Streptosporangium saharense sp. nov., an actinobacterium isolated from Saharan soil

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A novel actinobacterium, designated strain SG20 T, was isolated from a Saharan soil sample collected from Béni-isguen (Mzab), Ghardaïa province, southern Algeria. The micro-organism developed small roundish sporangia on aerial mycelium that were sessile or carried by very short sporangiophores. The cell-wall peptidoglycan contained meso-diaminopimelic acid and the whole-cell sugars comprised glucose, ribose and mannose, but madurose was not detected. The predominant menaquinones were MK-9(H 4), MK-9(H6) and MK-9(H 2). The major fatty acids were iso-C16 : 0 and C16 : 0. The phospholipids detected were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and unknown lipids. The phenotypic and chemotaxonomic characteristics of the novel strain resembled those of recognized members of the genus Streptosporangium. Moreover, phylogenetic analysis based on a 16S rRNA gene sequence generated from the strain identified its closest relative as Streptosporangium jomthongense BCC 53154T (98.5 % similarity), which produces single spores on aerial mycelium, but no sporangia. In hybridization experiments, the DNA–DNA relatedness values recorded between strain SG20 T and S. jomthongense DSM 46822T fell well below 70 %. On the basis of phenotypic and genotypic data, strain SG20 T can be distinguished as representing a novel species of the genus Streptosporangium, for which the name Streptosporangium saharense sp. nov. is proposed. The type strain is SG20 T (= DSM 46743T = CECT 8840T).

The genus Streptosporangium, belonging to the family Streptosporangiaceae, was first established by Couch (1955) with Streptosporangium roseum as the type species. Subsequently, Stackebrandt et al. (1994) and Intra et al. (2014) emended the description of the genus Streptosporangium. In the List of Prokaryotic names with Standing in Nomenclature (http://www.bacterio.cict.fr/index.html), 19 species and two subspecies of the genus Streptosporangium were cited at the time of writing, including the newly described species Streptosporangium nanhuense (He et al., 2014) and Streptosporangium jomthongense (Intra et al., 2014). The genus Streptosporangium as now defined comprises aerobic, Gram-positive, non-acid-fast, non-motile microorganisms that generate abundantly branched, non-fragmenting substrate mycelia which carry aerial hyphae that differentiate into spherical spore vesicles that enclose non-motile sporangiospores; however, non-motile single spores without sporangia may be produced (Intra et al., 2014). Chemotaxonomically, members of the genus Streptosporangium are characterized by the presence of meso-diaminopimelic acid in the cell wall, and usually madurose in the whole-cell hydrolysates (Stackebrandt et al., 1994). Strains in the genus Streptosporangium have di- and tetra-hydrogenated menaquinones with nine isoprene units, and diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and glucosamine-containing phospholipids as major phospholipids (Quintana & Goodfellow, 2012).
Strain SG20\textsuperscript{T} was isolated as part of a discovery and identification programme for rare actinobacteria from Saharan soils of Algeria that may produce novel metabolites with potential medical applications. Previous studies from the same programme reported the presence of novel strains of the genus *Streptosporangium* that produce novel compounds with both antibacterial and antifungal activities (Hacène *et al.*, 1998; Boudjella *et al.*, 2006, 2007, 2010). The aim of the present study was to determine the taxonomic status of an actinobacterium, strain SG20\textsuperscript{T}, isolated from a Saharan soil sample collected from Béni-isguen (Mzab), Ghardia province, southern Algeria. The micro-organism was the subject of a polyphasic taxonomic study, which showed that it represents a novel species of the genus *Streptosporangium*.

Strain SG20\textsuperscript{T} was isolated from a soil sample that was air-dried at room temperature for 10 days before being baked at 120 °C for 1 h (Hayakawa *et al.*, 1991). The soil was then suspended in sterile distilled water, serially diluted and spread-plated on chitin–vitamin B agar medium (Hayakawa & Nomoura, 1987) supplemented with penicillin (25 mg l\textsuperscript{-1}). After 21 days of incubation at 30 °C, the strain was transferred and purified on yeast extract-malt extract agar (ISP 2 medium of the International Streptomyces Project; Shirling & Gottlieb, 1966), and was maintained as working cultures on this medium.

General cell morphology was studied by light microscopy (model B1; Motic) and scanning electron microscopy (JSM-7100F; JEOL) of a 21-day-old culture of strain SG20\textsuperscript{T} grown on ISP 2 medium (Shirling & Gottlieb, 1966). The colours of substrate and aerial mycelium were determined as described by Minnikin *et al.* (1984) and were analysed by HPLC (Kroppenstedt, 1982, 1985). The cellular fatty acid composition was studied as described by Sasser (1990) using the Microbial Identification System (MIDI) Sherlock version 6.1 (TSBA40 database).

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 and purification of the PCR product were carried out following Rainey *et al.* (1996). 16S rRNA gene sequence similarities between strain SG20\textsuperscript{T} and the type strains of recognized species of the genus *Streptosporangium* were calculated on the basis of pairwise alignment using the EzTaxon-e server (Kim *et al.*, 2012). The almost-complete 16S rRNA gene sequence of strain SG20\textsuperscript{T} was determined and deposited under accession number KT581983 in the GenBank database. Phylogenetic analyses were conducted using MEGA version 5.05 (Tamura *et al.*, 2011). The 16S rRNA gene sequence of strain SG20\textsuperscript{T} was aligned against neighbouring nucleotide sequences using the CLUSTAL W program (with default parameters) (Thompson *et al.*, 1994). Phylogenetic trees were inferred using the neighbour-joining algorithm (Saitou & Nei, 1987) with the model of Jukes & Cantor (1969), maximum-likelihood algorithm (Felsenstein, 1981) with Kimura’s two-parameter model (Kimura, 1980) and maximum-parsimony algorithm (Fitch, 1977). The stability of the clades in the trees was appraised using a bootstrap analysis with 1000 repeats (Felsenstein, 1985).

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). DNA–DNA hybridization experiments were performed in duplicates in 2 × SSC in the presence of 10 % formamide at 71 °C.

Strain SG20\textsuperscript{T} exhibited good growth on ISP 2, oatmeal agar (ISP 3), peptone/yeast extract/iron agar (ISP 6) and tyrosine agar (ISP 7), but no growth on inorganic salts-starch agar (ISP 4). Strain SG20\textsuperscript{T} developed abundant, aerial mycelia on ISP 2, 3 and 7 media, but not on ISP 6 medium. The colour of the aerial mycelium was pale pink–beige on ISP 2, pinkish beige on ISP 3 and whitish beige on ISP 7 media. The micro-organism formed non-fragmenting substrate mycelium, yellow–brown on ISP 2, pale white to yellow on ISP 3, light brownish beige on ISP 6 and light beige on ISP 7 media. Cells developed small, roundish sporangia (measuring 1–3 μm in diameter) at the ends of very short sporangiophores on aerial mycelium (Fig. 1); sporangia may be also sessile. Sporangiospores were not motile. No diffusible pigment was produced on any of the media tested. Melanoid pigments were not produced on ISP 6 or 7 media. Strain SG20\textsuperscript{T} grew at...
20–42 °C, at pH 3.0–12.0 and in the presence of 0–1 % (w/v) NaCl. It was able to utilize L-arabinose, cellobiose, D-galactose, D-glucose, D-mannitol, D-mannose, L-rhamnose, sodium acetate, sodium pyruvate, trehalose and D-xylose as sole carbon sources. The novel strain was able to reduce nitrate, peptonize milk and hydrolyse casein, gelatin, Tween 80 and L-tyrosine. The detailed physiological characteristics of strain SG20\(^T\) are given in Table 1 and in the species description.

Several phenotypic characteristics differentiate the new isolate from its closest relative, \textit{S. jomthongense} BCC 53154\(^T\), such as growth and cultural characteristics (the colour of aerial and substrate mycelium) (Table 2), ability to hydrolyse gelatin and to grow at pH 3, 4 and 12, inability to utilize D-xylose, sucrose, raffinose, melibiose, D-mannitol, myo-inositol or D-fructose as sole carbon sources, to hydrolyse starch and to grow in the presence of 2 % (w/v) NaCl. These differences, together with the ability to produce sporangia on the aerial hyphae (strain SG20\(^T\) forms spores within sporangia while \textit{S. jomthongense} BCC 53154\(^T\) produces no sporangia, but single spores), confirmed the distinct species status of this strain. Physiological properties that distinguish strain SG20\(^T\) from its closely related species \textit{S. jomthongense} BCC 53154\(^T\) are presented in Table 1.

With regard to chemotaxonomic characteristics, strain SG20\(^T\) contained \textit{meso}-diaminopimelic acid (cell wall type III; Lechevalier & Lechevalier, 1970) as the cell-wall diamino acid. Whole-cell hydrolysates yielded glucose, ribose and mannose, but madurose, which usually characterizes recognized species of the genus \textit{Streptosporangium}, including \textit{S. jomthongense} BCC 53154\(^T\), was not detected. The polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylglycerolphosphatidylcholine, three unknown amino phospholipids, one unknown glycolipid, one unknown phospholipid, one unknown glycolipid and one unknown lipid were also detected (Fig. S1, available in the online Supplementary Material). The menaquinones of strain SG20\(^T\) were MK-9(H\(_4\)) (39 %), MK-9(H\(_6\)) (19 %), an unknown menaquinone (16 %) and MK-9(H\(_2\)) (12 %); small amounts of another unknown menaquinone (8 %) and MK-9(H\(_6\)) (3 %) were also present. The major cellular fatty acids (>5 %) were iso-C\(_{16}:0\), C\(_{16}:0\), 10-methyl C\(_{17}:0\), 10-methyl C\(_{16}:0\) and C\(_{18}:1\)ω9c (Table S1). Chemotaxonomic characteristics support the classification of strain SG20\(^T\) as a member of the genus \textit{Streptosporangium}.

An almost-complete 16S rRNA gene sequence (1452 nt) was obtained for strain SG20\(^T\) and compared with those available in public databases. The phylogenetic trees reconstructed using the neighbour-joining (Fig. 2), maximum-likelihood and maximum-parsimony algorithms (Fig. S2) showed the phylogenetic relationship between strain SG20\(^T\) and members of the genus \textit{Streptosporangium} with validly published names. These data revealed that strain SG20\(^T\) should be classified as a member of the genus \textit{Streptosporangium} and that it was clearly separated from its closest relatives. Strain SG20\(^T\) shared a 16S rRNA gene sequence similarity of 98.5 % with its nearest relative, \textit{S. jomthongense} BCC 53154\(^T\). Sequence similarities with all other members of the genus \textit{Streptosporangium} with validly published names were less than 98.2 %.

The taxonomic integrity of the test strain was supported by DNA–DNA relatedness data. Levels of DNA–DNA relatedness between strain SG20\(^T\) and \textit{S. jomthongense} DSM 46822\(^T\) were 39.9–44.7 %. These values are well below the 70 % cut-off point recommended for the delineation of genomic species (Wayne \textit{et al.}, 1987). DNA–DNA hybridization was not carried out between strain SG20\(^T\) and other species of the genus \textit{Streptosporangium} because

**Table 1.** Differential characteristics between strain SG20\(^T\) and \textit{S. jomthongense} BCC 53154\(^T\)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SG20(^T)</th>
<th>\textit{S. jomthongense} BCC 53154(^T)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Utilization of:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Fructose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Melibiose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td><strong>Hydrolysis of:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>pH range</td>
<td>3–12</td>
<td>5–11</td>
</tr>
<tr>
<td>Tolerance to NaCl (%)</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Data are from this study. +, Positive reaction; –, negative reaction.
they were positioned in different clusters in the phylogenetic tree and shared relatively low 16S rRNA gene sequence similarities (<98.2 %). In addition, previous studies have shown that some *Streptosporangium* species have high 16S rRNA gene sequence similarities, but have low DNA–DNA relatedness values (below the 70 % cut-off point). For example, the nearest neighbour *Streptosporangium sandarakinum* DSM 45763^T^ showed 16S rRNA gene sequence similarity of 99.9 % to *Streptosporangium pseudovulgare* DSM 43181^T^. This value (99.9 %) was also found between *Streptosporangium canum* DSM 45034^T^ and *Streptosporangium roseum* DSM 43021^T^, and between *Streptosporangium album* DSM 43023^T^ and *Streptosporangium vulgare* DSM 43802^T^, yet these strains shared low DNA–DNA hybridization (<70 %) (Zhang *et al.*, 2009; Kämpfer *et al.*, 2013). Moreover, the previously accepted value of 97.0 % 16S rRNA gene sequence similarity has been reassessed by statistical comparison of 16S rRNA.

### Table 2. Cultural characteristics of strain SG20^T^ and *S. jomthongense* BCC 53154^T^ grown on different media

<table>
<thead>
<tr>
<th>Medium</th>
<th>SG20^T^</th>
<th><em>S. jomthongense</em> BCC 53154^T^</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aerial mycelium</td>
<td>Substrate mycelium</td>
</tr>
<tr>
<td>ISP 2</td>
<td>Pale pink-beige</td>
<td>Yellow–brown</td>
</tr>
<tr>
<td>ISP 3</td>
<td>Pinkish beige</td>
<td>White to pale yellow</td>
</tr>
<tr>
<td>ISP 4</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>ISP 6</td>
<td>None</td>
<td>Light beige to brownish</td>
</tr>
<tr>
<td>ISP 7</td>
<td>White to beige</td>
<td>Light beige</td>
</tr>
</tbody>
</table>

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**Fig. 2.** Phylogenetic tree for species of the genus *Streptosporangium* calculated from almost complete 16S rRNA gene sequences (1452 nt) using Jukes & Cantor (1969) evolutionary distance methods and the neighbour-joining method of Saitou & Nei (1987). This illustrates the taxonomic position of strain SG20^T^ relative to the other species of the genus. Bootstrap values above 50 % (percentages of 1000 replications) are indicated. *Actinomadura madurae* DSM 43067^T^ was used as the outgroup. Bar, 0.005 nt substitutions per site.
gene sequence similarities and DNA–DNA reassociation values, and new thresholds, 98.20 and 98.65 %, were recommended by Meier-Kolthoff et al. (2013) and Kim et al. (2014), respectively, as the point at which DNA–DNA reassociation experiments should be mandatory for testing the genomic uniqueness of a novel species.

Based on data from the present polyphasic study, strain SG20T could be readily differentiated from other members of the genus Streptosporangium and is considered to represent a novel species, for which the name Streptosporangium saharense sp. nov. is proposed.

Description of Streptosporangium saharense sp. nov.

Streptosporangium saharense (sa.har.en’se. N.L. neut. adj. saharense pertaining to Sahara, the source of the soil from which the type strain was isolated).

Aerobic, Gram-stain-positive, mesophilic, non-motile actinobacterium that generates well-developed and branched substrate hyphae. Good growth occurs on ISP 2, 3, 6 and 7 media, but not on ISP 4 medium. Forms non-fragmenting, yellow–brown (on ISP 2), pale white to yellow (on ISP 3), light brownish beige (on ISP 6) and light beige (on ISP medium 7) substrate mycelia that bear pale pink–beige (on ISP 2), pinkish beige (on ISP 3) or white–beige (on ISP 7) aerial mycelia. Small, roundish ISP 3), light brownish beige (on ISP 6) and light beige (on ISP medium 7) substrate hyphae. Good growth occurs on ISP 2, 3, 6 and 7 media, but not on ISP 4 medium. Forms non-fragmenting, yellow–brown (on ISP 2), pale white to yellow (on ISP 3), light brownish beige (on ISP 6) and light beige (on ISP medium 7) substrate mycelia that bear pale pink–beige (on ISP 2), pinkish beige (on ISP 3) or white–beige (on ISP 7) aerial mycelia. Small, roundish sporangia (measuring 1–3 μm in diameter) containing non-motile, rod-shaped sporangiospores are formed on aerial mycelia. Sporangia are sessile or carried by very short sporangiophores. No diffusible pigment is detected on ISP 2 and 3 media. Melanoid pigments are not produced on ISP 6 or 7 media. Growth occurs at 20–42 °C (optimum, 25–37 °C) and pH 3.0–12.0 (optimum, pH 4–10). The maximum NaCl concentration for growth is < 1 %. Utilizes L-arabinose, cellobiose, D-galactose, D-glucose, D-mannitol, D-mannose, L-rhamnose, sodium acetate, sodium pyruvate, trehalose and D-xylene, but not adonitol, D-fructose, glycerol, myo-inositol, lactose, maltose, melezitose, melibiose, methyl α-D-glucoside, raffinose, D-ribose, salicin, D-sorbitol, sucrose, benzoate, butyrate, citrate, lactate, oxalate, propionate, succinate or tartrate. Hydrolyses casein, gelatin, Tween 80 and L-tyrosine but not aesculin, arbutin, adenine, cellulose, guanine, hypoxanthine, starch or xanthine. Positive for nitrate reduction and milk peptonization but negative for milk coagulation. The cell wall contains meso-diaminopimelic acid and the whole-cell sugars are ribose, glucose and mannose, but not madurose. The polar lipid profile contains diphasatidylglycerol, phosphatidylinositol and phosphatidylethanolamine. The predominant menaquinones are MK-9(H4), MK-9(H6), MK-9(H8) and one unknown menaquinone. The major fatty acids are iso-C16:0 and C16:0.

The type strain is SG20T (= DSM 46743T = CECT 8840T) isolated from a Saharan soil sample collected from southern Algeria.

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References


