Bacillus notoginsengisoli sp. nov., a novel bacterium isolated from the rhizosphere of Panax notoginseng

Meng-Yue Zhang,† Juan Cheng,† Ying Cai, Tian-Yuan Zhang, Ying-Ying Wu, Deene Manikprabhu, Wen-Jun Li and Yi-Xuan Zhang

Abstract

A Gram-stain-positive, rod-shaped, motile bacterium designated as SYP-B691T was isolated from rhizospheric soil of Panax notoginseng. Phylogenetic analysis indicated that SYP-B691T clearly represented a member of the genus Bacillus and showed 16S rRNA gene similarity lower than 97.0% with the type strains of species of the genus Bacillus, which indicates that it should be considered as a candidate novel species within this genus. The optimum growth of the strain was found to occur at 37°C and pH 7.0–9.0. The genomic DNA G+C content was determined to be 45.2 mol%. It contained meso-2,6-diaminopimelic acid in the cell-wall peptidoglycan. The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unknown phospholipid. MK-7 was the only menaquinone identified. The major cellular fatty acids of SYP-B691T were identified as iso-C15:0 and anteiso-C15:0. On the basis of phenotypic, chemotaxonomic and phylogenetic characteristics, SYP-B691T merits recognition as a representative of a novel species of the genus Bacillus, for which the name Bacillus notoginsengisoli sp. nov. is proposed, with SYP-B691T (=DSM 29196T=JCM 30743T) as the type strain.

The genus Bacillus was first proposed by Cohn [1] for aerobic or facultatively anaerobic, Gram-stain-positive, endospore-forming and rod-shaped bacteria to be included in the family Bacillaceae of the class Bacilli [2]. At present, there are 318 species described in the genus Bacillus (www.bacterio.net/bacillus.html). The many applications of representatives of this genus have led to the frequent description of novel species isolated from various ecosystems and reported in recent years, such as Bacillus crassostrear [3], Bacillus gobiensis [4], Bacillus lycopersici [5], Bacillus oleivorans [6], Bacillus oryzaeorticus [7], Bacillus paralicheniformis [8], Bacillus polymachus [9], Bacillus rigiliproduci [10], Bacillus shacheensis [11], Bacillus tianshenii [12] and Bacillus tianshenii [13]. The commonest peptidoglycan type is meso-diaminopimelic acid (DAP), and MK-7 is the major menaquinone in species of the genus Bacillus. DNA G+C contents of species within the genus Bacillus range from 32 to 66 mol% [14].

During a study of microbial diversity in the rhizospheric soil sample of Panax notoginseng collected from Yunnan province, western China, many potential strains were isolated and many of them were reported to represent novel species such as Flavobacterium notoginsengisoli [15], Luteimonas notoginsengisoli [16] and Sinomonas notoginsengisoli [17]. Among various potential isolates, strain SYP-B691T showed similar phylogenetic characteristics to those of members of the genus Bacillus, so it was selected for further identification. The 16S rRNA gene sequence of SYP-B691T showed highest similarity to that of Bacillus subterraneus (96.9%). This result encouraged us to establish the taxonomic position of SYP-B691T through phenotypic, chemotaxonomic and phylogenetic analyses.

SYP-B691T was isolated from a soil sample collected from the rhizosphere of Panax notoginseng in Yunnan province, PR China (23° 48’ 36.4” N, 103° 37’ 50.04” E). The soil suspension (1 g soil suspended in 9 ml sterile saline solution) was serially diluted and incubated on Luria–Bertani (LB) medium (Oxoid) supplemented with 50 mg l–1 nystatin (30°C, one week). Colonies were selected and re-streaked repeatedly onto LB agar (Oxoid) to obtain a pure culture. The reference strain B. subterraneus DSM 13966T was selected for the comparison of morphological, physiological, chemotaxonomic and phylogenetic characteristics. The reference strain was cultivated under the same culture conditions as the novel species; growth on LB medium at 37°C.

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Keywords: Bacillus notoginsengisoli sp. nov.; Gram-stain-positive; Panax notoginseng; rhizosphere.

Abbreviation: DAP, meso-diaminopimelic acid.

†These authors contributed equally to this work.

The 16S rRNA gene sequence of strain SYP-B691T has been deposited in GenBank/EMBL/DDBJ under the accession number KP076294.

Two supplementary figures and one supplementary table are available with the online Supplementary Material.
Cell morphology was observed by a light microscopy (BH-2; Olympus) and transmission electron microscope (JEM-2100, JEOL). For transmission electron microscopy, harvested cells were suspended with sterilized water and fixed with 2% sodium phosphotungstate. The cell specimens were observed with a transmission microscope. Gram staining was performed using standard Gram’s reaction. Flagella and endospores were examined according to the methods of Leifson and Schaeffer–Fulton, respectively [18]. Growth at various temperatures (4–55°C) and NaCl tolerance (0–12.0% w/v) were observed using LB as the basal medium. The pH range (pH 4.0–12.0, at intervals of 1.0 pH unit) of the basal medium was maintained using the buffer adjusted described by Xu et al. [19].

Catalase activity was determined by assessing the production of bubbles on addition of a drop of 3% (v/v) H2O2 on the bacterial culture. Oxidase activity was determined based on oxidation of tetramethyl-p-phenylenediamine [20]. H2S production, milk coagulation and peptonization were performed as described by Gonzalez et al. [21]. Other phenotypic and enzyme activities were tested using the API 20NE (bioMérieux), API ZYM (bioMérieux) and GEN III Micro Plate (Biolog) assays according to the manufacturer’s instructions.

SYP-B691T was Gram-stain-positive, rod-shaped and 0.4–0.7×1.1–5.9µm in size with ellipsoidal endospores located sub-terminally and centrally in swollen sporangia, and motile with peritrichous flagella (Fig. S1, available in the online Supplementary Material). The temperature for growth was in the mesophilic range (21–42°C) which was quite similar to that for B. subterraneus DSM 13966T (21–45°C). The NaCl tolerance was up to 3.0% (w/v) which was less than that for B. subterraneus DSM 13966T (8.0% w/v). The pH range for growth was (5.0–10.0) which was almost similar to that of B. subterraneus DSM 13966T (6.0–10.0). SYP-B691T was positive for catalase, nitrate reduction and hydrolysis of Tweens 40 and 60 but negative for oxidase, indole production, hydrolysis of cellulose, gelatin and starch, which were similar to the phenotype of B. subterraneus DSM 13966T. SYP-B691T was positive for urease but negative for milk peptonisation; in contrast, B. subterraneus DSM 13966T was negative for urease and positive for milk peptonisation. The API ZYM results indicated that SYP-B691T was positive for leucine arylamidase, trypsin and acid phosphatase, whereas B. subterraneus DSM 13966T was negative for them. The Biolog GEN III results indicated that SYP-B691T was negative for dextrin, lactose, D-mannitol, D-fructose, D-arabitol and glycerol, whereas B. subterraneus DSM 13966T was positive for them. The strain SYP-B691T was sensitive to 1% sodium lactate, nalidixic acid and D-serine, whereas B. subterraneus DSM 13966T was resistant to them. Features that differentiate SYP-B691T from B. subterraneus DSM 13966T are listed in Table 1.

The genomic DNA isolation and 16S rRNA gene sequencing were performed according to the methods reported by Cui et al. [22] and Li et al. [23]. Pairwise sequence similarities were calculated using a global alignment algorithm implemented at the EzTaxon-e server (www.ezbiocloud.net/eztaxon; [24]). Phylogenetic analysis was performed

Table 1. Differential characteristics of SYP-B691T and the type strains of related species of the genus Bacillus

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<tr>
<td>Temperature range for growth (°C)</td>
<td>21–42</td>
<td>21–45</td>
<td>10–45</td>
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<td>NaCl range (w/v)</td>
<td>0–3.0</td>
<td>0–8.0</td>
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<td>DNA G+C content (mol%)</td>
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<td>43*</td>
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<td>42.2</td>
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<td>42.8</td>
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<td>β-Galactosidase</td>
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<td>H2S production</td>
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<td>ND</td>
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<td>Nitrate reduction</td>
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<td>Starch hydrolysis</td>
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<td>α-D-Glucose</td>
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<td>L-Arabinose</td>
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<td>Maltose</td>
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*Data obtained from Kanso et al. [34].
using the software package MEGA 5 [25] after multiple alignments of sequence data by CLUSTAL X [26]. Three tree-making algorithms, namely the neighbour-joining [27], maximum-likelihood [28] and maximum-parsimony [29], were used for tree reconstruction using Kimura’s two-parameter model [30] with 1000 bootstrap replications [31]. The G+C content of the genomic DNA was determined by using reversed phase HPLC according to the protocol of Mesbah et al. [32], and the standard strain Escherichia coli DH5α was selected as a reference.

An almost complete 16S rRNA gene sequence (1530 bp) was obtained and submitted to GenBank under the accession number KP076294. The BLAST results showed that the SYP-B691T had highest similarity with B. subterraneus DSM 13966T (96.9 %) and less than 96.8 % with the other type strains of species of the genus Bacillus. It is generally accepted that for two bacterial species sharing less than 97.0 % 16S rRNA gene sequence similarity, their DNA–DNA relatedness should be less than 70 % [33]. So, it was not necessary to carry out DNA–DNA hybridization between the SYP-B691T and other strains of species of the genus Bacillus.

The neighbour-joining tree (Fig. 1) showed the SYP-B691T clustered closely with the members of the genus Bacillus. The cluster was found to be stable when the trees reconstructed by using maximum-parsimony and maximum-likelihood methods were examined. The genomic DNA G+C content of SYP-B691T was 45.2 mol%, which was higher than that of B. subterraneus DSM 13966T (43.0 mol%) [34].

Analysis of the isomer of diaminopimelic acid was performed according to the procedures described by Hasegawa et al. [35] and Lechevalier and Lechevalier [36]. Polar lipids were extracted as described by Minnikin et al. [37] and identified by two-dimensional TLC [38]. The quinones were extracted [39] and analyzed using HPLC [40]. For cellular fatty acid analysis, biomass was obtained from cell grown on tryptone soy agar (TSA; Difco) at 37 °C for 3 days. The analysis was performed by using the Microbial Identification System (Sherlock Version 6.1; MIDI database: TSBA6; [41]).

SYP-B691T contained meso-2,6-diaminopimelic acid (meso-DAP) as the cell-wall diamino acid. The polar lipids consisted of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and an

![Figure 1](https://example.com/figure1.png)

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of SYP-B691T and strains of other related species of the genus Bacillus. Bhargavaea beijingensis ge10T (EF371374) was used as an outgroup. Bootstrap values (expressed as percentages of 1000 replications) of above 50 % are shown at the branch points. Bar, 0.005 substitutions per nucleotide position. *Indicate that the clades that were conserved when maximum-parsimony and maximum-likelihood method were used to reconstruct the phylogenetic trees.
Cells are Gram-stain-positive, rod shaped (0.4 μm × 1.1–5.9 μm), have ellipsoidal endospores located sub-terminally and centrally in swollen sporangia, and are motile with peritrichous flagella. Growth occurs at 21–42 °C, pH 5.0–10.0 and with up to 3.0 % (w/v) NaCl. Optimal growth occurs at 37 °C, pH 7.0–9.0 and in the presence of 0–2.0 % NaCl (w/v). Positive for catalase, aesculin hydrolysis, urease, milk coagulation, nitrate reduction and hydrolysis of Tween 40 and 60 but negative for arginine dihydrolase, H₂S production, indole production, hydrolysis of casein, cellulose, gelatin or starch, methyl red and Voges–Proskauer tests, milk peptonisation, oxidase and hydrolysis of Tween 20 and 80. Tests for acid phosphatase, alkaline phosphatase, cystine arylamidase, leucine arylamidase, trypsin and valine arylamidase are positive. Positive for utilization of L-arabinose, D-fructose-6-phosphate, gelatin, α-D-glucose, N-acetyl-glucosamine, glucuronate, D-gluconic acid, L-lactic acid, maltose, glycyrl-γ-proline and trehalose but negative for ace- toacetic acid, L-arginine, D-arabitol, d-aspartic acid, α-keto-butyric acid, γ-aminobutyric acid, cellobiose, citric acid, dextrin, formic acid, D-fucose, L-fucose, N-acetyl-D-galactosamine, D-galacturonic acid, gentiobiose, 3-methyl glucose, α-ketoglutaric acid, L-histidine, myo-inositol, lactose, D-malic acid, D-mannose, D-mannitol, melibiose, masic acid, pectin, propionic acid, L-lyguluramic acid, quinic acid, raffinose, L-rhamnose, D-salicin, D-sorbitol, stachyose, sucrose and turanose. The whole-cell hydrolysates contain meso-DAP as the cell-wall diamino acid. The polar lipids are consisted of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and an unknown phospholipid (PL). MK-7 is the only menaquinone identified. The major cellular fatty acids are iso-C₁₅:₀ and anteiso-C₁₅:₀.

The type strain is SYP-B691ᵀ (=DSM 29196ᵀ=JCM 30743ᵀ), which was isolated from the rhizosphere of Panax notoginseng. The genomic DNA G+C content of the type strain is 45.2 mol%.

**DESCRIPTION OF BACILLUS NOTOGINSENGISOLI SP. NOV.**

*Bacillus notoginsengisoli* (no.to.gin.seng.iso’li. N. L. neut. n. notoginsengium notoginseng; L. n. solum soil; N. L. gen. n. notoginsengisoli of soil of a notoginseng root, the source of the organism).

Based on the phenotypic and chemotaxonomic characteristics, SYP-B691ᵀ is proposed to represent a novel species within the genus *Bacillus*, for which the name *Bacillus notoginsengisoli* sp. nov. is proposed.


43. Tiago I, Pires C, Mendes V, Morais PV, Da Costa MS. Separation of bacterial menaquinones by HPLC using reverse phase (RP18) and a silver loaded ion exchanger as stationary phases. *J Liq Chromatogr* 1982;5:2359–2367.

