A novel actinobacterium, designated strain NEAU-ND5\(^T\), was isolated from the rhizosphere of corn (Zea mays L.) collected in Heilongjiang Province, north-east China, and characterized using a polyphasic approach. 16S rRNA gene sequence analysis showed that strain NEAU-ND5\(^T\) was a member of the genus Nonomuraea, with highest sequence similarities to Nonomuraea jabiensis A4036\(^T\) (98.29 %), Nonomuraea rosea GW12687\(^T\) (98.25 %), Nonomuraea candida HMC10\(^T\) (98.22 %), Nonomuraea rhizophila YIM 67092\(^T\) (98.04 %) and Nonomuraea kuesteri NRRL B-24325\(^T\) (98.04 %). Similarities to other type strains of the genus Nonomuraea were lower than 98 %. Morphological and chemotaxonomic properties of strain NEAU-ND5\(^T\) were also consistent with the description of the genus Nonomuraea. The cell wall contained meso-diaminopimelic acid and the whole-cell sugars were glucose, ribose and madurose. The phospholipid profile consisted of diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, hydroxy-phosphatidylmonomethylethanolamine, hydroxy-phosphatidylglycerol, phosphatidylglycerol and phosphatidylglycerol mannoside. The major menaquinones were MK-9(H\(_2\)), MK-9(H\(_6\)) and MK-9(H\(_8\)). The predominant cellular fatty acids were iso-C\(_{15:0}\) and 10-methyl C\(_{17:0}\). A combination of DNA-DNA hybridization results and some phenotypic characteristics demonstrated that strain NEAU-ND5\(^T\) was clearly distinguished from its closely related Nonomuraea species. Consequently, it is concluded that strain NEAU-ND5\(^T\) represents a novel species of the genus Nonomuraea, for which the name Nonomuraea zeae sp. nov. is proposed. The type strain is NEAU-ND5\(^T\) (=CGMCC 4.7280\(^T\)=DSM 100528\(^T\)).

The genus Nonomuria (sic) was first proposed by Zhang et al. (1998) belonging to the family Streptosporangiaceae and the name was corrected to Nonomuraea by Chiba et al. (1999). The genus is characterized by extensively branched substrate and aerial mycelia, and the aerial hyphae differentiate into hooked, spiral or straight chains of spores, which show a folded, irregular, smooth or warty surface ornamentation (Nonomura & Ohara, 1971). At the time of writing, the genus comprises 37 species with validly published names and two subspecies according to the List of Prokaryotic names with Standing in Nomenclature website (http://www.bacterio.net), including the newly described Nonomuraea syzygii (Rachniyom et al., 2015). Two further species, ‘Nonomuraea indica’ (Quadri et al., 2015) and ‘Nonomuraea flavida’ (Chen et al., 2015), have also been recently described but the names are not yet validly published. In the course of investigation into novel actinobacteria from the rhizosphere of corn (Zea mays L.), strain NEAU-ND5\(^T\) was isolated. In this study, we performed a polyphasic taxonomy investigation of this strain and propose that it represents a novel species of the genus Nonomuraea.

Strain NEAU-ND5\(^T\) was isolated from the rhizosphere of corn collected in Heilongjiang Province, north-east China (45° 40’ N 127° 30’ E). The strain was isolated using the standard dilution plate method and grown on humic acid-vitamin agar (HV) (Hayakawa & Nonomura, 1987) supplemented with nystatin (50 mg l\(^{-1}\)) and nalidixic acid (20 mg l\(^{-1}\)). After 14 days of aerobic incubation at 28 °C, a colony
was transferred and purified on oatmeal agar [International Streptomyces Project (ISP) 3 medium] (Shirling & Gottlieb, 1966) and maintained as glycerol suspensions (20 %, v/v) at −80 °C.

Morphological characteristics were observed by light (Nikon ECLIPSE E200) and scanning electron microscopy (Hitachi S-3400N) using cultures grown on ISP 3 agar at 28 °C for 28 days. Cultural characteristics were determined on glucose-asparagine agar (Waksman, 1961), glucose-yeast extract (GY) agar (Jia et al., 2013), Czapek’s agar (Atlas, 2004), nutrient agar (Waksman, 1961) and ISP media 2–7 (Shirling & Gottlieb, 1966) after 14 days at 28 °C. Colour determination was done with colour chips from the ISCC-NBS colour charts standard samples No. 2106 (Kelly, 1964). Growth at different temperatures (4, 10, 15, 20, 28, 35, 37, 40 and 45 °C) was determined on ISP 3 medium after incubation for 14 days. Growth tests for pH range (pH 4–10, at intervals of 1 pH unit) were carried out by using the buffer system described by Xie et al. (2012), and NaCl tolerance was determined in GY medium supplemented with 0–7 % (w/v) NaCl at 28 °C for 14 days on a rotary shaker. Production of catalase, esterase and urease were tested as described by Smibert & Krieg (1994). The utilization of sole carbon and nitrogen sources, decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, peptonization of milk, liquefaction of gelatin and production of H₂S were examined as described previously (Gordon et al., 1974; Yokota et al., 1993).

Biomass for chemical studies was prepared by growing the strain in GY medium in shake flasks at 28 °C for 7 days and then cells were freeze-dried. The isomer of diaminopimelic acid in the cell-wall hydrolysates was derivatized according to McKerrow et al. (2000) and analysed using an HPLC method according to Jia et al. (2015). The whole-cell sugars were analysed according to the procedures developed by Lechevalier & Lechevalier (1980). The N-acetyl group of muramic acid in the peptidoglycan was determined according to method of Uchida et al. (1999). The presence of mycolic acids was checked by the acid methanolation method as described previously (Minnikin et al., 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 Collins (1985). Extracts were analysed using an HPLC-UV method (Wu et al., 1989). Fatty acid methyl esters were extracted from the biomass as described by Gao et al. (2014) and analysed by GC-MS using the method of Xiang et al. (2011).

The almost full-length 16S rRNA gene sequence of strain NEAU-ND5ᵀ (1515 bp) was obtained and aligned with multiple sequences obtained from the GenBank/EMBL/DDBJ databases using CLUSTAL X 1.83 software. Phylogenetic trees were reconstructed with the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms using MEGA software version 6.0 (Tamura et al., 2013). The stability of the topology of the phylogenetic trees was assessed using the bootstrap method with 1000 repetitions (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 (complete deletion option). 16S rRNA gene sequence similarities between strains were calculated on the basis of pairwise alignments using the EzTaxon-e server (Kim et al., 2012).

The G+C contents of the genomic DNA were determined by the thermal denaturation (Tm) method (Mandel & Mar, 1968) with Escherichia coli JM109 DNA as the control. DNA–DNA relatedness tests between strain NEAU-ND5ᵀ and closely related strains were carried out as described by De Ley et al. (1970) with consideration of the modifications described by Huss et al. (1983), using a model Cary 100 Bio UV/VIS spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with in situ temperature probe (Varian). The DNA samples used for hybridization were diluted to an OD₂₆₀ of around 1.0 using 0.1× SSC (solute sodium citrate buffer), then sheared using a JY92-II ultrasonic cell disruptor (ultrasonic time 3 s, interval time 4 s, 90 times). The DNA renaturation rates were determined in 2× SSC at 70 °C. The experiments were performed with three replications and the DNA–DNA relatedness values were expressed as the mean of the three values.

The morphological and cultural properties of strain NEAU-ND5ᵀ were consistent with its classification in the genus Nonomuraea. Strain NEAU-ND5ᵀ formed extensively branched substrate mycelia without fragmentation. Long spore chains were formed on the aerial mycelia and the spore surface was smooth (Fig. 1). The aerial mycelia were white on ISP 2 and ISP 3 agar. Cultural characteristics of strain NEAU-ND5ᵀ are shown in Table S1 (available in the online Supplementary Material). Strain NEAU-ND5ᵀ showed good growth on ISP 2, ISP 3, ISP 6 and nutrient agar, moderate growth on ISP 7,
Nonomuraea zeae sp. nov.

Fig. 2. Maximum-likelihood tree based on nearly complete 16S rRNA gene sequences (1515 nt) showing the relationship between strain NEAU-ND5T and members of the genus Nonomuraea. Amycolatopsis orientalis NBRC 12806T was used as an outgroup. Asterisks indicate branches of the tree that were also recovered using the neighbour-joining method. Bootstrap values >50% (based on 1000 replications) are shown at branch points. Bar, 0.01 substitutions per nucleotide position.

Strain NEAU-ND5T contained meeso-diaminopimelic acid as the cell-wall diamino acid, indicating that strain NEAU-ND5T is of cell-wall chemotype III (Lechevalier & Lechevalier, 1970). The whole-cell hydrolysates were glucose, ribose and madurose. The N-acetyl type of muramic acid in the peptido-glycan was acetyl. Mycolic acids were not detected. The predominant menaquinones detected were MK-9(H2) (72.3%), MK-9(H2) (16.6%) and MK-9(H6) (11.1%). The phospholipid profile was found to consist of diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, hydroxypolphatidylmonomethylethanolamine, hydroxy-phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannoside, corresponding to phospholipid type IV (Lechevalier et al., 1977) (Fig. S1, available in the online Supplementary Material). The cellular fatty acid profile comprised iso-C16:0 (24.9%), 10-methyl C17:0 (23.7%), C16:0 (9.3%), C17:0 ω7c (8.3%), C14:0 (7.0%), C17:0 (6.0%), 10-methyl C16:0 (6.0%), C16:1 ω7c (4.7%), C15:0 (4.5%), iso-C15:0 (2.7%), iso-C14:0 (1.5%) and C18:1ω7c (1.4%), corresponding
to fatty acid type 3b (Kroppenstedt, 1985). All these chemotaxonomic data indicate that strain NEAU-ND5\textsuperscript{T} can be assigned to the genus \textit{Nonomuraea}. The mean (±SD) DNA G+C content of strain NEAU-ND5\textsuperscript{T} was 69.7 ± 0.3 mol%.

EzTaxon-e analysis of the 16S rRNA gene sequence indicated that strain NEAU-ND5\textsuperscript{T} should be classified within the genus \textit{Nonomuraea}. Strain NEAU-ND5\textsuperscript{T} was found to be closely related to \textit{Nonomuraea jabiensis} A4036\textsuperscript{T} (98.29 % 16S rRNA gene sequence similarity), \textit{Nonomuraea rosea} GW12687\textsuperscript{T} (98.25 %), \textit{Nonomuraea candida} HMC10\textsuperscript{T} (98.22 %), \textit{Nonomuraea rhizophila} YIM 67092\textsuperscript{T} (98.04 %) and \textit{Nonomuraea kuesteri} NRII B-24325\textsuperscript{T} (98.04 %). The phylogenetic tree based on 16S rRNA gene sequences showed that strain NEAU-ND5\textsuperscript{T} formed a monophyletic clade with \textit{N. jabiensis} A4036\textsuperscript{T} in the maximum-likelihood tree (Fig. 2). However, this clade was not supported by a high bootstrap value with the neighbour-joining method (Fig. S2). To determine whether strain NEAU-ND5\textsuperscript{T} does indeed represent a novel genomic species, DNA–DNA hybridization was used to further clarify the relatedness between strain NEAU-ND5\textsuperscript{T} and \textit{N. jabiensis} DSM 45507\textsuperscript{T}, \textit{N. rosea} DSM 45177\textsuperscript{T}, \textit{N. candida} JCM 15928\textsuperscript{T}, \textit{N. rhizophila} DSM 45382\textsuperscript{T} and \textit{N. kuesteri} JCM 13854\textsuperscript{T}. The mean levels of DNA–DNA relatedness between them were 37.6±1.1, 32.3±0.5, 22.7±0.7, 27.9±0.3 and 23.5±0.9 %, respectively. These values are below the threshold value of 70 % recommended by Wayne et al. (1987) for assigning bacterial strains to the same genomic species.

Besides the genotypic evidence above, a comparison of phenotypic characteristics between strain NEAU-ND5\textsuperscript{T} and its closest neighbours, \textit{N. jabiensis} DSM 45507\textsuperscript{T}, \textit{N. rosea} DSM 45177\textsuperscript{T}, \textit{N. candida} JCM 15928\textsuperscript{T}, \textit{N. rhizophila} DSM 45382\textsuperscript{T} and \textit{N. kuesteri} JCM 13854\textsuperscript{T}, was performed to differentiate them (Table 1). Strain NEAU-ND5\textsuperscript{T} could be easily distinguished from \textit{N. jabiensis} DSM 45507\textsuperscript{T}, \textit{N. rosea} DSM 45177\textsuperscript{T}, \textit{N. candida} JCM 15928\textsuperscript{T}, \textit{N. rhizophila} DSM 45382\textsuperscript{T} and \textit{N. kuesteri} JCM 13854\textsuperscript{T} by the different colony colours on ISP 2 and ISP 3 after being incubated at 28 °C for 2 weeks. Moreover, strain NEAU-ND5\textsuperscript{T} was unable to utilize l-arginine, which distinguishes it readily from the closely related species. Other phenotypic differences such as the ability to reduce nitrate, growth with 3 % (w/v) NaCl, the temperature range for growth, decomposition of urea, degradation of starch, and utilization of certain carbon and nitrogen sources can also be used to differentiate the new isolate from closely related species.

Therefore, it is evident from the genotypic and phenotypic data that strain NEAU-ND5\textsuperscript{T} represents a novel species of the genus \textit{Nonomuraea}, for which the name \textit{Nonomuraea zeae} sp. nov. is proposed.

### Description of \textit{Nonomuraea zeae} sp. nov.

\textit{Nonomuraea zeae} (zeae. L fem. n. zeae a kind of grain; N.L. gen. n. zeae of the plant genus Zea).

Aerobic, Gram-stain-positive actinobacterium that forms extensively branched substrate mycelia that do not fragment. Long spore chains are formed on the aerial mycelia and the spore surface is smooth. Good growth is observed

<table>
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<td>l-Arabinose</td>
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<td>l-Serine</td>
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<td>Growth temperature range (°C)</td>
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<td>Growth with NaCl (3 %, w/v)</td>
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</table>
on ISP 2, ISP 3, ISP 6 and nutrient agar, moderate growth on ISP 7, glucose-yeast extract and Czapek’s agar, and poor growth on ISP 4, ISP 5 and glucose-asparagine agar. Colony colours vary from deep reddish orange to light yellowish pink. Positive for production of catalase and urease, and hydrolysis of aesculin and starch, but negative for reduction of nitrate, liquefaction of gelatin, decomposition of cellulose, production of H₂S and peptidation of milk. L-arabinose, D-fructose, D-glucose, myo-inositol, lactose, maltose, D-mannitol, D-mannose, D-raffinose, L-rhamnose, D-ribose, D-sorbitol and sucrose are utilized as sole carbon sources but D-galactose and D-xylene are not. L-Alanine, L-glutamic acid, L-glutamine, glycine, L-serine, L-threonine and L-tyrosine are utilized as sole nitrogen sources but L-arginine, L-asparagine and L-aspartic acid are not. The cell wall contains meso-diaminopimelic acid and the whole-cell sugars are glucose, ribose and madurose. The predominant menaquinones are MK-9(H₄), MK-9(H₂) and MK-9(H₆). The phospholipid profile contains diphasatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, hydroxy-phosphatidylmonomethylethanolamine, hydroxy-phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannoside. The predominant fatty acids are iso-C₁₆:0 and 10-methyl C₁₇:0.

The type strain is NEAU-NDSᵀ (=CGMCC 4.7280ᵀ=DSM 100528ᵀ), which was isolated from the rhizosphere of corn (Zea mays L.) collected in Heilongjiang Province, north-east China. The mean DNA G+C content of the type strain is 69.72±0.3 mol%.

Acknowledgements

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References


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for the extraction of bacterial isoprenoid quinones and polar lipids.


