Streptomyces scabrisporus sp. nov.

Xu Ping,† Yoko Takahashi,1,2 Akio Seino,1 Yuzuru Iwai1 and Satoshi Ōmura1,2

1Research Center for Biological Function, The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan
2Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

The taxonomic position was determined for a soil actinomycete, isolate KM-4927T, 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-4927T 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-4927T (= JCM 11712T = NRRL B-24202T).

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-4927T, 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-4927T did not form aerial mycelia on media provided for growth, its taxonomic affiliation could not be determined (Oiwa et al., 1989). Here, we report the identification of strain KM-4927T 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-4927T 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 I−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.
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$_{\text{nuc}}$ 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 Cytofluorr 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-sporo 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

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

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.
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-acetyl 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-4927T 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-4927T formed a distinct branch with the type strains of two other *Streptomyces* species, *Streptomyces sparsogenes* and *Streptomyces turgidiscabies*. The sequence similarity of strain KM-4927T was 95.0 % to *S. sparsogenes* NRRL-2940T and 94.0 % to *S. turgidiscabies* ATCC 700248T. The tree constructed by the maximum-likelihood method supported this result.

A number of phenotypic features can also be used to differentiate strain KM-4927T 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.](http://ijs.sgmjournals.org)
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 albificiens, Streptomyces albus, Streptomyces alvusenti, Streptomyces antimycinicus, Streptomyces cacaoi, Streptomyces graminifaciens, Streptomyces griseoflavus, Streptomyces humidus, Streptomyces hygrosopicus, Streptomyces lydicus, Streptomyces naraensis, Streptomyces nigellus, Streptomyces nigrescens, Streptomyces parvulus, Streptomyces rangoonensis, Streptomyces saraceticus, Streptomyces sclerotialus and Streptomyces sioyensis) 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 -bra -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 x 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-xylene, 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(H\(_4\)), MK-9(H\(_6\)), MK-9(H\(_2\)) 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.

**References**


