Nonomuraea purpurea sp. nov., an actinomycete isolated from mangrove sediment

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A polyphasic approach was used to verify the novel actinomycete, strain 1SM4-01T, isolated from mangrove sediment collected from Ranong Province, Thailand. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the organism was a member of the genus Nonomuraea and was most closely related to Nonomuraea syzygii GKU 164T (98.7 % sequence similarity), Nonomuraea rhizophila YIM 67092T (98.4 %), Nonomuraea solani NEAU-Z6T (98.4 %), Nonomuraea monospora PT708T (98.3 %) and Nonomuraea thailandensis KC-061T (98.2 %). The strain produced branching aerial mycelium which differentiated into straight chains of rough-surfaced spores borne at the end of a short sporophore. The whole-cell hydrolysates contained meso-diaminopimelic acid as the diagnostic diamino acid, with madurose, mannose and ribose as the main sugars. MK-9(H4) was a major menaquinone of this strain. The acyl type of peptidoglycan was N-acetyl. The predominant cellular fatty acids were C17:0 ω8c and iso-C16:0. Phospholipids consisted of diphosphatidylglycerol, hydroxy-phosphatidylethanolamine, hydroxy-phosphatidylcellulose, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylglycerol, G+C content of the genomic DNA was 70.4 mol%. On the basis of phenotypic characteristics, DNA–DNA relatedness and phylogenetic distinctiveness, strain 1SM4-01T represents a novel species of the genus Nonomuraea, for which the name Nonomuraea purpurea sp. nov. is proposed. The type strain is 1SM4-01T (=BCC 60397T=NBRC 109647T).

The genus Nonomuraea (sic) was first proposed by Zhang et al. (1998) and the spelling later corrected to Nonomuraea by Chiba et al. (1999). The genus Nonomuraea is a member of the family Streptosporangiaceae, which comprises aerobic, Gram-stain-positive, non-acid-fast, non-motile actinomycetes. The typical chemotaxonomic characteristics of the genus Nonomuraea are type III cell wall with type IV phospholipid pattern. Mycolic acids are absent. Predominant menaquinones are MK-9(H4) and MK-9(H2). The major fatty acids are iso-C16:0 and 10-methyl C17:0 (Goodfellow et al., 1990; Kämpfer, 2012). Members of the genus Nonomuraea form extensively branched substrate and aerial mycelia that further differentiate into hooked, spiral or straight chains of spores with smooth or rough surface (Kämpfer, 2012). At the time of writing, this genus comprises 38 species with validly published names and two subspecies (LPSN: http://www.bacterio.net/nonomuraea.html). Three additional species, ‘Nonomuraea guangzhouensis’ and ‘Nonomuraea harbinensis’ (Wang et al., 2014) and ‘Nonomuraea shaanxiensis’ (Zhang et al., 2014) have also been described but the names are not yet validated. The type species of the genus is Nonomuraea pusilla (Nonomura & Ohara, 1971; Zhang et al., 1998). Most members of the genus Nonomuraea have been discovered in soil from various habitats such as arid or acidic areas, island, coast, cave, mangrove, mine and rhizosphere, and also found as endophytes in many plants (Wang et al., 2013; Xi et al., 2011; Zhao et al., 2011; Li et al., 2012; Nakaew et al., 2012; Sripreechasak et al., 2013; Camas et al., 2013; Quadri et al., 2015; Rachniyom et al., 2015). Among these, three species...
have been isolated from Thailand, namely Nonomuraea monospora (Nakaew et al., 2012), Nonomuraea thailandensis (Sripreechasak et al., 2013) and Nonomuraea syzygii (Rachniyom et al., 2015), from cave soil, island soil and roots of a jambolan plum tree, respectively. In the present study, actinomycete strain 1SM4-01 T was also discovered in Thailand, its taxonomic position was determined using a polyphasic approach and it is proposed as representing a novel species of the genus Nonomuraea.

Strain 1SM4-01 T was isolated from mangrove sediment by the dilution plate technique during a study of microbial diversity in the mangrove forest, Ranong Province, Thailand (9° 22′ 50.1″ N 98° 24′ 8.1″ E). A 10-fold dilution of mangrove soil suspension was spread onto 10-fold-diluted marine broth and incubated for 7 days. Cultured cells were harvested by centrifugation, and the pellet was washed three times with sterile distilled water before freeze drying. The isomer forms of diaminopimelic acid were determined by the methods of Becker et al. (1965) and Hasegawa et al. (1983). Whole-cell sugars were analysed by TLC as described by Staneck & Roberts (1974). Cellular fatty acid methyl esters were determined by GLC according to the instructions of the Sherlock Microbial Identification System (Microbial ID; MIDI Version 6.1) (Sasser, 1990) and identified with the RTBSA6 database. The analysis was performed at the Faculty of Science, King Mongkut’s Institute of Technology Ladkrabang (KMITL), Thailand. Polar lipids were extracted from freeze-dried biomass for chemotaxonomic studies and examined by two-dimensional TLC according to the method of Tomiyasu (1982). The N-acyl types of muramic acid were determined using the method of Uchida & Aida (1984).

Phylogenetic analysis based on 16S rRNA gene sequences showed that strain 1SM4-01 T formed a distinct cluster with the type strains of species of the genus Nonomuraea that was confirmed by a bootstrap value of 98 % (Fig. 1). The almost-complete 16S rRNA gene sequence (1469 nt) of strain 1SM4-01 T was determined according to Shirling & Gottlieb (1966). The temperature range for growth was determined on ISP 2 using a temperature gradient incubator over the temperature range 10–40 °C. Optimal conditions for growth such as pH (4.0–11.0) at intervals of 1.0 pH unit using biological buffers) and NaCl concentration (0–6 % at intervals of 1%, w/v) were examined on ISP 2 after incubation for 14 days at 28 °C. Utilization of carbohydrates as sole carbon sources at a final concentration of 1 % (w/v) was investigated on ISP9 (Shirling & Gottlieb, 1966). The decomposition of (w/v) aesculin (0.1 %), allantoin (0.33 %), arbutin (0.1 %), casein (skimmed milk, 5.0 %), cellulose (1.0 %), gelatin (0.4 %), hypoxanthine (0.4 %), starch (1.5 %), Tween 80 (1.0 %, v/v), tyrosine (0.4 %), urea (1.8 %), xanthine (0.4 %) and xylan (0.4 %) was examined using standard procedures (Gordon & Mihn, 1957; Gordon et al., 1974). Catalase and oxidase tests were performed using 3 % (v/v) hydrogen peroxide and 1 % (w/v) tetramethyl-p-phenylenediamine dihydrochloride solution, respectively. Enzyme activities were determined using the API ZYM test kit (bioMérieux) according to the manufacturer’s instructions. Nitrate reduction was also studied following standard methods (Gordon et al., 1974), Nonomuraea syzygii GKI 164 T, Nonomuraea rhizophila DSM 45382 T, Nonomuraea solani DSM 45729 T, N. monospora PT708 T and N. thailandensis KC-061 T were used for morphological, physiological, chemotaxonomic and molecular taxonomic studies.

Biomass for chemotaxonomic studies was prepared by growing the strain in shaking flasks of GYE broth at 28 °C for 7 days. Cultured cells were harvested by centrifugation, and the pellet was washed three times with sterile distilled water before freeze drying. The isomer forms of diaminopimelic acid were determined by the methods of Becker et al. (1965) and Hasegawa et al. (1983). Whole-cell sugars were analysed by TLC as described by Staneck & Roberts (1974). Cellular fatty acid methyl esters were determined by GC according to the instructions of the Sherlock Microbial Identification System (Microbial ID; MIDI Version 6.1) (Sasser, 1990) and identified with the RTBSA6 database. The analysis was performed at the Faculty of Science, King Mongkut’s Institute of Technology Ladkrabang (KMITL), Thailand. Polar lipids were extracted from freeze-dried biomass for chemotaxonomic studies and examined by two-dimensional TLC according to the procedure developed by Minnikin et al. (1977). Cellular menaquinones were extracted from freeze-dried biomass using the procedure of Collins et al. (1977) and subsequently analysed by LC/MS (JMS-T100LP; JEOL) with a CAPCELL PAK C18UG120 column (Shiseido) using methanol/2-propanol (7:3). Mycolic acid methyl esters were examined by TLC according to the method of Tomiyasu (1982). The N-acyl types of muramic acid were determined using the method of Uchida & Aida (1984).

Genomic DNA extraction was performed as described by Mingma et al. (2014). The 16S rRNA gene was PCR-amplified from genomic DNA by using primers 1F (5′-TCA CGGAGAGTTGATCCTG-3′) and 1530R (5′-AAGGAGATCACCAGCCGCA-3′) (Kataoka et al., 1997) under the following conditions: initial denaturation for 5 min at 94 °C; 30 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 55 °C and extension for 1 min at 72 °C; and a final extension step for 10 min at 72 °C. PCR products were purified using a Gel/PCR DNA Fragment Extraction kit (Geneaid) then sent to Macrogen for DNA sequencing analysis. The assembled sequence was compared with other sequences of type strains in the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net) (Kim et al., 2012). Evolutionary trees were inferred using the maximum-likelihood (Fitch, 1971), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) tree-making algorithms drawn from MEGA 6 ( Tamura et al., 2013). An evolutionary distance matrix for the neighbour-joining algorithm was generated using the Jukes & Cantor (1969) model. The DNA G+C content was determined by HPLC according to the method of Tamaoka & Komagata (1984). Levels of DNA–DNA relatedness were measured on nylon membranes according to the method described by Wang et al. (2011).

The cultural and growth characteristics of strain 1SM4-01 T were examined on International Streptomyces Project (ISP; Shirling & Gottlieb, 1966) media 2, 3, 4, 5 and 7, Czapek’s Dox agar (Atlas, 2004) and GYE agar. The colours of substrate mycelia, aerial mycelia and soluble pigments were compared with the Color Harmony Manual (Mundie, 1995). Mycelium formation was observed under scanning electron microscopy (Quanta 450 FEI) after the strain was cultivated on ISP 2 for 4 weeks at 28 °C. The morphological characteristics of strain 1SM4-01 T were determined according to
comparisons and the result indicated that the closest relatives were *N. syzygii* GKV 164T (98.7% sequence similarity), *N. rhizophila* YIM 67092T (94.4%), *N. solani* NEAU-Z6T (98.4%), *N. monospora* PT708T (98.3%) and *N. thailandensis* KC-061T (98.2%). The genomic DNA G+C content of strain 1SM4-01T was 70.4 mol%. DNA–DNA relatedness determinations were examined independently twice and revealed that values between strain 1SM4-01T and each of its closely related species, *N. syzygii* GKV 164T, *N. rhizophila* DSM 45382T, *N. solani* DSM 45729T, *N. monospora* PT708T and *N. thailandensis* KC-061T, were 46.1±0.7, 36.7±0.5, 34.9±0.1, 42.5±0.1 and 34.7±1.4%, respectively. These values were well below the 70% cut-off value recommended for the assignment of bacterial strains to the same genomic species (Wayne et al., 1987).

Strain 1SM4-01T exhibited good growth on ISP 2, ISP 3 and GYE agar but poor growth on ISP 4, ISP 5, ISP 7 and Czapek’s Dox agar. A very dark purplish red colour of substrate mycelia and light brown spore pigment were produced on ISP 2, ISP 3 and GYE agar. Conversely, there was no evidence of soluble pigments on ISP 4, ISP 5 or ISP 7. Spore formation was not observed on ISP 4, ISP 5, ISP 7, Czapek’s Dox or GYE agar, but we found pearl-grey spores on ISP 2 and white spores on ISP 3. The strain was able to grow on ISP 2 at 14–34°C (optimum growth at 22–30°C) and exhibited growth at pH 6–8. The maximum NaCl concentration for growth was 2% (w/v). Morphological observations by scanning electron microscopy of 4-week-old culture grown on ISP 2 revealed aerial mycelia that further differentiated into straight chains of rough-surfaced spores (~0.8–0.9×1.0–1.2 μm in size) on a short sporophore (Fig. 2). There were significant differences in morphological, physiological and biochemical properties between strain 1SM4-01T and its closely related species of the genus *Nonomuraea* (Table 1). Only strain 1SM4-01T produced straight spore chains while other closely related species produced single spores or spiral spore chains. However, the spore surface of the novel strain was rough similar to those of *N. syzygii* GKV 164T, *N. rhizophila* DSM 45382T and *N. solani* DSM 45729T. Degradation of L-tyrosine and starch was negative for strain 1SM4-01T, whereas the other strains showed positive results. Strain 1SM4-01T was able to utilize glucose, raffinose, sucrose and D(-)-xylose, characteristics that were not seen in *N. syzygii* GKV 164T. Strain 1SM4-01T showed oxidase activity, whereas *N. monospora* PT708T and *N. thailandensis* KC-061T did not show this activity. Moreover, growth of the four most closely related species was detected at 37°C, but not for strain 1SM4-01T.

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain 1SM4-01T and its closest phylogenetic relatives. Asterisks indicate that the corresponding branches were also recovered in both maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) phylogenetic trees. Bootstrap percentages given at the nodes were based on 1000 re-samplings. The sequence of *Thermopolyspora flexuosa* DSM 43186T was used as an outgroup. Bar, 0.005 nt substitutions per nucleotide position.
Chemotaxonomic analysis of strain 1SM4-01<sup>T</sup> revealed meso-
diaminopimelic acid, madurose, mannose and ribose in its
whole-cell hydrolysates. The acyl type of the peptidoglycan
was N-acetyl. The menaquinones detected were MK-9(H<sub>4</sub>)(79.6 %), MK-9(H<sub>6</sub>)(8.7 %), MK-9(H<sub>2</sub>)(6.8 %), MK-9(H<sub>0</sub>)(3.7 %) and MK-10(H<sub>4</sub>)(1.2 %). The polar lipid profile
comprised diphosphatidylglycerol, hydroxy-phosphatidyleth-
anolamine, hydroxy-phosphatidylmonomethylethanolamine,

![Fig. 2. Scanning electron micrographs showing spore chains (a), and rough-surfaced spores (b) of strain 1SM4-01<sup>T</sup>, after
cultivation on ISP 2 agar at 28 °C for 4 weeks. Bars, (a) 5 µm; (b) 1 µm.](image)

**Table 1.** Phenotypic characteristic that differentiate strain 1SM4-01<sup>T</sup> from closely related species of the genus *Nonomuraea*

<table>
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<th>Characteristic</th>
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<td>Spore chain*</td>
<td>Straight</td>
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<td>Spiral&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Single&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Single&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Spiral&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td>Spore surface*</td>
<td>Rough</td>
<td>Rough&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Rough&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Rough&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>L-Tyrosine</td>
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<td>Xylan</td>
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<td>Sucrose</td>
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<td>Enzyme activities (API ZYM):</td>
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<td>Acid phosphatase</td>
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<td>α-Chymotrypsin</td>
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<td>Cystine arylamidase</td>
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<td>α-Fucosidase</td>
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<td>β-Glucosidase</td>
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<td>Naphthol-AS-BI-phosphohydrolase</td>
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<td>Valine arylamidase</td>
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<td>Oxidase activity</td>
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<td>Growth at 37 °C</td>
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*Data taken from: a, Rachniyom et al. (2015); b, Zhao et al. (2011); c, Wang et al. (2013); d, Nakaew et al. (2012); e, Sripreechasak et al. (2013).*
phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, aminophospholipids and unidentified lipids (Fig. S1, available in the online Supplementary Material). The major cellular fatty acids were C17:0-ω8c (18.4%), iso-C16:0 (13.1%), C16:0 (9.6%), summed feature 3 (comprising C16:0-ω7c and/or C16:0-ω6c; 8.7%), C16:0 2-ΟΗ (6.9%), 10-methyl C17:0 (6.6%), C14:0 (6.2%) and C18:0-ω9c (5.1%). The minor fatty acids were C17:0 (4.6%), iso-C15:0 (4.3%), summed feature 9 (comprising 10-methyl C16:0 and/or iso-C17:0-ω9c; 2.8%), C15:0 2-ΟΗ (2.5%), 10-methyl C18:0 (1.6%), C13:0 (1.5%) and C17:0 2-ΟΗ (1.1%). These chemotaxonomic characteristics of strain 1SM4-01T were similar to those of species of the genus Nonomuraea (Zhao et al., 2011; Nakaew et al., 2012; Wang et al., 2013; Sripreechasak et al., 2013; Rachniyom et al., 2015).

On the basis of the genotypic and phenotypic characteristics presented, it is evident that strain 1SM4-01T can be differentiated from previously described reference strains of species of the genus Nonomuraea. Strain 1SM4-01T should be classified as a representative of a novel species in the genus Nonomuraea, for which the name Nonomuraea purpurea sp. nov. is proposed.

**Description of Nonomuraea purpurea sp. nov.**

*Nonomuraea purpurea* (pur.pu’re.a. L. fem. adj. purpurea purple or purple–red, referring to the colour of the substrate mycelium of the organism).

Gram-stain-positive, aerobic actinomycete that forms extensively branched aerial and substrate mycelia. Aerial mycelia further differentiate into straight chains of rough-surfaced spores borne at the tip of a short sporophore on ISP 2, after incubation at 28 °C for 4 weeks. Good growth with very dark purplish red colour of substrate mycelia and light brown soluble pigment is produced on ISP 2, ISP 3 and GYE agar. Optimum growth occurs at 22–30 °C and pH 6–8. Tolerates up to 2% (w/v) NaCl. Catalase and oxidase are positive, but nitrate reduction is negative. Adonitol, L(+)-arabinose, (+)-cellobiose, D(-)-fructose, fucose, D(+)-galactose, glucose, myo-inositol, β-lactose, maltose, D(-)-mannitol, melibiose, rafinose, L(-)-rhamnose, D(-)-ribose, D(-)-sorbitol, sucrose, trehalose and D(+)-xylose are utilized as sole carbon sources, but sorbose and xylitol are not. Activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase are detected with the API ZYM enzyme assay whereas the activities of lipase (C14), valine arylamidase, cystine arylamidase, β-glucuronidase and α-fucosidase are not found. Able to hydrolyse aesculin, arbutin, casein, gelatin, Tween 80 and xylan, but not allantoin, cellulose, hypoxanthine, starch, L-tyrosine, urea or xanthine. Whole-cell hydrolysates contain meso-diaminopimelic acid. Madurose, mannose and ribose are the major whole-cell sugars. The acyl type of muramic acid in the peptidoglycan is acetyl. MK-9(H4) is a major menaquinone of the organism. Polar lipids are diphasatidylglycerol, hydroxy-phosphatidylethanolamine, hydroxy-phosphatidylmonomethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylglycocitol mannoside, aminophospholipids and unidentified lipids. The predominant cellular fatty acids are C17:0-ω8c and iso-C16:0.

The type strain is 1SM4-01T (=BCC 60397T=NBRC 109647T), which was isolated from soil in the mangrove forest at, Ranong Province, Thailand. The DNA G+C content of the type strain is 70.4 mol%.

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**References**


