**Nonomuraea syzygii** sp. nov., an endophytic actinomycete isolated from the roots of a jambolan plum tree (*Syzygium cumini* L. Skeels)

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A novel endophytic actinomycete, designated strain GKU 164T, was isolated from the roots of a jambolan plum tree (*Syzygium cumini* L. Skeels), collected at Khao Khitchakut National Park, Chantaburi province, Thailand. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the strain formed a distinct clade within the genus *Nonomuraea*, and was most closely related to *Nonomuraea monospora* PT708T (98.77 % 16S rRNA gene sequence similarity) and *Nonomuraea thailandensis* KC-061T (98.73 %). Strain GKU 164T formed a branched substrate and aerial hyphae that generated single spores with rough surfaces. The cell wall contained meso-diaminopimelic acid. The whole-cell sugars were madurose, galactose, mannose, ribose, rhamnose and glucose. The N-acyl type of muramic acid was acetyl. The predominant menaquinone was MK-9(H4) with minor amounts of MK-9(H6), MK-9(H2) and MK-9(H0). The phospholipid profile contained diphosphatidylglycerol, phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, phosphatidylglycerol, phosphatidylglycinol, phosphatidylinositol, phosphatidylmannosides, phosphatidylmonomethylethanolamine, hydroxy-phosphatidylmonomethylethanolamine, an unidentified aminophosphoglycolipid and four unknown phospholipids. The major fatty acids were iso-C16:0 and 10-methyl C17:0. The genomic DNA G+C content was 70.4 mol%. Significant differences in the morphological, chemotaxonomical, and biochemical data together with DNA–DNA relatedness values between strain GKU 164T and type strains of closely related species, clearly demonstrated that strain GKU 164T represents a novel species of the genus *Nonomuraea*, for which the name *Nonomuraea syzygii* sp. nov. is proposed. The type strain is GKU 164T (=BCC 70457T =NBRC 110400T).

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**Abbreviations**: DPG, diphosphatidylglycerol; ISP, International Streptomyces Project; OH-PE, hydroxy-phosphatidylethanolamine; OH-PME, hydroxy-phosphatidylmonomethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylglycinol; PIM, phosphatidylinositolmannoside; PME, phosphatidylmonomethylethanolamine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GKU 164T is KF667499.

Two supplementary figures and two supplementary tables are available with the online Supplementary Material.

The genus *Nonomuria* (sic) was firstly proposed by Zhang et al. (1998) belonging to the family *Streptosporangiaceae*, but the genus name was later corrected to *Nonomuraea* by Chiba et al. (1999). Members of the genus *Nonomuraea* form extensively branched substrate and aerial mycelia. The aerial mycelia differentiate into either hooked, spiral or straight chains of spores, or form a single spore, which shows a folded, irregular, smooth, warty or rough surface ornamentation (Quintana et al., 2003; Kämpfer et al., 2005;...
Zhao et al., 2011; Zhang et al., 2014b). The type species of the genus is *Nonomuraea pusilla* (Nonomura & Ohara, 1971; Zhang et al., 1998). At the time of writing, the genus comprises 35 species with validly published names and two subspecies, including the newly described *Nonomuraea muscovensis* (Ozdemir-Kocak et al., 2014) and *Nonomuraea fuscireosa* (Zhang et al., 2014a). Three further species, *Nonomuraea guangzhouensis* and *Nonomuraea harbinensis* (Wang et al., 2014), and *Nonomuraea shaanxiensis* (Zhang et al., 2014b), have also been recently described but the names are not yet validly published. Members of the genus *Nonomuraea* have been isolated from a wide range of natural habitats such as soils, including terrestrial and mangrove rhizospheric soil, coastal sediments and caves, and plants (Wang et al., 2011, 2013, 2014; Xi et al., 2011; Zhao et al., 2011; Nakaew et al., 2012; Zhang et al., 2014b). Some novel species have been also isolated from soil in Thailand, namely *Nonomuraea monospora* (Nakaew et al., 2012) and *Nonomuraea thaiandensis* (Srîpreechasak et al., 2013), and some species have been found as endophytes, for example *Nonomuraea antimicrobica* (Qin et al., 2009) and *Nonomuraea endophytica* (Li et al., 2011).

In this study, we performed a taxonomic analysis of strain GKU 164T using a polyphasic approach, and propose that the strain represents a novel species of the genus *Nonomuraea*.

As part of a program to discover novel endophytic actinomycetes from medicinal plants, strain GKU 164T, was recovered from the roots of a jambolan plum tree (*Syzygium cumini* L. Skeels) collected at Khao Khitchakut National Park, Chantaburi province, Thailand. The excised roots were surface-sterilized and endophytic actinomycetes were isolated using the modified method of Indananda et al. (2010). The sample roots were cut into small pieces of 2 cm in length and then rinsed with 0.1 % (v/v) Tween 20 solution for 5 min. The root surface was sterilized by soaking in 1 % sodium hypochlorite for 15 min and subsequently immersing in 70 % ethanol for 10 min. Surface-sterilized roots were then washed in sterile water three times. The root materials were then soaked in 10 % (w/v) NaHCO₃ solution for 10 min to delay the growth of fungi. The roots were then crushed in 1/4 Ringer’s solution and the resulting solution was spread onto starch-casein agar (SCA; Küster & Williams, 1964) supplemented with 100 μg ml⁻¹ ampicillin, 2.5 U ml⁻¹ penicillin G, 50 μg ml⁻¹ amphotericin B and 50 μg ml⁻¹ cycloheximide. The remaining root debris was also placed on SCA. Colonies of endophytic actinomycetes appeared on the agar after incubation at 28 °C for 3–4 weeks. Colonies of strain GKU 164T were purified on mannitol-soya (MS) agar (Hobbs et al., 1989). The pure culture was maintained in 20 % (v/v) glycerol suspensions at −80 °C and by lyophilized cells for long-term preservation.

Genomic DNA of strain GKU 164T was extracted by the method of Kieser et al. (2000). The 16S rRNA gene was amplified using primers STR1F (5'-TCACGGAGAGTT-GATCTTG-3') and STR1530R (5'-AAGGAGATCCAGCC-GCA-3') (Kataoka et al., 1997). The PCR program was as follows: initial denaturation at 94 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min 30 s and extension at 72 °C for 1 min, with a final extension step at 72 °C for 4 min. The PCR product was purified using a Gel/PCR DNA Fragment Extraction kit (Geneaid) and then sent to Macrogen (Korea) for DNA sequencing using primers STR1F and STR1530R, 926F (5'-AAACTCAAGGAATTGACGG-3') (Tajima et al., 2001) and ATT026R (5'-TGGACTACCA-GGTATCTAATC-3'). The resulting 16S rRNA gene sequence of strain GKU 164T was compared with corresponding sequences of type strains available in the EzTaxon-e server (Kim et al., 2012). A multiple sequence alignment of strain GKU 164T and other related taxa was performed using the CLUSTAL X program version 2.0 (Larkin et al., 2007). Phylogenetic trees were reconstructed using neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms in MEGA software version 6.0 (Tamura et al., 2013). The topology of the phylogenetic trees was evaluated with bootstrap analysis based on 1000 resamplings (Felsenstein, 1985). A distance matrix was generated using Kimura’s two-parameter model (Kimura, 1980). All positions containing gaps and missing data were eliminated from the dataset. The values of pairwise 16S rRNA gene sequence similarity among strain GKU 164T and other related species were determined using the EzTaxon-e server.

Comparison of the almost-complete 16S rRNA gene sequence (1448 nt) of strain GKU 164T with those of related strains indicated that strain GKU 164T was a member of the genus *Nonomuraea*. The most closely related strains were *N. monospora* PT708T (98.77 % 16S rRNA gene sequence similarity; 17 nt difference at 1380), *N. thailandensis* KC-061T (97.83 %; 18 nt difference at 1422), *Nonomuraea solani* NEAU-Zè1 (98.68 %; 19 nt difference at 1442), *Nonomuraea rhizophila* YIM 67092T (98.34 %; 24 nt difference at 1444), ‘*N. shaanxiensis*’ NEAU-st1 (98.20 %; 26 nt difference at 1442) and *Nonomuraea rosea* GW 12687T (98.04 %; 27 nt difference at 1375). 16S rRNA gene sequence similarity values of strain GKU 164T with strains of other species of the genus *Nonomuraea* were lower than 98 %. The neighbouring-joining tree was in agreement with the maximum-likelihood tree in which strain GKU 164T always formed a closely phylectic line with *N. monospora* PT708T and *N. thailandensis* KC-061T [Figs 1 and S1 (available in the online Supplementary Material)].

The morphological characteristics of strain GKU 164T were observed by light and scanning electron microscopy (model JSM-5410; JEOL) using cultures grown on Czapek’s agar (Hobbs et al., 1989). The pure culture was maintained in 20 % (v/v) glycerol suspensions at −80 °C and by lyophilized cells for long-term preservation.
(ISP 4), glycerol-asparagine agar (ISP 5), peptone-yeast extract-iron agar (ISP 6) and tyrosine agar (ISP 7). Colours were designated by comparison with colour strips from the Colour Harmony Manual (Jacobson et al., 1958). The range of temperature (5–50°C), pH (4–11) and NaCl (1–7 %) for growth tolerance of strain GKU 164^T was examined on NA for 14–21 days. Catalase and oxidase activities were observed with 3 % (v/v) hydrogen peroxide solution and 1 % (v/v) tetramethyl-p-phenylenediamine solution, respectively. Reduction of nitrate was observed using nitrate broth (Difco). Starch hydrolysis was examined on ISP 4 medium. Urease activity was determined based on a colour change in Stuart’s urea agar (Stuart et al., 1945). Gelatin liquefaction was evaluated on glucose-peptone-gelatin medium (2.0 % glucose, 0.5 % peptone, 20 % gelatin; pH 7.0). Coagulation and peptonization of milk were observed in 10 % (w/v) skimmed milk broth (Difco). Hydrolysis of DNA was tested on DNase agar (Difco). Production of H2S and melanin pigment were determined on ISP 6 and ISP 7 media, respectively. Citrate utilization was tested on Simmons’ citrate agar (Difco). Decomposition of casein, xanthine, hypoxanthine and L-tyrosine were
Substrate mycelia of strain GKU 164<sup>T</sup> were developed without fragmentation. Sporangiophores were not found. Strain GKU 164<sup>T</sup> showed good growth on ISP 2, ISP 3, ISP 4, MBA, NA and YS agar, but no growth was observed on ISP 5. Substrate mycelia were abundantly produced on most of the media tested and the colours were variable depending on the growth medium (Table S1). No soluble pigment was detected on any of the media tested. Aerial mycelium was absent on most of the media except for CZA, where poor growth was observed after incubation at 28 °C for 21 days. Non-motile spores were borne singly and the spore surface was rough (Fig. 2). The temperature and pH range for growth of strain GKU 164<sup>T</sup> were 10–36 °C and pH 5–9, with optimum growth at 18–32 °C and pH 7–8. Other physiological and biochemical characteristics of strain GKU 164<sup>T</sup> were compared to strains of closely related species and revealed the differences from <i>N. monospora</i> PT708<sup>T</sup>, <i>N. thailandensis</i> KC-061<sup>T</sup>, <i>N. solani</i> NEAU-Z6<sup>T</sup>, <i>N. rhizophila</i> YIM 67092<sup>T</sup> and ‘<i>N. shaanxiensis</i>’ NEAU-st1 but the spore surface is rough while others are smooth.

Biomass for chemotaxonomic studies was obtained from cultures grown in yeast extract-glucose broth (1 % yeast extract, 1 % glucose; pH 7.0) at 27 °C for 7 days and then cells were freeze-dried. The isomer of diaminopimelic acid in the cell wall was determined by TLC using whole-cell hydrolysates according to the method of Hasegawa <i>et al.</i> (1983). The whole-cell sugars composition was analysed by TLC using the method of Becker <i>et al.</i> (1965). The <i>N</i>-acyl type of muramic acid in the peptidoglycan was examined by the method of Uchida & Aida (1984). The presence of mycolic acids was monitored by TLC following the procedure of Tomiyasu (1982). Isoprenoid quinones were extracted and purified by using the method of Collins <i>et al.</i> (1977) and analysed by GC/MS (JSM-T100LP; JEOI). Analyses of phospholipids and whole-cell fatty acids using the freeze-dried cells were carried out by the service at Faculty of Science, King Mongkut’s Institute of Technology Ladkrabang (KMITL), Thailand. Phospholipids in cells were extracted and identified by two-dimensional TLC as described by Minnikin <i>et al.</i> (1984). Cellular fatty acids were extracted, methylated and analysed by using the Microbial Identification System (MIDI) according to the method of Sasser (1990) and the manufacturer’s instructions. Fatty acid methyl esters were analysed by GLC and using the Microbial Identification software package (Sherlock version 6.1; MIDI database RTSBA6). The G+C content of genomic DNA of strain GKU 164<sup>T</sup>, which was extracted according to the method of Marmur (1961), was determined by HPLC according to the procedure of Tamaoka & Komagata (1984).

Chemotaxonomic properties of strain GKU 164<sup>T</sup> revealed typical characteristics of members of the genus <i>Nonomuraea</i>. The strain contained <i>meso</i>-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan. Madurose, galactose, mannose, ribose, rhamnose and glucose were detected as the diagnostic sugars in the whole-cell hydrolysates. The presence of <i>meso</i>-diaminopimelic acid and madurose indicated that cell wall and whole-cell sugar of strain GKU 164<sup>T</sup> was of type III and type B, respectively (Lechevalier & Lechevalier, 1970). The <i>N</i>-acyl type of muramic acid in the peptidoglycan was acetyl. Mycolic acids were not detected. The predominant menaquinone was MK-9(<i>H</i><sub>4</sub>) (76 %), while minor amounts of MK-9(<i>H</i><sub>6</sub>) (10 %), MK-9(<i>H</i><sub>8</sub>) (9 %) and MK-9(<i>H</i><sub>0</sub>) (5 %) were also detected. The diagnostic phospholipids were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), hydroxyphosphatidylethanolamine (OH-PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylinositolmannosides (PIMs), phosphatidylmonomethylethanolamine (PME), hydroxyphosphatidylmonomethylethanolamine (OH-PME), an unidentified aminophosphoglycolipid and four unknown phospholipids (Fig. S2). The phospholipid pattern of strain GKU 164<sup>T</sup> corresponded to type IV (Lechevalier <i>et al.</i>, 1977). The major cellular fatty acids were iso-<i>C</i><sub>16:0</sub> (31.8 %) and 10-methyl <i>C</i><sub>17:0</sub> (25.0 %) which were generally similar to those of recognized members of the genus <i>Nonomuraea</i> (Qin <i>et al.</i>, 2009; Li <i>et al.</i>, 2011; Zhao <i>et al.</i>, 2011; Nakaew <i>et al.</i>, 2012; Sripreetchak <i>et al.</i>, 2013); the minor fatty acids are shown in Table S2. The genomic DNA G+C content of strain GKU 164<sup>T</sup> was 70.4 mol%.

According to phenotypic and genotypic results, strain GKU 164<sup>T</sup> was evidently different from type strains of...
closely related species. Therefore, DNA–DNA hybridization between GKU 164T and the closest strains, N. monospora PT708T and N. thailandensis KC-061T (Fig. 1) was fluorometrically determined using photobiotin-labelled DNA probes and microplate wells as described by Ezaki et al. (1989). The DNA–DNA relatedness values were examined from two independent determinations and revealed values of 40.11 ± 2.33% and 37.61 ± 0.23% for N. monospora PT708T and N. thailandensis KC-061T, respectively. The results clearly indicated that strain GKU 164T was distinguished from N. monospora PT708T and N. thailandensis KC-061T because the values were below the recommended threshold value of 70% for the definition of bacterial species (Wayne et al., 1987).

On the basis of data from this taxonomic study using a polyphasic approach, strain GKU 164T differs from closely related species of the genus Nonomuraea; therefore, strain GKU 164T represents a novel species of this genus, for which the name Nonomuraea syzygii sp. nov. is proposed.

### Description of Nonomuraea syzygii sp. nov.

Nonomuraea syzygii (sy.zy'gi.i. N.L. gen. n. syzygii of Syzygium cumini L. Skeels, the jambolan plum tree from which the type strain was isolated).

Aerobic, Gram-stain-positive, non-acid-fast actinomycete that forms branched, non-fragmenting substrate hyphae. Non-motile spores are borne singly on the aerial mycelia and the spore surface is rough. Sporangia are not detected. No soluble pigment is observed in any of the media tested. The optimal temperature and pH for good growth is 18–32 °C and pH 7–8. The NaCl tolerance range for growth is up to 3% (w/v). Catalase, oxidase, DNase, gelatin

### Table 1. Comparison of the phenotypic properties of strain GKU 164T and type strains of closely related species of the genus Nonomuraea

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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</thead>
<tbody>
<tr>
<td><strong>Spore morphology</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Arrangement</td>
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<td>Single</td>
<td>Spiral</td>
<td>Single</td>
<td>Spiral</td>
<td>Single</td>
</tr>
<tr>
<td>Ornamentation</td>
<td>Rough</td>
<td>Smooth</td>
<td>Smooth</td>
<td>Smooth</td>
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<td>Number of spores</td>
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<td>1</td>
<td>&gt;10</td>
<td>1</td>
<td>7-10</td>
<td>1</td>
</tr>
<tr>
<td>Growth on ISP 3</td>
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<td>Greyish-brown</td>
<td>Reddish-grey</td>
<td>White</td>
<td>White</td>
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<tr>
<td>Aerial mycelium</td>
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<td>Cuba</td>
<td>Reddish-brown</td>
<td>Violet brown</td>
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<tr>
<td>Substrate mycelium</td>
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<td>Reddish-brown</td>
<td>None</td>
<td>None</td>
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</tr>
<tr>
<td>Soluble pigment</td>
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<td>None</td>
<td>Reddish-brown</td>
<td>None</td>
<td>None</td>
<td>None</td>
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<tr>
<td>Temperature for growth (°C)</td>
<td>10–36</td>
<td>5–9</td>
<td>14–38</td>
<td>20–39</td>
<td>10–37</td>
<td>18–28</td>
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<td>pH range for growth</td>
<td>5–9</td>
<td>5–9</td>
<td>7–9</td>
<td>6–8</td>
<td>6–9</td>
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<td>3</td>
<td>3</td>
<td>2</td>
<td>7</td>
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<td>+</td>
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<td>–</td>
<td>+</td>
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<td>+</td>
<td>–</td>
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<tr>
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<td>w</td>
<td>–</td>
<td>–</td>
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<td>+</td>
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<td>Oxidase activity</td>
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<td>+</td>
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<td>Coagulation of milk</td>
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<td>–</td>
<td>–</td>
<td>ND</td>
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<td>Peptonization of milk</td>
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<td>w</td>
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<td>ND</td>
<td>+</td>
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<td>W</td>
<td>W</td>
<td>ND</td>
<td>+</td>
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<tr>
<td>L-Tyrosine</td>
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<td>+</td>
<td>+</td>
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<td>L-Arabinose</td>
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<td>+</td>
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<td>Cellobiose</td>
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<td>+</td>
<td>ND</td>
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<tr>
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<td>+</td>
<td>W</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>D-Fructose</td>
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<td>+</td>
<td>W</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>myo-Inositol</td>
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<td>–</td>
<td>W</td>
<td>+</td>
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<td>+</td>
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<td>W</td>
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<td>Sucrose</td>
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<tr>
<td>D-Xylose</td>
<td>–</td>
<td>+</td>
<td>–</td>
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</table>

Strains: 1, GKU 164T (data from this study); 2, N. monospora PT708T (this study); 3, N. thailandensis KC-061T (this study); 4, N. solani NEAU-Z6T (Wang et al., 2013); 5, N. rhizophila YIM 67092T (Zhao et al., 2011); 6, ‘N. shaanxiensis’ NEAU-st1 (Zhang et al., 2014b). ND, No data available; +, positive; –, negative; w, weakly positive.
liquefaction and starch hydrolysis are positive, but negative reactions are observed for urease, milk peptonization and coagulation, nitrate reduction, and production of \( \text{H}_2\text{S} \) and melanin. Casein, L-tyrosine and xanthine are degraded, while hypoxanthine is weakly degraded and citrate is not degraded. D-Glucose, inulin, D-mannitol, D-mannose, L-xylose and trehalose are utilized as sole carbon sources, but L-arabinose, cellobiose, dulcitol, D-fructose, myo-inositol, \( \beta \)-lactose, maltose, raffinose, D-sorbitol, sucrose, xylitol and D-xylose are not. According to the API ZYM system, \( N \)-acetyl-\( \beta \)-glucosaminidase, acid phosphatase, alkaline phosphatase, \( \alpha \)-chymotrypsin, esterase (C4), esterase lipase (C8), \( \alpha \)-galactosidase, \( \beta \)-galactosidase, \( \alpha \)-glucosidase, \( \beta \)-glucosidase, leucine arylamidase, \( \alpha \)-mannosidase, trypsin and valine arylamidase are positive; while, arylamidase, cysteine, \( \alpha \)-fucosidase and naphthol-AS-BI-phosphohydrolase are weakly positive, and \( \beta \)-glucuronidase and lipase (C4) are negative. The diagnostic diaminoc acid in the cell-wall peptidoglycan is \textit{meso}-diaminopimelic acid. The whole-cell hydrolysates contain madurose, galactose, mannos, ribose, rhamnose and glucose. The \( N \)-acyl type of muramic acid is acetyl. Mycolic acids are not detected. The predominant menaquinone is MK-9(\( H_4 \)), while minor amounts of MK-9(\( H_6 \)), MK-9(\( H_2 \)) and MK-9(\( H_0 \)) are also present. The phospholipid profile consists of DPG, PE, OH-PE, PG, PI, PIMs, PPE, OH-PME, an unidentified aminophosphoglycolipid and four unknown phospholipids. The major fatty acids are iso-C\textsubscript{16:0} and 10-methyl C\textsubscript{17:0}.

The type strain, GKVUB 164\textsuperscript{T} (=BCC 70457\textsuperscript{T}=NBRC 110400\textsuperscript{T}), was isolated from the roots of a jambolan plum tree (\textit{Syzygium cumini} L. Skeels) collected at Khao Khrichakut National Park, Chantaburi province, Thailand. The genomic DNA G+C content of the type strain is 70.4 mol%.

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### References


