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

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



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Attribution Non-Commercial License (http: //creativecommons.org/licenses/by-nc/4.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. 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 surfacesterilized in 30% H₂O₂ 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° C. 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:

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

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*.

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KNUE23N042 MT78229 Paraphacosphaetia sporulosa 100 1.27 KNUE223N043 MT864933 Tirichockrra dingkyae 99.83 1.27 KNUE23N046 MK275241 Ceratobasidium sp. 98.16 3.80 KNUE23N046 MK275241 Ceratobasidium sp. 98.16 3.80 KNUE23N057 MT70848 Naranin sp. 1 92.8 2.53 KNUE23N057 LC206655 Daporthe nobils 99.63 2.53 KNUE23N057 LC206655 Daporthe nobils 99.63 2.53 KNUE23N057 LC206655 Daporthe nobils 99.63 2.53 KNUE23N050 MT644300 Pestalotopsis microspora 100 1.27 KNUE23N07 OP741026 Kalmus ia negispora 99.82 1.27 KNUE23N07 OP741026 Kalmus ia negispora 99.82 1.27 KNUE23N07 OP741026 Kalmus ia negispora 99.83 1.27 KNUE23N07 OP741026 Kalmus ia negispora 99.81 3.80 KNUE23N100	KNUE23N039	MT446212	Periconia homothallica	99.64	1.27	
KNUE23N043 MH864593 Tirchoderna dingleyae 99.83 1.27 KNUE23N044 MH990457 Tirchoderna harzanann 99.35 3.80 KNUE23N040 MK752541 Caratobosidiam sp. 98.16 3.80 KNUE23N050 MK70048 Nernania sp. 1 99.28 2.53 KNUE23N057 LC20655 Diapotte nobils 99.63 2.53 KNUE23N060 OF099815 Sordaria lappae 99.82 1.27 KNUE23N07 MK64304 Matoria spin circospora 100 1.27 KNUE23N07 OF941026 Kalmissi kongispona 99.82 1.27 KNUE23N07 NF34757 Tirchoderma attorinide 100 1.27 KNUE23N07 NF3458 Kalmissi kongispona 99.81 3.80 KNUE23N107 <td< td=""><td>KNUE23N042</td><td>MT738229</td><td>Paraphaeosphaeria sporulosa</td><td>100</td><td>1.27</td><td></td></td<>	KNUE23N042	MT738229	Paraphaeosphaeria sporulosa	100	1.27	
KNUE23N04 MH930457 Tinchoderma harzanum 99.35 3.80 KNUE23N046 MK275241 Centobasidium sp. 98.16 3.80 KNUE23N050 MF770448 Naranii sp.1 99.28 2.53 KNUE23N050 MF770448 Naranii sp.1 99.63 2.53 KNUE23N057 LC20665 Diaporthe nobils 99.63 2.53 KNUE23N067 MT644300 Pestabtiopsis microspora 100 1.27 KNUE23N07 OM804348 Microcen sp. 100 1.27 KNUE23N07 OP741026 Kalmisi longispora 99.82 1.27 KNUE23N07 OP741026 Kalmisi longispora 99.82 1.27 KNUE23N07 OP741026 Kalmisi longispora 99.82 1.27 KNUE23N07 OP741026 Kalmisi longispora 99.81 3.80 KNUE23N07 OP741026 Kalmisi longispora 99.81 3.80 KNUE23N07 MT18737 Stocknicinnycetes sp.1 98.93 1.27 KNUE23N11 MT482502	KNUE23N043	MH864593	Trichoderma dingleyae	99.83	1.27	
KNUE23N046 MK275241 Ceratobasidiarmsp. 98.16 3.80 KNUE23N08 MN31171 Daldmin childiae 100 3.80 15.79 KNUE23N053 JN91483 Ardminium sp.1 99.28 2.53 10.53 KNUE23N057 LC206655 Diaporthe nobils 99.62 2.53 10.53 KNUE23N067 MT644300 Peasitorips in microspora 100 1.27 KNUE23N070 MK804348 Microcera sp. 100 1.27 KNUE23N070 OWS60252 Collectrichum simersee 100 1.27 KNUE23N070 OWS60252 Collectorichum simersee 100 1.27 KNUE23N070 OWS60252 Collectorichum simersee 100 1.27 KNUE23N070 NR50518 <i>Hypocra</i> sp. 98.60 1.27 KNUE23N10 NR_159861 <i>Hypocra</i> sp. 98.60 1.27 KNUE23N11 MT482502 <i>Fisarium tosporum</i> 99.81 3.80 KNUE23N13 KZ75448 Xylaria cabersis 100 1.27 </td <td>KNUE23N044</td> <td>MH930457</td> <td>Trichoderma harzianum</td> <td>99.35</td> <td>3.80</td> <td></td>	KNUE23N044	MH930457	Trichoderma harzianum	99.35	3.80	
KNUE23N048 MN341717 Dadinia childia 100 3.80 15.79 KNUE23N050 MF770484 Nernania sp. 1 9928 2.53	KNUE23N046	MK275241	Ceratobasidium sp.	98.16	3.80	
KNUE23N050 MF770848 Nernania sp. 1 99.28 2.53 KNUE23N057 JK914483 Arthrinium sp. 1 94.18 10.53 KNUE23N067 LC206655 Diaporthe nobils 99.63 2.53 KNUE23N067 DEgoothe nobils 99.82 1.27 KNUE23N067 MT64300 Pestalotiopsis microspora 100 1.27 KNUE23N071 OP808229 Collectorichum siamense 100 1.27 KNUE23N077 OP741026 Kalmissi longispora 99.82 1.27 KNUE23N070 OP808229 Collectorichum siamense 100 1.27 KNUE23N070 OP741026 Kalmissi longispora 99.82 1.27 KNUE23N070 NF055218 Hypocrea sp. 98.60 1.27 KNUE23N100 NR 159861 Fusarium oxyporum 99.81 3.80 KNUE23N131 MZ854248 Xybria cubensis 100 1.27 KNUE23N131 MZ854248 Xybria cubensis 100 1.27 KNUE23N131 MZ854248 Xybria	KNUE23N048	MN341717	Daldinia childiae	100	3.80	15.79
KNUE23N053 JX914483 Arthriniarrsp.1 94.18 1053 KNUE23N057 L206655 Diaporthe nobils 99.63 2.53 KNUE23N060 OP699815 Sordaria lappae 99.82 127 KNUE23N070 MK804348 Microcera sp. 100 127 KNUE23N070 OP862829 Collectrichum siamense 100 127 KNUE23N070 OP741026 Kalmusia longispora 99.82 127 KNUE23N070 OP741026 Kalmusia longispora 99.82 127 KNUE23N070 OP741026 Kalmusia longispora 99.82 127 KNUE23N070 MF862518 Hypocrea sp. 98.60 127 KNUE23N107 K15861 Fusarium tabinka 100 127 KNUE23N117 MT183734 Sordarian coysporum 99.83 127 KNUE23N131 MZ854248 Xylaria cubensis 100 127 KNUE23N133 K759458 Valas ordida 99.15 127 KNUE23N134 M280572 Nernania sepers 93.0 127 KNUE23N135 K1758843 <t< td=""><td>KNUE23N050</td><td>MF770848</td><td>Nemania sp. 1</td><td>99.28</td><td>2.53</td><td></td></t<>	KNUE23N050	MF770848	Nemania sp. 1	99.28	2.53	
KNUE23N057 LC206655 Diaporthe nobils 99.63 2.53 KNUE23N060 0P699815 Sordaria lappae 99.82 1.27 KNUE23N067 MT644300 Pestabolopsis microspora 100 1.27 KNUE23N070 OR68299 Colleotrichum siamense 100 1.27 KNUE23N071 OP741026 Kalmusia longispora 99.82 1.27 KNUE23N070 OP741026 Kalmusia longispora 99.82 1.27 KNUE23N071 OP741026 Kalmusia longispora 99.82 1.27 KNUE23N070 OP741026 Kalmusia longispora 99.82 1.27 KNUE23N000 NR 159861 Fusarium tabiral 100 1.27 KNUE23N100 NR 159861 Fusarium tabiral 100 1.27 KNUE23N131 MT854248 Sordariomycetes sp. 1 98.93 1.27 KNUE23N133 K739458 Valas sorda 99.15 1.27 KNUE23N179 OQ01032 Alternatria alternata 100 1.27 KNUE23N174 K1758843 Cyanodermella sp. 90.94 1.27 5.26 <	KNUE23N053	JX914483	Arthrinium sp. 1	94.18		10.53
KNUE23N060 OP699815 Sordaria lappae 99.82 1.27 KNUE23N067 MT644300 Pestalotiopsis microspora 100 1.27 KNUE23N070 MK804348 Microcera sp. 100 1.27 KNUE23N071 OP862829 Collectrichum siamense 100 1.27 KNUE23N070 OP741026 Kalmusia longispora 99.82 1.27 KNUE23N070 OP741026 Kalmusia longispora 99.82 1.27 KNUE23N096 MT341775 Trichoderma atrovinide 100 1.27 5.26 KNUE23N097 AF055218 Hypocrea sp. 98.60 1.27 KNUE23N101 MT18734 Sordaria moxysporum 99.81 3.80 KNUE23N131 MZ854248 Xylaria cubensis 100 1.27 KNUE23N133 K1739458 Valas sordida 99.15 1.27 KNUE23N148 MT1920572 Nerrania seprars 99.30 1.27 5.26 KNUE23N179 OQ001032 Al	KNUE23N057	LC206655	Diaporthe nobils	99.63	2.53	
KNUE23N067 MT644300 Pestalotiopsis microspora 100 127 KNUE23N070 MK804348 Microcera sp. 100 127 KNUE23N071 OP862829 Colletotrichum siamense 100 127 KNUE23N071 OP862829 Colletotrichum siamense 100 127 KNUE23N077 OP741026 Kalmusia longispora 9982 127 KNUE23N096 MT3411775 Trichockerma atroviride 100 127 KNUE23N097 AF055218 Hypocrea sp. 98.60 127 KNUE23N100 NR_159861 Fusarium tosysporum 99.81 380 KNUE23N111 MT482502 Fusarium oxysporum 99.81 380 KNUE23N131 MZ854248 Sylaria cubersis 100 127 KNUE23N133 K1739458 Valas sordida 99.15 127 KNUE23N134 MT920572 Nernania separs 99.30 127 526 KNUE23N180 LC431573 Nernania sp.2 99.47 127 KNUE23N200 MT18313 Sordariomycetes sp. 99.46 127 KNUE23	KNUE23N060	OP699815	Sordaria lappae	99.82	1.27	
KNUE23N070 MK804348 Microcer sp. 100 127 KNUE23N071 OP862829 Collectrichum siamense 100 127 KNUE23N077 OP741026 Kalmusia longispora 99.82 127 KNUE23N076 MT341775 Trichoderma attoviride 100 127 5.26 KNUE23N077 AF055218 Hypocrea sp. 98.60 1.27 5.26 KNUE23N100 NR_159861 Fusarium babinda 100 1.27 5.26 KNUE23N111 MT482502 Fusarium oxysporum 99.81 3.80 5.26 KNUE23N127 MT183734 Sordariomycetes sp. 1 98.93 1.27 5.26 KNUE23N133 KJ739458 Valas oordida 99.15 1.27 5.26 KNUE23N133 KJ739458 Valas oordida 99.15 1.27 5.26 KNUE23N179 OQ001032 Alternaria alternata 100 1.27 5.26 KNUE23N194 M1860399 Dotiora sp. 96.65 1.27 5.26 KNUE23N	KNUE23N067	MT644300	Pestalotiopsis microspora	100	1.27	
KNUE23N071 OP862829 Collectorichum siamense 100 127 KNUE23N077 OP741026 Kalmusia longispora 99.82 127 KNUE23N066 MT341775 Trichoderma attoviride 100 127 5.26 KNUE23N097 AF055218 Hypocrea sp. 98.60 127 5.26 KNUE23N100 NR_159861 Fusarium tobinda 100 127 5.26 KNUE23N100 NR_159861 Fusarium oxyporum 99.81 3.80 - KNUE23N131 MZ854248 Xylaria cubersis 100 1.27 - KNUE23N133 KJ739458 Valsa sordica 99.15 1.27 - KNUE23N133 KJ739458 Valsa sordica 99.15 1.27 - KNUE23N135 KT758843 Cyanodermella sp. 99.30 1.27 5.26 KNUE23N148 MT920572 Nernania sp.2 99.47 1.27 - KNUE23N149 OQ301032 Alternaria alternata 100 1.27 - K	KNUE23N070	MK804348	Microcera sp.	100	1.27	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	KNUE23N071	OP862829	Colletotrichum siamense	100	1.27	
KNUE23N096 MT341775 Trichockerna 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 KNUE23N131 MZ854248 Xylaria cubensis 100 1.27 KNUE23N137 K1739458 Valas sordida 99.15 1.27 KNUE23N137 K1758843 Cyanockernella sp. 90.94 1.27 5.26 KNUE23N179 OQ001032 Alternaria alternata 100 1.27 5.26 KNUE23N180 LC431573 Nemania sp.2 99.47 1.27 5.26 KNUE23N184 MH860399 Dotiora sp. 96.65 1.27 5.26 KNUE23N204 MK367476 Picosporales sp.2 100 5.26 5.26 KNUE23N215 N198467 Magrapo	KNUE23N077	OP741026	Kalmusia longispora	99.82	1.27	
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 cubersis 100 1.27 KNUE23N133 KJ739458 Valas sordida 99.15 1.27 KNUE23N133 KJ739458 Valas sordida 99.15 1.27 KNUE23N133 KJ739458 Valas sordida 99.15 1.27 KNUE23N134 MT920572 Nemania sepers 99.30 1.27 5.26 KNUE23N190 OQ01032 Alternaria alternata 100 1.27 5.26 KNUE23N144 MH860399 Dothiora sp. 96.65 1.27 5.26 KNUE23N144 MH860399 Dotiora sp. 99.46 1.27 5.26 KNUE23N204 MK367476 Picosporales sp. 2 100 5.26 5.26 KNUE23N217 OU89423 Paraphacophieria neglecta <	KNUE23N096	MT341775	Trichoderma atroviride	100	1.27	5.26
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 KNUE23N137 KT758843 Cyanodermella sp. 99.30 1.27 5.26 KNUE23N148 MT920572 Nemania seperas 99.30 1.27 5.26 KNUE23N180 LC431573 Nemania sp. 2 99.47 1.27 KNUE23N180 LC431573 Nemania sp. 2 99.46 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 KNUE23N217 OU989423 Paraphaeosphaeria neglecta 99.83	KNUE23N097	AF055218	Hypocrea sp.	98.60	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 Nernania serpens 99.30 1.27 5.26 KNUE23N179 OQ001032 Alternaria alternata 100 1.27 KNUE23N180 LC431573 Nernania sp. 2 99.47 1.27 KNUE23N180 LC431573 Nernania sp. 2 99.47 1.27 KNUE23N184 MH860399 Dothiora sp. 96.65 1.27 KNUE23N190 OP380745 Discosia artocreas 99.46 1.27 KNUE23N200 MT183813 Sordariomycetes sp. 2 100 5.26 KNUE23N204 MK367476 Pleosporales sp. 97.44 5.26 KNUE23N217 OU989423 Paraphaeosphaeria neglecta 99.83 5.26	KNUE23N100	NR 159861	Fusarium babinda	100	1.27	
KNUE23N127 MT183734 Sordariomycets 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 Sordariomycets sp. 2 100 5.26 KNUE23N204 MK367476 Pleosporales sp. 97.44 5.26 KNUE23N217 OU989423 Paraphaeosphaeria neglecta 99.83 5.26 KNUE23N225 LC206659 Pezicula neosponulosa 100 1.27 KNUE23N228 KP050630 Arthrinium sp. 2 93.95 5.26 <	KNUE23N111	 MT482502	Fusarium oxysporum	99.81	3.80	
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 sepens 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 KNUE23N217 OU989423 Paraphaeosphaeria neglecta 99.83 5.26 KNUE23N225 LC20659 Pezicula neosporulosa 100 1.27 KNUE23N227 MZ348514 Colletotrichum sp. 100 1.27 KNUE23N277 MT872061 Colletotrichum aenigma 100 5.26 <	KNUE23N127	MT183734	Sordariomycetes sp. 1	98.93	1.27	
KNUE23N133 KJ739458 Valas sordida 99.15 127 KNUE23N137 KT758843 Cyanodermella sp. 90.94 127 526 KNUE23N148 MT920572 Nemania serpens 99.30 127 526 KNUE23N179 OQ001032 Alternaria alternata 100 127 KNUE23N180 LC431573 Nemania sp. 2 99.47 127 KNUE23N180 LC431573 Nemania sp. 2 99.46 127 KNUE23N144 OP380745 Discosia artocreas 99.46 127 KNUE23N200 MT183813 Sordariomycetes sp. 2 100 526 KNUE23N204 MK367476 Pleosporales sp. 97.44 526 KNUE23N216 JN198467 Magnaporthales sp. 99.09 5.06 KNUE23N225 LC206659 Pezicula neosporulosa 100 127 KNUE23N227 MZ348514 Colletotrichum sp. 2 93.95 526 KNUE23N227 MT872061 Colletotrichum aenigma 100 127 KNUE23N277 MT872061 Colletotrichum aenigma 100 526 <	KNUE23N131	MZ854248	Xylaria cubensis	100	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 KNUE23N180 LC431573 Nemania sp. 2 99.46 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 KNUE23N227 MZ348514 Colletotrichum sp. 100 1.27 KNUE23N227 MT872061 Colletotrichum aerigma 100 5.26 Shannon's index (H) 3.51 2.33 5.26 Sh	KNUE23N133	KJ739458	Valsa sordida	99.15	1.27	
KNUE23N148 MT920572 Nemania serpens 9930 1.27 5.26 KNUE23N179 OQ001032 Alternaria alternata 100 1.27 KNUE23N179 OQ001032 Alternaria alternata 100 1.27 KNUE23N180 LC431573 Nemania sp. 2 9947 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 KNUE23N227 MZ348514 Colletotrichum sp. 100 1.27 KNUE23N277 MT872061 Colletotrichum aenigma 100 1.27 KNUE23N277 MT872061 Colletotrichum aenigma 100 1.27 KNUE23N277 MT872061 Colletotrichum aenigma 100 5.26	KNUE23N137	KT758843	Cvanodermella sp.	90.94	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 KNUE23N227 MZ348514 Collectorichum sp. 100 1.27 KNUE23N228 KP050630 Arthrinium sp. 2 93.95 5.26 KNUE23N277 MT872061 Collectorichum aenigma 100 1.27 KNUE23N277 MT872061 Collectorichum aenigma 100 5.26 Shannon's index (H') 3.51 2.33 0.93 0.94 Number of species 43 12	KNUE23N148	MT920572	Nemania serpens	99.30	1.27	5.26
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 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 2.33 Species evenness (E) 0.93 0.94 43 12	KNUE23N179	OO001032	Alternaria alternata	100	1.27	
KNUE23N184 MH860399 Dothiora sp. 96.65 1.27 KNUE23N194 OP380745 Discosia artocreas 99.46 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 KNUE23N277 MT872061 Colletotrichum aenigma 100 5.26 Shannon's index (H') 3.51 2.33 2.33 Species evenness (E) 0.93 0.94 Number of species 43 12	KNUE23N180	LC431573	Nemania sp. 2	99.47	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	KNUE23N184	MH860399	Dothiora sp.	96.65	1.27	
KNUE23N200 MT183813 Sordariomycetes sp. 2 100 526 KNUE23N204 MK367476 Pleosporales sp. 97.44 526 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	KNUE23N194	OP380745	Discosia artocreas	99.46	1.27	
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KNUE23N216 JN198467 Magnaporthales sp. 99.09 5.06 KNUE23N217 OU989423 Panaphaeosphaeria 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	KNUE23N204	MK367476	Pleosporales sp.	97.44		5.26
KNUE23N217 OU989423 Paraphaeosphaeria neglecta 99.83 526 KNUE23N225 LC206659 Pezicula neosporulosa 100 1.27 KNUE23N227 MZ348514 Colletotrichum sp. 100 1.27 KNUE23N228 KP050630 Arthrinium sp. 2 93.95 526 KNUE23N277 MT872061 Colletotrichum aenigma 100 526 Shannon's index (H') 3.51 2.33 Species evenness (E) 0.93 0.94 Number of species 43 12	KNUE23N216	JN198467	Magnaporthales sp.	99.09	5.06	
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	KNUE23N217	OU989423	Paraphaeosphaeria neglecta	99.83		5.26
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	KNUE23N225	LC206659	Pezicula neosporulosa	100	1.27	
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KNUE23N277 MT872061 Collectotrichum aenigma 100 526 Shannon's index (H') 3.51 2.33 Species evenness (E) 0.93 0.94 Number of species 43 12	KNUE23N228	KP050630	Arthrinium sp. 2	93.95		5.26
Shannon's index (H') 3.51 2.33 Species evenness (E) 0.93 0.94 Number of species 43 12	KNUE23N277	MT872061	Colletotrichum aenigma	100		5.26
Species evenness (E)0.930.94Number of species4312	Shannon's index (H')					2.33
Number of species 43 12	Species evenness (E)				0.93	0.94
	Number of species				43	12

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

^aRA: 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 $(2.73-) 3.17-3.45 (-3.96) \times (2.23-) 2.77-2.98 (-3.31) \,\mu\text{m}$ 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).





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 $4.1-4.3 \times 3.1-3.2 \mu$ m) [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 \pm 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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