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 in 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 LL-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 (=CCMM 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; Watev 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
Cultural and chemotaxonomic characteristics of strain S10T supported its classification as a member of the genus Streptomyces. 16S rRNA gene sequences from type strains of closely related Streptomyces species were retrieved from GenBank, aligned with CLUSTAL W (Larkin et al., 2007), 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).

For physiological characteristics, 16S rRNA gene sequence analysis, strain S10T was cultivated for 2 days at 28 °C with agitation in 500 ml flasks containing 100 ml of Hickey–Tresner medium, 1 g yeast extract 1−1, 1 g beef extract 1−1, 2 g NZamine A 1−1, 10 g dextrin 1−1, 20 mg CoCl2 · 6H2O 1−1 (Hopwood et al., 1985). Biomass was harvested by centrifugation (8000 g for 10 min) and washed twice with double-distilled water. Mycelia (200 mg) were used for DNA extraction as described by Liu et al. (2000). The 16S rRNA gene was amplified by PCR using the universal primers PA and PH. Amplification was carried out in 50 μl 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 althiaticus 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., 2007), 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).

For the chemotaxonomic characterization of strain S10T, 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 S10T 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 S10T showed that it displayed greater than 99% sequence similarity to the 16S rRNA gene sequences of many members of the genus Streptomyces. The highest degree of similarity was found with Streptomyces marokkonensis LMG 23016T (99.65%), S. almquistii NRRL B-1685T (99.58%), S. althioticus NBRC 12889T (99.51%), S. matensis NBRC 14892T (99.32%), Streptomyces lienomyctici NBRC 15425T (99.24%), Streptomyces coelescens DSM 40421T (99.20%), and Streptomyces violaceolatus DSM 40438T (99.18%). Five of these strains were selected for DNA–DNA hybridization experiments. Low levels of DNA–DNA relatedness to strain S10T were found for all five strains: S. coelescens DSM 40421T 4.6 ± 0.6%, S. aurantiogriseus DSM 40138T 6.7 ± 1.0%, S. lienomyctici DSM 41475T 9.3 ± 0.3%, S. violaceolatus DSM 40438T 10.5 ± 4.5% and S. marokkonensis DSM 41918T 33.4 ± 0.4%. When applying the recommended threshold of 70% DNA–DNA relatedness as proposed by Wayne et al. (1987), strain S10T could be differentiated from its five closest neighbours.

Phylogenetic analysis showed that strain S10T was most closely related to Streptomyces marokkonensis LMG 23016T and that both strains branched off separately from the S. violaceoruber species group (Fig. 1).

A comparison of the phenotypic characteristics of strain S10T and the strains with the top BLAST results is shown in Table 1. It is clear from these comparisons that strain S10T is phenotypically different from the most closely related 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 S10T represents a novel species within the genus Streptomyces. The name Streptomyces thinghirensis sp. nov. is proposed with strain S10T as the type strain.

### Description of Streptomyces thinghirensis sp. nov.

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-extract-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. H2S 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
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 pH 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 scabiei.

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

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References


