RESEARCH ARTICLE

Isolation and Characterization of Previously Undescribed Seventeen Fungal Species Belonging to the order Hypocreales in Korea

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ABSTRACT

Hypocreales is one of the largest orders within the class Sordariomycetes in Ascomycota. Several species of this order are cosmopolitan and have a broad range of habitats. Here, we isolated several fungal strains from environmental samples, including freshwater sediment and plant litter. The strains were identified via molecular and phylogenetic analyses of rDNA and other DNA markers, such as *TUB*, *RPB2*, and *EF1*. The morphological characteristics of the fungi were investigated using microscopy, and culture characteristics were assessed from their growth on several media. We identified 17 species previously unrecorded in Korea: *Dactylonectria hordeicola*, *Flavocillium bifurcatum*, *Fusarium luffae*, *Ilyonectria ilicicola*, *Ilyonectria qitaiheensis*, *Ilyonectria robusta*, *Lecanicillium aphanocladii*, *Nectria ulmicola*, *Neonectria lugdunensis*, *Ovicillium oosporum*, *Pseudonectria foliicola*, *Sarocladium spinificis*, *Scolecofusarium ciliatum*, *Trichoderma appalachiense*, *Trichoderma subviride*, *Trichoderma taiwanense*, and *Trichoderma tsugarense*.

Keywords: Environmental sample, Hypocreales, New records

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INTRODUCTION

Hypocreales is one of the largest orders within the class Sordariomycetes in Ascomycota. More than 2,647 species (seven families and 237 genera) belong to this order [1]. Fungal species in this order generally show brightly colored perithecial ascomata or spore-producing structures. They are found worldwide and in various habitats, including aquatic and terrestrial environments. As described in the National List of Species of Korea, 212 species in this order have been reported in Korea until 2020 [2].

The genus *Dactylonectria* belongs to the family Nectriaceae, one of the largest families of Hypocreales, Ascomycota. To date, 18 species belonging to this genus have been reported [3]. Several species of this genus have been reported as plant pathogens and endophytic fungi [4]. This genus is classified in terms of its anamorphic stage and conidial morphology [5].

In the genus *Ilyonectria* of the family Nectriaceae, 37 species have been reported in 2021 [3]. Various species of this genus have been reported as saprophytes on decaying wood [4]. This genus is classified according to the characteristics of its sexual stage and conidia morphology [5-7].

In the genus *Nectria* of the family Nectriaceae, 938 species have been reported in 2021 [3]. Several species of this genus have been reported as plant soilborne pathogens [4]. This genus is classified by characteristics such as sexual stage and mycological morphology, such as color and texture [8-10].

The genus *Neonectria* belongs to the family Nectriaceae. To date, 52 species belonging to this genus have been reported [3]. Several species of this genus are common in tropical and temperate regions and have been reported as woody plant pathogens [11]. Occasionally, they have been found in decaying materials [4,12]. This genus is classified according to characteristics such as sexual stage and conidial morphology [8].

In the genus *Pseudonectria* of the family Nectriaceae, 40 species have been reported to date [3]. Several species of this genus are well-known plant pathogens [13]. Occasionally, they have been found in decaying materials [4]. This genus is classified according to characteristics such as sexual stage and conidial morphology [4,14].

The genus *Fusarium* is one of the largest genera in the family Nectriaceae, and more than 1,000 species have been reported in 2021. Several species of this genus have been reported as plant pathogens in the air and soil [15]. This genus is classified according to the characteristics of sexual stage and morphology of asexual conidia [16].

The genus *Scolecofusarium* of the family Nectriaceae has been classified as a new combination in 2021. To date, only one species belonging to this genus has been reported [3]. This genus is classified according to its unique sexual stage and pionnotes on the agar surface [17].

The genus *Flavocillium* belongs to Cordycipitaceae, one of the largest families of Hypocreales, and only four species belonging to this genus have been reported [18]. All species of this genus were in the genus *Lecanicillium* until 2020 and have recently been introduced as a new genus by multigene phylogeny [18]. Some species of this genus have been reported as entomopathogenic fungi [18]. This genus is classified by its sexual and asexual stages [18].

In the genus *Lecanicillium* of the family Cordycipitaceae, more than 21 species have been reported [19]. Some species of this genus have been reported as entomopathogenic fungi [19] and have been used as commercial biological pesticides [20]. This genus is classified by an asexual stage [19].

The genus *Ovicillium* belongs to Bionectriaceae, one of the common families of Hypocreales, and was classified as a new combination in 2016 [21]. To date, only four species have been reported [3]. Fungal species of this genus are found in mushrooms, wood, plants, and soil [21]. This genus is classified according to characteristics such as asexual stage and conidial morphology [21].

The genus *Sarocladium* belongs to Sarocladiaceae, one of the families of Hypocreales, and was classified as a new combination in 2016 [21]. Thirty species have been reported to date [3]. Several species of *Sarocladium* have been reported as plant pathogens or as saprobes or human pathogens [22]. This genus is classified based on characteristics such as the asexual stage, conidial morphology, and molecular phylogeny [22].

The genus *Trichoderma* belongs to Hypocreaceae, one of the largest families of Hypocreales, and this genus is one of the largest genera in Hypocreales. To date, 483 species have been reported [3]. Several species of this genus are known to be potential biocontrol agents for plant pathogens, and studies on antimicrobial activities have been carried out for a long time [23]. This genus is classified based on the morphological characteristics of the asexual stage [24].

To the best of our knowledge, this is the first report of 17 Hypocreales species (*Dactylonectria hordeicola*, *Flavocillium bifurcatum*, *Fusarium luffae*, *Ilyonectria ilicicola*, *Ilyonectria qitaiheensis*, *Ilyonectria robusta*, *Lecanicillium aphanocladii*, *Nectria ulmicola*, *Neonectria lugdunensis*, *Ovicillium oosporum*, *Pseudonectria foliicola*, *Sarocladium spinificis*, *Scolecofusarium ciliatum*, *Trichoderma appalachiense*, *Trichoderma subviride*, *Trichoderma taiwanense*, and *Trichoderma tsugarense*) in Korea from environmental samples such as freshwater, plant litter, sediment in rivers, wetland, streams, and forest soil; the molecular, phylogenetic and morphological characteristics of these species were also investigated.

MATERIALS AND METHODS

Isolation of fungal strains and culture conditions

Fungal strains were collected from environmental samples, including freshwater and forests, in Korea. The collection information for all strains identified in this study is listed in Table 1. To isolate fungal strains, plant litter samples were washed with distilled water at least twice and incubated in a pretreatment liquid medium (0.05% 3-morpholinopropane-1-sulfonic acid [weight/volume (w/v)], 0.05% KNO_3 [w/v], 0.025% KH_2PO_4 [w/v], and 0.025% K_2HPO_4 [w/v]) at 20°C for 3 d. Thereafter, 100 µL of the pretreatment medium was spread on a 1% water agar plate and incubated at 20°C for 2 d. Hyphal tips and germinated conidia were isolated under a microscope, transferred onto a 24-well plate containing V8 agar (V8A; 8% V8 juice [v/v] and 1.5% agar [w/v], adjusted to pH 6.0, using 10 N NaOH), and incubated at 25°C in the dark. A dilution method was used to isolate fungal strains from sediment and soil. The diluted suspensions (200 µL) of sediment or soil with distilled water (1:200 and 1:2,000) were spread on potato dextrose agar (PDA; 3.9% PDA powder [w/v]; Difco, Sparks, MD, USA) containing 50 ppm streptomycin, and fungal strains were isolated in pure form after incubation for 4-5 d at 25°C by repeating this step. All strains identified in this study were grown on malt extract agar (MEA; 2% malt extract [w/v] and 2% agar [w/v]), oatmeal agar (OA; 7.25% OA powder [w/v]; Difco), synthetic nitrogen-poor or nutrient-poor agar (SNA; 0.02% sucrose [w/v], 0.02% glucose [w/v], 0.1% KNO_3 [w/v], 0.1% KH_2PO_4 [w/v], 0.05% $MgSO_4 \cdot 7H_2O$ [w/v], 0.05% NaCl [w/v], and 1.2% agar [w/v]), corn meal dextrose agar (CMDA; 2% cornmeal [w/v], 2% glucose [w/v], and 2% agar), and yeast extract peptone dextrose agar (YPDA; Duchefa Biochemie, Haarlem, Netherland).

Table 1. Information of strains used in this study.

Species	Strain no.	Source	Collection date	Location (GPS)
Dactylonectria hordeicola	NNIBRFG27733	freshwater	May 06, 2020	Hwajeon-dong, Taebaek-si, Gangwon-do (37°12'14"N, 128°56'34"E)
Flavocillium bifurcatum	NNIBRFG4608	soil	July 04, 2017	Gohan-ri, Gohan-eup, Jeongseon, Gangwon-do (37°8'57.4"N, 128°54'10.8"E)
Fusarium luffae	NNIBRFG28864	Plant root	August 21, 2020	Jipyeong-ri, Gonggeom-myeon, Sangju-si, Gyeongsangbuk-do (36°32'16.2"N, 128°6'16.2"E)
Ilyonectria ilicicola	NNIBRFG29120	freshwater	March 06, 2020	Songgang-ri, Sancheok-myeon, Chungju-si, Chungcheongbuk-do (37°7'10"N, 127°58'9.4"E)
Ilyonectria qitaiheensis	NNIBRFG535	Plant root	August 12, 2016	Gangjeong-dong, Seogwipo-si, Jeju-do (33°14'1.1"N, 126°29'13.5"E)
Ilyonectria robusta	NNIBRFG6883	Plant root	July 27, 2018	Neunggang-ri, Susan-myeon, Jecheon-si, Chungcheongbuk-do (36°59'27"N, 128°12'30"E)
Lecanicillium aphanocladii	NNIBRFG2172	freshwater	April 08, 2016	Gunwi-eup, Gunwi-gun, Gyeongsangbuk-do (36°13'55"N, 128°33'45.6"E)
Nectria ulmicola	NNIBRFG22085	freshwater	March 06, 2019	Namhansanseong-myun, Gwangju-si, Gyeonggi-do (37°28'30"N, 127°13'8.3"E)
Neonectria lugdunensis	NNIBRFG2160	freshwater	April 07, 2016	Hamaengbang-ri, Geundeok-myeon, Samcheok-si, Gangwon-do (37°23'6.5"N, 129°11'27.9"E)
Ovicillium oosporum	NNIBRFG4781	sediment in freshwater	September 20, 2017	Dumil-ri, Gapyeong-eup, Gapyeong-gun, Gyeonggi-do (37°49'42.7"N, 127°26'12.2"E)
Pseudonectria foliicola	NNIBRFG15097	sediment in freshwater	September 05, 2018	Maehyang-dong, Paldal-gu, Suwon, Gyeonggi-do (37°16'59.3"N, 127°1'4.9"E)
Sarocladium spinificis	NNIBRFG27318	sediment in freshwater	March 19, 2019	Seonhak-ri, Haeryong-myeon, Suncheon-si, Jeollanam-do (34°53'7"N, 127°30'34"E)
Scolecofusarium ciliatum	NNIBRFG22713	Plant litter	March 28, 2019	Nocheon-ri, Yeonggwimi-myeon, Hongcheon-gun, Gangwon-do (37°40'32"N, 128°1'57"E)
Trichoderma appalachiense	NNIBRFG15407	freshwater	December 12, 2018	Sanghyo-dong, Seogwipo-si, Jeju-do (33°17'59"N, 126°34'54"E)
Trichoderma subviride	NNIBRFG6329	sediment in freshwater	June 22, 2018	Ihwa-ri, Ubo-myeon, Gunwi-gun, Gyeongsangbuk-do (36°10'57"N, 128°40'15"E)
Trichoderma taiwanense	NNIBRFG20685	sediment in freshwater	December 5, 2018	Iyeon-ri, Danbuk-myeon, Uiseong-gun, Gyeongsangbuk-do (36°22'43"N, 128°23'27"E)
Trichoderma tsugarense	NNIBRFG23468	sediment in freshwater	April 18, 2019	Ongjeong-ri, Hanbando-myeon, Yeongwol-gun, Gangwon-do (37°13'7"N, 128°20'52"E)

DNA extraction, polymerase chain reaction (PCR), and DNA sequencing

Fungal genomic DNA was isolated using the NucleoSpin® Plant II DNA extraction kit (Macherey-Nagal, Düren, Germany). For molecular identification of fungi, PCR amplification was performed for the internal transcribed spacer (ITS) rDNA region using primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [25], for the large subunit of rDNA (LSU) using primers LR0R (5′-ACCCGCTGAACTTAAGC-3′) and LR7 (5′-TACTACCACCAAGATCT-3′) [26], for the beta-tubulin gene (TUB) using primers bt2a (5′-GGTAACCAAATCGGTGCTGCTTTC-3′) and bt2b (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′) [27], for the translation elongation factor 1 gene (EF1) using primers EF1-983F (5′-GCYCCYGGHCAYCGTGAYTTYAT-3′) and EF1-

1576R (5′-ACHGTRCCRATACCACCRATCTT-3′) [28], and for the RNA polymerase II gene (RPB2) using primers fRPB2-5F (5′-GAYGAYMGWGATCAYTTYGG-3′) and fRPB2-7cR (5′-CCCATRGCTTGYTTRCCCAT-3′) [29]. Amplicons were sequenced with the help of a DNA sequencing service (Macrogen Inc., Seoul, Korea) using the same primers as those used for amplification. A homology search of DNA sequences was performed using BLAST algorithms available from the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov).

Phylogenetic analysis

Previously published reference sequences [4-24,30-45] were obtained from the NCBI (Table 2). Sequences were edited using the DNAStar software package version 5.05 (DNAStar, Inc., Madison, WI, USA). The accession numbers of sequences used in this study are shown in phylogenetic trees (Figs. 1-6) and Table 2. Phylogenetic trees were constructed using the maximum likelihood (ML) anlalysis, which was performed using MEGA 7.1 [46] with the default settings of the program, except for replacement with the Tamura-Nei model. Bootstrapping analysis of 1,000 replicates was performed to test the robustness of each grouping.

Table 2. Taxa, collection numbers, and GenBank accession numbers used in the present study.

Species	Culture Collection no.	GenBank accession no.				
	(Isolate no.)	ITS	TUB	RPB2	LSU	EF1
Chlamydocillium cyanophilum	(T) CBS 246.74a	KU382145	-	-	KU382212	-
Chlamydocillium cyanophilum	CBS 599.93	KU382146	-	-	KU382214	-
Chlorocillium griseum	CBS 101243, IMI 185384	KU382149	-	-	KU382217	-
Dactylonectria estremocensis	CBS 129085T; CPC 19170	JF735320	JF735448	-	-	-
Dactylonectria hordeicola	CBS 162.89T	AM419060	AM419084	-	-	-
Dactylonectria hordeicola	**NNIBRFG27733**	OK090964	OK235331	-	-	-
Dactylonectria macrodidyma	CBS 112615T; CPC 3976	AY677290	AY677233	-	-	-
Dactylonectria torresensis	CBS 129086T; CPC 19171	JF735362	JF735492	-	-	-
Flavocillium acerosum	CBS 418.81	EF641893	-	-	KM283786	KM283810
Flavocillium subprimulinum	HKAS99548	MG585314	-	-	MG585315	MG585317
Flavocillium subprimulinum	HKAS99549	MG585318	-	-	MG585319	MG585321
Flavocillium bifurcatum	YFCC 6101 T	MN576833			MN576781	MN576951
Flavocillium bifurcatum	**NNIBRFG4608**	OK090968	-	-	OK090990	OK235318
Fusarium concolor	NRRL 13459	-	-	GQ505852	-	-
Fusarium luffae	NNIBRFG28864	-	-	OK235319	-	-
Fusarium luffae	CQ1038	MK280852	-	MK289723	-	-
Fusarium luffae	LC12167	MK280807	-	MK289754	-	-
Fusarium luffae	NRRL	GQ505701	-	GQ505790	-	-
Fusarium luffae	NRRL	GQ505697	-	GQ505786	-	-
Fusarium nanum	LC12168	MK280794	-	MK289755	-	-
Fusarium nanum	LC1384	MK280842	-	MK289764	-	-
Fusarium nanum	LC1385	MK280781	-	MK289765	-	-
Fusarium nanum	LC1516	MK280782	-	MK289766	-	-

Table 2. Continued.

Species	Culture Collection no.	GenBank accession no.				
	(Isolate no.)	ITS	TUB	RPB2	LSU	EF1
Fusarium nanum	NRRL 22244	GQ505685	-	GQ505774	-	-
Fusarium nanum	NRRL 32868	GQ505706	-	GQ505795	-	-
Fusarium nanum	NRRL 32993	GQ505709	-	GQ505798	-	-
Fusarium pernambucanum	URM 6801	MH668834	-	LS398513	-	-
Fusarium pernambucanum	URM 6810	MH668836	-	LS398515	-	-
Fusarium pernambucanum	URM 6812	MH668835	-	LS398514	-	-
Fusarium scirpi	NRRL 13402	GQ505681	-	GQ505770	-	-
Fusarium scirpi	NRRL 29134	GQ505694	-	GQ505783	-	-
Fusarium scirpi	NRRL 36478	GQ505743	-	GQ505832	-	-
Hypomyces tubariicola	CBS 225.84	KU382162	-	-	KU382220	-
Ilyonectria ilicicola	Cy-FO-224	KY676883	KY676877	-	-	-
Ilyonectria ilicicola	CBS 142828; Cy-FO-225	KY676884	KY676878	-	-	-
Ilyonectria ilicicola	Cy-FO-226	KY676885	KY676879	-	-	-
Ilyonectria ilicicola	**NNIBRFG29120**	OK090965	OK235332	-	-	-
Ilyonectria qitaiheensis	CGMCC 3.18787 = H309	MF350472	MF350418	-	-	-
Ilyonectria qitaiheensis	J919	MF350473	MF350419	-	-	-
Ilyonectria qitaiheensis	**NNIBRFG535**	OK090957	OK235325	-	-	-
Ilyonectria robusta	CBS 308.35	JF735264	JF735377	-	-	-
Ilyonectria robusta	CBS 129084	JF735273	JF735391	-	-	-
Ilyonectria robusta	J906	KM015300	KM015297	-	-	-
Ilyonectria robusta	**NNIBRFG6883**	OK090959	OK235326	-	-	-
Lecanicillium antillanum	CBS 350.85	AJ292392	-	-	AF339536	DQ522350
Lecanicillium aphanocladii	CBS 797.84	-	-	-	KM283787	KM283811
Lecanicillium aphanocladii	**NNIBRFG2172**	OK090967	-	-	OK090991	OK235317
Lecanicillium aranearum	CBS 726.73a	AJ292464	-	-	AF339537	EF468781
Lecanicillium araneicola	BTCC-F35	AB378506	-	-	-	-
Lecanicillium araneogenum	GZU1031Lea	-	-	-	-	KX845697
Lecanicillium attenuatum	CBS 402.78	AJ292434	-	-	AF339565	EF468782
Lecanicillium flavidum	CBS 300.70D	EF641877	-	-	KM283789	KM283813
Lecanicillium fungicola var. aleophilum	CBS 357.8	NR_111064	-	-	KM283791	KM283815
Lecanicillium fungicola var. fungicola	CBS 992.69	NR_119653	-	-	KM283792	KM283816
Lecanicillium psalliotae	CBS 532.81	JN049846	-	-	AF339560	EF469067
Lecanicillium tenuipes	CBS 309.85	JN036556	-	-	KM283802	DQ522341
Lecanicillium wallacei	CBS 101237	EF641891	-	-	AY184967	EF469073
Leptobacillium leptobactrum	CBS 703.86	EF641866	-	-	KU382226	-
Nectria balansae	CBS 123351; AR 4446	HM484552	HM484607	-	-	-
Nectria balansae	CBS 129349; AR 4635	JF832653	JF832908	-	-	-
Nectria nigrescens	CBS 125148T; AR 4211	HM484707	HM484806	-	-	-
Nectria ulmicola	CFCC 52117	MG231959	MG232043	-	-	-
Nectria ulmicola	CFCC 52118	MG231960	MG232044	-	-	-
Nectria ulmicola	**NNIBRFG22085**	OK090962	OK235329	-	-	-

Table 2. Continued.

Species	Culture Collection no.	GenBank accession no.				
	(Isolate no.)	ITS	TUB	RPB2	LSU	EF1
Neonectria candida	CBS 151.29; IMI 113894; MUCL 28083	AY677291	DQ789863	-	-	-
Neonectria lugdunensis	CBS 127475	KM515896	KM515888	-	-	-
Neonectria lugdunensis	**NNIBRFG2160**	OK090958	OK235327	-	-	-
Neonectria neomacrospora	CBS 324.61; DSM 62489	JF735312	DQ789875	-	-	-
Neonectria neomacrospora	CBS 198.62; BBA 9628; IMI 113890	AJ009255	DQ789866	-	-	-
Neonectria tsugae	CBS 788.69T	KM231763	KM232020	-	-	-
Ovicillium napiforme	(T) CBS 426.95	KU382192	-	-	KU382233	-
Ovicillium oosporum	**NNIBRFG4781**	OK090966	-	-	OK090989	-
Ovicillium oosporum	CBS 110152	KU382194	-	-	KU382234	-
Ovicillium subglobosum	(T) CBS 101963	KU382205	-	-	KU382235	-
Penicillium rubefaciens (outgroup)	CBS 145.83	MH861557	KJ834487	-	-	-
Protocrea pallida	CBS 121552	-	-	EU703944	-	-
Pseudonectria buxi	CBS 324.53	KM231778	KM232037	-	-	-
Pseudonectria buxi	CBS 114049; AR 2716	KM231779	KM232038	-	-	-
Pseudonectria foliicola	CBS 122566; AR 2709	KM231777	KM232036	-	-	-
Pseudonectria foliicola	CBS 123190T; CPC 15385	KM231776	KM232035	-	-	-
Pseudonectria foliicola	**NNIBRFG15097**	OK090960	OK235328	-	-	-
Sarocladium bacillisporum	CBS 425.67 T	HE608639	-	-	HE608658	-
Sarocladium bactrocephalum	CBS 749.69	HG965006	-	-	HQ231994	-
Sarocladium bifurcatum	UTHSC05-3311 T	HG965009	-	-	HG965057	-
Sarocladium brachiariae	CGMCC 2192 T	EU880834	-	-	KP715271	-
Sarocladium gamsii	CBS 707.73 T	HG965015	-	-	HG965063	-
Sarocladium glaucum	CBS 796.69 T	FN691454	-	-	HE608657	-
Sarocladium hominis	UTHSC04-1034 T	HG965012	-	-	HG965060	-
Sarocladium kiliense	CBS 122.29 T	FN691446	-	-	HQ232052	-
Sarocladium mycophilum	CBS 166.92 T	HG965024	-	-	HG965046	-
Sarocladium ochraceum	CBS 428.67 T	HG965025	-	-	HQ232070	-
Sarocladium oryzae	CBS180.74 ET	HG965026	-	-	HG965047	-
Sarocladium oryzae	CBS 399.73	HG965027	-	-	HG965048	-
Sarocladium oryzae	CBS 414.81	HG965028	-	-	HG965049	-
Sarocladium pseudostrictum	UTHSC 02-1892 T	HG965029	-	-	HG965073	-
Sarocladium spinificis	**NNIBRFG27318**	OK090969	-	-	OK090992	-
Sarocladium spinificis	Z0106	KF269096	-	-	KC920827	-
Sarocladium strictum	CBS 346.7 T	FN691453	-	-	HQ232141	-
Sarocladium subulatum	MUCL9939 T	HG965031	-	-	HG965075	-
Sarocladium summerbellii	CBS 430.7 T	HG965034	-	-	HG965078	-
Sarocladium terricola	CBS 243.59 T	FN706553	-	-	HE608659	-
Sarocladium zeae	CBS 800.69 T	FN691451	-	-	HQ232152	-
Scolecofusarium ciliatum	IHEM:2989	KJ125591	KJ125887	-	-	-
Scolecofusarium ciliatum	**NNIBRFG22713**	OK090963	OK235330	-	-	-
Simplicillium lanosoniveum	CBS 704.86	AJ292396	-	-	AF339553	DQ522358
Sphaerostilbella parabroomeana	(T) CBS 102308, G.J.S. 82-274	KU382208	-	-	U00756	-

Table 2. Continued.

Species	Culture Collection no. (Isolate no.)	GenBank accession no.				
		ITS	TUB	RPB2	LSU	EF1
Trichoderma appalachiense	**NNIBRFG15407**	-	-	OK235321	-	-
Trichoderma appalachiense	TC226	-	-	MF095868.1	-	-
Trichoderma asperellum	GJS 90-7	-	-	EU338337	-	-
Trichoderma austrokoningii	CBS 247.63	-	-	FJ442772	-	-
Trichoderma ghanense	G.J.S. 95-137_T	-	-	JN175559	-	-
Trichoderma ghanense	DAOM 165776	-	-	JN175560	-	-
Trichoderma hamatum	Hypo 648	-	-	KJ665275	-	-
Trichoderma hamatum	Hypo 647	-	-	KJ665274	-	-
Trichoderma koningii	S227	-	-	JN715609	-	-
Trichoderma koningii	S22	-	-	KC285749	-	-
Trichoderma koningiopsis	S359	-	-	KJ665285	-	-
Trichoderma longibrachiatum	S328	-	-	JQ685883	-	-
Trichoderma olivascens	S34	-	-	KC285751	-	-
Trichoderma olivascens	S475	-	-	KC285752	-	-
Trichoderma olivascens	Hypo 273	-	-	KC285750	-	-
Trichoderma orientale	CBS 131488	-	-	JQ685884	-	-
Trichoderma ovalisporum	DIS 70A	-	-	FJ442742	-	-
Trichoderma ovalisporum	DIS 172H	-	-	FJ442741	-	-
Trichoderma ovalisporum	G.J.S. 04-113	-	-	FJ442781	-	-
Trichoderma paratroviride	CBS 136489_T	-	-	KJ665321	-	-
Trichoderma paratroviride	S489	-	-	KJ665322	-	-
Trichoderma subviride	**NNIBRFG6329**	-	-	OK235320	-	-
Trichoderma subviride	8658	-	-	KU529142.1	-	-
Trichoderma subviride	8878	-	-	KU529143.1	-	-
Trichoderma taiwanense	**C.P.K. 416**	-	-	JN715608	-	-
Trichoderma taiwanense	**NNIBRFG20685**	-	-	OK235323	-	-
Trichoderma tsugarense	**TAMA 0203**	-	-	AB807659	-	-
Trichoderma tsugarense	**NNIBRFG23468**	-	-	OK235324	-	-
Trichoderma viridarium	S136 = CBS 132568 (T)	-	-	KC285760	-	-
Trichoderma viride	CBS 119325 = Hypo 292 (T)	-	-	EU711362	-	-

Bold letters indicate isolates and accession numbers determined in the present study.

CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; T, Type material; ITS, internal transcribed spacer; TUB, beta-tubulin; LSU, large subunit of rDNA; RPB2, RNA polymerase II; EF1, elongation factor 1.

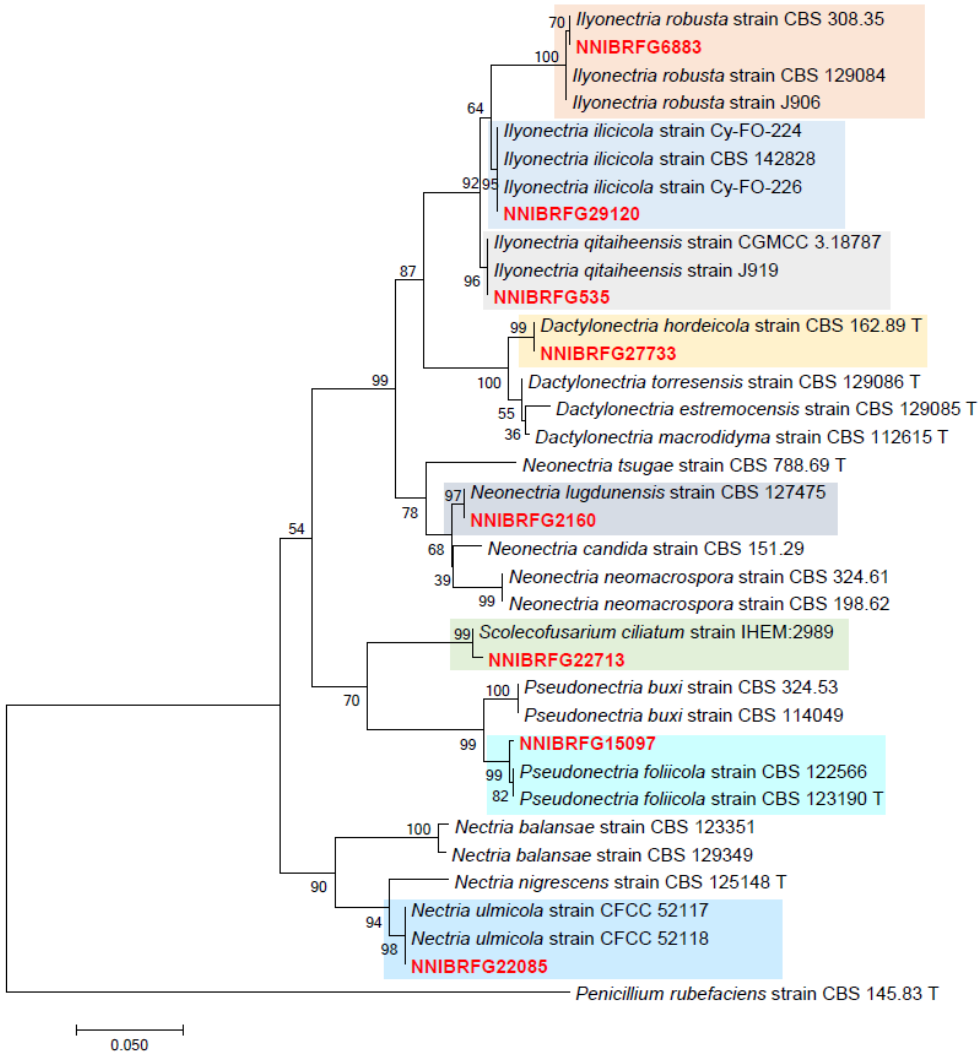

Fig. 1. Phylogenetic tree of *Dactylonectria, Ilyonectria, Nectria, Neonectria, Pseudonectria, Scolecofusarium* and their related species, based on maximum-likelihood analysis of the combination of internal transcribed region sequences and beta-tubulin gene sequences. Numbers at the nodes indicate the bootstrap values (>50%) from 1,000 replications. Red color font indicates fungal strains in this study.

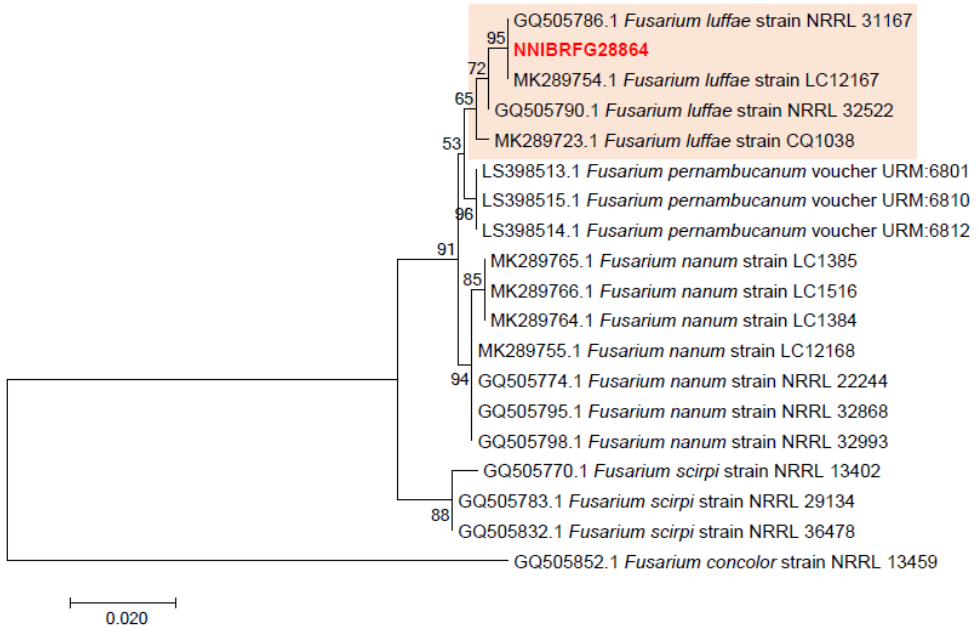

Fig. 2. Phylogenetic tree of *Fusarium* species, based on maximum-likelihood analysis of the RNA polymerase II second largest subunit (RPB2) sequences. Numbers at the nodes indicate the bootstrap values (>50%) from 1,000 replications. Red color font indicates fungal strain in this study.

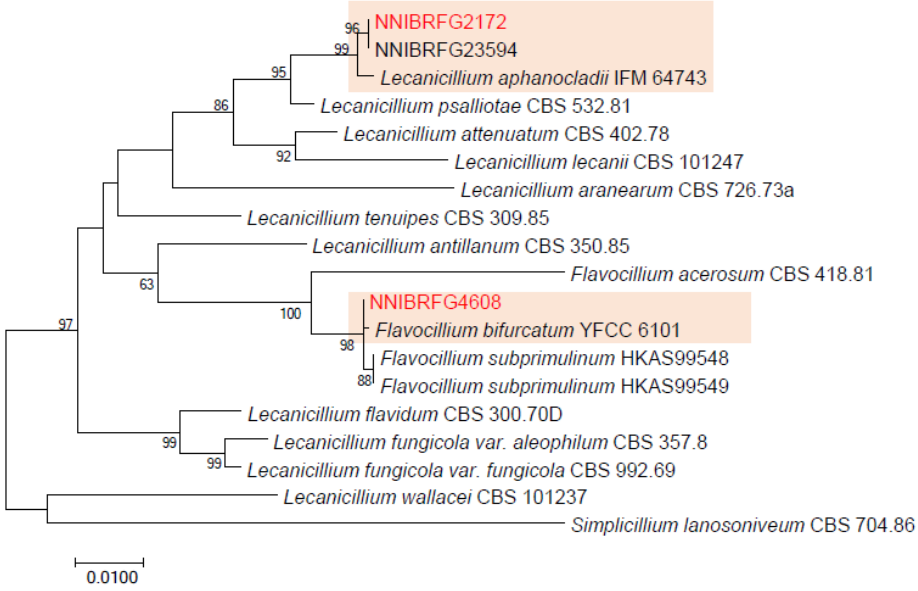

Fig. 3. Phylogenetic tree of *Lecanicillium, Flavocillium* and their related species, based on maximum-likelihood analysis of the combination of internal transcribed region sequences, the large subunit of rDNA sequences and elongation factor 1 sequence. Numbers at the nodes indicate the bootstrap values (>50%) from 1,000 replications. Red color font indicates fungal strains in this study.

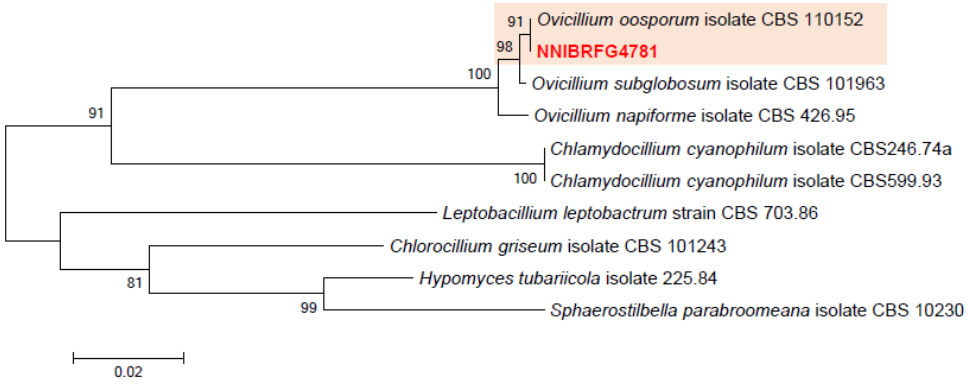

Fig. 4. Phylogenetic tree of *Ovicillium* and its related species, based on maximum-likelihood analysis of the combination of internal transcribed region sequences and the large subunit of rDNA sequences. Numbers at the nodes indicate the bootstrap values (>50%) from 1,000 replications. Red color font indicates fungal strain in this study.

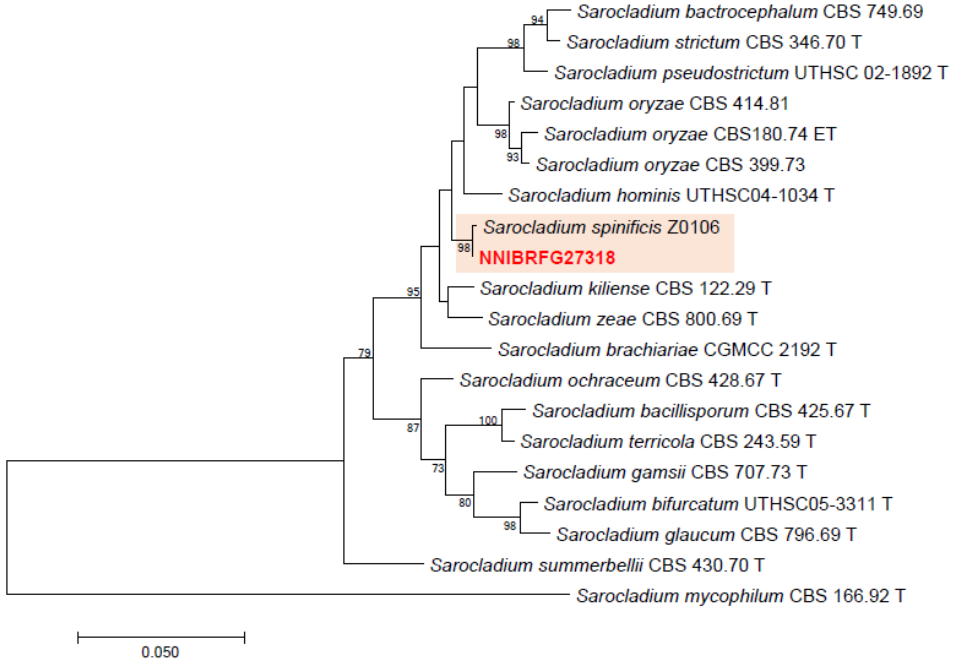

Fig. 5. Phylogenetic tree of *Sarocladium* and its related species, based on maximum-likelihood analysis of the combination of internal transcribed region sequences and the large subunit of rDNA sequences. Numbers at the nodes indicate the bootstrap values (>50%) from 1,000 replications Red color font indicates fungal strain in this study.

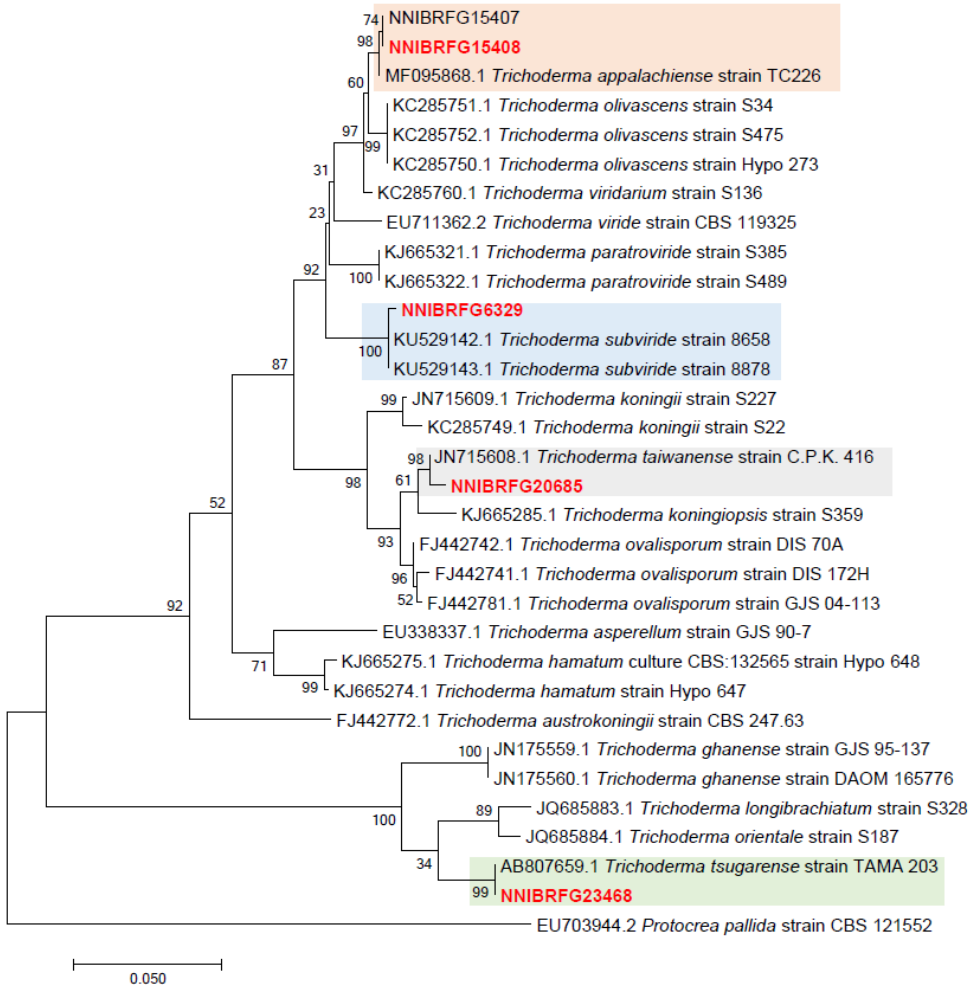

Fig. 6. Phylogenetic tree of *Trichoderma* and its related species, based on maximum-likelihood analysis of the RNA polymerase II second largest subunit sequences. Numbers at the nodes indicate the bootstrap values (>50%) from 1,000 replications. Red color font indicates fungal strains in this study.

Morphological analysis

Microstructures of fungal species were observed under an Eclipse Ni light microscope (Nikon, Tokyo, Japan) equipped with a Ds-Ri2 digital camera (Nikon). At least 50 individuals were examined for the observation and measurement of each structure. For scanning electron microscopy, we followed the protocol previously described by Alves et al. [47]. Photographs were obtained using a scanning electron microscope (SEM; SU8220, Hitachi, Japan).

Analysis of antifungal, enzyme, and plant growth-promoting activities

For the antifungal activity assay, we used two plant pathogens, *Fusarium oxysporum* and *F. solani*, from *Angelica gigas* in Youngju (Forest Medicinal Resources Research Center). Inhibition rates were measured using a dual culture assay at 7 dpi at 25℃. To determine chitinase activities, discoloration was observed

7 d after inoculation on media with the following supplements: 4.5 g colloidal chitin, 0.3 g $MgSO_4 \cdot 7H_2O$, 3 g NH_4SO_4, 1 g KH_2PO_4, 0.15 g bromocresol purple, 0.2 mL Tween 80, and 15 g/L agar [48]. To determine laccase activity, discoloration was observed 7 d after inoculation on media supplemented with 5 g malt extract, 0.2 g guaiacol, and 15 g/L agar [49]. To determine other enzyme activities, halo zones were measured on minimal MEA (1% malt extract [w/v] and 1.5% agar [w/v]) supplemented with the appropriate substrate after 2 weeks of incubation at 25°C. The supplemented substrates included 0.5% Tween 20 (w/v) for lipase activity and 1% skim milk (w/v) for protease activity in the presence of Congo red (500 ppm). For the phosphate solubilization assay, a clear zone was observed 7 d after inoculation on Pikovskaya agar (HiMedia, Mumbai, India) [50]. For the siderophore production assay, discoloration was observed 7 d after inoculation on CAS media [51].

RESULTS AND DISCUSSION

Phylogenetic analysis

Phylogenetic analysis was performed to identify the 17 fungal strains and infer their phylogenetic relationships with other similar species. ITS and TUB sequences were used for phylogenetic analysis of *Dactylonectria*, *Ilyonectria*, *Nectria*, *Neonectria*, *Pseudonectria*, *Scolecofusarium*, and their related species. As shown in Fig. 1, strain NNIBRFG6883 formed a clade with three other strains of *Ilyonectria robusta*. The sequences of NNIBRFG6883 showed high similarities (100% in ITS and 99.69% in TUB) with those of *I. robusta*. NNIBRFG29120 formed a clade with three other strains of *I. ilicicola*. The sequences of NNIBRFG29120 showed high similarity (100% in ITS and 99.63% in TUB) with those of *I. ilicicola*. NNIBRFG535 formed a clade with two other strains of *I. qitaiheensis*. The sequences of the NNIBRFG535 showed high similarities (100% in ITS and 99.69% in TUB) with those of *I. qitaiheensis*. NNIBRFG27733 formed a clade with a strain of *Dactylonectria hordeicola*. The sequences of NNIBRFG27733 showed high similarities (100% in ITS and TUB) with those of *D. hordeicola*. NNIBRFG2160 formed a clade with the *Neonectria lugdunensis* strain. The sequences of the NNIBRFG2160 showed high similarities (100% in ITS and TUB) with those of *N. lugdunensis*. NNIBRFG22713 formed a clade with the *S. ciliatum* strain. The sequences of NNIBRFG22713 showed high similarities (99.39% in ITS and 99.37% in TUB) with those of *S. ciliatum*. NNIBRFG15097 formed a clade with two other strains, including *Pseudonectria foliicola*. The sequences of NNIBRFG15097 showed high similarities (100% in ITS and 98.16% in TUB) with those of *Pseudonectria foliicola*. NNIBRFG22085 formed a clade with two other strains of *Nectria ulmicola*. The sequences of NNIBRFG22085 showed high similarities (100% in ITS and TUB) with those of *Nectria ulmicola*. As shown in Fig. 2, RPB2 sequences were used for the phylogenetic analysis of *Fusarium* species. NNIBRFG28864 formed a clade with four strains of *Fusarium luffae*. The sequences of NNIBRFG28864 showed a high similarity (100% in RPB2) with those of *F. luffae*. As shown in Fig. 3, a combination of ITS, LSU, and EF1 sequences was used for the phylogenetic analysis of *Lecanicillium*, *Flavocillium*, and their related species. NNIBRFG4608 formed a clade with *Flavocillium bifurcatum*. BLASTn analysis of

NNIBRFG4608 genes showed high similarities (99.8% [ITS], 99.87% [LSU], and 99.57% [EF1]) with those of *F. bifurcatum*. NNIBRFG2172 formed a clade with a strain of *L. aphanocladii*. BLASTn analysis of NNIBRFG2172 genes showed high similarities (100% [ITS], 100% [LSU], and 98.87% [EF1]) with those of *L. aphanocladii*. As shown in Fig. 4, a combination of ITS and LSU sequences was used for the phylogenetic analysis of *Ovicillium* and the related species. NNIBRFG4781 formed a clade with a strain of *Ovicillium oosporum*. The sequences of NNIBRFG4781 showed high similarities (100% in ITS and 99.64% in LSU) with those of *O. oosporum*. As shown in Fig. 5, a combination of ITS and LSU sequences was used for the phylogenetic analysis of *Sarocladium* and its related species. NNIBRFG27318 formed a clade with a strain of *Sarocladium spinificis*. The sequences of NNIBRFG27318 showed high similarities (100% in ITS and 99.80% in LSU) with those of *S. spinificis*. As shown in Fig. 6, RPB2 sequences were used for the phylogenetic analysis of *Trichoderma* species and the related species. NNIBRFG15408 formed a clade with a *T. appalachiense* strain. The sequences of NNIBRFG15408 showed a high similarity (99.89% in RPB2) with those of *T. appalachiense*. NNIBRFG6329 formed a clade with two strains of *T. subviride*. The sequences of NNIBRFG6239 showed a high similarity (99.77% in RPB2) with those of *T. subviride*. NNIBRFG20685 formed a clade with a *T. taiwanense* strain. The sequences of NNIBRFG20685 showed a high similarity (99.48% in RPB2) with those of *T. taiwanense*. NNIBRFG23468 formed a clade with a *T. tsugarense* strain. The sequences of NNIBRFG23468 showed a high similarity (100% in RPB2) with those of *T. tsugarense*.

Through the molecular identification, we identified 17 fungal species in Hypocreales. Moreover, we examined antifungal activities for plant pathogens, extracellular enzyme activities, and plant growth promotion activities (Table 3). These results suggested that many species of this study could be potential biocontrol agents or other industrial usage.

In this study, here we report the characterization of unrecorded fungal species from environmental samples. It could contribute to understanding fungal diversity and characterization of various habitat in Korea.

Table 3. Antifungal activities, enzyme activities, and plant growth promotion of the strains used in this study.

Strain Number	Species	Antifungal activity		Enzyme activity			Plant growth promotion	
		Fo[a]	*Fs*[b]	Chitinase	Laccase	Others	Phosphate solubility	Siderophore production
NNIBRFG535	*Ilyonectria qitaiheensis*	ND	ND	ND	ND		-	+
NNIBRFG2172	*Lecanicillium aphanocladii*	-	-	++	+	lipase	++	+++
NNIBRFG4608	*Flavocillium bifurcatum*	-	-	-	+		+++	+++
NNIBRFG22085	*Nectria ulmicola*	ND	ND	ND	ND		-	+++
NNIBRFG2160	*Neonectria lugdunensis*	ND	ND	ND	ND	protease	ND	ND
NNIBRFG15097	*Pseudonectria foliicola*	ND	ND	ND	ND		-	+++
NNIBRFG22713	*Scolecofusarium ciliatum*	+	+	ND	ND		+++	+
NNIBRFG15407	*Trichoderma appalachiense*	ND	ND	ND	ND		-	+
NNIBRFG6329	*Trichoderma subviride*	+	+	-	+		-	++
NNIBRFG20685	*Trichoderma taiwanense*	-	-	-	+		-	++
NNIBRFG23468	*Trichoderma tsugarense*	-	-	-	+		-	+

[a] *Fusasirum oxysporum*, [b] *Fusasirum solani*.

ND: Not determined.

Taxonomy

Dactylonectria hordeicola **L. Lombard & Crous, Phytopathologia Mediterranea 53 (3): 527 (2014) [MB#810146] (Figs. 7A and 8A-D)**

Description: Colonies grew slowly at 25 ℃ and reached 11 mm on CMDA, 11 mm on MEA, 19 mm on OA, 10 mm on PDA, 23 mm on V8A, and 19 mm on YPDA, 7 d after inoculation. The color of the colony was hyaline with a smooth aerial mycelial surface on CMDA, hyaline with a smooth aerial mycelial surface on MEA, creamy white with fluffy aerial mycelia on OA, creamy white to light yellow from the center with fluffy aerial mycelia on PDA, creamy white to brown with cottony aerial mycelia on V8A, and creamy white to yellow with fluffy aerial mycelia on YPDA. Microconidia were aseptate or 1 septate, hyaline, cylindrical, measuring 8.9-20.9 μm×2.9-5.8 μm (x=13.6±2.57 μm×4.1±0.62 μm, L/W ratio=3.32, n=50). Chlamydospores were brownish and globose or subglobose, measuring 7.7-15.0×7.2-13.7 μm (x=11.2±2.76 μm×10.1±2.53 μm, n=10).

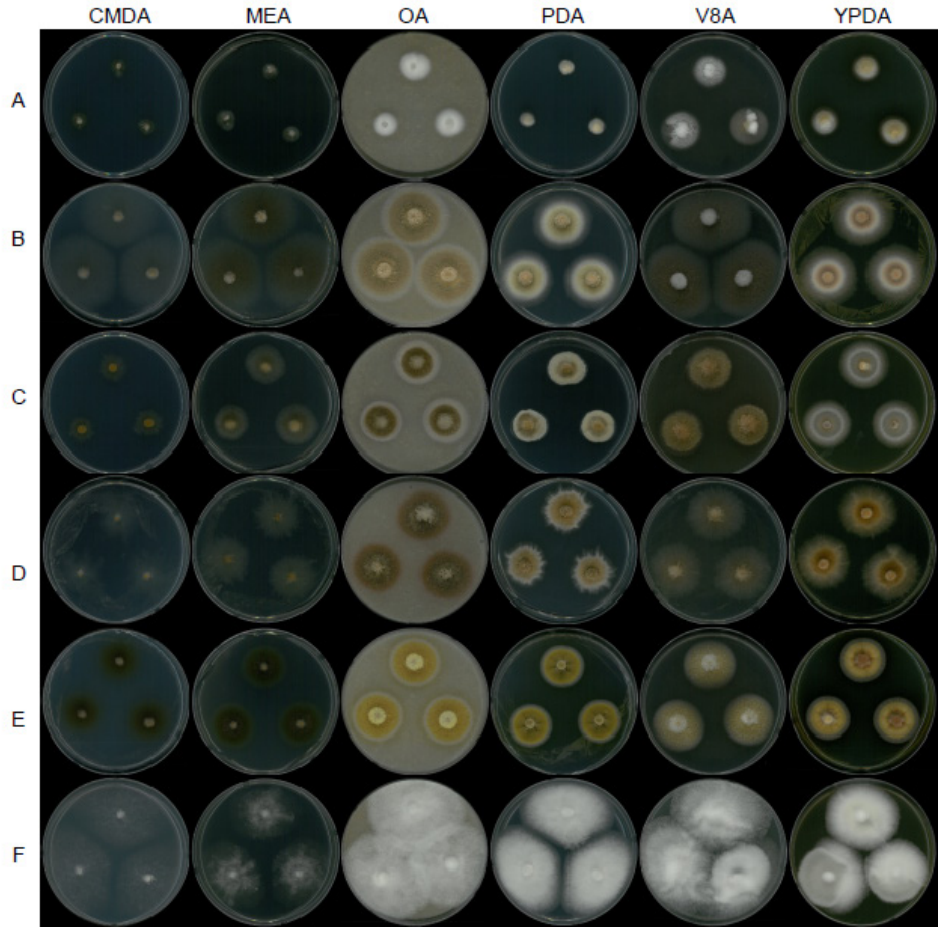

Fig. 7. Mycelial growth on corn meal dextrose agar (CMDA), malt extract agar (MEA), oatmeal agar (OA), potato dextrose agar (PDA), V8 agar (V8A), and yeast extract peptone dextrose agar (YPDA) for 7 days at 25℃. A, *Dactylonectria hordeicola* strain NNIBRFG27733; B, *Ilyonectria ilicicola* strain NNIBRFG29120; C, *Ilyonectria qitaiheensis* strain NNIBRFG535; D, *Ilyonectria robusta* strain NNIBRFG6883; E, *Neonectria lugdunensis* strain NNIBRFG2160; F, *Pseudonectria foliicola* strain NNIBRFG15097.

Habitat: Filtered freshwater in a stream

Specimen examined: Hwajeon-dong, Taebaek-si, Gangwon-do, 06 May, 2020, **NNIBRFG27733**, Nakdonggang National Institute of Biological Resources

Note: *Dactylonectria hordeicola* was first reported as an isolate from *Hordeum vulgare* [4]. In this study, NNIBRFG27733 was isolated from filtered freshwater streams.

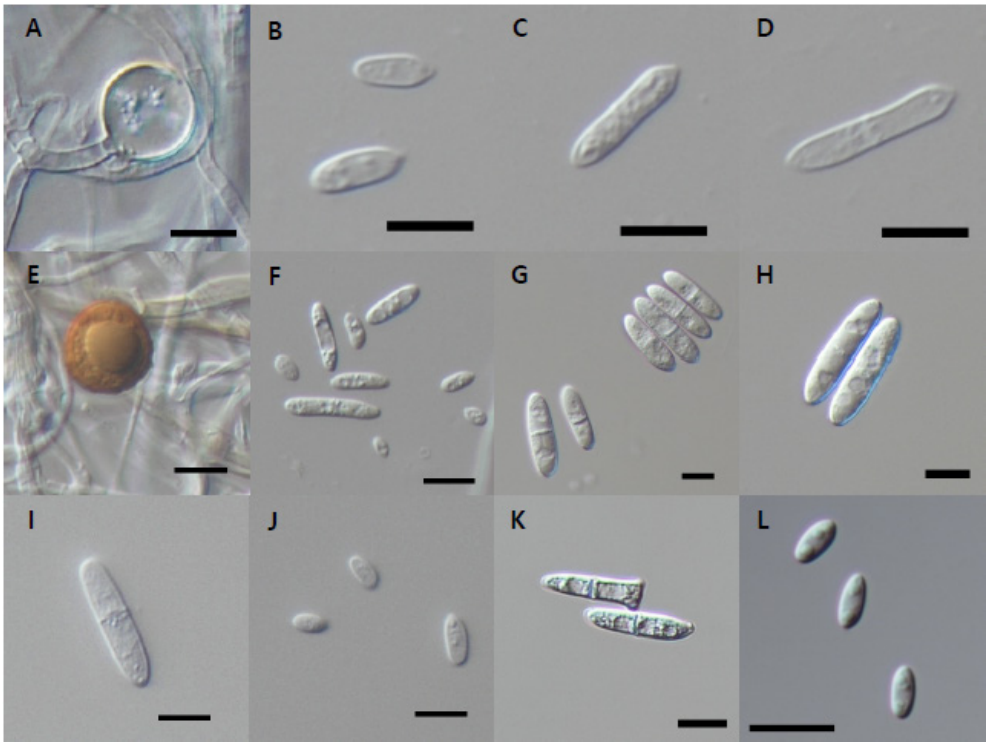

Fig. 8. Microscopic observation. A-D, Conidial morphology of *Dactylonectria hordeicola* strain NNIBRFG27733. Chlamydospore (A) and conidia (B-D); E-F, *Ilyonectria ilicicola* strain NNIBRFG29120. E, chlamydospore (E) and conidia (F); G-H, conidia morphology of *Ilyonectria qitaiheensis* strain NNIBRFG535; I-J, conidia morphology of *Ilyonectria robusta* strain NNIBRFG6883; K, conidia morphology of *Neonectria lugdunensis* strain NNIBRFG2160; L, conidia morphology of *Pseudonectria foliicola* strain NNIBRFG15097. (Scale bars: 10 μm).

Flavocillium bifurcatum **H. Yu, Y.B. Wang, Y. Wang, Q. Fan & Zhu L. Yang, Fungal Diversity 103: 20 (2020) [MB#833096] (Figs. 9A and 10A-B)**

Description: Colonies grew well at 25°C and reached 27 mm on CMD, 27 mm on MEA, 29 mm on OA, 26 mm on PDA, 31 mm on V8A, and 16 mm on MEA, 7 d after inoculation. The color of the colony was hyaline with a cottony aerial mycelial surface on CMDA, hyaline with a cottony aerial mycelial surface on MEA, creamy white with dense aerial mycelia on OA, creamy white with dense aerial mycelia on PDA, creamy white with fluffy aerial mycelia on V8A, and creamy white with dense aerial mycelia on YPDA. Following 10 d of growth on PDA, a diffusing pink-red pigment was observed. Conidia were hyaline, aseptate, and ovoid to ellipsoid, measuring 4.0-17.1 μm×1.8-4.0 μm (x=7.7±2.84 μm×2.7±0.51 μm, L/W ratio=2.86, n=50).

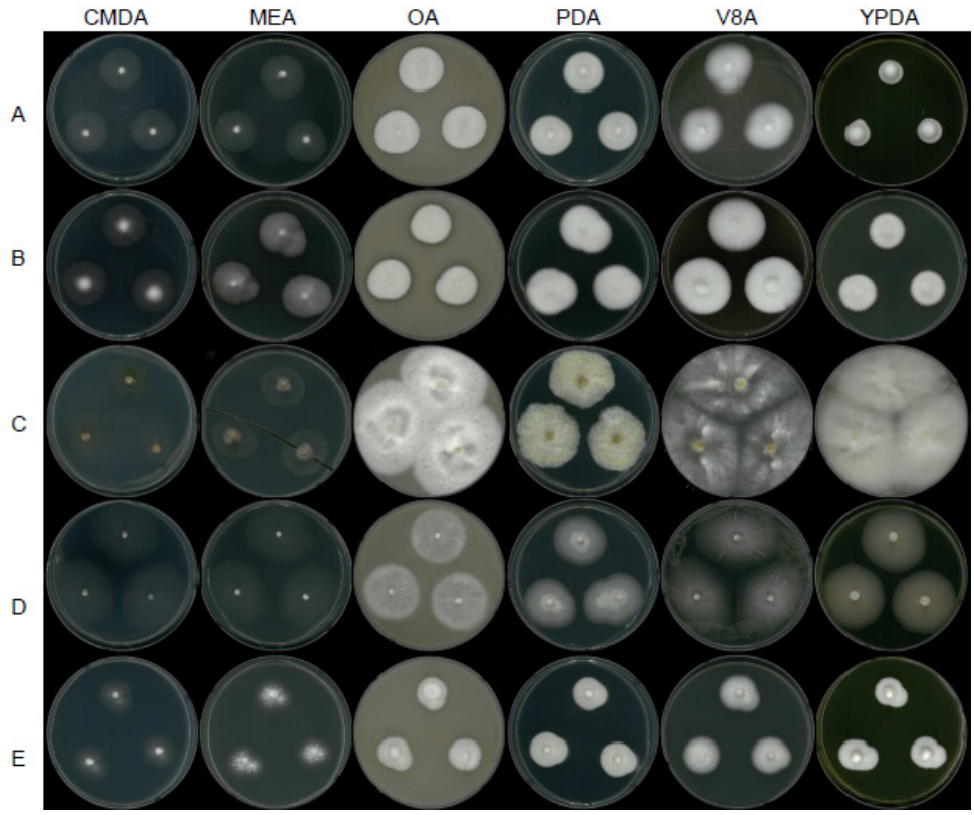

Fig. 9. Mycelial growth on corn meal dextrose agar (CMDA), malt extract agar (MEA), oatmeal agar (OA), potato dextrose agar (PDA), V8 agar (V8A), and yeast extract peptone dextrose agar (YPDA) for 7 days at 25℃. A, *Flavocillium bifurcatum* strain NNIBRFG4608; B, *Lecanicillium aphanocladii* strain NNIBRFG2172; C, *Fusarium luffae* strain NNIBRFG28864; D, *Ovicillium oosporum* strain NNIBRFG4781; E, *Sarocladium spinificis* strain NNIBRFG27318.

Habitat: Soil in forest

Specimen examined: Gohan-ri, Gohan-eup, Jeongseon, Gangwon-do, 04 July 2017, **NNIBRFG4608**, Nakdonggang National Institute of Biological Resources

Note: *Flavocillium bifurcatum* was isolated from larva of Noctuidae [18]. In this study, NNIBRFG4608 was isolated from soil in forests. Notably, NNIBRFG4608 showed enhanced siderophore production, phosphate solubility, and laccase activity (Table 3).

Fusarium luffae **M.M. Wang, Qian Chen & L. Cai, Persoonia 43: 85 (2019) [MB#829540] (Figs. 9C and 10E-F)**

Description: The colonies grown at 25℃ reached 16 mm on CMDA, 28 mm on MEA, 59 mm on OA, 41 mm on PDA, 73 mm on V8A, and 72 mm on YPDA, 7 d after inoculation. The color of the colony was hyaline with a few aerial mycelia on the surface of CMDA, hyaline to white from the center with a smooth aerial mycelial surface on MEA, light yellow with fluffy aerial mycelia on PDA, creamy white to light yellow from the center with fluffy aerial mycelia on PDA, hyaline and creamy white with cottony aerial mycelia on V8A, and creamy white with abundant aerial mycelia on YPDA. Macroconidia were 2 to 7 septate, hyaline, falcate, straight to curved, and measured 20.6-41.5 μm×3.9-6.1 μm (x=32.5±4.76 μm×4.9±0.52 μm, L/W ratio=6.62, n=50). Chlamydospores were not observed in this study.

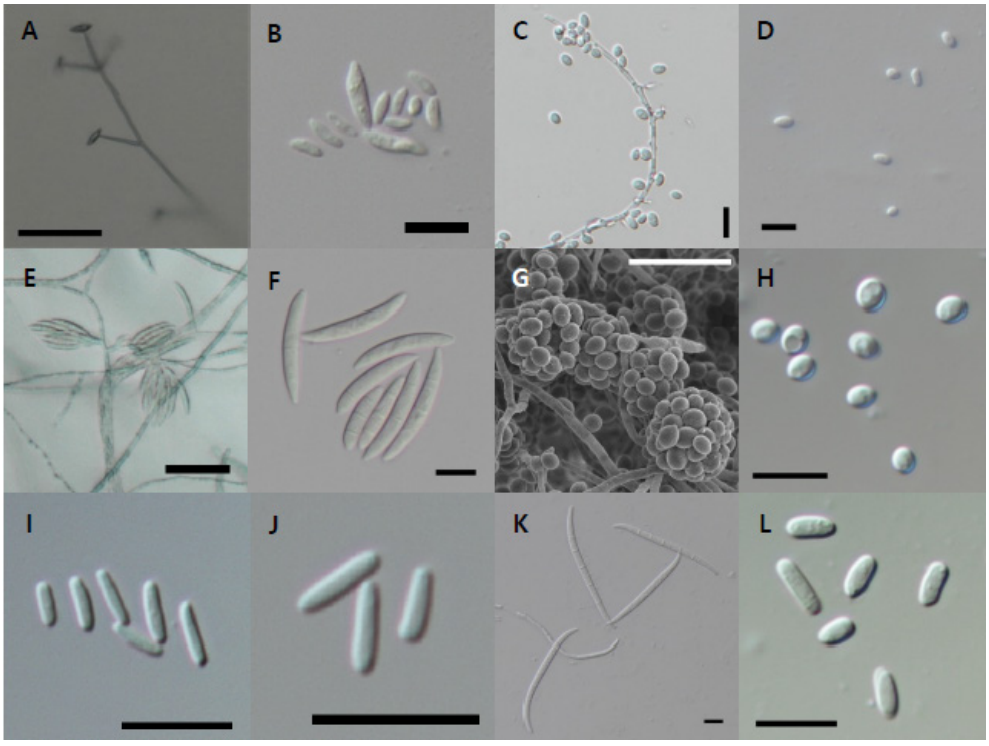

Fig. 10. Microscopic observation. A-B, Conidiophore (A) and conidia (B) morphology of *Flavocillium bifurcatum* strain NNIBRFG4608; C-D, conidiophore (C) and conidia (D) morphology of *Lecanicillium aphanocladii* strain NNIBRFG2172; E-F, conidiophore (E) and conidia (F) morphology of *Fusarium luffae* strain NNIBRFG28864; G-H, conidiophore (G) and conidia (H) morphology of *Ovicillium oosporum* strain NNIBRFG4781; I-J, conidia morphology of *Sarocladium spinificis* strain NNIBRFG27318; K, conidia morphology of *Scolecofusarium cilliatum* strain NNIBRFG22713; L, conidia morphology of *Nectria ulmicola* strain NNIBRFG22085. Scale bars are 10 μm (B-D, F-L) and 50 μm (A and E).

Habitat: Root of *Viola verecunda* in a stream

Specimen examined: Jipyeong-ri, Gonggeom-myeon, Sangju-si, Gyeongsangbuk-do, 21 August 2021, **NNIBRFG28864**, Nakdonggang National Institute of Biological Resources

Note: *Fusarium luffae* was initially isolated from *Luffa aegyptiaca* [16], but in this study, three strains were isolated from the roots of *Viola verecunda*.

***Ilyonectria ilicicola* B. Mora-Sala, A. Cabral, Armengol & Abad-Campos, Plant Disease 102 (11): 2095 (2018) [MB#822025] (Figs. 7B and 8E-F)**

Description: Colonies grew slightly fast at 25℃ and reached 50 mm on CMDA, 48 mm on MEA, 43 mm on OA, 37 mm on PDA, 50 mm on V8A, and 39 mm on YPDA, 7 d after inoculation. The color of the colony was hyaline with a cottony aerial mycelial surface on CMDA, hyaline to brown with a cottony aerial mycelial surface on MEA, white to dark goldenrod with fluffy aerial mycelia on OA, white to goldenrod from the center with fluffy aerial mycelia on PDA, grey-brown with cottony aerial mycelia on V8A, and creamy white to dark goldenrod with fluffy aerial mycelia on YPDA. Macroconidia were 1-3 septate, hyaline, cylindrical, and measured 18.0-32.6 μm×3.9-5.8 μm (x=23.0±4.48 μm×4.38±1.03 μm,

L/W ratio=5.2, n=50). Microconidia were aseptate or 1 septate, hyaline, fusiform, and measured 3.9-7.7 μm ×2.3-3.6 μm (x=5.9±1.05 μm×3.0±0.33 μm, L/W ratio=1.93, n=50). Chlamydospores were brownish and globose or subglobose, measuring 13.8-20.6 μm×12.9-19.4 μm (x=17.4±1.77 μm×16.2±1.70 μm, n=50).

Habitat: Filtered freshwater from a stream

Specimen examined: Songgang-ri, Sancheok-myeon, Chungju-si, Chungcheongbuk-do, Republic of Korea, 6 March 2020, **NNIBRFG29120**, Nakdonggang National Institute of Biological Resources

Note: *Ilyonectria ilicola* was isolated from the roots of *Ilex* sp. [6]. In this study, three strains were isolated from filtered freshwater from streams.

***Ilyonectria qitaiheensis* X. Lu & W. Gao, Journal of Ginseng Research 44: 514 (2020) [MB#823895] (Figs. 7C and 8G-H)**

Description: Colonies grew slightly slow at 25℃ and reached 16 mm on CMDA, 43 mm on MEA, 29 mm on OA, 30 mm on PDA, 19 mm on V8A, and 18 mm on YPDA at 25℃, 7 days after inoculation. The color of the colony was hyaline with a smooth aerial mycelial surface on CMDA, hyaline to grey-brown with cottony aerial mycelial surface on MEA, white to dark goldenrod from center with fluffy aerial mycelia on OA; creamy white to goldenrod from center with fluffy aerial mycelia on PDA, yellow brown with fluffy aerial mycelia on V8A, and creamy white to grey-brown with cottony aerial mycelia on YPDA. Macroconidia were 1 to 3 septate, hyaline, cylindrical, and measured 19.5-35.3 μm×4.6-8.3 μm (x=28.7 ±3.72 μm×6.8±0.74 μm, L/W ratio=4.17, n=50).

Habitat: Root of aquatic plant (*Umbelliterae* sp.) near a stream

Specimen examined: Gangjeong-dong, Seogwipo-si, Jeju-do, Republic of Korea, March 6, 2020, **NNIBRFG535**, Nakdonggang National Institute of Biological Resources

Note: *Ilyonectria qitaiheensis* was isolated from the roots of *Panax ginseng* in China [4]. In this study, NNIBRFG535 was isolated from the roots of aquatic plants (*Umbelliterae* sp.) near the stream.

***Ilyonectria robusta* (A.A. Hildebr.) A. Cabral & Crous, Mycological Progress 11 (3): 680 (2011) [MB#560113] (Figs. 7D and 8I-J)**

Description: Colonies grew slightly slow at 25℃ and reached 17 mm on CMDA, 30 mm on MEA, 36 mm on OA, 30 mm on PDA, 36 mm on V8A, and 36 mm on YPDA, 7 d after inoculation. The color of the colony was hyaline with rough margins on CMDA, hyaline to grey-brown with a cottony aerial mycelial surface on MEA, dark goldenrod with fluffy aerial mycelia on OA, brown from the center with an ivory rough margin on PDA, hyaline to light grey with cottony aerial mycelia on V8A, and light brown to dark goldenrod with dense aerial mycelia on YPDA. Macroconidia were 1-3 septate, hyaline, cylindrical with round ends, and measured 19.5-35.3 μm×4.6-8.3 μm (x=23.9±6.47 μm×6.4±1.17 μm, L/W ratio=3.97, n=50). The 1-septate macroconidia measured 9.6-32.0×2.7-8.3 μm (x=22.9±5.71 μm×5.9±1.16 μm, L/W ratio=3.87). The 2-septate macroconidia measured 25.9-38.1×5.2-8.5 μm (x=30.9±4.13 μm×6.8 ±1.13 μm, L/W ratio=4.52). The 3-septate macroconidia measured 30.7-38.6×5.6-8.9 μm (x=34.7±3.07

μm×7.1±0.88 μm, L/W ratio=4.89). Microconidia were aseptate or 1 septate, hyaline, ellipsoid, measuring 4.4-12.0 μm×2.2-4.8 μm (x=8.0±1.61 μm×3.5±0.59 μm, L/W ratio=2.25, n=50). Chlamydospores were brownish and globose or subglobose, measuring 10.8-17.6 μm×9.0-15.7 μm (x=13.7±1.69 μm×12.5±1.67 μm, n=50).

Habitat: Root of moss on rock near a stream

Specimen examined: Neunggang-ri, Susan-myeon, Jecheon-si, Chungcheongbuk-do, Republic of Korea, 7 July 2018, **NNIBRFG6883**, Nakdonggang National Institute of Biological Resources

Note: *Ilyonectria robusta* was isolated from the living root of *Panax quinquefolium* in Canada [7]. In this study, NNIRFG6883 was isolated from the root of moss in a rock near a stream.

***Lecanicillium aphanocladii* Zare & W. Gams, Nova Hedwigia 73 (1-2): 27 (2001) [MB#484541] (Figs. 9B and 10C-D)**

Description: Colonies grew well at 25℃ and reached 28 mm on CMD, 26 mm on MEA, 27 mm on OA, 30 mm on PDA, 36 mm on V8A, and 24 mm on MEA, 7 d after inoculation. The color of the colony was hyaline to creamy white with a fluffy aerial mycelial surface on CMDA, white with a cottony aerial mycelial surface on MEA, creamy white with dense aerial mycelia on OA, creamy white with dense aerial mycelia on PDA, creamy white with dense aerial mycelia on V8A, and creamy white to light pink from the margin with dense aerial mycelia on YPDA. After a 10 d incubation on PDA, a diffusing pink-red pigment was observed. Conidia were produced solitary at the tip of aphanophialides, were aseptate, oval to subglobose, and measured 3.0-8.5 μm×1.5-4.2 μm (x=5.3±1.26 μm×2.7±0.60 μm, L/W ratio=1.95, n=50).

Habitat: Freshwater from a stream

Specimen examined: Gunwi-eup, Gunwi-gun, Gyeongsangbuk-do, 08 April 2016, NNIBRFG2172, Nakdonggang National Institute of Biological Resources

Note: Several strains of *L. aphanocladii* were isolated from mushrooms and leaf litter [19]. In this study, NNIBRFG2172 was isolated from freshwater stream. Notably, NNIBRFG2172 showed enhanced siderophore production and phosphate solubility, as well as other extracellular enzyme activities, such as chitinase, laccase, and lipase (Table 3).

***Nectria ulmicola* C.M. Tian & Q. Yang, Phytotaxa 356 (3): 204 (2018) [MB#825504] (Figs. 10Land 11B)**

Description: Colonies grew slowly at 25℃ and reached 22 mm on MEA, 18 mm on OA, and 12 mm on PDA, 7 d after inoculation. The color of the colony was hyaline with a few aerial mycelia on the surface of MEA, hyaline to white with short cottony aerial mycelia on OA, creamy white with a rough margin, and short cottony aerial mycelia on PDA. Conidia were hyaline, cylindrical or ellipsoid, aseptate, and measured 4.8-8.7 μm×2.3-3.6 μm (x=6.4±0.89 μm×3.0±0.32 μm, L/W ratio=2.16, n=50).

Habitat: Filtered freshwater from a stream

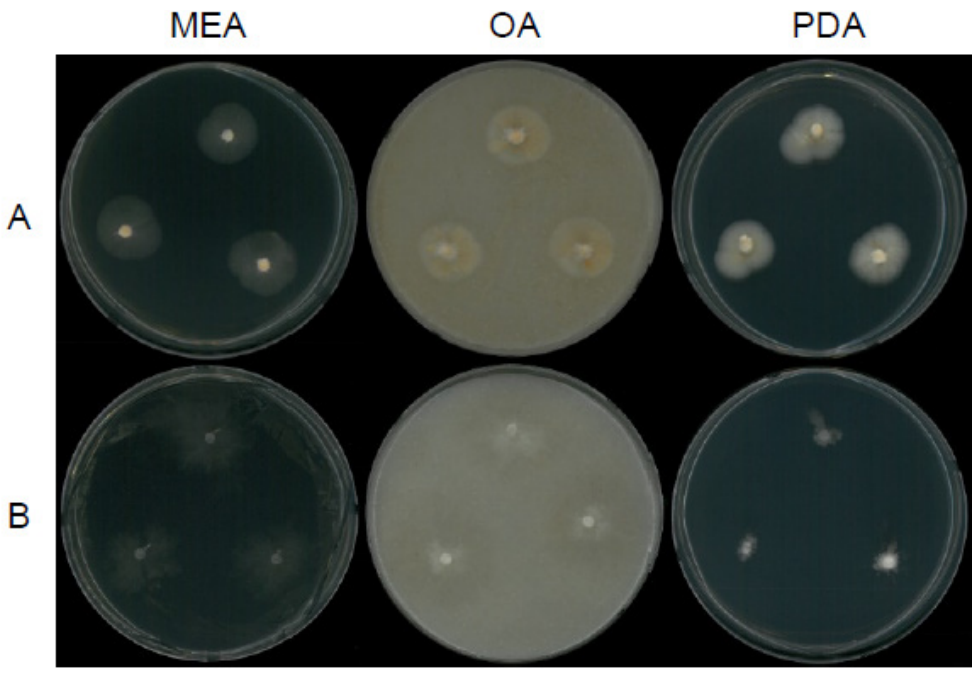

Fig. 11. Mycelial growth on malt extract agar (MEA), oatmeal agar (OA), and potato dextrose agar (PDA) for 7 days at 25℃. A, *Scolecofusarium cilliatum* strain NNIBRFG22713; B, *Nectria ulmicola* strain NNIBRFG22085.

Specimen examined: Namhansanseong-myun, Gwangju-si, Gyeonggi-do, 06 Mar 2019, **NNIBRFG22085**, Nakdonggang National Institute of Biological Resources

Note: *Nectria ulmicola* was isolated from twigs and branches of *Ulmus davidiana* var. *japonica* in China [10]. In this study, NNIBRFG22085 was isolated from filtered freshwater from a stream. Notably, NNIBRFG22085 showed enhanced siderophore production (Table 3).

***Neonectria lugdunensis* (Sacc. & Therry) L. Lombard & Crous, Phytopathologia Mediterranea 53 (3): 528 (2014) [MB#810155] (Figs. 7E and 8K)**

Description: Colonies grew well at 25℃ and reached 25 mm on CMD, 28 mm on MEA, 34 mm on OA, 28 mm on PDA, 34 mm on V8A, and 29 mm on MEA, 7 d after inoculation. The color of the colony was dark olive green with a cottony aerial mycelial surface on CMDA, dark olive green with a cottony aerial mycelial surface on MEA, yellow to light yellow from the center with fluffy aerial mycelia on OA, goldenrod to brown with dense aerial mycelia on PDA, light brown with fluffy aerial mycelia on V8A, and yellow orange to goldenrod from the center with dense aerial mycelia on YPDA. Macroconidia were hyaline, 0–3 septate, clavate with a widened subapical region, and measured 17.5-30.4 μm×3.7-6.4 μm (x=24.8±3.07 μm×5.2±0.76 μm, n=50).

Habitat: Filtered freshwater from a stream

Specimen examined: Hamaengbang-ri, Geundeok-myeon, Samcheok-si, Gangwon-do, 07 April 2016, **NNIBRFG2160**, Nakdonggang National Institute of Biological Resources

Note: *Neonectria lugdunensis* was first isolated from submerged decaying alder leaves in a stream bed of England [12]. In this study, NNIBRFG2160 was isolated from filtered freshwater from streams. Moreover, NNIBRFG2160 showed weak protease activity (Table 3).

***Ovicillium oosporum* Zare & W. Gams, Mycological Progress 15: 1022 (2016) [MB#815498] (Figs. 9D and 10G-H)**

Description: Colonies grew slightly fast at 25℃ and reached 43 mm on CMDA, 46 mm on MEA, 36 mm on OA, 35 mm on PDA, 51 mm on V8A, and 41 mm on MEA, 7 d after inoculation. The color of the colony was hyaline with a short cottony aerial mycelial surface on CMDA, hyaline with a cottony aerial mycelial surface on MEA, creamy white with fluffy aerial mycelia on OA, hyaline to creamy white from the center, with fluffy aerial mycelia on PDA, hyaline to white with cottony aerial mycelia on V8A, and white to light lemon with smooth aerial mycelia on YPDA. Conidia were hyaline, aseptate, and subglobose to globose, measuring 3.6-5.5 μm×2.6-4.5 μm (x=4.2±0.47 μm×3.6±0.46 μm, L/W ratio=1.18, n=50).

Habitat: Sediment in freshwater

Specimen examined: Dumil-ri, Gapyeong-eup, Gapyeong-gun, Gyeonggi-do, Republic of Korea, 20 September 2017, **NNIBRFG4781**, Nakdonggang National Institute of Biological Resources

Note: *Ovicillium oosporum* was isolated from *Theobroma gileri* in South America; however other strains were also identified, as this species had various isolation sources such as soil, humans, mushrooms, insects, etc., [21]. In this study, NNIBRFG4781 was isolated from sediment in streams.

***Pseudonectria foliicola* L. Lombard & Crous, Studies in Mycology 80: 219 (2015) [MB#810180] (Figs. 7F and 8L)**

Description: Colonies grew fast at 25℃ and reached 51 mm on CMDA, 37 mm on MEA, 61 mm on OA, 61 mm on PDA, 63 mm on V8A, and 45 mm on MEA, 7 d after inoculation. The color of the colony was hyaline to white with a cottony aerial mycelial surface on CMDA, hyaline to white with a cottony aerial mycelial surface and a rough margin on MEA, creamy white with abundant fluffy aerial mycelia on OA, creamy white with fluffy dense aerial mycelia on PDA, creamy white with fluffy aerial mycelia on V8A, and creamy white with fluffy dense aerial mycelia on YPDA. Conidia were hyaline, aseptate, and fusiform to ellipsoid, measuring 4.7-8.2 μm×2.1-3.6 μm (x=6.4±0.81 μm×2.9±0.33 μm, L/W ratio=2.22, n=50).

Habitat: sediment in freshwater

Specimen examined: Dumil-ri, Gapyeong-eup, Gapyeong-gun, Gyeonggi-do, Republic of Korea, 20 September 2017, **NNIBRFG4781**, Nakdonggang National Institute of Biological Resources

Note: *Pseudonectria foliicola* was isolated from the leaves of Buxus sempervirens in New Zealand [14] and reported as a pathogen of boxwood causing Volutella blight [13]. In this study, NNIBRFG15097 was isolated from sediment in streams. Notably, NNIBRFG15097 showed enhanced siderophore production (Table 3).

Sarocladium spinificis **Yu Hung Yeh & R. Kirschner, Botanical Studies 55 (25): 3 (2014) [MB#805250] (Figs. 9E and 10I-J)**

Description: Colonies grew slightly slow at 25℃ and reached 22 mm on CMDA, 24 mm on MEA, 24 mm on OA, 23 mm on PDA, 23 mm on V8A, and 23 mm on MEA, 7 d after inoculation. The color of the colony was hyaline to white from the center with a cottony aerial mycelial surface on CMDA, creamy white with a cottony aerial mycelial surface on MEA, creamy white with dense aerial mycelia on OA, creamy white with dense aerial mycelia on PDA, white with fluffy aerial mycelia on V8A, and creamy white with dense aerial mycelia on YPDA. Conidia were hyaline, aseptate, cylindrical or ellipsoid, and measured 3.9-8.3 μm×1.2-3.0 μm (x=5.7±0.88 μm×1.8±0.32 μm, L/W ratio=3.19, n=50).

Habitat: Sediment in freshwater

Specimen examined: Seonhak-ri, Haeryong-myeon, Suncheon-si, Jeollanam-do, Republic of Korea, 19 March 2019, NNIBRFG27318, Nakdonggang National Institute of Biological Resources

Note: *Sarocladium spinificis* was isolated as an endophytic fungus from the coastal grass *Spinifex littoreus* in Taiwan [40]. In this study, NNIBRFG2731s8 was isolated from sediment in streams.

Scolecofusarium ciliatum **(Link) L. Lombard, Sand.-Den. & Crous, Studies in Mycology 98 (no. 100116): 74 (2021) [MB#838677] (Figs. 10K and 11A)**

Description: Colonies grew slowly at 25℃ and reached 18 mm on PDA, 20 mm on OA, and 21 mm on MEA, 7 d after inoculation. The color of the colony was light yellow with a smooth aerial mycelial surface on PDA, hyaline with a smooth aerial mycelial surface on MEA, and apricot white to light goldenrod from the center with slightly fluffy aerial mycelia on OA. Macroconidia were slightly falcate, smooth-walled, and 3-7 septate, measuring 41.9-92.7 μm×3.1-5.3 μm (x=68.6±9.9 μm×3.9±0.4 μm, L/W ratio=17.47, n=50).

Habitat: Plant litter in stream

Specimen examined: Nocheon-ri, Yeonggwimi-myeon, Hongcheon-gun, Gangwon-do, 28 March 2019, **NNIBRFG22713**, Nakdonggang National Institute of Biological Resources

Note: Previous molecular phylogenetic studies have suggested that the position of *S. ciliatum* can be moved to another genus [34,36]. Several strains of *S. ciliatum* have been reported as isolates from the leaves of *Fagus sylvatica* [17]. In this study, NNIBRFG22713 was isolated from plant litter in streams.

Trichoderma appalachiense **Samuels & Jaklitsch, Persoonia 31: 135 (2013) [MB#803627] (Figs. 12A and 13A-B)**

Description: Colonies grew slightly slowly at 25℃ and reached 56 mm on PDA, 64 mm on CMDA, and 16 mm on SNA, 7 d after inoculation. The colony on PDA was hyaline to white, forming concentric rings with a cottony aerial mycelial surface, and its reverse side was white to light yellow from the center. The colony on CMDA was hyaline with little aerial mycelia, and its reverse side was hyaline. The colony on SNA was hyaline with a little cottony aerial mycelial surface, and its reverse side was hyaline. The conidiophores branched divergently. Phialides were lageniform, arising directly from the main axis or terminating lateral branches, solitary or in clusters, and measured 7.1-11.8 μm×2.3-4.3 μm at the widest point (x=9.8±1.33 μm×3.2±0.45 μm, L/W ratio=3.03, n=30). Conidia were green, aseptate, ellipsoid or

subglobose, and thick-walled, measuring 3.0-4.8 μm×2.7-4.0 μm (x=3.8±0.35 μm×3.3±0.27 μm, L/W ratio=1.14, n=50).

Habitat: Filtered freshwater from stream

Specimen examined: Sanghyo-dong, Seogwipo-si, Jeju-do, Republic of Korea, 19 March 2019, **NNIBRFG15407**, Nakdonggang National Institute of Biological Resources

Note: *Trichoderma appalachiense* was isolated from decorticated wood in the mid-Atlantic states of the USA [41]. In this study, NNIBRFG15407 was isolated from filtered freshwater from a stream in Jeju Island. Moreover, this strain exhibited weak siderophore production (Table 3).

Trichoderma subviride **W.T. Qin & W.Y. Zhuang, Scientific Reports 6 (no. 27074): 11 (2016) [MB#570245] (Figs. 12B and 13C-D)**

Description: Colonies grew fast at 25℃ and reached 87 mm on PDA, 87 mm on CMDA, and 87 mm on SNA, 4 d after inoculation. The colony on PDA was white to green, with a fluffy aerial mycelial surface, and its reverse side was white to light yellow. The colony on CMDA was hyaline with little aerial mycelia, and its reverse side was hyaline. The colony on SNA was hyaline with a little cottony aerial mycelial surface, and its reverse side was hyaline. The conidiophores branched divergently. Phialides were lageniform or ampulliform, arising directly from the main axis or terminating lateral branches, solitary or in clusters, measuring 4.3-12.5 μm×2.0-3.7 μm at the widest point (x=7.3±1.62 μm×2.8±0.42 μm, L/W ratio=2.56, n=30). Conidia were green, aseptate, subglobose or globose, and measured 3.0–4.5 μm × 2.8–3.9 μm (x=3.7±0.31 μm×3.4±0.27 μm, L/W ratio=1.07, n=50). Chlamydospores were aseptate, globose or subglobose, and thick-walled, measuring 7.0-11.2 μm×6.3-11.4 μm (x=9.3±1.31 μm×8.5±1.08 μm, L/W ratio=1.09, n=20).

Habitat: Sediment in freshwater

Specimen examined: Ihwa-ri, Ubo-myeon, Gunwi-gun, Gyeongsangbuk-do, Republic of Korea, 22 June 2018, **NNIBRFG6329**, Nakdonggsang National Institute of Biological Resources

Note: *Trichoderma subviride* was isolated from twigs in China [42]. In this study, NNIBRFG6329 was isolated from sediment in Wicheon. Moreover, this strain exhibited antifungal activity against *Fusarium oxysporum* and *F. solani*, extracellular laccase activity, and siderophore production (Table 3).

Trichoderma taiwanense **Samuels & M.L. Wu, Studies in Mycology 56: 130 (2006) [MB#501048] (Figs. 12C and 13E-F)**

Description: Colonies grew fast at 25℃ and reached 87 mm on PDA, 87 mm on CMDA, and 71 mm on SNA, 7 d after inoculation. The colony on PDA was white to dark green, with a fluffy aerial mycelial surface, and its reverse side was white to light yellow. The colony on CMDA was hyaline with little aerial mycelia, and its reverse side was hyaline. The colony on SNA was hyaline with a little cottony aerial mycelial surface, and its reverse side was hyaline. The conidiophores branched divergently. Phialides were lageniform, arising directly from the main axis or secondary branches, solitary or in clusters, and measured 5.1-13.0 μm×1.1-4.3 μm at the widest point (x=8.1±1.78 μm×2.0±0.70 μm, L/W ratio=4.01, n=20). Conidia were green, aseptate, and subglobose or globose, measuring 3.2-4.7 μm×2.4-4.1 μm (x=4.0±0.37 μm×3.1±0.28 μm, L/W ratio=1.29, n=50). Chlamydospores were not observed in this study.

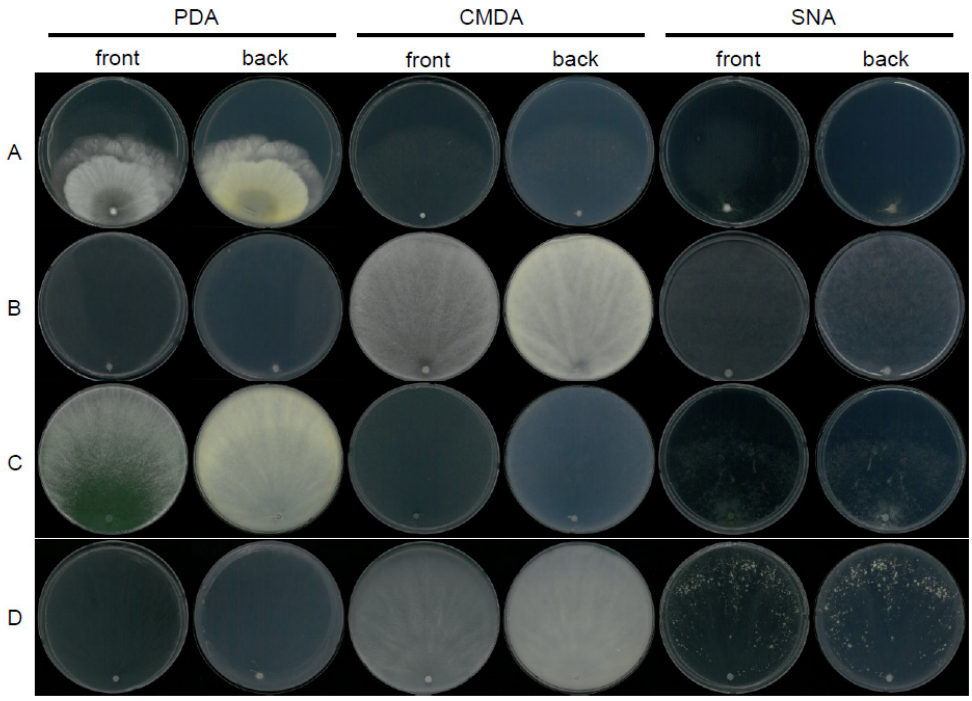

Fig. 12. Mycelial growth on potato dextrose agar (PDA), corn meal dextrose agar (CMDA), and synthetic nitrogen-poor or nutrient-poor agar (SNA) for 7 days at 25℃. A, *Trichoderma appalachiense* strain NNIBRFG15408; B, *Trichoderma subviride* strain NNIBRFG6329; C, *Trichoderma taiwanense* strain NNIBRFG20685; D, *Trichoderma tsugarense* strain NNIBRFG23468.

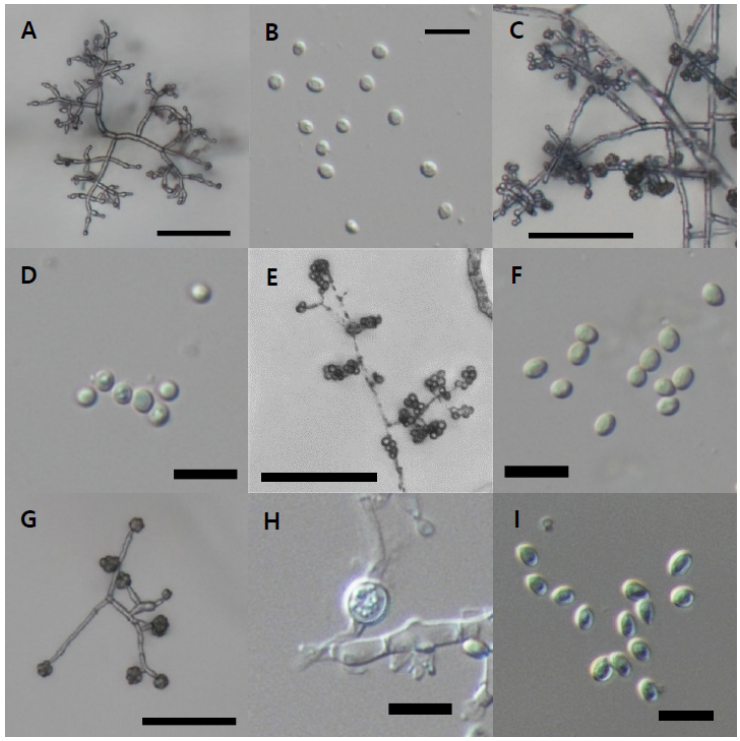

Fig. 13. Microscopic observation. A-B, conidiophore (A) and conidia (B) of *Trichoderma appalachiense* strain NNIBRFG15408; C-D, conidiophore (C) and conidia (D) of *Trichoderma subviride* strain NNIBRFG6329; E-F, conidiophore (E) and conidia (F) of *Trichoderma taiwanense* strain NNIBRFG20685; G-I, conidiophore (G), chlamydospore (H), and conidia (I) of *Trichoderma tsugarense* strain NNIBRFG23468. Scale bars indicate 10 μm (B, D, F, H-I) and 50 μm (A, C, E, G).

Habitat: Sediment in freshwater

Specimen examined: Iyeon-ri, Danbuk-myeon, Uiseong-gun, Gyeongsangbuk-do, Republic of Korea, 05 December 2018, **NNIBRFG20685**, Nakdonggang National Institute of Biological Resources

Note: *Trichoderma taiwanense* was isolated from barks in Taiwan [43]. In this study, NNIBRFG20685 was isolated from sediment in Wicheon. This strain showed extracellular laccase activity and siderophore production (Table 3).

***Trichoderma tsugarense* Yabuki & Okuda, Mycoscience 55(3): 209 (2014) [#MB 804140] (Figs. 12D and 13G-I)**

Description: Colonies grew fast at 25°C and reached 87 mm on PDA, 87 mm on CMDA, and 87 mm on SNA, 7 d after inoculation. The colony on PDA was white to beige, with a fluffy aerial mycelial surface, and its reverse side was white. The colony on CMDA was hyaline with little aerial mycelia, and its reverse side was hyaline. The colony on SNA was hyaline with light yellow pustules in the margins and a little cottony aerial mycelial surface, and its reverse side was hyaline. Phialides were lageniform, solitary or in clusters, and measured 7.3-12.8 μm×2.3-3.9 μm at the widest point (x=9.4±1.32 μm×3.6±0.59 μm, L/W ratio=2.63, n=20). Conidia were green, aseptate, and subglobose or globose, measuring 4.1-6.6 μm ×2.5-5.2 μm (x=5.3±0.58 μm×3.8±0.68 μm, L/W ratio=1.40, n=50). Chlamydospores were aseptate, globose or subglobose, and thick-walled, measuring 7.7-11.6 μm×6.6-9.2 μm (x=9.0±1.51 μm×7.6± 0.99 μm, L/W ratio=1.18, n=20).

Habitat: Sediment in freshwater

Specimen examined: Iyeon-ri, Danbuk-myeon, Uiseong-gun, Gyeongsangbuk-do, Republic of Korea, 05 December 2018, **NNIBRFG20685**, Nakdonggang National Institute of Biological Resources

Note: *Trichoderma tsugarense* was isolated from volcanic ash soil in Japan [44]. In this study, NNIBRFG23468 was isolated from sediment in Wicheon. This strain exhibited extracellular laccase activity and siderophore production (Table 3).

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