Description of \textit{Saccharomonospora xinjiangensis} sp. nov. based on chemical and molecular classification

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Comparative studies of morphology, physiology, biochemistry and chemical composition of cells, and phylogenetic analysis based on 16S rRNA gene sequences were carried out with strains XJ-54$^T$ and XJ-58 of the genus \textit{Saccharomonospora} and type strains of related genera. The results indicated that the two strains are different from known members of the genus \textit{Saccharomonospora}. A new species with the name \textit{Saccharomonospora xinjiangensis} sp. nov. is proposed. The new species is characterized by the presence of longitudinal pairs of spores on both the aerial and the vegetative hyphae and contains phosphatidyl ethanolamine, phosphatidyl choline and unknown glucosamine-containing phospholipids, and the major menaquinones MK-9$(H_2)$, MK-9$(H_4)$ and MK-7$(H_4)$.

**Keywords**: \textit{Saccharomonospora xinjiangensis} sp. nov., 16S rRNA

**INTRODUCTION**

The genus \textit{Saccharomonospora} was first described by Nonomura & Ohara (21). The genus is characterized by the formation of single spores on the vegetative hyphae, and occasionally pairs and short chains on aerial hyphae, and contains meso-diaminopimelic acid, arabinose and galactose in the peptidoglycan (wall chemotype IV). Only one species, \textit{Saccharomonospora viridis}, is described in \textit{Bergey’s Manual} (20). There are currently three validly described species of the genus, \textit{Saccharomonospora azurea} Hu 1987 (8), \textit{Saccharomonospora cyanea} Hu et al. 1988 (9) and \textit{Saccharomonospora glauca} Greiner-Mai et al. 1988 (4). Recently, Yoon et al. (25, 26) investigated the rapid identification of the genus by using genomic DNA fragments and rRNA gene probes, and the RFLP analysis of PCR-amplified 16S rRNA genes. Kim et al. analysed the phylogenetic relationship among \textit{Amycolatopsis}, \textit{Pseudonocardia}, \textit{Saccharomonospora} and \textit{Saccharopolyspora} (11).

**METHODS**

\textbf{Actinomycete strains.} Strains XJ-54$^T$ and XJ-58 were isolated with glycerol-asparagine agar and HV agar (7) from soil samples collected from Xinjiang, People’s Republic of China. Some of the type cultures for comparative studies were obtained from ATCC, NRRL and IFO.

**Morphology.** The media used for micromorphological studies were yeast extract-malt extract agar (ISP 2) and oatmeal agar (ISP 3) (23), and the incubation time was 14–21 d at 28 °C. Morphological observations of spores were made with a light microscope and a model EPMA-8705 electron microscope.

**Cultural, physiological and biochemical tests.** The media and procedures used for the study of the cultural and physiological characteristics of the strains were those described by Shirling & Gottlieb (23), Locci (17), Waksman (24), Gordon (5) and Gordon & Horan (6). Colour determinations were made by comparing the cultures with colour chips from the ISCC-NBS color charts standard samples no. 2106 (10).

**Cell wall preparation.** Cell walls were purified and analysed using the methods of Lechevalier & Lechevalier (15).

**Whole-cell analysis of diagnostic cell wall amino acids.** The procedures of Becker et al. (1) and Lechevalier & Lechevalier (14, 15) were used for whole-cell analysis.

**Phospholipid analysis.** The phospholipid analysis was carried out using the method of Lechevalier et al. (16).

**Menaquinone analysis.** Menaquinones were determined using the procedures of Collins (2).

**16S rRNA gene sequencing.** The chromosomal DNA was extracted using the procedures of Marmur (19), Jiang & Xu (12) and Zhou (27). The 16S rRNA gene was amplified by PCR (22) using Taq DNA polymerase (Sino-American
Biotechnology), primer A 7-26 (5'CCG TCG ACG AGC TCA GAG TTT GAT CCT GGC TCA G 3') and primer B 1523-1504r (5'CCC GGG TAC CAA GCT TAA GGA GGT GAT CCA GGC GCA 3'). The 1.5 kb amplified 16S rRNA gene fragment was purified by agarose gel electrophoresis. The purified fragments were directly sequenced using a FS-DNA sequencing kit (Applied Biosystems). The sequencing primers used were primer A, primer B, primer C 704-685r (5'TCT GCG CAT TTC ACC GCT AC 3') and primer D 1151-1130r (5'AGG GTT GCG CTC GTT G 3'). Sequencing was performed with a model 377 PRISM automatic sequencer (Applied Biosystems).

The 16S rRNA gene sequences of typical strains of related genera were obtained from GenBank.

Sequence alignment and phylogenetic analysis. The 16S rRNA gene sequences were manually aligned with the sequences of members of the order Actinomycetales by using the ae2 editor (18). Genetic distances were calculated by using PAUP 3.1. A phylogenetic tree was reconstructed by using treeing algorithms contained in the PHYLIP package.

RESULTS

Micromorphology

Strains XJ-54T and XJ-58 were Gram-positive and non-acid fast. The vegetative hyphae were well developed, long, irregularly branched, fine (0.3-0.6 μm in diameter) and did not fragment. A large number of paired spores were borne in longitudinal pairs on the vegetative hyphae. Most of spores were oval or ellipsoidal and 0.7-0.9 μm in diameter by 1.0-1.4 μm long; the spore surface was smooth. The aerial hyphae of strains XJ-54T and XJ-58 were abundant on many media. Their aerial mycelium was irregularly branched, and fine (0.4-0.6 μm in diameter). Spores were borne in longitudinal pairs or singly on aerial mycelium. The spores were spherical or oval and 0.6-0.9 μm in diameter; the spore surface was smooth. The spores on both vegetative and aerial mycelium were non-motile. No sporangia were observed (Fig. 1).

Cultural characteristics

Table 1 shows the degree of growth and colour of both aerial and vegetative hyphae of strain XJ-54T on various media tested. The aerial mycelium of strain XJ-54T was abundant and yellow-white in colour on most of the media used but was light green-grey on Czapek agar. The reverse sides of colonies were light yellow on most media used. The sporulation of vegetative hyphae and aerial mycelium was abundant on most media tested. The strain produced light yellow-brown diffusible pigment on potato extract-glucose agar and did not produce melanin on tyrosine agar (ISP 7).

Physiological characteristics

The physiological reactions of strain XJ-54T are shown in Table 2. Autolysis of strain XJ-54T was observed when it was cultured on yeast extract-malt extract agar and nutrient agar.

Chemotaxonomy

The cell walls of strains XJ-54T and XJ-58 contained meso-diaminopimelic acid. The whole-cell hydrolysates contained galactose and arabinose. No madurose was detected. Thus strains XJ-54T and XJ-58 can be considered to have a type IV cell wall. The

![Fig. 1. Scanning electron micrographs of spores of strain XJ-54T.](image)

<table>
<thead>
<tr>
<th>Agar medium</th>
<th>Growth</th>
<th>Aerial mycelium</th>
<th>Vegetative mycelium</th>
<th>Diffusible pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czapek agar</td>
<td>Good</td>
<td>Light grey green</td>
<td>Pale yellow</td>
<td>None</td>
</tr>
<tr>
<td>Glycerol-asparagine (ISP 5)</td>
<td>Moderate</td>
<td>None</td>
<td>Yellowish white</td>
<td>None</td>
</tr>
<tr>
<td>Inorganic salt-starch (ISP 4)</td>
<td>Poor</td>
<td>None</td>
<td>Yellowish brown</td>
<td>None</td>
</tr>
<tr>
<td>Oatmeal (ISP 3)</td>
<td>Good</td>
<td>None</td>
<td>Pale yellow</td>
<td>None</td>
</tr>
<tr>
<td>Potato extract-glucose</td>
<td>Good</td>
<td>Yellowish white</td>
<td>Pale yellow</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Nutrient</td>
<td>Good</td>
<td>Yellowish white</td>
<td>Yellowish white</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Yeast extract-malt extract (ISP 2)</td>
<td>Good</td>
<td>Yellowish white</td>
<td>Yellowish white</td>
<td>Light yellow</td>
</tr>
</tbody>
</table>
### Table 2. Physiological and biochemical characters of strain XJ-54<sup>T</sup>

<table>
<thead>
<tr>
<th>Character</th>
<th>Reaction</th>
<th>Character</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanin</td>
<td>+</td>
<td>Enzyme production:</td>
<td></td>
</tr>
<tr>
<td>Carbon source utilization:</td>
<td></td>
<td>Lecithinase</td>
<td></td>
</tr>
<tr>
<td>Adonitol</td>
<td>+</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;S production</td>
<td>-</td>
</tr>
<tr>
<td>Celllobiose</td>
<td>+</td>
<td>NO&lt;sub&gt;2&lt;/sub&gt; reduction</td>
<td>+</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>+</td>
<td>Autolysis</td>
<td>+</td>
</tr>
<tr>
<td>Inulin</td>
<td>+</td>
<td>Antibiosis:</td>
<td></td>
</tr>
<tr>
<td>d-mannitol</td>
<td>+</td>
<td>Aspergillus niger</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>Bacillus subtilis</td>
<td>+</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>Escherichia coli</td>
<td>-</td>
</tr>
<tr>
<td>D-xylose</td>
<td>+</td>
<td>Antibiotic resistance:</td>
<td></td>
</tr>
<tr>
<td>Inositol</td>
<td>+</td>
<td>Neomycin (50 μg ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>+</td>
</tr>
<tr>
<td>Nitrogen source utilization:</td>
<td></td>
<td>Rifampicin (50 μg ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>+</td>
</tr>
<tr>
<td>Alanine</td>
<td>+</td>
<td>Growth at 45 °C</td>
<td>+</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>+</td>
<td>50 °C</td>
<td>+</td>
</tr>
<tr>
<td>Proline</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degradation of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

unusual type PIV phospholipid pattern was found (phosphatidyl ethanolamine, phosphatidyl choline and unknown glucosamine-containing phospholipids). The major menaquinones of XJ-54<sup>T</sup> were MK-9(H<sub>2</sub>), MK-9(H<sub>4</sub>) and MK-7(H<sub>4</sub>).

**16S rRNA gene sequence comparisons and phylogenetic analysis**

The nearly complete 16S rRNA sequence (1468 nucleotides) of strain XJ-54<sup>T</sup> was determined. The sequence was compared with the corresponding sequences of the representative reference strains of cell wall types I–IV of the other genera of the order Actinomycetales. The results show that strain XJ-54<sup>T</sup>, *Saccharomonospora viridis* (21), *Saccharomonospora caesia* (3) and *Saccharoglycospora hirsuta* are clustered into a group. The evolutionary distances among the four strains were 0.0096–0.0448. Fig. 2 shows a neighbour-joining phylogenetic tree constructed on the basis of the evolutionary distances calculated by using the 1516 positions that could be aligned.

**DISCUSSION**

Morphological characteristics and phospholipid pattern of XJ-54<sup>T</sup> are the same as those of the genus *Actinobispora* (13). However, on the basis of the similarity of 16S rRNA gene sequences, strain XJ-54<sup>T</sup> should be placed in the genus *Saccharomonospora*. The two strains, XJ-54<sup>T</sup> and XJ-58 are clearly different from *Saccharomonospora azurea* and *Saccharomonospora cyanea* in morphological and cultural characteristics and growth temperatures. No aerial hyphae of strains XJ-54<sup>T</sup> and XJ-58 were observed on ISP 4 and ISP 5, spores were borne in pairs on vegetative hyphae, and growth temperature was between 45 and 50 °C while *Saccharomonospora azurea* and *Saccharomonospora cyanea* have white and dark blue aerial hyphae, no spores on vegetative hyphae and growth temperature of 28–40 °C. 16S rRNA gene sequences of strain XJ-54<sup>T</sup>, *Saccharomonospora viridis* and *Saccharomonospora glauca* are different from each other, and the evolutionary distances are greater than 0.04. Therefore we propose strains XJ-54<sup>T</sup> and XJ-58 as a new species named *Saccharomonospora xinjiangensis* sp. nov.

### Description of *Saccharomonospora xinjiangensis* sp. nov.

*Saccharomonospora xinjiangensis* (xin.jiang.en’sis. M.L. neut. adj. xinjiangensis pertaining to Xinjiang, a province of north-west China).

Light yellowish vegetative hyphae. Yellow-white aerial mycelium on most media tested, light green-grey on Czapek agar. The sporulation of both vegetative hyphae and aerial mycelium are abundant on most media tested. The spores are borne in longitudinal pairs on vegetative hyphae, and in longitudinal pairs (or in a few cases singly) on aerial hyphae. Light yellow-brown diffusible pigment is produced on potato extract-glucose agar and no melanin is produced on tyrosine agar. Adonitol, inulin, cellulose, fructose, rhamnose, mannitol, raffinose, xylose and inositol are utilized but no acid is produced from these carbon sources. Alanine, histidine and proline are utilized.
Actinoplanes philippinensis
Saccharomonospora chaleur
Dactylosporangium aurantiacum
Saccharothrix aurantiens
Actinomadura madurae
dMicrobispora chromogenes
Microbispora thermostoea
Microbispora thermohydrogenes
Trophosporosa glauca
Saccharothrix australiensis
Kutzneria viridogrisea
Nocardia asteroides
Nocardia otitidisaviarum
Nocardia seriae
Tsukamurella inchoensis
Pseudonocardia thermophila
Actinobispora yunnanensis
Actinobispora aurantiacae
Actinobispora alaniniphila
Actinobispora xingjiangensis
Saccharomonospora vinidn
Saccharomonospora caela

Saccharomonospora xingjiangensis XJ-54
Saccharopolyspora hirsuta
Promicromonospora enterophila
Nocardia de albus
Streptomyces thermocarboxydus
Streptomyces thermocarboxydivorans
Streptomyces thermoviscusae
Streptomyces coelicolor
Streptomyces albus
Streptomyces ladanatum subsp. ladanum
Streptomyces hygroscopus
Streptomyces olivoreticuli
Streptomyces lavendulae
Streptomyces griseus
Thermoactinomyces dichotomicus
Thermoactinomyces vulgaris
Thermoactinomyces candidus
Escherichia coli

Fig. 2. Phylogenetic tree showing the relationships between Saccharomonospora xingjiangensis, related species of the same genus and other taxa based on 16s rRNA gene sequences.

Starch is hydrolysed. Produces hydrogen sulfide. Degrades cellulose. Produces lecithinase. Autolysis of aerial hyphae is observed on yeast extract-malt extract agar and nutrient agar. Growth at 45 and 50 °C. Has been isolated from soil in Xinjiang, China. The type strain is XJ-54T.

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REFERENCES

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