Streptomyces jietaisiensis sp. nov., isolated from soil in northern China

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An actinomycete, strain FXJ46^T, was isolated from cypress forest soil in northern China and shown to have chemotaxonomic and morphological properties consistent with streptomycetes. It developed greyish aerial mycelium and pinkish-brown substrate mycelium on oatmeal agar. Phylogenetic analyses based on an almost complete 16S rRNA gene sequence of the strain and on the 120 nucleotide variable γ-region of this molecule showed that it formed a distinct (but closely associated) line with *Streptomyces griseoaurantiacus* DSM 40430^T in *Streptomyces* trees. However, the DNA–DNA relatedness between the two strains was only 48·8 %. A number of phenotypic properties also readily distinguished the isolate from *S. griseoaurantiacus* and related *Streptomyces* species with validly published names. It is proposed, therefore, that this organism be classified as a novel species of the genus *Streptomyces*, for which the name *Streptomyces jietaisiensis* sp. nov. is proposed. The type strain is FXJ46^T (=AS 4.1859^T=JCM 12279^T).

In the last decade, the taxonomy of the genus *Streptomyces* has undergone much improvement, in part due to increased interest in the identification of these organisms, particularly those from the soil environment. The use of molecular taxonomic methods such as 16S rRNA gene sequencing has done much to aid the classification of this complex group and the recognition of novel species (Kim & Goodfellow, 2002; S. B. Kim *et al.*, 1998, 2004; Al-Tai *et al.*, 1999; B. Kim *et al.*, 2000; Li *et al.*, 2002; Saintpierre *et al.*, 2003). However, due to the large number of species, the complete 16S rRNA gene sequences have not been determined for many type strains of this genus; incomplete sequence data may result in misclassification (Kataoka *et al.*, 1997; Anderson & Wellington, 2001). In this study, therefore, the partial sequence (120 nucleotides) covering the variable γ-region of the 16S rRNA gene was also analysed phylogenetically to classify strain FXJ46^T. While phylogenetic data showed that it was closely related to *S. griseoaurantiacus* DSM 40430^T, polyphasic studies based on a judicious combination of genotypic and phenotypic features revealed a novel species of *Streptomyces*.

Strain FXJ46^T was isolated on a yeast extract-starch agar (Emerson, 1958) plate supplemented with 50 μg cycloheximide ml^{-1}, which had been seeded with a soil sample suspension and incubated for 14 days at 28 °C. The soil sample was collected from a cypress forest, at Jietaisi, Beijing, China. The isolate was maintained on yeast extract-starch slopes at 4 °C and as glycerol suspensions (20 %, v/v) at −20 °C. Biomass for the chemotaxonomic and molecular systematic studies was prepared as described previously (Li *et al.*, 2002).

The morphological characteristics of strain FXJ46^T were examined by light and scanning electron microscopy of 14-day cultures grown on oatmeal agar and inorganic salts-starch agar (ISP medium 4). The coverslip technique (Zhou *et al.*, 1998; Kawato & Shinobu, 1959) was used to observe the hyphae and spore-chain characters by light microscope. Spore-chain morphology and spore-surface ornamentation were studied by examining gold-coated dehydrated specimens with a model FEI QUANTA electron microscope. The cultural features were observed on a number of standard media (Table 1) after 14 days incubation at 28 °C. The test strain was examined for a range of biochemical and physiological properties as described by Williams *et al.* (1983) and Kämpfer *et al.* (1991).

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains FXJ46^T and *S. griseoaurantiacus* DSM 40430^T are AY314783 and AY450561, respectively.

A micrograph showing aerial hyphae with *rectiflexibiles* spore chains and a neighbour-joining tree based on the 120 nt γ-region are available as supplementary material in IJSEM Online.

Table 1. Comparison of cultural characteristics of strain FXJ46^T and *Streptomyces griseoaurantiacus* DSM 40430^T

No pigments were formed on the listed agars.

Agar medium	Aerial mycelium		Substrate mycelium	
	FXJ46^T	DSM 40430^T	FXJ46^T	DSM 40430^T
Czapek–Dox	Grey, moderate	White, sparse	Pinkish cream	Pink
Glycerol-asparagine (ISP 5)	Grey	Reddish grey	Grey	Greyish yellowish pink
Inorganic salts-starch (ISP 4)	Pale grey, abundant	Brown, abundant	Orange–pink	Reddish brown
Nutrient	None	None	Light pinkish brown	Light brown
Oatmeal (ISP 3)	Smoky grey, abundant	Smoky grey, abundant	Light pinkish brown	Brownish yellow
Peptone-yeast extract-iron (ISP 6)	Grey, sparse	None	Yellowish brown	Cream
Tyrosine (ISP 7)	Pale grey, abundant	Greyish black, abundant	Whitish grey	Brownish black
Yeast extract-malt extract (ISP 2)	Mouse grey, abundant	White, moderate	Orange–brown	Reddish orange
Yeast extract-starch	Grey, abundant	Pale grey, abundant	Light vinaceous purple	Light reddish brown

The isomers of diaminopimelic acid and whole-organism sugars were analysed following the procedures developed by Hasegawa *et al.* (1983) and Lechevalier & Lechevalier (1980). Menaquinones were extracted and purified according to Collins (1985) and analysed by HPLC. Polar lipids were examined and identified using the method of Minnikin *et al.* (1984). The fatty acids were extracted, methylated and analysed by GC using the standard Sherlock MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The G + C content of the DNA of the test strain was determined using the thermal denaturation method (Marmur & Doty, 1962) with *Escherichia coli* AS 1.365 as a control.

Genomic DNA preparation and PCR amplification of the 16S rRNA gene of strain FXJ46^T were carried out using the procedure of Chun & Goodfellow (1995). The PCR products were sequenced using the method of Huang *et al.* (2001). CLUSTAL X version 1.8 (Thompson *et al.*, 1997) was used for multiple alignment with available almost-complete sequences of type strains of the family *Streptomycetaceae* and then with corresponding sequences of representative *Streptomyces* species; in each case, the reference sequences were retrieved from the DDBJ/EMBL/GenBank databases. The least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) algorithms from the PHYLIP package version 3.5c (Felsenstein, 1993) were used to infer the phylogenetic trees. Evolutionary distance matrices were generated as described by Kimura (1980). Tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. The partial sequence covering the variable γ-region (120 nt, positions 158–277) of the 16S rRNA gene sequence of strain FXJ46^T was also compared with corresponding nucleotide sequences of 485 *Streptomyces* type strains retrieved from GenBank. A phylogenetic tree based on these partial sequences was constructed using the neighbour-joining algorithm (Saitou & Nei, 1987).

Levels of DNA–DNA relatedness between strain FXJ46^T and *S. griseoaurantiacus* DSM 40430^T were determined according to the fluorometric micro-well method (Ezaki *et al.*, 1989), with the modification that detection of the DNA hybridization rate was tested by the addition of streptavidin-conjugate alkaline phosphatase acting on the substrate 4-methylumbelliferyl phosphate (Christensen *et al.*, 2000). Fluorescence intensities were measured using a Fluostar Optima microplate reader (BMG LABTECH) at a wavelength of 360 nm for excitation and 460 nm for emission. The DNA hybridization rate was calculated from quadruplicated hybridization experiments and expressed as a mean of the corresponding reciprocal values.

The organism exhibited a range of chemotaxonomic and phenotypic properties typical of members of the genus *Streptomyces*. It formed an extensively branched substrate mycelium, aerial hyphae which carried smooth-surfaced spores in *rectiflexibiles* spore chains (see Supplementary Fig. S1 available in IJSEM Online) and a greyish aerial spore mass on several standard media (Table 1). It contained LL-diaminopimelic acid in whole-organism hydrolysates, hexa-, octa- and a minor amount of tetrahydrogenated menaquinones with nine isoprene units [MK-9 (H_6, H_8 and H_4)] as isoprenologues, and diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides as major polar lipids (phospholipid type II *sensu* Lechevalier *et al.*, 1977). The fatty acid profile was composed mainly of saturated straight-chain and iso- and anteiso-branched-chain fatty acids (fatty acid type 2c *sensu* Kroppenstedt, 1985).

An almost complete 16S rRNA gene sequence (1426 nt) was determined for the organism. Primary sequence analysis with sequences of representatives of the family *Streptomycetaceae* confirmed that the unknown isolate was closely related to species of the genus *Streptomyces*. The highest 16S rRNA gene sequence similarity value was found with *Streptomyces nogalater* JCM 4799^T (97·5 %). However, in the phylogenetic tree based on the 120 nt γ-region, the strain

showed a very close affinity with *S. griseoaurantiacus*, supported by a 97 % bootstrap level, and was loosely related to other species (see Supplementary Fig. S2). The almost-complete 16S rRNA gene sequence (1458 nt) of *S. griseoaurantiacus* DSM 40430^T was therefore also determined in this study, and added to the 16S rRNA gene analysis. It is clear from Fig. 1 that strain FXJ46^T consistently formed a distinct phyletic line with *S. griseoaurantiacus* DSM 40430^T, suported by all four tree-making algorithms and by high bootstrap values. A 100 % bootstrap value with the neighbour-joining method was indicated. The divergence of the 16S rRNA gene sequences between the two strains was 0·2 % (3 nt differences at 1421 sites). The isolate also showed moderately low sequence divergence from other *Streptomyces* type strains, namely *S. nogalater* JCM 4799^T (2·2 %), *Streptomyces ambofaciens* ATCC 23877^T (2·2 %), *Streptomyces rutgersensis* subsp. *rutgersensis* DSM 40077^T (2·3 %), *Streptomyces intermedius* DSM 40372^T (2·3 %), *Streptomyces paradoxus* DSM 43350^T (2·3 %), *Streptomyces gougerotii* DSM 40324^T (2·4 %), *Streptomyces violaceolatus* DSM 40438^T (2·4 %), *Streptomyces collinus* DSM 40129^T (2·5 %) and *Streptomyces eurythermus* DSM 40014^T (2·6 %), respectively. Therefore, well-selected phenotypic traits and DNA–DNA pairing data are needed (Kim *et al.*, 1998; Labeda, 1988) to clarify the finer relationships between the isolate and the phylogenetically close species.

The DNA–DNA relatedness between strain FXJ46^T and *S. griseoaurantiacus* DSM 40430^T was 48·8 %, which is well below the 70 % cut-off point for recognition of genomic species (Wayne *et al.*, 1987), thus suggesting that the test strain should be considered as a separate species. Furthermore, the strain was distinguished from *S. griseoaurantiacus* DSM 40430^T by cultural characteristics on a number of standard media (Table 1), by spore-chain characters and by a set of physiological features (Table 2). The test strain can also be distinguished from all of the other phylogenetically close relatives using a combination of phenotypic properties (Table 2).

Based on the genotypic and phenotypic evidence, strain FXJ46^T warrants classification as the type strain of a novel species of the genus *Streptomyces*, for which the name *Streptomyces jietaisiensis* sp. nov. is proposed.

Description of *Streptomyces jietaisiensis* sp. nov.

Streptomyces jietaisiensis (jie.tai.si.en′sis. N.L. masc. adj. *jietaisiensis* pertaining to Jietaisi, a place in a suburb of Beijing, where the type strain was isolated).

The organism is aerobic, Gram-positive and mesophilic. The characteristics of aerial and substrate mycelium on various solid media is given in Table 1. Spore chains with 10 to 20 cylindrical spores are *rectiflexibiles*. The spore surface is smooth. Diffusible pigments are not produced, nor are melanin pigments formed on peptone-yeast extract-iron or tyrosine agars. Growth occurs between 10 and 40 °C, and between pH 5·0 and 10·0, but not at pH 4·0 or 11·0 or in the presence of streptomycin (10 μg ml^{-1}) or novobiocin (5 μg ml^{-1}). Adonitol, D-cellobiose, dextrin, D-galactose, D-glucose, inulin, glycogen, D-maltose, D-mannitol, D-mannose, D-melezitose, salicin, D-trehalose, D-xylose, (all at 1 %, w/v), L-alanine, L-arginine, L-aspartic acid, L-cysteine, L-glutamic acid, L-histidine, L-isoleucine,

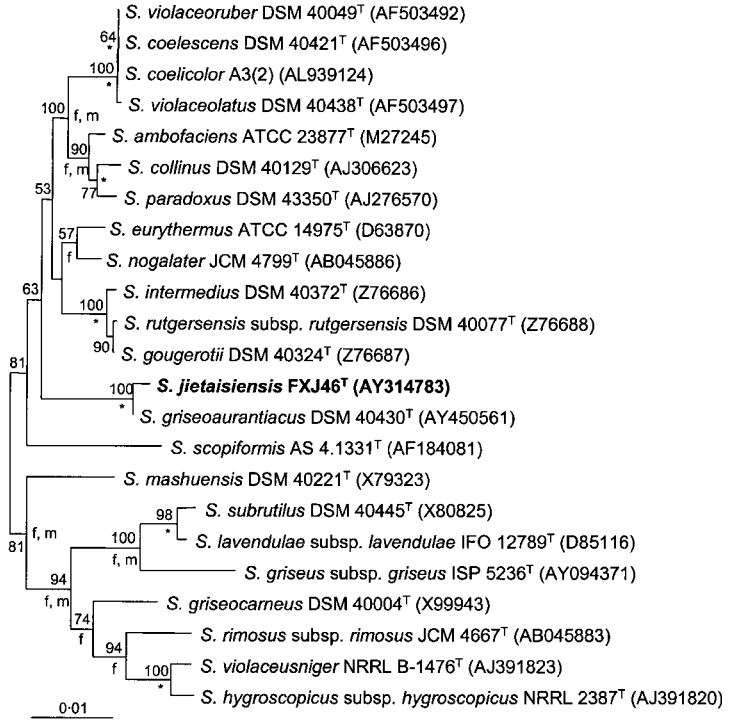

Fig. 1. Unrooted neighbour-joining tree (Saitou & Nei, 1987) based on almost-complete 16S rRNA gene sequences showing the phylogenetic relationships between *Streptomyces jietaisiensis* FXJ46^T and related *Streptomyces* species. Asterisks indicate branches that were recovered using least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) algorithms.; f and m respectively indicate branches that were also formed when the least-squares and maximum-likelihood tree-making algorithms were used. Bootstrap values (>50 %) based on 1000 replications are shown at nodes of the tree. Bar, 0·01 substitutions per nucleotide position.

Table 2. Phenotypic characteristics of strain FXJ46^T and related *Streptomyces* species

Strains: 1, strain FXJ46^T; 2, *S. ambofaciens* ATCC 23877^T; 3, *S. collinus* DSM 40129^T; 4, *S. eurythermus* DSM 40014^T; 5, *S. gougerotii* DSM 40324^T; 6, *S. griseoaurantiacus* DSM 40430^T; 7, *S. intermedius* DSM 40372^T; 8, *S. nogalater* JCM 4799^T; 9, *S. paradoxus* DSM 43350^T; 10, *S. rutgersensis* subsp. *rutgersensis* DSM 40077^T; 11, *S. violaceolatus* DSM 40438^T. All strains degrade Tween 60.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Aerial spore mass on oatmeal agar	Smoky grey	Light grey	Greyish white	Grey	Inadequate	Smoky grey	Pale greyish yellow or inadequate	Brownish grey	Greyish white	Greyish yellow	Brownish grey
Spore-chain morphology*	RF	SP	SP to RA	RA to SP	RF	SP	RF	RF to RA	RA	RF	SP
Spore surface	Smooth	Smooth to warty	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Melanin production	−	−	+	−	−	−	−	−	+	−	−
Production of diffusible pigments	−	Yellow or −	−	−	−	−	Yellow or −	Orange–yellow to red	−	−	Violet or red
Degradation of:											
Adenine	+	+	−	+	+	−	−	+	−	+	+
Arbutin	+	+	−	−	−	+	−	−	+	+	+
Elastin	−	−	−	+	+	−	+	+	+	+	−
Guanine	−	−	−	−	+	−	+	+	+	+	−
Hypoxanthine	−	+	+	+	+	−	+	+	+	+	+
Tween 20	+	−	+	+	+	+	+	+	−	+	+
Tween 40	+	+	+	+	+	+	+	+	−	+	+
Tween 80	+	−	+	+	−	+	−	−	−	+	+
Xanthine	−	+	−	+	+	−	−	+	+	+	−
Xylan	+	−	−	+	−	+	+	+	−	−	−
Growth on sole carbon sources (1·0 %, w/v):											
L-Arabinose	+	+	+	+	+	+	+	+	+	+	−
Methyl α-D-glucopyranoside	+	+	+	−	+	−	+	−	+	+	+
Inositol	+	+	+	−	+	+	−	+	+	+	−
D-Inulin	−	+	−	−	−	−	−	−	+	−	−
D-Lactose	+	+	−	+	+	+	−	+	+	+	−
D-Raffinose	+	+	+	+	−	+	−	+	+	−	−
L-Rhamnose	+	+	−	−	−	+	−	−	+	+	−
D-Ribose	+	+	+	+	+	+	+	+	+	+	−
D-Sorbitol	+	−	+	+	+	−	−	+	−	+	+
D-Sorbose	−	−	+	−	+	−	−	−	−	−	+
D-Sucrose	−	+	+	+	+	+	+	+	+	−	−
Sodium acetate (0·1 %)	+	+	−	+	−	+	+	+	−	+	−
Sodium citrate (0·1 %)	+	+	−	+	+	+	−	+	−	+	−
Sodium malonate (0·1 %)	−	+	−	+	+	+	+	+	+	+	−
Growth on:											
5 % NaCl	+	+	+	+	+	−	+	+	+	+	+
7 % NaCl	+	+	−	+	−	−	+	+	−	+	−
10 % NaCl	−	−	−	−	−	−	−	−	−	+	−
0·1 % Phenol	−	+	−	+	−	−	+	+	−	−	−
0·01 % NaN_3	+	−	−	+	+	+	+	−	+	+	−
Growth at 45 °C	−	+	+	+	−	−	−	+	+	−	−

*RA, Retinaculiaperti; RF, *rectiflexibiles*; SP, spiral.

L-phenylalanine, sodium oxalate, sodium pyruvate, L-threonine and L-valine (all at 0·1 %, w/v) are used as sole carbon sources for energy and growth, but not glycerol, glycine, xylitol (all at 1 %, w/v) or DL-aminobutyric acid (at 0·1 %, w/v). L-alanine, L-arginine, L-aspartic acid, L-glutamic acid and L-phenylalanine (all at 0·1 %, w/v) are metabolized as sole carbon and nitrogen sources, but not L-isoleucine (at 0·1 %, w/v). Cell wall type I, phospholipid type II and menaquinone MK-9 (H_6, H_8 and H_4). The fatty acid profile is composed of ai-$C_{15:0}$ (35·7 %), ai-$C_{17:0}$ (18·9 %), i-$C_{16:0}$ (14·8 %), ai-$C_{17:1}\omega9c$ (8·1 %), $C_{16:0}$ (6·2 %), i-$C_{16:1}$ (4·5 %), i-$C_{15:0}$ (4·3 %), $C_{16:1}\omega7c$ (2·33 %), i-$C_{17:1}\omega9c$ (1·8 %), i-$C_{17:0}$ (1·7 %) and i-$C_{14:0}$ (1·7 %). The G+C content of the DNA is 72·3 mol%. Additional characteristics are listed in Table 2.

The type strain is FXJ46^T (=AS 4.1859^T=JCM 12279^T), isolated from cypress forest soil collected at Jietaisi, Beijing, China.

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