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Actinopolyspora biskrensis sp. nov., a Novel Halophilic Actinomycete Isolated from Northern Sahara

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Abstract A novel halophilic, filamentous actinomycete, designated H254^T, was isolated from a Saharan soil sample collected from Biskra (Northern Sahara), and subjected to a polyphasic taxonomic characterization. The strain is Gram-positive, aerobic, and halophilic, and the optimum NaCl concentration for growth is 15–20 % (w/v). The cell-wall hydrolysate contained *meso*-diaminopimelic acid, and the diagnostic whole-cell sugars were arabinose and galactose. The diagnostic phospholipid detected was phosphatidylcholine, and MK-9(H_4) was the predominant menaquinone. The major fatty acid profiles were anteiso-$C_{17:0}$ (32.8 %), $C_{15:0}$ (28 %), and iso-$C_{17:0}$ (12.3 %). Comparative analysis of the 16S rRNA gene sequences revealed that the strain H254^T formed a well-separated sub-branch within the radiation of the genus *Actinopolyspora*, and the microorganism was most closely related to *Actinopolyspora saharensis* DSM 45459^T (99.2 %), *Actinopolyspora halophila* DSM 43834^T (99.1 %), and *Actinopolyspora algeriensis* DSM 45476^T (99.0 %). Nevertheless, the strain had relatively lower mean values for DNA–DNA relatedness with the above strains (57.2, 68.4, and 45.6 %, respectively). Based on phenotypic features and phylogenetic position, we propose that strain H254^T represents a novel species of the genus *Actinopolyspora*, for which the name *Actinopolyspora biskrensis* sp. nov. is proposed. The type strain of *A. biskrensis* is strain H254^T (=DSM 46684^T =CECT 8576^T).

The GenBank accession number for the 16S rRNA gene sequences of strains H254^T is KJ504178.

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Introduction

The genus *Actinopolyspora* was firstly described by Gochnauer et al. [9], with the description of *Actinopolyspora halophila* as the type species. At the time of writing, the genus comprises 12 recognized species, all of them are halophilic actinomycetes, namely *A. halophila* [10], *A. mortivallis* [35], *A. xinjiangensis* [11], *A. egyptensis* [14], *A. alba* and *A. erythraea* [34], *A. algeriensis* [24], *A. saharensis*, *A. righensis,* and *A. mzabensis* [25–27], *A. dayingensis* [13], and *A. lacussalsi* [12].

Typically, members of the genus are characterized by fragmentation of both aerial and substrate mycelia into rods and ovoid elements, a chemotype IVA cell-wall (*meso*-diaminopimelic acid without glycine, and arabinose and galactose as diagnostic whole-cell sugars) [21], a phospholipid type PIII (phosphatidylcholine) pattern [22], the presence of MK-9(H_4) and MK-10(H_4) as the

Table 1 Differential characteristics of strain H254^T compared with its closest relative recognized species of the genus *Actinopolyspora*

Characteristics	1	2	3	4
Growth on ISP 2	−	+	+	+
Utilization of				
D-Cellobiose	+	−	+	+
Erythritol	+	−	−	+
D-Galactose	+	−	+	+
D-Glucose	+	−	+	+
meso-Inositol	−	+	−	+
D-Lactose	+	−	+	+
D-Maltose	+	−	+	+
D-Mannitol	−	+	−	−
D-Mannose	+	−	+	+
Raffinose	+	−	−	+
Salicin	−	+	−	−
Sucrose	+	−	+	+
Decomposition of				
Casein	−	+	−	−
Hypoxanthine	+	+	+	−
Starch	+	−	+	−
Xanthine	+	−	+	−
Production of nitrate reductase	−	−	+	+
Resistance to lysozyme (0.005 % w/v)	+	−	+	+
Growth in the presence of 10 and 30 % of NaCl	+	+	−	+

The phenotypic properties of strains *A. biskrensis* H254^T, *A. halophila* DSM 43834^T, *A. saharensis* DSM 45459^T, and *A. algeriensis* DSM 45476^T grown under the same conditions were determined in the present study. Taxa 1 Strain H254^T, 2 *A. halophila* DSM 43834^T, 3 *A. saharensis* DSM 45459^T, and 4 *A. algeriensis* DSM 45476^T. + positive, − negative

predominant menaquinones, the presence of anteiso-$C_{17:0}$, iso-$C_{15:0}$, and iso-$C_{17:0}$ as the major fatty acids, and the absence of mycolic acids [10, 11, 34].

During an investigation on halophilic actinomycetes from Saharan soils in Biskra province (Northern Sahara), strain H254^T was isolated and identified by a polyphasic approach. Based on phenotypic and genotypic characteristics, it is proposed that the strain H254^T represents a novel species of the genus *Actinopolyspora*, for which the name *Actinopolyspora biskrensis* sp. nov. is proposed.

Materials and Methods

Isolation and Phenotypic Characterization

The strain H254^T was isolated from a Saharan soil sample collected from Biskra (34°38´North, 5°25´East) in northeast of Algeria, by serial dilution method on complex medium (CM) agar [3] supplemented with 15 % (w/v) NaCl and 50 mg l^{-1} actidione. After 3–4 weeks incubation at 30 °C, the strain H254^T was picked-up, purified, and preserved on CM agar medium at 4 °C. Strain H254^T was deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ) as strain DSM 46684^T and in Spanish Type Culture Collection (CECT) as strain CECT 8576^T.

The colors of substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the ISCC-NBS color charts [17]. Morphological characteristics were observed by light microscopy using 14 days old cultures grown on CM agar medium, International *Streptomyces* Project (ISP 4 and ISP 2) media, and nutrient agar. Growth was tested at pH 5.0–9.0 (at intervals of 1.0 pH units) and at 20, 30, 37, and 45 °C on nutrient agar. NaCl tolerance was studied on nutrient agar containing NaCl at final concentrations of 0–30 % (w/v) (at intervals of 5.0 %). Other physiological characteristics, including utilization of sole carbon and nitrogen sources, decarboxylation of nine organic acids, degradation of adenine, aesculin, arbutin, gelatin, guanine, hypoxanthine, starch, testosterone, Tween 80, tyrosine, and xanthine, and reduction of nitrate and sensibility to lysozyme, were assessed by the media and methods of Locci et al. [23].

Chemotaxonomic Characterization

Biomass for chemical and molecular studies was obtained by cultivation in shake flasks (250 rpm, 30 °C, 7 days) using complex medium broth (pH 7.0) supplemented with 15 % (w/v) NaCl. The whole-cell sugar pattern and the diagnostic isomers of diaminopimelic acid were analyzed according to the procedures developed by Becker et al. [1] and Lechevalier and Lechevalier [21]. Phospholipids were extracted and identified as described by Minnikin et al. [28]. The cellular fatty acid analysis was performed as described by Sasser [33] using the Microbial Identification System (MIDI). Menaquinones were extracted following the procedure of Minnikin et al. [30], and separated by HPLC [20]. Analysis of mycolic acids was performed using the method of Minnikin et al. [29].

16S rRNA Gene Sequence and Phylogenetic Analysis

The genomic DNA of strain H254^T was extracted with DNA extraction kit (MasterPure^{TM} Gram-Positive DNA Purification Kit, Epicentre® Biotechnologies, Madison). PCR amplification of the 16S rRNA gene was performed as described by Rainey et al. [31]. PCR products were purified with a PCR product purification kit (Qiagen, Germany). The primers used for sequencing were listed in Coenye et al. [4]. Multiple alignments with sequences of all species of the genus *Actinopolyspora* and calculations of levels of sequence similarity were carried out by the EzTaxon server [18]. Phylogenetic trees were constructed

using the neighbor-joining method [32] with Jukes and Cantor model [16], maximum-likelihood [6] with Kimura's two-parameter model [19], and maximum-parsimony methods [8]. The topology of the phylogenetic tree was evaluated using the bootstrap resampling method of Felsenstein [7] with 1,000 replicates.

DNA–DNA Hybridization

For DNA–DNA hybridization, cells were disrupted by a French pressure cell (Thermo Spectronic). DNA in the crude lysate was purified by chromatography on hydroxyapatite as described by Cashion et al. [2]. Genomic hybridization experiments between strain H254^T and *A. halophila* DSM 43834^T, *A. saharensis* DSM 45459^T or *A. algeriensis* DSM 45476^T were performed by the method described by De Ley et al. [5] under consideration of the modifications described by Huss et al. [15] 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 % (w/v) formamide at 71 °C.

Results and Discussion

The strain H254^T showed good growth on ISP 4 medium, moderate growth on nutrient agar and CM agar media, but no growth was observed on ISP 2 medium. The aerial mycelium was moderately produced with white to yellowish-white color on these media. The strain formed irregularly branched and fragmented substrate mycelium with dark brown color on ISP 4 medium, pinkish color on nutrient agar, and beige to reddish-orange color on CM agar medium. The diffusible pigments were produced on nutrient agar (pinkish) and ISP 4 (dark brown) media but not on CM agar medium. The aerial mycelium was irregularly branched and formed straight to flexuous chains of 5–15 spores (sometimes 20 spores) per chain. The spores were non-motile and rod-shaped. Strain H254^T growth occurring in the presence of 10–30 % (w/v) NaCl (optimum 15–20 %), at pH 6–8 (optimum pH 7.0) and at 20–37 °C (optimum 30 °C). The detailed physiological features are indicated in Table 1 and in the species description. Cell-wall hydrolysate of strain H254^T contained the *meso*-diaminopimelic acid isomer, but not glycine. Whole-cell sugars were arabinose, galactose, and ribose. This is typical of cell wall type IV and whole-cell sugar pattern type A [21]. The major menaquinones were MK-9(H_4) (62.3 %) and MK-10(H_4) (28.0 %), and minor amounts of MK-9(H_2) (3.2 %), MK-9(H_0) (2.1 %), MK-10(H_6) (1.2 %), and MK-10(H_2) (0.8 %) were also detected. The phospholipid profile contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, and three unknown glycolipids (Supplement Fig. 1). The fatty acid profiles were composed as follows: anteiso-$C_{17:0}$ (32.8 %), iso-$C_{15:0}$ (28.0 %), iso-$C_{17:0}$ (12.3 %), 9-methyl $C_{16:0}$ (8.6 %), anteiso-$C_{15:0}$ (7.9 %), and iso-$C_{16:0}$ (5.1 %).

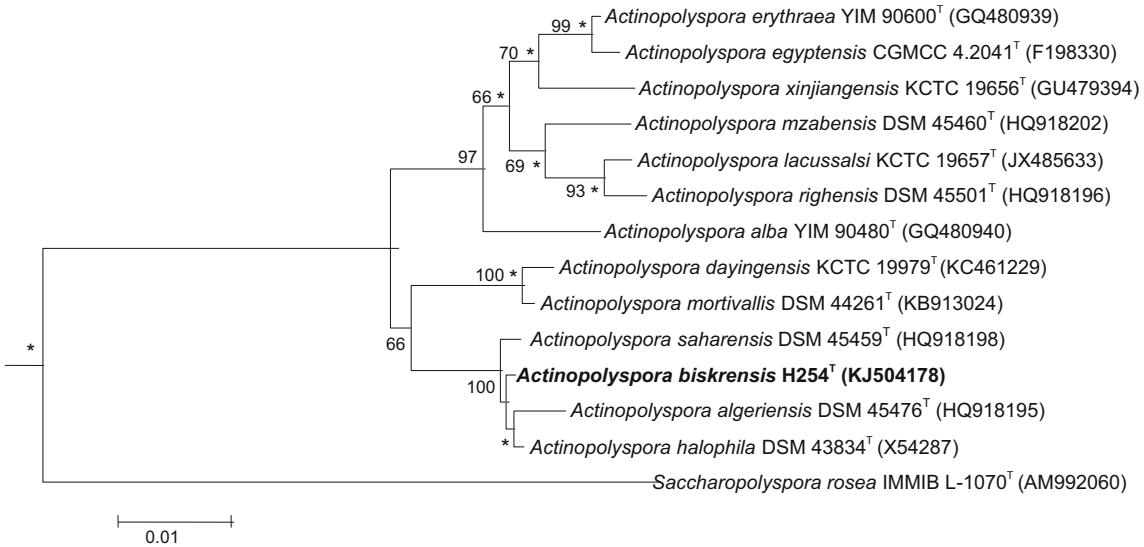

Fig. 1 Phylogenetic tree for species of the genus *Actinopolyspora* calculated from almost complete 16S rRNA gene sequences using Jukes and Cantor [16] evolutionary distance methods and the neighbor-joining method of Saitou and Nei [32]. This illustrates the taxonomic position of strain H254^T relative 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 nodes are bootstrap values, expressed as percentages of 1,000 resamplings (only values >50 % are shown). *Saccharopolyspora rosea* IMMIB L-1070^T was used as an outgroup. *Bar* 1 substitution per 100 nucleotides

Phylogenetic analysis of an almost complete 16S rRNA gene sequence (1,491 bp, GenBank accession number KJ574180) showed that strain H254^T was related to members of the genus *Actinopolyspora*. Moreover, the 16S rRNA sequence exhibited highest 16S rRNA gene sequence similarity to *A. saharensis* DSM 45459^T (99.2 %), *A. halophila* DSM 43834^T (99.1 %), and *A. algeriensis* DSM 45476^T (99.0 %). The phylogenetic relationship between strain H254^T and the other *Actinopolyspora* species is seen in the neighbor-joining (Fig. 1), maximum-parsimony (Supplement Fig. 2), and maximum-likelihood (Supplement Fig. 3) dendrograms.

DNA–DNA relatedness between strain H254^T and strains *A. saharensis* DSM 45459^T, *A. halophila* DSM 43834^T, and *A. algeriensis* DSM 45476^T has respective mean values of 57.2, 68.4, and 45.6 %, respectively.

Strain H254^T differs from these three closely related species of *Actinopolyspora* by several physiological characteristics, and also by some chemotaxonomical characteristics (Table 2). Strain H254^T differs from *A. halophila* DSM 43834^T, which is genetically the closest species (DNA–DNA relatedness of 68.4 %), by 17 physiological characteristics, by the percentage of fatty acid anteiso-$C_{15:0}$ (only 7.9 % for H254^T and 34.6 % for *A. halophila* DSM 43834^T) and anteiso-$C_{17:0}$ (32.8 % for H254^T and only 10.2 % for *A. halophila* DSM 43834^T), by the percentage of menaquinone MK-10(H_4) (28.0 % for H254^T and only 10.0 % for *A. halophila* DSM 43834^T), by the presence of diphosphatidylglycerol (DPG), and by the absence of lyso-DPG.

It is evident from the phenotypic, chemotaxonomic, and genetic data that strain H254^T represents a novel species in the genus *Actinopolyspora*, for which we propose the name *Actinopolyspora biskrensis* sp. nov.

Description of *Actinopolyspora biskrensis* sp. nov

Actinopolyspora biskrensis (bis.kren'sis, N.L. fem. adj. *biskrensis* pertaining to Biskra, where the type strain was isolated).

Gram-positive, aerobic, extremely halophilic actinomycete. Aerial mycelium is white to yellowish-white, and forms straight to flexuous chains of 5 to 15 (sometimes 20) rod-shaped and non-motile spores. The color of the substrate mycelium is pinkish on nutrient agar medium, dark brown on ISP 4 medium, and beige to reddish-orange on CM agar medium. The substrate mycelium is well developed and fragments with age into non-motile rods. The diffusible pigments are produced on nutrient agar (pinkish) and ISP 4 (dark brown) media, but not on CM agar medium. Temperature and pH ranges for growth are 20–37 °C (optimal at 30 °C) and pH 6.0–8.0 (optimal at pH 7.0). The NaCl concentration range for growth is 10–30 %, with optimal growth occurring at 15–20 %. Utilizes adonitol, L-arabinose, D-cellobiose, erythritol, D-fructose, D-galactose D-glucose, glycerol, D-lactose, maltose, D-mannose, D-raffinose, L-rhamnose, D-ribose, D-sorbitol, sucrose, D-trehalose, and D-xylose, as sole carbon sources, but not *meso*-inositol, mannitol, D-melezitose, and D-melibiose. Nitrates are not reduced. Aesculin, gelatin, hypoxanthine, starch, Tween 80, and xanthine are hydrolyzed; adenine, arbutin, casein, guanine, testosterone, and tyrosine are not decomposed. H_2S is not formed. Citrate was decarboxylated, but not acetate benzoate, butyrate, oxalate, propionate, pyruvate, succinate, and tartrate. L-serine was

Table 2 Cellular fatty acid, menaquinone, and phospholipid composition of *Actinopolyspora biskrensis* H254^T in comparison with the closely related species *A. halophila* DSM 43834^T, *A. saharensis* DSM 45459^T, and *A. algeriensis* DSM 45476^T

Chemotaxonomic characteristics	1	2	3	4
Fatty acid composition (%)				
iso-$C_{14:0}$	–	2.4	–	3.4
iso-$C_{15:0}$	28.0	24.1	20.4	17.8
anteiso-$C_{15:0}$	7.9	34.6	9.1	15.8
iso-$C_{16:0}$	5.1	9.3	15.6	15.6
$C_{16:0}$	–	4.7	–	0.5
9-Methyl-$C_{16:0}$	8.6	–	–	2.2
iso-$C_{17:0}$	12.3	7.4	9.3	3.8
anteiso-$C_{17:0}$	32.8	10.2	30.8	31.3
cis9-$C_{18:1}$	–	0.6	–	2.7
Menaquinone composition (%)				
MK-8(H_4)	–	4	5.9	3.4
MK-9(H_0)	2.1	–	–	–
MK-9(H_2)	3.2	2	5.3	9.1
MK-9(H_4)	62.3	64	22.4	39.1
MK-9(H_6)	–	3	–	–
MK-10(H_2)	0.8	–	9.4	5.7
MK-10(H_4)	28.0	10	29.4	32.1
MK-10(H_6)	1.2	–	1.9	–
MK-11(H_2)	–	–	3.0	–
MK-11(H_4)	–	–	8.3	–
Phospholipid composition				
PC	+	+	+	+
DPG	+	–	+	+
PG	+	+	+	+
PI	–	–	+	+
Lyso-DPG	–	+	–	–

Strains: 1, strain H254^T; 2, *A. halophila* DSM 43834^T; 3, *A. saharensis* DSM 45459^T; 4, *A. algeriensis* DSM 45476^T, values are percentages of total fatty acids and menaquinones, –, not detected. Data for fatty acids analyses of all strains were from this study. Data for menaquinones and phospholipids were from this study (strain H254^T), Gochnauer et al. [9] (*A. halophila* DSM 43834^T), Meklat et al. [25] (*A. saharensis* DSM 45459^T) and Meklat et al. [24] (*A. algeriensis* DSM 45476^T)

used as source of nitrogen, but not L-alanine and L-proline. Moreover, the growth occurs in the presence of erythromycin (15 g ml^{-1}) and 0.005 % (w/v) lysozyme, but not in the presence of tetracycline (30 g ml^{-1}) and nalidixic acid (30 g ml^{-1}). Contains *meso*-diaminopimelic acid, as cell-wall diamino acid, arabinose, and galactose as major whole-cell sugars (chemotype IVA). The diagnostic phospholipid is phosphatidylcholine. Mycolic acids are absent. The major menaquinones are MK-9(H_4) and MK-10(H_4). The major fatty acids are anteiso-$C_{17:0}$, iso-$C_{15:0}$, and iso-$C_{17:0}$.

The type strain is H254^T (=DSM 46684^T =CECT 8576^T) isolated from a Saharan soil sample collected from Biskra region (northern Sahara).

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Conflict of interest The authors declare that there is no conflict of interests regarding the publication of this paper.

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