**Abstract**

A Gram-staining-negative, non-motile and rod-shaped bacterium, designated strain h337\(^T\), was isolated from an arable soil sample of a tobacco field in Kunming, south-west China. The cells showed oxidase-positive and catalase-positive reactions. Growth was observed at 10–35 °C, at pH 6.0–9.0 and in the presence of up to 3 % (w/v) NaCl, with optimal growth at 30 °C, pH 7.0 and with 1–2 % (w/v) NaCl. The predominant isoprenoid quinone was MK-7. The major fatty acids were identified as iso-C\(_{15:0}\), iso-C\(_{17:0}\) 3-0H, summed feature 3 (C\(_{16:1}\)ω7c and/or C\(_{16:1}\)ω6c) and summed feature 4 (iso-C\(_{17:1}\) I and/or anteiso-C\(_{17:1}\) B). The cellular polar lipids contained phosphatidylethanolamine, sphingophospholipid, four unidentified phospholipids, five unidentified lipids and three unidentified aminophospholipids. The genomic DNA G+C content was 41.5 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain h337\(^T\) should be assigned to the genus *Sphingobacterium*. 16S rRNA gene sequence similarity analysis showed that strain h337\(^T\) was most closely related to *Sphingobacterium yamdrokense* 3-0-1 (98.8 %) and *Sphingobacterium yanglingense* CCNWSP36-1\(^T\) (98.5 %) and shared less than 97 % similarity with other species of the genus *Sphingobacterium*. DNA–DNA hybridization data indicated that the isolate represented a novel genomic species belonging to the genus *Sphingobacterium*. The characteristics determined in this polyphasic taxonomic study indicated that strain h337\(^T\) represents a novel species of the genus *Sphingobacterium*, for which the name *Sphingobacterium tabacisoli* sp. nov. (type strain h337\(^T\)=KCTC 52298\(^T\)=CCTCC AB 2017155\(^T\)) is proposed.

**TAXONOMIC DESCRIPTION**

The genus *Sphingobacterium* was first described by Yabuuchi *et al.* for a group of sphingophospholipid-containing Gram-staining-negative rods [1], and was subsequently assigned as the type genus of the family *Sphingobacteriaceae* [2]. At the time of writing, the genus contains 42 species (http://www.bacterio.net/sphingobacterium.html). Members of this genus are Gram-staining-negative, non-motile and rod-shaped, and chemotaxonomic features include MK-7 as the predominant isoprenoid quinone and iso-C\(_{15:0}\), iso-C\(_{17:0}\) 3-0H and summed feature 3 (C\(_{16:1}\)ω7c and/or C\(_{16:1}\)ω6c) as major fatty acids. Members of the genus *Sphingobacterium* have been reported from leaf tissues, soil, lakes, compost, insects and nodule surfaces of soybean [3–9]. This paper describes the isolation and characterization of another member of this genus, strain h337\(^T\), which we obtained during a study on the diversity of bacteria in arable soil of tobacco.

The soil sample for the study was collected from a tobacco field soil, located in Kunming, south-west China. Following inoculation of the soil suspension on International Streptomycetes Project (ISP) 2 agar plates [10], colonies of strain h337\(^T\) were obtained after incubation for 2 weeks at 30 °C. Purified strain h337\(^T\) was routinely maintained on ISP 2 agar slants and also preserved as glycerol suspensions (20 %, w/v) at −80 °C. Biomass of strain h337\(^T\) for chemical and molecular studies was obtained from cultures grown on ISP 2 medium at 30 °C for 4 days, unless otherwise mentioned. The reference strains ‘*Sphingobacterium yamdrokense*’ CGMCC 1.12560, *Sphingobacterium yanglingense* JCM 30166\(^T\) and *Sphingobacterium nematocida* M-SX103\(^T\) were obtained from the China General Microbiological Culture Collection Centre, Japan Collection of Micro-organisms and our laboratory, respectively. All type strains were also

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**Keywords:** *Sphingobacterium tabacisoli* sp. nov.; polyphasic taxonomy; arable soil; tobacco.

**Abbreviation:** ISP, International Streptomycetes Project.

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain h337\(^T\) is KX129934.

One supplementary table and three supplementary figures are available with the online Supplementary Material.

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*Sphingobacterium tabacisoli* sp. nov., isolated from a tobacco field soil sample

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cultivated under similar conditions for comparative analyses.

Colony morphology of strain h337\textsuperscript{T} was observed from cultures grown on ISP 2 medium. Cell morphology was observed by transmission electron microscopy (JEM-2100, JEOL) with 3-day-old growth on ISP 2 medium. The motility was determined on the basis of development/absence of turbidity by an inoculum of the strain in a tube containing semi-solid ISP 2 medium as described by Skerman et al. [11]. Gram-staining was performed by the standard Gram’s reaction and confirmed with the KOH lysis test [12]. The pH range for growth was determined between pH 4.0 and 13.0 (with intervals of 1.0 pH unit) in ISP 2 broth prepared using the buffer system as described by Xu et al. [13]. Salt tolerance was observed by supplementing up to 13 % (w/v) NaCl (at intervals of 1 %, w/v) in ISP 2 medium. The ability to grow at different temperatures was investigated by incubating related strains on ISP 2 medium at 5–55 °C (at intervals of 5 °C, except with one more at 37 °C). Oxidase activity was detected by using the bioMérieux oxidase reagent according to the manufacturer’s instructions. Catalase activity was tested by observing the formation of bubbles on addition of a drop of 3 % (v/v) \( \text{H}_2\text{O}_2 \). Hydrolysis of casein, cellulose, starch and Tweens 20, 40, 60 and 80 was carried out as described by Smibert and Krieg [14]. The Biolog Gen III microPlate system was used for testing carbon source utilization according to the manufacturer’s instructions.

Acid production from carbohydrates and other physiological and biochemical properties were investigated by using the API 50CH, API ZYM and API 20E test strips (bioMérieux) according to the manufacturer’s instructions. For use with the above test kits, strain h337\textsuperscript{T} and the related reference strains were incubated on ISP 2 medium at 30 °C for 2 days prior to the experiments.

Cells of strain h337\textsuperscript{T} were Gram-stain-negative, non-motile and rod shaped (0.4–0.6 µm in width and 0.8–1.8 µm in length) (Fig. 1). Colonies were yellow, smooth, circular and convex, measuring 2–6 mm in diameter. Growth of strain h337\textsuperscript{T} was observed at 10–35 °C and pH 6.0–9.0, optimally at 30 °C and pH 7.0. Strain h337\textsuperscript{T} was able to tolerate up to 3 % NaCl (w/v) with an optimum concentration of 1–2 % NaCl (w/v) on ISP 2 medium. Catalase and oxidase reactions were both positive. Hydrolysis of casein, starch and cellulose was negative, while that of Tweens 20, 40, 60 and 80 was positive. Distinctive physiological and biochemical characteristics of the isolate from the type species and related strains of species of the genus Sphingobacterium are shown in Table 1, while the detailed characteristics of strain h337\textsuperscript{T} are given in the species description below.

The respiratory quinones were extracted with chloroform/methanol (2:1, v/v) from lyophilized cells and purified by TLC as described by Collins et al. [15]. The purified extracts were analysed by reverse-phase HPLC [16]. Polar lipids were extracted as described by Minnikin et al. [17] and separated on two-dimensional silica TLC gels and identified by staining with appropriate detection reagents [18]. For fatty acids analysis, strain h337\textsuperscript{T} and the reference strains were cultured on TSA medium at 30 °C for 3 days. Cellular fatty acid analysis was carried out as described by Sasser [19] according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sherlock Version 6.1; MIDI database: TSB6A). For determination of DNA G+C content, the genomic DNA was degraded to nucleosides by using PI nuclease and bovine intestinal mucosa alkaline phosphatase as described by Mesbah et al. [20]. Nucleosides were separated by reversed-phase HPLC according to the method described by Tamaoka and Komagata [21]. The DNA G+C content was calculated from the ratio of deoxyguanosine to thymidine.

MK-7 was determined as the predominant menaquinone. Cellular polar lipids extracted contained phosphatidylethanolamine, sphingophospholipid, four unidentified phospholipids, five unidentified lipids and three unidentified aminophospholipids. (Fig. S1, available in the online Supplementary Material). The major fatty acids (>10 %) were iso-C\textsubscript{15:0}, 3-OH, summed feature 3 (C\textsubscript{16:1O7c} and/or C\textsubscript{16:1O6c}) and summed feature 4 (iso-C\textsubscript{17:1} I and/or anteiso-C\textsubscript{17:0} B). The fatty acid profiles of strain h337\textsuperscript{T} and other related reference strains are given in Table S1. The genomic DNA G+C content of strain h337\textsuperscript{T} was 41.5 mol%.

The genomic DNA extraction, PCR amplification and sequencing of the 16S rRNA gene were carried out using procedures described by Li et al. [22]. Multiple alignments with 16S rRNA gene sequences of members of the genus Sphingobacterium and calculations of levels of sequence similarity were carried out using the EzBioCloud server [23]. Phylogenetic analysis was performed using three tree-making algorithms, the neighbour-joining [24], maximum-likelihood [25] and maximum-parsimony [26] methods, by using the software MEGA 7 [27] to determine the taxonomic position of strain h337\textsuperscript{T}. Evolutionary distances were calculated using

Fig. 1. Transmission electron micrograph of strain h337\textsuperscript{T} grown on ISP 2 medium at 30 °C for 3 days. Bar, 1 µm.
Table 1. Distinctive characteristics between strain h337<sup>T</sup> and representatives of the most closely related species of the genus *Sphingobacterium*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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</thead>
<tbody>
<tr>
<td>Temperature range for growth (°C)</td>
<td>10–35</td>
<td>5–35</td>
<td>10–35</td>
<td>20–35</td>
<td>10–40</td>
<td>Not 5 or 42*</td>
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<tr>
<td>NaCl tolerance range (w/v, %)</td>
<td>0–3</td>
<td>0–4</td>
<td>0–3</td>
<td>0–2</td>
<td>0–6</td>
<td>ND</td>
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<td>Hydrolysis of:</td>
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<tr>
<td>Gelatinase</td>
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<td>+</td>
<td>–</td>
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<td>Valine arylamidase</td>
<td>+</td>
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<tr>
<td>d-Ribose</td>
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<td>ND</td>
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<tr>
<td>d-Galactose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>ND</td>
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<tr>
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<td>+</td>
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<td>+</td>
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<td>ND</td>
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<tr>
<td>Malose</td>
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<td>+</td>
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<td>+</td>
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<td>Trehalose</td>
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<td>Carbon source utilization</td>
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<tr>
<td>d-Fucose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Mucic acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Methyl pyruvate</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>d-Malic acid</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>41.5</td>
<td>42.5 [5]</td>
<td>41.1 [9]</td>
<td>40.6 [3]</td>
<td>45.5 [34]</td>
<td>40.1 [1]</td>
</tr>
</tbody>
</table>

*Temperature growth range for strain *S. spiritivorum* GIFU 3101<sup>T</sup> as reported by Holmes et al. [33].

Kimura’s two-parameter method [28]. The topologies of the resultant trees were evaluated by using the bootstrap resampling method of Felsenstein [29] with 1000 replicates. DNA–DNA hybridization tests were carried out by the fluorometric micro-well method [30, 31]. The hybridization temperature for the experiment was set at 37 °C.

An almost complete 16S rRNA gene sequence (1519 bp) was obtained from strain h337<sup>T</sup>. The result of sequence similarity analysis indicated that strain h337<sup>T</sup> shared 16S rRNA gene sequence identities of 98.8, 98.5 and 95.6% with 'S. yamdrokense' 3-0-1, *S. yanglingense* CCNWSP36-1<sup>T</sup>, and *S. nematocida* M-SX103<sup>T</sup> and *Sphingobacterium suaeae* T47<sup>T</sup>, respectively. The similarity values were, however, less than 95.0% with other members of the genus *Sphingobacterium*. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain h337<sup>T</sup> was affiliated to the genus *Sphingobacterium*. The neighbour-joining phylogenetic tree showed that strain h337<sup>T</sup> clustered with 'S. yamdrokense' 3-0-1 and *S. yanglingense* CCNWSP36-1<sup>T</sup> with a 99% bootstrap value (Fig. 2). A similar relationship was also indicated in phylogenetic trees reconstructed by the maximum-parsimony and maximum-likelihood algorithms (Figs S2 and S3). Strain h337<sup>T</sup> displayed a DNA–DNA relatedness of 52.0±0.8%
with 'S. yamdrokense' CGMCC 1.12560 and 35.9±0.7 % with S. yanglingense JCM 30166T. Both values were lower than 70 %, which is the threshold value for prokaryotic species delineation [32].

Data from morphological, physiological, biochemical and phylogenetic analyses showed that strain h337T exhibited characteristics of the genus Sphingobacterium: Gram-staining-negative, non-motile and rod-shaped cells, MK-7
as respiratory quinone, major fatty acids containing iso-C_{15:0}, iso-C_{17:0} 3-OH, summed feature 3 (C_{16:1} ω7c and/or C_{16:1} ω6c) and summed feature 4 (iso-C_{17:1} I or anteiso-C_{17:1} B), phosphatidylethanolamine and sphingophospholipid as its polar lipids. Furthermore, phylogenetic analysis based on 16S rRNA gene sequences indicated that strain h337T falls within the clade of the genus Sphingobacterium (Figs S2 and S3). These results indicated that strain h337T should be affiliated to the genus Sphingobacterium. However, strain h337T had some distinctive characteristics from its phylogenetically related neighbours and the type strain of the type species of the genus Sphingobacterium, *S. spiritivorum* GIFU 3101T [1, 33] (Table 1). The novel isolate was not able to grow at 5 °C, but *S. yamdrokense* CGMCC 1.12560 showed growth at this temperature. Strain h337T could hydrolyse Tween 80, but *S. yanglingense* JCM 30166T could not. Strain h337T was positive for gelatin hydrolysis, trypsin, α-chymotrypsin and α-mannosidase, but these characteristics were negative for *S. suadeae* T47T [34]. The isolate could also be differentiated from the type strain of the type species, *S. spiritivorum* GIFU 3101T, by its positive reactions for gelatin hydrolysis and α-mannosidase [1]. In API ZYM tests, the isolate showed positive reactions for cystine arylamidase and valine arylamidase, but *S. nematocida* M-SX103T was negative. These differentiating characteristics of strain h337T from the closely related strains and the type species of the genus Sphingobacterium are provided in Table 1. Additionally, the values of DNA–DNA hybridization between strain h337T and *S. yamdrokense* CGMCC 1.12560 and *S. yanglingense* JCM 30166T were lower than the threshold value (70 %) for prokaryotic species delineation, which indicated that strain h337T represents a novel genomic species. Based on the phylogenetic analysis, and phenotypic and molecular characteristics, it is concluded that strain h337T represents a novel species of the genus Sphingobacterium, for which the name *Sphingobacterium tabacisoli* sp. nov. is proposed.

### DESCRIPTION OF SPHINGOBACTERIUM TABACISOLI SP. NOV.

*Sphingobacterium tabacisoli* (ta.ba.ci.so’li. N.L. gen. neut. n. tabaci ol’ from tobacco; L. n. solum soil; N.L. gen. n. tabacisoli from tobacco field soil, the source of isolation of the type strain).

Cells are Gram-staining negative, non-motile and rod-shaped. Cells are 0.4–0.6 μm in width and 0.8–1.8 μm in length. Colonies are yellow, smooth, convex and circular with diameters of 2–6 mm after inoculation for 3 days on ISP 2 medium at 30 °C. Growth occurs at 10–35 °C and pH 6.0–9.0, optimally at 30 °C and pH 7.0. Cells are able to tolerate up to 3 % NaCl (w/v) on ISP 2 medium. Catalase-positive and oxidase-positive. Hydrolysis of Tween 20, 40, 60 and 80 is positive, while negative reactions for hydrolysis of casein, starch and cellulose are observed. In API 20E test strips, tryptophan deaminase, Voges–Proskauer reaction and gelatin hydrolysis are positive; negative for L-arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H_{2}S production, urease and indole reaction. According to the results from the API ZYM strips, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphthol-AS-Bl-phospho-hydroxidase, α-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase are positive; negative results for lipase (C14), β-galactosidase, β-glucoronidase and β-fucosidase activities. In API 50CH test strips, acid is produced from amygdalin, d-arabinose, arbutin, aesculin ferric citrate, L-fucose, gentiobiose, methyl α-L-glucopyranoside, d-glucose, lactose, d-mannose, melibiose, salicin and trehalose, but not from d-adonitol, l-arabinose, d-arabitol, dulcitol, erythritol, d-fructose, d-galactose, glucitol, glycogen, inositol, maltose, d-mannitol, potassium d-glucanate, potassium 2-ketogluconate, raffinose, d-ribose, d-sorbitol, L-sorbosse, sucrose, xylitol, d-xylene or L-xyllose. In the Biolog Gen III microPlate system, the following carbon sources are utilized: acetic acid, acetoacetic acid, L-alanine, L-aspartic acid, cellubiose, dextrin, d-fructose, L-fucose, N-acetyl-d-galactosamine, gelatin, gentiobiose, α-d-glucose, d-glucose 6-phosphate, methyl β-d-glucoside, L-glutamic acid, glycerol, l-histidine, l-lactic acid, lactose, d-malic acid, maltose, d-mannose, N-acetyl-β-d-mannosamine, mucic acid, pectin, glycy1-l-proline, L-rhamnose, d-salicin, L-serine, trehalose and turanose, but bromosuccinic acid, α-ketobutyric acid, formic acid, p-hydroxyphenylactic acid and propionic acid are not utilized. The predominant menaquinone is MK-7. The cellular polar lipids are phosphatidylethanolamine, sphingophospholipid, four unidentified phospholipids, five unidentified lipids and three unidentified aminophospholipids. The major fatty acids contain iso-C_{15:0}, iso-C_{17:0} 3-OH, summed feature 3 (C_{16:1} ω7c and/or C_{16:1} ω6c) and summed feature 4 (iso-C_{17:1} I or anteiso-C_{17:1} B).

The type strain, h337T (=KCTC 52298T=CCTCC AB 2017155T), was isolated from an arable soil sample of a tobacco field in Kunming, south-west China. The genomic DNA G+C content of the type strain is 41.5 mol%.

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### Conflicts of interest

The authors declare that there are no conflicts of interest.
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