RESEARCH ARTICLE

First report of six Sordariomycetes fungi isolated from plant litter in freshwater ecosystems of Korea

Jaeduk Goh*, Hye Yeon Mun, Yu-Jeong Jeon, Namil Chung, Young-Hwan Park, Sangkyu Park, Hyejin Hwang and Wonsu Cheon

Fungi Research Team, Nakdonggang National Institute of Biological Resources, Sangju 37242, Korea

***Corresponding author:** jdgoh@nnibr.re.kr

ABSTRACT

Freshwater ecosystems provide a complex environment for microorganisms. In this study, we isolated diverse fungal strains from plant litter in freshwaters. These strains were identified using molecular phylogenetic analyses of rDNA and/or other gene sequences (*TUB*, *GAPDH*, and *EF1*). In addition, we examined their morphological characteristics by microscopy and cultural characteristics on several media. We identified six previously unrecorded Sordariomycetes species in Korea, i.e., *Colletotrichum godetiae*, *Discosia rubi*, *Robillarda sessilis*, *Monochaetia dimorphospora*, *Idriella lunata*, and *Phialemoniopsis endophytica*. Of these, *D. rubi* and *M. dimorphospora* exhibited high extracellular amylase, lipase, and protease activities, suggesting that these fungal isolates might play an important role as decomposers in freshwater ecosystems. Plant litter could thus be a good source for isolating and investigating previously undocumented fungal species in freshwater environments.

Keywords: Freshwater, Plant litter, Sordariomycetes

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INTRODUCTION

Freshwater ecosystems encompass several habitat types, providing a diverse environment for microorganisms. Plant litter, such as fallen leaves and decaying wood, are typical habitats for several freshwater fungi. Freshwater fungi, which represent a poly-phylogenetic group, are taxonomically diverse organisms colonizing the substrate in aquatic or semi-aquatic environments [1-2]. To date, freshwater fungi have been identified in diverse substrates, including plant litter and sediment; however, their roles in aquatic ecosystems and their physiological and biochemical characteristics remain to be determined.

Sordariomycetes is one of the largest classes in the subdivision Pezizomycotina (Ascomycota) [3]. Fungi belonging to Sordariomycetes generally produce their asci in perithecial fruiting bodies. This class includes more than 1,300 genera with over 3,000 species, representing a wide range of ecologies, including plant pathogens and endophytes, animal pathogens, and mycoparasites [4]. The name "Sordariomycetes" is derived from the Latin "Sordes" (filth), as some of these species grow in animal feces, although growth

habits vary widely across this class. Some species of Sordariomycetes are found in aquatic habitats; however, most Sordariomycetes species occupy terrestrial habitats [4].

In this study, six Sordariomycetes species, i.e., *Colletotrichum godetiae*, *Discosia rubi*, *Robillarda sessilis*, *Monochaetia dimorphospora*, *Idriella lunata*, and *Phialemoniopsis endophytica*, were isolated from plant litter in Korean freshwaters for the first time, and their molecular phylogeny and morphological characteristics were investigated.

MATERIALS AND METHODS

Isolation of fungal strains and culture conditions

Fungal strains were collected from plant litter sampled from freshwater streams and rivers. The collection information of all strains used in this study is listed in Table 1. To isolate fungal strains, plant litter samples were washed with distilled water more than 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 three days. Then, 100 μL of the pretreatment medium was spread on a 1% water agar plate and incubated at 20°C for two days. Hyphal tip and germinated conidia were isolated under a microscope and 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. All strains used in this study were grown on potato dextrose agar (PDA; 3.9% potato dextrose agar powder [w/v]; Difco, Sparks, MD, USA), malt extract agar (MEA; 2% malt extract [w/v] and 2% agar [w/v]), oatmeal agar (OA; 7.25% oatmeal agar powder [w/v]; Difco, Sparks, MD, USA), potato carrot agar (PCA; 2.4% potato carrot agar powder [w/v]; HiMedia, Mumbai, India), corn meal agar (CMA; 3.9% corn meal agar powder [w/v]; Difco, Sparks, MD, USA), Czapek-dox solution agar (CDA; Difco Sparks, MD, USA), dichloran glycerol chloramphenicol (DG18; Merck Millipore, Billerica, MA, USA), and yeast extract peptone dextrose agar (YPDA; Duchefa Biochemie, Haarlem, Netherlands).

Table 1. Information of strains used in this study.

Species	Strain No.	Source	Collection date	Location (GPS)	GenBank acc. no.
Colletotrichum godetiae	NNIBRFG36	Plant litter	13 Oct 2015	Gangwon-do; Samcheok-si; Geundeok-myeon (37°23′14.4″ N, 129°11′53.1″ E)	KU751867[a]/ MT292313[d]
Discosia rubi	NNIBRFG201	Plant litter	23 Oct 2015	Gyeongsangbuk-do; Sangju-si; Namjang-dong (36°24'34.8"N, 128°06'50.8"E)	MT292314[e]
Idriella lunata	NNIBRFG113	Plant litter	13 Oct 2015	Gangwon-do; Samcheok-si; Geundeok-myeon (37°23′14.4″ N, 129°11′53.1″ E)	MT292315[e]
Monochaetia dimorphospora	NNIBRFG396	Plant litter	29 Oct 2015	Gyeongsangbuk-do; Andong-si; Dosan- myeon (36°43'21"N, 128°51'49.6"E)	MT271967[a]
Phialemoniopsis endophytica	NNIBRFG18	Plant litter	13 Oct 2015	Gangwon-do; Samcheok-si; Geundeok-myeon (37°23′14.4″ N, 129°11′53.1″ E)	MT27197[a]
Robillarda sessilis	NNIBRFG311	Plant litter	23 Oct 2015	Gyeongsangbuk-do; Sangju-si; Oeseo-myeon (36°27'55"N, 128°10'3.7"E)	KU892285[a]/ MT271966[b]

[a] internal transcribed spacer (ITS); [b] large subunit of ribosomal RNA (LSU) ; [c] elongation factor 1 (EF1); [d] glyceraldehyde-3-phosphate dehydrogenase (GAPDH); [e] beta tubulin (TUB).

Extracellular activities

To determine the extracellular 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 two weeks of incubation at 25°C. Supplemented substrates included 0.5% soluble starch [w/v] for amylase activity, 0.5% tween20 [w/v] for lipase activity, 0.5% carboxymethyl cellulose [w/v] for cellulase activity, and 1% skim milk [w/v] for protease activity in the presence of Congo red (500 ppm).

Morphological analysis

Conidiophores and conidia were observed under a model Eclipse Ni light microscope (Nikon, Tokyo, Japan) equipped with a Ds-Ri2 digital camera (Nikon, Tokyo, Japan). At least 50 individuals were measured for each structure.

DNA extraction, PCR, and sequencing

Fungal genomic DNA was isolated using NucleoSpin® Plant II DNA extraction kit (Macherey-Nagal, Düren, Germany). For molecular identification of the fungi, PCR amplifications were performed for an internal transcribed spacer (ITS) rDNA region using primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) [5], beta tubulin gene (*TUB*) using primers bt2a (5′-GGTAACCAAATCGGTGCTGCTTTC-3′) and bt2b (5′-ACCCTCAGTGTAGTGACCCTTGGC-3′) [6], glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*) using primers GDF1 (5′-GCCGTCAACGACCCCTTCATTGA-3′) and GDR1 (5′-GGGTGGAGTCGTACTTGAGCATGT-3′) [7], and translation elongation factor 1 gene (*EF1*) using primers EF1-983F (5′-GCYCCYGGHCAYCGTGAYTTYAT-3′) and EF1-1576R (5′-ACHGTRCCRATACCACCRATCTT-3′) [8]. Amplicons were sequenced by a DNA sequencing service (Macrogen Inc., Korea), with the same primers as used for the amplifications. A homology search of the DNA sequences was performed using BLAST algorithms available from the National Center for Biotechnology Information (NCBI).

Phylogenetic analysis

We obtained the sequences of reference species from NCBI (https://www.ncbi.nlm.nih.gov) for phylogenetic analyses, which are shown in Figs. 1-6. All reference species sequences have been reported previously [9-22]. The sequences were edited using DNAStar software package version 5.05 (DNAStar, Inc., Madison, WI, USA). Accession numbers of all sequences used in this study are shown in the phylogenetic trees (Figs. 1-6), constructed using the maximum likelihood (ML) method. The ML analysis was performed using MEGA 7.1 [23] 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.

RESULTS AND DISCUSSION

Phylogenetic analysis

Phylogenetic analyses were performed to identify and infer the phylogenetic relationships of the six Sordariomycetes species with other similar species. For genus *Phialemoniopsis* and related species, the ITS sequences were used for phylogenetic analysis. It is evident from Fig. 1 that NNIBRFG18 belonged to a clade with *Phialemoniopsis endophytica*. In BLASTn analysis, the ITS of NNIBRFG18 showed high similarity (99.79%) with that of *Phialemoniopsis endophytica* strain ACCC38980. For *Colletotrichum* species, a combination of ITS and *GAPDH* sequences was used for phylogenetic analysis. Fig. 2 shows that NNIBRFG36 formed a clade with four isolates of *Colletotrichum godetiae*, including the type material. In BLASTn analysis, NNIBRFG36 showed high similarities with the ITS (99.79%) and *GAPDH* (100%) sequences of *Colletotrichum godetiae*. For genus *Idriella* and related species, the *EF1* sequences were used for phylogenetic analysis. Fig. 3 shows that NNIBRFG113 formed a clade with two isolates of *Idriella lunata*. In BLASTn analysis, NNIBRFG113 showed high similarity with the *EF1* of *Idriella lunata* (98.3%). For *Discosia* species, the *TUB* sequences were used for phylogenetic analysis. Fig. 4 shows that NNIBRFG201 formed a clade with the type material of *Discosia rubi*. In BLASTn analysis, NNIBRFG201 was identical with the *TUB* sequences of *Discosia rubi* (100%). For *Robillarda* species, a combination of ITS and large subunit of ribosomal RNA (LSU) sequences was used for phylogenetic analysis. Fig. 5 shows that NNIBRFG311 formed a clade with four isolates of *Robillarda sessilis*, including the ex-epitype

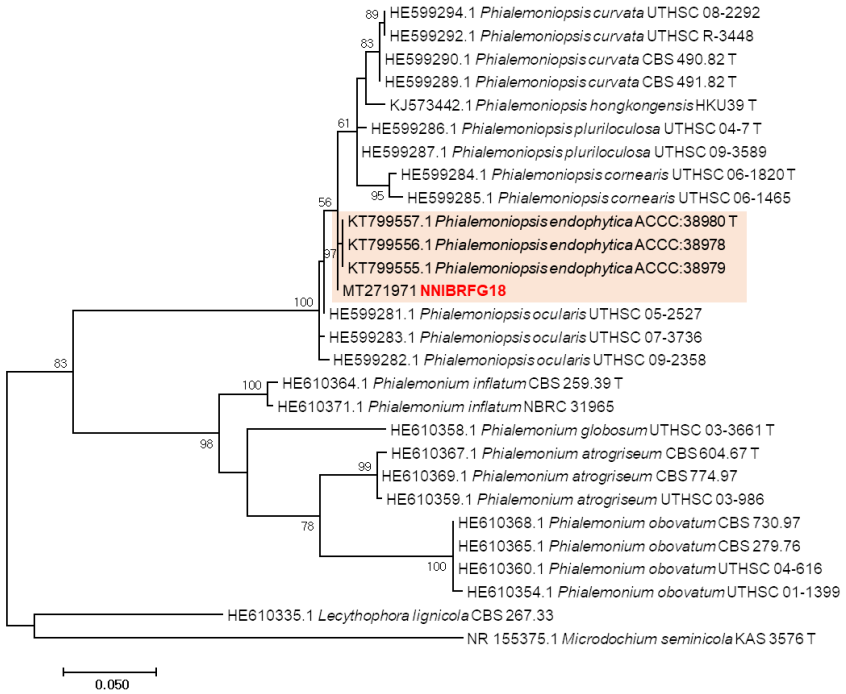

Fig. 1. Phylogenetic tree of *Phialemoniopsis endophytica* NNIBRFG18 and related species, based on maximum-likelihood analysis of the internal transcribed spacer (ITS) rDNA sequences. The sequence of *Microdochium seminicola* was used as an outgroup. Numbers at the nodes indicate the bootstrap values (>50%) from 1,000 replications. The bar indicates the number of substitutions per position. New isolate from the present study is shown in red.

material. In BLASTn analysis, NNIBRFG311 showed high similarity with the ITS (100%) and LSU (100%) of *Robillarda sessilis*. For *Monochaetia* species, the ITS sequences were used for phylogenetic analysis. Fig. 6 shows that NNIBRFG396 formed a clade with the type material of *Monochaetia dimorphospora.* In BLASTn analysis, NNIBRFG396 showed high similarity with the ITS of *M. dimorphospora* (100%).

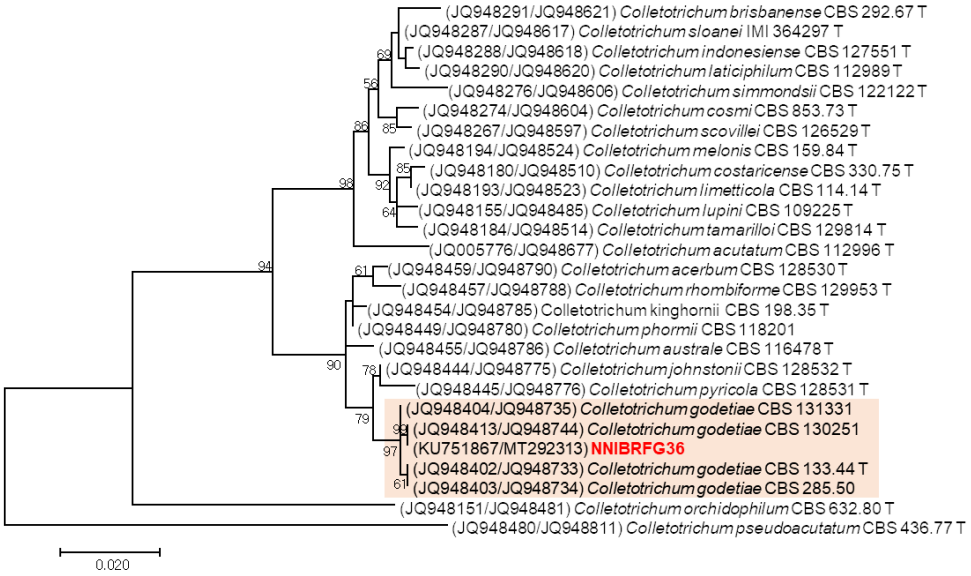

Fig. 2. Phylogenetic tree of *Colletotrichum godetiae* NNIBRFG36 and related species, based on maximum-likelihood analysis of a combination of internal transcribed spacer (ITS) rDNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene sequences. The sequence of *Colletotrichum pseudoacutatum* was used as an outgroup. Numbers at the nodes indicate the bootstrap values (>50%) from 1,000 replications. The bar indicates the number of substitutions per position. New isolate from the present study is shown in red.

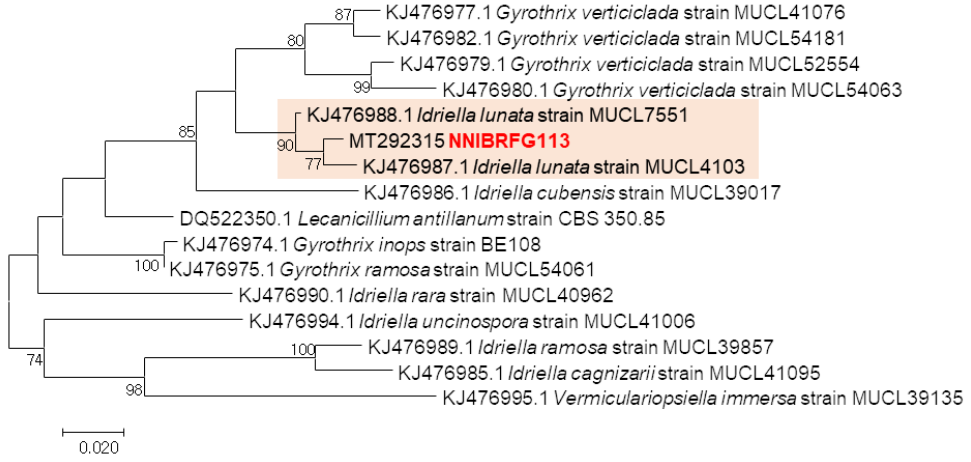

Fig. 3. Phylogenetic tree of *Idriella lunata* NNIBRFG113 and related species, based on maximum-likelihood analysis of the translation elongation factor 1 (EF1) sequences. The sequence of *Vermiculariopsiella immersa* was used as an outgroup. Numbers at the nodes indicate the bootstrap values (>50%) from 1,000 replications. The bar indicates the number of substitutions per position. New isolate from the present study is shown in red.

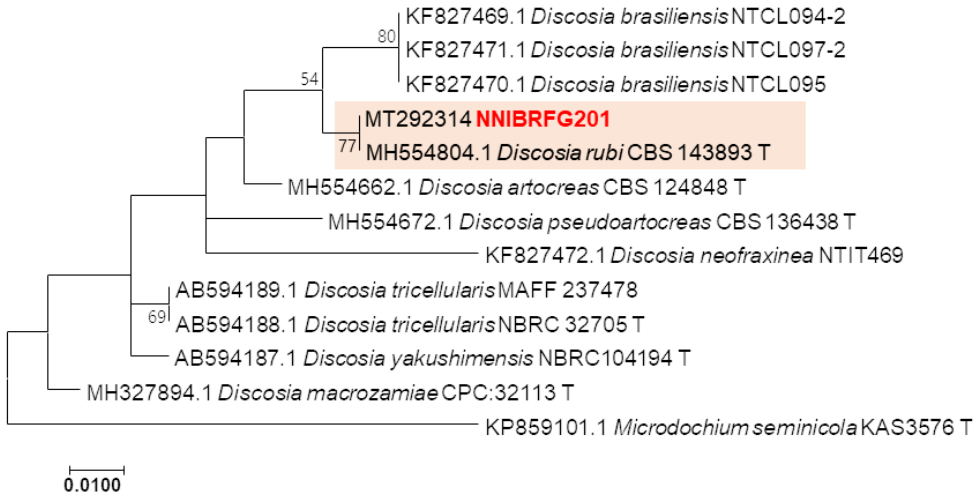

Fig. 4. Phylogenetic tree of *Discosia rubi* NNIBRFG201 and related species, based on maximum-likelihood analysis of beta-tubulin sequences. The sequence of *Microdochium seminicola* was used as an outgroup. Numbers at the nodes indicate the bootstrap values (>50%) from 1,000 replications. The bar indicates the number of substitutions per position. New isolate from the present study is shown in red.

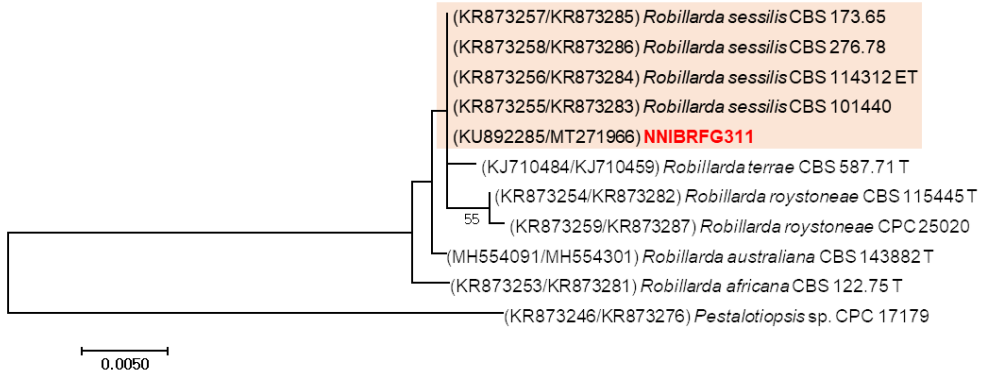

Fig. 5. Phylogenetic tree of *Robillarda sessilis* NNIBRFG311 and related species, based on maximum-likelihood analysis of a combination of internal transcribed spacer (ITS) rDNA and 28S rDNA sequences. The sequence of *Pestalotiopsis* sp. was used as an outgroup. Numbers at the nodes indicate the bootstrap values (>50%) from 1,000 replications. The bar indicates the number of substitutions per position. New isolate from the present study is shown in red.

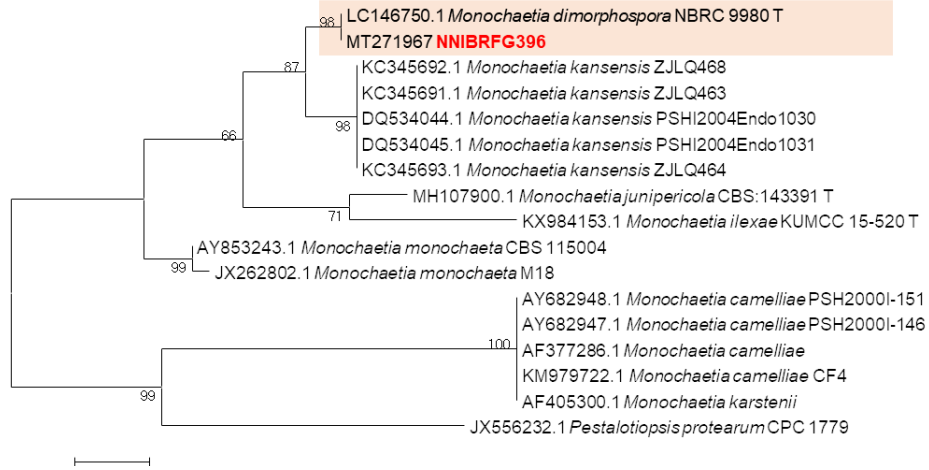

Fig. 6. Phylogenetic tree of *Monochaetia dimorphospora* NNIBRFG396 and related species, based on maximum-likelihood analysis of the internal transcribed spacer (ITS) rDNA sequences. The sequence of *Pestalotiopsis protearum* was used as an outgroup. Numbers at the nodes indicate the bootstrap values (>50%) from 1,000 replications. The bar indicates the number of substitutions per position. New isolate from the present study is shown in red.

Extracellular enzyme activities

Some of Sordariomycetes fungi are known to show activities in extracellular enzyme production and plant growth promoting [24]. To screen extracellular enzyme activities, we performed a growth test using minimal malt extract agar media containing various substrate. After 14 days of incubation at 25℃, enzyme activity was measured by size and discoloration of halo zone. Table 2 showed enzyme activity of four enzymes – cellulase, amylase, lipase, and protease. As a result, *Discosia rubi* (NNIBRFG201) and *Monochaetia dimorphospora* (NNIBRFG396) are shown in high amylase, lipase and protease activity. In previous studies, *Discosia* sp. showed amylase activity and plant growth promoting effects [24-25]. NNIBRFG201 showed not only amylase but also protease and lipase. In *Monochaetia* genus, there are no previous report about extracellular enzyme activities. These results suggested that *Monochaetia dimorphospora* and *Discosia rubi* might play a role as a decomposer in freshwater ecosystem.

Table 2. Enzyme activities of fungal strains.

Species	Strains	Cellulase	Amylase	Lipase	Protease
Phialemoniopsis endophytica	NNIBRFG18	-	-	-	-
Colletotrichum godetiae	NNIBRFG36	-	-	-	-
Idriella lunata	NNIBRFG113	-	-	-	-
Discosia rubi	NNIBRFG201	-	++	++	+++
Robillarda sessilis	NNIBRFG311	-	-	-	-
Monochaetia dimorphospora	NNIBRFG396	-	+	+++	+++

-, activity not detected; +, inhibition zone≤2 mm; ++, 2 mm<inhibition zone≤4 mm; +++, inhibition zone>4 mm.

Taxonomy

Based on the molecular phylogeny and morphological data, we identified six fungal species that have not previously been recorded in Korea: *Phialemoniopsis endophytica*, *Colletotrichum godetiae*, *Discosia rubi*, *Idriella lunata*, *Robillarda sessilis,* and *Monochaetia dimorphospora*.

***Phialemoniopsis endophytica* L. Su & Y.C. Niu, Mycological Progress 15 (5/48): 3 (2016) [MB#814529] (Figs. 1, 7, 8, 9A and 9B)**

Description: Colonies grew slightly slowly and reached 46 mm on PDA, 52 mm on MEA, 48 mm on OA, 51 mm on PCA, 58 mm on CMA, 46 mm on CDA, 50 mm on DG18A, and 39 mm on YPDA at 25℃, 10 days after inoculation. The optimal growth conditions were on PDA at 25℃. At temperatures higher than 35℃, no growth was observed on any medium. The color of the colony was brownish gray, turning olive-brown to black, on PDA; translucent white to olive-brown on MEA; white to light gray on OA; translucent white to creamish on PCA; hyaline white, with a smooth surface, on CMA; whitish, with soft, cottony aerial mycelium, turning olive-brown in the center, on CDA; white, with floccose aerial mycelium, on DG18A; and yellowish, with dense aerial mycelium, on YPDA. Hyphae and conidiophores were hyaline and smooth, and conidiogenous cells were monophialidic. Phialides of the aerial mycelium were straight to slightly bent, and had a cylindrical form, measuring 6.08-16.5 μm×0.95-2.8 μm (x=9.83 μm×1.7 μm, n=17). One-celled conidia were formed at the apex of the phialides, and were globose to ellipsoidal, measuring 2.2-4.1 μm×0.9-2.5 μm (x=3.03 μm×1.55 μm, n=50).

Habitat: Plant litter (fallen leaves) in streams

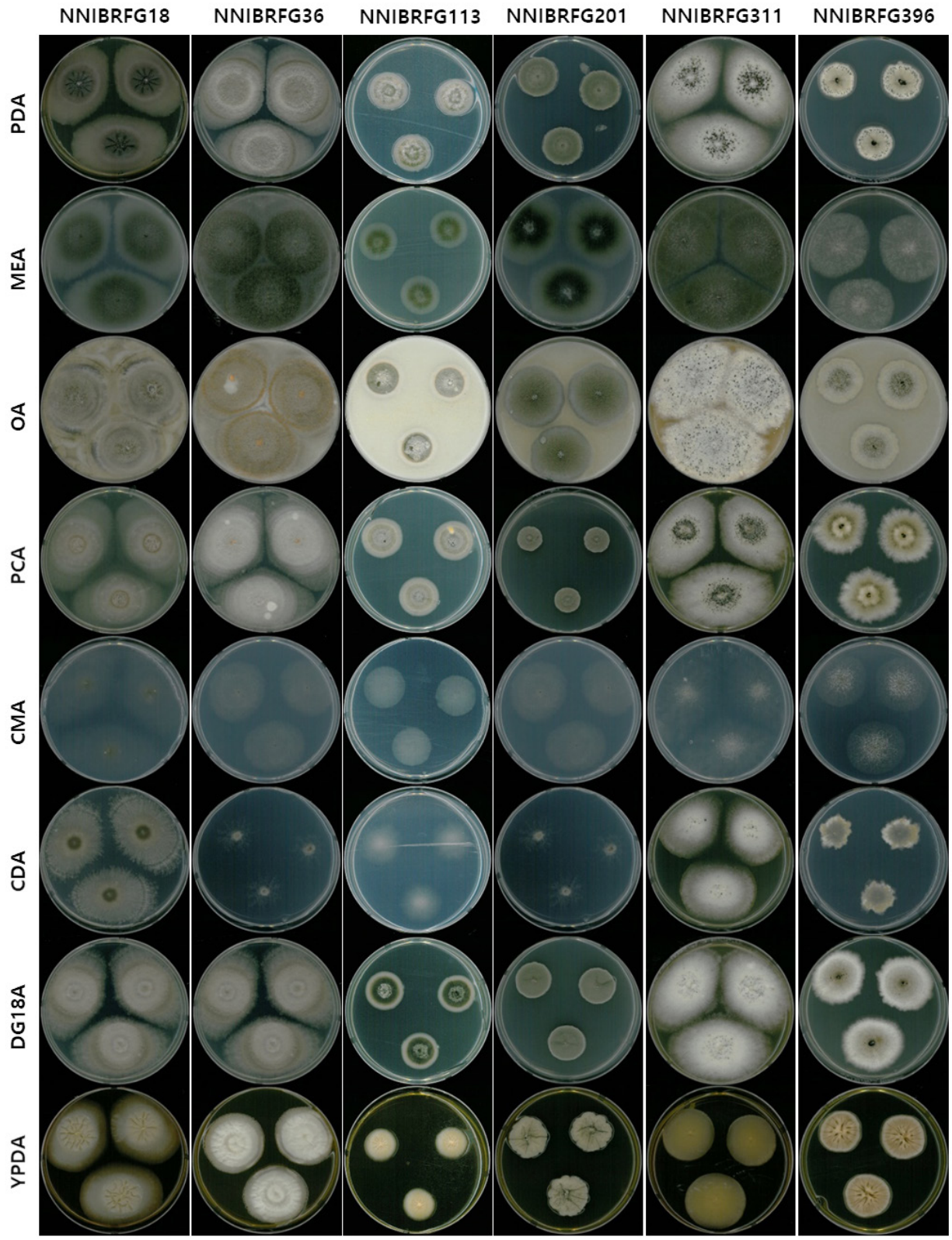

Fig. 6. Colony morphology of strains in this study. All strains were cultured at 25℃ for 10 days.

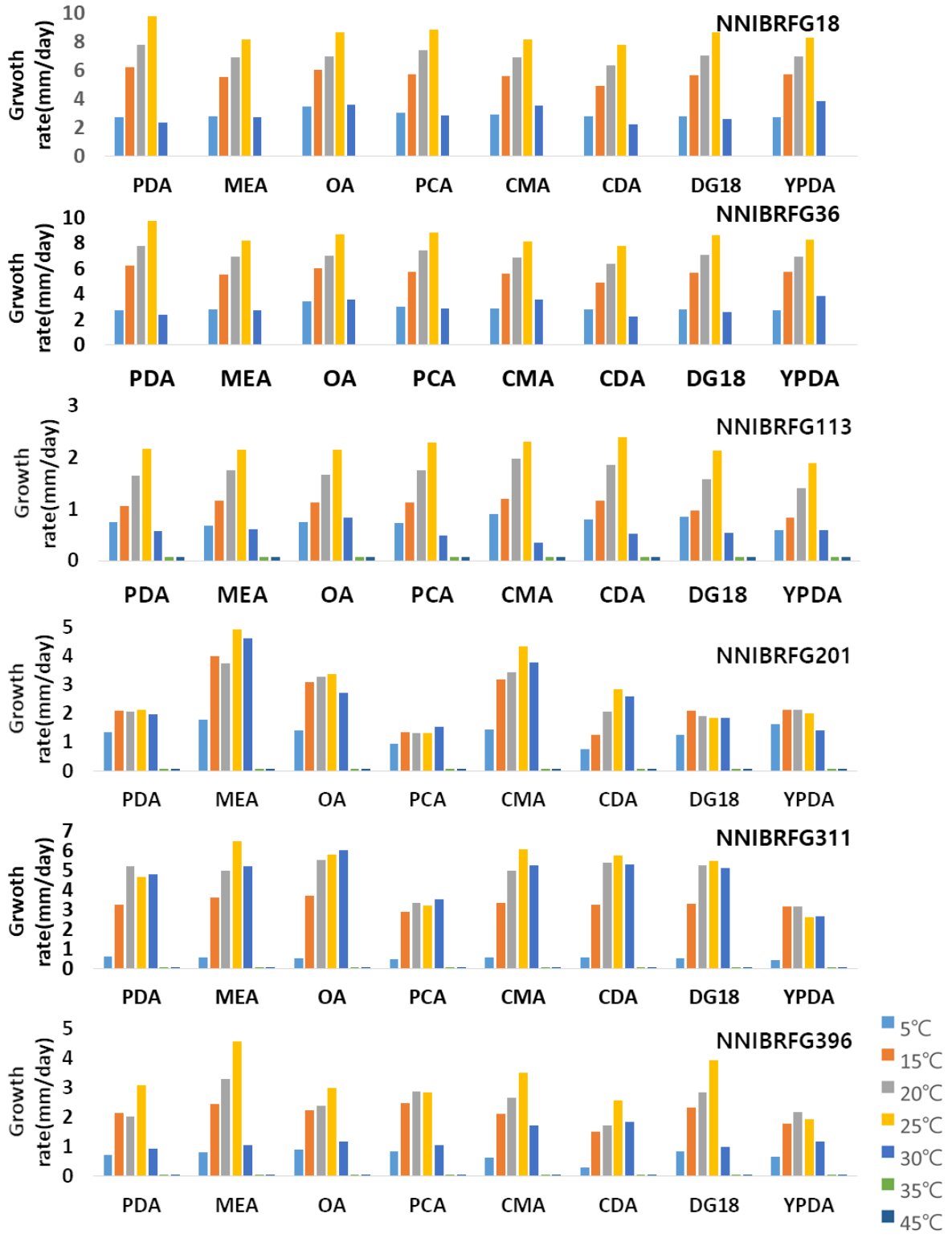

Fig. 8. Mycelial growth rate of strains in this study on eight different media (PDA, MEA, OA, PCA, CMA, CDA, DG18, and YPDA) and temperature conditions (5-45℃).

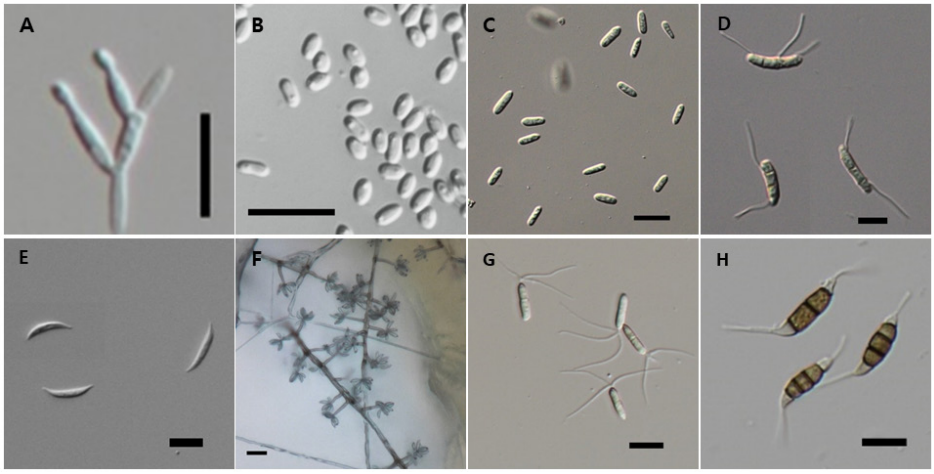

Fig. 9. Microscopic features of six Sordariomyctes fungi. A, B: conidiophores (A) and conidia (B) of *Phialemononiopsis endophytica* NNIBRFG18; C: conidia of *Collectotrichum godetiae* NNIBRFG36; D : conidia of Discosia rubi NNIBRFG201; E, F: conidia (E) and conidiophores (F) of *Idriella lunata* NNIBRFG113; G: conidia of *Robillarda sessilis* NNIBRFG311; H: conidia of *Monochaetia dimorphospora* NNIBRFG396. Scale bars: A-D, F-H=10 μm; E=20 μm.

Specimen examined: Sohancheon, Geundeok-myeon, Samcheok-si, Gangwon-do, Republic of Korea, 13 Oct 2015, NNIBRFG18, Nakdonggang National Institute of Biological Resources

Note: *Phialemoniopsis endophytica* was first reported as an endophytic fungus in China [16]. We isolated this strain from plant litter in a freshwater stream.

***Colletotrichum godetiae* Neerg., Friesia 4 (1-2): 72 (1950) [MB#440867] (Figs. 2, 7, 8, and 9C)**

Description: Colonies grew slightly slowly and reached 52 mm on PDA, 53 mm on MEA, 52 mm on OA, 57 mm on PCA, 35 mm on CMA, 25 mm on CDA, 51 mm on DG18A, and 33 mm on YPDA at 25℃, 10 days after inoculation. The optimal growth conditions were on PDA at 25℃. At higher temperatures (35 and 45℃), no growth was observed on any medium. The color of the colony was creamish to light gray, with dense floccose mycelium, on PDA; translucent white at the margin, turning dark olive-brown, on MEA; yellowish-brown on OA; hyaline white at the margin, turning creamish, on PCA; hyaline white, with a smooth surfaced-mycelium, on CMA; hyaline-white, with scant aerial mycelium, on CDA; white, with floccose aerial mycelium, turning light gray, on DG18A; and yellowish at the margin, with whitish dense aerial mycelium, on YPDA. Conidiation was observed 3-5 days after inoculation at 25℃. Conidia were hyaline, smooth-walled, aseptate, with a cylindrical to clavate form; measuring 9.8-17.5 μm×2.0-5.2 μm (x=12.12 μm×4.00 μm, n=50), with an L/W ratio of 3.02.

Habitat: Plant litter (fallen leaves) in streams

Specimen examined: Sohancheon, Geundeok-myeon, Samcheok-si, Gangwon-do, Republic of Korea, 13 Oct 2015, NNIBRFG36, Nakdonggang National Institute of Biological Resources

Note: *Colletotrichum godetiae* is one of the species belong to *C. acutatum* complex. This species is known as a plant pathogen on various fruits such as apple bitter, avocado and grapevine [9, 13, 21]. We isolated this strain from plant litter in a freshwater stream.

***Idriella lunata* P.E. Nelson & S. Wilh., Mycologia 48 (4): 547 (1956) [MB#298872] (Figs. 3, 7, 8, 9E, and 9F)**

Description: Colonies grew slowly and reached 20 mm on PDA, 19 mm on MEA, 19 mm on OA, 19 mm on PCA, 21 mm on CMA, 18 mm on CDA, 18 mm on DG18A, and 19 mm on YPDA at 25℃, 10 days after inoculation. The optimal growth conditions were on PDA at 25℃. The colony color was light gray to charcoal on PDA, with dense floccose mycelium distinct at the margin; translucent white at the margin, turning dark olive-brown, on MEA; white at the margin, turning gray, on OA; creamish at the margin, turning light gray, on PCA; hyaline white, with a smooth surfaced-mycelium, on CMA; hyaline-white, with scant aerial mycelium and an indistinct margin, on CDA; white at the margin, turning blackish-brown in the center, with dense, stubby aerial mycelium, on DG18A; and apricot-like, with dense aerial mycelium, on YPDA. Conidia were hyaline, aseptate, with a crescent form, measuring 13.6-19.3 μm×2.2-3.5 μm (x=15.55 μm×2.59 μm, n=50), with an L/W ratio of 5.99. Chlamydospores were brown.

Habitat: Plant litter (fallen leaves) in streams

Specimen examined: Sohancheon, Geundeok-myeon, Samcheok-si, Gangwon-do, Republic of Korea, 13 Oct 2015, NNIBRFG113, Nakdonggang National Institute of Biological Resources

Note: *Idriella lunata* was first reported as a plant pathogen causing root rot of strawberry [26]. We isolated this strain from plant litter in a freshwater stream.

***Discosia rubi* F. Liu, L. Cai & Crous, in Liu, Bonthond, Groenewald, Cai & Crous, Stud. Mycol. 92: 322 (2018) [2019] [MB# *828322*] (Figs. 4, 7, 8, and 9D)**

Description: Colonies grew moderately slowly and reached 24 mm on PDA, 49 mm on MEA, 39 mm on OA, 15 mm on PCA, 39 mm on CMA, 23 mm on CDA, 23 mm on DG18A, and 25 mm on YPDA at 25℃, 10 days after inoculation. The optimal growth conditions were on MEA at 25℃. The colony color was gray-green on PDA, with dense mycelium distinct at the margin; hyaline at the margin, turning dark green, on MEA; gray green on OA; gray green, with mycelium distinct at the margin, on PCA; hyaline, with soft cottony mycelium and a smooth surface, on CMA; hyaline-white, with scant aerial mycelium and an indistinct margin, on CDA; gray green and dense, with a distinct margin, on DG18A; and yellowish-brown, with a rough margin, on YPDA. Conidia were straight or slightly curved, almost colorless, mostly 3-septate, with a cylindrical form and 1-3 appendages (mostly 2), measuring 10.4-22.2 μm×2.1-4.5 μm (x=15.64 μm×3.46 μm, n=50). The appendages were 6.8.18.1 μm in length (x=13.61 μm, n=76).

Specimen examined: Bukcheon, Namjang-dong, Sangju-si, Gyeongsangbuk-do, Republic of Korea, 23 Oct 2015, NNIBRFG201, Nakdonggang National Institute of Biological Resources

Note: These strains showed high levels of extracellular amylase and protease enzymes. This is the first report on the extracellular enzyme activities of *Discosia rubi*.

***Robillarda sessilis* (Sacc.) Sacc., Sylloge Fungorum 3: 408 (1884) [MB#225336] (Figs. 5, 7, 8, and 9G)**

Description: Colonies reached 70 mm on PDA, 75 mm on MEA, 65 mm on OA, 62 mm on PCA, 66 mm on CMA, 47 mm on CDA, 64 mm on DG18A, and 34 mm on YPDA at 25℃, 10 days after inoculation. The optimal growth conditions were on MEA at 25℃. The color of the colony was white, with small black spots, on PDA; dark green on MEA; white, with small black spots, on OA; cream overall, with

an olive green center, on PCA; hyaline, with soft cottony mycelium, on CMA; whitish-cream, with fluffy aerial mycelium and a wide hyaline margin, on CDA; whitish-cream, with fluffy aerial mycelium, on DG18A; and murky yellow, with scant aerial mycelium and a distinct margin, on YPDA. Conidia were straight or slightly curved, almost colorless, mostly 2-septate, fusiform with 1-4 appendages (mostly 3), measuring 10.4-22.2 μm×2.1-4.5 μm (x=11.30 μm×3.32 μm, n=50). The appendages were 11.21-29.14 μm in length (x=20.64 μm, n=140).

Specimen examined: Oeseocheon, Oeseo-myeon, Sangju-si, Gyeongsangbuk-do, Republic of Korea, 23 Oct 2015, NNIBRFG311, Nakdonggang National Institute of Biological Resources.

Note: *Robillarda sessilis* is known to be found in various hosts and substratum types including bark, branches, leaves and seeds [11]. We isolated this strain from plant litter in a freshwater stream.

***Monochaetia dimorphospora* T. Yokoy., Transactions of the British Mycological Society 65 (3): 500 (1975) [MB#317826] (Figs. 6, 7, 8, and 9H)**

Description: Colonies grew moderately slowly and reached 23 mm on PDA, 43 mm on MEA, 28 mm on OA, 34 mm on PCA, 34 mm on CMA, 21 mm on CDA, 36 mm on DG18A, and 27 mm on YPDA at 25℃, 10 days after inoculation. The optimal growth conditions were on MEA at 25℃. The colony was creamish to light gray, with black spots near the margin, on PDA; hyaline, with soft cottony mycelium, on MEA; creamish to light gray on OA; white to yellow-green, with a rough and distinct margin, on PCA; hyaline, with scant aerial mycelium, on CMA; gray, with aerial mycelium and a rough white margin, on CDA; white, turning gray in the center, on DG18A; and grayish-mustard, with a rugose mycelium, on YPDA. Conidia were straight or slightly curved, almost brown, mostly 5-septate, fusiform with 1-2 appendages (mostly 2) at the apex, measuring 16.6-25.2 μm×4.5-6.5 μm (x=19.96 μm×5.43 μm, n=50). The shorter appendage on the upper side of the conidia was 4.5-11.7 μm in length (x=7.85 μm, n=50), and the longer appendage on the lower side of the conidia was 6.1-22.7 μm in length (x=15.02 μm, n=50).

Specimen examined: Nakdonggang-river, Dosan-myeon, Andong-si, Gyeongsangbuk-do, Republic of Korea, 29 Oct 2015, NNIBRFG396, Nakdonggang National Institute of Biological Resources.

Note: These strains showed high levels of extracellular lipase and protease enzymes. This is the first report on the extracellular enzyme activities of *Monochaetia dimorphospora*.

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