Streptosporangium algeriense sp. nov., an actinobacterium isolated from desert soil

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The taxonomic position of a novel actinobacterium, strain 169T, isolated from a sample of Algerian Saharan soil, was determined using a polyphasic taxonomic approach. The aerial mycelium produced non-motile, round- to oval-shaped spores, with a smooth surface, which were sessile or carried by short sporophores. Chemotaxonomically, isolate 169T showed the same results as members of the genus Streptosporangium, but madurose, the so far diagnostic sugar of the genus, was not detected in the whole-cell hydrolysate. Despite the absence of sporangia, the 16S rRNA gene sequence analysis confirmed that strain 169T was a member of the genus Streptosporangium. Strain 169T was most closely related to Streptosporangium jomthongense NBRC 110047T (99.3 % 16S rRNA gene sequence similarity), which is the only non-sporangia-forming species reported among the genus. However, DNA–DNA hybridization studies with this species showed 60 % relatedness. Based upon genotypic and phenotypic data, a novel species, Streptosporangium algeriense sp. nov., is proposed, with 169T (=DSM 45455T=MTCC 11561T=CCUG 62974T) as the type strain.

Streptosporangium, the type genus of the family Streptosporangiaceae, was proposed by Couch (1955) for the actinobacteria producing aerial mycelium with spherical sporangia that enclose non-motile sporangiospores. These sporangia are produced by 21 species of the genus Streptosporangium. However Intra et al. (2014) described a novel species of this genus with single roundish, smooth- surfaced-spores, which were formed through the intermediary of very short sporophores on aerial hyphae. This novel species, unlike the other members of the genus Streptosporangium, does not produce sporangia. Chemotaxonomically, the genus Streptosporangium is characterized by the presence of meso-diaminopimelic acid in the cell wall and madurose in the whole-cell hydrolysates, di- and tetra-hydrogenated menaquinones with nine isoprene units, phosphatidylethanolamine and phospholipids containing glucosamine as diagnostic phospholipids, and complex mixtures of iso-, anteiso-, saturated, unsaturated and 10-methyl-branched fatty acids (Quintana & Goodfellow, 2012).

During taxonomic and antibiotic studies of Saharan actinobacteria, strain 169T was isolated from a Saharan soil sample collected from Adrar palm grove in southern Algeria (latitude 27° 53’ N, longitude 0° 16’ E, altitude 262 m) by a dilution agar plating method using chitin–vitamin agar medium (Hayakawa & Nonomura, 1987) supplemented with actidione (50 µg ml⁻¹). The presence of members of the genus Streptosporangium in these soils has already been reported (Boudjella et al., 2006). The aim of this study was to identify strain 169T, which represents a novel species within the genus Streptosporangium, by using phenotypic, genotypic and phylogenetic approaches.

Cultural characteristics were investigated on media from the International Streptomyces Project (ISP) (Shirling & Gottlieb, 1966), nutrient agar and Bennett’s agar (Waksman, 1961). The cultural characteristics were recorded after 7, 14 and 21 days of incubation at 30 °C. The colours of substrate and
aerial mycelia and any soluble pigments produced were determined according to the ISCC-NBS centroid colour chart (Kelly & Judd, 1976). Spores and mycelia were examined by light microscope (B1 Series; Motic) and scanning electron microscope (model S450; Hitachi) after 2 weeks’ growth on ISP 2 medium (Shirling & Gottlieb, 1966).

Several physiological tests were used to characterize the actinobacterial strain. Peptone/yeast extract/iron agar (ISP 6) and tyrosine agar (ISP 7) (Shirling & Gottlieb, 1966) were used to determine melanoid pigment production. Degradation of adenine, gelatin, guanine, hypoxanthine, milk casein, starch, testosterone, Tween 80, tyrosine and xanthine was studied as described by Goodfellow (1971) and Marchal et al. (1987). Media and procedures used for determination of physiological features and carbon source utilization were those described by Gordon et al. (1974) and Williams et al. (1989). Growth at different temperatures (15, 30, 35 and 45 °C) and pH (5, 7 and 9), and in the presence of erythromycin (15 mg l\(^{-1}\)) was studied using biomass, and carbon source utilization were those described by Gordon et al. (1974) and Williams et al. (1989). Growth at different temperatures (15, 30, 35 and 45 °C) and pH (5, 7 and 9), and in the presence of erythromycin (15 mg ml\(^{-1}\)), chloramphenicol (30 mg ml\(^{-1}\)) and novobiocin (5 mg ml\(^{-1}\)) was determined on nutrient agar medium.

Chemotaxonomic analysis was performed using biomass, obtained at 30 °C after 4 days, of strain culture in shake flasks (250 r.p.m.) containing ISP 2 medium. The biomass was harvested by centrifugation and washed several times with sterilized distilled water. The isomeric form of diaminopimelic acid and predominant whole-cell sugars were detected following standard procedures described by Becker et al. (1964) and Lechevalier & Lechevalier (1970). Phospholipids were analysed using the procedure of Minnikin et al. (1977). The fatty acid profile was determined by the method of Sasser (1990), using the Microbial Identification System (MIDI) Sherlock version 6.1 (TSBA40 database). The menaquinones were extracted following the procedure of Minnikin et al. (1984) and analysed by HPLC (Kroppenstedt, 1982, 1985).

Genomic DNA was extracted with a DNA extraction kit (MasterPure Gram Positive DNA Purification kit; Epicentre Biotechnologies). PCR-mediated amplification of the 16S rRNA gene was performed as described by Rainey et al. (1996). The sequence was compared with sequences present in the public sequence databases as well as with those on the EzTaxon-e server (Kim et al., 2012). Phylogenetic analyses were conducted using MEGA version 5 (Tamura et al., 2011). The 16S rRNA gene sequence of strain 169\(^{T}\) was aligned against neighbouring nucleotide sequences using CLUSTAL W (with default parameters) (Thompson et al., 1994). Phylogenetic trees were reconstructed by using neighbour-joining (Saitou & Nei, 1987) with the model of Jukes & Cantor (1969), maximum-likelihood (Felsenstein, 1981) with Kimura’s two-parameter model (Kimura, 1980), and maximum-parsimony (Fitch, 1977). The topology of the phylogenetic trees was evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The G + C content of the chromosomal DNA of strain 169\(^{T}\) was determined by HPLC as described by Mesbah et al. (1989).

For DNA–DNA hybridization, cells were disrupted by using a French pressure cell (Thermo Spectronic). The DNA in the crude lysate 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) under consideration of the modifications described by Huss et al. (1983), using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multichannel changer and a temperature controller with in-situ temperature probe (Varian). The DNA–DNA hybridization experiment was done in duplicates in 2 × SSC (saline sodium citrate) buffer in the presence of 10 % formamide at 71 °C.

Strain 169\(^{T}\) showed very good growth with production of white and yellowish-white aerial mycelium, respectively, on ISP 2 and Bennett’s media, and moderate to good growth on nutrient agar and ISP 3 media (Shirling & Gottlieb, 1966) with yellowish-white aerial mycelium. Growth did not occur on ISP 4 medium (Shirling & Gottlieb, 1966), after 14 days of incubation at 30 °C. The strain did not produce diffusible pigments on the media tested. The colour of substrate mycelium was strong yellowish brown on ISP 2, yellowish white on ISP 3, strong reddish brown on Bennett’s and pale yellowish on nutrient agar. Spores and mycelia were examined by light microscope and scanning electron microscope after 2 weeks’ growth. On all media tested the aerial mycelium produced non-motile, round- to oval-shaped spores, with a smooth surface, which were sessile or carried by very short sporophores (Fig. 1).

Morphologically, strain 169\(^{T}\) was different from all the described species of the genus Streptosporangium, which

![Fig. 1. Scanning electron micrograph of spores of strain 169\(^{T}\) grown on yeast extract-malt extract agar (ISP 2 medium) for 2 weeks at 30 °C. The spores are sessile (ss) or carried by very short sporophores (sp). Bar, 5 μm.](http://ijs.microbiologyresearch.org/1035)
are characterized by the presence of spores in sporangia, except the type strain Streptosporangium jomthongense NBRC 110047^T described by Intra et al. (2014), which produces spores but no sporangium, just like strain 169^T.

The cell-wall hydrolysate of strain 169^T contained meso-diaminopimelic acid but not glycine, and the whole-cell hydrolysate contained glucose, ribose and mannose, but not madurose, which characterizes the species of the genus Streptosporangium. Phospholipids detected were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylaminositol, two uncharacterized glycocephospholipids, two uncharacterized aminophospholipids and one uncharacterized glycolipid (Fig. S1, available in the online Supplementary Material). The predominant fatty acids were iso-C_{16:0} (16.9 %), C_{16:0} (15.7 %), C_{17:1} \( \alpha c \) (10.1 %) and 10-methyl C_{17:0} (10.8 %). The detailed composition of fatty acids of strain 169^T in comparison with that of the most closely related species, S. jomthongense, is given in Table S1. The predominant menaquinones were MK-9(H_2) (51.8 %) and MK-9(H_4) (33.3 %). The chemotaxonomic properties of strain 169^T were consistent with those of members of the genus Streptosporangium, except for the absence of madurose.

Strain 169^T could be distinguished from S. jomthongense NBRC 110047^T, the type strain of the most closely related species, by the absence of madurose and arabinose in the whole-cell hydrolysate, the colours of aerial and substrate mycelia, the absence of growth on ISP 4 medium, the incapacity to degrade casein and starch, the inability to use fructose, inositol and raffinose as the sole carbon source, and the absence of growth at pH 5 and 9, as can be seen from the differential phenotypic characteristics given in Table 1. Complete physiological characteristics of strain 169^T are given in the species description.

The phylogenetic relationships between strains 169^T and the members of the genus Streptosporangium are shown in the neighbour-joining (Fig. 2), maximum-parsimony and maximum-likelihood dendrograms (Fig. S2). The similarity of the 16S rRNA gene sequence of strain 169^T (1505 bp) to those of the other species of the genus Streptosporangium ranged from 97.3 and 99.3 %, with S. jomthongense NBRC 110047^T the phylogenetically closest species. The other species of the genus Streptosporangium showed similarity values lower than 98.6 %, which is the threshold for differentiating two bacterial species as recently reported by Kim et al. (2014).

The G+C content of genomic DNA of strain 169^T was 70.7 mol%.

Strain 169^T was determined to have 60 % genomic DNA–DNA relatedness (based on a mean of duplicate determinations, 58 % and 62 %) with S. jomthongense NBRC 110047^T, which is phylogenetically the closest species of the genus Streptosporangium, and also the only species that micromorphologically resembles strain 169^T by the absence of sporangia. This value is clearly below the 70 % relatedness guideline proposed by Wayne et al. (1987) for delineation of separate species. All of the data support the designation of strain 169^T as representing a novel species of the genus Streptosporangium, for which we propose the name Streptosporangium algeriense sp. nov., with the type strain 169^T.

**Description of Streptosporangium algeriense sp. nov.**

Streptosporangium algeriense (al.ge.ri.en’se. N.L. neut. adj., algeriense pertaining to Algeria, from where the type strain was isolated).

Aerobic, Gram-stain-positive, filamentous actinobacterium. Aerial mycelium is yellowish white on ISP 3, Bennett’s and nutrient agar media, and white on ISP 2 medium. Non-motile, roundish to oval, smooth spores, sessile or carried by very short sporophores. The colour of substrate

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**Table 1. Differential phenotypic properties of strain 169^T compared with the most closely related species of the genus Streptosporangium**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>Colour of aerial mycelium</td>
<td>White to pink</td>
<td>White to yellowish white</td>
<td>White to pink</td>
<td>White to yellowish pink</td>
</tr>
<tr>
<td>Colour of substrate mycelium</td>
<td>Yellowish brown</td>
<td>Pink</td>
<td>Yellow</td>
<td>Yellowish pink</td>
</tr>
<tr>
<td>Diffusible pigment</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Production of sporangia</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Growth on ISP 4 medium</td>
<td>–</td>
<td>Good</td>
<td>Poor</td>
<td>Good</td>
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<td>Decomposition of:</td>
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<tr>
<td>Starch</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Casein</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Gelatin</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Carbon source utilization:</td>
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<tr>
<td>Arabinose</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>Fructose</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<td>Inositol</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Raffinose</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<td>Growth at:</td>
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<tr>
<td>pH 5</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>pH 9</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>15 °C</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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mycelium is strong yellowish brown on ISP 2, yellowish white on ISP 3, strong reddish brown on Bennett’s, and pale yellowish on nutrient agar. Does not produce diffusible pigments on any of the media tested. The optimal temperature for growth is 30°C; growth does not occur at 15 or 45°C. Utilizes cellobiose, D-galactose, D-glucose, glycerol, D-mannitol, D-mannose, melezitose, melibiose, L-rhamnose, D-sorbitol, sucrose, D-xylose, acetate, citrate, pyruvate, succinate and tartrate as carbon sources, but not adonitol, L-arabinose, D-fructose, myo-inositol, lactose, maltose, raffinose, D-ribose, salicin, benzoate, butyrate, oxalate or propionate. L-Alanine, L-proline and L-serine are used as source of nitrogen. Gelatin, tyrosine and Tween 80 are utilized, but aesculin, adenine, arbutin, casein, guanine, hypoxanthine, starch, testosterone and xanthine are not decomposed. Nitrate reductase is produced. Growth does not occur at pH 5 or pH 9 or in the presence of erythromycin or novobiocin, but occurs in the presence of chloramphenicol. Cell-wall hydrolysate contains meso-diaminopimelic acid, and whole-cell hydrolysate contains glucose, ribose and mannose. The diagnostic phospholipid is phosphatidylethanolamine. The predominant menaquinones are MK-9(H2) and MK-9(H4). The major fatty acids are iso-C16:0, C16:0, iso-C17:1ω8c and 10-methyl C17:0.

The type strain is 169T (=DSM 45455T=MTCC 11561T=CCUG 62974T), isolated from a Saharan soil sample collected from Southern Algeria. The G+C content of genomic DNA of the type strain is 70.7 mol%.

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References


