NOTE

***Streptosporangium subroseum* sp. nov., an actinomycete with an unusual phospholipid pattern**Li-ping Zhang,¹ Cheng-lin Jiang² and Wen-xin Chen³¹ College of Life Sciences, HeBei University, Baoding 071002, PR China² The Key Laboratory for Microbial Resources of Ministry of Education, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China³ Department of Microbiology, College of Life Sciences, China Agricultural University, Beijing 100094, PR China

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A strain of *Streptosporangium* with an unusual phospholipid pattern was isolated from Yunnan Province, a region in China. The isolate, designated CY-7113^T, was identified by morphological and physiological properties, cell chemistry, genomic DNA G+C content, DNA–DNA hybridization and phylogenetic analysis. Based on 16S rRNA gene sequencing, DNA–DNA hybridization, phenotypic characteristics and its unusual phospholipid pattern, it was concluded that strain CY-7113^T belongs to a novel *Streptosporangium* species, for which the name *Streptosporangium subroseum* sp. nov. is proposed. Strain CY-7113^T (= CCTCC 97008^T = CRC 16302^T) is the type strain.

Keywords: *Streptosporangium subroseum* sp. nov., 16S rDNA, actinomycete

The genus *Streptosporangium* (Couch, 1955) is the type genus of the family *Streptosporangiaceae* (Goodfellow *et al.*, 1990), emended by Ward-Rainey *et al.* (1996) and Stackebrandt *et al.* (1997) and created in the suborder *Streptosporangineae* by Stackebrandt *et al.* (1997). The family *Streptosporangiaceae* contains several genera, including *Streptosporangium*, *Herbidospira*, *Microbispora*, *Microtetraspora*, *Planobispora*, *Planomonospora*, *Planotetraspora*, *Nonomuraea* and *Acrocarpospora* (Tamura *et al.*, 2000). In the course of research on unknown microbial resources, some strains of *Streptosporangium* were isolated from samples collected in Yunnan Province, a region of south-western China that has specific geographical conditions, i.e. it is located between the Tibet Plateau and the plain of south-west China and, although it is in the subtropical zone, its climate is spring-like all year round. One of these, strain CY-7113^T, was identified by a polyphasic taxonomy approach.

Strains of *Streptosporangium* used for the comparative study were *Streptosporangium roseum* JCM 3005^T, *Streptosporangium album* JCM 3025^T, *Streptosporangium amethystogenes* JCM 3026^T, *Streptosporangium viridialbum* JCM 3027^T, *Streptosporangium vulgare* JCM 3028^T, *Streptosporangium nondiastaticum* JCM 3114^T, *Streptosporangium pseudovulgare* JCM 3115^T, *Streptosporangium longisporum* JCM 3106^T and *Streptosporangium violaceochromogenes* JCM 3281^T.

Strain CY-7113^T and the other strains were cultured for 3, 5, 7 and 14 days at 28 °C on International *Streptomyces* Project (ISP) media (Shirling & Gottlieb, 1966), such as oatmeal agar (ISP3) and HV agar (Hayakawa & Nonomura, 1987), and observed using Olympus light microscopy and a model KYKY-AMRAY-100B scanning electron microscopy. Cultural characteristics were studied using 14-day-old cultures grown at 28 °C on various agar media. Colour determinations were made by comparing the culture with colour strips from the standard samples of the Research Groups of Actinomycetes (1970). Physiological characteristics of the strains were tested according to the *Streptosporangium* standard (Nonomura, 1989).

Chemotaxonomic characteristics were determined as described previously. For biomass preparation, cultures were grown in shake flasks containing Bennett's broth (Jones, 1949). These cultures were incubated for 5–7 days at 28 °C. When maximum growth was observed, the broth cultures were checked for purity, harvested by centrifugation and washed three times with distilled water. Cell walls (from 10 g wet biomass) were purified. Amino acids of purified cell walls were analysed using the method of Lechevalier & Lechevalier (1980). Diagnostic amino acids and sugars of whole-cell hydrolysates were determined using the methods of Becker *et al.* (1964). Phospholipids were obtained from freeze-dried biomass (approx. 100 mg) and analysis was carried out by the method of Lechevalier *et al.* (1981). Menaquinones (from 100 mg

Abbreviation: ISP, International *Streptomyces* Project.

The GenBank accession number for the 16S rDNA sequence of strain CY-7113^T is AF191734.

Fig. 1. Neighbour-joining tree, based on nearly complete 16S rDNA sequences, showing the relations between strain CY-7113^T and members of the genus *Streptosporangium*. The numbers at the nodes indicate the levels of bootstrap support based on neighbour-joining analyses of 1000 resampled datasets; only values over 50% are given. The letters f, p, and m under the nodes represent the clades that were recovered in Fitch-Margoliash, maximum-parsimony and maximum-likelihood trees, respectively. Bar, 0.01 nt substitution per nt position.

freeze-dried biomass) were analysed as described previously by Collins (1985). Methyl esters of cellular fatty acids (from 10 mg freeze-dried biomass) were determined according to Miller (1982) and Kuykendall *et al.* (1988).

The G + C content of the DNA was determined from the melting point value of the thermal denaturation profile according to the equation of Marmur & Doty (1962), as modified by De Ley (1970), using a spectrophotometer (Ultrospec 2000) equipped with a programmable melting temperature control unit. Chromosomal DNA of strain CY-7113^T was prepared by following the method of Marmur (1961). The initial-reassociation-rate method (De Ley *et al.*, 1970) was used for determining the percentage of DNA-DNA hybridization.

For 16S rDNA sequence comparisons and phylogenetic analysis, chromosomal DNA was extracted using the procedure described by Marmur (1961) and Jiang & Xu (1990). The 16S rDNA was amplified by PCR (Saiki *et al.*, 1988) (PCR kit; Sino-American Biotechnology) with primers A 8-37f (5'-CG-GGATCCAGAGTTTGATCCTGGCTCAGAACG-AACGCT-3') and B 1479-1506r (5'-CGGGATCC-TACGGCTACCTTGTTACGACTTCACCCC-3') (underlined sequences are *Bam*HI restriction sites) and the 1.5 kb amplified 16S rDNA fragment was purified by 0.8% low-melting-point agarose gel electrophoresis using the method of Wieslander (1979). Purified rDNA and plasmid pUC18 were cut with *Bam*HI and ligated at 18 °C for 20 h. Ligated plasmids were transformed into *Escherichia coli* DH5 α (Wieslander, 1979) and transformants were selected on the basis of results of the blue-white screening procedure (Sambrook *et al.*, 1989). Plasmids were extracted and purified according to the method of Tiesman & Rizzino (1991). Purified plasmids containing 16S rDNA were sequenced with a model 377 Prism automatic sequencer; the sequencing primer was 5'-TTCAGCAGGGACGAAGTTGA-3'.

The 16S rDNA sequences of the strains were aligned manually using the CLUSTAL X program (version 1.64b; Thompson *et al.*, 1997) against corresponding nucleotide sequences of representatives of the genus *Streptosporangium* retrieved from GenBank. Evolutionary trees were inferred by the neighbour-joining (Saitou &

Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-parsimony (Fitch, 1972) treeing algorithms from the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices were generated as described by Jukes & Cantor (1969). Tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings of the neighbour-joining dataset using the SEQBOOT and CONSENSE options from the PHYLIP suite of programs.

The nearly complete 16S rDNA sequence (1436 nt) of strain CY-7113^T was determined. The sequence was aligned with corresponding sequences of the representative reference strains of *Streptosporangiaceae*. The results show that strain CY-7113^T, *S. amethystogenes* and *S. longisporum* clustered into a group. The evolutionary distances of strain CY-7113^T from *S. amethystogenes* and *S. longisporum* were 1.568 and 2.290%, respectively. Fig. 1 shows a neighbour-joining phylogenetic tree constructed on the basis of the evolutionary distances calculated using bootstrap analysis (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings. It is clear from phylogenetic analyses that strain CY-7113^T belongs to the genus *Streptosporangium* and represents a distinct phyletic line that can be equated with a genomic species (Stackebrandt & Goebel, 1994).

Strain CY-7113^T produced aerial mycelium with single or clustered spherical sporangia. The sporangia were commonly 4–10 μ m in diameter. Spores in the sporangium were non-motile and formed by the separation of unbranched hyphae within the spore vesicle (Fig. 2). The vegetative mycelia grew well in the various organic and inorganic media tested.

Strain CY-7113^T grew well on various inorganic and organic media, produced spherical sporangia and different coloured aerial and vegetative hyphae. The aerial mycelium was abundant and white-pinkish in colour on most of the media used. The substrate mycelium was yellowish-brown and did not produce pigment on the various media tested.

The identification of the isolate at the genus level was also supported by chemotaxonomic analysis. Purified cell walls of strain CY-7113^T contained *meso*-diaminopimelic acid. The whole-cell hydrolysates contained madurose, glucose, ribose and rhamnose. This strain

Fig. 2. Scanning electron micrograph (a; bar, 1 µm) and light micrograph (b; bar, 10 µm) of strain CY-7113^T grown on oatmeal agar for 14 days at 28 °C showing hyphae and sporangia.

had a type III cell wall. Phospholipids consisted only of phosphatidyl ethanolamine and belonged to the type PII phospholipid pattern based on Lechevalier & Lechevalier (1980). The major menaquinones of the strain were MK-9(H₀), MK-9(H₂) and MK-9(H₄). The predominant whole-cell fatty acids (determined as methyl ester derivatives) were C_{16:0} (5.82%), C_{17:0} (11.39%), C_{18:0} (32.11%) and C_{19:0} (18.64%).

The physiological reactions of strain CY-7113^T are given in the species description. This strain did not grow in Bennett's agar containing concentrations of NaCl up to 20% (w/v). Optimal growth temperature was 30 °C; growth occurred at 10 and 42 °C, but no growth occurred at 50 °C. The G+C content of the DNA of CY-7113^T was 71.52 mol%.

The levels of DNA–DNA relatedness of strain CY-7113^T with *S. roseum* JCM 3005^T, *S. album* JCM 3025^T, *S. amethystogenes* JCM 3026^T, *S. viridialbum* JCM 3027^T, *S. vulgare* JCM 3028^T, *S. nondiasticum* JCM 3114^T, *S. pseudovulgare* JCM 3115^T, *S. longisporum* JCM 3106^T and *S. violaceochromogenes* JCM 3281^T were 15.8, 21.6, 27.8, 20.4, 11.7, 6.7, 3.9, 8.9 and 4.6%, respectively.

Strain CY-7113^T should be classified in the genus *Streptosporangium* based on its morphological characteristics, menaquinone composition, predominant fatty acids, DNA base composition and 16S rDNA sequence similarity. Evolutionary distances between strain CY-7113^T and the type strains of *S. amethystogenes* and *S. longisporum* were 1.568 and 2.290%, respectively. DNA–DNA hybridization is the standard for designation of species and the criterion for members of the same species is ≥ 70% DNA–DNA relatedness (Wayne *et al.*, 1987). The values of DNA–DNA relatedness of strain CY-7113^T with related type strains were < 70%. Strain CY-7113^T contained only phosphatidyl ethanolamine. *S. amethystogenes* and *S. longisporum* contained phospholipids of unknown structure containing glucosamine and/or ninhydrin-positive and sugar-positive phospholipids. Strain CY-7113^T did not produce violet-coloured crystals and

required B vitamins for growth, but *S. amethystogenes* produced the crystals and B vitamins were not required. Sporangiospores of strain CY-7113^T are spherical, but sporangiospores of *S. longisporum* are rod-shaped. It is therefore proposed that strain CY-7113^T be classified as a novel species, *Streptosporangium subroseum* sp. nov.

Description of *Streptosporangium subroseum* sp. nov.

Streptosporangium subroseum (sub.ro'se.um. L. neut. adj. *subroseum* pale rose coloured).

Abundant and white-pinkish in colour on most media, such as glycerol-asparagine agar (ISP5), oatmeal agar (ISP3), oatmeal-yeast extract agar and Bennett's agar. The substrate mycelium is yellowish-brown and does not produce pigment on the various media tested. Produces spherical sporangia on aerial mycelium on HV agar and oatmeal agar. Sporangiospores are formed by separation of coiled unbranched hyphae; they are spherical or oval-shaped and non-motile. Cell walls contain *meso*-diaminopimelic acid, whole-cell hydrolysates contain madurose, glucose, ribose and rhamnose. Phosphatidyl ethanolamine is the only phospholipid. Major menaquinones are MK-9(H₀), MK-9(H₂) and MK-9(H₄). Predominant whole-cell fatty acids are C_{16:0} (5.82%), C_{17:0} (11.39%), C_{18:0} (32.11%) and C_{19:0} (18.64%). DNA G+C content is 71.52 mol%. Does not grow in Bennett's agar containing NaCl concentrations of up to 20% (w/v). Optimal growth temperature is 28–30 °C; growth occurs at 10 and 42 °C, but not at 50 °C. L-Arabinose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, L-rhamnose, D-sucrose, D-xylose, D-raffinose, cellobiose are utilized, but not inositol. Degradation of cellulose is positive. Degradation of starch is negative. Positive reaction for reduction of nitrate and liquefaction of gelatin. B vitamins not required for growth. Iodine production is negative. The type strain of *Streptosporangium subroseum* is CY-7113^T (= CCTCC 97008^T = CCRC 16302^T).

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