**Nonomuraea glycinis** sp. nov., a novel actinomycete isolated from the root of black soya bean [*Glycine max* (L.) Merr]

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**Abstract**

A novel actinomycete, designated strain NEAU-BB2C19¹, was isolated from the root of black soya bean [*Glycine max* (L.) Merr] collected from Harbin, Heilongjiang Province, China, and characterized using a polyphasic approach. The strain was an aerobic, Gram-stain-positive actinomycete that formed extensively branched substrate mycelium and aerial hyphae. The predominant menaquinones were MK-9(H₆) and MK-9(H₈). The major cellular fatty acid profile consisted of iso-C₁₆:0, 10-methyl C₁₇:0 and 10-methyl C₁₈:0. The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, phosphatidylglycerol, phosphatidylmannoside, and phosphatidylglycerol and glycolipid.

The DNA G+C content was 68.2±0.4 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain NEAU-BB2C19¹ should be assigned to the genus *Nonomuraea* and formed a distinct branch with its closest neighbour *Nonomuraea guangzhouensis* NEAU-ZJ3¹ (98.75% 16S rRNA gene sequence similarity). The morphological and chemotaxonomic properties of the strain were also consistent with those of members of the genus *Nonomuraea*. A combination of DNA–DNA hybridization results and some phenotypic characteristics indicated that strain NEAU-BB2C19¹ could be clearly differentiated from its closest phylogenetic relative. Thus, the strain is concluded to represent a novel species of the genus *Nonomuraea*, for which the name *Nonomuraea glycinis* sp. nov. is proposed. The type strain is NEAU-BB2C19¹ (=CGMCC 4.7430¹=DSM 104838¹).

The genus *Nonomuraea* belongs to the family *Streptosporangiaceae*, which was first proposed by Zhang et al. [1]. The name was subsequently corrected by Chiba et al. [2]. The type species is *Nonomuraea pusilla* [1, 3]. Members of the genus form extensively branched substrate and aerial mycelia. Aerial hyphae generally bear chains of spores which are hooked, spiral or straight, but single spores may be produced [4–6]. At the time of writing, the genus *Nonomuraea* contains 39 species and two subspecies with validly published names, including the latest described species *Nonomuraea purpurea* [7], *Nonomuraea ceiba* [8] and *Nonomuraea rhodomyclinica* [9]. Members of the family *Streptosporangiaceae* are widespread in soil [9], mangrove sediments [7], mushroom compost [10], rhizospheric soil [11] and also the endophytic environment, such as leaf of *Maytenus austroyunnanensis* [12], plant tissues of *Artemisia annua* L. [13], and the root of eggplant (*Solanum melongena* L.) [14] and jambolan plum tree (*Syzygium cumini* L. Skeels) [15]. In the course of investigation of novel actinomycetes from the root of black soya bean, an actinomycete strain, NEAU-BB2C19¹T, was isolated. Here we report on the taxonomic characterization and classification of the isolate and propose that NEAU-BB2C19¹T represents a novel species of the genus *Nonomuraea*.

Strain NEAU-BB2C19¹T was isolated from the root of black soya bean [*Glycine max* (L.) Merr] collected from Northeast Agriculture University, Harbin, Heilongjiang Province, China (44° 04' N 125° 42' E). The plant, which was stored in a clean plastic bag, was collected from a greenhouse and brought back to the laboratory. The root sample was air-

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**Keywords:** *Nonomuraea glycinis* sp. nov.; polyphasic taxonomy; 16S rRNA gene; black soya bean.

**Abbreviations:** CA, Crzapek’s agar; CGMCC, China General Microbiological Culture Collection Center; CPA, cellulose-proline agar; CSC, Chang Jiang Scholar Candidates Program; CSCP, Chang Jiang Scholar Candidates Program; DPG, diphosphatidylglycerol; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen; Gal, galactose; GC-MS, gas chromatography-mass spectrometer; GL, glycolipid; Glu, glucose; GTR, General Time Reversible; Gy, glucose-yeast extract powder; ISCC-NBS, Inter-society color council-national bureau of standards; ISP, International Streptomyces Project; Mad, madurose; MBA, Bennett’s agar; MEGA, Molecular Evolutionary Genetics Analysis; meso-DAP, meso-diaminopimelic acid; NA, nutrient agar; OHP, hydroxy-phosphatidylethanolamine; OH-PM, hydroxyphosphatidylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PME, phosphatidylmethylethanolamine; Rib, ribose; SSC, saline-sodium citrate.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NEAU-BB2C19¹ is KY328642.

One supplementary table and three supplementary figures are available with the online version of this article.
dried for 24 h at room temperature and then washed in water with an ultrasonic step (160 W, 15 min) to completely remove soil and adherent epiphytes. After drying, the sample was cut into pieces 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\textsuperscript{−1}) and nalidixic acid (20 mg l\textsuperscript{−1}), followed by a wash in sterile water, a 5 min wash in 5 % (v/v) NaClO, a 10 min wash in 2.5 % (w/v) Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3}, a 5 min wash in 75 % (v/v) ethanol, a wash in sterile water and a final rinse in 10 % (w/v) NaHCO\textsubscript{3} for 10 min, and then the rinsed root sample was dried at 100 °C for 15 min. Subsequently, the sample was cut up in a commercial blender and ground with a mortar and pestle, employing 1 ml of 0.5 M potassium phosphate buffer (pH 7.0) per 100 mg of tissue. Tissue samples were dried at 100 °C using ISP media 1–7 [16] supplemented with cycloheximide (50 mg l\textsuperscript{−1}) and nalidixic acid (50 mg l\textsuperscript{−1}). After 15 days of aerobic incubation at 28 °C, a colony was transferred and purified on oatmeal agar [International Streptomyces Project (ISP) 3 medium] [17] and maintained as glycerol suspensions (20 %, v/v) at −80 °C.

Morphological properties were observed by light microscopy (ECLIPSE E200; Nikon) and scanning electron microscopy (SU8010; Hitachi) using cultures grown on ISP 3 agar at 28 °C for 28 days. Cultural characteristics were determined after 14 days at 28 °C using ISP media 1–7 [17], nutrient agar (NA) [18], Czapek’s agar (CA) [19] and Bennett’s agar (MBA) [20]. ISCC-NBS colour charts [21] were used to determine colours of substrate and aerial mycelia. Growth at different temperatures (4, 10, 15, 20, 28, 35, 37 and 41 °C) was determined on ISP 3 medium after incubation for 14 days. Growth tests for 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) were done in GY medium at 28 °C using ISP media 1–7 [17]. Nutrient agar (NA) [18], Czapek’s agar (CA) [19] and Bennett’s agar (MBA) [20]. ISCC-NBS colour charts [21] were used to determine colours of substrate and aerial mycelia. Growth at different temperatures (4, 10, 15, 20, 28, 35, 37 and 41 °C) was determined on ISP 3 medium after incubation for 14 days. Growth tests for 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) were done in GY medium at 28 °C using ISP media 1–7 [17].

Fig. 1. Scanning electron micrograph of spore chains of strain NEAU-BB2C19\textsuperscript{T} grown on ISP 3 agar for 4 weeks at 28 °C. Bar, 5 µm.

and substrate mycelium on ISP 3 medium were white and vivid yellow, respectively (Table S1, Fig. S1, available in the online version of this article). No diffusible pigments or melanin were observed on any of the tested media. Strain NEAU-BB2C19\textsuperscript{T} grew at a temperature range of 10–37 °C (optimum 28 °C), pH 7–9 (optimum 7–8) and NaCl concentrations of 0–4 % (w/v). Detailed physiological characteristics are presented in the species description and Table 1.

Biomass for chemical studies was prepared by growing the strain in GY broth [25] in shake flasks at 28 °C for 7 days. The isomer of diaminopimelic acid in the cell wall was determined according to McKerrow et al. [26] and analysed by HPLC using an Agilent TC-C18 column (250×4.6 mm, i.d. 5 µm). The whole-cell sugars were analysed according to the procedures developed by Lechevalier and Lechevalier [27]. Polar lipids in cells were examined by two-dimensional TLC and identified using the method of Minnikin et al. [28]. Menaquinones were extracted from freeze-dried biomass, purified according to Collins [29] and analysed by HPLC-UV method as described previously [30]. To determine cellular fatty acid compositions, strain NEAU-BB2C19\textsuperscript{T} was cultivated in ISP 2 broth for 5 days in shake flasks at 28 °C. Fatty acid methyl esters were extracted from the biomass as described by Gao et al. [31] and analysed by GC-MS using the method of Xiang et al. [32].

Strain NEAU-BB2C19\textsuperscript{T} was found to exhibit a range of chemotaxonomic properties that are typical of members of the genus Nonomuraea. We found meso-diaminopimelic acid as the cell-wall diamino acid and whole-cell sugars included galactose and madurose. The polar lipid profile consisted of diphasphatidylglycerol, phosphatidylethanolamine, hydroxyphosphatidylethanolamine, phosphatidylglycerol and glycolipid. The colours of aerial mycelium and substrate mycelium on ISP 3 medium were white and vivid yellow, respectively (Table S1, Fig. S1, available in the online version of this article). No diffusible pigments or melanin were observed on any of the tested media. Strain NEAU-BB2C19\textsuperscript{T} grew at a temperature range of 10–37 °C (optimum 28 °C), pH 7–9 (optimum 7–8) and NaCl concentrations of 0–4 % (w/v). Detailed physiological characteristics are presented in the species description and Table 1.

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those of *Nonomureaea guangzhouensis* NEAU-ZJ3\textsuperscript{T} were MK-9(H\textsubscript{4}) (37.5 %), MK-9(H\textsubscript{2}) (33.3 %) and MK-9(H\textsubscript{0}) (21.7 %); MK-8(H\textsubscript{2}) (7.5 %) was also present [33]. The cellular fatty acid profile was composed of iso-C\textsubscript{16:0} (27.3 %), 10-methyl C\textsubscript{17:0} (17.6 %), 10-methyl C\textsubscript{18:0} (10.7 %), C\textsubscript{16:0} (8.3 %), C\textsubscript{17:1} \(\omega 7c\) (6.4 %), C\textsubscript{17:0} (5.0 %), C\textsubscript{18:0} (4.2 %), C\textsubscript{18:1} \(\omega 7c\) (4.1 %), C\textsubscript{15:0} (3.5 %), 10-methyl C\textsubscript{16:0} (3.8 %), anteiso-C\textsubscript{17:0} (2.9 %), iso-C\textsubscript{15:0} (2.0 %) and C\textsubscript{16:1} \(\omega 7c\) (1.9 %).

Extraction of chromosomal DNA and PCR amplification of the 16S rRNA gene sequence were carried out using the methods of Kim et al. [34]. The PCR product was purified and cloned into the vector pMD19-T (Takara) and sequenced using an Applied Biosystems DNA sequencer (model 3730XL). The almost full-length 16S rRNA gene sequence of strain NEAU-BB2C19\textsuperscript{T} (1514 bp) was obtained and aligned with multiple sequences obtained from the GenBank/EMBL/DDBJ databases using CLUSTAL X 1.83 software [35]. Phylogenetic trees were reconstructed with the neighbour-joining [36] and maximum-likelihood [37] algorithms using MEGA software version 6.06 [38]. The stability of the clades of the phylogenetic trees was assessed using the bootstrap method with 1000 replications [39]. Phylogenetic distances were calculated with the Kimura two-parameter model for the neighbour-joining method and General Time Reversible model with Invariant sites (GTR+G+I) for the maximum-likelihood method [40, 41]. In addition, a heuristic search was performed using nearest neighbour interchange branch swapping in maximum-likelihood inference. 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 alignment using the EzBioCloud server [42]. The G+C content of the genomic DNA was determined using the thermal denaturation (\(T_m\)) method [43] with *Escherichia coli* JM109 DNA used as the control. DNA–DNA relatedness tests between the new isolate and its closest related strain were carried out as described by de Ley et al. [44] under consideration of the modifications described by Huss et al. [45], 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 OD\textsubscript{260} of around 1.0 using 0.1× SSC (saline 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

Table 1. Differential characteristics between strain NEAU-BB2C19\textsuperscript{T} and the type strain of *Nonomureaea guangzhouensis*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth on ISP 3 medium</td>
<td>White</td>
<td>White</td>
</tr>
<tr>
<td>Aerial mycelium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substrate mycelium</td>
<td>Yellowish white</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>Temperature range for growth (°C)</td>
<td>10–37</td>
<td>4–32</td>
</tr>
<tr>
<td>pH range for growth</td>
<td>7–9</td>
<td>5–9</td>
</tr>
<tr>
<td>NaCl tolerance (% w/v)</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Decomposition of cellulose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Liquefaction of gelatin</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Reduction of nitrate</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Peptization of milk</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Production of catalase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\alpha)-Fructose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>(\alpha)-Ribose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Malose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>(\alpha)-Sorbitol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Phospholipids*</td>
<td>DPG, PE, OH-PE, PIM, PG, PI, GL</td>
<td>DPG, PIME,OH-PME, PE, OH-PE, PIM, PI, PG, GL</td>
</tr>
<tr>
<td>Major menaquinones</td>
<td>MK-9(H\textsubscript{2} \textsubscript{2}), o</td>
<td>MK-9(H\textsubscript{4} \textsubscript{2}, o)</td>
</tr>
<tr>
<td>Whole-cell sugars</td>
<td>Gal, Mad</td>
<td>Rib, Glu, Mad</td>
</tr>
<tr>
<td>Major fatty acids (&gt;10%)</td>
<td>iso-C\textsubscript{16:0}, 10-methyl C\textsubscript{17:0}, 10-methyl C\textsubscript{18:0}</td>
<td>C\textsubscript{16:0}, 10-methyl C\textsubscript{17:0}</td>
</tr>
</tbody>
</table>

PG, diphosphatidylglycerol; GL, glycolipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PI, one unknown phospholipid; PME, phosphatidylinositol monomethylethanolamine; OH-PE, hydroxy-phosphatidylethanolamine; OH-PME, hydroxy-phosphatidylmonomethylethanolamine.
Fig. 2. Neighbour-joining tree showing the phylogenetic position of strain NEAU-BB2C19<sup>T</sup> and related taxa based on 16S rRNA gene sequences. The out-group used was Thermopolyspora flexuosa DSM 43186<sup>T</sup> (AY039253). Only bootstrap values above 50% (percentages of 1000 replications) are indicated. Asterisks indicate branches also recovered in the maximum-likelihood tree. Bar, 0.005 nucleotide substitutions per site.
replications and the DNA–DNA relatedness values were expressed as the mean of the three values.

Based on the EzBiocloud analysis, strain NEAU-BB2C19\(^T\) was related to the genus *Nonomuraea* with the highest 16S rRNA gene sequence similarity to *N. guangzhouensis* NEAU-ZJ3\(^T\) (98.75 %), *Nonomuraea indica* DBQ-2\(^T\) (98.28 %), *Nonomuraea endophytica* YIM 65601\(^T\) (98.20 %), *Nonomuraea salmonnea* DSM 43678\(^T\) (98.12 %) and *Nonomuraea turkmenica* DSM 43926\(^T\) (98.02 %). The phylogenetic tree based on 16S rRNA gene sequences showed that strain NEAU-BB2C19\(^T\) formed a phyletic line with its closest neighbour *N. guangzhouensis* NEAU-ZJ3\(^T\), which was supported by a bootstrap value of 78 % in the neighbour-joining tree (Fig. 2). The tree topology in this region was also supported by the maximum-likelihood algorithm, with a bootstrap value of 77 % (Fig. S3). The DNA G+C content of strain NEAU-BB2C19\(^T\) was 68.2±0.4 mol%. DNA–DNA hybridization was employed to further clarify the relatedness between the new isolate and *N. guangzhouensis* NEAU-ZJ3\(^T\). The level of DNA–DNA relatedness between them was 36.3±0.6 %, which was below the threshold value of 70 % recommended by Wayne et al. [46] for assignment of strains to the same genomic species.

A comparison of phenotypic characteristics between strain NEAU-BB2C19\(^T\) and its closest neighbour, *N. guangzhouensis* NEAU-ZJ3\(^T\), was performed to differentiate the strains (Tables 1 and S1, Fig. S3). Differential cultural characteristics included: good growth observed on ISP 3, ISP 4 and ISP 7 media for strain NEAU-BB2C19\(^T\), in contrast to *N. guangzhouensis* NEAU-ZJ3\(^T\) where no growth was observed; NaCl tolerance of strain NEAU-BB2C19\(^T\) was up to 4 % (w/v), which is higher than that of *N. guangzhouensis* NEAU-ZJ3\(^T\); and strain NEAU-BB2C19\(^T\) could grow at 37 °C while *N. guangzhouensis* NEAU-ZJ3\(^T\) could not. Other phenotypic differences are shown in Table 1, including reduction of nitrate, peptonization of milk, liquefaction of gelatin, decomposition of cellulose, production of catalase, and utilization of D-fructose, maltose, raffinose, D-ribose, D-sorbitol and L-threonine. In addition, other chemotaxonomic characteristics, such as the absence of phosphatidymethylethanolamine in the polar lipid profile and the absence of MK-9(H\(_{4}\)) as a predominant menaquinone, could also be used to distinguish strain NEAU-BB2C19\(^T\) from *N. guangzhouensis* NEAU-ZJ3\(^T\).

In conclusion, it is evident from the genotypic and phenotypic data that strain NEAU-BB2C19\(^T\) represents a novel species of the genus *Nonomuraea*, for which the name *Nonomuraea glycinis* sp. nov. is proposed.

**DESCRIPTION OF NONOMUREA GLYCINIS SP. NOV.**

*Nonomuraea glycinis* (gly.c'i.nis. N.L. fem. gen. n. glycinis of Glycine max, the soybean).

Aerobic, Gram-stain-positive actinomycete that forms extensively branched substrate mycelium and aerial hyphae. The colours of aerial mycelium and substrate mycelium on ISP 3 medium are white and vivid yellow, respectively. Spinal spore chains are composed of 14–16 motile spores (0.82×1.00 μm) with a wrinkled surface that are borne directly on aerial mycelia. Sporangia are not detected. Growth on ISP 2, ISP 3, ISP 4, ISP 5, ISP 7, NA and MPA media, and moderate growth on ISP 1, ISP 6 and CA media. Growth occurs at pH 7–9 (optimum pH 7–8), at 10–37 °C (optimum 28 °C) and in the presence of 0–4 % (w/v) NaCl. Positive for hydrolysis of aesculin and urea and decomposition of cellulose, but negative for hydrolysis of Tweens (20, 40 and 80), production of H\(_2\)S, peptonization of milk, liquefaction of gelatin, production of catalase and reduction of nitrate. D-Galactose, D-glucose, inositol, lactose, maltose, D-mannitol, D-mannose, L-rhamnose, sucrose and D-xylose are utilized as sole carbon sources but not L-arabinose, D-fructose or D-ribose. L-Arginine, L-asparagine, L-aspartic acid, creatine, L-glutamic acid, L-glutamine and L-serine are utilized as sole nitrogen sources but not glycine, L-threonine or L-tyrosine. Cell walls contain meso-diaminopimelic acid as the diagnostic diamino acid and the whole-cell sugars are galactose and madurose. The predominant menaquinones are MK-9(H\(_{4}\)) and MK-9(H\(_{8}\)). The polar lipid profile comprises diphasphatidylglycerol, phosphatidylethanolamine, hydroxy-phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside, phosphatidylglycerol and glycolipid. The major cellular fatty acids are iso-C\(_{16:0}\), 10-methyl C\(_{17:0}\) and 10-methyl C\(_{18:0}\).

The type strain is NEAU-BB2C19\(^T\) (=CGMCC 4.7430\(^T\) =DSM 104838\(^T\)), isolated from root of black soybean [Glycine max (L.) Merr.] collected from Harbin, Heilongjiang Province, China. The DNA G+C content of the type strain is 68.2±0.4 mol%.

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**Conflicts of interest**

The authors declare that there are no conflicts of interest.

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