RESEARCH ARTICLE

Unrecorded Fungi Isolated from Fire Blight-controlled Apple Orchard Soil in Korea

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ABSTRACT

To explore fungal diversity in orchard soil where fire-blighted apple trees are buried, we collected soil samples from apple orchards in Chungju, Korea. Fungal isolates were obtained from DG18 agar and identified at the species level based on morphological features and phylogenetic analyses. The colony characteristics and microstructures were examined using a light microscope and a scanning electron microscope after culturing on potato dextrose agar (PDA), malt extract agar (MEA), Czapek yeast agar (CYA), and oatmeal agar (OA) The PCR-amplified products of the ITS1-5.8S-ITS2 region and 28S large subunit of the nuclear ribosomal RNA gene, as well as partial sequences of the β-tubulin, calmodulin, and translation elongation factor 1-α genes were sequenced and analyzed phylogenetically. Seven previously unknown fungal species were explored in Korea. All samples, including *Aspergillus aureolatus*, *Botryotrichum atrogriseum*, *Dactylonectria novozelandica*, *Fusarium denticulatum*, *Paecilomyces tabacinus*, *Sarcopodium tibetense* and *Talaromyces stollii*, had ascomycetes. Herein, we report their descriptions and features.

Keywords: Apple orchards, Ascomycota, Fire blight control, Unrecorded species

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INTRODUCTION

Apple (*Malus pulmina*) is a Rosaceae plant whose fruit is consumed worldwide fresh or processed. In Korea, apples are representative fruit trees. In 2018, the cultivation area of apples was 33,234 ha, accounting for 21.2% fruit tree cultivation area [1]. Recently, fire blight has damaged many apple trees in orchards across several provinces in Korea [2]. It is caused by *Erwinia amylovora*, a bacterium belonging to the Enterobacteriaceae [2]. According to the burial control regulations, fire-blighted apple trees are buried 4-5 m below the soil [3]. To ensure burial control safety, soil microorganisms in apple orchards must be explored to understand the environment of the burial soil.

Apple trees buried in the soil are mostly degraded and deteriorated by saprophytic soil fungi [4]. Thus, information on soil fungal diversity is important to understand the decay process of fire-blight-controlled apple trees. Saprophytic fungi produce enzymes that decompose cellulose, hemicellulose, and pectin present in plant materials. We investigated the fungal diversity in orchard soil where fire-blighted apple trees are buried. In this study, we report the description and morphological features of seven ascomycetes previously unreported in Korea.

MATERIALS AND METHODS

Soil sampling

Soil samples were collected by an excavator from three locations at each site in several apple orchards in Chungju where all diseased trees were buried for at least 24 months. Soil was collected with permission from the Rural Development Administration, Korea and in compliance with plant quarantine regulations. Approximately 300 g soil samples were collected using a sterile spade at approximately 3 m depth from the surrounding areas that had come into contact with the buried plants. The collected soil samples were placed in zipper bags in a low-temperature cooler and transported to the laboratory.

Isolation and morphological studies of fungi

The collected soil samples were analyzed in a biosafety level 2 licensed facility at the Dankook University. A portion of the soil sample was placed in a 50 mL sterile conical tube containing 20 mL sterile distilled water and then vortexed for 3 min to suspend the soil sample. The suspension was gradually diluted to 10^4 using sterile distilled water and 100 μL diluent was spread on Dichloran-Glycerol 18% (DG18) agar medium and cultured at 25°C for 5 days. The fungal mycelia were transferred to new media, and fungal flora that appeared different from each other were selected for identification. The selected fungal strains were assigned a Dankook University Culture Collection (DUCC) number and grown for 7-9 days on potato dextrose agar (PDA), malt extract agar (MEA), Czapek yeast agar (CYA), and oatmeal agar (OA) (BD Science, Franklin Lakes, USA) for morphological studies. The colony size, shape, and color were observed using a stereomicroscope (BX53; Olympus, Tokyo, Japan). Mycelial growth was recorded by measuring the colony diameters. The microstructures were observed using a phase-contrast microscope (Karl Zeiss, Axioskop 40). For scanning electron microscopy (SEM), the fungal isolate was grown for 5-7 days at 25°C on 2% PDA plates overlaid with cellophane (Bio-Rad Laboratories, Hercules, Canada) and the ultra-structures were examined with a Hitachi S-4300 scanning electron microscope operating at 15 kV [5].

Molecular analysis

For molecular analysis, fungi were grown on PDA for 5 days at room temperature, and fungal genomic DNA was extracted from the cultured fungal mycelia using a Direct DNA Prep kit (Navibiotech, Cheonan, Korea). Using the extracted DNA as a template, molecular taxonomic marker sequences were amplified by PCR (T100 Thermal Cycler; BIO-RAD, Hercules, USA) using the primers for ITS1-5.8S-ITS2 region (ITS region), 28S large subunit of the nuclear ribosomal RNA gene (LSU region), and partial sequences of the β-tubulin, calmodulin, and translation elongation factor 1-α genes (Bioneer Corp., Daejeon, Korea; Table 1) [6-12]. The PCR conditions are listed in Table 1. PCR amplicons were electrophoresed on 1% agarose gel to confirm the amplified DNA bands. The confirmed PCR amplicon was purified using the High Pure PCR Product Purification Kit (Roche, Indianapolis, USA) and submitted to Macrogen (Seoul, Korea) for determining the nucleotide sequence.

Table 1. PCR primer sequences and conditions.

Marker region	Primer	Primer sequence (5′-3′)	Orientation	PCR condition	Reference
ITS region	ITS1	GAAGTAAAAGTCGTAACAAGG	Forward	95°C 5 min; 35 cycles: 95°C 30 s, 56°C 30 s, 72°C 1 min; 72°C 5 min	[6]
	ITS4	TCCTCCGCTATTGATATGC	Reverse		
LSU region	LROR	ACCCGCTGAACTTAAGC	Forward	95°C 5 min; 35 cycles: 95°C 30 s, 56°C 30 s, 72°C 1 min; 72°C 5 min	[7]
	LR5	TCCTGAGGGAAACTTCG	Reverse		[8]
β-tubulin gene	Bt2a	GGTAACCAAATCGGTGCTGCTTTC	Forward	95°C 10 min; 35 cycles: 95°C 50 s, 52°C 50 s, 72°C 1 min; 72°C 5 min	[9]
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC	Reverse		
TEF-1α gene	TEF728	CATCGAGAAGTTCGAGAAGG	Forward	95°C 10 min; 34 cycles: 95°C 1 min, 57°C 1 min, 72°C 1 min; 72°C 5 min	[10]
	TEF1	GCCATCCTTGGAGATACCAGC	Reverse		[11]
Calmodulin gene	CL1	GA(GA)T(AT)CAAGGAGGCCTTCTC	Forward	95°C 10 min; 34 cycles: 95°C 50 s, 55°C 50 s, 72°C 1 min; 72°C 10 min	[12]
	CL2A	TTTTTGCATCATGAGTTGGAC	Reverse		

ITS: internal transcribed spacer; LSU: large subunit; TEF-1α: translation elongation factor 1 alpha.

The fungal homologs for nucleotide sequences were searched in the NCBI DB using the BLAST program at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov). The reference sequences were obtained from the NCBI GenBank DNA database (Table 2) and analyzed using the ClustalW alignment tool of the MEGA 11 program to obtain a phylogenetic tree for the phylogenetic analysis [13-14]. The maximum likelihood method was used to construct the phylogenetic tree based on aligned sequences [15]. The Kimura 2-parameter model was used to estimate genetic differences [16]. The reliability of the nodes in the phylogenetic tree was confirmed using 1,000 bootstraps.

Table 2. The fungal species used to construct the phylogenetic tree and GenBank accession numbers of the five marker sequences for phylogenetic analysis(continued).

Scientific name	Strain	Country	ITS region	LSU 28S rDNA	β-tubulin gene	Calmodulin gene	TEF1-α gene
Aspergillus amoenus	NRRL 35600	USA				JN854033	
Aspergillus askibugien	CFF 4716	Czech Republic				LN873965	
Aspergillus asperescens	NRRL 4738	Poland				EF652384	
Aspergillus aureolatus	DUCC20603	Korea				OP973111	
Aspergillus aureolatus	NRRL 5126	Yugoslavia				EF652413	
Aspergillus aureolatus	A15	Spain				LN873972	
Aspergillus creber	MUT<ITA>:2513	Ireland				MG832144	
Aspergillus cvjetkovicii	NRRL 230	China				JN854023	
Aspergillus fructus	MUT<ITA>:2175	Tunisia				MG832132	
Aspergillus fructus	MUT<ITA>:2200	Tunisia				MG832139	
Aspergillus protuberus	CCF 4980	Romania				LT594395	
Aspergillus protuberus	S804	Romania				LT594409	
Aspergillus purpureus	NRRL 6133	Egypt				EF652418	
Aspergillus puulaauensis	MUT<ITA>:2522	Ireland				MG832146	
Aspergillus stellatus	NRRL 4793	USA				LN873965	
Aspergillus subversicolor	NRRL 58999	India				JN854010	
Aspergillus sydowii	S865	Romania				LT594404	
Aspergillus tabacinus	S124	Romania				LT594401	

Table 2. The fungal species used to construct the phylogenetic tree and GenBank accession numbers of the five marker sequences for phylogenetic analysis.(continued)

Scientific name	Strain	Country	ITS region	LSU 28S rDNA	β-tubulin gene	Calmodulin gene	TEF1-α gene
Botryotrichum atrogriseum	DUCC15157	Korea			OR756452		
Botryotrichum atrogriseum	CBS 130.28	Netherlands			KX976931		
Botryotrichum atrogriseum	CBS 604.69	Canada			KX976932		
Botryotrichum murorum	CBS 173.68	Netherlands			KX976934		
Botryotrichum murorum	DTO 324-G9	China			KX976935		
Botryotrichum peruvianum	CBS 460.90	Spain			KX976937		
Botryotrichum piluliferum	CBS 654.79	Netherlands			KX976939		
Botryotrichum piluliferum	CBS 105.14	Unknown			KX976940		
Botryotrichum piluliferum	DTO 194-F7	Netherlands			KX976941		
Botryotrichum spirotrichum	CBS 211.55	USA			KX976943		
Botryotrichum spirotrichum	CBS 828.71	Algeria			KX976944		
Byssochlamys nivea	BCC84295	Thailand		MF780715			
Dactylonectria alcacerensis	JZB3310014	China					MN968324
Dactylonectria alcacerensis	JZB3310013	China					MN968323
Dactylonectria horedeicola	3S07	China					MF350509
Dactylonectria horedeicola	EFA 443	Spain					MH070095
Dactylonectria macrodidyma	JZB3310009	China					MN956383
Dactylonectria macrodidyma	JZB3310008	China					MN956382
Dactylonectria macrodidyma	JZB3310010	China					MN956384
Dactylonectria novozelandica	BV-0760	Spain					MK602815
Dactylonectria novozelandica	DUCC15141	Korea					OP753412
Dactylonectria torresensis	N37IL	Italy					KP411803
Dactylonectria torresensis	HQ2	China					MN561697
Fusarium concentricum	M8156R	Malaysia					MK414234
Fusarium denticulatum	CBS 407.97	African					MT011002
Fusarium denticulatum	DUCC20602	Korea					OP973113
Fusarium ficicrescens	CBS 125178	Unknown					MT011004
Fusarium foetens	CBS 110286	Unknown					MT011001
Fusarium foetens	NRRL 52749	Unknown					JF740825
Fusarium fujikuroi	NM214	USA					MN896944
Fusarium globosum	CBS 428.97	Unknown					MT010993
Fusarium napiforme	CBS 748.97	Unknown					MT011011
Fusarium nygamai	CBS 749.97	Unknown					MT011009
Fusarium oxysporum	Fus436	Unknown					MK906811
Fusarium proliferatum	NRRL 25028	India					JF740705
Fusarium pseudocircinatum	CBS 449.97	Unknown					MT011003
Fusarium thapsinum	NRRL 22049	Unknown					MN193871
Paecilomyces clematidis	BRNU 677844	Czech Republic		NG_149004			
Paecilomyces dactylethromorphus	CBS 368.70	U. K.		MH871467			
Paecilomyces fulvus	CBS 135.62	Switzerland		MH869700			
Paecilomyces fulvus	CBS 146.48	England		NG_063990			
Paecilomyces niveus	CBS 134.37	Unknown		MH867357			
Paecilomyces niveus	CBS 756.71	Finland		MH872089			
Paecilomyces tabacinus	CCF 5290	USA					
Paecilomyces tabacinus	DUCC16069	Korea		OL871280			
Paecilomyces tabacinus	CCF 5290	USA		LT548280			
Paecilomyces variotii	DUCC4134	Korea		MH819182			
Pseudonectria buxi	CBS 114049	Spain			KM232038		

Table 2. The fungal species used to construct the phylogenetic tree and GenBank accession numbers of the five marker sequences for phylogenetic analysis.

Scientific name	Strain	Country	ITS region	LSU 28S rDNA	β-tubulin gene	Calmodulin gene	TEF1-α gene
Pseudonectria foliicola	CBS 123190	New Zealand			KM232035		
Sarcopodium circinatum	CBS 100998	Brazil			KM232045		
Sarcopodium circinatum	CBS 587.92	Costa Rica			KM232046		
Sarcopodium circinosetiferum	CBS 100251	Argentina			KM232041		
Sarcopodium circinosetiferum	CBS 100252	Argentina			KM232040		
Sarcopodium durantae	DN1	Thailand			MT383672		
Sarcopodium flavolanatum	CBS 112283	Ecuador			KM232044		
Sarcopodium flavolanatum	CBS 128370	China			KM232043		
Sarcopodium macalpinei	CBS 115296	Hong Kong			KM232042		
Sarcopodium tibetense	11073	Tibet			MT648557		
Sarcopodium tibetense	DUCC15158	Korea			OP753414		
Sarcopodium vanillae	CBS 100582	Ecuador			KM232039		
Stachybotrys chartarum	CBS 129.13	Unknown			KM232127		
Talaromyces adpressus	NRRL 62466	USA			MH792961		
Talaromyces domesticus	NRRL 58121	USA			MH792927		
Talaromyces flavus	CBS 22566	Unknown			AY766252		
Talaromyces lentulus	NRRL 62119	Portugal			MH792935		
Talaromyces liani	NRRL 1009	Unknown			MH792902		
Talaromyces pratensis	NRRL 62126	USA			MH792937		
Talaromyces pratensis	NRRL 13548	USA			MH792916		
Talaromyces sayulitensis	BEOFB2601m	Serbia			MH780061		
Talaromyces sayulitensis	MUT<ITA>:3092	Italy			MF351750		
Talaromyces sayulitensis	MUT<ITA>:4410	Italy			MK067054		
Talaromyces stollii	DUCC20601	Korea			OR756453		
Talaromyces stollii	NRRL 62122	USA			MH792936		
Talaromyces stollii	NRRL 62298	USA			MH792959		
Talaromyces tumuli	NRRL 62469	USA			MH792962		

The seven fungal species identified in this study are indicated in bold.
ITS: internal transcribed spacer; LSU: large subunit; TEF-1α: translation elongation factor 1 alpha.

RESULTS AND DISCUSSION

The morphological characteristics and the results of the molecular phylogenetic analysis of the seven unrecorded soil fungi are described below. The nucleotide sequences of these seven DUCC fungal strains were registered in the GenBank database, and the accession numbers are listed in Table 1. The strains were deposited at the National Institute of Biological Resources of the Republic of Korea and received NIBRFGC numbers.

Aspergillus aureolatus

The diameters of *Aspergillus aureolatus* DUCC20603 (NIBRFGC000508675) colonies were 28 mm on PDA after 14 days and 11, 12, and 14 mm on MEA, CYA, OA, respectively, after 7 days. The colonies were olive green with a white border, white, green with a thick white border, and dark green with a thin white border on PDA, MEA, CYA, and OA, respectively (Fig. 1). The DUCC20603 strain has 3-4 μm spherical conidia and 5-6 μm elongated cylindrical phialide. When observed under SEM, the conidia had projections on the surface (Fig. 1). Based on the morphological characteristics and molecular phylogenetic

analysis, the DUCC20603 strain was identified as *Aspergillus aureolatus*, showing 99.84% calmodulin gene sequence similarity to that of *A. aureolatus* (GenBank accession no. LR593496.1; Fig. 2; Table 2). *A. aureolatus* was isolated from air and reported in Yugoslavia in 1964 [17].

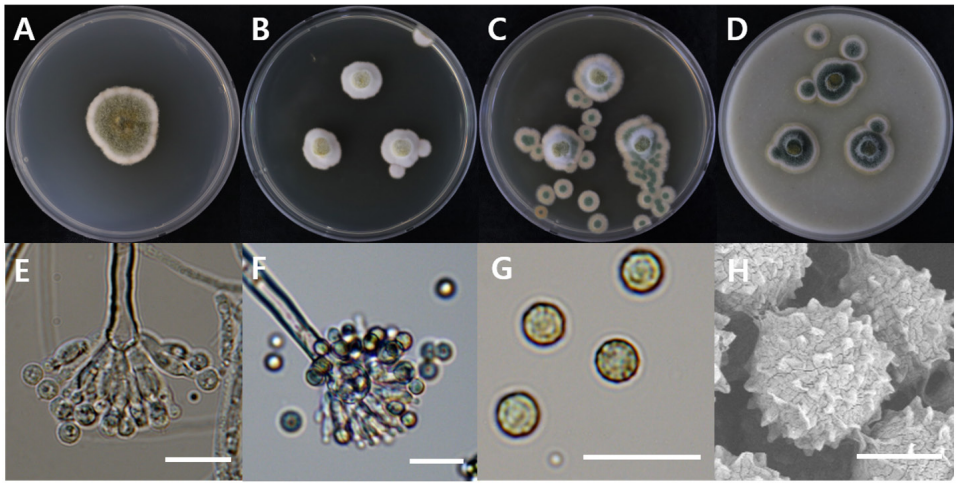

Fig. 1. Colony morphology of *Aspergillus aureolatus* DUCC20603 (NIBRFGC000508675) strain grown on different media and their microstructures. A: Potato dextrose agar (PDA). B: Malt extract agar (MEA). C: Czapek yeast agar (CYA). D: Oatmeal agar (OA). E-G: Light microscopic images of conidia, phialides, metulae, vesicle, and conidiophores. H: Scanning electron microscopy (SEM) image of conidia. Scale bar, E-G=10 μm, H=2 μm.

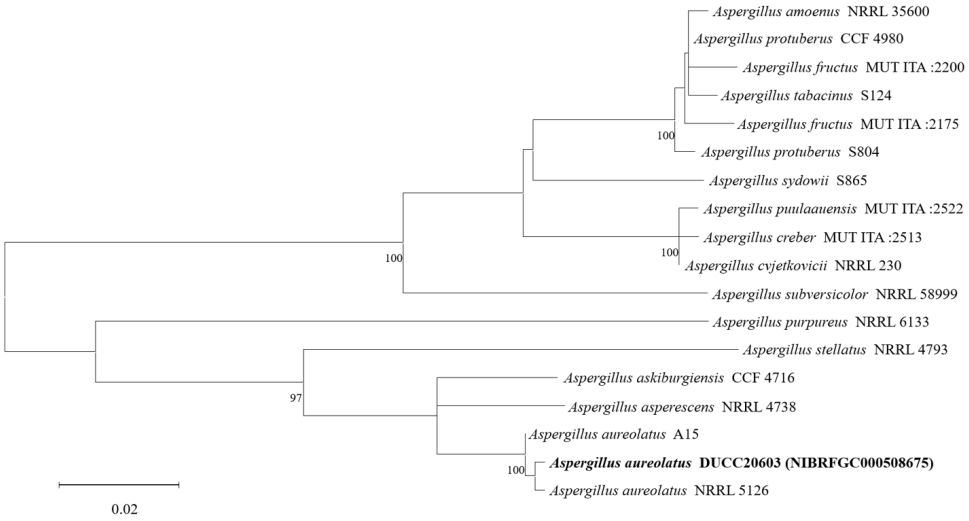

Fig. 2. Maximum likelihood phylogenetic tree based on the partial calmodulin gene sequence of *Aspergillus aureolatus* DUCC20603 (NIBRFGC000508675) strain. Fungal strain isolated in this study is indicated in bold. Only bootstrap value exceeding 80 is used.

Botryotrichum atrogriseum

Botryotrichum atrogriseum DUCC15157 (NIBRFGC000508662) colonies grew to 34, 45, 12, and 22 mm on PDA, MEA, CYA, and OA, respectively, after 14 days. The colonies were pale yellow, white, light yellow, and gray with a white border on PDA, MEA, CYA, and OA, respectively (Fig. 3). The

DUCC15157 strain has ≥130 μm diameter conidiophores and 21-25 μm light brown spherical conidia (Fig. 3). Based on the morphological characteristics and molecular phylogenetic analysis, the DUCC15157 strain was identified as *Botryotrichum atrogriseum*, showing 99.51% β-tubulin gene sequence similarity with that of *B. atrogriseum* (GenBank accession no. KX976932.1; Fig. 4; Table 2). *B. atrogriseum* was first reported in 1929 and may be used as a biological control agent against *Fusarium* wilt [18-19].

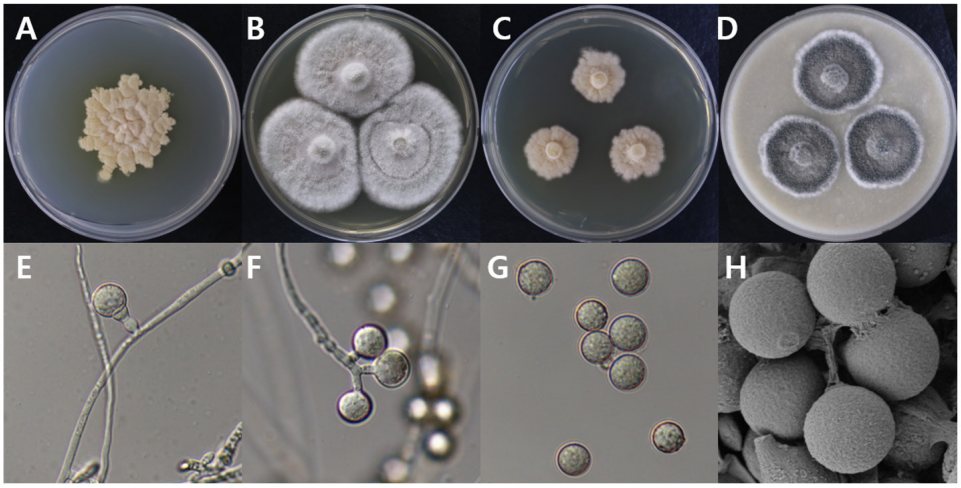

Fig. 3. Colony morphology of *Botryotrichum atrogriseum* DUCC15157 (NIBRFGC000508662) strain grown on different media and their microstructures. A: Potato dextrose agar (PDA). B: Malt extract agar (MEA). C: Czapek yeast agar (CYA). D: Oatmeal agar (OA). E-G: Light microscopic images of conidia and conidiophore. H: Scanning electron microscopy (SEM) image of conidia. Scale bar, E-G=10 μm, H=2 μm.

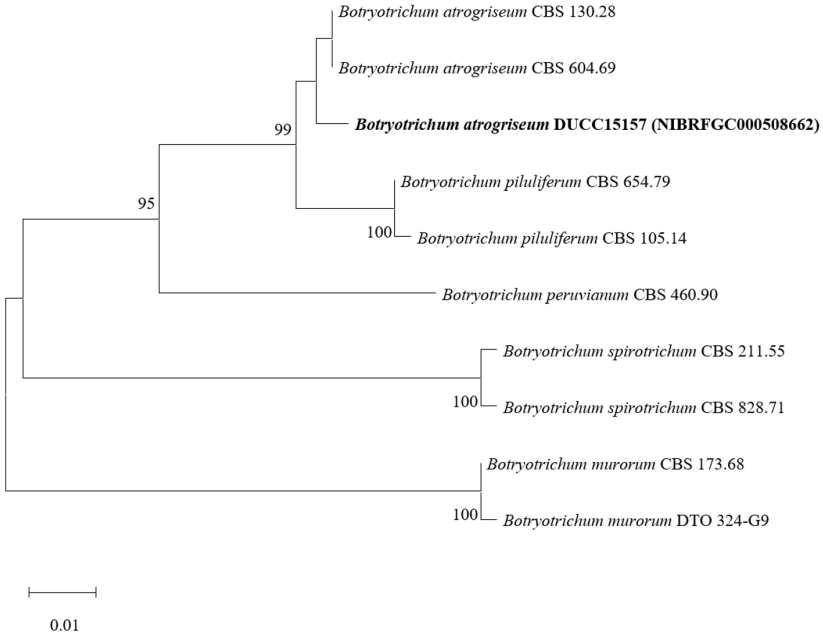

Fig. 4. Maximum likelihood phylogenetic tree based on the β-tubulin gene sequence of *Botryotrichum atrogriseum* DUCC15157 (NIBRFGC000508662) strain. Fungal strain isolated in this study is indicated in bold. Only bootstrap value exceeding 80 is used.

Dactylonectria novozelandica

Dactylonectria novozelandica DUCC15141 (NIBRFGC000508663) colonies grew to 23, 25, 19 and 21 mm in diameter on PDA, MEA, CYA, and OA, respectively, after 7 days. The colonies were yellow with a thick white border on PDA and CYA and brownish yellow with a white border on OA (Fig. 5). From the center, the colony color was yellow, purple, or brown on MEA. The DUCC15141 strain produced a brown secondary metabolite in all four media. The DUCC15141 strain has 40-100 μm conidiophores and 3.5-4.0×25-30 μm² rod-shaped conidia (Fig. 5). Based on the morphological characteristics and molecular phylogenetic analysis, the DUCC15141 strain was identified as *Dactylonectria novozelandica*, showing 99.65% translation elongation factor 1 alpha (TEF-1α) sequence similarity with that of *D. novozelandica* (GenBank accession no. MK602815.1; Fig. 6; Table 2). *D. novozelandica* is the basionym *of Ilyonectria novozelandica* and was isolated from *Vitis vinifera* in 2012 [20]. It also causes root and crown rot of strawberries in Uruguay [21].

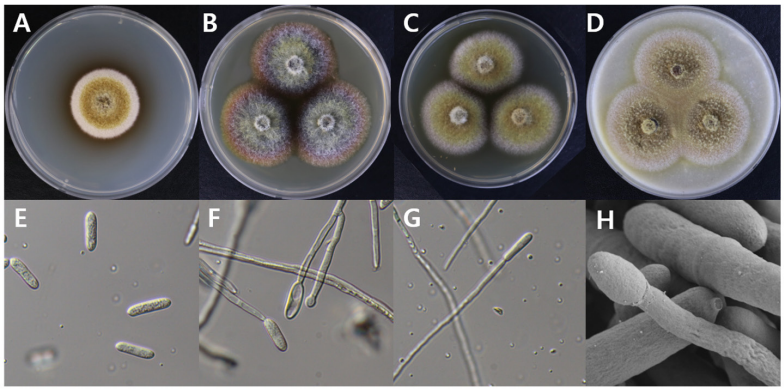

Fig. 5. Colony morphology of *Dactylonectria novozelandica* DUCC15141 (NIBRFGC000508663) strain grown on different media and their microstructures. A: Potato dextrose agar (PDA). B: Malt extract agar (MEA). C: Czapek yeast agar (CYA). D: Oatmeal agar (OA). E-G: Light microscopic images of conidia and conidiophore. H: Scanning electron microscopy (SEM) image of conidia and conidiophores. Scale bar, E-G=10 μm, H=2 μm.

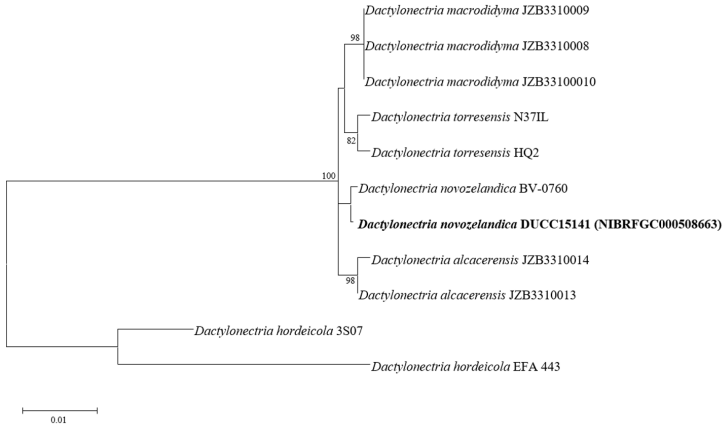

Fig. 6. Maximum likelihood phylogenetic tree based on the translation elongation factor 1 alpha (TEF1-α) gene sequence of *Dactylonectria novozelandica* DUCC15141 (NIBRFGC000508663) strain. Fungal strain isolated in this study is indicated in bold. Only bootstrap value exceeding 80 is used.

Fusarium denticulatum

Fusarium denticulatum DUCC20602 (NIBRFGC000508674) colonies grew to 47, 45, 47 and 21 mm on PDA, MEA, CYA, and OA, respectively, after 7 days. The colonies were white with mycelial band edges on PDA, light orange on MEA, and white on CYA and OA (Fig. 7). The DUCC20602 strain has 6-8 μm elliptical conidia (Fig. 7). Based on the morphological characteristics and molecular phylogenetic analysis, DUCC20602 strain was identified as *Fusarium denticulatum*, showing 99.78% TEF-1α sequence similarity with that of *F. denticulatum* (GenBank accession no. MT011002.1; Fig. 8; Table 2). *F. denticulatum* was isolated from *Ipomoea batatas* in Louisiana in 1998 and caused chlorotic leaf distortion in sweet potatoes in China [22-23].

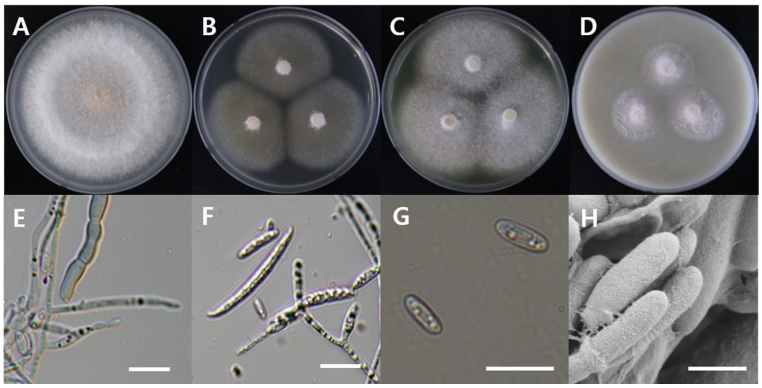

Fig. 7. Colony morphology of *Fusarium denticulatum* DUCC20602 (NIBRFGC000508674) strain grown on different media and their microstructures. A: Potato dextrose agar (PDA). B: Malt extract agar (MEA). C: Czapek yeast agar (CYA). D: Oatmeal agar (OA). E-G: Light microscopic images of conidia, conidiophore, and macroconidia. H: Scanning electron microscopy (SEM) image of conidia and conidiophores. Scale bar, E-G=10 μm, H=1 μm.

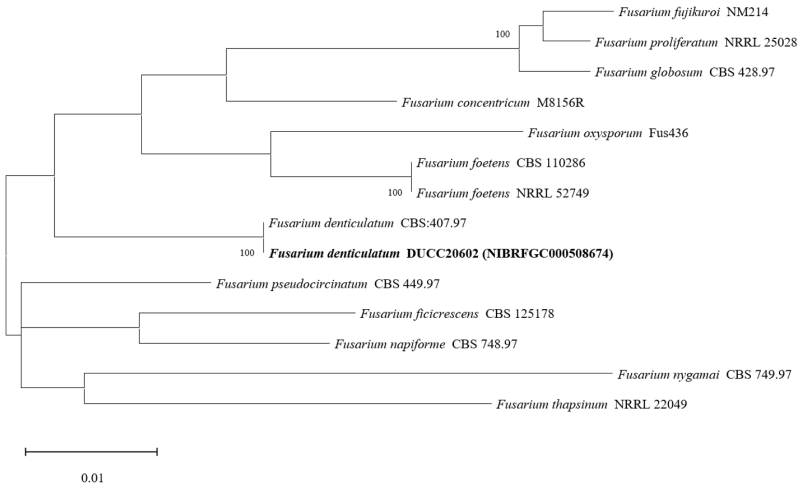

Fig. 8. Maximum likelihood phylogenetic tree based on the translation elongation factor 1 alpha (TEF1-α) sequence of *Fusarium denticulatum* DUCC20602 (NIBRFGC000508674) strain. Fungal strain isolated in this study is indicated in bold. Only bootstrap value exceeding 80 is used.

Paecilomyces tabacinus

Paecilomyces tabacinus DUCC16069 (NIBRFGC000508666) colonies grew to 35, 55, 39, and 41 mm on PDA, MEA, CYA, and OA, respectively, after 7 days. The colonies were light brown on PDA, white with a brown center on MEA and OA, and dark brown on CYA. The DUCC16069 strain has 2-6 μm oval or globular conidia (Fig. 9). Based on the morphological characteristics and molecular phylogenetic analysis of the LSU region, DUCC16069 strain was identified as *Paecilomyces tabacinus*, showing 100 region sequence similarity with that of *P. tabacinus* (GenBank accession no. LT548280.1; Fig. 10; Table 2). *P. tabacinus* was isolated from the leaves of *Nicotiana tabacum* in North Carolina, USA in 2016 [24].

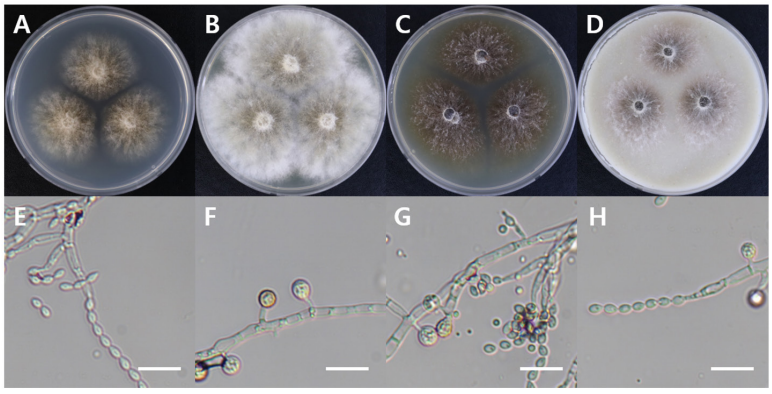

Fig. 9. Colony morphology of *Paecilomyces tabacinus* DUCC16069 (NIBRFGC000508666) strain grown on different media and their microstructures. A: Potato dextrose agar (PDA). B: Malt extract agar (MEA). C: Czapek yeast agar (CYA). D: Oatmeal agar (OA). E-H: Light microscopic images of conidia and conidiophores. Scale bar, 10 μm.

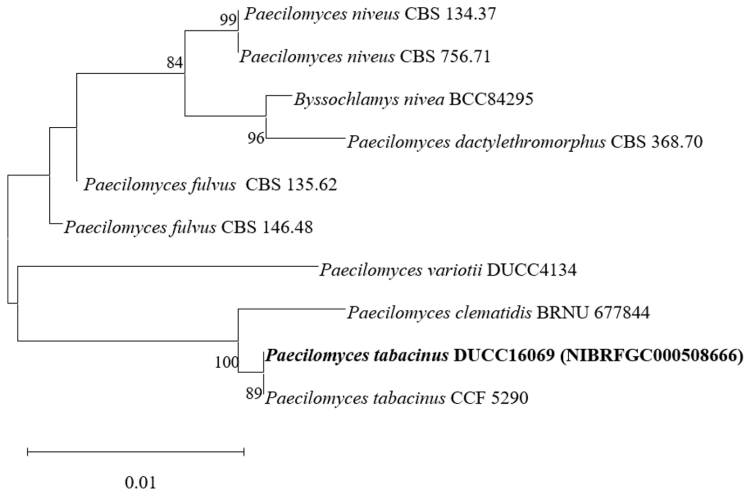

Fig. 10. Maximum likelihood phylogenetic tree based on the 28S large subunit of the nuclear ribosomal RNA gene sequence of *Paecilomyces tabacinus* DUCC16069 (NIBRFGC000508666) strain. Fungal strain isolated in this study is indicated in bold. Only bootstrap value exceeding 80 is used.

Sarcopodium tibetense

Sarcopodium tibetense DUCC15158 (NIBRFGC000508664) colonies grew to 21, 35, 36, and 23 mm on PDA, MEA, CYA, and OA, respectively, after 7 days. The colonies were dark yellow, pale yellow with a white border, mixed yellow and white, and yellow with a white border on PDA, MEA, CYA, and OA, respectively (Fig. 11). The DUCC15158 strain produces 1-1.5×3.5-4.0 μm² elliptical conidia from very dense (10 μm or more overlapped) and short (10-15 μm) as well as 4-5 μm circular conidia from undifferentiated conidiophores. When observed using SEM, the conidia appeared as slightly oval-like eggs (Fig. 11). Based on the morphological characteristics and molecular phylogenetic analysis of the β-tubulin gene, DUCC15158 was identified as *Sarcopodium tibetense*, showing 99.40% β-tubulin sequence similarity with that of *S. tibetense* (GenBank accession no. MT648557.1; Fig. 12; Table 2). *S. tibetense* was reported from China in 2021 [25].

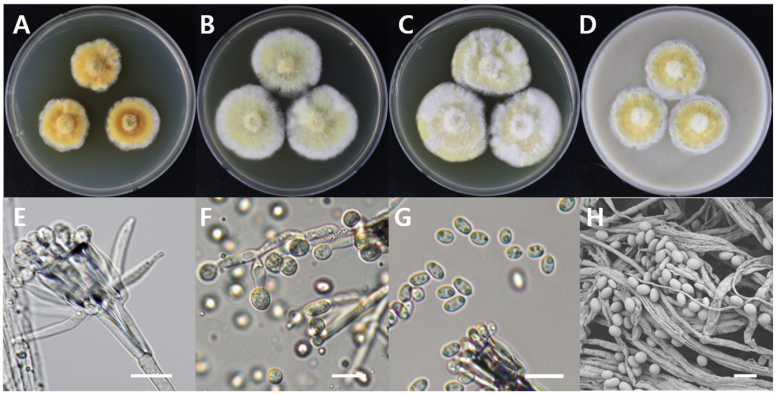

Fig. 11. Colony morphology of *Sarcopodium tibetense* DUCC15158 (NIBRFGC000508664) strain grown on different media and their microstructures. A: Potato dextrose agar (PDA). B: Malt extract agar (MEA). C: Czapek yeast agar (CYA). D: Oatmeal agar (OA). E-G: Light microscopic images of conidia and conidiophore. H: Scanning electron microscopy (SEM) image of conidia and conidiophores. Scale bar, E-G=10 μm, H=2 μm.

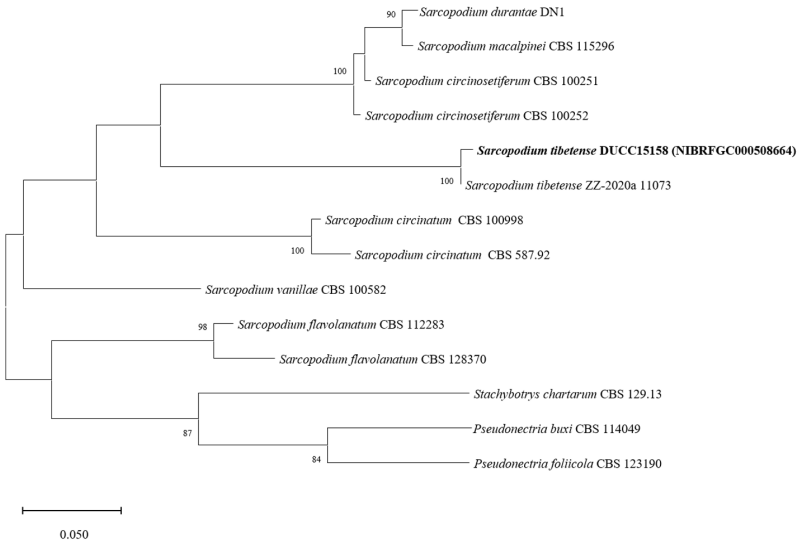

Fig. 12. Maximum likelihood phylogenetic tree based on the α-tubulin gene sequence of *Sarcopodium tibetense* DUCC15158 (NIBRFGC000508664) strain. Fungal strain isolated in this study is indicated in bold. Only bootstrap value exceeding 80 is used.

Talaromyces stollii

Talaromyces stollii DUCC20601 (NIBRFGC000508673) colonies grew to 32, 14, 27 and 33 mm on PDA, MEA, CYA, and OA, respectively, after 7 days. The colonies were green with a white border on PDA and CYA, white on MEA, and dark green with a white border on OA (Fig. 13). The DUCC20601 strain has 2-3 μm spherical conidia and 6-7 μm elongated cylindrical phialide. When observed under SEM, the conidia had a bumpy surface (Fig. 13). Based on the morphological characteristics and molecular phylogenetic analysis, DUCC20601 strain was identified as *Talaromyces stollii*, showing 99.87% β-tubulin gene sequence similarity to *T. stollii* (GenBank accession no. MH792959.1) (Fig. 14; Table 2). In 2012, *T. stollii* was isolated from a patient with acquired immunodeficiency syndrome in the Netherlands in 2012 and caused pineapple fruitlet core rot in 2020 [26-27].

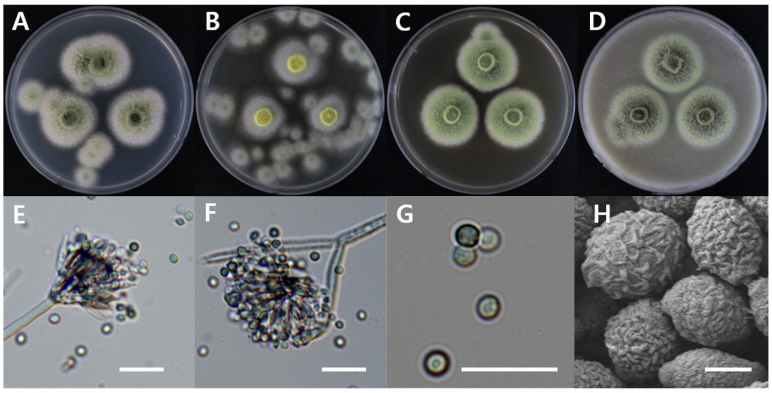

Fig. 13. Colony morphology of *Talaromyces stollii* DUCC20601 (NIBRFGC000508673) strain grown on different media and their microstructures. A: Potato dextrose agar (PDA). B: Malt extract agar (MEA). C: Czapek yeast agar (CYA). D: Oatmeal agar (OA). E-G: Light microscopic images of conidia, phialides, metule and conidiophore. H: Scanning electron microscopy (SEM) image of conidia. Scale bar, E-G=10 μm, H=1 μm.

Fig. 14. Maximum likelihood phylogenetic tree based on the β-tubulin gene sequence of T*alaromyces stollii* DUCC20601 (NIBRFGC000508673) strain. The fungal strain isolated in this study is indicated in bold. Only bootstrap value exceeding 80 is used.

CONFLICT OF INTERESTS

The authors declared no conflicts of interest.

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