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Streptomyces roseoalbus sp. nov., an actinomycete isolated from soil in Yunnan, China

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Abstract

An actinomycete strain was isolated from a soil sample collected from a secondary forest at Yongsheng of Yunnan province, China. The isolate, YIM 31634^T, was identified by a polyphasic approach. The 16S rDNA sequence analysis showed that the strain YIM 31634^T belongs to the genus *Streptomyces*, with closest similarity to *Streptomyces olivochromogenes* DSM 40451^T (97.66% similarity). Sequence similarities between strain YIM 31634^T and other *Streptomyces* species in the same subclade ranged from 97.59% (with *Streptomyces resistomycificus* DSM 40133^T) to 97.22% (with *Streptomyces mirabilis* ATCC 27447^T). Key phenotypic characteristics as well as chemotaxonomic features of the actinomyces were congruent with the description of the genus *Streptomyces*. On the basis of phenotypic and phylogenetic analyses, strain YIM31634^T was recognized as a new species of the genus *Streptomyces* for which the name *Streptomyces roseoalbus* sp. nov. is proposed. The strain YIM 31634^T has been deposited in the Chinese Center of Type Culture Collection as strain CCTCC M 203016^T and in the Deutsche Sammlung von Mikroorganismen (DSM 41833^T).

Introduction

Microorganisms are virtually unlimited sources of novel compounds with many medicinal and agricultural applications. Actinomycetes, among them, hold a prominent position due to their ability to produce numerous different metabolites such as antibiotics, enzymes and inhibitors. Further, the discovery of novel antibiotic and non-antibiotic lead compounds through microbial secondary metabolite screening is becoming increasingly important.

In recent years, there has been an increasing interest in discovering new agricultural antibiotics for the protection of our living environments. In the course of screening for agricultural antibiotics, one actinomycete strain, with anti-fungal activities was isolated from a soil sample collected from a secondary forest soil at Yongsheng of Yunnan province, China. The isolate was then identified using a polyphasic approach. The results showed that the strain YIM 31634^T belongs to the genus *Streptomyces*.

Materials and methods

Organism

Strain YIM 31634^T was isolated from a soil sample collected from a secondary forest at Yongsheng in Yunnan Province, China, using HV agar medium (Hayakawa and Nonomura 1987) after about 2 weeks incubation at 28 °C.

Morphological, cultural, physiological and biochemical characteristics

The medium used for morphological studies was yeast extract-malt extract agar (ISP 2) (Shirling and Gottlieb 1966). Morphology of strain YIM 31634^T was observed by using optical and scanning electronic microscopy (Model EPMA-8705, Shimadzu), following desiccation of cells and coating with a layer of gold before use.

To determine the cultural, physiological and biochemical characteristics of strain YIM 31634^T, it was grown on a number of culture media suggested by Williams et al. (1983) and Shirling and Gottlieb (1966). The color determinations were made by comparing the cultures with color chips from the ISCC-NBS COLOR CHARTS Standard Samples No. 2106 (Kelly 1964).

Chemotaxonomy

The cell wall fraction was purified and analyzed by the methods of Lechevalier and Lechevalier (1980). The procedures of Becker et al. (1964) and Lechevalier and Lechevalier (1980) were used for analyses of whole-cell chemical compositions. Phospholipid analysis was carried out as described by Lechevalier et al. (1981). Menaquinones were determined using the procedures of Collins (1985).

DNA base composition

DNA for the G + C content was obtained by the method of Marmur (1961). The G + C content was determined using the thermal denaturation method of Marmur and Doty (1962).

DNA preparation, amplification and determination of 16S rDNA sequence

Genomic DNA extraction of strain YIM 31634^T was performed according to the method of Xu et al. (2003). 16S rDNA was amplified by PCR using TaKaRa ExTaq as described previously (Cui et al. 2001) with primer A 8-27f (5′- AGAGT TTGATCCTGGCTCAG-3′) and primer B 1523-1504r (5′-AAGGAGGTG ATCCAGCCGCA-3′; primers are according to the *Escherichia coli* numbering system of Brosius et al. 1978). The PCR reaction parameters included: an initial 5 min for pre-denaturation at 95 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at 56 °C, 3 min at 72 °C and a final extension of 5 min at 72 °C and then cooled to 4 °C. The 1.5 kb amplified 16S rDNA fragment was separated by agarose gel electrophoresis. The purified fragment was directly sequenced using a *Taq* DyeDeoxy terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, California) and analyzed with an ABI PRISMTM 377 DNA sequencer (Applied Biosystems, Inc.). Sequencing primers used included primer A, primer B and primer C (5′-AGGGTTGCGCTCGTTG-3′).

Analysis of sequence data

The variable γ region (positions 158–277) of 16S rDNA from 445 known *Streptomyces* species obtained from the DDBJ databases and strain YIM 31634^T were aligned. The 16S rDNA sequence of strain YIM 31634^T was aligned manually with representative sequences of related *Streptomyces* from the GenBank database. The evolutionary tree, rooted with *Streptomyces megasporus* (Z68001) as the outgroup, was inferred by using the neighbor-joining method (Saitou and Nei 1987) from the evolutionary distance data corrected by Kimura's 2 parameter model (Kimura 1980). The topology of the resultant tree was evaluated by bootstrap analysis (Felenstein 1985) of the neighbor-joining method based on 1000 resamplings. The Clustal X program (Thompson et al. 1997) was used for multiple alignment and phylogenetic analysis. TreeView program (Page 1996) was used to display, edit and print phylogenetic trees.

Results and discussion

Morphological observation of the 7–15 days old culture of strain YIM 31634^T revealed that both aerial and vegetative hyphae were abundant, well-developed and not fragmented. Long spore chains in retinaculiaperti were borne on the aerial mycelium, the spores being rod-shaped with a smooth surface. The aerial mycelium was observed after the 15th day of incubation in all test media.

Cultural characteristics of strain YIM 31634^T are shown in Table 1. Aerial mycelium of strain YIM 31634^T was abundant, well-developed and varied from pink-white to yellow-white on different tested media. The substrate hyphae varied from pale-yellow to brown-yellow. Pale yellow diffusible pigments were produced on most test media, except nutrient agar, and melanin was not produced.

The cell wall of strain YIM 31634^T only contained LL-diaminopimelic acid (L-DAP) and glycine, indicating that strain YIM31634^T has a cell wall chemotype I (Lechevalier and Lechevalier 1970a,b). The whole cell hydrolysates contained galactose. The predominant menaquinones were MK-9(H_4), MK-9(H_6) and MK-9(H_8) and the diagnostic phospholipids were phosphatidylethanolamine and phosphatidyl choline. Thus, these phenotypic characteristics, along with the results of the cell wall type, clearly revealed that strain YIM 31634^T should belong to the genus *Streptomyces*.

The G + C content of the DNA of the strain YIM 31634^T was determined as 62.5%. Analysis of the 16S rDNA γ region sequences showed that strain YIM 31634^T was grouped into a branch with the type strain of *Streptomyces longisporoflavus* ISP 5165 (the nearest neighbor, similarity value of 95.8%, five nucleotide differences in 120 sites). The almost complete 16S rRNA gene sequence of strain YIM 31634^T was determined in this study and has been deposited in the GenBank datebase (accession number AY222322). A neighbor-joining tree (Saitou and Nei 1987) based on 16S rDNA gene sequences was constructed to show relationships between strain YIM 31634^T and some other related *Streptomyces* species (Figure 1). Analysis of 16S rDNA revealed that strain YIM 31634^T was phylogenetically related to members of the genus *Streptomyces*. The comparative analysis of the16S rRNA gene sequences

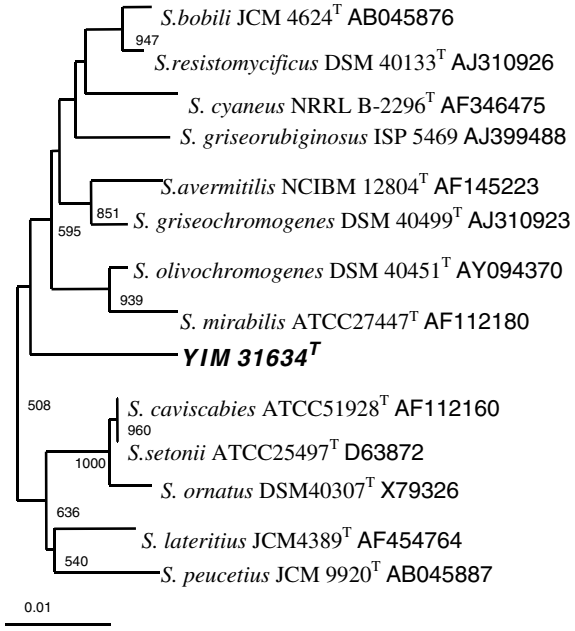

Figure 1. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rDNA sequences, showing the position of strain YIM 31634^T among phylogenetic neighbors. Numbers on branch nodes are bootstrap values (1000 resamplings). The sequence of *Streptomyces megasporus* (Z68100) was used as root. Bar, 1% sequence divergence.

Table 1. Cultural characteristics of strain YIM 31634^T

Media	Aerial mycelium	Substance mycelium	Soluble pigment
Czapek's agar	Pink white	Light orange yellow	Pale yellow
Glycerol/asparagine agar	Pale pink	Light orange yellow	Pale yellow
Inorganic salt-starch agar (ISP* medium 4)	Pink white	Pale Yellow pink	Pale yellow
Yeast extract-malt extract agar (ISP* medium 2)	Pink white	Brown yellow	Pale yellow
Oatmeal agar (ISP* medium 3)	Pink white	Yellow pink	Pale yellow
Nutrient agar	Yellow white	Pale yellow	Pale yellow

*ISP, International *Streptomyces* Project (Shirling and Gottieb 1966).
Note: Colors taken from ISCC-NBS COLOR CHARTS Standard samples No 2106 (Kelly 1964).

Table 2. Differentiating characteristics of strain YIM 31634^T and related species

Characteristics		*S.rosealbus* YIM31634^T	*S.olivochromogenes** DSM 40451^T	*S. resistomycificus** DSM 40133^T	*S.longisporoflavus** ISP 5165^T
Spore chain morphology		RA	SP to RF	SP to RA	SP to RA
Glycerol–asparagine agar	AM	Pale pink light	White–gray	Gray	Absent
(ISP 5 medium)	SM	Orange yellow	Yellow–grayish blue	Yellowish brown	Light grayish yellowish brown
	SP	Pale yellow	–	Yellowish brown	–
Inorganic salt/starch agar	AM	Pink white	White–dark gray	White	Yellow
(ISP 4 medium)	SM	Pale yellow pink	White–gray	Grayish yellow–pale yellow	Grayish yellow
	SP	Pale yellow	–	–	–
Nutrient agar	AM	Yellow white	White–light gray	Gray	
	SM	Pale white	Gray–blue gray	Dark brown	ND
	SP	–	Dark gray	Dark brown	
Gelatine liquification		–	+	+	ND
Milk coagulation		–	–	+	ND
Milk peptonization		–	–	ND	ND
Starch hydrolization		+	+	ND	ND
Nitrate reduction		+	+	ND	ND
Growth on cellulose		–	–	–	ND
H_2S Production		–	+	ND	ND
Melanin		–	+	+	–
Utilization:					
Lactose		–	+	–	ND
Mannose		+	+	–	ND
Raffinose		–	+	+	–
Inositol		–	+	+	D
Mannitol		+	+	+	D

Note: All the four type strains used glucose, arabinose and xylose as a sole carbon source.
Abbreviations: AM, Aerial mycelium; SM, Substrate mycelium; SP, Soluble pigment; RA, retinaculiaperti; RF, rectiflexibiles; SP, spirales.
Symbols: +, utilization; –, not utilization; D, doubtful; ND, not determined.
*Data for reference type species strains were taken from Shirling and Gottlieb (1968a,b; 1969).

and the estimation of phylogenetic relationships showed that strain YIM 31634^T formed a separate subclade in the tree and showed closest level of sequence similarity with *Streptomyces olivochromogenes* DSM 40451^T (97.66% similarity). The sequence divergence values between strain YIM 31634^T and other members of this tree ranged from 2.78% (*Streptomyces mirabilis* ATCC 27447^T) to 2.41% (*Streptomyces resistomycificus* DSM 40133^T), and these data indicate that strain YIM 31634^T may represent a novel species.

A comparative study between strain YIM 31634^T and closely related species of the genus *Streptomyces*, revealed that it differed from *S. longisporoflavus* ISP 5165^T, *S. olivochromogenes* DSM 40451^T and *S. resistomycificus* DSM 40133^T in morphological, cultural characteristics and physiological characteristics as summarized in Table 2.

The phenotypic and phylogenetic data presented in this study provide clear evidence that strain YIM 31634^T represents a new species of the genus *Streptomyces*. Therefore, we proposed the name *Streptomyces roseoalbus* sp. nov.

Description of *Streptomyces roseoalbus* sp. nov.

Streptomyces roseoalbus (ro.seo.al.bus N.L. fem. adj. *roseoalbus* red and white colored). Gram-positive, non-acid-fast and aerobic. Both vegetative and aerial hyphae are abundant, well-developed and not fragmented. Vegetative hyphae is extensively branched, and does not bear any spores. Aerial mycelium appears usually after 15 days of incubation and varies from pink-white to yellow-white in the test media. At maturity, it forms retinaculiaperti (RA) type spore chains. Spore surface is smooth and non-motile. Gelatin is not liquefied. Milk is not coagulated or peptonized. Pale-yellowish diffusible pigments are produced in most of test media (Table 1) except nutrient agar, and melanin is not produced. Starch is hydrolyzed but not cellulose. H_2S is not produced. Nitrate is reduced. Glucose, mannose, arabinose, xylose, ribose, mannitol, glycine, histidine and methionine are utilized as sole carbon and nitrogen sources, and lactose, raffinose, inositol and sorbitol are not. No acid is produced on these carbon sources. Optimal growth temperature is 28 °C. The purified cell wall contains L-DAP and glycine. Whole cell hydrolysates contain galactose. The predominant menaquinones are MK-9(H_4), MK-9(H_6) and MK-9(H_8) and the diagnostic phospholipids are phosphatidylethanolamine and phosphatidylcholine. The DNA G + C content is 62.5 mol%. The type strain, YIM 31634^T, was isolated from a soil sample collected from Yongsheng in Yunnan Province, China, and has been deposited in the Chinese Center of Type Culture Collection as strain CCTCC M 203016^T and in the Deutsche Sammlung von Mikroorganismen (DSM 41833^T).

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