**Sphingomonas endophytica** sp. nov., isolated from *Artemisia annua* L.

Hai-Yu Huang,1† Jie Li,2† Guo-Zhen Zhao,1 Wen-Yong Zhu,1 Ling-Ling Yang,1 Hai-Yun Tang,1 Li-Hua Xu1 and Wen-Jun Li1,3

1Key Laboratory of Microbial Diversity in Southwest China, Ministry of Education and Laboratory for Conservation and Utilization of Bio-Resources, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China
2Key Laboratory of Marine Bio-resources Sustainable Utilization CAS, RNAM Center for Marine Microbiology, Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou 510301, PR China
3Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Ürümqi 830011, PR China

A novel bacterium (strain YIM 65583T) belonging to the genus *Sphingomonas* was isolated from surface-sterilized tissue of *Artemisia annua* L., which was collected from Yunnan province, south-west China. Its morphology, physiology and biochemical features were consistent with those of members of the genus *Sphingomonas*. Analysis of the 16S rRNA gene sequence of strain YIM 65583T further confirmed that it should be classified as a member of the genus *Sphingomonas* and was most closely related to *Sphingomonas phyllosphaerae* FA2T (99.7 %) and *Sphingomonas yunnanensis* YIM 003T (98.3 %). The isolate was Gram-negative and formed yellow-pigmented colonies on ISP 2 medium. It grew optimally at pH 6.0–8.0, at 20–37 °C and in the presence of 0–1 % (w/v) NaCl. The major respiratory lipoquinone was ubiquinone-10; C\textsubscript{18}\textsubscript{1}ω7c, anteiso-C\textsubscript{16}\textsubscript{1}ω1c, C\textsubscript{14}\textsubscript{0}ω2OH, C\textsubscript{17}\textsubscript{1}ω6c, C\textsubscript{16}\textsubscript{0} and C\textsubscript{15}\textsubscript{0} were the major fatty acids. Major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and sphingoglycolipid. The G+C content of the genomic DNA was 63.3 mol%. The DNA–DNA relatedness values of the isolate YIM 65583T with *S. phyllosphaerae* FA2T and *S. yunnanensis* YIM 003T were 43.1 % and 37.9 %, respectively. Based on these features, it is concluded that the strain represents a novel species of the genus *Sphingomonas*, for which the name *Sphingomonas endophytica* sp. nov. is proposed, with YIM 65583T (=CCTCC AA 209035T = JCM 17394T) as the type strain.

The genus *Sphingomonas* was first proposed by Yabuuchi et al. (1990) and subsequently emended by Takeuchi et al. (2001), who divided the original *Sphingomonas* into four separate genera, namely *Sphingomonas*, *Sphingobium*, *Novosphingobium* and *Sphingopyxis*. Members of the genus *Sphingomonas* are yellow- or orange-pigmented, Gram-negative, aerobic, non-spore-forming, non-fermentative, motile or non-motile rods and are characterized chemotaxonomically by the presence of ubiquinone-10 and 2-hydroxy fatty acids and by the absence of 3-hydroxy fatty acids (Zhang et al., 2005). At the time of writing, the genus *Sphingomonas* comprised 48 species with validly published names, including some recently described species (Huang et al., 2009; Roh et al., 2009; Romanenko et al., 2009). In this study, we report on the detailed taxonomic characterization of a *Sphingomonas*-like bacterium, strain YIM 65583T, which was isolated from *Artemisia annua* L. in Yunnan province, south-west China.

Strain YIM 65583T was isolated from the roots of *A. annua* L., collected in Yunnan province, south-west China. Samples were washed in running water to remove soil particles and sterilized by the established procedure (Li et al., 2008). The surface-sterilization process was verified by rolling surface-sterilized plant material on isolation medium and tryptic soy agar (TSA; BD) and then incubating the plates at 28 °C for 7 days. The surface-sterilized root samples were sliced into pieces and 100 g plant material was boiled with 1 l water for 1 h. The suspension was collected, then evaporated under reduced pressure to yield 100 ml plant
Sphingomonas genus 65583T was phylogenetically related to members of the 16S rRNA gene sequence analysis indicated that strain YIM data based on 1000 resamplings (Felsenstein, 1985). Bootstrap analysis was used to evaluate tree topology of the neighbour-joining PhyML-Alrt (Guindon & Gascuel, 2003). Bootstrap analysis and maximum-parsimony (Fitch, 1971), whereas a max−using the neighbour-joining method (Saitou & Nei, 1987) and phylogenetic trees were constructed generated according to Kimura’s two-parameter model (Kimura, 1980) and phylogenetic analysis indicated the novel isolate, YIM 65583T, formed a separate branch with high bootstrap support using neighbour-joining algorithms with S. phyllosphaerae FA2T and S. yunnanensis YIM 003T, both with bootstrap values of 100% in Fig. 1 (smaller tree with the 14 phylogenetically most closely related members of the genus), and with bootstrap values of 99 and 95%, respectively, in Fig. S3 (tree with all taxa in the genus Sphingomonas). It was also supported by the maximum-parsimony and maximum-likelihood algorithms with higher bootstrap values (Figs S4 and S5).

DNA–DNA hybridizations of strain YIM 65583T with S. phyllosphaerae FA2T and S. yunnanensis YIM 003T were carried out according to the fluorometric micro-well method (Ezaki et al., 1989; Jahnke, 1992; He et al., 2005). The values obtained were 43.1 % and 37.9 % (repeated six times) for DNA–DNA relatedness of strain YIM 65583T with S. phyllosphaerae FA2T and S. yunnanensis YIM 003T, respectively. These values are 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 65583T, belongs to a different genomic species with respect to S. phyllosphaerae FA2T and S. yunnanensis YIM 003T.

The DNA G+C content was determined by the method of Mesbah et al. (1989) with the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. The DNA G+C content of strain YIM 65583T was 63.3 mol%, which is similar to values reported for other Sphingomonas species (Yabuuchi et al., 2002).

Strain YIM 65583T was incubated on ISP 2 (4.0 g yeast extract, 10 g malt extract, 4.0 g glucose and 20 g agar per litre tap water, pH 7.2) medium for observation of cells and colony morphology, respectively. The morphology of strain YIM 65583T was observed under a light microscope (model BH2; Olympus) and using a transmission electron

![Fig. 1. Neighbour-joining phylogenetic tree based on almost-complete 16S rRNA gene sequences, showing the relationships between strain YIM 65583T and the 14 closest phylogenetic members. Numbers at nodes are levels of bootstrap support for branch points, based on 1000 resamplings; only values greater than 50% are shown. The sequence of Rhodospirillum rubrum ATCC 11170T was used as the outgroup. Bar, 2% nucleotide substitutions per position.](http://ijs.sgmjournals.org)
The cells of strain YIM 65583<sup>T</sup> were Gram-negative. The isolate grew over the temperature range 12–45 °C, pH range 6.0–8.0 and NaCl concentrations range 0–3 % (w/v). Optimal growth was observed between 20–37 °C, between pH 6.0–8.0 and NaCl concentrations range 0–1 % (w/v). Strain YIM 65583<sup>T</sup> differed from <i>S. phyllosphaerae</i> FA2<sup>T</sup> and <i>S. yunnanensis</i> YIM 003<sup>T</sup> (Zhang et al., 2004; Rivas et al., 2004; Zhang et al., 2005). The fatty acid profile of YIM 65583<sup>T</sup> was similar to those of <i>S. phyllosphaerae</i> FA2<sup>T</sup> and <i>S. yunnanensis</i> YIM 003<sup>T</sup>. The isoprenoid quinones detected in strain YIM 65583<sup>T</sup> were ubiquinone-8 (12.1 %), ubiquinone-9 (5.2 %) and ubiquinone-10 (93.6 %). This predominant ubiquinone type was the same as those of Sphingomonas species (Yabuuchi et al., 1990; Rivas et al., 2004; Zhang et al., 2005). The fatty acid profile of YIM 65583<sup>T</sup> was similar to those of Sphingomonas species. Furthermore, <i>S. phyllosphaerae</i> FA2<sup>T</sup> has iso-C<sub>15</sub>:<sub>0</sub> (1.48 %), anteiso-C<sub>16</sub>:<sub>1</sub> (10.29 %), anteiso-C<sub>15</sub>:<sub>0</sub> (1.38 %) and anteiso-C<sub>17</sub>:<sub>0</sub> (1.12 %), which were not detected in strain YIM 65583<sup>T</sup>. The fatty acid characteristics of strains YIM 65583<sup>T</sup> and the phylogenetically most closely related species (i.e. <i>S. phyllosphaerae</i> FA2<sup>T</sup> and <i>S. yunnanensis</i> YIM 003<sup>T</sup>) are shown in Table S1.

### Table 1. Phenotypic differences among strain YIM 65583<sup>T</sup> and its two closest phylogenetic relatives, <i>S. phyllosphaerae</i> FA2<sup>T</sup> and <i>S. yunnanensis</i> YIM 003<sup>T</sup>

<table>
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<td>Hydrolysis of:</td>
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<td>Gelatin</td>
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<td>Tween 40</td>
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<td>Glycine</td>
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<td>l-Hydroxyproline</td>
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<td>l-Lysine</td>
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<td>l-Serine</td>
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<td>Xanthine</td>
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<td>Citrate</td>
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<td>L-Serine</td>
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<td>D-Sorbitol</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>63.3</td>
<td>67.5</td>
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The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and sphingoglycolipid (Fig. S2). The polar lipids of strain YIM 65583<sup>T</sup> were the same as those of <i>S. phyllosphaerae</i> FA2<sup>T</sup> and <i>S. yunnanensis</i> YIM 003<sup>T</sup>. Enzyme activities, acid production from different carbohydrates, assimilation of various substrates and growth on carbohydrates were determined using commercial systems, API 20NE and API 50CH kits, at 28 °C, respectively, according to instructions of the manufacturer (bioMérieux).

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All data from analysis of the 16S rRNA gene sequence, quinone system and major polar lipids indicated that strain YIM 65583\(^\text{T}\) is a member of the genus *Sphingomonas*. However, there were many physiological and biochemical features of isolate YIM 65583\(^\text{T}\) that differed from those of its relatives, such as differences in degradation of xanthine, hydrolysis of Tweens 20 and 40, and carbon source utilization patterns. The levels of DNA–DNA relatedness further support the proposal that strain YIM 65583\(^\text{T}\) represents a novel species of the genus *Sphingomonas*. Therefore, on the basis of the data presented, strain YIM 65583\(^\text{T}\) should be placed in the genus *Sphingomonas* as the type strain of a novel species, for which the name *Sphingomonas endophytica* sp. nov. is proposed.

**Description of Sphingomonas endophytica sp. nov.**

*Sphingomonas endophytica* (en.do.phy’ti.ca. Gr. pref. endo within; Gr. n. phyton plant; L. fem. suff. -ica adjectival suffix used with the sense of belonging to; N.L. fem. adj. endophytica within plant, pertaining to the original isolation from plant tissues).

Gram-negative, strictly aerobic, non-spore-forming, rod-shaped cells, 0.4–0.7 μm wide and 1.0–2.4 μm long in diameter. Motile by polar flagellation. Colonies on ISP 2 medium are circular convex, slimy, yellow, opaque and 2–3 mm in diameter after 5 days of growth at 28 °C. Temperature range for growth is 12–45 °C. Optimal growth occurs at 20–37 °C, at pH 6.0–8.0, and in 0–1 % (w/v) NaCl. Able to degrade L-alanine, L-arginine, L-asparagin, L-hypoxanthine, L-phenylalanine, L-tyrosine, L-valine and xanthine, but not glycine, L-hydroxyproline, L-lysine or L-serine. Positive for catalase and gelatinase activity, but oxidase-negative. Tweens 20 and 40, starch and cellulose are not hydrolysed. H2S and nitrate are not reduced. L-Arabinose, cellobiose, D-galactose, D-glucose, D-mannose, raffinose, L-rhamnose and sucrose are used as sole carbon sources, but citrate, D-fructose, galactitol, glycerol, inositol, D-mannitol, D-ribose, D-sorbitol, sodium oxalate and D-xylene cannot be used. Milk is not coagulated peptonized. Major respiratory lipoquinone is ubiquinone-10. Major fatty acids are C\(_{18:0}\):10\(\Delta^8\), anteiso-C\(_{16:1}\), C\(_{14:0}\), 2-OH, C\(_{17:1}\):10\(\Delta^7\), C\(_{16:0}\) and C\(_{15:0}\). Major polar lipids detected are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and sphingoglycolipid.

The type strain, YIM 65583\(^\text{T}\) (≡CCTCC AA 209035\(^\text{T}\)=JCM 17394\(^\text{T}\)), was isolated from tissue of *Artemisia annua* L. in Yunnan province, south-west China. The DNA G+C content of the type strain is 63.3 mol%.

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**References**


