Nonomuraea antimicrobica sp. nov., an endophytic actinomycete isolated from a leaf of Maytenus austroyunnanensis

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A novel actinomycete strain, designated YIM 61105T, was isolated from a leaf of Maytenus austroyunnanensis from the tropical rainforest in Xishuangbanna, Yunnan Province, south-west China. A 16S rRNA gene sequence analysis revealed that the organism belonged to the phylogenetic cluster of the genus Nonomuraea and was most closely related to Nonomuraea candida HMC10T (98.2 %), 'Nonomuraea aegyptia' S136 (97.9 %), Nonomuraea kuesteri GW 14-1925T (97.5 %) and Nonomuraea turkmeniaca DSM 43926T (97.4 %). The 16S rRNA gene sequence similarities to other Nonomuraea species were less than 97.4 %. The main chemotaxonomic properties of strain YIM 61105T, such as the principal amino acid of the peptidoglycan, the predominant menaquinone and the polar lipid profile, supported its classification within the genus Nonomuraea. Strain YIM 61105T was also readily differentiated from closely related species on the basis of a broad range of phenotypic properties and DNA–DNA hybridization values. Thus, this isolate is considered to represent a novel species of the genus Nonomuraea, for which the name Nonomuraea antimicrobica sp. nov. is proposed. The type strain is YIM 61105T (=DSM 45220T =CCTCC AA 208016T).

The genus Nonomuraea was proposed by Zhang et al. (1998) as a member of the family Streptosporangiaceae. Members of the genus Nonomuraea are aerobic, Gram-positive, non-acid-fast, non-motile actinomycetes that form an extensively branched substrate and aerial mycelium. The aerial hyphae differentiate into hooked, spiral or straight chains of spores, which show a folded, irregular, smooth or warty ornamentation. The genus is characterized chemotaxonomically by the presence of meso-diaminopimelic acid in the cell wall, madurose as a characteristic sugar in the whole-cell hydrolysates, considerable amounts of C17:0 10-methyl and iso-C16:0 branched-chain fatty acids, major proportions of di-, tetra- and hexahydrogenated menaquinones with nine isoprene units and major amounts of diphosphatidylglycerol, hydroxylated phosphatidylethanolamine, uncharacterized glycolipids and a glucosamine-containing phospholipid (Nonomura & Ohara, 1971; Zhang et al., 1998; Quintana et al., 2003).

At the time of writing, the genus Nonomuraea comprised 21 species with validly published names and 2 subspecies on the basis of a polyphasic approach. There is no record of the isolation of Nonomuraea species from the endophytic environment. Xishuangbanna is famous in China for its diverse flora, especially the rainforest plants, many of which have an indigenous pharmaceutical history. The rapidly diminishing tropical rainforests may hold the greatest possible resource for acquiring novel microorganisms and their products.

In the course of our study on endophytic actinomycetal diversity of the tropical-rainforest medicinal plants of Xishuangbanna, strain YIM 61105T was isolated from a healthy leaf of Maytenus austroyunnanensis. After being thoroughly washed in tap water, healthy leaf samples were air-dried at room temperature and the surfaces were sterilized according to the five-step sterilization procedure described by Qin et al. (2008). The surface-sterilized samples were pulverized in a ceramic mortar, distributed onto HV agar (Hayakawa & Nonomura, 1987) and incubated at 28 °C for 3 weeks. The isolate was maintained as mycelial fragments in a 20 % (v/v) glycerol suspension at −80 °C.

Extraction of genomic DNA and amplification of the 16S rRNA gene sequence from strain YIM 61105T were
performed as described by Li et al. (2007). An almost-full-length 16S rRNA gene sequence (1454 bp) was aligned with selected sequences obtained from the GenBank/EMBL/DDBJ databases using CLUSTAL X version 1.8 (Thompson et al., 1997). Neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) trees were constructed using MEGA version 3.1 (Kumar et al., 2004). The reliability of the tree topology was evaluated by bootstrap analysis (Felsenstein, 1985). The phylogenetic tree (Fig. 1) based on the 16S rRNA gene sequences revealed that the closest relatives of strain YIM 61105T were *Nonomuraea candida* HMC10T (98.2 %), *‘Nonomuraea aegyptia’ S136 (97.9 %), Nonomuraea kuesteri GW 14-1925T (97.5 %) and *Nonomuraea turkmenica* DSM 43926T (97.4 %). The 16S rRNA gene sequence similarity values between the isolate and other members of the genus *Nonomuraea* were lower than 97.4 %. Strain YIM 61105T formed a coherent and monophyletic clade with *‘N. aegyptia’ S136* that was supported by a high bootstrap value of 84 % in the neighbour-joining tree and also recovered with the maximum-parsimony algorithm.

Cultural and morphological characteristics were observed with strain YIM 61105T grown on yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4) and glycerol-asparagine agar (ISP 5; Shirling & Gottlieb, 1966), as well as potato-glucose agar (PDA; Difco), Czapek’s agar and nutrient agar (Waksman, 1967), for 3 weeks at 28 °C. The colour of both substrate and aerial mycelia and any soluble pigments produced were determined by comparison with chips from the colour charts of the Inter-Society Color Council (Kelly, 1964). The morphological features of the spore chains and mycelia were observed by using light microscopy (BH-2; Olympus) and scanning electron microscopy (JSM-5600LV; JEOL).

Biomass for quantitative fatty acid analysis was prepared by scraping colonies from tryptic soy agar (TSA; Difco) after incubation at 28 °C for 10 days. Biomass for other chemotaxonomic studies was obtained after cultivation at 28 °C for 7–10 days in shaken cultures with ISP 2. The antimicrobial activity of strain YIM 61105T was detected using the agar-well diffusion method (Hugo & Russell, 1983). *Staphylococcus aureus*, *Escherichia coli*, *Mycobacterium smegmatis* and *Candida albicans* were used as indicator organisms. Standard analytical procedures were used to extract and analyse the isomeric forms of diaminopimelic acid, whole-organism sugars (Hasegawa et al., 1983), isoprenoid quinones (Groth et al., 1997), polar lipids (Minnikin et al., 1979; Collins & Jones, 1980) and fatty acids (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). Strain YIM 61105T contained meso-diaminopimelic acid.
acid and madurose in whole-organism hydrolysates (cell-wall chemotype IIIB; Lechevalier & Lechevalier, 1970). The predominant menaquinone was MK-9(H₄) (78 %), and MK-9(H₂) (10 %), MK-9(H₆) (6 %) and MK-9 (6 %) were also present. The diagnostic phospholipids were phosphatidylinositol and a glucosamine-containing phospholipid (phospholipid type IV; Lechevalier et al., 1977). The detailed fatty acid profile was iso-C₁₄:₀ (3.9 %), C₁₄:₀ (4.2 %), iso-C₁₅:₀ (6.5 %), iso-C₁₆:₁ G (12.7 %), iso-C₁₆:₀ (32.2 %), C₁₆:₀ (11.3 %), C₁₇:₀ 10-methyl (17.1 %), anteiso-C₁₇:₀ (2.7 %), C₁₇:₀ (2.4 %), C₁₈:₀ω9c (3.0 %) and C₁₈:₀ 10-methyl (5.8 %). Strain YIM 661105ᵀ showed antimicrobial activity against Candida albicans.

The genomic DNA G+C content of strain YIM 61105ᵀ was 69.2 mol%, determined by using HPLC (Mesbah et al., 1989). DNA–DNA relatedness values were measured fluorometrically using the microplate hybridization method (Ezaki et al., 1989; He et al., 2005). Strain YIM 61105ᵀ showed relatively low DNA–DNA relatedness with N. candida DSM 45086ᵀ (37 %) and ‘N. aegyptia’ DSM 45082 (34 %). These results are below the 70 % cut-off point recommended for the delineation of genomic species (Stackebrandt & Goebel, 1994). It has been shown that Nonomuraea species exhibit high 16S rRNA gene sequence similarities (97.7–99.4 %), but low DNA–DNA relatedness values (<70 %) (Fischer et al., 1983; Poscher et al., 1985; Tamura et al., 2000; Kämpfer et al., 2005). Stackebrandt et al. (2001) reported DNA–DNA relatedness values between the type strains of Nonomuraea africana, N. dietziae and N. recticatena of 45–48 %, although these species shared 16S rRNA gene sequence similarities of 98.9–99.8 %. For this reason, DNA–DNA relatedness experiments between strain YIM 61105ᵀ and type strains with 16S rRNA gene sequence similarities that were less

Table 1. Comparison of the phenotypic properties of strain YIM 61105ᵀ and the nearest phylogenetically related species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>Spore chains</td>
<td>Spirals</td>
<td>Straight to hooked/curled</td>
<td>Hooked/curled</td>
<td>Spirals</td>
<td>Spirals</td>
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<tr>
<td>Growth on ISP 3</td>
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<td>Moderate brown</td>
<td>Yellowish brown</td>
<td>White (spare)</td>
<td>Cream</td>
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<td>30–45</td>
<td>ND</td>
<td>ND</td>
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<td>+</td>
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<td>+</td>
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<td>–</td>
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<td>H₂S production</td>
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<td>–</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hypoxanthine</td>
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<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Starch</td>
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<td>ND</td>
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<td>W</td>
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<tr>
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<td>W</td>
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<tr>
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<td>–</td>
<td>+</td>
<td>+</td>
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</table>

Fig. 2. Scanning electron micrograph of spiral spore chains of strain YIM 61105ᵀ after growth on ISP 2 medium at 28 °C for 3 weeks. Bar, 10 µm.
than 97.4 % were not carried out. Recently, Stackebrandt & Ebers (2006) recommended an increase of about 2 % for the 16S rRNA gene sequence similarity threshold value used to determine the taxonomic status of a strain, from 97 to 98.7–99 %, provided that these data are supported by clear phenotypic differences. Thus, strain YIM 61105T clearly represents a novel species in the genus Nonomuraea.

The morphological and chemotaxonomic characteristics of strain YIM 61105T are consistent with its classification in the genus Nonomuraea. However, strain YIM 61105T is phenotypically different from its closest phylogenetic neighbours, with differences, for example, in growth characteristics on ISP 3 medium, spore chain arrangement and biochemical and carbon-source utilization test results (Table 1). On the basis of the phenotypic and genotypic data, strain YIM 61105T represents a novel species within the genus Nonomuraea. In view of its antimicrobial activity towards Candida albicans, the name Nonomuraea antimicrobica sp. nov. is proposed.

**Description of Nonomuraea antimicrobica sp. nov.**

Nonomuraea antimicrobica (an.ti.mi.cro’bi.ca. Gr. prep. anti against; N.L. n. microbi um microbe; L. adj. suff.-icus -a -um suffix used with various meanings; N.L. fem. adj. antimicrobica antimicrobial).

Gram-positive, aerobic, non-acid-fast and non-acid–alcohol-fast actinomycete that forms extensively branched, brown substrate mycelia and white-to-pink aerial mycelia on ISP 2–5 and PDA media. Pink diffusible pigments are produced on ISP 5 and PDA media. After 14 days of incubation at 28 °C, spiral spore chains composed of smooth spores are observed on the aerial mycelium. Temperature range for growth is 15–37 °C, with optimal growth at 28 °C. pH range for growth is pH 6.0–9.0, with optimal growth at pH 7.0. No growth is observed with 5 % NaCl. Positive for catalase. Negative for oxidase, milk coagulation, milk peptonization, gelatin liquefaction, nitrate reduction. The diagnostic amino acid of the peptidoglycan is MK-9(H4); MK-9(H2), MK-9(H6) and MK-9 are also present. Major fatty acids (>10 %) are iso-C16:0, C17:0 10-methyl, iso-C16:1 G and C16:0. Additional phenotypic properties are shown in Table 1. The DNA G+C content of the type strain is 69.2 mol%.

The type strain, YIM 61105T (=DSM 45220T=CCTCC AA 208016T), was isolated from a surface-sterilized leaf of Maytenus austroyunnanensis from the tropical rainforest of Xishuangbanna, Yunnan Province, south-west China.

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**References**


