Plantactinospora soyae sp. nov., an endophytic actinomycete isolated from soybean root [Glycine max (L.) Merr]

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A novel actinomycete, designated strain NEAU-gxj3†, was isolated from soybean root [Glycine max (L.) Merr.] collected from Harbin, Heilongjiang Province, China, and characterized using a polyphasic approach. The 16S rRNA gene sequence of strain NEAU-gxj3† showed highest similarity to those of Micromonospora equina Y22† (98.2 %) and Plantactinospora endophytica YIM 68255† (98.0 %). Phylogenetic analysis based on the 16S rRNA gene and gyrB gene demonstrated that the isolate clustered with the members of the genus Plantactinospora. The chemotaxonomic properties of strain NEAU-gxj3† were also consistent with those of members of the genus Plantactinospora. The cell wall contained meso-diaminopimelic acid and whole-cell sugars were xylose, glucose and galactose. The predominant menaquinones were MK-10(H6), MK-9(H8), MK-10(H2) and MK-10(H4). The polar lipid profile consisted of diphasphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannoside. The major fatty acids were identified as anteiso-C₁₇:0, iso-C₁₆:0, iso-C₁₅:0 and C₁₅:0. A combination of DNA–DNA hybridization result and some phenotypic characteristics indicated that strain NEAU-gxj3† could be differentiated clearly from its closest phylogenetic relatives. Therefore, the strain is concluded to represent a novel species of the genus Plantactinospora, for which the name Plantactinospora soyae sp. nov. is proposed. The type strain is NEAU-gxj3† (=CGMCC 4.7221† =DSM 46832†).

The genus Plantactinospora, belonging to the family Micro- monosporaceae, was established by Qin et al. (2009) with Plantactinospora mayteni as the type species, and its description has been emended by Zhu et al. (2012). Most members of the genus lack aerial mycelium, except Plantactinospora mayteni YIM 61359†, which produces sparse aerial mycelium (Qin et al., 2009). Spores are borne directly on the substrate hyphae, singly or as conglomerates. The genus is identified chemotaxonomically by the presence of meso-diaminopimelic acid in cell wall and arabinose, galactose, glucose, mannose, xylose and rhamnose in the whole-cell hydrolysates, MK-10(H₆) or MK-9(H₆) as predominant isopenologues, phosphatidylethanolamine, diphasphatidylglycerol and phosphatidylglycerol as major phospholipids (phospholipid type PII), and iso-C₁₅:0, anteiso-C₁₇:0, iso-C₁₆:0 and anteiso-C₁₅:0 as major fatty acids (fatty acid type 2d). At the time of writing, the genus comprised five species with validly published names (http://www.bacterio.net/s/plantactinospora.html) including the recently described Plantactinospora veratri (Xing et al., 2015) and Plantactinospora sonchi (Ma et al., 2015).

Soybean [Glycine max (L.) Merr.] is one of the most important oil crops of the world, which also has tremendous importance as a food legume. Research into beneficial microorganisms in soybean has been primarily focused on
rhizobia, however, with relatively little attention paid to other bacterial and fungal species. As part of a programme to research the diversity of endophytic actinomycetes in soybean root and to discover novel actinomycetes and novel natural products, we have identified two novel species of the genus *Streptomyces*, which produce borrelidin and ikarugamycin, respectively (Liu et al., 2013a, b), a novel genus of the family *Micromonosporaceae* (Jia et al., 2013), and a novel species of the genus *Actinoplanes* (Shen et al., 2013). In this study, we performed polyphasic taxonomy on another strain, designated strain NEAU-gxj3<sup>T</sup>, and propose that this strain represents a novel species of the genus *Plantactinospora*, named *Plantactinospora soyae* sp. nov.

Strain NEAU-gxj3<sup>T</sup> was isolated from soybean root collected from Harbin, Heilongjiang province, north China (45° 54′ N 126° 52′ E). The root sample was processed as described by Liu et al. (2013b) and placed on a plate of dulcitol-proline agar (dulcitol 0.2 %, proline 0.05 %, K<sub>2</sub>HPO<sub>4</sub> 0.03 %, NaCl 0.03 %, CaCl<sub>2</sub> 2H<sub>2</sub>O 0.1 %, MgSO<sub>4</sub> 7H<sub>2</sub>O 0.1 %, agar 1.5 %, w/v, pH 7.2–7.4) supplemented with nystatin (50 mg l<sup>−1</sup>) and nalidixic acid (20 mg l<sup>−1</sup>). 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 (SEM) (Hitachi S-3400N) using cultures grown on ISP 3 agar at 28 °C for 28 days. Spore motility was assessed by light microscopic (Nikon ECLIPSE E200) observation of cells suspended in phosphate buffer (pH 7.0, 1 mM). Cultural characteristics were determined after 14 days at 28 °C using ISP media 2–7, nutrient agar, Czapek–Dox agar and Bennett’s agar (Shirling & Gottlieb, 1966; Jones, 1949; Waksman, 1961). 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, 20, 28, 30, 32, 35, 37, 40 and 45 °C) was determined on ISP 3 medium after incubation for 14 days. Growth at different pH (pH 4–11, at intervals of 1.0 pH unit, assessed by using the buffer system described by Xie et al., 2012) and NaCl concentrations (0–7 %, with an interval of 1 %, w/v) were tested in GY broth (Jia et al., 2013) at 28 °C for 14 days on a rotary shaker. Production of catalase and urease was 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 H<sub>2</sub>S were examined as described by Gordon et al. (1974) and Yokota et al. (1993).

Biomass for chemical studies was prepared by growing the strain in GY broth in shake flasks at 28 °C for 7 days. Cells were harvested by centrifugation, washed with distilled water and freeze-dried. The isomer of diaminopimelic acid in the cell-wall hydrolysates was derivatized according to the method of McKerrow et al. (2000) and analysed by a HPLC method using an Agilent TC-C18 column (250×4.6 mm i.d. 5 µm) with a mobile phase consisting of acetonitrile/0.05 mol phosphate buffer (1 : 1), pH 7.2 (15 : 85) at a flow rate of 0.5 ml min<sup>−1</sup>. An Agilent G1321A fluorescence detector was used for peak detection with 365 nm excitation and 455 nm longpass emission filters. The whole-cell sugars were analysed according to the procedures developed by Lechevalier & Lechevalier (1980). The polar lipids 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 a HPLC-UV method (Wu et al., 1989) using an 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). Mycolic acids were checked by the acid methanolysis method as described by Minnikin et al. (1980). To determine cellular fatty acid compositions, strain NEAU-gxj3<sup>T</sup> was cultivated in GY broth in shake flasks at 28 °C for 7 days. 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) and identified with the NIST MS Search 2.0 database.

Extraction of chromosomal DNA and PCR amplification of the 16S rRNA gene sequence were carried out using standard procedures (Kim et al., 2000). 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-gxj3<sup>T</sup> (1512 bp) was obtained and aligned with multiple sequences obtained from the GenBank/EMBL/DDBJ databases using CLUSTAL X 1.83 software. The strains belonging to species with validly published names that had a similarity with NEAU-gxj3<sup>T</sup> higher than 97 % were chosen to reconstruct phylogenetic trees. The alignments were verified manually and adjusted prior to the reconstruction of phylogenetic trees. Phylogenetic trees were reconstructed with neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms using MEGA software version 6.06 (Tamura et al., 2013). The stability of the clades of the phylogenetic trees was assessed using the bootstrap method with 1000 replications (Felsenstein, 1985). Phylogenetic distances were calculated with the Kimura two-parameter model for the neighbour-joining method and the General Time Reversible model with Invariant sites (GTR+G+I) for the maximum-likelihood method (Kimura, 1983; Tamura & Nei, 1993). 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 EzTaxon-e server (Kim et al., 2012). The almost full-length gyrB gene sequence of strain...
Macromonomospora cremae CR301 (FN658654)  
Macromonomospora coiiae NAR01 (AY784008)  
Macromonomospora endotricha DSM 44399T (A856039)  
Macromonomospora chersina DSM 44151T (X92628)  
Macromonomospora halotolerans CR18T (FN658652)  
Macromonomospora tubiflagi TVU1T (EU196562)  
Macromonomospora rosaria DSM 803T (X92631)  
Macromonomospora sagamiensis DSM 43912T (X92624)  
Macromonomospora inositolata DSM 43819T (X92610)  
Macromonomospora costi CS1-12T (AB981048)  
Macromonomospora fulviviridis DSM 43936T (X92620)  
Macromonomospora ebuea LK2-10T (AB107231)  
Macromonomospora narathaiensis BTG4-1T (AB193559)  
Macromonomospora siamensis TT2-4T (AB193565)  
Macromonomospora chokorensis 2-1960T (AB241454)  
Macromonomospora humi P0402T (GU459068)  
Macromonomospora aureus 2-30-b28T (AB241455)  
Macromonomospora maritima D10-9-5T (HQ704071)  
Macromonomospora marina JSM1-1T (AB196712)  
Macromonomospora aurantiaca ATCC 27029T (CP002162)  
Macromonomospora echinofusca DSM 43913T (X92625)  
Macromonomospora guersey DSM 43836T (X92603)  
Macromonomospora citrea DSM 43903T (X92617)  
Macromonomospora conreaula DSM 43143T (X92598)  
Macromonomospora chayaphumensis MCS-1T (AB196710)  
Macromonomospora aurantiaca JSM2-19T (AB159779)  
Macromonomospora palita DSM 43817T (X92608)  
Macromonomospora viridifaciens DSM 43909T (X92623)  
Macromonomospora echinaceata DSM 43904T (X92618)  
Macromonomospora nigra DSM 43818T (X92609)  
Macromonomospora yagouensis FX6.01T (GU002071)  
Plantactinospora soya NEAU-gx3T (KM359703)  
Plantactinospora siamensis CM2-8T (AB454379)  
Plantactinospora endophytica 'YM 882590T' (GQ494033)  
Plantactinospora szechuanica JCM 30345T (KM108473)  
Plantactinospora venturi DSM 46178T (KF888635)  
Plantactinospora mayenense YM 61356T (FJ214343)  
Micromomonospora equina Y22T (JP912511)  
Micromomonospora avicenniae 268506T (JQ867183)  
Micromomonospora polyrhachis NEAU-gxcm2T (KC139400)  
Micromomonospora sonneratiae 274745T (JQ619535)  
Micromomonospora pisii GUI 15T (AM944497)  
Micromonomospora rubra DSM 44944T (AB223089)  
Micromonomospora pattalaeensis J12-2T (AB275500)  
Micromonomospora olivasterospora DSM 43886T (X92619)  
Micromonomospora scabriplcia DSM 45730T (JG073709)  
Verrucosispora quiae NBRC 10684T (EU427445)  
Jishengella endophytica DSM 45430T (EU560726)  
Salinispora pacifica DSM 45820T (DQ224161)  
Salinispora arenicola DSM 44819T (AY040819)  
Salinispora tropicalis CBP-440T (CP002067)  

**Fig. 1.** Neighbour-joining tree showing the phylogenetic position of strain NEAU-gx3T and related taxa based on 16S rRNA gene sequences. Asterisks denote branches that were also recovered using the maximum-likelihood method. Bootstrap values above 50% (based on 1000 replications) are shown at branch points. Bar, 0.002 substitutions per nucleotide position.

NEAU-gx3T (1159 bp) was obtained and aligned with multiple sequences obtained from the GenBank/EMBL/DDBJ databases using CLUSTAL X 1.83 software. The gene encoding the DNA gyrase b-subunit, which has been widely used in bacterial taxonomy, was sequenced with primers GYF1 and GYR3B (Garcia et al., 2010) under the PCR programme for the 16S rRNA gene. Sequencing and phylogenetic analysis were performed as described above.
Fig. 2. Neighbour-joining phylogenetic tree showing the positions of strain NEAU-gg3<sup>T</sup> and related taxa based on gyrB gene sequences. *Actinoplanes regularis* NBRC 12514<sup>T</sup> was used as an outgroup. Asterisks indicate branches of the tree that were also recovered using the maximum-likelihood method. Numbers at branch points indicate bootstrap percentages (based on 1000 replicates); only values >50% are indicated. Bar, 0.02 substitutions per nucleotide position.

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The G+C contents of the genomic DNA were determined using the thermal denaturation \(T_m\) method (Mandel & Marmur, 1968) with *Escherichia coli* JM109 DNA used as the control. DNA–DNA relatedness tests between the novel isolate and closely related strains were carried out as described by De Ley et al. (1970) under consideration of the modifications described by Huss et al. (1983), using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6x6 multicell changer and a temperature controller with in situ temperature probe (Variian). The concentration and purity of DNA samples were determined by measuring the optical density at 260, 280 and 230 nm. The DNA samples used for hybridization were diluted to OD\(_{260}\) around 1.0 using 0.1 \(\times\) 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 \(\times\) SSC at 70 °C. The experiments were performed with three replications and the DNA–DNA relatedness values were expressed as mean of the three values.

Morphological observation of a 4-week-old culture of strain NEAU-gxj\(^T\) grown on ISP3 medium revealed that it formed extensively branched substrate mycelium without fragmentation. Aerial hyphae and sporangia were not present. Non-motile spores (0.6–0.7\(\times\)0.7–0.8 \(\mu\)m) were borne singly on the substrate mycelium and the spore surface was smooth (Fig. S1, available in the online Supplementary Material). Strain NEAU-gxj\(^T\) grew well on ISP 3, ISP 6, Czapek’s and Bennett’s agar, moderately on ISP 2, nutrient agar and glucose-aspargine agar, and poorly on ISP 4, ISP 5 and ISP 7 media. The colony colours of strain NEAU-gxj\(^T\) were observed to be pale yellow to strong orange-yellow. No diffusible pigments or melanin were observed on any of the media tested. Strain NEAU-gxj\(^T\) grew within a temperature range of 20–32 °C (optimum temperature 28 °C), at pH 6–10 (optimum pH 7–8) and with NaCl concentrations of 0–1 %. Detailed physiological characteristics are presented in the species description.

Chemotaxonomic analyses revealed that strain NEAU-gxj\(^T\) exhibited characteristics which are typical of members of the genus *Plantactinospora*. It contained meso-diaminopimelic acid as the cell-wall diamino acid and whole-cell sugars included xylose, glucose and galactose. The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannoside, corresponding to phospholipid type II of Lechevalier et al. (1977) (Fig. S2). The predominant menaquinones detected were MK-10(H\(_4\)) (42.2 %), MK-9(H\(_6\)) (16.5 %), MK-10(H\(_2\)) (13.7 %) and MK-10(H\(_4\)) (11.5 %), with minor amounts of MK-9(H\(_4\)) (9.0 %) and MK-9(H\(_6\)) (7.0 %). The cellular fatty acid profile was determined to be composed of anteiso-C\(_{17}:0\) (15.8 %), iso-C\(_{16}:0\) (15.6 %), iso-C\(_{15}:0\) (15.5 %), C\(_{15}:0\) (13.7 %), C\(_{16}:1\omega7c\) (9.3 %), C\(_{18}:0\) (4.5 %), C\(_{19}:0\) (4.5 %), C\(_{16}:0\) (3.9 %) and C\(_{18}:1\omega7c\) (3.6 %), which corresponds to fatty acid type 2d of Kroppenstedt (1985). Mycolic acids were not detected.

The nearly complete 16S rRNA gene sequence (1512 bp) of strain NEAU-gxj\(^T\) was subjected to EzTaxon-e analysis

**Table 1. Differential characteristics of strain NEAU-gxj\(^T\) and its most closely related species**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NEAU-gxj(^T)</th>
<th>*Plantactinospora endophytica YIM 68255(^T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour on ISP 3</td>
<td>Light yellow to pale greenish yellow</td>
<td>Orange red to brown</td>
</tr>
<tr>
<td>Growth temperature (°C)</td>
<td>20–32</td>
<td>10–45</td>
</tr>
<tr>
<td>Growth pH</td>
<td>6–10</td>
<td>5–10</td>
</tr>
<tr>
<td>Maximum NaCl tolerance (% w/v)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Decomposition of urea</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Fructose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>d-Galactose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>d-Mannitol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>d-Mannose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>d-Ribose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>d-Sorbitol</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>d-Xylose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Glycine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>70.3</td>
<td>73*</td>
</tr>
</tbody>
</table>

*Data from Zhu et al. (2012).*
together with members of the family *Micromonosporaceae* (>97.0 % similarities). The highest sequence similarities (>97.0 %) were between strain NEAU-gxj3T and members of the genera *Micromonospora*, *Plantactinospora*, *Polymerophossa*, *Xiangella*, *Salinispora*, *Verruicospora* and *Fushegnella* (97.0–98.2 %). The phylogenetic results based on 16S rRNA gene and gyrB gene sequences clearly showed that strain NEAU-gxj3T belonged to the genus of *Plantactinospora* (Figs 1, 2), with highest 16S rRNA gene and gyrB gene sequence similarities to *Plantactinospora endophytica* YIM 68255T (98.0 % and 93.2 %, respectively) in the genus *Plantactinospora*. DNA–DNA hybridization was employed to further clarify the relatedness between the isolate and *Plantactinospora endophytica* YIM 68255T; the level of DNA–DNA relatedness between them was 51.2±0.6 %, which was below the threshold value of 70 % recommended by Wayne et al. (1987) for assignment of strains to the same genomic species.

Phenotypic comparisons between strain NEAU-gxj3T and *Plantactinospora endophytica* YIM 68255T are shown in Table 1. Strain NEAU-gxj3T can decompose urea, while *Plantactinospora endophytica* YIM 68255T cannot. *Plantactinospora endophytica* YIM 68255T can grow with 3 % (w/v) NaCl, in contrast to the new isolate, which cannot. Other phenotypic characteristics that differentiate strain NEAU-gxj3T from *Plantactinospora endophytica* YIM 68255T include different colony colours on ISP 3 agar, pH and temperature ranges for growth and patterns of carbon and nitrogen source utilization.

In conclusion, it is evident from the genotypic and phenotypic data that strain NEAU-gxj3T represents a novel species of the genus *Plantactinospora*, for which the name *Plantactinospora soyae* sp. nov. is proposed.

**Description of *Plantactinospora soyae* sp. nov.**

*Plantactinospora soyae* (so'yae. N.L. gen. n. soyae of soy, of soybean, referring to the source of the isolate).

Aerobic, Gram-stain-positive actinomycete that forms well-developed substrate hyphae but no aerial hyphae. Single, non-motile and elliptical spores with a smooth surface are formed on substrate mycelium. Colony colours vary from pale yellow to strong orange–yellow. Positive for production of catalase and negative for hydrolysis of starch, production of H$_2$S and peptonization of milk. D-Glucose, meso-inositol, lactose, maltose, D-mannitol, D-mannose, raffinose, L-rhamnose, D-sorbitol and sucrose are utilized as sole carbon sources. L-Asparagine, L-aspartic acid, L-serine, L-threonine and L-tyrosine are utilized as sole nitrogen sources but L-glutamic acid is not. Growth occurs at pH 6–10 (optimum pH 7–8), 20–32 °C (optimum 28 °C) and in the presence to 0–1 % (w/v) NaCl. The diagnostic amino acid of the cell wall is meso-diaminopimelic acid, and whole-cell hydrolysates contain xylose, glucose and galactose. The predominant menaquinones are MK-10(H$_2$), MK-9(H$_8$), MK-10(H$_2$), and MK-10(H$_4$). The polar lipid profile comprises diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannoside. The fatty acids are iso-C$_{16:0}$, anteiso-C$_{17:0}$, C$_{18:0}$, C$_{19:0}$, C$_{15:0}$, iso-C$_{16:0}$, C$_{16:1}$, ω7c and C$_{18:1}$ω7c.

The type strain is NEAU-gxj3T (=CGMCC 4.7221T=DSM 46832T), isolated from soybean root ([*Glycine max* (L.) Merr.] collected from Harbin, Heilongjiang Province, China. The DNA G+C content of the type strain is 70.3±0.7 mol%.

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