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Saccharothrix hoggarensis sp. nov., an actinomycete isolated from Saharan soil

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An actinomycete, designated SA181^T, was isolated from Saharan soil in the Hoggar region (south Algeria) and was characterized taxonomically by using a polyphasic approach. The morphological and chemotaxonomic characteristics of the isolate were consistent with the genus *Saccharothrix*, and 16S rRNA gene sequence analysis confirmed that strain SA181^T was a novel member of the genus *Saccharothrix*. DNA–DNA hybridization values between strain SA181^T and its closest phylogenetic neighbours, the type strains of *Saccharothrix longispora*, *Saccharothrix texasensis* and *Saccharothrix xinjiangensis*, were clearly below the 70 % threshold. The genotypic and phenotypic data showed that the isolate represents a novel species of the genus *Saccharothrix*, for which the name *Saccharothrix hoggarensis* sp. nov. is proposed, with the type strain SA181^T (=DSM 45457^T =CCUG 60214^T).

The genus *Saccharothrix* was described for the first time by Labeda *et al.* (1984). Several species of this genus have been transferred to other new taxa, including the genera *Lentzea* (Yassin *et al.*, 1995), *Crossiella* (Labeda, 2001), *Lechevalieria* (Labeda *et al.*, 2001), *Goodfellowia* (Labeda & Kroppenstedt, 2006) and *Umezawaea* (Labeda & Kroppenstedt, 2007). Currently, 12 species of the genus *Saccharothrix* have been described based on chemical analysis, physiological properties, cellular fatty acid composition, phylogeny and DNA–DNA hybridization data. The genus is characterized by fragmentation of both substrate and aerial mycelia into rods and ovoid elements, type III cell-wall *meso*-diaminopimelic acid without glycine, galactose, rhamnose and small amounts of mannose as diagnostic whole-cell sugars, a phospholipid type PII (phosphatidylethanolamine) or PIV (phosphatidylethanolamine and glucosamine-containing phospholipids) pattern (Labeda & Lechevalier, 1989), the presence of MK-9(H_4) as the predominant menaquinone and the absence of mycolic acids (Labeda & Kroppenstedt, 2000).

During a study of Saharan actinomycetes, strain SA181^T was isolated from a soil sample from Tamanrasset (22° 47′ 0″ N 5° 31′ 0″ E), an arid area in the south of Algeria (Hoggar), by a dilution-plating method using humic acid-vitamin agar (Hayakawa & Nonomura, 1987) supplemented with 50 μg actidione ml^{-1}.

Cultural characteristics were investigated on media from the International *Streptomyces* Project (ISP; Shirling & Gottlieb, 1966), nutrient agar and Bennett's agar (Waksman, 1961). The degree of growth, aerial mycelia, pigmentation and other features were recorded after 7, 14 and 21 days of incubation at 30 °C. Peptone-yeast extract-iron agar (ISP 6) and tyrosine agar (ISP 7) were used to determine melanoid pigment production. 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). Strain SA181^T exhibited good growth on ISP 2, ISP 3, ISP 4, nutrient agar and Bennett's agar, with greyish blue, yellowish white and pinkish brown aerial mycelium on ISP 2, nutrient agar and Bennett's agar, respectively. No aerial mycelium was observed on ISP 3 or ISP 4. The colour of substrate mycelium was pale to light yellow on nutrient agar, moderate to deep yellowish brown on ISP 2 and ISP 4, light brown on Bennett's agar and dark brown on ISP 3. The substrate mycelium was fragmented into non-motile rods. The isolate did not produce diffusible or melanoid pigments. Spores and mycelia were examined by light

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SA181^T is HQ399564.

Two supplementary figures are available with the online version of this paper.

microscopy (B1 series; Motic) and scanning electron microscopy after 2 weeks on ISP 2. The substrate mycelium exhibited an abundant fragmentation on both solid and liquid media. The aerial mycelium was fragmented into rod-shaped spores ($0.8–1.0 \times 1.0–1.5$ μm). The spores had a smooth surface (Fig. 1) and were non-motile. No endospores, sporangia, sclerotia or synnemata were observed.

Chemotaxonomic characteristics were studied using biomass obtained after 4 days of incubation at 30 °C in shake flasks containing ISP 2, harvested by centrifugation and washed several times with 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 and mycolic acids were analysed using the procedure of Minnikin *et al.* (1977, 1980). The fatty acid composition was determined by the method of Sasser (1990), using the TSBA40 database in the Sherlock Microbial Identification System version (MIDI) version Sherlock 6.1. The menaquinones were extracted following the procedure of Minnikin *et al.* (1984) and analysed by HPLC (Kroppenstedt, 1982, 1985). Strain SA181^T contained *meso*-diaminopimelic acid with alanine and glutamic acid, but not glycine. The whole-cell hydrolysates contained galactose, rhamnose, traces of mannose and ribose, typical of cell-wall type IIIE (Stackebrandt *et al.*, 1994). The diagnostic phospholipid detected was phosphatidylethanolamine, which corresponds to phospholipid type PII (Lechevalier et *al.*, 1977) (Fig. S1, available in IJSEM Online). Mycolic acids were not detected. The predominant fatty acid was iso-branched hexadecanoate (iso-$C_{16:0}$), and significant amounts of iso-$C_{15:0}$, 9-methyl-$C_{16:0}$, anteiso-$C_{17:0}$, iso-$C_{16:1}$ H, iso-$C_{16:0}$ 2-OH, anteiso-$C_{15:0}$, iso-$C_{17:0}$, *cis*9-$C_{17:1}$ and anteiso-$C_{17:0}$ 2-OH were also detected (Table 1). Furthermore, a small fraction (3.04 %) of iso-$C_{15:0}$ 2-OH could be detected in summed feature 4. Strain SA181^T contained menaquinones MK-9(H_4), MK-7(H_4), MK-10(H_4), MK-10(H_0) and MK-11(H_4) (55, 12, 9, 3 and 2 %, respectively, with traces of MK-9(H_0), MK-9(H_2), MK-9(H_6) and MK-10(H_2). The morphological and chemical characteristics described above clearly support the placement of strain SA181^T within the genus *Saccharothrix*.

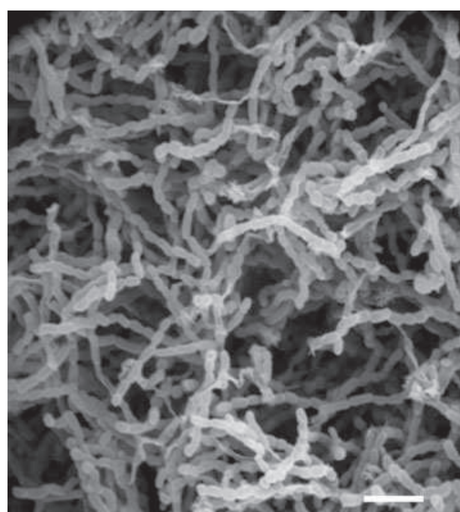

Fig. 1. Scanning electron micrograph of spore chains of strain SA181^T grown on yeast extract-malt extract agar (ISP 2) for 10 days at 30 °C. Bar, 5 μm.

Media and procedures for the determination of physiological features and carbon source utilization have been described by Gordon *et al.* (1974) and Williams *et al.* (1989). The results showed that strain SA181^T is physiologically different from its closest neighbours in the genus *Saccharothrix*, as can be seen from the differential characters given in Table 2. The physiological characteristics of strain SA181^T are given in the species description.

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 obtained was compared with sequences in public databases as well as in the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012), a web-based tool for the identification of

Table 1. Cellular fatty acid compositions of strain SA181^T and its most closely related neighbours in the genus *Saccharothrix*

Strains: 1, *Saccharothrix hoggarensis* sp. nov. SA181^T; 2, *S. longispora* DSM 43749^T; 3, *S. texasensis* DSM 44231^T; 4, *S. xinjiangensis* DSM 44896^T. All data were taken from this study. G and H indicate that the double bonds are in different locations. Fatty acid identities were determined using the TSBA40 database in the Sherlock Microbial Identification System version 6.1.

Fatty acid (%)	1	2	3	4
iso-$C_{14:0}$	1.34	5.40	2.76	2.80
$C_{14:0}$	–	3.17	–	0.15
iso-$C_{15:0}$	14.87	14.60	23.97	5.70
anteiso-$C_{15:0}$	4.03	5.06	5.63	7.93
iso-$C_{16:1}$ H	6.81	3.72	4.55	–
iso-$C_{16:1}$ G	–	–	–	5.67
iso-$C_{16:0}$	27.00	24.63	24.73	24.89
*cis*9-$C_{16:1}$	–	1.64	–	6.94
$C_{16:0}$	0.27	6.40	0.26	1.19
9-Methyl-$C_{16:0}$	9.45	2.13	4.59	2.66
iso-$C_{17:0}$	3.99	3.14	3.67	1.83
anteiso-$C_{17:0}$	9.38	4.37	4.35	9.04
*cis*9-$C_{17:1}$	3.72	6.60	4.90	13.18
iso-$C_{16:0}$ 2-OH	5.80	–	4.35	4.45
$C_{17:0}$	0.38	2.19	0.59	1.94
10-Methyl-$C_{17:0}$	1.03	0.47	3.55	1.50
*cis*9-$C_{18:1}$	0.46	7.71	0.22	3.41
anteiso-$C_{17:0}$ 2-OH	2.53	–	1.59	2.28
Summed feature 4	3.04	–	–	–

Table 2. Differential phenotypic characteristics of strain SA181^T and its most closely related neighbours in the genus *Saccharothrix*

Strains: 1, *Saccharothrix hoggarensis* sp. nov. SA181^T; 2, *S. longispora* DSM 43749^T; 3, *S. texasensis* DSM 44231^T; 4, *S. xinjiangensis* DSM 44896^T. All data were taken from this study.

Characteristic	1	2	3	4
Decomposition of:				
Adenine	−	+	−	−
Hypoxanthine	+	−	+	+
Production of:				
Nitrate reductase	−	+	+	+
Assimilation of:				
Butyrate	+	−	+	−
Citrate	−	+	−	−
Acid from:				
Inositol	−	−	+	+
Lactose	−	+	+	+
Mannitol	−	+	+	+
Melibiose	+	−	+	+
Methyl α-D-glucoside	−	−	+	+
Raffinose	−	−	+	+
Salicin	−	+	−	−
Sorbitol	+	−	−	−
Growth at/with:				
45 °C	+	−	−	−
4 % (w/v) NaCl	+	−	+	−
5 % (w/v) NaCl	+	−	+	−

prokaryotes based on 16S rRNA gene sequences from type strains. Phylogenetic analysis was conducted using MEGA version 5 (Tamura *et al.*, 2011). The 16S rRNA gene sequence of strain SA181^T was aligned with neighbouring sequences using CLUSTAL W (with default parameters; Thompson *et al.*, 1994). Phylogenetic trees were constructed 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). Bootstrap analysis (Felsenstein, 1985) was performed to evaluate the reliability of the tree topology.

The phylogenetic relationships between strain SA181^T and members of the genus *Saccharothrix* are shown in the neighbour-joining dendrogram (Fig. 2). Maximum-parsimony and maximum-likelihood calculations resulted in similar tree topologies (Fig. S2). The similarity of the 16S rRNA gene sequence of strain SA181^T to those of other members of the genus *Saccharothrix* ranged from 96.8 to 98.9 %. Strain SA181^T displayed highest 16S rRNA gene sequence similarity with *Saccharothrix longispora* NRRL B-16113^T (98.9 %), *Saccharothrix xinjiangensis* NBRC 101911^T (98.4 %) and *Saccharothrix texasensis* NRRL B-61634^T (98.2 %) and 16S rRNA gene sequence similarities with the other remaining members of the genus *Saccharothrix* were below 97.9 %.

For DNA–DNA hybridization, cells were disrupted by using a French pressure cell (Thermo Spectronic). 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 multicell changer and a temperature controller with *in-situ* temperature probe (Varian). DNA–DNA hybridization experiments were done in duplicate in 2× SSC in the presence of 10 % formamide at 71 °C. DNA of strain SA181^T was hybridized with that of its closest phylogenetic neighbours. Mean DNA–DNA relatedness values between strain SA181^T and *S. longispora* DSM 43749^T, *S. texasensis* DSM 44231^T and *S. xinjiangensis* DSM 44896^T were 16.05 % (14.7 and 17.4 %), 50.05 % (46.5 and 53.6 %) and 22.0 % (24.2 and 19.8 %), respectively, which are clearly below the 70 % threshold proposed by Wayne *et al.* (1987) for the delineation of separate species.

A comparison of physiological characteristics of the isolate and *S. longispora* DSM 43749^T showed differences in the utilization of adenine, hypoxanthine, sodium butyrate, sodium citrate, lactose, mannitol, melibiose, salicin and sorbitol, production of nitrate reductase, growth at 45 °C and with 4 and 5 % (w/v) NaCl. Furthermore, *S. texasensis* DSM 44231^T showed differences in the utilization of inositol, lactose, mannitol, methyl α-D-glucoside, raffinose and sorbitol, production of nitrate reductase and growth at 45 °C, and *S. xinjiangensis* DSM 44896^T showed differences in the utilization of sodium butyrate, inositol, lactose, mannitol, methyl α-D-glucoside, raffinose and sorbitol, production of nitrate reductase and growth at 45 °C and with 4 and 5 % (w/v) NaCl.

Recently, '*Saccharothrix yanglingensis*' was described (Yan *et al.*, 2012). Strain SA181^T differs from that species in several morphological and physiological characteristics, including colour of substrate and aerial mycelia, production of diffusible pigment, degradation of gelatin, hypoxanthine and tyrosine, reduction of nitrate, use of arabinose, lactose, melibiose, rhamnose, xylose and sorbitol and growth at pH 5, at 45 °C and in the presence of 4 % (w/v) NaCl. Unlike strain SA181^T, '*S. yanglingensis*' contains no rhamnose in its whole cell.

All data support the conclusion that strain SA181^T represents a novel species of the genus *Saccharothrix*, for which we propose the name *Saccharothrix hoggarensis* sp. nov.

Description of *Saccharothrix hoggarensis* sp. nov.

Saccharothrix hoggarensis (hog.gar.en′sis. N.L. fem. adj. *hoggarensis* pertaining to Hoggar, the source of the soil from which the type strain was isolated).

Aerobic, Gram-positive, filamentous actinomycete. Aerial mycelium is light greyish blue on ISP 2, yellowish white on

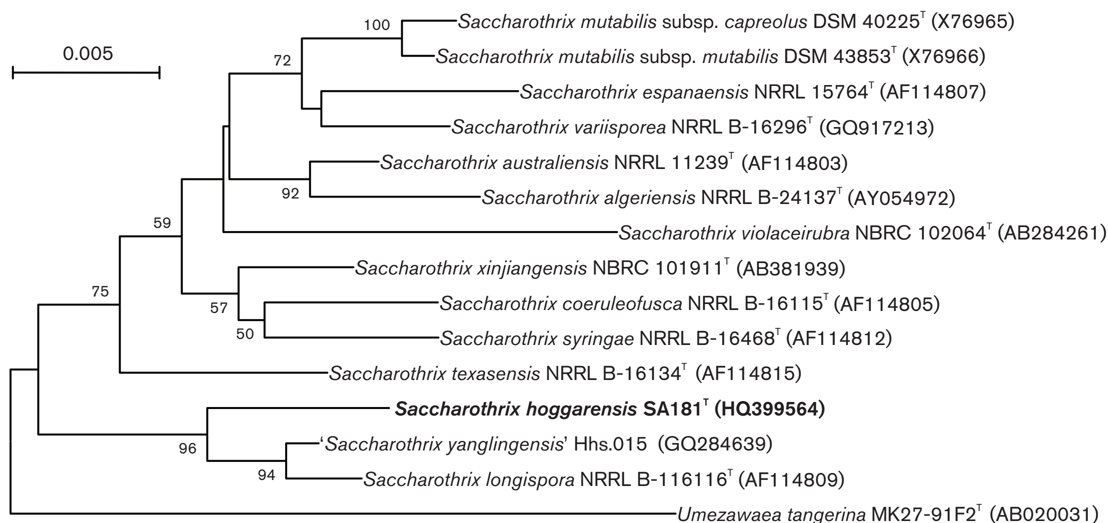

Fig. 2. Neighbour-joining phylogenetic tree based on almost-complete 16S rRNA gene sequences showing the position of strain SA181^T in the genus *Saccharothrix*. This illustrates the taxonomic position of strain SA181^T relative to the other species of the genus, including '*Saccharothrix yanglingensis*' (Yan *et al.*, 2012). Bootstrap values (>50 %) based on 1000 resamplings are shown at branch nodes. Bar, 0.005 substitutions per site.

nutrient agar and pinkish brown on Bennett's agar and is well fragmented into rod-shaped spores (0.8–1.0 × 1.0–1.5 μm). Substrate mycelium is pale to light yellow on nutrient agar, moderate to deep yellowish brown on ISP 2 and ISP 4, light brown on Bennett's agar and dark brown on ISP 3. The substrate mycelium fragments into non-motile rods. Diffusible and melanoid pigments are not produced. Growth occurs at 20–45 °C (optimum 30 °C), but not 10 °C, at pH 6–9 (optimum pH 7), but not at pH 5 or 11, and with 4–5 % (w/v) NaCl, but not with 7 %. Aesculin, casein, gelatin, hypoxanthine, Tween 80, starch, tyrosine and urea are degraded, but adenine, arbutin, testosterone and xanthine are not. Utilizes L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, glycerol, maltose, melibiose, D-ribose, L-rhamnose, D-sorbitol, sucrose, D-xylose, acetate, butyrate, lactate, propionate, pyruvate and succinate as carbon sources, but adonitol, lactose, D-mannitol, melezitose, *myo*-inositol, D-mannose, methyl α-D-glucoside, raffinose, salicin, trehalose, benzoate, citrate, oxalate and tartrate are not utilized. L-Proline is used as a source of nitrogen, but L-alanine and L-serine are not utilized. Nitrate reductase is not produced. Resistant to (μg ml^{-1}) chloramphenicol (30), erythromycin (15) and novobiocin (5). The cell wall is type IIIE (*meso*-diaminopimelic acid, galactose, mannose, rhamnose and ribose in whole-cell hydrolysates). Exhibits phospholipid type PII (phosphatidylethanolamine, with phosphatidylmethylethanolamine, phosphatidylinositol, phosphatidylinositol mannosides and diphosphatidylglycerol). The predominant menaquinones are MK-9(H_4), MK-7(H_4), MK-10(H_4), MK-10(H_0) and MK-11(H_4), with traces of MK-9(H_0), MK-9(H_2), MK-9(H_6) and MK-10(H_2). The major fatty acids are iso-$C_{16:0}$, iso-$C_{15:0}$, 9-methyl-$C_{16:0}$ and anteiso-$C_{17:0}$.

The type strain is SA181^T (=DSM 45457^T =CCUG 60214^T), isolated from a Saharan soil sample collected from Hoggar region (south Algeria).

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