A novel Gram-negative strain, designated M-SX103T, was isolated from a fresh leaf of *Nicotiana tabacum* Linn. collected at Yuxi City, Yunnan province, China, and subjected to a polyphasic taxonomic study. Growth occurred at 10–40 °C, at pH 6.0–9.0 and with 0–3 % (w/v) NaCl. The predominant isoprenoid quinone was MK-7. The major cellular fatty acids of strain M-SX103T were iso-C₁₅ : 0, iso-C₁₇ : 0 3-OH and summed feature 3 (C₁₆ : 1ω7c and/or C₁₆ : 1ω6c). The G+C content of the genomic DNA was 40.6 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate belongs to the genus *Sphingobacterium*, sharing 95.4–89.7 % sequence similarity with type strains of members of the genus *Sphingobacterium*; it was most closely related to *Sphingobacterium composti* T5-12T. On the basis of the taxonomic evidence, a novel species, *Sphingobacterium nematocida* sp. nov., is proposed. The type strain is M-SX103T (=JCM 17339T=CCTCC AB 2010390T).

During an investigation on the diversity of nematicidal endophytic micro-organisms in China, an endophytic bacterial strain, designated M-SX103T, was isolated from the leaf tissue of *Nicotiana tabacum* Linn. (HongHuaDaJinYuan variety) collected from Yuxi City, Yunnan province, China. A pure culture was obtained by repeated transfers of separate colonies on Luria–Bertani (LB) agar, then maintained at 4 °C and as glycerol suspensions (20 %, v/v) at −80 °C. A polyphasic approach was used to determine the taxonomic position and identity of strain M-SX103T.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were performed as described by Li et al. (2007). *BLAST* results obtained by comparing the 16S rRNA gene sequence (1390 bp) of strain M-SX103T to publicly available sequences showed that the closest relatives were members of the genus *Sphingobacterium*. Multiple alignments and sequence evolutionary distance calculations with closely related taxa were carried out using CLUSTAL_X (Thompson et al., 1997). Neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) phylogenetic analysis was performed using the MEGA version 4 (Tamura et al., 2007) software. Maximum-likelihood analysis was

Yabuuchi et al. (1983) proposed the genus *Sphingobacterium* based mainly on the presence of high concentrations of sphingophospholipids and, subsequently, the genus was assigned as the type genus of the family *Sphingobacteriaceae* (Steyn et al., 1998) in the phylum ‘Bacteroidetes’. The genus *Sphingobacterium* currently comprises 15 species with validly published names: *Sphingobacterium spiritivorum* (the type species), *S. anhuiense*, *S. antarcticum*, *S. bambusae*, *S. canadense*, *S. composti*, *S. daejeonense*, *S. faecium*, *S. kitahiroshimense*, *S. mizutaii*, *S. multivorum*, *S. shayense*, *S. siyangense*, *S. thalpophilum* and *S. wenxiniae* (Wei et al., 2006; Shibata et al., 1992; Duan et al., 2008; Liu et al., 2008; Matsuyama et al., 2006; Takeuchi & Yokota, 1992; Yu et al., 2003; He et al., 2010; Liu et al., 2008; Zhang et al., 2012).
performed by using PHYLIP version 3.6 (Felsenstein, 2002). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. The neighbour-joining phylogenetic tree showed that strain M-SX103T formed a coherent cluster with members of the genus Sphingobacterium and formed an intragenus branch with Sphingobacterium composti T5-12T (Fig. 1), and they exhibited the highest 16S rRNA gene sequence similarity (95.4 %) with strains of other Sphingobacterium species. The maximum-parsimony and maximum-likelihood phylogenetic trees showed similar topologies (Figs S1 and S2, available in IJSEM Online). These results indicate that strain M-SX103T probably represents a novel member of the genus Sphingobacterium.

Morphological and colonial properties were examined on LB agar for 48 h at 37 °C. Cell morphology was examined by light microscopy (BH-2; Olympus). Gliding motility of cells was examined by using the hanging-drop method (Bernardet et al., 2002). Gram staining was carried out by the standard Gram reaction combined with the KOH lysis method (Gregersen, 1978). Growth of strain M-SX103T at various temperatures (4, 10, 20, 28, 32, 37, 40, 45 and 50 °C), pH values (5, 6, 7, 8, 9, 10), and salt concentrations [0–10 % (w/v)] NaCl, at intervals of 0.5 %] were tested on LB agar. All tests were carried out by incubating the cultures at 37 °C, except for the investigation of the effect of temperatures. Oxidase activity was detected using API oxidase reagent according to the manufacturer’s instructions. Catalase activity was determined by production of bubbles after the addition of a drop of 3 % H2O2. Hydrolysis of casein, starch, and Tweens 20 and 80 was tested as described by Cowan & Steel (1965). Carbon source utilization was tested using Biolog GEN III (MicroPlate) according to the manufacturer’s instructions. Qualitative enzyme tests, acid production from carbohydrates and other physiological and biochemical properties were determined by using the API ZYM, API 50 CH and API 20E systems (bioMérieux) according to the manufacturer’s instructions. Cells of strain M-SX103T were Gram-negative, non-motile rods (0.4–0.8 μm in width and 1.0–1.5 μm in length). Colonies were yellow, circular, smooth and convex with entire margins on LB agar. The isolate grew at 10–40 °C, with optimum growth at 37 °C. Growth occurred at pH 6–9 and in the presence of 0–3 % (w/v) NaCl, with optimum growth at pH 7 and in the presence of 0–1 % (w/v) NaCl. Phenotypic characteristics that readily distinguish the novel strain from the closely related type strain S. composti T5-12T are the absence of growth in 4 % NaCl, citrate utilization, gelatin liquefaction and the ability to utilize a variety of carbon sources. Other differential phenotypic characteristics are shown in Table 1 and in the species description.

Isoprenoid quinones were extracted following established methods (Collins et al., 1977) and were separated by using HPLC (Groth et al., 1997). Polar lipids were extracted as described by Minnikin et al. (1984) and identified by two-dimensional TLC sprayed with specific reagents (Cowan & Jones, 1980). Biomass for quantitative fatty acid analysis of strain M-SX103T and S. composti KCTC 12578T was obtained from LB agar at 30 °C for 48 h. Cellular fatty acids were separated by an automated GC system (Agilent Technologies 7890A GC System) and identified by using the Microbial Identification software package (Sherlock Version 6.1; MIDI database TSBA6). The G+C content of the DNA was determined by using reversed-phase HPLC according to Mesbah et al. (1989), using E. coli DH5α to determine the standard deviation. The predominant isoprenoid quinone was MK-7 (95 %), which is the same as that described for members of the genus Sphingobacterium. The major polar lipid was phosphatidylglycerolphosphate and several unknown polar lipids were also detected (Fig. S3). Sphingolipid, which is characteristic of members of the genus Sphingobacterium, was present in strain M-SX103T (Fig. S3). Cellular fatty acids (>10 %) were iso-C15 : 0 (33.3 %), iso-C17 : 0 3-OH (13.4 %) and summed feature 3 (C16 : 1o7c and/or C16 : 1ω6c) 35.0 %). Compared with S. composti KCTC 12578T, significant differences in the relative amount of summed feature 3 (C16 : 1o7c and/or C16 : 1ω6c) were found in strain M-SX103T and a lower amount of summed feature 9 (iso-C17 : 1ω9c and/or 10-methyl C16 : 0) was observed in strain M-SX103T. These differences can be used to differentiate the isolate from the closely related species S. composti. The DNA G+C content of strain M-SX103T was 40.6 mol%, which was much higher than that of S. composti T5-12T.

On the basis of the phenotypic (Tables 1 and 2) and genotypic data presented above, we propose that strain

![Fig. 1. Neighbor-joining tree derived from aligned 16S rRNA sequences, showing the position of strain M-SX103T in the genus Sphingobacterium. The sequence of Flavobacterium subsaxonicum WB4.1-42T was used as an outgroup. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; values >50 % are shown. Bar, 0.02 substitutions per nucleotide position.](image)
M-SX103<sup>T</sup> represents a novel species of the genus Sphingobacterium, with the name Sphingobacterium nematocida sp. nov.

**Description of Sphingobacterium nematocida sp. nov.**

*Sphingobacterium nematocida* (ne.ma.to’ci.da. N.L. pl. n. *Nematoda* zoological name of a phylum; L. masc. neut. suffix -*cida* a killer; N.L. masc. n. *nematocida* a killer of nematodes).

Cells are rod-shaped, 0.4–0.8 μm in diameter and 1.0–1.5 μm long, non-motile and Gram-negative. Colonies grown on LB agar are yellow, circular and convex with entire margins. The temperature, pH and NaCl ranges for growth are 10–40 °C, pH 6–9 and 0–3 % NaCl, respectively. Catalase and oxidase are positive. Casein, starch, and Tweens 20 and 80 are not hydrolysed. β-Galactosidase test, citrate utilization, Voges–Proskauer reaction and gelatin hydrolysis are positive. l-Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H<sub>2</sub>S production, tryptophan deaminase, urease, nitrate and indole reaction are negative (API 20E system). When assayed with the API ZYM system, positive for alkaline phosphatase, esterase C4, esterase lipase C8, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosaminidase and α-D-glucosidase, N-acetyl-B-D-glucosaminidase and N-acetylgalactosaminidase; but negative for lipase C14, β-galactosidase, β-glucuronidase and fucosidase. Acid is produced from erythritol, D-arabinose, glucose, mannose, amygdalin, arbutin, aesculin, salicin, celllobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, and L-fucose, but not from sucrose, trehalose, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose, and L-fucose, but not from glycogen, L-arabinose, ribose, D-xylene, D-xylose, adonitol, methyl D-xlyopyranoside, galactose, fructose, sorbose, rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylgalactosamine, inulin, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate (API 50 CH system).

### Table 1. Differential characteristics of *S. nematocida* M-SX103<sup>T</sup> and the closely related species *S. composti*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth temperature (°C)</td>
<td>10–40</td>
<td>15–42</td>
</tr>
<tr>
<td>NaCl growth range (% w/v)</td>
<td>0–3</td>
<td>0–4</td>
</tr>
<tr>
<td>pH growth range</td>
<td>6.0–9.0</td>
<td>5.5–8.5</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tween 20</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Tween 80</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentiobiose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Stachyose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetyl-D-glucosamine</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Glucose 6-phosphate</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>l-Glutamic acid</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Gluconic acid</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Quinic acid</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>2-Ketoglutaric acid</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythritol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Xyloside</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Methyl α-D-mannopyranoside</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Methyl α-D-glucopyranoside</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetylgalactosamine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Inulin</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Xyitol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>40.6</td>
<td>35.4</td>
</tr>
</tbody>
</table>

M-SX103<sup>T</sup> and the closely related strain *S. composti* KCTC 12578<sup>T</sup>. All data were obtained from this study.

### Table 2. Cellular fatty acid composition of *S. nematocida* M-SX103<sup>T</sup> and the closely related strain *S. composti* KCTC 12578<sup>T</sup>

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branched fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;15&lt;/sub&gt;:0</td>
<td>33.3</td>
<td>34.0</td>
</tr>
<tr>
<td>anteiso-C&lt;sub&gt;15&lt;/sub&gt;:0</td>
<td>1.9</td>
<td>0.9</td>
</tr>
<tr>
<td>anteiso-C&lt;sub&gt;16&lt;/sub&gt;:0</td>
<td>–</td>
<td>0.6</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;14&lt;/sub&gt;:0</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>C&lt;sub&gt;16&lt;/sub&gt;:0</td>
<td>4.5</td>
<td>2.5</td>
</tr>
<tr>
<td>C&lt;sub&gt;18&lt;/sub&gt;:0</td>
<td>–</td>
<td>0.8</td>
</tr>
<tr>
<td>Unsaturated fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;15&lt;/sub&gt;:1 F</td>
<td>–</td>
<td>2.7</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;16&lt;/sub&gt;:0 3-OH</td>
<td>0.5</td>
<td>1.0</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;18&lt;/sub&gt;:0 3-OH</td>
<td>–</td>
<td>1.1</td>
</tr>
<tr>
<td>Hydroxy fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;15&lt;/sub&gt;:0 3-0H</td>
<td>2.2</td>
<td>2.0</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;16&lt;/sub&gt;:0 3-0H</td>
<td>–</td>
<td>0.5</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;18&lt;/sub&gt;:0 3-0H</td>
<td>3.3</td>
<td>0.5</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;17&lt;/sub&gt;:0 2-0H</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>iso-C&lt;sub&gt;17&lt;/sub&gt;:0 3-0H</td>
<td>13.4</td>
<td>15.0</td>
</tr>
<tr>
<td>Summed features*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>–</td>
<td>1.2</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>23.9</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>9</td>
<td>1.0</td>
<td>9.2</td>
</tr>
</tbody>
</table>

*Fatty acids that cannot be separated by GC using Microbial Identification System (Microbial ID) software are considered to be summed features. Summed feature 1 contains iso-C<sub>15</sub>:1 H and/or C<sub>13</sub>:0 3-OH. Summed feature 3 contains C<sub>16</sub>:1ω7c and/or C<sub>16</sub>:1ω6c. Summed feature 4 contains iso-C<sub>17</sub>:1 1 and/or anteiso-C<sub>17</sub>:1 3. Summed feature 9 contains iso-C<sub>15</sub>:1ω9c and/or 10-methyl C<sub>16</sub>:0.

20E system). When assayed with the API ZYM system, positive for alkaline phosphatase, esterase C4, esterase lipase C8, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase; but negative for lipase C14, β-galactosidase, β-glucuronidase and fucosidase. Acid is produced from erythritol, D-arabinose, glucose, mannose, amygdalin, arbutin, aesculin, salicin, celllobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, turanose and L-fucose, but not from glycerol, L-arabinose, ribose, D-xylene, D-xylose, adonitol, methyl D-Xlyopyranoside, galactose, fructose, sorbose, rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylgalactosamine, inulin, D-lyxose, D-tagatose, D-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate (API 50 CH system). The
following carbon sources are utilized: dextrin, maltose, cellulbiose, sucrose, raffinose, melibiose, d-salicin, N-acetyl-d-glucosamine, N-acetyl-beta-d-mannosamine, N-acetyl-beta-d-galactosamine, a-d-glucose, d-mannose, d-fructose, d-galactose, d-fucose, l-rhamnose, d-sorbitol, d-mannitol, glycerol, d-glucose 6-phosphate, d-fructose 6-phosphate, gelatin, glycyrl l-proline, l-glutamic acid, l-histidine, l-serine, pectin, d-galacturonic acid, l-galactonic acid lactone, d-gluconic acid, d-glucuronic acid, glucuronamide, quinic acid, z-ketoglutaric acid and acetic acid. Does not utilize trehalose, gentiobiose, turanose, stachyose, z-lactose, methyl beta-d-glucoside, N-acetyl neuraminic acid, 3-methyl glucose, l-fucose, inosine, D-arabitol, myo-inositol, d-aspartic acid, d-serine, L-alanine, L-arginine, L-aspartic acid, L-pyruvylglycamic acid, mucic acid, d-saccharic acid, p-hydroxyphenylacetic acid, methyl pyruvate, d-lactic acid methyl ester, l-lactic acid, citric acid, d-malic acid, l-malic acid, bromosuccinic acid, Tween 40, p-aminobutyrlic acid, z-hydroxybutyric acid, beta-hydroxy-DL-butyric acid, z-ketobutyric acid, acetoacetic acid, propionic acid or formic acid (Biolog GEN III). The predominant isoprenoid quinone is MK-7. The major polar lipid is phosphatidylethanolamine and several unknown polar lipids are present. Spingolipid is present. The major fatty acids are iso-C15:0, iso-C17:0 3-OH and summed feature 3 (C16:1&O7c and/or C16:0 9c).

The type strain, M-SX103T (=JCM 17339T=CCTCC AB 2010390T), was isolated from leaf tissue of Nicotiana tabacum Linn. (HongHuaDaJinYuan variety) collected from Yuxi City, Yunnan province, China. The DNA G+C content of the type strain is 40.6 mol%.

Acknowledgements

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References


