Streptomyces youssoufiensis sp. nov., isolated from a Moroccan phosphate mine

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A novel actinomycete, strain X4T, was isolated from a phosphate mine in Youssoufia, 100 km north of Marrakesh, Morocco. The taxonomic status of this strain was evaluated by a polyphasic approach. Strain X4T had white aerial mycelium with Rectiflexibiles spore chains bearing smooth-surfaced spores and did not produce diffusible pigments. Chemotaxonomic analysis showed that the cell wall of strain X4T contained L-diaminopimelic acid and glycine. Phylogenetic analysis based on the almost complete 16S rRNA gene sequence indicated that strain X4T belongs to the Group I streptomycetes, branching off next to Streptomyces ramulosus NRRL B-2714T and Streptomyces kasugaensis M338-M1T. DNA–DNA relatedness and phenotypic data enabled strain X4T to be distinguished from the phylogenetically most closely related type strains. It is therefore proposed that strain X4T represents a novel species of the genus Streptomyces, for which the name Streptomyces youssoufiensis sp. nov. is proposed; the type strain is X4T (=CCMM B709T =DSM 41920T).

Since the first description of plant-growth-promoting rhizobacteria (Kloeper & Schrot, 1978), the benefits attributed to these bacteria have been defined more precisely. Micro-organisms of the rhizosphere can contribute to plant fitness by increasing the efficiency of biological nitrogen fixation (Meunchang et al., 2006) or phosphate solubilization (Gyaneshwar et al., 2002; Caravaca et al., 2005; Duponnois et al., 2005). Reports in the literature indicate that Serratia marcescens EB 67, Pseudomonas sp. CDB 35 (Hameeda et al., 2006), Enterobacter sp. and Bacillus subtilis (Toro et al., 1997) contribute to an improvement in plant growth as rock-phosphate-solubilizing bacteria. Soil-borne micro-organisms can also be beneficial for plants by increasing the availability of trace elements such as iron, zinc, etc., in the rhizosphere (Cakmakci et al., 2006). Among soil micro-organisms, actinobacteria are of special interest because they possess many properties that can contribute to plant growth or fitness (Lehr et al., 2008), such as the production of phytohormones that stimulate plant growth (e.g. indole acetic acid; Vivas et al., 2006) and/or excretion of substances such as siderophores and/or anti-fungal agents (Jain & Jain, 2007; Hamdali et al., 2008a, b, c) that limit the development and/or infectivity of various plant pathogens. These mostly filamentous and sporulating bacteria strongly adhere to soil particles and may form endophytic relationships with plants (Cao et al., 2005; Conn et al., 2008).

In the course of our screening programme for rock-phosphate-solubilizing actinomycetes (Hamdali et al., 2008a), strain X4T was isolated from a Moroccan phosphate mine in Youssoufia, in the Marrakesh region of southern Morocco. The strain was identified using a polyphasic approach and the results showed that it should be classified as a representative of a novel species within the genus Streptomyces.

Strain X4T was isolated on soil extract agar as described by Ouhdouch et al. (2001). The strain was maintained on International Streptomyces Project (ISP) 2 agar slants (Shirling & Gottlieb, 1966) at 4°C and as 20% (v/v) glycerol stocks at −20°C. Biomass for chemical and molecular studies was obtained by growing strain X4T in shake flasks of ISP 2 broth (28°C, 1 week, 150 r.p.m.).

Physiological characteristics were determined after growth for 2 weeks at 28°C according to the methods described by the ISP (Shirling & Gottlieb, 1966). Morphological properties were examined by light microscopy (Olympus...
The colour of the aerial mycelium was determined from mature sporulating aerial mycelium according to the scale adopted by Prauser (1964) and the colour series was determined according to the system proposed by Nonomura (1974). Production of melanoid pigments was determined on ISP 6 and ISP 7 media. Analysis of cell wall diaminopimelic acid isomers and whole cell sugars was performed according to the protocol described by Lechevalier & Lechevalier (1980). Fatty acid methyl esters were prepared and analysed as described previously (Klatte et al., 1994) using the standard Microbial Identification system (MIDI) for automated GC analysis (Sasser, 1990).

Carbon source utilization was determined on ISP 9 medium supplemented with sterile carbon sources. Standard techniques were used for the determination of catalase, oxidase and nitrate reduction activities. Sensitivity to NaCl was established by the method of Tresner et al. (1968). Growth temperature range was determined on ISP medium (Olson, 1968) incubated at 28 °C for 21 h. The antimicrobial activity of strain X4T was determined by the plate diffusion method (Bauer et al., 1966). Bacterial test strains were incubated on nutrient agar at 37 °C for 24 h and fungal strains were incubated on Sabouraud agar medium at 28 °C for 24 h (yeasts) or 48 h (moulds).

For 16S rRNA gene sequence analysis, cells of strain X4T were cultivated for 2 days at 28 °C with agitation in 500 ml flasks containing 100 ml Hickey-Tresner medium (per litre: 1 g yeast extract, 1 g beef extract, 2 g NZamine A, 10 g Dextrin, 20 mg CoCl2.6H2O; 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 universal primers P (5’-AGAGTTGATCCTGGCGCCCA-3’) and PH (5’-AAGGAGGT-GATCCAGCCGCA-3’). Amplification was carried out in 50 μl reaction volumes containing 1.5 U AmpliTaq Gold Taq polymerase (Applied Biosystems), dNTPs (0.25 mM each), 1 μM each of 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 elongation step 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 the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). 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 the www.ncbi.nlm.nih.gov web server.

For DNA–DNA hybridization analysis, 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 carried out 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 multicl in charger and a temperature controller with an in situ temperature probe (Varian), as described by Wayne et al. (1987). All DNA–DNA hybridizations were done in duplicate and the reported results give the means of the two experiments.

For phylogenetic analysis, 16S rRNA gene reference sequences for the type strains of closely related species of the genus Streptomyces were retrieved from GenBank, aligned with CLUSTAL W2 (Thompson et al., 1994), 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).

![Fig. 1. Micrograph showing spore chains and spore shape of strain X4T grown on ISP 2 for 14 days at 28 °C. Bar, 5 μm.](image-url)
A BLAST search of the EzTaxon database showed that the similarities between the 1518 bp 16S rRNA gene sequence of strain X4T and 16S rRNA gene sequences of type strains of the most closely related members of the genus *Streptomyces* were less than 99%; highest similarities were observed with *Streptomyces coerulescens* NBRC 12758T (98.97 %), *Streptomyces lilacinus* NBRC 12884T (98.78 %), *Streptomyces abikoensis* NBRC 13860T (98.76 %), *Streptomyces ramulosus* NRRL B-2714T (98.72 %), *Streptomyces varsoviensis* NRRL B-3589T (98.66 %) and *Streptomyces kasugaensis* M338-M1T (98.43 %). DNA–DNA hybridizations were performed with *Streptomyces coerulescens* DSM 40146T, the organism with the highest degree of 16S rRNA gene sequence similarity, with *Streptomyces ramulosus* DSM 40100T and *Streptomyces kasugaensis* DSM 40819T, the closest neighbours in the phylogenetic tree, and with *Streptomyces varsoviensis* NRRL B-3589T, but not for the other top-scoring strains in the BLAST search (type strains of *Streptomyces lilacinus* and *Streptomyces abikoensis*), because these were not supported by the topology of the phylogenetic tree (Fig. 2). Low levels of DNA–DNA relatedness to strain X4T were found for the four strains studied: *Streptomyces coerulescens* NBRC 12758T (28.5 ± 0.5 %), *Streptomyces ramulosus* NRRL B-2714T (11.4 ± 4.8 %), *Streptomyces kasugaensis* M338-M1T (24 ± 4.4 %) and *Streptomyces varsoviensis* NRRL B-3589T (5.4 ± 4.0 %). When applying the recommended threshold of 70 % DNA–DNA relatedness as proposed by Wayne *et al.* (1987), strain X4T could be differentiated from its closest neighbours.

The phenotypic characteristics of strain X4T and the most closely related strains (highest BLAST results) are shown in Table 1. It is clear from these comparisons that strain X4T is phenotypically distinct from the most closely related species of the genus *Streptomyces*. Additional phenotypic properties of the isolate are given in the species description.

From the phenotypic and genotypic data obtained, it is proposed that strain X4T represents a novel species within the genus *Streptomyces*. The name *Streptomyces youssoufiensis* sp. nov. is proposed, with strain X4T as the type strain.

**Description of *Streptomyces youssoufiensis* sp. nov.**

*Streptomyces youssoufiensis* (you.sou.fi.en’sis. N.L. masc. adj. youssoufiensis of or belonging to Youssoufia, named after the rock phosphate mining town in Morocco where the strain was isolated).

Hyphae are abundant and well-developed. No diffusible pigments are produced on any test media and cream substrate mycelium and white aerial mycelium are visible on yeast-malt extract agar (ISP 2).

![Fig. 2. Unrooted maximum-likelihood phylogenetic tree based on 1518 aligned positions of the 16S rRNA gene showing the phylogenetic relationships between strain X4T and the most closely related type strains of the genus 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 any of the maximum-parsimony, neighbour-joining and least-squares (FITCH) inferences. Bar, 0.005 substitutions per nucleotide position.](https://www.microbiologyresearch.org)
The type strain is X4T, isolated from a phosphate mine in Youssoufia, 100 km north of Marrakesh, Morocco.

### Table 1. Physiological characteristics of strain X4T and its phylogenetic neighbours

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*W, White; G, cream; G, grey; Y–BR, yellow–brown; Y, yellow; B, beige; BR–B, brown–beige; P–GR, pale green.

is observed on peptone-yeast-extract-iron agar (ISP 6) or tyrosine agar (ISP 7). Good growth is observed on ISP 2 agar. Starch, cellulose and pectin are degraded. Gelatin is not liquefied. Milk is coagulated and peptonized. H2S is not produced. Nitrate is reduced. D-Fructose, D-glucose, lactose, D-mannitol, D-mannose and sucrose are utilized as sole carbon sources. Cellulose and maltose are utilized weakly, whereas D-arabinose, D-galactose, myo-inositol, raffinose, L-rhamnose, D-sorbitol and D-xylene are not utilized as sole carbon sources. Growth occurs at 28–42 °C, at pH 5–10 and in the presence of 7% (w/v) NaCl. Resistant to ampicillin (10 μg ml⁻¹), amoxicillin (10 μg ml⁻¹), nalidixic acid (30 μg ml⁻¹), penicillin G (10 μg ml⁻¹), sulfadime (25 μg ml⁻¹), gentamicin (10 μg ml⁻¹), cefalotin (30 μg ml⁻¹), bacitracin (10 μg ml⁻¹) and rifampicin (5 μg ml⁻¹), but sensitive to novobiocin (30 μg ml⁻¹), polymyxin B (300 U) and streptomycin (10 μg ml⁻¹). Active against moulds (Fusarium oxysporum f. sp. albedinis, Pythium ultimum, Mucor ramannianus and Verticillium dahliae), yeasts (Candida albicans and Candida tropicalis), bacteria (Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli, Micrococcus luteus, Rhodotorula rubra, Staphylococcus aureus and Streptomyces scabies).

Table 1. Physiological characteristics of strain X4T and its phylogenetic neighbours

| Strains: 1, X4T; 2, Streptomyces varsovienis DSM 40346T; 3, Streptomyces coerulens DSM 40146T; 4, Streptomyces ramosus DSM 40100T; 5, Streptomyces kasugaiensis DSM 40819T. +, Positive/ well utilized; ±, weakly positive/poorly utilized; −, negative/not utilized. All strains were negative for degradation of aesculin. All data were determined in this study.

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**References**


