Amycolatopsis samaneae sp. nov., isolated from roots of *Samanea saman* (Jacq.) Merr.

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A novel actinomycete, designated strain RM287^T, was isolated from surface-sterilized roots of *Samanea saman* (Jacq.) Merr., collected from Bangkok, Thailand. The status of the novel strain was determined using a polyphasic taxonomic approach. Phylogenetic analyses based on 16S rRNA gene sequences showed that the organism formed a distinct phyletic line within the radiation of the genus *Amycolatopsis*. The 16S rRNA gene sequence similarity indicated that strain RM287^T was most closely related to *Amycolatopsis mediterranei* IMSNU 20056^T (97.4%), *A. rifamycinica* DSM 46095^T (97.2%), *A. kentuckyensis* NRRL B-24129^T (97.2%), *A. pretoriensis* DSM 44654^T (97%) and *A. australiensis* DSM 44671^T (97%). The novel organism was found to have chemical properties typical of members of the genus *Amycolatopsis* such as meso-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan and arabinose and galactose as the diagnostic sugars. The major menaquinone was MK-9(H₄). The major fatty acids were iso-C_{16:0}, iso-C_{15:0}, iso 2-OH-C_{16:0} and iso-C_{17:0}. The DNA G + C content was 71.7 mol%. Phenotypic data clearly distinguished the novel isolate from its closest relatives. The combined genotypic and phenotypic data indicated that strain RM287^T represented a novel species of the genus *Amycolatopsis*. The proposed name for this organism is *Amycolatopsis samaneae* sp. nov., with the type strain RM287^T (=TISTR 1919^T=BCC 35842^T=NBRC 106095^T).

The genus *Amycolatopsis* comprises a large group of commercially and medically important actinomycetes. The genus was proposed by Lechevalier *et al.* (1986) and its description has been emended recently by Lee (2009). Many novel species of this genus have been isolated from diverse environments such as clinical material (Labeda *et al.*, 2003; Huang *et al.*, 2004), natural caves (Lee, 2006), ocean sediment (Bian *et al.*, 2009), soil (Tan *et al.*, 2006; Tseng *et al.*, 2006; Lee, 2009) and vegetable matter

(Goodfellow *et al.*, 2001). The present study was designed to determine the status of *Amycolatopsis* strain RM287^T, isolated from surface-sterilized plant roots, using a polyphasic taxonomic approach. It was evident from the data obtained that this organism represents a novel species of the genus *Amycolatopsis*.

As part of our discovery programme for endophytic actinomycetes, strain RM287^T was isolated from a leguminous plant using starch casein agar (Küster & Williams, 1964) supplemented with ketokonazole (100 µg ml⁻¹) and nalidixic acid (25 µg ml⁻¹). Plates that had been inoculated with surface-sterilized crushed root suspensions of *Samanea saman* were incubated at 28 °C for 14 days. The

Abbreviation: ISP, International *Streptomyces* Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain RM287^T is GQ381310.

organism was purified and maintained on glucose yeast extract agar slants [GYE agar; containing 1.0 % glucose (w/v), 1 % yeast extract (w/v) and 1.5 % agar (w/v)] at room temperature as the working culture. The pure culture was also preserved as mixtures of fragmented hyphae and spores in 20 % glycerol at -20°C or as lyophilized cells for long-term storage. Biomass for chemotaxonomic analysis was obtained by growing the novel strain in shake flasks of GYE broth for 3 days at 28°C . The cells were harvested by centrifugation. The resultant cells were washed three times with distilled water and freeze-dried.

The phylogenetic position of the new isolate was determined by 16S rRNA gene sequence analysis. Genomic DNA was extracted and purified following the method of Kieser *et al.* (2000). The 16S rRNA gene was amplified as described by Duangmal *et al.* (2005). The PCR products were sequenced (Macrogen) using universal primers (Lane, 1991). The almost complete 16S rRNA gene sequence (1417 bp) was aligned with the corresponding sequences of representatives of the genus *Amycolatopsis*, retrieved from the GenBank database, using the CLUSTAL_X (Thompson *et al.*, 1997) and PHYDIT programs (<http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred by the least-squares (Fitch & Margoliash, 1967), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms from the PHYLIP suite of programs (Felsenstein, 1993). The resultant tree topologies were evaluated using bootstrap analyses (Felsenstein, 1985) based on 1000 resampled datasets of the SEQBOOT and CONSENSE options from the PHYLIP package. The resultant phylogenetic trees were viewed by using the TreeView program (Page, 1996).

It was evident from the 16S rRNA gene phylogenetic tree that strain RM287^T formed a distinct phyletic line within members of the genus *Amycolatopsis* (Fig. 1). The 16S rRNA gene sequence similarities between strain RM287^T and its nearest neighbour, *Amycolatopsis mediterranei* IMSNU 20056^T showed that the two organisms shared a 16S rRNA gene sequence similarity of 97.4 %, a value that corresponded to 36 differences at 1412 locations. In addition, the novel isolate showed relatively low 16S rRNA gene sequence similarities with *Amycolatopsis rifamycinica* DSM 46095^T (97.2 %), *A. kentuckyensis* NRRL B-24129^T (97.2 %), *A. pretoriensis* DSM 44654^T (97 %) and *A. australiensis* DSM 44671^T (97 %). However, it was clear that strain RM287^T represented a novel subline within the genus *Amycolatopsis* as its position in the tree was well separated from these nearest phylogenetic neighbours and all other members of the genus (Fig. 1). DNA–DNA relatedness studies were not performed between the new isolate and its closest phylogenetic neighbours as it has been shown that members of the genus *Amycolatopsis* that share much higher 16S rRNA gene sequence similarities had DNA–DNA relatedness values well below the 70 % cut-off point (Wayne *et al.*, 1987) for strains that belonged to the same genomic species (Labeda *et al.*, 2003; Tan *et al.*, 2007).

The cultural characteristics of strain RM287^T were examined on ISP 2, 4, 5, 6 and 7 media (International *Streptomyces* Project; Shirling & Gottlieb, 1966) and GYE agar. The colour of the mycelium and soluble pigment were determined by comparing them with the colour chips from the Colour Harmony Manual (Jacobson *et al.*, 1958). Morphological characteristics were observed by light and scanning electron microscopy (JSM 5600 LV; JEOL) of 10 day old cultures grown on ISP 3 medium. Acid production from carbohydrates was determined using the media and methods described by Gordon *et al.* (1974). Enzyme activity profiles were examined using API ZYM (bioMérieux) tests. Urease activity was determined by a colour change on urea agar (Gordon *et al.*, 1974). The temperature range for growth was determined on GYE agar using a temperature gradient incubator (Tokyo Kagaku Sangyo) with low and high temperatures of 5 and 50°C , respectively. The production of hydrogen sulphide was detected using lead acetate strips. Hydrolysis of casein and gelatin and nitrate reduction were examined using the methods of Gordon & Mihm (1957). Production of melanoid pigment was according to Shirling & Gottlieb (1966). NaCl tolerance was studied on ISP 2 medium containing NaCl at a final concentration of 1–12 % (w/v) (at intervals of 1 %).

Standard procedures were used to determine the isomers of diaminopimelic acid (Hasegawa *et al.*, 1983). The acyl type of the cell wall was analysed according to the method of Uchida & Aida (1984). Whole-cell sugars were determined as described by Becker *et al.* (1965). Menaquinones were extracted and purified by the method of Collins *et al.* (1977), and isoprene units were subsequently analysed by LC/MS (JMS-T100LP; JEOL) with a PEGASIL ODS column (2.00×50 mm) using methanol/2-propanol (7 : 3). Mycolic acids were detected by TLC according to the method of Tomiyasu (1982). Polar lipids were examined by TLC using the method of Minnikin *et al.* (1977). The G + C content (mol %) of the DNA was determined by HPLC according to the method of Tamaoka & Komagata (1984). Analysis of the fatty acids was performed according to the procedures of the Sherlock Microbial Identification System (Microbial ID).

Strain RM287^T was a non-motile, aerobic, Gram-positive filamentous actinomycete. Mycelium developed well on ISP 2, 5, 6, 7 media and GYE agar with white aerial mycelium and yellowish brown substrate mycelium after 10 days at 28°C . Moderate growth occurred on ISP 3 and ISP 4 media. Light yellowish soluble pigments were produced on ISP 2, 5, 6, 7 media and GYE agar, but not on ISP 3 or ISP 4 media. Chains of fragmented rod-shaped elements of aerial mycelium were observed (Fig. 2).

The results from chemical analysis indicated that strain RM287^T had chemotaxonomic markers typical of the genus *Amycolatopsis*. The strain contained *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan and MK-9(H_4) as the major menaquinone. Arabinose and galactose were detected as the major

Fig. 1. Neighbour-joining (Saitou & Nei, 1987) phylogenetic tree based on nearly complete 16S rRNA gene sequences showing the position of strain RM287^T in the genus *Amycolatopsis*. Asterisks indicate branches that were also found using the least-squares (Fitch & Margoliash, 1967) and maximum-parsimony (Fitch, 1971) tree-making algorithms. Numbers at the nodes indicate the percentage bootstrap support from an analysis of 1000 resampled datasets (only values $\geq 50\%$ are shown). Bar, 0.1 substitutions per site.

components of sugars in the whole-cell hydrolysates. Polar lipid analysis showed that the organism contained diposphatidylglycerol, hydroxy-phosphatidylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannoside. The major fatty acids were iso-C_{16:0} (29.3%), iso-C_{15:0} (13.9%), iso 2-OH-C_{16:0} (10.4%) and iso-C_{17:0} (10.0%). Fatty acids found in smaller proportions included anteiso-C_{17:0} (6.9%), C_{16:0} (6.2%), 2-OH-C_{16:1} (3.7%), C_{18:0} (2.5%), anteiso 2-OH-C_{17:0} (2.1%), anteiso-C_{15:0} (1.5%) and anteiso 2-OH-C_{15:0} (1.4%). No mycolic acids were detected. The G + C content of the DNA was 71.7 mol%.

The assignment of strain RM287^T to the genus *Amycolatopsis* was supported by biochemical, morphological and physiological evidence. The novel strain was positive for allantoinase, catalase and urease. The temperature range for growth was 16–40 °C and good growth was observed at 25–34 °C.

Fig. 2. Scanning electron micrograph of fragmented aerial mycelia of strain RM287^T grown on ISP 3 medium for 10 days at 28 °C. Bar, 1 µm.

Table 1. Differential characteristics of strain RM287^T and closely related strains of the genus *Amycolatopsis*

Strains: 1, RM287^T; 2, *A. mediterranei* JCM 4789^T; 3, *A. rifamycinica* JCM 12674^T; 4, *A. kentuckyensis* JCM 12670^T; 5, *A. pretoriensis* JCM 12673^T; 6, *A. australiensis* JCM 15587^T. +, Positive; –, negative.

Characteristic	1	2	3	4	5	6
Acid production from:						
Adonitol	+	–	–	–	–	–
(+)-L-Arabinose	–	+	+	+	+	+
Maltose	–	+	–	+	+	+
Raffinose	–	+	–	+	+	+
(–)-D-Rhamnose	–	+	+	+	+	+
Sucrose	–	+	+	+	+	+
(+)-D-Xylose	–	+	+	+	+	+
Allantoinase production	+	–	–	+	–	+
Decomposition of xanthine	+	–	–	–	+	–
Growth at 10 % NaCl (w/v)	+	–	–	–	–	–

No growth was observed at or above 42 °C. The strain was able to grow at pH 5.0–11.0. The organism tolerated up to 10 % (w/v) NaCl. Melanin pigment was not produced. Strain RM287^T could be readily differentiated from its closest relatives *A. mediterranei* JCM 4789^T, *A. rifamycinica* JCM 12674^T, *A. kentuckyensis* JCM 12670^T, *A. pretoriensis* JCM 12673^T and *A. australiensis* JCM 15587^T on the basis of physiological properties (Table 1), in particular on the production of allantoinase and decomposition of xanthine which were both positive in the novel strain. These characteristics were negative for *A. mediterranei* and *A. rifamycinica*. No acid was produced from (+)-L-arabinose, (–)-D-rhamnose, sucrose or (+)-D-xylose, properties which were positive for *A. mediterranei*, *A. rifamycinica*, *A. kentuckyensis*, *A. pretoriensis* and *A. australiensis*. Moreover, growth at 10 % (w/v) NaCl was observed only for strain RM287^T.

It is apparent that on the basis of genotypic and phenotypic data, strain RM287^T could be distinguished from *A. mediterranei*, *A. rifamycinica*, *A. kentuckyensis*, *A. pretoriensis* and *A. australiensis*. It is concluded that strain RM287^T represents a novel species of the genus *Amycolatopsis*, for which the name *Amycolatopsis samaneae* sp. nov. is proposed.

Description of *Amycolatopsis samaneae* sp. nov.

Amycolatopsis samaneae [sa.ma.ne'ae. N.L. n. *Samanea* the name of a botanical genus; N.L. gen. n. *samaneae* of *Samanea*, isolated from root of *Samanea saman* (Jacq.) Merr.].

Aerobic, Gram-positive, non-acid–alcohol-fast, non-motile and catalase-positive actinomycete. Forms extensively branched vegetative mycelia. Aerial hyphae fragment into squarish rod-shaped elements. The aerial mycelium is white, with yellowish to yellowish brown substrate mycelium and soluble light yellow pigment production

on ISP 2, 5, 6 and 7 media and GYE agar. H₂S production and the nitrate reduction test are negative. Allantoinase production and urease are positive. Acid is produced from adonitol, (+)-D-cellobiose, fucose, (–)-D-fructose, (+)-D-galactose, (+)-D-glucose, glycerol, *myo*-inositol, lactose, (–)-D-mannitol, (+)-D-mannose, (–)-D-ribose and (+)-D-trehalose. No acid production from (+)-L-arabinose, maltose, melibiose, (–)-D-rhamnose, raffinose, (–)-D-sorbitol, sorbose, sucrose, xylitol or (+)-D-xylose. Aesculin, arbutin, casein, gelatin, hypoxanthine, L-tyrosine and xanthine are degraded. Adenine, cellulose, chitin, guanine and starch are not degraded. The pH range for growth is pH 5.0–11.0. Cells can grow in the presence of 0–10 % (w/v) NaCl. Acid phosphatase, alkaline phosphatase, chymotrypsin, cystine aminopeptidase, β-glucosidase, leucine aminopeptidase, lipase C8, lipase C14, α-mannosidase, N-acetyl-β-glucosaminidase and valine aminopeptidase are detected with the API ZYM enzyme assay; tests for esterase C4, α-galactosidase, β-galactosidase, α-fucosidase, α-glucosidase, β-glucuronidase, phosphoamidase and trypsin are negative. The diagnostic diamino acid of the cell-wall peptidoglycan is *meso*-diaminopimelic acid. Whole cell sugars are arabinose and galactose. The glycan moiety of the murein is acetylated. The major menaquinone is MK-9(H₄). Mycolic acids are not detected. The polar lipids are diphosphatidylglycerol, hydroxy-phosphatidylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannoside. The major fatty acids in the cellular fatty acid profile are iso-C_{16:0}, iso-C_{15:0}, iso 2-OH-C_{16:0} and iso-C_{17:0}.

The type strain, strain RM287^T (=TISTR 1919^T=BCC 35842^T=NBRC 106095^T), was isolated from roots of *Samanea saman* (Jacq.) Merr., collected from Kasetsart University, Bangkok, Thailand. The G+C content of the type strain DNA is 71.7 mol%.

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