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A novel actinobacterial strain, designated ACD12^T, was isolated from a Saharan soil sample collected from Adrar province, southern Algeria. A polyphasic study was carried out to establish the taxonomic position of this strain. Strain ACD12^T was observed to form extensively branched substrate mycelia. Aerial mycelium was absent or was weakly produced on all media tested, while spore chains were short with a hooked and irregular spiral form (2–3 turns). The dominant diaminopimelic acid isomer in the cell wall was *meso*-diaminopimelic acid. Glucose, ribose, galactose, mannose and madurose occured in whole-cell hydrolysates. The major phospholipid was diphosphatidylglycerol and phosphatidylinositol. The predominant menaquinone was MK-9 (H_6). The fatty acid profile was characterized by the presence of $C_{16:0}$, $C_{17:0}$, $C_{15:0}$, $C_{18:0}$, $C_{18:1}$ *cis*9 and iso-$C_{16:0}$. Results of 16S rRNA gene sequence comparisons revealed that strain ACD12^T shared the highest degree of 16S rRNA gene sequence similarity with *Actinomadura sputi* DSM 45233^T (98.3 %) and *Actinomadura hallensis* DSM 45043^T (97.8 %). All tree-making algorithms used also supported strain ACD12^T forming a distinct clade with its most closely related species. In addition, DNA–DNA hybridization indicated only 39.8 % relatedness with *A. sputi* DSM 45233^T and 18.7 % relatedness with *A. hallensis* DSM 45043^T. The combined phenotypic and genotypic data show that the novel isolate represents a novel species of the genus *Actinomadura*, for which the name *Actinomadura adrarensis* sp. nov., is proposed, with the type strain ACD12^T (=DSM 46745^T =CECT 8842^T).

The genus *Actinomadura*, a member of the family *Thermomonosporaceae*, was proposed by Lechevalier & Lechevalier (1968). The strains of species of the genus *Actinomadura* have been principally isolated from soil (Lu *et al.*, 2003; Quintana *et al.*, 2003; Ara *et al.*, 2008). However, some species have been isolated from patients, such as *Actinomadura sputi* (Yassin *et al.*, 2010). This genus is of great importance in several domains, including the production of new bioactive metabolites active against pathogenic microorganisms (Euanorasetr *et al.*, 2015). Species of the genus *Actinomadura* produce an extensively branched non-fragmenting substrate mycelium and, generally, aerial mycelium is moderately developed or absent. Spore chains are short and differentiate into straight, spiral or hooked forms. The strains of species of the genus *Actinomadura* are characterized by the presence of type III cell walls (*meso*-diaminopimelic acid without glycine). Whole-cell hydrolysates contain madurose as the diagnostic sugar. Cell membranes contain diphosphatidylglycerol and phosphatidylinositol as the diagnostic phospholipids, and MK-9(H_4) and MK-9(H_6) as the major menaquinones

The GenBank /EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ACD12^T is KU356942.

(Lechevalier *et al.*, 1977; Kroppenstedt *et al.*, 1990; Wink *et al.*, 2003; Cook *et al.*, 2005). Many species of the genus *Actinomadura* have been described in recent years and, at the time of writing, the genus comprises 53 species with validly published names (http://www.bacterio.net).

During our study on the actinobacterial diversity of Saharan soils, many new taxa were recorded (Saker *et al.*, 2014; Aouiche *et al.*, 2015; Boubetra *et al.*, 2015; Bouras *et al.*, 2015; Meklat *et al.*, 2015). In the present work, we describe a novel species of actinobacteria belonging to the genus *Actinomadura*.

Strain ACD12^T was isolated, using the standard dilution plate method, from a sandy loam soil sample collected from the palm grove of the Bouda region, adrar province, southern Algeria (27°52'N, 0°17'W), and grown on chitin-vitamin agar medium, which is recommended for isolating rare actinobacteria (Hayakawa & Nonomura, 1987). The medium was supplemented with 80 μg ml^{-1} of cycloheximide to inhibit the development of invasive fungi. After 21 days of incubation at 30 °C, colonies were transferred onto International *Streptomyces* Project (ISP) 2 medium (Shirling & Gottlieb, 1966) and purified strain ACD12^T was maintained at 4 °C.

Cultural characteristics were observed after 7, 14 and 21 days of incubation at 30 °C using several media: Bennett's agar (Waksman, 1961), yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4) (Shirling & Gottlieb, 1966) and humic acid-vitamin agar (Hayakawa & Nonomura, 1987). Morphological characteristics were determined using the naked-eye and by using light and scanning electron microscopes (Motic, B1 Series and JEOL, JSM-7100F, respectively). The ISCC-NBS colour name chart (Kelly & Judd, 1976) was used to determine the colours of the aerial mycelium, substrate mycelium and diffusible pigments.

Several physiological tests were used to characterize the actinobacterial strain. The utilization of carbohydrates and decarboxylation of organic acids were studied using the method of Gordon *et al.* (1974). Degradation of adenine, aesculin, arbutin, gelatin, guanine, hypoxanthine, starch, Tween 80, L-tyrosine and xanthine, reduction of nitrate, and also coagulation and peptonisation of milk, were evaluated according to the methods of Goodfellow (1971) and Marchal *et al.* (1987). Growth at different temperatures (15, 20, 25, 30, 37, 40 and 45 °C) and at different pH values (5, 6, 7, 8, 9, 10 and 11) was determined on ISP 2 medium.

Biomass for chemotaxonomic studies was obtained by growing strain ACD12^T on ISP 2 broth in flasks on a rotary shaker at 250 r.p.m at 30 °C for one week. Cells were harvested by centrifugation and washed several times with distilled water, than dried at 37 °C. Isomers of diaminopimelic acid and cell sugars were detected following the standard procedures described by Becker *et al.* (1964) and Lechevalier & Lechevalier (1970). Menaquinones were extracted and purified by using the methods of Minnikin *et al.* (1984) and were analysed by HPLC (Kroppenstedt, 1982, 1985). Polar lipids were extracted and identified by using two-dimensional TLC (Minnikin *et al.*, 1984). The fatty acid profile was determined by the method of Sasser (1990), using the Microbial Identification System (MIDI) Sherlock software version 6.1 (method TSBA40, TSBA6 database).

Genomic DNA was extracted with a DNA extraction kit (MasterPure Gram-Positive DNA Purification Kit, Epicentre Biotechnologies). PCR amplification of the 16S rRNA gene sequence of strain ACD12^T was carried out according to the procedures described by Rainey *et al.* (1996). The EzTaxon-e server (Kim *et al.*, 2012) was employed to identify phylogenetic neighbours and to calculate pairwise 16S rRNA gene similarities. The 16S rRNA gene sequence of strain ACD12^T was aligned against corresponding nucleotide sequences using the CLUSTAL W program (Larkin *et al.*, 2007) of representatives of the genus *Actinomadura* retrieved from the EzTaxon-e server. Phylogenetic trees were reconstructed with the neighbour-joining algorithm (Saitou & Nei, 1987) with the model of Jukes & Cantor (1969), the maximum-likelihood algorithm (Felsenstein, 1981) with the Kimura 2-parameter model (Kimura, 1980) and maximum-parsimony algorithm (Fitch, 1977) using molecular evolutionary genetics analysis, (MEGA version 5) (Tamura *et al.*, 2011). The topology of the phylogenetic trees was evaluated by bootstrap analysis (Felsenstein, 1985), based on 1000 resamplings of the neighbour-joining dataset.

For DNA–DNA relatedness studies, DNA was isolated by using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite, as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out in duplicate, as described by De Ley *et al.* (1970) with the modifications described by Huss *et al.* (1983).

Strain ACD12^T exhibited moderate growth on ISP 2, ISP 3, ISP 4 and Bennett's media. The isolate formed extensively branched substrate mycelium, which were light beige. No aerial mycelium was observed on the media tested, while a

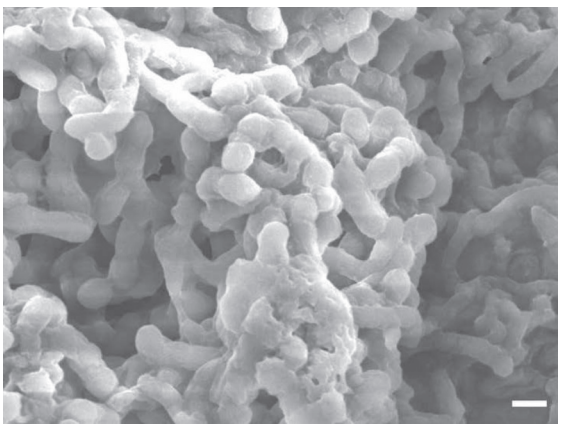

Fig. 1. Scanning electron micrograph of spore chains of strain ACD12T. The strain was grown onhumic acid-vitamin agar medium for 2 weeks at 30 °C. Bar, 1 μm.

very scanty white aerial mycelium was observed only on humic acid-vitamin agar medium. Spore chains (2–12 spores) were observed to be short with hooked and irregular spiral forms and with a smooth surface (Fig. 1). No diffusible pigments were detected on any of the media tested. No sporangia, sclerotia or synnemata were observed.

Strain ACD12^T was found to grow at 25–37 °C, at pH 7–10 and at 0–2 % (w/v) NaCl. Strain ACD12^T and its two most closely related reference type strains (*Actinomadura sputi* DSM 45233^T and *Actinomadura hallensis* DSM 45043^T) were positive for the utilization of aesculin and cellobiose, and negative for the utilization of adenine, xanthine and raffinose. However, the novel strain differed from the two reference type strains in terms of other physiological characteristics, as illustrated in Table 1. The complete physiological characteristics of strain ACD12^T are given in the species description.

Table 1. Phenotypic characteristics that differentiate strain ACD12^T from its most closely related species of the genus *Actinomadura*

Strains: 1, ACD12^T; 2, *A. sputi* DSM 45233^T; 3, *A. hallensis* DSM 45043^T. All data are from this study, except the spore-surface ornamentation of *A. sputi* DSM 45233^T (Yassin *et al.*, 2010) and *A. hallensis* DSM 45043^T (Lee & Jeong, 2006). +, Positive; −, negative.

Characteristic	1	2	3
Aerial mycelium on ISP2 medium	−	+	+
Spore-chains arrangement	Hooks, spirals	Straight	Hooks, spirals
Spore-surface ornamentation	Smooth	Smooth	Warty
Utilization of:			
Adonitol	+	−	−
L-Arabinose	−	−	+
D-Fructose	−	+	+
D-Glucose	−	−	+
D-Lactose	−	+	−
D-Maltose	+	−	+
D-Mannitol	−	−	+
L-Rhamnose	−	+	−
D-Sorbitol	−	+	−
D-Trehalose	+	−	+
D-Xylose	−	−	+
Decomposition of:			
Casein	+	−	+
Gelatin	+	−	+
Hypoxanthine	+	−	−
Tween 80	+	+	−
L-Tyrosine	−	+	−
Starch	−	+	+
Growth at:			
45 °C	−	−	+
Growth in 3 % (w/v) NaCl	−	+	+

Strain ACD12^T exhibited chemical markers typical of members of the genus *Actinomadura*. The cell wall of strain ACD12^T was found to contain *meso*-diaminopimelic acid as the diagnostic peptidoglycan diamino acid, but not glycine. The whole-cell hydrolysate was found to contain madurose as the diagnostic sugar, along with glucose, ribose, galactose and mannose. These results indicate that this strain has type IIIB (Lechevalier & Lechevalier, 1970). The predominant menaquinone was determined to be MK-9(H_6) (64.5 %) with small amounts of MK-9(H_4) (12.5 %), MK-9(H_8) (12 %) and MK-9(H_2) (2.3 %) also detected. The diagnostic phospholipids detected were diphosphatidylglycerol and phosphatidylinositol, which corresponds to phospholipid type PI (Lechevalier *et al.*, 1977); phosphatidylinositol mannosides and phosphatidylglycerol were also present (Fig. S1, available in the online Supplementary Material). The cellular fatty acids higher than 5 % were identified as $C_{16:0}$ (22.2 %), 10-methyl $C_{17:0}$ (15.2 %), $C_{17:1}\omega 9c$ (11.5 %), $C_{15:0}$ (11.1 %), 10-methyl $C_{18:0}$ (9.3 %) and $C_{18:1}\omega 9c$ (5 %). Details are given in Table S1 .

Phylogenetic analysis of the 16S rRNA gene sequence (1484 bp, GenBank accession KU356942) confirmed the placement of strain ACD12^T within the genus *Actinomadura*. High degrees of 16S rRNA gene sequence similarity were found between strain ACD12^T and its nearest neighbours, *A. sputi* DSM 45233^T (98.3 %) and *A. hallensis* DSM 45043^T (97.8 %).

The similarity of the 16S rRNA gene sequence of strain ACD12^T to those of other members of the genus *Actinomadura* were found to be lower than 97.7 %. The phylogenetic relationships between strain ACD12^T and members of the genus *Actinomadura* are demonstrated in the neighbour-joining (Fig. 2), maximum-parsimony and maximum-likelihood dendrograms (Fig. S2). The levels of DNA–DNA relatedness of strain ACD12^T with *A. sputi* DSM 45233^T and *A. hallensis* DSM 45043^T were 39.8 % and 18.7 %, respectively (standards deviations were 5.6 and 0.7 %, respectively). These values are well below the 70 % threshold proposed by Wayne *et al.* (1987) for the delineation of separate species.

Based on these phenotypic and genotypic data, strain ACD12^T is a member of the genus *Actinomadura* and represents a novel species, for which the name *Actinomadura adrarensis* sp. nov. is proposed.

Description of *Actinomadura adrarensis* sp. nov.

Actinomadura adrarensis (ad.rar. en'sis. N.L. fem. adj. *adrarensis* pertaining to Adrar, the source of the soil from which the type strain was isolated).

Aerobic, Gram-stain-positive, non-motile actinobacterium that forms an extensively branched, light beige substrate

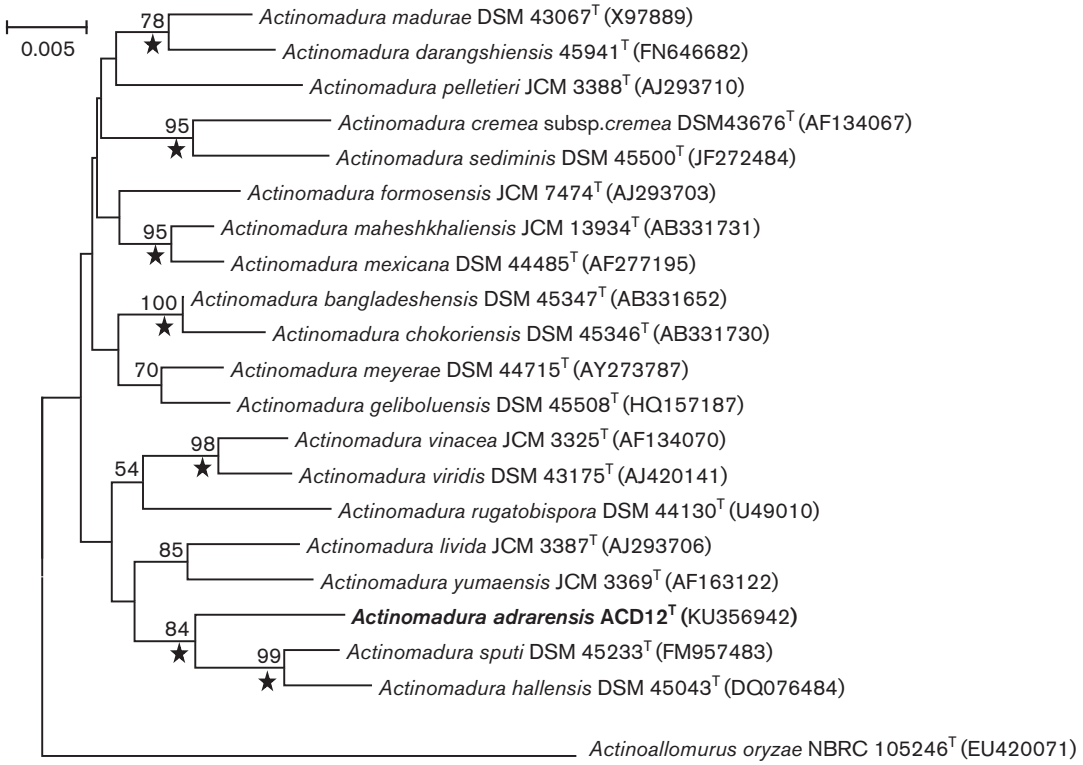

Fig. 2. Neighbour-joining phylogenetic tree based on almost complete 16S rRNA gene sequences showing the position of the strain ACD12T(1484 bp) in the genus *Actinomadura*, including the taxonomically not yet validated '*A.maheshkhaliensis*'. This illustrates the taxonomic position of strain ACD12T relative to the related species. Asterisks indicate branches of the tree that were also found using the maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1977) tree making algorithms. Bootstrap values (>50 %) based on 1000 resamplings are shown at branch nodes. *Actinoallomurus oryzae* NBRC 105246T was used as the out-group. Bar, 0.005 substitutions per site.

mycelium. No aerial mycelium is observed on ISP 2, ISP 3, ISP 4 and Bennett's media, while a very scanty white aerial mycelium is observed on humic acid-vitamin agar medium. Aerial mycelium bear chains of spores (2–12 spores) with hooked and irregular spiral forms. No diffusible pigments are detected on any of the media tested. The optimum growth temperature, pH and NaCl concentration are 30 °C, 8 and 0 % (w/v), respectively. Acetate, aesculin, casein, gelatin, hypoxanthine, pyruvate and Tween 80 are degraded, but adenine, arbutin, benzoate, guanine, oxalate, propionate, starch, succinate, tartrate, L-tyrosine and xanthine are not. Negative for nitrate reduction. Milk peptonisation is positive, while milk coagulation is negative. Adonitol, D-cellobiose, D-maltose, D-mannose, D-ribose and D-trehalose are utilized, but L-arabinose, D-fructose, D-galactose, D-glucose, D-lactose, D-mannitol, D-melibiose, D-raffinose, L-rhamnose, D-sorbitol and D-xylose are not decomposed. The diamino acid in the cell wall is *meso*-diaminopimelic acid. Madurose is the diagnostic sugar in whole-cell hydrolysates. The major phospholipids are diphosphatidylglycerol and phosphatidylinositol. The predominant menaquinone is MK-9(H_6). The major fatty acids are $C_{16:0}$, 10-methyl $C_{17:0}$, $C_{17:1}\omega 9c$, $C_{15:0}$ and 10-methyl $C_{18:0}$.

The type strain is ACD12^T (=DSM 46745^T =CECT 8842^T) isolated from a Saharan soil sample collected from Bouda region, Adrar province (South Algeria).

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