

Streptomyces hainanensis sp. nov., a novel member of the genus *Streptomyces*

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A novel isolate belonging to the genus *Streptomyces*, strain YIM 47672^T, was obtained from soil from Hainan, an island in China. The characterization of this isolate was performed by using a polyphasic approach. The strain formed long spore chains in the aerial mycelia. The cell wall contained L-diaminopimelic acid, traces of meso-diaminopimelic acid and glycine. Whole-cell hydrolysates contained galactose and xylose. The phospholipid was of type II. The 16S rRNA gene sequence similarities for YIM 47672^T with respect to the most closely related type strains of species of the genus *Streptomyces* were less than 96.3%. Therefore strain YIM 47672^T represents a novel member of the genus *Streptomyces*, for which the name *Streptomyces hainanensis* sp. nov. is proposed. The type strain is YIM 47672^T (=CCTCC AA 205017^T=DSM 41900^T).

The genus *Streptomyces* was proposed by Waksman & Henrici (1943) and emended by Rainey *et al.* in Stackebrandt *et al.*, 1997). Kim *et al.* (2003) proposed the emendation of the description of the family *Streptomycetaceae*, which comprises the genera *Streptomyces*, *Kitasatospora* (Zhang *et al.*, 1997) and *Streptacidiphilus* (Kim *et al.*, 2003). Members of the family *Streptomycetaceae* have attracted great attention because of their production of various natural products of considerable commercial value (Berdy, 2005).

In the course of our research on new sources of actinomycetes, we obtained morphological, physiological, chemical and phylogenetic data for isolate YIM 47672^T. Strain YIM 47672^T was shown to represent a novel species of the genus *Streptomyces*.

Strain YIM 47672^T was isolated, using the dilution plating method, from a soil sample collected in a forest (comprising evergreen broadleaved trees) on Wuzhi Mountain in Hainan Province, China. Selective isolation was achieved using trehalose-proline agar (Jiang *et al.*, 2006) and incubation at 28 °C for 35 days. Strain YIM 47672^T was maintained on YIM 38 medium [10 g malt extract, 4 g yeast extract, 4 g glucose, vitamin mixture (0.5 mg each of thiamine-HCl, riboflavin, niacin, pyridoxine-HCl, inositol, calcium pantothenate and p-aminobenzoic acid and 0.25 mg biotin), 20 g agar; pH 7.2]

(Hayakawa & Nonomura, 1987) at 4 °C and preserved in 20% (v/v) glycerol at –20 °C. Biomass for chemical and molecular systematic studies was obtained by growing the strain in shake flasks (at 180 r.p.m.) using YIM 38 broth at 28 °C for 4–7 days. Cultural characteristics were determined, after 2 weeks incubation at 28 °C, with the methods used in the International *Streptomyces* Project (Shirling & Gottlieb, 1966). Czapek's agar and nutrient agar were prepared as described by Dong & Cai (2001). Morphological properties were examined using a light microscope (BH-2; Olympus) and a scanning electron microscope (XL30 ESEM-TMP; Philips). Colour determination was performed using colour chips from the ISCC–NBS colour charts (standard samples, no. 2106) (Kelly, 1964).

Analyses of the amino acids and sugars in whole-cell hydrolysates were performed using the procedures described by Stanek & Roberts (1974) and analysis of the phospholipids was carried out according to Lechevalier *et al.* (1981). Menaquinones were determined using the procedures of Collins *et al.* (1977). Biomass for a quantitative fatty acid analysis was prepared by scraping cell mass from plates containing trypticase soy broth (BBL; 3%, w/v) and Bacto agar (Difco; 1.5%, w/v), that had been incubated for 5 days at 28 °C. The fatty acids were extracted, methylated and analysed by using the standard MIDI (Microbial Identification) system (Kämpfer & Kroppenstedt, 1996; Sasser, 1990). Chromosomal DNA of strain YIM 47672^T was extracted as described by Marmur (1961). The DNA G + C content was determined by HPLC

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YIM 47672^T is AM398645.

(Tamaoka & Komagata, 1984) with an Agilent 1100 LC system (IRIS Technologies).

The cell wall of strain YIM 47672^T contained LL-diaminopimelic acid, traces of meso-diaminopimelic acid and glycine. Whole-cell hydrolysates consisted of galactose and xylose. The analysis of the polar lipids revealed the presence of phosphatidylethanolamine and diphosphatidylglycerol, indicating that the phospholipid was of type II *sensu* Lechevalier *et al.* (1977). The major components of the fatty acid profile included iso-branched hexadecanoic acid (iso-C_{16:0}), hexadecanoic acid (C_{16:0}) and anteiso-branched heptadecanoic acid (C_{17:0}). These characteristics are consistent with membership of the family *Streptomycetaceae*. According to Kim *et al.* (2003), the major menaquinones of the genus *Streptomyces* are MK-9(H₆) and MK-9(H₈). Strain YIM 47672^T also contained MK-9(H₆) (14.0%) and MK-9(H₈) (13.6%), but the predominant menaquinones were MK-9(H₄) (45.4%) and MK-10(H₀) (27.0%). The G + C content of genomic DNA from strain YIM 47672^T was 73.4 mol%.

Strain YIM 47672^T grew well on most of the media tested (Table 1). Aerial mycelia developed well, were long and branched and ranged in colour from white to pink–white and pink–grey. Vegetative mycelia developed well, and ranged in colour from a pale to a deep orange–yellow. Light brown to yellowish orange soluble pigments were produced. Spore chains were spiral or *retinaculiaperti* (looped at the top) in nature (Fig. 1). Spores were elliptical or shaped like short rods (0.4–0.6 × 0.7–1.0 μm). The spore surfaces were smooth.

A range of phenotypic properties was examined using standard procedures (Goodfellow, 1971; Williams *et al.* 1983). In addition, acid production from carbohydrates was tested using the media and methods described by Gordon *et al.* (1974). The utilization of carbon and nitrogen sources was determined by using the methods of Gordon & Mihm (1962) and Tsukamura (1966). Strain YIM 47672^T was negative in tests for gelatin liquefaction, milk coagulation and peptonization, arginase activity, phenylalanine deaminase activity, DNase activity and melanin production. The strain was positive in tests for

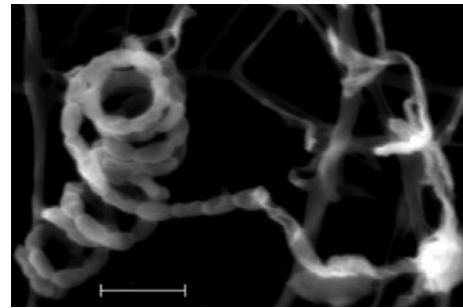


Fig. 1. Scanning electron micrograph of spiral spore chains of strain YIM 47672^T after growth on YIM 38 medium for 14 days. Bar, 2 μm.

starch hydrolysis, arginine decarboxylase activity, nitrate reduction, gas production from nitrate, growth on cellulose and H₂S production. Growth occurred at pH 6.0–9.0 (optimum, pH 7.0) and with 0–10% NaCl (optimum, 0% NaCl). Glucose, cellobiose, starch, aesculin, trehalose, β-galactoside and urea were utilized; acids were not produced from these carbon sources. A broad range of additional carbon sources, as well as three nitrogen sources, were tested and shown not to be utilized.

The almost-complete 16S rRNA gene sequence of strain YIM 47672^T was aligned with sequences of related type strains, as well as with sequences of representative *Streptomyces* species (obtained from the GenBank/EMBL/DDBJ and RDPII databases). The CLUSTAL_X program (Thompson *et al.*, 1997) was used for multiple alignments (corrected manually). In addition, a second alignment method, ARB, was used. Both alignment methods revealed the same tree topology (data not shown). The phylogenetic analysis was performed with the ARB alignment and the neighbour-joining method (Saitou & Nei, 1987) using the Kimura two-parameter model (Kimura, 1980) using the MEGA3.1 software package (Kumar *et al.*, 2004). The PHYML software package (Guindon *et al.*, 2005) was used to construct the maximum-likelihood tree, using the general time reversible (GTR) model. The topologies of the trees

Table 1. Cultural characteristics of strain YIM 47672^T

+, Poor; ++, moderate; +++, good; –, not present.

Media	Growth	Aerial mycelium		Substrate mycelium		Soluble pigment
		Presence	Colour	Presence	Colour	
Czapek's agar	++	++	Pink–grey	++	Orange–yellow	Light brown
Glycerol-asparagine agar (ISP 5)	+	–	None	+	Pale yellow	None
Inorganic salts–starch agar (ISP 4)	++	++	Pink–white	++	Light reddish brown	Pale orange–yellow
Yeast extract–malt extract agar (ISP 2)	++	++	Pink–white	++	Brilliant orange–yellow	Orange–yellow
Oatmeal agar (ISP 3)	+++	+++	Pale pink	+++	Deep orange–yellow	Orange–yellow
Nutrient agar	+	+	White	+	Soft orange–yellow	None

were evaluated by using bootstrap analysis (Felsenstein, 1985) based on 1000 resamplings (neighbour-joining) and 500 resamplings (maximum-likelihood), respectively. NJPLOT software (Perrière & Gouy, 1996) was used to display the phylogenetic trees.

The phylogenetic analysis demonstrated that strain YIM 47672^T belongs to the genus *Streptomyces*. The rooted phylogenetic tree for YIM 47672^T and related and representative type strains of the genus *Streptomyces*

indicated that this strain formed a distinct clade with an undescribed isolate, *Streptomyces* sp. D44 (Q. Gu, Y. Huang & Z. Liu, unpublished; GenBank/EMBL/DDJB accession no. DQ460471), derived from a traditional Chinese medicinal plant. *Streptomyces* sp. D44 was found to be the organism most closely related to strain YIM 47672^T (showing 99.9% gene sequence similarity). The separate branching of this clade was supported by the neighbour-joining tree (Fig. 2) and by the maximum-likelihood tree (data not shown). An analysis of the γ -region sequences of

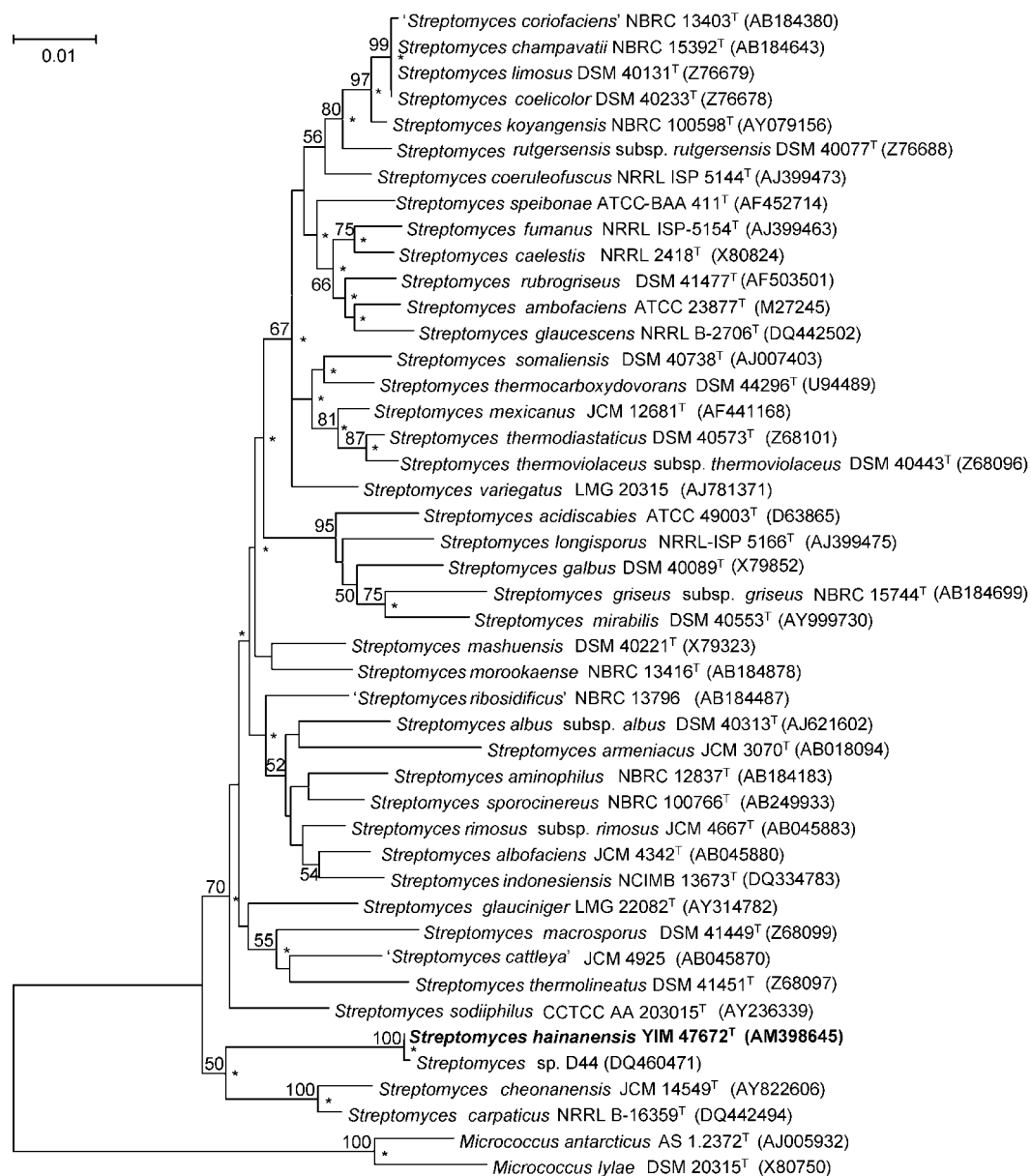


Fig. 2. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences (1412 bp, by omitting unaligned regions), showing the relationships between strain YIM 47672^T and related and representative species of the genus *Streptomyces*. Type strains of the genus *Micrococcus* were used as an outgroup. Numbers at branching points indicate bootstrap percentages (based on 1000 replications). Asterisks indicate branches that were also obtained using maximum-likelihood analysis. Bar, 0.01 substitutions per nucleotide position.

the 43 *Streptomyces* strains shown in the phylogenetic trees (positions 158–277 on the 16S rRNA gene; Kataoka *et al.*, 1997) confirmed the affiliation of strain YIM 47672^T to a separate branch (data not shown). *Streptomyces sodiiphilus* CCTCC AA 203015^T clustered with *Streptomyces rimosus* subsp. *rimosus* JCM 4667^T, *Streptomyces albus* subsp. *albus* DSM 40313^T and *Streptomyces albofaciens* JCM 4342^T. *Streptomyces cheonanensis* JCM 14549^T belonged to a cluster consisting of *Streptomyces carpaticus* NRRL B-16359^T, *Streptomyces armeniacus* JCM 3070^T, the suggested type strain of '*Streptomyces cattleya*' JCM 4925, *Streptomyces macrosporus* DSM 41449^T and *Streptomyces thermolineatus* DSM 41451^T.

The 16S rRNA gene sequence similarities between strain YIM 47672^T and the type strains of closely related *Streptomyces* species with validly published names were 96.2 % (for *S. sodiiphilus*; Li *et al.*, 2005), 95.95 % (for *S. carpaticus*; Gause *et al.*, 1983, 1986) and 95.81 % (for *S. cheonanensis*; Kim *et al.*, 2006). The levels of gene sequence similarity with respect to other members of the genus *Streptomyces* ranged from 93.40 to 95.50 %.

Strain YIM 47672^T showed some characteristics that differed from those of its closest relatives. Whole-cell hydrolysates of *S. sodiiphilus* contain mainly galactose and glucose, the predominant menaquinones are MK-9(H₄) (13%), MK-9(H₆) (68%) and MK-9(H₈) (19%) and the G+C content is 70.5 mol%. *S. cheonanensis* shows *rectiflexibiles* spore chains, the aerial mycelium is grey and the substrate mycelium is light yellow; it produces brown and dark grey soluble pigments, produces melanin, utilizes arabinose, mannitol, raffinose and xylose and the DNA G+C content is 75.5 mol%. The description of *S. carpaticus* focused mainly on its morphological and physiological characteristics (*Spirales* spore chains dark brown mycelium on ISP 4 agar, utilization of rhamnose, arabinose, mannitol, raffinose, fructose and xylose).

It can be concluded, therefore, that strain YIM 47672^T represents a novel member of the genus *Streptomyces*, for which the name *Streptomyces hainanensis* sp. nov. is proposed.

Description of *Streptomyces hainanensis* sp. nov.

Streptomyces hainanensis (hai.nan.en'sis. N.L. adj. *hainanensis* pertaining to Hainan, a province of south China, from where the type strain was isolated).

Aerial mycelia are white and pink–white to pink–grey. Spore chains are spiral or looped. Spores are elliptical or shaped like short rods. Spore surface is smooth. Vegetative mycelia are pale to deep orange–yellow. Produces light brown to orange–yellowish soluble pigments. Negative in tests for gelatin liquefaction, milk coagulation and peptonization, arginase activity, phenylalanine deaminase activity, DNase and melanin production. Positive for starch hydrolysis, arginine decarboxylase activity, nitrate reduction, gas production from nitrate, growth on cellulose and

H₂S production. Optimal growth occurs at pH 7.0 (range, pH 6.0–9.0) and without NaCl (range, 0–10% NaCl). Glucose, cellobiose, starch, aesculin, trehalose, β-galactoside and urea are utilized; acids are not produced from these carbon sources. Galactose, mannose, fructose, arabinose, xylose, ribose, rhamnose, sucrose, lactose, maltose, melibiose, raffinose, turanose, melezitose, sorbin, dextrin, salicin, adonitol, inositol, mannitol, sorbitol, xylitol, galactitol, erythritol, amygdaloside, sodium citrate, sodium acetate, gluconate, malonate, tartrate, lysine, ornithine and acetamide are not utilized. Resistant to the following antibiotics (μg, unless indicated otherwise): penicillin G (10 U), amoxicillin/clavulanic acid (20:10), novobiocin (30), rifampicin (5) and ampicillin (10). Sensitive to the following antibiotics (μg, unless indicated otherwise): erythromycin (15), gentamicin (10), kanamycin (30), tetracycline (30), vancomycin (30), midecamycin (15), clindamycin (2), sulfamethoxazole/trimethoprim (23.75:1.25), chloramphenicol (30), polymyxin B (300 U) and norfloxacin (10). Cell wall contains L-diaminopimelic acid, traces of meso-diaminopimelic acid and glycine. Whole-cell hydrolysates contain galactose and xylose. The diagnostic phospholipid is phosphatidylethanolamine. The predominant menaquinones are MK-9(H₄) (45.4%), MK-9(H₆) (14.0%), MK-9(H₈) (13.6%) and MK-10(H₀) (27.0%). Fatty acids comprise iso-C_{15:0} (1.1%), iso-C_{16:0} (30.9%), C_{16:1}ω7d/C_{16:1}ω6c (2.1%), C_{16:0} (13.7%), iso-C_{17:1}ω9c (1.4%), iso-C_{17:0} (2.8%), anteiso-C_{17:0} (10.8%), C_{17:1}ω8c (7.1%), C_{17:0} cyclo (1.4%), C_{17:0} (5.6%), anteiso-C_{18:0}/C_{18:2}ω6,9c (9.7%), C_{18:1}ω9c (4.9%) and C_{18:0} (1.2%). In addition, the sum of C_{16:1}ω7d/C_{16:1}ω6c was 2.1%, the sum of anteiso-C_{18:0}/C_{18:2}ω6,9c was 9.7% and the sum of iso-C_{17:1}ω9c and/or C_{16:0} 10-methyl was 1.4%. The G+C content of genomic DNA is 73.4 mol%.

The type strain, YIM 47672^T (=CCTCC AA 205017^T=DSM 41900^T), was isolated from soil collected from a forest (of evergreen broadleaved trees) on Wuzhi Mountain, Hainan Province, China.

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