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ORIGINAL ARTICLE

Isolation of endophytes from *Dioscorea nipponica* Makino for stimulating diosgenin production and plant growth

Shangni Dang[1] · Jiang Geng[2] · Ran Wang[1] · Yumei Feng[1] · Youzhi Han[1] · Runmei Gao[1]

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Abstract

Key message **Both bacterial and fungal endophytes exhibited one or more plant growth-promoting (PGP) traits. Among these strains, the *Paenibacillus peoriae* SYbr421 strain demonstrated the greatest activity in the direct biotransformation of tuber powder from *D. nipponica* into diosgenin.**

Abstract Endophytes play crucial roles in shaping active metabolites within plants, significantly influencing both the quality and yield of host plants. *Dioscorea nipponica* Makino accumulates abundant steroidal saponins, which can be hydrolyzed to produce diosgenin. However, our understanding of the associated endophytes and their contributions to plant growth and diosgenin production is limited. The present study aimed to assess the PGP ability and potential of diosgenin biotransformation by endophytes isolates associated with *D. nipponica* for the efficient improvement of plant growth and development of a clean and effective approach for producing the valuable drug diosgenin. Eighteen bacterial endophytes were classified into six genera through sequencing and phylogenetic analysis of the 16S rDNA gene. Similarly, 12 fungal endophytes were categorized into 5 genera based on sequencing and phylogenetic analysis of the ITS rDNA gene. Pure culture experiments revealed that 30 isolated endophytic strains exhibited one or more PGP traits, such as nitrogen fixation, phosphate solubilization, siderophore synthesis, and IAA production. One strain of endophytic bacteria, *P. peoriae* SYbr421, effectively directly biotransformed the saponin components in *D. nipponica*. Moreover, a high yield of diosgenin (3.50%) was obtained at an inoculum size of 4% after 6 days of fermentation. Thus, SYbr421 could be used for a cleaner and more eco-friendly diosgenin production process. In addition, based on the assessment of growth-promoting isolates and seed germination results, the strains SYbr421, SYfr1321, and SYfl221 were selected for greenhouse experiments. The results revealed that the inoculation of these promising isolates significantly increased the plant height and fresh weight of the leaves and roots compared to the control plants. These findings underscore the importance of preparing PGP bioinoculants from selected isolates as an additional option for sustainable diosgenin production.

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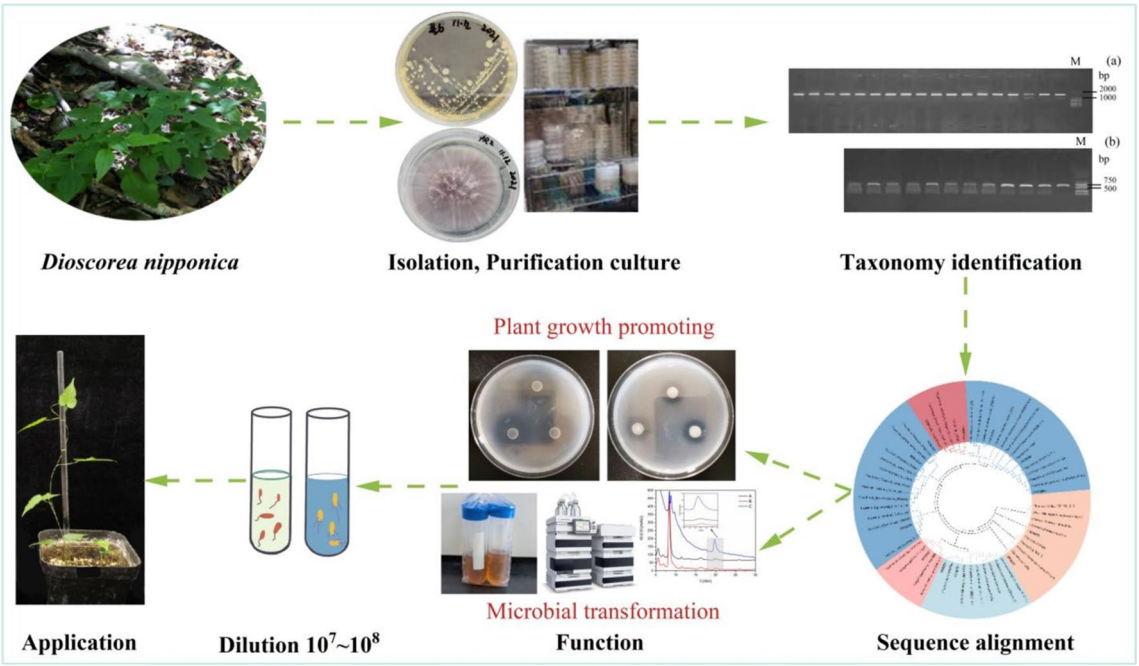
✉ Youzhi Han
hanyouzhi@sxau.edu.cn

✉ Runmei Gao
sxndgrm@163.com

[1] College of Forestry, Shanxi Agricultural University, Taigu, Shanxi, China

[2] Shanxi Province Cancer Hospital/Shanxi Hospital Affiliated to Cancer Hospital, Chinese Academy of Medical Sciences/Cancer Hospital Affiliated to Shanxi Medical University, Taiyuan, Shanxi, China

Graphical abstract



Keywords *Dioscorea nipponica* Makino · Endophytes · Diosgenin · Biotransformation · Plant growth promotion

Introduction

Dioscorea nipponica Makino, a pharmaceutical monocotyledon from the Dioscoreaceae family, is well known for its high content of diosgenin in the rhizome. Diosgenin (25*R*-spirost-5-en-3β-ol) primarily exists within plant tissues as a steroidal saponin, where glucose or rhamnose attaches to the aglycone through C–O glycosidic bonds at C-3 and C-26 (Zhu et al. 2010). Diosgenin serves as a precursor to dioscin (Li et al. 2021), which necessitates the addition of one glucose and two rhamnose groups at the C-3 position through UDP-glucosyltransferase and UDP-rhamnosyltransferase (Zhu et al. 2010). Consequently, dioscin can be cleaved by acid into sugar moieties and diosgenin (Xiang et al. 2018; Qi et al. 2009). Diosgenin holds significant pharmacological potential as a precursor to numerous steroid hormone drugs, offering diverse medicinal benefits, including anti-atherosclerosis, immune regulation, blood lipid reduction, antitumor, and anti-inflammatory properties (Xiang et al. 2018; Moalic et al. 2001; Higdon et al. 2001; AlHabori et al. 2001). The escalating demand for diosgenin due to the growth of the steroid hormone drug synthesis industry has led to the overexploitation of natural resources, resulting in significant germplasm degradation within the species (Chen et al. 2007). Moreover, diosgenin extraction generates wastewater and acids, posing substantial environmental pollution concerns and impeding the development of the *D. nipponica* processing industry (Xu et al. 2010; Pan et al. 2014; Yang et al. 2016; Hu et al. 2021). Therefore, there is an urgent need for sustainable and environmentally friendly methods for the production of diosgenin. Microorganisms offer a promising alternative, representing a green and easily industrialized approach to augment crop yield and enhance the production of secondary metabolites from various medicinal (Shukla et al. 2022; Kumari et al. 2023).

Endophytes (bacteria as well as fungi) are an endosymbiotic group of microorganisms that are ubiquitous in nature and are known to dwell inside the plant endosphere without causing any noticeable deleterious effects or triggering an immune response in the host (Hazarika et al. 2021; Compant et al. 2021). Studies indicate that endophytes play pivotal roles in supporting host plants by facilitating nutrient acquisition, promoting growth, mitigating pathogenic microorganisms, activating defense systems, and enhancing the production of secondary metabolites (Aeron et al. 2020). Owing to their gene exchange with plant cells, endophytic microbes yield therapeutically significant bioactive compounds that mirror those found in the host plant. Compounds such as Taxol, camptothecin, vincristine, vinblastine, huperzine, cinchonine, and podophyllotoxin are among the valuable bioactive molecules synthesized by these endophytes (Stierle et al. 1993; Gouda et al. 2016; Zhao et al. 2011).

In 1993, paclitaxel-producing endophytic fungi were isolated from the Pacific yew, *Taxus brevifolia*, sparked a significant surge of research focused on medicinal plant-derived endophytic fungi. In particular, given the current scenario of resource scarcity and environmental degradation, endophytes have emerged as promising alternative resource for medicinal plants. Leveraging the unique biocatalytic capabilities of endophytes, active compounds sourced from plants can be transformed into high-quality, pure natural drugs in an environmentally friendly manner (Guo et al. 2019). This approach not only diminishes the biological toxicity of natural products and enhances human absorption and utilization but also surmounts the technical barriers associated with chemically synthesized drugs. In addition, these findings can aid in better understanding the structural–conformational relationship between drug structures and pharmacodynamic activity, thereby offering new avenues for drug development. The literature on endophytes associated with *D. nipponica* is limited. Recent studies have focused primarily on generating bioactive compounds akin to host secondary metabolites. For instance, *Fusarium* sp. C39, an endophytic fungus, effectively biotransformed saponin components in *D. nipponica* (Huang et al. 2022). In addition, *Aspergillus oryzae* DLFCC-38 exhibited enzymatic catalytic ability to transform steroidal saponins into progenin III (Liu et al. 2013). Past research has predominantly concentrated on isolating endophytic fungi from Dioscoreaceae root tubers, with minimal attention given to other tissues and endophytic bacteria (Huang et al. 2022; Du et al. 2017). Studies on *Dioscorea zingiberensis* root tubers revealed a diverse array of endophytic fungi. These fungi yielded various metabolites, such as oligosaccharides from *Fusarium oxysporum* Dzf17 and polysaccharides/oligosaccharides from *Berkleasmium* sp. Dzf12. These metabolites exhibited enhancing effects on diosgenin production in cell and seedling cultures (Li et al. 2011; Özçakmak et al. 2012). Research on endophytic fungi associated with *Dioscorea bulbifera* L. highlighted their promising antagonistic properties, plant growth promotion, and extracellular enzymatic potential (Sharma et al. 2023). In light of the limited available information regarding the interaction between endophytes and *D. nipponica*, this study investigated the prevalent endophyte community in *D. nipponica.* This investigation employed molecular and phylogenetic approaches to identify endophytic strains, screened functional strains, and searched for endophytic strains with potential applications in plant growth and beneficial metabolite synthesis. This study contributes to establishing a theoretical foundation for leveraging endophytes to address reproductive issues, and enhance yield, and serve as a reference for further exploration of endophytes in *D. nipponica*.

Materials and methods

Isolation and molecular characterization of endophytes

Purification of endophytes

Fresh *D. nipponica* tissues were collected from the Zhong-Tiao Mountains, Shanxi Province, China. The plant samples (separated into stems, leaves, and seeds) were surface sterilized under aseptic conditions using the methods of Ouyabe et al. (2020) and Dang et al. (2022). The surface sterilization process for the root tubers was the same as that for the stems, leaves, and seeds, but the treatment time doubled (Xiang et al. 2018). The isolation of endophytic bacteria and fungi was performed by dilution smear and plate scribing methods on nutrient agar (NA) and potato dextrose agar (PDA) media. To verify the efficacy of surface sterilization of the samples, inoculation in the medium of water from the last rinse was used to confirm superficial disinfection (Laczeski et al. 2020). The plates were incubated at 26–28 °C for 3–14 days.

All potentially different colonies were transferred to separate plates with a sterile needle for further purification. Bacteria were cultured on NA plates, and mycelia from the fungal cultures were placed on PDA plates. All colonies were streaked for consecutive generations until no different traits appeared. Then under a microscope, all the uncontaminated colonies were preliminarily divided into bacteria and fungi based on colony morphology (Zou et al. 2021). Furthermore, fungal isolates were classified according to the phenotype of the colonies on the plates (including colony size, color, texture, shape, surface, margin, and medium color). Similarly, the bacterial cultures were classified based on shape, color, and texture. Individual colonies of these organisms were picked to preserve on their respective agar slants in 40% glycerol at − 20 °C for further study (Vinayarani and Prakash 2018; Xu et al. 2010; Jain et al. 2020; Shukla et al. 2022; Gupta et al. 2022).

Genomic DNA extraction and phylogenetic characterization

The first step was the isolation of DNA from the culture. Therefore, the quality of the samples was assessed on a 1% agarose gel, and a band of high-molecular-weight DNA (representing a single clone of the endophyte selected for the studies) was observed according to standard protocols (Pandey et al. 2017).

Genomic DNA was extracted using a bacterial genomic DNA extraction kit (Beijing Genesand Biotech, China;

Cat no: DE703-50) according to the manufacturer's protocol. A fragment of the 16S rDNA gene was amplified by PCR from the isolated DNA. Fungal DNA was extracted using a modified CTAB method. Molecular identification was performed by ITS rDNA sequence analysis (Zhang et al. 2013). The PCR products were subsequently sequenced using two primers by Sangon Biotech (Beijing, China). The primers and PCR conditions used are shown in Supplementary Table S1. The PCR amplicon bands of approximately 1500 bp and 750 bp were observed when resolved on agarose gels (Fig. S1). Phylogenetic tree and molecular evolutionary analyses were also performed using the maximum likelihood method of neighbor joining in MEGA 7.0 (Xiang et al. 2018).

Biological functions of endophytes

Biochemical assays for PGP traits

The PGP ability of the 30 representative strains was evaluated by assessing their nitrogenase activity, IAA production, siderophore production, and inorganic phosphate solubilization. The PGP activities of each bacterial and fungal endophytic isolate were screened using standardized procedures and selective growth media. Bacteria were cultured in 8 mL of NB, while fungi were cultured in 8 mL of PDB overnight in a shaking incubator set at 180 rpm and 28 °C. Unless otherwise specified, 10 µl of culture medium was used for all the assays. All the experiments were performed in triplicate.

Phosphate solubilization activity

Phosphate solubilization screening of the endophyte isolates was conducted following the procedure outlined by Qin et al. (2015) using NBRIP medium. After inoculation, the plates were incubated at 28 °C for 5 days. As a result of phosphate solubilization, a clear zone appeared around the bacterial and fungal colonies, which confirmed the phosphate solubilization capacity of the cultures (Shukla et al. 2022).

Nitrogen fixation activity

The nitrogen fixation capacity of the endophytic isolates was tested using nitrogen-free medium, which allows the growth of microorganisms that utilize only atmospheric nitrogen as their sole source of nitrogen (Laczeski et al. 2020; Hazarika et al. 2021; Shukla et al. 2022; Cueva-Yesquén et al. 2021). The isolates were inoculated into Ashby medium and incubated aerobically at 28 °C for 5 days. The strains exhibiting nitrogen fixation ability displayed a transparent halo surrounding their colonies on Ashby agar medium, indicating a positive result (Andriiuk. 1967) (Supplementary Fig. S6A).

Siderophore synthesis

A qualitative siderophore production assay was conducted for each strain on plates using MSA-CAS medium, as per the methods outlined by Liu et al. (2022). This medium comprises MSA medium, CAS dyeing solution, and phosphate buffer. Following incubation at 28 °C for 2 days, a positive signal was observed in the liquid medium, signified by its color change from blue to red, orange, or purple. The quantitative analysis of siderophore production was conducted following the protocol outlined by Wang et al. (2022). Siderophores produced by the isolates were assessed by calculating the A/Ar values, where Ar represents the absorbance of the reference and A represents the absorbance of the sample at 630 nm.

IAA production capability

Following cultivation, 20 ml of fermentation broth was subjected to centrifugation at 8000 *rpm* and 4 °C for 10 min to obtain a precipitate. The resulting supernatant was carefully transferred into a sterile triangular flask. The pH of the solution was adjusted to 2.0 with 2 M HC1. The mixture was then subjected to extraction using equal volumes of ethyl acetate, which was repeated three times. Following each extraction, the mixture was centrifuged at 8000 *rpm* and 4 °C for 10 min, after which the organic phase was obtained and subsequently concentrated using a rotary evaporator at 37 °C. The concentrated extract was dissolved in 1 ml of methanol, filtered through a 0.22 µm filter membrane, and stored in a − 20 °C refrigerator as a backup. Quantitative analysis of the methanol-dissolved samples was performed using HPLC.

IAA production was determined via HPLC. Initially, 2000 µg of IAA was dissolved in methanol to afford a 2 mL solution, which was subsequently diluted to concentrations of 1000, 500, 250, 125, and 100 $\mu g \cdot mL^{-1}$, respectively. The injection volume for each gradient was 20 µL, and this injection was repeated three times. The peak area–mass concentration standard curve was plotted according to the above chromatographic conditions (Supplementary Fig. S2A).

Screening isolates for diosgenin production

0.5 mL of suspension were inoculated to 20 mL PDB medium and 20 mL NB medium both containing a concentration of 1% tuber powder (fresh tubers were dried in an oven at 80 °C until constant weight, then pulverized and passed through an 80-mesh sieve) isolated from

D. nipponica. The ingredients were mixed thoroughly and sterilized at 121 °C for 30 min. The media were placed in 100 mL Erlenmeyer flasks and incubated at 28 °C on a rotary shaker (160 rpm) for 5 days. The fermentation broths were subsequently extracted three times with 10 mL of petroleum ether. The petroleum ether layer was collected by centrifugation and concentrated under reduced pressure. The products were characterized by thin-layer chromatography (TLC) using petroleum ether/ethyl acetate (7:3, v/v) and concentrated sulfuric acid/ethanol (1:9, v/v) as developing solvents and chromogenic agents.

Determination of diosgenin content

A diosgenin standard curve was drawn by high-performance liquid chromatography (HPLC). A total of 2000 µg diosgenin was dissolved in methanol to 2 mL and then diluted to 500, 250, 125, 100, 50, and 25 $\mu g \cdot mL^{-1}$, respectively. The injection volume for every gradient was 20 µL, and the injection was repeated three times. The peak area–mass concentration standard curve was plotted according to the above chromatographic conditions (Supplementary Fig. S2B). The calibration curve of diosgenin was obtained as follows:

$$Y = 12402.46x + 13.07$$

$$R^2 = 0.99524.$$

The diosgenin yield was calculated with the following equation (Xiang et al. 2018):

$$\text{Diosgenin yield (\%)} = \text{Diosgenin content (g)/tube powder (g)} \times 100\%.$$

The mass spectrometry (MS) conditions used were as follows: API3000 triple quadrupole mass spectrometer, electrospray ionization positive ion source (ESI^+), capillary voltage of 3 kV, multiple reaction monitoring mode (MRM), cone hole voltage of 30 V, and scan range of 100–600.

Optimization of fermentation conditions

Optimal fermentation conditions are the key to successfully obtaining high-yield fermentation products. To maximize the conversion rate of diosgenin, single-factor experiments were performed to optimize the fermentation time, inoculum volume, extraction solution, and substrate concentration. For instance, 0.5 mL of suspension was inoculated into 20 mL of NB medium containing a concentration of 1% tuber powder. The media were placed in 100 mL Erlenmeyer flasks and incubated for 5 days at 28 °C on a rotary shaker (160 rpm). The fermentation broth was then extracted three times with 10 mL of petroleum ether. The petroleum ether layer was collected by centrifugation and concentrated under reduced pressure. The crude extract of the screening strain was obtained, and the crude extract was fixed to 1 ml with chromatographically pure methanol and filtered through a 0.22 µm pore size microporous membrane to obtain a liquid fermentation sample, which was set aside. The response area of diosgenin was subsequently determined using liquid chromatography. Unless otherwise stated, twenty microliters of crude extract of the strain product was used in the HPLC assays.

The optimum extraction solution was screened based on the medium. The mixture was centrifuged to remove the precipitate, and the supernatant was isolated for further investigation. In addition, the combination of precipitate and supernatant was investigated. Similarly, the media were placed in 100 mL Erlenmeyer flasks and incubated at 28 °C for 2, 4, 6, or 8 days on a rotary shaker (160 rpm). The optimal fermentation time was determined by HPLC. Subsequently, the optimum inoculum volume, based on the medium, was investigated for strains suspended at 1%, 2%, 3%, and 4%. In addition, the effect of substrate concentration on the yield of diosgenin was investigated. The substrate concentrations were screened as 1%, 2%, 3%, 4%, or 5% after which the samples were sterilized. The optimal substrate concentrations were determined by HPLC.

In vitro and pot screening of isolates for growth promotion

In vitro and pot experiments were conducted in triplicate to elucidate the effects of endophytes on the physiological and morphological performance of *D. nipponica*. Since *D. nipponica* is propagated mainly by rhizome division and by seedlings, we selected these two materials for in vitro experiments (Yu et al. 2018). On the basis of the results of biochemical assays—phosphate solubilization, a nitrogen fixation halo zone diameter, a siderophore concentration, and an IAA production capability, five isolates exhibiting multiple PGP traits were selected. These strains were further assessed for their ability to promote the growth and germination of *D. nipponica* plants. The isolates were activated on solid plates and then were prepared by growing on PDB and NB liquid media at 28 °C for 24 h with shaking (120 rpm). The pellets were diluted to 1.0 OD_{600} to obtain the endophytic fermentation broth for backup. The amount of inoculum ($OD_{600=}$1.0) was 2% (v/v) in all the experiments unless otherwise stated (Liu et al. 2022; Laczeski et al. 2020).

In vitro culture experiment

The seeds and rhizomes of *D. nipponica* were surface sterilized using the method of Dang et al. The MS agar media for seed and rhizome growth contained 2.0 mg·L^{-1}6-BA + 0.2 mg·L^{-1} NAA, and 10 µL of fermentation solution was added to the other half of the Petri dish, while 10 µL of sterile water was added to the control for a total of 7 days of horizontal incubation.

Seed germination

The surface-sterilized seeds were immersed in endophyte fermentation broth for 24 h. Thirty seeds inoculated with each endophytic isolate were spread on two layers of moistened filter paper on Petri plates. Thirty surface-sterilized seeds treated with sterilized hypochlorite (2%) for the control treatment were also established (Cueva-Yesquén et al. 2021). Inoculated and control plates were incubated in a constant temperature incubator at 28 °C for 30 days. One milliliter of sterilized distilled water was added daily to ensure sufficient moisture for germination. After a 30-day period, plumule and radicle length, germination rate, and fresh weight were measured to evaluate and determine the growth-promoting characteristics of the plants.

Pot screening of isolates

In pot experiments, surface-sterilized seeds were inoculated into the selected isolates as described above. The following treatments were used for the inoculated single-strain and non-inoculated strain controls: (1) non-inoculated (CK), (2) inoculated with strain SYbr421, (3) inoculated with strain SYfl221, and (4) inoculated with strain SYfr1321. The bacterial (fungal spore) suspension was irrigated in potted soil every month (sterile water instead of suspension for the control). Seedlings were grown under controlled laboratory conditions with a growth chamber temperature of 27 °C and a photoperiod of 10 h (Laczeski et al. 2020). Plant samples were collected after 60 days of sowing. The following vegetative growth parameters were recorded after a separate root and shoot system was established: fresh weight of the leaf and tubers, plant height, number of leaves per plant, length of the leaf and tubers, and tuber volume. The morphological indices were measured manually at harvest, whereas the leaf area and tuber indices were determined by ImageJ and WinRHIZO software.

Statistical analysis

All the data are expressed as the mean values of the treatments. Differences between treatments were determined using analysis of variance (ANOVA). All the statistical analyses were performed using SPSS 22.0 software (SPSS, Chicago, IL, USA), and post hoc tests were performed using Duncan's multiple range test. P values < 0.05 were considered to indicate statistical significance. Interrelationships between treatments and vegetative parameters were assessed using principal component analysis (PCA) in the origin of 2022.

Results

Isolation and identification of endophytes

The isolated bacterial and fungal strains were confirmed to be endophytes, as no bacterial or fungal colonies were observed on the control plates. A total of 93 endophytes were isolated from the root tubers, stems, leaves, and seeds of *D. nipponica* seedlings. Culturable endophytes were most abundant in the root tubers (32 isolates), followed by the seeds (26 isolates), leaves (21 isolates), and stems (14 isolates). Subsequently, all the isolated strains were purified, subcultured, and stored for further in vitro testing. These isolates were grouped into 18 clusters of bacterial strains and 12 clusters of fungal strains based on SDS-PAGE analysis of whole-cell proteins and morphological characteristics.

From each cluster, one strain was selected as a representative, resulting in 30 representative strains used to construct a phylogenetic tree (Table S2). The results showed that the fungi were mainly *Fusarium* and that the bacteria were mainly *Bacillus* strains. These strains exhibited congruence with members of different genera, *Bacillus*, *Pseudomonas*, *Stenotrophomonas*, *Chaetomium*, *Alternaria*, *Colletotrichum*, and *Fusarium,* based on macro and morphological characteristics, as well as molecular identification. Among the bacterial endophytes, *Bacillus* was the most prevalent genus, followed by *Paenibacillus*, *Pseudomonas*, and *Priestia*, while *Stenotrophomonas* and *Serratia* were singletons. *Bacillus* and *Paenibacillus* were distributed in both the root tubers and seeds of the host plant (Fig. 1A). Among the 12 representative fungal isolates, the phylogenetic reconstruction identified *Fusarium*

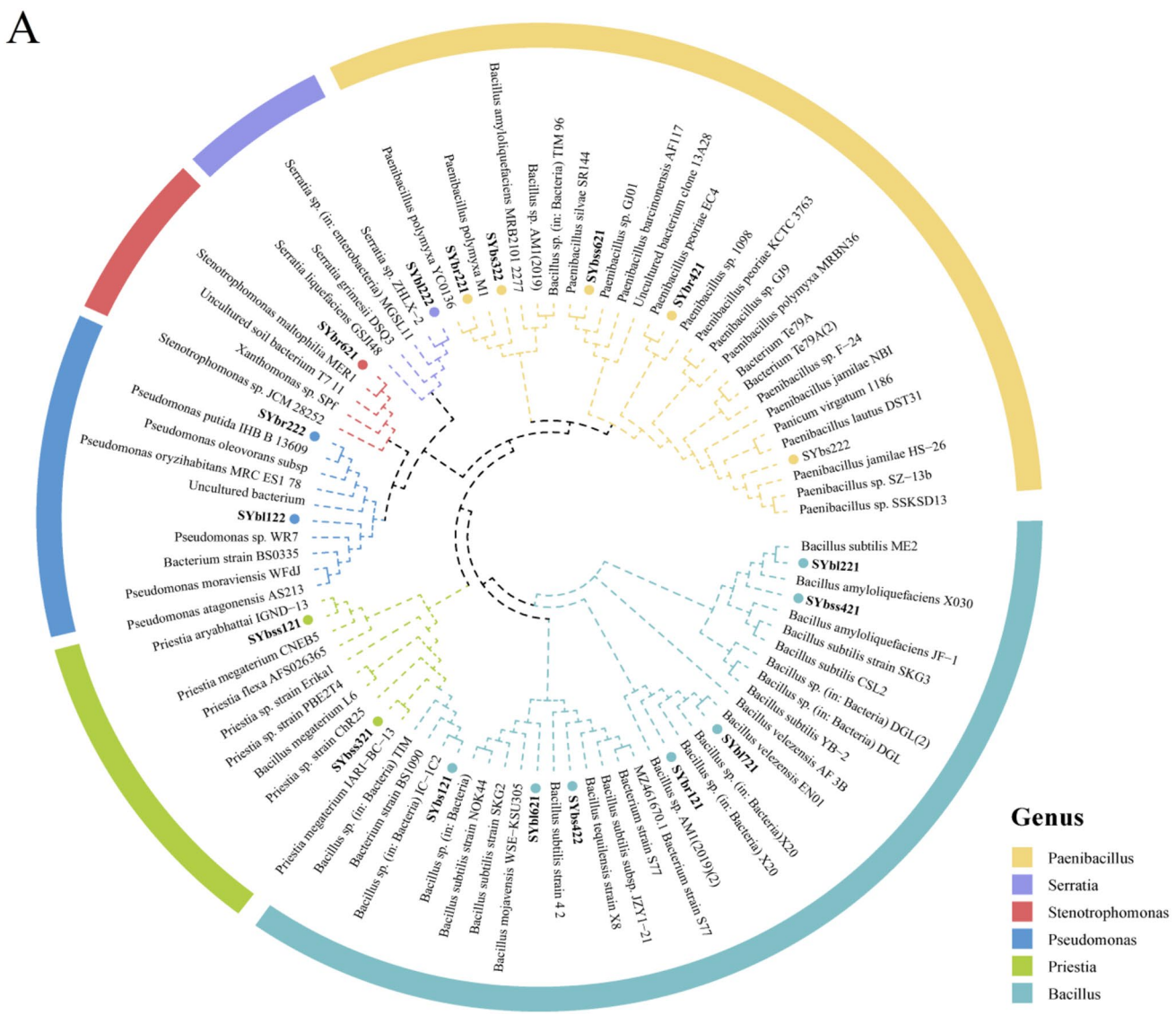


Fig. 1 Pure culture of endophytes isolated from *D. nipponica*. **A** Phylogenetic dendrogram of 16S rDNA gene sequences for all representative strains constructed using the neighbor-joining method and reference sequences from NCBI. **B** Neighbor-joining phylogenetic tree showing the locations of the 12 investigated fungal endophytes. The different branching colors indicate different bacterial and fungal genera

as the most common genus, followed by *Alternaria* and *Colletotrichum*, while *Arcopilus* and *Nectriaceae* were identified as singletons (Fig. 1B).

Figure 2 shows a summary of the tissue distributions of diverse culturable endophytes obtained from various plant parts of *D. nipponica*, revealing their prevalence at the genus level. In addition to comparing fungal and bacterial communities from different plant parts, a Venn diagram of shared and unique OTUs was generated (Fig. S5). The findings revealed that among the four plant parts, only two OTUs were shared, one each belonging to *Fusarium* sp. and *Bacillus* sp. Notably, the abundance of OTUs varied among these plant parts, with the highest species richness observed in the root tubers.

PGP activities of endophytes in vitro

The capacity of endophytes to enhance plant growth was evaluated using a phenotypic method (Cueva-Yesquén et al. 2021). Biochemical assays were designed to assess the potential of the strains to promote essential nutrients (phosphate solubilization, nitrogen fixation, and siderophore production) and plant growth regulator (IAA) synthesis. Thirty representative strains were isolated that exhibited one or more PGP properties (Fig. 3). Atmospheric nitrogen was

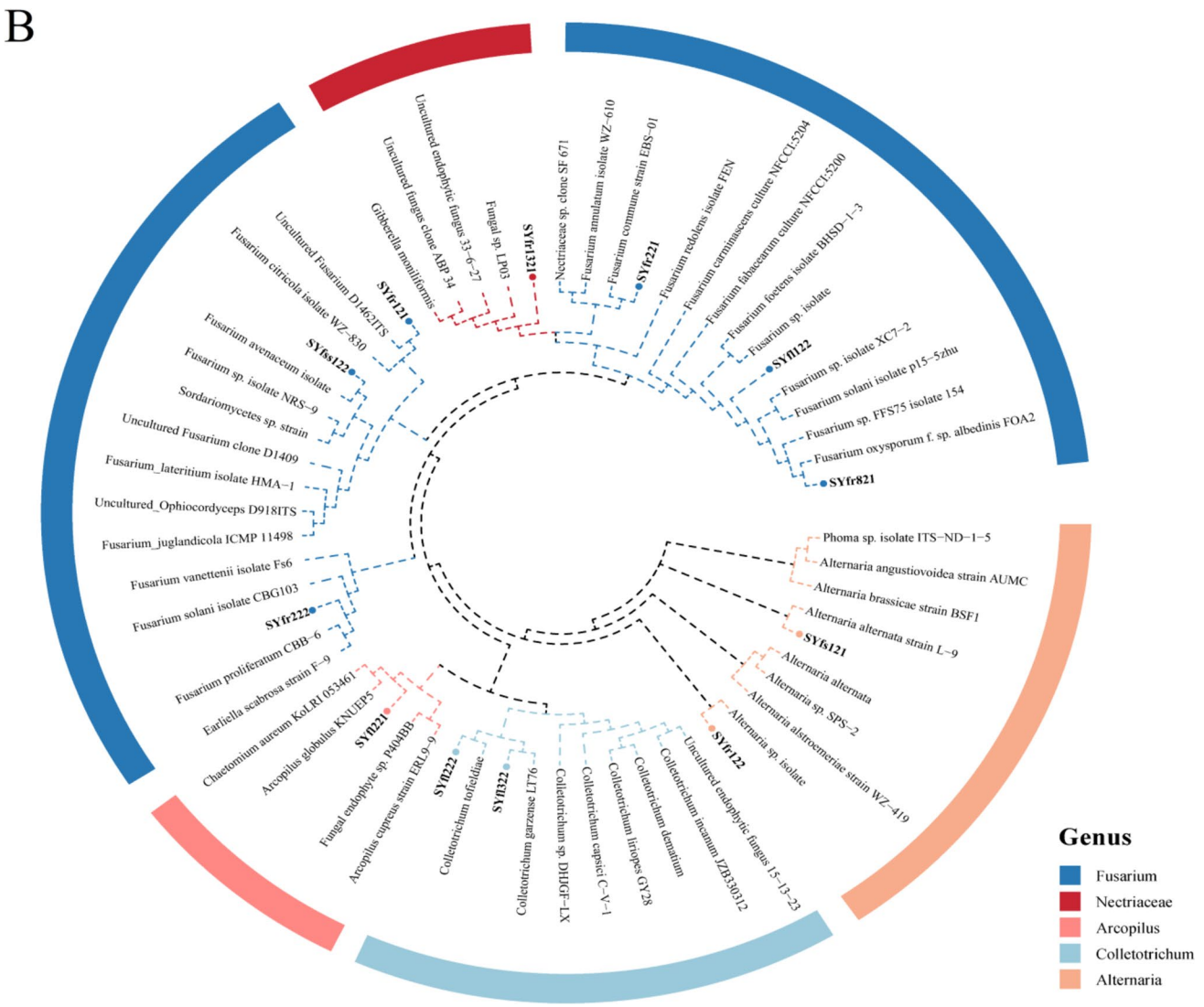


Fig. 1 (continued)

fixed in 24 isolates, and a clear zone was observed around colonies of strains SYbr221, SYbr621, and SYbr222, which is an indication of nitrogen fixation activity. For example, 28 endophytic strains were able to solubilize inorganic phosphates, with a markedly clear halo around the colony of strains SYbr421 and SYbr621. Furthermore, 17 isolates were confirmed to produce siderophores, which could promote plant growth by directly absorbing endophytic Fe^{3+} siderophore complexes (Fig. S6). The ability to biosynthesize IAA was observed in 17 isolates. The majority of the isolates that produced IAA were *Bacillus* (five isolates), followed by *Fusarium* (four isolates) and *Paenibacillus* (two isolates). Members of *Arcopilus*, *Alternaria*, *Pseudomonas*, *Priestia*, *Stenotrophomonas*, and *Nectriaceae* were represented by only one positive strain. (Fig. 3A). The quantitative IAA production ranged from 16.04 ± 0.51 to 206.64 ± 2.06 μg·mL^{-1}. Seven of these strains produced more than 30 μg·mL^{-1} (Table 1). On the basis of the combined evaluation of all four PGP traits, nine isolates (SYfl221, SYfr121, SYfr1321, SYbs422, SYbl221, SYbl721, SYbss121, SYbss621, and SYbr421) exhibited the capacity to produce all the traits shown in Fig. 3B.

Screening of active endophytes

TLC analysis was carried out to detect the presence of diosgenin in the extracts obtained from the 30 representative strains. Notably, three strains (SYbr421, SYfl221, and SYfss122) exhibited varying activities in hydrolyzing substrates into diosgenin. In particular, strain SYbr421 exhibited the highest hydrolysis activity on the tuber powder, yielding more diosgenin and fewer intermediates (Fig. 4A, B).

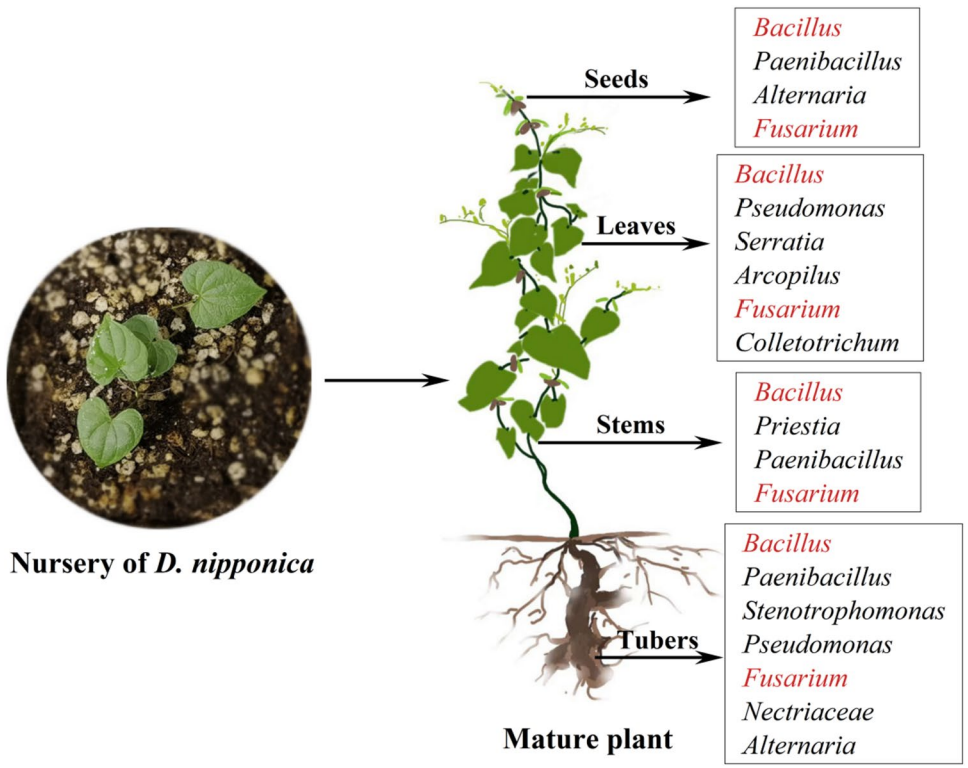


Fig. 2 Culturable endophyte diversity obtained from various plant parts of *D. nipponica*

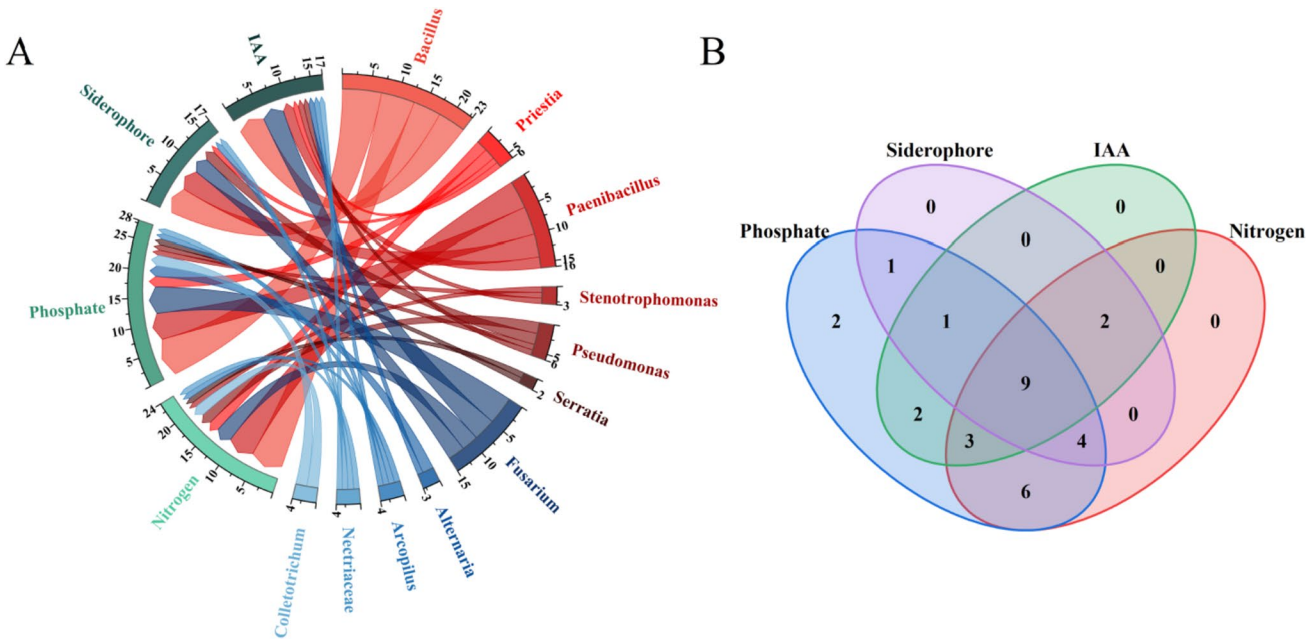


Fig. 3 A chord diagram showing the relationships between strains of different genera and growth-promoting trait indicators (**A**) and a Venn diagram representing the number of isolates positive for each test (**B**). A total of 30 isolates were evaluated for phosphate solubilization, nitrogen fixation, siderophore synthesis, and IAA production. The values indicate the number of positive isolates per test

Furthermore, HPLC was performed to confirm the presence of diosgenin in the fermentation broth. The results (Fig. 4D) revealed peaks matching the retention time of the diosgenin standard for all three strains identified in the initial screening. The UV absorption profile of this component was consistent with that of diosgenin, providing validation of the

Table 1 In vitro PGP traits shown by endophytic isolates

	Representative strains	Nitrogen fixation	Phosphorus solubilization	Siderophore production		IAA production ($\mu g \cdot mL^{-1}$)
Endophytic fungal isolates	SYfs121	ND[a]	+	ND		ND
	SYfl221	+	+	+	0.42	23.49 ± 0.24
	SYfl122	+	+	ND		32.46 ± 0.82
	SYfl222	+	+	ND		ND
	SYfl322	+	+	ND		ND
	SYfss122	ND	+	ND		26.08 ± 0.53
	SYfr121	+	+	+	0.47	31.23 ± 0.54
	SYfr221	+	ND	+	0.14	25.72 ± 0.51
	SYfr821	ND	+	+	0.12	ND
	SYfr1321	+	+	+	0.21	206.64 ± 2.06
	SYfr122	ND	+	ND		16.04 ± 0.51
	SYfr222	ND	+	ND		ND
Endophytic bacterial isolates	SYbs121	+	+	ND		16.67 ± 0.18
	SYbs222	+	+	+	0.43	ND
	SYbs322	+	+	+	0.21	ND
	SYbs422	+	+	+	0.29	28.96 ± 0.26
	SYbl221	+	+	+	0.55	22.99 ± 0.73
	SYbl621	+	+	+	0.50	ND
	SYbl721	+	+	+	0.46	17.01 ± 0.34
	SYbl122	+	ND	+	0.33	21.93 ± 0.50
	SYbl222	+	+	ND		ND
	SYbss121	+	+	+	0.28	33.91 ± 0.58
	SYbss321	+	+	ND		ND
	SYbss421	+	+	ND		ND
	SYbss621	+	+	+	0.25	63.69 ± 0.44
	SYbr121	ND	+	+	0.19	24.70 ± 0.50
	SYbr221	+	+	ND		ND
	SYbr421	+	+	+	0.34	65.83 ± 0.44
	SYbr621	+	+	ND		138.91 ± 2.88
	SYbr222	+	+	+	0.28	ND
	Total: 30	24	28	17		17

ND activity or product not detected in the assays

TLC findings and confirming the presence of diosgenin in the transformation broth.

The mass spectra of the diosgenin and SYbr421 extracts are displayed in Fig. 5. By analyzing the fragmentation pattern and referencing published reports (Huang et al. 2022), diosgenin ($C_{27}H_{42}O_3$) in the ESI^+ mode was found to typically exhibit an excimer ion at m/z 415 $[M+H]^+$ and the major secondary fragment ion was m/z 253.20 → 271. 21. The mass spectra of both the standard and the SYbr421 extract revealed signals at 415, 253, and 271, indicating their similarity (Fig. 5A, B). The ESI−MS data of the secondary fragments showed comparable or similar ion peaks to those of the standard. However, the signals of the latter ion peaks were notably weaker, likely due to lower concentrations of the analogs in the SYbr421 extract. These results confirmed the presence of the fundamental skeletal structure of diosgenin in the fermentation broth extract of SYbr421. This extract can be reliably identified as diosgenin, making it viable for use in microbiological preparation methods.

Optimization of fermentation for diosgenin preparation

Following screening experiments that revealed a greater transformation peak area for the bacterium SYbr421 than for the other two fungal strains, SYbr421 was chosen for condition optimization in further experiments. The HPLC analysis results are shown in Fig. 6. The analysis revealed that after SYbr421 treatment, the combination of supernatant and precipitate had the highest peak area compared

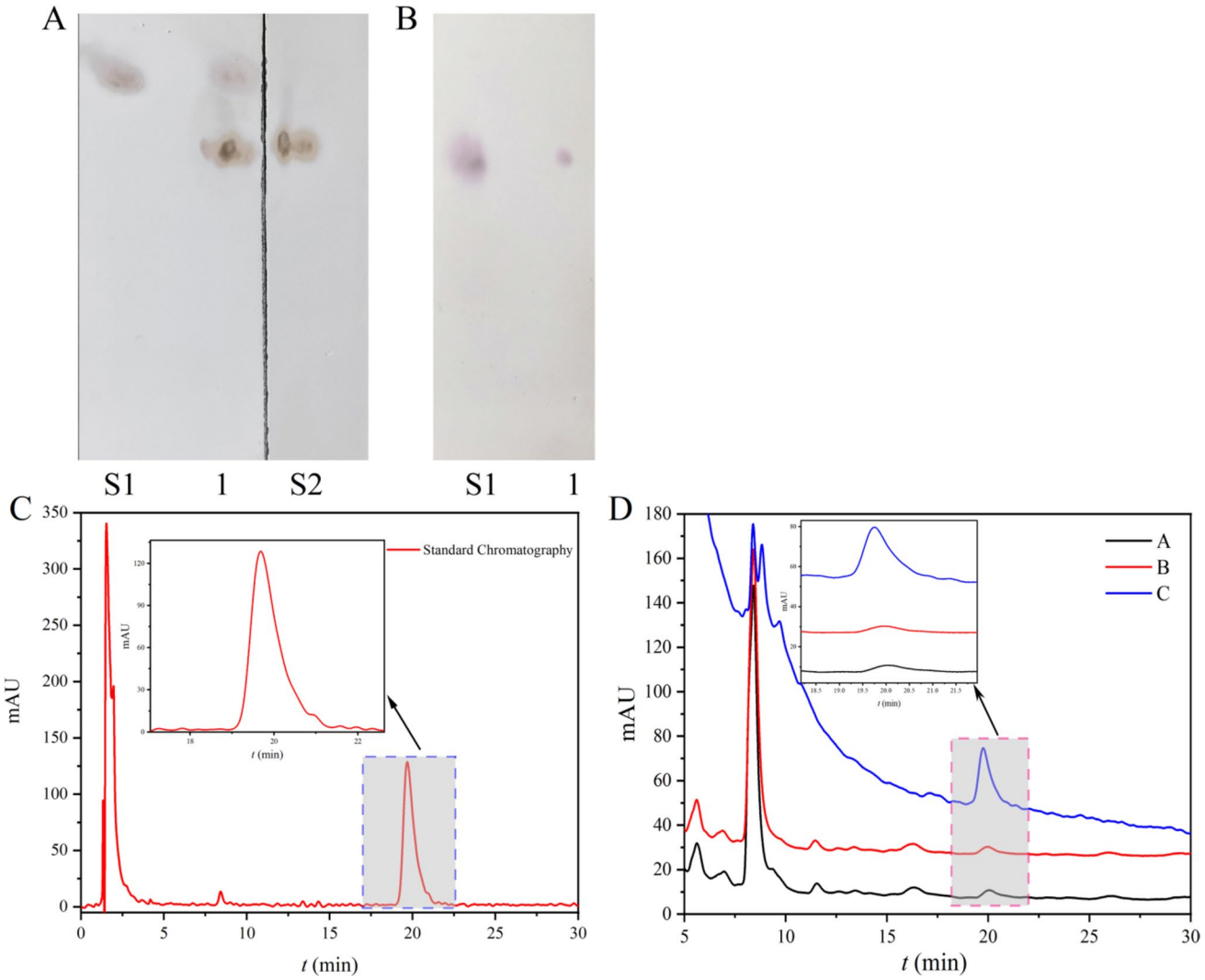


Fig. 4 TLC analysis of the products of tuber powder conversion by SYbr421. The developing solvents were concentrated sulfuric acid/ethanol (1:9, v/v). S1–S2, standard contrasts of dioscin and diosgenin. 1, products of tuber powder conversion by SYbr421 in the medium. HPLC chromatograms of the diosgenin standard (**C**) and fermentation products of the strains (**D**). **A** SYfss122; **B** SYfl221; **C** SYbr421

to that of the extracted supernatant or precipitate alone. This observation strongly suggested that the maximum diosgenin yield was obtained from the combined supernatant and precipitate. The impact of fermentation time on diosgenin yield is illustrated in Fig. 6B. The results indicated that the diosgenin yield at 4 and 6 days was notably greater than that at the other time points (0.64 and 1.06% yield, respectively). This finding suggested that a period between 4 and 6 days is more conducive to the rapid growth of SYbr421 in this medium and aligns closely with the optimal time for glycosidase activity. Subsequent experiments have focused primarily on this specific range of days. The quantity of inoculum significantly influences fermentation outcomes, particularly in determining the amount of glycosidase secreted. With an increase in inoculum from 1 to 4%, the diosgenin yield increased from 0.65 to 3.50%, likely attributable to its impact on SYbr421 growth (Fig. 6C). An increase in the enzyme concentration in the reaction system correlated with an increase in the inoculum concentration, enhancing the conversion rate. However, this increase tended to plateau after the inoculum reached 3% (Fig. S7C). Interestingly, the percentage of diosgenin-transformed plants exhibited a decreasing trend with increasing substrate concentration. At a lower substrate concentration of 1%, the secretion of glycosidase by SYbr421 appeared sufficient to completely convert the saponins (Fig. 6D). As the substrate concentration increased further, the system viscosity increased, limiting the oxygen content in the fermentation broth. This restriction hampered microbial growth and diosgenin conversion. Consequently, we identified a substrate concentration of 1% as the optimal level for transformation.

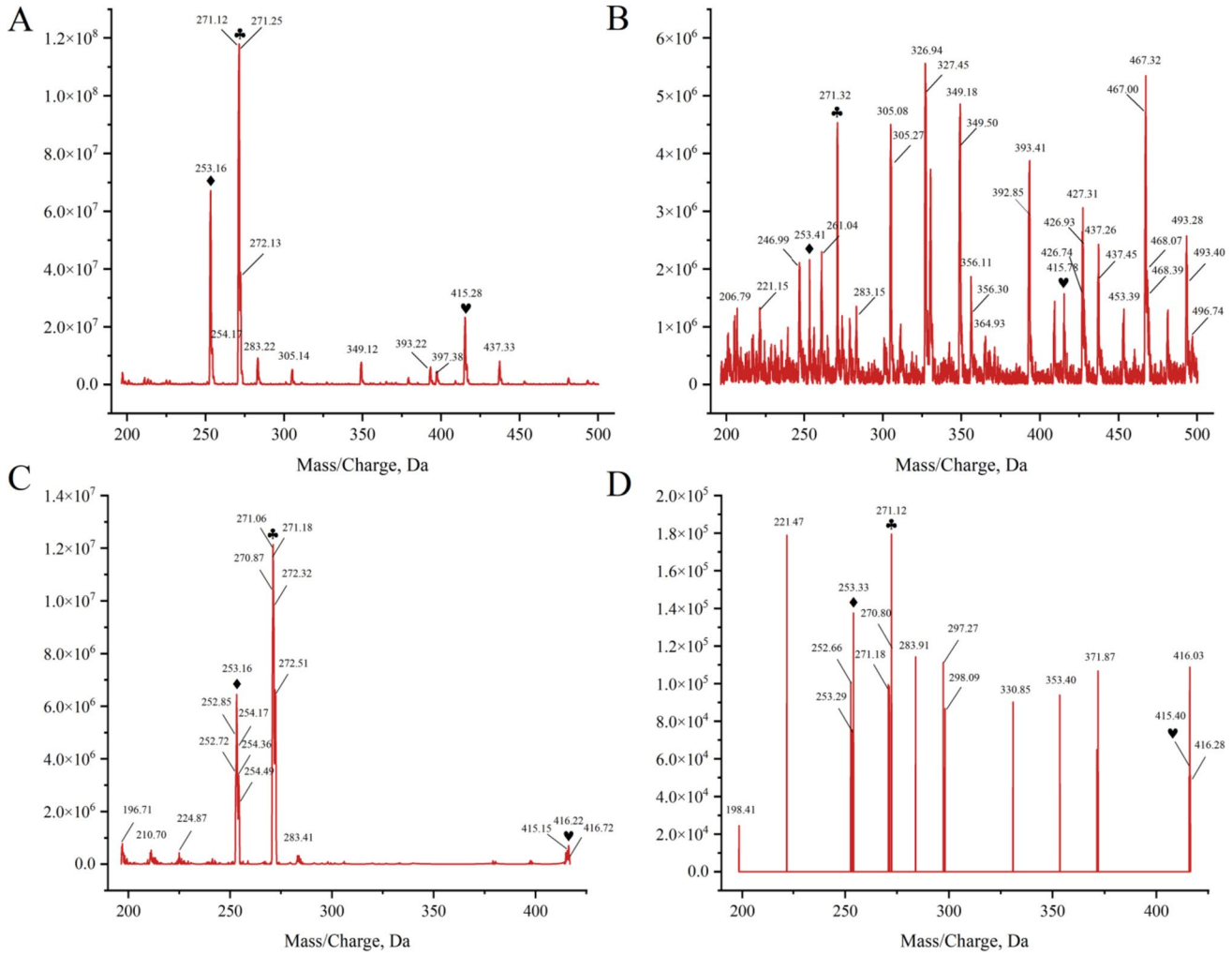


Fig. 5 Total ion chromatogram of LC−MS/ESI. Mass spectrometric analysis of diosgenin (**A**), SYbr421 extract (**B**), and m/z 415 full-scan production spectra of the secondary fragments of diosgenin (**C**) and SYbr421 fermentation products (**D**)

PGP activities of endophytes in vivo

Improved seed germination

Following the assessment of growth-promoting isolates, five specific endophytic strains, SYfl221 (*Chaetomium aureum*; 80%), SYfr121 (uncultured *Fusarium*; 76.67%), SYfr1321 (*Nectriaceae* sp.; 96.67%), SYbl221 (*Bacillus subtilis*; 63.33%), and SYbr421 (*Paenibacillus peoriae*; 99.73%), were identified for their significant impact on seed germination. These strains demonstrated notably greater germination percentages than non-inoculated seeds, which exhibited a 30% germination rate. Treatment with all the isolates significantly impacted the following seedling growth indices: plumule length, radicle length, and fresh weight (Fig. 7B). Notably, the expression of SYfl221, SYbr421, and SYfr1321 substantially increased in comparison to that in control plants. These strains markedly enhanced plumule length by 4.85–9.46-fold, radicle length by 1.78–3.38-fold, and fresh weight by 2.15–2.33-fold, indicating their potential as effective inoculants for *D. nipponica* seeds. The growth-promoting effects of the strains SYbr421, SYfr1321, and SYfl221 on *D. nipponica* were further validated, as shown in Fig. 7C.

Endophytic strains promoted plant growth

The strains SYbr421, SYfr1321, and SYfl221 were specifically chosen for assessment of their impact on plant growth through in vivo nursery experiments. After a 60-day growth period, the plants were harvested to assess their morphological attributes, including the fresh weight of the tuber/leaf, aboveground stem length, leaf number, and tuber volume. Various morphological indices were evaluated across all four treatments, and the collected data are presented in Fig. 8. Across the four treatments, notable variations were observed in the growth parameters. Compared with those in

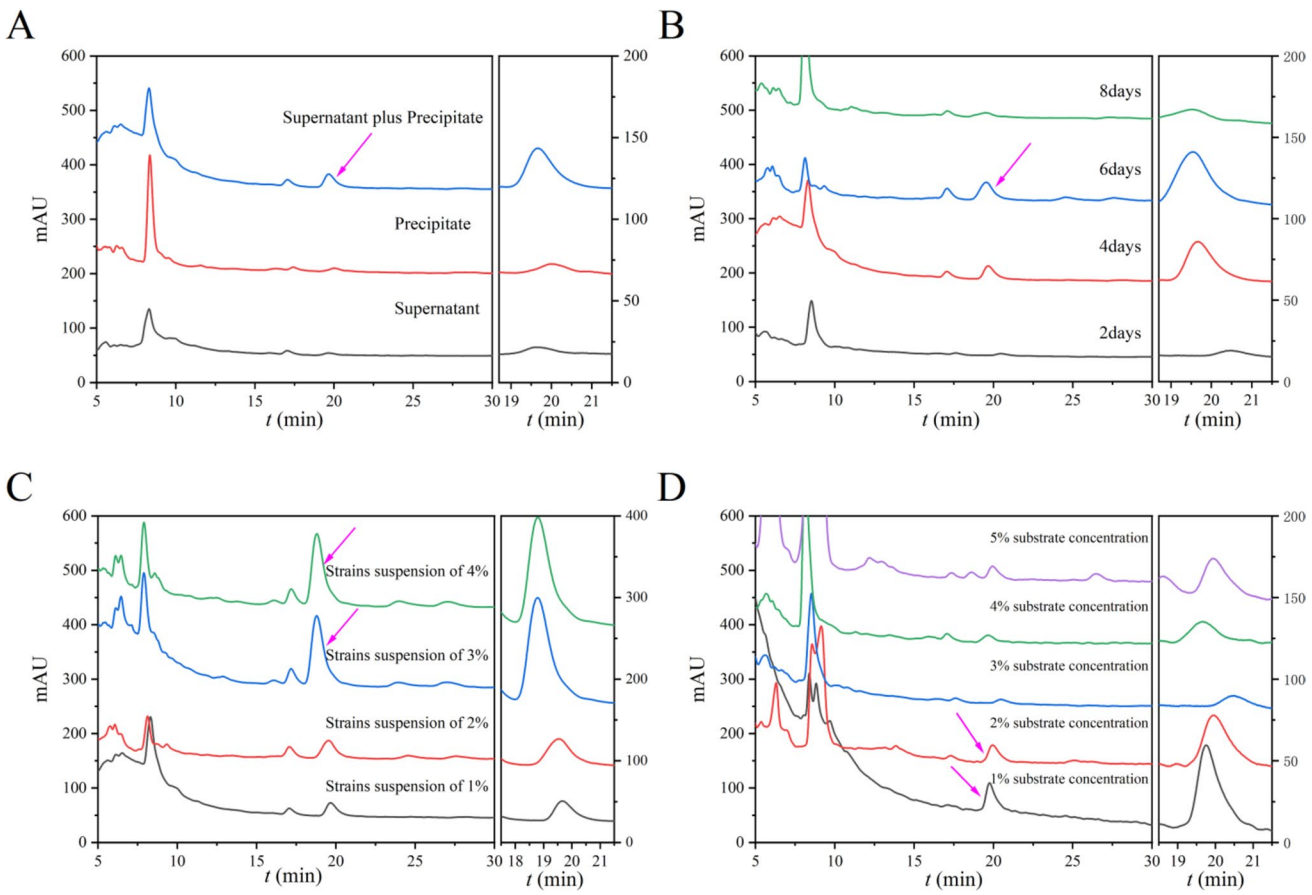


Fig. 6 HPLC chromatograms of SYbr421 extract at 204 nm. Options of extraction solution (**A**), effect of fermentation time on the yield of diosgenin (**B**), effect of inoculation on the yield of diosgenin (**C**), and effect of substrate concentration on the yield of diosgenin (**D**). The purity of the diosgenin product was calculated by the peak area ratio method

the control group, the plants treated with the specified strains exhibited greater biomass and aboveground stem length. Specifically, compared to those of the control plants, the leaf fresh weight of the SYbr421, SYfr1321, and SYfl221 strains significantly increased by 12.01%, 49.12%, and 38.16%, respectively. Moreover, compared with those of the control plants, the aboveground stem length of the plants treated with the same strains substantially increased by 27.18%, 82.61%, and 207.07%. Remarkable increments in tuber fresh weight were also noted, with the SYbr421, SYfr1321, and SYfl221 strains exhibiting significant increases of 57.18%, 144.83%, and 243.46%, respectively, compared to those of the control plants. While strains SYfr1321 and SYfl221 exhibited significant enhancements in tuber length and volume compared to those of the corresponding control plants, strain SYbr421 had contrasting effects. Specifically, the SYbr421-treated plants had shorter tubers than did the control plants. Surprisingly, despite these findings, the tuber volume was notably greater in the treatment group than in the control group. In addition, the underground structure of the plants treated with SYbr421 had fewer lateral roots but more robust primary roots, as depicted in Fig. 8B.

Correlations between a set of variables with a smaller set of linear combinations were summarized by PCA of different treatments and plant growth parameters (Fig. S8). A 3D plot of the plotted data for PC1, PC2, and PC3 represented 86.4% of the variance, approximately 57.5%, 16.3% and 12.6%, respectively, of the variance contributed by PC1, PC2 and PC3. Variables that showed strong correlations were in the same quadrant and were very close to each other. PC1 which included plant growth parameters such as the fresh weight of the leaf/tuber, aboveground stem length, leaf number, leaf area, leaf/tuber length, leaf width, tuber surface area, tuber volume, and tuber forks, demonstrated significant positive correlations. However, tuber diameter exhibited an inverse correlation with this parameter. On the other hand, PC2 revealed a negative correlation between leaf area, leaf length, leaf width, fresh weight of leaf, and other parameters. Finally, PC3 exhibited negative correlations with the fresh weight of the tuber, tuber length, tuber surface area, tuber volume, tuber forks, and other parameters.

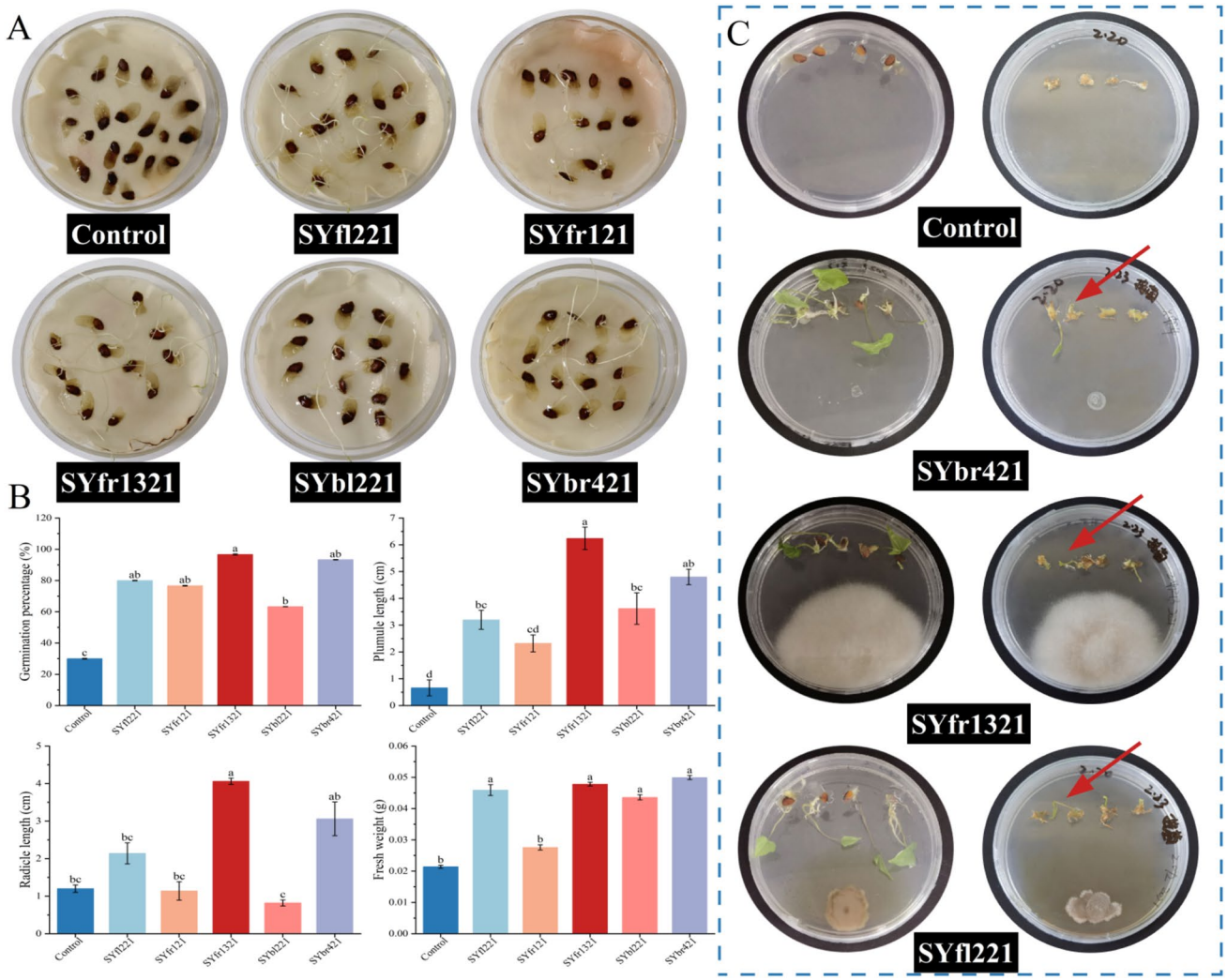


Fig. 7 Effects of endophytic strains on *D. nipponica* germination (**A**, **B**), **A** Representative pictures of germinated seeds and **B** bar chart of germination percentage (%), plumule and radicle length, and fresh weight exhibited by each bacterial strain. The columns represent the mean values ± standard deviations. Different letters indicate significant differences ($P < 0.05$). **C** Representative images showing the effect of endophytic fermentation broth on the germination of aseptic plants (seeds and tubers)

Discussion

Despite the importance of *D. nipponica* as a medicinal plant that has a precious cash value, its endophyte community has largely not been explored. Considering the ecological roles of endophytes in plant health, yield, mitigation of environmental stresses, and diversification, characterizing host microbiomes for sustainable cultivation of *D. nipponica* is imperative (Hardoim et al. 2015; Liu et al. 2017). Therefore, we investigated the biodiversity of endophytes in different tissues of healthy plants in *D. nipponica* plants by culture-dependent methods and obtained clues to improve growth. The results showed that endophytes colonized more abundantly on the roots than on the other tissues. Since tubers are nutrient-rich underground parts of plants, they provide favorable conditions for colonization by endophytes (Ahmad et al. 2022). The core microbiota plays a crucial role in the growth, development, and distribution of plants (Shade & Handelsman 2012). The core microbiome analysis of *D. nipponica* revealed the consistent presence of *Fusarium* and *Bacillus* across multiple tissues, including seeds, leaves, stems, and tubers. Particularly noteworthy was their widespread distribution in seeds and tubers. Thus, the interaction of *Bacillus* and *Fusarium* with *D. nipponica* may have a far-reaching influence on plant growth and development, as envisaged by the results of this study.

Previous studies have identified bacterial endophytes, predominantly belonging to the genus *Bacillus*, within various medicinal plants, such as ginseng (*Panax ginseng* C.A. Meyer) (Vendan et al. 2010), *Achillea fragrantissima*,

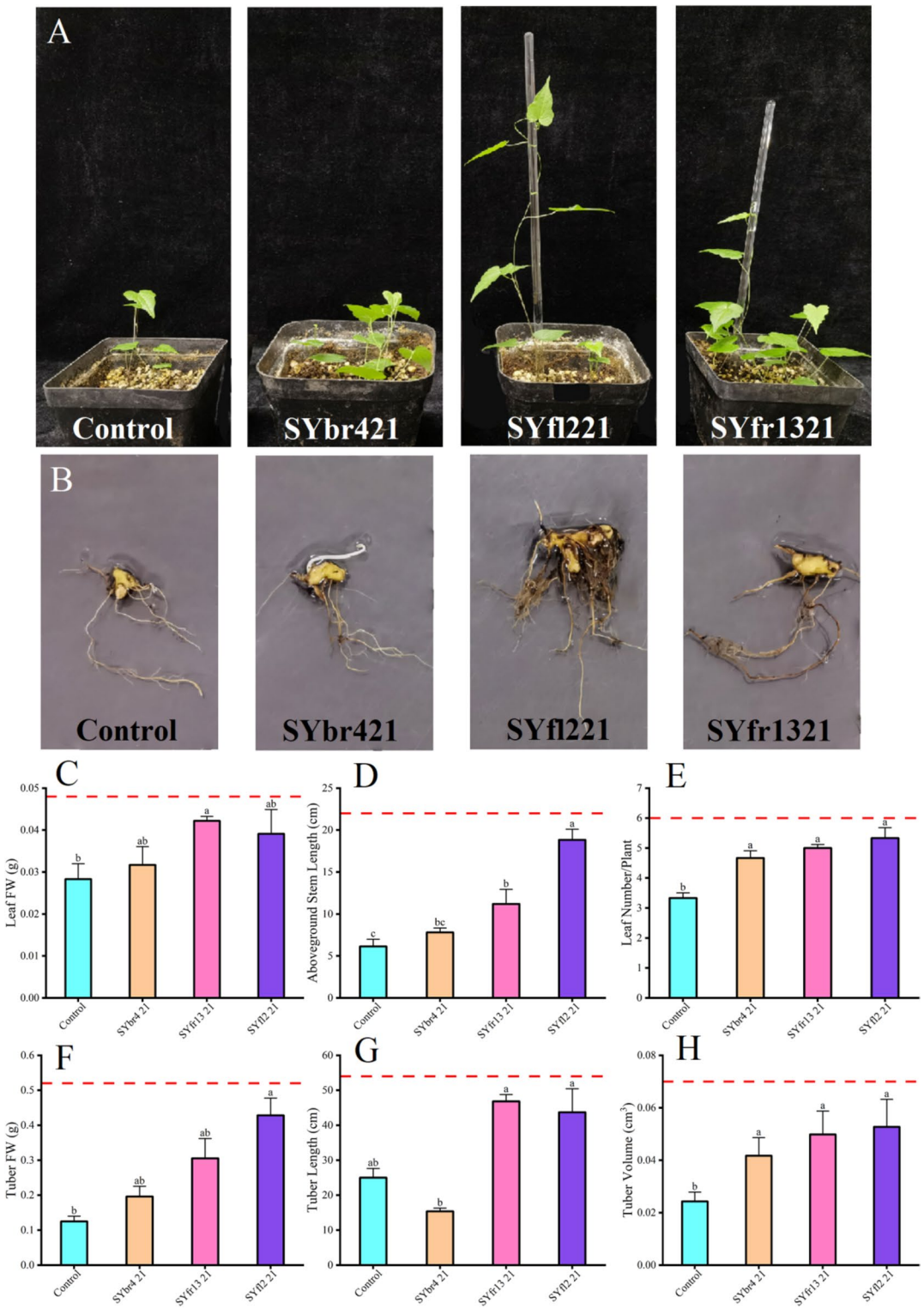


Fig. 8 The effect of endophytes on rooting and shooting of *D. nipponica* in greenhouse experiments. **A** Impact of the endophytes SYbr421, SYfr1321, and SYfl221 on the development of aboveground and **B** belowground parts of plants. Plots of the statistical analysis of plant growth parameters such as **C** leaf fresh weight (FW), **D** aboveground stem length, **E** number of leaves per plant, **F** tuber fresh weight (FW), **G** tuber length, and **H** tuber volume. The underground parts of rhizomes are collectively referred to in the text as "tubers"

Fagonia mollis (ALKahtani et al. 2020), *Taxus yunnanensis* (Miller et al. 2012), *Lonicera japonica* (Zhao et al. 2015), *Pinellia ternata* (Miller et al. 2012), and soybean (*Glycine max* L.) (Zhang et al. 2012). Notably, *Bacillus* was the dominant genus among the endophytic bacteria isolated from maize (Passari et al. 2016), consistent with our findings. In this study, *Fusarium* emerged as the dominant species, constituting nearly half of the total isolates. The genus *Fusarium* includes numerous pathogens known to induce severe diseases across a diverse array of crops and trees. However, evidence suggests that certain *Fusarium* species also exhibit endophytic behavior, fostering plant growth and bolstering resistance (Skiada et al. 2019). The growth and salinity resistance of *Liquidambar styraciflua* were proven to be significantly improved by *F. pseudograminearum* and *F. culmorum*, both of which were isolated from host plants (Pan et al. 2018). Furthermore, specific *Fusarium* species, *F. solani*, and *F. oxysporum* have been isolated from plants within the family Leguminosae, while *F. equiseti* showed a preference for *Lygeum spartum* (Gramineae) within mudflats and inland saline areas of the Mediterranean zone (Maciá-Vicente et al. 2008). Notably, *F. oxysporum* GG22 has been confirmed to positively impact the growth and secondary metabolism of *Rehmannia glutinosa*, thus demonstrating its potential application as a biofertilizer for *R. glutinosa* cultivation (Zhu et al. 2022).

Recently, there has been a push toward employing integrated agricultural techniques that incorporate microbial inoculants in the cultivation of medicinal plants to increase their productivity, focusing on biochemical constituents and biomass yield (Pandey et al. 2017). Since endophytes, like other beneficial microorganisms, promote plant growth by producing various PGP traits, the selection of potential strains for further studies was based on their ability to produce these traits (Hazarika et al. 2021). In this study, all the isolates exhibited one or more PGP activities in vitro. Therefore, among the 30 endophytic cultures, those demonstrating functional diversity and exhibiting varied PGP traits were chosen for further in vivo plant growth promotion experiments, guided by the outcomes of the in vitro assays. Treatment with the selected isolates increased the germination rate of *D. nipponica* seeds by 2.11–3.32-fold compared with that of the control after 30 days of incubation. PGP bacteria have been reported to positively influence seed germination synthesis (Delshadi et al. 2017). The isolates used in the germination assay showed the potential to synthesize one of the major plant hormones (IAA) associated with vegetative development. The results of pot experiments substantiated the ability of the SYbr421, SYfr1321, and SYfl221 strains to promote plant growth. These strains have previously shown potential for phosphate solubilization, nitrogen fixation, IAA synthesis, and siderophore production in biochemical assays. In addition to their role in promoting plant growth, endophytes influence the synthesis of bioactive molecules with high medicinal potential. Cultivable endophytes have been identified as a rich source of diverse bioactive chemicals with significant medicinal value, as highlighted in previous studies (Manganyi & Ateba 2020; Sharma & Kumar 2021).

In recent years, biotransformation has been widely used to modify natural products to obtain useful drug precursors (Qi et al. 2009). The effect of biotransformation on host compounds is one of the main ways for endophytic strains to mediate the production of pharmacologically active compounds in medicinal plants. This process involves the conversion of plant metabolites into new active substances or the enhancement of plant secondary metabolites through the action of intracellular or extracellular enzymes produced by endophytes. Recent research has focused on several key areas, including screening efficient strains, optimizing transformation conditions, and evaluating product activity (Zhang et al. 2022). Studies have highlighted the capacity of endophytic bacteria to transform major saponins within *Panax notoginseng*, yielding novel compounds and several trace components. Among these transformation products, vinaginsenoside R13, vinaginsenoside R22, pseudo-ginsenoside RT4, and vinaginsenoside R15, particularly rare ocotillol-type ginsenosides, were previously unreported in *P. notoginseng* (Luo et al. 2013). The *Astragalus* endophytic fungus *Neosartorya hiratsukae* has been instrumental in yielding three previously unknown neoruscogenin derivatives through biotransformation. This process results in the production of C-7 and C-12 hydroxylated compounds, as well as C-12 oxidized and C-1(O) glycosylated derivatives. These derivatives exhibit promising potential as sources for novel drugs possessing antitumor and anti-inflammatory properties (Özçinar et al. 2018). In addition, the endophytic *Penicillium* sp. JQ228238, isolated from *Polygonum cuspidatum*, has demonstrated the ability to convert resveratrol into pterostilbene. Pterostilbene exhibits enhanced metabolic stability, as well as stronger antioxidant and anti-inflammatory activities (Xu et al. 2020).

The conventional diosgenin production process encounters challenges due to the encapsulation of the saponin component within *D. nipponica* by starch and cellulose. The direct acid hydrolysis method not only yields low diosgenin output but also generates highly polluting acidic wastewater, posing significant environmental concerns. Moreover, meeting environmental standards necessitates substantial investment in wastewater treatment, becoming a bottleneck in the development of the diosgenin production industry. In contrast, direct biotransformation is an energy-efficient, environmentally friendly, and straightforward alternative to traditional methods. This innovative approach eliminates the need for the acidic hydrolysis, completely eradicating wastewater production and eliminating the necessity for

pretreatment (Dong et al. 2015; Liu et al. 2021; Xiang et al. 2018).

In our study, we attempted to obtain diosgenin through microbial transformation. Among the tested fungal and bacterial strains, strain SYbr421 showed the highest conversion capacity of 3.5% diosgenin within a short duration. The co-incubation environment of *D. nipponica* and strain SYbr421 produced certain special enzymes, such as amylase, glucanase, and glucosidase, to break down starch, and hydrolyze the sugar chain of the protodioscin, thereby liberating diosgenin, which provided a foundation for the development of an environmentally friendly bioprocess in the diosgenin extraction industry.

Conclusion

This study highlights the prevalence of dominant endophytes isolated from *D. nipponica,* which primarily belong to *Bacillus*, *Paenibacillus*, and *Fusarium*. These genera play pivotal roles in fostering plant growth and germination, highlighted by their potential for IAA production, nitrogen fixation, phosphate solubilization, and siderophore synthesis. Moreover, the isolated endophytic strains not only promoted plant growth in vitro but also promoted diosgenin biotransformation, particularly in *P. peoriae* SYbr421. This direct method provides a basis for further development of an environmentally friendly bioprocess in the diosgenin production. By harnessing their PGP attributes and biotransformation capabilities, these strains have the potential to contribute to the development of eco-friendly and economically viable strategies for diosgenin production.

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Author contributions SND performed the experiments, analyzed the data, and wrote the manuscript. JG facilitated the experiment by providing essential experimental equipment. RW and YMF contributed to the data analysis and manuscript refinement. RMG and YZH designed the study and provided advice. All the authors reviewed the manuscript.

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Data availability All data generated or analyzed during this study are included in this published article (and its supplementary information fles).

Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Aeron A, Dubey RC, Maheshwari DK (2020) Characterization of a plant-growth-promoting non-nodulating endophytic bacterium (*Stenotrophomonas maltophilia*) from the root nodules of *Mucuna utilis* var. *capitata* L. (Safed Kaunch). Can J Microbiol 66(11):670–677. https://doi.org/10.1139/cjm-2020-0196

Ahmad T, Farooq S, Mirza DN, Kumar A, Mir RA, Riyaz-Ul-Hassan S (2022) Insights into the endophytic bacterial microbiome of *crocus sativus*: functional characterization leads to potential agents that enhance the plant growth, productivity, and key metabolite content. Microb Ecol 83(3):669–688. https://doi.org/10.1007/s00248-021-01810-y

AlHabori M, Raman A, Lawrence MJ, Skett P (2001) *In vitro* effect of fenugreek extracts on intestinal sodium-dependent glucose uptake and hepatic glycogen phosphorylase A. Int J Exp Diab Res 2(2):91–99. https://doi.org/10.1155/EDR.2001.91

ALKahtani MD, Fouda A, Attia KA, Al-Otaibi F, Eid AM, Ewais EE, Hijri M, St-Arnaud M, Hassan SE, Khan N (2020) Isolation and characterization of plant growth promoting endophytic bacteria from desert plants and their application as bioinoculants for sustainable agriculture. Agronomy 10(9):1325. https://doi.org/10.3390/agronomy10091325

Andriiuk KI (1967) Nitrogen-fixing activity of soil actinomycetes and associative cultures. Mikrobiol Z 29(2):91–95

Chen FQ, Yang F, Wang DL, Xiang G, Wang L (2007) The effect of plant growth regulators and sucrose on the micropropagation and microtuberization of *Dioscorea nipponica* Makino. J Plant Growth Regul 26(1):38–45. https://doi.org/10.1007/s00344-005-0147-2

Compant S, Cambon MC, Vacher C, Mitter B, Samad A, Sessitsch A (2021) The plant endosphere world—Bacterial life within plants. Environ Microbiol 23(4):1812–1829. https://doi.org/10.1111/1462-2920.15240

Cueva-Yesquén LG, Goulart MC, Attili de Angelis D, Nopper Alves M, Fantinatti-Garboggini F (2021) Multiple plant growth-promotion traits in endophytic bacteria retrieved in the vegetative stage from passionflower. Front Plant Sci 11:621740. https://doi.org/10.3389/fpls.2020.621740

Dang SN, Gao RM, Zhang YQ, Feng YM (2022) *In vitro* regeneration and its histological characteristics of *Dioscorea nipponica* Makino. Sci Rep 12(1):18436. https://doi.org/10.1038/s41598-022-22986-4

Delshadi S, Ebrahimi M, Shirmohammadi E (2017) Influence of plant-growth-promoting bacteria on germination, growth and nutrients' uptake of *Onobrychis sativa* L. under drought stress. J Plant Interact 12:200–208. https://doi.org/10.1080/17429145.2017.1316527

Dong JZ, Lei C, Lu DY, Wang Y (2015) Direct biotransformation of dioscin into diosgenin in rhizome of *Dioscorea zingiberensis* by

Penicillium dioscin. Indian J Microbiol 55:200–206. https://doi.org/10.1007/s12088-014-0507-3

Du XW, Liu YP, Meng FJ, Wu JK, Yu D (2017) Isolation of endophytic fungi from *Dioscorea nipponica* Makino and analysis of its secondary metabolites. Acta Chin Med Pharmacol 5:004. https://doi.org/10.19664/j.cnki.1002-2392

Gao YG, Mo QQ, Zhao Y (2019) Microbial mediated accumulation of plant secondary metabolites and its action mechanism in medicinal plants: a review. J Southern Agric 50(10):2234–2240

Gouda S, Das G, Sen SK, Shin HS, Patra JK (2016) Endophytes: a treasure house of bioactive compounds of medicinal importance. Front Microbiol 7:1538. https://doi.org/10.3389/fmicb.2016.01538

Gupta R, Elkabetz D, Leibman-Markus M, Sayas T, Schneider A, Jami E, Kleiman M, Bar M (2022) Cytokinin drives assembly of the phyllosphere microbiome and promotes disease resistance through structural and chemical cues. ISME J 16(1):122–137. https://doi.org/10.1038/s41396-021-01060-3

Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79:293–320. https://doi.org/10.1128/MMBR.00050-14

Hazarika SN, Saikia K, Borah A, Thakur D (2021) Prospecting endophytic bacteria endowed with plant growth promoting potential isolated from *Camellia sinensis*. Front Microbiol 12:738058. https://doi.org/10.3389/fmicb.2021.738058

Higdon K, Scott A, Tucci M, Benghuzzi H, Tsao A, Puckett A, Cason Z, Hughes J (2001) The use of estrogen, DHEA, and diosgenin in a sustained delivery setting as a novel treatment approach for osteoporosis in the ovariectomized adult rat model. Biomed Sci Instrum 37:281–286

Hu Z, Wang C, Pan L, Han SY, Jin M, Xiang YS, Zheng LF, Li ZH, Gao R, Qin BF (2021) Identification and a phased pH control strategy of diosgenin bio-synthesized by an endogenous *Bacillus licheniformis* Syt1 derived from *Dioscorea zingiberensis* C. H Wright Appl Microbiol Biotechnol 105:9333–9342. https://doi.org/10.1007/s00253-021-11679-z

Huang N, Yu D, Huo J, Wu J, Chen Y, Du X, Wang X (2022) Study of saponin components after biotransformation of *Dioscorea nipponica* by endophytic fungi C39. J Anal Methods Chem. https://doi.org/10.1155/2022/2943177

Jain A, Chatterjee A, Das S (2020) Synergistic consortium of beneficial microorganisms in rice rhizosphere promotes host defense to blight-causing *Xanthomonas oryzae* pv. *oryzae*. Planta 252(6):106. https://doi.org/10.1007/s00425-020-03515-x

Kumari P, Shanker K, Singh A (2023) Insight into *Andrographis paniculata* associated bacterial endomicrobiome and assessment of culturable bacterial endophytes for enhancement of industrially important andrographolide content. Ind Crops Prod. https://doi.org/10.1016/j.indcrop.2023.116840

Laczeski ME, Onetto AL, Cortese IJ (2020) Isolation and selection of endophytic spore-forming bacteria with plant growth promoting properties isolated from Ilex paraguariensis St. Hil. (yerba mate). Anais Da Academia Brasileira De Ciencias 92(1):e20181381. https://doi.org/10.1590/0001-3765202020181381

Li P, Mao Z, Lou J, Li Y, Mou Y, Lu S, Peng Y, Zhou L (2011) Enhancement of diosgenin production in *Dioscorea zingiberensis* cell cultures by oligosaccharides from its endophytic fungus *Fusarium oxysporum* Dzf17. Molecules (basel, Switzerland) 16(12):10631–10644. https://doi.org/10.3390/molecules161210631

Li J, Mosongo I, Li H, Wu Y, Li C, Yang S, Zhang Y (2021) Identification and characterization of a trillin rhamnosyltransferase from *Dioscorea zingiberensis*. Front Plant Sci 12:713036. https://doi.org/10.3389/fpls.2021.713036

Liu T, Yu H, Liu C, Bao Y, Hu X, Wang Y, Liu B, Fu Y, Tang S, Jin F (2013) Preparation of progenin III from total steroidal saponins of *Dioscorea nipponica* Makino using a crude enzyme from *Aspergillus oryzae* strain. J Ind Microbiol Biotechnol 40(5):427–436. https://doi.org/10.1007/s10295-013-1246-x

Liu H, Carvalhais LC, Crawford MH, Singh E, Dennis PG, Pieterse CM, Schenk PM (2017) Inner plant values: diversity, colonization and benefits from endophytic bacteria. Front Microbiol 8:2552. https://doi.org/10.3389/fmicb.2017.02552

Liu X, Zhou ZY, Cui JL, Wang ML, Wang JH (2021) Biotransformation ability of endophytic fungi: from species evolution to industrial applications. Appl Microbiol Biotechnol 105(19):7095–7113. https://doi.org/10.1007/s00253-021-11554-x

Liu LH, Yuan T, Zhang JY, Tang GX, Lü H, Zhao HM, Li H, Li YW, Mo CH, Tan ZY, Cai QY (2022) Diversity of endophytic bacteria in wild rice (*Oryza meridionalis*) and potential for promoting plant growth and degrading phthalates. Sci Total Environ 806(Pt1):150310. https://doi.org/10.1016/j.scitotenv.2021.150310

Luo SL, Dang LZ, Li JF, Zou CG, Zhang KQ, Li GH (2013) Biotransformation of saponins by endophytes isolated from *Panax notoginseng*. Chem Biodivers 10(11):2021–2031. https://doi.org/10.1002/cbdv.201300005

Maciá-Vicente JG, Jansson HB, Abdullah SK, Descals E, Salinas J, Lopez-Llorca LV (2008) Fungal root endophytes from natural vegetation in Mediterranean environments with special reference to *Fusarium* spp. FEMS Microbiol Ecol 64(1):90–105. https://doi.org/10.1111/j.1574-6941.2007.00443.x

Manganyi MC, Ateba CN (2020) Untapped potentials of endophytic fungi: a review of novel bioactive compounds with biological applications. Microorganisms 8(12):1934. https://doi.org/10.3390/microorganisms8121934

Miller KI, Qing C, Sze DMY, Roufogalis BD, Neilan BA (2012) Culturable endophytes of medicinal plants and the genetic basis for their bioactivity. Microb Ecol 64:431–449. https://doi.org/10.1007/s00248-012-0044-8

Moalic S, Liagre B, Corbière C, Bianchi A, Dauça M, Bordji K, Beneytout JL (2001) A plant steroid, diosgenin, induces apoptosis, cell cycle arrest and COX activity in osteosarcoma cells. FEBS Lett 506(3):225–230. https://doi.org/10.1016/S0014-5793(01)02924-6

Ouyabe M, Tanaka N, Shiwa Y, Fujita N, Kikuno H, Babil P, Shiwachi H (2020) Rhizobium dioscoreae sp. nov., a plant growth-promoting bacterium isolated from yam (*Dioscorea* species). Int J Syst Evol Microbiol 70(9):5054–5062. https://doi.org/10.1099/ijsem.0.004381

Özçakmak S, Dervişoğlu M, Yilmaz A (2012) Effects of polysaccharides and oligosaccharides from endophytic fungus *Berkleasmium* sp. Dzf12 on diosgenin accumulation in *Dioscorea zingiberensis* cell and seedling cultures. Afr J Microbiol Res 6:3079–3084. https://doi.org/10.5897/AJMR12.599

Özçinar Ö, Tağ Ö, Yusufoglu H, Kivçak B, Bedir E (2018) Biotransformation of ruscogenins by *Cunninghamella blakesleeana* NRRL 1369 and neoruscogenin by endophytic fungus *Neosartorya hiratsukae*. Phytochemistry 152:1–9. https://doi.org/10.1016/j.phytochem.2018.04.002

Pan CX, Zhao Y, Liu GH, Dou GY, Ru ZG, Zhu K (2014) Development and demonstration of a cleaner process to produce diosgenin from *Dioscorea zingiberensis* based on physical separation. J Clean Prod 76:161–166. https://doi.org/10.1016/j.jclepro.2013.12.074

Pan XY, Sun HJ, Yuan ZL (2018) Toxin accumulation of three *Leymus mollis*-associated endophytic *Fusarium* isolates and their effects on growth and salt tolerance of *Liquidambar styraciflua* seedlings. Forest Res 31(5):64–73. https://doi.org/10.13275/j.cnki.lykxyj.2018.05.009

Pandey DK, Nazir A, Dey A (2017) Isolation and characterization of phosphate solubilizing bacteria from rhizosphere of *Dioscorea alata* stimulating growth and diosgenin production. Proc. Natl.

Acad. Sci. India Sect B Biol 87:1143–1152. https://doi.org/10.1007/s40011-015-0670-2

Passari AK, Mishra VK, Leo VV, Gupta VK, Singh BP (2016) Phytohormone production endowed with antagonistic potential and plant growth promoting abilities of culturable endophytic bacteria isolated from *Clerodendrum colebrookianum* Walp. Microbiol Res 193:57–73. https://doi.org/10.1016/j.micres.2016.09.006

Qi SS, Dong YS, Zhao YK, Xiu ZL (2009) Qualitative and quantitative analysis of microbial transformation of steroidal saponins in *Dioscorea zingiberensis*. Chroma 69:865–870. https://doi.org/10.1365/s10337-009-0978-2

Qin HL, Dong CM, Zhang AH, Zhao Y, Tang H, Ruan YZ (2015) Screening and identification of phosphate-solubilizing bacteria from rhizosphere soil in banana orchards in Hainan and its influence on growth of banana seedlings. South China Fruits 44(2):18–22. https://doi.org/10.13938/j.issn.1007-1431.20140644

Shade A, Handelsman J (2012) Beyond the Venn diagram: the hunt for a core microbiome. Environ Microbiol 14(1):4–12. https://doi.org/10.1111/j.1462-2920.2011.02585.x

Sharma P, Kumar S (2021) Bioremediation of heavy metals from industrial effluents by endophytes and their metabolic activity: Recent advances. Biores Technol 339:125589. https://doi.org/10.1016/j.biortech.2021.125589

Sharma S, Dhar MK, Kaul S (2023) Antagonistic, plant growth promoting and extracellular hydrolytic enzyme activity of fungal endophytes of *Dioscorea bulbifera* L. Biocatal Agric Biotechnol 50:1878–8181. https://doi.org/10.1016/j.bcab.2023.102694

Shukla N, Singh D, Tripathi A, Kumari P, Gupta RK, Singh S, Shanker K, Singh A (2022) Synergism of endophytic *Bacillus subtilis* and *Klebsiella aerogenes* modulates plant growth and bacoside biosynthesis in *Bacopa monnieri*. Front Plant Sci 13:896856. https://doi.org/10.3389/fpls.2022.896856

Skiada V, Faccio A, Kavroulakis N, Genre A, Bonfante P, Papadopoulou KK (2019) Colonization of legumes by an endophytic *Fusarium solani* strain FsK reveals common features to symbionts or pathogens. Fungal Genet Biol 127:60–74. https://doi.org/10.1016/j.fgb.2019.03.003

Stierle A, Strobel G, Stierle D (1993) Taxol and taxane production by Taxomyces andreanae, an endophytic fungus of Pacific yew. Science (new York) 260(5105):214–216. https://doi.org/10.1126/science.8097061

Vendan RT, Yu YJ, Lee SH, Rhee YH (2010) Diversity of endophytic bacteria in ginseng and their potential for plant growth promotion. J Microbiol (seoul, Korea) 48(5):559–565. https://doi.org/10.1007/s12275-010-0082-1

Vinayarani G, Prakash HS (2018) Growth promoting rhizospheric and endophytic bacteria from *Curcuma longa* L. as biocontrol agents against rhizome rot and leaf blight diseases. Plant Pathol J 34(3):218–235. https://doi.org/10.5423/PPJ.OA.11.2017.0225

Wang X, Meng JF, Ma R, He SW, Guo HB, Isolation ZXX (2022) Isolation, identification of an endophytic *Paraburkholderia kuruiensis* in rice and evaluation of its plant growth promotion. Soil Fertil Sci China 4:218–228. https://doi.org/10.11838/sfsc.1673-6257.20709

Xiang HB, Zhang T, Pang X, Wei YZ, Liu HY, Zhang YQ, Ma BP, Yu LY (2018) Isolation of endophytic fungi from *Dioscorea zingiberensis* C. H. Wright and application for diosgenin production by solid-state fermentation. Appl Microbiol Biotechnol 102:5519–5532. https://doi.org/10.1007/s00253-018-9030-5

Xu L, Wang J, Zhao J, Li P, Shan T, Wang J, Li X, Zhou L (2010) Beauvericin from the endophytic fungus, *Fusarium redolens*, isolated from *Dioscorea zingiberensis* and its antibacterial activity. Nat Prod Commun 5(5):811–814. https://doi.org/10.1177/1934578X1000500527

Xu Z, Tian J, Gan L, Tian Y (2020) Discovery of the endophytic fungi from *Polygonum cuspidatum* and biotransformation of resveratrol to pterostillbene by the endophyte *Penicillium* sp. F5. Appl Biochem Microbiol 56:313–320. https://doi.org/10.1134/S0003683820030163

Yang H, Yin H, Shen YP, Xia GH, Zhang B, Wu XY, Cai B, Tam JP (2016) A more ecological and efficient approach for producing diosgenin from *Dioscorea zingiberensis* tubers via pressurized biphase acid hydrolysis. J Clean Prod 131:10–19. https://doi.org/10.1016/j.jclepro.2016.05.030

Yu H, Yu M, Liu B, Zhao ZY, Wu RZ (2018) Rapid propagation of *Discorea nipponica* Makino via axillary bud proliferation. Agric Biotechnol 7(5):6–9

Zhang YZ, Chen WF, Li M, Sui XH, Liu HC, Zhang XX, Chen WX (2012) Bacillus endoradicis sp. nov., an endophytic bacterium isolated from soybean root. Int J Syst Evol Microbiol 62(2):359–363. https://doi.org/10.1099/ijs.0.028936-0

Zhang T, Xiang HB, Zhang YQ, Liu HY, Wei YZ, Zhao LX, Yu LY (2013) Molecular analysis of fungal diversity associated with three bryophyte species in the Fildes Region, King George Island, maritime Antarctica. Extremophiles 17(5):757–765. https://doi.org/10.1007/s00792-013-0558-0

Zhang H, Liu MM, Liu XN, Li ZY, Zhao LL, Yang QX (2022) Impact of endophytic microorganisms on pharmaco-active compounds production in medicinal plants: a review. Biotechnol Bull 38(8):41–51. https://doi.org/10.13560/j.cnki.biotech.bull.1985.2021-1487

Zhao J, Shan T, Mou Y, Zhou L (2011) Plant-derived bioactive compounds produced by endophytic fungi. Mini Rev Med Chem 11(2):159–168. https://doi.org/10.2174/138955711794519492

Zhao L, Xu Y, Lai XH, Shan C, Deng Z, Ji Y (2015) Screening and characterization of endophytic *Bacillus* and *Paenibacillus* strains from medicinal plant *Lonicera japonica* for use as potential plant growth promoters. Braz J Microbiol 46(4):977–989. https://doi.org/10.1590/S1517-838246420140024

Zhu YL, Huang W, Ni JR, Liu W, Li H (2010) Production of diosgenin from *Dioscorea zingiberensis* tubers through enzymatic saccharification and microbial transformation. Appl Microbiol Biotechnol 85:1409–1416. https://doi.org/10.1007/s00253-009-2200-8

Zhu YH, Shao YY, Li L, Zhao L, Zhang MJ, Dong CM (2022) The plant growth-promoting endophytic *Fusarium oxysporum* GG22 enhances *Rehmannia glutinosa* secondary metabolites accumulation. Ind Crops Prod. https://doi.org/10.1016/j.indcrop.2022.114881

Zou K, Liu X, Hu Q, Zhang D, Fu S, Zhang S, Huang H, Lei F, Zhang G, Miao B, Meng D, Jiang L, Liu H, Yin H, Liang Y (2021) Root endophytes and *Ginkgo biloba* are likely to share and compensate secondary metabolic processes, and potentially exchange genetic information by LTR-RTs. Front Plant Sci 12:704985. https://doi.org/10.3389/fpls.2021.704985