Bacillus solisilvae sp. nov., isolated from forest soil

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Abstract
A novel Gram-stain-positive, motile, endospore-forming, rod-shaped bacterial strain, NEAU-cbsb5T, was isolated from forest soil from Changbai Mountain, Heilongjiang Province, China. The isolate grew at 15–40 °C (optimum 30 °C), at pH 6.0–8.0 (optimum pH 7.0) and in the presence of up to 4 % (w/v) NaCl, although NaCl was not required for growth. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain NEAU-cbsb5T formed a distinct lineage within the genus Bacillus and was most closely related to Bacillus acidicycler DSM 18954T (99.1 % similarity) and Bacillus luciferensis JCM 12212T (99.0 %). 16S rRNA gene sequence similarity to sequences of the type strains of other Bacillus species was less than 96.0 %. Average nucleotide identity (ANI) values between NEAU-cbsb5T and its most closely related species were 78.72–84.75 % by ANIm, ANIb and OrthoANIu analysis. The in silico DNA–DNA hybridization values between strain NEAU-cbsb5T and its close relatives B. acidicycler DSM 18954T and B. luciferensis JCM 12212T were both 23.80 %, again indicating they belong to different taxa. The major cellular fatty acids of NEAU-cbsb5T were iso-C15:0, anteiso-C15:0 and C16:0. The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidyglycerol and an unknown aminophospholipid. The cell-wall peptidoglycan contained meso-diaminopimelic acid and the predominant menaquinones were MK-7 and MK-6. The genomic DNA G+C content was 33.0 mol%. Based on the phylogenetic, phenotypic and chemotaxonomic data, strain NEAU-cbsb5T was classified as a representative of a novel species in the genus Bacillus, for which the name Bacillus solisilvae sp. nov. is proposed. The type strain is NEAU-cbsb5T (=CGMCC 1.14993=JSM 10485).

The genus Bacillus, belonging to the phylum Firmicutes, was first proposed by Cohn [1] and currently represents a large group of rod-shaped, aerobic or facultatively anaerobic, Gram-stain-positive and endospore-forming bacteria with low DNA G+C content (32–66 mol%) [2, 3]. Major polar lipids of this genus are phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol [2, 3]. Menaquinone-7 (MK-7) and meso-diaminopimelic acid (meso-DAP) are the major respiratory quinone and the diagnostic amino acid of the cell-wall peptidoglycan, respectively [2]. Species of Bacillus have been found in a wide variety of environments including soils, marine sediment, hot springs, desert sands, hypersaline sites, and inner tissues of plants and animals, as their endospores can survive extreme environmental conditions [2–4]. At the time of writing, more than 300 species have been classified in the genus Bacillus (http://www.bacterio.net/bacillus.html).

Changbai Mountain is volcanic and is the highest mountain in north-eastern China [5]. It is one of few well-conserved natural ecosystems on Earth and the vertical distribution of vegetation here mirrors the horizontal vegetation types of temperate and cold zones in Eurasia [6], indicating unique microbial community composition and diversity. To study the culturable microbial diversity in forest soil from Changbai Mountain, one plant-growth-promoting bacterium designated NEAU-cbsb5T was isolated and assigned to a novel species of the genus Bacillus. In this study, the taxonomic status of strain NEAU-cbsb5T is reported based on phylogenetic, chemotaxonomic and phenotypic evidence.

Strain NEAU-cbsb5T was isolated from forest soil collected at an altitude of 1500 m from Changbai Mountain, Heilongjiang Province (42° 06′ N 128° 04′ E). The sample was suspended in sterilized water, serially diluted, spread on nutrient agar (NA) and incubated at 30 °C for 48 h [7]. Pure

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Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; meso-DAP, meso-diaminopimelic acid.
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The GenBank/EMBL/DDBJ accession numbers for the draft genomes of NEAU-cbsb5T and Bacillus acidicycler DSM 18954T are NETJ00000000 and NESS00000000, respectively. The accession number for the 16S rRNA gene sequence of strain NEAU-cbsb5T is KJ733017. One supplementary table and four supplementary figures are available with the online Supplementary Material.
cultures were obtained by isolation of several successive single colonies. The strain was maintained as glycerol suspensions (20%, v/v) at −80°C. *Bacillus acidocaldarius* DSM 18954<sup>T</sup> and *Bacillus licheniformis* JCM 12212<sup>T</sup> were used as reference strains for comparison in this study. Unless indicated otherwise, morphological, physiological, molecular and chemotaxonomic studies were performed with cells grown on NA at 30°C.

To characterize strain NEAU-cbsb5<sup>T</sup>, standard phenotypic tests were selected according to the recommendations of the minimal standards for describing new taxa of aerobic, endospore-forming bacteria [8]. The cellular morphology characteristics of strain NEAU-cbsb5<sup>T</sup> were studied by light microscopy (ECLIPSE E200; Nikon) and transmission electron microscopy (H-7650; Hitachi) with cells grown on NA at 30°C for 2 days. Sporulation was induced on NA containing 5 mg MnSO₄·H₂O, grown at 30°C for 48 h. The presence of endospores was investigated by using the Schaeffer–Fulton staining method [9]. Gram staining was performed as described by Smibert and Krieg [10]. Motility was determined based on the presence of turbidity throughout tubes containing semi-solid medium and was further confirmed by the hanging-drop method [11, 12]. Growth under anaerobic conditions was tested by incubating cultures on NA in Bacteron anaerobic chambers (Sheldon Manufacturing). The utilization of sole carbon and other energy sources, decomposition of cellulose, hydroslysis of starch and ascor-ulin, reduction of nitrate, peptonization of milk, liquefaction of gelatin and production of H₂S were analysed as recommended by Ventosa et al. [13]. Hydrolysis of Tweens 20, 40 and 80 and production of oxidase, catalase, esterase and urease were tested as described by Smibert and Krieg [10]. Other physiological and biochemical characteristics were determined using API 20E and API 50CHB test strips with API 50CHB suspension medium (bioMérieux) according to the manufacturer’s protocols. Growth at different temperatures (4, 15, 20, 28, 30, 35, 37, 40, 42 and 45°C) was determined on NA after incubation for 7 days. Growth tests to determine pH range (4–12 at 1 pH unit intervals) and NaCl tolerance up to 10% (w/v) at 1% intervals were performed in trypticase soy broth (TSB) at 30°C for 7 days on a rotary shaker. The buffer systems were: pH 4.0–5.0, 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0, 0.1 M KH₂PO₄/0.1 M NaOH; pH 9.0–10.0, 0.1 M NaHCO₃/0.1 M Na₂CO₃; pH 11.0–12.0, 0.2 M KH₂PO₄/0.1 M NaOH.

Strain NEAU-cbsb5<sup>T</sup> grew well on common media used for aerobic bacteria such as NA and TSB, but grew only moderately on trypticase soy agar (TSA). Colonies of strain NEAU-cbsb5<sup>T</sup> grown on NA after 2 days of incubation were 2.5–3.3 mm in diameter, cream-coloured, smooth, circular and convex with entire margins. Strain NEAU-cbsb5<sup>T</sup> was Gram-stain-positive, facultatively anaerobic, endospore-forming, rod-shaped (0.5–0.7×1.8–2.1 μm) and motile. Ellipsoidal endospores were observed at subterminal position in slightly swollen sporangia and the peritrichous flagella further confirmed the motility (Fig. S1, available in the online Supplementary Material). Strain NEAU-cbsb5<sup>T</sup> was able to tolerate NaCl concentrations of up to 4% (w/v), although it did not require NaCl for growth. The temperature range for growth was 15–40°C, with the optimum temperature being 30°C. Growth was observed at pH 6.0–8.0, with the optimum pH being 7.0. Results of API 50CH tests showed that strain NEAU-cbsb5<sup>T</sup> produced acid from arbutin, cellobiose, D-fructose, D-glucose, D-mannitol, glycogen, maltose, N-acetyl-D-glucosamine, salicin, aesculin, starch and trehalose, but not from glyceral, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylene, L-xylene, D-adonitol, methyl D-xylopyranoside, D-galactose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, lactose, melibiose, sucrose, inulin, melezitose, raffinose, xyitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. In API 20E tests, the ONPG reaction, activities of arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and tryptophan deaminase, citrate utilization, H₂S production, the Voges–Proskauer reaction, indole production and gelatin hydrolysis were negative. The physiological and biochemical properties useful for distinguishing strain NEAU-cbsb5<sup>T</sup> from its two closest phylogenetic neighbours are shown in Table 1 and the detailed characteristics of NEAU-cbsb5<sup>T</sup> are presented in the species description.

For DNA extraction, strain NEAU-cbsb5<sup>T</sup> was cultured in TSB to early stationary phase (~24 h) and harvested by centrifugation. The chromosomal DNA was extracted according to standard methods [14]. Amplification of the 16S rRNA gene was performed by using the universal bacterial primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1541R (5′-AAGGAGTTGATCCTGCGCA-3′) under conditions described previously [15, 16]. The PCR product was purified and cloned into the vector pMD19-T (Takara) and sequenced by using an Applied Biosystems DNA sequencer (model 3730XL) and software provided by the manufacturer. Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using EzBioCloud [17]. The almost full-length 16S rRNA gene sequence of strain NEAU-cbsb5<sup>T</sup> (1543 bp) was 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 [18], maximum-likelihood [19] and maximum-parsimony [20] algorithms using MEGA software version 6.06 [21]. Phylogenetic distances were calculated with the Kimura two-parameter model together with Gamma distribution and Invariant sites (K2 +G+I) for the neighbour-joining and maximum-likelihood methods, respectively [22, 23]. In addition, a heuristic search was performed using nearest-neighbour interchange branch swapping in maximum-likelihood inference. The stability of the clades in the trees was appraised using a bootstrap procedure with 1000 repeats [24]. All positions containing gaps and missing data were eliminated from the dataset (complete deletion
The root position of the trees was inferred by using Paenibacillus polymyxa DSM 36\textsuperscript{T} (GenBank accession no. AJ320493). EzBioCloud analysis of the 1543 bp 16S rRNA gene sequence demonstrated that strain NEAU-cbsb5\textsuperscript{T} is related to members of the genus Bacillus with the closest relatives being B. acidiceler CBD 119\textsuperscript{T} (=DSM 18954\textsuperscript{T}) [25] and B. luciferensis LMG 18422\textsuperscript{T} (=JCM 12212\textsuperscript{T}) [26] with 99.1 and 99.0\% gene sequence similarity, respectively. The sequence similarities between strain NEAU-cbsb5\textsuperscript{T} and other members of the genus Bacillus were less than 96.0\%. In the maximum-likelihood phylogenetic tree (Fig. 1), strain NEAU-cbsb5\textsuperscript{T} formed a robust cluster with the related species B. acidiceler CBD 119\textsuperscript{T} and B. luciferensis LMG 18422\textsuperscript{T} with a bootstrap value of 100\%, forming a distinct phylogenetic lineage within the genus Bacillus. The genus Bacillus is a large, phenotypically and phylogenetically heterogeneous group consisting of highly diverse organisms with Bacillus subtilis as the type species [1, 2]. Species of the genus Bacillus often form polyphyletic clusters, distributed among the species from other genera [27]. Although the Bacillus cereus and B. subtilis clades are two of the largest, statistically well-supported groups among the entire Bacillus genus, no direct relationship of the species of these two clades was observed in many phylogenetically orientated analyses [27, 28]. It is clear that the branch clade of strain NEAU-cbsb5\textsuperscript{T} is phylogenetically distantly related to B. subtilis DSM 10\textsuperscript{T}, which is separate from Solibacillus silvestris DSM 12223\textsuperscript{T}, but closely related to species of the B. cereus clade and other species of Bacillus (Fig. 1). Indeed, the level of 16S rRNA gene sequence similarity between strain NEAU-cbsb5\textsuperscript{T} and B. subtilis DSM 10\textsuperscript{T} is 91.9\%, a value lower than those obtained with species of the B. cereus clade (94.9–95.2\%). Similar phylogenetic relationships between these clades were also observed in the maximum-likelihood phylogenetic tree reconstructed by Miller et al. [29]. Therefore, we propose that strain NEAU-cbsb5\textsuperscript{T} might be a member of the genus Bacillus. Furthermore, the topology of the maximum-likelihood phylogenetic tree was also confirmed using the neighbour-joining and maximum-parsimony methods (Figs S2 and S3), which indicated the affiliation of strain NEAU-cbsb5\textsuperscript{T} to the genus Bacillus.

It has been proposed that the traditional metric of DNA–DNA hybridization should be replaced by genome-sequence-based digital DNA–DNA hybridization (dDDH) due to the lack of reproducibility [30]. To further distinguish strain NEAU-cbsb5\textsuperscript{T} from its closely related Bacillus species, the genome sequences of these strains were sequenced by Novogene Bioinformatics Technology using an Illumina HiSeq PE150 sequencer following the manufacturer’s suggested protocols. The resulting reads were quality trimmed to the Q20 confidence level. The draft genome was assembled using SOAP de novo version 2.04 [31] with default parameters. The estimated dDDH values were calculated using formula 2 at the Genome-to Genome Distance Calculation (GGDC) website (http://ggdc.dsmz.de/distcalc2.php) as described by Meier-Kolthoff et al. [32]. The dDDH value between strain NEAU-cbsb5\textsuperscript{T} (NCBI accession number NETJ0000000) and both B. acidiceler DSM 18954\textsuperscript{T} (NCBI accession number NESS0000000) and B. luciferensis JCM 12212\textsuperscript{T} (NCBI accession number GCA_001712755) was 23.80\%, below the 70\% DDH species boundary as recommended by Meier-Kolthoff et al. [32]. Average nucleotide

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**Table 1.** Differential phenotypic characteristics among strain NEAU-cbsb5\textsuperscript{T} and the type strains of its two closest phylogenetic neighbours

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Rods occurring singly or in pairs</td>
<td>Rods occurring in pairs or short chains with branches*</td>
<td>Rods occurring singly or in pairs†</td>
</tr>
<tr>
<td>Swollen sporangia</td>
<td>+</td>
<td>+*</td>
<td>†</td>
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<tr>
<td>Motility</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Oxidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Hydrolysis of:</td>
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<tr>
<td>Casein</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Gelatin</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Urea</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Starch</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Tween 80</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Methyl red test</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>Voges–Proskauer test</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Temperature range (°C)</td>
<td>15–40</td>
<td>20–37</td>
<td>20–37</td>
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<tr>
<td>NaCl (%)</td>
<td>0–4</td>
<td>0–2</td>
<td>0–1</td>
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<td>Utilization of:</td>
<td></td>
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<tr>
<td>Creatine</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>D-Galactose</td>
<td>–</td>
<td>W</td>
<td>+</td>
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<tr>
<td>Malose</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>D-Sorbitol</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>L-Arabinose</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>L-Glycine</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>L-Glutamic acid</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>L-Glutamine</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>L-Threonine</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Acid production from API 50CH:</td>
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<td></td>
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<tr>
<td>Amygdalin</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Sucrose</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Menaquinone types</td>
<td>MK-7 (54%), MK-7 (53%), MK-6 (31%), MK-8 (16%), MK-6 (64%), MK-7 (36%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data taken from Peak et al. [25].†Data taken from Logan et al. [26].
identity (ANI) calculations were performed using the JSpe-
cies software (http://jspecies.ribohost.com/jspeciesws/) [33].
The ANI-MUMmer (ANIm) values between strain NEAU-
cbsb5\textsuperscript{T} and the most closely related strains
B. acidiceler DSM 18954\textsuperscript{T} and B. luciferensis JCM 12212\textsuperscript{T}
were 84.65 and 84.75 %, respectively. The ANI-Blast (ANIb) value between
strain NEAU-cbsb5\textsuperscript{T} and the most closely related strains
B. acidiceler DSM 18954\textsuperscript{T} and B. luciferensis JCM 12212\textsuperscript{T}
were 78.72 and 78.65 %, respectively. The results were simi-
lar to those determined by OrthoANIu analysis [34], which
gave an OrthoANI value of 79.33 % to both
B. acidiceler DSM 18954\textsuperscript{T} and B. luciferensis JCM 12212\textsuperscript{T}. All the ANI
values are below the 94–96 % cut-off values previously pro-
posed for species delimitation [35], indicating that strain
NEAU-cbsb5\textsuperscript{T} does not belong to any of these related
species.

The sequenced genome data provided a DNA G+C content
of 33.0, 32.8 and 32.9 mol% for strain NEAU-cbsb5\textsuperscript{T},
B. acidiceler DSM 18954\textsuperscript{T} and B. luciferensis JCM 12212\textsuperscript{T},
respectively. However, the DNA G+C contents for B. acidici-
celer DSM 18954\textsuperscript{T} and B. luciferensis JCM 12212\textsuperscript{T} were
originally reported to be 37.3 mol% [25] and 33.0 mol%
[26], respectively, using wet lab methods for both. The dis-
crepancy between the original data and the genome data for
B. acidiceler DSM 18954\textsuperscript{T} suggests the conventional wet lab
method has higher variability. This phenomenon has also
been observed in other Bacillus species [36] and was sup-
ported by a recent study comparing data from wet lab meth-
ods and genome-sequencing studies, which shows the DNA
G+C content varies by 3–5 % within species with conven-
tional methods and within 1 % for whole-genome sequenc-
ing data [37, 38]. All the G+C content values of these three
strains fall into the range (32–66 mol%) observed for mem-
bers of the genus Bacillus [2, 3].

Chemotaxonomic characterization of strain NEAU-cbsb5\textsuperscript{T}
and the type strains of related species was done by using
cells grown in TSB for 2 days at 30 °C. Cells were har-
vested by centrifugation, washed with distilled water and

![Fig. 1. Maximum-likelihood tree showing the phylogenetic position of strain NEAU-cbsb5\textsuperscript{T} and related taxa based on 16S rRNA gene
sequences. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with both the neighbour-
joining and the maximum-parsimony methods. Bootstrap values >50 % (based on 1000 replications) are shown at branch points. Bar,
0.02 substitutions per nucleotide position.](image-url)
freeze-dried. The isomer of DAP in the whole-cell hydrolysates was derivatized according to McKerrow et al. [39] and analysed by HPLC using an Agilent TC-C18 column (250 × 4.6 mm, i.d. 5 μm) with a mobile phase consisting of 0.05 mol l⁻¹ phosphate buffer, pH 7.2 (0.2 M Na₂HPO₄/0.2 M NaH₂PO₄, 28:72, v/v)/acetonitrile (85:15, v/v) at a flow rate of 0.5 ml min⁻¹. Peak detection was made using an Agilent G1321A fluorescence detector with a 365 nm excitation and a 455 nm longpass emission filter. The polar lipids were examined by two-dimensional TLC and identified using the method of Minnikin et al. [40]. Menaquinones were analysed as described by Collins [41] using the HPLC-UV method [42]. Fatty acids were analysed by GC-MS using the method of Xiang et al. [43].

The peptidoglycan of strain NEAU-csbsb5ᵀ contained meso-DAP as the diagnostic diamino acid, and the predominant menaquinone was MK-7 (54 %), followed by MK-6 (46 %). The quinone and peptidoglycan diamino acid of strain NEAU-csbsb5ᵀ were typical of the large majority of members of the genus *Bacillus* [44–46]. However, strain NEAU-csbsb5ᵀ could be distinguished from the related strains given that *B. luciferensis* JCM 12212ᵀ contains a higher amount of MK-6 than MK-7 and *B. acidiciper* DSM 18954ᵀ contains MK-8 (Table 1). The phospholipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and an unknown aminophospholipid (Fig. S4). The presence of the three major lipids is in agreement with data for *B. subtilis* and other species of the genus *Bacillus* [47]. Strain NEAU-csbsb5ᵀ could be distinguished from its closely related strains by the presence of unknown phospholipids but the absence of phosphatidylglycerol in *B. acidiciper* DSM 18954ᵀ and the presence of an unknown lipid but the absence of unknown aminophospholipid in *B. luciferensis* JCM 12212ᵀ (Fig. S4). The total cellular fatty acid profiles showed a large amount of branched fatty acids in strain NEAU-csbsb5ᵀ, and the major components (>5 %) were iso-C₁₅:0 (39.8 %), anteiso-C₁₅:0 (14.7 %), C₁₆:0 (14.6 %), C₁₇:0 (8.3 %), C₁₆:₁ω7c (5.8 %) and C₁₈:₀ (5.2 %) (Table S1). These data are consistent with reports that iso- and anteiso-branched fatty acids of the 14–17 carbon series are typical of members of the genus *Bacillus* [48]. Major differences were found in the fatty acid compositions of strains NEAU-csbsb5ᵀ, *B. acidiciper* DSM 18954ᵀ and *B. luciferensis* JCM 12212ᵀ (Table S1). Strain NEAU-csbsb5ᵀ had large amounts of C₁₆:₀ (14.6 %) compared to *B. acidiciper* DSM 18954ᵀ and *B. luciferensis* JCM 12212ᵀ in which the contents were less than 5 %. The other significant (>5 %) fatty acids detected in strain NEAU-csbsb5ᵀ included C₁₇:₀, C₁₈:₀ and C₁₆:₁ω7c, which are absent in *B. acidiciper* DSM 18954ᵀ and *B. luciferensis* JCM 12212ᵀ. The fatty acid iso-C₁₆:₀ could not be detected in strain NEAU-csbsb5ᵀ, while the two reference strains had this component at >5 %. These differences in fatty acid composition clearly distinguished strain NEAU-csbsb5ᵀ from its phylogenetically closest relatives.

Based on phylogenetic, biochemical and chemotaxonomic criteria, it is proposed that strain NEAU-csbsb5ᵀ should be classified as the type strain of a novel species within the genus *Bacillus*, for which the name *Bacillus solisilvae* sp. nov. is proposed.

**DESCRIPTION OF BACILLUS SOLISILVAE SP. NOV.**

*Bacillus solisilvae* (so.li.si’lva’e. L. neut. n. solum soil; L. fem. n. silia forest; N.L. gen. n. solisilvae of forest soil).

Cells are Gram-stain-positive, facultatively anaerobic, motile, endospore-forming and rod-shaped (0.5–0.7×1.8–2.1 μm). Cells occur mainly singly or occasionally in pairs and flagella are peritrichous. Endospores are oval and subterminal and occur in slightly swollen sporangia after 2 days of incubation at 30 °C on NA containing MnSO₄ (5 mg l⁻¹). Colonies on NA are moist and loosely butyrous, cream-coloured, circular with entire margins and 2.5–3.3 mm in diameter after 2 days of incubation at 30 °C. Grows at 15–40 °C (optimum, 30 °C) and at pH 6.0–8.0 (optimum, 7.0). NaCl is not required for growth but up to 4.0 % (w/v) NaCl is tolerated. Positive for catalase (weakly), hydrolysis of aesculin, starch and Tween 20, 40 and 80, but negative for ONPG, cellulose, gelatin, casein, Voges–Proskauer, methyl red, citrate utilization, nitrate reduction, H₂S and indole production tests. Reactions for arginine dihydrolase, lysine decarboxylase, urease, ornithine decarboxylase and tryptophan deaminase are negative. The following compounds are utilized as sole source of carbon and energy: D-mannitol, raffinose, D-sorbitol, D-xylulose, L-aspartic acid, L-glutamic acid, L-glycine, L-proline, L-phenylalanine, L-tyrosine, malate, oxalate and sucrose. The following compounds are not utilized as sole source of carbon and energy: creatine, D-galactose, maltose, D-fructose, D-glucose, D-ribose, inositol, L-alanine, L-arabinose, L-arginine, L-asparagine, L-glutamine, L-serine, L-threonine, lactose, mannose and pyruvate. Acid is produced from arbutin, celllobiose, D-fructose, D-glucose, D-mannitol, glycogen, maltose, N-acetyl-D-glucosamine, salicin, starch, aesculin and trehalose, but not from glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylulose, L-xylulose, L-adenosine, methyl β-D-xylorynanoside, D-galactose, L-ribose, L-rhamnose, ducitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, lactose, melibiose, sucrose, inulin, melezitose, raffinose, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate or 5-ketogluconate. The major menaquinones are MK-7 and MK-6. The cell-wall peptidoglycan contains meso-DAP as the diagnostic diamino acid. The phospholipid profile consists of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and an unknown aminophospholipid. Major fatty acids (>10 %) are iso-C₁₅:0, anteiso-C₁₅:0 and C₁₆:₀.

The type strain, NEAU-csbsb5ᵀ (=CGMCC 1.14993ᵀ=DSM 100485ᵀ), was isolated from a forest soil collected from...
Changbai Mountain, Heilongjiang Province, north-east China. The DNA G+C-content of the type strain is 33.0 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References


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