**Viridibacillus soli** sp. nov., isolated from forest soil in Ailaoshan National Nature Reserve

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**Abstract**

A Gram stain-positive, rod-shaped, and subterminal endospore-forming bacterium, designated strain YIM B01967^T, was isolated from a forest soil sample collected in Ailaoshan National Nature Reserve, Yuxi City, Xinpin county, Yunnan province, China. Strain YIM B01967^T showed the highest 16S rRNA gene sequence similarity with *Viridibacillus arvi* (99.1%) and *Viridibacillus arenosi* (98.9%). Based on the phylogenetic and 16S rRNA gene sequence results, strain YIM B01967^T was affiliated to the genus *Viridibacillus*. The growth of YIM B01967^T was observed at 15–35 °C (optimum, 28 °C), pH 7.0–9.0 (optimum, pH 7.5) and in the presence of 0–2% (w/v) NaCl (optimum in 2% NaCl). The cell wall sugars include ribose, glucose, arabinose, galactose, and mannose. The quinone system consisted of the major compound MK-8 and moderate amounts of MK-7. The major fatty acids (> 10%) included iso-C₁₅:₀, anteiso-C₁₅:₀, C₁₆:₁ω₁₀c. The major polar lipids profile included DPG, PME. The cell wall peptidoglycan was most likely of the type A4α with an l-Lys-d-Asp interpeptide bridge. The genomic DNA G + C content of strain YIM B01967^T was 36.3 mol%. The ANI and digital DNA–DNA hybridization (dDDH) values between strain YIM B01967^T and *Viridibacillus arvi* DSM 16317^T, *Viridibacillus arenosi* DSM 16319^T were 61.0% and 32.1%, 60.0% and 33.1% based on the draft genome sequence. The results support the conclusion that strain YIM B01967^T represents a novel species of the genus *Viridibacillus*, for which the name *Viridibacillus soli* sp. nov., is proposed. The type strain is YIM B01967^T (= KCTC 43249^T = CGMCC 1.18436^T).

**Keywords** *Viridibacillus* sp. nov. · Forest soil · Genome analysis · Polyphasic taxonomy

**Abbreviations**

PCA Plate count agar  
PCB Plate count broth  
DPG Diphosphatidylglycerol  
PME Phosphatidylmethyl ethanolamine  
PG Phosphatidylglycerol  
PE Phosphatidylethanolamine  
PL Unidentified phospholipid

**Introduction**

The genus *Viridibacillus* belongs to the family *Plano- cocciaceae*, phylum *Bacillales* which was first proposed by Albert et al. (2007) to reclassify some species in the genus *Bacillus*. Up to now, this genus consists of 3 species, namely *Viridibacillus arenosi* (Heyrman et al. 2005; Albert et al. 2007), *Viridibacillus arvi* (Heyrman et al. 2005; Albert et al. 2007), and *Viridibacillus neidei* (Nakamura et al. 2002; Albert et al. 2007), all isolated from soil. In this study, to study the soil microbial species diversity in the extreme environment of the alpine mountainous area, the strain YIM B01967^T was isolated from a forest soil sample in Ailaoshan National Nature Reserve, Yuxi City, Xinpin county, Yunnan province, China. Through the study of polyphasic taxonomy,
strain YIM B01967T is considered to be a new member of the genus *Viridibacillus*, named *Viridibacillus soli* sp. nov.

**Methods and materials**

**Strain isolation and culture conditions**

Forest soil surface samples were collected from the Ailao Mountain National Nature Reserve in Yuxi City, Yunnan Province, China. Isolation was performed by the standard dilution plate method on plate count agar (PCA; Difco) at 28 °C for 4–7 days. The isolation procedure was performed as described by Liu et al. (2017). The pure culture of the randomly selected single colony strain YIM B01967T was stored on a PCA slant at 4 °C, and stored as a glycerol suspension (20%, w/v) at −80 °C. Besides, it was preserved in lyophilized form in skimmed milk at 4 °C temperature. The reference strain, *Viridibacillus arvi* DSM 16317T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ).

**Phylogenetic and genotypic analyses**

The preliminary identification of strain YIM B01967T was performed based on the 16S rRNA gene sequence and phylogenetic analysis. The extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene were carried out as described by Li et al. (2007). The closest phylogenetic neighbours and the corresponding similarity were determined by aligning the obtained 16S rRNA sequence against the bacterial type species recorded in the EzBioCloud server (https://www.ezbiocloud.net; Yoon et al. 2017). Multiple sequence alignments were performed with CLUSTAL_X (Thompson et al. 1997). Phylogenetic analysis was performed using the MEGA software package version 7.0 (Kumar et al. 2016). The phylogenetic trees were reconstructed using neighbor-joining (NJ) (Saitou and Nei 1987), maximum-parsimony (MP) (Fitch 1971) with MEGA 7 software (Felsenstein 2017). The stability of the topology and the phylogenetic tree was evaluated using bootstrap analysis (Felsenstein 1985), with 1000 replications. The 16S rRNA gene sequence of *Bhargavaea cecembensis* DSM 22132T was used as an outgroup.

The sequencing of the whole genome was performed on the HiSeq X-Ten platform (Illumina). The draft whole-genome sequencing of the strain YIM B01967T was performed by Majorbio (Shanghai, China). Average Nucleotide Identity (ANI) values were calculated using JSpeciesWS (http://jspecies.ribohost.com/jspeciesws/#analyse) (Richter et al. 2016). The Genome-to-Genome Distance Calculator, version 2.1 was used to calculate the digital DNA–DNA hybridization (dDDH) value (Meier-Kolthhoff et al. 2013). The dDDH results of recommended formula 2 (identities/HSP length) were used. A phylogenomic tree was constructed based on genomic data using the supermatrix method (Zhi et al. 2017).

**Morphological, physiological, and biochemical analyses**

To determine the differential phenotypic properties, strain YIM B01967T was subjected to morphological, physiological, and biochemical analyses. Phenotypic characteristics of strain YIM B01967T were observed using cells grown on PCA medium for 4 days at 28 °C. Cell morphology was examined using a transmission electron microscope (JEM 2100; JEOL). For transmission electron microscopy, cells were negatively stained with 1% phosphotungstic acid before observation. Colony morphology and pigmentation were observed on PCA medium incubated at 28 °C for 4 days. Growth at different temperatures (4, 10, 15, 20, 25, 28, 30, 35, 37, 40, and 45 °C) was examined after incubation on PCA medium for 4 days. Tolerance to NaCl between 0 and 10% (w/v, at intervals of 2%) in plate count broth (PCB; Difco) medium at 28 °C was recorded after 4 days. The ability of the strain to grow at different pH values (4.0–10.0, at 0.5 intervals using the buffer system described by Tang et al. 2010). Catalase activity was determined by the production of bubbles after adding 3% H2O2 to the tested bacteria (Tarrand and Gröschel 1982). Enzyme activities, production of acid, utilization of different compounds, and the other physiological functions were tested with API ZYM, API 20NE kits, API 50CHB kits, and the Biolog GEN III MicroPlates kits according to the manufacturers’ instructions. All tests were completed in duplicate.

**Chemotaxonomic characterization**

The isolation of the peptidoglycan and analysis of the peptidoglycan structure were done according to published protocols (Schumann 2011; Schleifer and Kandler 1972). Analyses of cell wall peptidoglycan and sugars of whole-cell hydrolysates were performed according to the procedures described by Lechevalier and Lechevalier (1970) and Tang et al. (2009). Cellular fatty acids were extracted, methylated, and analyzed using the Sherlock Microbial Identification System (MIDI) according to the manufacturer’s instructions. Fatty acid methyl esters were analyzed using the Microbial Identification Software Package (Sherlock Version 6.1; MIDI databaseTSBA6) (Sasser 1990). The respiratory quinones of YIM B01967T were extracted from lyophilized cells (Collins and Jones, 1980), purified by TLC, and then analyzed by HPLC according to the methods of Xie...
and Yokota (2003). Polar lipids were extracted, examined by two-dimensional TLC, and identified using the procedures described by Collins and Jones (1980) and Minnikin et al. (1979).

**Results and discussion**

**Molecular phylogenetic analysis**

The almost-complete 16S rRNA gene sequence of YIM B01967T was 1540 bp (GenBank accession number MW386301). Strain YIM B01967T showed the highest 16S rRNA gene sequence similarity with *Viridibacillus arvi* (99.05%) and *Viridibacillus arenosi* (98.92%). The NJ tree, MP tree, and ML tree for the 16S rRNA shared the same topology and are presented in Fig. 1, Fig S1, and Fig S2, respectively.

The draft genome of strain YIM B01967T contained 112 contigs, with a total length of 4,553,251 bp and an N50 length of 171,059 bp (GenBank accession number JAE0AH000000000), and genome coverage of 14.0×. The DNA G+C content of strain YIM B01967T was determined from the genome to be 36.3 mol%. Strain YIM B01967T genome was annotated with 4444 genes, included 4200 protein-coding genes, 60 tRNA genes, 50 rRNA genes, 5 ncRNA genes, and 184 pseudogenes. In contrast, the draft genome of the reference strain *Viridibacillus arvi* DSM 16317T consists of 4,758,570 bp with an N50 contig length of 244,670 bp and a G+C content of 35.0 mol%. The ANI values between strain YIM B01967T and its closely related strains, *Viridibacillus arvi* DSM 16317T and *Viridibacillus arenosi* DSM 16319T, were 61.0% and 60.0%, respectively, based on the draft genome sequence, which was lower than the 95.0% cut-off for species demarcation (Wayne et al. 1987; Richter et al. 2016). The dDDH values between strain YIM B01967T and *Viridibacillus arvi* DSM 16317T, *Viridibacillus arenosi* DSM 16319T were 32.1% and 33.1% based on the draft genome sequence, which was much lower than the threshold value (70%) recommended for distinguishing novel prokaryotic species (Goris et al. 2007; Chun et al. 2018). The phylogenomics tree of YIM B01967T with the closely related strains is presented in Fig. 2.

**Morphological, physiological, and biochemical analyses**

The YIM B01967T strain was Gram positive, sporulating and active rod shaped. The endospores were approximately round and are located in the sporangia with enlarged or slightly enlarged ends (Fig S1). Cells can grow at in the presence of 0–2% (w/v) NaCl (optimum in 2% NaCl). Other physiological characteristics are given in Table 1. Strain YIM B01967T was catalase-positive and oxidase-negative, and this feature was also present in *Viridibacillus arvi* DSM 16317T and *Viridibacillus arenosi* DSM 16319T.

**Chemotaxonomic characterization**

The cell wall amino acids of strain YIM B01967T contained aspartic acid, glutamic acid, alanine and lysine. As these amino acids matches those of the reference strain *Viridibacillus arvi* DSM 16317T, we deduce that the peptidoglycan was the type A4α (Schleifer and Kandler 1972) with an l-Lys-d-Asp interpeptide bridge (Fig S4). Ribose, glucose, arabinose, galactose, and mannose were the major whole-cell sugars of strain. The quinone system consisted of the major compound MK-8 and moderate amounts of
MK-7 in strain, which was identical to that found in members of the genus *Viridibacillus* (Albert et al. 2007). The cellular fatty acids profile consisted of iso-C₁₅:₀ (34.64%), anteiso-C₁₅:₀ (12.72%) and C₁₆:₀ ω10c (11.53%) as major fatty acids (>10%), and C₁₆:₁ ω7c alcohol (8.95%), anteiso-C₁₇:₀ (6.15%), iso-C₁₆:₀ (6.02%), Summed Feature 4 (iso I-C₁₇:₁/anteiso B) (5.02%), iso-C₁₄:₀ (3.68%), iso-C₁₇:₁ ω10c (3.06%), iso-C₁₇:₀ (2.94%) and C₁₆:₀ (2.21%) as minor fatty acids (>1%) (Table S1). The polar lipids of the strain YIM B01967ᵀ were diphosphatidylglycerol (DPG), phosphatidylmethylethanolamine (PME), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and two unidentified phospholipids (PL1, PL2). (Fig S5). Chemotaxonomic analyses including cell wall peptidoglycan, whole-cell fatty acids, cell wall sugars, and polar lipids exhibited the close similarity of strain YIM B01967ᵀ to type strains of the closely related species, which confirmed its affiliation to the genus *Viridibacillus*, with sufficient differences to warrant its proposal as representing a novel species of the genus *Viridibacillus*, for which the name *Viridibacillus soli* sp. nov. is proposed.

**Description of *Viridibacillus soli* sp. nov.**

*Viridibacillus soli* (so'li. L. gen. n. soli of soil, the source of the type strain)

Cells are straight, round-ended, Gram-positive, motile rods (0.6–0.8 × 2.5–3.0 μm), occurring singly and in pairs, the endospores are approximately round and are located in the sporangia with enlarged or slightly enlarged ends. Growth occurs at temperature range of 15–37 °C (optimum, 28 °C), pH 6.0–9.0 (optimum, pH 7.5) and in the presence of 0–2% (w/v) NaCl (optimum 2% NaCl). Catalase-positive and oxidase-negative. In API 20NE, positive for urease, β-glucosidase, and malic acid, nitrate is reduced, gelatin is hydrolyzed. In API ZYM, positive for alkaline phosphatase, esterase, lipid esterase, leucine aramidase, valine aramidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase. In the Biolog GEN III MicroPlate, positive for N-acetyl-d-glucosamine, N-acetyl-β-d-mannosamine, d-galactose, 3-methyl-glucose, inosine, d-mannitol, d-arabitol, myoinositol, glycerol, d-glucose-6-PO₄, d-fructose-6-PO₄, gelatin, l-alanine, l-arginine,
l-glutamic acid, l-histidine, l-pyroglutamic acid, l-serine, d-galacturonic acid, d-gluconic acid, d-gluconuronic acid, quinic acid, d-saccharic acid, d-lactic acid, methyl ester, l-lactic acid, α-keto-glutaric acid, l-malic acid, Tween 40, α-hydroxy-butyric acid, β-hydroxy-d, l-butyric acid, acetate acid, 1% sodium lactate, nalidixic acid, and aztreonam.

In the API 50CHB gallery, acid is not produced from any of the carbohydrate substrates. Cell wall peptidoglycan is presumably of the type A4α with an L-Lys-D-Asp interpeptide bridge. The cell wall sugars are ribose, glucose, arabinose, galactose, and mannose. The quinone system consists of the major compound MK-8 and moderate amounts of MK-7. The fatty acids (> 5% of total fatty acids) are iso-C15:0, anteiso-C15:0, C16:1ω10c, C16:1ω7c alcohol, anteiso-C17:0, iso-C16:0. The polar lipids profile include diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and moderate to minor amounts of two unknown phospholipids (PL1, PL2). The DNA G + C content of the type strain is 36.3 mol% based on the draft genome sequence.

The type strain, YIM B01967T (= KCTC 43,249 T = CGMCC 1.18436 T), was isolated from a soil sample collected from a forest in Ailaoshan National Nature Reserve, Yuxi City, Xinpin county, Yunnan province, China.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and the draft genome sequence are MW386301 and JAE0AH000000000, respectively.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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