Actinomadura adrarensis sp. nov., an actinobacterium isolated from Saharan soil

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A novel actinobacterial strain, designated ACD12T, 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 ACD12T 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 occurred in whole-cell hydrolysates. The major phospholipid was diphosphatidylglycerol and phosphatidylinositol. The predominant menaquinone was MK-9 (H4). The fatty acid profile was characterized by the presence of C16:0, C17:0, C18:0, C18:1 cis-9 and iso-C18:0. Results of 16S rRNA gene sequence comparisons revealed that strain ACD12T shared the highest degree of 16S rRNA gene sequence similarity with Actinomadura sputi DSM 45233T (98.3 %) and Actinomadura hallensis DSM 45043T (97.8 %). All tree-making algorithms used also supported strain ACD12T forming a distinct clade with its most closely related species. In addition, DNA–DNA hybridization indicated only 39.8 % relatedness with A. sputi DSM 45233T and 18.7 % relatedness with A. hallensis DSM 45043T. 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 ACD12T (=DSM 46745T =CECT 8842T).

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 (Euanoraset 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(H4) and MK-9(H6) as the major menaquinones.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ACD12T is KU356942.

Two supplementary figures and one supplementary table are available with the online Supplementary Material.
and peptonisation of milk, were evaluated according to the procedures described by Becker et al. (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 ACD12T 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 ACD12T 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 Cashon 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 ACD12T 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

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).

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Fig. 1. Scanning electron micrograph of spore chains of strain ACD12T. The strain was grown on humic 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<sup>T</sup> was found to grow at 25–37°C, at pH 7–10
and at 0–2% (w/v) NaCl. Strain ACD12<sup>T</sup> and its two most
closely related reference type strains (<i>Actinomadura sputi</i>
DSM 45233<sup>T</sup> and <i>Actinomadura hallensis</i> DSM 45043<sup>T</sup>)
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 char-
acteristics, as illustrated in Table 1. The complete

**Table 1. Phenotypic characteristics that differentiate strain
ACD12<sup>T</sup> from its most closely related species of the genus
<i>Actinomadura</i>**

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

physiological characteristics of strain ACD12<sup>T</sup> are given in
the species description.

Strain ACD12<sup>T</sup> exhibited chemical markers typical of
members of the genus <i>Actinomadura</i>. The cell wall of strain ACD12<sup>T</sup>
was found to contain meso-diaminopimelic acid
as the diagnostic peptidoglycan diamino acid, but not gly-
cine. The whole-cell hydrolysate was found to contain
madurose as the diagnostic sugar, along with glucose,
rbose, 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<sub>8</sub>) (64.5%) with small amounts of MK-9(H<sub>4</sub>) (12.5 %),
MK-9(H<sub>6</sub>) (12%) and MK-9(H<sub>2</sub>) (2.3%) also detected. The
diagnostic phospholipids detected were diphostatidy-
lyglycerol and phosphatidylinositol, which corresponds to
phospholipid type PI (Lechevalier et al., 1977); phosphati-
dylinositol mannosides and phosphatidylyglycerol were also
present (Fig. S1, available in the online Supplementary
Material). The cellular fatty acids higher than 5% were
identified as C<sub>16:0</sub> (22.2%), 10-methyl C<sub>17:0</sub> (15.2%),
C<sub>17:1ω9c</sub> (11.5%), C<sub>15:0</sub> (11.1%), 10-methyl C<sub>16:0</sub> (9.3 %)
and C<sub>18:1ω9c</sub> (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<sup>T</sup> within the genus <i>Actinomadura</i>.
High degrees of 16S rRNA gene sequence similarity were
found between strain ACD12<sup>T</sup> and its nearest neighbours,
<i>Actinomadura sputi</i> DSM 45233<sup>T</sup> (98.3%) and <i>Actinomadura hallensis</i>
DSM 45043<sup>T</sup> (97.8%).

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

Based on these phenotypic and genotypic data, strain
ACD12<sup>T</sup> is a member of the genus <i>Actinomadura</i> and repre-
sents a novel species, for which the name <i>Actinomadura adrarensis</i>
sp. nov. is proposed.

**Description of <i>Actinomadura adrarensis</i> sp. nov.**

<i>Actinomadura adrarensis</i> (ad.rar. en’sis. N.L. fem. adj. adrar-
ensis 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
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 D-myo-inositol, L-aventoin, adonitol, 1,1-diaminocyclohexane, 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, 7 and 0% (w/v), respectively. Acetate, ascorbic acid, 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-xylene 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 diphostatidylglycerol and phosphatidylinositol. The predominant menaquinone is MK-9(H_8). The major fatty acids are C_{16:0}, 10-methyl C_{17:0}, C_{17:1}ω9c, 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).

Acknowledgements

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Reference


Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGAS: molecular evolutionary genetics analysis using


