**Nonomuraea fuscirosea** sp. nov., an actinomycete isolated from the rhizosphere soil of rehmannia (*Rehmannia glutinosa* Libosch)

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A novel actinomycete, designated strain NEAU-dht8T, was isolated from the rhizosphere soil of rehmannia (*Rehmannia glutinosa* Libosch) and characterized using a polyphasic approach. The organism was found to have morphological and chemotaxonomic characteristics typical of the genus *Nonomuraea*. The G+C content of the DNA was 68.47 mol%. On the basis of 16S rRNA gene sequence similarity studies, strain NEAU-dht8T was most closely related to *Nonomuraea maheshkhaliensis* 16-5-14T (99.31%), *Nonomuraea kuesteri* GW 14-1925T (98.77%), *Nonomuraea coxensis* JCM 13931T (98.71%), *Nonomuraea wenchangensis* 210417T (98.44%), *Nonomuraea bangladeshensis* 5-10-10T (98.36%) and *Nonomuraea salmonea* DSM 43678T (98.0%). Similarities to other species of the genus *Nonomuraea* were lower than 98%.

Two tree-making algorithms based on 16S rRNA gene sequences showed that the isolate formed a phyletic line with its closest neighbour *N. maheshkhaliensis* 16-5-14T. However, the low level of DNA–DNA relatedness allowed the novel isolate to be differentiated from *N. maheshkhaliensis* 16-5-14T. Strain NEAU-dht8T could also be differentiated from other species of the genus *Nonomuraea* showing high 16S rRNA gene sequence similarity (98–98.77%) by morphological and physiological characteristics. Thus, strain NEAU-dht8T is considered to represent a novel species of the genus *Nonomuraea*, for which the name *Nonomuraea fuscirosea* sp. nov. is proposed. The type strain is NEAU-dht8T (=CGMCC 4.7104T = DSM 45880T).

The genus *Nonomuraea*, the name of which was corrected by Chiba et al. (1999) from the original spelling, *Nonomuria*, was originally proposed by Zhang et al. (1998) as a member of the family *Streptosporangiaceae* (Goodfellow et al., 1990; Stackebrandt et al., 1997). Members of the genus *Nonomuraea* are aerobic, Gram-staining-positive, non-acid-fast, non-motile actinomycetes that can form extensively branched substrate and aerial mycelia. The aerial hyphae differentiate into hooked, spiral or straight chains of spores, which show a folded, irregular, smooth or warty ornamentation (Quintana et al., 2003; Kämpfer et al., 2005). 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 (wall chemoype IIIb sensu Lechevalier & Lechevalier, 1970), di-, tetra- and hexa-hydrogenated menaquinones with nine isoprene units as predominant isoprenologues (Nonomura & Ohara, 1971; Zhang et al., 1998; Quintana et al., 2003), and major amounts of diphosphatidylglycerol, hydroxylated phosphatidylethanolamine, uncharacterized glycolipids and a glucosamine-containing phospholipid (phospholipid type IV sensu Lechevalier & Lechevalier, 1970). The type species of the genus is *Nonomuraea pusilla* (Nonomura & Ohara, 1971). At the time of writing, the genus comprised 36 species with validly published names. As part of a programme to discover actinomycetes with novel antibiotic production properties, an aerobic actinomycete, strain NEAU-dht8T, was isolated. In this study, we performed a polyphasic taxonomic study on strain NEAU-dht8T, and propose that the isolate represents a novel species of the genus *Nonomuraea*.

Strain NEAU-dht8T was isolated from the rhizosphere soil of rehmannia (*Rehmannia glutinosa* Libosch) collected...
from Shijiazhuang, Hebei Province, north China (38° 3’ N 114° 26’ E). The strain was isolated using the standard dilution plate method and grown on humic acid-vitamin agar (HV) (Hayakawa & Nonomura, 1987) supplemented with cycloheximide (50 mg l⁻¹) and nalidixic acid (20 mg l⁻¹). After 21 days of aerobic incubation at 28 °C, colonies were transferred and purified on oatmeal agar [International Streptomycetes Project (ISP) 3 medium; Shirling & Gottlieb, 1966] and maintained as glycerol suspensions (20 %, v/v) at −80 °C.

The morphological properties of the isolate were observed by light microscopy (Nikon ECLIPSE E200) and scanning electron microscopy (Hitachi S-3400N) using cultures grown on ISP3 agar at 28 °C for 16 days. For cultural characterization, the isolate was grown for 14 days at 28 °C on various agar media (Table S1, available in the online Supplementary Material) as described by Waksman (1950, 1961), Shirling & Gottlieb (1966) and Asano & Kawamoto (1986). ISCC-NBS colour charts (Kelly, 1964) were used to determine the colours of substrate and aerial mycelia. Growth at different temperatures (4, 16, 18, 22, 28, 37 and 40 °C) was determined on ISP3 medium after incubation for 14 days. pH range (pH 4, 5, 6, 7, 8, 9, 10, 11 and 12) and NaCl tolerance (0, 1, 2, 3, 4, 5 and 6 %, w/v) for growth were determined in modified YEME medium (yeast extract, 3 g; sucrose, 103 g; tryptone, 5 g; malt extract, 3 g; glucose, 10 g; distilled water, 1 l; pH 7.2) at 28 °C for 7–14 days on a rotary shaker. Production of catalase, esterase and urease were tested as described by Smibert & Krieg (1994). Decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, coagulation and peptonization of milk, production of H₂S and liquefaction of gelatin were assessed following the procedures described by Gordon et al. (1974) and Yokota et al. (1993). Utilization of carbohydrates as sole carbon sources was tested by using ISP medium 9 as a basal medium according to the method of Shirling & Gottlieb (1966). Nitrogen utilization experiment was performed as described by Williams et al. (1983). Production of melanin was examined using tyrosine agar (ISP medium 7; Shirling & Gottlieb, 1966).

Morphological observation of a 16-day-old culture of strain NEAU-dht8ᵀ on ISP3 agar revealed that it was consistent with those members of the genus Nonomurea. Aerial and substrate mycelium were well-developed without fragmentation. Spiral spore chains (Fig. S1) of strain NEAU-dht8ᵀ were composed of about 5 to 11 non-motile spores (0.92 × 0.78 μm) with a smooth surface that were borne directly on aerial mycelia (Fig. S1). Sporangia were not detected. Good growth occurred on ISP2, ISP3, ISP6, ISP7, nutrient and Seino agar media; moderate growth on ISP4, glucose-yeast extract, water and Bennett agar; poor growth on glucose-asparagine agar; no growth on ISP5 agar. The substrate mycelium colour varied from pale yellow to strong brown on the media tested. Whole aerial mycelia and sporulation occurred on ISP3, ISP4 and water agar after 14 days of incubation at 28 °C. No diffusible pigment was observed on any of the media tested (Table S1). Strain NEAU-dht8ᵀ grew well between pH 6.0 and 9.0, with optimal growth at pH 7.0. The temperature range for growth was 16–37 °C, with the optimum temperature being 28 °C. Growth was observed in the presence of 0–2.0 % (w/v) NaCl. Detailed physiological and biochemical properties are presented in the species description.

Biomass for chemotaxonomic studies was prepared by growing strain NEAU-dht8ᵀ in modified YEME medium on a rotary shaker at 250 r.p.m. for 7 days at 28 °C; cells were harvested by centrifugation, washed twice with distilled water, recentrifuged and freeze-dried. The isomer of diaminopimelic acid in the cell wall peptidoglycan was analysed by an HPLC method using an Agilent TC-C₁₈ column (250 × 4.6 mm, internal diameter 5 μm) with a mobile phase consisting of acetonitrile/0.05 M phosphate buffer (pH 7.2) (15:85) at a flow rate of 0.5 ml min⁻¹. The peak detection used an Agilent G1321A fluorescence detector with a 365 nm excitation and 455 nm longpass emission filters (McKerrow et al., 2000). The whole-cell sugars were analysed according to the procedures developed by Lechevalier & Lechevalier (1980). Phospholipids in cells were examined by two-dimensional TLC and identified using the method of Minnikin et al. (1984). Menaquinones were extracted from freeze-dried biomass and purified according to the protocol of Collins (1985). Extracts were analysed by an HPLC-UV method using an Agilent Extend-C₁₈ column (150 × 4.6 mm, internal diameter 5 μm), typically at 270 nm. The mobile phase was acetonitrile/propyl alcohol (60:40, v/v), the flow rate was set to 1.0 ml min⁻¹ and the run time was 60 min. The injection volume was 20 μl, and the chromatographic column was controlled at 40 °C (Wu et al., 1989). Mycolic acids were checked by the acid methanolyis method as described by Minnikin et al. (1980). Cellular fatty acids were analysed by GC-MS using the method of Xiang et al. (2011).
menaquinones detected were MK-9 (H4) (53.93 %), MK-9 (H2) (27.95 %), MK-8 (H6) (9.43 %) and MK-9 (H4) (8.69 %). The phospholipid profile was found to consist of diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, hydroxy-phosphatidylmonomethylethanolamine, hydroxy-phosphatidylylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside and two unknown phospholipids (phospholipid type IV; Lechevalier & Lechevalier, 1970) (Fig. S2). Mycolic acids were not detected. The cellular fatty acid profile was determined to be composed of C16:0 (39.08 %), C18:0 (20.78 %), 10-methyl C17:0 (17.17 %), 10-methyl C16:0 (17.02 %), C15:0 (4.64 %) and C14:0 (1.31 %) (Fig. S3). Strain NEAU-dht8T shared many chemotaxonomic characteristics with other species of the genus Nonomuraea. However, the fatty acid profile of strain NEAU-dht8T was evidently different from those of other species of the genus Nonomuraea by the absence of iso-C16:0. This result might be because of the GY medium used to culture cells for cellular fatty acid analysis. Another study in our lab also produced a similar result (Zhang et al., 2014).

Genomic DNA of strain NEAU-dht8T was extracted as described by Lee et al. (2003) and PCR amplification of the 16S rRNA gene was carried out using the method of Loqmam et al. (2009). The PCR product was purified and cloned into the vector pMD19-T (Takara), sequenced by using an Applied Biosystems DNA sequencer (model 3730XL) and software provided by the manufacturer. The almost full-length 16S rRNA gene sequence (1510 nt) was determined by the thermal denaturation (Tm) method as described by Mandel & Marmur (1968), and Escherichia coli JM109 was used as the reference strain. The genomic DNA G+C content of strain NEAU-dht8T was 68.47 ± 0.25 mol%, which is consistent with values seen for members of the genus Nonomuraea.

In conclusion, it is evident from the genotypic, chemotaxonomic and phenotypic data that strain NEAU-dht8T represents a novel species of the genus Nonomuraea, for which the name Nonomuraea fuscicrosea sp. nov. is proposed.

**Description of Nonomuraea fuscicrosea sp. nov.**

*Nonomuraea fuscicrosea* (fus.ci.ro’s.e.a. L. adj. fuscus dark-coloured, brown; L. adj. roseus pink; N.L. fem. adj. fuscicrosea brownish-pink).

Aerobic, Gram-staining-positive actinomycete that forms branched, non-fragmenting substrate mycelium. Abundant aerial mycelia are present on ISP3 agar. Spore chains are spiral with one or two turns (5–11 spores) and the spore (0.92 x 0.78 μm) surface is smooth. Sporangia are not found. No diffusible pigment or melanin is observed on any of the tested media. Temperature range for growth is 16–37 °C, with optimal growth at 28 °C. pH range for growth is 6.0–9.0, with optimal growth at pH 7.0. The NaCl tolerance range for growth is up to 2.0 % (w/v). Positive for production of catalase, hydrolysis of aesculin, decomposition of cellulose, nitrate reduction, milk coagulation and milk peptonization. Negative for production of...
urease, esterase and H₂S, liquefaction of gelatin and hydrolysis of starch. L-Arabinose, D-galactose, D-glucose, lactose, maltose, D-mannose, D-mannitol, D-raffinose, D-rhamnose and sucrose are utilized as sole carbon sources but inositol, D-fructose, D-ribose, D-sorbitol and D-xylose are not utilized. L-Alanine, L-asparagine, L-aspartic acid, L-creatine and L-serine are utilized as sole nitrogen sources but L-glutamine, L-threonine, L-tyrosine, L-arginine, glycine and L-glutamic acid are not. Cell walls contain meso-diaminopimelic acid as diagnostic diamino acid and the whole cell sugars are glucose and madurose. The predominant menaquinones are MK-9 (H₂) and MK-9 (H₄). The polar lipid profile contains diphosphatidylglycerol, phosphatidylinositol, hyroxy-phosphatidylethanolamine, hyroxy-phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylcholine, and phosphatidylethanolamine.

Fig. 2. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing the relationship between strain NEAU-dht8ᵀ and all the species of the genus Nonomuraea with validly published names. Thermopolyspora flexuosa DSM 43186ᵀ was used as an out-group. Asterisks indicate branches that were also recovered using the maximum-likelihood algorithm. Numbers at nodes indicate bootstrap percentages (based on 1000 replicates); only values above 50% are shown. Bar, 0.005 substitutions per nucleotide position.
Table 1. Differential characteristics of strain NEAU-dht8T and related type strains of species of the genus Nonomuraea

Strains: 1, NEAU-dht8T; 2, N. maheshkhaliensis 16-5-14T; 3, N. kuesteri GW 14-1925T; 4, N. coensis JCM 13931T; 5, N. wenchangensis 210417T; 6, N. bangladeshensis 5-10-10T; 7, N. salmonea DSM 43678T. Data for strains 1–3 are taken from this study. Data for strains 4–7 are from Wang et al. (2011) and Kämpfer et al. (2005). H, Hooks, curled; S, spirals of one or two turns; Str, straight; Sp, spirals of three to five turns; ND, no data available; +, positive; –, negative; ±, weakly positive.

<table>
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<th>Characteristic</th>
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<th>5</th>
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<td>Number of spores</td>
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<td>Trace</td>
<td>Pink to white</td>
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<td>Pink</td>
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<td>Substrate mycelium</td>
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<td>Light wheat</td>
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<td>Orange</td>
<td>Pale pink</td>
<td>Pale brown</td>
<td>Pink Red</td>
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phosphatidylinositol mannoside and two unknown phospholipids. Mycolic acids are absent. Major fatty acids are C_{16:0}, C_{18:0}, 10-methyl C_{17:0} and 10-methyl C_{16:0}.

The type strain, NEAU-dht8T (=CGMCC 4.7104T = DSM 45880T), was isolated from the rhizosphere soil of rehmannia (Rehmannia glutinosa Libosch) collected from Shijiazhuang, Hebei Province, China. The DNA G+C content of the type strain is 68.47 ± 0.25 mol%.

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References


Nonomuraea fuscioseae sp. nov.


