Planomonospora sp. PM18: ISOLATION AND TAXONOMY OF NEW ACTINOBACTERIAL STRAIN ISOLATED FROM ALGERIAN SAHARAN SOIL

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Abstract.- *An actinobacterial strain, designated PM18, was isolated from a Saharan soil sample by agar plate dilution method on chitin-vitamin agar supplemented with polymyxin after pretreatment of the soil sample at 120°C (dry heat) for 1 h. The taxonomic status of this strain was determined basis on morphological and physiological characteristics and phylogenetic analysis based on the 16S rRNA gene sequence. Strain PM18 developed an extensively branched and non-fragmented substrate mycelium. The isolate was characterized by the presence of cylindrical monosporous sporangia, which were produced only on the aerial mycelium. Sporangia were arranged closely in parallel double rows. The monosporous sporangia were motile. The morphological features of this isolate corresponded to those of members of the genus* Planomonospora. *Furthermore, phylogenetic analysis based on the 16S rRNA gene sequence revealed that strain PM18 is a member of the genus* Planomonospora. *The 16S rRNA gene sequence similarity showed that this strain was most closely related to* Planomonospora sphaerica *JCM 9374^T (99.2%) and* Planomonospora parontospora *subsp.* antibiotica *JCM 3094^T (98.8%). Based on phenotypic differences and the separate position of strain PM18 in the phylogenetic tree, this strain may be a representative of a putative novel species in the genus* Planomonospora.

Key words: Planomonospora, *Actinobacteria, taxonomy, Saharan soil, Microbial diversity.*

Planomonospora sp. PM18: ISOLEMENT ET TAXONOMIE D'UNE NOUVELLE SOUCHE D'ACTINOBACTÉRIE ISOLÉE DU SOL SAHARIEN ALGÉRIEN

Résumé.- *Une souche d'actinobactérie, désignée PM18, est isolée d'un échantillon de sol saharien par la méthode de suspensions-dilutions sur milieu chitine-vitamines agar additionné de polymyxine, après un prétraitement de l'échantillon de sol à 120°C (chaleur sèche) pendant 1 h. La position taxonomique de cette souche est déterminée sur la base des caractéristiques morphologiques et physiologiques, ainsi que sur l'analyse phylogénétique après séquençage du gène codant pour l'ARNr 16S. La souche PM18 produit un mycélium du substrat ramifié et non fragmenté et un mycélium aérien portant des sporanges cylindriques monosporés disposés en une double rangée parallèle. Les sporangiospores sont mobiles. Les caractéristiques morphologiques de cette souche correspondent à ceux des membres du genre* Planomonospora. *L'appartenance à ce genre a été confirmée par l'analyse phylogénétique. L'alignement de la séquence de la souche PM18 a montré un pourcentage de similarité de 99,2% avec* Planomonospora sphaerica *JCM 9374^T et 98,8% avec* Planomonospora parontospora. *sous espèce* antibiotica *JCM 3094^T. En se basant sur les*

différences phénotypiques et sur sa position distincte dans l'arbre phylogénétique, la souche PM18 pourrait une nouvelle espèce du genre Planomonospora.

Mots clés: Planomonospora, *Actinobactéries, taxonomie, sol saharien, diversité microbienne.*

Introduction

Research isolating rare actinobacterial genera from Saharan soils is based on the assumption that samples from widely diverse locations are more likely to yield novel microorganisms and therefore, hopefully, novel secondary metabolites as a result of the geographical (eco-pedological) variation. Besides, this approach is helpful in discovering new actinobacterial species which produce a wide range of bioactive substances from Saharan soils [1-5].

Planomonospora is a Gram positive and not acid fast genus in the family *Streptosporangiaceae* that forms cylindrical to clavate sporangium, which contains a single motile sporangiospore on the aerial mycelium [6]. Substrate and aerial mycelia develop on various agar culture media. Substrate mycelia (0.6-1.0 µm in diameter) of *Planomonospora* strains are irregularly branched, occasionally septate, and non-fragmenting. Aerial mycelia (0.4-1.1 µm in diameter) are sparsely branched and rarely septate. Cylindrical to clavate sporangia (0.9-1.5 µm wide × 3.5-5.5 µm long), each containing a single spore, are formed only on the aerial mycelium [7]. Generally, the isolated *Planomonospora* strains are chemoorganotrophic, aerobic and mesophilic micro-organisms (grows well between 28 and 37°C). Usually, the colonies of *Planomonospora* strains grown on complex agar media are raised or flat with rugose or smooth surface. The color of substrate mycelium is either rose to light orange or brown-violet to light brown. The aerial mycelium is white with a rose or grayish white. The peptidoglycan of the cell walls contains *meso*-diaminopimelic acid (*meso*-DAP), and madurose is the characteristic sugar of whole-cell hydrolysates.

The name *Planomonospora* is derived from ancient Greek: *Planos* meaning wanderer (or vagabond), *monos* meaning solitary (single), and *spora* meaning a spore (a seed). The name therefore describes a motile, single organism with a single endospore.

At the time of writing, the genus *Planomonospora* still comprised only six species (including two sub-species) with validly published names: *Planomonospora parontospora* subsp. *parontospora*, *Planomonospora parontospora* subsp. *antibiotica*, *Planomonospora venezuelensis*, *Planomonospora sphaerica*, *Planomonospora alba*, *Planomonospora corallina* and recently *Planomonospora algeriensis* as described by CHAABANE CHAOUCH *et al.* (2016) [8].

As far as we are aware, no reports are available on the diversity and the characteristics of the members of *Planomonospora* in Saharan soils. The current study was designed to describe the isolation, the taxonomic position of a new actinobacterium, strain PM18, belonging to the genus *Planomonospora* isolated from Algerian Saharan soil.

1. - Materials and methods

1.1. - Isolation of the actinobacterial strain

Three non-rhizospheric Saharan soil samples (5–20 cm of depth) were collected aseptically from Béni-Abbès (Béchar, Saoura region, South-West Algeria). The samples were placed in sterile polyethylene bags, closed tightly and stored at 4°C until analysis. The soil samples were air-dried at room temperature for 10 days before being baked at 120°C for 1 h [9]. The soil was then suspended in sterile distilled water, serially diluted and spread-plated on chitin-vitamin B agar medium [10] supplemented with polymyxin and/or penicillin each at 25 mg l^{-1}. The plates were incubated at 30°C for 28 days, and all colonies were examined directly by light microscopy to detect the *Planomonospora*-like isolates.

1.2. - Cultural and morphological characteristics of strain PM18

Morphological properties were observed by light microscopy (Model B1, Motic) using cultures grown on various International *Streptomyces* Project media (ISP 2, ISP 3, ISP 4, ISP 6 and ISP 7) [11], Glucose-Asparagine Agar [11], HTA: Hickey-Tresner-Agar [12] and Bennett's medium [13] at 30°C for 14 days. The color of substrate and aerial mycelia was recorded using ISCC–NBS color charts [14].

1.3. - Physiological characteristics of strain PM18

Production of melanoid pigments was tested on peptone yeast extract-iron agar (ISP 6) and tyrosine agar (ISP 7) media [11]. Sensitivities to sodium chloride (0–4%) (w/v) and growth at 20, 30, 40 and 45°C and pH 4.0–12.0 were evaluated on HTA (Hickey-Tresner-Agar) medium. Other physiological characteristics, including utilization of sole carbon sources, decarboxylation of organic acids, degradation of adenine, aesculin, arbutin, casein, cellulose, gelatin, guanine, hypoxanthine, starch, Tween 80, tyrosine and xanthine, reduction of nitrate, milk peptonization and milk coagulation, were assessed by the media and methods of GORDON *et al.* (1974) and WILLIAMS *et al.* (1989) [15, 16].

1.4. - DNA extraction, PCR amplification and 16S rRNA gene sequencing

The strain PM18 was grown at 30°C for 4 days with agitation (250 rpm) in a 500 ml flask containing 100 mL of ISP 2 medium. Biomass was harvested by centrifugation (8,000 rpm for 10 min) and washed twice with double-distilled water. The 16S rRNA was amplified by PCR using an Invitrogen kit and two universal primers: 27f (5'–AGAGTTT GATCCTGGCTCAG–3') and 1492r (5'–GGTTACCTTGTT ACGACTT–3'). The PCR amplification was conducted using a thermocycler (STRATAGENE RoboCycler Gradient 96) in 50 µl containing 1.25 U of *Taq* DNA polymerase, 1 µl (500 ng) of purified DNA, 1 × PCR buffer (10 mM of Tris–HCl, 50 mmol of KCl, pH 9.0 at 25°C), 1.5 mmol of $MgCl_2$, 200 µmol of each dNTP and 1 µmol of each primer. Reaction conditions were: 97°C for 4 min, followed by 35 cycles of 97°C for 45 s, 52°C for 45 s, and 72°C for 45 s, with a final elongation step at 72°C for 10 min. The amplified products were visualized on a 0.8% (w/v) agarose gel by ultraviolet (UV) fluorescence after ethidium bromide staining. PCR products were purified with a PCR product purification kit (Qiagen, Hilden, Germany). The PCR products were sequenced using the same primers as above on an automated sequencer (model 3130 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA)

using a Big Dye Terminator v 3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's instructions.

1.5. - Phylogenetic analysis

The identification of phylogenetic neighbors and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) [17], a web-based tool for the identification of prokaryotes based on 16S rRNA gene sequences from type strains. Multiple alignments with sequences from closely related species were performed by using the program CLUSTAL W (with default parameters) in MEGA version 6 [18]. Evolutionary distance was generated as described by Jukes and Cantor [19] and a phylogenetic tree was inferred by the neighbor-joining method [20]. Tree topologies were evaluated by bootstrap analysis [21], based on 1000 re-samplings of the neighbor-joining dataset.

2. - Results and discussion

In total, 14 actinobacterial *Planomonospora*-like isolates were harvested from Saharan soil samples collected in Béni-Abbès, Béchar, Saoura region, South-West Algeria (GPS coordinates 30° 08' N and 02° 10' W). Among them one strain, designated PM18, was isolated on chitin-vitamins agar medium supplemented by polymyxin (cyclic peptide antibiotic inhibits mainly Gran-negative bacteria) as a selective agent from a soil sample that was previously baked at 120°C for 1 h.

Strain *Planomonospora* sp. PM18 showed good growth on Hickey-Tresner agar (HTA) and Bennett's agar media, moderate growth on ISP 3, ISP 4 and ISP 7 and little growth on ISP 2 and Glucose-Asparagine Agar, but no growth occurred on ISP 6 medium. Strain PM18 forms an extensively branched and non-fragmented substrate mycelium which was orange on ISP 2 and ISP 3, pink-orange on ISP 4 and beige on ISP 7, Glucose-Asparagine Agar, Hickey-Tresner agar and Bennett's agar media. It produces scanty white aerial mycelia on ISP 3, ISP 4, Glucose-Asparagine Agar, Hickey-Tresner agar and Bennett's agar with cylindrical sporangia arranged in double parallel rows (Figure 1).

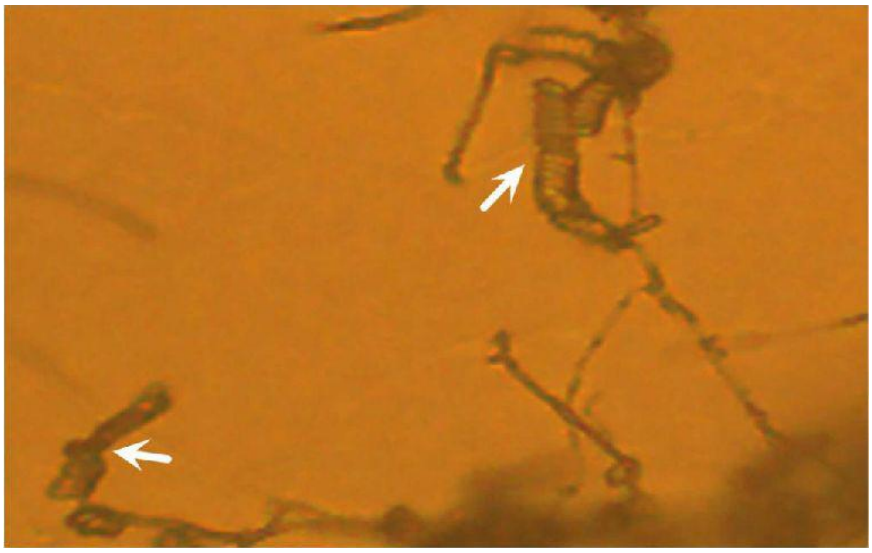

Figure 1.- Micromorphology with light microscopy of strain PM18 grown on Hickey-Tresner agar (HTA) medium for two weeks at 30°C. The arrows indicate the sporangia.

Each one contains a single motile sporangiospore. No spherical bodies were produced on the aerial mycelia on ISP 4 medium. No diffusible pigments or melanoid pigments were observed on any media tested. The cultural characteristics of strain *Planomonospora* sp. PM18 are summarized in Table I.

Table I.- Macromorphological characteristics of strain PM18 on different media after 14 days of incubation (+: weak, ++: moderate, +++: well, –: no one)

Agar medium	Growth	Production and color of:	
		Aerial mycelium	Substrate mycelium
ISP 2	+	–	Orange
ISP 3	++	+ white	Orange
ISP 4	++	+ white	Pink–orange
ISP 6	–	–	–
SP 7	++	–	Beige
GAA	+	+ white	Pale beige
HTA	+++	+ white	Beige

With regard to physiological characteristics, strain PM18 could utilize L-arabinose, D-cellobiose, D-galactose, D-glucose, maltose, D-mannitol, D-mannose, L-rhamnose, salicin, trehalose, D-xylose, sodium acetate, sodium butyrate, sodium lactate, sodium pyruvate and sodium succinate as sole carbon source, but not adonitol, cellulose, D-fructose, glycerol, *myo*-inositol, lactose, melezitose, melibiose, α-Methyl-D-glucoside, raffinose, D-ribose, sorbitol, sucrose and sodium salts of the following organic acids: benzoate, citrate, oxalate, propionate and tartrate. It is positive for milk peptonization, nitrate reduction and decomposition of aesculin, arbutin, casein, gelatin, starch, L-tyrosine and Tween 80, but negative for milk coagulation and decomposition of adenine, hypoxanthine and xanthine.

Strain PM18 grew between 20-45°C, pH 5.0-11.0 and in the presence of 0-1% (w/v) NaCl. Table II shows the results of physiological tests of strain PM18 in comparison with the most closely related species *Planomonospora sphaerica* DSM 44632^T.

Table II.- Physiological characteristics of strain PM18 compared with the most closely related species *Planomonospora sphaerica* DSM 44632^T (1: strain PM18; 2: *Planomonospora sphaerica* DSM 44632^T; +: Positive reaction; –: negative reaction; ND: not determined; *: Data from Mertz [22])

Characteristics	1	2*	Characteristics	1	2
Growth on sole carbon sources			Citrate	–	–
Adonitol	–	–	Lactate	+	+
L-Arabinose	+	+	Oxalate	–	–
D-Cellobiose	+	+	Propionate	–	–
Cellulose	–	–	Pyruvate	+	+
D-Fructose	–	+	Succinate	+	–
D-Galactose	+	+	Tartrate	–	–
D-Glucose	+	+	*Decomposition of:*		
Glycerol	–	–	Aesculin	+	–
Myo-Inositol	–	–	Adenine	–	–
Lactose	–	–	Arbutin	+	ND
Maltose	+	+	Casein	+	+
D-Mannitol	+	+	Gelatin	+	+

D-Mannose	+	+	Hypoxanthine	–	–
Melezitose	–	–	Starch	+	+
Melibiose	–	–	L-Tyrosine	+	+
α-Methyl-D-glucoside	–	–	Xanthine	–	–
L-Rhamnose	+	+	Tween 80	+	ND
Raffinose	–	–	Milk coagulation	–	ND
D-Ribose	–	–	Milk peptonization	+	ND
Salicin	+	+	pH range	5–11	ND
Sorbitol	–	–	Nitrate reduction	+	+
Sucrose	–	+	Growth at 45°C	+	–
Trehalose	+	+	Growth at 2% NaCl (w/v)	–	+
D-Xylose	+	+			
Decarboxylation of sodium					
Acetate	+	+			
Benzoate	–	–			
Butyrate	+	+			

The almost-complete 16S rRNA gene sequence (1461 nt) of strain PM18 was determined. EzTaxon-e analysis of the 16S rRNA gene sequence confirmed that strain PM18 belonged to the genus *Planomonospora.* The 16S rRNA sequence of strain *Planomonospora* sp. PM18 exhibited the highest similarities, 99.23% with *Planomonospora sphaerica* JCM 9374^T and 98.81% with *Planomonospora parontospora* subsp. *antibiotica* JCM 3094^T. A phylogenetic tree was constructed based on 16S rRNA gene sequences to show the comparative relationship between strain *Planomonospora* sp. PM18 and other *Planomonospora* species (fig. 2). The phylogenetic tree based on the neighbor-joining algorithm showed that strain *Planomonospora* sp. PM18 lies in a clade with its closest neighbor *P. sphaerica* JCM 9374^T at a bootstrap value of 99%.

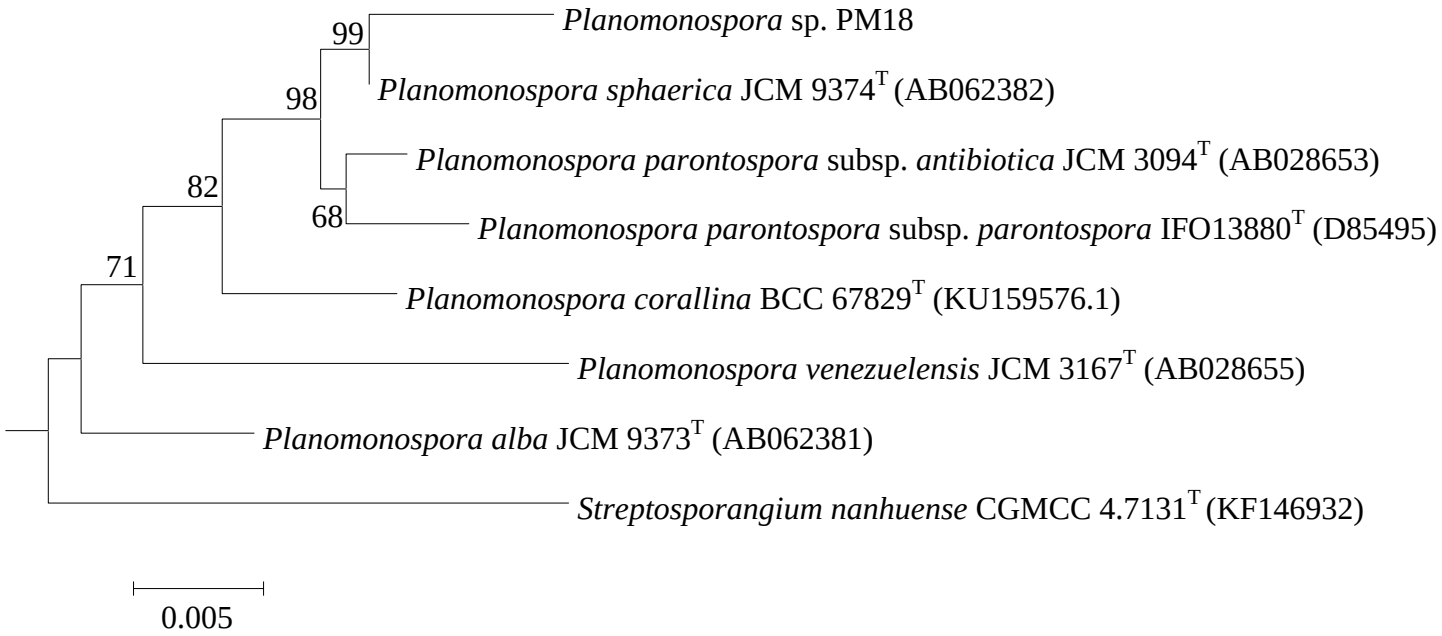

Figure 2.- Neighbor-joining tree [18] (based on almost-complete 16S rRNA gene sequences showing the position of strain *Planomonospora* sp. PM18 amongst its phylogenetic neighbors. *Streptosporangium nanhuense* CGMCC 4.7131^T was used as an outgroup. Numbers at nodes indicate levels of bootstrap support (%); only values ≥ 50% are shown. GenBank accession numbers are given in parentheses. Bar, 0.005 substitutions per site.

Besides of the separate position of strain PM18 in the phylogenetic tree, it differs with *Planomonospora sphaerica* JCM 9374^T in a number of morphologic characteristics such as the absence of the spherical bodies and physiologic features (tab. II). Hence, it is clear that this strain may be a new species of *Planomonospora*. Still, DNA/DNA hybridization experiments and chemotaxonomic analysis need to be performed to confirm this separate taxonomic status.

It is evident from this current research that rare actinobacteria from Algerian Saharan soils notably *Planomonospora* strains may be an excellent source of novel taxa.

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