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Streptomyces nanningensis sp. nov. a novel Streptomycete from forest soil

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Abstract

A strain YIM 33098^T (= CCTCC AA001027^T = DSM 41831^T) was isolated from a forest soil sample collected from Nanning in Guangxi Province, China, in the course of screening for producers of new drug lead compounds. This strain was identified by using a polyphasic approach. The results showed that it should be assigned to the genus *Streptomyces*. An almost complete 16S rRNA gene sequence of the strain was determined and compared with those of representative *Streptomyces* species. Strain YIM 33098^T was clustered in the same subclade with *Streptomyces tendae* ATCC19812^T and *Streptomyces eurythermus* ATCC14975^T. Similarities of strain YIM 33098^T with the two strains were 97.35% and 97.42%, respectively. Based on the phenotypic and genotypic evidence, it is therefore proposed that strain YIM 33098^T should be classified in the genus *Streptomyces* as a new species under the name of *Streptomyces nanningensis* sp. nov.

Introduction

Actinomycetes have attracted great attention since these microorganisms produce various natural compounds, such as antibiotics, inhibitors, and enzymes. The genus *Streptomyces* is the largest producer of bioactive compounds (Chun et al. 1997; Labeda et al. 1997). It remains important to discover new leader compounds from Streptomycetes for drug development. In the course of the screening for producers of lead compounds from actinomycetes, strain YIM 33098^T was selected with an inhibiting activity against acetylcholinesterase (data not shown). This paper reports a taxonomic analysis of strain YIM33098^T.

Materials and methods

Organism

Strain YIM 33098^T was isolated from a soil sample collected from Qingxiu mountain of Nanning, Guangxi Province, China using HV agar medium (Hayakawa and Nonomura 1987) after 2 weeks incubation at 28 °C. The strain was maintained by cultivation on #38 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. Stain YIM 33098^T was deposited in

the Collection Center of Typical Cultures, China (CCTCC) as strain CCTCC AA 001027^T, and DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen (GmbH) as strain DSM 41831^T.

Phenotypic characteristics

The medium used for morphological studies was yeast extract–malt extract agar (ISP 2) (Shirling and Gottlieb 1966) and the incubation time of the pure culture was 7–15 days at 28 °C. Morphological observations were made by using optical and electron microscopy (JEOL model JSM 5600LV). Cultural and Physiological characteristics of strain YIM 33098^T were determined according to the methods proposed by Shirling and Gottlieb (1966) and Williams et al. (1983). Color determinations were carried out 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.

16S rDNA sequencing

The chromosomal DNA of strain YIM 33098^T was isolated as described by Hopwood et al. (1985). 16S rDNA was amplified by PCR using a PCR kit (Sino-American Biotechnology Co., Beijing), primer A 8-27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and primer B 1523-1504r (5′-AAGGAGGTGATCCAGCCGCA-3′; primers are according to the *Escherichia coli* numbering system of Brosius et al. 1978). The conditions used for thermal cycling were as the follows: denaturation at 95 °C for 5 min followed by 35 cycles consisting of denaturation at 95 °C for 1 min, primer annealing at 56 °C for 1 min, and primer extention at 72 °C for 3 min. At the end of the cycles, the reaction mixture was kept at 72 °C for 5 min and then cooled to 4 °C. The 1.5-kb amplified 16S ribosomal DNA (rDNA) fragment was separated by agarose gel electrophoresis. The purified fragment was directly sequenced by using a *Taq* DyeDeoxy terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, California) and analyzed with an ABI PRISM^{TM} 377 DNA sequencer (Applied Biosystems, Inc.). Sequencing primers used included KMSO98PB1r (5′-TAAGGAGGTGATCCAGCC-3′), KMS584P1r (5′-TGCTGGCAACACAGAACAAG-3′) and KMS584P2r (5′-ACTCTGCCTGCCCGTATCG-3′).

Sequence alignment and phylogenetic analysis

The variable γ region (positions 158–277) of the 16S rDNA from 445 known *Streptomyces* species obtained from the DDBJ database and that of strain YIM 33098^T were aligned. The almost complete 16S rDNA sequence of strain YIM 33098^T was aligned manually with representative sequences of related Streptomycetes from the GenBank database. The evolutionary tree, rooted with *Streptomyces megasporus* (Z68100) 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 resultant tree was evaluated by bootstrap analysis (Felsenstein 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.

Nucleotide sequence accession number

The almost complete 16S rDNA sequence of strain YIM33098^T (1457 nucleotides) has been deposited in the GenBank database. The Accession No. for strain YIM 33098^T is AY222320.

Results and discussion

Morphological observation of the 7–15 day old culture of strain YIM 33098^T grown on yeast extract–malt extract agar (ISP2) (Shirling and

Table 1. Cultural characteristics of strain YIM 33098^T

Medium	Aerial mycelium	Substrate mycelium
Czapek's agar	Yellowish white	Yellowish white
Glycerol–asparagine agar	Light gray	Pale yellow
Inorganic salt–starch agar (ISP 4[a])	Yellowish white	Yellowish white
Yeast extract–malt extract agar (ISP 2[a])	Olive gray	Gray brown
Oatmeal agar (ISP 3[a])	Light gray	Light gray
Nutrient agar	Light olive gray	Light yellowish brown

[a]ISP, International *Streptomyces* Project Shirling and Gottieb (1966).

Gottlieb 1966) revealed that both aerial and vegetative hyphae were abundant, well-developed and not fragmented. Spore chains were lightly spiral. The spores had a short rod shape (0.5–0.7 × 1.0–1.2 μm) and smooth surfaces (Figure 1). Cultural characteristics of strain YIM 33098^T are shown in Table 1. Aerial mycelium was abundant, well-developed and varied from yellowish white to light gray on different test media. The substrate hyphae varied from yellowish white to light gray brown. Diffusible pigments were not produced on any test media and melanin was not produced.

The physiological and biochemical characteristics of strain YIM 33098^T are indicated in Table 2 and in the species description.

The cell wall peptidoglycan of strain YIM 33098^T contained only LL-diaminopimelic acid and glycine, indicating that strain YIM 33098^T has a chemotype cell wall type I (Lechevalier and Lechevalier 1970a, b). The whole-cell hydrolysates contained galactose and xylose.

Analysis of the γ region sequences of the 16S rDNA from *Streptomyces* species showed that strain YIM 33098^T was grouped into a branch with *Streptomyces xantholiticus* ISP 5244^T (D44413) (similarity value of 91.6%; 10 nucleotide differences in 120 sites) and *Streptomyces yogyakartensis* C4R3 (similarity value of 89.1%; 13 nucleotide differences in 120 sites), the two closest neighbors.

The almost complete 16S rRNA gene sequence of strain YIM 33098^T was determined in this study and has been deposited in the GenBank datebase (Accession No. AY222320). This sequence was subjected to similarity searches against public databases to infer a possible phylogenetic relationships of strain YIM 33098^T. This analysis revealed that strain YIM 33098^T was a member of the genus *Streptomyces*. A neighbor-joining tree (Saitou and Nei 1987) (Figure 2) based on 16S rDNA

Table 2. Phenotypic properties separating strain YIM 33098^T from closely related *Streptomyces* species based on the nearly complete 16S rDNA sequences

Characteristic	YIM 33098^T	*S. tendae* ATCC19812^T [a]	*S. eurythermus* ATCC14975^T [a]
Colony color on ISP2	Olive gray	Gray	Gray
Spore shape	Rod shape	Oval	Spherical or oval
Spore chain morphology	Spare spiral	Rectinaculiaperti to spiral	Rectinaculiaperti
Production of diffusible pigment	–	+	–
Melanoid pigment	–	–	+
Milk coagulation	–	ND	+
Milk peptonization	–	ND	+
Utilization of:			
Lactose	–	ND	ND
Mannose	+	ND	ND
Arabinose	–	+	+
Xylose	+	–	+
Raffinose	–	–	v
Ribose	+	ND	ND
Inositol	+	+	v
Mannitol	–	+	+
Sorbitol	–	ND	ND

Note: All the three type strains used glucose as a sole carbon source.
Symbols: +, utilization; –, non-utilization; v, variable; ND, not determined.
[a] Data for reference type species strains were taken from Shirling and Gottlieb (1968).

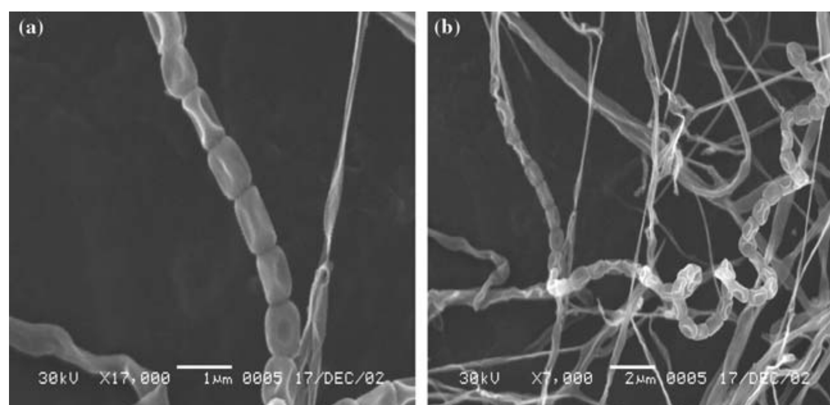

Figure 1. Scanning electron micrographs (a and b) showing strain YIM 33098^T short rod sparse spores and spiral spore chains. The organism was grown on yeast extract–malt extract agar (ISP 2) at 28 °C for 15 days.

gene sequences was constructed to show relationships between strain YIM 33098^T and some other related *Streptomyces* species. Bootstrap analysis was used to evaluate the tree topology of the neighbor-joining data by performing 1000 resamplings (Felsenstein 1985). Phylogenetically, strain YIM 33098^T was clustered in the same subclade (Figure 2) as *Streptomyces tendae* ATCC 19812^T (Ettlinger et al. 1958) and *Streptomyces eurytherumus* ATCC 14975^T (Corbaz et al. 1957). The 16S rDNA nucleotide sequence similarities of strain YIM33098^T with these two type strains were 97.25 and 97.42%, respectively.

A comparative study between strain YIM 33098^T and closely related species of the genus *Streptomyces* revealed that it differed from *S. tendae* ATCC 19812^T and *S. eurytherumus* ATCC 14975^T in morphological, cultural, and physiological characteristics as summarised in Table 2. In addition, the aerial mycelium of strain YIM 33098^T varied from yellowish white to light gray and soluble pigments were not produced. In contrast, the substrate mycelium of *S. tendae* ATCC 19812^T is yellow to red and soluble orange or brown yellowish pigments are produced. The aerial mycelium of *S. eurythermus* ATCC 14975^T is gray and the substrate mycelium is yellow to brown; yellowish to brown soluble pigments are produced.

Furthermore, DNA–DNA relatedness tests were performed between strain YIM 33098^T, *S. tendae* ATCC 19812^T and *S. eurytherumus* ATCC 14975^T using the thermal renaturation method (De Ley et al. 1970; Huss et al. 1983; Jahnke 1992). The low DNA–DNA relatedness between strain YIM 33098^T and *S. tendae* ATCC 19812^T (28.6%) and *S. eurytherumus* ATCC 14975^T (19.2%) also confirmed that they are different species.

Thus, based on the results of the above phenotypic and genotypic analyses, strain YIM 33098^T should represent a novel species of the genus *Streptomyces*, for which we propose the name *Streptomyces nanningensis* sp. nov.

Description of *Streptomyces nanningensis* sp. nov.

Streptomyces nanningensis (nan.ning.en'sis. M.L. neut. adj. *nanningensis* pertaining to Nanning, a city in the south of China). Both vegetative and aerial hyphae were abundant and well-developed. Aerial mycelium varied from yellowish white to light gray. The substrate hyphae from yellowish white to light gray brown. Diffusible pigments were not produced on any test media. Gelatine liquification and starch hydrolysis were positive. Milk coagulation and peptonization, nitrate reduction, growth on cellulose, and H_2S and melanin production were negative. Glucose, mannose, xylose, ribosoe, inositol, and histidine are utilized but not lactose, arabinose, raffinose, mannitol, sorbitol, glycine, and methionine. The cell wall peptidoglycan contains LL-diaminopimelic acid and glycine (chemotype I cell wall). The whole-cell hydrolysates contain galactose and xylose. The type strain YIM 33098^T = CCTCC AA 001027^T = DSM 4183^T was isolated from soil

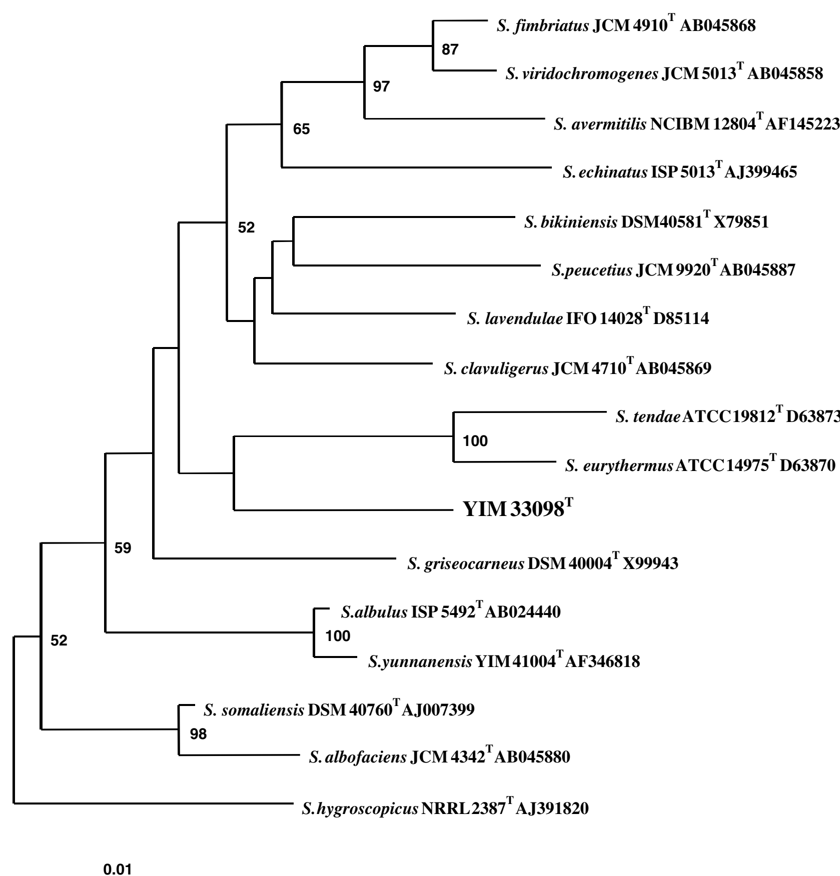

Figure 2. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rDNA sequences, showing the position of strain YIM 33098^T among phylogenetic neighbors. Numbers on branch nodes are bootstrap values (1000 resamplings). The sequence of *Streptomyces megasporus* DSM 41476^T (Z68100) was used as root. Bar, 1% sequence divergence.

sample collected from Nanning in Guangxi Province, China.

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