Streptomyces thinghirensis sp. nov., isolated from rhizosphere soil of Vitis vinifera

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A novel actinomycete, strain S10T, was isolated from rhizosphere soil of wild Vitis vinifera from Thinghir, Ouarzazate Province, Southern Morocco. The taxonomic status of this strain was established using a polyphasic approach. Strain S10T had white–grey aerial mycelium with long, spiral spore chains bearing smooth surfaced spores and produced a yellow diffusible pigment. Chemotaxonomic analyses showed that the cell wall of strain S10T contained L-L-diaminopimelic acid and glycine. Phylogenetic analysis based on the almost complete 16S rRNA gene sequence indicated that strain S10T belonged to the Group I streptomycetes, branching off next to Streptomyces marokkonensis LMG 23016T from the Streptomyces violaceoruber group. DNA–DNA relatedness and phenotypic data distinguished strain S10T from the phylogenetically closest related type strains. It is therefore proposed that strain S10T (≡CCM B35T≡DSM 41919T) represents the type strain of a novel species of the genus Streptomyces, for which the name Streptomyces thinghirensis sp. nov. is proposed.

Over the past decades, interest in the discovery of new sources of secondary metabolites with applications in medicine (Newman et al., 2003) and agriculture (Copping & Menn, 2000) has significantly increased. Microorganisms are an almost unlimited source of novel compounds. Among them, actinomycetes hold a prominent position due to their ability to produce various secondary metabolites, including antibiotics (Lazzarini et al., 2000; Wate et al., 2001; Donadio et al., 2002), antitumour agents (Maskey et al., 2003) and enzymes (Breccia et al., 1995; Ko et al., 2005). Actinomycetes are Gram-positive, aerobic bacteria. They form branching substrate and aerial mycelia that bear spores and possess DNA with a high G+C content. Many species of the genus Streptomyces are known to produce antibiotics (Chun et al., 1997; Labeda et al., 1997). Actinomycetes represent a high proportion of the soil microbial biomass and appear to be of importance among the microbial flora of the rhizosphere (Sardi et al., 1992). Associations between actinomycetes and plant organs can be deleterious or beneficial for the host. While some actinomycetes secrete herbicidal compounds (Tanaka & Omura, 1993) or cause plant diseases (Locci, 1994), others can fix atmospheric nitrogen symbiotically (Oakley et al., 2004) or protect plants against fungal infections (Cao et al., 2005). Several descriptive reports have shown that actinomycetes are a promising group of fungus-antagonistic and root-colonizing microbes. They protect several different plants from soil-borne fungal pathogens to various degrees (El-Tarabily & Sivasithamparam, 2006).

In the course of our screening programme for actinomycetes from Moroccan habitats that are active against many phytopathogens (Loqman et al., 2009), one actinomycete strain, strain S10T, was isolated from the rhizosphere soil of wild, healthy Vitis vinifera plants, collected from Thinghir, Ouarzazate Province, Southern Morocco. The strain was identified using a polyphasic approach.

Strain S10T was isolated on soil extract agar as described in Ouhdouch et al. (2001). The strain was maintained on

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S10T is FM202482.

Micrographs showing spore chains and the spore shape of Streptomyces thinghirensis sp. nov. and a table detailing the fatty acid pattern of the novel strain and closely related species of the genus Streptomyces are available with the online version of this paper.
Amplification was carried out in 50 ml reaction volumes containing 1.5 U of AmpliTaq Gold Taq polymerase (Applied Biosystems), dNTPs (0.25 mM each), 1 μM each primer and 100 ng genomic DNA. Reaction conditions were: 97 °C for 4 min, followed by 35 cycles of 97 °C for 45 s, 52 °C for 45 s and 72 °C for 45 s and a final incubation at 72 °C for 10 min. The amplified products were visualized on a 0.8 % (w/v) agarose gel stained with ethidium bromide. Sequencing reactions were performed by Macrogen. The primers used for sequencing are listed in Coenye et al. (1999). The sequences obtained were compared with sequences present in the public sequence databases as well as with EzTaxon, a web-based tool for the identification of prokaryotes based on 16S rRNA gene sequences from type strains (Chun et al., 2007). BLAST analysis was performed at www.ncbi.nlm.nih.gov.

DNA–DNA hybridization analysis was performed between strain S10T and its closest relatives based on the degree of 16S rRNA gene similarity and the inferred phylogeny. DNA–DNA hybridization with the high scoring (in EzTaxon) but ambiguous sequences for Streptomyces alniquistii NRRL B-1685T (GenBank accession no. AY999782), Streptomyces althioticus NRRL B-3981T (AY999791) and Streptomyces matensis NBRC 12889T (AB184221) was not deemed necessary because of their distant location in the phylogenetic tree (Fig. 1). DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion et al. (1977). DNA–DNA hybridization was performed as described by De Ley et al. (1970), incorporating the modifications described by Huß et al. (1983), using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with in situ temperature probe (Varian), as described by Wayne et al. (1987). All DNA–DNA hybridizations were conducted in duplicate and the reported results give the mean of the two experiments.

For phylogenetic analysis, 16S rRNA gene sequences for the type strains of closely related Streptomyces species were retrieved from GenBank, aligned with CLUSTAL W (Larkin et al., 1999), and analysed using the neighbour-joining, maximum-parsimony and maximum-likelihood tools from the PHYLIP package, version 3.6 (Felsenstein, 2005). Phylogenetic trees were visualized using Dendroscope (Huson et al., 2007).

Strain S10T had morphological characteristics that were consistent with members of the genus Streptomyces. From light and electron microscopic observations, it was found that strain S10T had Spirales-type spore chains (see Supplementary Fig. S1a in IJSEM Online) with smooth spore surface ornamentation (Supplementary Fig. S1b). The chemotaxonomic characteristics of strain S10T supported its classification as a member of the genus Streptomyces. 16S-Diaminopimelic acid and glycine were detected in the cell-wall peptidoglycan. As usually found for streptomycetes, the fatty acid profile was comprised mainly of fatty acids with a length of 14–18 carbon atoms (Lechevalier, 1977), in particular saturated iso- and anteiso-branched chain fatty acids: ai-C15:0 (23.7 %), i-C16:0 (19.5 %), ai-C17:0 (13.7 %), i-C15:0 (11.0 %), i-C17:0 (6.5 %) and i-C14:0 (2.5 %), with only a few unbranched fatty acids, C16:0 (5.6 %), C15:0 (2.3 %), C17:0 (0.7 %) and C14:0 (0.2 %). A compar-
ison of the fatty acid profiles of strain S10\textsuperscript{T} and closely related species is available in Supplementary Table S1 (available in IJSEM Online).

A BLAST search with the 1462 bp 16S rRNA gene sequence of strain S10\textsuperscript{T} showed that it displayed greater than 99% sequence similarity to the 16S rRNA gene sequences of many members of the genus \textit{Streptomyces}. The highest degree of similarity was found with \textit{Streptomyces marokkonensis} LMG 23016\textsuperscript{T} (99.65\%), \textit{S. almquistii} NRRL B-1685\textsuperscript{T} (99.58\%), \textit{S. althioticus} NBRC 12889\textsuperscript{T} (99.51\%), \textit{S. matensis} NBRC 12889\textsuperscript{T} (99.51\%), \textit{Streptomyces aurantiogriseus} NRRL B-3981\textsuperscript{T} (99.32\%), \textit{S. lienomycini} NBRC 15425\textsuperscript{T} (99.24\%), \textit{Streptomyces coelescens} DSM 40421\textsuperscript{T} (99.20\%) and \textit{Streptomyces violaceolatus} DSM 40383\textsuperscript{T} (99.18\%). Five of these strains were selected for DNA–DNA hybridization experiments. Low levels of DNA–DNA relatedness to strain S10\textsuperscript{T} were found for all five strains: \textit{S. coelescens} DSM 40421\textsuperscript{T} 4.6\% ± 0.6\%, \textit{S. aurantiogriseus} DSM 40138\textsuperscript{T} 6.7\% ± 1.0\%, \textit{S. lienomycini} DSM 41475\textsuperscript{T} 9.3\% ± 0.3\%, \textit{S. violaceolatus} DSM 40438\textsuperscript{T} 10.5\% ± 4.5\% and \textit{S. marokkonensis} DSM 41918\textsuperscript{T} 33.4\% ± 0.4\%. When applying the recommended threshold of 70% DNA–DNA relatedness as proposed by Wayne \textit{et al.} (1987), strain S10\textsuperscript{T} could be differentiated from its five closest neighbours.

Phylogenetic analysis showed that strain S10\textsuperscript{T} was most closely related to \textit{Streptomyces marokkonensis} LMG 23016\textsuperscript{T} and that both strains branched off separately from the \textit{S. violaceoruber} species group (Fig. 1).

A comparison of the phenotypic characteristics of strain S10\textsuperscript{T} and the strains with the top BLAST results is shown in Table 1. It is clear from these comparisons that strain S10\textsuperscript{T} is phenotypically different from the most closely related \textit{Streptomyces} species. Additional phenotypic properties of the new isolate are given in the species description.

From the phenotypic and genotypic data obtained, it is proposed that strain S10\textsuperscript{T} represents a novel species within the genus \textit{Streptomyces}. The name \textit{Streptomyces thinghirensis} sp. nov. is proposed with strain S10\textsuperscript{T} as the type strain.

**Description of \textit{Streptomyces thinghirensis} sp. nov.**

\textit{Streptomyces thinghirensis} (thin.ghi.ren’sis. N.L. masc. adj. thinghirensis of Thinghir, named after the town in Southern Morocco where the strain was isolated).

Hyphae are abundant and well-developed. A yellow diffusible pigment is produced on all test media and yellow substrate mycelium and white–grey aerial mycelium are visible. No melanin production is observed on peptone–yeast–iron agar (ISP 6) or tyrosine agar (ISP 7). Good growth is observed on ISP 2 agar. Gelatin is not liquefied. Milk is coagulated and peptonized. \(\text{H}_2\text{S}\) is not produced. Nitrate is reduced. D-Fructose, D-galactose, D-glucose, D-mannitol, D-mannose, myo-inositol, L-rhamnose and D-sorbitol are utilized as sole carbon sources. D-Sucrose, maltose, D-lactose and cellobiose are weakly

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**Fig. 1.** Unrooted maximum-likelihood phylogenetic tree based on 1436 aligned positions of the 16S rRNA gene showing the phylogenetic relationships between strain S10\textsuperscript{T} and the most closely related type strains of the genus \textit{Streptomyces}. Bootstrap values (%) above the branches were derived from 1000 replications of maximum-likelihood inferences, those below the branches give the maximum support by 1000 replications of maximum-parsimony, neighbour-joining and least squares (FITCH) inferences. Bar, 0.001 substitutions per nucleotide position.
Table 1. Physiological characteristics of strain S10T and its phylogenetic neighbours

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>Aerial mass colour</td>
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<td>G</td>
<td>G</td>
<td>Br</td>
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<td>RG</td>
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<td>G</td>
<td>GBr</td>
<td>Gr</td>
<td>Cs</td>
<td>B</td>
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<td>+</td>
<td>–</td>
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<td>Diffusible pigment</td>
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<td>+</td>
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<td>Melanin pigments</td>
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<td>Utilization of d-arabinose</td>
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<td>+</td>
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<td>Raffinose</td>
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</table>

Table 2. Phylogenetic trees and its phylogenetic neighbours

Tata: 1, S10T; 2, S. marokkonensis DSM 23016T; 3, S. coelescens DSM 40421T; 4, S. violaceolatus DSM 40348T; 5, S. liemneycini DSM 41475T; 6, S. aurantiogriseus DSM 40138T. Well utilized/present; w, weakly utilized; –, not utilized/absent; B, blue; Br, brownish; Cs, colourless; G, grey; GBr, grey–brown; RG, red–grey; WG, white–grey; Y, yellow.

utilized, while d-arabinose, d-xylose and raffinose are not utilized as sole carbon sources. Growth occurs from 28 to 42 °C, from pH 5 to 10, and in the presence of 7 % (w/v) NaCl. Resistant to (μg ml⁻¹): ampicillin (10), amoxicillin (10), nalidixic acid (30), penicillin G (10), sulfamide (25) and rifampicin (5), but sensitive to novobiocin (30), gentamicin (10) and streptomycin (10). Active against the moulds Aspergillus niger, Fusarium oxysporum f. sp. albedinis, Pythium ultimum, Sclerotium rolfsii and Verticillium dahliae, the yeasts Candida albicans, Candida tropicalis and Saccharomyces cerevisiae, and the bacteria Bacillus subtilis, Bacillus cereus, Escherichia coli and Streptomyces scabies.

The type strain, S10T (=CCMM B35T=DSM 41919T), was isolated from the rhizosphere soil of wild Vitis vinifera plants.

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References


