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Streptomyces daliensis sp. nov. from soil

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Abstract

A novel actinomycete strain YIM 31724^T was isolated from a soil sample collected from Dali, Yunnan Province, People's Republic of China. The strain is characterized by white to yellow white aerial mycelia, spiral spore chains and smooth spore surface. The cell wall of strain YIM 31724^T contained LL-diaminopimelic acid (A_2pm) and traces of *meso*-A_2pm. Whole-cell hydrolysates contained mainly glucose and small amounts of galactose and xylose. The menaquinones were MK-9(H_6) (31%) and MK-9(H_8) (69%). Phosphatidylethanolamine was the diagnostic phospholipid. The DNA G+C content of strain YIM 31724^T was 67.2 mol%. Phylogenetic analysis indicated that the strain belongs to the genus *Streptomyces*, with highest similarity to *Streptomyces rimosus* subsp. *rimosus* JCM 4667^T (rRNA gene sequence similarity value of 98.9%) and *Streptomyces erumpens* DSM 40941^T (rRNA gene sequence similarity value of 98.7%). Based on its phenotypic and genotypic characteristics, including low DNA–DNA hybridization results, strainYIM 31724^T(= CCTCC AA204020^T = KCTC 19076^T) is proposed as the type strain of a novel species, *Streptomyces daliensis* sp. nov.

Introduction

The genus *Streptomyces* was proposed by Waksman and Henrici (1943) for aerobic, spore-forming actinomycetes. *Streptomyces* are a rich source of bioactive compounds, notably antibiotics, enzymes, enzyme inhibitors and pharmacologically active agents (Bérdy 1995; Chun et al. 1997; Labeda et al. 1997). In the course of our screening program for new antibiotics, several actinomycete strains which contained both type I and type II polyketide bio-synthesis pathway genes were isolated from soil samples collected from Yunnan province of China (Xu et al. 2003b). This paper reports a taxonomic analysis of strain YIM 31724^T.

Materials and methods

Organism

Strain YIM 31724^T was isolated from a soil sample after 2 weeks incubation at 28 °C on glycerol–asparagine agar (ISP medium 5, Shirling and Gottlieb, 1966). The strain was maintained by cultivation 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, incubated at 28 °C for 7–15 days. Strain YIM 31724^T was deposited in the Collection Center of Typical Cultures, China (CCTCC) as strain CCTCC AA 204020^T and the Korean Collection for Type Cultures as strain KCTC 19076^T.

Phenotypic characteristics

Biomass for molecular systematic and most of the chemotaxonomic studies was obtained after incubation at 28 °C for 3 days by growing in shake flasks of ISP 2 broth supplemented with the vitamin mixture of HV medium (Hayakawa and Nonomura 1987). Cultural characteristics were determined after 2 weeks at 28 °C by methods used in the International *Streptomyces* Project (ISP, Shirling and Gottlieb 1966). Morphological observations of spores and mycelia were made by light microscopy (Olympus microscope BH-2) and scanning electron-microscopy (JEOL model JSM 5600LV). The test strain was examined for a range of phenotypic properties using standard procedures (Shirling and Gottlieb 1966; Williams et al. 1983). In addition, acid production from carbohydrates was carried out using media and methods described by Gordon et al. (1974). Tolerance to temperature (10 °C, 27 °C, 30 °C, 37 °C and 45 °C), sodium chloride (4%, 7%, 10% and 13%) and phenol (0.1%, 0.2%, 0.5% and 1.0%) was tested using modified Bennett's agar (Williams et al. 1983). Colors and hues were determined according to the color chips from the ISCC-NBS Color Charts Standard Samples no.2106 (Kelly 1964).

Chemotaxonomy

Cell walls were purified and the peptidoglycan amino acids were analyzed by TLC (Lechevalier and Lechevalier 1980; Jiang et al. 2001). Analysis of whole-cell sugar composition followed procedures described by Becker et al. (1965) and Lechevalier and Lechevalier (1980). Phospholipid analysis was carried out as described by Lechevalier et al. (1981). Menaquinones were determined using the procedures of Collins et al. (1977). 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

Genomic DNA was extracted and the 16S rRNA gene sequence amplified as described by Xu et al. (2003a). The nearly complete resultant 16S rRNA gene sequence (1520 nucleotides, GenBank accession number AY785161) was aligned manually with corresponding almost-complete sequences of representative *Streptomyces* species retrieved from the DDBJ, EMBL and GenBank databases by using the BLAST (Altschul et al. 1997) and Blast 2 search tools (Tatusova and Madden 1999). Evolutionary trees were constructed by using the least-squares (Fitch and Margoliash 1967), maximum-likelihood (Felsenstein 1993) and neighbor-joining (Saitou and Nei 1987) tree-making algorithms and from the PHYLIP package (Felsenstein, 1993). Evolutionary distances matrices were generated as described by Kimura (1980). The topology of the tree was evaluated by performing a bootstrap analysis (Felsenstein 1985) using 1000 resamplings. *Actinoplanes philippinensis* (GenBank sequence accession no. D85474) was used as an outgroup.

A partial nucleotide sequence (120 nt) of the tested strain based on the variable γ-region was compared with all corresponding nucleotide sequences of *Streptomyces* strains retrived from the DDBJ database. A phylogenetic tree based on these sequences was generated using the neighbor-joining algorithm.

The chromosomal DNA for genomic DNA G + C content analysis was extracted as described by Marmur (1961). The DNA G + C base content of strain YIM 31724^T was determined by the thermal denaturation method (Mandel and Marmur 1968).

DNA–DNA hybridization experiments between strains YIM 31724^T, *Streptomyces rimosus* subsp. *rimosus* ISP 5260^T (= JCM 4667^T), *Streptomyces erumpens* DSM 40941^T (= NRRL B-3163), *Streptomyces violens* ISP 5597^T (= DSM 40597^T) and *Streptomyces ochraceiscleroticus* ISP 5594^T

(= DSM 40594^T) were carried out according to the thermal renaturation method (De Ley et al. 1970; Huss et al. 1983).

Results and discussion

Substrate mycelium of strain YIM 31724^T developed well and branched irregularly on both complex and synthetic media; fragmentation of mycelium did not occur. Figure 1 shows a scanning electron micrograph of aerial spores of strain YIM 31724^T. The multi-spore chains were spiral; the spores were cylindrical and not motile. The spore surface was smooth. Sporangia or zoospores and sclerotia were not observed. Soluble pigment was not produced on any of the media tested. Growth was moderate or good on the media tested. Aerial mycelia are white to yellow white on various media and the color of vegetative mycelia is media-dependent (Table 1). The physiological features are indicated in Table 2 and in the species description.

Strain YIM 31724^T contained LL-diaminopimelic acid (A_2pm) and trace amounts of *meso*-A_2pm. Whole-cell hydrolysates contained mainly glucose and small quantities of xylose and galactose [cell wall chemotype I sensu Lechevalier and Lechevalier (1970)]. The menaquinones were MK-9(H_6) (31%) and MK-9(H_8) (69%), and the diagnostic phospholipid was phosphatidylethanolamine [phospholipid type II sensu Lechevalier and Lechevalier (1970)]. The predominant cellular fatty acids were i-$C_{14:0}$ (4.6%), i-$C_{15:0}$ (9.6%), ai-$C_{15:0}$ (22.5%), i-2OH-$C_{15:0}$ (5.7%), i-$C_{16:0}$ (24.7%), $C_{16:0}$ (5.0%), i-$C_{17:0}$ (4.6%) and ai-$C_{17:0}$ (14.2%). The G + C content of DNA was 67.2 mol%. The chemical and morphological properties of strain YIM 31724^T are clearly consistent with its assignment to the genus *Streptomyces* (Williams et al. 1989).

It is clear from the phylogenetic tree (Figure 2) that strain YIM 31724^T forms a single sub-clade separate from other representatives of the genus *Streptomyces*. The isolate was closest to the type strains of *Streptomyces rimosus* subsp. *rimosus* JCM 4667^T (similarity value of 98.9%; 17 nucleotide

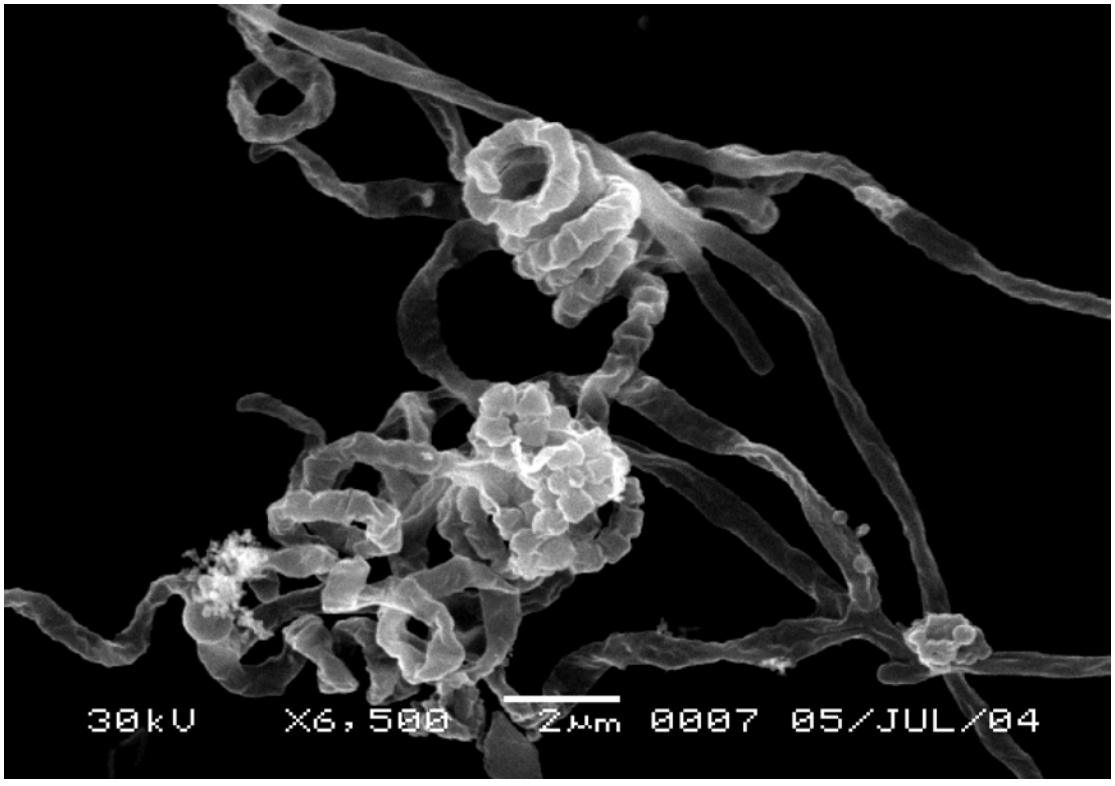

Figure 1. Scanning electron micrograph of the spore chains of *Streptomyces daliensis* sp. nov. (YIM 31724^T) grown on yeast-malt extract agar (ISP 2) for 14 days at 28 °C. Bar, 2 μm.

Table 1. Culture characteristics of strain YIM 31724^T on various media.

Medium	Growth	Sporulation	Color of colony[2] Aerial mycelium	Substrate mycelium
Yeast-malt extract agar (ISP[1] medium 2)	Good	Moderate	Yellow White	Soft Yellow Brown
Oatmeal agar (ISP[1] medium 3)	Good	Moderate	Yellow White	Pale Yellow
inorganic salt-starch agar (ISP[1] medium 4)	Good	Good	Yellow White	Pale Yellow
Glycerol–asparagine agar (ISP[1] medium 5)	Good	Moderate	Yellow White	Soft Yellow
Potato agar	Good	Good	White	Moderate Olive Yellow
Nutrient agar	Good	Poor	Yellow White	Soft Orange Yellow
Czapek's agar	Moderate	Moderate	Yellow White	Pale Yellow

[1]ISP, International *Streptomyces* Project (Shirling & Gottieb 1966).[2] The ISCC-NBS COLOR CHARTS Standard Samples No 2106 (Kelly 1964).

differences in 1486 sites) and *Streptomyces erumpens* DSM 40941^T (similarity value of 98.7%; 20 nucleotide differences in 1498 sites). 16S rRNA gene sequence similarities and nucleotide differences within these ranges have been recorded for the type strains of several streptomycete species (Kim et al. 1998; Sembiring et al. 2000; Kim and Goodfellow 2002; Manfio et al. 2003; Saintpierre et al. 2003) which showed DNA–DNA relatedness values below the 80% cut-off point recommended for the recognition of genomic species of *Streptomyces* (Labeda and Lyons 1992; Labeda 1993, 1998). Strain YIM 31724^T also shared relatively high 16S rRNA gene sequence similarity values with the type strains of *Streptomyces niger* DSM 43049^T (98.5%), *Streptomyces olivaceiscleroticus* DSM 40595^T (98.5%), *Streptomyces sclerotialus* DSM 43032^T (98.5%), *Streptomyces chrestomyceticus* DSM 40545^T (98.1%), *Streptomyces rimosus* subsp. *paromomycinus* DSM 41429^T (98.1%), *Streptomyces platensis* JCM 4662^T (98.1%), *Streptomyces purpurogeneiscleroticus* DSM 43156^T (97.9%), *Streptomyces albofaciens* JCM 4342^T (97.9%), *Streptomyces violens* DSM 40597^T (97.9%), *Streptomyces catenulae* DSM 40258^T (97.8%), and *Streptomyces kasugaensis* M338-M1^T (97.8%). The high 16S rRNA gene sequence similarities of strain YIM 31724^T with the sequences of the type strains of these *Streptomyces* species was underpinned by the results from the 120 nt sequence analysis (data not shown) and the isolate was once again shown to be closely related to the type strains of *S. rimosus* subsp. *rimosus* JCM 4667^T and *S. violens* JCM 3072^T and to *Streptomyces ochraceiscleroticus* JCM 4801^T. DNA–DNA hybridisation experiments revealed low DNA–DNA relatedness between strain YIM 31724^T and *S. rimosus* subsp. *rimosus* ISP 5260 (47.1%), *S. erumpens* DSM 40941^T (38.4%), *S. violens* ISP 5597^T (25.6%) and *S. ochraceiscleroticus* ISP 5594^T (33.2%). These data confirmed that strain YIM 31724^T can be considered as a novel taxon. Furthermore, as shown in Table 2, strain YIM 31724^T can also be distinguished from the type strains of *S. rimosus* subsp. *rimosus* ISP 5260^T (=JCM 4667^T), *S. erumpens* DSM 40941^T, *S. violens* ISP 5597^T (=DSM 40597^T) and *S. ochraceiscleroticus* ISP 5594^T (=DSM 40594^T) and some other related type strains by using a combination of phenotypic properties. The differential morphological and pigmentation features expressed on oatmeal and peptone-yeast iron agars are especially significant for the delineation of members of phylogenetically related streptomycete species (Labeda and Lyons 1991; Labeda et al. 1997; Kim et al. 1998; Kim et al. 2000; Manfio et al. 2003). Strain YIM 31724^T and *S. rimosus* subsp. *rimosus* JCM 4667^T had markedly different phenotypic profiles: only *S. rimosus* subsp. *rimosus* uses D-cellobiose, D-trehalose, adonitol, sorbitol and sodium citrate as sole carbon sources for growth; can reduce nitrate; does not produce melanin pigments on peptone-yeast extract-iron agar; and has looped or spiral spore chains. In contrast, strain YIM 31724^T uses melezitose, L-rhamnose, D-sucrose, D-xylose and salicin as sole carbon sources for growth; nitrate reduction is negative; and has long and tight spiral spore chains (Table 2; Williams et al. 1983). In addition to differences in the utilization of D-raffinose, D-sucrose and D-xylose as sole carbon sources for growth, strain YIM 31724^T and *S. erumpens* DSM 40941^T can be

Table 2. Phenotypic properties separating strain YIM 31724^T from related *Streptomyces* species

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Color of aerial mass on oatmeal agar[1]	W/G	G	WG	Gn	G	W[2]	R	G	G	G	W P	LBG	G		YR	GY	G	W[2]	W	YW	W[2]		G	G	YW
Spore chain ornamentation	S	S	S	S	S	S	S	r	r	r	S	S	r	S	S	S	S	S		S	S	S	r	S	S
Spore chain morphology	sp	ra	sp	sp	sp	l/sp	V	sp	sp	sp	sp	sp	sp	sp	sp	sp	sp	sp	sp	sp/ra	sp	RF	sp	F	sp
Production of diffusible pigments	–	+	+	–			–	–	+	+	+	–	–	v	–	–	–	+	–	–	–	+	–	–	–
Melanin production	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Growth on sole carbon sources (1%,w/v):																									
L-Arabinose	+	–	–	+	+	–	–	+	+	v	–	+	+	+	+	+	v	+	–	+	+	+	+	–	+
D-Fructose	+	+	+	+	+		+	+	+	v	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
meso-Inositol	+	–	+	+	+		–	v	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
D-Mannitol	+	+	+	+	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	–	+
D-Raffinose	+	–	+	v	–		–	–	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+
L-Rhamnose	–	–	–	–	+		–	+	–	–	–	v	+	+	+	+	–	+	–	–	+	+	+	–	+
D-Sucrose	–	–	+	–	–		+	–	+	+	–	+	v	+	+	+	+	+	–	–	+	+	+	–	+
D-Xylose	v	–	+	v	–	–		v			–	+	+	+	+	+	v	+	v	v	+	+	+	–	+
Adonitol	+	–	–	+		–								+	+			+	+	+	+	+			–
D-Cellobiose	+		+	+		–	–	+					+	+	+			+	+	+	+	+	+		–
D-trehalose	+	+	+	+		+		+				+	+	+	+		+	+	+	+	+	+	+		–
Degradation of Hypoxanthine	+	+	–	+		+	+					+		+	+		+	+		+	+	+		–	+
Xanthine			–			+	+	–					–	+	+			+			+	+	–	–	+
Adenine		+	+			–	–	+	–	+		+	+	+	+		+	+			+	+	+		+
Tween 80	+	+	+	+		–	+	+	+	+		+	+	+	+		+	+		+	+	+	+	+	+
Starch	+	+	+			–	+	+			–	+	+	+	+		+	+	+	+	+	+	+	+	+
H_2S production		+						+				–	+	+	+		–	+			+	+	+		–
Grow in 4%(w/v) NaCl	+	+	+	+		–	–					+		+	+		+	+		+	+	+			+
0.1%(w/v) phenol		+	+			–	–	–				–	–	+	+			+			+	+	–		+
Grow at 45 °	+	–	–			+	–	+	–	–	–	–	–		–		–			–			+	–	–
Resistance to:																									
Gentamicin sulphate		–	–			–		–				–	–	–	+		–	–			–	–	–		+
Streptomycin sulphate	+	–	–	+	–	–								–	+			–		+	–	–			–

1. *S.albofaciens* ISP 5268^T, 2. *S. catenulae* DSM 40258^T, 3.*S. chattanoogensis* DSM 40002^T, 4. *S. chrestomyceticus* DSM 40545^T, 5. *S. erumpens* DSM 40941^T, 6. *S. ferratilis* SFOp68^T, 7. *S.griseocarneus* ISP 5004^T, 8. *S.hygroscopicus* NRRL 2387^T, 9. *S.indonesiensis* A4R2^T, 10. *S.javensis* B22P3 (B26)^T, 11. *S.kasugaensis* M338-M1^T, 12. *S.lydicus* ISP 5461^T, 13. *S.melanosporofaciens* ISP 5318^T, 14. *S.niger* DSM 43049^T, 15. *S.ochraceiscleroticus* ISP 5594^T, 16. *S. olivaceiscleroticus* DSM 40595^T, 17. *S.platensis* ISP 5041^T, 18. *S.purpurogeneiscleroticus* DSM 43156^T, 19. *S. rimosus* subsp.*paromomycinus* DSM 41429^T , 20. *S.rimosus* subsp. *rimosus* ISP 5260^T, 21. *S. sclerotialus* DSM 43032^T, 22. *S.violens* ISP 5597^T, 23. *S.violaceusniger* ISP 5563^T, 24. *S. yeochonensis* CN732^T, 25. YIM 31724^T.

Abbreviations: B, brown; G, gray; Gn, green; GY, grayish yellow; LBG, Light brownish gray; PY, pale yellow; R, reddish; WG, white gray; WP, white putty; Y, yellow; YG, yellowish gray; YW, yellowish white. r, Rugose, S, smooth. F, flexuous; l, looped; ra, retinaculiaperti; RF, rectiflexibiles; sp, spirales; V, verticillati. Symbols: +, positive or utilization; –, negative or not utilization; v, variable or trace grow; ND, not detected. Data for reference type species strains were taken from Coffey et al. (1959); Shirling and Gottlieb (1968, 1972); Williams et al. (1983, 1989); Hamada et al. (1995); Sembiring et al. (2000); Kim et al. (2004) and Saintpierre-Bonaccio et al. (2004).*Note*: [1]on oatmeal agar; [2]on the other media.

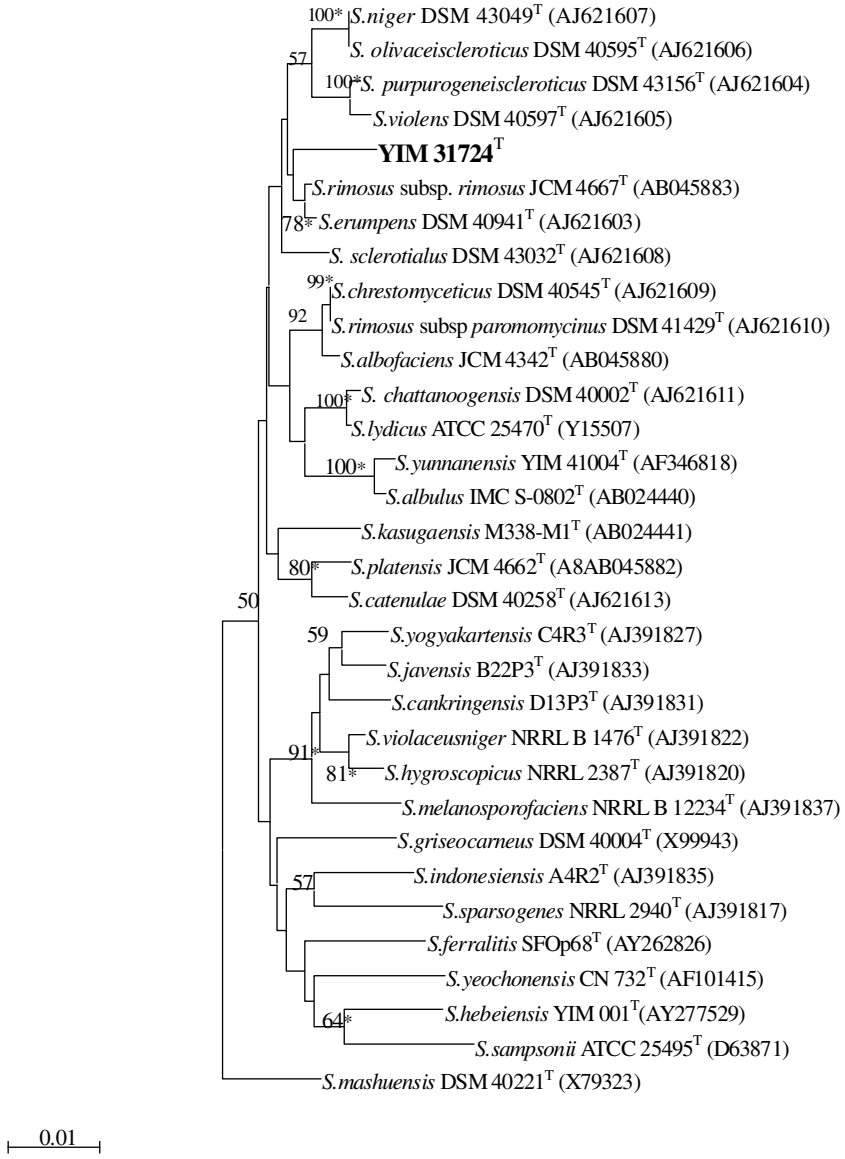

Figure 2. Phylogenetic dendrogram obtained by neighbor-joining analysis based on 1450 bp of 16S rDNA sequences, showing the position of strain YIM 31724^T among its phylogenetic neighbors. Bootstrap values greater than 50% are indicated at the nodes (1000 replications). Sequence accession numbers are given in parentheses. The sequence of *Actinoplanes philippinensis* IFO13878^T (D85474) was used as root. Asterisks indicate branches that were also recovered using the least-squares (Fitch and Margoliash 1967) and maximum-likelihood (Felsenstein 1993) tree-making algorithms. Bar, 0.01 substitutions per nucleotide position.

distinguished with the color of aerial spore mass on oatmeal agar (Table 2). In conclusion, the genotypic, chemotaxonomic and phenotypic data show that strain YIM 31724^T forms a novel species of the genus *Streptomyces*, for which we propose the name *Streptomyces daliensis* sp. nov.

Description of *Streptomyces daliensis* sp. nov.

Streptomyces daliensis (da.li.en'sis. N.L. masc. adj. *daliensis* pertaining to Dali, a city in Yunnan province in the south of China where the sample was collected.)

The culture grows well at 27 °C, 30 °C and 37 °C , but does not grow at 45 °C or 10 °C . Substrate mycelia are well-branched. Aerial mycelia form complete spiral chains of spores. The spores are not motile. No synnemata, sclerotia or sporangia are observed. Spores are cylindrical in shape, and the spore surface is smooth. Aerial mycelia are white to yellow white on various media and the color of vegetative mycelia is media-dependent (Table 1). Diffusible pigments are not formed.

In addition to the properties shown in Table 2, ribose, galactose, glucose, mannose, lactose, melibiose, maltose, melezitose, dextrin, glycerol, xylitol, dulcitol, salicin, malonate and oxalate are utilized as sole carbon and energy sources, but not arabitol, sorbitol, acetate, citrate, galacturonate, 5-ketogluconate and tartrate. Acid is not formed from these carbon sources tested. L-Alanine, L-asparagine, L-arginine, glycine, L-histidine, L-hydroxyproline, L-lysine, phenylalanine, L-proline, L-threonine, L-valine and acetamide are used as sole carbon and nitrogen sources, but not L-cysteine, L-tryptophane, L-tyrosine, L-glutamic acid or glucosamine. Allantoin, urea, chitin, aesculin, amygdalin and keratin are hydrolysed. Tweens 20 and Tweens 80 are degraded, but not cellulose and DNA. Tests for gelatin and resistance to KCN are positive, but tests for nitrate reduction, milk coagulation and peptonization are negative. Strains grow in the presence of 7% sodium chloride, from pH6 to pH9 and in the presence of phenol at 0.1%. Resistant to lysozyme, penicillin G, polymyxin B, erythromycin, oleandomycin, tobramycin and nalidixic acid, but sensitive to chloramphenicol, vancomycin, aureomycin, terramycin, tetracycline, amikacin, novobiocin, kanamycin, netilmicin and ciprofloxacin. Diagnostic amino acid of peptidoglycan is LL-A_2pm and trace amounts of *meso*-A_2pm. Whole-cell hydrolysates contain glucose and small quantities of xylose and galactose. The menaquinones are MK-9(H_6) (31%) and MK-9(H_8) (69%), and phosphatidylethanolamine is the diagnostic phospholipid. The major cellular fatty acids are i-$C_{14:0}$, (4.6%); i-$C_{15:0}$, (9.6%); ai-$C_{15:0}$, (22.5%); i-2OH-$C_{15:0}$, (5.7%); i-$C_{16:0}$, (24.7%); $C_{16:0}$, (5.0%); i-$C_{17:0}$, (4.6%); ai-$C_{17:0}$, (14.2%). The G + C contents of the DNA is 67.2 mol%.

It was isolated from soil sample collected from Dali, Yunnan Province, China. The type strain is strainYIM 31724^T(= CCTCC AA204020^T = KCTC 19076^T).

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