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ARTICLE

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Endophytic Fungus *Paraconiothyrium cyclothyrioides* Associated with *Larix sibirica* Ledeb.: Molecular Identification and Functional Characterization

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Abstract
Endophytic fungi play important roles in plant growth, development, and adaptation to environmental stress through the production of bioactive metabolites. This study aimed to isolate and characterize an endophytic fungal strain associated with the roots of *Larix sibirica* Ledeb. and to evaluate its plant growth-promoting potential. The fungal strain T1R7 was isolated from root tissues and identified as *Paraconiothyrium cyclothyrioides* based on internal transcribed spacer (ITS) region sequencing, showing 100% similarity to reference sequences in the GenBank database. Functional characterization revealed that the strain did not exhibit phosphate-solubilizing activity on Pikovskaya's medium. However, it produced indole-3-acetic acid (IAA), a key phytohormone involved in plant growth regulation, at a concentration of 6.93 ± 1.96 mg L^{-1}, which was significantly higher than that of the control (1.13 ± 0.17 mg L^{-1}; $p < 0.05$). These results indicate that *P. cyclothyrioides* possesses plant growth-promoting potential through IAA production and may contribute to the growth and development of its host plant. Further studies are required to evaluate its ecological role and potential applications in forestry and plant biotechnology.

Keywords: forest microbiome, root endophyte, phytohormone production, fungal biotechnology, plant–microbe interaction

Introduction

Larix sibirica Ledeb. (Siberian larch) is one of the dominant coniferous tree species in the boreal forests of Mongolia, Russia, and Northeast Asia [1]. It plays a critical ecological role in carbon sequestration, timber production, soil stabilization, and ecosystem functioning. The species is well adapted to harsh environmental conditions, including low temperatures, drought, and nutrient-deficient soils [1], [2]. Despite its ecological and economic importance, relatively little is known about the microbial communities associated with *L. sibirica*, particularly endophytic fungi that may contribute to host growth, adaptation, and stress tolerance.

Endophytic fungi inhabit internal plant tissues without causing visible disease symptoms and may establish mutualistic, commensal, or latent pathogenic relationships with their hosts [3]. These microorganisms are increasingly recognized as important components of plant microbiomes due to their ability to enhance plant growth and resilience through the production of phytohormones,

secondary metabolites, and other bioactive compounds [4]–[6]. In forest ecosystems, endophytic fungi may contribute to nutrient acquisition, stress tolerance, and protection against pathogens, thereby influencing host survival and productivity.

One of the most important plant growth-promoting mechanisms of endophytic fungi is the production of indole-3-acetic acid (IAA), the predominant auxin in plants. IAA regulates numerous physiological processes, including cell division, cell elongation, root initiation, and vascular tissue development [7], [8]. Many fungal endophytes are capable of synthesizing IAA through tryptophan-dependent pathways, thereby promoting root growth and facilitating plant–microbe interactions [9], [10].

Another important functional trait of beneficial fungi is phosphate solubilization. Although phosphorus is an essential macronutrient required for plant growth and metabolism, a large proportion of soil phosphorus exists in insoluble forms that are unavailable for plant uptake. Phosphate-solubilizing fungi can increase phosphorus availability through the secretion of organic acids and other metabolites that mobilize insoluble phosphate compounds [11], [12]. However, the ability to solubilize phosphate varies considerably among fungal species and isolates.

The genus *Paraconiothyrium* comprises a diverse group of fungi that have been isolated from a wide range of ecological niches, including plants, soil, and marine environments. Several species have been reported as endophytes and producers of biologically active metabolites, suggesting potential roles in plant growth promotion and environmental adaptation. Nevertheless, information regarding the functional characteristics of *Paraconiothyrium cyclothyrioides* associated with *L. sibirica* remains limited.

Therefore, the objective of this study was to isolate and identify an endophytic fungal strain associated with *L. sibirica* roots and to evaluate its plant growth-promoting traits, including indole-3-acetic acid production and phosphate solubilization under laboratory conditions. We hypothesized that endophytic fungi isolated from *L. sibirica* possess at least one plant growth-promoting characteristic that may contribute to host development and adaptation.

Materials and methods

Isolation of Endophytic Fungi

Root samples of *Larix sibirica* Ledeb. were collected in 2023 from a larch forest in Erdene soum, Tuv Province, Mongolia (47°44′50.0″ N, 107°38′27.0″ E), located within the Khentii Mountain taiga and Mongol-Daguur forest-steppe region.

The root samples were thoroughly washed under running tap water to remove adhering soil and debris. Surface sterilization was performed according to Daru et al. [13] with minor modifications. Root tissues were sequentially immersed in 95% ethanol for 5 s, 0.5% sodium hypochlorite for 2 min, and 70% ethanol for 2 min, followed by three rinses with sterile distilled water. Following surface sterilization, the root tissues were air-dried on sterile filter paper, cut into approximately 0.5 × 0.5 cm segments using sterile scalpels, and placed on potato dextrose agar (PDA) supplemented with chloramphenicol (0.05 g L^{-1}) to suppress bacterial growth. The plates were incubated at 26 ± 2 °C for 7–14 days. Emerging fungal colonies were repeatedly sub-cultured to obtain pure isolates, which were maintained on PDA at 4 °C for further analyses.

Phosphate Solubilization Assay

Phosphate-solubilizing activity was evaluated on Pikovskaya's (PVK) agar medium containing tricalcium phosphate (TCP) as the insoluble phosphorus source [14]. Mycelial plugs (5 mm diameter) obtained from actively growing PDA cultures were inoculated onto PVK agar plates and incubated at 25 °C for 5 days. Each assay was performed in triplicate.

Phosphate solubilization was assessed by the formation of a clear halo zone surrounding the fungal colony. Colony diameter and halo-zone diameter were measured, and the phosphate solubilization index (SI) was calculated according to the following equation:

$$\textbf{Solubilization Index } (SI) = \frac{\textbf{Colony Diameter} + \textbf{Halo Zone Diameter}}{\textbf{Colony Diameter}}$$

Indole-3-acetic acid (IAA) production

Indole-3-acetic acid (IAA) production by the fungal isolate was determined using the Salkowski colorimetric method [15]. The isolate was cultured in Potato Dextrose Broth (PDB) supplemented with 1% (w/v) L-tryptophan and incubated at 25 °C with shaking at 150 rpm for 7 days.

Following incubation, 1.5 mL of culture broth was centrifuged at 10,000 rpm for 10 min. One milliliter of the resulting supernatant was mixed with 2 mL of Salkowski reagent containing $FeCl_3$ in sulfuric acid and incubated in the dark at room temperature for 30 min. The development of a pink coloration indicated the presence of IAA.

Absorbance was measured at 535 nm using a spectrophotometer. IAA concentration was quantified using a calibration curve prepared with standard IAA solutions at concentrations of 10, 20, 40, 60, 80, and 100 mg L^{-1} and expressed as µg mL^{-1}.

Molecular Identification

Genomic DNA was extracted from the endophytic fungal isolate T1R7 grown on potato dextrose agar (PDA) using a cetyltrimethylammonium bromide (CTAB)-based protocol. Fresh mycelium was harvested from actively growing cultures, lysed in CTAB extraction buffer, and purified by chloroform–isoamyl alcohol extraction followed by isopropanol precipitation. The DNA pellet was washed with 70% ethanol, resuspended in Tris–EDTA (TE) buffer, and stored at −20 °C until analysis.

The internal transcribed spacer (ITS) region of fungal rDNA was amplified using the universal primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT G-3′) [16]. PCR amplification was carried out in a 25 µL reaction mixture containing GoTaq® Green Master Mix (Promega, USA), primers, genomic DNA, and nuclease-free water. Thermal cycling consisted of an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. Amplification products were verified by electrophoresis on 1.5% agarose gels stained with GelRed®.

PCR products were purified using the High Pure PCR Product Purification Kit (Roche Diagnostics, Germany) according to the manufacturer's instructions and submitted to Macrogen Japan (Tokyo, Japan) for Sanger sequencing. The obtained ITS sequence was compared with reference sequences available in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLASTn) to determine the closest taxonomic affiliation of the isolate.

Phylogenetic Analysis

Phylogenetic analysis was performed using the ITS sequence of isolate T1R7 and related reference sequences retrieved from the National Center for Biotechnology Information (NCBI) database. Sequences were aligned using MEGA11 software [17]. A phylogenetic tree was constructed using the Maximum Likelihood (ML) method based on the Kimura two-parameter substitution model. The robustness of the inferred phylogeny was assessed using 1,000 bootstrap replications, and bootstrap values were displayed at the corresponding branch nodes.

Statistical Analysis

All experiments were conducted in triplicate, and data are expressed as mean ± standard deviation (SD). Statistical analyses were performed using R software. Differences between the endophytic fungal isolate T1R7 and the uninoculated control in IAA production were assessed using Welch's two-sample *t*-test. Differences were considered statistically significant when $p < 0.05$.

Results

Colony Characteristics and Growth of the Endophytic Isolate

The endophytic fungal isolate T1R7, identified as *Paraconiothyrium cyclothyrioides*, exhibited moderate growth on potato dextrose agar (PDA) supplemented with chloramphenicol (0.05 g L^{-1}) at 25 °C. After 7 days of incubation, colonies reached a mean diameter of 54.0 ± 4.32 mm (n = 3).

Colony morphology changed during the incubation period. At 5 days, colonies were white with dense aerial mycelium (Figure 1a, b). After 7 days, the colony surface gradually became pale grey to brownish-grey while maintaining a compact, velvety texture with slightly lobate margins (Figure 1c).

The reverse side of the colony was dark brown and no diffusible pigment production was observed throughout the incubation period (Figure 1d).

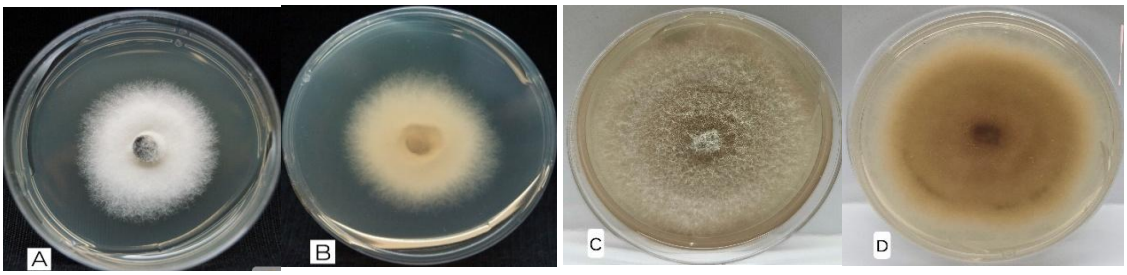

Figure 1. Colony morphology of *Paraconiothyrium cyclothyrioides* isolate T1R7 cultured on PDA supplemented with chloramphenicol at 25 °C. (a) Colony surface after 5 days of incubation; (b) reverse side after 5 days; (c) colony surface after 7 days; and (d) reverse side after 7 days.

Phosphate solubilization ability

The phosphate-solubilizing activity of isolate T1R7 was evaluated on Pikovskaya's agar medium containing tricalcium phosphate as the sole insoluble phosphorus source. After 5 days of incubation at 25 °C, the isolate exhibited colony growth on the medium but did not produce a visible halo zone surrounding the colony (Figure 2). Accordingly, the phosphate solubilization index (SI) remained 1.0 throughout the incubation period.

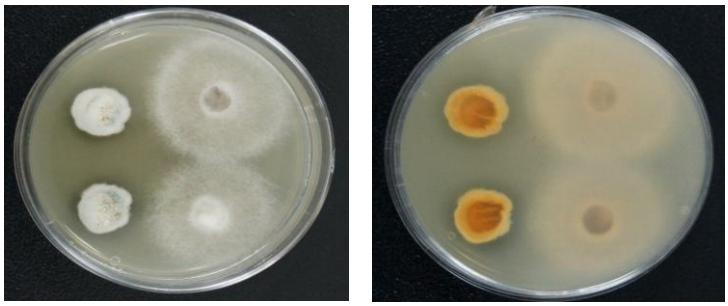

Figure 2. Phosphate-solubilizing activity of fungal isolates on Pikovskaya's agar after 5 days of incubation at 25 °C. Isolate T1R7 (*Paraconiothyrium cyclothyrioides*) (left) showed no halo-zone formation, whereas the phosphate-solubilizing reference isolate (right) produced a distinct clear zone surrounding the colony.

Indole-3-Acetic Acid production

A visible pink coloration developed in the culture supernatant of isolate T1R7 following reaction with Salkowski reagent, indicating the presence of indole-3-acetic acid (IAA) (Figure 3). Based on the standard calibration curve, the isolate produced 6.93 ± 1.96 mg L^{-1} IAA (n = 3).

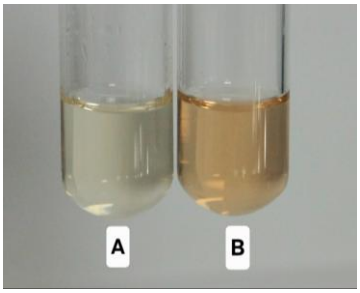

Figure 3. IAA production by the uninoculated control (a) and isolate T1R7 (b) after reaction with Salkowski reagent for 30 min. The pink coloration observed in T1R7 indicates positive IAA production, whereas no color change was observed in the control.

In contrast, the uninoculated control contained 1.13 ± 0.17 mg L^{-1} IAA. The concentration of IAA produced by isolate T1R7 was significantly higher than that of the control according to Welch's two-sample *t*-test ($p < 0.05$) (Table 1).

Table 1.

Indole-3-acetic acid (IAA) concentrations produced by *Paraconiothyrium cyclothyrioides* isolate T1R7 and the uninoculated control

Treatment	IAA concentration (mg/L^{-1})
Control	1.13 ± 0.17
T1R7 (*P. cyclothyrioides*)	6.93 ± 1.96

Values are presented as mean ± standard deviation (n = 3).

Molecular Identification

The internal transcribed spacer (ITS) region of the rDNA of isolate T1R7 was successfully amplified using the universal primers ITS1 and ITS4. BLAST analysis of the obtained sequence against the NCBI database revealed 100% sequence similarity to *Paraconiothyrium cyclothyrioides* (GenBank accession no. PX458088).

Phylogenetic analysis based on ITS sequences placed isolate T1R7 within the *Paraconiothyrium cyclothyrioides* cluster together with closely related reference sequences retrieved from GenBank. *Tricholoma giganteum* was used as the outgroup in the analysis (Figure 4).

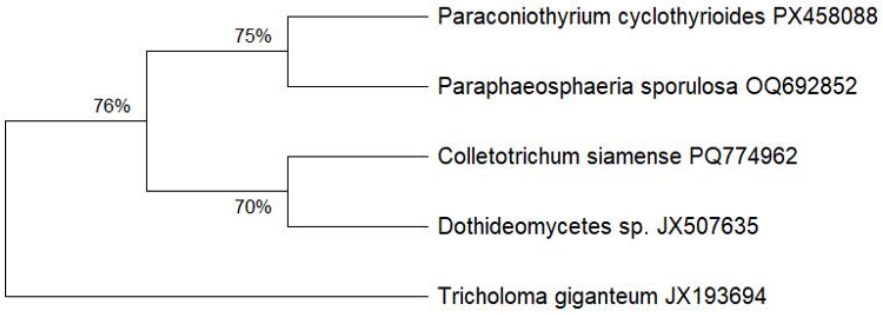

Figure 4. Phylogenetic tree of isolate T1R7 based on ITS sequences constructed using the Maximum Likelihood method in MEGA11. Bootstrap values based on 1,000 replications are shown at the branch nodes. *Tricholoma giganteum* was used as the outgroup.

Discussion

In this study, the endophytic fungal isolate T1R7 obtained from the roots of *Larix sibirica* was identified as *Paraconiothyrium cyclothyrioides* based on ITS sequence analysis. The isolate exhibited characteristic colony morphology and produced indole-3-acetic acid (IAA) at a concentration of 6.93 ± 1.96 mg L^{-1}. In contrast, no phosphate-solubilizing activity was detected on Pikovskaya's agar.

IAA is one of the most important phytohormones involved in plant growth and development, regulating processes such as cell division, cell elongation, root initiation, and vascular differentiation [20]–[22]. Numerous endophytic fungi have been reported to synthesize IAA and contribute to plant growth through hormone-mediated mechanisms [7], [8], [26], [27]. The ability of isolate T1R7 to produce IAA suggests that this strain may possess plant growth-promoting potential.

The concentration of IAA produced by T1R7 was within the range reported for several plant-associated fungi and bacteria that positively influence plant development [20]–[23]. Previous studies have shown that moderate concentrations of auxin can stimulate root growth and biomass accumulation, whereas excessive concentrations may inhibit growth responses [22], [23]. However, the effects of fungal-derived IAA on *L. sibirica* have not yet been evaluated and require further investigation.

IAA biosynthesis in fungi is commonly associated with tryptophan-dependent pathways, in which tryptophan serves as a precursor for auxin production [26]. Because the culture medium used in this study was supplemented with L-tryptophan, the observed IAA production by T1R7 may have

resulted from a similar biosynthetic pathway. Comparable mechanisms have been reported in other endophytic fungi, including *Piriformospora indica*, where fungal-derived IAA contributes to plant growth promotion [27].

Unlike IAA production, isolate T1R7 did not exhibit phosphate-solubilizing activity. The absence of a halo zone on Pikovskaya's agar indicates that the isolate was unable to solubilize tricalcium phosphate under the conditions tested. Similar variability in phosphate-solubilizing capacity has been reported among endophytic fungal species, suggesting that this trait is strain dependent [11], [12]. Therefore, any potential plant growth-promoting effect of T1R7 is more likely to be associated with phytohormone production than with phosphorus mobilization.

Previous studies have reported that certain *Paraconiothyrium* species possess additional beneficial properties, including antagonistic activity against plant pathogens and nematodes. However, these characteristics were not examined in the present study. Further research is needed to evaluate the ecological functions of *P. cyclothyrioides* associated with *L. sibirica* and to determine its potential applications in forestry and plant biotechnology.

Conclusion

An endophytic fungal isolate obtained from the roots of *Larix sibirica* was identified as *Paraconiothyrium cyclothyrioides* based on ITS sequence analysis. The isolate produced indole-3-acetic acid (6.93 ± 1.96 mg L^{-1}) but did not exhibit phosphate-solubilizing activity under the conditions tested. These findings indicate that *P. cyclothyrioides* possesses a plant growth-promoting trait through phytohormone production. Further studies are required to evaluate its effects on larch seedling growth, development, and stress tolerance under greenhouse and field conditions.

Author contribution

O.O. conducted the fungal isolation experiments, analyzed the data, and interpreted the results. E.V. and G.A. conceived and supervised the study, contributed to the experimental design, performed the literature review, and participated in manuscript preparation. Ts.Lkh. contributed to the literature review and manuscript editing. E.Z. and A.T. carried out field surveys and collected root samples. U.B performed the molecular identification and phylogenetic analysis of the fungal isolate. U.J. assisted with polymerase chain reaction (PCR) analysis. All authors read and approved the final manuscript.

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