RESEARCH ARTICLE

Diversity and Screening of Endophytic Fungal Strains from *Prunus* × *yedoensis* for Inhibition of *Taphrina wiesneri* Growth

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ABSTRACT

Prunus × *yedoensis*, which is widely cultivated for ornamental purposes, is highly susceptible to witches' broom disease caused by *Taphrina wiesneri*. Conventional physical and chemical control measures have certain limitations due to environmental concerns, thereby highlighting the need for alternative approaches. In this study, we sought to isolate and identify endophytic fungi from *Prunus* × *yedoensis* and evaluate their potential as biological control agents against *T. wiesneri* using dual culture assays. A total of 45 endophytic fungal species were isolated and identified based on an analysis of internal transcribed spacer sequences. Fungal species composition was found to differ between leaves and twigs, with *Diaporthe eres* and *Nothophoma quercina* being the most prevalent species. Dual culture assays revealed that *Aspergillus flavus,* and *Trichoderma guizhouense* have significant inhibitory effects on *T. wiesneri* (inhibition index > 0.5). These findings indicate that endophytic fungi could be harnessed to facilitate the suppression of witches' broom disease in *Prunus* × *yedoensis*

Keywords: Biological Control Agent, Dual Culture, Endophytic fungi, *Prunus* × *yedoensis*, *Taphrina wiesneri*, Witches' broom

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INTRODUCTION

Prunus × *yedoensis* Matsum., a plant prized for its ornamental value, is widely planted in urban landscapes and public parks [1]. However, its susceptibility to witches' broom disease caused by the fungus *Taphrina wiesneri* poses a serious threat to its health and longevity [2]. Infected twigs tend to be characterized by abnormal shoot proliferation and leaf expansion, leading to suppressed flowering and reduced tree vitality. In severe cases, twig dieback occurs within a few years, and prolonged infection can result in tree mortality [3,4].

Taphrina wiesneri has been established to colonize host tissues and produce phytohormones, such as indole-3-acetic acid and cytokinin, that contribute to disrupting the host's hormonal balance and inducing

disease symptoms [5,6]. Current control strategies include the physical removal of infected twigs and the application of triazole fungicides, such as tebuconazole and difenoconazole [7]. However, these approaches are often insufficient for long-term disease management and raise ecological concerns relating to chemical use and pathogen resistance [8,9].

Endophytic fungi, which reside within plant tissues without causing disease, have garnered attention for their potential as biological control agents [10,11]. These fungi can inhibit plant pathogens via competition, mycoparasitism, or the production of antifungal compounds, and the findings of several studies have provided evidence of the antagonistic activity of endophytes against important phytopathogens, thereby highlighting their potential utility in sustainable plant disease management [12–14].

In this study, we sought to isolate and identify endophytic fungi from healthy *Prunus* × *yedoensis* tissues and to evaluate their antagonistic activity against *T. wiesneri* using dual culture assays. By identifying potential biocontrol candidates, our findings in this study will provide a basis for developing eco-friendly alternatives for managing witches' broom disease in cherry trees.

MATERIALS AND METHODS

Sample collection and isolation of endophytic fungi

In April 2024, samples of healthy leaves and twigs were collected from 12 *Prunus* × *yedoensis* trees in Seoul, and in September 2024, from five trees in Cheongju. Having initially washed under running tap water, these samples were sequentially surface-sterilized using 35% hydrogen peroxide (H_2O_2) for 2 min, followed by 70% ethanol for 30 s, and were then rinsed three times with sterile water [15]. The surface-sterilized tissues were thereafter cut into 10 × 10 mm (leaves) or 10 mm (twigs) segments and placed on potato dextrose agar (PDA; Kisan Bio, Seoul, Korea). Plates were incubated at 25°C and monitored for the emergence of fungal colonies, which were subsequently sub-cultured onto fresh PDA to obtain pure isolates.

Molecular identification of fungal isolates

Genomic DNA was extracted from the isolates using a HiGene™ Genomic DNA Prep Kit for Fungi (BioFACT, Korea). The internal transcribed spacer (ITS) region, including ITS1, 5.8S rDNA, and ITS2 sequences, was amplified using the primer pair ITS1-F and ITS4 [16,17]. Additional DNA regions, namely, the large subunit (LSU) and translation elongation factor-1-α (*Tef1α*), were also amplified, using LR0R/LR5 [18] and EF1-983F/EF1-1567R [19], respectively. Sequences were identified on the basis of BLAST (Basic Local Alignment Search Tool) searches against the NCBI (National Center for Biotechnology Information) GenBank database, and have been submitted to this database.

Dual culture assays

Dual culture assays were performed to assess the antagonistic activity of endophytic fungal isolates

against *Taphrina wiesneri* strain KACC45487, which was obtained from the Korean Agricultural Culture Collection (KACC). A 7-mm-diameter agar plug of *T. wiesneri* was placed 10 mm from the edge of 90-mm-diameter PDA plates and incubated at 25°C for 7 days. Subsequently, a plug of an endophytic fungal isolate was placed on the opposite side of plates, and the plates were incubated at 25°C for a further 21 days. Each assay was performed in triplicate. As a control, *T. wiesneri* was cultured alone under the same conditions. The area of *T. wiesneri* colonies was measured using ImageJ software [20], and the inhibition index (I) was calculated using the following formula:

$$I = (A_{control} - A_{treatment})/A_{control}$$

where $A_{control}$ is the colony area of *T. wiesneri* in control plates, and $A_{treatment}$ is the colony area in dual-culture plates [21]. Statistical analysis of the inhibitory effects was conducted using Student's *t*-test in R (v. 4.2.2), for which, p-values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Diversity of endophytic fungi

A total of 204 fungal strains were isolated from the leaves and twigs of *Prunus* × *yedoensis*. On the basis of BLAST analysis of the ITS sequences of these isolates against accessions in the GenBank database, strains with ≥ 98% similarity were identified at the species level, whereas those with lower similarity were assigned to the genus level (Table 1). A total of 45 endophytic fungal species from 21 genera were identified from *Prunus* × *yedoensis*, among which 33 species from 19 genera were isolated from leaves, and 25 species from nine genera were obtained from twigs. Common species found in both organs included *Alternaria alternata*, *Aureobasidium pullulans*, and *Colletotrichum fioriniae* (Table 1). *Nothophoma quercina* was identified as the most frequently isolated species from leaves (41.2%), followed by *Paraconiothyrium brasiliense* (35.3%) and *Dothiorella gregaria* (29.4%), whereas *Diaporthe eres* was the most common species detected in twigs (70.6%), followed by *Botryosphaeria dothidea* (52.9%) and *Diaporthe nobilis* (41.2%) (Table 1). Statistical analysis of species diversity indices revealed that values of the Shannon–Wiener diversity index were significantly higher for fungi in twigs than those in leaves ($p < 0.05$) (Fig. 1). However, we detected no significant differences between leaves and twigs with respect to species richness and evenness. These findings accordingly reveal differences in the composition and diversity of endophytic fungi in the leaves and twigs of *Prunus* × *yedoensis*, which is consistent with previously reported findings indicating that fungal communities differ depending on plant tissue and environmental conditions [21,22].

Table 1. Frequency of endophytic fungi isolated from the leaves and twigs of *Prunus* × *yedoensis*

Endophytic fungal species	Representative strain	GenBank Accession No (ITS)	Frequency of isolates (%)	
			Leaves	Twigs
Alternaria alternata	24N0182	PV668950	5.88	17.65
Anteaglonium sp.	24N0144	PV791132	5.88	0.00
Aspergillus flavus	24N0267	PV682710	11.76	0.00
Aspergillus niger	24N0281	PV791150	5.88	0.00
Aureobasidium melanogenum	24N0137	PV790968	5.88	0.00
Aureobasidium pullulans	24N0265	PV791115	11.76	5.88
Botryosphaeria dothidea	24N0282	PV791156	17.65	52.94
Cephalotheca foveolate	24N0279	PV791148	5.88	0.00
Cladosporium cladosporioides	24N0149	PV791003	23.53	0.00
Colletotrichum fioriniae	24N0262	PV791020	5.88	17.65
Colletotrichum gigasporum	24N0300	PV791160	5.88	0.00
Colletotrichum gloeosporioides	24N0181	PV791007	0.00	11.76
Colletotrichum siamense	24N0230	PV791010	0.00	5.88
Collophorina rubra	24N0139	PV791002	5.88	0.00
Diaporthe alnea	24N0287	PV791154	5.88	0.00
Diaporthe amygdali	24N0193	PV791179	17.65	23.53
Diaporthe capsica	24N0238	PV791012	0.00	5.88
Diaporthe celeris	24N0312	PV791165	5.88	5.88
Diaporthe cotoneastri	24N0234	PV791011	0.00	17.65
Diaporthe eres	24N0261	PV791018	11.76	70.59
Diaporthe fukushii	24N0218	PV791008	0.00	5.88
Diaporthe fusicola	24N0317	PV791169	5.88	11.76
Diaporthe garethjonesii	24N0314	PV791168	0.00	5.88
Diaporthe nobilis	24N0309	PV791166	5.88	41.18
Diaporthe perseae	24N0306	PV791162	0.00	17.65
Diaporthe phaseolorum	24N0199	PV791180	0.00	5.88
Diaporthe phragmitis	24N0241	PV791014	0.00	11.76
Diaporthe vaccinii	24N0208	PV791006	5.88	11.76
Dothiorella gregaria	24N0255	PV791017	29.41	11.76
Epicoccum nigrum	24N0248	PV791016	0.00	5.88
Fusarium solani	24N0277	PV791143	5.88	0.00
Fusarium verticillioides	24N0116	PV668820	5.88	0.00
Kalmusia longispora	24N0248	PV791142	5.88	0.00
Nothophoma quercina	24N0273	PV791116	41.18	17.65
Paraconiothyrium brasiliense	24N0114	PV790601	35.29	0.00
Penicillium brevicompactum	24N0119	PV790967	5.88	0.00
Penicillium griseofulvum	24N0280	PV791149	5.88	0.00
Pestalotiopsis chamaeropis	24N0166	PV791004	0.00	5.88
Pestalotiopsis cocculin	24N0264	PV791021	5.88	0.00
Pestalotiopsis kenyana	24N0224	PV791009	0.00	5.88
Pestalotiopsis microspora	24N0130	PV668952	11.76	17.65
Phyllosticta capitalensis	24N0297	PV791161	5.88	0.00
Quixadomyces sp.	24N0115	PV809772	5.88	0.00
Trichoderma guizhouense	24N0293	PV682713	5.88	0.00
Xylaria primorskensis	24N0276	PV791133	5.88	0.00

ITS, internal transcribed spacer.

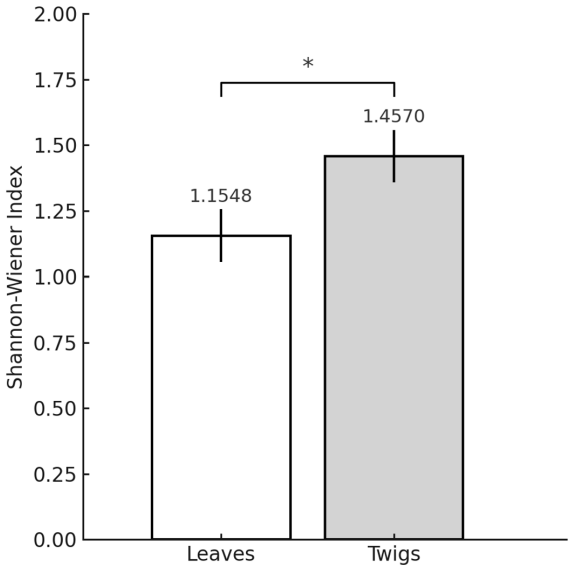

Fig. 1. Comparison of the Shannon–Wiener diversity index values obtained for endophytic fungi isolated from the leaves and twigs of *Prunus* × *yedoensis*. Values of the Shannon–Wiener diversity index for twig isolates were approximately 0.138- to 0.464-fold higher than those for leaf isolates (t = -2.614, p = 0.016). * Indicates $p < 0.05$.

Dual culture assays with *T. wiesneri*

Twelve endophytic fungal species, selected based on their prevalence in both sampling regions and plant organs, were tested against *T. wiesneri* KACC45487 using dual culture assays. The results revealed that although none of the assessed endophytic fungi produced zones of inhibition of *T. wiesneri* growth, within 7 days, some fungal isolates had overgrown the pathogen colony. Among these, five isolates were found to have caused a significant suppression of pathogen growth ($p < 0.05$) (Fig. 2), with *Aspergillus flavus* 24N0267 and *Trichoderma guizhouense* 24N0293 isolated from leaves being characterized by inhibition indices (I) exceeding 0.5 (Table 2).

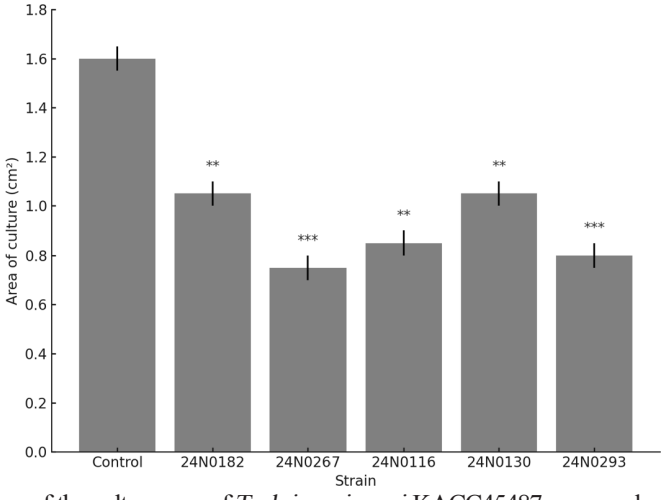

Fig. 2. Comparison of the culture area of *Taphrina wiesneri* KACC45487 measured on the 21st day of dual culture with selected endophytic fungi. Bars represent mean areas with standard errors. Asterisks indicate significant differences compared with the control (KACC45487) based on analysis using Student's t-test: ** p <0.01, *** $p < 0.001$.

Table 2. Molecular identification and the inhibition index of five endophytic fungal strains against *Taphrina wiesneri* KACC45487

Fungal strains	GenBank accession numbers submitted			Inhibition index (I)
	ITS	LSU	*Tef1α*	
Alternaria alternata 24N0182	PV668950	PV682695	PV691783	0.34
Aspergillus flavus 24N0267	PV682710	PV682711	PV691784	0.56
Fusarium verticillioides 24N0116	PV668820	PV668962	PV691782	0.49
Pestalotiopsis microspora 24N0130	PV668952	PV682712	PV684600	0.38
Trichoderma guizhouense 24N0293	PV682713	PV682714	PV691785	0.51

ITS, internal transcribed spacer; LSU, large subunit; *Tef1α*, translation elongation factor-1.

Aspergillus flavus is widely encountered as an endophytic fungus in woody and herbaceous plants worldwide [23,24]. It produces mycotoxins, such as aflatoxin B1 (AFB1) and aspergillic acid, along with extracellular hydrolytic enzymes, including pectinase and protease, which contribute to fungal defense mechanisms [25,26]. These enzymes can potentially degrade the cell walls of other fungi, thereby contributing to antagonistic activity of this species. However, AFB1 has been established to be a potent carcinogen that contaminates crops such as peanuts and corn, and thus further studies are necessary to assess ecological safety of *A. flavus* for biocontrol applications [27]. Similarly, *Alternaria alternata* produces AAL- and AF-toxins and can act as an opportunistic pathogen in several crops [28,29]. Moreover, *Fusarium verticillioides* induces wilt and rot in maize [30]. Therefore, additional studies are required to reduce the toxicity and enhance the stability of these fungi for use as biocontrol agents.

In contrast, *Pestalotiopsis microspora* has been established to produce pestacin, an antifungal compound, and taxol, an anticancer agent [31,32], whereas *Trichoderma guizhouense* is an efficient producer of cellulase and has been applied to enhance crop productivity [33]. It is accordingly speculated that its potential utility as a biocontrol agent may involve mycoparasitism mediated via the production of cell wall-degrading enzymes. On the basis of the evidence obtained to data, these two species are thus considered promising candidates for safe and effective biological control applications [34].

Given that *T. wiesneri* resides within host tissues and induces disease symptoms, employing endophytic fungi that naturally inhabit the same niche without harming the plant represents a sustainable and ecologically safe strategy for disease management. If further experiments confirm the inhibitory effects of selected endophytic fungi on *T. wiesneri* in vivo, this approach could be practically applied in disease control. Moreover, if the five fungal species identified in this study are found at significantly lower frequencies in diseased trees than in healthy ones, this would provide additional evidence in support of their role as potential biocontrol agents against witches' broom disease.

In this study, we identified 45 endophytic fungal species isolated from *Prunus* × *yedoensis* and assessed their potential as biocontrol agents against *T. wiesneri*. Among these fungal isolates, five strains were demonstrated to have significant inhibitory effects against *T. wiesneri* in dual culture assays. Further studies should evaluate the field efficacy and ecological safety of these strains to facilitate the development of sustainable biological control strategies against witches' broom disease.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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