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ORIGINAL PAPER

Streptomyces serianimatus sp. nov., isolated from a rhizophere soil

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Abstract A novel actinomycete strain, designated YIM 45720^T, was isolated from a *Cephalotaxus fortunei* rhizophere soil sample collected from Yunnan Province, southwest China. The strain formed well-differentiated aerial and substrate mycelia. Chemotaxonomically, it contained LL-diaminopimelic acid in the cell wall. The cell-wall sugars contained ribose, mannose, and galactose with traces of glucose and xylose. Phospholipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol. MK-9 (H_8) was the predominant menaquinone. The major fatty acids (>10%) were iso-$C_{16:0}$, iso-$C_{15:1}$ and anteiso-$C_{15:0}$. The G + C content of the DNA was 70 mol%. Phylogenetic analysis data based on 16S rRNA gene sequence showed that strain YIM 45720^T formed a distinct branch with the type strain of *Streptomyces scabrisporus* JCM 11712^T within the genus *Streptomyces*. On the basis of the phenotypic and genotypic characteristics, strain YIM 45720^T (=DSM 41883^T = CCTCC AA 206006^T) is proposed as the type strain of a novel species, *Streptomyces serianimatus* sp. nov.

Keywords *Streptomyces serianimatus* sp. nov. · Polyphasic taxonomy · 16S rRNA

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Introduction

The family *Streptomycetaceae*, which comprises the genus *Streptomyces*, *Kitasatospora* and *Streptacidiphilus*, was first proposed by Waksman and Henrici (1943) and emended by Kim et al. (2003). The genus *Streptomyces* was described by Waksman and Henrici (1943) simultaneously for aerobic and spore-forming actinomycetes. The streptomycetes, producers of more than half of the 10,000 documented bioactive compounds, such as antibiotics, enzymes, inhibitors and pharmacologically active agents, have offered over 50 years of interest to industry and academia (Anderson and Wellington 2001; Bérdy et al. 2005). In the course of our screening program, strain YIM 45720^T was isolated from a rhizophere soil sample obtained in Yunnan, China and provisionally assigned to the genus *Streptomyces* using chemotaxonomic and morphological properties. In this paper, we report the phenotypic and

genotypic characteristics of the novel isolate and it is proposed that it represents a novel species of the genus *Streptomyces*.

Materials and methods

Organism

Strain YIM 45720^T was isolated from a *Cephalotaxus fortunei* rhizophere soil sample collected from Xishuangbanna, Yunnan Province, southwest of China. A modification of Suzuki's and co-workers method (Suzuki et al. 1999) was used for isolation. The soil sample was dry in air for about 7 days. One gram of the soil sample was first suspended in 9 ml MOPS solution (10 mM) containing 1% keratin for 2 h, then incubated at 45°C for 1 h with vigorous shaking in order to kill fast-growing bacteria and promote actinomycete spore germination. This culture was centrifuged and 0.1 ml supernatant re-suspended in 9 ml sterile water before spread onto glycerol–asparagine agar (ISP 5 medium; Shirling and Gottlieb 1966). The incubation for isolation was performed at 28°C for 21 days. The strain was maintained on ISP 2 agar medium that contained (per liter): 4 g glucose, 4 g yeast extract, 5 g malt extract and vitamin-amino acid mixture (1 mg vitamin B_1; 1 mg vitamin B_2; 1 mg vitamin B_6; 1 mg biotin; 1 mg nicotinic acid; 1 mg phenylalanine; 0.3 g alanine) at pH 7.2. Strain YIM 45720^T was deposited in the Collection Center of Typical Cultures, China (CCTCC) as strain CCTCC AA 206006^T and the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH as strain DSM 41883^T.

Phenotypic characteristics

After incubation on ISP 2 medium at 28°C for 21 days, morphological features of spores and mycelia were examined by Olympus BH-2 microscope and scanning electron microscopy (Philips XL30; ESEM-TMP). All cultural characteristics were determined after 2 weeks at 28°C by methods used in the International *Streptomyces* Project (ISP, Shirling and Gottlieb 1966). In addition, carbon source utilization was as those described by Shirling and Gottlieb (1966) and Locci (1989). Temperature and pH tolerance were tested using modified Bennett's agar (Williams et al. 1983) as described by Xu et al. (2005). Susceptibility to antibiotics was examined by the method described by Groth et al. (2004). Colors and hues were determined according to the color chips from the ISCC-NBS Color Charts Standard Samples no.2106 (Kelly 1964).

Chemotaxonomy

Biomass for most of the chemotaxonomic studies and molecular systematic was obtained after incubation at 28°C for 1 week by growing in shake flasks of ISP 2 broth. Procedures for identification of cell-wall amino acids and sugars followed those described by Staneck and Roberts (1974). Analysis of whole-cell sugar compositions followed procedures described by Becker et al. (1965) and Lechevalier and Lechevalier (1980). Polar lipids were extracted, examined by two-dimensional TLC and identified using the procedures described by Minnikin et al. (1984). Menaquinones were isolated using the methods of Minnikin et al. (1984) and separated by HPLC (Kroppenstedt 1982). Biomass for the quantitative fatty acid analysis was prepared by scraping growth from TSB agar plates [trypticase soy broth (BBL), 3%(w/v); Bacto agar (Difco), 1.5% (w/v)] that had been incubated for 7 days at 28°C. The fatty acids were extracted, methylated and analysed using the standard MIDI (Microbial Identification) system (Sasser 1990; Kämpfer and Kroppenstedt 1996).

Molecular analysis

The chromosomal DNA for genomic DNA G + C content analysis was extracted as described by Marmur (1961). The G + C content of the DNA was determined using the HPLC method (Mesbah et al. 1989).

Genomic DNA was extracted and the 16S rRNA gene was amplified as described by Xu et al. (2003). The nearly complete 16S rRNA gene sequence (1410 nucleotides, GenBank Accession No. DQ997046) of strain YIM 45720^T was obtained and compared with those most

related type strains within the genus *Streptomyces* (downloaded from the GenBank/EMBL/DDBJ database). Phylogenetic analysis was performed using the software packages PHYLIP (Felsenstein 1993) and MEGA version 2.1 (Kumar et al. 2001) after multiple alignments of the data using CLUSTAL X (Thompson et al. 1997). Distances (using distance options according to the Kimura two-parameter model; Kimura 1980, 1983) were calculated and clustering was performed with the neighbour-joining method (Saitou and Nei 1987). Bootstrap analysis (1000 resamplings) was used to evaluate the tree topology of the neighbour-joining data (Felsenstein 1985). *Streptosporangium roseum* DSM 43021^T (GenBank sequence accession no. X89947) was used as the outgroup.

Results and discussion

Substrate mycelium of strain YIM 45720^T developed well and branched irregularly on all media tested; fragmentation of mycelium did not occur. Figure 1 shows a scanning electron micrograph of aerial spores of strain YIM 45720^T. The long spore chains were flexuous to straight; the oval spores are non-motile and the spore surface is smooth but shrunken. Sporangia or zoospores were not observed. Soluble pigments were not produced on any of the media tested. Aerial mycelium occurred late, and were produced after cultivation for 2 weeks on all media tested. Yellow-white aerial mycelia were slowly formed on ISP 5, ISP3 and Czapek's agar media; minor amounts of grey-white aerial mycelia were formed on ISP 2 and ISP 4. The color of the vegetable mycelia was deep orange yellow on ISP 2, yellow on ISP 3, yellowish brown on ISP 4 and yellow white on ISP 5 and Czapek's agar media. The physiological features are indicated in Table 1 and in the species description.

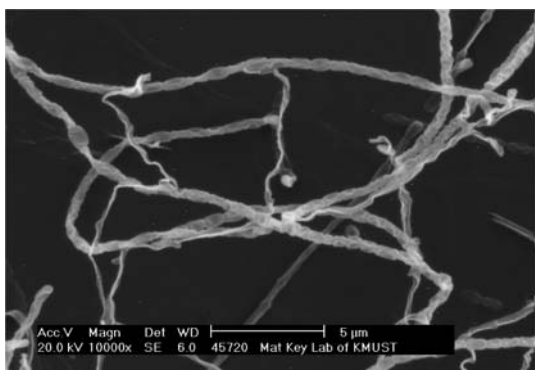

Fig. 1 Scanning electron micrograph of spore chains of strain YIM 45720^T grown on ISP 2 medium for 21 days at 28°C. Bar, 5 µm

Amino acids in the peptidoglycan layer of strain YIM 45720^T were LL-diaminopimelic acid, alanine, glycine and glutamic acid, indicating a type I wall chemotype according to the classification of Lechevalier and Lechevalier (1970). Whole-cell hydrolysates contained ribose, mannose and galactose, with traces of glucose and xylose. The major menaquinone detected was MK-9 (H_8) (87.8%) with minor components MK-9 (H_4) (1.9%) and MK-9 (H_6) (10.3%). Phospholipids of strain YIM 45720^T were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine phosphatidylinositol. Fatty acids present included iso-$C_{16:0}$ (24.5%), iso-$C_{15:1}$ (15.5%), anteiso-C15:0 (11.5%), iso-$C_{14:0}$ (7.7%), $C_{16:0}$ (7.3%), iso-$C_{17:1}$ (4.5%), anteiso-$C_{17:0}$ (4.2%), iso$C_{16:1}$H (4.2%), anteiso-$C_{17:1}$w9c (1.8%), $C_{14:0}$ (1.7%), $C_{17:1}$w8c (1.2%), $C_{18:1}$w9c (0.9%), $C_{16:1}$w9c (0.7%), $C_{17:0}$ (0.7%), iso$C_{13:0}$ (0.5), anteiso-$C_{15:1}$ (0.5%), $C_{18:0}$ (0.5%),

Table 1 Phenotypic properties that separate strain YIM 45720^T and its closest phylogenetic neighbour, *S. scabrisporus* JCM 11712^T

Characteristic	YIM 45720^T	*S. scabrisporus* JCM 11712^T*
Spore chain	Flexuous	Spiral
Spore surface	Smooth	Rugose
Aerial spore-mass colour	Grey-white	Grey
Hydrolysis of starch	–	+^{w}
Liquefaction of gelatin	+	–
Coagulation of milk	–	+^{w}
Utilization of inositol	–	+
Sucrose	+	d
L-Arabinose	+	d
Salicin	–	d
Rhamnose	–	+
D-xylose	–	+

Note: Both strains are negative for melanin production, positive for reduction of nitrate; the strains cannot utilize D-mannitol, raffinose and melibiose as carbon sources

+, Positive, utilized; –, negative, not utilized; d, doubtful; w, weak

* Data for the reference species were taken from Xu et al. (2004)

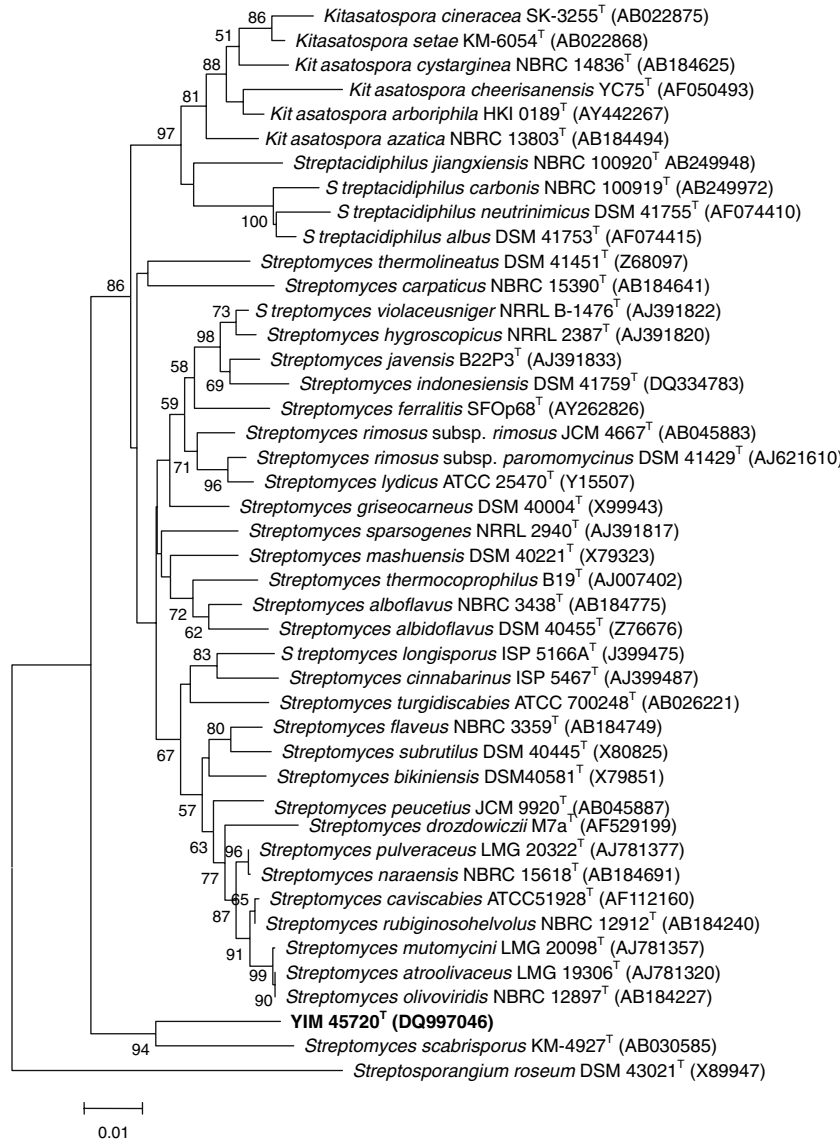

Fig. 2 Neighbour-joining dendrogram based on 16S rRNA gene sequences showing the positions of strain YIM 45720^T and related strains. Only bootstrap values above 50%, expressed as percentages of 1000 replications, are shown at the branch points. Bar, 0.01 substitution per nucleotide position

10-methyl $C_{17:0}$ (0.4%), iso-$C_{18:\ 1}$ (0.4%), $C_{13:0}$ (0.3%), $C_{18:0}$ (0.3%) and anteiso-$C_{\ 13:0}$ (0.3%). An major unidentifiable fatty acid (10.5%) was also detected.

Based on the chemotaxonomic data of strain YIM 45720^T, including its isomer of DAP, predominant phospholipids and predominant menaquinone, the organism could be placed in either the genera *Streptomyces* or *Streptacidiphilus*. However, its pH optimum for growth (7.0–8.0) is consistent with it belonging to the genera *Streptomyces* rather than *Streptacidiphilus.* Moreover, BLAST sequence analysis of the 16S rRNA gene sequence of strain YIM 45720^T points to it belonging to *Streptomyces* rather than *Streptacidiphilus*. It is evident from the phylogenetic tree (Fig. 2) that strain YIM 45720^T forms a distinct branch with the type strain of *Streptomyces scabrisporus* JCM 11712^T within the family *Streptomycetaceae*. The 16S rRNA sequence similarity value between them was 95.6%. It is generally accepted that organisms displaying 16S rRNA sequence similarity values of 97% or less belong to different species (Stackebrandt and Goebel 1994).

Furthermore, as shown in Table 1, strain YIM 45720^T can also be distinguished from the type

strain of *S. scabrisporus* JCM 11712^T in many phenotypic characteristics. For example, both strains have different types of spore chain and spore surface. *S. scabrisporus* JCM 11712^T could use inositol, D-xylose, and rhamnose as sole carbon source for growth, whereas the new isolate YIM 45720^T could not utilize these.

Thus, on the basis of the above phenotypic and genotypic data, we consider strain YIM 45720^T to represent a novel species of the genus *Streptomyces*, for which we propose the name *Streptomyces serianimatus* sp.nov. However, given the outlying position of YIM 45720^T and *S. scabrisporus* JCM 11712^T in our phylogenetic tree (Fig. 2), it may eventually be possible to describe these taxa as members of a novel genus within the family *Streptomycetaceae.*

Description of *Streptomyces serianimatus* sp. nov.

Streptomyces serianimatus (se'ri.an.i.ma.tus N.L adj serus slow, animatus N.L. adj animatus growing, referring to the aerial mycelia growing slowly on most media tested).

Gram-positive. Catalase-positive. Urease-positive. No H_2S is produced. Nitrate is reduced to nitrite. Substrate mycelia are well branched. Yellow-white to grey-white aerial mycelia are slowly formed on media tested, which matures into flexuous to straight long spore chains. The oval spores are non-motile and the spore surface is smooth but shrunken. No synnemata, sclerotia or sporangia are observed. No diffusible pigment is produced on all media tested. In addition to the properties shown in Table 1, aesculin, cellobiose, galactose, glucose, glycerol, maltose, ribose and salicin can be used as sole carbon sources, but not dextrin, mannose and mannitol. Acid is not formed from these carbon sources tested. Acetamide, adenine, L-alanine, L L-asparagine, casein, L-cysteine L-histidine, L-hydroxyproline, hypoxanthine, phenylalanine, L-proline, L-threonine, DL-tyrosine, urea, L-valine and xanthine can be used as sole nitrogen sources, but not L-arginine, glucosamine, L-glutamic acid, glycine, L-lysine, L-tryptophane or L-tyrosine. Gelatin is hydrolysed. Tween 20 and Tween 80 are degraded, but not cellulose and DNA. Tests for milk coagulation and peptonization are negative. The optimal growth temperature is 28°C with optimal pH 7.0–8.0. Growth occurs in the presence (μg ml^{-1}) of amikacin (32), amoxicillin (32), ampicillin (10), rifampin (5), clindamycin (2), sulfamethoxazole(23), trimethoprim (1.25), chloramphenicol (30), but not in presence of gentamicin sulphate (16), kanamycin sulphate (16), midecamycin (4), penicillin G (16 IU), streptomycin sulphate (16), tetracycline hydrochloride (32) ,erythromycin (8) or novobiocin (8). The diagnostic amino acid of the cell wall is LL-A_2pm. The whole-cell hydrolysates contain ribose, mannose and galactose, with traces of glucose and xylose. The menaquinones are MK-9 (H_4) (1.9%), MK-9 (H_6) (10.3%) and MK-9 (H_8) (87.8%). Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine phosphatidylinositol are the diagnostic phospholipids. The major fatty acids (>10%) were iso-$C_{16:0}$ (24.53%), iso-$C_{15:1}$ (15.48%) and anteiso-$C_{15:0}$ (11.52%). DNA G + C content is 70 mol%.

Strain YIM 45720^T (=DSM 41883^T = CCTCC AA 206006^T) was isolated from a Tropical Plant rhizosphere soil sample from Xishuangbanna, Yunnan, southwest of China.

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