Streptomyces rubrisoli sp. nov., neutrotolerant acidophilic actinomycetes isolated from red soil

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Three neutrotolerant, acidophilic actinomycete strains, designated FXJ1.526, FXJ1.725T and FXJ1.726, were isolated from red soil collected from Liujiazhan, Jiangxi Province, China. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the three strains clustered together and their closest relative was Streptomyces ferralitis CGMCC 4.1985T (98.9–99.0 % similarity). Multilocus sequence analysis confirmed their relationship to S. ferralitis and separated these strains as representing a novel species. Mean DNA–DNA hybridization values among strains FXJ1.526, FXJ1.725T and FXJ1.726 were 81.6±3.5–87.2±3.8 %, and the values between the three strains and S. ferralitis CGMCC 4.1985T were well below 70 %. The three strains also shared several phenotypic characteristics that were distinct from the closely related species. They grew at 21–50 °C, at pH 4.0–9.0 (with an optimal pH of 5.0) and with 0–3 % (w/v) NaCl, and the major cellular fatty acids were anteiso-C15 : 0, iso-C15 : 0 and iso-C16 : 0. On the basis of data from this polyphasic taxonomic study, it is proposed that strains FXJ1.526, FXJ1.725T and FXJ1.726 be classified as representatives of a novel species of the genus Streptomyces, with the name Streptomyces rubrisoli sp. nov. The type strain is FXJ1.725T (=CGMCC 4.7025T=DSM 42083T).

The genus Streptomyces was proposed by Waksman & Henrici (1943), and contained over 660 species with validly published names at the time of writing (http://www.bacterio.net/streptomycesa.html; Ezéby, 1997). As the most extensively studied representatives within actinomycetes, species of the genus Streptomyces have complex developmental life cycles (Flärtdh & Buttner, 2009) and are prolific producers of natural bioactive compounds, accounting for 70–80 % of secondary metabolites that are used in human medicine as anti-infective, anti-tumour and immnosuppressant drugs (Challis & Hopwood, 2003). During a project on the selective isolation of diverse actinomycetes from red soil regions, strains FXJ1.526, FXJ1.725T and FXJ1.726 were isolated from a red soil (pH 4.6) of the Red Soil Ecology Experimental Station of the Chinese Academy of Sciences at Liujiazhan (28° 15’ 20” N 116° 55’ 30” E), south-eastern Jiangxi Province, China. The soils of this region were derived from Quaternary red clay and are classified as Udic Ferralsols in the Chinese Soil Taxonomy and Ferric Acrisols in the FAO classification system (Jiang et al., 2014), characterized by low organic carbon, high content of iron oxides and acidity. The isolates showed chemotaxonomic characteristics of the genus Streptomyces, but were phenotypically and genotypically different from recognized members of this genus. We thus propose that the three strains represent a novel species of the genus Streptomyces.

Strains FXJ1.526, FXJ1.725T and FXJ1.726 were isolated on 100-fold diluted modified mineral-medium agar containing 0.5 % sorbitol (Lee et al., 2000) supplemented with cycloheximide, nystatin, nalidixic acid (each at 50 μg ml⁻¹), and novobiocin (at 25 μg ml⁻¹), which had been inoculated

Abbreviations: MLSA, multilocus sequence analysis; DDH, DNA–DNA hybridization.


Three supplementary tables and three supplementary figures are available with the online Supplementary Material.
with a soil suspension pretreated in a water bath sonicator (model KQ-100DB, 40 kHz, 100 W; Kunshan Ultrasonic instruments) for 2 min at 30 °C, and incubated at 28 °C for 3–4 weeks. *Streptomyces ferralitis* CGMCC 4.1985 T (Saintpierre-Bonaccio et al., 2004) was used as a reference strain for the investigation of phenotypic properties under the same laboratory conditions. The strains were maintained on ISP 2 (International Streptomyces Project medium no. 2; Shirling & Gottlieb, 1966) agar slants at 4 °C and as glycerol suspensions (20 %, v/v) at −20 °C.

Morphological properties of the strains were examined on ISP 3 agar (Shirling & Gottlieb, 1966) after 2 weeks at 28 °C by scanning electron microscopy (Quanta 200; FEI). Cultural characteristics were determined after 2 weeks of incubation at 28 °C on ISP media (Shirling & Gottlieb, 1966). Colours were determined as described by Kelly (1964). The Gram reaction was performed using the non-staining method described by Buck (1982). Motility was examined by the wet-mount method, and spore formation was analysed using the staining method of Schaeffer & Fulton (1933). Standard physiological tests were performed using the well-established procedures of Williams et al. (1983) and Kämpfer et al. (1991). Carbon-source utilization was tested using ISP 4 (Shirling & Gottlieb, 1966) without soluble starch, supplemented with each carbon source at a 1 % final concentration. Growth at different temperatures (4, 16, 21, 28, 37, 45, 50 and 55 °C) and pH (3.5, and 4.0–11.0 at intervals of 1.0 pH unit) were assessed on ISP 2 agar plates. KH₂PO₄/HCl, KH₂PO₄/K₂HPO₄ and K₂HPO₄/NaOH buffer systems were used to maintain the pH values of the medium. NaCl tolerance was determined on ISP 2 agar plates (pH 5.5, 28 °C) supplemented with 0–5 % (w/v) NaCl (increments of 1 %).

Biomass for chemical and molecular systematic studies was obtained after incubation at 28 °C for 5–8 days in shaken flasks (about 180 r.p.m.) of trytycose soy broth. Standard procedures were used to determine cell-wall amino acids and sugars in whole-cell hydrolysates (Staneck & Roberts, 1974). Polar lipids were extracted as described by Minnikin et al. (1984) and identified using two-dimensional TLC and spraying with specific reagents (Collins & Jones, 1980). Fatty acids were extracted, methylated and analysed using the Microbial Identification system (MIDI, Sherlock version 6.0). MIDI database TSBA 6 was used for peak identification. Menaquiones were extracted according to the method of Collins et al. (1977) and analysed by HPLC as described by Tamaoka et al. (1983). Mycolic acids were determined by the acid methanalysis method of Minnikin et al. (1980). The G+C contents of the genomic DNAs were determined by reversed-phase HPLC of nucleosides according to Mesbah et al. (1989).

Extraction of genomic DNA and PCR amplification and sequencing of the 16S rRNA gene were performed using methods described elsewhere (Tamura et al., 2001). PCR amplification and sequencing of five housekeeping genes, *atpD*, *recA*, *rpoB*, *trpB* and *gyrB*, were performed using primers and amplification conditions described by Guo et al. (2008) and Rong et al. (2009). Phylogenetic trees based on the almost-complete 16S rRNA gene sequences and on the concatenated protein-coding sequences were reconstructed by using three tree-making algorithms, neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971). These algorithms were used from the MEGA 5.0 package (Tamura et al., 2011). The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) with 1000 resampled datasets. The calculation of pairwise
Table 1. Physiological and biochemical characteristics that differentiate the new isolates from the type strain of S. ferralitis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
<th>Strain 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>w</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Decomposition of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Urea</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tween 20</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Tween 40</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Tween 80</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>+</td>
<td>w</td>
<td>w</td>
<td>–</td>
</tr>
<tr>
<td>Dextrin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>α-Lactose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>α-Xylose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>w</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

Growth ranges:

- pH: 4.0–9.0
- Temperature (°C): 21–50
- NaCl tolerance (w/v, %): ≤3

Table 2. Chemotaxonomic characteristics separating the new isolates from the type strain of S. ferralitis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 1</th>
<th>Strain 2</th>
<th>Strain 3</th>
<th>Strain 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-cell sugars*</td>
<td>Glc, Man, Xyl, Rib</td>
<td>Glc, Man, Xyl, Rib</td>
<td>Glc, Man, Xyl, Rib</td>
<td>Ara, Glc, Xyl</td>
</tr>
<tr>
<td>Menaquinones (%)</td>
<td>MK-9(H₂) 5.8</td>
<td>TR</td>
<td>TR</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>MK-9(H₄) 7.5</td>
<td>4.7</td>
<td>3.6</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>MK-9(H₆) 20.3</td>
<td>25.6</td>
<td>28.1</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>MK-9(H₈) 63.4</td>
<td>66.4</td>
<td>64.6</td>
<td>39.9</td>
</tr>
<tr>
<td>Major fatty acids (%)†</td>
<td>iso-C₁₄ : 0 6.39</td>
<td>8.97</td>
<td>3.79</td>
<td>15.29</td>
</tr>
<tr>
<td></td>
<td>iso-C₁₅ : 0 24.17</td>
<td>21.84</td>
<td>21.55</td>
<td>12.71</td>
</tr>
<tr>
<td></td>
<td>iso-C₁₆ : 0 20.18</td>
<td>19.17</td>
<td>14.24</td>
<td>31.35</td>
</tr>
<tr>
<td></td>
<td>iso-C₁₆ : 1 H 4.52</td>
<td>5.50</td>
<td>4.22</td>
<td>10.21</td>
</tr>
<tr>
<td></td>
<td>anteiso-C₁₇ : 0 4.00</td>
<td>2.81</td>
<td>4.21</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td>anteiso-C₁₇ : 0 27.15</td>
<td>27.54</td>
<td>30.03</td>
<td>17.82</td>
</tr>
<tr>
<td></td>
<td>DNA G + C content (mol%)</td>
<td>74.4</td>
<td>72.9</td>
<td>75.6</td>
</tr>
</tbody>
</table>

*Ara, Arabinose; Glc, glucose; Man, mannose; Xyl, xylose; Rib, ribose.
†Bold type indicates the most abundant fatty acid (>25%).
acids, L-l-diaminopimelic acid, glucose, mannose, xylose and ribose. The polar lipids of the isolates contained diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside, and several unidentified aminolipids, glycolipids and phospholipids (Fig. S1). An unidentified aminophospholipid was detected in strain FXJ1.526 (Fig. S1). The menaquinones of strains FXJ1.526, FXJ1.725T and FXJ1.726 were MK-9 (H₄) (63.4, 66.4 and 64.6 %, respectively), MK-9(H₄) (20.3, 25.6, 28.1 %), MK-9(H₂) (7.5, 4.7, 3.6 %) and MK-9(H₂) (5.8 %, trace, trace) (Fig. S2). The major fatty acids (>10 %) were anteiso-C₁₅ : 0, iso-C₁₅ : 0 and iso-C₁₆ : 0. The DNA G+C contents were 72.9–75.6 mol%.

Almost-complete 16S rRNA gene sequences (1490 nt) were determined for strains FXJ1.526, FXJ1.725T and FXJ1.726, which were almost identical with only 2 or 3 nt differences in base composition among each other. These isolates stably formed a distinct monophyletic line, which was clustered with S. ferralitis CGMCC 4.1985T in the Streptomyces 16S
rRNA gene tree irrespective of the tree-making algorithm used (Fig. 2). The three strains also showed highest 16S rRNA gene sequence similarity (98.9–99.0 %) to *S. ferralitis* CGMCC 4.1985\(^\mathrm{T}\). 16S rRNA gene sequence similarities of the new isolates to all other recognized species of the genus *Streptomyces* were <98.6 %, lower than the 98.65 % threshold of 16S rRNA gene sequence similarity that corresponds to 95–96 % average nucleotide identity of whole-genome sequences for species delineation of prokaryotes (Kim *et al.* 2014). The phylogenetic tree based on the five protein-coding gene sequences (Table S2) available for the related species in the 16S rRNA gene tree supported five protein-coding gene sequences (Table S2) available for the related species in the 16S rRNA gene tree supported the close association of strains FXJ1.526, FXJ1.725\(^\mathrm{T}\) and FXJ1.726 to *S. ferralitis* CGMCC 4.1985\(^\mathrm{T}\), and good congruence was found between multitocus sequence analysis (MLSA) trees reconstructed using different tree-making algorithms (Fig. S3). The isolates formed a distinct clade in the MLSA tree, supported by a 100 % bootstrap value and with 0.001 MLSA evolutionary distance between one another. The MLSA distance between the isolates and *S. ferralitis* CGMCC 4.1985\(^\mathrm{T}\) was 0.063–0.064, which was well above the cut-off point of 0.007 recommended by Rong & Huang (2010, 2012) for the assignment of strains to the same *Streptomyces* species.

DNA–DNA hybridization (DDH) tests were performed among the new isolates and *S. ferralitis* CGMCC 4.1985\(^\mathrm{T}\). According to the results (Table S3), strains FXJ1.526, FXJ1.725\(^\mathrm{T}\) and FXJ1.726 shared 81.6 ± 3.5–87.2 ± 3.8 % relatedness. Mean DDH values between the new isolates and *S. ferralitis* CGMCC 4.1985\(^\mathrm{T}\) were 24.3 ± 1.6–25.8 ± 0.3 %, which confirmed that the isolates belong to a distinct genomic species (Wayne *et al.*, 1987). On the basis of the data presented, we suggest that the three strains represent a novel species of the genus *Streptomyces*, for which the name *Streptomyces rubrisoli* sp. nov. is proposed.

**Description of *Streptomyces rubrisoli* sp. nov.**

*Streptomyces rubrisoli* (ru.bri.so.li. L. adj. ruber red; L. n. solum soil; N.L. gen. n. rubrisoli of red soil).

Cells are Gram-reaction-positive, aerobic and non-motile. Good growth occurs on ISP 2, 3 and 4 agar media, forming pink to yellowish colonies and extensively branched substrate mycelium. Abundant aerial hyphae are produced on ISP 3 and 4 agar media, which differentiate into tight, spiral spore chains containing elliptical smooth spores. Growth occurs between pH 4.0 and 9.0, and between 21 and 50 °C, with optimal growth at pH 5.0 and 25–30 °C. The maximum NaCl concentration for growth is 3 % (w/v). Positive for decomposition of adenine, hypoxanthine and L-tyrosine, but negative for liquefaction of gelatin, production of H\(_2\)S, and decomposition of casein, starch, guanine and xanthine. Utilizes cellobiose, chitin, dextrin, D-galactose, D-glucose, maltose, D-mannitol, L-rihamnone, sucrose and trehalose as sole carbon sources, but not L-arabinose, glycerogen, D-lactose, melezitose, methyl D-glucopyranoside, D-xylose, salicin or D-xylitol. Whole-cell hydrolysates contain meso-diaminopimelic acid, L-l-diaminopimelic acid, glucose, mannosse, xylose and ribose. The major polar lipids are diphostidyglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside, and unidentified aminolipids, glycolipids and phospholipids. The major fatty acids are anteiso-C\(_{15}\):0, iso-C\(_{15}\):0 and iso-C\(_{16}\):0. The predominant menaquinones are MK-9(H\(_{4}\)) and MK-9(H\(_{8}\)). The G+C content of the DNA is 72.9–75.6 mol%.

The type strain, FXJ1.725\(^\mathrm{T}\) ( =CGMCC 4.7025\(^\mathrm{T}\)=DSM 42083\(^\mathrm{T}\)), was isolated from a red soil of the Red Soil Ecology Experimental Station of the Chinese Academy of Sciences at Liujiazhan, Jiangxi Province, China. The G+C content of the type strain is 72.9 mol%. FXJ1.526 and FXJ1.726 are other strains of the species.

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**References**


