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## *Actinomadura algeriensis* sp. nov., an actinobacterium isolated from Saharan soil

Abdelhadi Lahoum · Nouredine Bouras · Florence Mathieu · Peter Schumann · Cathrin Spröer · Hans-Peter Klenk · Nasserline Sabaou

**Abstract** During the course of a screening programme for new taxa of actinobacteria, a strain designated ACD1<sup>T</sup>, was isolated from a Saharan soil in the Hoggar region (Algeria). The taxonomic position of this strain was determined using a polyphasic taxonomic approach. The strain was observed to form extensively branched, non-fragmenting substrate mycelium, and aerial mycelium with straight to flexuous, hooked and irregular spirals (1–2 turns) forming short chains of spores. The diamino acid present in the cell wall is *meso*-diaminopimelic acid. Galactose, glucose, madurose,

mannose and ribose occur in whole-cell hydrolysates. The diagnostic phospholipids detected were diphosphatidylglycerol and phosphatidylinositol. The major menaquinones were identified as MK-9 (H<sub>4</sub>) and MK-9 (H<sub>2</sub>). The major fatty acids were found to be C<sub>16:0</sub>, C<sub>18:1</sub> *cis*9, iso-C<sub>16:0</sub> and 10-methyl C<sub>18:0</sub>. Phylogenetic analysis based on the 16S rRNA gene showed that the strain belongs to the genus *Actinomadura*, and is closely related to *Actinomadura sediminis* DSM 45500<sup>T</sup> (98.5 % similarity) and *Actinomadura cremea* subsp. *cremea* DSM 43676<sup>T</sup> (98.3 % similarity). However, DNA–DNA hybridization revealed only 48.0 % relatedness with *A. sediminis* DSM 45500<sup>T</sup> and 33.2 % relatedness with *A. cremea* subsp. *cremea* DSM 43676<sup>T</sup>. The combined phenotypic and genotypic data showed that the strain represents a novel

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species of the genus *Actinomadura*, for which the name *Actinomadura algeriensis* sp. nov. is proposed, with the type strain ACD1<sup>T</sup> (= DSM 46744<sup>T</sup> = CECT 8841<sup>T</sup>).

**Keywords** *Actinomadura* · *Actinomadura algeriensis* sp. nov. · Actinobacteria · Saharan soil · Polyphasic taxonomy

## Introduction

The genus *Actinomadura* was proposed by Lechevalier and Lechevalier (1968), and is part of family *Thermomonosporaceae*. The strains of the genus *Actinomadura* produce extensively branched non-fragmenting substrate mycelium. Generally, the aerial mycelium is moderately developed. Spores chains are short (or sometimes long) and differentiate into straight, spiral or hooked form. Cell walls contain *meso*-isomer (DL) of diaminopimelic acid without glycine. Whole-cell hydrolysates contain madurose as the diagnostic sugar, diphosphatidylglycerol and phosphatidylinositol as the diagnostic phospholipids, and MK-9 (H<sub>4</sub>) and MK-9 (H<sub>6</sub>) as major menaquinones (Lechevalier et al. 1977; Kroppenstedt et al. 1990; Wink et al. 2003; Cook et al. 2005). The principal reservoir of the genus *Actinomadura* is the soil (Lu et al. 2003; Quintana et al. 2003; Ara et al. 2008). However, some species are isolated from patients, for example *Actinomadura sputi* (Yassin et al. 2010). In addition, several *Actinomadura* species were transferred to other new genera, including *Nonomuraea* (Zhang et al. 1998), *Actinocorallia* (Inuma et al. 1994) and *Actinoallomurus* (Tamura et al. 2009). At the time of writing, the genus *Actinomadura* encompasses 52 species with validly published names (<http://www.bacterio.net>).

During our study on the diversity and taxonomy of actinobacteria in Saharan soils, many new taxa of non-halophilic actinobacteria (Aouiche et al. 2015; Boubeta et al. 2015; Bouras et al. 2015) and halophilic actinobacteria (Boudjelal et al. 2015; Meklat et al. 2015; Saker et al. 2015) have been reported. In the present work, we describe a new species of non-halophilic actinobacteria belonging to the genus *Actinomadura*. This is the first time that a new species of this genus is reported from Saharan soil.

## Materials and methods

### Isolation and maintenance of strain

Strain ACD1<sup>T</sup> was isolated from a soil sample collected from Hoggar region, Tamanrasset province (22°47'N, 5°31'E), South of Algeria, by the dilution agar plating method using a chitin-vitamin B medium recommended for isolation of rare actinobacteria (Hayakawa and Nonomura 1987). The medium was supplemented with 80 mg l<sup>-1</sup> of cycloheximide to inhibit development of invasive fungi. The strain was purified and maintained at 4 °C on International *Streptomyces* Project (ISP) 2 medium (Shirling and Gottlieb 1966).

Strain ACD1<sup>T</sup> has been deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ), Germany, as strain DSM 46744<sup>T</sup> and in Spanish Type Culture Collection (CECT), Spain, as strain CECT 8841<sup>T</sup>.

### Phenotypic characterization

Morphological characteristics were observed using several media, namely yeast extract/malt extract agar (ISP 2), oatmeal agar (ISP 3) and inorganic salts/starch agar (ISP 4) (Shirling and Gottlieb 1966), and also Bennett's medium (Waksman 1961). After incubation at 30 °C for 7, 14 and 21 days, morphological characteristics were recorded by the naked-eye and by using a light microscope (Motic, B1 Series, Hong Kong). The ISCC–NBS colour name chart (Kelly and Judd 1976) was used to determine the colours of aerial mycelium, substrate mycelium and diffusible pigments.

Several physiological tests were used to characterize the actinobacterial strain. Degradation of adenine, aesculin, arbutin, gelatin, guanine, hypoxanthine, starch, Tween 80, L-tyrosine, xanthine and reduction of nitrate, and also coagulation and peptonisation of milk, were evaluated according to the methods of Goodfellow (1971) and Marchal et al. (1987). Utilization of carbohydrates and decarboxylation of organic acids were examined as described by Gordon et al. (1974). Temperature range (15–45 °C), pH range (5–10), tolerance to NaCl (0–15 %, w/v) and growth in the presence of novobiocin and rifampicin (10 mg l<sup>-1</sup>) were determined on ISP 2 medium.

## Chemical analysis of cell constituents

For chemotaxonomic study, strain ACD1<sup>T</sup> was grown on ISP 2 broth in flasks on a rotary shaker at 250 rpm and 30 °C for 5 days. Biomass was harvested by centrifugation and washed several times with distilled water, then dried at 37 °C. Isomer of diaminopimelic acid and cell sugars were detected following standard procedures described by Becker et al. (1964) and Lechevalier and 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 Sherlock software version 6.1 (method TSBA40, TSBA6 database).

## Phylogenetic analyses

Genomic DNA was extracted with DNA extraction kit (MasterPure<sup>TM</sup> Gram Positive DNA Purification Kit, Epicentre<sup>®</sup> Biotechnologies, Madison). PCR amplification of the 16S rRNA gene sequence of strain ACD1<sup>T</sup> was performed as described by Rainey et al. (1996). The sequence obtained was compared for similarity with the reference strains in the public sequence databases and with the EzTaxon-e server (Kim et al. 2012). Phylogenetic analyses were conducted using MEGA version 5 (Tamura et al. 2011). The 16S rRNA gene sequence of strain ACD1<sup>T</sup> was aligned using the CLUSTAL W program (Larkin et al. 2007) against corresponding nucleotide sequences of representatives of the *Actinomadura* genus retrieved from EzTaxon-e server. Phylogenetic trees were reconstructed by using the neighbour-joining method (Saitou and Nei 1987) with the model of Jukes and Cantor (1969), maximum-likelihood (Felsenstein 1981) with Kimura 2-parameter (Kimura 1980) model and maximum-parsimony (Fitch 1977) methods. Topology of the phylogenetic tree was evaluated by bootstrap analysis (Felsenstein 1985), based on 1000 resamplings of the neighbour-joining dataset.

For the DNA-DNA relatedness study, 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) under consideration of the modifications described by Huss et al. (1983).

## Results and discussion

Strain ACD1<sup>T</sup> was found to show good growth on ISP 2, ISP 4 and Bennett's media and moderate growth on ISP 3 medium. The aerial mycelium (white to pinkish) was found to be abundant on ISP 2 medium, moderately developed on ISP 3 medium and absent on ISP 4 and Bennett's media. The substrate mycelium was observed to be a red purplish colour on all tested media. A red diffusible pigment was found to be produced on all tested media. The strain ACD1<sup>T</sup> was found to form extensively branched non-fragmenting and sterile substrate mycelium. However, the aerial mycelium was found to bear short chains of spores that are straight to flexuous, hooked and irregular spirals (1–2 turns).

Strain ACD1<sup>T</sup> grows at 20–37 °C (but not at 15, 40 and 45 °C) and at pH 6.0–9.0 (but not at pH 5 and 10.0). Detailed results of the physiological analyses are given in Table 1 (in comparison with the closest species) and in the species description.

Strain ACD1<sup>T</sup> exhibited typical chemical markers of members of the genus *Actinomadura*. The cell wall of strain ACD1<sup>T</sup> was found to contain *meso*-diaminopimelic acid but not glycine. The whole-cell hydrolysate was found to contain madurose as diagnostic sugar, along with glucose, ribose, galactose and mannose. These results indicate that this strain has cell-wall type III and whole cell sugar pattern type B (Lechevalier and Lechevalier 1970). The predominant menaquinones were determined to be MK-9 (H<sub>4</sub>) (49 %) and MK-9 (H<sub>2</sub>) (32 %); small amounts of MK-9 (H<sub>6</sub>) (10 %) and MK-9 (H<sub>0</sub>) (7 %) were also detected. The diagnostic phospholipids detected were diphosphatidylglycerol and phosphatidylinositol, which corresponds to phospholipid type PI (Lechevalier et al. 1977); phosphatidylinositol mannosides, phosphatidylglycerol, an unidentified phospholipid, an unidentified glycolipid and an unidentified aminolipid were also present (Fig. S1). The cellular fatty acids (>1 %) were identified as C<sub>16:0</sub> (32.9 %), C<sub>18:1 cis9</sub> (24.6 %), iso-C<sub>16:0</sub> (14.1 %), 10-methyl C<sub>18:0</sub> (13.9 %), C<sub>16:1 cis9</sub> (2.8 %), C<sub>17:0</sub> (2.1 %), iso-C<sub>18:0</sub> (2.1 %), C<sub>18:0</sub> (1.9 %) and 10-methyl C<sub>17:0</sub> (1.2 %).

**Table 1** Phenotypic characteristics that differentiate the strains ACD1<sup>T</sup> from their closest relative recognized species of the genus *Actinomadura* (*A. sediminis* DSM 45500<sup>T</sup> and *A. cremea* subsp. *cremea* DSM 43676<sup>T</sup>)

Characteristics	Type strains		
	1	2	3
Colour of aerial mycelium	White pinkish	White to pale yellow–pink	White to yellow
Colour of substrate mycelium	Red purplish	White to grey–brown	Colourless
Diffusible pigments	Red	Deep brown	–
Decomposition of			
Gelatin	+	–	+
Hypoxanthine	–	+	+
Starch	–	–	+
L-Tyrosine	–	+	+
Xanthine	–	+	–
Utilization of			
Cellobiose	+	–	+
Galactose	–	+	+
Glucose	+	–	+
Maltose	+	–	+
Mannose	–	–	+
Ribose	+	–	+
Sorbitol	+	–	–
Trehalose	+	–	+
Growth at/with			
20 °C	+	–	+
45 °C	–	+	–
4 % (w/v) NaCl	–	+	+

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

Strains 1 *Actinomadura algeriensis* ACD1<sup>T</sup>, 2 *A. sediminis* DSM 45500<sup>T</sup>, 3 *A. cremea* subsp. *cremea* DSM 43676<sup>T</sup>, + positive, – negative

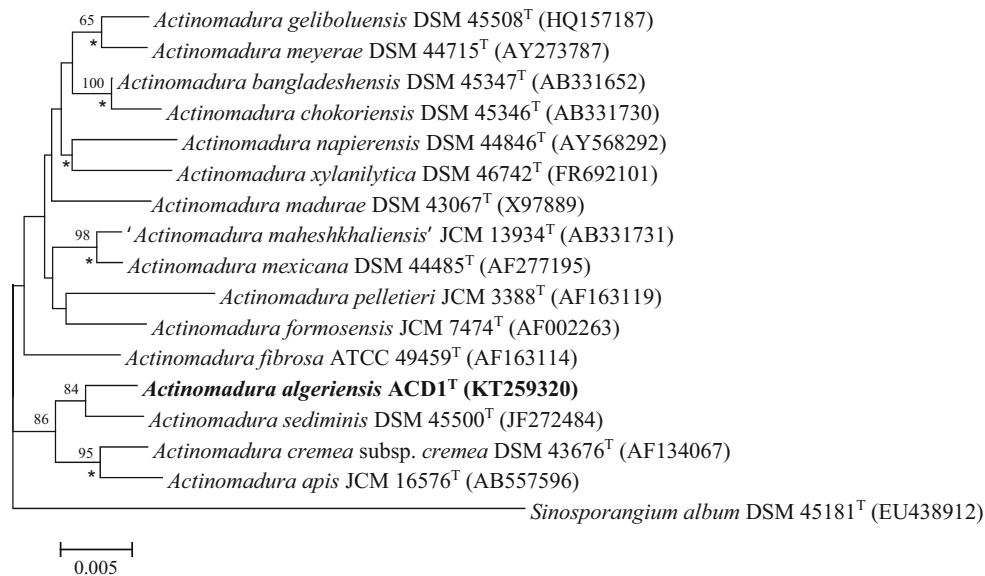
The morphological and chemical characteristics described above clearly support the placement of strain ACD1<sup>T</sup> within the genus *Actinomadura*.

Phylogenetic analysis of the 16S rRNA gene sequence (1446 bp, GenBank accession number KT259320) showed that strain ACD1<sup>T</sup> is related to members of the genus *Actinomadura* and exhibits high 16S rRNA gene sequence similarity to *A. sediminis* DSM 45500<sup>T</sup> (98.5 %) and *A. cremea* subsp. *cremea* DSM 43676<sup>T</sup> (98.3 %). The phylogenetic relationship between strain ACD1<sup>T</sup> and the closely related *Actinomadura* species is seen in the neighbour-joining (Fig. 1), maximum parsimony (Fig. S2) and maximum-likelihood dendrograms (Fig. S3).

Mean DNA–DNA relatedness values between strain ACD1<sup>T</sup> and *A. sediminis* DSM 45500<sup>T</sup> and *A. cremea* subsp. *cremea* DSM 43676<sup>T</sup> were 48.0 ± 1.3 % and 33.2 ± 3.1 %, respectively. These values are below the 70 % cut-off point recommended for the assignment of strains to the same genomic species (Wayne et al. 1987).

In addition, a comparison of phenotypic characteristics of strain ACD1<sup>T</sup> and *A. sediminis* DSM 45500<sup>T</sup> showed differences in the colour of aerial and substrate mycelia, colour of diffusible pigments, utilization of cellobiose, galactose, lactose, maltose, ribose, sorbitol and trehalose as sole carbon sources, decomposition of gelatin, hypoxanthine, L-tyrosine and xanthine, and growth at 20 and 45 °C and with 4 % (w/v) NaCl. Furthermore, *A. cremea* subsp. *cremea* DSM 43676<sup>T</sup> showed differences in the colour of aerial and substrate mycelia, production of diffusible pigments, utilization of galactose, lactose, mannose and sorbitol as sole carbon sources, decomposition of hypoxanthine, starch and L-tyrosine, and growth with 4 % (w/v) NaCl.

In addition, it is interesting to note the presence of large amount of menaquinone MK-9 (H<sub>2</sub>) in the cell membrane of strain ACD1<sup>T</sup>, while other *Actinomadura* species are characterized mainly by the presence of MK-9 (H<sub>6</sub>), MK-9 (H<sub>4</sub>) and MK-9 (H<sub>8</sub>) (Kroppenstedt et al. 1990).



**Fig. 1** Neighbour-joining phylogenetic tree based on almost complete 16S rRNA gene sequences showing the position of the strain ACD1<sup>T</sup> (1446 bp) in the genus *Actinomadura*, including the taxonomically not yet validated '*A. maheshkhaliensis*'. This illustrates the taxonomic position of strain ACD1<sup>T</sup> 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. *Sinosporangium album* DSM 45181<sup>T</sup> was used as the out-group. Bar 0.005 substitutions per site

Based on the phenotypic and genotypic data, strain ACD1<sup>T</sup> is concluded to be a member of the genus *Actinomadura* and to represent a novel species, for which the name *Actinomadura algeriensis* sp. nov. is proposed.

#### Description of *Actinomadura algeriensis* sp. nov

*Actinomadura algeriensis* (al.ger.i.en'sis. N.L. fem. adj. *algeriensis* referring to Algeria, the country from where the type strain was isolated).

Aerobic, Gram-stain positive, non-motile actinobacterium that forms an extensively branched and non-fragmented substrate mycelium. Aerial mycelium, when present, is white and becomes progressively pinkish with age on ISP 2 and ISP 3 media. A red diffusible pigment is produced on all tested media. Growth occurs at 20–37 °C and at pH 6–9. The NaCl tolerance range for growth is 0–3 % (w/v). Aesculin, arbutin, casein, gelatin and Tween 80 are degraded, but not adenine, guanine, hypoxanthine, starch, L-tyrosine and xanthine. Acetate, pyruvate and succinate are decarboxylated, but not benzoate, oxalate, propionate and L-tartrate. Nitrate reduction is positive. Milk peptonisation is positive, while milk coagulation is negative. Utilizes adonitol, L-arabinose, cellobiose,

fructose, glucose, maltose, mannitol, rhamnose, ribose, sorbitol, trehalose and xylose, but not galactose, lactose, mannose, melibiose and raffinose. Growth does not occur in the presence of novobiocin and rifampicin. 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 major menaquinones are MK-9 (H<sub>4</sub>) and MK-9 (H<sub>2</sub>). The major fatty acids are C<sub>16:0</sub>, C<sub>18:1 cis9</sub>, iso-C<sub>16:0</sub> and 10-methyl C<sub>18:0</sub>.

The type strain is ACD1<sup>T</sup> (= DSM 46744<sup>T</sup> = CECT 8841<sup>T</sup>) isolated from a Saharan soil sample collected from Hoggar region (South Algeria). The 16S rRNA gene sequence of strain ACD1<sup>T</sup> has been deposited in Genbank under the accession number KT259320.

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