Amycolatopsis ultiminotia sp. nov., isolated from rhizosphere soil, and emended description of the genus Amycolatopsis

Soon Dong Lee

Department of Science Education, Cheju National University, Jeju 690-756, Republic of Korea

A novel actinomycete, designated strain RP-AC36\(^T\), was isolated from a cliff-associated plant (*Peucedanum japonicum* Thunb.) in the Republic of Korea and its taxonomic status was determined by using a polyphasic approach. Phylogenetic analyses based on 16S rRNA gene sequence analysis showed that the organism formed a distinct clade within the radiation of the genus *Amycolatopsis*. The chemotaxonomic properties supported the assignment of the isolate to the genus *Amycolatopsis*. High levels of 16S rRNA gene sequence similarity were found with *Amycolatopsis sulphurea* (98.2 %), *Amycolatopsis halotolerans* (97.5 %) and *Amycolatopsis jejuensis* (97.1 %). DNA–DNA relatedness data, together with phenotypic differences, clearly distinguished the isolate from its closest relative *A. sulphurea*. Based on the phenotypic and genotypic evidence, it is suggested that the organism be assigned as representing a novel species of the genus *Amycolatopsis*, for which the name *Amycolatopsis ultiminotia* sp. nov. is proposed. The type strain is RP-AC36\(^T\) (=NRRL B-24662\(^T\)=DSM 45180\(^T\)).

The genus *Amycolatopsis* is one of the well-defined taxa in the family *Pseudonocardiaecae*, based on chemotaxonomic characteristics (Lechevalier *et al.*, 1986; Kim & Goodfellow, 1999; Yassin *et al.*, 1993; Groth *et al.*, 2007) and phylogenetic evidence based on the comparison of 16S rRNA gene sequences (Lee *et al.*, 2000; Tan *et al.*, 2006). In the recently emended genus description (Groth *et al.*, 2007), some species contain MK-11(H\(_4\)), instead of MK-9(H\(_4\)), as the predominant menaquinone. At the time of writing, the genus consists of 38 recognized species and many novel species have been described from diverse environments such as soil, vegetation, human and animal clinical sources, fresh water, rock and subterranean sites. The present polyphasic study was designed to determine the taxonomic status of an *Amycolatopsis* strain that was isolated from a rhizosphere soil sample.

Strain RP-AC36\(^T\) was isolated from a cliff-associated plant (*Peucedanum japonicum* Thunb.) on Mara Island, Jeju, Republic of Korea. For bacterial isolation, 10-fold dilutions of rhizosphere soil samples with sterile distilled water were inoculated on starch-casein agar and incubated at 30 °C for 14 days, as described by Lee (2006). The organism was maintained as mycelial fragments in 20 % (v/v) glycerol at –80 °C.

Morphological and cultural characteristics of strain RP-AC36\(^T\) were examined on various media: ISP 2, ISP 3, ISP 4, ISP 5, ISP 6 and ISP 7 (Shirling & Gottlieb, 1966), oatmeal-nitrate agar (Prauser & Bergholz, 1974) and HV agar (Nonomura & Ohara, 1969). The results were recorded after incubation for 14 days at 30 °C. For electron microscopy, cells were prepared as described previously (Lee, 2006) and observed with a scanning electron microscope (JSM 5410LV; JEOL). For genetic comparison, *Amycolatopsis sulphurea* IMSNU 20060\(^T\) was grown on ISP 2 medium as the basal medium. The initial pH for growth was examined over the range pH 4.1–12.1 (with intervals of 1.0 unit). Acid production from carbohydrates, degradation of casein, hypoxanthine, starch, DL-tyrosine, urea and xanthine, nitrate reduction, gelatin liquefaction

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RP-AC36\(^T\) is FM177516.

The phospholipid profile of cells of strain RP-AC36\(^T\) and fatty acid compositions of strain RP-AC36\(^T\) and its phylogenetic neighbours are available as supplementary material with the online version of this paper.

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and catalase activity were examined as described previously (Lee et al., 2006). Hydrolysis of chitin (0.5 %, w/v), CM-cellulose (0.5 %, w/v; Sigma) and elastin (0.4 %, w/v) was examined on ISP 2 medium. DNA hydrolysis was determined by using DNase test agar (Difco). Data for physiological properties are shown in the species description and Table 1.

Biomass for chemotaxonomic characterization was obtained from cultures grown in trypticase soy broth (Difco) for 3 days at 30 °C with shaking. Analyses of the isomer of diaminopimelic acid (Staneck & Roberts, 1974) and sugars (Saddler et al., 1991) in whole-cell hydrolysates, mycolic acids (Minnikin et al., 1980) and polar lipids (Minnikin et al., 1975; 1984) were performed as described previously (Lee et al., 2006). Menaquinones were extracted by using the method of Minnikin et al. (1984) and analysed by HPLC (Kroppenstedt, 1985). Cellular fatty acid methyl esters were prepared by alkaline methanolysis and analysed by GC with an Agilent model 6850 gas chromatograph, as described previously (Lee & Hah, 2001).

The chemotaxonomic characteristics of strain RP-AC36T were typical of the genus Amycolatopsis in having a type IV cell-wall composition (meso-diaminopimelic acid, arabinose and galactose in whole-cell hydrolysates), MK-9(H4) as the major menaquinone and a polar lipid profile including phosphatidylmethylthanolamine and an unknown ninhydrin-positive phospholipid (see Supplementary Fig. S1, available in IJSEM Online). Mycolic acids were not present. The cellular fatty acids of the isolate were represented by considerable amounts of saturated and branched-chain acids, with small amounts of unsaturated or hydroxy fatty acids. The major fatty acids were C17:0, C15:0, i-C16:0 and i-C15:0. The cellular fatty acid profiles of strain RP-AC36T and its phylogenetic neighbours, Amycolatopsis jejuensis N7-3T and A. sulphurea IMSNU 20060T, are given in Supplementary Table S1 (in IJSEM Online). Strain RP-AC36T showed significant differences from A. jejuensis N7-3T and A. sulphurea IMSNU 20060T in the relative amounts of C15:0, C16:0, C17:0, C18:0, i-C14:0, i-C17:0 and ai-C15:0 fatty acids. The DNA G+C content was analysed by HPLC (Mesbah et al., 1989) and measured to be 67.5 mol%.

Extraction of chromosomal DNA and the amplification of the 16S rRNA gene by PCR were performed as described previously (Lee, 2006). The resultant PCR product was purified using a Wizard Genomic DNA Purification kit (Promega) according to the manufacturer’s instructions and subjected to direct sequencing as described previously (Lee, 2006). The CLUSTAL_X program (Thompson et al., 1997) was used for multiple sequence alignments. Phylogenetic analyses, tree construction and bootstrap analysis were carried out by using several programs contained in the PHYLIP package (Felsenstein, 1993).

A partial 16S rRNA gene sequence (1413 nt) of strain RP-AC36T was compared with the corresponding sequences of representatives of the genus Amycolatopsis. The neighbour-joining tree (Fig. 1) revealed that the organism belongs to the genus Amycolatopsis and forms a branch between A. sulphurea and A. jejuensis. The phylogenetic position was supported by a high bootstrap value (82 %) and was also found in trees constructed using maximum-parsimony and maximum-likelihood algorithms (not shown). The closest relatives were A. sulphurea IMSNU 20060T (98.2 % 16S rRNA gene sequence similarity) followed by Amycolatopsis halotolerans NRRL B-24428T (97.5 %) and A. jejuensis NRRL B-24427T (97.1 %). Strain RP-AC36T shared 16S rRNA gene sequence similarities between 94.8 and 97.0 % with the type strains of other representatives of the genus Amycolatopsis.

DNA–DNA hybridization of strain RP-AC36T was performed against the closest relative, A. sulphurea IMSNU 20060T. Genomic DNA was isolated using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite as described previously (Cashion et al., 1977). DNA–DNA hybridization was

### Table 1. Phenotypic characteristics that differentiate strain RP-AC36T from its phylogenetic neighbours

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<th>3</th>
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<tbody>
<tr>
<td>Growth at 37 °C</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Growth with 5 % (w/v) NaCl</td>
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<td>−</td>
<td>−</td>
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<td>Nitrate reduction</td>
<td>−</td>
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<td>+</td>
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<td>Acid production from:</td>
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<tr>
<td>Adonitol</td>
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<td>+</td>
<td>−</td>
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<td>Cellobirose</td>
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<td>+</td>
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<tr>
<td>Dextran</td>
<td>+</td>
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<td>D-Fructose</td>
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<td>+</td>
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<tr>
<td>D-Glucose</td>
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<td>−</td>
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<tr>
<td>Glycerol</td>
<td>+</td>
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</tr>
<tr>
<td>myo-Inositol</td>
<td>+</td>
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<tr>
<td>Lactose</td>
<td>+</td>
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<td>Maltose</td>
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<tr>
<td>R-Rhamnose</td>
<td>−</td>
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<td>Sucrose</td>
<td>+</td>
<td>−</td>
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<td>Trehalose</td>
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<td>+</td>
<td>−</td>
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<tr>
<td>D-Xylose</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<td>Degradation of:</td>
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<td>Aesculin</td>
<td>+</td>
<td>ND</td>
<td>−</td>
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<td>Hypoxanthine</td>
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<td>−</td>
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<td>Urea</td>
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<td>W</td>
<td>−</td>
</tr>
<tr>
<td>Xanthine</td>
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carried out using the spectrophotometric method as described by De Ley et al. (1970), with the modifications described by Huß et al. (1983). Strain RP-AC36\textsuperscript{T} had a DNA–DNA relatedness of 26.6\% (31.0\% in the duplicate measurement) with \textit{A. sulphurea} IMSNU 20060\textsuperscript{T}. According to the criterion recommended by Wayne et al. (1987), a DNA–DNA hybridization value less than 70\% indicates that the isolate can be assigned to a different species. Differential phenotypic characteristics of the isolate from its phylogenetic neighbours, \textit{A. jejuensis} and \textit{A. sulphurea}, are given in Table 1. Strain RP-AC36\textsuperscript{T} differed from both strains in nitrate reduction, xanthine degradation and in acid production from cellobiose, D-fructose, lactose, L-rhamnose and D-xylose.

The phenotypic and DNA–DNA hybridization data presented here suggest that the isolate can be assigned as a novel species of the genus \textit{Amycolatopsis}, for which the name \textit{Amycolatopsis ultiminotia} sp. nov. is proposed.

**Emended description of the genus \textit{Amycolatopsis} Lechevalier et al. 1986**

\textit{Amycolatopsis} (A.my.co.la.top’sis. M.L. fem. n. \textit{Amycolata} genus belonging to the order \textit{Actinomycetales}; Gr. n. \textit{opsis} appearance; M.L. fem. n. \textit{Amycolatopsis} that which appears similar to \textit{Amycolata}).

The description of the genus \textit{Amycolatopsis} Lechevalier et al. 1986 is emended as follows. The diagnostic phospholipid is phosphatidylethanolamine (with or without phosphatidylmethyl ethanolamine) or phosphatidylmethyl ethanolamine (type II phospholipid pattern).

**Description of \textit{Amycolatopsis ultiminotia} sp. nov.**

\textit{Amycolatopsis ultiminotia} (ul.ti.mi.no’ti.a. L. sup. adj. ultimus farthest, extreme; L. fem. adj. notia southern; N.L. fem. adj. ultiminotia farthest southern, implying that
the type strain was isolated from the southernmost parts of the Republic of Korea).

Aerobic, Gram-positive, non-acid-alcohol-fast, catalase-positive. The aerial mycelium is white and the vegetative mycelium is cream to yellow. Both aerial and substrate hyphae fragment into rod-shaped elements. Growth occurs between 10 and 37 °C, with optimal growth at 20–37 °C. Growth does not occur at 42 °C. Initial pH for growth is pH 5.1–12.1, with optimal growth at pH 5.1–9.1. Growth occurs in the presence of up to 5 % (w/v) NaCl. Acid is produced from dulcitol but not from D-arabinose, inulin, methyl D-mannoside or D-ribose. DNA and elastin are hydrolysed but chitin or CM-cellulose are not hydrolysed. Other phenotypic features are given in Table 1. Major fatty acids are C₁₇:₀ (23.7 %), C₁₅:₀ (19.8 %), i-C₁₆:₀ (13.2 %) and i-C₁₅:₀ (10.0 %). The DNA G+C content of the type strain is 67.5 mol%.

The type strain is strain RP-AC36T (=NRRL B-24662T =DSM 45180T), isolated from the rhizosphere of a cliff-associated plant (Peucedanum japonicum Thunb.).

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References


