Micromonospora rifamycinica sp. nov., a novel actinomycete from mangrove sediment

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An actinomycete strain, AM105T, that produces rifamycin, was isolated from mangrove sediment samples collected from the South China Sea. The strain showed closest 16S rRNA gene sequence similarity to Micromonospora matsumotoense (98.0 %). Chemotaxonomic characteristics of the isolate coincided with members of the genus Micromonospora. The value of DNA–DNA relatedness to M. matsumotoense (53.6 %) and phenotypic differences from phylogenetically related Micromonospora species indicated that this isolate belongs to a novel species, for which the name Micromonospora rifamycinica sp. nov. is proposed. The type strain is AM105T (CGMCC 4.2495T = DSM 44983T).

Mangroves are a unique woody plant community of intertidal coasts in the tropical and subtropical zones, which are regarded as highly productive ecosystems and abode to large unexplored microbial diversity (Bashan & Holguin, 1997). Strain AM105T was isolated from mangrove sediment samples from the South China Sea. Isolation was carried out by the standard dilution-plating technique on modified Gause inorganic agar (Gause et al., 1983), which contained 20 g soluble starch, 1 g KNO₃, 0.5 g K₂HPO₄, 0.5 g MgSO₄ . 7H₂O, 0.01 g FeSO₄ . 7H₂O, 15 g agar, 1 l old sea water (pH 7.2–7.4). Plates were incubated for 30 days at 28 °C. A pure culture was maintained in a glycerol suspension (20 %, w/v) at −70 °C.

Cultural features were observed on oatmeal agar (DSMZ medium 609), glycerol–asparagine agar (Shirling & Gottlieb, 1966), GYM agar (DSMZ medium 65), potato dextrose agar (DSMZ medium 129), Sauton’s agar (Mordarska et al., 1972) and Gause inorganic agar after 7, 14 and 21 days incubation at 28 °C. Cell morphology and spore production were observed by light and scanning electron microscopy using 6- and 20-day-old cultures grown on various agar media. The ability to grow on sole carbon sources (1 %, w/v) was tested as described by Williams et al. (1983). NaCl tolerance (0–4.5 %, w/v) and temperatures (4–45 °C) for growth were tested on GYM agar. Methods and media for other physiological tests and the assays for enzymic activities were performed according to Wang (1986).

For chemotaxonomic studies, the strain was grown in GYM broth in a shaking incubator at 200 r.p.m. and 28 °C for 5 days. Biomass was harvested by centrifugation and washed with distilled water. Isomers of diaminopimelic acid (DAP) and sugars were determined in whole-cell hydrolysates by TLC on microcrystalline cellulose (Wang, 1986).

Genomic DNA was isolated as described by Pitcher et al. (1989). The DNA G + C content was determined using the thermal melting method (Mandel & Marmur, 1968). PCR amplification of the 16S rRNA gene and sequencing of the purified PCR product were done as described previously (Rivas et al., 2003). The sequence of isolate AM105T was aligned and compared with representative sequences of members of the genus Micromonospora obtained from GenBank using the CLUSTAL_X 1.8 program (Thompson et al., 1997). Phylogenetic analysis was performed with the MEGA version 2.1 program (Kumar et al., 2001). Phylogenetic trees were constructed using three tree-making algorithms, namely, neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Kidd & Sgaramella-Zonta, 1971; Rzhetsky & Nei, 1993) and maximum-parsimony (Fitch, 1972); bootstrap analysis was also conducted. DNA–DNA relatedness experiments were performed by the method of Ezaki et al. (1989). DNA–DNA relatedness values were calculated according to Christensen et al. (2000).

Strain AM105T showed good growth and no diffusible pigments on all of the media used. Colonies were orange, but turned dark brown on sporulation after 14–21 days on oatmeal agar and Gause inorganic agar. Spores were smooth rod-shaped, ovoid or spherical. Spores were single or in short chains with two to five spores, which borne on...
short or long sporophores. Some spores were sessile (Fig. 1). Aerial mycelium, at times visible only on Sauton's agar and Gause inorganic agar, was scanty and milk-white. Other cultural and morphological characters are given in the species description.

An almost-complete 16S rRNA gene sequence (1475 bp) was obtained. The 16S rRNA-based phylogenetic analysis showed a close relationship of the isolate to members of the genus *Micromonospora*. The highest 16S rRNA gene sequence similarities were found with *Micromonospora matsumotoense* DSM 44100T (98.0 %, 30 nt differences), *Micromonospora carbonacea* DSM 43168T (97.8 %, 32 nt differences), *Micromonospora mirobrigensis* DSM 44830T (97.2 %, 34 nt differences) and *Micromonospora siamensis* JCM 12729T (96.9 %, 41 nt differences). Various tree-making algorithms yielded very similar tree topologies (only some positions of the branches varied slightly) and the isolate always grouped with *M. matsumotoense* (data not presented). The reduced tree showing the position of strain AM105T among the closest phylogenetic relatives is given as Fig. 2.

Strain AM105T contained meso-DAP in the cell wall. The diagnostic cell-wall sugars (Lechevalier & Lechevalier, 1970) were arabinose and xylose. The G+C content of the DNA was 71 %. The DNA–DNA relatedness between strain AM105T and *M. matsumotoense* DSM 44100T was 53.6 % (the means of triplicate analyses), which are below the threshold value of 70 % for the definition of a bacterial species according to Wayne et al. (1987).

Strain AM105T grew between 20 and 37 °C, but no growth was found at 4, 15 or 45 °C after 3 weeks. Tolerates NaCl up to 3 % and no growth occurred at 4.5 %. Among the carbohydrates tested, only D-glucose was utilized well. Other physiological and biochemical characters are listed in the species description; characters differentiating the strain from related species are given in Table 1.

For analyses of antibiotic production, the strain was cultured in a medium containing 5 g glucose, 5 g yeast extract, 15 g starch, 10 g soy meal, 1 l sea water, pH 7.0, at 200 r.p.m. and 28 °C. After 6 days of cultivation, fermentation broth was centrifuged and the supernatant was assayed for an antimicrobial activity using the paper disc diffusion methods (Isnansetyo & Kamei, 2003). A clear antimicrobial activity was revealed against *Staphylococcus aureus* CGMCC 1.89, *Staphylococcus aureus* OY84 (a meticillin-resistant clinical isolate), *Bacillus subtilis* CGMCC 1.88, but not against *Escherichia coli* CGMCC 1.747, *Candida albicans* CGMCC 2.538 or *Piricularia oryzae* CGMCC 3.3283. The active ethyl acetate extract from the supernatant was separated by silica gel G250 column, and the bioactive fraction was eluted with petroleum ether/ethyl acetate (3 : 1), concentrated, and further separated by TLC (chloroform/methanol, 98 : 2). The bioactive compound was then purified through Sephadex LH-20 (chloroform/methanol, 1 : 1) and analysed by HPLC and various spectroscopic methods (Traxler et al., 1981; Hermann & Zuordnung, 1973; Gallo et al., 1974; Cellai et al., 1982; Arora & Arjunan, 1992). In HPLC analysis, the compound showed one peak but two sets of proton and carbon signals were observed in the 1H- and 13C-NMR spectra. Further identification using 1H-NMR, 13C-NMR, heteronuclear multiple bond correlation, heteronuclear single quantum coherence, rotary overhauser effect spectroscopy, electrospray ionization mass spectrometry and IR spectroscopy showed that the active compound (purple amorphous powder) consisted of rifamycin S and its isomer (Huang et al., 2006), while *M. carbonacea* was reported to produce everninomicin (Luedemann & Brodsky, 1965). No data are available for antibiotic production by other related species, *M. matsumotoense*, *M. mirobrigensis* and *M. siamensis*.

**Fig. 1.** Scanning electron micrograph of cells of strain AM105T after growth on oatmeal agar for 21 days at 28 °C. Bar, 5 μm.

**Fig. 2.** Phylogenetic neighbour-joining tree based on the 16S rRNA gene sequences of strain AM105T and other *Micromonospora* species. Bootstrap values (1000 replicates) are shown as percentages at each node for values above 50 %. Bar, 0.005 nucleotide substitutions per position.
and Gause inorganic agar. Diffusible pigments are not scanty and milk-white, at times produced on Sauton’s agar sporophores. Some spores are sessile. Aerial mycelium, with two to five spores, and borne on short or long shaped, ovoid or spherical, smooth, single or in short chains on oatmeal agar and Gause inorganic agar. Spores are rod-shaped, ovoid or spherical, smooth, single or in short chains with two to five spores, and borne on short or long sporophores. Some spores are sessile. Aerial mycelium, scanty and milk-white, at times produced on Sauton’s agar and Gause inorganic agar. Diffusible pigments are not produced. Growth occurs in 0–3% NaCl and 20–37 °C. D-Glucose is utilized as a sole carbon source; L-arabinose, D-xylose, D-fructose, L-rhamnose, mannitol, sucrose, D-raffinose and inositol are not utilized. Able to degrade starch and cellulose, but not gelatin or casein. Nitrate reductase and tyrosinase are produced but hydrogen sulfide (H2S) is not. Produces rifamycin S and its isomer. Whole-cell hydrolysates contain meso-DAP and the main sugars xylose and arabinose. The DNA G+C content is 71%. The type strain, AM105T (=CGMCC 4.2495T=DSM 44983T), was isolated from mangrove sediment from the South China Sea.

### Table 1. Physiological and biochemical characteristics that differentiate strain AM105T from related Micromonospora species

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>4</th>
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<tr>
<td>Growth on sole carbon source (1%, w/v) of:</td>
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<tr>
<td>L-Arabinose</td>
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<td>−</td>
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<tr>
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<td>+</td>
<td>ND</td>
<td>W</td>
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<tr>
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<td>−</td>
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<tr>
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<td>+</td>
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<td>Everninomicin</td>
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</table>

Thus, the data obtained in this study clearly show that strain AM105T merits description as a novel species of the genus Micromonospora, for which the name Micromonospora rifamycinica sp. nov. is proposed.

### Acknowledgements

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### References


