RESEARCH ARTICLE

Species Diversity of Endophytic Fungi Isolated from *Taxus cuspidata* Inhabiting Mt. Hallasan, Korea

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ABSTRACT

Several endophytic fungal strains were isolated from *Taxus cuspidata* and identified by molecular analysis of the internal transcribed spacer and RNA polymerase II second largest subunit. This study aimed to determine the relative abundance and compare the species diversity of endophytic fungal communities within needle leaves and twigs. We identified a total of 49 endophytic fungal species. Notably, two species, *Trichoderma dingleyae* and *Xylaria cubensis,* were discovered to be previously unrecorded in Korea. The fungal communities in both plant tissues demonstrated distinct species composition. Differences were observed in the relative abundance and species diversity index between needle leaves and twigs. Our findings suggest that the host plant tissues influence the species diversity of endophytic fungal communities.

Keywords: Endophytic fungi, Jeju island, Species diversity, *Trichoderma dingleyae, Xylaria cubensis*

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INTRODUCTION

Endophytic fungi are symbiotic with plants [1]. They live in plant tissues without causing diseases [2]. These fungi chemically defend their host plants against herbivores, insects, and external pathogens [3,4]. Additionally, they enhance the host plants' resistance to environmental stressors [5]. Endophytic fungi form symbiotic relationships with a range of plants across different climatic zones [6,7]. Their presence is noted in almost all plants, including bryophytes, ferns, conifers, evergreen broad-leaved trees, and deciduous broad-leaved trees [8-12]. These fungi colonize a variety of plant tissues, from vegetative organs, such as roots, stems, and leaves, to reproductive organs, such as flowers and fruits [7]. The relative abundance, species diversity, and community structure of endophytic fungi can vary based on the specific plant tissue [13,14].

Taxus cuspidata Siebold et Zucc. is an evergreen coniferous tree that is geographically distributed in the Far East of Russia, northeastern China, Japan, the Korean Peninsula, and Jeju Island. *T. cuspidata* grows at altitudes ranging from 700 to 2,500 meters above sea level from Mt. Sungjeoksan in North Korea to Mt. Hallasan on Jeju Island, South Korea [15]. Various tissues of *T. cuspidata* such as the stem bark, root bark, fibrous roots, twigs, and leaves, produce the anticancer substance taxol (paclitaxel) [16]. Furthermore, endophytic fungi, such as *Pestalotiopsis* spp. isolated from *T. cuspidata*, have been shown to produce taxol [17,18].

In this study, we isolated and identified the endophytic fungal strains from *T. cuspidata* inhabiting Yeongsil area in Mt. Hallasan, Jeju Island. We attempted to confirm the species diversity and community structure according to the plant tissue parts from where the endophytic fungi were isolated.

MATERIAL AND METHODS

Sample collection and fungal isolation

Two to three twig samples with needle leaves of *T. cuspidata* were collected per tree from the Yeongsil area on Mt. Hallasan in December 2022. We collected samples from 18 individuals and these samples were transported to the laboratory within 24 h. Healthy tissues without disease were selected and surface-sterilized in 30% H_2O_2 solution for 2 min and 70% EtOH for 1 min [19]. Three sterilized pieces of the same tissue were placed on potato dextrose agar (PDA; Difco Lab., Detroit, MI, USA) medium. Two media with leaf samples and two media with twig samples were prepared per each individual, and observed while culturing at 25℃. Once the hyphae were confirmed to extend from the tissue, they were sub-cultured in fresh PDA medium for pure culture of the fungal strain.

Morphological characterization

The morphology of the colonies was observed after 7 d of incubation in PDA. The morphology of the unrecorded species was further observed by culturing on malt extract agar (MEA; Kisan Bio, Seoul, Korea) for 7 d. The spores were observed under an optical microscope (Axio Imager A2; Carl Zeiss, Oberkochen, Germany).

Molecular identification

To identify fungal strains, genomic DNA was extracted from the mycelia using the HiGene Genomic DNA Prep Kit (BioFACT, Daejeon, Korea). The internal transcribed spacer (ITS) region containing the 5.8S region of rDNA was amplified using the fungal-specific primers ITS1F and ITS4 [20]. For a more accurate identification of the previously unrecorded fungal species, we amplified the RNA polymerase II second largest subunit (*RPB2*) region with the specific primers fRPB2-5f and fRPB2-7cR [21]. Polymerase chain reaction products were electrophoresed on a 1.5% agarose gel for 20 min. When an adequate DNA fragment size was confirmed, DNA sequencing was performed (SolGent Co., Ltd., Daejeon, Korea). Fungal species were identified by matching their DNA sequence similarity to that of previously recorded species using BLAST from the National Center for Biological Information. Phylogenetic analysis was conducted to identify unrecorded species. The Neighbor-joining (NJ) method was used for the ITS and RPB2 regions. The Kimura-2-parameter model and 1,000 bootstrap replications were adjusted using the NJ method in MEGA7 [22].

Species diversity

After the species identification, we calculated the relative abundance as follow:

$$\text{Relative abundance}(\%) = \frac{\text{population of specific fungal species}}{\text{population of all isolated fungal species}} \times 100$$

To determine species diversity, we calculated Shannon's diversity index (H´) and species evenness (E) by using MVSP 3.2 software (Kovach Computing Services, Anglesey, Wales, UK). Finally, we compared the relative abundance and the species diversity indices between the needle leaves and the twigs of *T. cuspidata*.

RESULTS AND DISCUSSION

We isolated a total of 98 endophytic fungal strains from the needle leaves and twigs of *T. cuspidata*. Molecular identification revealed 49 fungal species across 33 genera (Fig. 1). From needle leaves, 12 species representing 10 genera were isolated, while from the twigs, 43 species from 29 genera were isolated (Table 1).

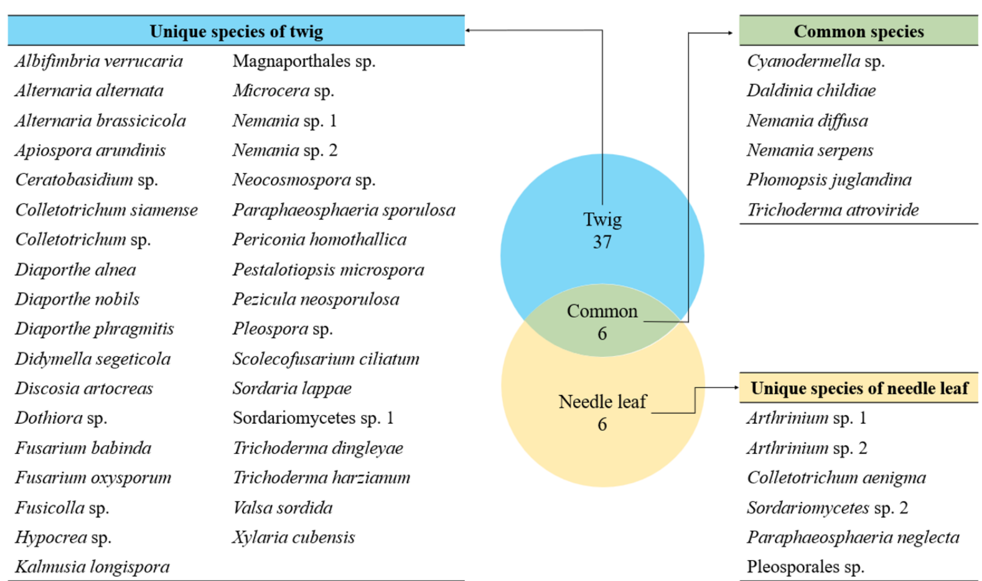

Fig. 1. Comparison of the isolated endophytic fungal species between the needle leaf and the twig in *Taxus cuspidata*.

Table 1. The identified endophytic fungal species and the species diversity of endophytic fungal communities.

Strain No.	Accession No.	Endophytic fungal species	Similarity (%)	RA[a] (%)	
				Twig	Needle leaf
KNUE23N002	MN341420	*Apiospora arundinis*	99.82	6.33	
KNUE23N004	KX866870	*Diaporthe phragmitis*	100	6.33	
KNUE23N006	LC505117	*Nemania diffusa*	99.28	6.33	21.05
KNUE23N007	MT635275	*Alternaria brassicicola*	100	1.27	
KNUE23N008	MK396602	*Diaporthe eres*	99.64	10.13	
KNUE23N009	MH861015	*Fusicolla* sp.	99.81	2.53	
KNUE23N015	OP163476	*Didymella segeticola*	99.61	2.53	
KNUE23N019	MF379326	*Diaporthe amygdali*	100	2.53	
KNUE23N020	FJ481946	*Pleospora* sp.	89.29	1.27	
KNUE23N022	EF211127	*Albifimbria verrucaria*	99.13	1.27	
KNUE23N023	NR_171058	*Neocosmospora* sp.	99.82	1.27	
KNUE23N025	KU837233	*Phomopsis juglandina*	99.82	2.53	10.53
KNUE23N036	OP163537	*Diaporthe alnea*	100	1.27	
KNUE23N038	OK090963	*Scolecofusarium ciliatum*	99.62	1.27	
KNUE23N039	MT446212	*Periconia homothallica*	99.64	1.27	
KNUE23N042	MT738229	*Paraphaeosphaeria sporulosa*	100	1.27	
KNUE23N043	MH864593	*Trichoderma dingleyae*	99.83	1.27	
KNUE23N044	MH930457	*Trichoderma harzianum*	99.35	3.80	
KNUE23N046	MK275241	*Ceratobasidium* sp.	98.16	3.80	
KNUE23N048	MN341717	*Daldinia childiae*	100	3.80	15.79
KNUE23N050	MF770848	*Nemania* sp. 1	99.28	2.53	
KNUE23N053	JX914483	*Arthrinium* sp. 1	94.18		10.53
KNUE23N057	LC206655	*Diaporthe nobils*	99.63	2.53	
KNUE23N060	OP699815	*Sordaria lappae*	99.82	1.27	
KNUE23N067	MT644300	*Pestalotiopsis microspora*	100	1.27	
KNUE23N070	MK804348	*Microcera* sp.	100	1.27	
KNUE23N071	OP862829	*Colletotrichum siamense*	100	1.27	
KNUE23N077	OP741026	*Kalmusia longispora*	99.82	1.27	
KNUE23N096	MT341775	*Trichoderma atroviride*	100	1.27	5.26
KNUE23N097	AF055218	*Hypocrea* sp.	98.60	1.27	
KNUE23N100	NR_159861	*Fusarium babinda*	100	1.27	
KNUE23N111	MT482502	*Fusarium oxysporum*	99.81	3.80	
KNUE23N127	MT183734	Sordariomycetes sp. 1	98.93	1.27	
KNUE23N131	MZ854248	*Xylaria cubensis*	100	1.27	
KNUE23N133	KJ739458	*Valsa sordida*	99.15	1.27	
KNUE23N137	KT758843	*Cyanodermella* sp.	90.94	1.27	5.26
KNUE23N148	MT920572	*Nemania serpens*	99.30	1.27	5.26
KNUE23N179	OQ001032	*Alternaria alternata*	100	1.27	
KNUE23N180	LC431573	*Nemania* sp. 2	99.47	1.27	
KNUE23N184	MH860399	*Dothiora* sp.	96.65	1.27	
KNUE23N194	OP380745	*Discosia artocreas*	99.46	1.27	
KNUE23N200	MT183813	Sordariomycetes sp. 2	100		5.26
KNUE23N204	MK367476	Pleosporales sp.	97.44		5.26
KNUE23N216	JN198467	Magnaporthales sp.	99.09	5.06	
KNUE23N217	OU989423	*Paraphaeosphaeria neglecta*	99.83		5.26
KNUE23N225	LC206659	*Pezicula neosporulosa*	100	1.27	
KNUE23N227	MZ348514	*Colletotrichum* sp.	100	1.27	
KNUE23N228	KP050630	*Arthrinium* sp. 2	93.95		5.26
KNUE23N277	MT872061	*Colletotrichum aenigma*	100		5.26
Shannon's index (H´)				3.51	2.33
Species evenness (E)				0.93	0.94
Number of species				43	12

[a]RA: relative abundance, the blanks indicate the 0% value.

Among the isolated endophytes, only *Ceratobasidium* sp. belonged to the phylum Basidiomycota, and all other species belonged to the phylum Ascomycota. Six species (*Cyanodermella asteris, Daldinia childiae, Nemania diffusa, N. serpens, Phomopsis juglandina*, and *Trichoderma atroviride*) were isolated from both needle leaves and twigs. The remaining species were isolated only from either needle leaves or twigs (Fig. 1). Among the species isolated from needle leaves, *N. diffusa* showed the highest relative abundance at 21.05%. In twigs, *Diaporthe eres* showed the highest relative abundance of 10.13%. This suggests that the endophytic fungal communities in needle leaves and twig have distinct species composition, indicating that the plant tissue sites can influence the structure of endophytic fungal community [23].

Of the fungal species isolated, two had not been previously unrecorded in Korea. We describe the morphological characteristics and phylogenetic analysis results for these two fungal strains.

Trichoderma dingleyae Samuels & Dodd, Studies in Mycology 56: 108 (2006) [MB#501036]

Morphological characteristics: The diameter of the colonies on both PDA and MEA was approximately 45 mm. The colonies on PDA (Figs 2A and E) and MEA (Figs 2B and F) were entirely pale beige or light gray; however, on PDA, the margins showed a paler color pattern because the fluffy aerial mycelium was concentrated in the center. The mycelia exhibited an undulating growth pattern from the center to the margin on PDA, whereas a radial growth pattern was observed on MEA. The colonies showed flat elevations on PDA and MEA. The conidia were hyaline, colorless, or sometimes pigmented and yellowish-brown. They appeared to grow in clusters and exhibited an aseptate, ovoid shape (Figs 2I and J). The conidia were (2.73-) 3.17-3.45 (-3.96)×(2.23-) 2.77-2.98 (-3.31) μm in diameter (n=20).

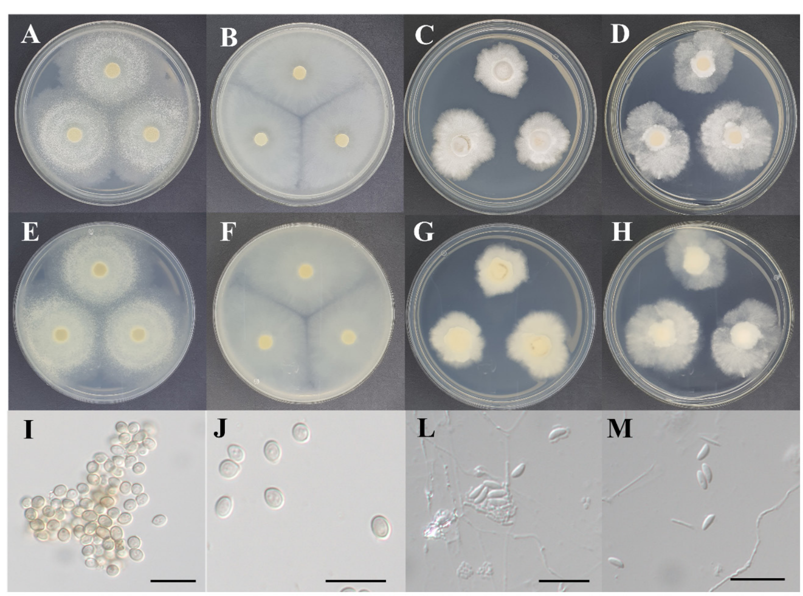

Fig. 2. Cultural characteristics of two fungal strains. Colonies of *Trichoderma dingleyae* KNUE23N043 grown for 7 days on potato dextrose agar (PDA) (A: surface, E: reverse) and malt extract agar (MEA) (B: surface, F: reverse), conidia (I, J); Colonies of *Xylaria cubensis* KNUE23N131 grown for 7 days on PDA (C: surface, G: reverse) and MEA (D: surface, H: reverse), conidia (L, M) (Scale bars=10 μm).

Specimen examined: Yeongsil, Mt. Hallasan, Seogwipo-si, Jeju-do, Republic of Korea; N33°18′54.67″, E126°28′31.058″, December 02, 2022, isolated from the twig of *Taxus cuspidata*, strain KNUE23N043, NIBR No. NIBRFGC000510444; GenBank accession No. OR689232 (ITS) and OR715106 (RPB2).

Phylogenetic analysis: The DNA sequence from the ITS region of KNUE23N043 showed 99.83% similarity with that of MT530250, whereas the sequence from the *RPB2* regene showed 98.07% similarity with that of EU341803. The combined DNA sequence formed a monophyletic group with *T. dingleyae* strain CBS119056 in the NJ phylogenetic tree (Fig. 3).

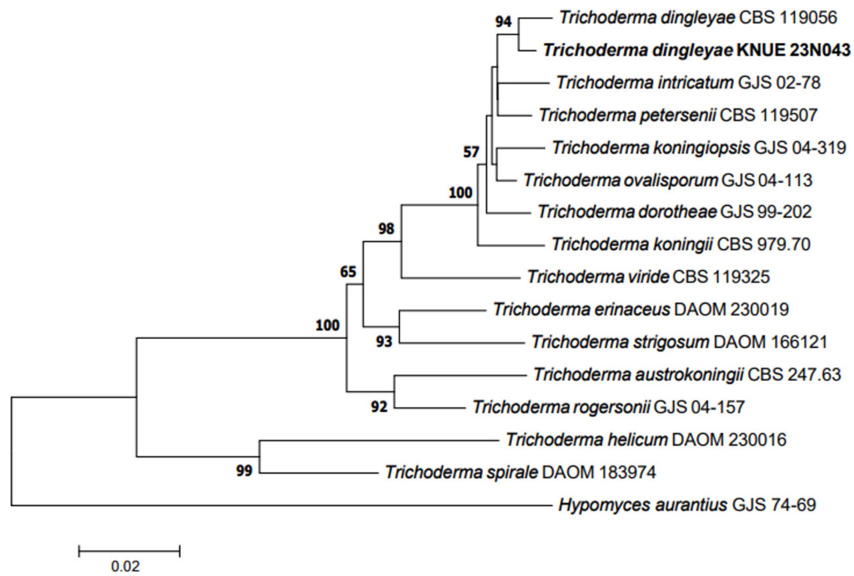

Fig. 3. Neighbor-joining phylogenetic tree of *Trichoderma dingleyae* KNUE23N043 based on the internal transcribed spacer (ITS) and RNA polymerase Ⅱ second largest subunit (*RPB2*) DNA sequences. Test of phylogeny was 1,000 replicated with a bootstrap method. *Hypomyces aurantius* denotes an outgroup. The fungal strain isolated in this study is in bold.

Note: *Trichoderma dingleyae* was initially isolated from the bark of *Nothofagus* spp. in New Zealand. This species was an anamorph of *Hypocrea dingleyae*. This species was derived from various phenotypes of *Trichoderma koningii*, and was reported as a new species in 2006 [24]. The conidiophores arise laterally from the hyphae and produce broadly ellipsoidal conidia (approximately 4.1-4.3×3.1-3.2 μm) [24]. In the present study, we did not observe any conidiophores; however, the overall morphological characteristics of the conidia we observed were consistent with the original description. The phylogenetic analysis confirmed that *T. dingleyae* KNUE23N043 can be distinguished from other morphologically related species such as *T. koningii*, *T. dorotheae*, and *T. intricatum* [24]. Based on these analyses, we identified the strain KNUE23N043 as *T. dingleyae*.

Xylaria cubensis (Mont.) Fr., Nova Acta Regiae Societatis Scientiarum Upsaliensis Ser. 3, 1: 126 (1851) [MB#179243]

Morphological characteristics: The diameter of the colonies on PDA was 29.83±2.30 mm. The colonies were bright white on the surface and beige to ivory on the reverse. The colonies grew radially with irregular margins and increased elevation (Figs 2C and G). The diameter of the colonies on MEA was 37.03 ±1.95 mm. The colonies on both the surface and the reverse were light white. The woolly aerial mycelia were concentrated at the center of the colony, and the substrate mycelia grew radially. The colonies had irregular margins and flat elevations (Figs 2D and H). The conidia were hyaline and colorless and occurred from the lateral side of the hyphae and seemed to form layers because they grew in a sector form (Fig. 2L). They showed an aseptate, ellipsoidal to fusiform shape and were usually curved (Fig. 2M). The conidia were (2.79-) 3.18-3.43 (-3.84)×(1.13-) 1.38-1.51 (-1.81) μm in diam (n=20).

Specimen examined: Yeongsil, Mt. Hallasan, Seogwipo-si, Jeju-do, Republic of Korea; N33°20′19.583″, E126°29′6.673″, 2nd December, 2022, isolated from the twig of *Taxus cuspidata*, strain KNUE23N131, NIBR No. NIBRFGC000510445; GenBank accession No. OR690434 (ITS) and OR715107 (*RPB2*).

Phylogenetic analysis: The DNA sequence from the ITS region of KNUE23N131 showed 99.08% similarity with that of MF682325, whereas the sequence from the *RPB2* regene showed 98.22% similarity with that of MN917802. The combined DNA sequences formed a monophyletic group with *X. cubensis* voucher 515 in the NJ phylogenetic tree (Fig. 4).

Note: *Xylaria cubensis* was initially reported as *Hypoxylon cubense* in 1840, and was later recombined

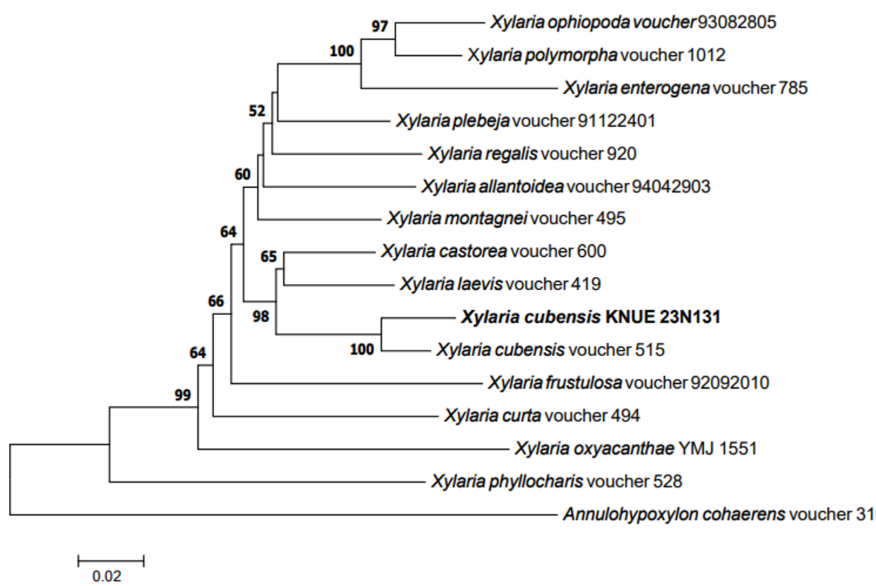

Fig. 4. Neighbor-joining phylogenetic tree of *Xylaria cubensis* KNUE23N131 based on the internal transcribed spacer (ITS) and *RPB2* DNA sequences. Test of phylogeny was 1,000 replicated with a bootstrap method. *Annulohypoxylon cohaerens* denotes an outgroup. The fungal strain isolated in this study is in bold.

with the genus *Xylaria* in 1851 [25]. The conidia of *X. cubensis* are hyaline, either obovate, or ellipsoidal, 1-celled, and grow with sympodial branching from the conidiophore [26]. The conidia observed in the present study also exhibited these morphological characteristics. *Xylaria cubensis* has been isolated as an endophyte from *Litsea akoensis* in Taiwan [27], as well as mangroves in Thailand [28]. This species produces secondary metabolites such as xylaritriol, isosclerone, and akotriol. These metabolites can be used as antimicrobial and anti-inflammatory agents [29].

Pestalotiopsis microspora, *Alternaria alternata*, and *A. brassicola* were isolated from the twigs. Both *P. microspora* and *A. alternata* have been reported to produce the taxol [18,30]. While *Alternaria brassicola* can also produce taxol, its known host plant is *Terminalia arjuna* [31]. Beyond *Pestalotiopsis* spp. and *Alternaria* spp., other endophytic fungal species from *T. cuspidata* also have the potential to produce taxol [17]. Therefore, further screening for taxol production is warranted for the other endophytic fungal species isolated in this study.

The Shannon's index was higher for twigs (H′=3.51) than for needle leaves (H′=2.33). Conversely, species evenness was slightly higher in needle leaves (E=0.93) compared to twigs (E = 0.94), though the difference was not significant. A previous study found higher number of fungal strains in leaves; however, some particular species were dominant and thus, species diversity was found to be higher in the lignified branch bark than in the leaves [13].

In the present study, *Ceratobasidium* sp., typically found in soil or plant roots, was isolated from a twig of *T. cuspidata*. This species is commonly recognized as a mycorrhizal or endophytic symbiont in plant roots [32]. While endophytic fungi often undergo horizontal transmission between host plants [33], and some fungal species prefer specific plant tissue parts [34]; however, the presence of *Ceratobasidium* sp. in twigs suggests potential involvement of vertical transmission in the distribution of endophytic fungi [35,36].

Endophytic fungi and their host plants have a very close evolutionary history, co-evolving through mutual interactions [37]. Studies on the relationship between host plants and endophytic fungal community structures will provide a basis for understanding the interactions between endophytic fungi and their host plants.

CONFLICT OF INTERESTS

No conflict of interest was reported by the authors.

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REFERENCES

1. Carroll G. Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. Ecology 1988;69:2-9.
2. Gao FK, Dai CC, Liu XZ. Mechanisms of fungal endophytes in plant protection against pathogens. Afr J Microbiol Res 2010;4:1346-51.

3. Bouton J, Gates R, Belesky D, Owsley M. Yield and persistence of tall fescue in the southeastern coastal plain after removal of its endophyte. Agron J 1993;85:52-5.
4. Rowan DD, Latch GC, Bacon C, White J. Utilization of endophyte-infected perennial ryegrasses for increased insect resistance. Biotechnology of Endophytic Fungi of Grasses. Boca Raton: CRC Press; 1994. p. 169-83.
5. Arachevaleta M, Bacon CW, Hoveland CS, Radcliffe DE. Effect of the tall fescue endophyte on plant response to environmental stress. Agron J 1989;81:83-90.
6. Zhang HW, Song YC, Tan RX. Biology and chemistry of endophytes. Nat Prod Rep 2006;23:753-71.
7. Eo JK, Choi JW, Eom AH. Diversity, distribution, and host plant of endophytic fungi: a focus on Korea. Mycobiology 2022;50:399-407.
8. Choi HS, Park H, Eo JK, Eom AH. Unrecorded endophytic fungi isolated from *Mnium heterophyllum* and *Hypnum plumaeforme* in Korea: *Biscogniauxia petrensis* and *Cercophora thailandica*. Kor J Mycol 2020;48:55-61.
9. Oh SY, Park KH, Baldrian P, Fong JJ, Kwon HJ, Kim SY, Lim YW. Fungal diversity living in the root and sporophore of the endemic Korean fern *Mankyua chejuense*. Fungal Ecol 2021;50:101038.
10. Eo JK, Eom AH. Diversity of foliar endophytic fungi inhabiting coniferous trees in Korea. Kor J Mycol 2018;46:205-11.
11. Gwon JH, Park H, Lee JC, Choi JW, Eom AH. Unreported endophytic fungi isolated from the leaves of evergreen woody plants in Korea: *Muyocopron lithocarpi* and *Didymocyrtis cladoniicola*. Kor J Mycol 2021;49:373-8.
12. Kim CK, Eo JK, Eom AH. Diversity of foliar endophytic fungi isolated from *Lindera obtusiloba* in Korea. Kor J Mycol 2012;40:136-40.
13. Kumar DSS, Hyde KD. Biodiversity and tissue-recurrence of endophytic fungi in *Tripterygium wilfordii*. Fungal Divers 2004;17:69-90.
14. Park H, Jung CR, Eom AH. Species diversity and antifungal activity of endophytic fungi isolated from *Angelica gigas* Nakai. Kor J Mycol 2021;49:497-505.
15. Cho MG, Chung JM, Jung HR, Kang MY, Moon HS. Vegetation structure of *Taxus cuspidata* communities in subalpine zone. J Agric Life Sci 2012;46:1-10.
16. Fang W, Wu Y, Zhou J, Chen W, Fang Q. Qualitative and quantitative determination of taxol and related compounds in *Taxus cuspidata* Sieb et Zucc. Phytochem Anal 1993;4:115-9.
17. Kim SU, Strobel G, Ford E. Screening of taxol-producing endophytic fungi from *Ginkgo biloba* and *Taxus cuspidata* in Korea. J Appl Biol Chem 1999;42:97-9.
18. Kumaran RS, Kim HJ, Hur BK. Taxol promising fungal endophyte, *Pestalotiopsis* species isolated from *Taxus cuspidata*. J Biosci Bioeng 2010;110:541-6.
19. Choi JW, Lee JM, Park SY, Eom AH. *Acrodontium burrowsianum* and *Pestalotiopsis humicola*: two previously unrecorded fungal species isolated from conifer leaves in Korea. Kor J Mycol 2022;50:311-8.
20. Gardes M, Bruns TD. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol Ecol 1993;2:113-8.
21. Liu YJ, Whelen S, Hall BD. Phylogenetic relationships among ascomycetes: evidence from an RNA polymerse II subunit. Mol Biol Evol 1999;16:1799-808.
22. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 2016;33:1870-4.

23. Singh DK, Sharma VK, Kumar J, Mishra A, Verma SK, Sieber TN, Kharwar RN. Diversity of endophytic mycobiota of tropical tree *Tectona grandis* Linn. f.: Spatiotemporal and tissue type effects. Sci Rep 2017;7:3745.
24. Samuels GJ, Dodd SL, Lu BS, Petrini O, Schroers HJ, Druzhinina IS. The *Trichoderma koningii* aggregate species. Stud Mycol 2006;56:67-133.
25. Rogers JD. *Xylaria cubensis* and its anamorph *Xylocoremium flabelliforme*, *Xylaria allantoidea*, and *Xylaria poitei* in continental United States. Mycologia 1984;76:912-23.
26. Hashemi SA, Khodaparast SA, Zare R, Elahinia SA. Contribution to the identification of *Xylaria* species in Iran. Rostaniha 2014;15:153-66.
27. Fan NW, Chang HS, Cheng MJ, Hsieh SY, Liu TW, Yuan GF, Chen IS. Secondary metabolites from the endophytic fungus *Xylaria cubensis*. Helv Chim Acta 2014;97:1689-99.
28. Klaiklay S, Rukachaisirikul V, Sukpondma Y, Phongpaichit S, Buatong J, Bussaban B. Metabolites from the mangrove-derived fungus *Xylaria cubensis* PSU-MA34. Arch Pharm Res 2012;35:1127-31.
29. Macías-Rubalcava ML, Sánchez-Fernández RE. Secondary metabolites of endophytic *Xylaria* species with potential applications in medicine and agriculture. World J Microbiol Biotechnol 2017;33:1-22.
30. Fu Y, Li X, Yuan X, Zhang Z, Wei W, Xu C, Song J, Gu C. *Alternaria alternata* F3, a novel taxol-producing endophytic fungus isolated from the fruits of *Taxus cuspidata*: isolation, characterization, taxol yield improvement, and antitumor activity. Appl Biochem Biotech 2023;2023:1-24.
31. Gill H, Vasundhara M. Isolation of taxol producing endophytic fungus *Alternaria brassicicola* from non-Taxus medicinal plant *Terminalia arjuna*. World J Microbiol Biotechnol 2019;35:1-8.
32. Mosquera-Espinosa AT, Bayman P, Prado GA, Gómez-Carabalí A, Otero JT. The double life of *Ceratobasidium*: orchid mycorrhizal fungi and their potential for biocontrol of *Rhizoctonia solani* sheath blight of rice. Mycologia 2013;105:141-50.
33. Fisher PJ, Petrini OSBC, Sutton BC. A comparative study of fungal endophytes in leaves, xylem and bark of *Eucalyptus* in Australia and England. Sydowia 1993;45:338-45.
34. Win PM, Matsumura E, Fukuda K. Diversity of tea endophytic fungi: cultivar-and tissue preferences. Appl Ecol Environ Res 2018;16:677-95.
35. Eo JK, Lee BH, Eom AH. Diversity of endophytes isolated from *Thuja koraiensis* Nakai in the Korean Peninsula. Kor J Mycol 2016;44:113-7.
36. Shahzad R, Khan AL, Bilal S, Asaf S, Lee IJ. What is there in seeds? Vertically transmitted endophytic resources for sustainable improvement in plant growth. Front Plant Sci 2018;9:1-10.
37. Saikkonen K, Wäli P, Helander M, Faeth SH. Evolution of endophyte–plant symbioses. Trends Plant Sci 2004;9:275-80.