**Streptomyces luteus** sp. nov., an actinomycete isolated from soil

Xiao-xia Luo,* Liang Kai, Yang Wang, Chuan-xing Wan and Li-Li Zhang*

### Abstract

A Streptomyces-like strain, designated TRM 45540T, was isolated from soil of the Loulan area (89° 22′ 22″ E 40° 29′ 55″ N), Xinjiang Province, north-west China, and was characterized taxonomically by using a polyphasic study. Phylogenetic analysis of the 16S rRNA gene sequence revealed that strain TRM 45540T shared 99.87% similarity with *Streptomyces mutabilis* NBRC 12800T (GenBank accession number A8184156). The strain was aerobic, Gram-stain-positive, with an optimum NaCl concentration for growth of 5% (w/v). The isolate formed white aerial mycelium that was long filamentous with few branches; the substrate mycelium possessed long, smooth-surfaced spore chains bearing smooth spores and produced a yellow diffusible pigment. The strain contained iso-C₁₅:₀, anteiso-C₁₅:₀, anteiso-C₁₇:₀ and C₁₆:₀ as major cellular fatty acids. The predominant menaquinones of the strain were MK-9(H₄), MK-9(H₄) and MK-9(H₄). The whole-cell sugar pattern contained glucose and ribose. The polar lipid pattern of the strain consisted of phosphatidylethanolamine, diphostatidylglycerol, phosphatidylinositol, phosphatidylglycerol and phosphatidylinositolmannosides. Genotypic and phenotypic data confirmed that strain TRM 45540T represents a novel species, clearly different from related species of the genus *Streptomyces*, and for which the name *Streptomyces luteus* (type strain TRM 45540T=CCTCC AA 2014003=NRRL B-59117T) is proposed.

The genus *Streptomyces* was first described by Waksman and Henrici [1] and, at the time of writing, consists of 787 species with validly published names [2]. Species of the genus *Streptomyces* are aerobic actinomycetes and most of them are able to form an extensively branched substrate mycelium and aerial hyphae that typically differentiate into chains of spores [3] and possess DNA rich in G+C [4, 5].

Strain TRM 45540T was isolated from soil of the Loulan area (89° 22′ 22″ E 40° 29′ 55″ N), Xinjiang Province, southeastern China. The strain was isolated on mannitol-casein acid hydrolysis (GW1) medium [6] with 5% (w/v) NaCl. The composition of the GW1 medium was (per litre distilled water): 1.0 g mannitol, 0.3 g casein, 2.0 g KNO₃, 2.0 g MgSO₄·7H₂O, 1.0 g K₂HPO₄·3H₂O, 3.0 g NaHCO₃, 2.0 g (NH₄)₂SO₄ and 18 g agar. The organism was grown and maintained on ISP4 medium [7] containing 5% (w/v) NaCl for 5–7 days at 37°C. A culture of the isolated strain was stored at −80°C in the presence of 20% (v/v) glycerol.

Culture characteristics were determined after incubation at 37°C for 7 days on media (ISP2, ISP3, ISP4, ISP5, nutrient agar and Czapek’s agar plates) described by Shirling and Gottlieb [8] amended with 5% (w/v) NaCl. Growth was good on all of the media tested. Morphological observations of spores and mycelia were conducted by light microscopy (Olympus CX21) and scanning electron microscopy (Hitachi High-Technologies) of cultures grown on ISP4 agar [7] incubated at 37°C for 7 days. Carbon source utilization tests were performed according to the methods described by Shirling and Gottlieb [8], and using the basal medium recommended by Pridham and Gottlieb [9]. Growth at various NaCl concentrations (0–10%) (0, 1, 3, 5, 8 and 10%, w/v) and different temperatures (4–55°C) (4, 10, 15, 20, 25, 30, 37, 40, 45, 50 and 55°C) was examined by growing the strain on Gause’s plates as the basal medium. Growth was tested over the pH range 4–10 (4, 5, 6, 7, 8, 9 and 10) as described by Xu et al. [10] on Gause’s medium. Biomass for chemical and molecular studies was obtained by cultivation in ISP4 medium on a shaker at 180 r.p.m. and 37°C for 7 days. Other physiological characteristics of strain TRM 45540T were assessed by using the media and methods of Gordon et al. [11]. The aim of this study was to identify the exact taxonomic status of strain TRM 45540T by using a polyphasic approach.

Colonies of strain TRM 45540T were grey on some media tested (Gause’s, ISP4 and ISP2), and the strain formed well...
developed yellow substrate mycelium, with aril hyphae that differentiated into smooth-surfaced spores borne on straight chains (Fig. 1). Growth of strain TRM 45540<sup>T</sup> occurred at pH 7–10 and with 0–10% (w/v) NaCl, and the temperature range for growth was 16–40°C, with the optimum temperature being 37°C. Other physiological characteristics of strain TRM 45540<sup>T</sup> are given in the species description and in Table 1.

For 16S rRNA gene sequence analysis, strain TRM 45540<sup>T</sup> was cultivated for 7 days at 37°C. Biomass was harvested from ISP4 agar plates, and genomic DNA extraction and PCR amplification of the 16S rRNA gene were performed according to the methods of Cui [12]. The sequence was aligned with the most closely related species of the genus *Streptomyces* and calculation of levels of sequence similarity were carried out on the EzTaxon-e server [13]. Phylogenetic trees were generated using the maximum-parsimony [14], maximum-likelihood [15] and neighbour-joining [16] algorithms drawn from the MEGA 6 program [17]. All phylogenetic trees were evaluated by the bootstrap resampling method of Felsenstein [18] with 1000 replications. As the topologies of these trees were similar (Figs S1 and S2, available in the online Supplementary Material), only the neighbour-joining tree is shown (Fig. 2).

The phylogenetic tree (Fig. 2) based on the neighbour-joining algorithm showed that strains TRM 45540<sup>T</sup> and *Streptomyces mutabilis* NRRL 12800<sup>T</sup> (AB184156) form two monophyletic lines within the genus *Streptomyces*. Highest 16S rRNA gene sequence similarity was found with *S. mutabilis* NBRC 12800<sup>T</sup> (99.65%), *Streptomyces africanus* CPJVR-H<sup>T</sup> (98.71%), *Streptomyces malachitospinus* NBRC 101004<sup>T</sup> (98.63%) and *Streptomyces speibonae* PK-Blue<sup>T</sup> (97.98%). These relationships were supported by the other two tree-making methods used in this study (Figs S1 and S2). The taxonomic integrity of the isolate was also supported by DNA–DNA hybridization data. It shared relatively low levels of DNA–DNA relatedness with *S. mutabilis* NRBL 12800<sup>T</sup> (26.97%), *S. africanus* CPJVR-H<sup>T</sup> (34.74%), *S. speibonae* PK-Blue<sup>T</sup> (36.96%), *S. anandii* NRRL B-3590<sup>T</sup> (20.57%) and *S. malachitospinus* NBRC 101004<sup>T</sup> (37.74%).

The cell-wall sugars in whole-cell hydrolysates were identified by using the method described by Hasegawa [19]. Menaquinoles were extracted according to the method of Shah and Collins [20] and analysed by HPLC [21]. Polar lipids were extracted, examined by two-dimensional TLC and identified with 10% ethanolic molybdophosphoric acid using the procedures of [22]. The cellular fatty acid composition was determined as described by Collins (1980) using the Microbial Identification System (MIDI Sherlock version 6.0). The DNA G+C content of strain TRM 45540<sup>T</sup> was determined by using the HPLC method [23].

Isolate TRM 45540<sup>T</sup> showed a range of chemotaxonomic properties consistent with its classification in the genus *Streptomyces* [24]. The predominant fatty acids were iso-C<sub>16:0</sub> (36.85%), anteiso-C<sub>15:0</sub> (15.14%), anteiso-C<sub>17:0</sub> (10.42%), C<sub>16:0</sub> (8.58%), iso-C<sub>15:0</sub> (4.84%), iso-C<sub>17:0</sub> (3.39%), anteiso-C<sub>17:0</sub>:<sub>0:0</sub>-9 (2.25%), C<sub>17:0</sub> (1.81%), iso<sub>16:1</sub>:<sub>h</sub> (1.99%) and cyclo-C<sub>17:0</sub> (1.88%). The major cell-wall diamino acid was LL-diaminopimelic acid, and the major cell-wall sugars were glucose and ribose. The major menaquinones were MK-9(H<sub>4</sub>), MK-9(H<sub>2</sub>) and MK-9(H<sub>10</sub>). The major polar lipids were phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylinositol (PI), phosphatidylglycerol (PG) and phosphatidylinositolmannosides (PIM) (Figs S3–S5). The DNA G+C content of strain TRM 45540<sup>T</sup> was 62 mol%.

Strain TRM 45540<sup>T</sup> was different from members of other species of the genus *Streptomyces* in some morphological and physiological properties: scanning electron microscopic observations of strain TRM 45540<sup>T</sup> showed white aerial mycelium that was long and filamentous with few branches, and the substrate mycelium possessed long smooth-surfaced spore chains bearing smooth spores and produced a yellow diffusible pigment; the pH range, temperature range and carbon source utilization range enabled it to be differentiated from *S. mutabilis* NBRC 12800<sup>T</sup> (Table 1). Strain TRM 45540<sup>T</sup> exhibited chemotaxonomic differences from members of species of the genus *Streptomyces* (Table 1): it contained PE, DPG, PI, PG and PIM, and MK-9(H<sub>4</sub>), MK-9(H<sub>2</sub>) and MK-9(H<sub>10</sub>) as the major menaquinones. It contained iso-C<sub>16:0</sub> and anteiso-C<sub>15:0</sub> and anteiso-C<sub>17:0</sub> (10.42) as major cellular fatty acids, which is distinctly different from *S. mutabilis* NBRC 12800<sup>T</sup>, *S. africanus* CPJVR-H<sup>T</sup>, *S. speibonae* PK-Blue<sup>T</sup> and *S. anandii* NcRRL B-3590<sup>T</sup>. *S. malachitospinus* NRRL12800<sup>T</sup> contained DPG, PE, PC, PG, PI and PL, *S. africanus* CPJVR-H<sup>T</sup>...
Table 1. Growth and cultural characteristics of strain TRM 45540T and phylogenetically related *Streptomyces* species

Strains: 1, TRM 45540T; 2, *S. mutabilis* NBRC 12800T; 3, *S. africanus* CPJVR-H1T; 4, *S. speibonae* PK-BlueT; 5, *S. anandii* NRRL B-3590T. Strain TRM 45540T utilized l-rhamnose, d-glucose, l-arabinose, d-mannitol, d-ribose, d-mycose and d-melanin as carbon sources, but not d-sorbitol or d-saligenin. Starch hydrolysis, H2S production, nitrate reduction, oxidase, catalase and urease were positive, while melanin, oxidase, lipase (Tweens 20, 40 and 60) and gelatin reaction were negative. *S. mutabilis* NBRC 12800T utilized l-rhamnose, d-glucose, l-arabinose, d-mannitol, d-ribose and d-saligenin, but not d-sorbitol, raffinose or d-mycose. Starch hydrolysis, H2S production, nitrate reduction and lipase reaction were positive, while urease, gelatin, oxidase and catalase reaction were negative. *S. africanus* CPJVR-H1T utilized raffinose, l-rhamnose, d-glucose, l-arabinose and d-mannitol, but not d-ribose, d-sorbitol, d-saligenin and d-mycose. Starch hydrolysis, lipase, oxidase and urease reaction were negative. *S. speibonae* PK-BlueT utilized l-rhamnose, d-glucose, l-arabinose, d-mannitol, d-ribose and d-sorbitol, but not raffinose, d-saligenin or d-mycose. Gelatin, lipase, melanin, H2S and nitrate reductase reaction were positive, while urease, gelatin, oxidase and catalase reaction were negative. *S. anandii* NRRL B-3590T utilized l-rhamnose, d-glucose, l-arabinose, d-mannitol and d-ribose, but not d-sorbitol, raffinose, saligenin or d-mycose. Melanin, H2S production, nitrate reductase and lipase reaction were positive, while urease, gelatin, oxidase and catalase reaction were negative. +, Positive; –, negative. DAP, diaminopimelic acid. DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PL phosphatidylglycerol; PIM, phosphatidylinositolmannosides; PC, phosphatidylcholine; PL phospholipid.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>Aerial spore mass colour on ISP4</td>
<td>White</td>
<td>Grey</td>
<td>Blue</td>
<td>Grey</td>
<td>Grey</td>
</tr>
<tr>
<td>Reverse side of colony on ISP4</td>
<td>Yellow</td>
<td>Brown</td>
<td>Yellow</td>
<td>Blue</td>
<td>Yellow</td>
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<tr>
<td>Diffusible pigment</td>
<td>Yellow</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Spore chain</td>
<td>Straight</td>
<td>Hooks, spirale</td>
<td>Spire</td>
<td>Spire</td>
<td>Spire</td>
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<tr>
<td>Spore surface</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Spiny</td>
<td>Hairy</td>
<td>Smooth</td>
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<tr>
<td>NaCl (% w/v) for growth</td>
<td>&lt;8</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;4</td>
<td>&lt;4</td>
</tr>
<tr>
<td>pH</td>
<td>7.5–8</td>
<td>4.3</td>
<td>6–7</td>
<td>6–7</td>
<td>6–7</td>
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<tr>
<td>Major cell-wall diamino acid</td>
<td>L-l-DAP</td>
<td>meso-D AP</td>
<td>L-l-DAP</td>
<td>L-l-DAP</td>
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</tr>
<tr>
<td>Major whole-cell sugars</td>
<td>Glucose, ribose</td>
<td>Xylose, galactose, glucose</td>
<td>Ribose, xylose</td>
<td>Galactose, mannose</td>
<td>Galactose, mannose</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>DPG, PE, PL, PC, PG, PIM</td>
<td>DPG, PE, PC, PG, PL</td>
<td>DPG, PE, PL, PG, PC, PL</td>
<td>DPG, PE, PIM, PG, PL</td>
<td>DPG, PE, PIM, PL</td>
</tr>
<tr>
<td>Predominant cellular fatty acids</td>
<td>iso-C16:0, anteiso-C15:0, anteiso-C17:0</td>
<td>iso-C16:0, iso-C16:1, anteiso-C15:0, C16:0</td>
<td>iso-C16:0, cyclo-C17:0, anteiso-C15:0, anteiso-C17:0</td>
<td>iso-C14:0, anteiso-C15:0, iso-C16:0</td>
<td>iso-C15:0, anteiso-C13:0</td>
</tr>
<tr>
<td>Predominant menaquinones</td>
<td>MK-9(H12), MK-9(He), MK-9 (H10)</td>
<td>MK-9(H12), MK-8(He), MK-9(H8), MK-9(H6)</td>
<td>MK-9(H2), MK-9(He), MK-9(H6)</td>
<td>MK-8(He), MK-9(H8), MK-9(H6)</td>
<td>MK-9(He), MK-9(H8), MK-9(H6), MK-9(H2)</td>
</tr>
</tbody>
</table>
On the basis of a combination of phylogenetic distinctness and differences in chemotaxonomic and morphological characteristics, strain TRM 45540 \(^\text{T}\) contained DPG, PE, PI, PG, phosphatidylcholine (PC) and phospholipid (PL), \(S.\) speibonae PK-Blue \(^T\) contained DPG, PE, PIM, PG, PI and PL, and \(S.\) anandii NRRL B-3590 \(^\text{T}\) contained DPG, PE, PIM and PI. Regarding major menaquinones, \(S.\) mutabilis NBRC 12800 \(^\text{T}\) contained MK-9(H\(_6\)), MK-8(H\(_6\)), MK-9(H\(_8\)) and MK-9(H\(_9\)), \(S.\) africanus CPJVR-H \(^T\) contained MK-9(H\(_6\)) and MK-9(H\(_8\)), \(S.\) speibonae PK-Blue \(^T\) contained MK-8(H\(_8\)), MK-9(H\(_8\)) and MK-9(H\(_9\)), and \(S.\) anandii NRRL B-3590 \(^\text{T}\) contained MK-9(H\(_8\)), MK-9(H\(_9\)) and MK-9(H\(_9\)). Regarding major fatty acids, \(S.\) mutabilis NBRC 12800 \(^\text{T}\) contained iso-C\(_{16:0}\), iso-H\(_{16:1}\), anteiso-C\(_{15:0}\) and C\(_{16:0}\). \(S.\) africanus CPJVR-H \(^T\) contained iso-C\(_{16:0}\), cyclo-C\(_{17:0}\), anteiso-C\(_{15:0}\) and anteiso-C\(_{17:0}\). \(S.\) speibonae PK-Blue \(^T\) contained iso-C\(_{14:0}\), anteiso-C\(_{15:0}\) and iso-C\(_{16:0}\), and \(S.\) anandii NRRL B-3590 \(^\text{T}\) contained iso-C\(_{15:0}\) and anteiso-C\(_{15:0}\).

On the basis of a combination of phylogenetic distinctness and differences in chemotaxonomic and morphological characteristics, strain TRM 45540 \(^\text{T}\) represents a novel species in the genus \(Streptomyces\), for which the name \(Streptomyces luteus\) sp. nov. is proposed.

**DESCRIPTION OF STREPTOMYCES LUTEUS SP. NOV.**

\(Streptomyces luteus\) (lu’t’e.us. L. masc. adj. luteus yellow, describing the yellow substrate mycelium).

Aerobic, Gram-strain-positive actinomycete. Forms white aerial mycelium with few branches. Spore surface is smooth. Substrate mycelium is yellow. A bright yellow soluble pigment is produced. Utilizes L- rhamnose, D-glucose, L-arabinose, D-mannitol, D-ribose, D-mycose and D-melanin as carbon sources, but not D-sorbitol or D-saligenin. Positive for starch hydrolysis, milk coagulation and peptonization, nitrate reduction, oxidase, catalase, urease and lipase (Tweens 20, 40, 60) reactions, and \(\text{H}_2\text{S}\) production. Negative for gelatin liquefaction, oxidase reaction and melanin production. Growth occurs between 25 and 40 °C but not at 45 °C, and the optimum growth temperature is 37 °C. The pH range for growth is pH 6.0–10.0 and the optimum pH is 7.5–8.0. The NaCl tolerance range for growth is 0–8 % (w/v) NaCl and the optimum concentration for growth is 5 % (w/v) NaCl. The whole-cell sugar pattern consists of glucose and ribose. The polar lipid pattern consists of PE, DPG, PI, PG and PIM. The predominant menaquinones are MK-9(H\(_6\)), MK-9(H\(_8\)) and MK-9(H\(_9\)). The major cellular fatty acids are iso-C\(_{16:0}\), anteiso-C\(_{15:0}\), anteiso-C\(_{17:0}\), cyclo-C\(_{17:0}\), iso-C\(_{15:0}\), iso-C\(_{17:0}\), anteiso-C\(_{17:0}\) and anteiso-C\(_{16:0}\), and cyclo-C\(_{17:0}\).

The type strain, TRM 45540 \(^\text{T}\) (=CCTCC AA 2014003 \(^\text{T}\) =NRRL B-59117), was isolated from soil of Loulan, Xinjiang Province, north-west China. The G+C content of the genomic DNA of the type strain is 62 %.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

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