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Cultivation Mode Reshapes Root Fungal Endophyte Communities and Links to Medicinal Compound Accumulation in *Dendrobium officinale* (Orchidaceae)

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Abstract

Background: Symbiotic fungi play essential roles throughout the entire cycle of orchid plants, including seed germination, seedling development, and maturation. *Dendrobium officinale Kimura & Migo* (Orchidaceae) (*D. officinale*) is a rare and highly valued traditional Chinese medicinal herb. Currently, artificial breeding using tissue culture technology is widely adopted and essential in the Dendrobium industry; however, this approach may impair or disrupt the plant's ability to establish and maintain symbiotic relationships with mycorrhizal fungi; **Methods**: In this study, the fungal endophyte community (FEC) in the roots of *D. officinale* cultivated under four different modes was analyzed using high-throughput sequencing. Correlation analyses were also carried out to examine the relationships between bioactive compounds and the FEC; **Results:** 1) the FEC in *D. officinale* roots was dominated by Ascomycota and Basidiomycota, with significant differences in abundance, diversity, and community structure among cultivation modes; 2) the FEC under greenhouse cultivation mode differed significantly from those under tree epiphytic cultivation in terms of fungal nutritional types and dominant taxa; 3) six major mycorrhizal fungal taxa were identified in Dendrobium roots, although non-mycorrhizal fungi accounted for approximately 97% of the community; and 4) the main bioactive compounds were positively correlated with variations in the FEC; **Conclusions:** this study provides a foundation for understating the growth of *D. officinale* under different cultivation modes and highlights the relationship between bioactive compound accumulation and mycorrhizal fungal communities.

Keywords: *Dendrobium officinale*; cultivation modes; fungal endophyte; polysaccharides

1. Introduction

Mycorrhizas refers to the symbiotic association between plants and fungi, which is thought to have originated in early-diverging land plants (e.g., mosses, Haplomitiriopsida) and Mucoromycotina fungi [1,2]. The 4 major types of mycorrhizae include arbuscular mycorrhizas (AM), ectomycorrhizas (EcM), orchid mycorrhizas (OM), and ericoid mycorrhizas (ErM). Mycorrhizal fungi can supply up to 80% of plant nitrogen (N) and phosphorus (P) requirements [3]. In orchids, mature seeds lack endosperm and therefore rely on symbiotic fungi for germination, which provide carbon and inorganic nutrients under natural conditions. During both seedling and adult stages, orchids fully or partially depend on orchid mycorrhizal fungi (OMF) [4]. Members of the families Tulasnellaceae, Ceratobasidiaceae and Serendipitaceae are among the most common and evolutionarily ancient OMF, collectively referred to as "*Rhizoctonia*-like" Basidiomycetes in wild

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orchids [5–8]. These fungi form specialized peloton (like *Rhizoctonia*) within orchid root cortex cells and facilitate the transfer of fungal-derived nutrients to the host plant, thereby contributing to the wide distribution of orchids across habitats with diverse nutrient availability [4,9,10].

Dendrobium officinale is a perennial herb and a highly valued traditional Chinese medicinal plant, known for its pharmacological properties, including anti-oxidation, anti-inflammation, immunomodulatory effects, free radical scavenging, and inhibition of tumorigenesis and metastasis [11]. However, wild populations of *D. officinale* have been severely depleted, and the surviving wild individuals are extremely rare and classified as endangered under Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) due to habitat destruction and overharvesting [12]. To meet commercial demand, large-scale cultivation of *Dendrobium* has been practiced in southern China for several decades, facilitated by advances in plant tissue culture technology [11]. However, propagation under sterile conditions, combined with reduced environmental selection pressure and the frequent use of fungicides, may impair the ability of Dendrobium to establish and maintain symbiotic relationships with mycorrhizal fungi and plant-growth-promoting rhizobacteria [13,14].

The establishment of obligate symbiotic relationships outside the native habitat of orchids presents a significant challenge for large-scale cultivation and population restoration [15]. Studies comparing endophytic fungal communities in wild and cultivated crops, such as rice and wheat, have shown that beneficial endophytes are often absent or underrepresented in domesticated systems [16,17]. Similarly, preliminary studies suggest that the fungal endophytic communities (FEC) reconstructed in cultivated orchids differ from those in wild populations. Following transplantation, fungi belonging to Atractiellales, Auriculariales, Ceratobasidiaceae, and Fusarium tend to increase, whereas the abundance of Tulasnellaceae and Pyronemataceae decreases [18–20]. This shift indicates a constrained reassembly and a potential loss of key OMF taxa [21]. Such alterations may ultimately affect the quality and medicinal value of cultivated orchids. Previous studies have demonstrated interactions between the plant metabolites and microbial communities within and surrounding *Dendrobium* plants [22,23]. However, knowledge of the fungal endophytic communities established by artificially propagated *Dendrobium* plants under cultivation conditions remains limited.

This study aimed to characterize the fungal endophytic communities (FFC) in the roots of *D. officinale* roots under 4 different cultivation modes and to evaluate how simulated natural habitat conditions influence these communities and their potential benefits to plant growth. The findings will provide a foundation for understanding the plasticity of orchid-endophyte associations for developing potential applications of fungal-based fertilizers in *Dendrobium* cultivation.

2. Materials and Methods

2.1. Sample Collection

Six samples, including roots, stems of *D. officinale* or cultivation substrate, were collected from Zhejiang Juyoupin Biotechnology Co. Ltd., located in the Dendrobium ecological cultivation base in Yueqing County (28.41394N, 121.17977E), a native habitat and one of the most suitable cultivation areas for *D. officinale* in Wenzhou, China. The region is characterized by a typical mild and humid climate (sampling-day temperature: 26-31 °C; relative humidity: ~70%). Of the four *D. officinale* samples, three root and stem samples were collected from epiphytic cultivation (EC) modes, in which plants were attached to the main trunks of living Ginkgo (GEC), Jujube (JEC), or Pear (PEC) trees, respectively (Figure 1). One additional root and stem sample was obtained from a raised bed cultivation (RBC) system in a greenhouse, which utilized crushed pine bark as the substrate and maintained approximately 80% air humidity and temperatures of 23-25 °C. The remaining two samples were non-root substrate samples collected from the same RBC system: rhizosphere substrate (RS), which was in direct contact with Dendrobium roots, and non-rhizosphere substrate (NRS), which was not associated with the roots.

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Figure 1. Different cultivation modes of *Dendrobium Officinale* in an ecological base in Yueqing County, Wenzhou, Zhejiang Province, China. (a), Raised-bed cultivation in a greenhouse (RBC); (b-d), Epiphytic cultivation. *D. officinale* was fixed to the living tree trunks of Jujube (b), pear (c) and ginkgo (d), respectively.

Healthy two-year-old *D. officinale* plants were selected, and roots (for fungal community analysis) and stems (for polysaccharide content determination) were harvested. The samples were thoroughly washed under running water, followed by surface sterilization using 1% sodium hypochlorite solution for 4 min. Subsequently, samples were rinsed twice with sterile water and dried using sterile filter paper. All samples were quickly frozen in liquid nitrogen and stored at –80 °C until subsequent analysis.

2.2. Genome DNA Extraction and Target Fragment Sequencing

DNA was extracted from the roots of *D. officinale* and associated cultivation substrates using the cetyltrimethylammonium bromide (CTAB) method. DNA concentration and quality were assessed using Nanodrop (Thermo Scientific, NC2000) and by 1.2% agarose gel electrophoresis analysis, respectively.

The internal transcribed spacer 1 (ITS1) region of fungal 16S rDNA was amplified using the forward primer ITS1F 5′-CTTGGTCATTTAGAGGAAGTAA-3′ and the reverse primer ITS2 5′-GCTGCGTTCTTCATCGATGC-3′. Sample-specific 7-nucleotide barcodes were incorporated into the primers to enable multiplex sequencing. The PCR reaction mixture (25 mL total volume) consisted of 5 mL of 5× buffer, 0.25 μL of Fast pfu DNA polymerase (5U/μL), 2 μL (2.5 mM) of dNTPs, 1 μL (10 mM) of each forward and reverse primer, 1 μL of DNA template, and 14.75 μL of double-distilled water (ddH2O). The PCR thermal cycling conditions were as follows: initial denaturation at 98 °C for 2 minutes; 30 cycles of denaturation at 98 °C for 15s, annealing at 55 °C for 30s, and extension at 72 °C for 45s; followed by a final extension at 72 °C for 5 min.

PCR amplicons were purified using Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After individual quantification, amplicons were pooled in equimolar amounts and sent to Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China) for pair-end 2×250 bp sequencing using the Illumina NovaSeq platform (NovaSeq 6000 SP Reagent Kit).

2.3. Sequencing Data Processing and Analysis

The DADA2 pipeline was used for primer removal, quality filtering, denoising, sequence merging, and chimera removal [24]. The FrameBot software (V1.2) was used to correct insertion-deletion (indel) errors in DNA sequences based on reference protein sequences downloaded from the

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RDP database (https://github.com/rdpstaff /Framebot). The corrected sequences were analyzed for their length distribution to ensure consistency with the expected fragment size. Taxonomic annotation was performed using the UNITE database (Release 8.0), and the amplicon sequence variant (ASV) abundance table was rarefied to 95% of the minimum sequencing depth across samples using the "feature-table rarefy" function in QIIME2 (2019.4). Statistical analyses of fungal endophytic community (FEC) composition were conducted at multiple taxonomic levels.

Alpha (a)-diversity indices were calculated using QIIME2 (2019.4), and statistical significance was assessed using the Kruskal-Wallis test followed by Dunn's post-hoc test. Beta (β)-diversity was calculated in QIIME2, and non-metric multidimensional scaling (NMDS) analysis based on the Bray-Curtis distance matrix was performed using R scripts. Linear discriminant analysis effect size (LEfSe) was conducted using the LEfSe package in Python. The FUNGuild database was used to predict the functional guilds within the fungal communities associated with Dendrobium roots.

2.4. Measurement of Bioactive Components and Ecophysiological Indicators

Polysaccharides from *D. officinale* stems were extracted and quantified according to the Chinese Pharmacopoeia [25]. Briefly, samples were subjected to water-bath reflux extraction, followed by ethanol precipitation and quantification using the phenol-sulfuric acid colorimetric assay. A glucose standard curve was prepared for calibration. The polysaccharide content was calculated using the following formula: mg/g = C×(Vt/V)×D×0.001/m, where C represents the sugar concentration determined from the standard curve, Vt is the volume used for measurement, V is the total extraction volume, D is the dilution factor, and m is the sample weight (g) (Nanjing Convinced-Test Company, Nanjing, China).

For the measurement of ethanol-soluble crude extractives (ESE), samples were dried to a constant weight and extracted by refluxing with ethanol, followed by evaporation to dryness and weighing after cooling. The ESE content was calculated based on dry weight [25].

The photosynthetic photon flux density (PPFD) was measured in situ at the sampling Dendrobium cultivation sites using a quantum PAR meter (AZ8584, Hengxin, Taiwan). The morphological and physiological traits of *D. officinale* were measured under laboratory conditions, including stem length (from the second internode to the base), stem diameter (measured at the middle of the stem), and chlorophyll content (measured on fully expanded young leaves and the two leaves immediately below) using a chlorophyll meter (SPAD-502 Plus, Konica Minolta).

2.5. RDA and Correlation Analysis

The distribution of endophytic fungal genera in relation to ecophysiological factors was analyzed using Redundancy Analysis (RDA). The correlations between endophytic fungal genera and ecophysiological variables were visualized using a heatmap. Both analyses were performed using the Wekemo Bioincloud platform (www.bioincloud.tech).

3. Results

3.1. a-Diversity of Fungal Communities in Dendrobium Roots and Cultivation Substrates

The ITS amplification libraries from fungal communities in Dendrobium roots under four different cultivation modes and associated cultivation substrates were analyzed using Illumina MiSeq PE250 high-throughput sequencing, which generated 2,171,876 quality-filtered reads and 1,983,040 chimera-free reads, with an average length of 230 to 323 bp. A total of 550 ASVs were identified, of which 388 were derived from root samples and 274 from cultivation substrate samples.

Alpha-diversity analysis showed that the rhizosphere substrate (RS) sample had the highest number of ASVs (217), with a Shannon index of 4.92, while the RBC sample had the lowest number of ASVs (121), with a Shannon index of 3.32. The RS samples show significantly higher α-diversity, with Chao1 and observed species indices of 330 and 325, respectively, compared with 112 and 111 in RBC samples ($P<0.05$). Compared with RBC samples, the living tree-epiphytic cultivation samples

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exhibited slightly higher α-diversity; however, these differences were not statistically significant (Table 1).

Table 1. Alpha diversity of fungal communities in *Dendrobium* roots and substrates.

Sample	Chao1	Goods_coverag	Observed_species	Pielou_evenness	Shannon	Simpson
NRS	296.79±5.43^{ab}	1.00±0	292.23±6.79^{ab}	0.59±0.08^{a}	4.83±0.64^{a}	0.84±0.09^{a}
RS	329.61±29.46^{a}	1.00±0	324.80±28.91^{a}	0.59±0.12^{a}	4.92±1.06^{a}	0.88±0.13^{a}
RBC	112.55±35.77^{b}	1.00±0	111.07±34.07^{b}	0.49±0.10^{a}	3.32±0.66^{b}	0.76±0.14^{a}
GEC	210.76±62.55^{ab}	1.00±0	207.10±61.71^{ab}	0.58±0.04^{a}	4.43±0.44^{ab}	0.89±0.03^{a}
JEC	191.35±73.48^{ab}	1.00±0	186.73±70.63^{ab}	0.58±0.10^{a}	4.33±0.76^{ab}	0.85±0.09^{a}
PEC	165.30±6.08^{ab}	1.00±0	163.03±6.45^{ab}	0.52±0.04^{a}	3.85±0.29^{ab}	0.86±0.04^{a}

Note: Statistical significance of differences was determined by the t-test. Lowercase letters denote significant differences ($p < 0.05$).

3.2. β-Diversity, Venn and LEfSe Analysis of Fungal Communities in Dendrobium Roots and Cultivation Substrates

The stress value of the NMDS analysis was 0.102, indicating that the ordination adequately represented the differences among fungal communities across the six samples. The fungal communities of the four Dendrobium root samples and two cultivation substrate samples clustered in distinct regions along the NMDS1 axis. In addition, the communities in roots from the three epiphytic cultivation modes were positioned closer to each other along the NMDS2 axis, whereas the RBC samples were more distant from the three epiphytic samples (Figure 2a).

A core set of 26 genera was identified as shared among all samples (Figure 2b). An additional eight fungal genera were shared exclusively among Dendrobium root samples across the four cultivation modes and were absent from the two cultivation substrate samples (Table S1). LEfSe analysis identified a total of 63 biomarkers that differed significantly among the four Dendrobium root samples and two cultivation substrate samples (Figure 2c). The highest number of differential taxa was detected in NRS (28), whereas RS contained the fewest (2).

3.3. Fungal Community Composition in Dendrobium Roots and Cultivation Substrates

The ASV sequences obtained in this study were taxonomically annotated using the UNITE database, which revealed that the endophytic fungi in Dendrobium roots were classified into 5 phyla, 22 classes, 85 orders, 176 families, and 276 genera. The dominant phyla in Dendrobium roots were Ascomycota and Basidiomycota, with a combined mean relative abundance (RA) of 89%. At the order level, the dominant orders were Pleosporales, Hypocreales, and Eurotiales, with a combined mean RA of 44% (Figure 3a). At the genus level, the dominant genera were Pyrenochaetopsis, Talaromyces, and Fusarium, with a combined mean RA of 30%. Among these, Pyrenochaetopsis exhibited the highest average RA (13.1%) (Figure 3b).

The RA of Ascomycota and Basidiomycota varied significantly among samples. The RBC sample was characterized by a low Ascomycota/Basidiomycota ratio (58%/17%), whereas the PEC sample showed a ratio of 86%/2%. At the genus level, the top 10 fungal genera identified in *D. officinale* roots were collectively distributed across 27 genera, with each sample exhibiting distinct dominant genera. The cultivation substrate samples did not share the same top 10 genera as the root samples. Instead, Resinicium, Subulicystidium, and Trichoderma were the most abundant genera in the substrates (Table S2)

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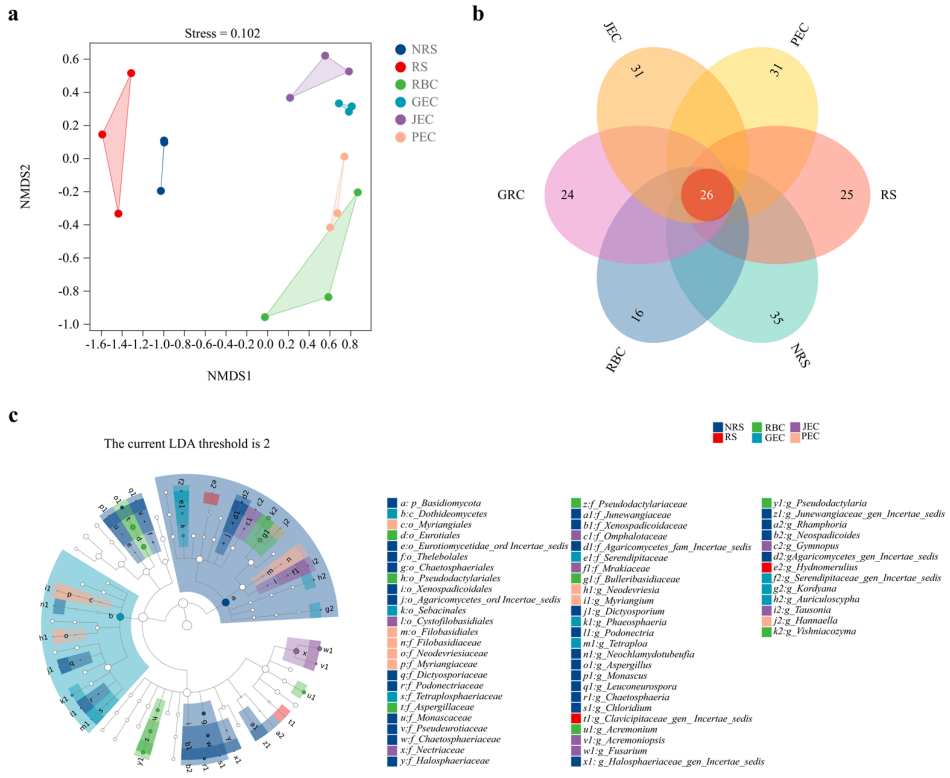

Figure 2. Analysis of fungal community structure, core taxa, and differential taxa of the fungal communities from different cultivation modes and substrates. (a) Results of NMDS analysis using Bray-Curtis distance; (b) Venn diagram showing the number of fungal genera uniquely detected in each sample; (c) Results of LEfSe analysis. The taxonomic cladogram displays the classification hierarchy of major taxa (from phylum to genus, inner to outer circles) in the sample community. Hollow nodes represent taxa with no significant intergroup differences, while nodes of other colors indicate taxa that exhibit significant intergroup differences. Colored regions represent different cultivation modes and substrates. The size of each node is proportional to the abundance of the taxonomic unit. The LDA threshold value is set at 2.

3.4. Functional Analysis of Fungal Communities in Dendrobium Roots and Cultivation Substrates

The FUNGuild database was used to predict the functional guilds within the fungal communities associated with Dendrobium roots. The analysis showed that Dendrobium roots were primarily inhabited by pathotrophic, symbiotrophic, and saprotrophic fungi. Additionally, 12.37%–30.15% of the fungal taxa could not be assigned into specific functional guilds (Figure 4a). Further subdivision of the functional categories revealed that the fungal community in Dendrobium roots was mainly composed of saprotrophs (45.68%), endophytes (36.20%), lichen parasites (31.85%), and plant pathogens (22.64%) (Figure 4b).

The RA values of fungi classified as pathotrophic and symbiotrophic in the epiphytic cultivation samples were 2.98-fold and 4.25-fold higher, respectively, than those in RBC samples ($P<0.05$). The sum of the RA values across different nutritional types exceeded 100% in the epiphytic cultivation samples, which was significantly higher than that in the RBC samples, indicating that the endophytic fungi in Dendrobium roots under epiphytic cultivation exhibited more complex nutritional modes, with many fungal taxa displaying multiple trophic lifestyles.

Three ASVs were annotated as orchid mycorrhizal fungi (OMF): an unclassified fungus belonging to Sebacinaceae, Serendipita vermifera (Serendipitaceae), both within the order Sebacinales, and an unclassified fungus assigned to Meliniomyces (order Helotiales). The two Sebacinales fungi were detected at very low RA (0.00022%) and low detection frequency (8%), whereas the unclassified Meliniomyces was detected in 50% of the samples with an RA of 0.064%.

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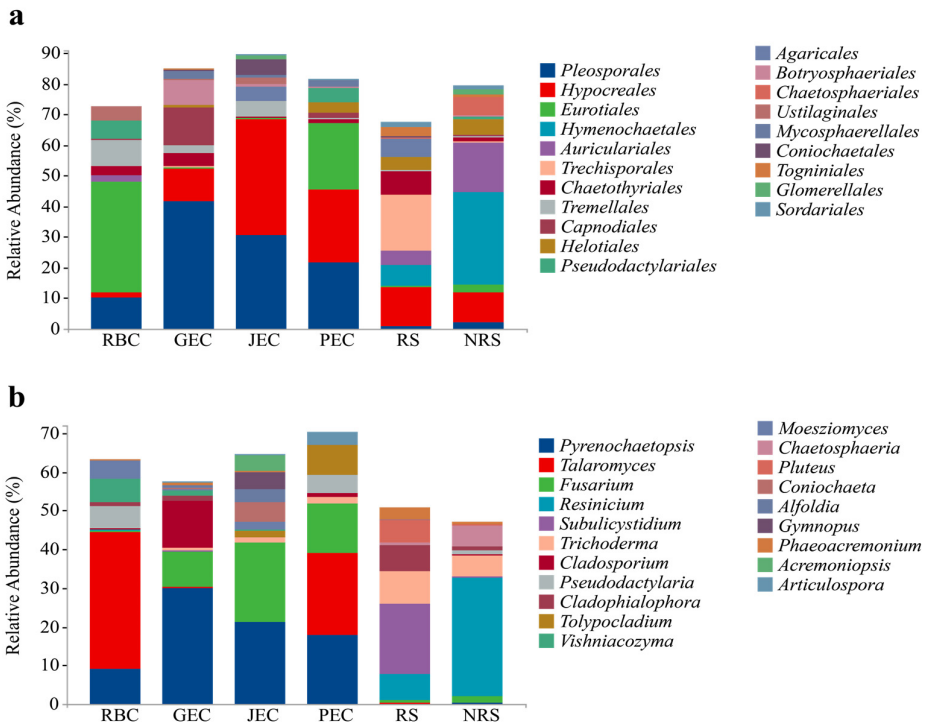

Figure 3. Taxonomic composition of fungal communities in *Dendrobium officinal* roots and cultivation substrates. (a) The top 20 fungal orders; (b) the top 20 fungal genera.

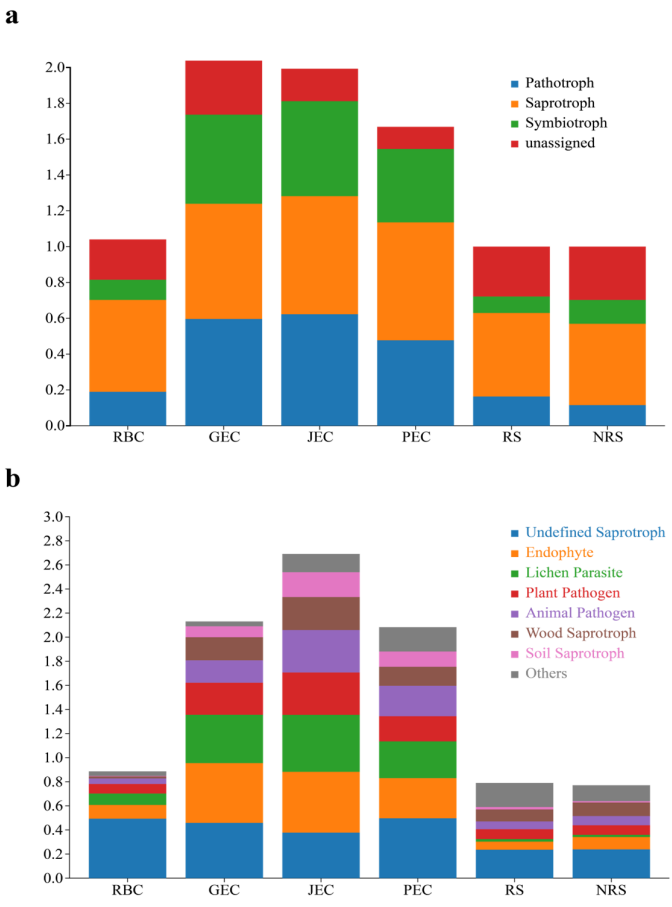

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Figure 4. Nutritional function classification of fungal communities in *Dendrobium officinal* roots from different cultivation modes and substrates. (a) Nutritional type classification; (b) detailed guild classification.

Given that the number of reported OMF taxa has continued to increase, we expanded the search for potential mycorrhizal fungi based on taxa reported in previous studies [26,27]. Three additional groups were identified as putative OMF: Herpotrichiellaceae (including Cladophialophora, Exophiala, and seven other genera; present in all samples, RA 2.48%), Psathyrellaceae (detected almost exclusively in the epiphytic cultivation samples, RA 0.01%), and Russulaceae (detected almost exclusively in the substrate samples, RA 0.08%).

3.5. D. officinale Stem Bioactive Components and Morphological Indicators

Bioactive components and phenotypic traits of *D. officinale* under different cultivation methods were determined as described in the Methods section. In this study, the polysaccharide content in Dendrobium stems was significantly higher in the samples from the RBC cultivation than in those from the EC modes ($P < 0.01$). The ethanol-soluble extractives (ESE) content, chlorophyll content, and stem length were also significantly higher in the samples from RBC compared with those from EC modes (Table 2). Among the three EC samples, the samples from GEC showed higher values of ESE content, stem length, and stem diameter, whereas samples from PEC exhibited higher polysaccharide and chlorophyll contents.

Table 2. Bioactive components and phenotypic characteristics of *Dendrobium officinale* under different cultivation methods.

Cultivation methods	Polysaccharide /mg/g(DW)	Ethanol-soluble extract/%（DW)	Chlorophyll /SPAD	Stem Length/cm	Stem Diameter/mm
RBC	194.2±20.5^{A}	3.74±0.67^{a}	61.1±4.2^{A}	21.3±2.2^{A}	5.0±0.0^{a}
GEC	98.7±22.1^{B}	2.57±1.18^{ab}	36.0±4.0^{C}	19.7±3.0^{A}	5.3±0.6^{a}
JEC	99.4±12.9^{B}	2.15±0.60^{b}	36.2±4.1^{C}	13.2±1.9^{B}	4.7±0.6^{a}
PEC	119.0±15.5^{B}	1.71±0.35^{b}	42.5±3.4^{B}	11.7±1.5^{B}	4.7±0.6^{a}

Notes: The samples include 4 *Dendrobium officinale* roots and 2 cultivation substances. Statistical significance of differences was determined by the t-test. Capital letters denote extremely significant differences ($p < 0.01$), whereas lowercase letters denote significant differences ($p < 0.05$).

3.6. Relationship Between Endophytic Fungi and Stem Characteristics

To explore the relationship between the abundance of fungal genera and the ecophysiological index of Dendrobium, RDA analysis was performed using the top 50 genera. Factors including stem length, stem diameter, chlorophyll content, stem polysaccharide content, ESE content, and photosynthetic photon flux density (PPFD) were included in the analysis. The RDA results showed that the first two axes explained 51.22% of the total variance in the fungal community structure. Among these factors, chlorophyll content ($R2 = 0.91$, $P< 0.001$) was the most significant variable influencing the microbial community, followed by stem length ($R2 = 0.59$, $P<0.01$) and polysaccharide content ($R2 = 0.59$, $P<0.01$). Several genera were positively correlated with stem polysaccharide content, including Meira, Aspergillus, Vishniacozyma, Acremonium, Moesziomyces, Talaromyces, Exophiala, Pseudodactylaria, and Fellomyces. In contrast, genera positively correlated with light intensity (PPFD) included Phaeoacremonium, Pyrenochaetopsis, Vexillomyces, and Auriculoscypha (Figure 5).

The main bioactive components in *D. officinale* were located in the stem. The stem diameter, internode length, and bioactive component content varied among different cultivation modes (Table

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2). Correlation analysis between stem characteristics and FEC showed that Moesziomyces and Rhinocladiella were positively correlated with stem diameter, whereas Meria and Acremonium were positively correlated with internode length. Exophiala and Talaromyces were significantly and positively correlated with the stem polysaccharide, while Pseudodactylaria and Fellomyces were highly significantly and positively correlated with the stem polysaccharide ($p < 0.01$) (Figure S1).

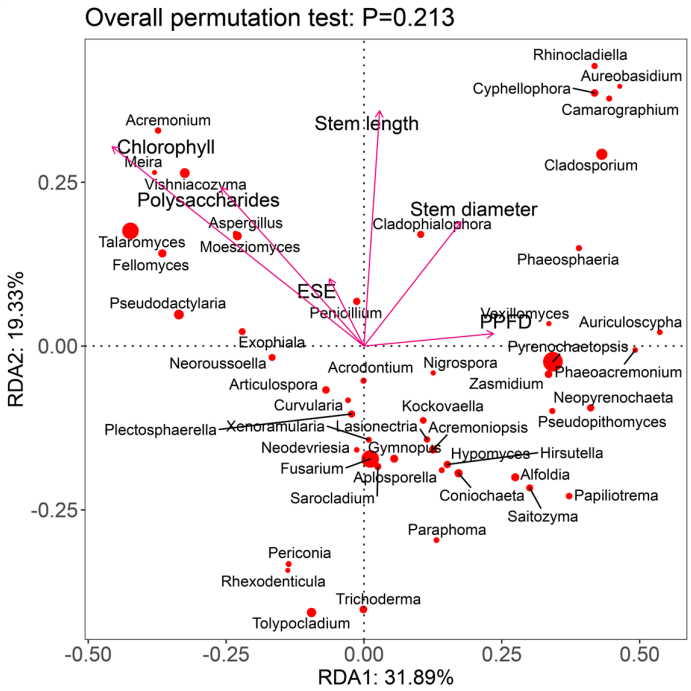

Figure 5. RDA analysis of endophytic fungi at the genus level in Dendrobium under different cultivation methods. The top 50 most abundant genera are shown with red solid circles, and the size of each circle indicates the relative abundance of that genus. The red arrows represent photosynthetic photon flux density (PPFD), stem length, stem thickness, leaf chlorophyll content, stem polysaccharide content, and alcohol-soluble extractives content (ESE) respectively. The analysis was performed using Wekemo Bioncloud (www.bioincloud.tech).

4. Discussion

4.1. FEC Structure in D. officinale Roots Under Different Cultivation Modes

Fungal community diversity was found to vary among cultivation modes. Fungal richness and diversity under the three epiphytic cultivation modes were higher than those under the RBC mode (Table 1). This pattern is consistent with the ecological characteristics of epiphytic orchids, which rely heavily on mycorrhizal fungi throughout their life cycle in acquiring water and nutrients under limiting conditions. Similarly, Leroy (2021) reported higher endophytic fungal diversity in the roots of epiphytic bromeliads than in those of terrestrial and lithophytic bromeliads [28]. The diversity values observed under RBC conditions (Chao1 113, Shannon index 3.3) were comparable to those reported for *D. officinale* cultivated in bark-based substrate across multiple regions (Chao1 114–185, Shannon index 2.5–3.2) [22,29,30]. Beta (β)-diversity analysis further showed that fungal communities in *D. officinale* roots under epiphytic cultivation were clearly separated from those under RBC conditions (Figure 2a), consistent with previous findings that orchid-associated fungal communities vary with environmental conditions [8,22,31].

Among the shared taxa, 34 fungal genera were identified across cultivation modes. Of these, six genera have so far been reported only in soil and freshwater environments, whereas the remaining 28 have been widely documented in orchid-associated microbiomes [32–35]. Notably, genera such as

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Cladosporium, Resinicium, and Fusarium are considered part of the core plant microbiome [28,29,33,36,37]. The combined mean RA of these shared genera reached 72.47%, with no significant differences among cultivation modes, suggesting strong adaptability to the endophytic niche and a potentially important role in host adaptation.

Several biomarkers identified by LEfSe, including Aspergillus and members of Nectriaceae, have also been reported as dominant endophytes in other orchids. For example, Aspergillus dominates in tropical terrestrial orchids (20–30%) and in Calanthe (10.9%) [38,39], while Nectriaceae is prevalent in *D. officinale* and Neuwiedia singapureana [29,35]. These patterns suggest that cultivated Dendrobium may preferentially recruit fungal taxa that are broadly adapted to orchid hosts.

4.2. FEC Composition in D. officinale Roots from Different Cultivation Modes

At the phylum level, fungal endophytic communities in cultivated *D. officinale* roots were dominated by Ascomycota and Basidiomycota, consistent with previous studies [23,29]. However, the RA ratio of Ascomycota to Basidiomycota was markedly higher in the epiphytically cultivated samples (85%/7%) than in the RBC samples (58%/17%) and previously reported values (46%/28%) [29]. This pattern may reflect environmental differences, as Ascomycota are often associated with stress-tolerant strategies, whereas Basidiomycota are typically linked to nutrient-rich conditions [40].

At the genus level, dominant taxa included Pyrenochaetopsis (RA 19%, the most common genus in epiphytic cultivation), Fusarium (RA 14%), Talaromyces (RA 12%, the most common genus in the RBC mode), Cladosporium (RA 4%), along with less abundant genera such as Vishniacozyma (RA 2%) and Pseudodactylaria (RA 2%) (Figure 3b). Many of these taxa, including Fusarium, Cladosporium, and members of Aspergillaceae, are commonly reported as dominant orchid endophytes [29,33,41]. In contrast, the ecological roles of other genera remain less well understood, although they have been identified as endophytes in other plant species [37,42,43].

4.3. Functional Groups of Endophytic Fungi in D. officinale Roots from Different Cultivation Modes

Tulasnellaceae is widely recognized as the most prevalent OMF in wild *D. officinale*, accounting for up to 82.98%–95.2% of OMF sequences [7,44]. However, this group was not detected in the present study. Recent studies suggest that Tulasnellaceae requires organic nitrogen for symbiosis, and mineral nitrogen conditions may disrupt this association [45,46]. Similar patterns have been observed in cultivated orchid Cypripedium and other ericoid plant [45,47]. In addition to Tulasnellaceae, other common mycorrhizal taxa such as Ceratobasidiaceae and Serendipita. indica (which also relies on organic nitrogen), were also absent. These results suggest that cultivation practices may alter fungal recruitment and disrupt typical mycorrhizal associations.

Although classical “rhizoctonia-like” fungi were largely absent, several non-rhizonctonia fungal taxa with potential mycorrhizal functions were detected. These fungi are typically saprobic or ectomycorrhizal and may contribute to nutrient transfer under certain conditions [48,49]. In this study, these taxa were present at low relative abundance but may represent newly recruited fungal partners from the surrounding environment.

Non-mycorrhizal fungi (ONF) accounted for the majority of the fungal endophytic community (~97%), consistent with previous reports [33,35,50]. These fungi occupy ecological niches within plant roots without forming classical mycorrhizal structures and may contribute to stress tolerance and nutrient acquisition [51]. The dominance of orders such as Pleosporales and Hypocreales is consistent with studies on grasses and non-mycorrhizal plant systems [52,53]. Seasonal and environmental factors, such as temperature and drought, may further influence their prevalence [54].

Overall, in cultivated *D. officinale*, classical OMF were largely absent, whereas diverse ONF and potential alternative mycorrhizal fungi were detected. This flexible recruitment strategy may allow orchids to adapt to varying environmental conditions and maintain functional resilience under cultivation.

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4.4. Comparative Analysis of Main Components of Dendrobium from Different Cultivation Modes

Polysaccharides and ethanol-soluble extractives (ESE) are major bioactive components of medicinal *D. officinale*. In this study, both polysaccharide and ESE contents were significantly higher in the RBC samples than in the epiphytic cultivation samples ($P < 0.01$) (Table 2), consistent with previous findings [57]. These results suggest that controlled greenhouse conditions may promote the accumulation of bioactive compounds in *D. officinale* (Table2). Such differences may be attributed to environmental factors. RBC systems provide relatively stable conditions, including optimized temperature, humidity, light, and nutrient availability, which are known to influence metabolite accumulation [58]. However, some studies report higher polysaccharide content under more stressful conditions, such as rock epiphytic environments [59], indicating that metabolite accumulation is influenced by complex interactions between environmental stress and phycological regulation.

4.5. Correlation Between Dendrobium Polysaccharides and Endophytic Fungi

Polysaccharides are key bioactive compounds responsible for the antioxidant, anti-tumor, and immune-boosting properties of *D. officinale* [11,60]. In this study, RDA analysis showed a significant correlation between stem polysaccharide content and fungal community composition (R^2= 0.58, p= 0.004) (Figure 5), consistent with previous correlation studies [22,29] and co-culture experiments. Previous studies show that mycorrhizal fungi such as Mycena spp., Tulasnella spp., and other endophytes can enhance polysaccharide accumulation in Dendrobium [61,62]. In the present study, several fungal taxa, including Exophiala, were positively correlated with polysaccharide content (Figure S1). Exophiala has been identified as both an orchid-associated fungus and a dark septate endophyte (DSE), which is known to enhance plant nutrient uptake and stress tolerance [63,64].

In addition, several non-mycorrhizal fungi, including Talaromyces, Pseudodactylaria, and Fellomyces, showed significant positive correlations with polysaccharide content in Dendrobium stems (Figure S1). Talaromyces is widely distributed in plant roots and is known to facilitate phosphorus solubilization and plant growth [65,66], and its metabolites have been shown to stimulate bioactive compound accumulation in Dendrobium [67]. The ecological roles of Pseudodactylaria and Fellomyce remain less well characterized, although they have been reported in other plant-associated (e.g., ferns and lichens) systems [68,69].

4.6. Futural Research and Application in Practice

Dendrobium officinale is a highly valuable medicinal herb with multiple health-promoting functions. This study focused on the abundance and taxonomic classification of fungi interacting with the roots of *D. officinale* under four cultivation modes. The following aspects of mycorrhizal interactions with *D. officinale* should be considered in future studies. First, the nutrient metabolism of *D. officinale* in association with specific fungal populations under different cultivation modes should be investigated. Second, the precise correlation among the accumulation of bioactive compounds (e.g., polysaccharides, dendrobine, and flavonoids), specific fungal populations associated with *D. officinale*, and the cultivation modes, should be determined. Third, more suitable host tree species should be identified for wild-simulated cultivation of *D. officinale*, as the commercial and medicinal value of *D. officinale* from wild-simulated cultivation is significantly higher than that from greenhouse cultivation. Fourth, the symbiotic fungal banks associated with *D. officinale* should be established for potential future applications [70]. Finally, eco-friendly fungal fertilizers should be developed and applied for the cultivation management of *D. officinale* and other Orchidaceae plants based on the findings of this study.

5. Conclusions

This study revealed significant differences in the abundance, diversity, and community structure of endophytic fungi in *Dendrobium officinale* roots across four cultivation modes. The greenhouse cultivation mode (RBC) promoted plant growth and enhanced the accumulation of key bioactive

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compounds, including stem polysaccharides and ethanol-soluble extractives (ESE). These bioactive components, together with selected plant phenotypic traits, were positively associated with variations in microbial community structure. The fungal endophytic community in *D. officinale* roots was dominated by non-mycorrhizal fungi (ONF, ~97%), whereas only a small proportion (~3%) was attributed to orchid mycorrhizal fungi (OMF), represented by six taxa. These findings highlight the potential importance of recruiting locally adapted fungal partners and establishing novel root-fugus associations to enhance the adaptability of Dendrobium to agricultural ecosystems, particularly when classic OMF are absent under artificial breeding and cultivation conditions.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org. Figure S1: Correlation analysis between the composition and abundance of top 50 endophytic fungi and OMF and the morphological traits, light density, and compound contents of *Dendrobium officinale*; Table S1: Thirty-four fungal genera shared in *Dendrobium* roots across four different cultivation modes; Table S2: The Top 10 fungal genera found in *Dendrobium* roots and substrates.

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Abbreviations

The following abbreviations are used in this manuscript:

RBC	Raised bed cultivation
GEC	Ginkgo tree-epiphytic cultivation
JEC	Jujube tree-epiphytic cultivation
PEC	Pear tree-epiphytic cultivation
RS	Rhizosphere substrate
NRS	Non-rhizosphere substrate
ESE	Ethanol-soluble crude extractives
OMF	Orchid mycorrhizal fungi
ASV	Amplicon sequence variant
FEC	Fungal endophytic community
LEfSe	Linear discriminant analysis effect size
RDA	Redundancy Analysis
RA	Relative abundance

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