Streptomyces glauciniger sp. nov., a novel mesophilic streptomycete isolated from soil in south China

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A polyphasic study was undertaken to establish the taxonomic status of a soil isolate. The organism, strain FXJ14^T, was found to have chemical and morphological properties characteristic of streptomycetes. Phylogenetic analyses based on an almost complete 16S rRNA gene sequence of the strain and on the 120 nt variable γ-region of the 16S rRNA molecule showed that it formed a distinct phyletic line within the range of variation encompassed by the genus *Streptomyces*. The sharp separation of the organism from representatives of the genus *Streptomyces* was strengthened by the fact that its BOX-PCR and RFLP of 16S rDNA-ITS fingerprints differed from those of over 450 recognized *Streptomyces* species. The isolate also had a profile of phenotypic properties that readily distinguished it from the genotypically close type strains. It is evident from the combination of genotypic and phenotypic data that strain FXJ14^T (=AS 4.1858^T=JCM 12278^T=LMG 22082^T) should be classified as the type strain of a novel species of the genus *Streptomyces*, for which the name *Streptomyces glauciniger* sp. nov. is proposed.

The genus *Streptomyces* remains a focus of systematic research, not only because streptomycetes are still the most promising source of commercially significant compounds, but also because current molecular biological methods are having an increasing impact on conventional streptomycete systematics that are based on phenotypic characteristics (Williams *et al.*, 1983; Kämpfer *et al.*, 1991). While molecular systematic data show that, based on recognized species, the genus is clearly overspeciated (Hatano *et al.*, 2003; Lanoot *et al.*, 2002, 2004), polyphasic studies based on a judicious combination of genotypic and phenotypic features continue to bring us novel species and indicate that the genus *Streptomyces* as a whole is underspeciated (Labeda *et al.*, 1997; S. B. Kim *et al.*, 1998; Al-Tai *et al.*, 1999; B. Kim *et al.*, 2000; Kim & Goodfellow, 2002; Li *et al.*, 2002; Saintpierre *et al.*, 2003). The present study describes a distinct mesophilic actinomycete, strain FXJ14^T, as a novel *Streptomyces* species based on a polyphasic approach.

Strain FXJ14^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 suspension and incubated at 28 °C for 2 weeks. The soil sample was collected from willow woods in Nanning City, Guangxi Province, China. The isolate was maintained on Bennett's agar (Jones, 1949) slopes at 4 °C and as glycerol suspensions (20 %, v/v) at −20 °C. Biomass for chemotaxonomic and molecular systematic studies was prepared as described by Li *et al.* (2002).

The arrangement of hyphae and spore chains were observed on modified Bennett's agar and oatmeal agar (ISP medium 3) after 14 days at 28 °C using the coverslip technique of Kawato & Shinobu (1959). Spore chain morphology and spore surface ornamentation were observed by examining gold-coated dehydrated specimens with a model FEI QUANTA electron microscope. Cultural characteristics were observed on a number of standard media (Table 1)

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *S. cuspidosporus* AS 4.1686^T and *S. glauciniger* FXJ14^T are AY589505 and AY314782, respectively.

A scanning electron micrograph, a neighbour-joining tree based on γ-region sequences and dendrograms generated from RFLP and BOX-PCR results are available as supplementary material in IJSEM Online.

Table 1. Growth and cultural characteristics of strain FXJ14^T

No soluble pigments were produced on the listed agars.

Agar medium	Growth	Aerial mycelium colour; abundance	Substrate mycelium colour
Modified Bennett's	Abundant	Dark greyish brown; abundant	Greenish black
Czapek Dox	Abundant	White/grey; moderately abundant	Yellowish white
Glycerol/asparagine (ISP 5)	Poor	Greyish brown; moderately abundant	Greyish olive
Inorganic salts/starch (ISP 4)	Moderate	Light greyish brown; moderately abundant	Yellowish white
Nutrient agar	Abundant	Greyish white; moderately abundant	Yellow
Oatmeal (ISP 3)	Abundant	Greyish brown; abundant	Not distinctive
Peptone/yeast extract/iron (ISP 6)	Abundant	Grey; sparse	Yellow
Tyrosine (ISP 7)	Abundant	Greyish cream; moderately abundant	Yellowish cream
Yeast extract/malt extract (ISP 2)	Abundant	Dark greyish brown; abundant	Dark brown

after 14 days at 28 °C. Strain FXJ14^T was examined for a range of physiological properties using established procedures described by Williams *et al.* (1983) and Kämpfer *et al.* (1991).

The isomers of diaminopimelic acid and whole-organism sugars were analysed according to the procedures developed by Hasegawa *et al.* (1983) and Lechevalier & Lechevalier (1980). Polar lipids were examined and identified using the method of Minnikin *et al.* (1984). Menaquinones were extracted and purified following Collins (1985) and then analysed by HPLC (Wu *et al.*, 1989). Fatty acids were extracted, methylated and analysed by GC using the standard Sherlock MIDI (Microbial Identification) system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). DNA G+C content of the tested strain was determined using the thermal denaturation method of Marmur & Doty (1962), with *Escherichia coli* AS 1.365 as a control.

Genomic DNA preparation and PCR amplification of the 16S rRNA gene sequence of strain FXJ14^T were performed as described by Chun & Goodfellow (1995). The PCR product was purified and directly sequenced as described by Huang *et al.* (2001). The resultant sequence was aligned manually using CLUSTAL X version 1.8 (Thompson *et al.*, 1997) 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 final dataset consisted of information on 23 strains. Phylogenetic trees were inferred by using 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). Evolutionary distance matrices were generated following the method of Kimura (1980). The resultant unrooted tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings. A partial sequence covering the variable γ-region (120 nt, positions 158–277) of the 16S rRNA gene sequence of strain FXJ14^T was also aligned with the corresponding nucleotide sequences of nearly 500 *Streptomyces* type strains retrieved from GenBank. A phylogenetic tree based on these partial sequences was generated using the neighbour-joining algorithm (Saitou & Nei, 1987).

BOX-PCR fingerprinting was carried out following the method of Lanoot *et al.* (2004). For RFLP of 16S rDNA-ITS (internally transcribed spacer), a universal primer set (PA, 5′-AGAGTTTGATCCTGGCTCAG-3′; BL235R, 5′-GCGCCCTTAAAAACTTGG-3′) was used to amplify both the 16S rRNA gene and the adjacent 16S–23S rDNA ITS region in one PCR. Digested PCR products, using restriction enzymes *Bst*UI and *Hae*III, were separated on 8 % polyacrylamide gels. The fingerprinting patterns were compared with corresponding in-house databases containing more than 450 recognized *Streptomyces* species using the software package BIONUMERICS version 2.5 (Applied Maths).

Morphological and chemical features of strain FXJ14^T were consistent with its assignment to the genus *Streptomyces* (Williams *et al.*, 1989; Manfio *et al.*, 1995). The organism formed an extensively branched substrate mycelium, aerial hyphae that carried smooth-surfaced spores in spiral spore chains (see Supplementary Fig. A in IJSEM Online) and a greyish aerial spore mass on several standard media (Table 1). It contained major amounts of LL-diaminopimelic acid in whole-organism hydrolysates, hexa- and octahydrogenated menaquinones with nine isoprene units [MK-9(H_6, H_8)] as predominant isoprenologues and diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides as typical polar lipids (phospholipid type II *sensu* Lechevalier *et al.*, 1977), but lacked characteristic sugars and mycolic acids. The fatty acid profile comprised mainly saturated straight-chain and iso- and anteiso-branched-chain fatty acids (fatty acid type 2c *sensu* Kroppenstedt, 1985).

The assignment of strain FXJ14^T to the genus *Streptomyces* was also supported by 16S rRNA gene sequence data. An almost complete 16S rRNA gene sequence (1428 nt) was determined for the organism. Preliminary phylogenetic

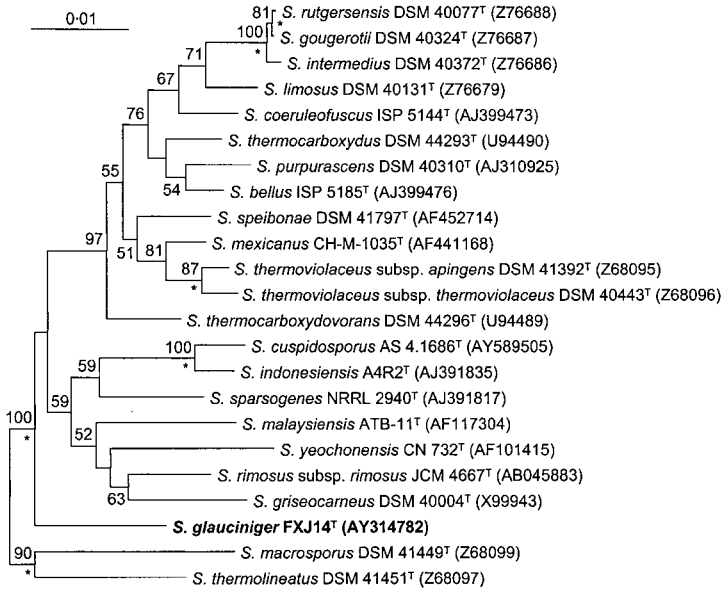

Fig. 1. Unrooted neighbour-joining tree (Saitou & Nei, 1987) based on almost complete 16S rRNA gene sequences showing the position of strain FXJ14^T in the *Streptomyces* tree. Asterisks indicate branches that were recovered using least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) algorithms. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50 % are given. Bar, 0·01 substitutions per nucleotide position.

analysis, which included available, almost full-length sequences of type strains of the family *Streptomycetaceae*, showed that strain FXJ14^T fell within the evolutionary radiation encompassed by the genus *Streptomyces* (data not shown). Despite the fact that the organism was found to be closely associated with some members of the genera *Kitasatospora* and *Streptacidiphilus* in a comparison of 16S rRNA gene sequences using a standard nucleotide–nucleotide BLAST search (Altschul *et al.*, 1997) against DDBJ/EMBL/GenBank, specific nucleotide signatures of these two genera (Zhang *et al.*, 1997; Kim *et al.*, 2003) were absent in strain FXJ14^T. It is clear from Fig. 1 that the tested strain forms a distinct phyletic line in the 16S rRNA gene sequence *Streptomyces* tree. Sequence similarity values between strain FXJ14^T and its nearest neighbours, namely *Streptomyces rimosus* subsp. *rimosus* JCM 4667^T, *Streptomyces malaysiensis* ATB-11^T, *Streptomyces sparsogenes* NRRL 2940^T and *Streptomyces yeochonensis* CN 732^T, were 97·3 (39 nt differences at 1428 sites), 97·2 (40 nt differences at 1415 sites), 97·2 (40 nt differences at 1425 sites) and 97·2 % (40 nt differences at 1425 sites), respectively. Values in this range are well below the range recorded for many recognized *Streptomyces* species (S. B. Kim *et al.*, 1998; Al-Tai *et al.*, 1999; B. Kim *et al.*, 2000; Sembiring *et al.*, 2000; Kim & Goodfellow, 2002; Li *et al.*, 2002; Saintpierre *et al.*, 2003), nor are the relationships between the tested strain and the type strains supported by good bootstrap levels. A number of phenotypic properties also separated strain FXJ14^T from its most closely related type strains (Table 2).

Phylogenetic analysis based on the 120 nt γ-region of the 16S rRNA gene sequence showed that the organism was loosely clustered with the type strains of *Streptomyces indigoferus* and *Streptomyces herbaricolor* (Supplementary Fig. B). However, the latter two strains can be distinguished readily from strain FXJ14^T using phenotypic properties (Table 2). Although RFLP of 16S rDNA ITS fingerprints revealed that strain FXJ14^T was most closely associated with the type strain of *Streptomyces cuspidosporus* (Supplementary Fig. C), the two organisms were distinguished from one another on the basis of almost complete 16S rRNA gene sequence analysis (Fig. 1), sharing a relatively low sequence similarity of 96·9 %. With respect to BOX-PCR fingerprints, strain FXJ14^T had a unique pattern when compared with corresponding data on 473 *Streptomyces* type strains (Supplementary Fig. D shows a partial dendrogram, including the neighbouring type strains), thereby confirming its distinct position in the genus *Streptomyces*.

Based on a combination of genotypic and phenotypic data, strain FXJ14^T merits recognition as the type strain of a novel species in the genus *Streptomyces*, for which we propose the name *Streptomyces glauciniger* sp. nov.

Description of *Streptomyces glauciniger* sp. nov.

Streptomyces glauciniger (glau′ci.ni.ger. L. adj. *glaucus* greenish grey; L. adj. *niger* black; N.L. masc. adj. *glauciniger* greenish black, referring to the colour of colony reverse on modified Bennett's agar).

Aerobic, Gram-positive mesophilic actinomycete that forms an extensively branched substrate mycelium and aerial hyphae that differentiate into long spiral spore chains with 15–20 cylindrical spores per chain. Spore surface is smooth. Soluble pigments are not produced, nor are melanin pigments formed on peptone/yeast extract/iron or tyrosine agars. Additional cultural characteristics on various agar media are given in Table 1. Growth occurs at 10–35 °C and pH 5·0–10·0, but not at 40 °C or at pH 4·0 or 11·0. Growth also occurs in the presence of phenol (0·1 %, w/v) but not in the presence of NaCl (5 %, w/v), novobiocin (5 μg ml^{-1}) or streptomycin (10 μg ml^{-1}). In addition to the properties listed in Table 2, the organism

Table 2. Phenotypic properties that separate strain FXJ14^T from related *Streptomyces* species

Strains: 1, strain FXJ14^T; 2, *S. cuspidosporus* IFO 12378^T (=AS 4.1686^T); 3, *S. herbaricolor* ISP 5123^T (=ATCC 23922^T=JCM 4138^T); 4, *S. indigoferus* ISP 5124^T (=ATCC 23924^T=JCM 4646^T); 5, *S. malaysiensis* ATB-11^T (=DSM 41697^T); 6, *S. rimosus* subsp. *rimosus* ISP 5260^T (=JCM 4667^T=NRRL 2234^T); 7, *S. sparsogenes* ISP 5356^T (=DSM 40356^T=NRRL 2940^T); 8, *S. yeochonensis* CN 732^T (=KCTC 9926^T=NRRL B-24245^T). Data for reference strains are taken from previous studies (Shirling & Gottlieb, 1968, 1969; Williams *et al.*, 1983; Lonsdale, 1985; Al-Tai *et al.*, 1999; Kim *et al.*, 2004). +, Positive; −, negative; d, doubtful.

Character	1	2	3	4	5	6	7	8
Aerial spore mass on oatmeal agar	Greyish brown	Grey	Grey; sparse	Grey; sparse	Smokey black	Yellow/ white	Grey	Grey
Spore chain	Spiral	Spiral	*Rectiflexibiles*	*Rectiflexibiles*	Spiral	Spiral	Spiral	*Rectiflexibiles*
Spore surface	Smooth	Warty	Smooth	Smooth	Rugose	Smooth	Spiny	Smooth
Melanin production	−	−	−	+	+	−	−	−
Production of diffusible pigments	−	+	−	−	+	+	−	−
Growth on sole carbon sources (1 %, w/v):								
L-Arabinose	+	+	+	+	+	+	+	−
D-Fructose	+	+	+	−	+	+	+	−
meso-Inositol	+	+	−	−	+	+	d	−
D-Mannitol	+	+	−	−	+	+	+	−
D-Raffinose	+	+	+	−	+	+	+	−
L-Rhamnose	+	+	−	−	+	−	+	−
D-Sucrose	+	+	+	−	−	−	+	−
D-Xylose	+	+	+	d	+	d	+	−

degrades adenine, casein, hypoxanthine, starch and xanthine, but not cellulose or elastin. It uses dextrin, D-galactose, D-glucose (all at 1 %, w/v), sodium acetate and sodium citrate (both at 0·1 %, w/v), but not D-maltose (1 %, w/v), as sole carbon sources for energy and growth. Nitrate is reduced. Gelatin is not liquefied. It shows antimicrobial activity against strains of *Bacillus subtilis* and *Candida albicans*, but not against strains of *Aspergillus niger*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* or *Staphylococcus aureus*. Cell wall is of type I, phospholipid type II and menaquinone MK-9(H_6, H_8). The fatty acid profile is composed of iso-$C_{16:0}$ (25·4 %), anteiso-$C_{17:0}$ (17·4 %), anteiso-$C_{15:0}$ (16·7 %), iso-$C_{15:0}$ (9·5 %), $C_{16:0}$ (7·7 %), iso-$C_{17:0}$ (3·8 %), iso-$C_{14:0}$ (3·5 %), iso-$C_{17:1}\omega 9c$ (3·3 %), anteiso-$C_{17:1}\omega 9c$ (3·0 %), iso-$C_{16:1}$ (2·9 %), $C_{15:0}$ (2·8 %) and $C_{17:0}$ cyclo (2·7 %). DNA G+C content is 67·0 mol%.

The type strain, FXJ14^T (=AS 4.1858^T=JCM 12278^T=LMG 22082^T), was isolated from soil in a willow wood collected in Nanning City, Guangxi Province, China.

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