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## Streptomonospora algeriensis sp. nov., a halophilic actinomycete isolated from soil in Algeria

Atika Meklat · Noureddine Bouras · Amar Riba · Abdelghani Zitouni · Florence Mathieu · Manfred Rohde · Peter Schumann · Cathrin Spröer · Hans-Peter Klenk · Nasserdine Sabaou

**Abstract** A halophilic actinomycete strain, designated H27<sup>T</sup>, was isolated from a soil sample collected from a hypersaline habitat in Djelfa Province (North-Central Algeria), and then investigated using a polyphasic taxonomic approach. The strain was observed to produce poor aerial mycelium, which formed short chains of oval to cylindrical-shaped spores at maturity, and non fragmented substrate mycelium. The optimum NaCl concentration for growth was found to be 10–15 % (w/v) and the optimum growth temperature and pH were found to be 28–37 °C and 6–7, respectively. The diagnostic diamino acid in the cell-wall peptidoglycan was identi-

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Laboratoire de Génie Chimique, UMR 5503 (CNRS/ INPT/UPS), Université de Toulouse, INPT-ENSAT, 1 Avenue de l'Agrobiopôle, BP 32607, 31326 Castanet-Tolosan, Auzeville Tolosane, France fied as meso-diaminopimelic acid. The predominant menaquinones of strain H27<sup>T</sup> were identified as MK-11 (H<sub>4</sub>) and MK-10 (H<sub>6</sub>). The major fatty acids were found to be iso- $C_{16:0}$ , anteiso- $C_{17:0}$ , 10 methyl  $C_{17:0}$  and 10 methyl C<sub>16:0</sub>. The diagnostic phospholipids detected were phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and phosphatidylinositol. The chemotaxonomic properties of strain  $H27^{T}$  are consistent with those shared by members of the genus Streptomonospora. 16S rRNA gene sequence analysis indicated that strain H27<sup>T</sup> is most closely related to Streptomonospora alba DSM 44588<sup>T</sup> (98.8 %) and Streptomonospora flavalba DSM 45155<sup>T</sup> (98.7 %) whereas the DNA-DNA relatedness values between strain  $H27^{T}$  and the two type strains were 17.1 and 57.9 %, respectively. Based on the combined

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P. Schumann · C. Spröer · H.-P. Klenk Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Brunswick, Germany genotypic and phenotypic evidence, it is proposed that strain H27<sup>T</sup> should be classified as representative of a novel species, for which the name *Streptomonospora algeriensis* sp. nov. is proposed. The type strain is H27<sup>T</sup> (=DSM 45604<sup>T</sup> =CCUG 63369<sup>T</sup> =MTCC 11563<sup>T</sup>).

**Keywords** Streptomonospora · Streptomonospora algeriensis sp. nov · Halophilic actinomycete · Algerian soil · Polyphasic taxonomy

#### Introduction

The genus Streptomonospora was described by Cui et al. (2001) and was affiliated with the family Nocardiopsaceae of the order Streptosporangiales (Zhi et al. 2009). Currently, the genus contains eight recognized species, Streptomonospora salina (Cui et al. 2001), Streptomonospora alba (Li et al. 2003), Streptomonospora halophila (Cai et al. 2008), Streptomonospora amylolytica and Streptomonospora flavalba (Cai et al. 2009), Streptomonospora sediminis, Streptomonospora nanhaiensis and Streptomonospora arabica (Zhang et al. 2013). The genus is characterized by cell walls of type IIIC (mesodiaminopimelic acid without diagnostic sugar); a type PII phospholipid pattern (phosphatidylethanolamine); the presence of menaquinones with nine or ten isoprenoid chains and a varying degree of hydrogenation as predominant menaquinone; and the presence of  $C_{16:0}$ , C<sub>17:0</sub>, iso-C<sub>15:0</sub> and iso-C<sub>16:0</sub> as the major fatty acids. The G + C content of the genomic DNA of members of the genus is 70.7-74.4 mol%.

During our study of the taxonomy of halophilic actinomycetes (Meklat et al. 2011), an isolate H27<sup>T</sup>, was isolated from Djelfa region in Algeria. Based on data from the present polyphasic taxonomic research, it is proposed that strain H27<sup>T</sup> represents a novel species of the genus *Streptomonospora*, named *Streptomonospora algeriensis* sp. nov. The type strain is H27<sup>T</sup> (=DSM 45604<sup>T</sup> =CCUG 63369<sup>T</sup> =MTCC 11563<sup>T</sup>).

#### Materials and methods

Isolation and maintenance of strain

Strain H27<sup>T</sup> was isolated from a soil sample collected from Djelfa Province (Algeria). Isolation was carried

out by a dilution-plate method using complex medium (CM) agar (Chun et al. 2000) supplemented with actidione (50  $\mu$ g ml<sup>-1</sup>) and 20 % (w/v) NaCl and incubated for 5 weeks. The strain was purified and maintained at 4 °C on CM slants agar containing 20 % (w/v) NaCl and at -20 °C as 20 % (v/v) glycerol suspensions.

Strain H27<sup>T</sup> was deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ), Germany, as strain DSM 45604<sup>T</sup>; in the Culture Collection, University of Göteborg (CCUG), Sweden, as strain CCUG 63369<sup>T</sup> and in the Microbial Type Culture Collection (MTCC), India, as strain MTCC 11563<sup>T</sup>.

#### Phenotypic characterization

Cultural characteristics were investigated after 7, 14 and 21 days of incubation at 30 °C on media of the International *Streptomyces* Project, ISP 2 and ISP 4 (Shirling and Gottlieb 1966), and also on CM agar (Chun et al. 2000) and nutrient agar (NA) (Waksman 1961). The degree of growth was determined and 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). All media used for morphological characteristics contained 15 % (w/v) NaCl. The morphological characteristics of strain H27<sup>T</sup>, 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 strain H27<sup>T</sup>. 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 NA medium.

#### Chemotaxonomy

Amino acid and sugar analyses of whole-cell hydrolysates were performed according to the procedures described by Becker et al. (1964) and Lechevalier and Lechevalier (1970). Phospholipids were analyzed according to the method developed by Minnikin et al. (1977). The fatty acid profile was determined by the method of Sasser (1990), using the Microbial Identification System (MIDI). Menaquinones were isolated according to Minnikin and O'Donnell (1984) and were analyzed by HPLC (Kroppenstedt 1982, 1985).

#### Phylogenetic analyses

Strain H27<sup>T</sup> was grown in CM broth supplemented with 15 % (w/v) NaCl and genomic DNA was extracted with a DNA extraction kit (MasterPure Gram Positive DNA Purification kit; Epicentre Biotechnologies). PCR 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). The primers used for sequencing were as listed in Coenye et al. (1999). 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). Phylogenetic analyses were conducted using MEGA version 5 (Tamura et al. 2011). The 16S rRNA gene sequence of strain H27<sup>T</sup> was aligned against neighbouring nucleotide sequences using CLUSTAL W (with default parameters) (Larkin et al. 2007). Phylogenetic trees were reconstructed by using the neighbour-joining method (Saitou and Nei 1987) with the model of Jukes and Cantor (1969), the maximumlikelihood method (Felsenstein 1985) with Kimura's 2-parameter (Kimura 1980) model and maximumparsimony (Fitch 1977) methods. The topology of the trees was evaluated by bootstrap analysis based on 1,000 replicates (Felsenstein 1985).

#### DNA-DNA hybridization

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  $2 \times$  SSC buffer in the presence of 10 % formamide at 71 °C.

#### **Results and discussion**

Morphological observation of a 3 weeks old culture of strain H27<sup>T</sup> revealed that the substrate mycelium was

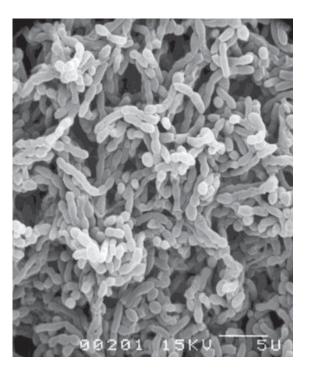


Fig. 1 Scanning electron micrograph of aerial mycelium of strain  $H27^{T}$  grown on CM agar medium containing 15 % (w/v) NaCl for 28 days at 30 °C. *Bar* 5  $\mu$ m

abundant, well developed and non-fragmented but the aerial mycelium was poor. Strain H27<sup>T</sup> was found to show good growth on CM agar and NA media but to exhibit poor growth on ISP 2 and ISP 4 media. Yellowwhite aerial mycelium was found to be produced on CM agar and NA media but no aerial mycelium was formed on ISP 2 and ISP 4 media. The substrate mycelium was pale observed to be yellow on all tested media. No diffusible pigment was detected on any tested media. The aerial mycelium was found to form short chains of spores at maturity, which were straight to flexuous; spores were observed to be oval to cylindrical-shaped with smooth surfaces and to be non-motile (Fig. 1). The substrate mycelium was found to produce, very rarely, non-motile isolated ovoid spores (Fig. S1), which were observed only on CM agar medium.

Strain H27<sup>T</sup> was determined to contain *meso*-diaminopimelic acid (but not glycine) in its cell wall. Whole-cell hydrolysates were found to contain galactose (in addition to glucose), which is typical of cell-wall type III and wholecell sugar pattern type C (Lechevalier and Lechevalier 1970). Strain H27<sup>T</sup> was found to possess phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and two

Table 1Differential physiologic and chemotaxonomic char-<br/>acteristics of strain  $H27^T$  (*Streptomonospora algeriensis* sp.<br/>nov.) compared with its closest relative recognized species of<br/>the genus *Streptomonospora* 

Characteristics	Type strains		
	1	2	3
Utilization of			
Arabinose	_	-	+
Cellobiose	+	-	_
Maltose	+	_	+
Rhamnose	_	-	+
Alanine	_	-	+
Proline	_	-	+
Serine	_	-	+
Hydrolysis of			
Starch	_	-	+
Tyrosine	_	-	+
Production of melanoid pigments	-	-	+
Production of nitrate reductase	-	+	-
NaCl range of growth (%, w/v)	7–20	5–25	5–25
pH range of growth	5–8	5–9	5–9
Temperature optimum (°C)	30	28	37
Menaquinone composition			
MK-9 (H <sub>4</sub> )	_	+	_
MK-9 (H <sub>8</sub> )	_	+	+
MK-10 (H <sub>2</sub> )	_	+	_
MK-10 (H <sub>4</sub> )	+	+	_
MK-10 (H <sub>6</sub> )	+	_	+
MK-10 (H <sub>8</sub> )	_	_	+
MK-11 (H <sub>4</sub> )	+	_	_
Phospholipid composition			
PE	+	+	_
MPE	_	+	_
PS	_	+	_
PIM	_	_	+
Sugar in whole-cell hydrolysates (with galactose)	Glucose	Arabinose	Glucose

All data presented in this table were realized under the same conditions

Strains 1 S. algeriensis H27<sup>T</sup>; 2 S. alba DSM 44588<sup>T</sup>; 3 S. flavalba DSM 45155<sup>T</sup>; + Positive; – negative; *PE* phosphatidylethanolamine; *MPE* methylphosphatidylethanolamine; *PS* phosphatidylserine; *PIM* phosphatidylinositol mannosides

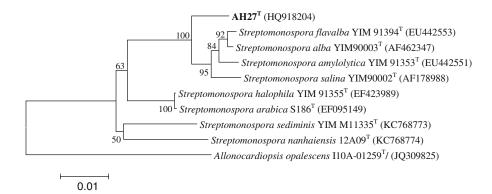
glycolipids (Fig. S2). The predominant menaquinones were determined to be MK-11 (H<sub>4</sub>) (41.0 %) and MK-10 (H<sub>6</sub>) (23.9 %); MK-10 (H<sub>4</sub>) (2.0 %) was also detected. The fatty acids profile was composed as follows: iso-C<sub>16:0</sub> (39.2 %), anteiso-C<sub>17:0</sub> (28.9 %), 10 methyl C<sub>17:0</sub> (6.7 %), 10 methyl C<sub>16:0</sub> (6.2 %) and anteiso-C<sub>15:0</sub> (4.1 %). The morphological and chemical characteristics described above clearly support the placement of strain H27<sup>T</sup> within the genus *Streptomonospora*.

Good growth was found to occur at 28–37 °C, pH 6.0–7.0 and in the presence of 10–15 % of NaCl. The organism was found to be resistant to kanamycin (5  $\mu$ g ml<sup>-1</sup>), erythromycin (10  $\mu$ g ml<sup>-1</sup>), streptomycin (10  $\mu$ g ml<sup>-1</sup>) and penicillin (25  $\mu$ g ml<sup>-1</sup>), but sensitive to chloramphenicol (25  $\mu$ g ml<sup>-1</sup>) and lysozyme (0.005 % w/v). 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 H27<sup>T</sup> from the nearest recognized species *S. alba* and *S. flavalba*.

Phylogenetic analysis of an almost complete 16S rRNA gene sequence (GenBank accession number HO918204) showed that strain  $H27^{T}$  is related to members of the genus Streptomonospora and exhibits highest 16S rRNA gene sequence similarity to S. alba (98.8 %) and S. flavalba (98.7 %). The phylogenetic relationship between strain H27<sup>T</sup> and the other *Strep*tomonospora species is seen in the neighbour-joining dendrogram (Fig. 2). DNA-DNA relatedness between strain H27<sup>T</sup> and the type strains S. alba DSM  $44588^{T}$ and S. flavalba DSM 45155<sup>T</sup> were respectively mean values of 17.1 % (14.7 and 19.6 %) and 57.9 % (58.3 and 57.5 %). These hybridization values are 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 H27<sup>T</sup> represents a novel species of the genus Streptomonospora, for which the name Streptomonospora algeriensis sp. nov. is proposed.

#### Description of Streptomonospora algeriensis sp. nov

*Streptomonospora 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).



**Fig. 2** Phylogenetic tree for species of the genus *Streptomo-nospra* 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 H27<sup>T</sup>

Halophilic filamentous actinomycete that forms well-developed and non-fragmented substrate mycelium. Aerial mycelium is not abundant and shows a yellow-white colour on CM agar and NA media. Produces short chains of non-motile spores, which are straight to flexuous. Substrate mycelium produces, very rarely, non-motile isolated spores, which are ovoid.

Diffusible pigments are not produced on CM agar, NA media, ISP2 and ISP 4 media. Temperature and pH ranges for growth are 20-45 °C and pH 5.0-8.0, with optima at 28-37 °C and pH 6.0-7.0. The NaCl concentration range for growth is 7-20 %, with optimal growth occurring at 10-15 %. Utilizes cellobiose, erythritol, fructose, galactose, glucose, maltose and mannose, but not adonitol, arabinose, glycerol, inositol, lactose, mannitol, melezitose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose and xylose. Positive for casein and Tween 80 hydrolysis, but negative for adenine, gelatin, guanine, hypoxanthine, starch, testosterone, tyrosine and xanthine hydrolysis. Acetate, citrate and pyruvate are decarboxylated, but not benzoate, butyrate, oxalate, propionate, succinate and tartrate. L-alanine, L-proline and L-serine are not used as a source of nitrogen. Nitrate reductase is not produced. Whole-cell hydrolysates contain meso-diaminopimelic acid, galactose and glucose. Polar lipids consist of phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and two glycolipids. The predominant menaquinones are 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). *Allonocardiopsis opalescens* DSM 45601<sup>T</sup> was used as the outgroup. *Bar* 0.01 nucleotide substitution per site

MK-11 (H<sub>4</sub>) and MK-10 (H<sub>6</sub>). Major fatty acids are iso- $C_{16:0}$ , anteiso- $C_{17:0}$ , 10 methyl  $C_{17:0}$  and 10 methyl  $C_{16:0}$ .

The type strain,  $H27^{T}$  (=DSM 45604<sup>T</sup> =CCUG 63369<sup>T</sup> =MTCC 11563<sup>T</sup>), was isolated from an Algerian soil sample collected from Djelfa Province. The GenBank accession number for the 16S rRNA gene sequence of strain H27<sup>T</sup> is HQ918204.

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