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

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A Gram-negative bacterium, YIM 003T, 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 003T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, sphingoglycolipid and one unidentified phospholipid. The major fatty acids were C18:1ω7c (59.8 %), C16:0 (9.9 %), ai-C17:0 (5.3 %), i-C17:0 (4.4 %) and C14:0 2-OH (15.8 %). The G+C content of the genomic DNA was 67.5 mol%. Strain YIM 003T exhibited levels of 16S rRNA gene sequence similarity of 98.2 % to Sphingomonas phyllosphaerae FA2T and 98.0 % to Sphingomonas adhaesiva DSM 7418T 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 FA2T and S. adhaesiva DSM 7418T were 59 and 26 %, respectively. The phenotypic characteristics and genotypic data indicate that strain YIM 003T should be distinguished from S. phyllosphaerae FA2T and S. adhaesiva DSM 7418T. 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 003T (= CCTCC AB 204064T = KCTC 12346T).

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 003T, which was picked from a contaminated plate in our laboratory. The 16S rRNA gene sequence of strain YIM 003T, 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 003T, 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 003T was incubated on ISP2 and nutrient agar media for observation of cells and colony morphology,
respectively. The morphology of strain YIM 003T 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 003T 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 003T 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 003T is largely like that of reference strain Sphingomonas phyllosphaerae FA2T (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 003T was measured by using the thermal denaturation method (Marmur & Doty, 1962), with a Shimadzu-1601 spectrophotometer.

The major respiratory lipoquinone of strain YIM 003T was ubiquinone Q-10. The phosphate-containing lipids detected were diphostatidglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, sphingoglycolipid and one unidentified phospholipid (see the supplementary figure in IJSEM Online). The fatty acid profile of strain YIM 003T was composed mainly of C18:1ω7c.

Table 1. Phenotypic differences among strain YIM 003T and its two closest phylogenetic relatives, S. phyllosphaerae FA2T and S. adhaesiva DSM 7418T

Data for reference strains were taken from Rivas et al. (2004) (S. phyllosphaerae FA2T) and Yabuuchi et al. (1990) (S. adhaesiva DSM 7418T). 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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>S. yunnanensis YIM 003T</th>
<th>S. phyllosphaerae FA2T</th>
<th>S. adhaesiva DSM 7418T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrite from nitrate</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gelatinase</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acid from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Salicin</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Trehalose</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Tween 20 esterase</td>
<td>+</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Milk peptonization and coagulation</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Polar lipids*</td>
<td>PE, PG, DPG, PC,</td>
<td>PE, PG, DPG, PC,</td>
<td>PME, PE, PG, DPG, PDE, PC,</td>
</tr>
<tr>
<td></td>
<td>SGL, PL</td>
<td>SGL, PL1, PL2</td>
<td>SGL, APL1, PL1, PL2, PL3, GL2</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>67·5</td>
<td>61</td>
<td>67·2</td>
</tr>
</tbody>
</table>

*Abbreviations: APL1, unidentified aminophospholipid; DPG, diphostatidglycerol; GL2, unidentified glycolipid; PC, phosphatidylcholine; PDE, phosphatidyl dimethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, PL1, PL2, PL3, unidentified phospholipids; PME, phosphatidymonoethanolamine; 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} 2-0H (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_{\text{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 
\textit{S. phyllosphaerae} FA2^T and \textit{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 \textit{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 \textit{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 \textit{Sphingomonas}.

DNA–DNA hybridizations were carried out among strains YIM 003^T, \textit{S. phyllosphaerae} FA2^T and \textit{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 \textit{S. phyllosphaerae} FA2^T and \textit{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 \textit{S. phyllosphaerae} FA2^T and \textit{S. adhaesiva} DSM 7418^T.

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, \( \beta \)-glucosidase, \( \beta \)-galactosidase, \( \alpha \)-galactosidase, \( x \)-glucosidase, \( x \)-maltohydrolase, amylopectin, maltose, rhamnose, inositol, palatinose, cellobiose and xylose are used. The major respiratory lipoquinone is ubiquinone Q-10. The polar lipids contain diphosphatidylethanolamine, phosphatidylglycerol and one unidentified phospholipid. The cellular fatty acid profile of strain YIM 003^T is composed mainly of C_{16 : 1} \( \omega \)-7c (59·8 %), C_{16 : 0} (9·9 %), ai-C_{17 : 0} (5·3 %), i-C_{17 : 0} (4·4 %) and C_{14 : 0} 2-0H (15·8 %). The DNA G+C content is 67·5 mol%.

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

### Description of \textit{Sphingomonas yunnanensis} sp. nov.

\textit{Sphingomonas yunnanensis} (yun.nan.en’s is. N.L. fem. adj. \textit{yunnanensis} pertaining to Yunnan, a province of south-west China).

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**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 \textit{Erythrobacter longus} DSM 6997^T was used as the outgroup. Bar, 1 % sequence divergence.
The type strain, YIM 003<sup>T</sup> (= CCTCC AB 204064<sup>T</sup> = KCTC 12346<sup>T</sup>), was isolated from a contaminated plate. CCTCC is the China Center of Type Culture Collection (Wuhan City, Hubei Province, China).

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**References**


