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, Bechar (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, phosphatidylmethylmethanolamine, phosphatidylethanolamine, phosphatidyldihydroxymethylmethanolamine, phosphatidyldihydroxyethanolamine, phosphatidylglycerol, phosphatidylglycerolphosphate and phosphatidylinositol mannosides. The predominant cellular fatty acids were C17:0 3-OH, C17:1 ω8c and C17:0 10-methyl. The 16S rRNA gene sequence similarity analysis 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 %), ‘Streptosporangium fragile’ DSM 43847T (98.6 %) and ‘Streptosporangium sandarakinum’ DSM 45763T (98.5 %). Phylogenetic analyses based on 16S rRNA 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’
Strain SG1\textsuperscript{T} was isolated by a dilution plating method on chitin–vitamin agar medium (Hayakawa & Nonomura, 1987) supplemented with polymyxin (25 mg L\textsuperscript{-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 Streptomycetes Project; Shirling & Gottlieb, 1966) and then was maintained as working cultures on ISP 2 agar slants at 4 °C.

The phenotypic characteristics of strain SG1\textsuperscript{T} 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 peptidization 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-dimensional 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 SG1\textsuperscript{T} 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 SG1\textsuperscript{T} and related type strains, ‘S. subfuscum’ DSM 46724, Streptosporangium pseudovulgare DSM 43181\textsuperscript{T}, Streptosporangium fragi-ile DSM 43847\textsuperscript{T} and Streptosporangium sandarakinorum DSM 45763\textsuperscript{T}, were determined. 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 multiecell changer and a temperature controller with in situ temperature probe (Varian). DNA–DNA hybridization experiments were performed in duplicate in 2×SSC in the presence of 10 % formamide at 71°C.

Strain SG1T had characteristics typical of the genus Streptosporangium. It developed globose sporangia at the ends of short sporangiophores on aerial mycelium. The sporangiophores were not motile. Aerial and substrate mycelium were well developed without fragmentation. Substrate mycelium colour of strain SG1T was 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. The aerial mycelium was white to pinkish white on ISP 2 and pink on the remaining media tested, except on ISP 6 medium in which aerial mycelium was not produced (Table 1). Diffusible pigments were not detected on any of the media tested. Melanoid pigments were not produced on ISP 6 or ISP 7 media. Strain SG1T grew at 20–42°C (optimum 25–37°C), at pH 6.0–12.0 (optimum pH 7.5–8.5) and with 0–2% (w/v) NaCl. The phenotypic properties that can be used to distinguish strain SG1T from S. subfuscum DSM 46724 and S. pseudovulgare DSM 43181T are shown in Tables 1 and 2. The main characteristics of strain SG1T are summarized in the species description.

The cell-wall diamino acid in the peptidoglycan layer of strain SG1T 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 nondiastaticum 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 SG1T was composed of two major components, MK-9(H4) (62.8 %) and MK-9(H8) (22.7 %), with minor amounts of MK-9(H0) (5.6 %), MK-9(H4) (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, 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 subfuscum DSMT66.

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>3</th>
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<tbody>
<tr>
<td>Growth on sole carbon sources (1.0%, w/v)</td>
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<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>D-Galactose</td>
<td>+</td>
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<td>–</td>
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<tr>
<td>myo-Inositol</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>D-Lactose</td>
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<tr>
<td>D-Mannitol</td>
<td>+</td>
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<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>D-Sorbitol</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>Sucrose</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
<td>D-Xylose</td>
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<tr>
<td>Hydrolysis of gelatin</td>
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<td>Hydrolysis of starch</td>
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<tr>
<td>Hydrolysis of Tween 80</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<td>Production of nitrate reductase</td>
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<td>Growth at 50°C</td>
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Table 1. Cultural characteristics on different media of strain SG1T and its closest relatives, ‘Streptosporangium subfuscum’ DSM 46724 and Streptosporangium pseudovulgare DSM 43181T.

Data are from this study. AM, aerial mycelium; MS, substrate mycelium.

Table 2. Differential characteristics between strain SG1T and its closest related species.

<table>
<thead>
<tr>
<th>Growth at sole carbon sources (1.0%, w/v)</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>L-Arabinose</td>
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<td>+</td>
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<tr>
<td>D-Galactose</td>
<td>+</td>
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<tr>
<td>myo-Inositol</td>
<td>–</td>
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<td>D-Lactose</td>
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<td>D-Mannitol</td>
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<td>L-Rhamnose</td>
<td>+</td>
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<td>D-Sorbitol</td>
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<td>Sucrose</td>
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<td>Production of nitrate reductase</td>
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</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 SG1\(^T\) 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 43067\(^T\) was used as the outgroup. Bar, 0.005 nucleotide substitutions per site.

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**Table S1. All chemotaxonomic properties of strain SG1\(^T\) were consistent with its classification in the genus *Streptosporangium.*

Analysis of the almost-complete 16S rRNA gene sequence of strain SG1\(^T\) (1436 bp) using the EzTaxon-e server revealed highest similarities to *S. subfuscum* DSM 46724 (99.7\%), *S. pseudovulgare* DSM 43181\(^T\) (98.7\%), *S. fragile* DSM 43847\(^T\) (98.6\%) and *S. sandarakinum* DSM 45763\(^T\) (98.5\%). The phylogenetic tree based on the neighbour-joining algorithm (Fig. 1) showed that strain SG1\(^T\) formed a distinct phyletic line with its closest neighbour *S. subfuscum* 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. subfuscum* DSM 46724 (58.1 ± 0.1 %), *S. pseudounguere* 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 sporangiophores. 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-xylose, acetate, L-lactate, pyruvate, propionate and succinate as sole carbon source, but not adonitol, glycerol, *myo*-inositol, D-lactose, melezitose, melibiоз, methyl α-D-glucoside, raffinose, D-ribose, D-sorbitol, sucrose, trehalose, benzoate, butyrate, citrate, oxalate or D-tartrate. Hydrolysates casein, gelatin, starch, L-tyrosine, ascinil 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 diphosphatidylglycerol, phosphatidylglycerol, phosphatidylmethylthanolamine, phosphatidylethanolamine, phosphatidylhydroxymethylthanolamine, phosphatidylglyceroxethanolamine, 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 (H6), MK-9(H8), MK-10(H6) and MK-10(H4) are also detected.

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

**Acknowledgements**

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**References**


