A novel actinomycete, designated Z4\textsuperscript{T}, was isolated from soil in Yaan, Sichuan Province, south China. The taxonomic status of this strain was established using a polyphasic approach. The morphological and chemotaxonomic characteristics of the organism are typical of the members of the genus \textit{Streptomyces}. Phylogenetic analysis based on the almost complete 16S rRNA gene sequence indicated that strain Z4\textsuperscript{T} belonged to the genus \textit{Streptomyces}, branching off next to \textit{Streptomyces durhamensis} ATCC 23194\textsuperscript{T} (98.7\%), \textit{Streptomyces punicicabiae} KACC 20253\textsuperscript{T} (98.7\%) and \textit{Streptomyces filipinensis} ATCC 23905\textsuperscript{T} (98.6\%). However, DNA–DNA hybridization studies and phenotypic differences between strain Z4\textsuperscript{T} and closely related species of the genus \textit{Streptomyces} suggested that strain Z4\textsuperscript{T} represented a different genomic species. It is therefore proposed that Z4\textsuperscript{T} (=CGMCC 4.7035\textsuperscript{T}=KCTC 29111\textsuperscript{T}) represents the type strain of a novel species of the genus \textit{Streptomyces}, for which the name \textit{Streptomyces yaanensis} sp. nov. is proposed.

Micro-organisms are an abundant source of novel compounds and play an important role in the discovery of new sources of secondary metabolites with applications in medicine (Newman \textit{et al.}, 2003) and agriculture (Copping \\& Menn, 2000). Among them, streptomycetes are used extensively in industry because of their ability to generate a number of chemical compounds, including antibiotics, enzyme inhibitors, antitumour agents, antifungal compounds (Zhao \textit{et al.}, 2010) and enzymes, such as lipase (Vishnupriya \textit{et al.}, 2010), chitinase (Subramaniam \textit{et al.}, 2012) and xylanase (Liu \textit{et al.}, 2013). The genus \textit{Streptomyces} was originally proposed by Waksman \\& Henrici (1943). Strains of species of the genus \textit{Streptomyces} are aerobic and Gram-stain-positive actinomycetes with relatively high G+C content. They form extensively branched substrate and aerial mycelia that produce rod-shaped spores. L-L-diaminopimelic acid is present in the cell wall and characteristic sugars are absent in whole-cell hydrolysates (Otoguro \textit{et al.}, 2009). The genus \textit{Streptomyces} contains more than 600 species and subspecies with validly published names at this time and species of the genus \textit{Streptomyces} are abundant in soil.

In the course of an investigation of actinomycetes, strain Z4\textsuperscript{T} was isolated from soil collected from Yaan, Sichuan Province, south China. For isolation, the soil sample (1 g) was suspended in distilled water (100 ml) and spread on plates of International Streptomyces Project (ISP) 2 medium (Shirling \\& Gottlieb, 1966) after serial dilution. The plates were incubated at 28 °C for 7 days. The purified strain Z4\textsuperscript{T} was picked and maintained on ISP 2 agar slants at 4 °C and as 20 % (w/v) glycerol suspensions at −20 °C.

The morphological characteristics of strain Z4\textsuperscript{T}, including spore-chain morphology and surface ornamentation, were assessed by light microscopy (BH-2; Olympus) and scanning electron microscopy (JSM-5600LV; JEOL) of 8-day-old cultures on ISP 3 medium. Aerial mycelium colour, substrate mycelium pigmentation and coloration of the diffusible pigments of strain Z4\textsuperscript{T} were recorded on ISP media (Shirling \\& Gottlieb, 1966), Czapek’s agar and nutrient agar prepared as described by Dong \\& Cai (2001). Colour determination was assessed by comparison with the colour chips from Inter-Society Color Council–National Bureau of Standards (ISCC–NBS) Colour Charts standard sample no.2106 (Kelly, 1964).

From light and electron microscopic observations, it was found that strain Z4\textsuperscript{T} had an extensively branched substrate mycelium and aerial hyphae that carried smooth-surfaced spores in spiral spore chains (see Fig. S1, available in IJSEM Online). Strain Z4\textsuperscript{T} was observed to grow well on some of the media tested, including Czapek’s agar, potato–glucose agar, ISP 2, ISP 3 (oatmeal agar), ISP 4 (inorganic salts-starch.
agar) and ISP 5 (glycerol–asparagine agar), but growth was poor on nutrient agar. Culture characteristics of strain Z4\(^T\) are shown in Table 1. The aerial mycelium was white on some test media. The substrate mycelium was yellow or purple. Furthermore, we found that soluble pigments (brown–red) were produced only on ISP 3 agar.

Carbon source utilization was determined on ISP 9 medium supplemented with sterile carbon sources. Standard techniques were used for the determination of catalase, oxidase and nitrate reduction activities. Acid production from carbohydrates was assessed as described by Gordon et al. (1974). The effects of various temperatures (4–65 °C), pH (4.0–10.0) at intervals of 1.0 pH unit; determined at 28 °C and NaCl concentrations [0, 1, 2, 3, 4, 5, 10, 15, 20 and 25 % (w/v); determined at 28 °C] on growth were determined on cells grown on ISP 2 for 3–4 weeks (Zhao et al., 2009). Other phenotypic characteristics were tested using standard procedures (Goodfellow, 1971; Williams et al., 1983). Antibiotic resistance was examined by the disc diffusion method on ISP 2 medium with plates incubated at 28 °C for 7 days. The antimicrobial activity of strain Z4\(^T\) was determined by the double-layer agar method (Li et al., 2007). The test strains, purchased from the China General Microbiological Culture Collection Center (CGMCC), were Escherichia coli CGMCC 1.797, Staphylococcus aureus CGMCC 1.89, Bacillus subtilis CGMCC 1.1849 and Aspergillus niger CGMCC 3.2915. Bacterial test strains were incubated on nutrient agar at 37 °C for 24 h and the fungal strain was incubated on PDA medium [potato (infusion form), 200 g; glucose, 20 g; agar, 15 g; pH 5.6] at 28 °C for 24 h. The physiological and biochemical characteristics of strain Z4\(^T\) are given in Table 2 and in the species description.

For chemical analysis, biomass for chemotaxonomic studies was obtained after incubation at 28 °C for 7 days in shake flasks of ISP 2. Isomers of diaminopimelic acid and sugars of whole-cell hydrolysates were analysed according to the procedures developed by Hasegawa et al. (1983) and Lechevalier & Lechevalier (1970). Menaquinones were extracted and purified following the protocol of Collins et al. (1987); purified menaquinones were determined by reverse-phase HPLC (Wu et al., 1989). Polar lipids were examined by two-dimensional TLC and identified using the method of Minnikin et al. (1984). The cellular fatty acid profile was determined by growing the strain on TSBA (Difco) at 28 °C for 24 h. After growth, bacteria were saponified and the cellular fatty acids were extracted, purified, methylated and quantified by GC using the standard Microbial Identification System (MIDI; Sasser, 1990; Kämpfer & Kroppenstedt, 1996), the database used was TSBA40. The G + C content of the DNA was determined by means of the thermal denaturation method (Marmur & Doty, 1962) with E. coli AS 1.365 as a reference.

Strain Z4\(^T\) contained LL-diaminopimelic acid as the major diamino acid. Whole-cell hydrolysates contained glucose, galactose, ribose and mannose. The menaquinones were MK-9(H\(_6\)) (43.0 %), MK-9(H\(_8\)) (34.7 %) and MK-9(H\(_4\)) (14.5 %). The phospholipids were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol mannosides (PIM) and one unknown phospholipid (see Fig. S2). The major fatty acids found were iso-C\(_{16:0}\) (15.15 %), anteiso-C\(_{15:0}\) (14.44 %), C\(_{16:0}\) (12.17 %) and iso-C\(_{15:0}\) (11.07 %). The DNA G + C content of strain Z4\(^T\) was 68.7 mol%. All morphological and chemical features of strain Z4\(^T\) were consistent with its assignment to the genus Streptomyces.

Chromosomal DNA preparation, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product from strain Z4\(^T\) were carried out after the methods of Rainey et al. (1996) and the PCR product was
sequenced directly using the method of Lu et al. (2001). The EzTaxon server (http://eztaxon-e.ezbiocloud.net/) was used for identifying the closest known relatives of strain Z4T and calculating their sequence similarities. Multiple alignments with corresponding sequences of representatives of the genus Streptomyces (retrieved from the GenBank/EMBL/DDBJ database) and calculations of levels of sequence similarity were carried out using the CLUSTAL_X 1.8 software (Thompson et al., 1997). Phylogenetic trees were reconstructed with the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) methods using the MEGA5 program (Tamura et al., 2011), with bootstrap values based on 1000 replications.

The almost-complete 16S rRNA gene sequence (1438 bp) for strain Z4T was obtained. The closest BLAST hit for the 16S rRNA gene sequence is to Streptomyces diastatochromogenes subsp. luteus NBRC 13814 and these two sequences share 1425/1438 identical sites. This strain is not a type strain. So, it was not used for reconstructing the phylogenetic tree and the phylogenetic analysis. The phylogenetic tree (Fig. 1) reconstructed on the basis of 16S rRNA gene sequences indicated that strain Z4T was phylogenetically most closely affiliated to the genus Streptomyces. Strain Z4T shared greatest similarity to Streptomyces durhamensis ATCC 23194T (98.7 %), Streptomyces puniciscabiei KACC 20253T (98.7 %) and Streptomyces filipinensis ATCC 23905T (98.6 %). In view of the high level of 16S rRNA gene sequence similarities between strain Z4T and the most closely related Streptomyces species, DNA–DNA relatedness between them was studied using a thermal denaturation procedure (De Ley et al., 1970; Huß et al., 1983) with a UV-1206 spectrophotometer (Shimadzu) fitted with a TB-85 thermostab and standard software (Jahnke, 1992). Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values were quoted as DNA–DNA relatedness values. The DNA–DNA relatedness values of strain Z4T with Streptomyces durhamensis ATCC 23194T, Streptomyces puniciscabiei KACC 20253T and Streptomyces filipinensis ATCC 23905T were 40.6, 38.0 and 30.3 %, respectively, which were significantly lower than 70 %, the threshold value for the delineation of genomic species (Wayne et al., 1987), suggesting that strain Z4T should be considered to represent a different genomic species of the genus Streptomyces.

Besides the genotypic evidence, there are many phenotypic differences between strain Z4T and the most closely related species of the genus Streptomyces (Table 2). Therefore, on the basis of the data presented above, strain Z4T represents a novel species of the genus Streptomyces, for which the name Streptomyces yaanensis sp. nov. is proposed.
Description of *Streptomyces yaanensis* sp. nov.

*Streptomyces yaanensis* (ya.an.en’sis. N.L. masc. adj. *yaanensis* pertaining to Yaan, Sichuan Province, south China, the site from which the type strain was isolated).

Aerobic, Gram-stain-positive actinomycete. Forms spiral spore chains. Spores are smooth. Grows well on potato–glucose agar, Czapek’s agar media and ISP media 2, 3, 4 and 5 but poorly on nutrient agar. Aerial mycelium is white, substrate mycelium is white to yellow or purple. Soluble pigments are produced on ISP3 agar. No melanin production is observed on peptone–yeast extract–iron agar (ISP 6) or tyrosine agar (ISP 7). Gelatin is not liquefied. Milk is coagulated and peptonized. H₂S is produced. Nitrate is not reduced. Negative for Voges–Proskauer, methyl red tests and oxidase reaction. Starch, urea and Tween 20 are hydrolysed, but cellulose, Tween 40 and Tween 80 are not hydrolysed. Temperature range for growth is 15–45 °C (optimum 28 °C). Growth occurs at pH 5.0–11.0 and in the presence of 0–4% NaCl. Utilizes L-arabinose, fructose, galactose, D-glucose, *myo*-inositol, lactose, rhamnose, ribose, sucrose and D-xylose as carbon sources, weakly utilizes mannitol, but not raffinose. Adenine, L-alanine, L-asparagine, glycine, hypoxanthine, L-leucine, L-lysine, L-phenylalanine, L-tyrosine and L-tyrvaline, but not L-arginine or L-tryptophan, can be used as sole nitrogen sources. Acid is produced from L-arabinose, fructose, D-glucose, mannitol, ribose, starch and D-xylose and weakly from trehalose. Sensitive to ampicillin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), novobiocin (5 μg), penicillin G (20 IU), rifampicin (5 μg), streptomycin (50 μg) and tetracycline (30 μg), but resistant to nalidixic acid (30 μg) and sulfamethoxazole/trimethoprim (23.75/1.25 μg). Shows no antimicrobial activities against the four strains tested (*E. coli* CGMCC 1.797T, *Staphylococcus aureus* CGMCC 1.893T, *Bacillus subtilis* CGMCC 1.1849 and *Aspergillus niger* CGMCC 3.2915T). The fatty acid profile is composed mainly of iso-C₁₅∶₀, anteiso-C₁₅∶₀, iso-C₁₆∶₀, iso-C₁₇∶₀, anteiso-C₁₇∶₀, C₁₈∶₁ω₇c, C₁₈∶₀ and C₁₇∶₀ω₇c. Polar lipids are *diphosphatidylglycerol, phosphatidylethanolamine*, *phosphatidylinositol, phosphatidylglycerol, phosphatidylinositol mannosides* and one unknown phospholipid. The diagnostic amino acid in the peptidoglycan is *L*-diaminopimelic acid, and glucose, galactose, ribose and mannose are present in whole-cell hydrolysates. The menaquinones are MK-9(H₄), MK-9(H₆) and MK-9(H₈).

The type strain, Z₄T (=CGMCC 4.7035T =KCTC 29111T), was isolated from soil sample from Yaan, Sichuan Province, south China. The DNA G+C content of the type strain is 68.7 mol%.

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References


