Baia soyae gen. nov., sp. nov., a mesophilic representative of the family Thermoactinomycetaceae, isolated from soybean root [Glycine max (L.) Merr]

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A mesophilic, endophytic, filamentous bacterium, designated strain NEAU-gxj18T, was isolated from soybean root [Glycine max (L.) Merr.] collected from Harbin, Heilongjiang Province, China and characterized using a polyphasic approach. Growth was observed at 20–40 °C (optimum 37 °C). Aerial mycelium was absent on all the media tested. Substrate mycelia were well-developed and formed abundant single endospores with smooth surfaces. The only menaquinone was MK-7. The diagnostic diamino acid was meso-diaminopimelic acid. The whole-cell sugars were ribose, glucose and galactose. The major fatty acids were iso-C15 : 0, C13 : 0 and iso-C17 : 0. The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, one unidentified aminophospholipid and one unidentified phospholipid. The DNA G+C content was 49.9 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain NEAU-gxj18T was phylogenetically related to members of the family Thermoactinomycetaceae, with the highest sequence similarity to Geothermomicrobium terrae YIM 77562T (93.35%). On the basis of morphological and chemotaxonomic characteristics, phylogenetic analysis and characteristic patterns of 16S rRNA gene signature nucleotides, strain NEAU-gxj18T represents a novel species of a new genus within the family Thermoactinomycetaceae, for which the name Baia soyae gen. nov., sp. nov. is proposed. The type strain of the type species is NEAU-gxj18T (=CGMCC 4.7223T =DSM 46831T).

The family Thermoactinomycetaceae, which consisted of six genera Laceyella, Thermoflavimicrobium, Thermoactinomyces, Seinonella, Planifilum and Mechercharimyces and was affiliated to the order Bacillales, was proposed by Matsuo et al. (2006). At the time of writing, this family comprised 18 genera and 32 recognized species, including the recently described genera Melghirimyces (Addou et al., 2012, 2013; Li et al., 2013), Polycladomyces (Tsubouchi et al., 2013), Hazenella (Buss et al., 2013), Geothermomicrobium (Zhou et al., 2014), Salinithrix (Zarparvar et al., 2014), Marinithermofilum (Zhang et al., 2015) and Novibacillus (Yang et al., 2015). Members of the family Thermoactinomycetaceae are aerobic, Gram-stain-positive and show filamentous growth. Some members are able to form a single, non-stalked spore on the aerial or substrate hyphae, while others form consecutive spores on straight or branched sporophores. Most species of the family Thermoactinomycetaceae are thermophilic, growing at 30–60 °C. However, several members of the genera Seinonella, Mechercharimyces, Shimazuella, Marininema, Hazenella and Marinithermofilum are mesophilic, only growing below 45 °C. Members of this family have been isolated

†These authors contributed equally to this work.

Abbreviations: DAP, diaminopimelic acid; ISP, International Streptomyces Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NEAU-gxj18T is KM359702.

Four supplementary figures and a supplementary table are available with the online Supplementary Material.
from various sources, such as salt lake, a clinical sample, marine sediments, mushroom compost, mouldy hay, sugar cane, humidifiers of air-conditioning systems and other clinical and environmental sources.

During an investigation on the ecological diversity of symbiotic micro-organisms from soybean root, another mesophilic member of the family *Thermoactinomycetaceae* was isolated. In this taxonomic study, we performed a polyphasic analysis on this strain, and conclude that the isolate represents a novel species in a new genus of the family *Thermoactinomycetaceae*.

Strain NEAU-gxj18<sup>T</sup> was isolated from soybean root collected from Harbin, Heilongjiang province, north China (45° 34’ N 126° 52’ E). The root sample was processed as described by Liu et al. (2013) and placed on a plate of dulcitol-proline agar (DPA), which contained 1 g l<sup>−1</sup> distilled water: dulcitol 2.0, proline 0.5, K<sub>2</sub>HPO<sub>4</sub> 0.3, NaCl 0.3, CaCl<sub>2</sub>·2H<sub>2</sub>O 1.0, MgSO<sub>4</sub>·7H<sub>2</sub>O 1.0 and agar 15) supplemented with cycloheximide (50 mg l<sup>−1</sup>) and nalidixic acid (50 mg l<sup>−1</sup>). After 14 days of aerobic incubation at 37 °C, colonies were transferred and purified on International *Streptomyces* Project (ISP) medium 3 (Shirling & Gottlieb, 1966) and maintained as glycerol suspensions (20 %, v/v) at −80 °C.

Morphological characteristics were observed by light microscopy (ECLIPSE E200; Nikon) and scanning electron microscopy (S-3400N; Hitachi) using cultures grown on ISP 3 medium at 37 °C for 3 days. Cultural characteristics were determined on ISP media 2–7 (Shirling & Gottlieb, 1966), nutrient agar (NA) (Waksman, 1961), Czapek’s agar (CA), potato-glucose agar (PDA) prepared as described by Dong & Cai (2001), and tryptic soy agar (TSA) after 14 days at 37 °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, 20, 28, 30, 35, 37, 40, 45 and 50 °C) was determined on ISP 3 medium after incubation for 14 days. Growth tests for pH range (pH 3–12) and NaCl tolerance (0–7 % NaCl, w/v) were performed in modified Bennett’s medium (Williams et al., 1983) at 37 °C for 14 days on a rotary shaker. Hydrolysis of Tween 80 and production of catalase and urease were tested as described by Snibert & 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 previously by Gordon et al. (1974) and Yokota et al. (1993).

Biomass for chemical studies was prepared by growing strain NEAU-gxj18<sup>T</sup> in tryptic soy broth (TSB) in shake flasks at 37 °C for 7 days. Cells were harvested by centrifugation, washed with distilled water and freeze-dried. The isomer of diaminopimelic acid (DAP) in the cell-wall hydrolysates was derivatized and analysed by a HPLC method (McKerrow et al., 2000) using an Agilent TC-C<sub>18</sub> Column (250 × 4.6 mm i.d. 5 µm) with a mobile phase consisting of acetonitrile/0.05 M phosphate buffer pH 7.2 (15 : 85, v/v) at a flow rate of 0.5 ml min<sup>−1</sup>. An Agilent G1321A fluorescence detector with 365 nm excitation and 455 nm longpass emission filters was used for peak detection. The whole-cell sugars were analysed according to the procedures developed by Lechevalier & Lechevalier (1980). Polar lipids were examined by two-dimensional TLC and identified using the method of Minnink et al. (1984). Menaquinones were extracted from freeze-dried biomass and purified according to Collins (1985). Extracts were analysed by a HPLC-UV method using an Agilent TC-C<sub>18</sub> Column (250 × 4.6 mm i.d. 5 µm), typically at 270 nm. The mobile phase was acetonitrile/2-propanol (60 : 40, v/v), the flow rate was set to 1.0 ml min<sup>−1</sup> 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). To determine cellular fatty acid compositions, strain NEAU-gxj18<sup>T</sup> was cultivated in TSB medium in shake flasks at 37 °C and harvested at the exponential growth phase. Fatty acid methyl esters were extracted from the biomass as described by Gao et al. (2014) and were analysed by GC-MS using the method of Xiang et al. (2011).

Extraction of chromosomal DNA and PCR amplification of the 16S rRNA gene sequence were carried out using a standard procedure (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-gxj18<sup>T</sup> (1499 bp) was obtained and aligned with multiple sequences obtained from the GenBank/EMBL/DDJB databases using CLUSTAL X 1.83 software. Phylogenetic trees were reconstructed with neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) algorithms using MEGA software version 6.06 (Tamura et al., 2013). All the species of genera in the family *Thermoactinomycetaceae* were included in the phylogenetic trees. The stability of the topology of the phylogenetic tree 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). The 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 G+C contents of the genomic DNA were determined using the thermal denaturation (T<sub>m</sub>) method (Mandel & Marmur, 1968) with *Escherichia coli* JM109 DNA used as the control. The experiments were performed with three replications.

Nucleotides and nucleotide pairs in the 16S rRNA gene of strain NEAU-gxj18<sup>T</sup> and the genera of the family *Thermoactinomycetaceae* were determined after manual verification of the CLUSTAL X sequence alignment (Zhi et al., 2009). Nucleotide positions were numbered according to the
corresponding position in the 16S rRNA gene sequence of *E. coli* (Brosius et al., 1978).

Morphological observation of a 14-day-old culture of strain NEAU-gxj18<sup>T</sup> grown on ISP 3 agar revealed that it formed extensive substrate mycelium, but no aerial mycelium was detected. Single endospores (1.1–1.3 × 1.1–1.3 μm) were formed on the substrate mycelium; the spores were non-motile and smooth (Fig. 1 and Fig. S1, available in the online Supplementary Material). Strain NEAU-gxj18<sup>T</sup> grew well on ISP 3, NA and PDA, moderately on ISP 2, ISP 6 and TSA, but no growth was observed on ISP 4, ISP 5, ISP 7 or CA media. The colour of substrate mycelium was moderate orange–yellow on ISP 3, vivid redish-orange on ISP 2 and ISP 6, brilliant orange–yellow on NA, vivid orange on TSA, and strong orange on PDA. Aerial mycelium and diffusible pigment were not observed on any of the tested media. Strain NEAU-gxj18<sup>T</sup> grew well between pH 6.0 and 8.0, with optimal growth at pH 7.0. The range of temperature for growth was determined to be 20–40 °C, with optimum growth at 37 °C. Strain NEAU-gxj18<sup>T</sup> could tolerate up to 1.0 % (w/v) NaCl, and exhibited optimum growth with no added NaCl. Detailed physiological characteristics are presented in the species description.

Strain NEAU-gxj18<sup>T</sup> contained meso-diaminopimelic acid in the cell wall. The whole-cell hydrolysate contained ribose, glucose and galactose. The polar lipid profile consisted of phosphatidyglycerol, phosphatidylethanolamine, phosphatidylglycerol, one unidentified aminophospholipid and one unidentified phospholipid (Fig. S2). The only menaquinone detected was MK-7. The cellular fatty acid profile was determined to be composed of iso-C<sub>15</sub>:<sub>0</sub> (44.2 %), C<sub>13</sub>:<sub>0</sub> (13.0 %), iso-C<sub>17</sub>:<sub>0</sub> (11.2 %), iso-C<sub>14</sub>:<sub>0</sub> (9.4 %), C<sub>17</sub>:<sub>0</sub> (8.8 %), C<sub>14</sub>:<sub>0</sub> (5.3 %), anteiso-C<sub>13</sub>:<sub>0</sub> (3.1 %), C<sub>15</sub>:<sub>0</sub> (2.8 %), iso-C<sub>16</sub>:<sub>0</sub> (1.7 %) and C<sub>16</sub>:<sub>0</sub> (0.5 %). The DNA G+C content was 49.9±0.3 mol%. All the morphological characteristics and chemotaxonomic data showed that strain NEAU-gxj18<sup>T</sup> should be assigned to the family *Thermoactinomycesaceae*.

The almost-complete 16S rRNA gene sequence of strain NEAU-gxj18<sup>T</sup> (1499 bp) was determined in this study. Based on the EzTaxon-e analysis, strain NEAU-gxj18<sup>T</sup> was affiliated to the family *Thermoactinomycesaceae*, with the highest 16S rRNA gene sequence similarity to *Geothermomonobium terrae* YIM 77562<sup>T</sup> (93.35 %). 16S rRNA gene sequence similarity values between strain NEAU-gxj18<sup>T</sup> and other members of the family *Thermoactinomycesaceae* were less than 92.2 %: *Hazenella coriacea* DSM 45707<sup>T</sup> (92.18 %), *Lihuxella thermophila* YIM 77831<sup>T</sup> (91.76 %), *Laceyella* (91.18–91.45 %), *Shimazuella kribbensis* KCTC 9933<sup>T</sup> (91.17 %), *Marinimicrobium thermophilum* (90.37–90.71 %), *Thermomonospora* (90.26–90.47 %), *Thermoflavimicrobium dichotomicum* KCTC 3667<sup>T</sup> (90.01 %), *Seinonella peptonophila* KCTC 9740<sup>T</sup> (90.01 %), *Megthermisspora* (87.93–89.73 %), *Salinitrichia halophila* R458<sup>T</sup> (87.49 %), *Kroppenstedtia* (87.25–89.75 %), *Desmospora* (87.05–90.32 %), *Marinithrix halophila* SC511157<sup>T</sup> (86.94 %), *Polychlamydomyces abyssicus* JIR-001<sup>T</sup> (85.68 %), *Planifilum* (85.28–88.84 %) and *Novibacillus thermophilus* SG-1<sup>T</sup> (84.58 %). The phylogenetic tree (Fig. 2) based on 16S rRNA gene sequences indicated that the novel isolate formed a coherent clade with the genera *Geothermomonobium*, *Hazenella*, *Shimazuella* and *Seinonella*, an association that was supported by a bootstrap value of 76 % in the neighbour-joining tree and also recovered by the maximum-likelihood and maximum-parsimony algorithms (Figs S3 and S4). However, the relatively high sequence divergence values (>6.65 %) showed that the isolate was distantly related to the described taxa. Patterns of 16S rRNA gene signature nucleotides analysis demonstrated that strain NEAU-gxj18<sup>T</sup> contained the signature nucleotides pattern defined for the family *Thermoactinomycesaceae*, namely, 415–428 (C–G), 440–493 (C–G), 682–708 (G–C) and 694 (G) (Yassin et al., 2009). However, when the signature nucleotide positions of strain NEAU-gxj18<sup>T</sup> were compared with those of genera of the family *Thermoactinomycesaceae*, there were several nucleotide pair differences from them (Table 1 and Table S1).

Apart from the phylogenetic analysis based on 16S rRNA gene sequences, several phenotypic characteristics also support the distinctiveness of strain NEAU-gxj18<sup>T</sup> from other related genera (Table 1). Strain NEAU-gxj18<sup>T</sup> differs from member of the genus *Geothermomonobium* by being mesophilic. Moreover, the absence of aerial mycelium differentiates strain NEAU-gxj18<sup>T</sup> from members of the genera *Shimazuella* and *Seinonella*. The menaquinone profile differentiates the isolate from members of the genera *Shimazuella*, *Hazenella* and *Seinonella*. Strain NEAU-gxj18<sup>T</sup> has the highest 16S rRNA gene sequence similarity with the type strain of *Geothermomonobium terrae*, which is the type and sole species of the genus *Geothermomonobium*. However, strain NEAU-gxj18<sup>T</sup> can be
easily distinguished from \textit{Geothermocystium terrae} YIM 77562\textsuperscript{T} on basis of phenotypic and chemotaxonomic properties. Strain NEAU-gxj18\textsuperscript{T} does not grow above 40 °C, in contrast to \textit{Geothermocystium terrae} YIM 77562\textsuperscript{T}, which grows well at 50–55 °C; the endospores of strain NEAU-gxj18\textsuperscript{T} are smooth, while those of \textit{Geothermocystium terrae} YIM 77562\textsuperscript{T} are warty; and strain NEAU-gxj18\textsuperscript{T} contains C\textsubscript{13} : 0 and iso-C\textsubscript{17} : 0 as major fatty acids, which are not major fatty acids of \textit{Geothermocystium terrae} YIM 77562\textsuperscript{T}. Furthermore, strain NEAU-gxj18\textsuperscript{T} can be differentiated from \textit{Geothermocystium terrae} YIM 77562\textsuperscript{T} based on the absence of phosphatidylethanolamine and unidentified polar lipids in the polar lipid profile. These results suggested that the isolate represents a novel species in a new genus of the family \textit{Thermoactinomycetaceae}, for which the name \textit{Baia soyae} gen. nov., sp. nov. is proposed.

**Description of \textit{Baia} gen. nov.**

\textit{Baia} (Bai’ a. N.L. fem. n. \textit{Baia} named after Hua Bai, a Chinese microbiologist).

Cells are Gram-stain-positive, aerobic, mesophilic and show filamentous growth. Aerial mycelium is not produced. The substrate mycelia are well-developed. Single smooth endospores are formed on the substrate mycelium, and the spores are non-motile. Cell walls contain meso-DAP, and ribose, glucose and galactose are the whole-cell sugars. The only menaquinone is MK-7. The major cellular fatty acids are iso-C\textsubscript{15} : 0, C\textsubscript{13} : 0 and iso-C\textsubscript{17} : 0. The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, one unidentified aminophospholipid and one unidentified phospholipid. The DNA G+C content is 49.9 mol%.

Fig. 2. Neighbour-joining tree based on 16S rRNA gene sequences (1499 bp) showing the relationships between strain NEAU-gxj18\textsuperscript{T} and members of the family \textit{Thermoactinomycetaceae}. \textit{Bacillus subtilis} NCIMB 3610\textsuperscript{T} was used as an outgroup. Numbers at nodes are bootstrap values (percentages of 1000 replications); only values >50 % are indicated. Asterisks denote branches that were also recovered using the maximum-parsimony and maximum-likelihood methods. Bar, 0.01 nt substitutions per site.
The type species is *Baia soyae*.

**Description of *Baia soyae* sp. nov.**

*Baia soyae* (so’ya.e. N.L. gen. n. *soyae* of soya, of soybean, referring to the source of the isolate).

Displays the following characteristics in addition to those given in the genus description. Grows well on ISP 3, NA and PDA media, moderately on ISP 2, ISP 6 and TSA media, but no growth is observed on ISP 4, ISP 5, ISP 7 and CA media. The colony colours are in the orange colour-series. Aerial mycelium and diffusible pigment are not produced on any of the tested media. Negative for reduction of nitrate; production of catalase, cellulase, urease and H$_2$S; liquefaction of gelatin; hydrolysis of aesculin, starch and Tween 80; and peptonization of milk. D-Glucose, D-mannitol, D-mannose and L-rhamnose are utilized as sole carbon sources, but L-arabinose, D-fructose, D-galactose, inositol, lactose, maltose, raffinose, D-ribose, D-sorbitol, sucrose and D-xylose are not utilized. L-Alanine, L-arginine, L-asparagine, L-aspartic acid, L-glutamic acid and L-glutamine are utilized as sole nitrogen sources, but creatine, glycine, L-serine, L-threonine and L-tyrosine are not. Growth occurs at 20-40 °C, pH 6.0–8.0, and at NaCl concentrations not more than 1.0 %. Optimal temperature and pH for growth are 37 °C and pH 7.0, respectively.

The type strain is NEAU-gxj18$^\text{T}$ (CGMCC 4.7223$^\text{T}$, DSM 46831$^\text{T}$), which was isolated from soybean root collected from Harbin, Heilongjiang Province, north China. The DNA G+C content is of the type strain 49.9 mol%.

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*DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PME, phosphatidylmethylethanolamine; PE, phosphatidylethanolamine; PL, unidentified phospholipid; UL, unidentified polar lipid; APL, unidentified aminophospholipid.

**Table 1. Differential characteristics of strain NEAU-gxj18$^\text{T}$ and related genera of the family Thermoactinomycetaceae**

Taxa: 1, strain NEAU-gxj18$^\text{T}$ (data from this study); 2, *Geothermomicrobium* (Zhou et al., 2014); 3, *Shimazuella* (Park et al., 2007); 4, *Hazenella* (Buss et al., 2013); 5, *Seinonella* (Yoon et al., 2005). All taxa contained meso-DAP as the diamino acid. +, Positive; -, negative; ND, no data available.
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References


tuberculosis, and emended description of the family Thermoa


