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Posted Date: 17 November 2023

doi: 10.20944/preprints202311.1157.v1

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Article

Species Diversity, Nitrogen Fixation and Nutrient Solubilization Activities of Endophytic Bacteria in Pea Embryos

Junjie Hao ^{1,*†}, Xiaoyan Zhang ^{1,†}, Shizuo Qiu ¹, Fengjing Song ¹, Xianghua Lyu ¹, Yu Ma ² and Hao Peng ^{3,*}

¹ Qingdao Academy of Agricultural Sciences, Qingdao, Shandong 266100, China; zxysea@126.com (X.Z.); 1548622910@qq.com (S.Q.); 471885194@qq.com (F.S.); lvxhua01@163.com (X.L.)

² Department of Horticulture and Crop Science, The Ohio State University, Columbus, OH 43210, USA; ma.2578@osu.edu

³ USDA-ARS, San Joaquin Valley Agricultural Sciences Center, Parlier, CA 93648, USA

* Correspondence: haojunj@126.com (J.H.); hao.peng@usda.gov (H.P.)

† These authors contributed equally to this work.

Featured Application: Endophytic bacteria identified in this research can be used as biofertilizers to improve nutrient utilization in plants.

Abstract: Endophytic bacteria, especially those participate in nitrogen fixation, play critical roles in supplying essential nutrients for legume plant growth. Compared to the numerous bacterial microbiome reports targeting legume roots and nodules, little is known about embryonic bacteria that facilitate plant nutrient utilization after seed germination. Here, we collected and investigated endophytic bacterial microbiomes in edible pea (*Pisum sativum*) embryos using five representative cultivars and a pea sprout control. Twenty-six nitrogen-fixing bacteria (NFBs) were isolated from pea embryos, with three strains found in fresh pea (FGP) and snow pea (SP) exhibiting the strongest nitrogenase activity. Some NFB isolates are also potassium-solubilizing bacteria (KSBs) or phosphorus-solubilizing bacteria (PSBs) utilizing inorganic and/or organic phosphorus. All 26 NFBs showed variable levels of indole-3-acetic acid (IAA) secretion. The nutrient-solubilizing NFBs identified in our research are potential targets for biofertilizer development. At the microbiome level, high throughput 16S ribosomal RNA (rRNA) sequencing of 40 bacterial collections from pea embryos revealed similar community structures and species abundances and diversities in most samples. Our embryo-derived endophytic bacterial pool provides a microbiome platform for seed dormancy and germination research of edible peas.

Keywords: bacterial diversity; edible peas; plant embryo microbiome; endophytic bacteria; nitrogen-fixing bacteria; plant growth-promoting bacteria

1. Introduction

The edible pea (*Pisum sativum*) is a major grain legume crop with about 7.5 million hectares of planting area worldwide [1]. Major edible pea cultivars in the market include dry grain pea (DGP), fresh pea (FGP), snow pea (SP), sweet crispy pea (SCP), and pea tip (SEP). In average, the pea grain consists of 55-60% starch, 20-25% protein, and 6-10% crude fiber, making it a healthy food resource of plant proteins, dietary fiber, vitamins, and essential minerals [2]. Like soybeans [3] and peanut [4,5], pea growth and nutrient uptake require growth-promoting endophytic bacteria [6], especially those involved in nitrogen fixation [7].

Endophytic bacteria thrive inside plant tissues and organs with no immediate impact on plant normal growth [8]. Over time, they may either cause damage to plants or promote plant growth under normal and stressful conditions [9]. Growth-promoting endophytic bacteria can enhance plant nutrient utilization via multiple approaches. Legume-symbiotic nitrogen-fixing bacteria (NFBs) have nitrogenase activity capable of transforming atmospheric nitrogen into plant-usable inorganic compounds [10]. Phosphorus-solubilizing bacteria (PSBs) [11] and potassium-solubilizing bacteria

(KSBs) [12] can solubilize soil inorganic/organic phosphorus and potassium, respectively. These nutrients can be easily utilized by plants in their soluble forms. Endophytic bacteria may also produce auxin [13], cytokinin [14], and/or gibberellin [15] to modulate plant hormonal homeostasis. Nitrogen fixation, nutrient solubilization, and phytohormone production activities are not mutually exclusive in growth-promoting endophytic bacteria, with some strains playing two or more roles in promoting plant nutrient uptake and utilization [9].

Cultivable endophytic bacteria only account for 0.01-10% of the total bacterial species. They can be studied by morphology observation, Gram staining, biochemical and physiological assays, and sequencing of their 16S ribosomal RNAs [16,17]. High throughput 16S RNA sequencing enables an accurate and complete investigation of endophytic bacterial community structure, species diversity and abundances at the microbiome level, with no need for individual strain isolation and culture [18–20].

Endophytic bacteria can be found in plant seeds, leaves, flowers, roots, and root nodules [21]. Seed endophytic bacteria can be horizontally transferred through multiple generations of plants and help to keep seed dormancy status, protect seed from pathogen infection, promote seed germination in the soil, and improve seedling tolerance to abiotic stress [22–24]. Leaf endophytic bacterial microbiomes have high variation associated with plant species, leaf sub-locations, and growth stages. They can suppress pathogen attachment on leaves and enhance plant growth and biomass accumulation [25–27]. Flower endophytic bacteria reside in stigmas, pollens, petals, and nectars to function in pollination [28]. Roots harbor far more endophytic bacterial species and abundance than any other organs. These bacteria are highly diverse in both community structure and functions [29]. Both rhizobia and nonrhizobial endophytic bacteria in root nodules are critical for legume growth and resilience [30].

Most endophytic bacterial studies in pea cultivars focus on roots and root nodules due to their legume and symbiotic nitrogen fixation nature [31–33]. In contrast, little is known about endophytic bacteria in plant embryos [34], which may harbor bacteria transmitted from flowers and seeds, and facilitate plant nutrient utilization after seed germination. The embryo bacterial microbiome also shapes the blueprint of bacterial communities in the next generation of plant body. We previously reported the community structure and associated networks of endophytic bacteria in pea roots throughout plant life cycle [35]. Here, we collected and investigate the endophytic bacterial microbiomes in edible pea embryos using five representative cultivars and a pea sprout control. Twenty-six NFBs were isolated from pea embryos. Some of the NFB isolates are also KSBs or PSBs. All 26 NFBs showed variable levels of indole-3-acetic acid (IAA) secretion. High throughput 16S RNA sequencing of 40 bacterial collections from pea embryos revealed similar community structures, species abundances and diversities in most samples. Our work identified nutrient-solubilizing NFBs with biofertilizer potential and established an embryo-derived endophytic bacterial pool for seed dormancy and germination research of edible peas.

2. Materials and Methods

2.1. Plant materials and sampling

Edible pea seeds were obtained from Qingdao Academy of Agricultural Sciences. Five pea cultivars with different fiber and sugar contents were used in this research, including dry grain pea (DGP), fresh pea (FGP), snow pea (SP), sweet crispy pea (SCP), and pea tip (SEP). Twenty pea accessions were sown with three to four accessions included in each cultivar and a pea sprout (SHP) accession used as the control. Pea plants were grown from March to June 2018. Samplings were carried out at seedling, flowering, grain-filling, and seed maturity stages. All collected samples were stored at -20°C.

2.2. Isolation and purification of cultivable endophytic bacteria

Pea embryos were isolated from de-coated seeds, sterilized with 70% ethanol for 3 min, and rinsed/washed with sterilized water. Adequate sterilization was tested by spreading 100 µl water from last rinse to Trypticase Soy Agar (TSA) plates (15 g/l tryptone, 5.0 g/l soybean peptone, 30 g/l

NaCl, 15 g/l agar). Embryo sterilization was considered successful if no microbe grew after 72-h incubation of the TSA plates at 28°C. Plastic-wrapped sterilized embryos were crushed and transferred to 1.5-ml Eppendorf tubes. After adding 1 ml saline solution to each tube, embryo samples were sonicated for 1 min. After 10-fold dilution, 100- μ l suspension was spread on either TSA or Ashby's nitrogen-free (20 g/l mannitol, 0.2 g/l K_2HPO_4 , 0.2 g/l MgSO_4 , 0.2 g/l NaCl, 0.1 g/l K_2SO_4 , 5.0 g/l CaCO_3 , 15 g/l agar, pH 7.4 \pm 0.2) plate. After incubation at 28°C for 72 h, single bacterial colonies were picked for multiple rounds of streak cultivation. Purified isolates were cultured and stored at -80°C. Bacteria strains isolated from the last round of streak were classified based on colony sizes, colors, transparency, and morphology.

2.3. Nitrogenase activity and nutrient solubilization assays of plant growth-promoting bacteria

Nitrogenase activity was measured by adding 1 ml NFB culture to a 13-ml glass vial and replacing 1 ml air with the same volume of acetylene (99.99% purity). After cultured at 28°C for 24 h, the bacterial sample was mixed with 2 ml of saturated NaCl solution. 200- μ l mixture (10% gas concentration) was then extracted and added to the absorption buffer (5.0 g/l CuSO_4 , 5.3 g/l ammonia, 23 g/l $\text{NH}_2\text{OH}\cdot\text{HCl}$, 0.9 g/l gelatin, 31.6% ethanol). The optical density (OD) was measured at 548 nm after shaking the mixture for 5 min.

To measure inorganic phosphorus solubilization, bacterial strains were inoculated to inorganic phosphorus screen medium (10 g/l glucose, 0.5 g/l $(\text{NH}_4)_2\text{SO}_4$, 0.2 g/l NaCl, 0.2 g/l KCl, 0.03 g/l $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 0.03 g/l MnSO_4 , 0.003 g/l FeSO_4 , 0.5 g/l yeast extract, 5.0 g/l $\text{Ca}_3(\text{PO}_4)_2$, 15 g/l agar, pH 6.8-7.0) and cultured at 30°C for 3 d. Inorganic phosphorus solubilization was indicated by the emergence of clear halos on the plates, with halo sizes positively correlated to solubilization activity. Similarly, organic phosphorus solubilization was tested by bacterial inoculation to egg yolk medium (10 g/l tryptone, 3.0 g/l beef extract, 5.0 g/l NaCl, 15 g/l agar, pH 7.0-7.5; add 3 ml of fresh egg yolk to 50-ml medium plate before use), incubation at 30°C for 3 d, and subsequent formation of turbid halos.

To measure potassium solubilization, bacterial strains were inoculated to silicate medium (10 g/l sucrose, 0.5 g/l yeast extract, 1.0 g/l $(\text{NH}_4)_2\text{SO}_4$, 2.0 g/l Na_2HPO_4 , 0.5 g/l $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 1.0 g/l CaCO_3 , 1.0 g/l potash feldspar powder, 15 g/l agar) and cultured at 30°C for 3 d. The emergence of clear halos on the plates indicated solubilization of inorganic potassium, with halo sizes positively correlated to bacterial solubilization activity.

Bacterial IAA production was quantified by using the Salkowski reagent (fresh mixture of 0.5 M FeCl_3 and 35% HClO_4) to generate a standard curve [36]. 50- μ l bacterial culture at the logarithmic growth phase was inoculated to beef extract liquid medium containing 0.5 g/l tryptophan. After shaking at 140 rpm and 28°C for 36 h, 50- μ l activated bacterial culture at the logarithmic growth phase were mixed with the Salkowski reagent and incubated (avoid light) at 25°C for 30 min. Bacterial IAA production was indicated by the emergence of pink color. IAA-producing bacterial cultures were then centrifuged at 8000 rpm for 5 min. 2-ml supernatant from each strain was mixed with 4-ml Salkowski reagent and incubated (avoid light) at 25°C for 30 min. IAA production was quantified by measuring ODs at 530 nm with three biological replicates for each sample. The standard curve was generated by using 8 IAA standards of gradient concentrations for Salkowski reactions and OD measurements.

2.4. Bacterial DNA extraction and sequencing

Genomic DNA samples were extracted from endophytic bacterial collections from pea embryos. Their 16S rDNA V3/V4-specific fragments (16S) were amplified using the high-fidelity TaKaRa Ex Taq DNA Polymerase (TaKaRa, Dalian, China) and two specific barcoded primers 343F (5'-TACGGRAGGCAGCAG-3') and 798R (5'-AGGGTATCTAATCCT-3'). PCR products were sequenced by OE Biotech (Shanghai, China).

2.5. Bioinformatic analysis of high-throughput DNA sequencing data

The raw bacterial DNA sequences in FASTQ format were processed by Trimmomatic [37] to remove low-quality reads. Optimized paired-end reads were assembled using FLASH [38]. Shorter-than-normal reads and reads containing ambiguous or single repetitive bases were discarded to improve assembly accuracy. At the same time, UCHIME [39] was used to detect and remove chimeric sequences. The high-quality sequences generated afterwards were processed by VSEARCH [40] to define operational taxonomic units (OTUs), with sequences sharing at least 97% identities being clustered as an OTU. Representative 16S OTU sequences were selected using QIIME [41] for blast searches against Greengenes [42] and SILVA [43] databases. Blast results with confidence intervals larger than 0.7 were used for species annotation using Ribosomal Database Project (RDP) Classifier [44].

3. Results

3.1. Morphological identification of cultivable endophytic bacteria in pea embryos

Twenty pea accessions were divided into six cultivar groups, including DGPS (dry grain pea samples S18P01-04), FGPS (fresh pea samples S18P05-08), SPS (snow pea samples S18P09-12), SCPS (sweet crispy pea samples S18P13-16), SEPS (pea tip samples S18P17-19), and SHPS (pea sprout sample S18P20) used as a control. Twenty-seven putative NFB isolates were identified from pea embryos based on their sizes, shapes, texture, and colors (Table S1; Figure 1a). These strains were somewhat evenly distributed in DGPS (S18P01-1, S18P01-2, S18P02-1, S18P02-2, S18P02-3, S18P03-1, S18P03-2, S18P04-1, S18P04-2, and S18P04-3), FGPS (S18P05-1, S18P05-2, S18P06-1, S18P06-2, S18P06-3, S18P07-1, S18P07-2, S18P07-3, S18P08-1, S18P08-2, and S18P08-3), and SPS (S18P09, S18P10, S18P11-1, S18P11-2, S18P12-1, and S18P12-2), with no NFB isolates being identified from SCPS, SEPS, or SHPS.

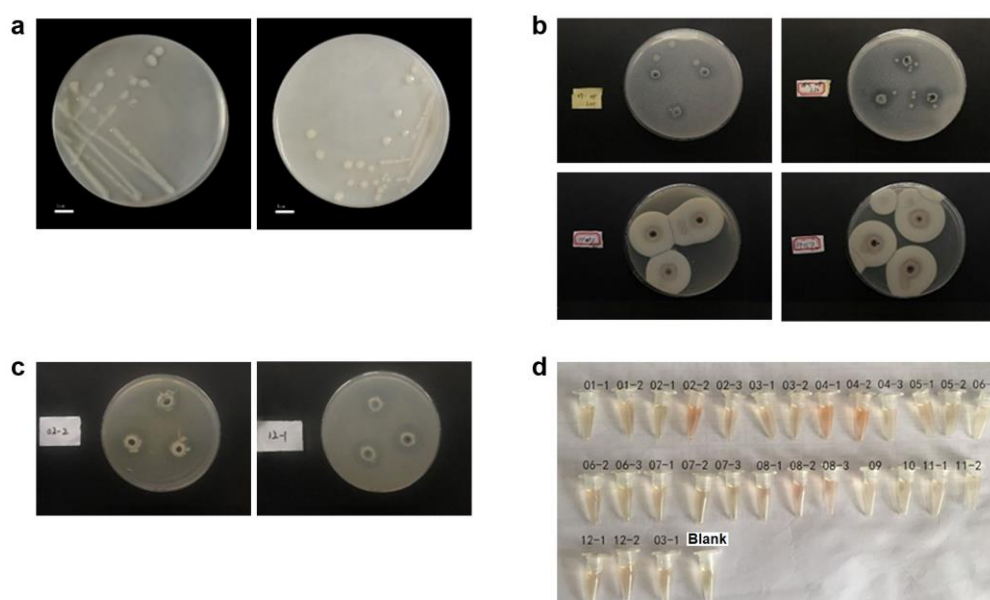


Figure 1. Isolation of nitrogen-fixing bacteria (NFB) from pea embryos and their nutrient solubilization activities. (a) Colonial morphology of two (S18P11-1 and S18P09) representative endophytic bacterial strains. 27 culturable endophytic bacterial strains isolated in this research may possess putative nitrogen fixation capability. (b) Two NFB isolates S18P05-2 and S18P08-3 are also phosphorus-solubilizing bacteria (PSB) capable of solubilizing both inorganic and organic phosphorus. Inorganic phosphorus screen plates (top) and egg yolk plates (bottom) plates were used to test bacterial abilities to solubilize inorganic and organic phosphorus, respectively. The emergence of clear (top) and turbid (bottom) halos indicates bacterial solubilization of inorganic and organic

phosphorus, respectively. (c) Among 13 isolated NFB that are also potassium-solubilizing bacteria (KSB), S18P02-2 (left) and S18P12-1 (right) have the highest potassium solubilization capacities. Bacterial potassium solubilization capability was measured by the emergence of clear halos on silicate plates. Both S18P02-2 and S18P12-1 generated clear halos with diameters as large as 2.2 cm. (d) Quantification assays using the Salkowski reagent show that all isolated NFB secrete IAA, with the highest and lowest levels of IAA production from S18P02-2 and S18P11-1, respectively.

3.2. Nutrient utilization activities of nitrogen-fixing bacteria (NFB) from pea embryos

Nitrogenase activities of the 27 putative NFB cultures ranged from 10.67 to 89.45 nmol C₂H₄/mL/h and averaged at 71.95. They were classified into four nitrogenase activity categories of less than 40 (S18P02-1 [10.67]), 40-60 (S18P11-1 [54.37]), 60-80 (21 isolates), and above 80 (S18P05-1 [85.02], S18P08-2 [89.45], and S18P12-1 [85.86]). The three strongest NFBs came from FGPS (S18P05-1 and S18P08-02) and SPS (S18P12-1). Overall, 26 out of 27 isolates, excluding S18P02-1, were verified as NFBs with considerable nitrogen fixation capability.

PSBs solubilize soil phosphorus for easy absorption by plants [45,46]. Eleven out of 26 NFB isolates generated clear halos on inorganic phosphorus screen plates, demonstrating their ability to solubilize inorganic phosphorus. On the other hand, only 3 out of 26 isolates were organic phosphorus solubilizers, as revealed by their ability to generate turbid halos on egg yolk plates. Two overlappers of the inorganic and organic PSB groups, S18P05-2 and S18P08-3, could solubilize both inorganic and organic phosphorus (Figure 1b).

KSBs catabolize potash feldspar powder in the silicate medium. They also degrade apatite and aluminosilicate minerals in the soil [47]. These soluble nutrients can then be utilized by plants. Thirteen out of 26 NFB isolates generated clear halos on silicate plates, demonstrating that they are also KSBs. Among them, S18P02-2 and S18P12-1 were the two strongest potassium solubilizers whose clear halos diameters were as large as 2.2 cm (Figure 1c).

Tests using the Salkowski reagent showed that all NFB isolates secreted IAA (Figure 1d). They were classified into four categories based on IAA production capacity (mg/ml) [48], including "below 2" (S18P11-1 [0.41] and S18P12-2 [1.97]), "2-4" (18 isolates), "4-6" (5 isolates), and "above 6" (S18P02-2 [7.10]). Isolates with the highest (S18P02-2) and the lowest (S18P11-1) IAA production levels came from FGPS and SPS, respectively.

3.3. Quality control of bacterial DNA sequencing data and operational taxonomic unit (OTU) clustering

23449 to 37104 (33793 in average) effective DNA sequencing reads were obtained from 20 pea embryo bacterial collections (two replicates per collection) after removing low-quality sequences (Table S2). Most of these high-quality reads are 410-450 base pairs (bp) in lengths (Figure S1). A total of 4234 OTUs were obtained using 97%-identity as the threshold for clustering high-quality effective reads (valid tags). OTUs per sample varied from 271 (S18P02.1) to 690 (S18P01.1), with 396 OTUs being annotated on average.

3.4. The community structure of endophytic bacteria in pea embryos

Based on OTU annotation results, endophytic bacteria isolated from the embryos of 20 pea accessions were clustered into 28 phyla, with *Bacteroidetes* (27.82%), *Firmicutes* (26.60%), *Proteobacteria* (17.92%), and *Actinobacteria* (4.10) exhibiting the highest abundances in average (Figure 2a). These OTUs were further clustered into 517 genera (Figure 2b). Overall, dominant genera include *Bacteroides*, *Enterococcus*, *Bacillus*, *Lactobacillus*, *Lachnospiraceae_NK4A136_group*, *Alloprevotella*, *Prevotellaceae_UCG_001*, *Streptococcus*, *Neisseria*, and *Fusobacterium*. Different from most samples, S18P07.2 and S18P10.2/S18P20.2/S18P11.2 harbored the most dominant genus of *Streptococcus* and *Enterococcus*, respectively. In the heat map of bacterial genus abundance (Figure 3), OTUs were somewhat evenly distributed across different genera in most samples except for S18P03.2 (low abundances of *Lactobacillus* and *Bacteroides* and high abundances of *Prevotella_7*, *Streptococcus*, and *Rothia*), S18P01.1 (high abundances of *Bacillus*, *Blautia*, *Prevotellaceae_NK3B31_group*, *Neisseria*, and

Fusobacterium), S18P011.2 (very high abundance of *Enterococcus* and low abundances of all other genera), and S18P14.2 (high abundances of *Bacteroides* and *Lachnospiraceae_NK4A136_group*).

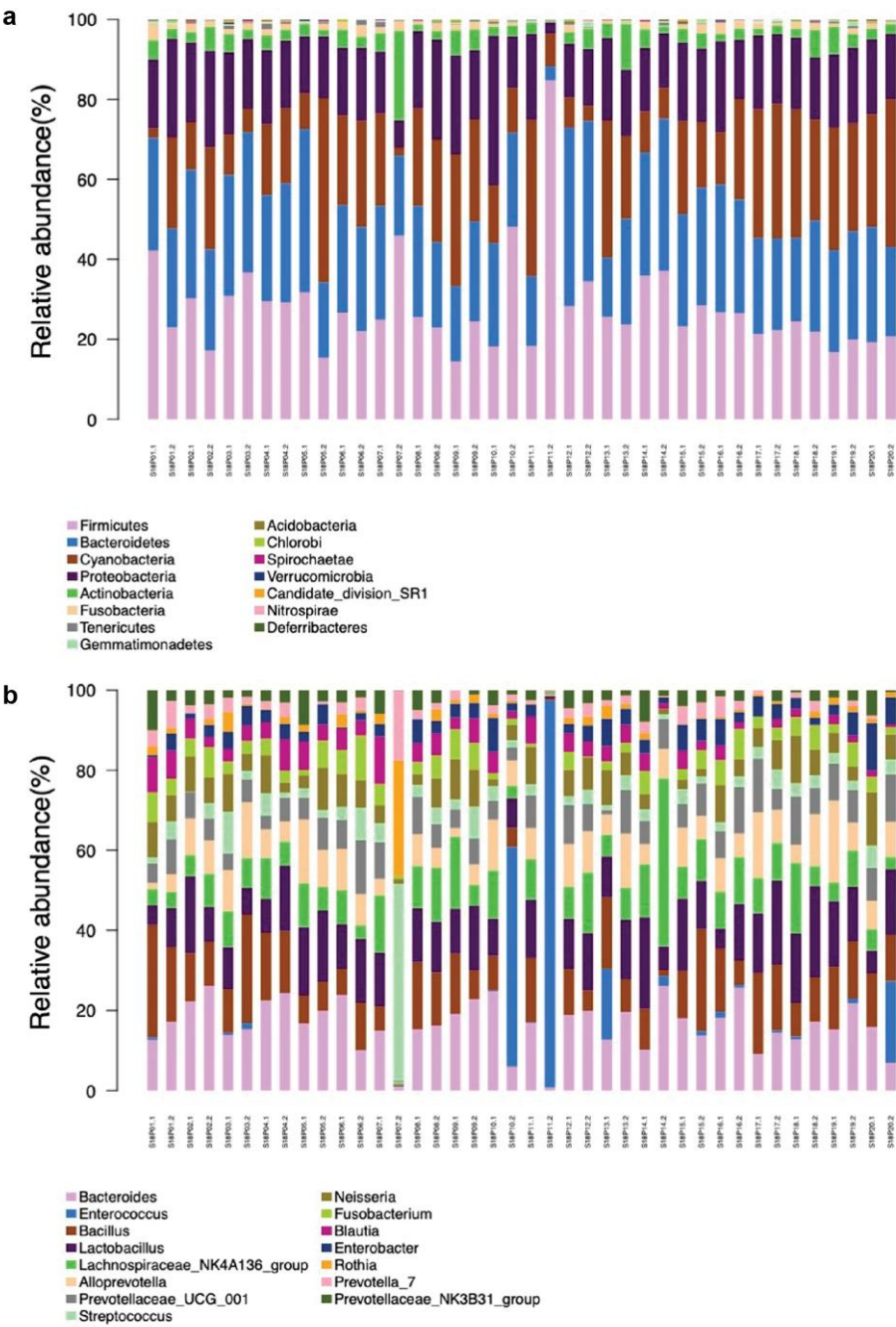


Figure 2. Horizontal community structure of endogenous bacteria in pea embryos at phylum (a) and genus (b) levels.

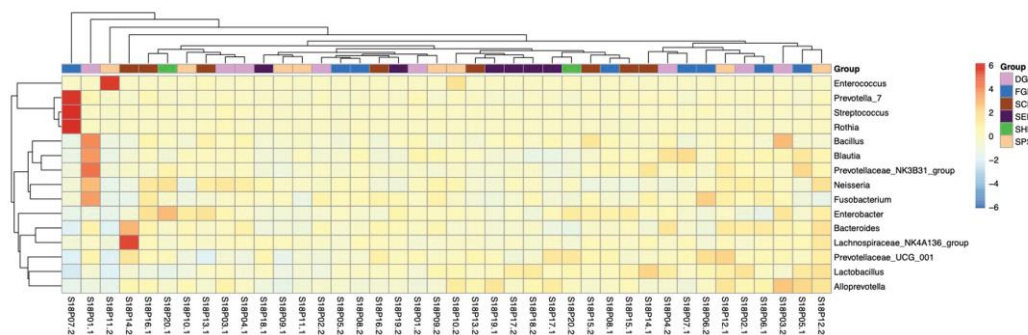


Figure 3. Heat map of horizontal abundance of bacterial genus in pea embryos. Red/orange and blue colors indicate relatively high and low abundances, respectively. DGPS: dry grain pea samples; FGPS: fresh pea samples; SPS: snow pea samples; SCPS: sweet crispy pea samples; SEPS: pea tip samples; SHPS: pea sprout samples.

3.5. Alpha diversity analysis of endophytic bacteria in pea embryos

Meaningful microbial community diversity and abundance analysis requires sufficient sequencing depth to cover most bacterial species in the samples. Constructed from the diversity indexes of different sequencing volumes, the Shannon-Wiener dilution curves [49] all went flat (Figure 4) with sequencing depths above 99% (Table S3), indicating that our sequencing data sizes are large enough to accurately reflect the endophytic bacterial microbiome in pea embryos.

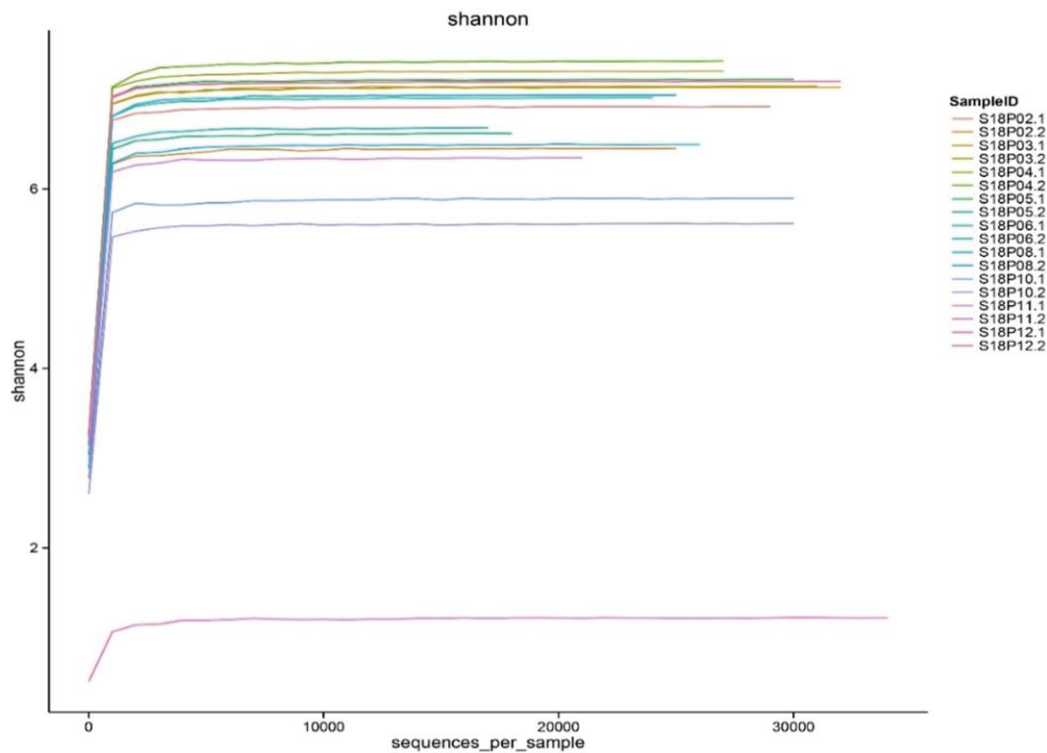


Figure 4. Dilution curves of bacterial diversity indexes in pea embryos.

Chao1, Shannon, and Simpson indexes [50] were calculated (Table S3) to estimate the Alpha (within-sample) diversity [51] of endophytic bacteria in pea embryos. The Chao1 index is the estimation of total OTUs in a sample by counting OTUs that appear only one to two times. High Shannon index values indicate high diversity and relatively even distribution of bacterial species. The Simpson index represents the possibility that two randomly selected bacterial isolates are different species. Simpson values are positively correlated to species diversities in samples. To

3.6. Principal component analysis (PCA) of endophytic bacteria in pea embryos

Figure 5. Principal component analysis (PCA) of endophytic bacteria in six pea embryo cultivar groups. DGPS: dry grain pea samples; FGPS: fresh pea samples; SPS: snow pea samples; SCPS: sweet crispy pea samples; SEPS: pea tip samples; SHPS: pea sprout samples.

3.7. Linear discriminant analysis (LDA) coupled with effect size measurement (LEfSe) analysis of endophytic bacteria in pea embryos

Linear discriminant analysis (LDA) coupled with effect size measurement (LEfSe) analysis was carried out to analyze endophytic bacterial difference in pea embryos. Relatively abundant endophytic bacterial species (LDA score > 3) were identified from all six cultivar groups of DGPS (*Bifidobacterium*, *Bifidobacteriales*, *Bifidobacteriaceae*, and *Blautia*), FGPS (*Prevotella_7*, *Eubacterium_coprostanoligenes_group*, *Entomoplasmatales*, *Entomoplasmatales_Incertae_Sedis*, *Candidatus_Hepatoplasma*, *Spirochaetae*, *Spirochaetaceae*, *Spirochaetales*, *Spirochaetes*, and *Treponema_2*), SPS (*Geodermatophilaceae*), SCPS (*Marvinbryantia*), SEPS (*Ruminococcus_2*, *Incertae_Sedis*, and *Peptoclostridium*), and SHPS (*Peptostreptococcaceae*, *Romboutsia*, *Flavisolibacter*, *Ruminococcaceae_UCG_013*, *Deferribacteraceae*, *Deferribacteres*, *uncultured_bacterium*, *Deferribacterales*, *Mucispirillum*, *Blirii41*, *Flammeovirgaceae*, *Ambiguous_taxa*, *Phaselicystidaceae*, *Phaselicystis*, *Candidatus_Nitrosoarchaeum_limnia_SFB1*, and *SAR324_clade_Marine_group_B*) (Figure 6).

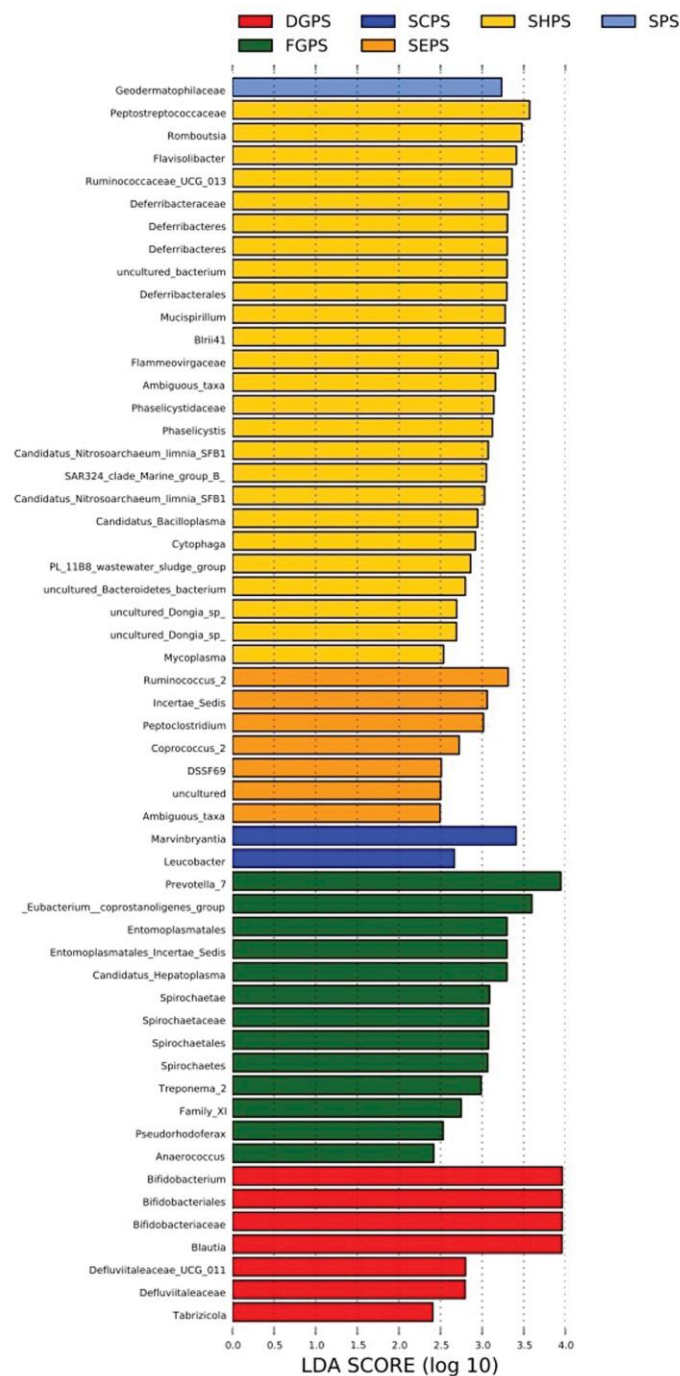


Figure 6. Linear discriminant analysis (LDA) coupled with effect size measurement (LEfSe) analysis of endophytic bacteria in six pea embryo cultivar groups. DGPS: dry grain pea samples; FGPS: fresh pea samples; SPS: snow pea samples; SCPS: sweet crispy pea samples; SEPS: pea tip samples; SHPS: pea sprout samples.

4. Discussion

In our research, the three NFB isolates with strongest nitrogenase activity are from FGPS (S18P05-1 and S18P08-02) and SPS (S18P12-1). Although they are embryo-localized and may not actually participate in nitrogen fixation at the current generation of peas, their transmission and subsequent symbiosis with the root nodules of offspring pea plants can contribute to nitrogen fixation. Pea lines with improved nitrogen fixation capacity can often be selected from nodulation mutants [52]. Crossing of the selected mutant lines with popular pea cultivars generate hybrid lines pertaining the trait of higher nitrogen fixation potential [53]. Alternatively, the strong NFB isolates isolated in this research can be inoculated to pea root nodules as biofertilizer. Their high nitrogenase activity is expected to improve the nitrogen fixation potential of host pea plants.

In our NFB collection, the number of inorganic PSB isolates are almost three folds more than that of organic PSBs. This observation is consistent with the soil origination of most endophytic bacteria in embryos. Most soil PSBs tend to solubilize and utilize inorganic phosphorus [54,55]. Since organic PSBs are scarce, they can be valuable biofertilizer resources supplementing with the more common inorganic PSBs that are already possessed by plants. Particularly, we obtained two dual-role PSB isolates (S18P05-2 and S18P08-3) capable of solubilizing both inorganic and organic phosphorus. When used as biofertilizer, they may help plants utilize broad types of soil phosphorus. Their potential to solubilize other forms of inorganic and organic phosphorus should be tested in the future.

Most soil potassium exists in mineral forms such as aluminosilicate, which cannot be directly utilized by plants. Endophytic KSBs play a critical role in the generation of soluble potassium for plant utilization [56]. Bacteria from the *Klebsiella* genus have been previously reported to be KSBs [57]. Consistently, one of the strongest KSB we obtained, S18P02-2, is a *Klebsiella* bacterium.

Members from the *Pseudomonas* genus are well known for their growth promotion and IAA production activities [58,59]. No surprise, A *Pseudomonas* isolate (S18P02-2) is the strongest IAA producer of all 26 NFBs in our collection.

Data analysis of high-throughput 16S sequencing results found little variation in endophytic bacterial abundance and diversity in most embryo samples, indicating that the bacterial community structure is not significantly affected by pea cultivars and accessions. The abundant endophytic bacteria identified in this research will be the targets for further biochemical, physiological, and functional investigations due to their strong presence in the pea embryo bacterial microbiome.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Figure S1: Morphological description of the 27 putative nitrogen-fixing bacterial (NFB) strains identified from pea embryos; Table S1: Morphological description of the 27 putative nitrogen-fixing bacterial (NFB) strains identified from pea embryos; Table S2: Summary of effective DNA sequencing reads from endophytic bacteria in pea embryos and derived operational taxonomic units (OTUs); Table S3: Sequencing depths and diversity indexes of endophytic bacterial samples in pea embryos.

Author Contributions: Conceptualization, J.H., H.P. and Y.M.; methodology, X.Z., S.Q., F.S. and X.L.; validation, X.Z., S.Q., F.S. and X.L.; formal analysis, X.Z., S.Q., F.S. and X.L.; investigation, X.Z., S.Q., F.S. and X.L.; resources, J.H., H.P. and Y.M.; data curation, X.Z., S.Q., F.S. and X.L.; writing—original draft preparation, H.P., X.Z., Y.M. and J.H.; writing—review and editing, H.P., X.Z., Y.M. and J.H.; visualization, X.Z., S.Q., F.S. and X.L.; supervision, J.H., H.P. and Y.M.; project administration, J.H., H.P. and Y.M.; funding acquisition, J.H. and H.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the internal grant of Qingdao Academy of Agricultural Sciences (J.H.) and the USDA-ARS appropriated project 2034-22000-015-000D (H.P.).

Data Availability Statement: The data presented in this study are available in the article.

Acknowledgments: Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not constitute endorsement by USDA. USDA is an equal opportunity provider and employer.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

DGP: Dry grain pea; DGPS: Dry grain pea samples; FGP: Fresh pea; FGPS: Fresh pea samples; IAA: Indole-3-acetic acid; KSB: Potassium-solubilizing bacteria; LDA: Linear discriminant analysis; LEfSe: Linear discriminant analysis (LDA) coupled with effect size measurement; NFB: Nitrogen-fixing bacteria; OTU: Operational taxonomic unit; PCA: Principal component analysis; PSB: Phosphorus-solubilizing bacteria; SCP: Sweet crispy pea; SCPS: Sweet crispy pea samples; SEP: Pea tip; SEPS: Pea tip samples; SHP: Pea sprout; SHPS: Pea sprout samples; SP: Snow pea; SPS: Snow pea samples; TSA: Trypticase Soy Agar.

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