


Fungal Diversity in Nam River and Their Biodegradative Activities

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ABSTRACT

145 fungal isolates were obtained from three sampling sites situated within the Nam River basin, located in the southern region of South Korea. Through ITS sequence analysis, the fungal isolates were identified to comprise 55 species of ascomycetes and 11 species of basidiomycetes. The 55 species of ascomycetes exclusively belong to the phylum Pezizomycotina, comprising 33 species of Dothideomycetes, 6 species of Eurotiomycetes, and 16 species of Sordariomycetes. Regarding their plant pathogenicity, an investigation into the fungi's ability to penetrate solid media revealed *Nigrospora chinensis* as displaying the highest growth, followed by *Pseudopezalotiopsis theae*, various *Curvularia* species, *Diaporthe* species, and *Alternaria alternata*. Further research associating this penetration ability with fungal pathogenicity is deemed necessary. Among the 10 fungal species exhibiting penetration abilities, an examination of their capability to degrade biological polymers revealed that two strains of *D. phaseolorum* displayed exceptional polymer degradation. These strains exhibited remarkable abilities in decomposing malachite green and crystal violet, both recalcitrant dyes. This study underscores the potential utilization of fungal diversity in freshwater environments as a foundational approach to address freshwater pollution issues.

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1. Introduction

Biodiversity provides various functions and productivity to the Earth's environment and serves as a crucial element in the resilient adaptation of ecosystems to environmental changes [1]. Fungi, primary decomposers in ecosystems, play a pivotal role as a key link in the global carbon cycle, returning carbon accumulated in plants back into the atmosphere. Paradoxically, their role intertwines with carbon emissions from fossil fuels, contributing to atmospheric carbon accumulation. Climate change due to atmospheric carbon accumulation significantly impacts fungal biodiversity. Fungi adapt their growth to habitat temperatures and are closely associated with plant ecosystems, making them susceptible to the effects of temperature rise and subsequent changes in plant ecology. The reduction in fungal diversity, the emergence of heat-adapted fungi, and the introduction of non-native fungal species from tropical and subtropical regions are expected to become major topics in the context of fungal diversity in the era of global warming. Consequently, comprehensive studies on fungal diversity and

conservation across various habitats become essential to address these challenges.

In terrestrial ecosystems, freshwater habitats serve as reservoirs of fungal diversity, hosting over 3000 species, including various Ascomycetes, Basidiomycetes, Chytridiomycetes, and Oomycetes, in diverse freshwater environments [2]. Although systematic studies on freshwater fungal habitats in Korea are limited, specific fungi such as *Mortierella fluviae*, *Paraconiothyrium estuarinum*, and *Pyrenochaetopsis paucisetos* have been identified in samples from the Yeongsan River and Suncheon Bay wetlands [3,4]. Additionally, widespread distribution of genera like *Aspergillus*, *Cladosporium*, *Penicillium*, *Epicoccum*, *Paraconiothyrium*, *Septoriella*, and *Talaromyces*, regardless of aquatic conditions or marine environment, have been reported [5]. These fungal clusters exhibit diverse compositions and sizes based on seasonal and environmental variables, serving as valuable biological indicators of environmental changes [6]. Furthermore, fungi isolated from ecosystems represent crucial resources due to their potential for various beneficial purposes. Species belonging to genera such as *Trichoderma*, *Aspergillus*, *Fusarium*, *Penicillium*, and

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Candida are utilized not only as indicators of environmental pollution but also to investigate the lifespan or persistence of pollutants, integrating past, present, and future ecological conditions [7].

As mentioned above, fungi, as primary decomposers in ecosystems, externally produce various hydrolytic and oxidative enzymes. Among the hydrolytic enzymes produced by fungi are cellulase, protease, amylase, pectinase, and xylanase, which are utilized for breaking down biological polymers constituting plant matter. The oxidative enzymes primarily include laccase, lignin peroxidase, manganese peroxidase, and versatile peroxidase, which are extensively used in the breakdown of wood lignin and crystalline cellulose. The ability to decompose polymeric substances has long been a subject of applied research in various fields such as traditional food production, organic pollutant remediation in freshwater and soil environments, and the utilization of biomass. Fungi capable of degrading pollutants are diverse, including species like *Phanerochaete chrysosporium*, *Lasiodiplodia* sp., and *Diaporthe schini* [8–11]. Recently discovered *Diaporthe schini* has been reported to efficiently adsorb and remove crystal violet (CV), a dye present in wastewater, at a high efficiency of 87% [11]. Additionally, fungi such as *A. fumigatus* and *A. niger* have been reported to effectively decolorize CV [12,13]. Therefore, fungal diversity research holds significant importance not only in understanding fungi but also from the perspective of harnessing fungal resources.

The Nam River, originating from the Namdeogyu Mountains, is a tributary of the Nakdong River, extending 189.8 kilometers in length. Flowing through Jinju City, it serves as a national waterway and plays a critical role as a significant source of drinking water and industrial water in the western Gyeongsangnam-do region, South Korea. This study aimed to investigate fungi in the Nam River area, analyzing their physiological and biochemical characteristics to provide fundamental data regarding fungal diversity and applicability within the freshwater ecosystem of the Nam River region. Furthermore, this research explored the environmental remediation function of fungi in the Nam River basin by examining the degradation capabilities of isolated fungal strains using highly toxic and recalcitrant substances, malachite green (MG), and CV.

2. Materials and methods

2.1. Collection and isolation of fungi

Three locations around the Nam River (Location 1: Latitude 35.15311/Longitude 128.10396, Location 2:

Latitude 35.181084/Longitude 128.097619, Location 3: Latitude 35.159235/Longitude 128.031318) were selected for the collection of airborne, aquatic, and marshland soil fungi from April to August 2022. Airborne fungi were collected by leaving DRBC agar plates (glucose 10 g/L, peptone 5 g/L, KH_2PO_4 1 g/L, $\text{MgSO}_4 \cdot 6\text{H}_2\text{O}$ 0.5 g/L, Rose Bengal 0.025 g/L, dichloran 0.002 g/L, chloramphenicol 0.1 g/L, and agar 1.5%) open for 5 min at each location. Aquatic fungi were collected by spreading samples obtained from the river on DRBC agar. Soil fungi were collected by suspending 0.1 g of soil sample in 1 ml of distilled water, centrifuging it, and then spreading the supernatant on DRBC agar. Fungi grown on DRBC were transferred to PDA and incubated at 25 °C for further cultivation.

2.2. ITS sequence analysis

The fungal mycelia grown on PDA medium were frozen in liquid nitrogen and ground into a fine powder using a mortar and pestle. Genomic DNA was then extracted using the Genomic DNA Prep Kit (BIOFACT, Daejeon, Korea). The extracted DNA was used as a template to amplify the internal transcribed spacer (ITS) region. The obtained sequences were subjected to BLAST analysis for fungal species identification. Samples obtained from different environments were labeled as A (air), W (water), or S (soil), and isolated fungal strains from the same location were numbered sequentially. The ITS sequences of the isolated fungi underwent multiple sequence alignment using the MAFFT service [14], followed by the construction of a phylogenetic tree using the Neighbor-Joining method (with 1,000 Bootstrap resampling iterations). The generated phylogenetic tree was visualized using the Phylo.io program [15], which was omitted here but summarized in Table 1.

2.3. Measurement of vertical growth

To assess the ability of isolated strains to colonize substrates, their vertical growth on a solid medium was measured. PDA was poured into 15-ml culture tubes, and the fungal mycelium of selected strains was inoculated. After culturing for a total of 21 d, the length of mycelial growth was measured from each sample. For further experimentation with selected strains, PDA was loaded into serological pipette tips (25 ml, 34.5 cm), and the mycelium was inoculated vertically. The mycelial growth was then measured over a period of 28 d.

Table 1. List of fungal isolates.

Isolate number ^a	Species name	GenBank accession number	Similar sequence (Identity%) ^b	Subclass	Class	Phyllum
A1_1, A1_2, A1_3	<i>Alternaria alternata</i>	PP069833, PP069834, PP069835	OR144097.1	Pleosporomycetidae	Dothideomycetes	Ascomycota
A2_1	<i>Curvularia sacchari-officinorum</i>	PP069870	MN215708.1(99.76%)			
A3_3, A3_6, A3_8	<i>Curvularia geniculata</i>	PP069907, PP069910, PP069912	MK729138.1			
A2_2	<i>Curvularia intermedia</i>	PP069871	GU073102.1(99.65%)			
A2_16	<i>Curvularia nodulosa</i>	PP069884	MH857738.1			
A2_5	<i>Curvularia</i> sp. 296	PP069873	KM877487.1			
A2_7, A2_8, A2_21	<i>Curvularia kusanoi</i>	PP069875, PP069876, PP069888	MG847354.1			
A3_1	<i>Corynespora cassiicola</i>	PP069905	MN393240.1			
A1_19, A1_21	<i>Lophiostoma macrostomum</i>	PP069850, PP069852	MT323120.1 KX611244.1			
A3_15	<i>Periconia epilithographicola</i>	PP069919	OR602954.1			
A3_20	<i>Periconia macrospinosa</i>	PP069923	MG576113.1			
W3_2, W3_8	<i>Letendreaa</i> sp.	PP069930, PP069934	MT366771.1			
A3_9	<i>Microdiplodia miyakei</i>	PP069913	FJ462760.1			
A2_10, A2_11, A2_12, A2_13, A3_11, A3_14, A3_18, W3_9	<i>Chrysosporium sanyaense</i>	PP069878, PP069879, PP069880, PP069881, PP069915, PP069918, PP069922, PP069935	MW830131			
A1_10	<i>Paracamarosporium hawaiiense</i>	PP069841	ON332164.1(99.81%)			
S1_3	<i>Microsphaeropsis arundinis</i>	PP069855	KY827354 (99.82%)			
A2_4, A2_6, A2_9, A3_17	<i>Pithomyces chartarum</i>	PP069872, PP069874, PP069877, PP069921	MH042801			
A2_14, A3_10	<i>Pseudopithomyces sacchari</i>	PP069882, PP069914	HG933799			
A3_12, A3_13	<i>Periconia byssoides</i>	PP069916, PP069917	MK446311.1(99.8%)			
S2_2	<i>Pseudopithomyces chartarum</i>	PP069894	OW983643.1			
A1_12	<i>Keissleriella</i> sp.	PP069843	ON705354.1			
A3_4	<i>Phoma herbarum</i>	PP069908	MN452486.1			
A1_9	<i>Cercospora canescens</i>	PP069840	MN400274.1(99.8%)			
W2_3, W2_4	<i>Pyrenochaetopsis</i> sp.	PP069899, PP069900	OR072742.1(99.79%)			
W3_5	<i>Neopyrenochaeta</i> sp.	PP069932	OL692437.1(98.81%)			
A2_17, A2_26	<i>Setophaeosphaeria microspora</i>	PP069885, PP069893	MK329132.1			
S3_1	<i>Phoma laundoniae</i>	PP069925	MT153728.1			
A2_25	<i>Dendryphiella</i> sp.	PP069892	KY399876.1			
A2_24	<i>Didymocyrtis brachylaenae</i>	PP069891	MH327821.1(97.69%)			
S2_7	<i>Aureobasidium melanogenum</i>	PP069896	ON207836.1(99.81%)			
A1_6, A1_7, A2_22, A2_23, A3_21	<i>Cladosporium cladosporioides</i>	PP069838, PP069839, PP069889, PP069890, PP069924	OR544489.1	Dothideomycetidae		
W3_3, W3_7	<i>Cladosporium tenuissimum</i>	PP069931, PP069933	MN429197.1(99.8%)			
W1_7	<i>Cladosporium halotolerans</i>	PP069865	MK762600.1			
A3_7	<i>Aspergillus brunneoviolaceus</i>	PP069911	MG543687.1	Eurotiomycetidae	Eurotiomycetes	
S1_1	<i>Talaromyces purpurogenum</i>	PP069853	MK460821.1			
S3_7	<i>Penicillium koreense</i>	PP069929	MK450699.1			
S3_5	<i>Penicillium raperi</i>	PP069928	MT529662.1			
S1_11	<i>Eurotiomycetes</i> sp.	PP069859	KX908576.1(85.71%)			
S1_8	<i>Aequabiliella effusa</i>	PP069857	KX586145.1(86%)			
A1_4	<i>Fusarium solani</i>	PP069836	MN481202.1	Hypocreomycetidae	Sordariomycetes	
S1_2, A2_19	<i>Fusarium oxysporum</i>	PP069854, PP069886	MT530243.1(99.8%)			
S1_5, S1-10	<i>Purpureocillium lilacinum</i>	PP069856, PP069858	MT609913.1			
S3_3	<i>Paramyrothecium roridum</i>	PP069926	LR963480.1(99.82%)			
A1_20	<i>Acremonium polychromum</i>	PP069851	JX076941.1			
A2_15	<i>Hypocreales</i> sp.	PP069883	HQ328023.1(99.2%)			
W2_1, W2_8	<i>Diaporthe phaseolorum</i>	PP069897, PP069903	MN650843.1	Diaporthomycetidae		
S3_4	<i>Diaporthe eres</i>	PP069908	OP326159.1			
S1_13	<i>Fusidium griseum</i>	PP069860	EF029217.1(92.08%)	Xylariomycetidae		
A3_5	<i>Nigrospora chinensis</i>	PP069909	OR826643.1			
W2_2	<i>Beltrania</i> sp.	PP069898	MK848670.1(99.81%)			
W2_7	<i>Eutypella</i> sp.	PP069902	MK120868.1			
A3_2	<i>Pseudopestalotiopsis theae</i>	PP069906	MT252050.1			
W3_11, W3_12	<i>Pestalotiopsis parva</i>	PP069936, PP069937	MH855908.1(86.56%)			
W1_10, S2_5	<i>Myrmecidium schulzeri</i>	PP069868, PP069895	MK808788.1			
A1_18	<i>Biscogniauxia petrensis</i>	PP069849	MN341576.1			

(Continued)

Table 1. Continued.

Isolate number ^a	Species name	GenBank accession number	Similar sequence (Identity%) ^b	Subclass	Class	Phylum
A1_5	<i>Coprinellus radians</i>	PP069837	MT507812.1	Agaricomycetes		Basidiomycota
W1_3, W1_6	<i>Emmia lacerata</i>	PP069862, PP069864	KJ152169.1			
W1_5	<i>Irpex lacteus</i>	PP069863	MK829156.1			
W1_11	<i>Steccherinum tenuissimum</i>	PP069869	MW204584.1			
A1_13, W2_5, W2_11	<i>Candolleomyces candolleanus</i>	PP069844, PP069901, PP069904	MH567059.1(99.22%)			
W1_8	<i>Psathyrella</i> sp.	PP069866	OM474045.1(99.38%)			
W1_2	<i>Phlebia acerina</i>	PP069861	MK404458.1			
A1_15	<i>Crepatura ellipsospora</i>	PP069846	OM955825.1			
A1_14, A1_16, A1_17, A2_20, A3_16	<i>Phanerochaete conrescens</i>	PP069845, PP069847, PP069848, PP069887, PP069920	LC387810.1			
W1_9	<i>Peniophora incarnata</i>	PP069867	MN328050.1			
A1_11	<i>Peniophora</i> sp.	PP069842	JF925333.1(99.14%)			

^aThe letters preceding the numbers denote the isolation environment: A for air, S for soil, and W for water.

^bWhen the sequence identity is greater than 99.9%, it was not separately noted.

2.4. Degradation of biological polymers

The ability to degrade biological polymers such as cellulose, starch, skim milk, polygalacturonic acid, and xylan, was assessed by measuring fungal growth on minimal medium (Congo Red 1g/L, yeast nitrogen base 1g/L, and agar 1.5%) supplemented with each substrate at a concentration of 5g/L [16,17]. The degradation capacity of malachite green (MG) and crystal violet (CV) was observed by adding concentrations ranging from 10 to 40ppm of each dye to PDA and culturing at 25°C.

3. Results

3.1. Partial identification by ITS sequence analysis

The total number of isolated fungal strains at three locations in the Nam River basin amounted to 145 strains (Table 1). Among these, airborne fungi totaled 82 strains, soil-based fungi comprised 27 strains, and water-based fungi accounted for 36 strains. After excluding duplicates in the analysis of their ITS sequences for partial identification, a final count of distinct species amounted to 66 (Table 1). Of these, 11 were Basidiomycetes, while 55 belonged to Ascomycetes, indicating significantly higher diversity within the Ascomycetes group. The isolated Basidiomycete strains were observed to belong to the Agaricomycetidae, Polyporales, and Russulales, typical mushroom strains found in South Korea's general environment. For instance, *Emmia lacerata* (W1_3 and W1_6) was identified in respiratory infections among domestic hospital patients [18], while *Phanerochaete conrescens* was reported as an endophytic fungus in pine trees from domestic mountainous regions [19]. *Peniophora incarnata* (W1_9)

has been noted for its Mn-peroxidase, which effectively degrades recalcitrant substances like anthracene [20].

The 55 species of isolated Ascomycetes all belong to the phylum Pezizomycotina, comprising 33 species of Dothideomycetes, 6 species of Eurotiomycetes, and 16 species of Sordariomycetes upon analysis. Based on the phylogenetic tree analysis using ITS sequences, among the Dothideomycetes, fungi belonging to the Dothideomycetidae subclass, such as *Cladosporium cladosporioides* (A1_6, five isolated strains), *Cladosporium halotolerans* (W1_7), *Cladosporium tenuissimum* (W3-3 and W3_7), often reported as plant pathogenic fungi [21–23], were grouped with fungi from the Eurotiomycetes and Sordariomycetes, indicating that the ITS sequence-based phylogenetic tree did not accurately reflect the evolutionary relationships among these fungi (Table 1). This phenomenon has been observed in other fungal isolation studies as well [24]. *Microdiplodia miyakei* (A3_9) has been observed in dust samples from North America but has not been reported in South Korea [25]. Among the Dothideomycetes, the fungi belonging to the Pleosporomycetidae subclass were the most abundant, with 29 species. This group includes fungi reported in literature such as *Alternaria alternata* (A1_1, A1_2, and A1_3), *Periconia macrospinoso* (A3_20), *Curvularia intermedia* (A2_2), *Corynespora cassiicola* (A3_1) as plant pathogens [23,26,27], and *Phoma herbarum* (A3_4) known for synthesizing Gibberellin [28].

3.2. Vertical growth characteristics

Upon observing the growth of fungi in petri dishes containing PDA medium, it was noted that a total

of 30 fungal strains deeply penetrated the surface of the solid medium, growing toward the bottom. Most of these strains, considered predominantly plant-pathogenic fungi, exhibited the ability to penetrate the inner layers of the solid medium, akin to their capacity to invade plant tissues. To assess this similarity, PDA was filled into a serological pipette (25 ml), and after 28 days of cultivation, the length of growth was measured (Figure 1). *Nigrospora chinensis* (A3_5) displayed the most substantial vertical growth, reaching 24.4 cm from the inoculation point, followed by *Pseudopestalotiopsis theae* (A3_2), *Curvularia* species (A2_1, A3_3, and A2_8), *Diaporthe* species (S3_4, W2_1, and W2_8), and *Alternaria alternata* (A1_1), showing the next highest growth rates. In South Korea, *N. chinensis* has been isolated as an endophyte or plant pathogen on woody plants [29], while various *Nigrospora* species have recently been discovered in coastal algae [30]. This fungus is reported to produce

various substances from the terpenoid group [31,32]. Meanwhile, *Beltrania* sp. (W_2-2) has been previously isolated as an endophyte in fir needles in South Korea [33], but in a solid medium, it predominantly grew near the surface. Among the *Pithomyces chartarum* strains, strains A2_4 and A2_6 did not penetrate into the medium, whereas strains A2_9 and A3_10 exhibited relatively better penetration. The ability of fungal mycelia to penetrate the interior of solid organic matter is an essential characteristic for both endophytic and saprophytic fungi. Mycelia penetrating the substrate absorb nutrients at their tips, transport intracellular substances from rear hyphae to tips, and facilitate gas exchange for respiration, necessitating efficient oxygen-carbon dioxide exchange at depth. Therefore, a more in-depth investigation is required to understand the solid medium penetration ability of these fungi from the perspective of material movement and exchange.

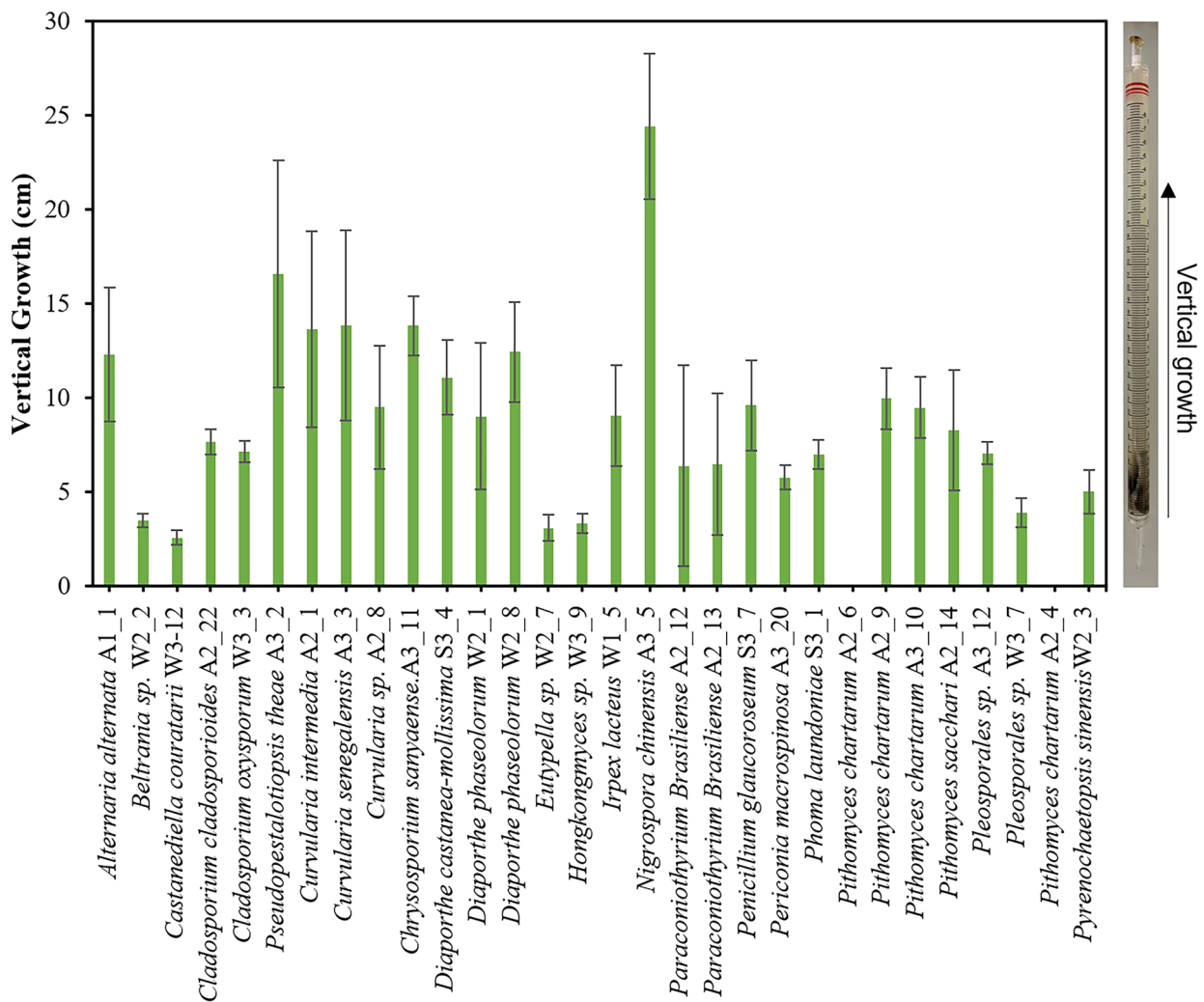


Figure 1. Vertical growth characteristics of the fungal isolates. Fungal isolates were inoculated at the tip of the serological pipette (25 ml, 34.5 cm) which was filled with PDA medium. The vertical growth was measured from the tip to the mycelial front propagated inside the pipette after 3 weeks of incubation at 25 °C.

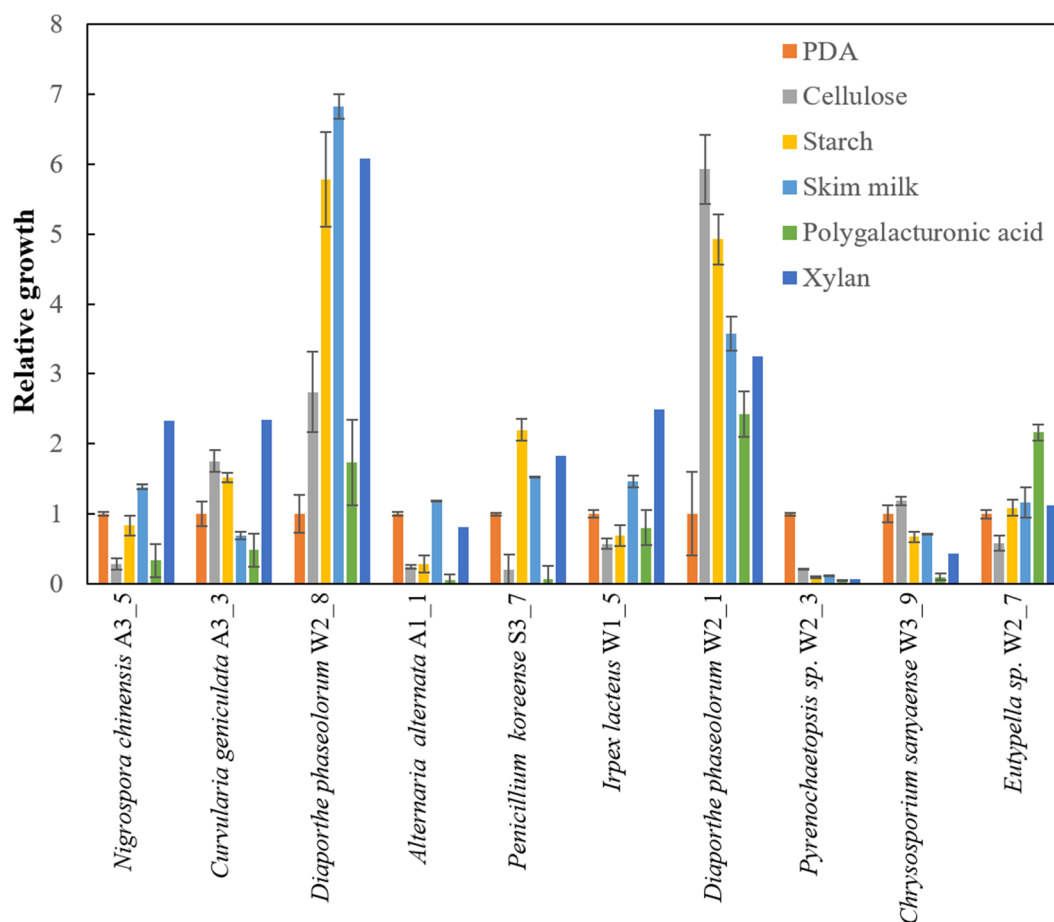


Figure 2. Growth of the fungal isolates on the minimal agar plates containing 5 g/L biological polymers, including cellulose, starch, skim milk, poly galacturonic acid and xylan. The relative growth was calculated by dividing the mycelial colony diameter grown on the biological polymers by the mycelial diameter grown on PDA.

3.3. Degradation of biological polymers and recalcitrant

The ability to penetrate solid media is presumed to be closely related to fungi's capacity for material degradation. To investigate this, the ability of 10 fungal strains to degrade various biological polymers such as cellulose, starch, skim milk, polygalacturonic acid, and xylan was measured by comparing the growth on minimal media to their growth on PDA. The results indicated that the growth of most strains was similar on both PDA and minimal media. However, in the case of *Diaporthe phaseolorum* W2_8 and W2_1 strains, their growth on biological polymers was over 3-7 times superior compared to their growth on PDA (Figure 2). This suggests that the ability to penetrate solid media and the capacity for material degradation might not have a direct correlation, indicating that within the fungal mycelium, the movement of substances like oxygen through cellular connections might be more critical.

Meanwhile, since the strains W2_8 and W2_1 demonstrated exceptionally superior abilities in degrading biological polymers, their potential for

decomposing recalcitrant substances, such as MG and CV, was investigated by adding 40 ppm of MG and CV to composite media. The results revealed that most fungi could not grow due to the toxicity of MG and CV. However, the strains W2_8 and W2_1 were able to decompose and grow in the presence of MG and CV similar to their capabilities in degrading biological polymers (Figure 3(A))

Based on these findings, the decomposition extent of MG and CV by the strains W2_8 and W2_1 was further investigated at concentrations of 20 ppm and 40 ppm over time. Both strains almost completely decomposed 20 ppm MG after 21 d of cultivation. At a concentration of 40 ppm MG, their growth was slower due to higher toxicity (Figure 3(B)). The degradation of MG by fungi is presumed to be mediated by oxidative enzymes secreted by fungi, as the surrounding area becomes transparent as MG breaks down. Enzymes such as laccase and peroxidase, known as oxidative enzymes produced by fungi, are thought to be responsible for MG decomposition [34]. CV, similar to MG, is a triphenylmethane dye. Unlike MG, fungi internalize and decompose CV

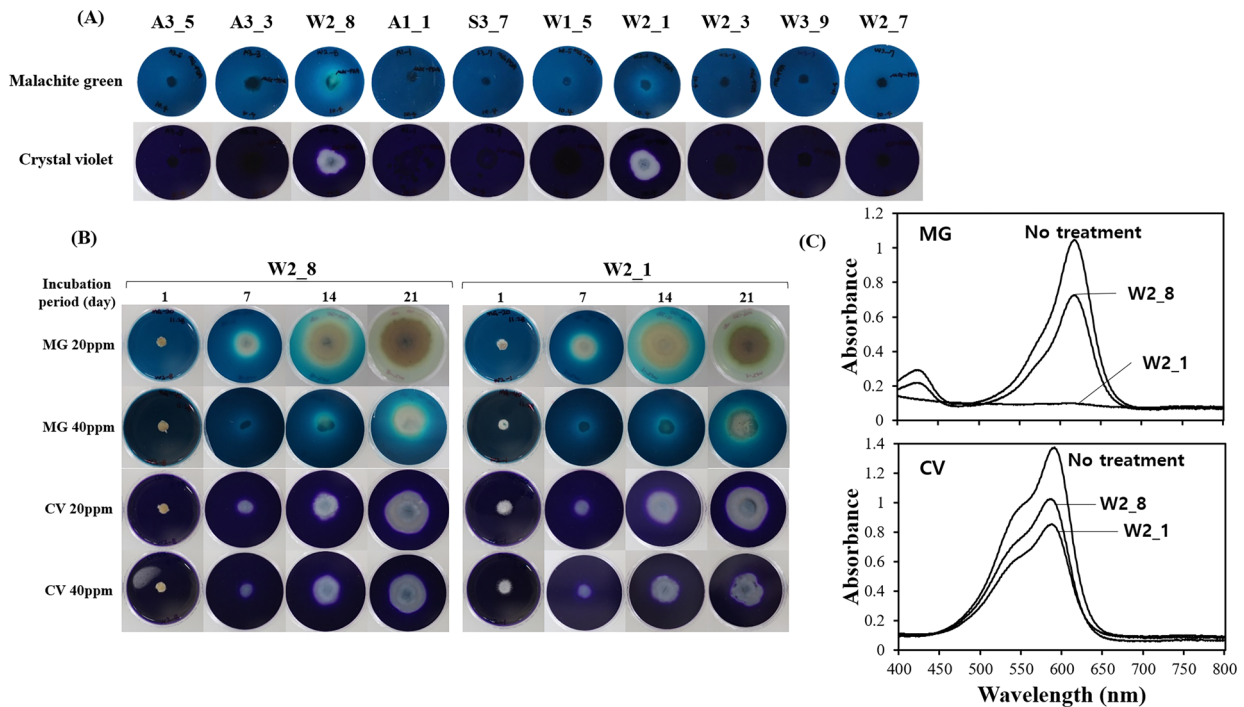


Figure 3. Degradation of malachite green (MG) and crystal violet (CV) by the fungal isolates. (A) Growth of some selected fungal isolates on PDA containing 40 ppm MG or CV. The plates were incubated 2 weeks at 25°C. (B) Growth of *Diaporthe phaseolorum* W2_8 or W2_1 in different concentration of MG or CV. (C) Degradation of MG and CV by *D. phaseolorum* W2_8 or W2_1 in liquid culture. The mycelial cells were grown in PDA medium containing 20 ppm of MG or CV for 21 d at 25°C. The culture supernatants, clarified by centrifugation for 5 min at 3000 rpm, were subjected to the UV-Vis spectral analysis.

within their mycelial cells without displaying a clear halo around the colonies.

Subsequently, 20 ppm concentrations of MG and CV in liquid media were examined for their decomposition using spectrophotometric analysis (Figure 3(C)). Analyzing the absorption spectra of the cultivation medium after 20 days revealed that the strain W2_1 completely decomposed MG, while the strain W2_8 decomposed only about 30%. For CV, approximately 61% and 77% remained in the strains W2_1 and W2_8, respectively, indicating that W2_1 had a slightly higher decomposition ability compared to W2_8, though lower than that observed for MG.

4. Discussion

The diverse fungi in freshwater ecosystems play a crucial role in organic matter decomposition and environmental purification. Therefore, the diversity of fungi in freshwater ecosystems can serve as an indicator of the health of these aquatic systems. However, studies on fungal diversity can vary significantly depending on climate conditions, physicochemical characteristics of sampling locations, sampling methods, sample processing, and culture methods. Unlike biodiversity studies of visible fauna and flora, studying fungal diversity is quite

challenging. In an effort to investigate fungal diversity in freshwater systems, this study aimed to isolate fungi from air, soil, and water samples in the Nam River basin using a DRBC medium and analyzed their diversity through ITS sequencing. As a result, a total of 145 strains were isolated from three locations, revealing the presence of 66 species consisting of 55 ascomycetes and 11 basidiomycetes. The number of these fungal species is likely underestimated as it is limited to culturable fungi on DRBC, indicating a potentially larger fungal diversity overall.

During the cultivation of isolated fungi, approximately 30 strains were discovered capable of penetrating and deeply infiltrating the interior of solid media, most of which were plant pathogenic fungi. Assessing their ability to penetrate solid media revealed that fungi known for their plant pathogenicity [29] such as *N. chinensis*, *P. theae*, *Curvularia*, and *A. alternata* exhibited high penetrating growth capabilities. This suggests a potential correlation between fungal plant pathogenicity and their ability for invasive mycelial growth, although further rigorous experimentation is required to substantiate this.

The material degradation capacity of fungi is associated with their production of extracellular

hydrolytic and oxidative enzymes, which are linked to the organic matter degradation in freshwater ecosystems. Investigating the degradative abilities of biological polymers such as cellulose, starch, skim milk, polygalacturonic acid, xylan, and the toxic triphenylmethane dyes, MG and CV, revealed that *D. phaseolorum* strains W2_8 and W2_1, revealed to have high biological polymer degradation activity, effectively decomposed MG and CV. *D. phaseolorum* is a fungus causing stem canker in soybeans and plant stems, with its metabolites known to induce seedling inhibition and wilting diseases in plants [35–37]. Its related species, *D. longicolla*, has been reported to secrete laccase enzymes capable of decomposing the persistent environmental hormone bisphenol A [38,39], while *D. schini* efficiently adsorbs and removes CV found in dye-contaminated wastewater [11]. Recent discoveries have revealed the production of lignin-degrading enzymes such as lignin peroxidase, Mn peroxidase, and laccase by these fungi [34]. Consequently, the degradation of MG and CV by *D. phaseolorum* observed in this study is likely attributed to the lignin-degrading enzymes produced by these fungi. In conclusion, this research marks the initial paper shedding light on the fungal diversity in the Nam River basin, potentially contributing to the study of fungal diversity in domestic freshwater environments and aiding research on environmental purification using freshwater fungi.

Author contributions

YJC and HSR conceived the project. JYP, JLP, and SC conducted sampling, isolation, characterization, and PCR analysis. YJC, HE, and HSR prepared the manuscript. All the authors have read and approved the final version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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