INTRODUCTION

On the basis of phylogenetic analysis of 16S rRNA gene sequences, Stackebrandt et al. (1997) proposed the family Streptosporangiaceae, containing the type genus Streptosporangium Couch 1955 as well as the genera Herbidospora Kudo et al. 1993, Microbispora Nonomura and Ohara 1957 emend. Zhang et al. 1998, Microtetraspora Thiemann et al. 1968 emend. Zhang et al. 1998, Planobispora Thiemann and Beretta 1968, Planomonospora Thiemann et al. 1967, Planotetraspora Hu et al. 1993 and Nonomuraea Zhang et al. 1998. In addition, the genus Acrocarpospora was proposed by Tamura et al. (2000) as another member of the Streptosporangiaceae. Description of taxa of each genus was based on phenotypic, genotypic and phylogenetic properties.

In the course of research on unknown microbial resources and microbiological biodiversity of actinomycetes, we isolated some strains of Streptosporangium from Yunnan Province, a region of south-western China that has specific geographical conditions that contribute to its great microbiological diversity. These isolated strains were identified using a polyphasic approach employing phenotypic, genotypic and phylogenetic techniques, such as study of morphological and physiological properties, cell chemistry, G+C content of the genomic DNA, DNA–DNA hybridization and phylogenetic analysis. The strains belong to two novel species of Streptosporangium on the basis of 16S rRNA gene sequencing. The results of morphological, physiological and biochemical investigations and DNA–DNA hybridization indicated that the two strains are different from known members of the genus Streptosporangium. The names Streptosporangium yunnanense sp. nov. (type strain CY-11007T = CCTCC AA 97009T = CCRC 16307T = DSM 44663T) and Streptosporangium purpuratum sp. nov. (type strain CY-15110T = CCTCC AA 97010T = CCRC 16308T = DSM 44688T) are proposed. They have been deposited in CCTCC in Wuhan.

Streptosporangium yunnanense sp. nov. and Streptosporangium purpuratum sp. nov., from soil in China

Li-Ping Zhang,1 Cheng-Lin Jiang2 and Wen-Xin Chen3

1College of Life Sciences, Hebei University, Baoding 071002, PR China
2The Key Laboratory for Microbial Resources of Ministry of Education, PR China, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China
3Department of Microbiology, College of Life Sciences, China Agricultural University, Beijing 100094, PR China

Two strains of Streptosporangium were isolated from Yunnan Province, a region of China with specific geographical conditions that contribute to its great microbiological diversity. They were identified using a polyphasic approach employing phenotypic, genotypic and phylogenetic techniques, such as study of morphological and physiological properties, cell chemistry, G+C content of the genomic DNA, DNA–DNA hybridization and phylogenetic analysis. The strains belong to two novel species of Streptosporangium on the basis of 16S rRNA gene sequencing. The results of morphological, physiological and biochemical investigations and DNA–DNA hybridization indicated that the two strains are different from known members of the genus Streptosporangium. The names Streptosporangium yunnanense sp. nov. (type strain CY-11007T = CCTCC AA 97009T = CCRC 16307T = DSM 44663T) and Streptosporangium purpuratum sp. nov. (type strain CY-15110T = CCTCC AA 97010T = CCRC 16308T = DSM 44688T) are proposed. They have been deposited in CCTCC in Wuhan.

METHODS

Bacterial strains. Test strains CY-11007T and CY-15110T used in this study were isolated from soil samples collected from Yunnan Province, China. Type strains used for comparative studies were Streptosporangium violaceochromogenes JCM 3281T (= DSM 43849T), Streptosporangium longisporum JCM 3106T (= DSM 43180T), Streptosporangium roseum JCM 3005T (= DSM 43021T), Streptosporangium vulgare JCM 3028T (= DSM 43022T), Streptosporangium nondaissaticum JCM 3114T (= DSM 43848T), Streptosporangium amethystogenes JCM 3026T (= DSM 43179T), Streptosporangium album JCM 3025T (= DSM 43023T), Streptosporangium fragile JCM 6242T (= DSM 43847T), Streptosporangium pseudovulgare JCM 3115T (= DSM 43181T) and Streptosporangium viridialbum JCM 3027T (= DSM 43801T).
Morphology. Strains CY-11007<sup>T</sup>, CY-15110<sup>T</sup> and other type strains were cultured for 3, 5, 7 and 14 days at 28 °C on International Streptomyces project medium 3 (ISP3; oatmeal agar) (Shirling & Gottlieb, 1966) and HV agar (Hayakawa & Nonomura, 1987) and observed by light microscopy (Olympus) and scanning electron microscopy (model KYKY-AMRAY-100B).

Cultural, physiological and biochemical tests. Cultural characteristics were studied by using 14-day-old cultures grown at 28 °C on various agar media. Colours were determined by comparing the cultures with colour chips from the ISCC-NBS colour charts standard samples no. 2106 (Kelly, 1964). The physiological characteristics of the strains were tested according to the standards for Streptosporangium (Nonomura, 1989).

Analysis of chemotaxonomic characteristics. Cultures of the test strains were grown in shake flasks containing Bennett’s broth to prepare biomass. These cultures were incubated for 5–7 days at 28 °C. Cell walls were purified from 10 g wet biomass. Amino acids of purified cell walls were analysed by the methods of Lechevalier & Lechevalier (1980). Amino acids and sugars of whole-cell hydrolysates were determined by the methods of Becker et al. (1964). Phospholipids were obtained from freeze-dried biomass (approx. 100 mg) and analysed by the methods of Lechevalier et al. (1981). Menaquinone analysis (from 100 mg freeze-dried biomass) was performed as described previously by Collins (1985). Methyl esters of cellular fatty acids (from 10 mg freeze-dried biomass) were determined by the methods of Miller (1982) and Kuykendall et al. (1989).

DNA base composition. The G + C content of the chromosomal DNA was determined from the melting point value of the thermal denaturation profile using a spectrophotometer (Ultrrospec 2000) equipped with a programmable temperature-control unit by the equation of Marmur & Doty (1962), as modified by De Ley (1970).

DNA–DNA hybridization. Chromosomal DNA of strains CY-11007<sup>T</sup> and CY-15110<sup>T</sup> was prepared as described by Marmur (1961). The initial reassociation rate method (De Ley et al., 1970) was used for determining percentage DNA–DNA hybridization.

16S rRNA gene sequencing. Chromosomal DNA was extracted as described by Marmur (1961) and Jiang & Xu (1990). 16S rRNA genes were amplified by PCR (Saiki et al., 1988) using a PCR kit (Sino-American Biotechnology Co.) with primers A 8–38f (5’-CG-GGATCCAGAGTTTGATCCTGGCTCAGAACGAACGCT-3’) and B 1479–1506r (5’-CGGGATCTACGGCTACCTTGTTACGACTTCA-GAGTTTACGACTTCA-CCC-3’) and the 1.5 kb amplified fragment was purified by 0.8% low-melting-point agarose gel electrophoresis, by the methods of Wieslander (1979). Purified PCR products and plasmid pUC18 vector were cut with BamHI and ligated at 18 °C for 20 h. Ligated plasmids were transformed into Escherichia coli DH5α (Wieslander, 1979) and transformants were selected by blue-white selection (Sambrook et al., 1989). Plasmids were extracted and purified according to the methods of Tiessen & Rizzino (1991). Purified plasmids containing PCR products were sequenced with a model 377 Prism automatic sequencer by American Cybersyn Company. The sequencing primer was 5’-TTCAGGCGGCGAGATTTGA-3’. The 16S rRNA gene sequences of type strains of species of related genera were obtained from GenBank.

Phylogenetic analysis. The 16S rRNA gene sequences were aligned by the CLUSTAL X program (Thompson et al., 1997) with corresponding nucleotide sequences of representatives of the genus Streptosporangium retrieved from GenBank (Benson et al., 1997). Phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-parsimony (Fitch, 1971) algorithms from the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices were generated according to the model of Kimura (1980). Tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the neighbour-joining dataset using SEQBOOT and CONSENSE options from the PHYLIP suite of programs.

RESULTS AND DISCUSSION

Morphology

Strain CY-11007<sup>T</sup> produced aerial mycelium with single or clustered spherical sporangia. The sporangia were generally 4–20 μm in diameter. Spores within the sporangium were non-motile and formed by the septation of unbranched hyphae within the spore vesicle. Sporangiospores were spherical or oblate in shape (Fig. 1a, b). Strain CY-15110<sup>T</sup> produced globose, single or clustered sporangia, usually 2–5 μm in diameter on the aerial mycelium. Sporangiospores were formed by septation of a coiled, unbranched mycelium. Sporangiospores within the sporangium were spherical and non-motile (Fig. 1c, d).

Cultural characteristics

Strains CY-11007<sup>T</sup> and CY-15110<sup>T</sup> grew well on various inorganic or organic media and produced spherical sporangia; the vegetative and aerial mycelium grew well on the media tested. Table 1 shows the degree of growth and colour of both aerial and vegetative hyphae of strains CY1100T and CY-15110<sup>T</sup> on various media. The aerial mycelium of CY-11007<sup>T</sup> was abundant and pale-pink (7. p. Pink; Research Group of Actinomycetes, 1970) to yellowish-pink (31. p.y. Pink) on most of the media used. The substrate mycelium was brownish and produced pigment on some media tested. Colonies of strain CY-15110<sup>T</sup> were deep red (13. deep Red) to deep purplish-red (257. v.deep p. R), lacking visible aerial mycelium by eye.
on organic media and poor aerial mycelium on inorganic media used. The strain did not produce soluble pigment.

Physiological characteristics

The physiological and biochemical reactions of strains CY-11007T and CY-15110T are shown in Table 2. The optimal growth temperatures of strains CY-11007T and CY-15110T were respectively 30 and 28°C and optimal pH for both strains was 7-2.

Chemotaxonomy

Pure cell walls of strains CY-11007T and CY-15110T contained meso-diaminopimelic acid (meso-DAP). Whole-cell hydrolysates contained madurose as the diagnostic sugar. The two strains can be considered to have a type III/B cell wall. Phospholipids consisted of phosphatidylethanolamine (PE), phosphatidylinositol (PI), diphosphatidylglycerol (DPG), phosphatidylmethylethanolamine (PME, only found

| Table 2. Comparison of physiological characteristics of strains CY-11007T and CY-15110T and related strains |
| Strains: 1, CY-11007T; 2, S. nondiastaticum JCM 3114T; 3, S. pseudovulgate JCM 3115T; 4, CY-15110T; 5, S. longisporum JCM 3106T. All strains are positive for utilization of D-glucose and D-sucrose and hydrolysis of succinate and uric acid and negative for utilization of inositol, L-rhamnose, lactose and methyl α-D-glucoside and iodinin production. |
| Property | 1 | 2 | 3 | 4 | 5 |
| Carbon source utilization: | | | | | |
| L-Arabinose | – | + | + | – | + |
| D-Galactose | – | + | + | + | – |
| D-Fructose | – | + | + | + | – |
| D-Mannose | – | + | + | – | – |
| D-Mannitol | – | + | + | – | – |
| D-Xylose | – | + | + | + | + |
| D-Raffinose | – | + | + | – | – |
| D-Cellobiose | – | + | + | – | – |
| Degradation of: | | | | | |
| Cellulose | + | – | + | – | – |
| Starch | + | – | + | – | – |
| Gelatin liquefaction | + | + | + | – | – |
| Requirement for B vitamins | – | + | + | – | – |
| Nitrite from nitrate | – | + | + | – | – |
| Melanin produced | + | + | + | – | – |
| Uricase | – | – | – | – | – |
| Hydrolysis of: | | | | | |
| Malate | + | + | – | – | – |
| Citrate | + | + | – | – | – |
| Hippurate | + | + | – | – | – |
in strain CY-11007<sup>T</sup>) and phospholipids of unknown structure containing glucosamine (GluNus), and belong to the type PIV phospholipid pattern <i>senso</i> Lechevalier <i>et al.</i> (1981). The major menaquinones of strain CY-11007<sup>T</sup> were MK-9(H<sub>0</sub>), MK-9(H<sub>2</sub>) and MK-9(H<sub>4</sub>) (in descending order of abundance) and for strain CY-15110<sup>T</sup> were MK-9(H<sub>2</sub>), MK-9(H<sub>0</sub>) and MK-9(H<sub>4</sub>). The predominant fatty acids in whole-cell methyl esters were C<sub>16:0</sub> (14-73 %), C<sub>17:0</sub> (4-95 %), C<sub>18:0</sub> (10-24 %), C<sub>19:0</sub> (29-64 %) and C<sub>16:3</sub> (2-54 %) (strain CY-11007<sup>T</sup>) and C<sub>15:0</sub> (23-74 %), C<sub>16:0</sub> (42-41 %), C<sub>17:0</sub> (24-84 %), C<sub>18:0</sub> (0-88 %) and C<sub>17:3</sub> (0-66 %) (strain CY-15110<sup>T</sup>).

**DNA base composition and DNA hybridization**

The G + C contents of the DNA of strains CY-11007<sup>T</sup> and CY-15110<sup>T</sup> were respectively 71-06 and 69-10 mol%. The levels of DNA–DNA relatedness with type strains of <i>Streptosporangium</i> ranged from 9 to 56-8 % (Table 3).

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

The nearly complete 16S rRNA gene sequences of strains CY-11007<sup>T</sup> (1436 nucleotides) and CY-15110<sup>T</sup> (1422 nucleotides) were determined. The sequences were compared with the corresponding sequences of representatives of the <i>Streptosporangiaceae</i>. The results showed that strains CY-11007<sup>T</sup> and CY-15110<sup>T</sup> were clustered into a group. The evolutionary distance between the two strains was 2-956 %. Fig. 2 shows a neighbour-joining phylogenetic tree constructed on the basis of evolutionary distances calculated by using the method of Kimura (1980).

**Taxonomic conclusions**

The typical characteristics of members of the genus <i>Streptosporangium</i> are as follows. They produce globose/spherical sporangia on aerial mycelium. Sporangiospores are formed by septation of a coiled, unbranched hypha within the sporangium; they are spherical, oval or rod-shaped and non-motile. Cell walls contain N-acetylated muramic acid and meso-DAP but no characteristic sugars. Whole-cell hydrolysates contain madurose. Major phospholipids include unknown glucosamine-containing compounds, but no phosphatidylcholine or phosphatidylglycerol (PG). They are Gram-positive, aerobic and mesophilic, and a few species are thermotolerant. The G + C content of the DNA is 69-5–71 mol% (T<sub>m</sub>) (Nonomura, 1989).

The two strains CY-11007<sup>T</sup> and CY-15110<sup>T</sup> should be placed in the genus <i>Streptosporangium</i> based on their morphological characteristics, menaquinones, predominant fatty acids in whole-cell methyl esters and DNA base composition and the similarity of their 16S rRNA gene sequences. The results of phylogenetic analysis based on 16S rRNA gene sequences show that strains CY-11007<sup>T</sup> and CY-15110<sup>T</sup> were clustered into a group, and the evolutionary distance between them was below 5 %. The novel isolates and other representatives of <i>Streptosporangium</i> appear to be relatively distantly related. DNA–DNA hybridization is the standard criterion for designation of species, and the criterion for a species is ≥70 % DNA–DNA relatedness (Wayne <i>et al.</i>, 1987). The DNA–DNA relatedness of strains CY-11007<sup>T</sup> and CY-15110<sup>T</sup> with related type strains was < 70%. Cultural characteristics, physiological properties and biochemical reactions indicated that strain CY-11007<sup>T</sup> is different from strain CY-15110<sup>T</sup>. We therefore propose that the two isolates represent novel species, <i>Streptosporangium yunnanense</i> sp. nov.
Two novel species of *Streptosporangium* from soil

**Table 4. Differences between isolates CY-11007\textsuperscript{T} and CY-15110\textsuperscript{T} and related strains of *Streptosporangium***

All species, including the novel isolates, contain MK-9(H\textsubscript{0}), MK-9(H\textsubscript{2}), MK-9(H\textsubscript{4}) as predominant menaquinones and produce non-motile sporangiospores.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>Streptosporangium</em> species</th>
<th>CY-11007\textsuperscript{T}</th>
<th>CY-15110\textsuperscript{T}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporangia</td>
<td>Globose/spherical</td>
<td>Spherical</td>
<td>Spherical</td>
</tr>
<tr>
<td>Sporangiospore shape</td>
<td>Spherical, oval or rod</td>
<td><em>meso</em>-DAP</td>
<td><em>meso</em>-DAP</td>
</tr>
<tr>
<td>Cell wall</td>
<td>N-acetylated muramic acid, <em>meso</em>-DAP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole-cell hydrolysates</td>
<td>Madurose</td>
<td>Madurose, glucose, rhamnose</td>
<td>Madurose, glucose, rhamnose</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>GluNus</td>
<td>PE, PME, DPG, PI, GluNus</td>
<td>PE, DPG, PI, GluNus</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>69–71</td>
<td>71-06</td>
<td>69-10</td>
</tr>
<tr>
<td>Fatty acids</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(CY-11007\textsuperscript{T}) and *Streptosporangium purpuratum* sp. nov. (CY-15110\textsuperscript{T}). Differences between isolates CY-11007\textsuperscript{T} and CY-15110\textsuperscript{T} and related strains of *Streptosporangium* are shown in Table 4.

**Description of *Streptosporangium yunnanense* sp. nov.**

*Streptosporangium yunnanense* (yun.nan.en’se. N.L. neut. adj. *yunnanense* pertaining to Yunnan, a province of southwest China).

Aerobic, Gram-positive. Abundant aerial mycelium is pale-pink (7.p. Pink) to yellowish-pink (31.p. Pink) on most of the media tested such as glycerol-asparagine agar (ISP5), oatmeal agar (ISP3), oatmeal-yeast extract agar and Bennett’s agar. Substrate mycelium is brownish and pigment is produced on various media tested. Spherical sporangia are produced on aerial mycelium on HV agar and oatmeal agar. Sporangiospores are formed by septation of coiled, unbranched hyphae and are spherical and non-motile. Cell walls contain *meso*-DAP; whole-cell hydrolysates contain madurose, glucose and rhamnose. Phospholipids consist of PE, PME, DPG, PI and GluNus; PG is not detected. Major menaquinones are MK-9(H\textsubscript{0}), MK-9(H\textsubscript{2}) and MK-9(H\textsubscript{4}). Predominant fatty acids in whole-cell methyl esters are 16:0 (14-73 %), 17:0 (4-95 %), 18:0 (10-24 %), 19:0 (29-64 %) and 16:3 (2-54 %). G+C content of the DNA of the type strain is 71-06 mol%. Optimal growth temperature is 30 °C; growth occurs at 10 and 42 °C but not at 50 °C. Optimal pH for growth is 7-2. D-Glucose, D-sucrose and D-cellobiose are utilized; L-arabinose, D-galactose, D-fructose, D-mannose, D-mannitol, L-rhamnose, inositol, D-raffinose, D-xylene, sorbitol, sorbose, erythrose, lactose, melibiose, methyl α-D-glucoside and dextrin are not utilized. Degradation of cellulose and starch is positive. Reduction of nitrate and liquefaction of gelatin are positive. Vitamin B is not required for growth and iodinin production is negative. Melanin is produced. Uricase is negative. Succinate, malate, citrate, uric acid and hippurate are hydrolysed.

The type strain is CY-11007\textsuperscript{T} (=CCTCC AA 97009\textsuperscript{T} = CCRC 16307\textsuperscript{T} = DSM 44663\textsuperscript{T}).

**Description of *Streptosporangium purpuratum* sp. nov.**

*Streptosporangium purpuratum* (pur.pur.at’um. L. neut. adj. *purpuratum* clad in purple-violet, referring to the colony colour).

Aerobic, Gram-positive. Colonies are deep red (13. deep Red) to deep purplish-red (257. v. deep p. R) on most media tested such as glycerol-asparagine agar (ISP5), oatmeal agar (ISP3), oatmeal-yeast extract agar and Bennett’s agar. Aerial mycelium is very poor so that it is invisible by eye. Substrate mycelium is deep red and soluble pigment is not produced on various media tested. Spherical sporangia are produced on aerial mycelium on HV agar and oatmeal agar, usually 2–5 μm in diameter. Sporangiospores are formed by septation of coiled, unbranched hyphae; they are spherical or oblate in shape and non-motile. Cell walls contain *meso*-DAP; whole-cell hydrolysates contain madurose, glucose and rhamnose. Phospholipids consist of PE, DPG, PI and GluNus. Major menaquinones are MK-9(H\textsubscript{2}), MK-9(H\textsubscript{0}) and MK-9(H\textsubscript{4}). Predominant fatty acids in whole-cell methyl esters are 15:0 (23-74 %), 16:0 (42-41 %), 17:0 (24-84 %), 18:0 (0-88 %) and 17:3 (0-66 %). Optimal growth temperature is 28 °C; grows at 42 and 50 °C. Optimal pH for growth is 7-2. D-Glucose, D-sucrose, D-cellobiose, D-fructose and D-xylene are utilized; L-arabinose, D-galactose, D-mannose, D-mannitol, inositol, D-raffinose, L-rhamnose, sorbitol, sorbose, erythrose, lactose, melibiose, methyl α-D-glucoside and dextrin are not utilized. Degradation of cellulose and starch is positive. Reduction of nitrate and liquefaction of gelatin are negative. Vitamin B is not required for growth and iodinin and melanin are produced. Uricase is negative. Succinate, uric acid, malate, citrate and hippurate are hydrolysed.

The type strain is CY-15110\textsuperscript{T} (=CCTCC AA 97010\textsuperscript{T} = CCRC 16308\textsuperscript{T} = DSM 44688\textsuperscript{T}).
ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (no. 30270002) and the Key Laboratory for Microbial Resources of Ministry of Education, PR China.

REFERENCES


