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, Shimazuela, 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.

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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-gxj18T 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 g l−1 distilled water: dulcitol 2.0, proline 0.5, K2HPO4 0.3, NaCl 0.3, CaCl2·2H2O 1.0, MgSO4·7H2O 1.0 and agar 15) supplemented with cycloheximide (50 mg l−1) and nalidixic acid (50 mg l−1). 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. Colony coloration 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. Hydration 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 H2S were examined as described previously by Gordon et al. (1974) and Yokota et al. (1993).

Biomas for chemical studies was prepared by growing strain NEAU-gxj18T 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-C18 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−1. 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 Minnikin 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-C18 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−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). To determine cellular fatty acid compositions, strain NEAU-gxj18T 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-gxj18T (1499 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 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 (Tm) 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-gxj18T 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
Strain NEAU-gxj18T contained species description. Detailed physiological characteristics are presented in the International Journal of Systematic and Evolutionary Microbiology. Scanning electron micrograph showing spores of strain NEAU-gxj18T grown on ISP 3 medium for 3 days at 37 °C.

Strain NEAU-gxj18T 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 reddish-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-gxj18T 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-gxj18T 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-gxj18T contained meso-diaminopimelic acid in the cell wall. The whole-cell hydrolysate contained ribose, glucose and galactose. The polar lipid profile consisted of diphosphatidylglycerol, 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_{15}:0 (44.2 %), C_{13}:0 (13.0 %), iso-C_{17}:0 (11.2 %), iso-C_{14}:0 (9.4 %), C_{17}:0 (8.8 %), C_{14}:0 (5.3 %), anteiso-C_{13}:0 (3.1 %), C_{15}:0 (2.8 %), iso-C_{16}:0 (1.7 %) and C_{16}:0 (0.5 %). The DNA G+C content was 49.9±0.3 mol%. All the morphological characteristics and chemotaxonomic data showed that strain NEAU-gxj18T should be assigned to the family Thermocatino-mycetaceae.

The almost-complete 16S rRNA gene sequence of strain NEAU-gxj18T (1499 bp) was determined in this study. Based on the EzTaxon-e analysis, strain NEAU-gxj18T was affiliated to the family Thermocatino-mycetaceae, with the highest 16S rRNA gene sequence similarity to Geothermomicribium terrae YIM 77562^{T} (93.35 %). 16S rRNA gene sequence similarity values between strain NEAU-gxj18T and other members of the family Thermocatino-mycetaceae were less than 92.2 %: Hazenella coriacea DSM 45707^{T} (92.18 %), Lihuaxuella thermophila YIM 77831^{T} (91.76 %), Laceylla (91.18–91.45 %), Shimazuella kribbensis KCTC 9933^{T} (91.17 %), Marinimema (91.15–91.38 %), Thermainomyces (90.37–90.71 %), Mechercharimyces (90.26–90.47 %), Thermainovimicrobium dichotomicum KCTC 3667^{T} (90.01 %), Seinonella peptonophila KCTC 9740^{T} (90.01 %), Meghirimycetes (87.93–89.73 %), Salinitrich halophila R458^{T} (87.49 %), Kroppenstedtia (87.25–89.75 %), Desmospora (87.05–90.32 %), Marinithermophillum abyssi SCSIO 11157^{T} (86.94 %), Polycladomyces abyssica JIR-001^{T} (85.68 %), Planfilum (85.28–88.84 %) and Novibacillus thermophilus SG-1^{T} (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 Geothermomicribium, Hazenella, Shimazuella and Seinonella, which is the type and sole species of the genus Geothermomicribium. However, strain NEAU-gxj18T can be

Fig. 1. Scanning electron micrograph showing spores of strain NEAU-gxj18T grown on ISP 3 medium for 3 days at 37 °C. Single spores with smooth surface (1.1–1.3 × 1.1–1.3 μm) were observed on substrate mycelium. Bar, 1 μm.
easily distinguished from Geothermomicriobium terrae YIM 77562T on basis of phenotypic and chemotaxonomic properties. Strain NEAU-gxj18T does not grow above 40 °C, in contrast to Geothermomicriobium terrae YIM 77562T, which grows well at 50–55 °C; the endospores of strain NEAU-gxj18T are smooth, while those of Geothermomicriobium terrae YIM 77562T are warty; and strain NEAU-gxj18T contains C13:0 and iso-C17:0 as major fatty acids, which are not major fatty acids of Geothermomicriobium terrae YIM 77562T. Further, strain NEAU-gxj18T can be differentiated from Geothermomicriobium terrae YIM 77562T based on the absence of phosphatidylmethylethanolamine 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 Thermoactinomycetaceae, for which the name Baia soyae gen. nov., sp. nov. is proposed.

**Description of Baia gen. nov.**

Baia (Bai’ā N.L. fem. n. 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-C15:0, C13:0 and iso-C17:0. 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%.
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 H2S; 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-gxj18T (=CGMCC 4.7223T = DSM 46831T), 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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<th>Table 1. Differential characteristics of strain NEAU-gxj18T and related genera of the family Thermoactinomycetaceae</th>
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<td>Taxa: 1, strain NEAU-gxj18T (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.</td>
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<td><strong>Characteristic</strong></td>
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<td>Colour of aerial mycelium</td>
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<td>Degradation of:</td>
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<td>Optimal growth temperature</td>
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<td>DNA G + C content (mol%)</td>
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<td>Predominant Menaquinone</td>
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*DPG, diphtosphatidylglycerol; PG, phosphatidylglycerol; PME, phosphatidymethylethanolamine; PL, unidentified phospholipid; UL, unidentified polar lipid; APL, unidentified aminophospholipid.

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