NOTE

***Amycolatopsis rubida* sp. nov., a new *Amycolatopsis* species from soil**

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The taxonomic position of a soil isolate, strain 13.4^T, was established using a polyphasic approach. The organism was found to have chemical and morphological properties consistent with its classification in the genus *Amycolatopsis*. Phylogenetic analysis of the strain based on its 16S rDNA sequence showed that it forms a distinct phyletic line within members of the genus *Amycolatopsis*. The organism was also readily distinguished from the type strains of all validly described *Amycolatopsis* species by its phenotypic features. The name *Amycolatopsis rubida* sp. nov. is proposed for this new species. The type strain is strain 13.4^T (= AS 4.1541^T = JCM 10871^T).

Keywords: *Amycolatopsis rubida* sp. nov., polyphasic taxonomy, 16S rDNA sequencing

The genus *Amycolatopsis* was proposed by Lechevalier *et al.* (1986) to accommodate nocardioform actinomycetes having type IV cell wall composition, type PII phospholipid pattern, and lacking mycolic acid. This taxon has been classified in the family *Pseudonocardaceae* since the application of the polyphasic taxonomic approach to actinomycete systematics (Embley *et al.*, 1988; Warwick *et al.*, 1994; Stackebrandt *et al.*, 1997; Kim & Goodfellow, 1999). Meanwhile, another two genera, *Actinosynnema* and *Saccharothrix*, have been excluded from the family *Pseudonocardaceae* (Warwick *et al.*, 1994), and been classified in a new family, *Actinosynnemataceae*, by Labeda & Kroppenstedt (2000).

The genus *Amycolatopsis* was established with four species: *Amycolatopsis orientalis*, *Amycolatopsis mediterranei*, *Amycolatopsis rugosa* and *Amycolatopsis sulphurea*. Since then, another seven species and one subspecies have been validly published (Henssen *et al.*, 1987; De Boer *et al.*, 1990; Mertz & Yao, 1993; Labeda, 1995; Goodfellow *et al.*, 1997, 2001; Chun *et al.*, 1999), and *Amycolatopsis rugosa* has been reclassified as *Prauserella rugosa* (Kim & Goodfellow, 1999). Representatives of the genus form a distinct phyletic line within the evolutionary radiation encom-

passed by the family *Pseudonocardaceae* (Embley *et al.*, 1988; Warwick *et al.*, 1994). In the present investigation, a soil isolate, strain 13.4^T, was subject to a polyphasic study designed to establish its taxonomic position. Genotypic and phenotypic data show that strain 13.4^T represents a new species of *Amycolatopsis*, for which we propose the name *Amycolatopsis rubida*.

A soil sample was collected from a conifer forest in Guangxi Province, China. Strain 13.4^T was isolated on a glucose-asparagine agar (GAA; glucose, 10 g; L-asparagine, 0.5 g; K₂HPO₄, 0.5 g; distilled water, 1 l; pH 7.2) plate, which had been seeded with a soil suspension and incubated at 28 °C for 14 d. The isolate was maintained on modified Sauton's agar (Mordarska *et al.*, 1972) slants at 4 °C and as a glycerol suspension (20 %, v/v) at –20 °C. Biomass for studies was prepared according to Chun *et al.* (1999). The cultural and morphological properties were examined by using standard procedures and procedures of Williams *et al.* (1983) after growth on GAA and glucose-yeast extract agar (GYEA; Gordon & Mihm, 1962) for 7–10 d at 28 °C. Phenotypic tests were carried out following the procedures of Goodfellow *et al.* (1997) and Gordon *et al.* (1974). The ability of the test strain to grow in the presence of five antibiotics was examined by placing 6 mm sensitivity discs (Difco) centrally in plates of Sauton's agar seeded with a loopful of 7 d Sauton's broth culture and inhibition zones observed after 3–5 d incubation at 28 °C were scored negative. Chemotaxonomy characters were

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The GenBank accession number for the 16S rDNA sequence of strain 13.4^T (= AS 4.1541^T = JCM 10871^T) is AF222022.

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost complete 16S rDNA sequences showing relationships between *Amycolatopsis rubida* sp. nov. and representatives of the family Actinosynnemataceae, Pseudonocardiaceae and related taxa. Asterisks indicate branches that were also recovered using the least-squares and maximum-likelihood methods. The numbers at the nodes indicate the levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled data sets; only values over 50% are given. Scale bar, 0.01 substitutions per nucleotide position.

determined as described previously (Minnikin *et al.*, 1980, 1984; Lechevalier & Lechevalier, 1980; Hasegawa *et al.*, 1983; Collins, 1985; Wu *et al.*, 1989).

Genomic DNA was extracted employing the method of Chun & Goodfellow (1995). The G + C content of the DNA was determined using the thermal denaturation (T_m) method (Marmur & Doty, 1962) with *Escherichia coli* AS1.365 as the control. PCR of the 16S rDNA was performed as described by Chun & Goodfellow (1995). The amplified product was directly sequenced using a *Taq* DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) and universal primers named 27f (5'-GAGAGTTTGATCCTGGC-TCAG-3'), 704f (5'-GTACGGTGAARTGCGCA-GA-3'), 765r (5'-CTGTTCGCTCCCCACGCTTTC-3') and 1495r (5'-CTACGGCTACCTTGTTACGA-3'). The nucleotide sequence was automatically obtained by using an Applied Biosystems 373A DNA sequencer according to the manufacturer's protocols. The resultant 16S rDNA sequence was aligned manually using the CLUSTAL X program (version 1.64b; Thompson *et al.*, 1997) against corresponding sequences retrieved from the GenBank database. Evolutionary trees were inferred by using three tree-making algorithms, namely, neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981). Evolutionary distance matrices were generated as described by Jukes & Cantor (1969). The resultant unrooted tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings. The PHYLIP package

(Felsenstein, 1993) was used for all phylogenetic analyses.

An almost complete 16S rDNA sequence was determined for strain 13.4^T (1402 nucleotides). Comparison of this sequence with those of representative reference strains of the families Actinosynnemataceae (Labeda & Kroppenstedt, 2000) and Pseudonocardiaceae shows that the organism belongs to the genus *Amycolatopsis* (Fig. 1). The nucleotide sequence also serves to distinguish it from other *Amycolatopsis* in that strain 13.4^T forms a monophyletic clade in the phylogenetic tree, supported by the 80% bootstrap value recorded using the neighbour-joining method. The 16S rDNA sequence similarity between strain 13.4^T and its nearest neighbour, *Amycolatopsis orientalis*, is 97.18%; this value corresponds to 39 differences out of 1383 nucleotide positions compared. Similarity values with the type strains of the remaining validly published *Amycolatopsis* species range from 93.76% to 96.91% (Table 1).

The chemotaxonomic data of the test strain are consistent with its assignment to the genus *Amycolatopsis* (Lechevalier *et al.*, 1986). Strain 13.4^T contains meso-diaminopimelic acid as the wall diamino acid, arabinose and galactose as major wall sugars, and hexahydrogenated menaquinones with nine isoprene units as the predominant isoprenoid quinone. It also contains phosphatidylethanolamine (taxonomically significant phospholipids), diphosphatidylglycerol, phosphatidylinositol mannosides and phosphatidylmethylethanolamine as diagnostic polar lipids but it lacks mycolic acids; the G + C content of the DNA is

Table 1. 16S rDNA similarity values between *Amycolatopsis rubida* sp. nov. and representatives of the genus *Amycolatopsis*

<i>Amycolatopsis</i> species	Similarity to <i>A. rubida</i> (%)	No. nucleotide differences/total no. nucleotides compared
<i>A. alba</i> DSM 44262 ^T	96.91	43/1383
<i>A. azurea</i> JCM 3275 ^T	96.50	44/1257
<i>A. coloradensis</i> DSM 44225 ^T	96.86	43/1369
<i>A. fastidiosa</i> IFO 14105 ^T	93.76	80/1282
<i>A. japonica</i> DSM 44213 ^T	96.41	50/1393
<i>A. mediterranei</i> JCM 4789 ^T	96.43	48/1345
<i>A. methanolica</i> IFO 15065 ^T	94.63	71/1322
<i>A. orientalis</i> subsp. <i>orientalis</i> JCM 4235 ^T	97.18	39/1383
<i>A. sulphurea</i> DSM 46092 ^T	96.71	46/1398
<i>A. thermoflava</i> IFO 14333 ^T	94.71	74/1399

Fig. 2. Scanning electron micrograph showing squarish fragments of mycelium of *Amycolatopsis rubida* sp. nov. grown on GAA for 8 d at 28 °C. Bar, 3.8 µm.

67.4 mol %. This chemical profile serves to distinguish strain 13.4^T from members of all other wall chemotype IV taxa, except those classified in the genus *Amycolatopsis* (Lechevalier *et al.*, 1986; Henssen *et al.*, 1987; Goodfellow & Lechevalier, 1989; Embley, 1992; Holt *et al.*, 1994). The classification of strain 13.4^T in the genus *Amycolatopsis* is also supported by phenotypic and morphological properties. The organism is aerobic, non-motile, Gram-positive, non-acid-

Table 2. Phenotypic characters of *Amycolatopsis rubida* sp. nov.

+, Positive or present; w, weak positive; —, negative or absent. Source of data on *Amycolatopsis* species: Chun *et al.* (1999).

Character	Result
Acid production from:	
Adonitol	+
L(+)Arabinose	+
D(+)Cellobiose	+
Dextrin	—
meso-Erythritol	+
D(+)Fructose	+
D(+)Galactose	+
meso-Inositol	+
D(+)Lactose	—
D(+)Maltose	—
D(—)Mannitol	+
D(+)Melezitose	—
D(+)Melibiose	—
Methyl α-D-glucoside	—
D(+)Raffinose	—
L(+)Rhamnose	+
Salicin	+
D(—)Sorbitol	—
Sucrose	+
D(+)Trehalose	+
D(+)Xylose	+
Presence of aerial mycelium	+
Colour of aerial mycelium:	
Blue	—
Purple	—
White	+
White to olive buff	—
Yellowish green	—
Production of soluble pigment	+
Decomposition of:	
Allantoin	—
Casein	+
Aesculin	+
Gelatin	w
Hypoxanthine	+
Xanthine	+
Growth at:	
10 °C	+
45 °C	—
Growth in presence of 5% (w/v) NaCl	+

alcohol-fast and produces branching substrate mycelium which fragments into squarish elements; aerial mycelium is formed and also fragments into squarish elements (Fig. 2). The organism can be distinguished from representatives of all validly published *Amycolatopsis* species using a combination of phenotypic properties (Table 2).

The chemical, genotypic and phenotypic data show that strain 13.4^T merits species status in the genus *Amycolatopsis*. Therefore, it is proposed that this organism be classified in the genus *Amycolatopsis* as *Amycolatopsis rubida* sp. nov.

Description of *Amycolatopsis rubida* sp. nov.

Amycolatopsis rubida (ru.bi'da. L. adj. *rubida* reddish).

The organism forms a branching white to yellowish substrate mycelium which fragments into squarish elements (0.4–0.5 × 1.2–3.0 µm); abundant white aerial mycelium is produced and it also fragments into squarish elements. A reddish diffusible pigment is produced on glucose-asparagine agar. It is positive for urease, lipase, catalase, phosphatase and nitrate reductase but negative for amylase, oxidase, cellulase, chitinase and xylanase. It grows on 7% NaCl and at 10–40 °C. Tolerant to lysozyme (0.005, w/v), penicillin G (10 international units), novobiocin (5 µg) and streptomycin sulphate (30 µg) but not to erythromycin (15 µg) or chloramphenicol (30 µg). Other phenotypic properties are given in Table 2. Cell wall contains major amounts of *meso*-diaminopimelic acid, arabinose and galactose. The organism contains phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol mannosides and phosphatidyl-methylethanolamine as major polar lipids and hexahydrogenated menaquinones with nine isoprene units as principal isoprenologue, but does not contain mycolic acids. The G+C content of the DNA is 67.4 mol% (*T_m* method). Isolated from a conifer forest soil sample collected in Guangxi Province, China. The type strain is strain 13.4^T (= AS 4.1541^T = JCM 10871^T).

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