

RESEARCH ARTICLE

Four Unrecorded Endophytic Fungal Species Belonging to Chaetomiaceae and Didymellaceae Isolated from Potatoes in Korea

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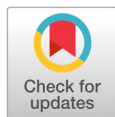
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ABSTRACT

In this study, endophytic fungal strains were isolated from the leaves, roots, and stems of potato plants from spring-cultivated potato fields in Gangwon-do and Jeju-do, Korea. Morphological characteristics of the isolates were observed, and molecular analyses were conducted using the internal transcribed spacer, large subunit rDNA, and second-largest subunit of RNA polymerase II regions. Four endophytic fungal species, previously unrecorded in Korea and belonging to the families Chaetomiaceae and Didymellaceae, were identified. The morphological and phylogenetic analyses of these species have also been reported.

Keywords: *Collariella*, *Epicoccum*, *Neosascochyta*, *Ovatospora*, *Solanum tuberosum*



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INTRODUCTION

Potato (*Solanum tuberosum* L.) is the second most-produced food crop in Korea [1], and spring-cultivated potatoes constitute approximately 63% of national production. Gangwon-do and Jeju-do are the leading provinces in spring-cultivated potato yield (kg/10 a) [2].

Fungal endophytes live symbiotically within plant tissues without causing disease. They protect the host plants from external pathogens by secreting secondary metabolites [3]. Several studies have reported endophytic fungi in diverse potato tissues [4–6], and some fungal species confer resistance against major potato diseases, such as late blight [5] or brown leaf spot [6]. Consequently, endophytic fungi have been investigated as potential biological control agents for reducing dependence on chemical pesticides. In this study, we have reported the morphological characteristics and phylogenetic analyses of four endophytic fungal species previously unrecorded in Korea, belonging to the families Chaetomiaceae and Didymellaceae, and isolated from spring-cultivated potatoes in Gangwon-do and Jeju-do.

MATERIAL AND METHODS

Sampling and isolation

Sampling was conducted in spring potato cultivation areas in Gangneung-si (Gangwon-do, 37°46'44.7"N, 128°55'34.8"E) and Jeju-si (Jeju-do, 33°28'43.9"N, 126°47'02.0"E) between May and June 2025. Healthy potato plants with no disease symptoms were collected, transported to the laboratory within 24 h, and rinsed with distilled water. Leaf and stem tissues were surface-sterilized in 1% NaOCl for 1 min and 70% ethanol for 2 min, excised into small pieces, and placed on potato dextrose agar (PDA) [7]. Root tissues were surface-sterilized in 3% NaOCl for 3 min and 70% ethanol for 2 min, treated with streptomycin (100 µg·mL⁻¹) for 10 min, and placed on water agar (WA) [8].

Morphological and molecular identification

When mycelia emerged from the tissue during cultivation at 25°C, they were sub-cultured on PDA. Morphological characteristics of the colonies, mycelia, and spores were observed (Table 1) on PDA, malt extract agar (MEA), and oatmeal agar (OA) by using an optical microscope (Nikon Eclipse E600, Nikon, Tokyo, Japan).

Table 1. Morphological characteristics of endophytic fungal strains isolated in this study

Strains	Colonies				Spore
	Cultural condition	Color	Size	Shape	
<i>Collariella bostrychodes</i>					
Strain CBS 163.73 [13]	OA, 25°C, 7 days	White	34–40 mm in diam.	Sparse white aerial hyphae, entire margin	(ascospores) olivaceous when mature, limoniform, bilaterally flattened, $6-7(-7.5) \times (5-)5.5-6.5 \times (4-)4.5-5.5 \mu\text{m}$
Strain HARI25E108	OA, 25°C, 7 days	Initially white, turn to gray as a somata mature	Avg. 43.9 mm in diam.	Entire margin, convex colony	(ascospore) initially colorless, light brown when mature, limoniform to ovate, $(4.00-)4.96(-5.88) \times (2.98-)4.10(-5.36) \mu\text{m}$
<i>Ovatospora brasiliensis</i>					
Strain CBS 140.50 [13]	OA, 25°C, 7 days	Olivaceous gray or honey-colored	41–47 mm in diam.	Entire or slightly undulate margin, concentric	(ascospores) olivaceous brown when mature, ovate, bilaterally flattened, $(6.5-)7-7.5(-8) \times (5.5-)6-6.5(-7) \times (4.5-)5-5.5(-6) \mu\text{m}$
Strain HARI25E097	OA, 25°C, 7 days	Olivaceous gray, darker than on PDA	Avg. 48.0 mm in diam.	Slightly undulate margin, concentric, raised colony	(ascospore) initially colorless, brown when mature, hyaline, fusiform to ovate, $(4.94-)5.65(-6.42) \times (2.90-)3.89(-4.86) \mu\text{m}$
<i>Epicoccum mackenziei</i>					
Strain KY45 [15]	MEA, 25°C, 5 days	White to olivaceous gray	Reaching 20 mm in diam.	Irregular at margin, white mycelial groups in the colony, middle black gummy substances	(chlamydospores) irregular, unicellular or multicellular, intercalary or terminal, solitary or in chains, $9-16 \mu\text{m} \times 7-15 \mu\text{m}$
Strain HARI25D082	MEA, 25°C, 7 days	Light brown	Avg. 63.6 mm in diam.	Sparse and radial mycelium, irregular erose margin, flat colony	(chlamydospores) irregular, ellipsoidal to obovate, hyaline, 2-4 spores in chains, $(8.16-)10.85(-14.76) \times (6.18-)8.31(-11.92) \mu\text{m}$
<i>Neosascochyta desmazieri</i>					
Strain CBS 297.69 [17]	MEA, 25°C, 7 days	Whitish, gray greenish olivaceous near the center	35–40 mm in diam.	Regular margin, felty	(pycnidia) pseudoparenchymatous, 2-4(-5)-layered, 15–28 μm thick, composed of oblong to isodiametric cells
Strain HARI25E015	MEA, 25°C, 7 days	Dark olivaceous, light gray in center	Avg. 57.2 mm in diam.	Irregular undulate margin, convex colony	(pycnidia) dark brown, ellipsoidal (pycniospores) hyaline, colorless, cylindrical to subglobose, $(1.84-)2.17(-2.67) \times (3.09-)4.16(-5.96) \mu\text{m}$

OA: oatmeal agar; PDA: potato dextrose agar; MEA: malt extract agar.

Genomic DNA was extracted from the mycelia by using the DiaStar™ Direct Multiplex PCR Kit (Solgent Co., Ltd., Daejeon, Korea), and PCR amplifications were performed. The internal transcribed spacer (ITS) region was amplified with ITS1F/ITS4 primers [9], the large subunit (LSU) rDNA with LR0R/LR16 primers [10], and the RNA polymerase II second-largest subunit (*RPB2*) region with fRPB2-5f/fRPB2-7cR primers [11]. PCR products were electrophoresed on a 1.5% agarose gel. Each DNA band was confirmed, and DNA sequencing was performed by Solgent Co., Ltd. (Daejeon, Korea). The resulting DNA sequences were compared against the NCBI BLAST database to determine the closest species matches, and alignments with related taxa were used to construct neighbor-joining phylogenetic trees in MEGA 7 [12]. All DNA sequences obtained in this study were registered in GenBank (accession numbers provided in the Results section), and the isolates were deposited in the Korean Agricultural Culture Collection (KACC), National Institute of Agricultural Sciences.

RESULTS

Family Chaetomiaceae

Collariella bostrychodes (Zopf) X. Wei Wang & Samson, Stud. Mycol. 84: 179 (2016) [MB#818862]

This strain was isolated from a potato root collected from Gangneung-si. On PDA incubated at 25°C for 7 days, colonies reached an average diameter of 37.1 mm; colonies were pinkish-white, flat, and exhibited an entire margin due to densely radiating mycelia (Fig. 1A). On MEA, the colonies grew slower (avg. 27.0 mm), displaying an undulate margin and sparser mycelia. The colony was light white or gray and flat on the plate (Fig. 1B). On OA, growth was the fastest (avg. 43.9 mm); the colonies were white at the margin, gray toward the center, and darkened as the ascomata matured (Fig. 1C). Asci were produced in a broom-like arrangement from the ascomata, containing lemon-shaped to ovoid ascospores with a pointed end; pigmentation ranged from light to dark brown at maturity (Fig. 1D & 1E). The size was (4.00–) 4.96 (–5.88) × (2.98–) 4.10 (–5.36) µm in diameter (n = 20).

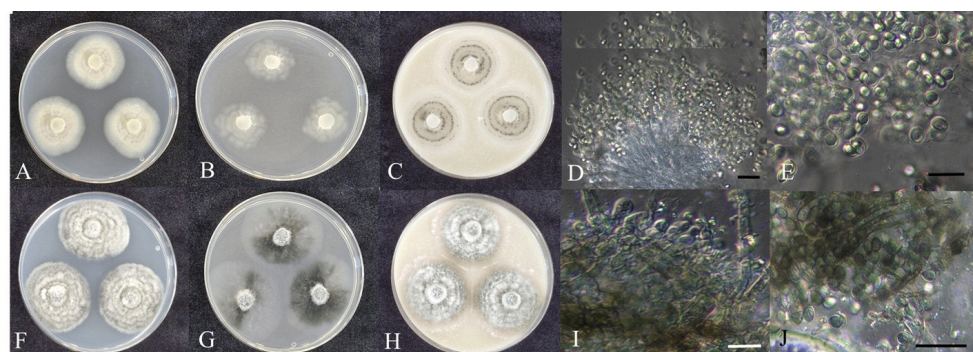


Fig. 1. Cultural characteristics of endophytic fungal strains belonging to Chaetomiaceae. Colonies of *Collariella bostrychodes* strain HARI25E108 grown for 7 days on PDA (A), MEA (B), and OA (C) and ascus and ascospores (D, E); Colonies of *Ovatospora brasiliensis* strain HARI25E097 grown for 7 days on PDA (F), MEA (G), and OA (H) and ascomata (I) and ascospores (J) (scale bars: D, I = 20 µm; E, J = 10 µm). PDA: potato dextrose agar; MEA: malt extract agar; OA: oatmeal agar.

Phylogenetic analysis. ITS sequence analysis showed 99.64% similarity to *C. bostrychodes* strain CBS 586.83 (MH861661.1), and the LSU sequence showed 97.07% similarity to *C. bostrychodes* strain CBS 586.83 (MH873374.1). In the concatenated ITS+LSU neighbor-joining tree, strain HARI25E108 formed a monophyletic clade with strains CBS 163.73 and CBS 586.83 (Fig. 2).

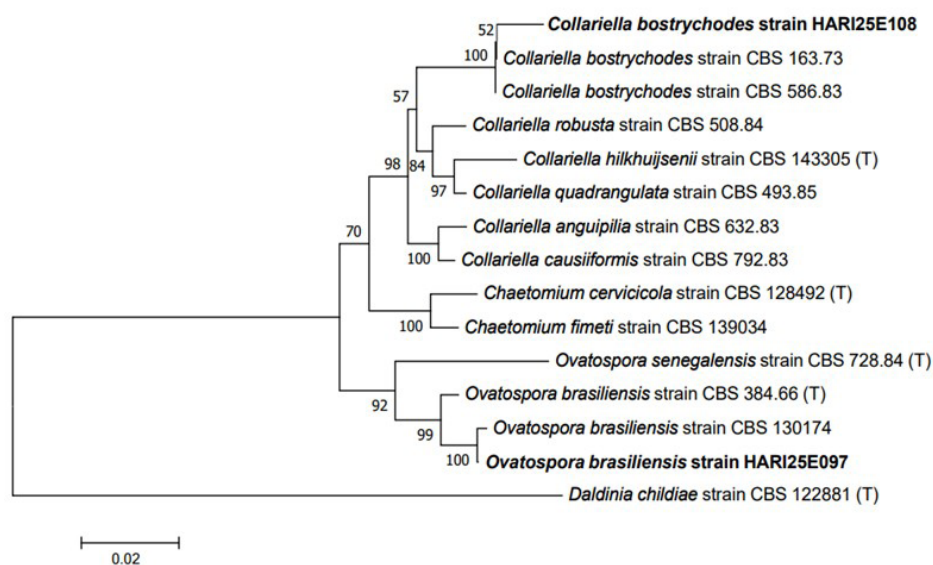


Fig. 2. Neighbor-joining phylogenetic tree of *C. bostrychodes* and *O. brasiliensis* on the basis of a concatenated alignment of internal transcribed spacer (ITS) and large subunit (LSU) rDNA sequences. *Daldinia childiae* was used as an outgroup. Numbers on branches indicate bootstrap values (1,000 replicates). Fungal strains isolated in this study are in bold. (T) indicates the type strains.

Specimen examined. Korea: Gangneung-si, Gangwon-do, 37°47'03.1"N, 128°55'25.6"E, June 29, 2025, isolated from healthy root of *Solanum tuberosum*, strain HARI25E108, KACC411096 (deposit No.), GenBank No. PX285310 (ITS), PX285311 (LSU).

Note. On OA, strain HARI25E108 was white, as described in the original description, but the color turned gray as the ascomata matured. The colonies were slightly larger than strain CBS 163.73 from the original description [13]. The asci darkened as the ascospores matured and had a liminiform shape, which was consistent with strain CBS 163.73; however, among the ascospores observed in this study, ovate shapes were rarely observed. These were thought to be immature ascospores. The asci formed a radial collar-like arrangement. Together with molecular phylogenetic evidence, these features support the identification of *C. bostrychodes*.

Ovatospora brasiliensis (Bat. & Pontual) X. Wei Wang & Samson, Stud. Mycol. 84: 207 (2016) [MB#818851]

This strain was isolated from a potato root collected from Gangneung-si. After 7 days at 25°C, colonies on PDA averaged 41.1 mm in diameter; colonies were initially dark beige with dark-gray concentric growth and a generally smooth margin due to dense hyphal growth and were slightly raised on the plate (Fig. 1F). On MEA, colonies averaged 48.6 mm and displayed black aerial mycelia over a translucent vegetative mycelium; colonies were flat on the plate and showed a smooth margin (Fig. 1G). On OA, average colony

diameter was 48.0 mm; overall shape was similar to that on PDA, but the concentric rings were less distinct and coloration tended toward olivaceous gray (Fig. 1H). Numerous irregular black perithecia were present, interconnected by ascomatal hairs curved into whorls or coils. Obovate asci were observed within the perithecia (Fig. 1I), releasing fusiform to ovoid ascospores that were initially hyaline but became pigmented brown at maturity (Fig. 1J). Ascospore size was $(4.94\text{--}5.65\text{--}6.42) \times (2.90\text{--}3.89\text{--}4.86)$ μm in diameter ($n = 20$).

Phylogenetic analysis. ITS sequence analysis showed 99.62% similarity to *O. brasiliensis* strain CBS 130714 (MH865522.1), and the LSU sequence showed 99.51% similarity to *O. brasiliensis* strain CBS 130714 (MH876958.1). In the concatenated ITS+LSU neighbor-joining tree, strain HARI25E097 formed a monophyletic clade with strains CBS 163.73 and CBS 586.83 (Fig. 2).

Specimen examined. Korea: Gangneung-si, Gangwon-do, $37^{\circ}46'44.7''\text{N}$, $128^{\circ}55'34.8''\text{E}$, June 29, 2025, isolated from healthy root of *Solanum tuberosum*, strain HARI25E097, KACC411095 (deposit No.), GenBank No. PX285304 (ITS), PX285305 (LSU).

Note. On OA, the colony size and color, concentric growth pattern, and mainly ovate ascospores were consistent with those of *O. brasiliensis* strain CBS 140.50 from the original description [13]. The morphology of the ascospores was similar to that of the closely related species *Ovatospora senegalensis*, but the ascospores of CBS 728.84 (type strain) had a noticeably pointed tip [13]; thus, they were clearly distinct from those of strain HARI25E097 in this study. The presence of ascomatal hairs forming connections between the perithecia and obovate asci in our isolate was also consistent with strain CBS 140.50 [13], supporting its identification.

Family Didymellaceae

Epicoccum mackenziei Jayasiri, Camporesi & K.D. Hyde, Mycosphere 8 (8): 1093 (2017) [MB#552362]

The strain was isolated from a potato leaf collected from Gangneung-si. After 7 days on PDA at 25°C , colonies averaged 60.1 mm in diameter and were light yellow-brown, slightly raised with a smooth margin on the plate (Fig. 3A). On MEA, colonies averaged 63.6 mm, exhibited sparser mycelia than those on PDA; the colonies were light brown and flat on the plate. It showed an erose margin due to irregular radial mycelial growth (Fig. 3B). On OA, colonies averaged 62.7 mm, appeared bright white with dense central aerial mycelia giving an umbonate appearance in the center; the margin was irregular (Fig. 3C). Asexual chlamydospores were hyaline, ellipsoidal to obovate, and formed hyphae in chains of 2–4 (Fig. 3D & 3E). The size was $(8.16\text{--}10.85\text{--}14.76) \times (6.18\text{--}8.31\text{--}11.92)$ μm in diameter ($n = 20$).

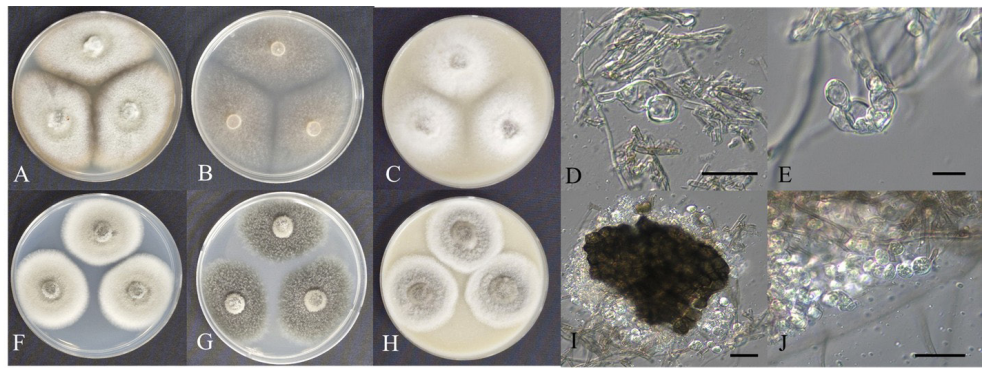


Fig. 3. Cultural characteristics of endophytic fungal strains belonging to Didymellaceae. Colonies of *Epicoccum mackenziei* HARI25D082 grown for 7 days on PDA (A), MEA (B), and OA (C) and chlamydospore (D, E); Colonies of *Neoascochyta desmazieri* HARI25E015 grown for 7 days on PDA (F), MEA (G), and OA (H) and pycnidial cell and pycnidia (I, J) (scale bars: D = 20 μ m; E, I = 10 μ m; J = 5 μ m). PDA: potato dextrose agar; MEA: malt extract agar; OA: oatmeal agar.

Phylogenetic analysis. ITS, LSU, and *RPB2* sequences showed 100% (strain KY45, OP550314.1), 99.62% (strain KY45, OP563622.1), and 98.88% (strain KY45, OP594302.1) similarity to *E. mackenziei*, respectively. In the concatenated ITS+LSU+*RPB2* neighbor-joining tree, strain HARI25D082 formed a monophyletic clade with *E. mackenziei* strain KY45 [14] (Fig. 4).

Specimen examined. Korea: Gangneung-si, Gangwon-do, 37°50'02.5"N, 128°52'11.3"E, May 29, 2025, isolated from healthy leaf of *Solanum tuberosum*, strain HARI25D082, KACC411093 (deposit No.), GenBank No. PX285299 (ITS), PX285300 (LSU), PX289874 (*RPB2*).

Note. In the original description of *E. mackenziei*, ascospores were reported at the sexual stage, whereas asexual conidia were not observed and only chlamydospores were noted [15]. In this study, the sexual stage was not observed and only chlamydospores were confirmed, mirroring the original description. Phylogenetic analysis revealed that it was closely related to *Epicoccum italicum* (Fig. 4). However, while *E. italicum* clearly formed clavate-shaped conidiomata on MEA [16], no such morphology was observed in strain HARI25D082. Therefore, this strain was identified as *E. mackenziei* based on its chlamydospore morphology (spores in chain, size, and irregular hyaline shape) and molecular biology.

Neoascochyta desmazieri (Cavara) Qian Chen & L. Cai, Stud. Mycol. 82: 198 (2015) [MB#814142]

This strain was isolated from a potato stem collected from Jeju-si. After 7 days at 25°C, colonies on PDA averaged 49.5 mm in diameter and were grayish green with a smooth margin. The colonies were slightly raised on the plate (Fig. 3F). On MEA, colonies averaged 57.2 mm and were predominantly dark olivaceous with a lighter gray center; the margin was undulate, and the colony was raised (Fig. 3G). On OA, colonies averaged 56.7 mm, appearing white overall with light gray-green central aerial mycelia; the margin was smooth, and the colony was convex (Fig. 3H). Dark brown, oval pycnidia containing dozens to hundreds of pycniospores released upon pycnidial opening (Fig. 3I). Pycniospores were hyaline, colorless, and cylindrical (Fig. 3J); size was $(1.84\text{--}2.17\text{--}2.67) \times (3.09\text{--}4.16\text{--}5.96)$ μ m in diameter ($n = 20$).

Phylogenetic analysis. The ITS sequences showed 99.59% similarity to *N. desmazieri* strain CBS 297.69 (NR_136130), the LSU sequence showed 100% similarity to *N. desmazieri* strain CBS 297.69 (NG_069315), and the *RPB2* sequence showed 99.71% similarity to *N. desmazieri* strain CBS 297.69

(NG_069315). In the concatenated ITS+LSU+RPB2 neighbor-joining tree, strain HARI25E015 formed a monophyletic clade with *N. desmazieri* strain CBS 247.79 and CBS 297.69 (Fig. 4).

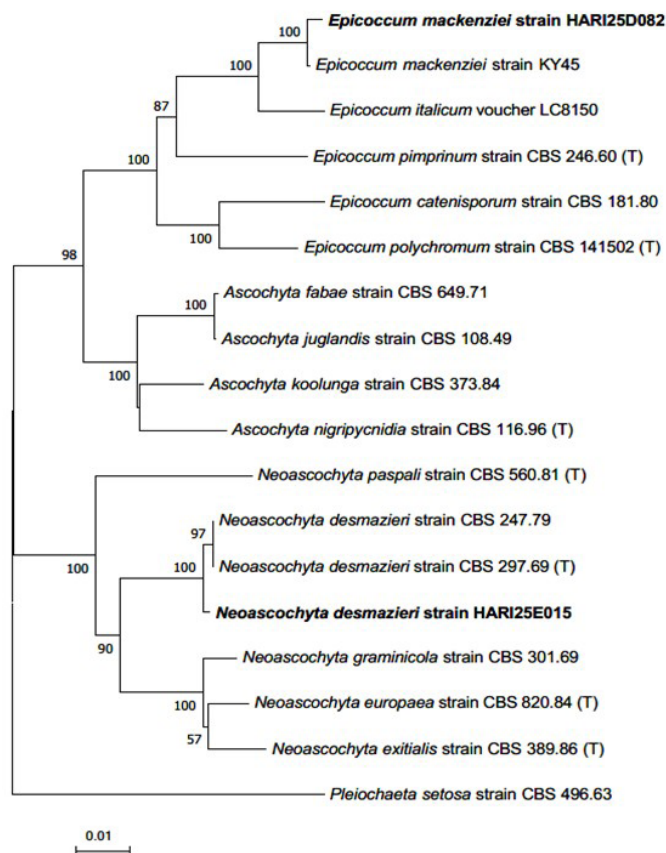


Fig. 4. Neighbor-joining phylogenetic tree of *E. mackenziei* and *N. desmazieri* on the basis of a concatenated alignment of internal transcribed spacer (ITS), large subunit (LSU) rDNA, and RNA polymerase II second-largest subunit (*RPB2*) DNA sequences. *Pleiochaeta setosa* was used as an outgroup. Numbers on branches indicate bootstrap values (1,000 replicates). Fungal strains isolated in this study are in bold. (T) indicates the type strains.

Specimen examined. Korea: Jeju-si, Jeju-do, 33°28'43.9"N, 126°47'02.0"E, May 19, 2025, isolated from healthy stem of *Solanum tuberosum*, strain HARI25E015, KACC411094 (deposit No.), GenBank No. PX285301 (ITS), PX285303 (LSU), PX289875 (*RPB2*).

Note. Phylogenetic analysis revealed a clear distinction between *Neoascochyta* and *Ascochyta* clades (Fig. 4). Although conidia were not observed in our material, the presence of ellipsoidal pycnidia and pycniospores, and molecular data support the identification of *N. desmazieri* [17].

DISCUSSION

Species belonging to the family Chaetomiaceae are commonly found in diverse environments, such as soil, indoor habitats, plant saprophytes, and endophytes [18,19]. These species, owing to their ability to produce unique enzymes and secondary metabolites, are widely used in biotechnology and agriculture [20–22]. They are typified by pigmented ascospores in fasciculate, evanescent asci [23,24], and, in many species, only the sexual stage is commonly observed [25,26].

Didymellaceae is one of the largest families within Dothideomycetes and includes the genera *Ascochyta*, *Didymella*, *Epicoccum*, and *Phoma* [27]. Species that belong to the family Didymellaceae have traditionally been recognized as plant pathogens that affect a broad host range, causing leaf and stem diseases [16]; however, some species have recently been reported as endophytes [4,28].

Collariella bostrychodes is the type species of the genus *Collariella*. Previously placed in *Chaetomium*, *C. bostrychodes* was transferred to *Collariella* on the basis of its ascomatal morphology; the genus name refers to the collar-like arrangement of developing asci [13]. Although originally described as an indoor airborne fungus [13], *C. bostrychodes* has since been reported as an endophyte in woolly plectranthus (*Coleus forskohlii*) [29] and Mongolian pine (*Pinus sylvestris* var. *mongolica*) [30]. The antagonistic activity of this species against *Rigidoporus microporus*, the causative agent of white root disease in rubber trees (*Ficus elastica*), has also been reported [31].

Ovatospora brasiliensis, the type species of *Ovatospora*, was formerly included in *Chaetomium* and later segregated on the basis of its ovate ascospore morphology [13]. Although originally isolated as a saprophyte from jute cloth [13], *O. brasiliensis* has been recovered as an endophyte from turmeric (*Curcuma caesia*) and has been implicated in the bioconversion of curcumin into calebin-A [32]. There has also been a report of the strong anti-inflammatory activity of *O. brasiliensis* isolated as an endophyte from moss [33].

Epicoccum mackenziei was first reported as a saprophytic fungus from dead branches of restharrow (*Ononis spinosa*) [15]; it has also been isolated from tea tree leaves (*Camellia sinensis*) as both a pathogen [14] and an endophyte [34].

Neosascochyta desmazieri was originally assigned to *Ascochyta* but was transferred to *Neosascochyta* on the basis of molecular evidence [17]. This species was first described as a Poaceae pathogen (e.g., *Lolium perenne*) [33]; however, this species was isolated from healthy potato stems, so we considered it an endophyte of potatoes.

CONCLUSION

In this study, endophytic fungal strains were isolated from healthy potato plant tissues collected from spring potato cultivation areas in Korea, and four endophytic fungal species previously unrecorded in Korea were identified. Several of the identified species have been reported to produce secondary metabolites and exhibit anti-fungal or anti-inflammatory activities, suggesting their potential utility in biological disease control. Given concerns regarding pesticide residues and the development of fungicide resistance,

evaluation of endophytic fungi as biological control agents is increasingly important. The strains described here warrant further study to evaluate their antagonistic activity against potato pathogens and their suitability for development as biocontrol agents.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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