**Streptosporangium becharense** sp. nov., an actinobacterium isolated from desert soil

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The taxonomic position of a novel actinobacterium, strain SG1ᵀ, isolated from a Saharan soil sample collected from Béni-Abbès, Béchar (south-west Algeria), was established by using a polyphasic approach. The micro-organism had morphological and chemical features that were consistent with its classification in the genus *Streptosporangium*. The cell-wall peptidoglycan contained meso-diaminopimelic acid. The whole-cell sugars contained ribose and glucose, but not madurose. The predominant menaquinones were MK-9(H₂) and MK-9(H₄). The polar lipid profile contained diphosphatidylglycerol, phosphatidylmethyllethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylhydroxyethanolamine, phosphatidylglycerol, phosphatidylglycerol, phosphatidylserine and phosphatidylglycerol mannosides. The predominant cellular fatty acids were C₁₇:₀, C₁₇:₁ω₈c, iso-C₁₆:₀, 10-methyl C₁₇:₀, C₁₈:₁ω₇c and C₁₈:₀. 16S rRNA gene sequence similarity analyses supported the classification of the isolate in the genus *Streptosporangium* and indicated that it was related most closely to *Streptosporangium subfuscum* DSM 46724 (99.7% similarity), *Streptosporangium pseudovulgare* DSM 43181ᵀ (98.7%) and *Streptosporangium fragile* DSM 43847ᵀ (98.6%) and *Streptosporangium sandarakinum* DSM 45763ᵀ (98.5%). Phylogenetic analyses based on 16S rDNA gene sequences showed that strain SG1ᵀ formed a cluster with its closest relative *S. subfuscum* DSM 46724. However, DNA–DNA relatedness as well as physiological and chemotaxonomical analyses showed that strain SG1ᵀ could be differentiated from its closest phylogenetic relatives. Therefore, it is proposed that strain SG1ᵀ should be classified as representing a novel species in the genus *Streptosporangium*, for which the name *Streptosporangium becharense* sp. nov. is proposed. The type strain is SG1ᵀ (=DSM 46887ᵀ=CECT 8961ᵀ).

The genus *Streptosporangium*, belonging to the family *Streptosporangiaceae* and the order *Streptosporangiales* (Quintana & Goodfellow, 2012), was proposed by Couch (1955) with *Streptosporangium roseum* as the type species. The description of the genus has been emended first by Stackebrandt *et al.* (1994) and more recently by Intra *et al.* (2014). At the time of writing, the genus *Streptosporangium* comprised 21 recognized species and two subspecies with validly published names (http://www.bacterio.cict.fr/index.html), including the recently described species *Streptosporangium nanhuense* (He *et al.*, 2014), *Streptosporangium jomthongense* (Intra *et al.*, 2014), *Streptosporangium algeriense* (Boubetra *et al.*, 2016) and *Streptosporangium saharense* (Chaabane Chaouch *et al.*, 2016). However, eight other species have been proposed in the meantime, including *Streptosporangium shenghense* (Zhang *et al.*, 2014), *Streptosporangium subfuscum*...
(Zhou et al., 2014) ‘Streptosporangium kronopoliitis’ and ‘Streptosporangium sonchi’ (Ma et al., 2015), ‘Streptosporangium terrae’ (Vaddavalli et al., 2015) and ‘Streptosporangium luteolentum’, ‘Streptosporangium fen-ghuangense’ and ‘Streptosporangium corydalii’ (Fang et al., 2016), but these names have not yet been validated. Although most Streptosporangium species have been isolated from soil samples, the recently described species Streptosporangium oxazolinicum, ‘S. sonchi’ and ‘S. corydalii’ have been isolated from plant roots and ‘S. kronopoliitis’ from a millipede (Inahashi et al., 2011b; Ma et al., 2015; Fang et al., 2016).

Streptosporangium is one of the actinobacterial genera that have been of great interest as new potential producers of diverse secondary metabolites with significant biological activities (Lazzarini et al., 2000). The genome of the type species S. roseum has been sequenced completely. The genome consists of a 10 341 314 bp long chromosome and a small 28 204 bp plasmid with its 9421 protein-coding and 80 RNA genes. It has been found to be the second largest microbial genome sequence yet deciphered (Nolan et al., 2010). It seems that for this reason, members of the genus Streptosporangium can produce enormous bioactive metabolites such as platymycins (Takasawa et al., 1975), sinefugin (Cooper et al., 1990), angucyclinone (Boudjella et al., 2010), spoxazomicin (Inahashi et al., 2011a), iodinin (Sletta et al., 2014), and sporaridin and sporazepin (Kornsakulkarn et al., 2014). Therefore, the isolation of novel species of this genus from different extreme environments should provide access to new bioactive products with significant therapeutic potential and contribute to an understanding of their ecological roles. Actinobacteria diversity and their biological activities are also a prospective field of research in the Saharan soils of Algeria as several new taxa of this phylum have been reported from these soils (Bouras et al., 2014) and a significant number of antibiotic patents have recently been registered (Lamari et al., 2002a, b; Zitouni et al., 2004, 2005; Badji et al., 2006, 2007; Bouras et al., 2008; Merrouche et al., 2010; Boubetra et al., 2012) including those produced by Streptosporangium strains (Hacène et al., 1998; Boudjella et al., 2006, 2007, 2010). In the same context, a novel actinobacterium, with both antibacterial and antifungal activities, designated strain SG1T, was isolated from a Saharan soil sample collected from Béni-Abbès, Béchar (Saoura region, south-west Algeria). To discriminate and identify strain SG1T, a polyphasic approach was used. The results of phenotypic, chemotaxonomic and phylogenetic analyses indicated that the isolate represents a novel species in the genus Streptosporangium.

Strain SG1T was isolated by a dilution plating method on chitin–vitamin agar medium (Hayakawa & Nonomura, 1987) supplemented with polymyxin (25 mg l−1) after 21 days of incubation at 30 °C. The novel strain was transferred and purified on yeast extract-malt extract agar (ISP 2 medium of the International Streptomyces Project; Shirling & Gottlieb, 1966) and then was maintained as working cultures on ISP 2 agar slants at 4 °C.

The phenotypic characteristics of strain SG1T were examined by using several standard methods. For the determination of morphological characteristics, cells were grown on ISP media 2, 3, 4, 6 and 7 (Shirling & Gottlieb, 1966) at 30 °C for 28 days, and were observed under a light microscope (Model B1; Motic). ISCC-NBS colour charts were used for determining colony colour (Kelly & Judd, 1976).

The decomposition of adenine, aesculin, arbutin, cellulose, gelatin, guanine, hypoxanthine, milk casein, starch, Tween 80, l-tyrosine and xanthine, and also coagulation and peptonization of milk and reduction of nitrate were evaluated using the media of Gordon et al. (1974). Carbon source utilization was tested according to Gordon et al. (1974) and Williams et al. (1989). The temperature and pH ranges for growth and NaCl tolerance were determined on ISP 2 medium.

The isomer of diaminopimelic acid and whole-cell sugars were determined using standard procedures (Becker et al., 1964; Lechevalier & Lechevalier, 1970). Polar lipids were extracted, examined and identified using the two-dimen-
sional TLC procedure as described by Minnikin et al. (1977). Menaquinones were extracted and purified according to the method of Minnikin et al. (1984) and were analysed by HPLC (Kroppenstedt, 1982, 1985). Fatty acids were identified as described by Sasser (1990) using the Microbial Identification System (MIDI Sherlock version 6.1 (TSBA40 database).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product were carried out following the protocols of Rainey et al. (1996).

The almost-complete 16S rRNA gene sequence (1436 nt) of strain SG1T was determined and deposited under accession no. KU593506 in the GenBank data library. The identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon-e server (Kim et al., 2012). Multiple alignment with sequences from closely related species was performed by using the program CLUSTAL W (with default parameters) in MEGA version 6 (Tamura et al., 2013). Phylogenetic trees were reconstructed with the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1977) and maximum-likelihood (Felsenstein, 1981) algorithms in MEGA version 6 (Tamura et al., 2013). Evolutionary distances were calculated using the model of Jukes & Cantor (1969). Topologies of the resultant trees were evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

DNA–DNA relatedness values between strain SG1T and related type strains, ‘S. subfuscum’ DSM 46724, Streptosporangium pseudovulgare DSM 43181T, Streptosporangium fragi-ile DSM 43847T and Streptosporangium sandarakinorum DSM 45763T, were determined. DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion
The phenotypic properties that can be used to distinguish characteristics of strain SG1
vulgare DSM 43181.

More recently, Boubetra et al. (1983), using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multiclamp changer and a temperature controller with in situ temperature probe (Varian). DNA–DNA hybridization experiments were performed in duplicate in 2 % (w/v) NaCl.

The absence of glucosamine-containing phospholipids, but glucosamine-containing phospholipids were not found (Fig. S1, available in the online Supplementary Material). The absence of glucosamine-containing phospholipids has been also reported for some species of Streptosporangium, such as Streptosporangium subfuscum and S. pseudovulgare (Quintana & Goodfellow, 2012), Streptosporangium

Table 1. Cultural characteristics on different media of strain SG1 \( ^{T} \) and its closest relatives, ‘Streptosporangium subfuscum’ DSM 46724 and Streptosporangium pseudovulgare DSM 43181 \( ^{T} \)

<table>
<thead>
<tr>
<th>Medium</th>
<th>AM</th>
<th>SM</th>
<th>AM</th>
<th>SM</th>
<th>AM</th>
<th>SM</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISP 2</td>
<td>White to pinkish white</td>
<td>Brownish yellow</td>
<td>Pinkish white</td>
<td>Brownish orange</td>
<td>White</td>
<td>Orange</td>
</tr>
<tr>
<td>ISP 3</td>
<td>Pink</td>
<td>Yellow orange</td>
<td>Pinkish white</td>
<td>Brownish pink</td>
<td>Pink</td>
<td>Yellowish brown to orange</td>
</tr>
<tr>
<td>ISP 4</td>
<td>Pink</td>
<td>Light pink</td>
<td>None</td>
<td>Vivid red</td>
<td>White</td>
<td>Cream</td>
</tr>
<tr>
<td>ISP 6</td>
<td>None</td>
<td>Brown</td>
<td>None</td>
<td>Brownish orange</td>
<td>None</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>ISP 7</td>
<td>Pink</td>
<td>Cream to pinkish cream</td>
<td>Pinkish white</td>
<td>Light yellowish pink</td>
<td>White</td>
<td>Light pink</td>
</tr>
</tbody>
</table>

The cell-wall diamino acid in the peptidoglycan layer of strain SG1 \( ^{T} \) was meso-diaminopimelic acid. Glucose and ribose were the only sugars found in the hydrolysates. Madurose, the usually diagnostic sugar in Streptosporangium species, was not detected. Stackebrandt et al. (1994) reported that madurose was absent from S. fragile, Streptosporangium nondastaticum and Streptosporangium violaceochromogenes; however, it was present only in trace amounts in Streptosporangium album. More recently, Boubetra et al. (2016) and Chaabane Chaouch et al. (2016) reported the absence of this sugar in S. algeriense and S. saharense, respectively. The menaquinone profile of strain SG1 \( ^{T} \) was composed of two major components, MK-9(H4) (62.8 %) and MK-9(H6) (22.7 %), with minor amounts of MK-9(H8) (5.6 %), MK-9(H9) (1.7 %), MK-10 (H4) (0.8 %) and MK-10(H2) (0.4 %). The polar lipids comprised diphosphatidylglycerol, phosphatidylglycerol, phosphatidylmethylethanolamine, phosphatidylethanolamine, phosphatidylhydroxymethylethanolamine, phosphatidylhydroxyethanolamine, phosphatidylinositol, phosphatidylinositol mannosides, an unknown phospholipid and unknown lipids, such as Streptosporangium subfuscum (Quintana & Goodfellow, 2012), Streptosporangium subfuscum DSM 46724 and S. pseudovulgare DSM 43181 \( ^{T} \).

Table 2. Differential characteristics between strain SG1 \( ^{T} \) and its closest related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth on sole carbon sources (1.0 %, w/v)</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Lactose</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Sorbitol</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D-Xylose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis of gelatin</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis of starch</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis of Tween 80</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Production of nitrate reductase</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Growth at 50 °C</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>
Streptosporangium becharense sp. nov.

Fig. 1. Phylogenetic tree of species of the genus Streptosporangium calculated from almost-complete 16S rRNA gene sequences (1436 nt) using Jukes & Cantor (1969) evolutionary distance methods and the neighbour-joining method of Saitou & Nei (1987). This illustrates the taxonomic position of strain SG1T relative to other species of the genus. Asterisks indicate branches of the tree that were also found using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1977) tree-making algorithms. Bootstrap values above 50% (percentages of 1000 replications) are indicated. Actinomadura madurae DSM 43067T was used as the outgroup. Bar, 0.005 nucleotide substitutions per site.

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Table S1. All chemotaxonomic properties of strain SG1T were consistent with its classification in the genus Streptosporangium.

Analysis of the almost-complete 16S rRNA gene sequence of strain SG1T (1436 bp) using the EzTaxon-e server revealed highest similarities to 'S. subfuscinum' DSM 46724 (99.7%), S. pseudovulgare DSM 43181T (98.7%), S. fragile DSM 43847T (98.6%) and S. sandarakinum DSM 45763T (98.5%). The phylogenetic tree based on the neighbour-joining algorithm (Fig. 1) showed that strain SG1T formed a distinct phyletic line with its closest neighbour 'S. subfuscumin' DSM 46724 at a bootstrap value of 100% in the
neighbour-joining tree; corresponding bootstrap values in the maximum-likelihood and maximum-parsimony trees were 92 and 98 %, respectively (Fig. S2).

The taxonomic status of strain SG1T was further investigated by DNA–DNA hybridization. The results confirmed that this strain belongs to a novel species; indeed, low DNA–DNA relatedness values were found between strain SG1T and ‘S. subfuscinum’ DSM 46724 (58.1 ± 0.1 %), S. pseudoduvugnye DSM 43181T (38.8 ± 2.8 %), S. fragile DSM 43847T (18.5 ± 3 %) and S. sandarakinum DSM 45763T (18.2 ± 1.6 %), which were significantly lower than the 70 % cut-off limit for delineating bacterial species (Wayne et al., 1987).

The phenotypic and genotypic data from this study indicate that strain SG1T merits classification as representative of a novel species of the genus Streptosporangium, for which the name Streptosporangium becharense sp. nov. is proposed.

Description of Streptosporangium becharense sp. nov.

Streptosporangium becharense sp. nov. (be.char.en’se. N.L. neut. adj. becharense pertaining to Béchar, south-west Algeria, the source of the soil from which the type strain was isolated).

Aerobic, Gram-stain-positive, mesophilic, non-motile, filamentous actinobacterium. Forms branched non-fragmenting substrate mycelium, brownish yellow on ISP 2, yellow orange on ISP 3, light pink on ISP 4, brown on ISP 6 and cream to pinkish cream on ISP 7. Aerial mycelium is abundant and produces spherical sporangia at the ends of short sporangiospores. The sporangiospores are not motile. Aerial mycelium is non-fragmenting, white to pinkish white on ISP 2 medium and pink on ISP 3, ISP 4 and ISP 7 media. Diffusible pigments are not produced. Melanoid pigments are not detected on ISP 6 or ISP 7 media. The pH range for growth is 6.0–12.0, with optimum growth at pH 7.5–8.5. The temperature range for growth is 20–42 °C, with optimal growth at 25–37 °C. The NaCl tolerance range is 0–2 % (w/v). Utilizes L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannitol, D-mannose, L-rhamnose, salicin, D-xylene, acetate, L-lactate, pyruvate, propionate and succinate as sole carbon source, but not adonitol, glycerol, myo-inositol, D-lactose, melezitose, melibiose, α-D-galactoside, raffinose, D-ribose, D-sorbitol, sucrose, trehalose, benzoate, butyrate, citrate, oxalate or D-tartrate. Hydrolyses casein, gelatin, starch, L-tyrosine, ascelin and arbutin, but not adenine, cellulose, guanine, hypoxanthine, Tween 80 or xanthine. Positive for nitrate reduction and milk peptonization, but negative for milk coagulation. Contains ribose and glucose as whole-cell sugars, but not madurose. The polar lipid profile contains diphasphatidylglycerol, phosphatidylglycerol, phosphatidyldimethylethanolamine, phosphatidylethanolamine, phosphatidylhydroxymethylethanolamine, phosphatidylhydroxyethanolamine, phosphatidylinositol and phosphatidylinositol

mannosides. The major fatty acids are C17:1ω8c, iso-C16:0, 10-methyl C17:0 and C18:1ω9c. The predominant menaquinones are MK-9(H2) and MK-9(H4). Small amounts of MK-9 (H8), MK-9(H6), MK-10(H8) and MK-10(H12) are also detected.

The type strain, SG1T (=DSM 46887=CECT 8961T), was isolated from a Saharan soil sample collected from Béni-Abbès, Béchar (south-west Algeria).

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References


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