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Biodiversity of Culturable Endophytic Actinobacteria Isolated from High Yield *Camellia oleifera* and Their Plant Growth Promotion Potential

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Abstract: *Camellia oleifera* Abel. is one of the world's four famous woody oil trees and has drawn increasing attention because of its high commercial value. Endophytes are microorganisms inhabiting inside plant tissues, and their functions vary with the change of host status and environmental parameters. To deepen our understanding of the interactions between *C. oleifera* and their endophytic actinobacteria, the present study investigated the four endophytic actinobacterial composition-residing high-yield *C. oleifera* trees. A total of 156 endophytic actinobacterial isolates were obtained distributed in 17 genera. Among them, *Streptomyces* was the dominant endophytic actinobacteria, followed by *Nocardia*, *Amycolatopsis*, *Microbiospora*, *Micromonospora* and other rare actinobacteria genera. Soil characteristics including soil pH and organic matter were found to play crucial roles in shaping the endophytic actinobacterial community composition. Furthermore, all isolates were studied to determine their plant growth-promotion traits, 86.54% could produce Indole 3-Acetic Acid, 16.03% showed nitrogen-fixing, 21.15% showed phosphorus solubilizing, and 35.26% produced siderophore. Under the glasshouse condition, some isolates exhibited growth promotion effects on *C. oleifera* seedlings with significant increase in spring shoot length and ground diameter. Altogether, this study demonstrated that *C. oleifera* plants harbored a high diversity and novelty of culturable endophytic actinobacteria, which represent important potential as natural biofertilizers for the high production of *C. oleifera*.

Keywords: *Camellia oleifera*; endophytic actinobacteria; biodiversity; plant growth promotion

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1. Introduction

Camellia oleifera Abel., a member of the Theaceae, is an edible oil tree species of China. It is widely distributed in southern China especially in Hunan, Jiangxi and Guangxi province [1,2]. *C. oleifera* has a long high-yielding period (approximately one hundred years), and the secondary metabolites it produces, such as camellia seed oil, saponins, polyphenols and flavonoids, are widely used in daily life [3]. Especially camellia seed oil, also well known as "eastern olive oil" [4], contains more than 90% unsaturated fatty acid content and can boost human immunity, reduce blood pressure, prevent cardiovascular and cerebrovascular diseases [5,6]. Moreover, *C. oleifera* generally grows in the mountainous areas of subtropical regions, thus playing important roles in the conservation of water and soil and keeping ecological balance [7,8]. To develop the great value of this excellent economic and ecological tree, the exploration of the relationship between *C. oleifera* and

associated microorganisms, and then the management involved plant microbiome selection, are gaining increasing interests for plant traits improvement [9].

Endophytes are plant-associated microorganisms that colonize inside the plant tissues without causing visible symptoms of disease [10]. It has been shown that endophytes exert a multitude of functions to their host plant, including increasing nutrient availability [11], controlling plant pathogens [12], and improving stress tolerances [13], ultimately enhancing plant growth and productivity. Compared with microorganisms derived from soil, endophytes have intense communications with their plant host and rhizobacteria due to their specific biological niche, thus they have profound effects on soil and plant simultaneously [14]. Furthermore, endophytes are rich resources of novel bioactive substances and thus have enormous potentials in pharmaceutical, agricultural, and biotechnological industries [15]. Especially the phylum *Actinobacteria* are in the foreground due to their production of secondary metabolites with multiple functions, for example an antibacterial, antifungal and antitumoral [16]. Therefore, endophytic actinobacteria are considered promising agents for agriculture and industry [17].

The endophytes isolated from oil-producing trees, such as olive and oil palms, have been reported [18–20], while only a few studies have observed *C. oleifera* endophytes. These limited studies mainly focused on endophytic fungi and bacteria [3,21]. For example, the endophytic bacteria *Bacillus subtilis* 1-L-29 from *C. oleifera* demonstrated antagonistic activity against anthracnose pathogen and exhibited growth-promotion potential to *C. oleifera* [1]. Almost no attention has been paid to endophytic actinobacteria in *C. oleifera*, much less the investigation of the interaction between endophytic actinobacteria and *C. oleifera*.

Cultivation-independent analyses using 16S and ITS rRNA gene-based methods give us insight into the abundant microorganisms including novel and uncultured taxa inhabiting in *C. oleifera* forestland [7,22]. Meanwhile, isolating pure culture are indispensable techniques for the identification and acquisition of strains with potential as biological fertilizers or pesticides [23]. Analyzing culturable microbial diversity can help us understand the behaviors and functions of microorganisms, as well as utilization and expansion of microbial resources. Hence, to address the knowledge gap of the endophytic actinobacteria inside *C. oleifera*, four high-yielding and locally promoted cultivars of *C. oleifera* were sampled to isolate the endophytic actinobacteria by a culture-dependent approach. The composition of associated endophytic actinobacteria influenced by soil conditions and their host plants were analyzed. Finally, the potential growth-promoting traits of the endophytic actinobacteria based on their metabolites producing behaviors and in vivo test under glasshouse condition were assessed. This is the first study to describe the endophytic actinobacteria in *C. oleifera* and their growth-promoting potentials to host plant.

2. Materials and Methods

2.1. Plant Materials

Four twelve-year-old *Camellia oleifera* trees, namely, *Camellia oleifera* cv. 'Xianglin No. 1' (XL1), 'Xianglin No. 97' (XL97), 'Xianglin No. 210' (XL210), and 'Xianglin No. 27' (XL27), were used in this study. These Oil-tea were cultivated in the Oil-tea germplasm repository of Oil-tea Research Institute of Hunan Academy of Forestry Sciences (113°01′ east longitude and 28°06′ north latitude in Changsha, China). These four trees belong to four different cultivars, and these cultivars are superior varieties that recommended and widely cultivated in China [24]. XL1 is an excellent variety with flowering stage from late October to late December, and fruit maturation period in late October. The fresh seed rate is 46.8%, and the seed kernel oil rate is 35.0%. XL210 is a good cultivar which has a flowering stage from late October to late December. The fresh seed rate is 44.8%, and the seed kernel oil rate is 36.4%. XL97 is with 21–40 fruits per 500 g, the fresh seed rate is 43.41–46.4%, seed kernel oil rate is 50.5%. XL27 is with 15–28 fruits per 500 g, the fresh seed rate is 50–56%, and the seed kernel oil rate is 34–37%. The detailed information about these four cultivars is available in Table 1 [2,24]. In the sampling area, the soil is red earth with organic fertilizer applied every December.

Table 1. Characters and yield performances of various cultivars of *C. oleifera*.

	XL1	XL97	XL210	XL27
Fruit diameter/mm	30–44	26–37	33–48	30–45
Fruit number per 500 g	15–30	21–40	15–30	15–28
Fresh seed rate/%	46.8	43.4–46.4	44.8	50–56
Seed kernel oil rate/%	35.0	50.5	36.4	34.7
Fruit oil rate/%	8.9	10.9	5.8	10.7
Oil production/kg ha^{-1}	772.5	901.5	618.8	995.4

2.2. Collection of Samples

C. oleifera samples were collected in the middle of April in 2020 for endophytes isolation. For each tree, 5–10 g branch samples and fifty leaves between 2 and 3 m from the ground from different directions of the tree were collected and mixed up as one tree sample, respectively. A 60 cm section was excavated to collect the roots (5–10 g) from the four directions of the tree. Plant samples were sealed in vacuum bags and kept at −20 °C. The rhizosphere soil adhering to the roots were gently shaken and mixed, then stored at 4 °C.

2.3. Determination of Rhizosphere Soil Characteristics

The rhizosphere soils were passed through a 2-mm sieve and the plant tissues and stones were remove. After dried in the shade, soils were sent for physicochemical characteristics determination. Soil pH was measured by a potentiometer in distilled water (1:2.5, *w*/*v*) [25]. Organic matter was determined by dichromate wet combustion and visible spectrophotometry [26]. The total nitrogen (N), phosphorus (P) and potassium (K) was determined via the Kjeldahl method [27], the ignition method [28] and a digestion method using a flame photometer to test [29], respectively. Available N was determined according to Shi [30]. Available P was measured by the molybdenum antimony colorimetric method [31]. Available K was determined by a flame photometer [32]. Iron (Fe), Calcium (Ca), Aluminum (Al) were extracted with perchloric acid and determined by inductively coupled plasma optical emission spectrometry (ICP-OES) using an ICP PE8300 [33].

2.4. Isolation of Endophytic Actinobacteria

The plant samples of leaf, branch and root tissues were washed thoroughly under running water, dried overnight and subjected to a surface sterilization procedure as described by Xiong [34]. Briefly, samples were ultrasonic washed for 1 min in 124 mM $Na_2HPO_4 \cdot 12H_2O$ solution, immersed in absolute ethanol for 1 min and 4% (*v*/*v*) NaClO for 6 min, then washed with absolute ethanol for 30 s, followed by rinsing in sterile water for 30 s and immersing in 5% (*w*/*v*) $Na_2S_2O_3$ for 5 min. Finally, samples were rinsed 5 times in sterilized water for 10 min each time and dried under laminar airflow for 3–4 h. Tissues were cut into 1-cm pieces using sterile scissors and transferred onto four isolation media: humic acid vitamin B agar (HV), mannitol soybean agar (MS), tap water yeast extract agar (TWYE) and water agar (WA) medium. Each medium was added with 20 mg L^{-1} nalidixic acid and 50 mg L^{-1} benomyl as antibacterial and antifungal agents, respectively. To verify the surface sterilization efficiency, 200 µL of the final rinse of water was spread onto potato dextrose agar (PDA) plates to detect possible contaminants. No microbial growth on these plates meant that the surface sterilization was successful. Isolation plates were incubated at 30 °C and checked regularly. The emerged actinobacteria colonies were recorded and transferred to PDA for purification. Several media were used to compare the colony morphology including aerial mycelium, substrate mycelium, soluble pigment and spore production, and the exactly same isolates were combined as one. The obtained different pure cultures were preserved at 4 °C for temporary storage or in 50% (*v*/*v*) glycerol at −80 °C for long-term storage.

2.5. Classification of Endophytic Actinobacteria

The endophytic actinobacteria were further classified based on 16S rRNA gene sequencing analysis. Actinobacteria were cultured for 5 days at 28 °C at 170 rpm on International *Streptomyces* Project (ISP)2 liquid medium. The mycelia were collected by centrifugation and washed with sterile water. Genomic DNA was extracted from mycelia using a commercial DNA extraction kit (Sangon Rapid Bacterial Genomic DNA Isolation Kit, Shanghai Sangon Biotech Co., Ltd., Shanghai, China) following the manufacturer's protocol. The 16S rRNA gene was amplified by the polymerase chain reaction (PCR) using the primer pairs 27f and 1492r on the following thermal cycling condition: 95 °C for 5 min; followed by 30 cycles of 94 °C for 1 min; 54 °C for 30 s; 72 °C for 90 s; and finally 72 °C for 10 min. The PCR product was sequenced using Sanger method at Sangon Biotechnology Co., Ltd., Shanghai, China and the resultant sequences were blasted online using BLAST at the National Center for Biotechnology Information website (https://www.ncbi.nlm.nih.gov/, accessed date: 11 January 2021). The standard blastn (nucleotide-nucleotide) algorithm was used with the default settings. Microorganisms with a high level of identity (97–100%) were selected as the closest match, and the actinobacterial isolates were classified to the genus level according to the matched microbes, respectively.

2.6. In Vitro Qualitative Assessment of Plant-Growth Promotion (PGP) Traits of Puried Endophytic Actinobacteria

The purified endophytic actinobacterial isolates were qualitatively assessed for their PGP traits, including N-fixing, P-solubilizing, siderophore and IAA production. Actinobacterial spore suspension (10 µL of 10^7 spores mL^{-1}) was spotted on different medium plates and incubated at 30 °C for 7 days. Pikovskaya's (PVK) agar medium containing tricalcium phosphate ($Ca_3[PO_4]_2$) [35], Chrome azurol S (CAS) agar medium [36] and Ashby N-free agar medium [37] were used for evaluation of P-solubilization, siderophore production and N-fixation, respectively. P-solubilization and siderophore production were qualitatively evaluated by the presence of a halo around the colony, whereas a positive growth on the medium indicate the N-fixing activity. All experiments were performed in triplicate. The IAA production was detected in Yeast Mannitol Broth (YMB) supplemented with 0.2% L-tryptophan. Individual actinobacterial spore suspension (5 µL of 10^7 spores mL^{-1}) were inoculated in YMB at 150 rpm at 28 °C for 7 days, and the supernatant obtained by centrifuging at 4000 rpm were reacted with Salkowski's reagent and kept in dark for 30 min. The development of a pink color indicates IAA production by the actinobacterial isolates [36]. Further quantification of IAA was measured under a spectrophotometer at the absorbance of 530 nm.

2.7. Effects of the Potential Endophytic Actinobacteria on the Growth of C. oleifera Seedlings

The isolates exhibiting high PGP potentials were selected for further assessment of their effects on *C. oleifera* seedling growth under glasshouse condition. Two-year-old *C. oleifera* seedlings with uniform height and ten pots with one seedling per pot in each treatment were used in this study. Forty milliliters of spore suspension (10^7 spore mL^{-1}) of each strain was applied to the soil at the base of each seedling. Seedlings applied with water were considered as the control. All pots were completely randomized in the glasshouse with their position changed every week. Plants in the glasshouse had natural light and grown during December 2020 to May 2021. After 5 months of growth, plant growth was evaluated by measuring seedling heights, spring shoot lengths and ground diameters.

2.8. Statistical Analysis

Venn diagrams, redundancy analysis were performed using the OmicShare online platform (http://www.omicshare.com/tools, accessed date: 6 April 2021). Data were analyzed by a one-way analysis of variance (ANOVA) with a least significant difference (LSD) test, and the means were compared at a significant level of $p < 0.05$. The SPSS statistical software (IBM SPSS, Chicago, IL, USA) was used for statistical analysis.

3. Results

3.1. Isolation of Culturable Endophytic Actinobacteria

The surface sterilization was generally efficient, seeing as no bacteria grew on the test plates, indicating that the isolates obtained were endophytes [38]. A total of 876 strains of endophytic actinobacteria colonies were observed on the tissue samples of four high-yielding *C. oleifera* trees. After preliminary distinguish and purified cultivation, 156 pure isolates were obtained, which included 105 isolates originating from XL97, 21 isolates from XL210, 16 from XL1, and 14 from XL27. The extent of colonization of endophytic actinobacteria differed among the host plants ranging from the maximum in XL97 and minimum in XL27. Among them, the endophytic actinobacteria from the root of *C. oleifera* accounted for the largest proportion, while only a small amount of actinobacteria were from branches and leaves.

3.2. Impact of Culture Media on Isolation

To obtain endophytic actinobacteria as much as possible, four selective isolation media suitable for endophytic actinobacteria were used simultaneously in this study. As shown in Figure 1, the frequencies of obtained actinobacteria were different from various culture media. In XL27 and XL97, the highest isolation rates were from the nutrient-rich medium MS, while in XL1, that was from the HV medium. The two nutrient-poor media, WA and TWYE, gave the lowest isolation frequency in these three samples. However, in XL210 samples, TWYE exhibited the highest isolation frequency, whereas MS and WA gave the lowest.

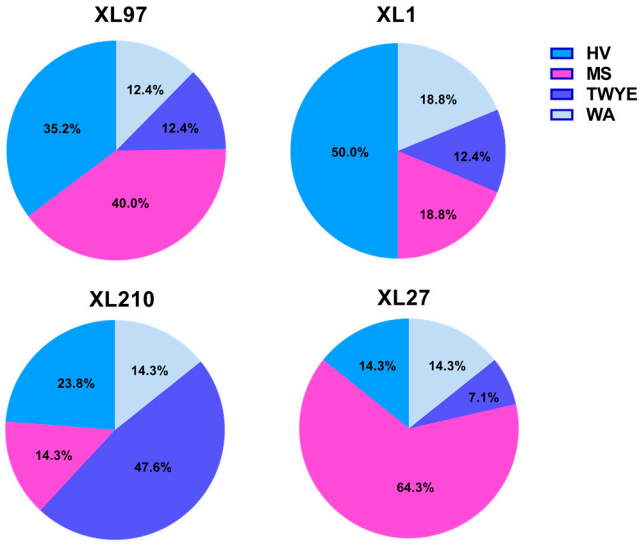

Figure 1. Numbers of endophytic actinobacteria isolated from different media across four different *C. oleifera* samples (HV: humic acid vitamin B agar; MS: mannitol soybean agar; TWYE: tap water yeast extract agar, WA: water agar medium).

3.3. Composition of Endophytic Actinobacteria

Based on the 16S rRNA gene sequencing results, 156 actinobacterial isolates were divided into 17 genera (Figure 2A,B). Obviously, respective genera abundance was different among all *C. oleifera* samples and *Streptomyces* was the most abundant genera in all the *C. oleifera* trees (Figure 2A,B). For example, in XL97, a total of 105 actinobacterial strains were retrieved, where *Streptomyces* and *Nocardia* represented the major abundance, with 44.76% and 35.24%, respectively (Figure 2B). As for 14 strains from XL27, *Streptomyces* was the most abundant genera (64.29%), followed by *Amycolatopsis* with 21.43%, *Micromonospora* and *Saccharopolyspora* with 7.14%.

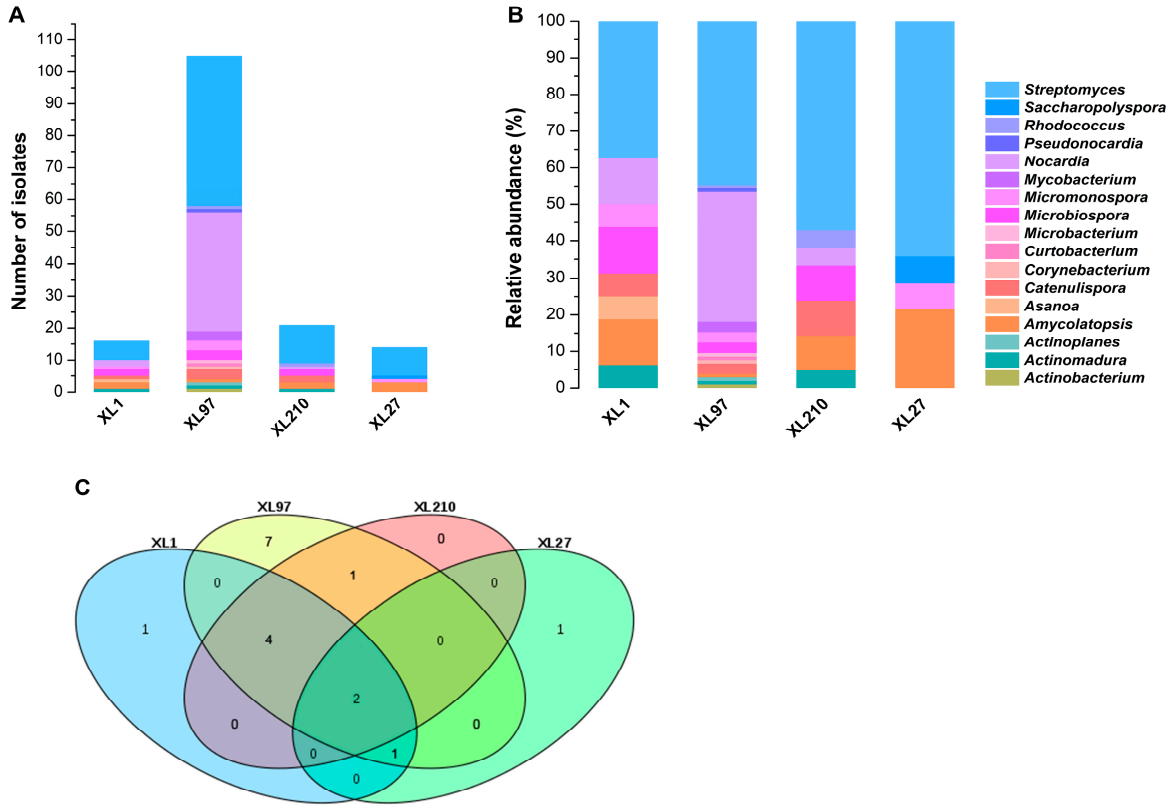

Figure 2. Culturable composition of endophytic actinobacteria in *C. oleifera* samples showing number (**A**) and relative abundance (**B**) at the genus level, and exclusive and shared numbers of genera (**C**) among four *C. oleifera* samples.

The shared and unique actinobacterial genera in different *C. oleifera* samples are shown in the Venn diagram (Figure 2C). *Streptomyces* and *Amycolatopsis* were two mutual groups present in four *C. oleifera* trees. Besides, isolates of *Catenulispora* sp., *Actinomadura* sp., *Microbiospora* sp. and *Nocardia* sp. were shared in XL1, XL97 and XL210, isolates of *Rhodococcus* sp. were obtained both from XL97 and XL210, isolate of *Micromonospora* sp. was found in XL1, XL97 and XL27. Additionally, unique genera were retrieved from each *C. oleifera* tree. XL97 demonstrated to present the most notable richness with 7 unique genera, isolates of *Actinobacterium* sp., *Actinoplanes* sp., *Corynebacterium* sp., *Curtobacterium* sp., *Microbacterium* sp., *Mycobacterium* sp. and *Psedonocardia* sp. were endemic to XL97. Isolates of *Asanoa* sp. were found only in XL1, while *Saccharopolyspora* sp. was retrieved from XL27 (Figure 2C).

3.4. Soil Characteristics

The rhizosphere soil of the four *C. oleifera* planting spots was collected to determine their nutrient status, and there were some differences in soil characteristics among these samples (Table 2). The pH value of XL27 soil was significantly lower than those of other samples. The total phosphorus (TP), total potassium (TK), available nitrogen (AN), available phosphorus (AP), calcium (Ca) and aluminum (Al) concentrations in the rhizosphere soil where XL27 planted were significantly higher than other samples, and XL97 soil had the highest organic matter (OM) and total nitrogen (TN) concentrations, whereas XL210 soil had the lowest TP, TK, AN, AP, Ca and Al concentrations.

Table 2. Rhizosphere soil properties across different *C. oleifera* trees. Values are the means ± standard deviation (n = 3). Different letters within a row indicate significant differences at a level of $p < 0.05$ between clone types (ANOVA followed by Fisher's LSD test).

	XL1	XL97	XL210	XL27
pH	5.71 ± 0.03 a	5.05 ± 0.03 b	5.07 ± 0.24 b	4.73 ± 0.02 c
Organic matter (g kg−1)	34.86 ± 1.48 c	57.33 ± 2.24 a	55.37 ± 3.11 a	41.01 ± 2.52 b
Total nitrogen (g kg−1)	2.68 ± 0.01 b	3.41 ± 0.27 a	2.63 ± 0.04 b	2.63 ± 0.23 b
Total phosphorus (g kg−1)	0.61 ± 0.13 b	0.59 ± 0.05 b	0.42 ± 0.04 b	1.16 ± 0.14 a
Total potassium (g kg−1)	5.24 ± 0.14 b	4.53 ± 0.05 bc	4.11 ± 0.08 c	7.69 ± 0.52 a
Available nitrogen (mg kg−1)	170.67 ± 15.73 b	171.88 ± 7.02 b	124.16 ± 15.44 c	215.37 ± 13.24 a
Available phosphorus (mg kg−1)	11.81 ± 0.74 bc	12.96 ± 0.72 b	10.90 ± 0.24 c	15.21 ± 2.49 a
Available potassium (mg kg−1)	107.74 ± 2.03 b	86.76 ± 4.67 c	88.39 ± 6.81 c	133.88 ± 3.78 a
Calcium (g kg−1)	0.53 ± 0.06 b	0.74 ± 0.09 a	0.40 ± 0.05 c	0.77 ± 0.09 a
Iron (g kg−1)	14.87 ± 0.56 d	23.23 ± 0.73 b	18.23 ± 1.53 c	30.25 ± 1.31 a
Aluminum (g kg−1)	1.10 ± 0.17 a	1.11 ± 0.13 a	0.96 ± 0.04 a	1.12 ± 0.16 a

3.5. Effects of Soil Condition on Endophytic Actinobacterial Community Distribution

To identify the rhizosphere soil properties contributing to endophytic actinobacterial community, we carried out redundancy analysis (RDA) which showed the possible correlations between soil parameter variables and actinobacterial community represented by the top 10 taxonomic groups at the genus level in four *C. oleifera* trees (Figure 3). As shown in Figure 3, X-Axis indicated 69.15% of the genus-level variability, which was negatively correlated with pH, organic matter and total nitrogen. Y-Axis explained a further 21.32% of the variability which was positively correlated with pH. The RDA analysis had 90.47% of the total variation explained by the first two axes. TN and pH had a positive influence on endophytic actinobacterial distribution, with TN and OM had a significant influence on XL97, pH had a significant influence on XL1. *Microbiospora*, *Catenulispora*, *Actinomadura* were positively related to pH, *Nocardia* was positively related to pH, TN and OM, while pH, TN and OM had negative relationships with *Streptomyces*, *Amycolatopsis*, *Saccharopolyspora*.

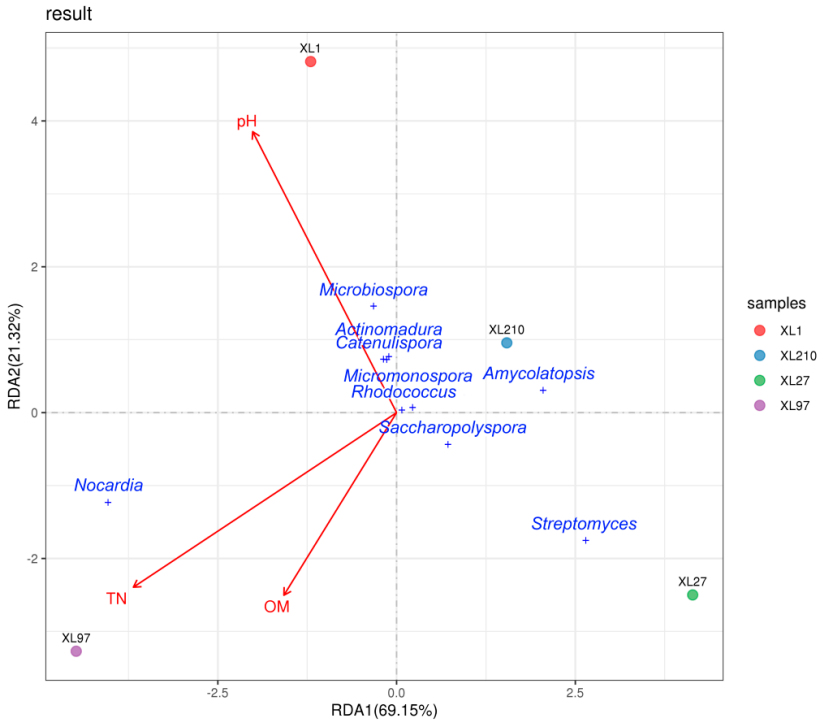

Figure 3. Redundancy analysis (RDA) for endophytic actinobacterial community and physiochemical properties of rhizosphere soil samples across different *C. oleifera* samples.

3.6. In Vitro Evaluation of Endophytic Actinobacteria with Plant Growth Promoting Traits

The production of active secondary metabolites is a prominent character of many plant growth promoting (PGP) microorganisms. All 156 endophytic actinobacterial isolates were qualitatively assessed for their PGP traits such as N-fixation, IAA production, mineral P-solubilization and siderophore production. As shown in Figure 4, most of the actinobacterial isolates have PGP activity: 135 isolates (86.54%) had the ability to produce IAA, 55 isolates (35.26%) had siderophore production activity, 33 isolates (21.25%) were able to solubilize insoluble phosphate and 25 isolates (16.03%) possessed N-fixation activity.

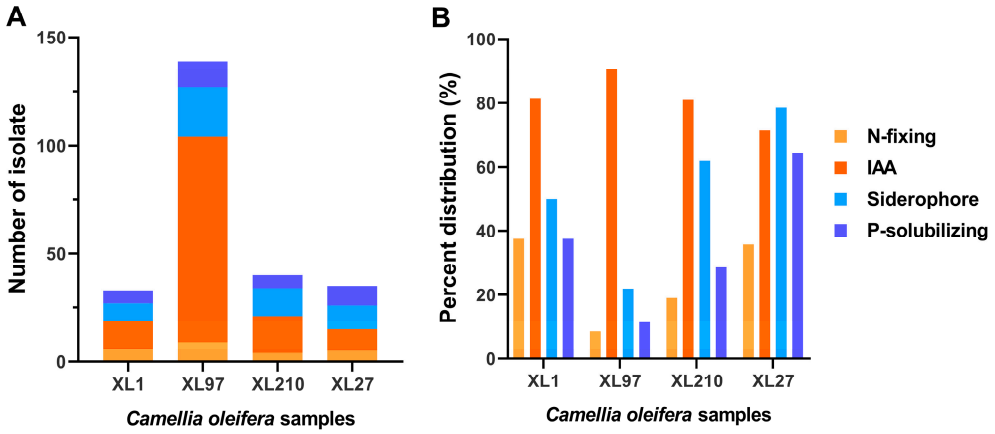

Figure 4. Numbers (**A**) and percent distribution (**B**) of culturable endophytic actinobacterial isolates purified from different *C. oleifera* samples with plant growth promotion traits.

The number and proportion of endophytic actinobacterial with PGP traits differed in four *C. oleifera* samples (Figure 4). In XL97, higher numbers of IAA producers (90.47%) were recorded, while the lowest population with N-fixation (8.57%), P-solubilization (11.4%) and siderophore-production activities (21.9%) were found. Interestingly, though the lowest number of actinobacterial isolates were obtained from XL27, the isolates with different PGP traits were uniformly distributed. The isolates with P-solubilization and siderophore-producing activities were exhibited high proportions in XL27 with 64.30% and 78.6%, respectively (Figure 4B).

3.7. In Vivo Effects of Endophytic Actinobacteria on the Growth of C. oleifera Seedlings

Five endophytic actinobacteria strains with high PGP potential were selected to evaluate their effects on the growth of *C. oleifera* seedlings under glasshouse condition. The results showed that all the tested endophytes increased the growth of seedlings at different levels compared to the control. Among these strains, the improvement of *C. oleifera* seedling treated with *Streptomyces* sp. 2GM57 represented significant with promotion of 32.99% for spring shoot length and 34.56% for ground diameter over untreated control (Figure 5). The spring shoot length of all endophytic actinobacteria treated seedlings exhibited significant increase over the control, and the ground diameter of *Streptomyces* sp. 2GM57, *Streptomyces* sp. 3GT17 and *Streptomyces* sp. 4GM3 inoculated seedlings were significantly increased in comparison with the control seedlings (Figure 5B,C).

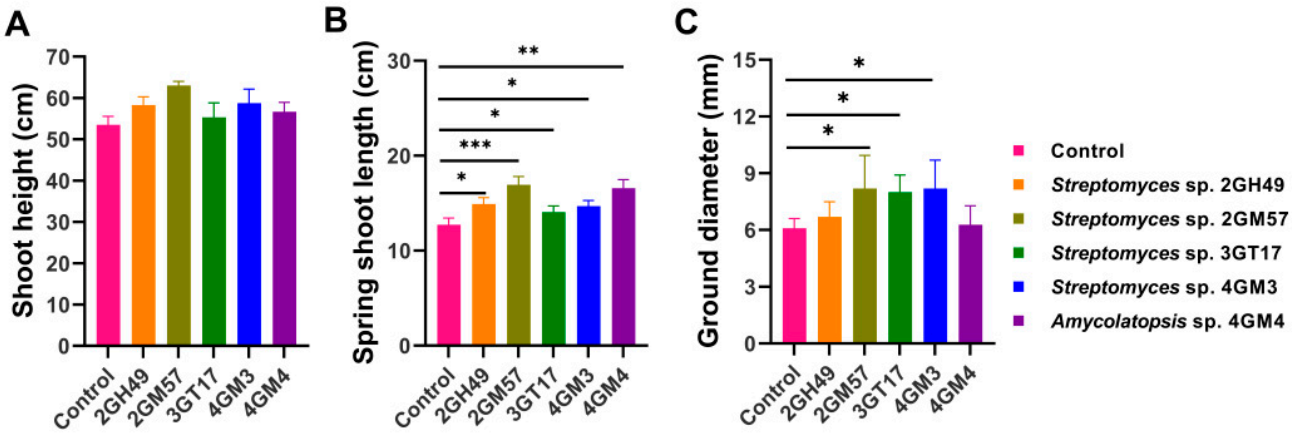

Figure 5. Effects of endophytic actinobacteria on the growth of *C. oleifera* seedlings under glasshouse conditions. (**A**): shoot height; (**B**): Spring shoot length; (**C**): ground diameter. Data are represented as the mean ± SE (n = 10). Statistical comparisons with the control were made by ANOVA (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$).

4. Discussion

Camellia oleifera is of agricultural significance in the southern part of China as the source of tea oil currently, but acid red soil in *C. oleifera* forestland lacks nutrient contents especially in nitrogen and phosphate, necessitating adaptations in plants for living in such barren soil [16]. Those plants that do survive are expected to harbor beneficial microbes that contribute to the host fitness in demanding habitats [39,40]. Previous studies have shown that some endophytic bacteria and fungi in *C. oleifera* might possess the potential of plant-growth promotion and disease-resistance. However, the diversity and function of endophytic actinobacteria in *C. oleifera* have been rarely studied. In the present study, we isolated and analyzed the culturable endophytic actinobacteria from four *C. oleifera* trees collected from Hunan Province. To the extent of our knowledge, this study is the first-ever to characterize and compare the endophytic actinobacterial communities in four high-yielding *C. oleifera* trees.

In our study, a total of 156 endophytic actinobacteria isolates were obtained from *C. oleifera* samples. Among these, most were from *C. oleifera* roots, only a few were from leaves and branches, suggesting that endophytic actinobacteria primarily colonized in root tissues of *C. oleifera*. Many investigations suggest that rhizosphere is rich in microbial community and plants may release nutrient substances from root into the rhizosphere which can attract rhizospheric actinobacteria [16]. Therefore, rhizosphere actinobacteria can easily enter plant roots and switch to endophytic lifestyles, shaping the specific microbial assemblages of the plant [41,42].

It is reported that the diversity of endophytes is determined by environmental conditions including climate, temperature, moisture and nutrients [43], as well as host plant characteristics such as genotype, growth period, plant tissues, plant stress and phytopathogen populations [35]. In our study, although the *C. oleifera* trees were planted in the same plantations which had been subjected to similar management practices, there were apparent differences in soil properties among the four corresponding spots over 10 years' cultivation (Table 2). This is owing to the fact that soil nutrients are significantly affected by plant root exudates and soil microbial community [7]. In addition, the *C. oleifera* samples were collected at the same time, thus the host plant characteristics and soil conditions were considered as the key factors influencing the population structure of endophytic actinobacteria.

As shown in Figure 3 of the redundancy analysis (RDA) analysis, 11 environmental factors, including soil pH, organic matter and other nutrient elements were calculated, among which soil organic matter, total nitrogen and pH significantly affected the endophytic actinobacterial community. In these factors, organic matter had a positive influence on endophytic actinobacterial distribution in XL97 (Figure 3). Correspondingly, the XL97

cultivated soil showed the highest organic matter concentration of 57.33 g kg^{-1} (Table 2), and most endophytic actinobacteria were isolated from XL97. This was in accordance with the earlier report that the soil organic matters present an important factor shaping the endophytic actinobacterial community [41]. Hence, most of the isolates in XL97 were from MS medium, the most nutrient-rich medium among the four isolation media (Figure 1). In addition, the fact that endophytic actinobacteria preferring organic matter were prevalent in XL97 indicates that XL97 might offer an inner organic-rich habitats that favor the existence of actinobacteria. This was consistent with the previous study that the actinobacteria were predominantly found in organic-rich habitats [42].

The soil pH in *C. oleifera* forestland ranged from 4.73–5.71, and the AP concentrations were at a low level (Table 2). These were the significant features of red acid soil [4]. The lowest pH of 4.73 was found in XL27 rhizosphere soil; this might be the reason of the minimum microbial number of endophytic actinobacteria retrieved from XL27 among the four trees. However, the proportion of endophytic actinobacteria with N-fixing, P-solubilizing and siderophore production abilities in XL27 were much higher than that of other trees (Figure 4). It was reported that most of the microorganisms with growth promoting traits are acidogenic heterotrophic microflora, which could reduce soil pH to a certain degree, thus activating insoluble element solubilization such as P [22]. Therefore, there might be a similar mechanism operating in the rhizosphere for XL27. In addition, bacterial siderophores not only influence the uptake of minor nutrients [43] but also act as determinants of induced systemic resistance against plant disease [44] and induced systemic tolerance against abiotic stresses respectively [45]. Thus, the more P-solubilizing and siderophore-producing endophytes in XL27 indicated that plant hosts might have prominent roles in recruiting beneficial endophytes that were more favorable for nutrient absorbing and host resilience improving by *C. oleifera* grown in red soil.

The TP, TK, AN, AP and TK concentrations in XL210 rhizosphere soil were significantly lower than those in other soils (Table 2), similar results were found that the soil fertility declined in XL210 soil over time [22]. This low nutrient status possibly resulted in the much less amount of endophytic actinobacteria in XL210. It was also reported that XL210 possessed strong adaptability and resistance to various harsh environments [24]. This indicated that XL210 has a strong nutrient absorption capacity, resulting in soil properties alteration after a period of planting. Therefore, only the actinobacteria surviving in nutrient-deficient environment could form a symbiotic relationship with this stress-tolerance cultivar, XL210. Correspondingly, higher frequency of endophytic actinobacteria of XL210 was from minimal media like TWYE (Figure 1), reflecting the endophytic actinobacteria community in XL210 were low-nutrition required.

Among the obtained isolates, *Streptomyces* and *Amycolatopsis* were two shared groups that occurred in four *C. oleifera* samples, suggesting that the actinobacteria of these two genera are the core members of *C. oleifera* endophytes (Figure 2). Notably, *Streptomyces* was the most universal genus among endophytic actinobacteria in various crops and trees [16,46], suggesting its high adaptability to a wide range of plants and environments [47]. In addition, endophytic *Streptomyces* are well-known for their capacity to produce various bioactive metabolites [47], which could serve as beneficial agents towards plant development and health [48,49]. Up to now, there has been no application regarding endophytic *Streptomyces* from *C. oleifera*. Thus, the *Streptomyces* strains in our study exerted new and broad application potential as growth-promotion agents for the woody plant. *Amycolatopsis* is a rare actinobacteria with little known about the number and kinds of *Amycolatopsis* species in natural habitats [50]. *Amycolatopsis* is also considered as a feature of harsh infertile environments because the strains in this genus are commonly found in hyper-arid desert soil [51,52] and plant endosphere [46,53,54]. In this study, both in XL210 and XL27, the proportion of *Amycolatopsis* is ranked second after *Streptomyces* (Figure 2). Considering that XL27 grows in the most acidic soil and XL210 in the most nutritionally impoverished soil, we expect that *Amycolatopsis* may have a helpful role to XL27 and XL210 in the harsh condition. Moreover, *Amycolatopsis* strains are also known as a rich source

of novel secondary metabolites [55]; hence, endophytic *Streptomyces* and *Amycolatopsis*, especially the ones with PGP traits, will be paid extreme attention to assess their potential as PGP agents in greenhouse/field conditions, and to better understand their interactions with *C. oleifera* and the environment.

Besides, there were other genera such as *Actinomadura*, *Microbiospora*, *Micromonospora* and *Nocardia* also shared in two or three *C. oleifera* samples. Studies have reported that these actinobacterial genera showed potential for use as biofertilizers in other crops including chickpea [56], alfalfa [57], *Casuarina glauca* [58]. These suggested that the endophytic actinobacteria from *C. oleifera* possessed wide range of application and great development value.

Apart from the shared genera, XL97 presents a notable richness with 7 unique genera, whereas XL1 and XL27 only harbor 1 unique genus (Figure 2C). The lower overlap of endophytic genus between the four *C. oleifera* trees reflects the unique endophytic communities selected by the host and their habitats, supporting the idea that endophytic communities were host-specific [40]. These unique genera, including *Actinobacterium*, *Actinoplanes*, *Corynebacterium*, *Curtobacterium*, *Microbacterium*, *Mycobacterium*, *Saccharopolyspora*, *Asanoa* and *Psedonocardia* were isolated less frequently with abundance below 2%, and most of them were represented as the so-called rare actinobacteria. The presence of these rare actinobacteria isolates in *C. oleifera* suggests that *C. oleifera* endosphere represents potential in bio-discovery [47].

Endophytic actinobacteria are currently gaining considerable attention for their involvement in PGP. In our study, most of actinobacterial isolates possessed in vitro function of PGP traits such as IAA production, siderophore production, N-fixation and P-solubilization (Figure 4). Pot assay under glasshouse condition confirmed that some isolates could boost the growth of *C. oleifera* seedlings, with the spring shoot length and ground diameter significantly increased (Figure 5). We noticed that there was no significant promotion in shoot height for all isolates, this might be because that *C. oleifera* is a woody plant which grows more slowly than other crops, so it is hard to show the obvious height increase within six months in this study. Hence, long-term effects of these isolates on the growth of *C. oleifera* should be evaluated. Among these isolates, *Streptomyces* sp. 2GM57 exhibited the highest promoting effect in seedling height (no significance), spring shoot length ($p < 0.005$) and ground diameter ($p < 0.01$), therefore showing great potential as biofertilizers. As such, field trials should be conducted for further evaluation of the effect on both growth and fruit yield of *C. oleifera*, and the function and mechanisms of the potential actinobacterial isolates involved in the growth promotion remain to be further investigated.

5. Conclusions

The present study elucidated the composition and functions of the culturable endophytic actinobacterial community associated with the Chinese endemic oil plant *Camellia oleifera* with high yield traits. Our study suggested that both the *C. oleifera* and the soil environment are able to shape the community composition of endophytic actinobacteria. Many isolates possessed PGP potentials such as nitrogen fixation, phosphate solubilization, IAA and siderophore production, and some isolates showed boosting effects on the growth of *C. oleifera* seedlings in vivo. Our findings revealed that the endophytic actinobacteria from *C. oleifera* exhibited plant growth promotion potential, which could be further exploited to develop commercial biofertilizers for the future management of *C. oleifera* forestland.

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