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ORIGINAL ARTICLE

Saccharopolyspora ghardaiensis sp. nov., an extremely halophilic actinomycete isolated from Algerian Saharan soil

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A novel halophilic actinomycete, strain designated H53^T, was isolated from a Saharan soil sample collected from Chaâbet Ntissa, Béni-isguen, Ghardaïa (South of Algeria) and was characterized taxonomically by means of polyphasic approach. Optimal growth was found to occur at 30–35 °C, pH 6–7 and in the presence of 15–25% (w/v) NaCl. The strain was observed to produce abundant aerial mycelium, which formed long chains of rod-shaped spores at maturity, and well developed and fragmented substrate mycelium. The cell wall was determined to contain *meso*-diaminopimelic acid; the diagnostic whole-cell sugars were arabinose and galactose. The predominant menaquinones were found to be MK-9(H_4) and MK-9(H_6). The predominant cellular fatty acids were determined to be iso- and anteiso-$C_{17:0}$, iso-$C_{15:0}$, and *cis*9 iso-$C_{17:1}$. The diagnostic phospholipid detected was phosphatidylcholine. The morphological and chemotaxonomic characteristics of the strain were consistent with those of members of the genus *Saccharopolyspora*. Phylogenetic analyses on the basis of the 16S ribosomal RNA (rRNA) gene sequence showed that this strain formed a distinct phyletic line within the radiation of the genus *Saccharopolyspora*. The 16S rRNA sequence similarities between strain H53^T and other members of the genus *Saccharopolyspora* ranged from 92.1 to 94.3%. The DNA G + C content of strain H53^T was 72.6%. The genotypic and phenotypic data showed that the strain H53^T represents a novel species of the genus *Saccharopolyspora*, for which the name *Saccharopolyspora ghardaiensis* sp. nov. is proposed, with the type strain H53^T (= DSM 45606^T = CCUG 63370^T = CECT 8304^T).

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INTRODUCTION

The genus *Saccharopolyspora* was first described by Lacey and Goodfellow[1] for actinomycetes isolated from spontaneously heated sugarcane bagasse, and was assigned to the family *Pseudonocardiaceae*.[2] Nevertheless, most species of this genus were isolated from soil samples. At the time of writing, the genus comprises 23 recognized species, with *Saccharopolyspora hirsuta* as the type species.[1] Most species of the genus *Saccharopolyspora* were non-halophilic, and some of them were halotolerant. However, only these species of this genus were reported to be extremely halophilic: *S. halophila*,[3] *S. qijiaojingensis*[4] and *S. lacisalsi*.[5]

Members of the genus *Saccharopolyspora* were characterized by extensively branched substrate mycelium that may fragment into rod-shaped and non-motile elements, and aerial mycelium that may segment into bead-like chains of spores. The strains of this genus were characterized chemotaxonomically by the presence of *meso*-diaminopimelic acid in the cell wall, arabinose and galactose as diagnostic sugars in whole-cell hydrolysates (wall chemotype IVA[6]), iso and anteiso-branched chain fatty acids, major amounts of phosphatidylcholine (phospholipid pattern PIII[7]), and MK-9(H_4) as the predominant menaquinone, but lack mycolic acids.[8] The DNA base compositions of members of this genus fall within the range of 66–77 mol% G + C.[9] Several strains and species belonging to the genus *Saccharopolyspora* produce different bioactive secondary metabolites, such as the enzyme inhibitor CL307-24[10] and the antimicrobial compounds erythromycin[11] and spinosyns.[12]

During our investigation of actinomycetes from Saharan soils, the strain H53^T was isolated. This strain showed a good antibacterial and antifungal activity, especially against *Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (ATCC 9314) and *Mucor ramannianus* (NRRL 1829). In this paper we describe the characterization of new species in the genus *Saccharopolyspora*.

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MATERIALS AND METHODS

Isolation of actinomycete strain

During our investigations on extremophilic actinomycetes diversity in Algerian Saharan soils, the halophilic strain H53^T was isolated from a non-saline soil sample (electrical conductivity = 0.15 mS cm^{-1}) collected from Chaâbet Ntissa (32°27′52″N, 3°40′19″E), Béni-isguen (Mzab), Ghardaïa province (South of Algeria), by a dilution-plate agar method using humic acid–vitamin agar medium[13] supplemented with actidione (50 mg l^{-1}) and 20% (w/v) NaCl at 30 °C for 45 days. The strain, which formed a white colony, was purified and maintained at 4 °C on complex medium (CM) agar[14] containing 20% (w/v) NaCl. Strain H53^T was deposited in the German Collection of Microorganisms and Cell Cultures as strain DSM 45606^T, in the Culture Collection, University of Göteborg, as strain CCUG 63370^T, and in the Spanish Type Culture Collection as strain CECT 8304^T.

Cultural and micro-morphological characteristics

Cultural characteristics of strain H53^T were investigated after 7, 14 and 21 days of incubation at 30 °C using the media of the International *Streptomyces* Project, ISP 2 and ISP 4,[15] CM agar[14] and nutrient agar (bacteriological peptone, 5 g; meat extract, 1 g; yeast extract, 2 g; NaCl, 5 g; agar, 18 g; in 1000 ml deionized water; pH 7.2). The colors of the substrate, and aerial mycelia and any soluble pigments produced were determined by comparison with ISCC–NBS color charts.[16] The morphological characteristics of strain H53^T, including spore size, spore-chain morphology and surface ornamentation, were examined by light microscope (Motic, B1 Series, Hong Kong) and scanning electron microscope (model S450; Hitachi, Japan) after two weeks growth on ISP 2 medium at 30 °C. All media used for morphological characteristics contained 15% (w/v) NaCl.

Physiological characterization

Physiological tests were used to characterize the actinomycete strain H53^T in comparison with its closest relative halophilic species (*S. lacisalsi* TRM 40133^T and *S. qijiaojingensis* YIM 91168^T). Growth at different temperatures (10, 15, 20, 25, 28, 30, 32, 35, 40, 45 and 47 °C), various pH values (5, 6, 7, 8 and 9) and NaCl concentrations (0, 7, 10, 15, 20, 25, 28, 30, 32 and 35%; w/v), and also in the presence of antibiotics, were determined by using nutrient agar medium, with the cultures incubated for 21 days at 30 °C. All media used for physiological tests contained 15% (w/v) NaCl (except for the NaCl concentration test). Production of acid from carbohydrates, and decarboxylation of organic acids were studied using the method of Gordon *et al.*[17] Degradation of different other organic compounds was evaluated as described by Goodfellow.[18] Lysozyme sensitivity and production of nitrate reductase were determined according to the methods of Gordon and Barnett[19] and Marchal *et al.*,[20] respectively. The production of melanoid pigments was evaluated on ISP 6 and ISP 7 media as recommended by Shirling and Gottlieb.[15]

Chemotaxonomic characterization

For the chemotaxonomic analyses, strain H53^T was grown in CM broth containing 15% (w/v) NaCl at 30 °C for 10 days on a rotary shaker (250 r.p.m.). Biomass was harvested by centrifugation at 3500 r.p.m. and washed several times with distilled water. The isomeric form of diaminopimelic acid and the presence (or absence) of glycine in the cell wall were realized as described by Becker *et al.*[21] The composition of whole-cell sugars was determined as described by Lechevalier and Lechevalier.[6] Polar lipids were determined according to the method described by Minnikin *et al.*[22] and separated by two dimensional TLC. The menaquinones were extracted according to the procedure of Minnikin *et al.*[23] and were analyzed by HPLC.[24,25] For extraction and analysis of cellular fatty acids, the physiological age of the strain was standardized by consistently choosing the same factor (the last quadrant streaked) on (CM + YE medium, see DSMZ web pages, https://www.dsmz.de/catalogues/catalogue-microorganisms/culture-technology/list-of-media-for-microorganisms.html) incubated at 28 °C for 10 days. The analysis was conducted using the Microbial Identification System (MIDI) Sherlock software version 4.5 (method TSBA40, TSBA6 database) as described by Sasser.[26] The analysis of mycolic acids was performed using the method of Minnikin *et al.*[27]

Determination of 16S ribosomal RNA gene sequence and phylogenetic analyses

For DNA analysis, the strain H53^T was grown on the CM broth supplemented with 15% (w/v) NaCl. The genomic DNA was extracted with DNA extraction kit (JetFlex, Hannover, Germany) according to the method of Liu *et al.*[28] PCR-mediated amplification of the 16S ribosomal RNA (rRNA) gene and sequencing of the purified PCR products were carried out as described by Rainey *et al.*[29] PCR products were purified with a PCR product purification kit (Qiagen, Hilden, Germany). The primers used for sequencing are listed in Coenye *et al.*[30] The 16S rRNA sequence has been deposited in the GenBank data library and assigned the accession number KC427277. The sequences obtained were compared with sequences present in the public sequence databases as well as with the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/;[31]), a web-based tool for the identification of prokaryotes based on 16S rRNA gene sequences from type strains. Phylogenetic analyses were conducted using MEGA version 5.[32] The 16S rRNA sequence of strain H53^T was aligned using the CLUSTAL W[33] against corresponding nucleotide sequences retrieved from GenBank. Phylogenetic trees were constructed by using the neighbor joining[34] with Jukes and Cantor[35] model, maximum likelihood[36] with Kimura two-parameter[37] model and maximum-parsimony[38] methods. The topology of the tree was evaluated by bootstrap analysis based on 1000 replicates.[39]

Determination of G + C content of DNA

The G + C content of the chromosomal DNA was determined by HPLC according to Mesbah *et al.*[40]

RESULTS AND DISCUSSION

Morphological characteristics

Strain H53^T was observed to grow well on ISP 2, nutrient agar and CM agar media, and the aerial mycelium was moderately abundant with white color on these media. However, poor growth was observed on ISP 4 medium. Substrate mycelium was dark reddish orange color on ISP 2 and nutrient agar media, and was light yellow on CM agar and ISP 4 media. Melanoid pigments and other diffusible pigments were not produced on tested media (ISP 6, ISP 7 and other media).

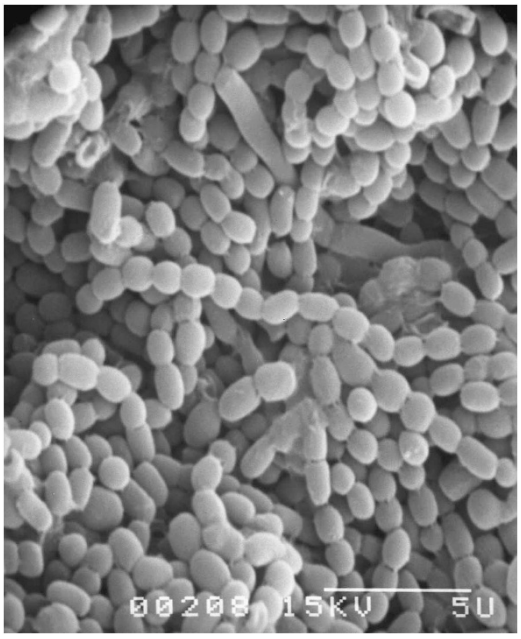

Figure 1 Scanning electron micrograph of strain H53^T showing long spore chains of smooth surface spores after 15 days growth at 30 °C on yeast extract–malt extract agar (ISP 2) containing 15% (w/v) NaCl. Bar = 5 μm.

The substrate mycelium was well developed and fragmented into non-motile cocci. It exhibited an abundant fragmentation on both solid and liquid media. Aerial mycelia form long chains of spores that are non-motile, smooth surfaced, oval or spherical in shape (Figure 1). No morphological forms or structures resembling sporangia, sclerotia or synnemata were observed.

Chemotaxonomic characteristics

Strain H53^T was determined to contain *meso*-diaminopimelic acid (but not glycine) in its cell wall. Whole-cell hydrolysates were found to contain arabinose and galactose (chemotype pattern IVA of Lechevalier and Lechevalier).[6] Furthermore, the presence of ribose was also confirmed. Mycolic acids were not detected. The phospholipids detected were phosphatidylcholine (in very small amount), diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol, as well as several glycolipids and unknown phospholipid (Supplementary Figures S1a and b). This polar lipid pattern corresponds to phospholipid type III.[7] The predominant menaquinones were determined to be MK-9 (H_4) (88.9%) and MK-9 (H_6) (4.6%), and minor amounts of MK-9 (H_2), MK-9 (H_8), as well as unidentified menaquinones were also detected. The fatty acid's profile was composed as follows: anteiso-$C_{17:0}$ (27.8%), iso-$C_{15:0}$ (26.2%), iso-$C_{17:0}$ (14.0%), *cis*9 iso-$C_{17:1}$ (11.7%), iso-$C_{16:0}$ (5.6%) and anteiso-$C_{15:0}$ (3.5%) (Supplementary Table S1). The morphological and chemical characteristics described above clearly support the placement of strain H53^T within the genus *Saccharopolyspora*.

Physiological characteristics

The strain H53^T grew in a wide range of NaCl concentrations (7–32% w/v on nutrient agar medium) and was strictly halophilic. It used the majority of sugars and other organic compounds for its growth. The organism is resistant to kanamycin (5 μg ml^{-1}), erythromycin (10 μg ml^{-1}), streptomycin (10 μg ml^{-1}), penicillin (25 μg ml^{-1}) and lysozyme (0.005% w/v), but sensitive to chloramphenicol (25 μg ml^{-1}). The complete physiological characteristics of strain H53^T are given in the species description.

Phylogenetic analysis based on 16S rRNA gene sequence comparison and G + C content of DNA

Phylogenetic analysis of an almost complete 16S rRNA gene sequence (1491 bp, GenBank accession number KC427277) showed that strain

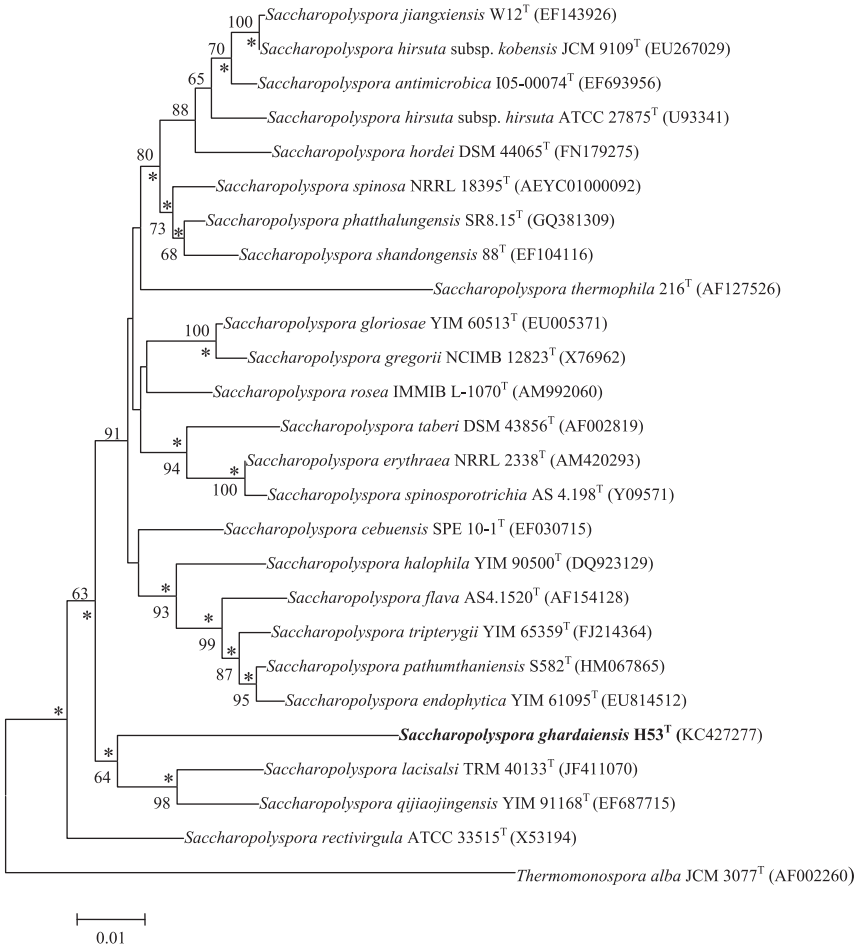

Figure 2 Phylogenetic tree for species of the genus *Saccharopolyspora* calculated from almost complete 16S rRNA gene sequences using Jukes and Cantor[35] evolutionary distance methods and the neighbor-joining method of Saitou and Nei.[34] This illustrates the taxonomic position of strain H53^T relative to the other species of the genus. Asterisks indicate branches that are conserved when the neighbor-joining, maximum-parsimony and maximum-likelihood methods were used in constructing phylogenetic trees. Numbers at the nodes are bootstrap values, expressed as a percentage of 1000 resamplings (only values > 50% are shown). Bar = 0.01 nucleotide substitutions per site.

H53^T was related to members of the genus *Saccharopolyspora*. Moreover, the 16S rRNA sequence similarities between strain H53^T and other species of the genus *Saccharopolyspora* ranged from 92.1 to 94.3%. The phylogenetic relationship between strain H53^T and the other *Saccharopolyspora* species is seen in the neighbor-joining dendrogram (Figure 2). Maximum parsimony and maximum-likelihood calculation resulted in a similar tree topology.

The strain H53^T has a DNA G + C content of 72.6 mol%.

CONCLUSION

The results of the morphological and chemotaxonomic investigations and phylogenetic analysis supported the affiliation of the strain H53^T to the genus *Saccharopolyspora*. The comparison with *S. lacisalsi* TRM 40133^T showed differences in the color of substrate mycelium, the G + C content, the decomposition of adenine, hypoxanthine, gelatin, starch and tyrosine, the growth at 45 °C, and the growth with 28, 30, 32 and 35% NaCl (w/v) on nutrient agar medium as shown in Table 1. Furthermore, the differences in the production of acids from the utilization of L-arabinose, D-fructose, D-lactose, D-mannitol and D-xylose were observed. In addition, a comparison with the *S. qijiaojingensis* YIM 91168^T showed differences in the color of substrate mycelium, the G + C content, the decomposition of adenine, gelatin, starch, Tween 80, tyrosine and xanthine, the production of nitrate reductase, the growth at 45 °C, and the growth with 25, 28, 30, 32 and 35% NaCl (w/v) on nutrient agar medium as shown in Table 1. Moreover, the differences in the production of acids from the utilization of L-arabinose, D-cellobiose, D-fructose, D-mannitol, L-rhamnose, sucrose and xylose were also observed.

All of the data support the designation of strain H53^T as representing a novel species of the genus *Saccharopolyspora*, for which the name *Saccharopolyspora ghardaiensis* sp. nov. is proposed.

Table 1 Differential phenotypic characteristics of strain H53^T compared with its closest relative halophilic species (*S. lacisalsi* TRM 40133^T and *S. qijiaojingensis* YIM 91168^T)

Characteristics	*1*	*2*	*3*
Color of substrate mycelium on ISP 2 and nutrient agar media	Dark reddish orange, light yellow	White-yellow	White-yellow
Utilization of			
L-Arabinose	+	−	−
D-Cellobiose	+	+	−
D-Fructose	+	−	−
D-Lactose	+	−	+
D-Mannitol	−	+	+
L-Rhamnose	−	−	+
Sucrose	+	+	−
D-Xylose	+	−	−
Degradation of			
Adenine	+	−	−
Hypoxanthine	+	−	+
Gelatin	−	+	+
Starch	+	−	−
Tween 80	+	+	−
Tyrosine	+	−	−
Xanthine	−	−	+
Production of nitrate reductase	+	+	−
Temperature range (°C)	25–45	25–40	20–40
pH range (%, w/v)	5–8	6–8	5–8
NaCl range (%, w/v)	7–32	5–25	6–22
DNA G + C content (mol%)	72.6	68.2	70.1

Abbreviations: −, negative; +, positive.
Strain: 1, H53^T; 2, *S. lacisalsi* TRM 40133^T; 3, *S. qijiaojingensis* YIM 91168^T.
Data were from this study, except those of *S. qijiaojingensis* YIM 91168^T: taken from Tang *et al.*[4]

Description of *S. ghardaiensis* sp. nov.

S. ghardaiensis (ghar.da.i.en'sis. N.L. fem. adj. *ghardaiensis*, pertaining to Ghardaïa, the source of the soil from which the type strain was isolated).

Halophilic filamentous actinomycete, aerial mycelium is white color on ISP 2, nutrient agar and CM agar media. The color of the substrate mycelium is dark reddish orange on ISP 2 and nutrient agar media, and is light yellow on CM agar medium. The substrate mycelium was well developed and fragmented into non-motile cocci. The aerial mycelia form long chains of non-motile, smooth-surfaced and oval (or spherical) spores. Melanoid and other diffusible pigments are not produced in tested media. Growth occurs at 25, 28, 30, 35, 40 and 45 °C (but not at 10, 15, 20 and 47 °C), and 30 to 35 °C is the optimal temperature among the tested ones. Moreover, the growth occurs at pH 5, 6, 7 and 8 (but not at pH 4 and 9), and pH 6–7 is the optimal pH among the tested ones. Acetate, adenine, citrate, hypoxanthine, starch, testosterone and Tween 80 are degraded. Casein, gelatin, guanine and xanthine are not decomposed. Utilizes L-arabinose, D-cellobiose, erythritol, D-fructose, D-galactose, D-glucose, glycerol, maltose, adonitol, D-lactose, *meso*-inositol, D-mannose, D-trehalose, sucrose and D-xylose as carbon sources for growth and acid production, but not D-mannitol, D-melezitose, melibiose, D-raffinose, L-rhamnose, D-ribose, salicin and D-sorbitol. Benzoate, butyrate, oxalate, propionate, pyruvate, succinate and tartrate were not decarboxylated. L-alanine, L-proline, L-serine and tyrosine are used as a source of nitrogen. Nitrate reductase is produced. Growth occurs on nutrient agar medium in the presence of NaCl at 7, 10, 15, 20, 25, 28, 30 and 32%, but not at 0 and 35% (w/v), and 15–25% NaCl (w/v) is the optimal concentration among the tested ones. Moreover, the growth occurs in the presence of kanamycin (5 μg ml^{-1}), erythromycin (10 μg ml^{-1}), streptomycin (10 μg ml^{-1}) and penicillin (25 μg ml^{-1}), and also in the presence of 0.005% lysozyme, but not in the presence of chloramphenicol (25 μg ml^{-1}). Chemotype IVA (*meso*-diaminopimelic acid, arabinose and galactose in whole-cell hydrolysates), the diagnostic phospholipid, is phosphatidylcholine. The predominant menaquinones are MK-9(H$_4$) and MK-9(H$_6$). The predominant cellular fatty acids are anteiso-C$_{17:0}$, iso-C$_{15:0}$, iso-C$_{17:0}$ and *cis*9 iso-C$_{17:1}$ The DNA G + C content of strain H53^T was 72.6%. The type strain H53^T (= DSM 45606^T = CCUG 63370^T = CECT 8304^T) is isolated from a Saharan soil sample collected from Ghardaïa province (South Algeria). The GenBank accession number for the 16S rRNA gene sequence of strain H53^T is KC427277.

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