Streptomyces hebeiensis sp. nov.

Ping Xu,[1,2] Wen-Jun Li,[1] Wen-long Wu,[1] Dong Wang,[1] Li-Hua Xu[1] and Cheng-Lin Jiang[1]

[1]The Key Laboratory for Microbial Resources of Ministry of Education, Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan, 650091, PR China

[2]New Drug R & D, North China Pharmaceutic Corp., Shijiazhuang, 050015, PR China

Correspondence
Cheng-Lin Jiang
lihxu@ynu.edu.cn *or*
liact@hotmail.com

A novel actinomycete strain, YIM 001^T, was isolated from a soil sample collected from Hebei province, People's Republic of China. The strain was characterized by white to grey aerial mycelium. Long spore chains, borne on the aerial mycelium, were straight to *Rectiflexibiles*; the spore chains were composed of non-motile and coccoid spores with a warty surface. The cell wall of strain YIM 001^T contained LL-diaminopimelic acid (A_2pm) and traces of *meso*-A_2pm. Whole-cell hydrolysates contained mainly glucose and small amounts of xylose, galactose and arabinose. The menaquinones were MK-9(H_4) (4·6 %), MK-9(H_6) (60 %), MK-9(H_8) (30·7 %) and MK-9(H_{10}) (4·7 %). Phosphatidylethanolamine was the diagnostic phospholipid. The DNA G+C content of strain YIM 001^T was 71·4 mol%. Phylogenetic analysis indicated that strain YIM 001^T belongs to the genus *Streptomyces*. Based on its phenotypic and genotypic characteristics, strain YIM 001^T (=CCTCC AA 203005^T=CIP 107974^T=DSM 41837^T) is proposed as the type strain of a novel species, *Streptomyces hebeiensis* sp. nov.

The genus *Streptomyces* was proposed by Waksman & Henrici (1943) for aerobic, spore-forming actinomycetes. Members of *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 programme for new antibiotics, several actinomycete strains that contained both type-I and type-II polyketide biosynthesis pathway genes were isolated from soil samples collected from Hebei province of China.

Strain YIM 001^T was isolated from a soil sample after 2 weeks incubation at 28 °C on glycerol-asparagine agar [International *Streptomyces* Project (ISP) medium 5; Shirling & Gottlieb, 1966]. 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 & Nonomura, 1987). Cultural characteristics were determined after 2 weeks at 28 °C by methods used in the ISP (Shirling & Gottlieb, 1966). Morphological observations of spores and mycelia were made by light microscopy (Olympus microscope BH-2) and scanning electron microscopy (model EPMA-8705).

Abbreviations: A_2pm, diaminopimelic acid; ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain YIM 001^T is AY277529.

16S rDNA variable γ-region and full 16S rDNA sequence similarities are available as supplementary material in IJSEM Online.

The test strain was examined for a range of phenotypic properties using standard procedures (Shirling & Gottlieb, 1966; Williams *et al.*, 1983). In addition, acid production from carbohydrates was assessed using media and methods described by Gordon *et al.* (1974). Tolerance of temperature (10, 27, 30, 37 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). Colours and hues were determined according to colour chips from the ISCC-NBS Color Charts standard samples no. 2106 (Kelly, 1964).

Cell wells were purified and amino acids of peptidoglycan were analysed by TLC (Lechevalier & Lechevalier, 1980; Jiang *et al.*, 2001). Analysis of whole-cell sugar composition followed procedures described by Becker *et al.* (1965) and Lechevalier & 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).

Genomic DNA was extracted and 16S rDNA amplified as described by Cui *et al.* (2001). The DNA G+C base content of strain YIM 001^T was determined by the thermal denaturation method (Mandel & Marmur, 1968). The variable γ region (positions 158–277) of the 16S rDNA from 379 known *Streptomyces* species obtained from the DDBJ databases and strain YIM 001^T were aligned. The nearly complete resultant 16S rDNA sequence (1520 nucleotides) was aligned manually with corresponding almost-complete sequences of representative *Streptomyces* species retrieved

from DDBJ, EMBL and GenBank by using BLAST (Altschul *et al.*, 1997) and BLAST 2 Sequences (Tatusova & Madden, 1999). CLUSTAL W (Thompson *et al.*, 1994) was used to estimate evolutionary distances (the K_{nuc} value of Kimura, 1980) and similarity values were used to reconstruct the phylogenetic tree by the neighbour-joining method (Saitou & Nei, 1987). The topology of the tree was evaluated by performing a bootstrap analysis (Felsenstein, 1985) using 1000 resamplings. *Actinoplanes philippinensis* (GenBank/EMBL/DDBJ accession no. D85474) was used as an outgroup. DNA–DNA hybridization experiments using strain YIM 001^T and comparative strains were carried out according to the thermal renaturation method (De Ley *et al.*, 1970; Huß *et al.*, 1983).

Strain YIM 001^T developed well on several media including ISP 2, ISP 4, potato agar and Czapek's agar and showed moderate growth on ISP 3, ISP 5 and nutrient agar (Table 1). Diffusible pigments were produced on all media tested. The pigment(s) is not a pH indicator or changed only slightly with addition of 0·05 M HCl in ISP 5 and ISP 6. Morphological features were observed on ISP 2, ISP 4 and ISP 5. Aerial mycelium and substrate mycelium were well developed (Fig. 1). At maturity, the aerial mycelium formed long, straight to *Rectiflexibiles* spore chains. The spores were non-motile. The physiological features are indicated in Table 2 and in the species description. Strain YIM 001^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, galactose and arabinose. The menaquinones were MK-9(H_4) (4·6 %), MK-9(H_6) (60 %), MK-9(H_8) (30·7 %) and MK-9(H_{10}) (4·7 %) and the diagnostic phospholipid was phosphatidylethanolamine. The G+C content of the DNA was 71·4 mol%. The chemical and morphological properties of strain YIM 001^T, except for the detection of a small amount of the menaquinone MK-9(H_{10}), are clearly consistent with its assignment to the genus *Streptomyces* (Williams *et al.*, 1989).

Analysis of γ-region sequences showed that strain YIM 001^T was grouped into a branch with *Streptomyces beijiangensis* YIM 6^T (the nearest neighbour; 93·3 % similarity, 8 nucleotide differences in 120 sites), *Streptomyces niveus* ISP 5088^T (92·5 %) and *Streptomyces spheroides* ISP 5292^T (91·7 %) (see Supplementary Table A in IJSEM Online). The low DNA–DNA relatedness between strain YIM 001^T and *S. niveus* ISP 5088^T (13·2 %) and *S. spheroides* ISP 5292^T (17·8 %) also confirmed that they are different species.

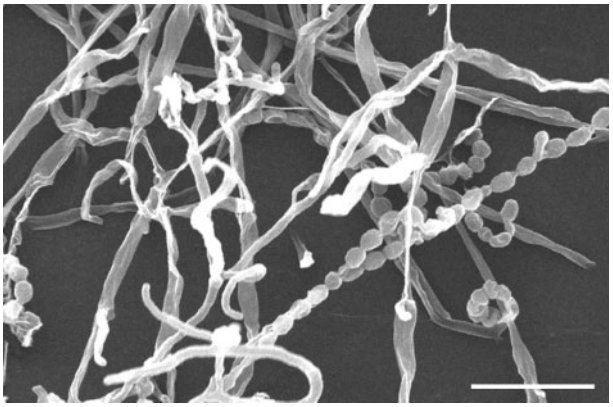

Fig. 1. Scanning electron micrograph of spore chains of *Streptomyces hebeiensis* sp. nov. YIM 001^T grown on yeast-malt extract agar (ISP 2) for 14 days at 28 °C. Bar, 5 μm.

16S rDNA sequence similarities between YIM 001^T and *Streptomyces* type strains are shown in Supplementary Table B. It is clear from the phylogenetic tree that strain YIM 001^T forms a single branch separate from other representatives of the genus *Streptomyces* (Fig. 2), notably from its nearest neighbour *Streptomyces sampsonii* ISP 5394^T (97·57 % similarity; 37 nucleotide differences in 1521 sites) and the closely related species *Streptomyces rutgersensis* DSM 40077^T (97·56 %; 36/1476), *Streptomyces gougerotii* DSM 40324^T (97·49 %; 37/1476), *Streptomyces tuirus* DSM 40505^T (97·46 %; 37/1457), *Streptomyces albidoflavus* ISP 5445^T (97·43 %; 38/1478), *Streptomyces coelicolor* ISP 5233^T (97·43 %; 38/1478), *Streptomyces odorifer* ISP 5347^T (97·43 %; 38/1478), *Streptomyces felleus* ISP 5130^T (97·43 %; 38/1478), *Streptomyces limosus* ISP 5131^T (97·43 %; 38/1478) and *Streptomyces canescens* ISP

Table 1. Culture characteristics of strain YIM 001^T on various media

Medium	Growth	Sporulation	Diffusible pigment	Colony colour	
				Aerial mycelium	Substrate mycelium
Yeast-malt extract agar (ISP 2)	Good	Good	Deep yellow-brown	Pale yellow–grey	Deep grey–yellow-brown
Oatmeal agar (ISP 3)	Moderate	Moderate	Soft yellow-brown	Deep yellow–grey	Deep grey–yellow
Inorganic salts-starch agar (ISP 4)	Good	Good	Deep yellow-brown	Deep grey–yellow	Deep yellow
Glycerol-asparagine agar (ISP 5)	Moderate	Moderate	Light yellow	White	Yellow–white
Potato agar	Good	Good	Moderate olive-brown	Light yellow–grey	Moderate olive-brown
Nutrient agar	Moderate	Moderate	Pale yellow	White	Pale yellow
Czapek's agar	Good	Good	Moderate reddish-brown	Yellow–grey	Light grey–reddish

Table 2. Phenotypic properties that separate strain YIM 001^T from related *Streptomyces* species

Strains: 1, strain YIM 001^T; 2, *S. beijiangensis* YIM 6^T; 3, *S. niveus* ISP 5088^T; 4, *S. spheroides* ISP 5292^T; 5, *S. albidoflavus* ISP 5445^T; 6, *S. coelicolor* ISP 5233^T; 7, *S. odorifer* ISP 5347^T; 8, *S. felleus* ISP 5130^T; 9, *S. limosus* ISP 5131^T; 10, *S. sampsonii* ISP 5394^T; 11, *S. canescens* ISP 5001^T; 12, *S. gougerotii* ISP 5324^T; 13, *S. rutgersensis* ISP 5077^T; 14, *S. tuirus* ISP 5505^T. All strains used D-xylose as a sole carbon source. V, Variable; ND, not detected. Data for reference strains were taken from Shirling & Gottlieb (1968, 1972), Williams *et al.* (1983) and Li *et al.* (2002).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Colony colour on ISP 2*	Pale YG	Pale W	Y	Y	WG	Y	Y	Y	Y	Pale Y	Y	WY	Y	G
Spore surface†	W	S	S	S	S	S	S	S	S	S	S	S	S	S
Spore chain morphology‡	ST to RF	RF to RA	SP	SP	RF	RF	RF	RF	RF	RF	RF	RF	RA RF	VSP
Production of diffusible pigment	+	−	−	−	−	+	+	−	−	−	−	−	−	+
Melanoid pigment	+	ND	−	−	−	−	−	−	−	−	−	−	−	+
Utilization of:														
Arabinose	+	−	V	+	+	+	+	+	+	+	V	V	+	+
Mannitol	+	−	V	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	−	+	+	+	+	+	+	+	+	V	+	+	+
Rhamnose	+	−	V	+	−	−	−	−	−	−	−	V	−	+
Sucrose	+	−	−	+	−	−	+	−	−	−	−	−	−	+
Raffinose	+	−	−	−	−	−	−	−	−	−	−	−	−	+
Inositol	+	ND	−	−	−	−	−	−	−	−	−	V	−	+

*G, Grey; W, white; WG, white–grey; WY, white–yellow; Y, yellow; YG, yellow–grey.

†W, Warty; S, smooth.

‡RA, *Retinaculaperti*; RF, *Rectiflexibiles*; SP, *Spirales*; ST, straight; VSP, verticillati and *Spirales*.

5001^T (97·43 %; 38/1478). Strain YIM 001^T can be readily distinguished from *S. beijiangensis* YIM 6^T (95·88 %; 61/1481), which neighboured strain YIM 001^T in the phylogenetic tree generated using γ-region sequences.

DNA–DNA hybridization studies were carried out between strain YIM 001^T and closely related strains selected on the basis of their 16S rDNA sequence similarity and phylogenetic positions. The low DNA–DNA relatedness between strain YIM 001^T and *S. sampsonii* ISP 5394^T (11·7 %), *S. rutgersensis* DSM 40077^T (21·2 %), *S. gougerotii* DSM 40324^T (15·3 %), *S. tuirus* DSM 40505^T (13·2 %), *S. albidoflavus* ISP 5445^T (15·2 %), *S. coelicolor* ISP 5233^T (8·1 %), *S. odorifer* ISP 5347^T (17·1 %), *S. felleus* ISP 5130^T (21·3 %), *S. limosus* ISP 5131^T (9·2 %) and *S. canescens* ISP 5001^T (13·5 %) confirmed that strain YIM 001^T can be considered as a novel taxon. This is also supported by phenotypic data, as at least seven differences in phenotypic properties were observed between strain YIM 001^T and its closest neighbours (Table 2). Comparison of the phenotypic characteristics of strain YIM001^T and *S. tuirus* reveals significant differences. Strain YIM001^T has straight to *Rectiflexibiles* spore chains, while *S. tuirus* has verticillati and *Spirales* spore chains. They can also be distinguished by colony colour and spore surface texture (Table 2).

In conclusion, the genotypic, chemotaxonomic and phenotypic data show that strain YIM 001^T represents a novel species of the genus *Streptomyces*, for which we propose the name *Streptomyces hebeiensis* sp. nov.

Description of *Streptomyces hebeiensis* sp. nov.

Streptomyces hebeiensis (he.bei.en′sis. N.L. masc. adj. *hebeiensis* pertaining to Hebei, a province in northern China where the sample yielding the type strain was collected).

Grows well at 27, 30 and 37 °C but does not grow at 45 or 10 °C. Aerial mycelium and substrate mycelium are well developed. Aerial mycelium at maturity forms long, straight to *Rectiflexibiles* spore chains composed of non-motile and coccoid spores with a warty surface. Diffusible pigments are produced on several media. The pigment(s) is not a pH indicator or is changed only slightly with addition of 0·05 M HCl in ISP 5 and ISP 6. Colony colour is medium-dependent (Table 1). In addition to the properties shown in Table 2, galactose, lactose, mannose, maltose, xylose, sorbitol, sodium citrate, sodium acetate, oxalate, starch and glycerol are utilized as sole carbon and energy sources, but cellulose and xylan are not. Acid is formed from mannose and starch but not from arabinose, fructose, galactose, glucose, inositol, lactose, mannitol, maltose, rhamnose, raffinose, sucrose, sorbitol, xylose, sodium citrate, sodium acetate, oxalate or glycerol. L-Histidine and L-hydroxyproline can be used as sole carbon and nitrogen sources. Casein and xanthine can be metabolized, but adenine and pectin can not. Tests for gelatin, nitrate reduction and melanin production are positive and tests for H_2S production, peptonization of milk are negative. Grows in the presence of 4 or 7 % NaCl

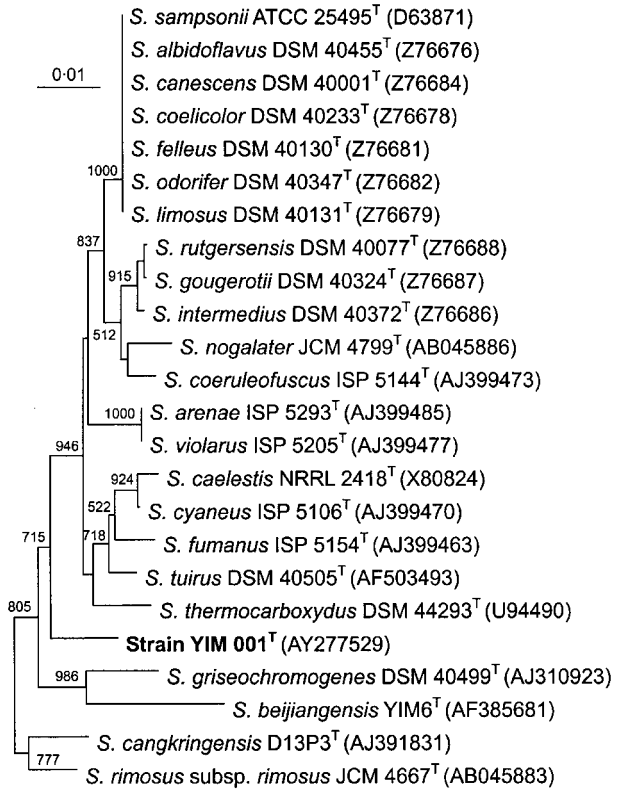

Fig. 2. Phylogenetic dendrogram obtained by neighbour-joining analysis based on 1443 bp of 16S rDNA sequences, showing the position of strain YIM 001^T among its phylogenetic neighbours. Numbers on branch nodes are bootstrap values (1000 resamplings). Sequence accession numbers are given in parentheses. The sequence of *Actinoplanes philippinensis* IFO 13878^T (D85474) was used as the root. Bar, 0·01 substitutions per nucleotide position.

and 0·1 % phenol. Diagnostic amino acid of peptidoglycan is LL-A_2pm with trace amounts of *meso*-A_2pm. Whole-cell hydrolysates contain glucose and small quantities of xylose, galactose and arabinose. The menaquinones are MK-9(H_4) (4·6 %), MK-9(H_6) (60 %), MK-9(H_8) (30·7 %) and MK-9(H_{10}) (4·7 %) and phosphatidylethanolamine is the diagnostic phospholipid. The G+C content of the DNA is 71·4 mol%.

The type strain, strain YIM 001^T (=CCTCC AA 203005^T=CIP 107974^T=DSM 41837^T), was isolated from a soil sample collected from Hebei Province, northern China.

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