

Sphingomonas yunnanensis sp. nov., a novel Gram-negative bacterium from a contaminated plate

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A Gram-negative bacterium, YIM 003^T, which was isolated from a contaminated plate in the laboratory, was subjected to a polyphasic taxonomic study. The organism had short-rod-shaped, motile cells, formed yellow-pigmented colonies on ISP2 medium and its optimum growth pH was 7.0–7.5. The major respiratory lipoquinone was ubiquinone Q-10. The phosphate-containing lipids detected in strain YIM 003^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, sphingoglycolipid and one unidentified phospholipid. The major fatty acids were C_{18:1 ω 7c} (59.8%), C_{16:0} (9.9%), ai-C_{17:0} (5.3%), i-C_{17:0} (4.4%) and C_{14:0} 2-OH (15.8%). The G+C content of the genomic DNA was 67.5 mol%. Strain YIM 003^T exhibited levels of 16S rRNA gene sequence similarity of 98.2% to *Sphingomonas phyllosphaerae* FA2^T and 98.0% to *Sphingomonas adhaesiva* DSM 7418^T but showed less than 97.0% similarity with respect to other species with validly published names. The DNA–DNA relatedness values of the isolate with *S. phyllosphaerae* FA2^T and *S. adhaesiva* DSM 7418^T were 59 and 26%, respectively. The phenotypic characteristics and genotypic data indicate that strain YIM 003^T should be distinguished from *S. phyllosphaerae* FA2^T and *S. adhaesiva* DSM 7418^T. Therefore, on the basis of the polyphasic taxonomic data presented, a novel species of the genus *Sphingomonas*, *Sphingomonas yunnanensis* sp. nov., is proposed, with the type strain YIM 003^T (= CCTCC AB 204064^T = KCTC 12346^T).

The genus *Sphingomonas* was first proposed by Yabuuchi *et al.* (1990) and its description was later emended several times by Takeuchi *et al.* (1993, 2001), Yabuuchi *et al.* (2002) and Busse *et al.* (2003). Members of the genus *Sphingomonas* are yellow-pigmented, Gram-negative, aerobic, non-spore-forming, non-fermentative, motile or non-motile rods and are characterized chemotaxonomically by the presence of ubiquinone Q-10 and 2-hydroxy fatty acids and by the absence of 3-hydroxy fatty acids. In this paper, we report the results of our polyphasic taxonomic study on strain YIM

003^T, which was picked from a contaminated plate in our laboratory. The 16S rRNA gene sequence of strain YIM 003^T, containing the signature nucleotides that are specific for the genus *Sphingomonas* (Takeuchi *et al.*, 2001), combined with the morphological, physiological and chemotaxonomic characteristics and phylogenetic analyses demonstrated that the strain should be assigned to a novel species of the genus *Sphingomonas*.

During the conservation of some useful microbes, with different bioactivities, in a refrigerator in our laboratory, one yellow-pigmented colony was found on an ISP2 agar plate. The strain, YIM 003^T, grew well on ISP2 plates (Shirling & Gottlieb, 1966) at 28 °C. Cells used for biochemical and molecular systematic analysis were grown in shake flasks (about 150 r.p.m.) containing ISP2 medium at 28 °C for 4 days. Stock cultures were maintained at 4 °C using ISP2 slants and as glycerol suspensions (20%, v/v) at –20 °C.

Strain YIM 003^T was incubated on ISP2 and nutrient agar media for observation of cells and colony morphology,

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 003^T is AY894691.

A two-dimensional thin-layer chromatogram of the phosphate-containing lipids of strain YIM 003^T is available as a supplementary figure in IJSEM Online.

respectively. The morphology of strain YIM 003^T was observed under a light microscope (model BH 2; Olympus) and using a transmission electron microscope (Hitachi H-800) after 48 h growth on ISP2 agar medium. The cells of strain YIM 003^T were aerobic, motile, non-spore-forming and short-rod-shaped (about 0.4–0.6 µm wide and 0.8–1.0 µm long), each bearing a single polar flagellum. Strain YIM 003^T formed yellow colonies with a maximum diameter of about 5 mm after incubation at 28 °C for 48 h on ISP2 agar. Colonies on ISP2 medium were circular, slightly convex and opaque. No diffusible pigments were observed on any medium. The cellular morphology of strain YIM 003^T is largely like that of reference strain *Sphingomonas phyllosphaerae* FA2^T (Rivas *et al.*, 2004).

Gram-staining was determined as described by Moaledj (1986), with 48 h cultures. All of the other physiological and biochemical tests were performed at 28 °C as described previously (Li *et al.*, 2004). The pH, NaCl concentration and temperature ranges for growth were pH 6.5–8.0, 0–5 % and 0–40 °C using ISP2 as the basic medium; the optimum pH, NaCl concentration and temperature range for growth were pH 7.0–7.5, 0–1 % and 28 °C. The isolate was catalase- and oxidase-positive. Methyl red and Voges–Proskauer tests and urease, melanin, tyrosinase and Tween 80 esterase production were negative, while milk peptonization and coagulation, nitrate reduction and Tween 20 esterase tests

were positive. Details of the physiological and biochemical properties are given in Table 1 and in the species description.

The respiratory isoprenoid quinones were isolated, purified and analysed as described by Lee *et al.* (2001). Only the phosphate-containing fraction was analysed according to the method of Ventosa *et al.* (1993), using molybdenum blue as the spray reagent; the designations were as referred to by Busse *et al.* (1999) and Rivas *et al.* (2004). Fatty acid analysis was performed using the standard method of Sasser (1990) and the results were compared with the database of fatty acids in the Sherlock Microbial Identification System (MIDI). Genomic DNA was isolated and purified by using the method of Marmur (1961). The DNA G + C content of strain YIM 003^T was measured by using the thermal denaturation method (Marmur & Doty, 1962), with a Shimadzu-1601 spectrophotometer.

The major respiratory lipoquinone of strain YIM 003^T was ubiquinone Q-10. The phosphate-containing lipids detected were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, sphingoglycolipid and one unidentified phospholipid (see the two-dimensional thin-layer chromatogram available as a supplementary figure in IJSEM Online). The fatty acid profile of strain YIM 003^T was composed mainly of C_{18:1}ω7c

Table 1. Phenotypic differences among strain YIM 003^T and its two closest phylogenetic relatives, *S. phyllosphaerae* FA2^T and *S. adhaesiva* DSM 7418^T

Data for reference strains were taken from Rivas *et al.* (2004) (*S. phyllosphaerae* FA2^T) and Yabuuchi *et al.* (1990) (*S. adhaesiva* DSM 7418^T). All three strains are aerobic, Gram-negative, yellow-pigmented, non-spore-forming, motile rod-shaped bacteria that contain sphingoglycolipid and have ubiquinone Q-10 as the major respiratory lipoquinone. +, Positive; –, negative; W, weakly positive; ND, not determined.

Characteristic	<i>S. yunnanensis</i> YIM 003 ^T	<i>S. phyllosphaerae</i> FA2 ^T	<i>S. adhaesiva</i> DSM 7418 ^T
Nitrite from nitrate	+	–	–
Gelatinase	–	–	+
Acid from:			
Glycerol	–	–	+
Maltose	–	+	+
Rhamnose	–	+	+
Salicin	–	–	+
Galactose	+	+	–
Trehalose	–	+	+
Tween 20 esterase	+	–	ND
Oxidase	+	W	+
Milk peptonization and coagulation	+	–	–
Polar lipids*	PE, PG, DPG, PC, SGL, PL	PE, PG, DPG, PC, SGL, PL1, PL2	PME, PE, PG, DPG, PDE, PC, SGL, APL1, PL1, PL2, PL3, GL2
DNA G + C content (mol%)	67.5	61	67.2

*Abbreviations: APL1, unidentified aminophospholipid; DPG, diphosphatidylglycerol; GL2, unidentified glycolipid; PC, phosphatidylcholine; PDE, phosphatidyltrimethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, PL1, PL2, PL3, unidentified phospholipids; PME, phosphatidylmonomethylethanolamine; SGL, sphingoglycolipid.

(59.8%), C_{16:0} (9.9%), ai-C_{17:0} (5.3%), i-C_{17:0} (4.4%) and C_{14:0} 2-OH (15.8%). The DNA G+C content was determined to be 67.5 mol%.

The 16S rRNA gene sequence of the isolate was amplified by a PCR using conserved primers close to the 3' and 5' ends of the gene, as described previously (Cui *et al.*, 2001). Multiple alignments with sequences of a broad selection of members of the family *Sphingomonadaceae*, and calculations of levels of sequence similarity, were carried out using CLUSTAL X software (Thompson *et al.*, 1997). A phylogenetic tree was reconstructed using the neighbour-joining method of Saitou & Nei (1987) from K_{nuc} values (Kimura, 1980, 1983). The topology of the phylogenetic tree was evaluated by using Felsenstein's bootstrap resampling method (Felsenstein, 1985) with 1000 replicates.

A nearly-complete 16S rRNA gene sequence (1415 bp) for strain YIM 003^T was obtained and subjected to a comparative analysis. Phylogenetically, strain YIM 003^T was closest to *S. phyllosphaerae* FA2^T and *Sphingomonas adhaesiva* DSM 7418^T, and the sequence similarities to the latter two type strains were 98.2 and 98.0%, respectively. They formed a distinct subclade in the phylogenetic tree of all members of the genus *Sphingomonas* (see Fig. 1; not all of the relatives are shown). Additionally, the 16S rRNA gene sequence of strain YIM 003^T, containing the signature nucleotides specific for the genus *Sphingomonas* cluster I (Takeuchi *et al.*, 2001), such as 52–359 (C–G), 134 (G), 593 (G), 987–1218 (G–C), 990–1215 (U–G), confirmed that the tested strain should be classified in the genus *Sphingomonas*.

DNA–DNA hybridizations were carried out among strains YIM 003^T, *S. phyllosphaerae* FA2^T and *S. adhaesiva* DSM 7418^T by applying the optical renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983; Jahnke, 1992) and using a UV-Vis spectrophotometer (model UV1601; Shimadzu) under optimal hybridization conditions. The values obtained were respectively 59 and 26% (repeated twice) for DNA–DNA relatedness between strain YIM 003^T and *S. phyllosphaerae* FA2^T and *S. adhaesiva* DSM 7418^T. Both values were lower than 70%, which is the value considered to be the threshold for the delineation of genospecies (Stackebrandt & Goebel, 1994), and clearly indicated that the novel isolate, YIM 003^T, belonged to a different genomic species with respect to *S. phyllosphaerae* FA2^T and *S. adhaesiva* DSM 7418^T.

On the basis of morphological, phylogenetic and chemotaxonomic data, strain YIM 003^T should be placed in the genus *Sphingomonas*. The differences between strain YIM 003^T and the two most closely related species of the genus *Sphingomonas* justify the description of a novel species, for which the name *Sphingomonas yunnanensis* sp. nov. is proposed.

Description of *Sphingomonas yunnanensis* sp. nov.

Sphingomonas yunnanensis (yun.nan.en'sis. N.L. fem. adj. *yunnanensis* pertaining to Yunnan, a province of south-west China).

Cells are aerobic, motile, non-spore-forming and short-rod-shaped and about 0.4–0.6 µm wide and 0.8–1.0 µm long with single polar flagella. Forms yellow-pigmented colonies with a maximum diameter of about 5 mm after incubation at 28 °C for 48 h on ISP2 agar. Colonies on ISP2 medium are circular, slightly convex and opaque. The optimum growth pH, NaCl concentration and temperature are 7.0–7.5, 0–1% and 28 °C, respectively. Catalase- and oxidase-positive. Starch is not decomposed. Positive for lipase, β-glucosidase, β-galactosidase, α-galactosidase, α-glucosidase, α-maltosidase, arylamidase, milk peptonization and coagulation, nitrate reduction, Tween 20 esterase and hydrolysis of aesculin. Negative for ornithine decarboxylase, arginine dihydrolase, lysine decarboxylase, urease, indole production, β-glucuronidase and gelatinase, in methyl red and Voges–Proskauer tests and for tyrosinase, Tween 80 esterase, melanin, H₂S and indole production. Acetamide, malonate, glucose, galactose, mannose, xylose, ribose, lactose, dextrin, amygdalin, fructose and *N*-acetyl-α-glucosamine can be utilized as sole carbon sources. Acid is produced from acetamide, lactose, galactose and mannose, whereas L-arabitol, D-arabitol, L-arabinose, galacturonate, phenol red, mannitol, 5-ketogluconate, maltose, sucrose, trehalose, rhamnose, inositol, palatinose, cellobiose and sorbitol are not used. The major respiratory lipoquinone is ubiquinone Q-10. The polar lipids contain diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, sphingoglycolipid and one unidentified phospholipid. The cellular fatty acid profile is composed mainly of C_{18:1ω7c} (59.8%), C_{16:0} (9.9%), ai-C_{17:0} (5.3%), i-C_{17:0} (4.4%) and C_{14:0} 2-OH (15.8%). The DNA G+C content is 67.5 mol%.

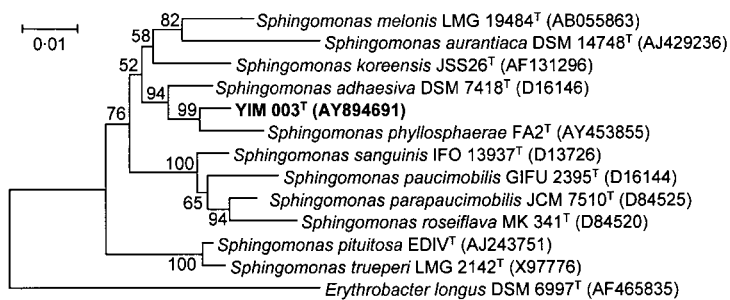


Fig. 1. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rRNA gene sequences, showing the position of strain YIM 003^T among phylogenetic neighbours. Numbers on branch nodes are bootstrap values (1000 resamplings). The sequence of *Erythrobacter longus* DSM 6997^T was used as the outgroup. Bar, 1% sequence divergence.

The type strain, YIM 003^T (= CCTCC AB 204064^T = KCTC 12346^T), was isolated from a contaminated plate. CCTCC is the China Center of Type Culture Collection (Wuhan City, Hubei Province, China).

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