Streptomyces scopiformis sp. nov., a novel streptomycete with fastigiate spore chains

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A distinct actinomycete strain was isolated from rhizosphere soil of Tsuga chinensis. The isolate, designated A25T, was assigned to the genus Streptomyces on the basis of morphological and chemotaxonomic criteria and was examined by using a polyphasic approach. An almost complete 16S rDNA sequence of the isolate was determined and compared with sequences of representative streptomycetes. The 16S rDNA data not only supported classification of the strain in the genus Streptomyces, but also showed that it formed a separate phyletic line. DNA–DNA hybridization between strain A25T and closely related reference strains confirmed that strain A25T is a novel taxon of Streptomyces. It is proposed, therefore, the strain A25T (＝ AS 4.1331T = LMG 20251T) is classified in the genus Streptomyces as Streptomyces scopiformis sp. nov.

Keywords: Streptomyces scopiformis sp. nov., polyphasic taxonomy, 16S rDNA sequence, DNA–DNA hybridization

INTRODUCTION

The genus Streptomyces was proposed by Waksman & Henrici (1943) for aerobic, spore-forming actinomycetes and is classified in the family Streptomyceseae on the basis of morphological and cell-wall chemotaxonomic characters. The taxon currently accommodates aerobic, Gram-positive bacteria that have high DNA G+C % content (69–78 mol%), present L,L-diaminopimelic acid and the absence of characteristic sugars in the cell wall (cell-wall type I, according to Lechevalier & Lechevalier, 1970) and produce extensively branched substrate mycelium and aerial hyphae (Williams et al., 1983, 1989; Embley & Stackebrandt, 1994). With more than 500 validly described species and subspecies, the taxon currently contains the largest number of species in the domain Bacteria (Hain et al., 1997).

Molecular-systematic methods, notably 16S rDNA phylogenetic analysis, are having an increasing impact on Streptomyces systematics (Kim et al., 1998). Of the large number of species, complete 16S rDNA sequences have not been determined for many type strains. Incomplete sequence data may result in misclassification. It has been reported that the γ variable region of 16S rDNA could be used to resolve inter- and intraspecies relationships within the streptomycetes (Kataoka et al., 1997; Anderson & Wellington, 2001). Hence, partial sequences covering the γ region (positions 158–276) of 485 streptomycetes have been sequenced and deposited in GenBank by K. Ueda, T. Kudo, T. Seki, T. Yoshida and M. Kataoka. Phylogenetic analysis including these sequences can provide useful information for classification and reduce the risk of incorrect identification.

Isolate A25T was reported previously as a novel taxon, ‘Streptomyces scopiformis’, that produced distinct broom-like spore chains (Liu & Zhang, 1996), but the name was not validated. In the present study, strain A25T was subjected to a polyphasic taxonomic analysis, an almost complete 16S rRNA gene (rDNA) sequence was obtained and its phylogenetic relationships were examined using different tree-making methods. DNA–DNA hybridization of isolate A25T and its closest neighbours was studied in order to reveal whether the isolate represents a novel taxon within the genus Streptomyces.
METHODS

Organisms and cultural conditions. Strain A25T was isolated on a glycerol/asparagine agar plate (glucose, 10 g; t-asparagine, 0.5 g; agar, 20 g; distilled water 1000 ml; pH 7.2–7.4) seeded with a soil sample suspension and incubated for 14 days at 28 °C. The soil sample was collected from the rhizosphere of Chinese hemlock (Tsuga chinensis) in Lushan Mountain, Jiangxi province, China. The novel isolate and reference strains were maintained on slopes of Bennett’s agar (Williams et al., 1983) at 4 °C and as glycerol suspensions (20 %, v/v) at −20 °C. Biomass for chemotaxonomic and molecular studies was prepared by growing organisms in 250 ml flasks containing 80 ml Bennett’s medium (prepared in 0.01 M phosphate buffer, pH 7.0) with shaking for 4 days at 28 °C. The flasks were inoculated by transferring a 4-day-old culture from Bennett’s agar slopes (prepared in 0.01 M phosphate buffer, pH 7.0) with 2 ml 0.1 M phosphate buffer (pH 7.0). Cells for chemical studies were washed in distilled water and freeze-dried; those for molecular systematic investigations were washed twice in distilled water and Tris/EDTA (0.03 M Tris/HCl, 0.1 M EDTA, pH 8.0) and stored at −20 °C until required.

Cultural and morphological properties. The morphology of aerial hyphae, substrate mycelium and spore chains was examined by light and scanning electron microscopy of a 14 day culture grown on Bennett’s agar and inorganic salts/starch agar (ISP medium 4). The cover-slip technique (Zhou et al., 1998; Kawato & Shinobu, 1959) was used to observe hyphae and spore chains by light microscopy. Spore morphology was studied by examining gold-coated dehydrated specimens with a model HITACHI-570 electron microscope. The cultural characteristics were observed on a number of media for 14 days at 28 °C.

Biochemical and physiological properties. The test strain were examined for a broad range of biochemical and physiological characteristics as described by Kämpfer et al. (1991), Williams et al. (1983) and Al-Tai et al. (1999).

Chemotaxonomy. The diagnostic isomers of dianimonipelic acid (DAP) and whole-organism sugars of the test strain were examined for a broad range of biochemical and molecular studies was prepared by growing organisms in 250 ml flasks containing 80 ml Bennett’s medium (prepared in 0.01 M phosphate buffer, pH 7.0) with shaking for 4 days at 28 °C. The flasks were inoculated by transferring a 4-day-old culture from Bennett’s agar slopes (prepared in 0.01 M phosphate buffer, pH 7.0) with 2 ml 0.1 M phosphate buffer (pH 7.0). Cells for chemical studies were washed in distilled water and freeze-dried; those for molecular systematic investigations were washed twice in distilled water and Tris/EDTA (0.03 M Tris/HCl, 0.1 M EDTA, pH 8.0) and stored at −20 °C until required.

DNA G + C content. The G + C content of DNA of the test strain was determined using the thermal denaturation method of Marmur & Doty (1962) with Escherichia coli AS1.365 as a control.

16S rDNA sequencing. Genomic DNA of test strains was isolated using the procedure of Chun & Goodfellow (1995). PCR amplification of 16S rDNA was carried out as described previously (Kim et al., 1996). The PCR products were sequenced using the method of Lu et al. (2001) on an Applied Biosystems DNA Sequencer (model 373A) and the software provided by the manufacturer.

Phylogenetic analysis. The almost complete 16S rDNA sequence of strain A25T was aligned manually with available streptomycete nucleotide sequences retrieved from EMBL/GenBank and the RDP (Maidak et al., 1997) using CLUSTAL x version 1.8 (Thompson et al., 1997). Phylogenetic trees were inferred by using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), Fitch–Margoliash (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) algorithms from the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices were generated as described by Kimura (1980). Tree topologies were evaluated by carrying out bootstrap analysis based on 1000 resamplings of the neighbour-joining dataset using the programs SEQBOOT and CONSENSE provided in the PHYLIP package (Felsenstein, 1993).

The partial sequence covering the γ region (120 bp) from the 16S rDNA sequence of strain A25T was compared with all sequences available in GenBank. The partial sequence was also aligned with partial γ-region sequences of 485 streptomycetes and used for construction of a neighbour-joining tree.

DNA–DNA relatedness studies. Levels of DNA–DNA relatedness between strain A25T and the type strains of Streptomyces ambifaciens, Streptomyces coeruleus, Streptomyces caelestis, Streptomyces nogaler, Streptomyces intermedius, Streptomyces albidoflavus and Streptomyces lateritius were determined according to the thermal renaturation method (De Ley et al., 1970; Hub et al., 1983; Yassin et al., 1993) using a UV-1206 spectrophotometer (Shimadzu) equipped with a TB-85 thermobath.

RESULTS AND DISCUSSION

The almost complete 16S rDNA sequence of strain A25T (1402 nt) was determined. The sequence data clearly showed that strain A25T is a member of the genus Streptomyces. The chemotaxonomic data confirmed the generic assignment. The presence of LL-DAP and the absence of characteristic sugars in whole-cell hydrolysates showed that strain A25T had wall type I (Lechevalier & Lechevalier, 1970). The strain also contained tetra- and hexahydroxycetone menaquinones with nine isoprene units as the predominant isoprenologue. The phospholipid pattern is type PI (containing phosphatidylethanolamine and diphasphatidylglycerol). Strain A25T showed a fatty acid profile typical of Streptomyces (type IIc according to Kroppestedt, 1985), with iso-C15:0 (21.5 %), anteiso-C15:0 (13.2 %), iso-C16:0 (18.9 %) and C16:0 (13.5 %) predominant. The DNA G+C content of strain A25T is 71 mol %.

The phenotypic properties are also consistent with the classification of strain A25T in the genus Streptomyces. Strain A25T grew poorly on inorganic media, while it grew well on organic media, producing abundant branching mycelium. The substrate mycelium is light blue/Paris blue to brown blue, consists of septate and swollen elements and does not fragment. The aerial mycelium tends to be blue grey/light blue grey and differentiates into long, rectiflexibles spore chains with spiny surfaces. The spore chains are derived from the point of a sporosphore and are often arranged in a fastigiate structure (Fig. 1). No verticillate structure is differentiated into long, rectiflexibles spore chains with spiny surfaces. The spore chains are derived from the point of a sporosphore and are often arranged in a fastigiate structure (Fig. 1). No verticillate structure is
The discriminative power of 16S rRNA sequencing is limited when closely related organisms are being inspected (Yassin et al., 1997), especially for members of the genus *Streptomyces*. Carefully selected phenotypic traits and DNA–DNA pairing data are therefore needed to differentiate *Streptomyces* species that share very similar 16S rDNA sequences (Kim et al., 1998; Labeda, 1988). In order to clarify the finer relationships, DNA–DNA association studies were carried out between strain A25\(^T\) and closely related strains selected on the basis of their 16S rDNA sequence similarity and phylogenetic positions. The low DNA–DNA relatedness between strain A25\(^T\) and *S. ambofaciens* AS 4\(^{-1}\)528\(^T\) (13-1\%), *S. coeruleus* JCM 4358\(^T\) (34-3\%), *S. caelestis* JCM 4218\(^T\) (23-4\%), *S. nogalater* JCM 4799\(^T\) (31-4\%), *S. intermedius* JCM 4483\(^T\) (26-3\%) and *S. albidoflavus* JCM 4446\(^T\) (19-6%) confirmed that strain A25\(^T\) can be considered as a novel taxon. This is also supported by phenotypic data, as at least five differences in phenotypic properties were observed between strain A25\(^T\) and its closest neighbours *S. ambofaciens*, *S. coeruleus* and *S. coelicolor* (Table 1).

Analysis of partial \(\gamma\) region sequences showed that strain A25\(^T\) was grouped into a branch with *S. lateritius* JCM 4389\(^T\) and *Streptomyces flavochromogenes* JCM 4752 (data not shown), but analysis of almost complete 16S rDNA sequences showed that *S. lateritius* JCM 4389\(^T\) fell into a branch with *Streptomyces venezuelae* that was distinct from strain A25\(^T\). The low DNA–DNA relatedness (17-4\%) between *S. lateritius* JCM 4389\(^T\) and strain A25\(^T\) also confirmed that they are different species.

In conclusion, the genotypic, chemotaxonomic and phenotypic data show that strain A25\(^T\) forms a novel taxon.

### Table 1. Diagnostic characteristics of strain A25\(^T\) and related strains

<table>
<thead>
<tr>
<th>Character</th>
<th>Strain A25(^T)</th>
<th><em>S. ambofaciens</em> JCM 4342(^T)</th>
<th><em>S. coeruleus</em> JCM 4389(^T)</th>
<th><em>S. coelicolor</em> DSM 40233(^T)</th>
</tr>
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<tbody>
<tr>
<td>Spore chain morphology*</td>
<td></td>
<td>RF</td>
<td>SP</td>
<td>RF</td>
</tr>
<tr>
<td>Spore surface</td>
<td>Spiny</td>
<td>Smooth</td>
<td>Spiny</td>
<td>Smooth</td>
</tr>
<tr>
<td>Melanoid pigment</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Diffusible pigment</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>pH indicator</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(L)-Rhamnose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Raffinose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(D)-Mannitol</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(i)-Inositol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Sucrose</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<td>Starch</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Growth at 45 °C</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

*RF, Rectiflexibiles; SP, spirales.*

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**Fig. 1.** Scanning electron micrograph of strain A25\(^T\) grown on Bennett’s agar for 14 days at 28 °C. Bar, 6 μm.
Description of \textit{Streptomyces scopiformis} sp. nov.

\textit{Streptomyces scopiformis} (sco.pi.for’mis. L. fem. n. \textit{scopa} a broom; L. fem. n. \textit{forma} form; N.L. n. \textit{scopiformis} in the form of a broom, referring to the structure of the spore chains).

Aerobic, mesophilic, Gram-positive. Substrate hyphae are extensively branching, septate and swollen. Recti-flexible chains of roundish, spiny-surfaced spores (0.7–0.8 μm) are arranged in fastigiate form. Spore mass is grey or blue-grey, the reverse is blue to grey-blue. Diffusible pigment is not formed. Cell-wall type I, phospholipid type PII and menaquinone MK9 (H\textsubscript{4}L\textsubscript{6}) are detected. The fatty acids are type II. Mycolic acids are not present. The G+C content of the DNA is 71 mol\%. Grows in presence of penicillin G, biotin, phenol and ethanol, at 20–45 °C and at pH 5–10 but not in the presence of bacteracin, lycosyme, sodium azide or methyl violet or at 10 or 50 °C or at pH 40 or 11-0. It utilizes L-arabinose, D-fructose, D-galactose, D-glucose, D-malose, L-rhamnose, D-xyllose, D-sucrose, dulcitol, \textit{meso}-inositol, melibiose, trehalose, sodium acetate and sodium citrate as sole carbon sources but not D-mannitol, D-raffinose, adonitol, methyl a-glucoside, L-erythritol or inulin. Tests for aesculin, starch, dextrin, elastin, nitrate reduction and gelatin are positive and tests for hippurate, cellulose and lipolysis are negative.

The type strain, A25\textsuperscript{T}, has been deposited at the Chinese General Microbiological Collection Centre as strain AS 4.1331\textsuperscript{T} (= LMG 20251\textsuperscript{T}).

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REFERENCES


Fig. 2. Neighbour-joining tree based on nearly complete 16 rDNA sequences of 45 streptomycetes. Branches labelled ‘f’ and ‘m’ were also recovered when the Fitch–Margoliash and maximum-likelihood methods were used. Numbers at nodes indicate percentages of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets. Only values >50% are given. Bar, 0.01 substitutions per nucleotide position.


