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Sphingomonas starnbergensis sp. nov. isolated from a prealpine fresh water lake

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Footnote

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 382^T = DSM 25077^T = LMG 26763^T is JN591314.

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ABSTRACT

A novel type of freshwater bacterium was isolated from the prealpine mesotrophic Starnberger See (Bavaria, southern Germany). Cells of strain 382^T were Gram-negative and rod-shaped, motile, and creamy white. The isolate was strictly aerobic, catalase- and oxidase- positive, and grew at pH values of 6 to 9 (optimum, pH 7) and temperatures of 10 to 37 °C (optimum, 28 °C). The genomic G+C content of strain 382^T was 64.1 mol%. Based on 16S rRNA gene sequence analyses strain 382^T belongs to the family *Sphingomonadaceae* and clusters within the genus *Sphingomonas*. *Sphingomonas histidinilytica* UM 2^T and *Sphingomonas wittichii* DSM 6014^T were the closest relatives as indicated by highest 16S rRNA gene sequence similarities (97.1 % and 96.8 %, respectively). *Sphingomonas paucimobilis* DSM 1098^T (the type species of genus *Sphingomonas*) exhibited 95.3 % sequence similarity. This affiliation of strain 382^T to the genus *Sphingomonas* is confirmed by the presence of Q-10 as the major respiratory quinone, two sphingoglycolipids, $C_{14:0}$ 2-OH as the major 2-hydroxy fatty acids, and *sym*-homospermidine as the major polyamine. The main cellular fatty acids of strain 382^T were $C_{18:1}\omega7c$ (39 %), $C_{16:1}\omega7c$ (21 %), $C_{16:0}$ (10 %) and $C_{14:0}$ 2OH (10 %). Based on the phylogenetic distance to other species of the genus *Sphingomonas* and its unusually high $C_{16:1}\omega7c$ content, strain 382^T represents a new species of the genus *Sphingomonas* for which the name *Sphingomonas starnbergensis* is proposed. The type strain is 382^T (=DSM 25077^T=LMG 26763^T).

Members of the genus *Sphingomonas* were initially recognized by the presence of specific sphingoglycolipids (Yabuuchi *et al.*, 1990). The genus was later divided into the four genera *Sphingomonas sensu stricto*, *Sphingobium*, *Novosphingobium* and *Sphingopyxis* based on phylogenetic, chemotaxonomic and physiological evidence (Takeuchi *et al.*, 2001). Although Yabuuchi *et al.* (2002, 2005) did not accept the latter three genera, the dissection of the genus *Sphingomonas* into four genera (Takeuchi *et al*., 2001) has found wide acceptance in the literature. Recently, three additional genera were classified within the family *Sphingomonadaceae*, namely *Sphingosinicella* (Maruyama *et al*., 2006), *Stakelama* (Chen *et al*., 2010) and *Sphingomicrobium* (Kämpfer *et al*., in press). All members of the family *Sphingomonadaceae* tested for their presence have been shown to contain sphingoglycolipids, Q-10 as the major quinone, $C_{18:1}$ as the dominant fatty acid, and $C_{14:0}$ 2-OH as the major 2-hydroxy fatty acid whereas 3-hydroxy fatty acids are missing (Takeuchi *et al*., 2001).

Members of the genus *Sphingomonas sensu stricto* differ from the other genera by characteristic signatures that are found at positions 52:359 (C: G), 134 (G), 593 (G), 987:1218 (A:U) and 990:1215 (U:G) of the 16S rRNA (Takeuchi *et al.*, 2001) and from *Sphingobium*, *Novosphingobium* and *Sphingopyxis* by the presence of *sym*-homospermidine as the major polyamine. Typically, *Sphingomonas* species stain Gram-negative, are rod-shaped, non-motile or motile by means of single polar flagella, strictly aerobic, chemoheterotrophic, and form yellow-pigmented colonies. They contain $C_{18:1}$, $C_{16:0}$ and/or $C_{17:1}$ as major fatty acids as well as $C_{14:0}$ 2-OH or $C_{15:0}$ 2-OH as major 2-hydroxy fatty acids (Takeuchi *et al.*, 2001). The type species of the genus is *Sphingomonas paucimobilis* (Yabuuchi *et al.*, 1990). At the time of writing the genus *Sphingomonas* comprised about 50 recognized species. Here we present a polyphasic study describing a novel strain, designated as 382^T, of the genus *Sphingomonas* which was isolated from lakewater and formed creamy white colonies.

Strain 382^T was isolated from a water sample obtained from mesotrophic prealpine lake Starnberger See (584 m above sea level; maximum water depth, 128 m). Water was collected on December 20, 2007, from a pier located on the eastern shore near the village of Ammerland (47°55′N, 11°02′E) at a depth of 1 m using a bilge pump (Overmann *et al.*, 1998). Primary enrichments were established in basic synthetic freshwater medium buffered with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Bartscht *et al*., 1999) and

supplemented with 20 amino acids, glucose, pyruvate, citrate, 2-oxoglutarate, succinate (200 µM each), Tween 80 (0.001 % v/v), formate, acetate and propionate (200 µM each), trace element solution SL 10, and 10-vitamin solution (Jaspers *et al*., 2001). Signal molecules (cAMP, *N*-butyryl homoserine lactone, *N*-oxohexanoyl-DL-homoserine lactone, ATP) were added at 10 µM final concentration for growth stimulation (Bruns *et al*., 2002). For purification of the strains, agar plates were prepared with washed agar (3 times washed with distilled water), basic synthetic freshwater medium and 1:10 diluted HD (0.05 % casein peptone, 0.01 % glucose, 0.025 % yeast extract, w/v). Type strains of the species *Sphingomonas paucimobilis* DSM 1098^T, *Sphingomonas wittichii* DSM 6014^T, *Sphingomonas panni* DSM 15761^T, *Sphingomonas echinoides* DSM 1805^T, and *Sphingomonas alaskensis* DSM 13593^T were obtained from the DSMZ collection. At the time of experimentation authentic cultures of *Sphingomonas histidinilytica* strain UM2^T (= MTCC 9473^T = CCM 7545^T) were not available for comparison.

All strains were routinely cultivated on medium DSM 830 (R2A medium, containing 0.05 % w/v yeast extract, 0.05 % peptone, 0.05 % casamino acids, 0.05 % glucose, 0.05 % starch, 0.03 % sodium pyruvate, 0.03 % K_2HPO_4, 0.005 % $MgSO_4$, 1.5 % agar; pH, 7.2) (Reasoner & Geldreich, 1985) and at an incubation temperature of 28 °C for comparative physiological testing. Growth was tested between pH values of 5 and 10 (at intervals of 1). Temperature dependence of growth was assessed between 8 °C and 45 °C (at intervals 3°C) using a Model TN-3 temperature gradient incubator (Sangyo Co., LTD). Cell morphology was determined by phase-contrast microscopy using agar-coated slides (Pfennig & Wagener, 1986) and by transmission electron microscopy (TEM). Cells were fixed with 2 % glutaraldehyde and 5 % formaldehyde in growth medium, washed with TE buffer (10 mM Tris-Cl, pH 7.5; 1 mM EDTA) and negatively stained with 4% aqueous uranyl acetate using a carbon film on mica. Samples were examined in a TEM910 transmission electron microscope (Carl Zeiss, Oberkochen) at an acceleration voltage of 80 kV. Cell motility was evaluated using light microscopy and soft agar (containing 0.1 % w/v yeast extract, 0.01 % K_2HPO_4, 0.2 % agar).

The Gram-type was determined using Bactident amino peptidase and confirmed by KOH testing. Catalase activity was assessed using 10 % (v/v) H_2O_2 and oxidase activity using N, N, N′, N′-tetramethyl-ρ-phenylenediamine. Physiological and biochemical characteristics, and enzyme activities were determined by using API 20NE, API ZYM, API 50CH (bioMérieux), and Biolog

Gen III microplates (BiOLOG, Hayward, CA, USA) according to the instructions of the manufacturer. Susceptibility to antibiotics was determined on R2A agar plates using the disc diffusion method (CLSI, 2007) and the following antibiotics (in µg per disc): penicillin G (10), oxacillin (5), ampicillin (10), ticarcillin (75), cefalotin (30), mezlocillin (30), cefazolin (30), cefotaxim (30), aztreonam (30), chloramphenicol (30), tetracyclin (30), imipenem (10), gentamycin (10), amikacin (30), vancomycin (30), erythromycin (15), lincomycin (15), ofloxacin (5), colistin (10), norfloxacin (10), pipemidic (20), bacitracin (10), polymyxin B (300), nitrofurantoin (100), neomycin (30), kanamycin (30), doxycycin (30), clindamycin (10), ceftriaxone (30), fosfomycin (50), nystatin (100), linezolid (10), moxifloxacin (5), quinupristin/dalfopristin (15), piperacillin/tazobactam (40), and teicoplanin (30).

Fatty acids, respiratory quinones, polar lipids, and polyamines were extracted from cells grown on R2A medium. Standardization of the physiological age of the cultures was obtained by choosing a sector from a quadrant streak of the agar plates. About 40 mg wet weight of fresh cells were harvested and extracted according to the standard protocol (Sasser, 1990) of the Microbial Identification System (MIDI Inc.; version 6.1) for fatty acids analysis. Compounds were identified against the TSBA40 peak naming table database. Respiratory quinones were extracted from 200 mg freeze-dried cell material and analysed according to the method described by Tindall (1990a, b; 2005). Respiratory quinones were first separated into their structural classes (such as menaquinones, ubiquinones) by thin layer chromatography (TLC). The resulting bands were eluted and further separated and identified by HPLC, using an RP_{18} column (Tindall, 1996). Polar lipids were extracted from 100 mg freeze-dried cell material, the lipids separated by two-dimensional chromatography and identified by their R_F values in combination with their reaction with specific staining reagents (Tindall 1990a, b). Polyamines were extracted from freeze-dried cells that were harvested at the late exponential growth phase according to Busse & Auling (1988). Analysis of polyamines were carried out using the equipment described by Stolz *et al.* (2007).

Genomic DNA was extracted using the High Pure PCR template preparation Kit (Roche). Primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Lane, 1991) were used to amplify the almost complete 16S rRNA gene. The resulting PCR products were purified with the QIAquick PCR purification

kit (Qiagen) and sequenced by the dideoxynucleotid method on an AB 3730 DNA Analyzer (Applied Biosystems) using the AmpliTaq FS Big Dye terminator cycle sequencing kit. Sequences were edited and assembled with the Vector NTI computer package (Invitrogen). Additional 16S rRNA gene sequences of the type strains of all species of *Sphingomonas* and of *Sphingobium yanoikuyae* DSM 7462^T as an out-group organism were retrieved from the GenBank database (Altschul *et al.*, 1997) and imported into the ARB program package (Ludwig *et al.*, 2004). Automated alignments were produced with the Fast Aligner tool and corrected manually according to secondary structure information. This yielded an alignment of approx. 1500 bp. A phylogenetic tree was constructed with the FastdnaML maximum likelihood algorithm as implemented in the ARB software package. Sequence accession numbers are provided in Fig. 1. The mol% G+C content of DNA was determined as described by Mesbah *et al.* (1989).

After 5 days of incubation at 28°C, strain 382^T had formed creamy white, circular, domed, convex and smooth colonies on both, R2A agar and 1:10 diluted HD agar. Cells were Gram-negative and asporogenous. The rod-shaped cells were 1.1-1.8 µm long and 0.4-0.65 µm wide (Fig. S1 in the supplementary material). The new isolate was strictly aerobic, catalase- and oxidase-positive and grew between pH values of 6 to 9 (optimum pH 7) and temperatures of 10 to 37°C (optimum 28°C). Additional characteristics of strain 382^T are given in the species description and Tables 1 and S1. It was not possible to compare strain 382^T with the type strain of the species *Sphingomonas histidinilytica* strain UM2^T (= MTCC 9473^T = CCM 7545^T), because the latter was not available in the MTCC and an incorrect deposit was present in the CCM, further indicating the need for clarity in the way the issue of the confirmation deposit of type material is handled (Tindall, 2008). Therefore all data of strain UM2^T had to be taken from Nigam *et al.* (2010).

Based on the nearly complete (1482 bp) 16S rRNA gene sequence of strain 382^T (JN591314), a comprehensive phylogenetic comparison with all type strains of *Sphingomonas* species was conducted. Based on this analysis, strain 382^T falls within the genus *Sphingomonas*. *Sphingomonas histidinilytica* UM 2^T, *Sphingomonas wittichii* DSM 6014^T, *Sphingomonas haloaromaticamans* DSM 13477^T and *Sphingmonas fennica* DSM 13665^T were the closest phylogenetic relatives and exhibited sequence similarities of 97.1 %, 96.6 %, 96.0 % and 95.8 %,

respectively (Fig. 1). The similarity between strain 382^T and the type strain of the type species of the genus, *Sphingomonas paucimobilis* DSM 1098^T, was 95.3 %. The genomic G+C content of strain 382^T was 64.1 mol%.

The assignment of strain 382^T to the genus *Sphingomonas* is supported by the results of the chemotaxonomic analyses. The respiratory lipoquinone of strain 382^T was ubiquinone-10 (Q-10). Diphosphatidylglycerol, phosphatidylglycerol, phosphatidyldimethylethanolamine, phosphatidylethanolamine, phosphatidylcholine, phosphatidylmonomethylethanolamine and sphingoglycolipids were identified as the major polar lipids (Fig. S2 in the supplementary material). The presence of sphingoglycolipids is reported to be characteristic for members of the genus *Sphingomonadaceae,* although their presence has not always been confirmed. Similar to other members of the genus *Sphingomonas,* the major polyamine of strain 382^T was *sym*-homospermidine [29.3 µmol (g dry mass)$^{-1}$], which was accompanied by small amounts of spermidine, spermine, putrescine and 1,3-diaminopropane [4.7, 1.5, 0.1 and 0.1 µmol (g dry mass)$^{-1}$, respectively], and trace amounts of cadaverine and norspermidine. Major fatty acids were $C_{18:1}\omega7c$ (39 %), summed feature 3 (21 %), and $C_{16:0}$ (10 %). $C_{14:0}$ 2-OH (10 %) was the major 2-hydroxy fatty acid (Table 2). Based on a combination of the absolute retention times, the biosynthetic pathways involved in fatty acid biosynthesis and the information provided by MIDI Inc., the fatty acid contained in the summed feature 3 was identified as $C_{16:1}\omega7c$ and will be referred to as such in this text. In addition, $C_{17:1}\omega6c$ (4 %), $C_{14:0}$ (4 %), $C_{15:0}$ (3 %), $C_{19:0}$ cyclo $\omega8c$ (2 %), 11-methyl $C_{18:1}\omega7c$ (2 %) and $C_{16:1}\omega5c$ (1 %) were identified as minor cellular fatty acids. Together with the phylogenetic distance, the different composition of cellular fatty acids with unusually high concentrations of $C_{16:1}\omega7c$, and the additional phenotypic differences to *S. histidinilytica* UM2^T indicate that strain 382^T represents a new species of *Sphingomonas*. Therefore we propose the novel species *Sphingomonas starnbergensis* sp. nov.

Description of *Sphingomonas starnbergensis* sp. nov.

Sphingomonas starnbergensis (starn.ber.gen´sis. N.L. fem. adj. *starnbergensis* from or pertaining to Lake Starnberg, Bavaria, Germany, from where the organism was isolated).

Colonies are creamy white, circular, domed, and convex on R2A agar or 1:10 diluted HD agar. Cells are Gram-negative and asporogenous, rod-shaped, 1.1-1.8 µm long and 0.4-0.65 µm

wide. Strictly aerobic, catalase- and oxidase-positive. Grows at pH values between 6 and 9 (optimum pH 7) and temperatures between 10 and 37 °C (optimum 28°C). Does not reduce nitrate to nitrite or nitrite to N_2. Produces alkaline phosphatase, leucine arylamidase, and β-glucosidase, but not acid phosphatase, arginine dihydrolase, α-chymotrypsine, cystine arylamidase, esterase (C4), esterase lipase (C8), α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase, naphthol-phosphohydrolase, protease, trypsine, urease, and valine arylamidase. Adonitol, L-arabinose, cellobiose, esculin, D-fructose, D-fucose, galactose, D-glucose, glycerol, D-maltose, D-mannitol, D-mannose, N-acetyl-glucosamine rhamnose, D-sorbitol, and D-xylose are utilized. Tests negative for utilization of adipic acid, amygdalin, D-arabinose, D-arabitol, L-arabitol, arbutin, dulcitol, erythritol, L-fucose, gelatin, β-gentiobiose, gluconate, glycerol, glycogen, inulin, inositol, 2-keto-gluconate, 5-keto-gluconate, D-lyxose, melizitose, melibiose, α-methyl-D-glucoside, α-methyl-D-mannoside, β-methyl-D-xyloside, phenylacetic acid, potassium gluconate, D-raffinose, ribose, salicin, L-sorbose, starch, sucrose, D-tagatose, trisodium citrate, D-turanose, xylitol, L-xylose. Cells are sensitive (inhibition zones >30 mm; no. in parenthesis give μg per disk) to penicillin G (10), ticarcillin (75), mezlocillin (30), cefazolin (30), cefotaxim (30), chloramphenicol (30), tetracyclin (30), imipenem (10), gentamycin (10), amikacin (30), vancomycin (30), erythromycin (15), ofloxacin (5), norfloxacin (10), pipemidic (20), nitrofurantoin (100), neomycin (30), kanamycin (30), doxycycin (30), ceftriaxone (30), linezolid (10), moxifloxacin (5). They are resistant to aztreonam (30), lincomycin (15), pipemidic (20), fosfomycin (50), nystatin (100), quinupristin/dalfopristin (15) and piperacillin/tazobactam (40).

Q-10 is the respiratory quinone. The major fatty acids are $C_{18:1}\omega7c$ (39 %), $C_{16:1}\omega7c$ (21 %), $C_{16:0}$ (10 %). $C_{14:0}$ 2-OH (10 %) is the major 2-hydroxy fatty acids. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidyldimethylethanolamine, phosphatidylethanolamine, phosphatidylcholine, phosphatidylmonomethylethanolamine and sphingoglycolipids. The major polyamine is *sym*-homosperimidine. The genomic DNA G+C content of strain 382^T is 64.1 mol%. The type strain is 382^T (= DSM 25077^T = LMG 26763^T) and was isolated from a fresh water lake (Starnberger See, Bavaria, Germany).

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FIGURE LEGENDS

Fig. 1. Rooted maximum likelihood phylogenetic tree based on 16 rRNA gene sequences showing the relationships among strain 382^T and type strains of other *Sphingomonas* species. The tree was constructed using the ARB software package (Ludwig *et al.*, 2004). Numbers at nodes indicate the level of bootstrap support (in %) based on 1000 resamplings of the dataset. *Sphingobium yanoikuyae* DSM 7462^T was used as the outgroup.

LEGENDS TO SUPPLEMENTARY FIGURE

Figure S1. A. Phase-contrast photomicrograph cells of strain 382^T. **B.** Transmission electron micrograph of negatively stained cells of strain 382^T.

Figure S2. Polar lipid pattern of strain 382^T after separation by two-dimensional thin-layer chromatogram (TLC) , detected with anis aldehyde (for all lipids), ninhydrin (for amino groups), *cis*-aconitin acid-anhydride (for phosphorus-containing lipids), meta-periodate/schiff (for vicinal hydroxy groups), molybdenum blue (for phosphor groups).
DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PMME, phosphatidylmonomethylethanolamine; PDME, phosphatidyldimethylethanolamine; AL1, unidentified aminolipids; L1-L4: unidentified lipids; SGL = sphingoglycolipids.

Table 1. Differential phenotypic and physiological characteristics of strain 382^T and closely related type strains of species in genus *Sphingomonas* and *Sphingopyxis alaskensis*.

Strains: 1, strain 382^T; 2, *Sphingomonas histidinilytica* UM2^T (all data taken from Nigam *et al.*, 2010); 3, *Sphingomonas panni* DSM 15761^T, 4, *Sphingomonas paucimobilis* DSM 1098^T; 5, *Sphingomonas echinoides* DSM 1805^T; 6, *Sphingopyxis alaskensis* DSM 13593^T. +, positive; -, negative; V, weak reaction; ?, borderline reaction; CW, creamy white; CC, cream-colored; Y: yellow; ND, not determined. For strains 3 to 6, data for cell width and length, motility, and G+C content were obtained from Yabuuchii & Kosako (2005).

All strains tested negative for: arginine dihydrolase, cystine arylamidase, α-fucosidase, α-galactosidase, β-glucuronidase, lipase (C14), α-mannosidase, protease, urease, fermentation of glucose, Gram-staining, indole production, nitrite reduction to N_2, adipic acid, D-arabinose, D-arabitol, L-arabitol, dulcitol, erythritol, L-fucose, gelatin, β-gentiobiose, gluconate, glycerol, glycogen, inulin, inositol, 2-keto-gluconate, 5-keto-gluconate, β-methyl-D-xyloside, phenylacetic acid, potassium gluconate, ribose, L-sorbose, starch, D-tagatose, xylitol, L-xylose. No data on these characteristics are available for *Sphingomonas histidinilytica* UM2^T.

All strains tested positive for: alkaline phosphatase, leucine arylamidase, catalase, β-glucosidase, aerobic growth, cellobiose, D-fucose, maltose, D-xylose.

Substrate or test	1	2	3	4	5	6
Cell width (μm)	0.4-0.65	0.4	0.5-0.7	0.7	0.8	0.2-0.5
Cell length (μm)	1.1-1.8	1.4	1.2-2.0	1.4	1.9	0.5-3
Motility	-	+	-	+	+	+
G+C content (mol%)	64.1	66.9	ND	65	65.8	65
Pigmentation of colonies	CW	CC	Y	Y	Y	Y
Growth in the presence of 4% NaCl	+	ND	+	+	+	-
Nitrate reduction to nitrite	-	V	-	-	-	-
N-Acetyl-β-glucosaminidase	-	ND	+	+	-	-
Acid phosphatase	-	ND	+	+	+	+
α-Chymotrypsine	-	ND	-	+	-	-
Esterase (C4)	-	ND	-	+	V	V
Esterase lipase (C8)	-	ND	+	-	V	V
β-Galactosidase	-	ND	+	+	?	-
α-Glucosidase	-	ND	+	+	+	+
Naphthol-phosphohydrolase	-	ND	V	-	V	V
Oxidase	+	-	-	+	+	-
Trypsine	-	ND	-	V	-	+
Valine arylamidase	-	ND	+	+	+	-
L-Arabinose	+	+	+	+	+	-
D-Fructose	+	ND	+	+	-	-
Galactose	+	-	+	+	+	-
D-Glucose	+	-	+	+	+	+

Glycerol	+	ND	-	-	-	-
Lactose	?	-	+	+	V	-
D-Lyxose	-	ND	+	V	+	V
D-Maltose	+	ND	-	+	+	+
D-Mannitol	+	ND	-	-	-	-
D-Mannose	+	+	+	+	-	-
Melezitose	-	ND	-	+	-	-
Melibiose	-	+	-	+	-	-
D-Raffinose	-	ND	-	+	-	-
Rhamnose	+	ND	+	-	-	-
Sucrose	-	-	+	+	+	-
Trehalose	V	-	+	+	+	-
D-Turanose	-	ND	V	+	+	-
Adonitol	+	ND	-	-	-	-
Amygdalin	-	ND	+	+	-	-
Arbutin	-	ND	+	V	-	-
Salicin	-	ND	-	V	-	-
D-Sorbitol	+	ND	-	-	-	-
α-Methyl-D-glucoside	-	ND	-	+	-	-
α-Methyl-D-mannoside	-	ND	-	+	-	-
N-Acetyl-glucosamine	+	ND	+	+	+	-
Capric acid	V	ND	-	+	-	-
Malic acid	V	ND	+	+	-	+
Trisodium citrate	-	ND	+	-	-	-

Table 2. Cellular fatty acid profiles of strain 382^T and type strains of the related species of the genus *Sphingomonas* and *Sphingopyxis alaskensis*.

Strains: 1, strain 382^T; 2, *Sphingomonas histidinilytica* UM2^T (taken from Nigam *et al.*, 2010); 3, *Sphingomonas paucimobilis* DSM 1098^T; 4, *Sphingomonas wittichii* DSM 6014^T; 5, *Sphingomonas panni* DSM 15761^T; 6, *Sphingomonas echinoides* DSM 1805^T; 7, *Sphingopyxis alaskensis* DSM 13593^T. All data except for 2 were generated in this study. Values shown are percentages of the total fatty acids. -, not detected; trace (<1%).

Strains	1	2**	3	4	5	6	7
$C_{14:0}$	4	1	tr	2	tr	tr	-
$C_{14:0}$ 2OH	10	8	7	8	4	8	1
$C_{15:0}$	3	-	-	-	3	-	3
$C_{15:0}$ 2OH	tr	-	-	-	1	-	9
$C_{15:1}$ ω6c	tr	-	-	-	-	-	tr
$C_{16:1}$ ω7c	21	8#	5	11	13	2	7
$C_{16:1}$ ω5c	1	4	tr	2	2	tr	tr
$C_{16:0}$	10	12	9	16	9	12	5
$C_{16:0}$ 2OH	-	-	-	-	-	-	1
$C_{16:1}$ 2OH	-	-	-	-	-	tr	-
$C_{17:1}$ ω8c	-	tr	-	-	1	-	9
$C_{17:1}$ ω6c	4	9	tr	-	12	tr	39
$C_{17:0}$	tr	1	-	-	2	tr	4
$C_{18:1}$ ω7c	39	33§	75	54	52	64	20
$C_{18:1}$ ω5c	-	2	2	-	tr	1	tr
$C_{18:0}$	tr	tr	tr	-	-	tr	tr
11-Methyl $C_{18:1}$ω7c	2	4	-	3	-	9	2
$C_{19:0}$ cyclo ω8c	2	16	-	4	-	tr	-
Summed feature 7 *	-	tr	-	-	-	-	tr

* Summed feature 7 contains C_{19} Cyclo ω10c and/or C_{19} ω6c.

** In addition, *Sphingomonas histidinilytica* UM2^T contains trace amounts of $C_{12:0}$, $C_{13:0}$ 2OH, $C_{18:0}$ ISO, $C_{20:0}$ ISO, $C_{20:2}$ ω6,9c, summed feature 4 (consists of $C_{17:1}$ ISO I and/or $C_{17:1}$ anteiso B), summed feature 9 (consists of $C1_{6:0}$ 10-methyl and/or $C_{17:1}$ω9c iso).

\# Listed as summed feature 3 C16 : 1 ω 7c and/or C16 : 1 ω 6c (at the time of writing the annotation used in many of the MIDI series 6 peak naming tables). Without access to the absolute retention times or the strain the identity of this peak as C16:1 ω7c cannot be confirmed.

§ Listed as summed feature 8 C18 : 1 ω 6c and/or C18 : 1 ω 7c. Without access to the absolute retention times or the strain the identity of this peak as C18:1 ω7c cannot be confirmed.

Supplementary Table S1. Susceptibilities of strain 382^T against 36 antibiotic agents.

Strains: 1, strain 382^T; 2, *Sphingomonas histidinilytica* UM2^T (taken from Nigam *et al.*, 2010); 3, *Sphingomonas paucimobilis* DSM 1098^T; 4, *Sphingomonas wittichii* DSM 6014^T; 5, *Sphingomonas panni* DSM 15761^T; 6, *Sphingomonas echinoides* DSM 1805^T; 7, *Sphingomonas alaskensis* DSM 13593^T. All data except for (2) were generated in this study. R, resistant; IM, intermediate; S, susceptible; ND, not detected.

antibiotic	1	2	3	4	5	6	7
Penicillin G	S	S	R	IM	IM	IM	S
Oxacillin	IM	ND	R	R	R	R	S
Ampicillin	IM	S	IM	S	IM	S	S
Ticarcillin	S	ND	IM	S	IM	IM	S
Cefalotin	IM	ND	IM	R	R	R	S
Mezlocillin	S	ND	R	IM	IM	R	S
Cefazolin	S	ND	IM	R	R	R	S
Cefotaxim	S	ND	S	IM	IM	S	S
Aztreonam	R	ND	R	R	IM	R	S
Chloramphenicol	S	S	S	S	R	S	S
Tetracyclin	S	ND	S	S	S	S	S
Imipenem	S	ND	S	S	IM	S	S
Gentamycin	S	S	IM	IM	IM	IM	IM
Amikacin	S	S	IM	S	IM	S	S
Vancomycin	S	S	S	S	S	S	S
Erythromycin	S	ND	S	IM	S	IM	S
Lincomycin	R	ND	R	R	R	R	R
Ofloxacin	S	ND	S	IM	R	S	S
Colistin	IM	ND	IM	IM	IM	IM	IM
Norfloxacin	S	ND	IM	IM	R	S	S
Pipemidic	R	ND	R	R	R	R	IM
Bacitracin	IM	ND	IM	R	IM	R	IM
Polymyxin B	IM	S	IM	IM	IM	IM	IM
Nitrofurantoin	S	ND	R	R	IM	IM	IM
Neomycin	S	R	IM	S	IM	S	IM
Kanamycin	S	S	IM	S	IM	S	S
Doxycycin	S	ND	S	S	S	S	S
Clindamycin	IM	ND	IM	R	R	R	IM
Ceftriaxone	S	ND	IM	IM	IM	IM	S
Fosfomycin	R	ND	IM	S	IM	S	R
Nystatin	R	ND	R	R	R	R	R
Linezolid	S	ND	IM	R	R	IM	IM
Moxifloxacin	S	ND	S	IM	R	IM	S
Quinupristin/dalfopristin	R	ND	IM	R	S	IM	S
Piperacillin/tazobactam	R	ND	IM	IM	IM	IM	S
Teicoplanin	IM	ND	IM	IM	IM	IM	S

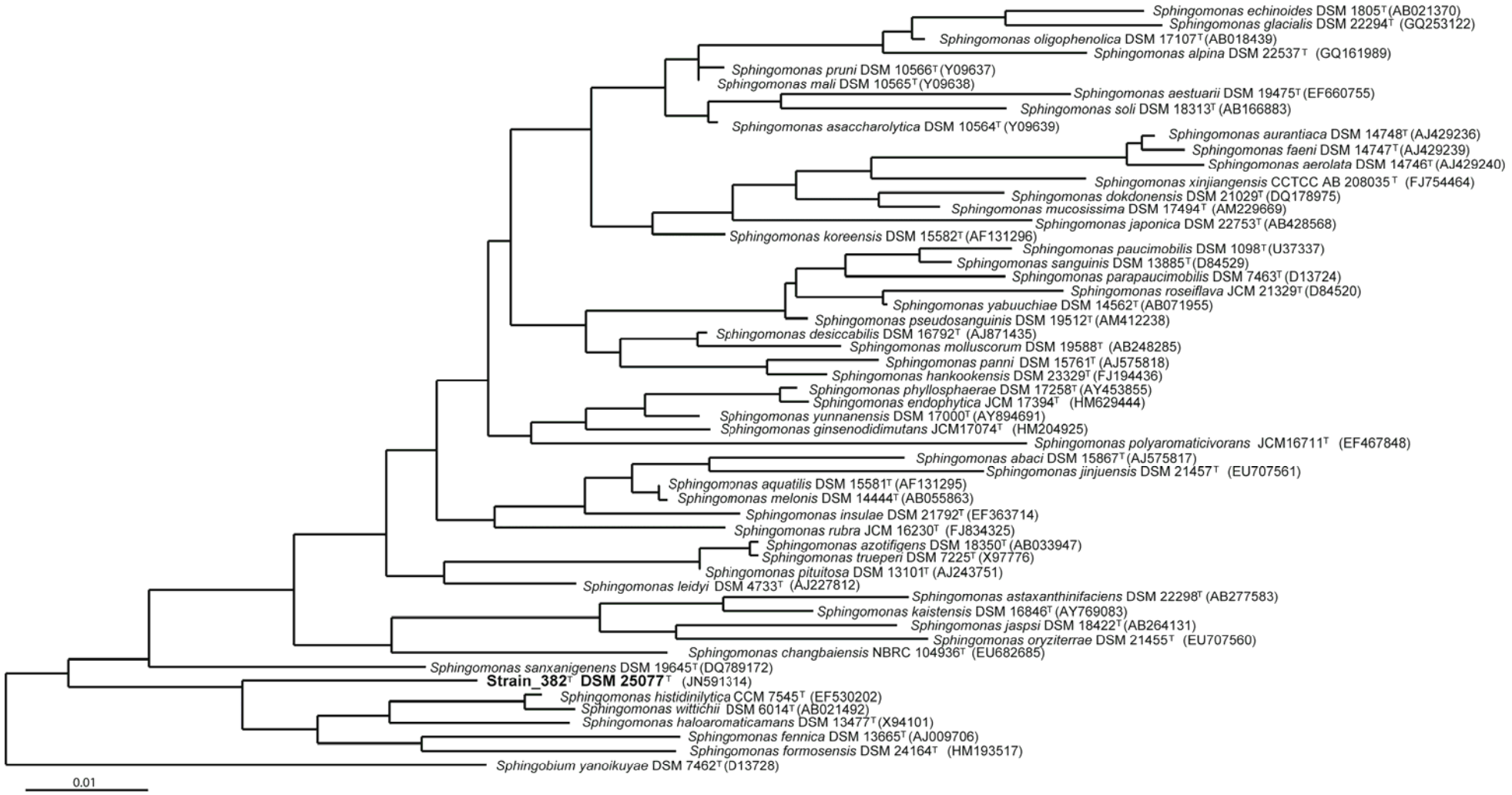

Figure 1
Chen et al. (2012)