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Paenibacillus hubeiensis sp. nov., a New Selenium-Resistant Bacterium Isolated from the Rhizosphere of *Galinsoga parviflora* in a Selenium-Rich Area of Enshi, Hubei Province

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Abstract: A Gram-stain-positive, motile, aerobic, rod-shaped strain ES5-4^T was isolated from the rhizosphere of *Galinsoga parviflora* growing in the selenium-rich ore area of Enshi, Hubei Province, China. This strain could grow at pH 5.0–10.0 and 4–42 °C, with optimal growth at pH 7.0 and 28 °C. It resisted NaCl up to 5% (w/v), with an optimal growth condition of 0.5–1.0%. It tolerated up to 5,000 mg/L of Se^{4+}. The major fatty acids of strain ES5-4^T were *anteiso*-$C_{15:0}$ (46.5%) and $C_{16:0}$ (21.7%). The predominant respiratory quinone was MK-7. The polar lipids were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and an unidentified phospholipid (PL). The 16S rRNA gene sequence implied that ES5-4^T belonged to a member of the genus *Paenibacillus*, with a highest sequence similarity of 98.4% to *Paenibacillus pabuli* NBRC 13638^T. The bac120 tree also verified that the strain was within the genus *Paenibacillus*. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values between ES5-4^T and closely related members of the genus *Paenibacillus* were all below the cutoff levels of 95%–96% and 70%, respectively. Therefore, based on phenotypic, chemotaxonomic, and phylogenetic analyses, strain ES5-4^T represents a novel *Paenibacillus* species, for which the name *Paenibacillus hubeiensis* sp. nov. is proposed. This type strain is ES5-4^T (= GDMCC 1.3540^T = KCTC 43478^T).

Keywords: *Paenibacillus hubeiensis* sp. nov.; polyphasic analysis; bac120 tree

1. Introduction

The genus *Paenibacillus* was originally described by Ash et al. based on the 16S rRNA gene analysis [1], and later emended by Shida et al., who proposed six new species in the genus [2]. The continuous emergence of new members increased the number of species belonging to *Paenibacillus*. At the time of writing (June 2025), the genus contains 323 species with validly published and correct names (https://lpsn.dsmz.de/genus/paenibacillus). Some members of this genus are rod shaped, aerobic or facultative anaerobic, motile or nonmotile, and endospore-form bacteria [3–5].

Paenibacillus species can be found in many ecological niches, such as fish gut [6], river otter [7], seashore land [8], hot spring [9], salt lake [10], digestive syrup [11], plants [12], and soil [13]. Among the genus, groups of species play a vital role in agricultural application through nitrogen fixation, phosphate solubilization, production of ACC deaminase, and IAA secretion [14–16], which are regarded as plant growth-promoting bacteria/rhizobacteria (PGPB/PGPR) [17–19].

Selenium (Se), an essential trace nutrient, has multiple biological functions, including roles in male reproductive health, the endocrine system, muscle function, the central nervous and

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cardiovascular systems, and immune syestems [20]. Research on Se has attracted extensive focus, partly because of selenocysteine, the constitutive part of the 21st amino acid, making it the only trace nutrient incorporated into proteins through genetic coding [21]. For example, certain Se functional microorganisms, called Se-respiring bacteria, play a pivotal role in the selenium cycle. Some selenate- or selenite-respiring bacteria are capable of producing Se nanoparticles [22]. Lin et al. [23] reported that *Priestia* sp. LWS1, a Se-resistant PGPR, could promote plant growth by 19% and Se accumulation by 75% in *Oryza sativa*.

Therefore, screening and isolating agricultural beneficial Se-resistant microorganisms from various habitats is imperative. In this study, a selenium-resistant PGPR, ES5-4^T was isolated from the rhizosphere of *Galinsoga parviflora* and characterized using polyphasic analysis; the results indicate that it is likely a novel species of the genus *Paenibacillus*.

2. Materials and Methods

2.1. Sample Collection and Isolation of Bacterial Strain

G. parviflora is the numerous species growing in selenium-rich ore area of Enshi, Hubei Province, China (30°8′43′′N 109°49′32′′E). Therefore, the rhizobacterial soil was collected in October 2020. The selenium (Se) content of the soil is 3.57 mg/kg, higher than 0.4 mg/kg, which was considered selenium-rich soil based on the national standard for soil selenium grade (GB/T 44971-2024). The bacterial isolation was based on the method described by Li et al. [24]. Briefly, the soil sample was diluted to 10^{-3} to 10^{-5} with sterile ddH_2O using a 10-fold dilution gradient and then smeared on Trypticase soy broth agar (TSA) medium supplemented with 20 mg/L of Se solution (Na_2SeO_3). After incubation for 3 days at 28 °C in an incubator, the single colony was selected, purified, and incubated on TSA plates with higher Se concentrations successively (50, 100, 200, 500, 1000, and 2000 mg/L). Finally, the strain ES5-4^T was obtained and maintained on TSA and preserved in 30% glycerol suspension at −80°C.

2.2. 16. S rRNA Gene Sequencing and Phylogenetic Analysis

The strain was cultivated in Trypticase soy broth (TSB) medium for 12 h to the exponential growth stage at 180 rpm in a rotary shaker (ZWY-2101C) at 28 °C. Next, the cell suspensions were used for the extraction of genomic DNA using a DNA isolation kit (Sangon Biotech, Shanghai, China), according to the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using the universal primers 27F and 1492R [25]. The PCR products were purified and sequenced by Sangon Biotech. The obtained 16S rRNA gene sequence was analyzed using the EzBioCloud server (https://www.ezbiocloud.net/) [26]. Multiple sequence alignments were performed using ClustalW software (http://www.clustal.org/clustal2/) [27]. Next, phylogenetic analysis of the 16S rRNA gene of strain ES5-4^T was conducted based on neighbor-joining (NJ), maximum-parsimony (MP), and maximum likelihood (ML) algorithms using MEGA 7 software [28]. Phylogenetic distances were calculated according to Kimura's two-parameter model [29]. The bootstrap values were set as 1000 replications, as described by Felsenstein [30].

2.3. Genomic Characterization

Strain ES5-4^T was incubated to the exponential growth stage as mentioned above. The cell suspension was then centrifuged at 4 °C at 8000 × *g*, the supernatant was discarded, the mycelium was rinsed three times with sterile deionized water, and the mycelium was collected and used for genome sequencing. A draft genome sequence of strain ES5-4^T was conducted using the Illumina HiSeq X platform (Personalbio, Shanghai, China). Raw sequence reads were filtered and quality controlled using FastQC [31]. The high-quality reads were *de novo* assembled using SPAdes v3.15.3 [32] in the UGENE software package [33]. The G+C content of the genomic DNA was directly determined using the draft genome sequence. Values of average nucleotide identity (ANI) based on

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BLAST (OrthoANIb) between strain ES5-4^T and its close relatives (*Paenibacillus illinoisensis* NBRC 15959^T, *Paenibacillus pabuli* NBRC 13638^T, *Paenibacillus taichungensis* DSM 19942^T, and *Paenibacillus xylanivorans* A59^T) was calculated on the EzBioCloud server [26]. The Genome-to-Genome Distance Calculator (GGDC) online service (http://ggdc.dsmz.de/distcalc2.php) was used to calculate the digital DNA–DNA hybridization (dDDH) values by applying Formula 2 [34]. Annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline [35] and online eggnog-mapper v2 server (https://eggnog-mapper.embl.de/) [36].

To confirm the taxonomic status of the type strain, a genome-based ML phylogenetic tree (bac120 tree) [37] was constructed based on the protein sequences of the genus *Paenibacillus*, along with the outgroup. Generally, genomic information of type strains in the genus *Paenibacillus* was collected using scripts (https://github.com/zdf1987/Usefull_Scripts/GetTypeStrainGenomes) and the proteomes were downloaded using the NCBI software datasets (https://www.ncbi.nlm.nih.gov/datasets/docs/v2/download-and-install/). Other genomic datasets of strains that closely related to strain ES5-4^T were downloaded manually. Gene sequences of each gene cluster were aligned using MUSCLE [38], and the ML tree was constructed using FastTree 2.1 [39] based on the common genes in the bac120 set after alignment trimming and concatenation. The bac120 tree was constructed automatically using EasyCGTree v4.2 (https://github.com/zdf1987/EasyCGTree4) [40].

2.4. Phenotypic and Biochemical Characterization

The phenotypic characteristics of strain ES5-4^T were evaluated on TSA. Gram staining was conducted using a Gram-staining kit (DM0015, Leagene, Beijing, China). Cell morphology of the strain was visualized using scanning (Sirion 200) and transmission electron microscopes (SEM, Sirion 200; TEM, Tecnai G2 spirit Bio-Twin) [41]. The effect of temperature (4, 10, 20, 28, 37, and 45 °C) on bacterial growth was observed on the TSA after 3 days of incubation. The pH in the range of 4.0 to 10.0 with an increment of 1.0 for bacterial growth was also assessed. The pH was adjusted with 1 M HCl or NaOH. Salt tolerance was evaluated using various NaCl concentrations (0%, 0.5%, 1.0%, 2.0%, 3.0%, 5%, 7%, and 10% w/v). The bacterial motility experiment was conducted by observing cell growth in test tubes with semisolid TSB with 0.5% agar after 3 days of incubation in an incubator at 28 °C. Other physiological and biochemical characteristics of the strain were determined using API 50CH (bioMérieux, France) and Biology GEN III micro test systems (Biology, USA). The reference strains (*Paenibacillus oceanisediminis* JCM17814^T, *Paenibacillus dongdonensis* KCTC 33221^T) used in the study were provided by Marine Culture Collection of China (MCCC). All experiments were performed in triplicate.

2.5. Chemotaxonomic Analysis

Strain ES5-4^T and its reference type strains were cultivated in TSB until to the logarithmic phase at 180 rpm in a rotary shaker (ZWY-2101C) at 28 °C. The cells were collected, washed, and lyophilized in a freeze dryer (Labconco, Kansas City, USA) [42]. The dried cells were used for cellular fatty acids, respiratory quinones, and polar lipid analysis. Cellular fatty acids were extracted and analyzed using the Sherlock Microbial Identification System (MIDI) following Sasser's protocol [43]. Quinones were purified and analyzed using high-performance liquid chromatography [44]. Polar lipids were first extracted based on the method of Minnikin et al. [45], and identified using two-dimensional thin-layer chromatography, according to Komagata and Suzuki [46].

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2.6. Effects of Se^{4+} Concentration on the Growth of Strain ES5-4^T

To assess the effect of Se^{4+} (supplied as Na_2SeO_3) on the cell growth of strain ES5-4^T, TSA and TSB media with various Se concentrations (0, 20, 50, 100, 200, 500, 1,000, 2,000, and 5,000 mg/L) were prepared. For the TSB test, 600 μL of overnight cultured cell suspension, equivalent to 3% inoculation volume (v/v), were added to 50-mL sterile Erlenmeyer flasks containing 20 mL of TSB with aforementioned Se^{4+} concentration and cultured for 2 days in a rotary shaker (ZWY-2101C) at 180 rpm at 28 ℃. Next, the optical density at the 600 nm wavelength (OD_{600}) was measured using a Multiskan Go microplate spectrophotometer (Thermo Fisher Scientific, CA, USA). Cell growth on TSA plates containing equivalent Se^{4+} concentrations was also performed. Each plate was evenly divided into three compartments, and 0.5 μL of overnight cultured cell suspension was dripped into each compartment, cultured in a biochemical incubator (Xinmiao, Shanghai, China) for 2 days at 28 ℃, and the growth of strain ES5-4^T was recorded. The diameter of the colonies of strain ES5-4^T was measured using a digital vernier caliper (DWGR-2954, DELIXI, China). All experiments were performed in triplicate.

2.7. Identification of the Functional Gene for Selenocompound Metabolism

Twenty-two genes involved in selenocompound metabolism (map00450) in bacteria of the genus *Paenibacillus* were identified according to a strategy similar to that previously described [47]. Briefly, profile hidden Markov models (HMMs) of the 22 relevant genes were retrieved from the KOfam database (https://www.genome.jp/ftp/db/kofam/) [48]. These HMMs were merged and adjusted to meet the requirements of the HMMER v3.0 software (http://hmmer.org), which was used by the analysis pipeline based on EasyCGTree v4.2 (https://github.com/zdf1987/EasyCGTree4) [49]. Subsequently, the "Gene_Prevelence.pl" script within the EasyCGTree software package was used to search for homologous genomes using customized HMMs with an e-value cutoff of 1e-80. All candidate genes were further submitted to the online annotation tool KofamKOALA (https://www.kegg.jp/kofamkoala/) [48] for homolog verification. Finally, the online tool ImageGP2 (https://www.bic.ac.cn/BIC/#/) was employed to generate a heatmap of the gene distribution.

2.8. Statistical Analysis

Data were analyzed using a one-way ANOVA. Pairwise differences among treatments were performed using Duncan's multiple range test. Data were visualized using SigmaPlot 14.0 (Systat Software, San Jose, CA, USA). All statistical analyses were conducted using SPSS 20.0 (IBM, Armonk, NY, USA), with a significance level set at $p < 0.05$.

3. Results and Discussion

3.1. Phylogenetic Analysis of the 16S rRNA Gene

The 16S rRNA gene sequence of the strain ES5-4^T was 1,403 bp in length, spanning the complete gene sequence (1552 bp; RefSeq accession NZ_JAVYAE020000044) with three mismatches, and has been deposited at GenBank/EMBL/DDBJ (accession number: OR584159). Strain ES5-4^T shared the highest 16S rRNA gene sequence similarity (98.4%) with *P. pabuli* NBRC 13638^T based on the results from EzBioCloud server [26], followed by *P. dongdonensis* KUDC 0114^T (98.3%) [50], *P. taichungensis* BCRC 17757^T (98.3%) [51] and *P. xylanivorans* A59^T (98.0%) [52]. Alignment of the full-length 16S rRNA gene on the genome against the genome database (refseq_genomes and wgs) on NCBI suggested that there were some genomes from uncharacterized bacteria sharing 16S rRNA gene identities of > 98.5% to strain ES5-4^T, including *Paenibacillus* sp. FSL W8-0426 (100%, NZ_CP150203.1), *P. pabuli* E1 (98.8%, NZ_CP073714.1), *P. xylanilyticus* W4 (98.7%, NZ_CP044310.1), *Paenibacillus* sp. 11B (98.6%, NZ JAUKNS010000005.1), *P. xylanilyticus* L-2 (98.6%, NZ CP182582.1), *Paenibacillus* sp. 1781tsa1 (98.58%, NZ JAMYWY010000001.1), *Paenibacillus* sp. PvP094 (98.5%, NZ JBEPOM010000001.1), and *Paenibacillus* sp. FSL R5-0713 (98.5%, NZ_CP150229.1).

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Phylogenetic analysis using ML method indicated that strain ES5-4^T clustered with *P. dongdonensis* KUDC 0114^T (Figure 1). The MP method revealed that the strain clustered with *P. illinoisensis* NRRL NRS-1356^T and *P. xylanilyticus* XIL 14^T (Figure S1). The above two topologies could not be reproduced in the trees using the NJ method (Figure S2). In addition, this phylogeny was not well supported (bootstrap value < 70%) in all three trees. This meant phylogenetic analysis based on the 16S rRNA gene in the genus *Paenibacillus* was not reliable. *P. dongdonensis* KUDC 0114^T (= KCTC 33221^T) and *P. oceanisediminis* L 10^T (= JCM 17814^T) were used as reference strains in the following tests because they have no available genomes for genome-based distinguishing from strain ES5-4^T.

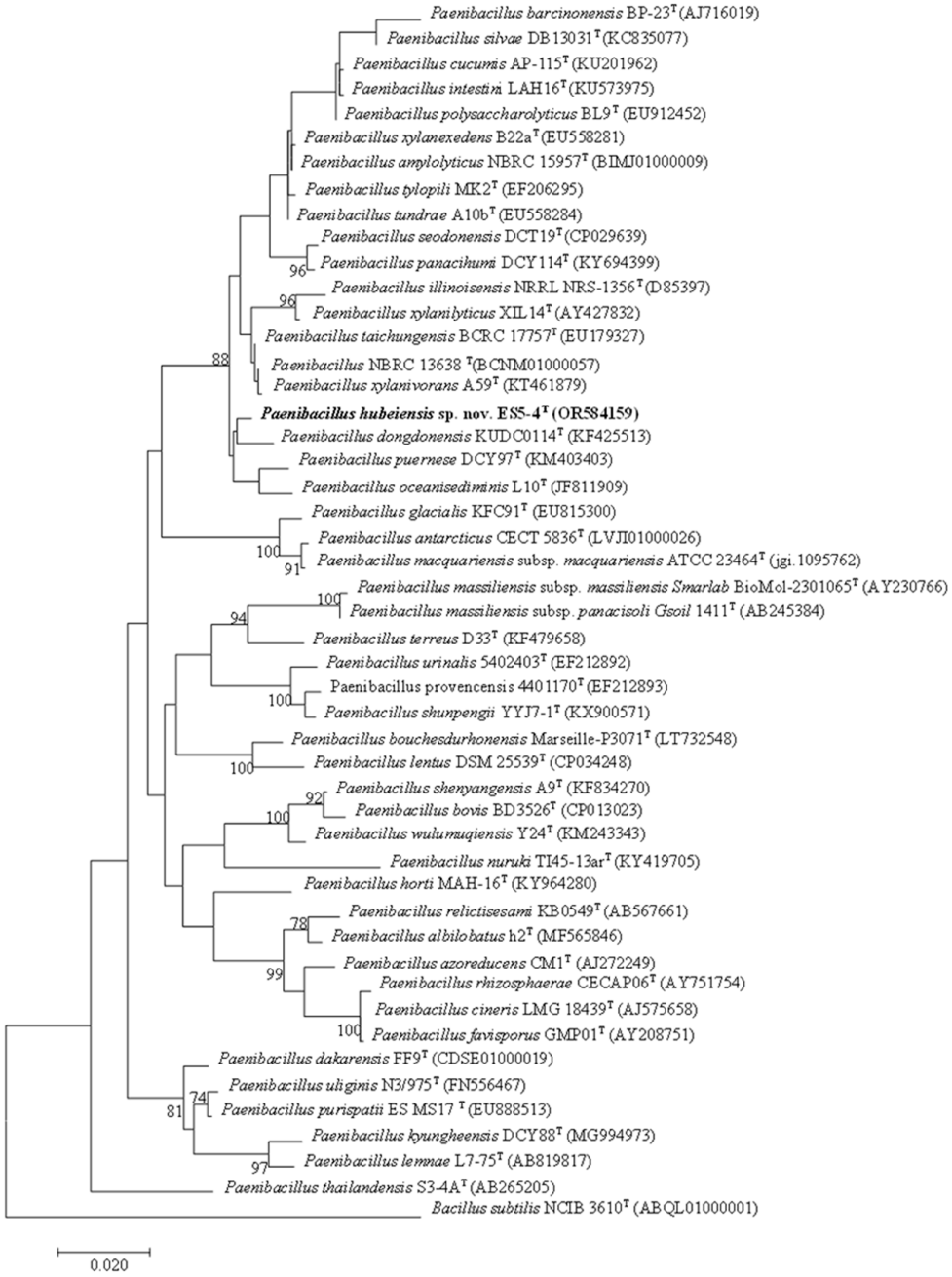

Figure 1. Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences showing the position of strain ES5-4^T. Bootstrap values (expressed as percentages of 1,000 replications) of > 70 (%) are shown at the branch nodes. GenBank accession numbers are indicated in brackets at the end of the tip labels. *Bacillus subtilis* NCIB 3610^T is used as the out group. Bar, 0.02 substitutions per nucleotide position.

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3.2. Genome-Based Characteristics

The whole Genome Shotgun project of strain ES5-4^T has been deposited at GenBank/EMBL/DDBJ with the accession number JAVYAE000000000. The whole draft genome contained 58 contigs, with a total length of 7,190,355 bp, and the genome G + C content was 50.5%. A total of 6,300 protein coding genes were predicted, of which 642, 3, 046 and 5, 370 genes were assigned to the Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Cluster of Orthologous Groups (COG) databases, respectively. Further KEGG pathway analysis revealed 34 genes involved in flagellar assembly (ko02040), 3 genes (*narI*, *narJ*, and *narH*) associated with nitrate reduction, 3 genes (*cysI*, *cysJ*, and *nirB*) with sulfite reduction, the nitrogen fixation gene *nifU*, and 317 ABC transporters; no photosynthesis gene cluster was detected.

According to the taxa of the 16S rRNA gene tree, 205 *Paenibacillus* genomes (including the seven aforementioned atypical strains together with genomes of strain ES5-4^T and *Staphylococcus aureus* NCTC 8325^T (GCA000013425.1, as outgroup) were used to infer a robust tree based on the bac120 gene set (Figure 2). The bac120 tree was well supported on all branches. Strain ES5-4^T was closely related to *Paenibacillus* sp. FSL W8-0426 (GCA_037969725.1) and was subsequently clustered with ten *Paenibacillus* species, including *P. pabuli* NBRC 13638^T, *P. taichungensis* DSM 19942^T, and *P. xylanivorans* A59^T. The results of the 16S rRNA gene tree and bac120 tree both demonstrated that the strain was within the genus *Paenibacillus*.

ANIb values between strain ES5-4^T and the closely related strains *P. pabuli* NBRC 13638^T (GCA004000925.1), *P. taichungensis* DSM 19942^T (GCA013359905.1), *P. xylanivorans* A59^T (GCA001280595.1) and *P. illinoisensis* NBRC 15959^T (GCA004000925.1) were 76.4%, 76.2%, 76.3% and 76.8%, respectively, whereas the corresponding dDDH values were 21.2%, 20.9%, 20.6% and 21.6%, respectively (Table 1). These values were lower than the proposed and acceptable species boundaries of 95-96% for ANI and 70% for dDDH [53,54]. In addition, strain ES5-4^T showed an ANI value of 98.8% with strain *Paenibacillus* sp. FSL W8-0426 (GCA_037969725.1), which had a 16S rRNA gene similarity of 100%. Taken together, these findings imply that the strain ES5-4^T represents a novel *Paenibacillus* species.

Table 1. Average nucleotide identity based on BLAST (OrthoANIb) and digital DNA–DNA hybridization (dDDH) analysis among ES5-4^T and its closely related species.

Strain	1	2	3	4	5
1	-	76.4	76.2	76.3	76.8
2	21.2	-	88.6	88.4	80.5
3	20.9	36.3	-	91.8	80.4
4	20.6	35.9	45.6	-	80.5
5	21.6	23.8	29.9	23.6	-

Note: 1, strain ES5-4^T; 2, *Paenibacillu pabuli* NBRC 13638^T; 3, *Paenibacillu taichungensis* DSM 19942^T; *Paenibacillu xylanivorans* A59^T; *Paenibacillu illinoisensis* NBRC 15959^T. OrthoANIb results are shown above the diagonal, and dDDH results are shown below the diagonal. The values are shown as percentages.

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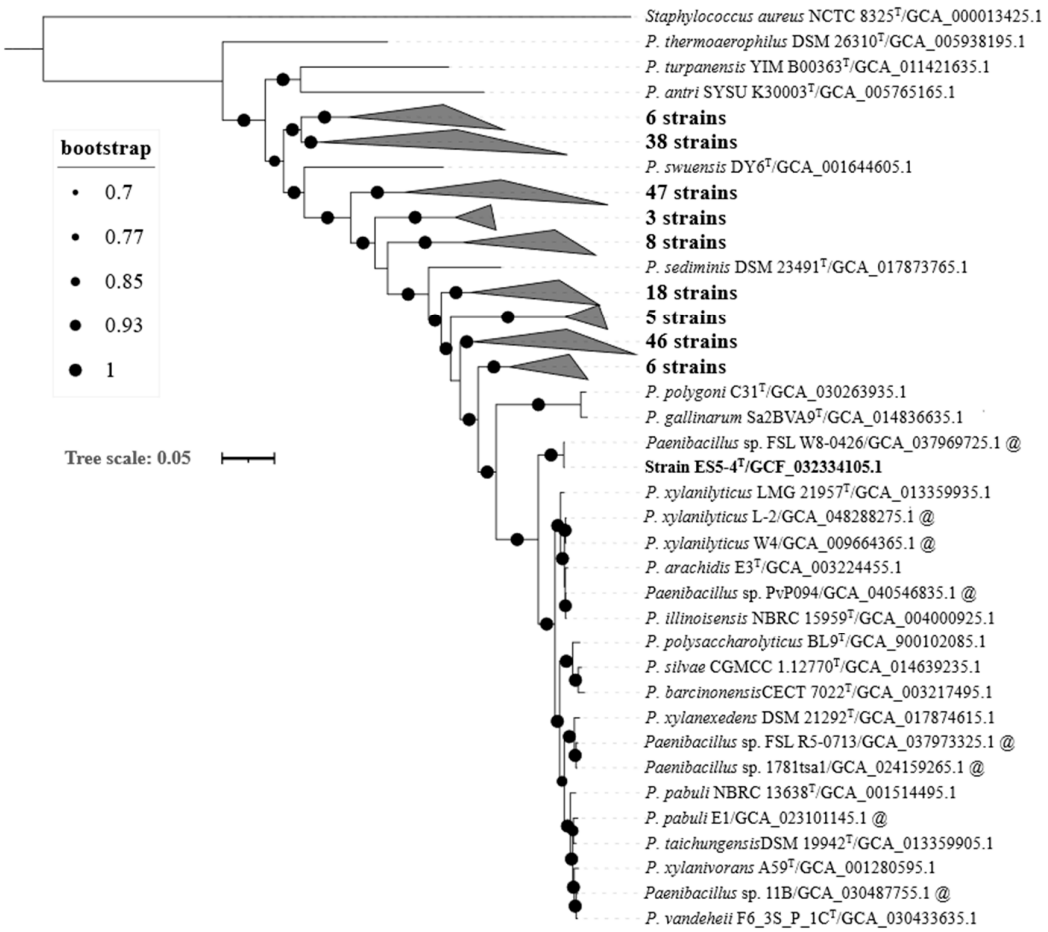

Figure 2. A maximum likelihood tree based on protein sequences of the bac120 gene set, showing the phylogenetic relationship of ES5-4^T in the genus *Paenibacillus*. Bootstrap values are displayed at the branch nodes. The GenBank assembly accession number is indicated in brackets. The symbol @ at the end of labels indicates an atypical strain that showed 16S rRNA gene similarities of > 98.5%. *Staphylococcus aureus* NCTC 8325^T (GCA000013425.1) is used as an outgroup. Bar, 0.1 substitutions per amino acid position.

3.3. Phenotypic and Biochemical Characteristics

Strain ES5-4^T was found to be Gram positive, aerobic, and motile with flagella (Figure 3A), and the features were consistent with the description of *Paenibacillus flagellatus* [55]. SEM image of strain ES5-4^T cells showed a rod-shaped profile with 0.4–0.6 μm width and 1.0–2.5 μm length (Figure 3B). Colonies on TSA were white, circle, moist, and smooth after incubation for 72 h at 28 °C. Growth occurred at NaCl concentrations of 0–5.0% (w/v), pH of 5.0–10.0, and temperature of 4–42°C. The optimal growth conditions for strain ES5-4^T were 0.5%–1.0% (w/v) NaCl, pH of 5.0–7.0, and 28 °C; those for *P. dongdonensis* KCTC 33221^T were 0.5%–2.0% (w/v) NaCl, pH of 6.0–7.0, 28 °C; and those for for *P. oceanisediminis* JCM17814^T were 0.5–1.0% (w/v) NaCl, pH of 7.0–8.0, and 28 °C (Table 2).

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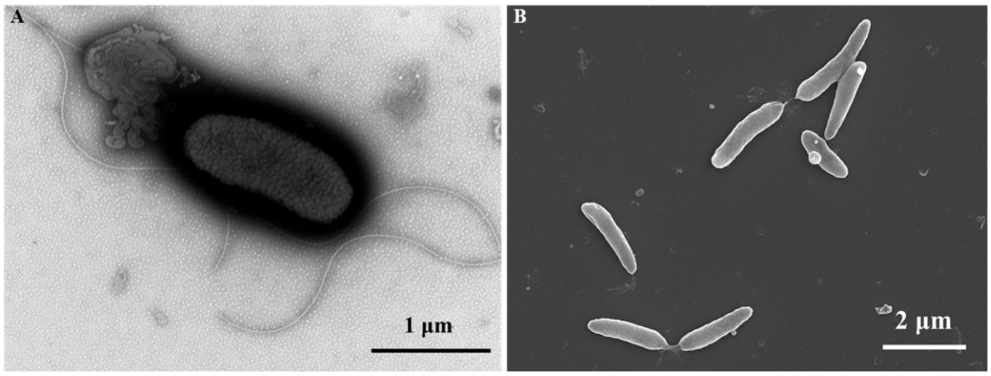

Figure 3. Electron microscopy images of strain ES5-4^T. A: TEM images showing the cell morphology of strain ES5-4^T cultured on TSA media at 28 ℃ for 2 days; B: SEM images showing the cell morphology of strain ES5-4^T cultured on TSA media at 28 ℃ for 1 day.

Table 2. Differential characteristics between strain ES5-4^T and its closely related species.

Characteristic	1	2	3
Motile	+	-	-
Optimal temperature (℃)	28	28	28
Optimal NaCl (%, w/v)	0.5–1.0	0.5–2.0	0.5–1.0
Optimal growth pH	5.0–7.0	6.0-7.0	7.0–8.0
H_2S production	+	+	-
Starch hydrolysis	-	+	+
Cellulose hydrolysis	-	+	+
Tween 20/40/60	-/+/+	+/+/+	-/+/-
API 50CH test			
L-Arabinose	-	+	+
Mannose	-	-	+
D-Turanose	-	w	-
Assimilation of:			
D-Maltose	-	+	+
D-Raffinose	-	-	+
L-Aspartic Acid	-	+	-
Fusidic Acid	+	-	-
D-Galacturonic acid	+	-	+
L-Glutamic Acid	+	+	-
Polar lipids	DPG, PG, PE, PL	ND	L, DPG, PG, PE, APL, PL
Major fatty acids (> 10%)	*anteiso*-$C_{15:0}$, $C_{16:0}$	*anteiso*-$C_{15:0}$, $C_{16:0}$	*anteiso*-$C_{15:0}$, *iso*-$C_{16:0}$, *iso*-$C_{14:0}$
DNA G+C content (%)	50.5	44.3	44.0

Note: 1, strain ES5-4^T; 2, *Paenibacillus dongdonensis* KCTC 33221^T; 3, *Paenibacillus oceanisediminis* JCM 17814^T. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PL, unidentified phospholipid; L, unidentified lipid; APL, Unidentified aminophospholipid. +, Positive; w, weakly positive; -, negative; ND, not determined. Data for taxa 1–3 were collected at the same time in the study.

In the API 50CH tests, strain ES5-4^T was only positive for esculin, and negative for L-arabinose, ribose, D-xylose, β- methyl-D-xyloside, galactose, glucose, fructose, rhamnose, mannitol, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, saccharose, trehalose, raffinose, and gluconate, unlike reference strain *P. oceanisediminis* JCM17814^T and *P. dongdonensis* KCTC 33221^T, which displayed positive reactions in these tests. Compared with the reference strain *P. dongdonensis* KCTC 33221^T, which was positive for H_2S production, hydrolysis of starch, cellulose, Tween 20, 40, and 60,

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strain ES5-4^T was positive only for H_2S production and hydrolysis of Tween 40 and 60. *P. oceanisediminis* JCM17814^T was positive for hydrolysis of starch, cellulose, and Tween 40 and negative for other relative tests (Table 2).

In the BIOLOG GEN III tests, strain ES5-4^T was positive for α-D-glucose, D-mannose, D-fructose, D-galactose, D-fucose, L-glutamic acid, L-serine, D-galacturonic acid, L-galactomic acid lactone, D-gluconic acid, D-glucuronic acid, glucuronamide, mucic acid, quinic acid, citric acid, and L-malic acid. In comparion, *P. oceanisediminis* JCM17814^T was negative for utilization of D-fucose, L-glutamic acid, L-serine, glucuronamide, mucic acid, and citric acid whereas *P. dongdonensis* KCTC 33221^T was negative for L-serine and glucuronamide. These differential characteristics among the three strains are summarized in Table 2.

3.4. Effects of Se^{4+} Concentration on the Growth of Strain ES5-4^T

The effect of Se^{4+} concentration on the growth of strain ES5-4^T was investigated using TSB and TSAs. As Se^{4+} concentration increased, the cell suspension color changed from yellow to deep orange red and then to light orange red in the TSB (Figure S3), indicating that the white Se^{4+} was converted to red selenium nanoparticles (SeNPs) by strain ES5-4^T [56]. The optical density values at 600 nm (OD_{600}) were disturbed by the red color caused by SeNPs except for strain concentration caused by Se^{4+} solution. Therefore, the values showed a trend of first decreasing, then increasing, and then decreasing again, reaching their maximum at a Se^{4+} concentration of 500 mg/L (Figure S3). The cell colors changed from white to red when strain ES5-4^T grew on TSA (Figure S4). In addition, with the increase of Se^{4+} concentration, the colony diameter seemed to decrease significantly ($p < 0.05$) (Figure 4). *Bacillus* sp. KW3, *Corynebacterium* sp. VS5, and *Pseudomonas* sp. VW7 have been reported to be resistant to Se^{4+} above 200 mM [57]. The minimal inhibitory concentrations of *Lysinibacillus xylanilyticus* and L. *macrolides* for Se^{4+} were 120 and 220 mM, respectively [58]. Strain ES5-4^T can be resistant to Se^{4+} concentration at least 5,000 mg/L, approximately responding to 30 mM.

Besides strain ES5-4^T, many other *Paenibacillus* species could also reduce white Se^{4+} to red SeNPs. For example, *Paenibacillus selenitireducens* ES3-24^T isolated from a selenium mineral soil was able to reduce Se to red elemental selenium [59]. Long et al. [60] reported the same function of *Paenibacillus motobuensis* LY5201.

SeNPs are versatile in their application, such as antioxidant [61], antibacterial [62], anticancer and immunomodulatory [63], bioremediation [64], detoxification of heavy metals, nutraceutical functions, as well as the fortification of selenium dietary especially in selenium deficiency areas [65]. Strain ES5-4^T might also be applicable in the aforementioned aspects. However, this requires further research.

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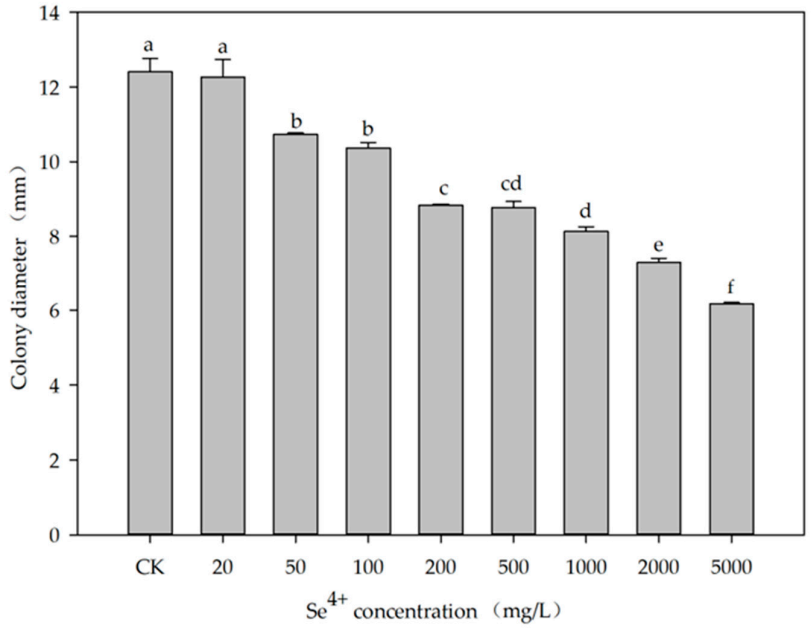

Figure 4. Effects of Se^{4+} concentration (20–5,000 mg/L) on colony diameter of strain ES5-4^T cultured on TSA media at 28 ℃ for 2 days.

3.5. Genetic Features of Paenibacillus for Selenocompound Metabolism

Given strain ES5-4^T's resistance to Se^{4+}, the distribution of the 22 genes involved in selenocompound metabolism was analyzed in the genus *Paenibacillus* and 14 genes were detected (Figure 5, Dataset S1). Genes *trxB*/*TRR*, *metC*, *patB*/*malY*, *metE*, *MARS*/*metG*, *sufS*, *yitJ*, and *metH*/*MTR* were almost universal in these strains, with *metH*/*MTR* absent in *Paenibacillus shenyangensis* A9. *selD*/*SEPHS* was only present in *P. shenyangensis* A9, which catalyzes selenophosphate synthesis using inorganic selenium and ATP as substrates [66]. *metB*, which can catalyze selenocysteine to selenocystathionine [67], was present only in *P. xylanexedens* DSM 21292, *P. shenyangensis* A9, and *Paenibacillus* sp. 1781tsa1. The gene for the reaction (*E4.4.1.11*), capable of producing methaneselenol from selenomethionine [68], was only found in *Paenibacillus* sp. FSL W8−0426, *P. silvae* CGMCC 1.12770, and strain ES5-4^T (Figure 5). The thioredoxin reductase gene *trxB*/*TRR* catalyzed selenite (SeO_3^{2-}) to hydrogen selenide (H_2Se), which was spontaneously oxidized by oxygen to become Se (0) and eventually formed SeNPs [69–71]. Cystathionine-β-lyase coded by *metC* catalyzes selenocystathionine to selenohomocysteine [72,73]. Together, the results suggest that species in the genus *Paenibacillus* were versatile in selenocompound metabolism. Notably, strain ES5-4^T carries two copies of *metC* and *trxB*/*TRR*, which likely confers its selenium resistance (Figure 5).

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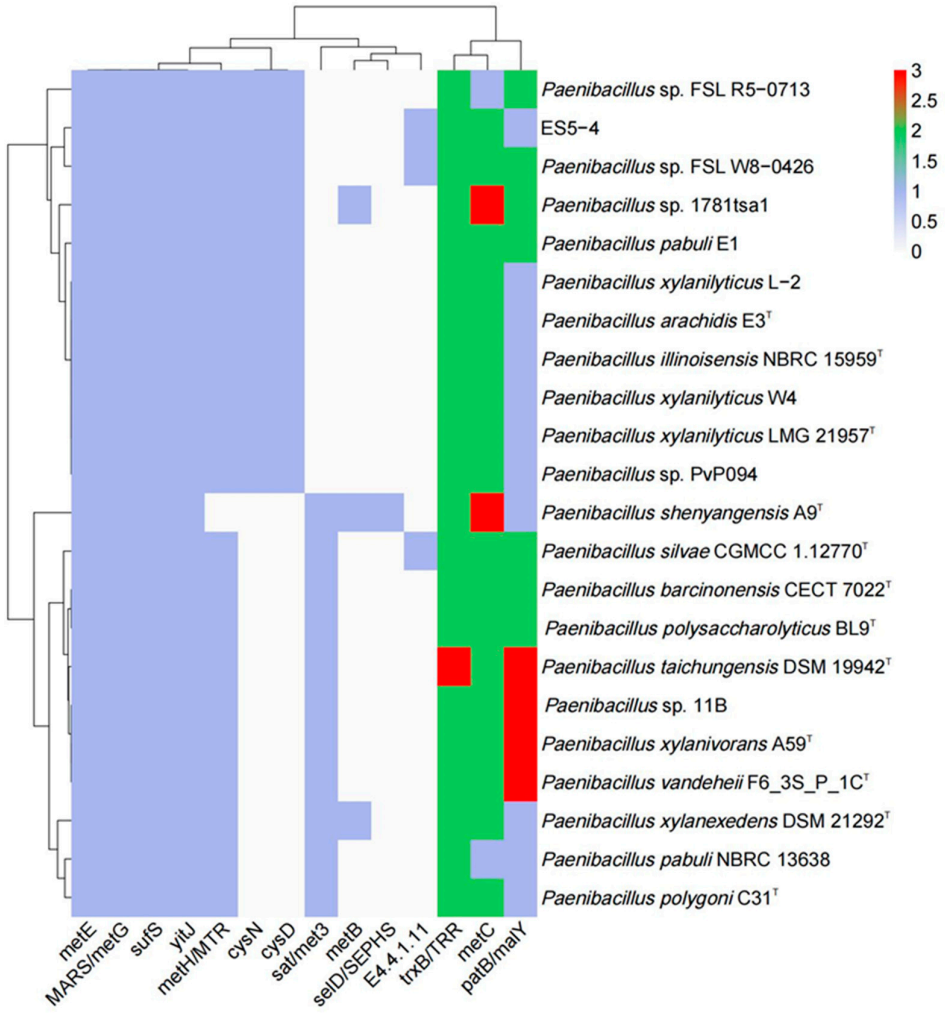

Figure 5. Distribution of genes for selenocompound metabolism within the genus *Paenibacillus*. Type strains are marked in bold. Gradient colors indicate gene numbers (0–3) detected in the relevant gene family.

3.6. Chemotaxonomic Characteristics

The major cellular fatty acids (> 10%) were *anteiso*-$C_{15:0}$ (46.5%) and $C_{16:0}$ (21.7%) for strain ES5-4^T, *iso*-$C_{14:0}$ (10.1%), *anteiso*-$C_{15:0}$ (45.6%), and *iso*-$C_{16:0}$ (20.0%) for *P. oceanisediminis* JCM 17814^T and $C_{16:0}$ (18.3%), and *anteiso*-$C_{15:0}$ (50.3%) for *P. dongdonensis* KCTC 33221^T (Table S1). The polar lipids of strain ES5-4^T mainly comprised diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and unidentified polar lipid (PL), which is similar to that of the closely related strain *P. oceanisediminis* JCM17814^T (Figure S5). The predominant respiratory quinone of strain ES5-4^T was MK-7, in conformity to the genus *Paenibacillus* [74,75].

4. Conclusions

A strain designated ES5-4^T was isolated from rhizosphere (with Se content of 3.57 mg/kg) of *G. parviflora* growing in the selenium-rich ore area of Enshi, Hubei Province, China. 16S rRNA and bac120 gene trees indicated that strain ES5-4^T formed a distinct clade in the genus *Paenibacillus*. The ANI and dDDH values between ES5-4^T and its closely related species were below the boundaries for ANI of 95%–96% and dDDH of 70%. Furthermore, strain ES5-4^T differed from the reference strains *P. oceanisediminis* JCM17814^T and *P. dongdonensis* KCTC 33221^T in phenotype and chemotaxonomic characteristics, such as growth conditions, motile, starch and cellulose hydrolysis, utilization of some

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substrates, polar lipids, and cellular fatty acids. Based on these phenotypic, chemotaxonomic and genotypic features, strain ES5-4^T can be considered a novel *Paenibacillus* species, for which the name *Paenibacillus hubeiensis* sp. nov. is proposed.

Description of Paenibacillus hubeiensis sp. nov.

Paenibacillus hubeiensis (hu. bei. en'sis. N.L. masc. adj. *hubeiensis*, pertaining to the province of Hubei, where the selenium-rich ore is located, from which the strain was isolated)

The strain (ES5-4^T) is Gram-staining positive, motile, aerobic, and rod shaped. The colonies on TSA are milky, circle, moist and smooth after incubation for 3 days at 28°C. The cells can be grown at 4–42 °C (optimum, 28 °C), pH 5.0–10.0 (optimum, 5.0-7.0) and is tolerated to 5% (optimum, 0.5% – 1.0%) of NaCl. It can tolerate up to 5,000 mg/L Se^{4+} and can produce SeNPs. It is only positive for esculin in the API 50CH analysis. In the BIOLOG GEN III tests, it is positive for 16 types of organic carbon sources. The major fatty acids are *anteiso*-$C_{15:0}$ (46.5%) and $C_{16:0}$ (21.7%). The predominant respiratory quinone is MK-7. The polar lipids mainly comprise diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and an unidentified phospholipid (PL).

The type strain, ES5-4^T (= GDMCC 1.3540^T = KCTC 43478^T), was isolated from the rhizosphere of *G. parviflora*, collected from a selenium-rich ore area of Enshi, Hubei Province, China (30°8′43′′N 109°49′32′′E). The genomic G + C content of the strain was 50.5%. The 16S rRNA gene sequence and genome were deposited at GenBank/EMBL/DDBJ with the accession number of OR584159 and JAVYAE000000000, respectively.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Maximum-parsimony phylogenetic tree based on 16S rRNA gene sequences showing the position of strain ES5-4^T. Bootstrap values (expressed as percentages of 1,000 replications) of > 70 (%) are shown at the branch nodes. *Bacillus subtilis* NCIB 3610^T is used as the out group; Figure S2: Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain ES5-4^T. Bootstrap values (expressed as percentages of 1,000 replications) of > 70 (%) are shown at the branch nodes. *Bacillus subtilis* NCIB 3610^T is used as the out group. Figure S3: Effects of Se^{4+} concentration (20–5,000 mg/L) on the growth of strain ES5-4^T cultured in TSB media at 28 ℃ for 2 days; Figure S4: Effects of Se^{4+} concentration (20–5,000 mg/L) on the growth of strain ES5-4^T cultured on TSA media at 28 ℃ for 2 days; Figure S5: Two-dimensional thin-layer chromatogram of polar lipids. Note: a, *Paenibacillus enshiensis* ES5-4^T; b, *Paenibacillus oceanisediminis* JCM17814^T. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PL, unidentified phospholipid; L, unidentified lipid; APL, Unidentified aminophospholipid; Table S1: Cellular fatty acid profiles (% of the totals) of strain ES5-4^T and its closely related type strains of the genus *Paenibacillus*.; Dataset S1: Annotated selenocompound metabolism gene profiles for the genus *Paenibacillus*.

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Data Availability Statement: The data supporting the conclusions of this article are included within the article and its additional files. The 16S rRNA gene sequence of strain ES5-4^T was deposited at NCBI database with the accession numbers of OR584159. The whole genome sequence of strain ES5-4^T was deposited at GenBank/EMBL/DDBJ with the accession number of JAVYAE000000000.

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Abbreviations

The following abbreviations are used in this manuscript:

ANI	Average nucleotide identity
ANIb	Average nucleotide identity calculated by the alignment algorithms BLAST +
APL	Unidentified aminophospholipid
bp	Base pairs
COG	Cluster of Orthologous Groups
dDDH	The digital DNA–DNA hybridization
DPG	Diphosphatidylglycerol
GGDC	Genome-to-Genome Distance Calculator
GO	Gene ontology
ICNP	International Code of Nomenclature of Prokaryotes
KEGG	Kyoto Encyclopedia of Genes and Genomes
L	Unidentified lipid
MCCC	Marine Culture Collection of China
ME	Minimum evolution
MIDI	Microbial identification system
ML	Maximum-likelihood
NCBI	National Center of Biotechnology Information
NJ	Neighbor-joining
PCR	Polymerase chain reaction
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PGPB	Plant growth-promoting bacteria
PGPR	Plant growth-promoting rhizobacteria
PL	Unidentified phospholipid
Se	Selenium
SEM	Scanning electron microscopy
sp. nov	Species novum
TEM	Transmission electron microscopy
TSA	Trypticase Soy Agar
TSB	Trypticase Soy Broth

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