Streptomyces scabrisporus sp. nov.

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The taxonomic position was determined for a soil actinomycete, isolate KM-4927^T, that produced the antibiotic hitachimycin. The strain was assigned to the genus *Streptomyces* on the basis of 16S rDNA analysis, where it formed a separate clade. The strain is characterized by grey aerial cell mass, spiral spore chains and a rugose spore surface, menaquinones of the MK-9(H₄, H₂, H₆) types and cell-wall chemotype I. DNA–DNA reassociation with 21 phylogenetically neighbouring *Streptomyces* type strains showing similar morphological characteristics to strain KM-4927^T indicated that this isolate is only moderately related to other *Streptomyces* species. On the basis of genomic and physiological properties, the novel species *Streptomyces scabrisporus* sp. nov. is proposed; the type strain is strain KM-4927^T (=JCM 11712^T=NRRL B-24202^T).

The genus *Streptomyces* was proposed by Waksman & Henrici (1943) for aerobic and spore-forming actinomycetes. The genus is made up of aerobic, Gram-positive bacteria with a DNA with a high G+C content (69–73 mol%), containing LL-diaminopimelic acid and lacking diagnostic sugars in the cell wall [wall chemotype I according to Lechevalier & Lechevalier (1970) and Williams *et al.* (1989)].

In the course of our screening programme for new antibiotics, a novel actinomycete, strain KM-4927^T, which produced the antibiotic hitachimycin, was isolated from a soil sample collected at Ushiku-cho, Ibaragi Prefecture, Japan (Ōmura *et al.*, 1982). As strain KM-4927^T did not form aerial mycelia on media provided for growth, its taxonomic affiliation could not be determined (Oiwa *et al.*, 1982). Here, we report the identification of strain KM-4927^T by conventional taxonomic procedures and phylogenetic methods.

Media used were those recommended by Shirling & Gottlieb (1966) in the International *Streptomyces* Project (ISP) and by Waksman (1961). Mycelium was observed

after incubation at 27 °C for 2 weeks. Colours and hues were determined according to Taylor *et al.* (1958). The morphology of strain KM-4927^T was observed by light microscopy and scanning electron microscopy (SEM) (JSM 5600; JEOL). Samples used for SEM had been cultured for 14 days on agar medium and then fixed overnight with osmium tetroxide vapour, freeze-dried and sputter-coated with gold palladium. Carbohydrate utilization was determined by growth on carbon utilization medium (ISP 9) (Pridham & Gottlieb, 1948) supplemented with 1 % carbon sources at 27 °C. The temperature range for growth was determined on inorganic salts/starch agar (ISP 4) using a temperature gradient incubator (Tokyo Kagaku Sangyo). Hydrolysis of starch and milk was evaluated by using the media of Gordon *et al.* (1974). Reduction of nitrate and production of melanoid pigment were determined by the method of the ISP (Shirling & Gottlieb, 1966). Liquefaction of gelatin was evaluated by using the method of Waksman (1961). All cultural characteristics were recorded after 14 days.

Cells used for chemotaxonomic analysis were obtained after incubation at 27 °C for 3 days in yeast extract/glucose broth (pH 7.0) containing 10 g yeast extract and 10 g glucose l⁻¹. Isomers of diaminopimelic acid in the whole-cell hydrolysate were determined by TLC according to the method of Becker *et al.* (1965) and Hasegawa *et al.* (1983). Whole-cell sugars were analysed according to the method of Becker *et al.* (1965). Mycolic acids were detected by TLC according to the method of Tomiyasu (1982). The acyl types of muramic acid were determined by the method of Uchida & Aida (1984). Cell phospholipids were extracted

Published online ahead of print on 31 October 2003 as DOI 10.1099/ijs.0.02692-0.

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Abbreviation: ISP, International *Streptomyces* Project.

The DDBJ accession number for the partial 16S rDNA sequence of strain KM-4927^T is AB030585.

Fig. 1. Scanning electron micrograph of spore chains of strain KM-4927^T grown on oatmeal agar for 14 days at 27 °C. Bar, 1 µm.

and identified by the method of Minnikin *et al.* (1977). Menaquinones were extracted and purified by the method of Collins *et al.* (1977), and isoprene units were analysed by HPLC using a CAPCELL PAK C18 column (Shiseido) (Tamaoka *et al.*, 1983). Chromosomal DNA was prepared following the method of Marmur (1961). The G + C content of DNA was determined by HPLC according to the method of Tamaoka & Komagata (1984). The methods used for PCR amplification of the 16S rRNA gene and gene sequencing were described previously (Tajima *et al.*, 2001). The resulting 16S rDNA of strain KM-4927^T was aligned manually against representative sequences of *Streptomyces* obtained from the DDBJ databases. Reference strains were chosen from BLAST (Altschul *et al.*, 1997) search results. CLUSTAL W (Thompson *et al.*, 1994) was then used to

generate evolutionary distances (the K_{nuc} value of Kimura, 1980) and similarity values and to reconstruct the phylogenetic tree by the neighbour-joining method (Saitou & Nei, 1987). In order to generate a phylogenetic tree by the maximum-likelihood method, PAUP* version 4.0 beta 8 (Swofford, 2001) was used. The topology of the tree was evaluated by performing a bootstrap analysis (Felsenstein, 1985) using 1000 resamplings. The phylogenetic tree was drawn using the TREEVIEW software. The tree was rooted with *Arthrobacter globiformis*. DNA relatedness was determined fluorometrically by the method of Ezaki *et al.* (1989). Fluorescence intensity in the cells was measured with a Cytofluor Multi-WELL plate reader series 4000 (Perseptive Biosystems) microplate reader.

Substrate mycelium of strain KM-4927^T developed well and branched irregularly on both natural and synthetic media; fragmentation of the mycelium did not occur. Fig. 1 shows a scanning electron micrograph of aerial spores of strain KM-4927^T. The multi-spore chains were spiral; the spores were cylindrical, 0.6–0.8 µm in diameter and 1.2–1.7 µm in length and non-motile. The spore surface was rugose. Sporangia or zoospores and sclerotia were not observed. Soluble pigment was not produced on any of the media tested; melanin was not produced. Growth was moderate or good and the substrate mycelium was colourless or light ivory (Table 1). Grey aerial mycelium was observed on some nutrition-poor agar media such as water agar, oatmeal agar and 1/10 V8 juice agar. Physiological characteristics and utilization of carbon sources of strain KM-4927^T are shown in Table 2. The temperature range for growth was 18–36 °C.

While LL-diaminopimelic acid was detected in whole-cell hydrolysates of strain KM-4927^T, galactose was lacking and

Table 1. Cultural characteristics of strain KM-4927^T

Colour names and codes (in parentheses) are taken from Taylor *et al.* (1958). Strain KM-4927^T did not produce soluble pigments on any of the media listed.

Medium	Growth	Aerial mycelium
Yeast extract/malt extract agar (ISP 2)	Good, wrinkled, light ivory (2ca)	—
Oatmeal agar (ISP 3)	Moderate, penetrating, colourless	+, Ivy (24ml)
Inorganic salts/starch agar (ISP 4)	Moderate, penetrating, pearl (2ba)	—
Glycerol/asparagine agar (ISP 5)	Good, penetrating, pearl (2ba)	—
Peptone/yeast extract/iron agar (ISP 6)	Good, wrinkled, pearl (2ba)	—
Tyrosine agar (ISP 7)	Moderate, raised, bamboo (2gc)	—
Glucose/asparagine agar	Good, raised, pearl (2ba)	—
Sucrose/nitrate agar	Good, penetrating, pearl (2ba)	—
Glucose/nitrate agar	Moderate, raised, light ivory (2ca)	—
Glycerol/calcium malate agar	Moderate, penetrating, colourless	—
Glucose/peptone agar	Good, raised, cream (1ca)	—
Nutrient agar	Good, raised, pearl (2ba)	—
Water agar	Moderate, penetrating, cream (1ca)	+, Mistletoe grey (24 1/2 ih)
1/10 V8 juice agar	Moderate, penetrating, light ivory (2ca)	+, Silver grey (3fe)

Table 2. Phenotypic properties of strain KM-4927^T and its phylogenetic neighbours, *S. sparsogenes* and *S. turgidiscabies*

Data for reference species were taken from Shirling & Gottlieb (1969) and Miyajima *et al.* (1998). +, Positive, utilized; –, negative, not utilized; ±, ambiguous; ND, not determined. All taxa are negative for melanin production.

Characteristic	KM-4927 ^T	<i>S. sparsogenes</i> ISP 5356 ^T	<i>S. turgidiscabies</i> IFO 10680 ^T
Spore chain	Spiral	Spiral	Flexuous
Spore surface	Rugose	Spiny	Smooth
Aerial spore-mass colour	Grey	Yellowish grey	Grey
Reduction of nitrate	+	–	+
Hydrolysis of starch	+	±	+
Liquefaction of gelatin	–	+	ND
Coagulation of milk	+	+	–
Utilization of:			
Inositol	+	–	+
Sucrose	±	+	+
L-Arabinose	±	+	+
Salicin	±	–	+
D-Mannitol	–	+	+
Raffinose	–	+	+
Melibiose	–	ND	+

arabinose was detected in traces only [chemotype I, according to Lechevalier & Lechevalier (1970)]. Phosphatidylethanolamine and an unidentified phospholipid containing glucosamine were the major phospholipids, while phosphatidylcholine and phosphatidylglycerol were absent [phospholipid type II, according to Lechevalier & Lechevalier (1970)]. MK-9(H₄), MK-9(H₂) and MK-9(H₆) were the predominant menaquinones, in the ratio 12:4:3. The *N*-acyl type of muramic acid in peptidoglycan was acetyl. No mycolic acid was detected. The G+C content of the DNA was 70.6 mol%. Strain KM-4927^T exhibited morphological and chemotaxonomic characteristics typical of the genus *Streptomyces*.

The almost complete 16S rDNA sequence (1479 nt) was compared with those of *Streptomyces* species deposited in public databases. Positions with any gaps and alignment uncertainty were omitted from the analysis. A total of 1422 unambiguous nucleotides was used for computing evolutionary distance. The rooted phylogenetic tree (Fig. 2) indicated that strain KM-4927^T formed a distinct branch with the type strains of two other *Streptomyces* species, *Streptomyces sparsogenes* and *Streptomyces turgidiscabies*. The sequence similarity of strain KM-4927^T was 95.0% to *S. sparsogenes* NRRL-2940^T and 94.0% to *S. turgidiscabies* ATCC 700248^T. The tree constructed by the maximum-likelihood method supported this result.

A number of phenotypic features can also be used to differentiate strain KM-4927^T from *S. sparsogenes* and *S. turgidiscabies* (Table 2), not least the ability of the former to form spiral spore chains and rugose-surfaced spores (Fig. 1), as opposed to spiny spores (*S. sparsogenes*) or

Fig. 2. Neighbour-joining dendrogram based on 1422 bp of 16S rRNA gene sequences. The tree was validated by a bootstrap analysis (1000 replications); values greater than 500 are indicated at nodes. Bar, 0.01 substitutions per nucleotide position.

flexuous morphology and smooth spores (*S. turgidiscabies*). The three strains can also be distinguished by their physiological properties.

Kataoka *et al.* (1997) reported that 120 bp of the 16S rRNA (rDNA) containing a variable region could be used for identification of streptomycete species. Phylogenetic analysis of KM-4927^T and 358 ISP strains with 120 bp 16S rRNA sequences was carried out. The result (not shown) confirmed the phylogenetic distinctness of the isolate, which appears closely related to the type strains of *Streptomyces chrestomyceticus*, *Streptomyces clavuligerus* and *Streptomyces misakiensis*.

Taxonomic characterization of strain KM-4927^T with ISP strains according to the classification key of Nonomura (1974) and the ISP description by Shirling & Gottlieb (1968a, b, 1969, 1972) revealed the isolate to be closely related to 18 *Streptomyces* species (*Streptomyces albobacillus*, *Streptomyces albus*, *Streptomyces almquisti*, *Streptomyces antimycopicus*, *Streptomyces cacaoi*, *Streptomyces graminofaciens*, *Streptomyces griseoplanus*, *Streptomyces humidus*, *Streptomyces hygroscopicus*, *Streptomyces lydicus*, *Streptomyces naraensis*, *Streptomyces nigellus*, *Streptomyces nigrescens*, *Streptomyces parvulus*, *Streptomyces rangoonensis*, *Streptomyces saraceticus*, *Streptomyces sclerotialis* and *Streptomyces sioyaensis*) on the basis of formation of spiral spore chains, smooth, warty or rugose surface of the spores, formation of a grey or white aerial mass and lack of melanin production. DNA reassociation was performed with labelled DNA from strain KM-4927^T and DNA from 21 reference strains including *S. chrestomyceticus*, *S. clavuligerus* and *S. misakiensis*. The results indicated relatedness values lower than 70 %. The highest value was 54 %, for *S. chrestomyceticus*.

Strain KM-4927^T is clearly differentiated from all other species of genus *Streptomyces* with validly published names based on a combination of phenotypic characteristics and genotypic data. We conclude that KM-4927^T represents a novel species of *Streptomyces*, for which the name *Streptomyces scabrisporus* sp. nov. is proposed.

Description of *Streptomyces scabrisporus* sp. nov.

Streptomyces scabrisporus (scab.ri.spor'us. L. adj. *scaber* -*brum* scabby, rough; N.L. n. *spora* spore; N.L. masc. adj. *scabrisporus* referring to the rugose surface of the spores).

Substrate and aerial mycelia are produced. The reverse sides of colonies are colourless to light ivory. The aerial mass on some agar media, such as water agar, oatmeal agar and 1/10 V8 juice agar, is grey. Mature spore chains are spiral, with more than 20 spores per chain. Spores are cylindrical in shape, 0.6–0.8 × 1.2–1.6 µm in diameter; the spore surface is rugose. Mycelia do not fragment into coccoid or bacillary elements. Melanoid or soluble pigments are not produced on any medium tested. Starch is weakly hydrolysed, gelatin is not liquefied and milk is weakly coagulated. D-Glucose,

D-fructose, D-xylose, *myo*-inositol and rhamnose are utilized for growth, but D-mannitol, raffinose and melibiose are not; little if any growth is observed with sucrose, L-arabinose or salicin. Menaquinone composition is MK-9(H₄), MK-9(H₂), MK-9(H₆) in the ratio 12:4:3. The G + C content of the DNA of the type strain is 70.6 mol%.

The type strain, strain KM-4927^T (=JCM 11712^T=NRRL B-24202^T), was isolated from a soil sample collected from Ushiku-cho, Ibaraki Prefecture, Japan.

Acknowledgements

We would like to thank Professor Cheng-Lin Jiang (Yunnan University), Mayumi Satoh (The Kitasato Institute) and Atsuko Matsumoto (Kitasato University) for their help. We also thank Professor Erko Stackebrandt (DSMZ) for revising our English text.

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