Saccharopolyspora subtropica sp. nov., a thermophilic actinomycete isolated from soil of a sugar cane field

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A novel thermophilic actinomycete, designated strain T3T, was isolated from a soil sample of a sugar cane field. The strain grew at 25–60 °C (optimum 37–50 °C), at pH 6.0–11.0 (optimum 7.0–9.0) and with 0–12.0 % (w/v) NaCl (optimum 0–7 %). The aerial mycelium was white and the vegetative mycelium was colourless to pale yellow. The substrate mycelium fragmented into rod-shaped elements after 4–5 days at 50 °C. The aerial mycelium formed flexuous chains of 5–20 spores per chain; the oval-shaped spores had spiny surfaces and were non-motile. The organism contained meso-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. The whole-cell sugars consisted of arabinose, galactose and ribose. The cellular fatty acid profile consisted mainly of anteiso-C₁₇ : ₀, iso-C₁₇ : ₀ and iso-C₁₆ : ₀. The quinone system was composed predominantly of MK-9(H₄). The phospholipids detected were diphosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylmethylethanolamine and ninhydrin-positive glycophospholipids. The DNA G+C content of strain T3T was 71.3 mol%. The organism showed a combination of morphological and chemotaxonomic properties typical of members of the genus Saccharopolyspora. In the 16S rRNA gene tree of Saccharopolyspora it formed a distinct phylogenetic line and was related most closely to Saccharopolyspora thermophila 216T. However, the phenotypic characteristics of strain T3T were significantly different from those of S. thermophila 216T and DNA–DNA hybridization revealed a low level of relatedness (28.6–32.3 %) between them. Based on the phenotypic and phylogenetic data, strain T3T represents a novel species in the genus Saccharopolyspora, for which the name Saccharopolyspora subtropica sp. nov. is proposed. The type strain is T3T (=DSM 46801T=CGMCC 4.7206T).

The genus Saccharopolyspora was established by Lacey & Goodfellow (1975) based on the type strain of Saccharopolyspora hirsuta and the description was emended by Warwick et al. (1994). Currently, the genus Saccharopolyspora belongs to the family Pseudonocardiaceae (Embley et al., 1988; Stackebrandt et al., 1997; Zhi et al., 2009) and contains 26 species with validly published names (http://www.bacterio.net/saccharopolyspora.html). Strains in the genus Saccharopolyspora possess meso-diaminopimelic acid as the cell-wall diamino acid, contain arabinose and galactose in their whole-cell hydrolysates, have MK-9(H₄) as the predominant menaquinone, and have major amounts of phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine and phosphatidylmethylethanolamine. The G+C content of the DNA is 66–77 mol%.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain T3T is KM114900.

Three supplementary figures are available with the online Supplementary Material.

Guangxi is located in a subtropical area of China and enjoys a humid monsoon climate; it is the largest sugar cane producing province in the country. During a survey of the biodiversity of rare actinomycetes in Guangxi province, a thermophilic actinomycete strain was isolated from a soil sample of a sugar cane field from Nanning (22°39’04” N 108°11’36” E). The soil samples were air-dried for 1 week after collection and isolated with the dilution plate technique, grown on modified HV agar (Hayakawa & Nonomura, 1987) (with 200 g of mushroom compost extract to replace each 1 g of humic acid in HV agar) and incubated at 50 °C in the dark for 5 days under aerobic conditions. Strain T3T was cultivated and maintained on modified (addition of 0.2 g Na₂HPO₄ per litre) ISP (International Streptomyces Project) 2 medium (Shirling & Gottlieb, 1966) at 4 °C and as 20 % (w/v) glycerol suspensions at −80 °C.

Cultural characteristics of strain T3T were tested on ISP2, ISP3, ISP4, ISP5, ISP6 and ISP7 media, Czapek’s agar, potato dextrose agar (Waksman, 1961), Gause’s asparagine
Saccharopolyspora subtropica sp. nov.

agar (Gause et al., 1983), Bennett’s agar (Jones, 1949), HV agar and water agar (15.0 g agar, 1000 ml tap water) after incubation for 3 and 7 days at 50 °C. Morphological characteristics were observed under light microscopy (Nikon 80i) and scanning electron microscopy (VEGA3 SBU) after incubation for 5 days on ISP2 medium at 50 °C. The colours of the substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the ISCC-NBS colour charts (Kelly, 1964). Growth temperature was tested on ISP2 medium at 4, 16, 25, 30, 37, 40, 45, 50, 55, 60 and 65 °C and observed after 14 days. Tolerance to between 0 and 15 % (w/v) NaCl (at intervals of 1 %) was tested on ISP2 and observed after 7 and 14 days at 30 °C. The pH range and the optimum pH for growth were examined on Bennett’s agar with the pH range between pH 4.0 and 11.0 (at intervals of 1.0 pH unit) using the following buffer system: pH 4.0–5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M KH₂PO₄/0.1 M NaOH; pH 9.0–10.0, 0.1 M NaHCO₃/0.1 M Na₂CO₃; pH 11.0, 0.05 M Na₂HPO₄/0.1 M NaOH. After 7 and 14 days of incubation at 50 °C, growth was scored as a positive result. Carbon source utilization for growth was examined on ISP9 as described by Shirling & Gottlieb (1966). The media and procedures used for determination of physiological features were as described by Williams et al. (1983).

Strain T3T grew well on HV agar, Bennett’s agar, ISP2, ISP3, ISP4, ISP5, ISP7, Czapek’s agar and Gause’s asparagus agar, and exhibited moderate growth on ISP6, potato dextrose agar and water agar. It formed well-developed aerial mycelium and vegetative mycelium on all the media tested. The aerial mycelium was white and the vegetative mycelium was colourless to pale yellow. The substrate mycelium fragmented into rod-shaped elements after 4–5 days at 50 °C. The aerial mycelium formed flexuous chains of 5–20 spores per chain, and the oval-shaped spores had spiny surfaces and were non-motile (Figs S1 and S2, available in the online Supplementary Material). The strain did not produce diffusible pigment on any of the media tested. No sporangia structures were observed. Strain T3T grew at 25–60 °C and with 0–12 % (w/v) NaCl (optimum 7.0–9.0) and with 0–12 % (w/v) NaCl (optimum 0–7 %). Other data for physiological and biochemical properties are given in the species description.

Biomass for chemotaxonomic studies was obtained by centrifugation of the culture in Bennett’s broth for 3 days at 50 °C and freeze-dried. The cell-wall diamino acid of strain T3T was determined from whole-cell hydrolysates as described by Hasegawa et al. (1983). Whole-cell sugars were analysed according to the procedures developed by Lechevalier & Lechevalier (1980). Polar lipids were extracted and examined by two-dimensional TLC and identified according to the method of Minnikin et al. (1984). Menaquinones were extracted and purified using the method of Collins et al. (1987) and separated by HPLC (Kroppenstedt, 1982). Extraction and analysis of mycolic acids followed the procedure described by Minnikin et al. (1980). Cellular fatty acid methyl esters were prepared and analysed by GC according to the instructions of the Microbial Identification System (version 2.11; MIDI), using the MI7H10 (version 3.8) database for the identification of fatty acids. The DNA G+C content of the genomic DNA was determined by reversed-phase HPLC according to Mesbah et al. (1989). DNA–DNA hybridization between strain T3T and Saccharopolyspora thermophila CGMCC 4.1511T (Lu et al., 2001) was performed on nylon membranes using the method described by Wang et al. (2011).

The whole-cell hydrolysates of strain T3T contained meso-diaminopimelic acid as the diagnostic diamino acid of the cell-wall peptidoglycan and the whole-cell sugars were arabinose, galactose and ribose. The phospholipids detected were diphosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylmethylethanolamine, ninhydrin-positive glycopospholipids, an unknown phospholipid and a glycolipid (Fig. S3). The predominant menaquinone of strain T3T was MK-9(H₄). The fatty acid profile consisted of major amounts of anteiso-C₁₇:₀ (28.1 %), iso-C₁₇:₀ (25.1 %) and iso-C₁₆:₀ (23.6 %), and the other components were iso-C₁₅:₀ (4.5 %), C₁₇:₀ (4.5 %), C₁₈:₀ (3.5 %), C₁₆:₁ (3.2 %), iso-C₁₈:₁ (1.8 %) and anteiso-C₁₅:₀ (1.0 %). The DNA G+C content of strain T3T was 71.3 mol%.

Genomic DNA for PCR amplification was prepared according to the method of Li et al. (2007) and the 16S rRNA gene was amplified using primers 27f (5'–GAGTTTGATCCTGGGTCAG-3') and 1525r (5'–AGAAAGGAGGTGATCCAGCC–3'). The PCR product was purified with a Gel extraction kit (Biomiga) and sequenced on an automatic DNA sequencer (model 3730x; Applied Biosystems). An almost full-length 16S rRNA gene sequence (1438 bp) was aligned and compared with available sequences in the GenBank/EMBL/DDBJ databases using BLAST searches (Altschul et al., 1997) and analysed using the EzTaxon server (http://www.ezbiocloud.net/eztaxon; Kim et al., 2012), revealing that the isolate was a member of the genus Saccharopolyspora. Multiple alignments with sequences from all recognized species in the genus Saccharopolyspora were carried out using CLUSTAL X (Thompson et al., 1997). Phylogenetic trees were reconstructed with representative sequences using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A neighbour-joining tree was reconstructed by using the software package MEGA version 6.0 (Tamura et al., 2013) and by using distances corrected according to Kimura’s two-parameter model (Kimura, 1980). Bootstrap analysis was performed by using 1000 neighbour-joining datasets (Felsenstein, 1985).

The 16S rRNA gene sequence of strain T3T showed highest similarity to members of the genus Saccharopolyspora (93.1–96.9 %). Highest sequence similarity was shown to
S. thermophila 216T (96.9 %). In the neighbour-joining tree (Fig. 1) based on the 16S rRNA gene sequences of all recognized species in the genus Saccharopolyspora, showing the evolutionary divergence between strain T3T and the type strains of Saccharopolyspora species, strain T3T formed a distinct phylogenetic lineage with the closely related S. thermophila 216T (99 % bootstrap support). However, the mean DNA–DNA relatedness values between strain T3T and S. thermophila CGMCC 4.1511T were 28.6–32.3 %, well below the 70 % cut-off point recommended for the assignment of strains to the same species (Wayne et al., 1987), and indicating that strain T3T represents a different species.

The chemotaxonomic and morphological characteristics of strain T3T also indicated that it belongs to the genus Saccharopolyspora and can be distinguished from its nearest phylogenetic neighbour S. thermophila 216T. The spore chains of strain T3T are flexuous and the spores have a spiny surface, contrasting with the hooks or flexuous hyphae and smooth spores of S. thermophila 216T. The two strains can clearly be distinguished from each other.

**Fig. 1.** Neighbour-joining tree derived from aligned 16S rRNA gene sequences showing the phylogenetic position of strain T3T among all recognized species in the genus Saccharopolyspora. Nonomuraea africana IFO 14745T was used as an outgroup. Asterisks indicate the corresponding branches supported by both the maximum-likelihood (Felsenstein, 1981) and the maximum-parsimony (Fitch, 1971) methods. Bootstrap values are based on 1000 resamplings; only values above 50 % are shown. Bar, 0.01 substitutions per nucleotide position.
based on morphology. Strain T3T was also different from *S. thermophila* 216T in the following physiological characteristics: it grew at 25–60 °C with an optimum temperature range of 37–55 °C and growth occurred in the presence of 12 % (w/v) NaCl; it was positive for reduction of nitrate, gelatin liquefaction and hydrolysis of hypoxanthine and negative for hydrolysis of adenine, aesculin and xylan. L-Arabinose was utilized as a sole carbon source, but cellobiose, lactose, salicin, propionate, raffinose and rhamnose were not. Furthermore, strain T3T exhibited some different chemotaxonomic properties from *S. thermophila* 216T: it contained MK-9(H4) as the predominant menaquinone, and diphosphatidylglycerol, phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine and phosphatidylmethylethanolamine as diagnostic phospholipids (Fig. S3). Differential morphological, physiological and chemotaxonomic properties between strain T3T and *S. thermophila* 216T are shown in Table 1.

Based on morphological characteristics, chemotaxonomic profile and phylogenetic analysis, it is confirmed that strain T3T belongs to the genus *Saccharopolyspora*. Our analysis also demonstrates that strain T3T and *S. thermophila* 216T are genotypically and phenotypically distinct from each other. Several lines of evidence confirm the distinct nature of strain T3T, most notably including the results of DNA–DNA hybridization as well as cultural and physiological tests. The major distinctions observed between strain T3T and *S. thermophila* 216T include differences in the type of spore arrangement and spore surface, temperature and NaCl content ranges that allow for growth, utilization of sole carbon sources, degradative properties and enzyme activities. On the basis of a combination of phylogenetic distinctiveness and differences in chemotaxonomic and morphological characteristics, strain T3T represents a novel species in the genus *Saccharopolyspora*, for which the name *Saccharopolyspora subtropica* sp. nov. is proposed.

**Description of *Saccharopolyspora subtropica* sp. nov.**

*Saccharopolyspora subtropica* (sub.tro.pi.ca. N.L. fem. adj. *subtropica* pertaining to the subtropical zone, the origin of the soil sample from which the type strain was isolated). Aerobic, Gram-stain-positive, non-acid-fast, non-motile, thermophilic actinomycete. Grows well on HV medium, Bennett’s agar, ISP2, ISP3, ISP4, ISP5, ISP7, Czapek’s agar and Gause’s asparagine agar; exhibits moderate growth on ISP6, potato dextrose agar and water agar. It forms well-developed aerial mycelium and vegetative mycelium on all media tested. The aerial mycelium is white and the vegetative mycelium is colourless to pale yellow. The substrate mycelium fragments into rod-shaped elements after 4–5 days at 50 °C. The aerial mycelium forms flexuous chains of 5–20 spores per chain, and the oval-shaped spores have spiny surfaces.

### Table 1. Differential characteristics between strain T3T and *S. thermophila* CGMCC 4.1511T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>T3T</th>
<th><em>S. thermophila</em> CGMCC 4.1511T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spore arrangement</td>
<td>Flexuous</td>
<td>Hooks or flexuous hyphae</td>
</tr>
<tr>
<td>Spore surface ornamentation</td>
<td>Spiny</td>
<td>Smooth</td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>25–60</td>
<td>45–55</td>
</tr>
<tr>
<td>Tolerance of NaCl (%)</td>
<td>0–12</td>
<td>0–7</td>
</tr>
<tr>
<td>Degradation of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Aesculin</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Xylan</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Reduction of nitrate</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Growth on sole carbon sources:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Salicin</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Propionate</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Menaquinone(s)</td>
<td>MK-9(H4)</td>
<td>MK-9(H4), MK-9(H6)</td>
</tr>
<tr>
<td>Polar lipids*</td>
<td>DPG, PC, PE, PI, PME, NPG, PL</td>
<td>DPG, PE, PME, PC, NPG, PL</td>
</tr>
</tbody>
</table>

*DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PME, phosphatidylmethylethanolamine; NPG, ninhydrin-positive glyrophospholipids; PL, unknown phospholipid; GL, unknown glycolipid.*
and are non-motile. Does not produce diffusible pigment on any of the media tested. No sporangia structures are observed. Grows at 25–60 °C with an optimum temperature range of 37–50 °C, and at pH 6.0–11.0 (optimum 7.0–9.0) and with 0–12 % (w/v) NaCl (optimum 0–7 %). Hydrolyses L-tyrosine, starch, hypoxanthine, gelatin, guanine, allantoin and urea but not casein, aesculin, adenine, xylan or xanthine. Positive for catalase, nitrate reduction and milk coagulation and peptonization but negative for oxidase and H₂S production. Tweens 20, 40, 60 and 80 are hydrolysed. Utilizes L-arabinose, glucose, sucrose, inositol, mannotol, D-fructose, D-mannose, ribose, trehalose, D-galactose, xyitol, D-sorbitol, maltose, sodium acetate, sodium citrate and sodium oxalate as sole carbon sources but not lactose, D-xylene, inulin, dextrin, raffinose, L-rhamnose, salicin, propionate or cellobiose. The whole-cell hydrolysates contain meso-diaminopimelic acid as the diagnostic cell-wall diamino acid and the whole-cell sugars are arabinose, galactose and ribose. The phospholipids detected are diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycinol, phosphatidylethanolamine, phosphatidylmethylethanolamine, ninhydrin-positive glycocephospholipids, an unknown phospholipid and a glycolipid (Fig. S3). The predominant menaquinone is MK-9(H₄). The fatty acid profile consists of major amounts of anteiso-C₁₇:₀, iso-C₁₇:₀ and iso-C₁₆:₀ and the other components are iso-C₁₅:₀, C₁₇:₀, C₁₈:₀, C₁₆:₀, iso-C₁₈:₀ and anteiso-C₁₅:₀.

The type strain is T3T (= DSM 46801T=CGMCC 4.7206T), isolated from soil of a sugarcane field in Nanning, Guangxi province, south-western China. The DNA G+C content of the type strain is 71.3 mol%.

Acknowledgements

We thank Professor Yunpeng Lian for his encouragement and invaluable discussion on morphological identification of the strains. This research was supported by Guangxi Natural Science Foundation Key Programs (No. 2010GXNSFD013027) and Project of Guangxi Innovation Team of China Agriculture Research System (CARS-GXIT-08).

References


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