Saccharopolyspora gloriosae sp. nov., an endophytic actinomycete isolated from the stem of Gloriosa superba L.

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A Gram-stain-positive, aerobic actinomycete, strain YIM 60513T, was isolated from the stem of Gloriosa superba L. collected from tropical rainforest at Xishuangbanna, Yunnan Province, southwest China. 16S rRNA gene sequence analysis indicated that strain YIM 60513T belonged to the genus Saccharopolyspora and was closely related to Saccharopolyspora gregorii NCIB 12823T (99.1 % similarity) and Saccharopolyspora cebuensis SPE 10-1T (97.3 % similarity). Data for the predominant quinone [MK-9(H4)], major fatty acids (iso-C16 : 0, anteiso-C17 : 0 and C17 : 1 cis9) and G + C content of the genomic DNA (71.6 mol%) were similar to those for members of the genus Saccharopolyspora. The level of DNA–DNA relatedness between strain YIM 60513T and S. gregorii NCIB 12823T was 43 %. The combination of phylogenetic analysis, phenotypic differences, chemotaxonomic characteristics and DNA–DNA hybridization data supported the view that strain YIM 60513T should be distinguished from S. gregorii NCIB 12823T and S. cebuensis SPE 10-1T. Strain YIM 60513T therefore represents a novel species of the genus Saccharopolyspora, for which the name Saccharopolyspora gloriosae sp. nov. is proposed. The type strain is YIM 60513T (=KCTC 19243T =CCTCC AA 207006T).

The genus Saccharopolyspora was first described by Lacey & Goodfellow (1975) and was assigned to the family Pseudonocardiaeae (Warwick et al., 1994). The genus encompasses aerobic, non-acid-fast organisms that form extensively branched substrate hyphae that may fragment into rod-shaped elements and aerial hyphae that may segment into bead-like chains of spores. Members of the genus are characterized chemotaxonomically by the presence of meso-diaminopimelic acid in the cell wall, arabinose and galactose as characteristic sugars in whole-cell hydrolysates, iso-branched and anteiso-branched fatty acids, major amounts of phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine and phosphatidylmethyllethanolamine and MK-9(H4) as the predominant menaquinone (Embley et al., 1987; Goodfellow et al., 1989; Lechevalier et al., 1977). At the time of writing, the genus comprises 19 recognized species: Saccharopolyspora hirsuta (Lacey & Goodfellow, 1975), S. erythraea (Labeleda, 1987), S. taberi (Korn-Wendisch et al., 1989), S. gregorii (Goodfellow et al., 1989), S. hordei (Goodfellow et al., 1989), S. rectivirga (Korn-Wendisch et al., 1989), S. spinosa (Mertz & Yao, 1990), S. spinosporotrichia (Zhou et al., 1998), S. flava (Lu et al., 2001), S. thermophila (Lu et al., 2001), S. cebuensis (Pimentel-Elardo et al., 2008), S. shandongensis (Zhang et al., 2008), S. antimicrobica (Yuan et al., 2008), S. halophila (Tang et al., 2009a), S. endophytica (Qin et al., 2008b), S. jiangxiensis (Zhang et al., 2009), S. rosea (Yassin, 2009), S. qijiaojingensis (Tang et al., 2009b) and S. tripterygii (Li et al., 2009).

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 60513T is EU005371.

A scanning electron micrograph showing curved chains of smooth-surfaced spores of strain YIM 60513T is available as supplementary material with the online version of this paper.
Endophytic actinomycetes have attracted attention in recent years, with increasing reports of isolates from a range of plant types (Araújo et al., 2002; Coombs & Franco, 2003; Gu et al., 2006; Qin et al., 2008a), but are relatively unstudied as potential sources of novel natural products for medical and commercial exploitation. The present study forms part of our long-term investigations into the diversity and bioactivity of endophytic actinomycetes from tropical rainforest medicinal plants of Yunnan Province, China. In this paper, an endophytic actinomycete, designated strain YIM 60513T, was isolated. Based on data from the present polyphasic taxonomic research, this strain is considered to represent a novel species of the genus Saccharopolyspora.

Strain YIM 60513T was isolated from stem samples of Gloriosa superba L., a traditional Chinese medicinal plant, collected from the tropical rainforest in Xishuangbanna, Yunnan Province, south-west China. The strain was isolated by using the following procedure. Fresh stem samples were air-dried for 48 h and then washed thoroughly as described by Coombs & Franco (2003). Subsequently, stems were subjected to a modified five-step surface sterilization procedure according to Qin et al. (2008a): a 3-min wash in 5 % NaOCl, followed by a 10-min wash in 2.5 % Na2S2O3, a 5-min wash in 75 % ethanol, a wash in distilled water and a final rinse in 10 % NaHCO3 for 10 min. Surface-sterilized stems were then broken aseptically into small fragments after air-drying at 80 °C for 1 h and placed in tap water-yeast extract agar (Crawford et al., 1993), followed by incubation at 28 °C for 4 weeks. The isolate was routinely cultured on yeast extract-malt extract agar (ISP 2; Shirling & Gottlieb, 1966) and was maintained as a glycerol suspension (20 %, v/v) at −80 °C.

Cultural characteristics were observed after growth at 28 °C for 2 weeks according to Shirling & Gottlieb (1966) as well as by using potato-glucose agar, Czapek’s medium and nutrient agar (Waksman, 1967). Colony colour was determined according to Kelly (1964). Cell morphology was examined by light microscopy and scanning electron microscopy (JSM 5600LV; JEOL) after 20–30 days incubation on ISP 2 medium. Growth was tested at pH 4.0–11.0 (at intervals of 0.5 pH units) and at 0, 4, 7, 10, 12, 15, 18, 20, 22, 25, 28, 30, 32, 35, 37, 40, 45, 50, 55 and 60 °C on ISP 2 medium. NaCl tolerance (0–20 %, w/v) was also checked by using ISP 2 medium. Other physiological characteristics, including utilization of sole carbon and nitrogen sources for energy and growth and decomposition of test substances, were assessed by using the media and methods of Gordon et al. (1974).

Biomass for chemical and molecular systematic studies was obtained after incubation of strain YIM 60513T at 28 °C for 7 days in shaken flasks (about 150 r.p.m.) of trypticase soy broth. Analyses of amino acids and sugars were carried out by using the methods of Hasegawa et al. (1983). Menaquinones were extracted and purified by using the method described by Collins et al. (1977) and were analysed by HPLC (Groth et al., 1997). Polar lipids were extracted and identified by two-dimensional TLC (Minnikin et al., 1979). Fatty acids were analysed according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification system (Sasser, 1990; Kämpfer & Kropfenstedt, 1996).

Extraction of genomic DNA, PCR-mediated amplification of the 16S rRNA gene and sequencing of PCR products were carried out as described by Li et al. (2007). The 16S rRNA gene sequence of strain YIM 60513T was multiply aligned with selected sequences obtained from the GenBank/EMBL/DDBJ databases by using CLUSTAL_X software (Thompson et al., 1997). The alignment was verified manually and adjusted prior to the construction of a phylogenetic tree. Phylogenetic trees were constructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) methods with MEGA 3.1 (Kumar et al., 2004). Bootstrap analysis (Felsenstein, 1985) was performed to evaluate the reliability of the tree topology.

Chromosomal DNA was isolated according to Marmur (1961). The G+C content was determined by the HPLC method (Mesbah et al., 1989). DNA–DNA hybridization was carried out by using photobiotin-labelled probes in microplate wells as described by Ezaki et al. (1989) and He et al. (2005). A microplate spectrofluorometer (Gemini XPS; Molecular Devices) was employed for fluorescence measurements.

Strain YIM 60513T was an aerobic, Gram-positive, non-acid–alcohol-fast, non-motile actinomycete that formed extensively branched but non-fragmented substrate mycelium. It developed well on most test media, including ISP 2, ISP 3 and ISP 5 agar. Weak growth was observed on potato-glucose agar. No diffusible pigments were produced on any media tested. White to pale orange–yellow substrate mycelium and white aerial hyphae, which bore chains of spores arranged in hooks and curves, were produced on the above media. The surface of the spores was smooth (see Supplementary Fig. S1 in IJSEM Online). Strain YIM 60513T grew at 10–32 °C, with optimum growth at 28 °C. Growth occurred at pH 6.0–8.0 and in the presence of 0–11 % NaCl (w/v), with optimum growth at pH 7.0 and in the presence of 0–5 % NaCl (w/v). The results of other phenotypic tests are listed in the species description and in Table 1.

The assignment of strain YIM 60513T to the genus Saccharopolyspora was also supported by chemotaxonomic data. It contained meso-diaminopimelic acid as the cell-wall diamino acid and whole-cell hydrolysates contained arabinose, galactose and ribose (wall chemotype pattern IV of Lechevalier & Lechevalier, 1970). Phosphatidylcholine was detected as the diagnostic phospholipid (phospholipid type III of Lechevalier et al., 1977). The predominant menaquinone was MK-9(H4) (92 %), and MK-9(H6) (8 %) was detected as a minor component. The cellular fatty acid composition of strain YIM 60513T was iso-C16:0 (29.2 %),

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anteiso-C<sub>17:0</sub> (14.9 %), C<sub>17:1 cis</sub>9 (14.4 %), iso-C<sub>15:0</sub> (8.1 %), C<sub>17:0</sub>10-methyl (7.2 %), anteiso-C<sub>15:0</sub> (5.2 %), iso-C<sub>17:0</sub>1 (5.1 %), C<sub>16:1 cis</sub>9 (3.3 %), iso-C<sub>16:1</sub> H (3.1 %), iso-C<sub>14:0</sub> (2.7 %), C<sub>16:0</sub> 9-methyl (2.7 %), C<sub>17:0</sub> (2.7 %) and C<sub>15:0</sub> (1.8 %).

The almost-complete 16S rRNA gene sequence of strain YIM 60513<sup>T</sup> (1456 bp) was determined in this study. Comparison of the 16S rRNA gene sequence with available sequences in public databases indicated that strain YIM 60513<sup>T</sup> belongs to the genus Saccharopolyspora. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YIM 60513<sup>T</sup> was related closely to S. gregorii NCIB 12823<sup>T</sup> (99.1 % similarity) and S. cebuensis SPE 10-1<sup>T</sup> (97.3 %). These three strains formed a distinct clade in the phylogenetic tree with high bootstrap support (Fig. 1). Levels of 16S rRNA gene sequence similarity between strain YIM 60513<sup>T</sup> and the type strains of other recognized Saccharopolyspora species were less than 97 %. It has been suggested that bacterial strains sharing less than 97 % 16S rRNA gene sequence similarity are members of different genomic species (Stackebrandt & Liesack, 1993).

The DNA G+C content of strain YIM 60513<sup>T</sup> was 71.6 mol%. Given the high level of similarity of their 16S rRNA gene sequences, DNA–DNA hybridization was performed to differentiate between YIM 60513<sup>T</sup> and its closest neighbour, S. gregorii NCIB 12823<sup>T</sup>. The level of DNA–DNA relatedness between strain YIM 60513<sup>T</sup> and S. gregorii NCIB 12823<sup>T</sup> was 43 ± 1.7 % (mean ± SD of five determinations), which is well below the accepted 70 % threshold level for species differentiation (Stackebrandt & Goebel, 1994). DNA–DNA hybridization experiments between strain YIM 60513<sup>T</sup> and S. cebuensis SPE 10-1<sup>T</sup> were not carried out because of the lower level of 16S rRNA gene sequence similarity.

A comparison of the phenotypic characteristics of strain YIM 60513<sup>T</sup> and its closest relatives is shown in Table 1. It is clear from the comparisons that strain YIM 60513<sup>T</sup> is phenotypically distinct from S. gregorii and S. cebuensis.

Table 1. Comparison of the physiological properties of strain YIM 60513<sup>T</sup> and its nearest phylogenetic neighbours

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>YIM 60513&lt;sup&gt;T&lt;/sup&gt;</th>
<th>S. gregorii NCIB 12823&lt;sup&gt;T&lt;/sup&gt;</th>
<th>S. cebuensis SPE 10-1&lt;sup&gt;T&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spore arrangement</td>
<td>Hooks and curved hyphae</td>
<td>Hooks or flexuous hyphae</td>
<td>Straight</td>
</tr>
<tr>
<td>Substrate mycelium</td>
<td>Branched</td>
<td>White</td>
<td>Fragments</td>
</tr>
<tr>
<td>Colour of aerial mycelium</td>
<td>White</td>
<td>White-yellow</td>
<td>Fragments</td>
</tr>
<tr>
<td>Colour of substrate mycelium</td>
<td>White, yellowish white, pale orange–yellow</td>
<td>Colourless, buff</td>
<td>White</td>
</tr>
<tr>
<td>Colour of soluble pigment</td>
<td>None</td>
<td>+</td>
<td>Brown</td>
</tr>
<tr>
<td>Degradation of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine</td>
<td>+</td>
<td>–*</td>
<td>–</td>
</tr>
<tr>
<td>Casein</td>
<td>–</td>
<td>+*</td>
<td>–</td>
</tr>
<tr>
<td>Chitin</td>
<td>–</td>
<td>+*</td>
<td>–</td>
</tr>
<tr>
<td>Gelatin</td>
<td>–</td>
<td>+*</td>
<td>ND</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>+</td>
<td>+*</td>
<td>–</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>+*</td>
<td>ND</td>
</tr>
<tr>
<td>Xanthine</td>
<td>+</td>
<td>–*</td>
<td>ND</td>
</tr>
<tr>
<td>NaCl tolerance (% w/v)</td>
<td>&lt;11</td>
<td>&lt;13</td>
<td>2.5–12.5</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>10–32</td>
<td>10–35</td>
<td>15–37</td>
</tr>
<tr>
<td>Utilization as sole carbon source of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Galactose</td>
<td>–</td>
<td>+*</td>
<td>+</td>
</tr>
<tr>
<td>Glycogen</td>
<td>+</td>
<td>–*</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>–</td>
<td>–*</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>+</td>
<td>+*</td>
<td>–</td>
</tr>
<tr>
<td>Raffinose</td>
<td>–</td>
<td>+*</td>
<td>+</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>+*</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>+*</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>–*</td>
<td>ND</td>
</tr>
<tr>
<td>Utilization of L-serine as sole nitrogen source</td>
<td>+</td>
<td>–*</td>
<td>ND</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>71.6</td>
<td>74.0</td>
<td>72.6</td>
</tr>
</tbody>
</table>

*Data from the present study.

The almost-complete 16S rRNA gene sequence of strain YIM 60513<sup>T</sup> (1456 bp) was determined in this study. Comparison of the 16S rRNA gene sequence with available sequences in public databases indicated that strain YIM 60513<sup>T</sup> belongs to the genus Saccharopolyspora. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain YIM 60513<sup>T</sup> was related closely to S. gregorii NCIB 12823<sup>T</sup> (99.1 % similarity) and S. cebuensis SPE 10-1<sup>T</sup> (97.3 %). These three strains formed a distinct clade in the phylogenetic tree with high bootstrap support (Fig. 1). Levels of 16S rRNA gene sequence similarity between strain YIM 60513<sup>T</sup> and the type strains of other recognized Saccharopolyspora species were less than 97 %. It has been suggested that bacterial strains sharing less than 97 % 16S rRNA gene sequence similarity are members of different genomic species (Stackebrandt & Liesack, 1993).
**Description of Saccharopolyspora gloriosae sp. nov.**

Saccharopolyspora gloriosae (glo.ri.o’sae. N.L. fem. gen. n. gloriosae of the plant genus Gloriosa, referring to the isolation of the type strain from a stem of *Gloriosa superba*).

Aerobic, Gram-stain-positive, non-acid–alcohol-fast, non-motile actinomycetes that forms extensively branched substrate mycelium. Smooth spores are arranged in curved and hooked chains on aerial mycelium. Grows well on ISP 2, ISP 3 and ISP 5 media, moderately well on ISP 4, nutrient agar and Czapek’s agar, but poorly on potato-dextrose agar. No diffusible pigment is produced.

**Temperature range for growth is 10–32 °C. Growth on pH 6.0–8.0 and in the presence of 0–11 % NaCl (optimum growth at 0–5 % NaCl). **

Utilizes a variety of organic compounds as sole carbon sources, including adonitol, D-arabinose, L-arabinose, D-arabitol, D-xylose. Utilizes L-adenine, D-arginine, L-asparagine, L-leucine, L-histidine, L-ornithine, L-proline, L-serine and L-threonine as sole nitrogen sources. Degrades adenine, hypoxanthine, starch, tyrosine and xanthine, but not inorganic nitrogen sources. Degrades adenine, hypoxanthine, starch, tyrosine and xanthine, but not inorganic nitrogen sources.

**or chitin. Negative for oxidase and reduction of nitrate. The cell wall contains meso-diaminopimelic acid as the diagnostic diamino acid. Whole-cell hydrolysates contain arabinose, galactose and ribose. The predominant menaquinone is MK-9(H4). The major cellular fatty acids (≥10 %) are iso-C16:0, anteiso-C17:0 and C17:1 ω9.**

The phospholipids comprise phosphatidylycholine, phosphatidylethanolamine, phosphatidylmethylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and several unknown phospholipids. The DNA G+C content of the type strain is 71.6 mol%.

The type strain, YIM 60513^T^ (=KCTC 19243^T^ =CCTCC AA 207006^T^), was isolated from a surface-sterilized stem of *Gloriosa superba* L. collected from the tropical rainforest of Xishuangbanna, Yunnan Province, south-west China.

**References**


