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Actinopolyspora righensis sp. nov., a novel halophilic actinomycete isolated from Saharan soil in Algeria

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Abstract A novel halophilic actinomycete strain, H23^T, was isolated from a Saharan soil sample collected in Djamâa (Oued Righ region), El-Oued province, South Algeria. Strain H23^T was identified as a member of the genus *Actinopolyspora* by a polyphasic approach. Phylogenetic analysis showed that strain H23^T had 16S rRNA gene sequence similarities ranging from 97.8 % (*Actinopolyspora xinjiangensis* TRM 40136^T) to 94.8 % (*Actinopolyspora mortivallis* DSM 44261^T). The strain grew optimally at pH 6.0–7.0, 28–32 °C and in the presence of 15–25 % (w/v) NaCl. The substrate mycelium was well developed and fragmented with age. The aerial mycelium produced long, straight or flexuous spore chains with non-motile, smooth-surfaced and rod-shaped spores. Strain H23^T had MK-10 (H_4) and MK-9 (H_4) as the predominant menaquinones. The whole microorganism hydrolysates mainly consisted of *meso*-diaminopimelic acid, galactose and arabinose. The diagnostic phospholipid detected was phosphatidylcholine. The major cellular fatty acids were anteiso-$C_{17:0}$ (37.4 %), iso-$C_{17:0}$ (14.8 %), iso-$C_{15:0}$ (14.2 %), and iso-$C_{16:0}$ (13.9 %). The genotypic and phenotypic data show that the strain represents a novel species of the genus *Actinopolyspora*, for which the name *Actinopolyspora righensis* sp. nov. is proposed, with the type strain H23^T (=DSM 45501^T = CCUG 63368^T = MTCC 11562^T).

Keywords *Actinopolyspora righensis* sp. nov. · halophilic actinomycete · Algerian Sahara · Polyphasic taxonomy

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Introduction

The genus *Actinopolyspora*, which belongs to the family *Actinopolysporaceae* (Zhi et al. 2009), was originally established by Gochnauer et al. (1975) and the description was later emended by Tang et al. (2011). The genus currently contains 8 recognized species: *Actinopolyspora halophila* (Gochnauer et al. 1975), *A. mortivallis* (Yoshida et al. 1991), *A. xinjiangensis* (Guan et al. 2010), *A. egyptensis* (Hozzein and Goodfellow 2011), *A. alba* and *A. erythraea* (Tang et al. 2011), *A. algeriensis* (Meklat et al. 2012) and *A. saharensis* (Meklat et al. 2013). The two species '*A. egyptensis*' and '*A. saharensis*' were described recently and have not yet been validated.

The genus *Actinopolyspora* was shown to exhibit distinct chemotaxonomic characteristics: cell-wall chemotype IVA (*meso*-diaminopimelic acid, arabinose and galactose in whole-cell hydrolysates; Lechevalier and Lechevalier 1970), phospholipid type PIII (phosphatidylcholine as characteristic phospholipid; Lechevalier et al. 1977), MK-9 (H_4) and MK-10 (H_4), MK-9 (H_4) and MK-9 (H_2) or MK-6 as the predominant menaquinones, iso-$C_{16:0}$ and anteiso-$C_{17:0}$ as the major fatty acids and absence of mycolic acids (Gochnauer et al. 1989; Guan et al. 2010; Tang et al. 2011). During an investigation of the soil actinobacterial community in the Sahara desert of Algeria, an *Actinopolyspora*-like strain was isolated. The aim of the present study was to determine the taxonomic position of this strain using polyphasic taxonomic approach.

Materials and methods

Strain and culture conditions

Strain H23^T was isolated from moderately saline soil (electrical conductivity = 2.3 mS cm^{-1}) of Oued Righ region (El-Oued) during an investigation of actinobacteria diversity in Saharan soils (Meklat et al. 2011). Serially diluted sample was plated on complex medium (CM) agar (Chun et al. 2000) supplemented with 20 % (w/v) NaCl and incubated for 5 weeks at 30 °C.

After primary isolation and purification, the isolate was preserved both on slants of CM agar at 4 °C and as 20 % (v/v) glycerol suspensions at −20 °C. Strain H23^T was deposited in the German Collection of Microorganisms and Cell Cultures as strain DSM 45501^T, in the Culture Collection, University of Göteborg as strain CCUG 63368^T, and in the Microbial Type Culture Collection as strain MTCC 11562^T.

Phenotypic characterization

Cultural characteristics of the new isolated strain were determined after 3 weeks incubation at 30 °C on the International *Streptomyces* Project media (Shirling and Gottlieb 1966), CM agar (Chun et al. 2000) and nutrient agar (Waksman 1961). The ISCC–NBS colour name charts (Kelly and Judd 1976) were used to determine colony colours. Morphological characteristics were observed under light microscopy (B1, Motic) and scanning electron microscopy (Hitachi, S450) using cultures grown on CM agar at 30 °C for 4 weeks. Temperature range (10–45 °C), pH range (5.0–9.0) and NaCl (0–35 %, w/v) tolerance for growth were determined on nutrient agar after incubating for 21 days at 30 °C. Utilization of carbohydrates, milk coagulation and peptonization, and decarboxylation of organic acids were evaluated using the method of Gordon et al. (1974). Degradation of other organic compounds was studied using protocol as described by Goodfellow (1971). Lysozyme sensitivity, methyl red and Voges–Proskauer tests, H_2S production and reduction of nitrate were determined according to the methods of Gordon and Barnett (1977) and Marchal et al. (1987), respectively.

Chemotaxonomy

Biomass for chemotaxonomy studies was obtained by cultivating the cell in shake flasks (250 rpm, 30 °C, 10 days) using CM broth containing 15 % NaCl (w/v). Analysis of cell-wall amino acids and sugars in whole-cell hydrolysates were carried out according to the methods described by Becker et al. (1964) and Lechevalier and Lechevalier (1970). Phospholipids were extracted, examined and identified by using the procedure developed by Minnikin et al. (1977). The cellular fatty acid composition was determined as described by Sasser (1990) using the microbial identification system (MIDI). Cellular menaquinones were extracted and purified as described by Minnikin et al. (1984) and were analyzed by HPLC (Kroppenstedt 1982, 1985). Analysis of mycolic acids was performed using the method of Minnikin et al. (1980).

Phylogenetic analyses

Extraction of chromosomal DNA of H23^T strain was carried out as described by Liu et al. (2000). Amplification of the 16S rRNA gene by PCR and the sequencing of the purified PCR products were performed as described previously by Rainey et al. (1996). The resulting 16S rRNA gene sequence was compared with sequences obtained from public databases to determine the most closely related species using EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al. 2012). Multiple alignments were performed using the CLUSTAL W program (Larkin et al. 2007). Phylogenetic trees were constructed using the neighbour-joining (Saitou and Nei 1987) with Jukes and Cantor (1969) model, maximum-likelihood (Felsenstein 1981) with Kimura 2-parameter (Kimura 1980) model and maximum-parsimony (Fitch 1977) methods. Bootstrap analysis was performed with 1,000 replicates (Felsenstein 1985) to validate the tree topology of the neighbour-joining method.

Results and discussion

Strain H23^T showed good growth on nutrient agar and CM agar media, while no growth was observed on ISP2 and ISP4 media. Strain formed irregularly branched substrate mycelia with yellowish colour on CM and nutrient agar. White aerial mycelium was observed on CM agar and nutrient agar media. Straight or flexuous chains of fragmented rod-shaped spores of aerial mycelium were observed (Fig. 1). The surface of spores was smooth. No diffusible pigments were produced on the tested media. Strain H23^T had morphological properties typical of members of the genus *Actinopolyspora*. It could grow at 20–40 °C, pH 5.0–8.0 and 10–30 % NaCl with optimal NaCl concentration for growth at 15–25 % (w/v). The optimal temperature and pH for growth were 28–32 °C and 6.0–7.0, respectively. Detailed results of the physiological and biochemical analyses are given in the species description and in Table 1.

The chemotaxonomic characteristics of strain H23^T supported its classification as a member of the genus *Actinopolyspora*. The *meso*-diaminopimelic acid was detected in its cell wall and the whole cell hydrolysates contained arabinose, galactose and small amount of

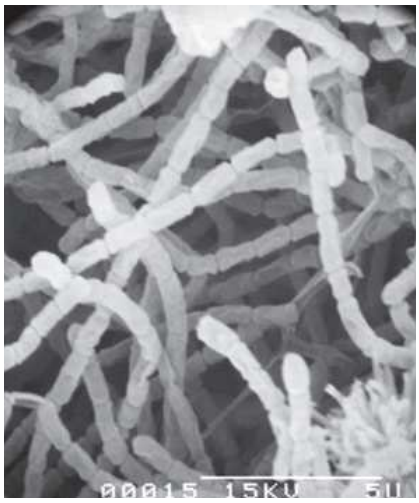

Fig. 1 Scanning electron micrograph of strain H23^T grown on complex medium agar containing 15 % (w/v) NaCl for 4 weeks at 30 °C. *Bar* 5 μm

ribose. Mycolic acids were not detected. The phospholipid profile contained diphosphatidylglycerol, phosphatidylcholine, phosphatidylinositol phosphatidylethanolamine, three unknown glycophospholipids and three unknown phospholipids (Fig. S1). The menaquinones detected were MK-10 (H4) (51.2 %), MK-9 (H4) (26.4 %), and minor amounts of MK-9 (5.2 %), MK-10 (H_6) (5.0 %), MK-9 (H_6) (2.4 %), MK-10 (H_2) (1.6 %), MK-9 (H_2) (1.7 %) and MK-10 (H_8) (0.7 %) were also detected. The fatty acids profile was composed as follows: anteiso-$C_{17:0}$ (37.4 %), iso-$C_{17:0}$ (14.8 %), iso-$C_{15:0}$ (14.2 %), iso-$C_{16:0}$ (13.9 %), *cis*9-$C_{17:1}$ (8.8 %) and anteiso-$C_{15:0}$ (4.5 %).

Phylogenetic analysis based on 16S rRNA gene sequences (Fig. 2 and Fig. S2) revealed that strain H23^T belonged to the genus *Actinopolyspora*; levels of similarity between strain H23^T and the type strains of recognized *Actinopolyspora* species ranged from 94.8 to 97.8 %. Strain H23^T was related most closely to *A. xinjiangensis* TRM 40136^T (97.8 %), *A. erythraea* DSM 45583^T (97.7 %) and *A. alba* DSM 45004^T (97.5 %). DNA–DNA relatedness experiments were not carried out between strain H23^T and its closest phylogenetic neighbours as the type strains of several *Actinopolyspora* species share 16S rRNA gene similarities within the range of 97.6–98.8 %, but have levels of DNA–DNA relatedness well below the 70 % cut-off point recommended by Wayne et al. (1987) for the recognition of genomic species. For example, the

Table 1 Differential physiologic and chemotaxonomic characteristics of strain H23^T and its closest relative recognized species of the genus *Actinopolyspora*

Characteristics	Type strains			
	1	2	3	4
Utilization of				
Fructose	+	+	−	−
Galactose	+	+	+	−
Lactose	+	ND	−	+
Maltose	+	−	+	+
Mannitol	−	−	−	+
Mannose	+	−	+	+
Xylose	+	+	+	−
Decomposition of				
Casein	+	ND	+	−
Starch	−	−	+	+
Tween 80	+	+	+	−
NaCl range (%, w/v)	10–30	8–25	10–25	10–25
NaCl optimum (%, w/v)	15–25	10–15	15–20	15–20
Temperature range (°C)	20–40	25–50	25–45	25–45
Temperature optimum (°C)	28–32	30–37	30–37	30–37
pH range	5–8	6–9	6–8	6–8
pH optimum	6–7	7–7.5	7–8	7–8
Menaquinone composition (%)				
MK-6	−	51.2	−	−
MK-7	−	13.5	−	−
MK-9	5.2	−	7.5	−
MK-9 (H_2)	1.7	2.0	25.5	3.7
MK-9 (H_4)	26.4	12.0	51.9	44.9
MK-10 (H_4)	51.2	14.3	8.1	43.9
MK-10 (H_6)	5.0	−	2.6	2.6
Phospholipid composition				
PG	−	+	−	−
PI	+	−	+	+
PE	+	−	−	−
PIM	−	−	+	+

Data for physiological tests were from this study, except those of *A. xinjiangensis* TRM 40136^T (taken from Guan et al. 2010). Data for chemotaxonomical analyses were realized under the same conditions

+ Positive, – negative, *ND* not determined, *PG* phosphatidylglycerol, *PI* phosphatidylinositol, *PE* phosphatidylethanolamine, *PIM* phosphatidylinositol mannoside. Strains: 1, *A. righensis* H23^T; 2, *A. xinjiangensis* TRM 40136^T; 3, *A. erythraea* DSM 45583^T; 4, *A. alba* DSM 45004^T

type strain *A. saharensis* DSM 45459^T exhibited 16S rRNA gene sequence similarity values of 98.8 and 98.5 % respectively with its nearest neighbours *A. algeriensis* DSM 45476^T and *A. halophila* DSM 43834^T, however shared a DNA–DNA relatedness value of 30.5 and 55.1 % (Meklat et al. 2013). The type strains *A. algeriensis* DSM 45476^T and *A. halophila* DSM 43834^T shared also a 16S rRNA gene sequence similarity of 98.5 %, but had a DNA–DNA relatedness of only 24.7 % (Meklat et al. 2012). Furthermore, the type strains of *A. alba* DSM 45004^T and *A. erythraea* DSM 45583^T with a 16S rRNA similarity value of 97.6 % shared a DNA–DNA relatedness value of 46.8 % (Tang et al. 2011). In addition, Stackebrandt and Ebers (2006) recommend a 16S rRNA gene sequence similarity threshold range of 98.7–99.0 % as the point at which DNA–DNA reassociation experiments should be mandatory for testing the genomic uniqueness of novel isolates. Furthermore, Meier-Kolthoff et al. (2013) showed that the DNA–DNA hybridization should be mandatory only above a similarity percentage of 98.2 % (based on 16S rRNA gene). For instance, the error probability is very insignificant (0.025 %) in a percentage of 97.8 % (as in our case).

In addition, the strain H23^T differs from other closely related species of *Actinopolyspora* not only by physiological characteristics (utilization of fructose, galactose, lactose, maltose, mannitol, mannose, xylose, casein, starch and Tween 80), but also by chemotaxonomical characteristics. Indeed, the predominant menaquinones were MK-10 (H_4) and MK-9 (H_4) for H23^T and *A. alba*, MK-9 (H_2) and MK-9 (H_4) for *A. erythraea*, and MK-6 for *A. xinjiangensis*. Moreover, phosphatidylethanolamine was present only in H23^T, while phosphatidylglycerol was present only in *A. xinjiangensis*. In addition, the strain H23^T differs from *A. xinjiangensis* by the presence of phosphatidylinositol, and from *A. erythraea* and *A. alba* by the presence of phosphatidylinositol mannoside.

On the basis of the phenotypic, chemotaxonomic and phylogenetic data presented, strain H23^T should be placed in the genus *Actinopolyspora* as the type strain of a novel species, for which we propose the name *Actinopolyspora righensis* sp. nov.

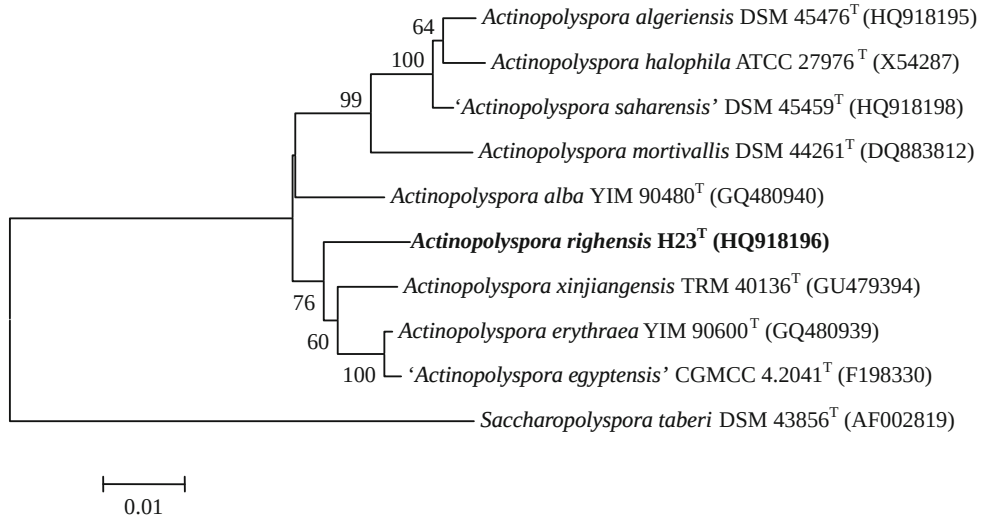

Fig. 2 Phylogenetic tree for species of the genus *Actinopolyspora* calculated from almost complete 16S rRNA gene sequences using Jukes and Cantor (1969) evolutionary distance methods and the neighbour-joining method of Saitou and Nei (1987). This illustrates the taxonomic position of strain H23^T relative to the other species of the genus. *Numbers* at the nodes are bootstrap values, expressed as a percentage of 1,000 resamplings (only values >50 % are shown). *Bar* 0.01 nucleotide substitution per site

Description of *Actinopolyspora righensis* sp. nov.

Actinopolyspora righensis (ri.ghen'sis, N.L. fem. adj. *righensis* pertaining to Oued Righ, where the type strain was isolated).

Gram-positive, extremely halophilic, filamentous, aerobic actinomycete; forms white aerial mycelium and yellow substrate mycelium. Good growth occurs on CM agar and nutrient agar media. Diffusible pigments are not produced. Substrate hyphae are well developed and fragment with age. Long, straight or flexuous spore chains are borne on aerial hyphae, which fragment into rod-shaped non-motile spores (0.5–0.6 × 1.2–1.8 μm) with smooth surfaces. Grows optimally at 28–32 °C and pH 6–8 and in the presence of 15–25 % (w/v) NaCl. Growth is not observed in the absence of NaCl. Able to reduce nitrate to nitrite. Degrades adenine, casein, gelatin, guanine, hypoxantine, Tween 80 and tyrosine, but not starch, testosterone or xanthine. The following compounds are utilized: adonitol, arabinose, glucose, glycerol, cellobiose, erythritol, fructose, galactose, lactose, maltose, mannose, melezitose, rhamnose, ribose, sorbitol, sucrose, xylose and proline. The following substances are not utilized: mannitol, melibiose, raffinose, salicin, trehalose, alanine and serine. Acetate, citrate and pyruvate are decarboxylated but not, benzoate, butyrate, oxalate, propionate, succinate and tartrate. Milk was coagulated and peptonized. H_2S was not produced. Methyl red and Voges–Proskauer tests were negative. 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 susceptible to chloramphenicol (25 μg ml^{-1}). Contains *meso*-diaminopimelic acid, as cell-wall diamino acid, and arabinose and galactose as major whole-cell sugars. The diagnostic phospholipid is phosphatidylcholine. The major fatty acids are anteiso-$C_{17:0}$ (37.4 %), iso-$C_{17:0}$ (14.8 %), iso-$C_{15:0}$ (14.2 %), and iso-$C_{16:0}$ (13.9 %). MK-10 (H_4) and MK-9 (H_4) are the predominant menaquinones. Mycolic acids are not detected.

The type strain is H23^T (=DSM 45501^T =CCUG 63368^T =MTCC 11562^T) isolated from a Saharan soil sample collected from Oued Righ region (South Algeria). The 16S rRNA gene sequence of strain H23^T has been deposited in GenBank under the accession number HQ918196.

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