Nonomurae solani sp. nov., an actinomycete isolated from eggplant root (Solanum melongena L.)

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A novel actinomycete, designated strain NEAU-Z6T, was isolated from eggplant (Solanum melongena L) root. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain NEAU-Z6T belonged to the genus Nonomuraea, with highest sequence similarity to Nonomuraea monospora PT 708T (98.83 %), Nonomuraea rosea GW 12687T (98.55 %) and Nonomuraea rhizophila YIM 67092T (98.02 %). Sequence similarities between strain NEAU-Z6T and other species of the genus Nonomuraea ranged from 97.94 % (Nonomuraea candida HMC10T) to 96.30 % (Nonomuraea wenchangensis 210417T). Key morphological, physiological and chemotaxonomic characteristics of strain NEAU-Z6T were congruent with the description of the genus Nonomuraea. The G+C content of the genomic DNA was 64.51 mol%. DNA–DNA relatedness and comparative analysis of physiological, biochemical and chemotaxonomic data allowed genotypic and phenotypic differentiation of strain NEAU-Z6T from closely related species. Thus, strain NEAU-Z6T represents a novel species of the genus Nonomuraea, for which the name Nonomuraea solani sp. nov. is proposed. The type strain is NEAU-Z6T (=CGMCC 4.7037T=DSM 45729T).

The genus Nonomuraea was proposed by Zhang et al. (1998) and is part of the family Streptosporangiaceae. Members of the genus Nonomuraea are aerobic, Gram-positive, non-acid-fast, non-motile actinomycetes which 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 surface ornamentation. The genus is characterized chemotaxonomically by the presence of meso-diaminopimelic acid in the cell wall, madurose as a characteristic sugar in whole-cell hydrolysates, di-, tetra- and hexa-hydrogenated menaquinones with nine isoprene units as the predominant isoprenologues (Nonomura & Ohara, 1971; Zhang et al., 1998; Quintana et al., 2003). At the time of writing, the genus comprised 32 species with validly published names (Ara et al., 2007a, b; Camas et al., 2013; Cao et al., 2012; Chiba et al., 1999; Kämpfer et al., 2005, 2010; Le Roes & Meyers, 2008; Li et al., 2011, 2012; Nakaew et al., 2012; Qin et al., 2009; Stackebrandt et al., 2001; Wang et al., 2011; Zhang et al., 1998; Zhao et al., 2011). During a study on the ecological diversity of actinomycetes from eggplant root, a total of 13 isolates of endophytic actinomycetes were isolated from eggplant root sample. Out of 13 isolates, Streptomyces was the dominant genus (n=9, 69.23 % of isolates), followed by species of the genus Micromonospora (n=3, 23.08 %) and species of the genus Nonomuraea (n=1, 7.69 %). In this study, we performed polyphasic taxonomy on strain NEAU-Z6T, a novel isolate of the genus Nonomuraea.

Strain NEAU-Z6T was isolated from eggplant root (Solanum melongena L) collected from Harbin, Heilongjiang province, north China (45° 45’ N, 126° 41’ E). The plant was tagged outdoors and stored in a clean plastic bag until used (approximately 24 h). The root sample was air-dried for 24 h at room temperature and then washed in water with an ultrasonic step (160 W, 15 min) to remove the surface soils and adherent epiphytes completely. After drying, the sample was cut into pieces of 5–10 mm in length and then subjected to a seven-step surface sterilization procedure: a 60 s wash in sterile tap water containing cycloheximide (100 mg l−1) and nalidixic acid (20 mg l−1), followed by a wash in sterile water, a 5 min wash in 5 % (v/v) NaOCl, a 10 min wash in

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NEAU-Z6T is JQ073731.

Three supplementary figures and a supplementary table are available with the online version of this paper.
2.5 % (w/v) Na₂S₂O₃, a 5 min wash in 75 % (v/v) ethanol, a wash in sterile water and a final rinse in 10 % (w/v) NaHCO₃ for 10 min, and then the rinsed root sample was dried at 100 °C for 15 min. After that, the sample was then cut up in a commercial blender and ground with a mortar and pestle, employing 1 ml 0.5 M potassium phosphate buffer (pH 7.0) per 100 mg tissue. Tissue particles were allowed to settle at 4 °C for 20–30 min, and the supernatant was spread on a plate of humic acid–vitamin agar (HV) (Hayakawa & Nonomura, 1987) supplemented with cycloheximide (50 mg l⁻¹) and nalidixic acid (20 mg l⁻¹). After 14 days of aerobic incubation at 28 °C, colonies were transferred and purified on International Streptomyces Project (ISP) 3 medium (Shirling & Gottlieb, 1966) and incubated at 28 °C for 2–3 weeks.

Genomic DNA of strain NEAU-Z6ᵀ was extracted from biomass obtained from shaking incubation in modified YEME medium [yeast extract 0.3 %, sucrose 10.3 %, tryptone 0.5 %, malt extract 0.3 % and glucose 1.0 % (w/v), pH 7.2] as described previously by Lee et al. (2003) and PCR amplification of the 16S rRNA gene was carried out according to the procedures described by Loqman et al. (2009). The resulting 16S rRNA (1514 nt) gene sequence was determined and compared with the corresponding sequences of other bacterial strains from the GenBank database. Multiple alignments with sequences from the most closely related actinobacteria and calculations of levels of sequence similarity were carried out using CLUSTAL_X 1.83 software. Phylogenetic trees were reconstructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms using MEGA 5.05 (Tamura et al., 2011). The topology of the phylogenetic trees was evaluated using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates. A distance matrix was evaluated using the bootstrap resampling method of Felsenstein (1981) with 1000 replicates. A distance matrix was evaluated using the bootstrap resampling method of Felsenstein (1981) with 1000 replicates. A distance matrix was evaluated using the bootstrap resampling method of Felsenstein (1981) with 1000 replicates.

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain NEAU-Z6ᵀ was a member of the genus Nonomuraea. The closest phylogenetic relatives were Nonomuraea monospora PT708ᵀ (98.83 %), Nonomuraea rosea GW 12687ᵀ (98.55 %) and Nonomuraea rhizophila YIM 67092ᵀ (98.02 %). 16S rRNA gene sequence similarities between strain NEAU-Z6ᵀ and other species of the genus Nonomuraea were lower than 98 %, ranging from 96.30 % (Nonomuraea wenchangensis 210417ᵀ) to 97.94 % (Nonomuraea candida HMC10ᵀ). The phylogenetic tree (Fig. 1) based on 16S RNA gene sequences showed that strain NEAU-Z6ᵀ formed a distinct phyletic line with N. monospora PT708ᵀ, N. rosea GW 12687ᵀ and N. rhizophila YIM 67092ᵀ, an association that was supported by maximum-likelihood algorithm employed (Fig. S1, available in IJSEM Online) and by a 97 % bootstrap value in the neighbour-joining analysis. To establish the precise taxonomic position of strain NEAU-Z6ᵀ, DNA–DNA hybridizations performed between the novel isolate and N. monospora PT708ᵀ, N. rosea GW 12687ᵀ and N. rhizophila YIM 67092ᵀ; the levels of DNA–DNA relatedness between them were 44.16 ± 0.67, 35 ± 0.70 and 60 ± 3.43 %, respectively. These values were below the threshold value of 70 % recommended by Wayne et al. (1987) for assignment of strains to the same species.

Morphological properties were observed by light microscopy (ECLIPSE E200; Nikon) and scanning electron microscopy (S-3400N; Hitachi) using cultures grown on ISP 3 agar for 14 days. Cultural characteristics were determined after 2 weeks at 28 °C by methods used in the ISP (Shirling & Gottlieb, 1966). The temperature range for growth was determined on ISP 3 medium after incubation for 21 days. The pH range and NaCl tolerance for growth were determined on a modified YEME medium at 28 °C for 14–21 days on a rotary shaker. Catalase, oxidase, urease and gelatinase activities, starch hydrolysis and nitrate reduction were assessed as described by Smibert & Krieg (1994). Other physiological and biochemical tests were performed as described by Gordon et al. (1974).

Morphological observation of a 14 day-old culture of strain NEAU-Z6ᵀ grown on ISP 3 agar revealed it had the typical characteristics of species of the genus Nonomuraea. Aerial and substrate mycelium were well-developed without fragmentation. Non-motile spores (0.81 ± 1.43 μm) were borne singly on the aerial mycelia and the spore surface was smooth (Fig. S3). Sporangia were not found. Cultural characteristics of strain NEAU-Z6ᵀ are shown in Table 1. Strain NEAU-Z6ᵀ grew well on ISP 2 and ISP 3 agar; moderate growth was recorded on ISP 4 and ISP 7 agar and poor growth on ISP 5 and ISP 6 agar. A white aerial mycelium was produced on ISP 3 agar but no aerial mycelium was formed on ISP 2, ISP 4, ISP 5, ISP 6 and ISP 7 agar. The substrate mycelium colour varied from white to brown–yellow on the media tested. No diffusible pigment was observed on any of the media tested. Growth of strain NEAU-Z6ᵀ occurred in the pH range 7–9 and 0–2 % NaCl (w/v), with optimum growth at pH 7.0 and 0 % NaCl (w/v). The temperature range for growth was 20–39 °C, with the optimum temperature being 28 °C. Detailed physiological and biochemical properties are presented in the species description. The morphological and physiological characteristics that differentiate strain NEAU-Z6ᵀ from the type strains of N. monospora, N. rosea and N. rhizophila are presented in Table 2.

Biomass for chemical studies was prepared by growing the strain in tryptic soy broth (TSB: tryptone, 15 g; soya peptone, 5 g; NaCl, 0.5 g; distilled water, 1 l; pH 7.0–7.4) in Erlenmeyer flasks for 5 days at 28 °C. Cells were harvested by centrifugation, washed with distilled water and freeze-dried. The isomers of diaminoimidic acid (DAP) in the whole-cell hydrolysates were derivatized according to McKerrow et al. (2000) and analysed by
**Fig. 1.** Neighbour-joining tree based on nearly complete 16S rRNA gene sequences (1514 nt) showing the relationship between strain NEAU-Z6ᵀ and species of the genus *Nonomuraea*. Asterisks denote branches that were also recovered using the maximum-likelihood method. Bootstrap values ≥ 50% (based on 1000 replications) are shown at branch points. Bar, 0.005 substitutions per nucleotide position.

HPLC using an Agilent TC-C₁₈ column (250 × 4.6 mm i.d. 5 μm) with a mobile phase consisting of acetonitrile: 0.05 mol l⁻¹ 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. The whole-cell sugars were analysed

**Table 1.** Growth and cultural characteristics of strain NEAU-Z6ᵀ

<table>
<thead>
<tr>
<th>Agar medium</th>
<th>Growth</th>
<th>Aerial mycelium</th>
<th>Substrate mycelium</th>
<th>Diffusible pigment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract/malt extract (ISP 2)</td>
<td>Good</td>
<td>None</td>
<td>Yellow–brown</td>
<td>None</td>
</tr>
<tr>
<td>Oatmeal (ISP 3)</td>
<td>Good</td>
<td>White</td>
<td>White</td>
<td>None</td>
</tr>
<tr>
<td>Inorganic salts/starch (ISP 4)</td>
<td>Moderate</td>
<td>None</td>
<td>White</td>
<td>None</td>
</tr>
<tr>
<td>Glycerol/asparagine (ISP 5)</td>
<td>Poor</td>
<td>None</td>
<td>White</td>
<td>None</td>
</tr>
<tr>
<td>Peptone/yeast extract/iron (ISP 6)</td>
<td>Poor</td>
<td>None</td>
<td>Yellow</td>
<td>None</td>
</tr>
<tr>
<td>Tyrosine (ISP 7)</td>
<td>Moderate</td>
<td>None</td>
<td>Yellow</td>
<td>None</td>
</tr>
</tbody>
</table>
Table 2. Differential phenotypic properties of strain NEAU-Z6\textsuperscript{T} and the most 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>
</tr>
</thead>
<tbody>
<tr>
<td>Spore arrangement</td>
<td>Single</td>
<td>Single</td>
<td>Spirals</td>
<td>Spirals</td>
</tr>
<tr>
<td>Substrate mycelium (on ISP 3 medium)</td>
<td>White</td>
<td>Vivid red</td>
<td>Pink–red</td>
<td>Brown–yellow</td>
</tr>
<tr>
<td>Decomposition of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Urea</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l-Arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>d-Mannitol</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>d-Xylose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>l-Asparagine</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>l-Alanine</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tolerance of NaCl (% w/v)</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>7</td>
</tr>
</tbody>
</table>

Strains: 1, NEAU-Z6\textsuperscript{T}; 2, \textit{N. monospora} PT708\textsuperscript{T}; 3, \textit{N. rosea} GW 12687\textsuperscript{T}; 4, \textit{N. rhizophila} YIM 67092\textsuperscript{T}. All data are from this study. +, Positive; −, negative.

Chemotaxonomic analyses revealed that strain NEAU-Z6\textsuperscript{T} exhibited characteristics which were typical of members of the genus Nonomuraea, such as the presence of meso-diaminopimelic acid and the presence of madurose and glucose as whole-cell sugars. The major menaquinones were MK-9(H\textsubscript{4}) (55.05 %), MK-9(H\textsubscript{6}) (35.67 %), MK-9(H\textsubscript{8}) (7.71 %) and MK-9(H\textsubscript{10}) (1.56 %). The phospholipids consisted of diphasphatidylglycerol (DPG), phosphatidylmonomethylethanolamine (PME), phosphatidylethanolamine (PE), hydroxyphosphatidylmonomethylethanolamine (OH-PME), hydroxyphosphatidylethanolamine (OH-PE), phosphatidylglycerol (PG), phosphatidylglycerol (PIM) and an unknown phospholipid (PL) (Figs S1, S2). The cellular fatty acid profile was composed of C\textsubscript{16:0} (32.74 %), C\textsubscript{18:0} (9.10 %), C\textsubscript{18:1ω9c} (8.39 %), C\textsubscript{16:1ω7c} (7.99 %), C\textsubscript{17:0} 10-methyl (7.39 %), C\textsubscript{18:0} 10-methyl (6.77 %), C\textsubscript{17:1ω7c} (5.74 %), C\textsubscript{16:0} 3-OH (3.91 %), C\textsubscript{16:0} 2-OH (2.30 %), C\textsubscript{15:0} (2.05 %), C\textsubscript{17:0} (1.98 %), C\textsubscript{18:1ω7c} (1.91 %), iso-C\textsubscript{17:0} 3-OH (1.39 %), iso-C\textsubscript{18:0} (1.08 %), C\textsubscript{16:0} 10-methyl (1.00 %) (Table S1).

The DNA G+C content of the genomic DNA was determined by the thermal denaturation method as described by Mandel & Marmur (1968), and \textit{Escherichia coli} JM109 was used as the reference strain. The DNA G+C content of strain NEAU-Z6\textsuperscript{T} is 64.51 ± 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-Z6\textsuperscript{T} represents a novel species of the genus Nonomuraea, for which the name Nonomuraea solani sp. nov. is proposed.

Description of \textit{Nonomuraea solani} sp. nov.

\textit{Nonomuraea solani} (so.la’ni. L. n. solanum name of a plant and also a botanical generic name (\textit{Solanum}); L. gen. n. solani of Solanum, referring to the isolation of the first strain from \textit{Solanum melongena}).

Aerobic, Gram-positive actinomycete that forms branched, non-fragmenting substrate hyphae. No diffusible pigment is observed on any of the tested media. Non-motile spores (0.81 × 1.43 μm) are borne singly on the aerial mycelia and the spore surface is smooth. Sporangia are not found. Grows at 20–39 °C (optimum 28 °C) and pH 7–9 (optimum pH 7.0). The NaCl tolerance range for growth is up to 2 % (w/v). Positive for catalase, gelatin liquefaction, hydrolysis of starch, aesculin and cellulose, but negative for oxidase, H\textsubscript{2}S production, Tween 80 hydrolysis, urea hydrolysis and nitrate reduction. L-arabinose, D-fructose, D-galactose, D-glucose, inositol, lactose, maltose, D-mannose, D-mannitol, D-raffinose, L-rhamnose, D-sorbitol, sucrose and D-xylose are utilized as sole carbon sources, but D-ribose is not utilized.
The type strain is NEAU-Z6T (=CGMCC 4.7037 = DSM 45729T), isolated from eggplant root collected from Harbin, Heilongjiang province, north China (45° 45′ N 126° 41′ E). The DNA G + C content of the type strain is 64.51 ± 0.25 mol.

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References


