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Actinopolyspora algeriensis sp. nov., a novel halophilic actinomycete isolated from a Saharan soil

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

Abstract A halophilic actinomycete strain designated H19^T, was isolated from a Saharan soil in the Bamendil region (Ouargla province, South Algeria) and was characterized taxonomically by using a polyphasic approach. The morphological and chemotaxonomic characteristics of the strain were consistent with those of members of the genus *Actinopolyspora*, and 16S rRNA gene sequence analysis confirmed that strain H19^T was a novel species of the genus *Actinopolyspora*. DNA–DNA hybridization value between strain H19^T and the nearest *Actinopolyspora* species, *A. halophila*, was clearly below the 70 % threshold. The genotypic and phenotypic data showed that the organism represents a novel species of the genus *Actinopolyspora* for which the name *Actinopolyspora algeriensis* sp. nov. is proposed, with the type strain H19^T (= DSM 45476^T = CCUG 62415^T).

A. Meklat · N. Bouras · A. Zitouni · N. Sabaou (✉)
Laboratoire de Biologie des Systèmes Microbiens (LBSM),
Ecole Normale Supérieure de Kouba, B.P. 92,
16 050 Kouba, Alger, Algeria
e-mail: sabaou@yahoo.fr

A. Meklat
e-mail: atika_mek@yahoo.fr

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

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

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Introduction

The genus *Actinopolyspora* was described for the first time by Gochnauer et al. (1975), which represents the only genus in the family *Actinopolysporaceae* (Zhi et al. 2009). This extremely halophilic genus comprises currently only five species with validly published names, namely *A. halophila* (Gochnauer et al. 1975), *A. mortivallis* (Yoshida et al. 1991), *A. xinjiangensis* (Guan et al. 2010), *A. alba* and *A. erythraea* (Tang et al. 2011). The species *A. egyptensis* was described by Hozzein and Goodfellow (2011), but not yet validated. The strains of this genus were characterized by long chains of spores on aerial mycelium, fragmentation of the substrate mycelium, type IVA cell wall (*meso*-diaminopimelic acid without glycine, arabinose and galactose as diagnostic whole cell sugars), type PIII (phosphatidylcholine) phospholipid pattern, type 2e fatty acid pattern, and G + C contents of the DNA from 64.2 to 68 mol % (Gochnauer et al. 1975; Yoshida et al. 1991; Kroppenstedt and Evtushenko 2006). Different predominant menaquinone patterns were reported: MK-6, MK-10(H₄), MK-7 and MK-9(H₄) for *A. xinjiangensis* TRM 40136^T (Guan et al. 2010), MK-9 (H₄) and MK-10 (H₄) for *A. alba* YIM 90480^T, *A. halophila* DSM 43834^T and *A. mortivallis* DSM 44261^T (Tang et al. 2011), or MK-9 (H₄) and MK-9 (H₂) for *A. erythraea* (Tang et al. 2011). In this study, a halophilic actinomycete, designated strain H19^T, was isolated. Based on data from the present polyphasic taxonomic research, this strain is considered to represent a novel species of the genus *Actinopolyspora*.

Materials and methods

Actinomycete strain

The strain H19^T was isolated from a saline soil sample (electrical conductivity = 3.6 mS cm⁻¹), collected from Bamendil palm grove (Ouargla province, South Algerian Sahara), by a dilution agar plating method using humic acid–vitamin agar (Hayakawa and Nonomura 1987) supplemented with actidione (50 mg l⁻¹) and 20 % (w/v) NaCl at 30 °C for 25 days. The strain was purified and maintained at 4 °C on complex medium (CM) agar (Chun et al. 2000) containing 20 % (w/v) NaCl.

Cultural and micro-morphological characteristics

Cultural characteristics were investigated after 7, 14 and 21 days of incubation at 30 °C on media from the International *Streptomyces* Project (ISP 2 and ISP 4) (Shirling and Gottlieb 1966), CM agar (Chun et al. 2000) 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 colours of substrate and aerial mycelia and any soluble pigments produced were determined according to the ISCC-NBS centroid colour chart (Kelly and Judd 1976). The production of melanoid pigments was evaluated on ISP6 and ISP7 media as recommended by Shirling and Gottlieb (1966). Spores and mycelia were examined by light microscope (Motic, B1 Series) and scanning electron microscope (model S450; HITACHI, Japan) after two weeks growth on ISP 2 medium. All media used for morphological characteristics contained 15 % (w/v) NaCl.

Physiological characterization

Several physiological tests were used to characterize the strain H19^T in comparison with *Actinopolyspora halophila* DSM 43834^T, the most closely related species. Growth and production of acid from 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. Growth at different temperatures, pH and NaCl concentrations, and in the presence of antibiotics was determined on nutrient agar medium. All media used for physiological tests contained 15 % (w/v) NaCl (except for the NaCl concentration test).

Chemotaxonomic characterization

For chemotaxonomic analyses, strain H19^T was grown in complex medium (CM) broth containing 15 % (w/v) NaCl at 30 °C for 10 days on a rotary shaker (250 rpm). Biomass was harvested by centrifugation at 3500 rpm and washed several times with demineralized water. The isomeric form of diaminopimelic acid and the presence (or not) of glycine in the cell wall were realized as described by Becker et al. (1964). The composition of whole-cell sugars was determined as described by Lechevalier and Lechevalier (1970). Phospholipids were analyzed using the procedure of Minnikin et al. (1977). The fatty acid profile was determined by the method of Sasser (1990), using the TSBA40 method on a Microbial Identification System (MIDI) version Sherlock 6.1. The menaquinones were extracted following the procedure of Minnikin et al. (1984), and were analyzed by HPLC (Kroppenstedt 1982, 1985).

Determination of 16S rRNA gene sequence, phylogenetic analysis and DNA–DNA hybridization

The strain H19^T was grown on the CM broth supplemented with 15 % (w/v) NaCl. Genomic DNA was extracted with DNA extraction kit (JetFlex, Germany). PCR-mediated amplification of the 16S rRNA gene was performed as described by Rainey et al. (1996). PCR products were purified with a PCR product purification kit (Qiagen, Germany). The primers used for sequencing are listed in Coenye et al. (1999). The 16S rRNA sequence has been deposited in the GenBank data library and assigned the accession number HQ918195. 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. Phylogenetic analyses were conducted using MEGA version 5 (Tamura et al. 2011). The 16S rRNA gene sequence of the strain H19^T was aligned against neighbouring nucleotide sequences using the CLUSTAL W (with default parameters) (Thompson et al. 1994). Phylogenetic tree was constructed by using the neighbour-joining (Saitou and Nei 1987) with Jukes and Cantor (1969) model. Bootstrap analysis (Felsenstein 1985) was performed to evaluate the reliability of the tree topology.

For DNA–DNA hybridizations, cells were disrupted by using a French pressure cell (Thermo Spectronic). The 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 as duplicates in 2 × SSC in the presence of 10 % formamide at 71 °C.

Results and discussion

Morphological and biochemical characteristics

Strain H19^T exhibited good growth on ISP 2, nutrient agar and CM agar media, and the aerial mycelium was moderately abundant with yellowish white colour on these media. However, no growth was observed on ISP 4 medium. Substrate mycelium was light yellow colour on ISP 2, nutrient agar and CM agar media. Melanoid pigments and other diffusible pigments were not produced on tested media. The substrate mycelium was well developed and fragmented into non-motile rods. The substrate mycelium exhibited an abundant fragmentation on both solid and liquid media. The aerial mycelium was irregularly branched and formed straight to flexuous chains of 10–30 spores per chain. The spores (0.5–0.6 × 1.2–1.8 μm) were non-motile, smooth-surfaced and rod-shaped (Fig. 1). No morphological forms or structures resembling sporangia, sclerotia or synnemata was observed. The strain H19^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

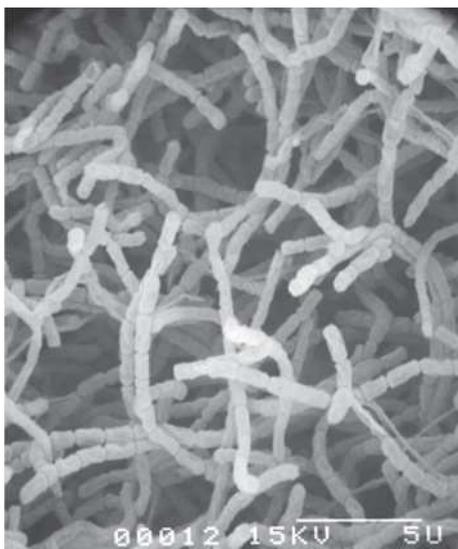


Fig. 1 Scanning electron micrograph of spore chains of strain H19^T grown on yeast extract/malt extract agar (ISP 2) containing 15 % (w/v) NaCl for 15 days at 30° C. Bar 5 μm

growth. It was resistant to some antibiotics tested. The morphological, chemical and physiological characteristics described above are consistent with those of the genus *Actinopolyspora*. Furthermore, the results showed that the strain H19^T is physiologically different from the nearest recognized *Actinopolyspora* species (*A. halophila*), as can be seen from the differential physiological characters given in Table 1. The complete physiological characteristics of strain H19^T are given in the species description below.

Chemotaxonomic characteristics

Strain H19^T contained *meso*-diaminopimelic acid (but not glycine) in cell wall. Whole-cell hydrolysates contained arabinose and galactose. This is typical of cell wall type IV and whole-cell sugar pattern type A (Lechevalier and Lechevalier 1970). Diagnostic phospholipid detected was phosphatidylcholine, corresponding to phospholipids type PIII (Lechevalier et al. 1977). The predominant fatty acids were anteiso-C_{17:0}, (31.3 %), iso-C_{15:0} (17.8 %), anteiso-C_{15:0} (15.8 %), iso-C_{16:0} (15.6 %) and iso-C_{17:0} (3.8 %)

Table 1 Differential phenotypic characteristics of *Actinopolyspora algeriensis* sp. nov. compared with the nearest recognized species of the genus *A. halophila* DSM 43834^T Strain: 1, H19^T; 2, *A. halophila* DSM 43834^T

Characteristic	1	2
Growth and production of acid		
D-Cellobiose	+	–
D-Galactose	+	–
D-Lactose	+	–
D-Maltose	+	–
D-Mannitol	–	+
D-Mannose	+	– ^a
Salicin	–	– ^a
D-Glucose	+	– ^a
Raffinose	+	–
Sucrose	+	–
D-Xylose	+	–
Decomposition of:		
Casein	–	+
Hypoxanthine	–	+
Production of nitrate reductase	+	–
Resistance to erythromycin (10 μg ml ⁻¹)	–	+
Growth at 45 °C	+	–
Growth on nutrient agar medium in the presence of:		
7 % NaCl	+	–
32 % NaCl	+	–

+, positive; –, negative. All data are from the present study

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

(Table 2). This fatty acid pattern belongs to fatty acid type 2e (Kroppenstedt and Evtushenko 2006). The predominant menaquinones were MK-9 (H₄) (39.1 %) and MK-10 (H₄) (32.1 %). The menaquinones MK-9 (H₂) (9.1 %), MK-10 (H₂) (5.7 %) and MK-8 (H₄) (3.4 %) were also detected.

Phylogenetic analysis based on 16S rRNA gene sequence comparison and DNA–DNA relatedness

The phylogenetic relationship between strain H19^T and the other species of *Actinopolyspora* with validly

Table 2 Cellular fatty acid composition of *Actinopolyspora algeriensis* strain H19^T in comparison with the closely related species *A. halophila* DSM 43834^T Taxa: 1, H19^T; 2, *A. halophila* DSM 43834^T Values are percentages of total fatty acids

Fatty acid	1	2
iso-C _{14:0}	3.4	2.4
iso-C _{15:0}	17.8	24.1
anteiso-C _{15:0}	15.8	34.6
iso-C _{16:1} H	1.1	–
iso-C _{16:0}	15.6	9.3
cis9-C _{16:1}	1.6	–
C _{16:0}	0.5	4.7
9-Methyl-C _{16:0}	2.2	–
anteiso-C _{17:1} C	1.3	–
iso-C _{17:0}	3.8	7.4
anteiso-C _{17:0}	31.3	10.2
cis9-C _{17:1}	1.3	–
cis9-C _{18:1}	2.7	0.6

– not detected

C and H indicate that the double bonds are in different locations fatty acid identities were determined by using the TSBA40 method on a Microbial Identification System (MIDI) version Sherlock 6.1

published names is seen in the neighbour-joining dendrogram (Fig. 2). The similarity level was 98.5 % to *Actinopolyspora halophila* DSM 43834^T (Gochnauer et al. 1975), the most closely related species. However, the 16S rRNA gene sequence similarities between strain H19^T and other remaining *Actinopolyspora* species were below 97 %. DNA of strain H19^T was hybridized with that of *A. halophila* DSM 43834^T (Gochnauer et al. 1975), the closest phylogenetic neighbour. The level of DNA–DNA relatedness with this strain was 43.6 % (based on the mean of duplicate determinations, 40.6 and 46.7 %), which is clearly below the 70 % relatedness guideline proposed by Wayne et al. (1987) for delineation of separate species.

Taxonomic conclusion

The results of the morphological and chemotaxonomic investigations and phylogenetic analysis supported the affiliation of the strain H19^T to the genus *Actinopolyspora*. In addition, a comparison with the physiology of the nearest species *A. halophila* DSM 43834^T, showed differences in the utilization of casein, hypoxanthine cellobiose, galactose, lactose, maltose, mannitol, mannose and salicin, in the production of nitrate reductase, in the growth at 10 and 20 °C, in the growth with 7 and 32 % NaCl (w/v) on nutrient agar medium, and in resistance to erythromycin (10 mg l⁻¹) as shown in Table 1. Furthermore, the differences in the production of acids from the utilization of glucose, maltose, raffinose, sucrose and xylose were observed. All of the data support the designation of strain H19^T as representing a novel species of the genus *Actinopolyspora*, for which the name *Actinopolyspora algeriensis* sp. nov. is proposed.

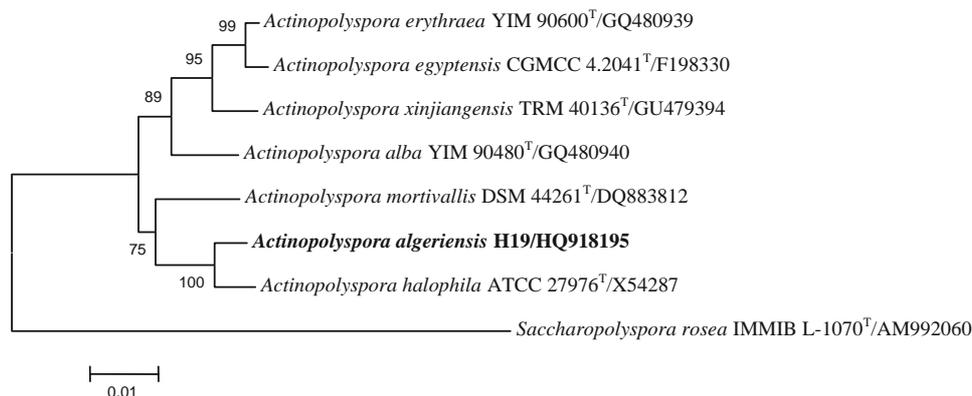


Fig. 2 Phylogenetic tree for species of the genus *Actinopolyspora* calculated from almost complete 16S rRNA gene sequences using Jukes and Cantor (1969) evolutionary distance methods and the neighbour-joining method of Saitou and Nei (1987). This illustrates the taxonomic position of strain H19^T relative to the other species of

the genus, including the taxonomically not yet validated *A. egyptensis* (Hozzein and Goodfellow 2011). 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

Description of *Actinopolyspora algeriensis* sp. nov.

Actinopolyspora algeriensis (al.ger.i.en'sis. N.L. fem. adj. *algeriensis*, pertaining to Algeria, the source of the soil from which the type strain was isolated.

Halophilic filamentous actinomycete, aerial mycelium is yellowish white colour on ISP 2, nutrient agar and CM agar media), and is irregularly branched and formed straight to flexuous chains of 10–30 rod-shaped spores ($0.5\text{--}0.6 \times 1.2\text{--}1.8 \mu\text{m}$) per chain. The colour of the substrate mycelium is light yellow on all tested culture media. The substrate mycelium was well developed and fragmented into non-motile rods. Melanoid and other diffusible pigments are not produced. Growth occurs at 25, 30, 35 and 40 °C (but not at 10, 15, 20 and 45 °C), and 30 °C is the optimal temperature among the tested ones. Moreover, the growth occurs at pH 5, 6, 7 and 8 (but not at pH 9), and pH 7 is the optimal pH among the tested ones. Gelatin, guanine and Tween 80 are degraded. Adenine, casein, starch, testosterone, hypoxanthine and xanthine are not decomposed. Utilizes: L-arabinose, D-cellobiose, erythritol, D-fructose, D-galactose, D-glucose, glycerol, maltose, L-rhamnose, D-ribose, adonitol, D-lactose, *meso*-inositol, D-mannose, D-raffinose, D-trehalose, sucrose and D-xylose as carbon sources for growth and acid production, but not D-mannitol, D-melezitose, melibiose, salicin and D-sorbitol. Acetate, benzoate, butyrate, citrate, oxalate, propionate, pyruvate, succinate and tartrate were not decarboxylated. L-alanine and L-proline are used as source of nitrogen but not L-serine and tyrosine. 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). Moreover, the growth occurs in the presence of kanamycin ($5 \mu\text{g ml}^{-1}$), streptomycin ($10 \mu\text{g ml}^{-1}$) and penicillin ($25 \mu\text{g ml}^{-1}$), and also in the presence of 0.005 % lysozyme, but not in the presence of erythromycin ($10 \mu\text{g ml}^{-1}$) and chloramphenicol ($25 \mu\text{g ml}^{-1}$). Type IVA cell wall (*meso*-diaminopimelic acid, arabinose and galactose in whole-cell hydrolysates). Phospholipids type PIII (phosphatidylcholine). The predominant menaquinones are MK-9 (H_4) and MK-10 (H_4). The predominant fatty acids are anteiso- $C_{17:0}$, iso- $C_{15:0}$, anteiso- $C_{15:0}$, iso- $C_{16:0}$ and iso- $C_{17:0}$.

The type strain is H19^T (= DSM 45476^T = CCUG 62415^T) isolated from a Saharan soil sample collected from Ouargla region (South Algeria).

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