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

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A novel actinomycete, designated strain NEAU-Z6^T, was isolated from eggplant (*Solanum melongena* L.) root. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain NEAU-Z6^T belonged to the genus *Nonomuraea*, with highest sequence similarity to *Nonomuraea monospora* PT 708^T (98.83%), *Nonomuraea rosea* GW 12687^T (98.55%) and *Nonomuraea rhizophila* YIM 67092^T (98.02%). Sequence similarities between strain NEAU-Z6^T and other species of the genus *Nonomuraea* ranged from 97.94% (*Nonomuraea candida* HMC10^T) to 96.30% (*Nonomuraea wenchangensis* 210417^T). Key morphological, physiological and chemotaxonomic characteristics of strain NEAU-Z6^T 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-Z6^T from closely related species. Thus, strain NEAU-Z6^T represents a novel species of the genus *Nonomuraea*, for which the name *Nonomuraea solani* sp. nov. is proposed. The type strain is NEAU-Z6^T (=CGMCC 4.7037^T=DSM 45729^T).

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-Z6^T, a novel isolate of the genus *Nonomuraea*.

Strain NEAU-Z6^T 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-Z6^T is JQ073731.

Three supplementary figures and a supplementary table are available with the online version of this paper.

2.5 % (w/v) $\text{Na}_2\text{S}_2\text{O}_3$, a 5 min wash in 75 % (v/v) ethanol, a wash in sterile water and a final rinse in 10 % (w/v) NaHCO_3 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-Z6^T 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 generated using Kimura's two-parameter model (Kimura, 1980). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). DNA–DNA relatedness test was performed in triplicate using the optical renaturation method as described by De Ley *et al.* (1970).

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain NEAU-Z6^T was a member of the genus *Nonomuraea*. The closest phylogenetic relatives were *Nonomuraea monospora* PT708^T (98.83 %), *Nonomuraea rosea* GW 12687^T (98.55 %) and *Nonomuraea rhizophila* YIM 67092^T (98.02 %). 16S rRNA gene sequence similarities between strain NEAU-Z6^T and other species of the genus *Nonomuraea* were lower than 98 %, ranging from 96.30 % (*Nonomuraea wenchangensis* 210417^T) to 97.94 % (*Nonomuraea candida* HMC10^T). The phylogenetic tree (Fig. 1) based on 16S rRNA gene sequences showed that strain NEAU-Z6^T formed a distinct phyletic line with *N. monospora* PT708^T, *N. rosea* GW 12687^T and *N. rhizophila* YIM 67092^T, 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-Z6^T, DNA–DNA hybridizations performed between the novel isolate and *N. monospora* PT708^T, *N. rosea* GW 12687^T and *N. rhizophila* YIM 67092^T; 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-Z6^T 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 ($0.81 \times 1.43 \text{ }\mu\text{m}$) were borne singly on the aerial mycelia and the spore surface was smooth (Fig. S3). Sporangia were not found. Cultural characteristics of strain NEAU-Z6^T are shown in Table 1. Strain NEAU-Z6^T 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-Z6^T 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-Z6^T 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 diaminopimelic 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-Z6^T and species of the genus *Nonomuraea*. Asterisks denote branches that were also recovered using the maximum-likelihood method. Bootstrap values $\geq 50\%$ (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-Z6^T

Agar medium	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigment
Yeast extract/malt extract (ISP 2)	Good	None	Yellow–brown	None
Oatmeal (ISP 3)	Good	White	White	None
Inorganic salts/starch (ISP 4)	Moderate	None	White	None
Glycerol/asparagine (ISP 5)	Poor	None	White	None
Peptone/yeast extract/iron (ISP 6)	Poor	None	Yellow	None
Tyrosine (ISP 7)	Moderate	None	Yellow	None

Table 2. Differential phenotypic properties of strain NEAU-Z6^T and the most closely related species of the genus *Nonomuraea*

Strains: 1, NEAU-Z6^T; 2, *N. monospora* PT708^T; 3, *N. rosea* GW 12687^T; 4, *N. rhizophila* YIM 67092^T. All data are from this study. +, Positive; –, negative.

Characteristic	1	2	3	4
Spore arrangement	Single	Single	Spirals	Spirals
Substrate mycelium (on ISP 3 medium)	White	Vivid red	Pink–red	Brown–yellow
Decomposition of:				
Gelatin	+	+	+	–
Starch	+	+	+	–
Urea	–	–	–	+
Utilization of:				
L-Arabinose	+	+	+	–
D-Mannitol	+	+	–	+
Raffinose	+	–	+	+
Sucrose	+	+	–	–
D-Xylose	+	+	+	–
L-Asparagine	+	–	–	+
L-Alanine	+	+	–	+
Tolerance of NaCl (% w/v)	2	3	5	7

according to the procedures developed by Lechevalier & Lechevalier (1980). The phospholipids 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 method of Collins (1985). Extracts were analysed by the HPLC–UV method using Agilent Extend-C₁₈ column (150 × 4.6 mm, i.d. 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). Biomass for quantitative fatty acid analysis of strain NEAU-Z6^T was produced by growing the strain in TSB in Erlenmeyer flasks for 5 days at 28 °C. Cells were harvested by centrifugation and washed with distilled water. Fatty acids were analysed by GC–MS using the method of Xiang *et al.* (2011).

Chemotaxonomic analyses revealed that strain NEAU-Z6^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(H₄) (55.05 %), MK-9(H₆) (35.67 %), MK-9(H₈) (7.71 %) and MK-9(H₂) (1.56 %). The phospholipids consisted of diphosphatidylglycerol (DPG), phosphatidylmonomethylethanolamine (PME), phosphatidylethanolamine (PE), hydroxyphosphatidylmonomethylethanolamine (OH-PME), hydroxyphosphatidylethanolamine (OH-PE), phosphatidylinositol (PI), phosphatidylinositol mannoside (PIM) and an unknown phospholipid (PL) (Fig. S2). The cellular fatty acid profile was composed of C_{16:0} (32.74 %), C_{18:0} (9.10 %), C_{18:1}ω9c (8.39 %), C_{16:1}ω7c (7.99 %), C_{17:0} 10-methyl (7.39 %), C_{18:0} 10-methyl (6.77 %), C_{17:1}ω7c (5.74 %), C_{14:0} (3.91 %), C_{16:0} 2-OH (2.30 %), C_{15:0} (2.05 %), C_{17:0} (1.98 %), C_{18:1}ω7c

(1.91 %), iso-C_{17:0} (1.39 %), iso-C_{18:0} (1.08 %), C_{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 *Escherichia coli* JM109 was used as the reference strain. The DNA G + C content of strain NEAU-Z6^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^T represents a novel species of the genus *Nonomuraea*, for which the name *Nonomuraea solani* sp. nov. is proposed.

Description of *Nonomuraea solani* sp. nov.

Nonomuraea solani (so.la'ni. L. n. *solanum* name of a plant and also a botanical generic name (*Solanum*); L. gen. n. *solani* of *Solanum*, referring to the isolation of the first strain from *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, H₂S 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.

L-alanine, L-arginine, L-asparagine, creatine, L-glutamine, glycine, L-threonine and L-tyrosine can be used as sole nitrogen sources. The diagnostic amino acid of the peptidoglycan is *meso*-diaminopimelic acid. Cell hydrolysates contain madurose and glucose. The phospholipids include diphosphatidylglycerol (DPG), phosphatidylmonomethylethanolamine (PME), phosphatidylethanolamine (PE), hydroxy-phosphatidylmonomethylethanolamine (OH-PME), hydroxy-phosphatidylethanolamine (OH-PE), phosphatidylinositol (PI), phosphatidylinositol mannoside (PIM) and an unknown phospholipid. The major menaquinones are MK-9(H₄) and MK-9(H₆). Major fatty acids are C_{16:0}, C_{18:0}, C_{18:1ω9c}, C_{16:1ω7c}, C_{17:0} 10-methyl, C_{18:0} 10-methyl and C_{17:1ω7c}.

The type strain is NEAU-Z6^T (=CGMCC 4.7037^T=DSM 45729^T), 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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