Actinomadura rupiterra sp. nov., isolated from cliff soil

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A novel actinomycete strain, designated CS5-AC15T, was isolated from a soil sample collected from a cliff on Mara Island, Jeju, Republic of Korea, and subjected to a polyphasic taxonomic analysis. The isolate produced well-developed, yellow substrate mycelium and white aerial mycelium that differentiated into straight or flexuous chains of smooth-surfaced spores. 16S rRNA gene sequence analyses showed that the organism belonged to the family Thermomonosporaceae and formed a tight cluster with the type strain of Actinomadura oligospora (97.4% sequence similarity). Chemotaxonomic characteristics were consistent with its assignment to the genus Actinomadura in that the isolate had meso-diaminopimelic acid as the diagnostic diamino acid in the cell wall, madurose as the characteristic sugar, N-acetyl type of murein in the peptidoglycan, MK-9(H₆) and MK-9(H₈) as major menaquinones and a polar lipid profile containing diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and unknown phospholipids. Mycolic acids were not detected. The predominant fatty acids were C₁₆:0, C₁₈:1ω₉c and iso-C₁₆:0. The DNA G+C content was 70.9 mol%. DNA relatedness of strain CS5-AC15T and A. oligospora JCM 10648T was 37.9 ± 0.7%. On the basis of the phenotypic, phylogenetic and DNA–DNA hybridization data, strain CS5-AC15T is assigned to a novel species of the genus Actinomadura, for which the name Actinomadura rupiterra sp. nov. is proposed (type strain CS5-AC15T =KCTC 19559T =DSM 45251T).

The genus Actinomadura (Lechevalier & Lechevalier, 1970b) belongs to the family Thermomonosporaceae Stackebrandt et al. 1997 emend. Zhang et al. 2001, together with the genera Actinoallomurus, Actinocorallia, Spirillospora and Thermomonospora, and at the time of writing consisted of 46 recognized species, including the recently described species Actinomadura miaoliensis (Tseng et al., 2009), A. keratinilytica (Puhl et al., 2009), A. flavialba (Qin et al., 2009), A. spati (Yassin et al., 2010), A. scrupuli (Lee & Lee, 2010), A. apis, A. rifamycina (Promnuan et al., 2011) and A. meridiana (Lee, 2012). Members of the genus contain meso-diaminopimelic acid as the diagnostic diamino acid and madurose as the characteristic sugar in the cell wall, with a murein structure of the acetyl type, and possess predominant menaquinones MK-9(H₆), MK-9(H₈) and MK-9(H₉), phospholipid type PI (diphosphatidylglycerol and phosphatidylglycerol present as major phospholipids), fatty acid type 3a [branched saturated and unsaturated fatty acids and tuberculostearic acid (TBSA)] and DNA G+C contents of 65–73 mol% (Kroppenstedt & Goodfellow, 1991; Meyer, 1989). Morphologically, the genus is characterized by the production of well-developed, non-fragmenting vegetative hyphae and aerial mycelium, on the tips of which spore chains of various lengths are arranged in straight, hooked or spiral form. The smooth, spiny or warty-surfaced spores are non-motile, oval or rod-shaped. This genus can be readily differentiated from the other genera of the family Thermomonosporaceae on the basis of a combination of morphological and chemotaxonomic characteristics (Kroppenstedt & Goodfellow, 2006). In this paper, the classification and identification of an Actinomadura strain that was isolated from soil is described by a polyphasic taxonomic approach.

Strain CS5-AC15T was isolated from a soil sample taken from a cliff on Mara Island, Jeju, Republic of Korea. Bacterial isolation was performed by using the procedure described by Lee & Jeong (2006) and starch-casein agar (Küster & Williams, 1964) as the isolation medium. The pure culture was maintained on International Streptomyces Project (ISP) medium 2 (Shirling & Gottlieb, 1966) and as mycelial fragments or spores in 20% (v/v) glycerol at –80 °C. For phenotypic analysis and DNA–DNA hybridization, A. oligospora JCM 10648T was grown on ISP medium 2 at 30 °C. Growth and pigmentation of strain CS5-AC15T were investigated on various media: yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5), peptone-yeast extract-iron agar (ISP 6) and tyrosine agar (ISP 7).
(Shirling & Gottlieb, 1966). Results for cultural and morphological characterization were recorded after 2 weeks of incubation at 30 °C. Spore chain morphology and spore ornamentation were observed by using scanning electron microscopy (JSM-6500; JEOL). The specimen for electron microscopy was fixed, dehydrated, critical-point-dried and coated with gold as described by Lee & Jeong (2006) before observation.

Strain CS5-AC15T grew well on ISP media 2, 3 and 4 and moderately on ISP 5, but poor or no growth was observed on ISP media 6 and 7. Substrate mycelium was well developed, branched and light to strong yellow in colour. White aerial mycelium was produced abundantly on ISP media 3 and 4 and differentiated into straight or flexuous chains of smooth-ornamented spores. Ten or more spores were present per chain (Fig. 1). No soluble pigments were produced on any of the tested media.

Unless otherwise indicated, physiological properties were determined using ISP 2 as the basal medium. Growth was examined at 4, 10, 20, 30, 37, 42 and 45 °C and at pH 4–12 (at intervals of 1 pH unit). The results were recorded after 14 days of incubation at or below 30 °C and after 5 days of incubation at or above 37 °C. Tolerance of NaCl for growth was determined on ISP 2 supplemented with 1–9 % (w/v) NaCl (at intervals of 1.0 %). Tolerance of pH and NaCl for growth was observed on plates incubated at 30 °C for 14 days. Decomposition of hypoxanthine, DL-tyrosine and xanthine was tested as described by Gordon et al. (1974). Oxidase activity was determined according to the method of MacFaddin (1980). Catalase and urease activities, H2S production, nitrate reduction and hydrolysis of casein and gelatin were tested as described by Lee & Jeong (2006). Cellulose hydrolysis was determined on ISP medium 2 supplemented with 0.5 % (w/v) CM-cellulose (Sigma). Aesculin hydrolysis was tested on agar medium containing 0.3 % (w/v) yeast extract, 0.05 % (w/v) ferric ammonium citrate, 1 % (w/v) aesculin and 0.75 % (w/v) agar, and blackening of the medium was recorded as positive after incubation for 14 days at 30 °C. Hydrolysis of DNA and starch was determined using DNase test agar (Difco) and starch agar (Difco), respectively. Utilization of substrates as sole carbon and energy sources was determined using ISP 9 supplemented with carbohydrates and alcohols at final concentrations of 1 % (w/v) and organic acids at final concentrations of 0.1 % (w/v). The physiological properties of strain CS5-AC15T are given in the species description and in Table 1.

Chromosomal DNA extraction, amplification of the 16S rRNA gene by PCR and its sequencing were performed as described previously (Lee, 2006). The CLUSTAL_X program (Thompson et al., 1997) was used for multiple alignments of related sequences. Phylogenetic analyses were performed using several programs contained in the PHYLIP package (Felsenstein, 1993).

Table 1. Characteristics that differentiate strain CS5-AC15T from the type strain of A. oligospora

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tr>
<td>Growth on ISP media 3 and 4</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Aerial mycelium on ISP media 3 and 4</td>
<td>Abundant</td>
<td>Trace</td>
</tr>
<tr>
<td>Growth at 2 % (w/v) NaCl</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Decomposition of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>DNA</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adonitol</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Dextrin</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>
| D-Galactose                     | −   | +   *
| Lactose                         | +   | −   |
| Melezitose                      | +   | −   |
| D-Mannitol                      | +   | −   |
| D-Ribose                        | −   | +   |
| L-Sorbose                       | −   | +   |
| Sucrose                         | +   | −   |

*Different response reported previously (Mertz & Yao, 1986).

Strains: 1, strain CS5-AC15T; 2, A. oligospora JCM 10648T. All data were obtained in this study, except for the cultural characteristics of A. oligospora, which were taken from Mertz & Yao (1986).
and was closely related to *A. oligospora* (97.4 % sequence similarity to the type strain). This branching pattern was supported by a moderate bootstrap value (69 %) and was also found in trees generated by the maximum-parsimony and maximum-likelihood algorithms. Strain CS5-AC15T showed 16S rRNA gene sequence similarity values lower than 96.8 % to other species of the genus *Actinomadura* and related taxa.

The following chemotaxonomic characteristics were analysed, with biomass obtained from cultures grown in ISP 2 broth for 7 days at 30 °C: the isomer of diaminopimelic acid (DAP) and 3-O-methyl-D-glucose (3-OMe-D-Glc).
A phylogenetic tree (Fig. 2) showed that strain CS5-AC15<sup>T</sup> contained meso-diaminopimelic acid as the diagnostic diamino acid and madurose and glucose as whole-cell sugars, showing that it possessed cell-wall type III (Lechevalier & Lechevalier, 1970a) and whole-cell sugar pattern B (Lechevalier, 1968). The acyl type of murein in the peptidoglycan was acetyl. The menaquinones of strain CS5-AC15<sup>T</sup> were MK-9(H<sub>4</sub>) (62%), MK-9(H<sub>6</sub>) (31%) and MK-9(H<sub>8</sub>) (7%). A. oligospora JCM 10648<sup>T</sup> contained a similar menaquinone profile, at a peak ratio of 71:27:2 in this study. The polar lipids were diphasphatidylglycerol, phosphatidylglycerol, phosphatidyl-

The cell wall of strain CS5-AC15<sup>T</sup> contained saturated, unsaturated iso-branched and 10-methyl-branched fatty acids. The predominant fatty acids contained saturated, unsaturated iso-branched and 10-methyl-branched fatty acids. The predominant fatty acids were C<sub>16</sub>:0 (31%), C<sub>18:1<sup>ω9c</sup></sub> (21.5%) and iso-C<sub>16</sub>:0 (11.1%). A. oligospora JCM 10648<sup>T</sup> showed a similar profile, but differed from strain CS5-AC15<sup>T</sup> in the relative proportions of 10-methyl C<sub>18:0</sub> (TBSA) and iso-C<sub>16:0</sub> (see Table S1, available in IJSEM Online). The G+C content of the DNA was 70.9 mol%, as determined by HPLC (Mesbah et al., 1989).

A phylogenetic tree (Fig. 2) showed that strain CS5-AC15<sup>T</sup> belongs to the family Thermomonosporaceae and is closely related to A. oligospora. The combination of morphological and chemotaxonomic data supports the assignment of the strain to the genus Actinomadura. Phenotypic features that differentiate strain CS5-AC15<sup>T</sup> from A. oligospora JCM 10648<sup>T</sup> are shown in Tables 1 and S1. Strain CS5-AC15<sup>T</sup> showed good growth and produced abundant aerial mycelium on ISP media 3 and 4, in contrast to A. oligospora (Mertz & Yao, 1986). Strain CS5-AC15<sup>T</sup> can be further differentiated from A. oligospora by utilization of carbohydrates and hydrolysis of casein and DNA (Table 1).

DNA–DNA hybridization experiments were performed according to the photobiotin-labelling method of Ezaki et al. (1989). Genomic DNA was extracted and purified by the method of Hopwood et al. (1985). Five replications were carried out for each DNA pairing and the highest and lowest scores for each DNA pairing were excluded. Finally, DNA relatedness values were recorded as mean ± SD values for the remaining three scores (Yoon et al., 2007). DNA–DNA relatedness between strain CS5-AC15<sup>T</sup> and A. oligospora JCM 10648<sup>T</sup> was 37.9±0.7 %, well below the 70% cut-off value recommended for the delineation of bacterial genospecies (Wayne et al., 1987).

Based on the phenotypic, phylogenetic and DNA–DNA hybridization data presented here, strain CS5-AC15<sup>T</sup> is considered to represent a novel species of the genus Actinomadura, for which the name Actinomadura rupiterrae sp. nov. is proposed.

**Description of Actinomadura rupiterrae sp. nov.**

*Actinomadura rupiterrae* (ru.pi.ter’rae. L. fem. n. rupes cliff; L. fem. n. terra soil; N.L. gen. n. rupiterrae of cliff soil, referring to the sample from which the type strain was isolated).

Catalase-positive. Oxidase-negative. Grows well on ISP 2, 3 and 4 media. Substrate mycelium is well-developed, branched and light to strong yellow in colour. Aerial mycelium is produced abundantly on ISP 3 and 4 and differentiates into straight to flexuous chains of ten or more spores. The spore surface is smooth. Growth occurs at 20–42 °C (optimum, 37–42 °C), at pH 5–11 (optimum, pH 6–8) and in the presence of up to 1 % (w/v) NaCl. Aesculin and gelatin are hydrolysed, but casein, DNA, CM-cellulose, starch and urea are not. Hypoxanthine, DL-tyrosine and xanthine are not decomposed. H<sub>2</sub>S production and nitrate reduction are not observed. Citrate, dextran, D-glucose, lactose, D-mannitol, melezitose and sucrose are utilized as sole carbon sources. Acetate, adonitol, D- and L-arabinose, benzoate, 2,3-butanediol, cellobiose, dulcitol, meso-erythritol, formate, D-fructose, D-galactose, glycerol, myo-inositol, inulin, DL-malate, maltose, methyl α-D-glucoside, methyl α-D-mannoside, 1,2-propanediol, raffinose, L-rhamnose, D-ribose, salicin, D-sorbitol, L-sorbose, succinate, trehalose, DL-tartrate, D-xylitol and D-xylulose are not utilized. *meso*-Diaminopimelic acid is the diagnostic diamino acid and madurose is the characteristic sugar (type III/B cell wall). The acyl type of muramic acid is acetyl. The major menaquinones are MK-9(H<sub>6</sub>) and MK-9(H<sub>8</sub>). The polar lipids are diphasphatidylglycerol, phosphatidyglycerol, phosphatidylinositol and unknown phospholipids. Mycolic acids are absent. The predominant fatty acids are C<sub>16:0</sub>, C<sub>18:1<sup>ω9c</sup></sub> and iso-C<sub>16:0</sub>.

The type strain, CS5-AC15<sup>T</sup> (=KCTC 19559<sup>T</sup> =DSM 45251<sup>T</sup>), was isolated from a soil sample collected from a cliff on Mara Island, Jeju, Republic of Korea. The G+C content of the DNA of the type strain is 70.9 mol%.

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References


