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Actinopolyspora saharensis sp. nov., a novel halophilic actinomycete isolated from a Saharan soil of Algeria

Atika Meklat · Nouredine Bouras · Abdelghani Zitouni · Florence Mathieu ·
Ahmed Lebrihi · Peter Schumann · Cathrin Spröer · Hans-Peter Klenk ·
Nasseridine Sabaou

Abstract A novel halophilic actinomycete, strain H32^T, was isolated from a Saharan soil sample collected in El-Oued province, south Algeria. The isolate was characterized by means of polyphasic taxonomy. Optimal growth was determined to occur at 28–32 °C, pH

6.0–7.0 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 fragmented substrate mycelium. The cell wall was determined to contain *meso*-diaminopimelic acid and the characteristic whole-cell sugars were arabinose and galactose. The predominant menaquinones were found to be MK-10(H₄) and MK-9(H₄). The predominant cellular fatty acids were determined to be anteiso C_{17:0}, iso-C_{15:0} and iso-C_{16:0}. The diagnostic phospholipid detected was phosphatidylcholine. Phylogenetic analyses based on the 16S rRNA gene sequence showed that this strain formed a distinct phyletic line within the radiation of the genus *Actinopolyspora*. The 16S rRNA gene sequence similarity indicated that strain H32^T was most closely related to '*Actinopolyspora algeriensis*' DSM 45476^T (98.8 %) and *Actinopolyspora halophila* DSM 43834^T (98.5 %). Furthermore, the result of DNA–DNA hybridization between strain H32^T and the type strains '*A. algeriensis*' DSM 45476^T, *A. halophila* DSM 43834^T and *Actinopolyspora mortivallis* DSM 44261^T demonstrated that this isolate represents a different genomic species in the genus *Actinopolyspora*. Moreover, the physiological and biochemical data allowed the differentiation of strain H32^T from its closest phylogenetic neighbours. Therefore, it is proposed that strain H32^T represents a novel species of the genus *Actinopolyspora*, for which the name *Actinopolyspora saharensis* sp. nov. is proposed. The type strain is H32^T (=DSM 45459^T=CCUG 62966^T).

A. Meklat · N. Bouras · A. Zitouni · N. Sabaou (✉)
Laboratoire de Biologie des Systèmes Microbiens
(LBSM), Ecole Normale Supérieure de Kouba, Alger,
Algeria
e-mail: sabaou@yahoo.fr

A. Meklat
Département de Biologie, Faculté des sciences
Agronomiques, Vétérinaires et Biologiques,
Université Saâd Dahleb de Blida, Blida, Algeria

F. Mathieu · A. Lebrihi
Laboratoire de Génie Chimique, UMR 5503
(CNRS/INPT/UPS), INPT-ENSAT, Université de
Toulouse, 1 Avenue de l'Agrobiopôle, Auzeville
Tolosane, BP 32607, 31326 Castanet-Tolosan, France

A. Lebrihi
Université Moulay Ismail, Marjane 2, BP 298 Meknes,
Morocco

P. Schumann · C. Spröer · H.-P. Klenk
Leibniz Institute DSMZ, German Collection
of Microorganisms and Cell Cultures, Inhoffenstraße 7B,
38124 Braunschweig, Germany

Keywords *Actinopolyspora* · *A. saharensis* sp. nov. · Halophilic actinomycete · Sahara · Polyphasic taxonomy

Introduction

The genus *Actinopolyspora* was proposed by Gochnauer et al. (1975) and its description has been emended recently by Tang et al. (2011). It currently encompasses the species *Actinopolyspora halophila* (Gochnauer et al. 1975), *Actinopolyspora mortivallis* (Yoshida et al. 1991), *Actinopolyspora xinjiangensis* (Guan et al. 2010), *Actinopolyspora egyptensis* (Hozzein and Goodfellow 2011), *Actinopolyspora alba* and *Actinopolyspora erythraea* (Tang et al. 2011) and *Actinopolyspora algeriensis* (Meklat et al. 2012). The two species '*A. egyptensis*' (Hozzein and Goodfellow 2011) and '*A. algeriensis*' (Meklat et al. 2012) were described recently but these names have not yet been validated. The genus is characterized by a cell wall of type IVA (*meso*-diaminopimelic acid, arabinose and galactose), a type PIII phospholipid pattern (phosphatidylcholine), the presence of MK-9 (H₄) and MK-10 (H₄) or MK-9 (H₄) and MK-9 (H₂) as the predominant menaquinones, the presence of iso-C_{16:0} and anteiso-C_{17:0} as the major fatty acids and the absence of mycolic acids (Gochnauer et al. 1989; Tang et al. 2011). The G+C contents of the genomic DNA are 64–69 mol%.

During a study on halophilic actinomycetes from Saharan soils in El-Oued province (south Algeria), strain H32^T was isolated and purified. The present study was carried out to determine the taxonomic status of this strain by using a polyphasic approach. Based on phenotypic and genotypic evidence, it is proposed that the strain H32^T represents a novel species of the genus *Actinopolyspora*, for which the name *Actinopolyspora saharensis* sp. nov. is proposed.

Materials and methods

Isolation and maintenance of isolate

During an investigation of actinomycete diversity in Saharan soils (Meklat et al. 2011), strain H32^T was isolated from a soil sample collected from El-Oued province (33°19'59"N, 6°52'59"E), south Algeria. Isolation was carried out by a dilution-plate method

using humic acid-vitamin agar medium (Hayakawa and Nonomura 1987) supplemented with actidione (50 µg ml⁻¹) and 20 % (w/v) NaCl. After 4 weeks of incubation at 30 °C, the isolate, which formed a white colony, was transferred and purified on complex medium (CM) agar (Chun et al. 2000) supplemented with 20 % (w/v) NaCl. The purified strain was maintained on CM agar slants at 4 °C and as 20 % (v/v) glycerol suspensions at -20 °C. Strain H32^T was deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ) as strain DSM 45459^T, and in the Culture Collection, University of Göteborg, Sweden (CCUG) as strain CCUG 62966^T.

Phenotypic characterization

Characterization of strain H32^T was determined after growth at 30 °C for 3 weeks using the media of the International *Streptomyces* Project, ISP 2 and ISP 4 (Shirling and Gottlieb 1966), and also CM agar (Chun et al. 2000) and nutrient agar (Waksman 1961). The colours of the substrate and aerial mycelia and any soluble pigments produced were determined by comparison with ISCC-NBS colour charts (Kelly and Judd 1976). The morphological characteristics of strain H32^T, including spore-chain morphology, spore size and surface ornamentation, were assessed by light microscopy (B1, Motic) and scanning electron microscopy (Hitachi S450). Several physiological tests were used to characterize actinomycete strain. Growth at different temperatures (10, 15, 25, 28, 30, 32, 35, 40 and 45 °C), various pH values (5.0, 6.0, 7.0, 8.0 and 9.0 using the buffer system described by Xu et al. 2005) and NaCl concentrations (0, 7, 10, 15, 20, 25, 28, 30, 32 and 35 %; w/v) were determined by using nutrient agar medium, with the cultures incubated for 21 days at 30 °C. Utilization of carbohydrates and decarboxylation of organic acids were evaluated using the method of Gordon et al. (1974). Degradation of different other organic compounds was studied as described by Goodfellow (1971). Lysozyme sensitivity and production of nitrate reductase were determined according to the methods of Gordon and Barnett (1977) and Marchal et al. (1987), respectively.

Chemotaxonomy

For the chemotaxonomic analyses, strain H32^T was grown in complex broth medium containing 15 % (w/v)

NaCl at 30 °C for 10 days on a rotary shaker (250 rpm). Biomass was harvested by centrifugation at 3,500 rpm and washed several times with distilled water. Analysis of diaminopimelic acid and whole-cell sugars was carried out using the methods of Becker et al. (1964) and Lechevalier and Lechevalier (1970). Phospholipids were analyzed according to the procedures developed by Minnikin et al. (1977). Menaquinones were isolated according to Minnikin and O'Donnell (1984) and were analyzed by HPLC (Kroppenstedt 1982, 1985). The cellular fatty acid composition was studied as described by Sasser (1990) using the microbial identification system.

Phylogenetic analyses

The genomic DNA of H32^T was extracted according to the method of Liu et al. (2000). PCR amplification of the 16S rRNA gene and sequencing of the purified PCR products were carried out as described by Rainey et al. (1996). 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/>; Kim et al. 2012), a web-based tool for the identification of prokaryotes based on 16S rRNA gene sequences from type strains. The 16S rRNA sequence of strain H32^T was aligned using the CLUSTAL W (Larkin et al. 2007) against corresponding nucleotide sequences retrieved from GenBank. Phylogenetic trees were constructed by 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. The topology of the tree was evaluated by bootstrap analysis based on 1,000 replicates (Felsenstein 1985). DNA–DNA hybridization was carried out as described by De Ley et al. (1970) incorporating the modifications described by Huss et al. (1983). The experiments were done as duplicates in 2 × SSC in the presence of 10 % formamide at 71 °C.

Results and discussion

Strain H32^T was observed to grow well on CM agar, NA and ISP 2 media, with a well-developed white-yellow aerial mycelium that fragmented into rod-

shaped spores. The spores were determined to have a smooth surface (Fig. 1) and be non-motile. Growth was found to be weak on ISP 4 medium, with the aerial mycelium poorly developed. The substrate mycelium showed light yellow or pale yellow colouration and exhibited a good fragmentation. A light yellowish diffusible pigment was found to be produced on CM agar medium after incubation for three weeks.

Strain H32^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 (in addition to ribose), which is typical of cell-wall type IV and whole-cell sugar pattern type A (Lechevalier and Lechevalier 1970). The diagnostic phospholipid detected was phosphatidylcholine, corresponding to phospholipid type PIII (Lechevalier et al. 1977). Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two unknown phospholipids and three unknown glycolipids were also detected (Supplementary Fig. S1a, b and c). The predominant menaquinones were determined to be MK-10 (H₄) (29.4 %) and MK-9 (H₄) (22.4 %) and minor amounts of MK-10 (H₂) (9.4 %), MK-11



Fig. 1 Scanning electron micrograph of strain H32^T grown on ISP 2 medium containing 15 % (w/v) NaCl for 28 days at 30 °C. Bar 5 µm

(H₄) (8.3 %), MK-8 (H₄) (5.9 %), MK-9 (H₂) (5.3 %), MK-11 (H₂) (3.0 %) and MK-10 (H₆) (1.9 %) were also detected. The fatty acids profile was composed as follows: anteiso-C_{17:0}, (30.8 %), iso-C_{15:0} (20.4 %), iso-C_{16:0} (15.6 %), iso-C_{17:0} (9.3 %) and anteiso-C_{15:0} (9.1 %). The morphological and chemical characteristics described above clearly support the placement of strain H32^T within the genus *Actinopolyspora*.

Good growth was found to occur at 28–32 °C, pH 6.0–7.0 and in the presence of 15–25 % of NaCl. 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). Detailed results of the physiological and biochemical analyses are given in Table 1 and in the species description. It is evident from Table 1 that there are several phenotypic characteristics that clearly separate strain H32^T from the nearest recognized species ‘*A. Algeriensis*’ and ‘*A. halophila*’.

Phylogenetic analysis of an almost complete 16S rRNA gene sequence (GenBank accession number HQ918198) showed that strain H32^T was related to members of the genus *Actinopolyspora* and exhibited highest 16S rRNA gene sequence similarity to ‘*A. Algeriensis*’ (98.8 %) and *A. halophila* (98.5 %), whereas the sequence similarities between strain H32^T and other members of the genus *Actinopolyspora* ranged from 96.5 to 97.2 %. The phylogenetic relationship between strain H32^T and the other *Actinopolyspora* species is seen in the neighbour-joining dendrogram (Fig. 2). Maximum parsimony and maximum-likelihood calculation resulted in a similar tree topology (Supplementary Fig. S2). DNA–DNA relatedness between strain H32^T and the type strains ‘*A. Algeriensis*’ DSM 45476^T, *A. halophila* DSM 43834^T and *A. mortivallis* DSM 44261^T were respectively mean values of 30.5 % (30.4 and 30.6 %), 55.1 % (54.3 and 56.0 %) and 31.5 % (28.1 and 34.1 %). These hybridization values were significantly less than 70 %, the threshold value for the delineation of genomic species (Wayne et al. 1987). Thus, on the basis of polyphasic taxonomic evidence, it is suggested that strain H32^T represents a novel species of the genus *Actinopolyspora*, for which the name *Actinopolyspora saharensis* sp. nov. is proposed.

Description of *A. saharensis* sp. nov.

Actinopolyspora saharensis (sa.ha.ren’sis, N.L. fem. adj. *saharensis* pertaining to Sahara, where the type strain was isolated).

Halophilic filamentous actinomycete that forms well-fragmented substrate mycelium. Aerial mycelium is well-developed white-yellow colour on ISP 2, nutrient agar and CM agar media, and is irregularly branched and forms straight to flexuous chains of 10–30 rod-shaped spores. A light yellowish soluble pigment is produced on CM agar medium.

Table 1 Differential characteristics of strain H32^T (*Actinopolyspora saharensis* sp. nov.) compared with its closest relative recognized species of the genus *Actinopolyspora*

Characteristics	Type strains		
	1	2	3
Utilization of			
D-cellobiose	+	–	+
Erythritol	–	–	+
D-galactose	+	–	+
D-glucose	+	–*	+
Inositol	–	+	+
D-lactose	+	–	+
D-maltose	+	–	+
D-mannitol	–	+	–
D-mannose	+	–*	+
Raffinose	–	–	+
Salicin	–	+	–
Sucrose	+	–	+
D-xylose	+	–	+
Decomposition of			
Casein	–	+	–
Hypoxanthine	+	+	–
Starch	+	–	–
Xanthine	+	–	–
Production of nitrate reductase	+	–	+
Resistance to lysozyme (0.005 % w/v)	+	–	+
Growth in the presence of 7 and 32 % of NaCl	–	–	+

The phenotypic properties of strains *A. saharensis* H32^T, *A. halophila* DSM 43834^T and ‘*A. Algeriensis*’ DSM 45476^T grown under the same conditions were determined in the present study. Strains: 1 *A. saharensis* H32^T, 2 *A. halophila* DSM 43834^T, 3 ‘*A. Algeriensis*’ DSM 45476^T, + positive, – negative

* Variable or doubtful results were obtained by Yoshida et al. (1991), Guan et al. (2010), Hozzein and Goodfellow (2011) and Tang et al. (2011)

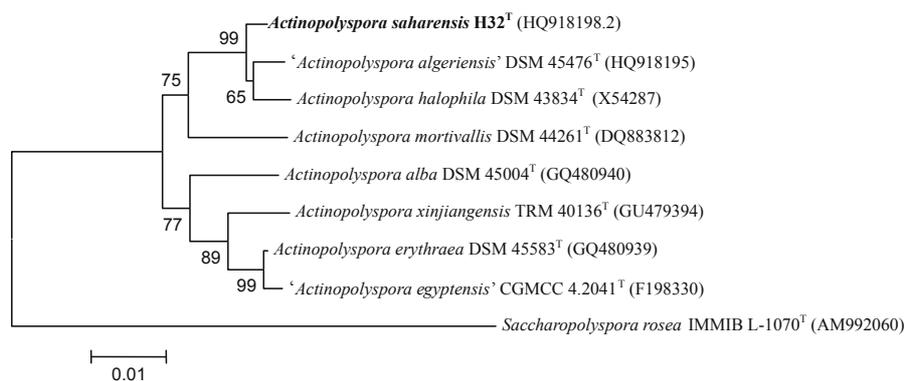


Fig. 2 Phylogenetic tree for species of the genus *Actinopolyspora* calculated from almost complete 16S rRNA gene sequences using Jukes and Cantor (1969) evolutionary distance methods and the neighbour-joining method of Saitou and Nei (1987). This illustrates the taxonomic position of strain H32^T

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

Temperature and pH ranges for growth are 20–35 °C and pH 5.0–8.0, with optima at 28–32 °C and pH 6.0–7.0. The NaCl concentration range for growth is 10–30 %, with optimal growth occurring at 15–20 %. Utilizes adonitol, arabinose, cellobiose, fructose, galactose, glucose, glycerol, lactose, maltose, mannose, rhamnose, sucrose, trehalose and xylose but not erythritol, inositol, mannitol, melezitose, melibiose, raffinose, ribose, salicin and sorbitol. Positive for adenine, gelatin, hypoxanthine, starch, Tween 80 and xanthine hydrolysis but negative for casein, guanine, testosterone and tyrosine hydrolysis. Citrate is decarboxylated but not acetate, benzoate, butyrate, oxalate, propionate, pyruvate, succinate and tartrate. L-proline is used as a source of nitrogen but not L-alanine and L-serine. Nitrate reductase is produced. Whole-cell hydrolysates contain *meso*-diaminopimelic acid, arabinose and galactose. The diagnostic phospholipid is phosphatidylcholine (type PIII sensu Lechevalier et al. 1977). The predominant menaquinones are MK-10 (H₄) and MK-9 (H₄). The major fatty acids are anteiso-C_{17:0}, iso-C_{15:0}, iso-C_{16:0}, iso-C_{17:0} and anteiso-C_{15:0}.

The type strain, H32^T (=DSM 45459^T=CCUG 62966^T), was isolated from a Saharan soil sample collected from El-Oued province (south Algeria). The GenBank accession number for the 16S rRNA gene sequence of strain H32 is HQ918198.

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