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Bioprospecting Plant-Growth-Promoting Endophytic Bacteria Isolated from Moso Bamboo (*Phyllostachys edulis*) Shoots

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Abstract: Endophytic bacteria improve plant health and are sometimes necessary to fight against adverse conditions. Bamboo shoots can be eaten as a vegetable and grow into culm wood. However, few studies have focused on the colonization characteristics of endophytic bacteria in Moso bamboo shoots at different tissue sites and different growth developmental phases. Endophytic bacteria were isolated from the top, the base, and the root of Moso bamboo shoots during three different growth and development stages (winter shoot period underground (S1), spring shoot period at 50 cm aboveground (S2), and fast growth shoot period at 200 cm aboveground (S3)), and the highest content of isolable endophytes were in roots in the S3 stage. A total of 253 different endophytic bacteria strains were totally isolated and then evaluated in term of their PGP (plant growth promoting) traits such as inorganic phosphorus solubilization, organic phosphorus solubilization, hormone production, siderophore production, ACC deaminase, and biological nitrogen fixation. Among those newly isolated strains, *Pseudomonas rhodesiae* RD7-4 had the strongest phosphorus solubilizing ability, *Burkholderia pyrrocinia* BD24-2 had the strongest nitrogen fixing ability, and *Pseudomonas edaphica* TD33-1 had the strongest IAA production ability. Inoculation with all three strains significantly increased the content of soluble starch in *Arabidopsis*, and *B. pyrrocinia* BD24-2 significantly increased the fresh weight and promoted the growth of the root system. Furthermore, in pot experiments, *B. pyrrocinia* BD24-2 significantly increased the biomass, root length, and leaf area. Our study contributes to understanding the endophytes in Moso bamboo shoots, and indicates that the strain BD24-2 can be potentially acted as a plant growth promoter.

Keywords: Moso bamboo shoots; endophytic bacteria; PGPB; plant growth; growth stages

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1. Introduction

Plant microbial endophytes are typically characterized as non-pathogenic bacteria and fungi that exist in plant tissues without causing significant harm to the host [1]. Endophytic bacteria, an important component of the plant microbiome, have been isolated from a wide variety of plants, with some demonstrating significant application potential. Some of these bacteria can boost plant growth and expedite seed germination in various ways, including nitrogen fixation, phosphate solubilization, siderophore production, and bioactive enzyme secretion [2]. Because plant endosphere ecological niches present unique habitats, bacterial endophytes may have different functions, specializations, adaptations, and capabilities [3,4]. Furthermore, it is likely that only specialized endophytic strains are able to colonize and survive in reproductive plant organs [5]. Only bacteria with competitive and adaptive settlement characteristics can inhabit shoots and seeds [6].

Bamboo is worldwide known as one of the most important non-timber forest products thanks to its dual uses for culm timber and edible shoot. Bamboo shoots/culms and underground rhizomes/shoots are connected together in a tightly interconnected system

for transport and sharing of nutrients, which allows them to compete for light with taller trees in the forest through an astonishing phase of "rapid stem elongation" [7]. *Phyllostachys edulis* (Moso bamboo) is one of the most important bamboo species in China, with an area of 4.67 million hm^2, accounting for 72.96% of the total bamboo forests. From late summer to early fall, the lateral buds of the mature underground rootstocks of Moso bamboo develop and grow into so-called winter shoots that are physiologically dormant during the winter. The winter shoots are broken in dormancy and begin to grow out from the ground into so-called spring shoots in suitable temperature and humidity conditions. The spring shoots grow rapidly and up to around 20 m high within 2 months [8,9]. Bamboo shoots, as an early stage form in the asexual reproduction process of Moso bamboo, have special anatomical structures and hormone space distributions at their tips and bases to guarantee their rapid growth [10,11]. Based on previous studies, the endophytic bacterial community of bamboo shoots differs significantly from the tissue flora of other stages of Moso bamboo in terms of colony number and colony structure [12], which might also be compatible with its rapid elongation characteristics. However, few studies have been conducted on the functional characterization and plant growth promotion of isolable endophytic bacteria at various growth and development stages of bamboo shoots.

Therefore, this study aims to isolate the endophytic bacteria strains in Moso bamboo shoots, evaluate endophytic bacterial associations at different growth stages and plant tissues, and assess the plant growth-promoting bacteria (PGPB) traits of those endophytic bacteria in the laboratory and pot experiment.

2. Materials and Methods

2.1. Plant Sample Collection

The Moso bamboo shoots were categorized into three unique growth and developmental stages (winter shoot period underground (S1), spring shoot period at 50 cm above ground (S2), and fast growth shoot period at 200 cm above ground (S3)) based on their growth and developmental features. In the Wuxing District, Huzhou City, Zhejiang Province, China (30°48′ N, 119°59′ E), Moso bamboo shoots of three identically sized and developmental stages were harvested on 4 January 2023, 1 March 2023, and 27 April 2022, respectively. Bamboo shoots were used as samples to isolate endophytes. All samples were put inside sterile self-sealing bags and brought to the lab at a low temperature, where they were kept at 4 °C until needed.

2.2. Isolation of Endophytes

One gram of each of the tips, bases, and roots of Moso shoots was taken and soaked in 70% ethanol for 1 min, then in 2% sodium hypochlorite for 5 min, followed by three washes with sterile water. To ensure successful surface sterilization, the last rinse solution was inoculated on the surface of an R2A agar medium (Qingdao Hope Bio-Technology Co. Ltd., Qingdao, China) and kept at 30 °C for 72 h. The absence of bacterial growth indicated that the surface sterilization was successful. The samples were then diluted by adding 9 mL of sterile water, and the suspensions were shaken at 120 rpm for 15 min. Next, the samples were diluted once more, and 100 µL of the suspensions were applied to a Luria Bertani agar (LB) medium (Qingdao Hope Bio-Technology Co. Ltd., Qingdao, China) once and to R2A media three times. The plates were stored at 30 °C for three days, and colonies with visually different morphology were selected for further analysis [13].

Bacterial endophytes were screened on the basis of morphological factors such as size, form, color, margin, and texture. The purified bacterial forms were kept in storage at −80 °C and as working cultures in the respective medium slants at 4 °C.

2.3. Plant-Growth-Promoting (PGP) Traits

The strains were grown on inorganic phosphorus agar (IPA) and organic phosphorus agar (OPA) media to evaluate their phosphorus solubilizing activity qualitatively [14]. The formation of halos around the bacterial colonies indicates phospholysis. The protease

production capacity of endophytic bacterial isolates was determined using skim milk agar (SMA) [15]. According to the method of Schwyn et al., siderophore production of isolated endophytic bacterial isolates was achieved using a chromium asphaltene sulfonate (CAS) agar medium [16]. The ability was assessed by its D/D value on the medium, where D = total diameter (colony + halo) and D = colony diameter.

The capacity of endophytic bacteria to grow on nitrogen-free plates was examined to ascertain the potential use of atmospheric nitrogen sources [17]. The nitrogen fixation capacity was comprehensively evaluated by observing the colony production of hyaline rings, colony ecology, and growth rate.

To determine whether the isolate produces 1-aminocyclopropane-1-carboxylate (ACC) deaminase, the isolates were inoculated into 25 mL of a PAF medium for 24 h and then into a DF medium and ADF medium and shaken at 150 rpm for 72 h. To determine the growth of the strains by watching how they developed in each of the three media, we measured the OD600nm value of each culture solution using a spectrophotometer. When the growth of the strain in the ADF (DF replacing $(NH_4)_2SO_4$ with ACC) medium was significantly better than that in the DF medium, it indicated that the strain was able to grow with ACC as the only nitrogen source, i.e., the strain was able to produce ACC deaminase [18].

2.4. Quantitative Estimation

The phosphorus solubilization ability of bacteria was quantified using NBRI phosphate medium broth (NBRI-PM) [19]. Culture suspension was centrifuged at 12,000× *g* for 15 min to estimate solubilized phosphorus in the supernatant using the ascorbic acid method [20].

Nitrogen-fixing capacity was quantified using Ashby's medium. The culture suspension was centrifuged at 12,000× *g* for 15 min, and the dissolved phosphorus in the supernatant was estimated using the alkaline potassium persulfate digestion UV spectrophotometric method [21]. Bacterial isolates were grown in a nutrient broth supplemented with 1 mg/mL L-tryptophan and incubated at 150 rpm for 72 h at 30 °C.

The quantification of indole-3-acetic acid (IAA) produced by the isolates was performed using Salkowski's reagent [22].

The CAS (Chromeazurol S) assay was performed to measure the quantitative production of siderophore. The percentage of siderophore units produced was calculated using the formula below [23]:

$$\text{Siderophore units}(\%) = \frac{a - A}{a} \times 100$$

where A = absorbance of CAS reagent and a = absorbance of the sample at 630 nm.

2.5. Molecular Identification of Bacterial Isolates

Identification of endophytic bacteria was performed based on 16S rRNA gene sequences. Prior to genomic DNA extraction, the strains were inoculated in an LB medium and incubated for 24 h at 28 °C with shaking (180 rpm). Using universal primers 27F (forward) 5′-AGAGTT TGATCATGCTCAG-3′ and 1492R (reverse) 5′-GGT ACCTTGTTACGACTT-3′ on the 16S rRNA gene sequences (approximately 1500 base pairs) were amplified via PCR. The PCR products were then visualized using electrophoresis, purified using kits from TransGen Biotech Co., Ltd. (Beijing, China), and sent to DynaPro Biotech (Wuhan, China) for DNA sequencing. Homologous DNA sequences were retrieved and analyzed using the BLAST algorithm. For the assignment of taxonomic affiliation at the species level, 16S rRNA gene sequences with greater than 99% identity to a particular bacterial species were sufficient to give the strain a tentative scientific name. In contrast, sequences with greater than 99% identity to two or more bacterial species or less than 99% identity to two or more bacterial species represented unidentified species designated as genus plus "sp". All sequences are available at the GenBank under the accession numbers OR533977 to OR533996. The final identification of bacterial strains was based on a neighbor-joining phylogenetic tree of bacterial 16S rRNA gene sequences created by the software MEGA (v.11.0.13, Mega Limited, Auckland, New Zealand).

2.6. Aminocyclopropane-1-Carboxylate (ACC) Deaminase Gene Identification (acdSf3 and acdSr4)

A single bacterial colony was picked into 25 mL of an LB medium, incubated at 28 °C with shaking (180 rpm for 24 h) until the logarithmic stage, an appropriate amount of bacterial liquid was taken to centrifuge the bacterial body at 5000× *g*, and then to use the Bacterial Genome Extraction Kit for extracting the genome according to the procedure. The primer pair *acdSf3* and *acdSr4* [24] was used, and a 50 µL PCR amplification system was used: 0.5 µL of each of the upstream and downstream primers, 2 µL of the DNA template, 25 µL of 2× Taq-MasterMix and 22 µL of deionized water. PCR was performed by touchdown-PCR: pre-transformation at 94 °C for 10 min, denaturation at 94 °C for 40 s, annealing at 65 °C for 50 s, extension at 72 °C for 1 min, annealing temperature was lowered by 0.4 °C for each cycle, annealing temperature was lowered to 58 °C after 15 cycles, 20 cycles were performed, and extension was performed at 72 °C for 10 min, and stored at 4 °C. PCR amplification was performed directly on 1% agar. The PCR-amplified products were directly detected by 1% agarose gel electrophoresis.

2.7. In Planta Assay for Plant Growth Promotion

2.7.1. Effect of Endophytic Bacteria on the Growth of Arabidopsis

Surface-sterilized Arabidopsis seeds were vernalized and placed in Petri dishes, and grown in culture chambers. Arabidopsis seedlings grown for 5 d were transferred into a 1/2 MS medium and incubated for 12 d with 20 µL of a 5 × 10^8 (cfu/mL) bacterial suspension and 20 µL of sterile water for the control [25]. After 12 days of inoculation, all horizontally cultured Arabidopsis thaliana were removed from the Petri dishes, and the aboveground parts were measured with an analytical balance (0.001 g) to determine the weight of each plant, i.e., fresh weight. Meanwhile, the root length of each plant was averaged after measuring with a vernier caliper. Total soluble sugar content was determined according to a slightly modified method from [26].

2.7.2. Effect of Endophytic Bacteria on the Growth of Moso Bamboo Potted Plants

Endophytic bacteria were used to evaluate the growth-promoting properties of endophytic bacteria using annual Moso bamboo seedlings in a nursery, and the pot placement was changed every three days during the experiment. Two hundred milliliters of bacterial culture prepared in LB medium and conditioned to a density of 5 × 10^7 (cfu/mL) with sterile distilled water was applied to each pot of annual Moso bamboo seedlings. Seedlings inoculated with water were used as controls. Ten pots with three plants per pot were used for each treatment. Applications were made at 10-day intervals for a total of three applications, and 30 days after the end of inoculation, 10 seedlings were randomly selected from each treatment to measure parameters related to plant growth. Roots were washed, and the number of shoots sprouting from their roots was recorded as the total root length and leaf area were measured using the WinRHIZO Root Analysis System. Then, the plants were dried to measure the dry weight.

2.8. Data Analysis

Statistical analyses were performed using a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test for independent samples or Student's *t*-test (SPSS version 17.0; IBM Corp., Armonk, NY, USA) when comparing two means. In all cases, confidence coefficients were set at $p < 0.05$. Data were processed and plotted using R 4.3.1 and Origin Pro 2023B.

3. Results

3.1. Tissue and Growth Stage Specific Culturable Endophytic Bacteria

Endophytes were isolated and enumerated from the tips, bases, and roots of shoots at the winter shoot period underground (S1), spring shoot period at 50 cm aboveground (S2), and fast growth shoot period at 200 cm aboveground (S3) (Figure 1).

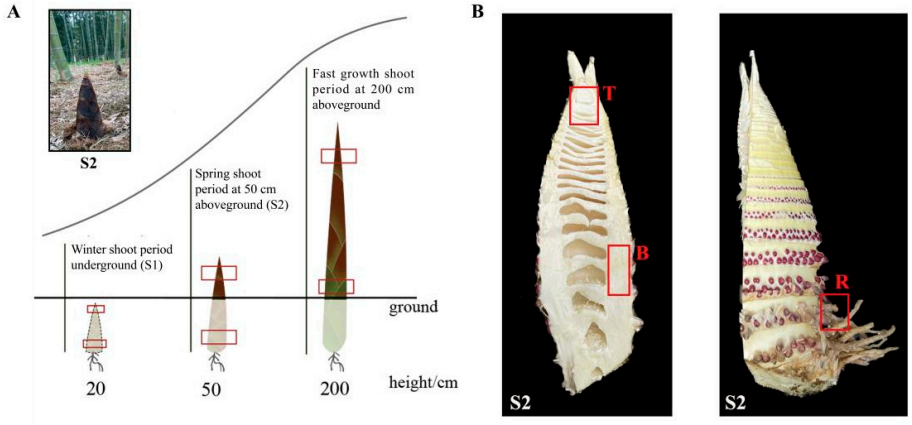

Figure 1. Schematic diagram of sampling stages and tissue sites of Moso bamboo shoots. (**A**) Stages of Moso bamboo rhizome buds were characterized by the bud or shoot length: winter shoot period underground (S1), spring shoot period at 50 cm aboveground (S2), and fast growth shoot period at 200 cm aboveground (S3). The red boxes show where the shoots were sampled. (**B**) Shoot tip biogenic tissue (T), shoot base mature tissue (B), and shoot root (R).

The endophyte loads were observed from 0.1×10^3 to 4.36×10^4 cfu/g in the tip tissues of Moso bamboo shoots on different media; however, the endophyte loads varied from 0.64×10^3 to 1.68×10^4 cfu/g in their basal and root parts (Figure 2). The R2A medium was found to be more suitable for deciphering the maximum endophytic bacterial diversity compared to LB (Table S1). The total amount of endophytes in roots and basal was always significantly higher than that in apices in the period of S1, S2, and S3, and for different tissue parts, the endophyte loads were always elevated with the growth stages (Figure 2).

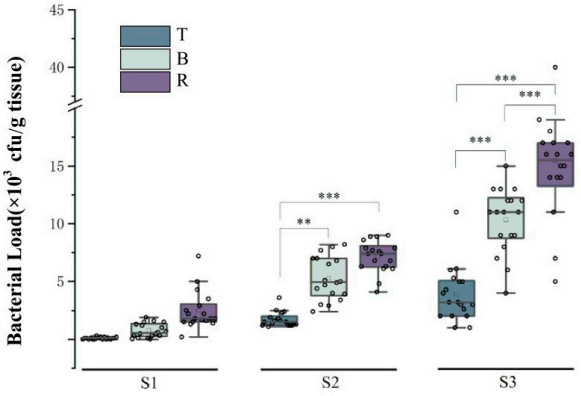

Figure 2. Total number of endophytic bacteria at different times. Significance was calculated using Tukey's test (** $p > 0.01$,*** $p > 0.001$).

3.2. Functional Annotation of Selected Endophytic Bacteria Based on Qualitative PGP Traits

A total of 235 morphologically diverse endophytes were isolated based on the colony morphology. Regarding the number of endophytic bacteria isolated from each part of the Moso bamboo shoot samples in the period of S1, S2, and S3 (Figure 3A), it can be seen that 91 bacteria were found to be the most abundant in the S3 period, while 71 and 73 strains were found in the S1 and S2 periods, respectively. In each period, it can be seen that the tip had the lowest number of endophytes with different morphologies, with an average of 17 strains, while the root had the highest number of isolable endophytes, with an average of 34 strains.

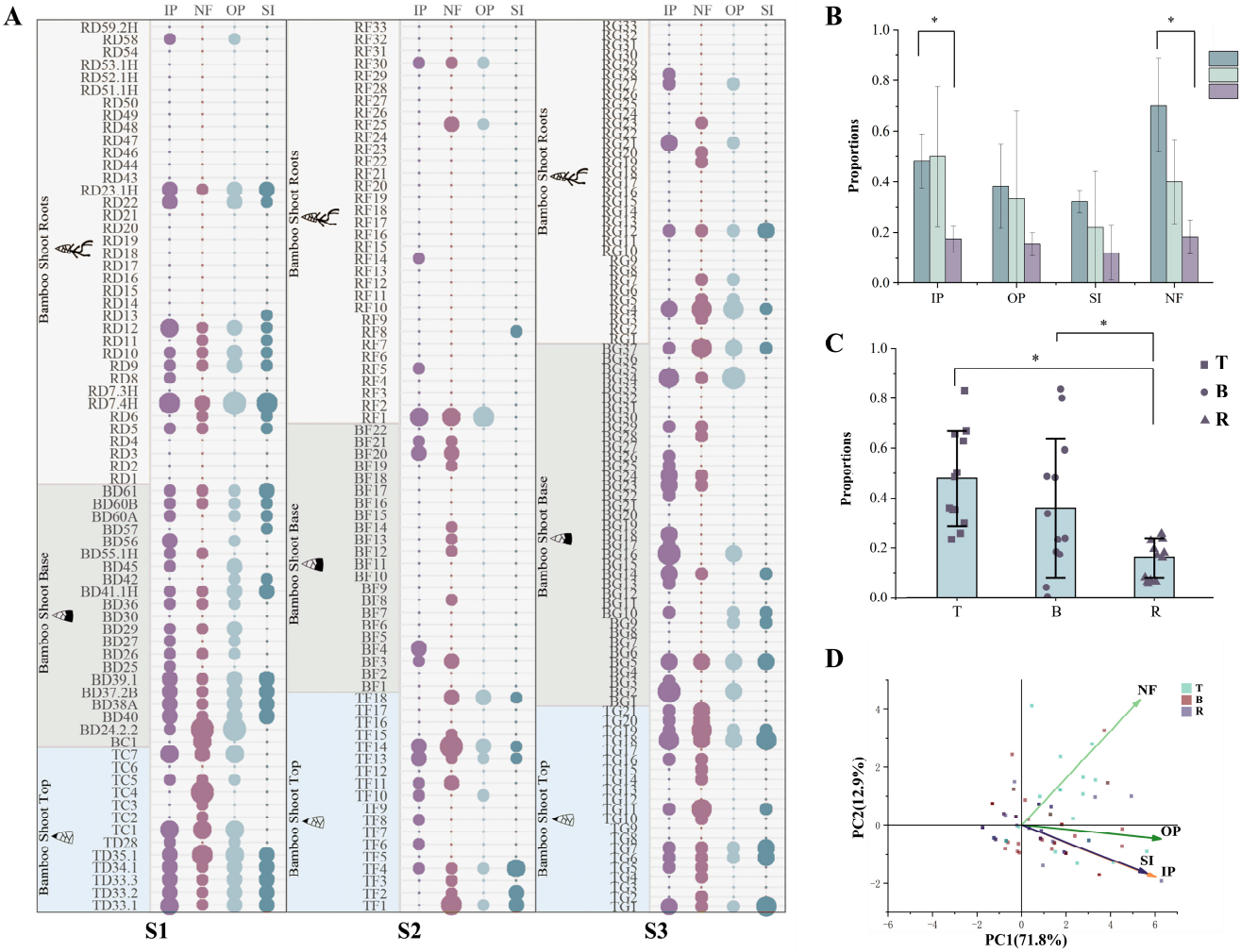

Figure 3. The isolation and functional primary screening of endophytes from Moso bamboo shoots. (**A**) The 71, 73, and 91 endophytic bacterial strains were isolated at three developmental periods, S1, S2, and S3, and the ability of inorganic phosphate (IP) dissolution, organic phosphate (OP) dissolution, nitrogen fixation (NF), and siderophore production (SI) were evaluated. The ratio of their hydrolysis circle and colony diameter (D/d) was used as a criterion for evaluating the magnitude of the degree of functionality, with "5" indicating D/d > 4:1, "4" indicating D/d > 3, "3 "5" meaning D/d > 4:1, "4" meaning D/d > 3, "3" meaning D/d > 2, "3" meaning D/d > 2, "2" meaning D/d > 1, and "1" meaning D/d > 0.5. (**B**) Three growth periods were used as replicates to count the proportion of isolates with some PGP functions in each tissue site to the total isolates. (**C**) The four PGP functions of the three growth periods were used as 12 replicates, and the proportion of isolates with a certain PGP function to total isolates was counted in each tissue site, respectively. (**D**) Principal component analysis of the functions of 235 isolates. Significance was calculated using Tukey's test (* $p > 0.05$).

Various plant-growth-promoting (PGP) traits were screened for 235 isolates purified during the period of S1, S2, and S3, in which 20 isolates were selected for further studies. We evaluated the PGP traits of these 235 endophytic bacteria in terms of inorganic phosphorus-organophosphorus solubilization assay, nitrogen-fixing capacity assay, and siderophore production assay (Figure 3A). It can be seen that most of the endophytic bacteria isolated from the tip portion possessed the ability to solubilize both organic and inorganic phosphorus, especially for the S1 stage; 9 out of 13 isolates possessed the ability to solubilize inorganic phosphorus, and 11 did the ability to solubilize organic phosphorus.

As can be seen from Figure 3B, the proportion of the isolates with inorganic phosphorus–organophosphorus solubilizing capacity in the tips of Moso bamboo shoots to the total

number of the isolates was significantly higher (48%) than in the roots (17%) in all three periods. Similarly, the isolates that could grow on Ashby's nitrogen-free medium, which we consider as the bacteria with the ability to transform nitrogen from the air, accounted for an average of 70.3% of the shoot tip isolates in all three periods, which was significantly higher than the percentage of nitrogen-fixing bacteria in the roots (Figure 3A). For the production of iron carriers, which is a PGP trait, 45 out of 235 isolates were positive for the production of a distinctive orange halo in the CAS solid medium (Figure 3A). These iron-carrier-positive strains were relatively concentrated at the tip of the Moso bamboo shoots, and their proportion of the total isolates in this ecotope showed a significant decrease in order of the tip, base, and root.

Considering strains with one or more of these four functions as growth-promoting, it can be seen that the proportion of isolates of growth-promoting bacteria at the tip of the Moso bamboo shoots was significantly higher than that at the root, while the proportion of the isolates of growth-promoting bacteria at the base was significantly higher than that at the root in all the three periods (Figure 3C).

It was found that 21% of the isolates that produced iron carriers were also phosphorus-solubilizing bacteria. According to the principal component analysis, the two PGP characteristics of producing iron carriers and solubilizing inorganic phosphorus were significantly correlated for the endophytic isolates of Moso bamboo shoots (Figure 3D).

3.3. Molecular Identification of Promastigotes and Their Phylogenetic Tree

Twenty strains of PGPB were screened out based on their PGP properties. Based on the 16S rDNA sequences, we could identify the 20 endophytic isolates to be 7 genera, namely *Burkholderia*, *Serratia*, *Pseudomonas*, *Collimonas*, *Pantoea*, *Rouxiella*, and *Paracoccus*. By analyzing their functional properties (Table S2), the three isolates with the strongest nitrogen fixation, phosphorus solubilization, and IAA production were screened. And the phylogenetic tree of the three endophytes was constructed (Figure 4).

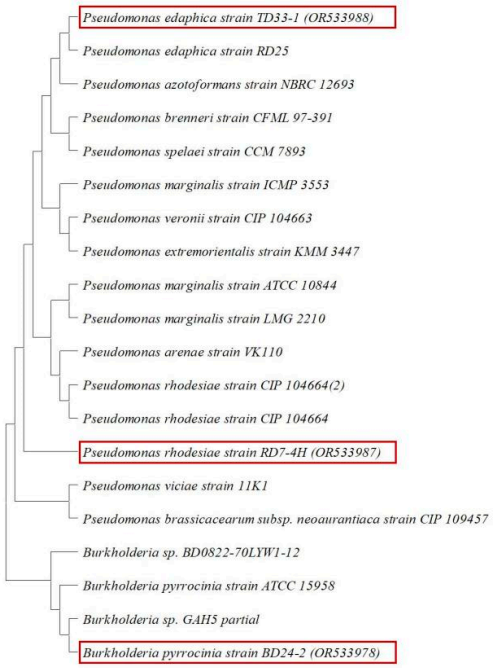

Figure 4. Phylogenetic tree showing the relationship among 3 bacterial isolates, 16S rDNA gene sequences with reference sequences obtained through BLAST analysis.

3.4. Quantitative Estimation of PGP Traits

The quantitative determination of the PGP capacity of three isolates is seen in Table 1. Regarding the phosphate solubilization capacity, the IP solubilization capacity ranged

between 60.69 ± 12.14 mg/L and 25.65 ± 2.05 mg/L, with isolate *Pseudomonas rhodesiae* RD7-4 (60.69 ± 12.14 mg/L) being the largest. Regarding the solubilizing capacity of IP, the isolates had a relatively small solubilizing capacity for OP, ranging from 17.33 ± 3.32 mg/L to 14.50 ± 0.94 mg/L, with *Burkholderia pyrrocinia* BD24-2 being the most solubilizing. *Pseudomonas edaphica* TD33-1 can produce large amounts of IAA, 10.02 ± 1.52 μL/mL in the presence of 1 mg/mL tryptophan. Isolated *B. pyrrocinia* BD24-2 showed the highest amount of siderophore (0.24%) production and the highest amount of nitrogen fixation (2.31 ± 0.06 μg/mL). Moreover, *B. pyrrocinia* BD24-2 had the ACC deaminase encoding genes ardSf3/ardSf4 and can produce ACC deaminase. All three isolates can produce proteases.

Table 1. Multiple PGP activities of endophytic bacterial isolates.

Function	Bacteria		
	***B. pyrrocinia* BD24-2**	***P. edaphica* TD33-1**	***P. rhodesiae* RD7-4**
IP (mg/L)	40.02 ± 5.43	25.65 ± 2.05	60.69 ± 12.14
OP (mg/L)	17.33 ± 3.32	18.33 ± 2.63	14.50 ± 0.94
NF (μg/mL)	2.31 ± 0.06	1.92±0.05	1.85 ± 0.04
Si Production(%)	0.24 ± 0.06	−	0.15 ± 0.05
IAA Production (μg/mL)	6.34 ± 0.92	10.02 ± 1.52	3.76 ± 0.77
ACC deaminase	+	−	−
Protease	+	+	+
acdSf3/acdSr4[1]	+	−	−

[1] ACC Deaminase Identification of Genes.

3.5. In Planta Assay for Plant Growth Promotion

Because *B. pyrrocinia* BD24-2, *P. edaphica* TD33-1, and *P. rhodesiae* RD7-4 are endophytic bacteria of Moso bamboo shoots and have excellent growth-promoting functions, we propose that they might be able to promote the growth of Arabidopsis. To test this hypothesis, Arabidopsis and the endophytes were co-cultured for 12 days, and the results showed that the pro-phytic bacterium *B. pyrrocinia* BD24-2 was the most effective in promoting the growth of leaves compared with the control, which increased the aboveground fresh weight fraction of Arabidopsis by 2.17-fold, whereas *P. rhodesiae* RD7-4 and *P. edaphica* TD33-1 also increased the aboveground fresh weight fraction of Arabidopsis by 1.71-fold, respectively, and 1.29-fold (Figure 5A). However, the growth of the belowground fraction varied considerably. *P. edaphica* TD33-1 had an inhibitory effect on the length of the primary roots of Arabidopsis, and *B. pyrrocinia* BD24-2-2 and *P. rhodesiae* RD7-4 increased the length of the primary roots of Arabidopsis by 2.25- and 1.38-fold, respectively (Figure 5B). In summary, *B. pyrrocinia* BD24-2 and *P. rhodesiae* RD7-4 were more effective in promoting growth in fresh weight and root length of Arabidopsis.

The experimental results showed that the accumulation of soluble sugars was significantly increased in all three endophyte strains (Figure 5C). Among them, *P. edaphica* TD33-1, *B. pyrrocinia* BD24-2-2, and *P. rhodesiae* RD7-4 increased Arabidopsis soluble sugars by 1.99, 1.29, and 1.41-fold, respectively. In a word, the endophytes of Moso bamboo shoots can promote the growth of Arabidopsis by increasing the accumulation of soluble sugars.

In parallel with the studies on aboveground and belowground growth of Arabidopsis, the effects of three probiotic bacteria isolated from the Moso bamboo shoot on the root architecture of Arabidopsis were also investigated. As shown in Figure 5E, the reduction in primary root length compared with the control, especially in seedlings exposed to *P. edaphica* TD33-1, demonstrated the inhibitory effect of *P. edaphica* TD33-1 on root elongation. In addition, we found that the inhibitory effect on primary root elongation was not significantly found until the sixth day of the treatment. In contrast, *B. pyrrocinia* BD24-2 and *P. rhodesiae* RD7-4 promote primary root development. The number and density of lateral roots in endophytic isolate-treated seedlings increased rapidly with growth time, whereas

the number and density of lateral roots in control plants were found to increase slightly (Figure 5E).

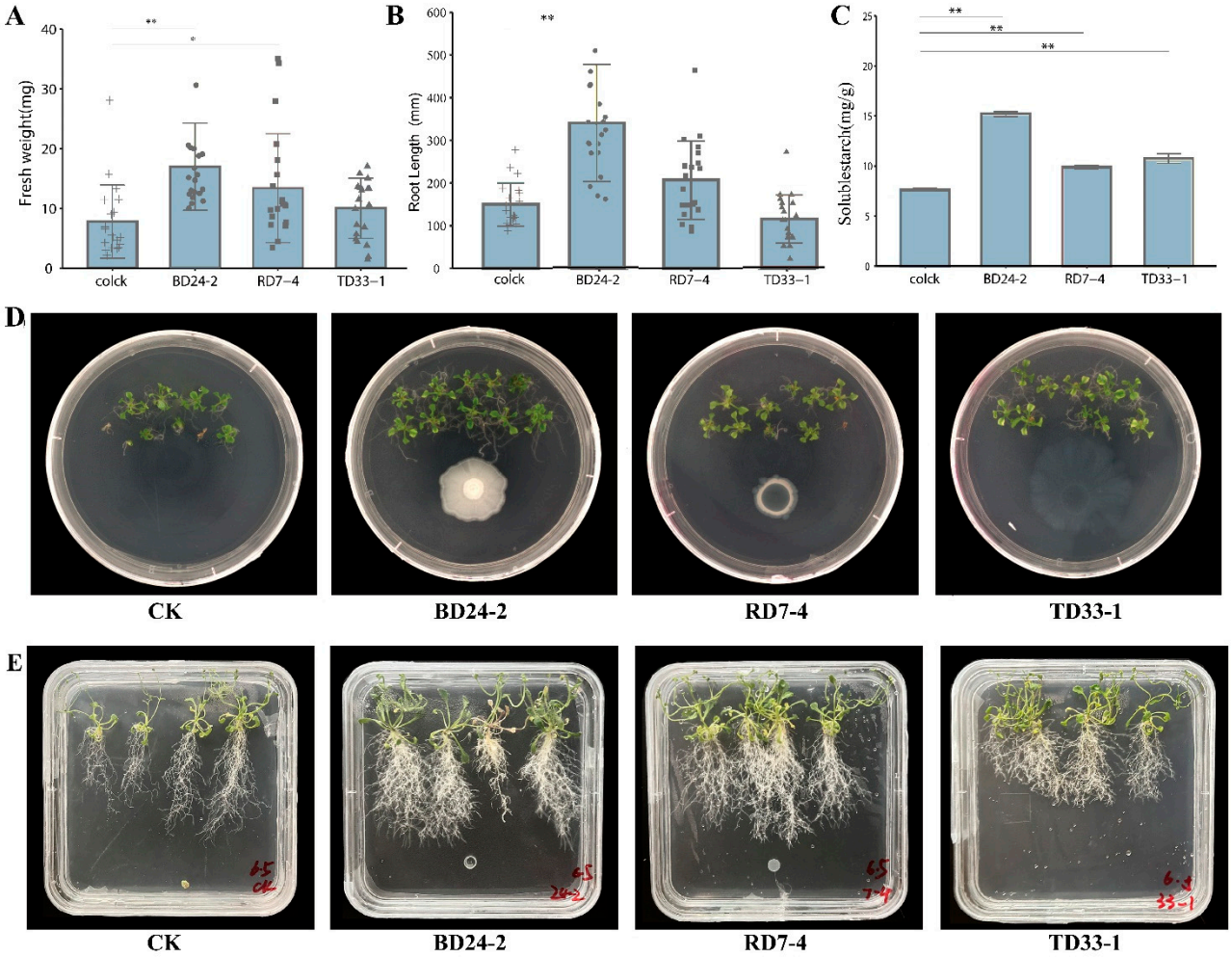

Figure 5. Images and analysis of growth-promoting conditions using selected growth-promoting bacteria co-cultured with Arabidopsis. (**A**) Comparison of fresh weights of Arabidopsis seedlings cultured horizontally with 30 replicates for each treatment. (**B**) Comparison of primary root length of horizontally cultured Arabidopsis seedlings. (**C**) The soluble sugar content of aboveground parts of horizontally cultured Arabidopsis seedlings. (**D**) Horizontally cultured Arabidopsis seedlings. (**E**) Vertically cultured Arabidopsis seedlings. Significance was calculated using Tukey's test (* $p > 0.05$, ** $p > 0.01$).

We further evaluated the effects of endophytes on plants in a complex microbial ecosystem in a Moso bamboo pot experiment (Figure 6). Unlike the Arabidopsis thaliana growth-promoting experiment, only *B. pyrrocinia* BD24-2 showed significant growth-promoting effects compared with water-treated CK. BD24-2-treated seedlings showed significantly longer roots, aboveground dry weight, belowground dry weight, leaf area, and number of shoots produced than the control ($p < 0.01$), with aboveground dry weight and belowground dry weight increased by 59% and 56%.

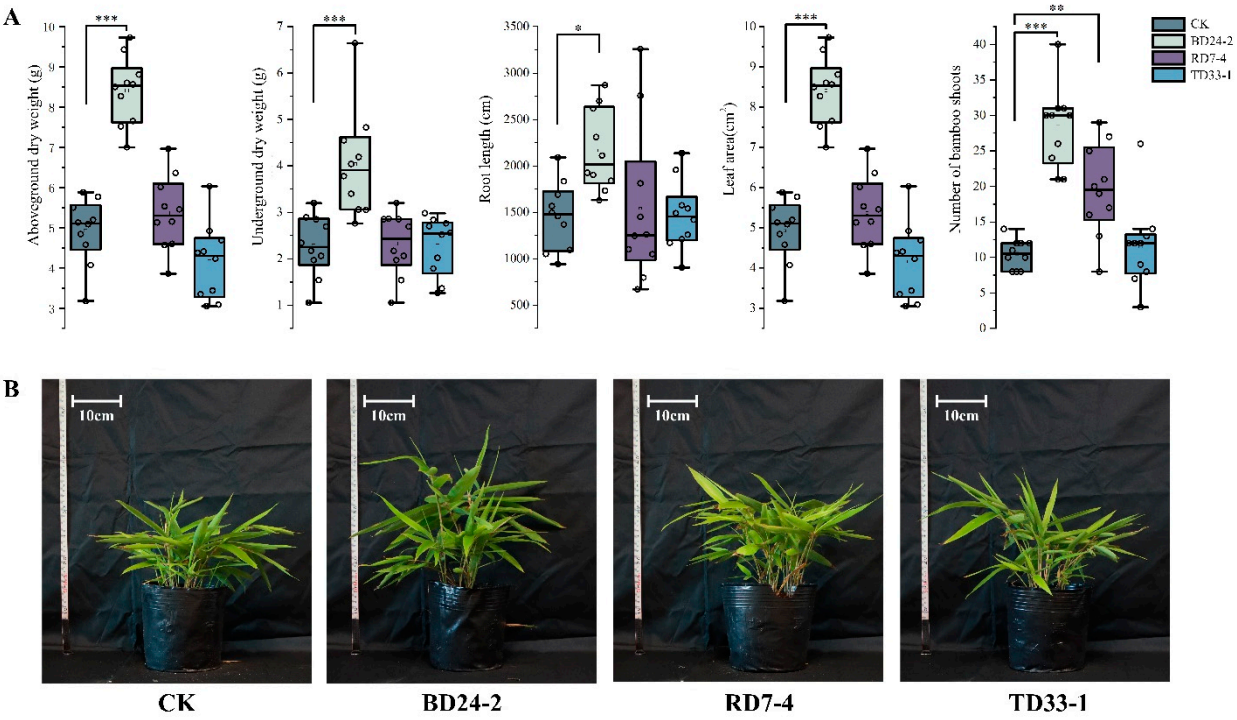

Figure 6. Images and analysis of growth parameters of Moso bamboo inoculated with selected growth-promoting bacteria. Significance was calculated using Tukey's test (* $p > 0.05$, ** $p > 0.01$, *** $p > 0.001$).

4. Discussion

4.1. Specific Distribution of Endophytic Flora in Moso Bamboo Shoots

We have shown that the winter shoots have low endophytic bacterial loads, and their endophytic bacterial loads gradually increase with their rapid growth process. As reproductive organs of Moso bamboo, bamboo shoots have a microbial ecosystem, which could be quite similar to that of plant seeds [27]. However, after the bamboo plant develops an underground rhizome and root system, the inter-root microorganisms enter the bamboo system and are multiplied, which leads to a gradual increase in bacterial loads in all the different tissue parts of the shoot. This is attributed to the fact that microorganisms move from the bulk soil towards the surface of the root system (rhizosphere), form microcolonies on the surface of the root system, and enter the inner part of the root system (endodermis) through cracks, wounds and others [28,29]. Therefore, with the growth of Moso bamboo shoots, the inter-root endophytes gradually enter the root system and colonize various tissue sites.

Isolation of endophytic bacteria from tissues of different sites at three stages showed that the highest endophytic bacterial loads were found in the roots of mauve bamboo shoots, followed by the bases, and the lowest in the tips at all growth stages. In contrast, the proportion of PGPB in the flora was highest at the tip, followed by the base, and lowest at the tip. In other words, the microbial community structure in the tips of Moso bamboo shoots was dominated by PGPB. Regarding the different biohabitats and colonization pathways in roots, culms, and leaves that could explain the differences in endophyte abundance and community structure, this has been observed in crops such as cotton [28], potato [30], maize [31], and rice [32].

In a previous study, the tip of Moso bamboo shoots, the active meristematic tissue, showed a specific accumulation of phyto-hormone [10], and some transcripts involved in cell formation and DNA synthesis were also enriched at this location [9,10]. While phyto-hormones are essentially central to all biological processes, phytohormones are co-regulated by both the plant itself and the plant's flora [33]. For example, *Pseudomonas fluorescens*

Pf4, *Pseudomonas aeruginosa* [5], or *Bacillus amyloliquefaciens* LJ02 [34] was inoculated into *Arabidopsis*, which triggered an increase in endogenous salicylic acid levels in different plant parts; the growth-promoting bacteria were inserted into rice, after which the expression of hormone-related genes in roots was upregulated [35]. In other words, the formation of a special ecological niche at the shoot tip site during the rapid growth of Moso bamboo shoots, such as rapid cell elongation and the accumulation of large amounts of growth hormone, led to the formation of this specific microbial community at the tip of Moso bamboo shoots relatively small in number, with a high percentage of beneficial microorganisms.

4.2. Plant-Growth-Promoting Bacteria (PGPB)

Phosphates are major essential macronutrients for biological growth and development, but the concentration of soluble phosphate in soil is usually low. In this regard, phosphate-solubilizing bacteria may play a crucial role in agricultural systems by solubilizing inorganic and organic phosphates in soil [36]. Siderophores are Fe-chelating biological compounds that bacteria synthesize; chelate Fe makes it available to the plant system [37]. In the study, 21% of the isolates produced siderophores and were also phosphorus solubilizers. Moreover, PCA analysis showed that the two functions of phosphorus solubilization and siderophore production were significantly correlated for the endophytic bacteria of Moso bamboo (Figure 3D). CoT10, an endophyte isolated from oil tea by Kunpeng Cui et al., had a high solubility of $FePO_4$ by its iron carrier, which confirmed that the Fe—P mobilization function of CoT10 was directly mediated by its siderophores [38].

Indole acetic acid (IAA) is one of the most important growth hormones synthesized by a variety of endophytic bacteria [39]. This phytohormone promotes plant cell division, differentiation, and cell elongation, stimulates seed germination, and also influences metabolite biosynthesis, pigment formation, and photosynthesis [40]. Microorganisms have the ability to synthesize IAA through various pathways using tryptophan as the main precursor. Microorganisms are able to synthesize IAA through various pathways using tryptophan as the main precursor, and the amount of IAA produced is related to the concentration of tryptophan. TD33-1 isolated from the tip of Moso bamboo shoots could secrete a high concentration of IAA (10.02 ± 1.52 μg/L) at 1 mg/mL of tryptophan.

The 1-aminocyclopropane-1-carboxylate (ACC) deaminase can degrade ACC, the direct precursor of ethylene, function by reducing ethylene concentration and promoting plant growth and stress tolerance. In this study, we screened ACC deaminase-producing strains by the traditional use of ACC as the sole nitrogen source and amplified the acdSf gene fragment encoding ACC deaminase in bacteria by PCR using acdSf3/acdSr4 primers [41]. Among the endophytes isolated, *B. pyrrocinia* BD24-2 from the base during the S 1 period exhibited ACC deaminase activity.

A total of 20 strains of endophytic bacteria were screened out from 235, and their PGP properties were analyzed as IAA production, solubilization of inorganic phosphorus and organic phosphorus, nitrogen fixation, and siderophore and protease and amylase production, three strains of endophytic bacteria were examined in a quantitative and functional way. *B. pyrrocinia* BD24-2 has high nitrogen fixation capacity and can dissolve phosphorus and produce siderophore and ACC deaminase; *P. rhodesiae* RD7-4 can dissolve organic and inorganic phosphorus with high efficiency; *P. edaphica* TD33-1 can secrete large amounts of IAA and also has a wide range of PGP traits.

4.3. Plant-Growth-Promotion by Bacteria Isolated from Moso Bamboo Shoots

It has been shown that many PGPB strains can alter the root architecture of host plants by promoting secondary root formation and enhancing soil exploration by roots [42]. The ability of inter-root PGPB to induce plant biomass production and systemic resistance has been extensively studied [43,44]. The use of biotrophic bacteria is an environmentally friendly way to promote plant growth and resistance to stress. We showed that all three probiotic bacteria stimulated Arabidopsis growth to a varying degree (Figure 5D), and in particular, *B. pyrrocinia* BD24-2 increased Arabidopsis fresh weight and root length in

more than 2-fold and also significantly increased soluble sugar content. Previously, it was shown that Pseudomonas fluorescens WCS417 promotes Arabidopsis growth by inhibiting primordial root elongation and promoting lateral root and root hair formation [42]. Similarly, our results showed that *P. edaphica* TD33-1 inhibited primary root elongation and promoted lateral root formation, and *B. pyrrocinia* BD24-2 and *P. rhodesiae* RD7-4 promoted root hair growth.

However, inoculating microorganisms in potted plants is completely different from the laboratory environment. It found that by the pot experiment, various antagonistic and synergistic interactions existed between microorganisms, which come from a stable microbial ecology with mutual checks and balances [45]. When we added PGPB to try to break that balance and allow PGPB to exist as the dominant flora of that ecology and to colonizing bamboo plant tissues, we found that only *B. pyrrocinia* BD24-2 continued its growth-promoting effects.

5. Conclusions

The present study provides basic information on the diversity and PGP traits of endophytic bacteria of Moso bamboo shoots during three different growth periods. It shows that more endophytic bacteria are favorably colonized at a rapid growth stage and root tissues. However, the endophytic flora of the tip tissues of Moso bamboo shoots, which are far away from the complex microbial environment, were more inclined to probiotic flora. *Burkholderia pyrrocinia* shows excellent growth-promoting properties and could be potentially used as an excellent biofertilizer in the future.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/f14102061/s1, Table S1: Total viable counts of endophytic bacterial population ($\times 10^3$ cfu/g tissue) from Moso bamboo shoots on different growth media; Table S2: Characterization of endophytic bacteria according to PGP traits.

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