Mycobiology

Research Article

Diversity of Endophytic Fungi Associated with the Roots of Four Aquatic Plants Inhabiting Two Wetlands in Korea

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Abstract A total of 4 aquatic plants, *Eleocharis kuroguwai* Ohwi, *Hydrocharis dubia* Backer, *Salvinia natans* All., and *Zizania latifolia* Turcz., were sampled from representative two wetlands of South Korea. A total of 38 endophytic fungal strains were isolated from aquatic plants native to the Daepyeong wetland, and 27 strains were isolated from the Jilnal wetland. The internal transcribed spacer regions of fungal isolates were sequenced and a phylogenetic analysis was performed. In addition, endophytic fungal diversity from each wetland and host plant species was deduced. A total of 25 fungal genera were purely isolated, and 16 fungal genera were isolated from each of the two wetlands. Commonly isolated genera from both wetlands were *Aspergillus*, *Cladosporium*, *Clonostachys*, *Fusarium*, *Leptosphaeria*, *Penicillium*, and *Talaromyces*. This study revealed that fungal diversity varied with environmental conditions and by host plant in representative two wetlands.

Keywords Aquatic plant, Diversity index, Endophytic fungi, Fresh water, Wetland

Wetlands are now known to be global controllers of their surrounding environments, due to their roles in natural purification, remediation, material cycling, and buffering [1]. Freshwater marsh always undergoes biological or microbial succession, which aids in the maintenance of biodiversity [2]. Furthermore, diverse aquatic plants have colonized areas surrounding well-developed wetlands, which were formed long before the agricultural stage in Korea [3].

Aquatic plants in wetlands carry out photosynthesis as primary producers in the ecosystem, with blooms at the surfaces of freshwater. Thus, they affect the penetration of sunlight into the freshwater bed, the concentration of dissolved oxygen and CO_2 concentration, and the structure of the aquatic ecosystem. Therefore, aquatic plants are considered useful resources because of their ability to purify water [4-6].

Meanwhile, endophytic fungi distributed in the leaves or roots of plants exhibit symbiotic relationships with their host plant. The plant growth promoting activity and the induction of systemic resistance (ISR) by these fungi in their host plants has been widely researched [7-11]. Despite the potential of these microbial resources, research regarding the endophytic fungi of aquatic plants native to the wetlands of Korea has not been conducted [11].

The aim of this study was to identify the distribution and diversity of fungi from 4 representative aquatic plant species native to the Daepyeong and Jilnal wetlands, which were designated as natural monuments for the purpose of wetland preservation. Fungal colonies from the roots of each aquatic plant were isolated and identified by the amplification of the internal transcribed spacer (ITS) region of genomic DNA. Fungal strains were then categorized into several groups based on the phylogeny. Furthermore, the fungal diversity of each plant was assessed and comparatively analyzed. This study is the first research that provides basic data on the relationship between aquatic plants and their endophytic fungi. Promising microbial resources with benefits for aquatic plants that purify water environments

Mycobiology 2015 September, **43**(3): 231-238
http://dx.doi.org/10.5941/MYCO.2015.43.3.231
pISSN 1229-8093 • eISSN 2092-9323

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Received May 27, 2015
Revised June 18, 2015
Accepted August 31, 2015

can be identified by this study.

MATERIALS AND METHODS

Sampling and isolation of endophytic fungi. A total of 4 representative aquatic plants, *Eleocharis kuroguwai* Ohwi (belonging to the order Cyperales), *Hydrocharis dubia* Backer (belonging to the order Psammophytes), *Salvinia natans* All. (a pteridophyte belonging to the order Polypodiales), and *Zizania latifolia* Turcz. (a gramineous plant, belonging to the order Braminales) are native to the Daepyeong and Jilnal wetlands. These species commonly live at the edge of water or float at the surface of freshwater (Table 1). Sampled plants (16 individuals per each species) were harvested along with freshwater from their habitats to minimize physiological changes. Sterile distilled water (SDW) and sterilized 0.1% Tween 80 solution (Sigma-Aldrich, St. Louis, MO, USA) was sprayed on the surface of samples to eliminate suspended solids or normal microflora on the plant surfaces. The plants were submerged in 1.0% perchloric acid ($HClO_4$) 2 times for 10 min each, and were subsequently washed with SDW 3~4 times. Residual water was eliminated with dried, sterile gauze and 50 pieces of root from a plant sample was cut to a length of 3~4 cm. Pre-treated samples were loaded into Hagem minimal medium containing 80 ppm of streptomycin (Sigma-Aldrich) to exclude root bacteria, and incubated at 25°C for 15 days [11]. Sub-culturing of endophytic fungi for pure isolation was performed with the same media and conditions. Finally, pure isolates were incubated on potato dextrose agar (Difco, Detroit, MI, USA) and selected based on morphological differences.

Extraction of genomic DNA and polymerase chain reaction (PCR). All endophytic fungi from the 4 aquatic host plants were inoculated into potato dextrose broth (Difco) media and incubated at 25°C for 7 days with 120 rpm using a rotary shaker. Filtered mycobionts were lyophilized for 2 days. The DNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA) was used for the extraction of genomic DNA from lyophilized mycobionts and primers targeting the ITS regions, ITS1 and ITS4 were used for amplification [12].

The PCR conditions were pre-denaturation (94°C, 4 min), denaturation (94°C, 1 min), annealing (55~58°C, 1 min), and extension (72°C, 2 min) for a total of 35 cycles, followed by a final extension (72°C, 2 min).

The PCR products were confirmed by electrophoresis (1.5% agarose gel, stained with ethidium bromide) and observation of the resulting band pattern under a UV transilluminator. The AccuPrep PCR & Gel Extraction Kit (Bioneer, Daejeon, Korea) was used for the purification of PCR products, and an ABI 3730XL DNA analyzer (Applied Biosystems, Carlsbad, CA, USA) was used for the sequencing of ITS regions [10].

Phylogenetic analysis and examination of the diversity of endophytic fungi. The ITS region sequences of endophytic fungi were compared with sequences of other fungal species showing similarity over 99%, as determined by analyzing data from the GenBank databases of National Center for Biotechnology Information (NCBI). Phylogenetic relationships were analyzed using the MEGA program ver. 6.0 with an alignment of sequences that was prepared using ClustalW software [13]. The phylogenetic trees were inferred with the neighbor-joining algorithm with the Kimura 2-parameter. The stability of relationships was evaluated by a bootstrap analysis with a resampling of 1,000 times [13]. The diversity of the endophytic fungi from each sampling site was analyzed and compared. Diversity at the genus level was revealed using the Margalef's richness index (Dmg) [14] and Mehinick's index (Dmn) [15].

RESULTS AND DISCUSSION

Isolation of endophytic fungi and phylogenetic analysis. Plant community of *E. kuroguwai* and *H. dubia*, around the Daepyeong and Jilnal wetlands, had floral axes of about 70~80 cm. *S. natans* had a floral axis of about 70 cm in length, and *Z. latifolia* has an axis of about 80~90 cm with a leaf width of 3~4 cm. Sampling information of aquatic plants is presented in Table 1.

Based on the phylogenetic analysis, we found the following fungal genera represented in the Daepyeong wetland. A total of 8 strains isolated from *E. kuroguwai* belonged to the genera *Cladosporium*, *Clonostachys*, *Fusarium*, *Leptosphaeria*,

Table 1. Information of aquatic plants collected from the wetlands

Sampling region	Scientific name of plants	Plant code	No. of isolates	Geographical position	GPS information
Daepyeong wetland	*Eleocharis kuroguwai* Ohwi	EK	8	Daesong-ri, Haman-gun	35°20′23.82″ N, 128°20′7.25″ E
	Hydrocharis dubia Backer	HD	11		35°20′23.40″ N, 128°20′9.57″ E
	Salvinia natans All.	SN	8		35°20′23.64″ N, 128°20′10.92″ E
	Zizania latifolia Turcz.	ZL	11		35°20′23.65″ N, 128°20′11.03″ E
Jilnal wetland	*Eleocharis kuroguwai* Ohwi	EK	6	Ugeo-ri, Haman-gun	35°19′16.82″ N, 128°20′55.79″ E
	Hydrocharis dubia Backer	HD	4		35°19′17.00″ N, 128°20′56.80″ E
	Salvinia natans All.	SN	9		35°19′16.85″ N, 128°20′56.73″ E
	Zizania latifolia Turcz.	ZL	8		35°19′16.88″ N, 128°20′55.86″ E

EK, *Eleocharis kuroguwai* Ohwi; HD, *Hydrocharis dubia* Backer; SN, *Salvinia natans* All.; ZL, *Zizania latifolia* Turcz.

Table 2. Endophytic fungi isolated from aquatic plants in the Daepyeong wetland

Plant code	Fungal isolates	NCBI blast search	Similarity (%)	Accession No.
EK	R1EK01	*Clonostachys rogersoniana* (KC806290)	99	KR091772
	R1EK02	*Fusarium graminearum* (KP689197)	99	KR091773
	R1EK03	*Cladosporium cladosporioides* (KM877468)	100	KR091774
	R1EK04	*Pestalotiopsis mangiferae* (KM510402)	100	KR091775
	R1EK05	*Fusarium circinatum* (NR_120263)	100	KR091776
	R1EK06	*Leptosphaeria* sp. (JX076952)	100	KR091777
	R1EK07	*Cladosporium tenuissimum* (KM357322)	100	KR091778
	R1EK08	*Plectosphaerella cucumerina* (JQ796755)	99	KR091779
HD	R1HD01	*Talaromyces funiculosus* (KM012003)	99	KR091780
	R1HD02	*Mucor circinelloides* f. *lusitanicus* (NR_126127)	100	KR091781
	R1HD03	*Cladosporium cladosporioides* (KP689250)	100	KR091782
	R1HD04	*Fusarium graminearum* (KP689197)	100	KR091783
	R1HD05	*Trichoderma harzianum* (KM079608)	99	KR091784
	R1HD06	*Myxotrichum deflexum* (KC460884)	99	KR091785
	R1HD07	*Fusarium verticillioides* (KM396284)	100	KR091786
	R1HD08	*Fusarium succisae* (KF889112)	100	KR091787
	R1HD09	*Talaromyces pinophilus* (KM100863)	100	KR091788
	R1HD10	*Pestalotiopsis mangiferae* (JX305692)	99	KR091789
	R1HD11	*Penicillium westlingii* (JN617668)	99	KR091790
SN	R1SN01	*Cladosporium cladosporioides* (KP689250)	100	KR091791
	R1SN02	*Pseudocercosporella fraxini* (GU214682)	100	KR091792
	R1SN03	*Diaporhte* sp. (KC763095)	99	KR091793
	R1SN04	*Aspergillus lentulus* (EF669970)	99	KR091794
	R1SN05	*Plectosphaerella cucumerina* (JX431888)	99	KR091795
	R1SN06	*Pestalotiopsis mangiferae* (KM510402)	100	KR091796
	R1SN07	*Fusarium chlamydosporum* (KP186124)	100	KR091797
	R1SN08	*Fusarium equiseti* (KJ371094)	100	KR091798
ZL	R1ZL01	*Talaromyces flavus* (JQ768266)	99	KR091799
	R1ZL02	*Talaromyces* sp. (KF741984)	99	KR091800
	R1ZL03	*Fusarium verticillioides* (KM396284)	100	KR091801
	R1ZL04	*Penicillium janthinellum* (KF906546)	99	KR091802
	R1ZL05	*Talaromyces flavus* (JN624905)	99	KR091803
	R1ZL06	*Leptosphaeria* sp. (JN618369)	99	KR091804
	R1ZL07	F*usarium equiseti* (KJ412506)	100	KR091805
	R1ZL08	*Alternaria tenuissima* (KP171633)	100	KR091806
	R1ZL09	*Penicillium* sp. (GU446637)	100	KR091807
	R1ZL10	*Leptosphaeria* sp. (HQ658112)	99	KR091808
	R1ZL11	*Acremonium cellulolyticus* (JN624892)	100	KR091809

EK, *Eleocharis kuroguwai* Ohwi; HD, *Hydrocharis dubia* Backer; SN, *Salvinia natans* All.; ZL, *Zizania latifolia* Turcz.

Pestalotiopsis, and *Plectosphaerella*. A total of 11 strains from *H. dubia* belonged to genera *Cladosporium*, *Fusarium*, *Mucor*, *Myxotrichum*, *Penicillium*, *Pestalotiopsis*, *Talaromyces*, and *Trichoderma*. A total of 8 strains from *S. natans* belonged to 7 genera, including *Aspergillus*, *Cladosporium*, *Diaporhte*, *Fusarium*, *Pestalotiopsis*, *Plectosphaerella*, and *Pseudocercosporella*. Finally, 11 strains from *Z. latifolia* belonged to 6 genera, including *Acremonium*, *Alternaria*, *Fusarium*, *Leptosphaeria*, *Penicillium*, and *Talaromyces* (Table 2).

Similarly, we found the following fungal genera represented in the Jilnal wetland. A total of 6 strains isolated from *E. kuroguwai* belonged to 4 genera, including *Clonostachys*, *Leptosphaeria*, *Massarina*, and *Penicillium*, and 4 strains from *H. dubia* belonged to 3 genera, including *Aspergillus*, *Fusarium*, and *Leptosphaeria*. A total of 9 strains from *S. natans* belonged to 8 genera, including *Cladosporium*, *Fusarium*, *Gibberella*, *Leptosphaeria*, *Paraphaeosphaeria*, *Phoma*, *Sarocladium*, and *Talaromyces*, and 8 strains from *Z. latifolia* belonged to 6 genera, including *Acephala*, *Cephalosporium*, *Clohesyomyces*, *Fusarium*, *Talaromyces*, and *Zalerion* (Table 3).

The fungi sampled varied by wetland. A total 38 fungal strains from 16 genera were isolated from the Daepyeong wetland, while 27 strains from 16 genera were isolated from the Jilnal wetland. The 65 total strains from the two wetlands belonged to 25 genera. Common isolates from both the Daepyeong and the Jilnal wetlands were identified as belonging to the species *Aspergillus*, *Cladosporium*, *Clonostachys*, *Fusarium*, *Leptosphaeria*, *Penicillium*, and *Talaromyces*. On the other hand, there were 9 unique genera from the Daepyeong wetland, including *Acremonium*, *Alternaria*, *Diaporthe*, *Mucor*, *Myxotrichum*, *Pestalotiopsis*,

Table 3. Endophytic fungi isolated from aquatic plants in Jilnal wetland

Plant code	Fungal isolates	NCBI blast search	Similarity (%)	Accession No.
EK	R2EK01	*Penicillium pinophilum* (EU910587)	100	KR091810
	R2EK02	*Massarina* sp. (JX076953)	99	KR091811
	R2EK03	*Leptosphaeria* sp. (HQ658112)	99	KR091812
	R2EK04	*Clonostachys rogersoniana* (KC806287)	100	KR091813
	R2EK05	*Penicillium glabrum* (JX140796)	100	KR091814
	R2EK06	*Leptosphaeria* sp. (JX076952)	99	KR091815
HD	R2HD01	*Fusarium verticillioides* (KF624791)	100	KR091816
	R2HD02	*Aspergillus japonicus* (KF031031)	100	KR091817
	R2HD03	*Leptosphaeria* sp. (HQ658112)	99	KR091818
	R2HD04	*Aspergillus lentulus* (EF669970)	99	KR091819
SN	R2SN01	*Talaromyces helicus* (AF033396)	98	KR091820
	R2SN02	*Fusarium incarnatum* (KF255427)	100	KR091821
	R2SN03	*Cladosporium tenuissimum* (KP689183)	100	KR091822
	R2SN04	*Paraphaeosphaeria verruculosa* (JX496080)	99	KR091823
	R2SN05	*Gibberella zeae* (DQ459832)	100	KR091824
	R2SN06	*Cladosporium cladosporioides* (KM877468)	100	KR091825
	R2SN07	*Leptosphaeria microscopica* (FN386274)	99	KR091826
	R2SN08	*Phoma* sp. (KF852596)	99	KR091827
	R2SN09	*Sarocladium strictum* (GQ376096)	100	KR091828
ZL	R2ZL01	*Zalerion varium* (AF169303)	100	KR091829
	R2ZL02	*Clohesyomyces aquaticus* (KM589855)	96	KR091830
	R2ZL03	*Acephala* sp. (HG530746)	99	KR091831
	R2ZL04	*Cephalosporium* sp. (KF367533)	98	KR091832
	R2ZL05	*Fusarium circinatum* (KC464621)	100	KR091833
	R2ZL06	*Talaromyces flavus* (HQ191279)	100	KR091834
	R2ZL07	*Fusarium kyushuense* (AF414971)	98	KR091835
	R2ZL08	*Fusarium graminearum* (KM513614)	100	KR091836

EK, *Eleocharis kuroguwai* Ohwi; HD, *Hydrocharis dubia* Backer; SN, *Salvinia natans* All.; ZL, *Zizania latifolia* Turcz.

Plectosphaerella, *Pseudocercosporella*, and *Trichoderma*. In contrast, there were 8 unique genera from the Jilnal wetland, including *Acephala*, *Cephalosporium*, *Clohesyomyces*, *Gibberella*, *Massarina*, *Paraphaeosphaeria*, *Phoma*, *Sarocladium*, and *Zalerion* (Tables 2 and 3).

The ITS sequences of endophytic fungal strains from each wetland were registered into the GenBank database of NCBI, including isolates of *E. kuroguwai* (KR091772~KR091779), *H. dubia* (KR091780~KR091790), *S. natans* (KR091791~KR091798), and *Z. latifolia* (KR091799~KR091809) from the Daepyeong wetland, and isolates of *E. kuroguwai* (KR091810~KR091815), *H. dubia* (KR091816~KR091819), *S. natans* (KR091820~KR091828), and *Z. latifolia* (KR091829~KR091836) to the Jilnal wetland. Phylogenetic trees of endophytic fungi isolated from the roots of aquatic plants native to the each wetland were constructed (Fig. 1A and 1B).

Diversity of endophytic fungi. The richness of fungal isolates from the Daepyeong and Jilnal wetlands was analyzed at the genus level using Mehinick's index (Dmn) and Margalef's richness index (Dmg). In terms of generic richness calculated by Margalef's index, the fungal biota from each aquatic plant was as follows: *E. kuroguwai* (2.404, 1.674), *H. dubia* (2.919, 1.443), *S. natans* (2.885, 3.186), and *Z. latifolia* (2.085, 2.404). Using Mehinick's index, the generic richness was calculated as follows: *E. kuroguwai* (2.121, 1.633), *H. dubia* (2.412, 1.500), *S. natans* (2.475, 2.667), and *Z. latifolia* (1.809, 2.121) (Table 4).

E. kuroguwai and *H. dubia* from the Daepyeong wetland showed higher diversity values than those from the Jilnal wetland. In contrast, *S. natans* and *Z. latifolia* from the Jilnal wetland showed higher values than those from the Daepyeong wetland. The high values of fungal diversity from *E. kuroguwai* and *H. dubia* in the Daepyeong wetland may be due to the greater number of isolates or variety of confirmed genus than from the Jilnal wetland. Similarly, the high values of fungal diversity from *S. natans* and *Z. latifolia* in the Jilnal wetland may be due to the greater number of confirmed genera than from the Daepyeong wetland. Mehinick's index is similar, conceptually, to Margalef's richness index for analyzing species richness. The deduced diversity values from each of the two indices showed similar patterns in this study. Because of the endophyte sample size, Shannon's diversity index (*H'*) [16] and Simpson's diversity index (D) [17] were not used.

Phylogenetic and diversity analyses of the endophytic fungi were conducted. Isolated fungi were found to belonging to 7 genera, and *Aspergillus*, *Cladosporium*, *Fusarium*, and *Penicillium* were commonly isolated. Some fungal species belonging to the genus *Fusarium* or *Leptosphaeria* have been revealed as plant pathogens, but the genus *Clonostachys* has

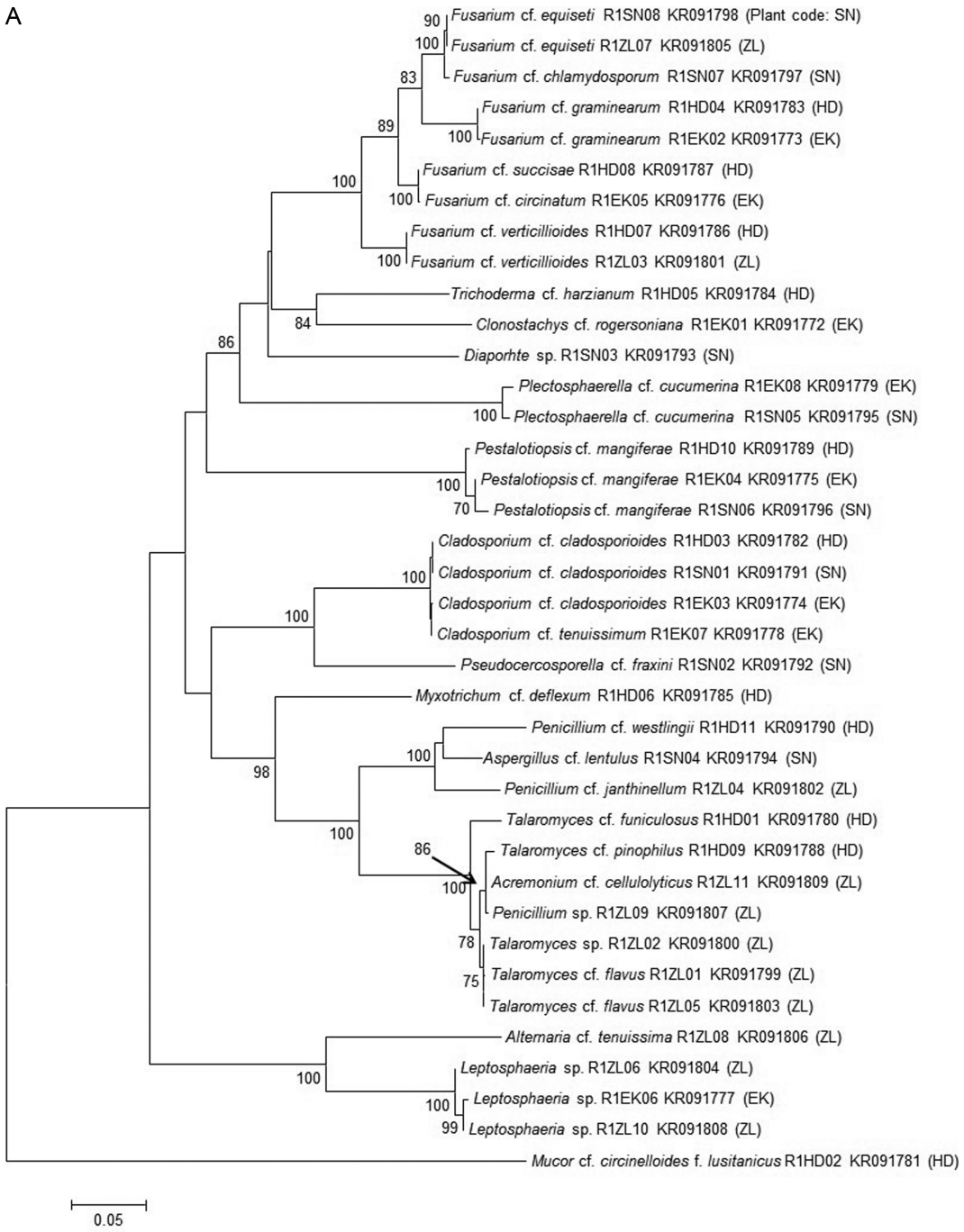

Fig. 1. Phylogenetic analysis of endophytic fungi isolated from the aquatic plants in Daepyeong and Jilnal wetlands. This phylogenetic tree was constructed by using the neighbor-joining method (1,000 bootstrap replications). Bootstrap values (70%) are indicated at relevant nodes. Dendrogram of endophytic fungi isolated from the aquatic plants in Daepyeong (A) and Jilnal wetlands (B).

not been studied well. Other genera have been isolated from diverse environments. Each strain belonging to the 9 genera that can be differentiated by the source wetland is thought that result from the unique environment features of the two habitats. This result indicates that fungal biota of host plants can be differentiated by habitat location, even if they are from the same plant species, and that this could be a result of adaptation to their unique environments. Hydroecological endophytes are well known for producing effective metabolites in their host plants [2, 18]. These positive roles might be applied to aquatic plants to the purpose of effective water purifying [1, 7]. First, endophyte diversity from water purifying aquatic plants native to freshwater marshes must be secured prior to this tactic [9].

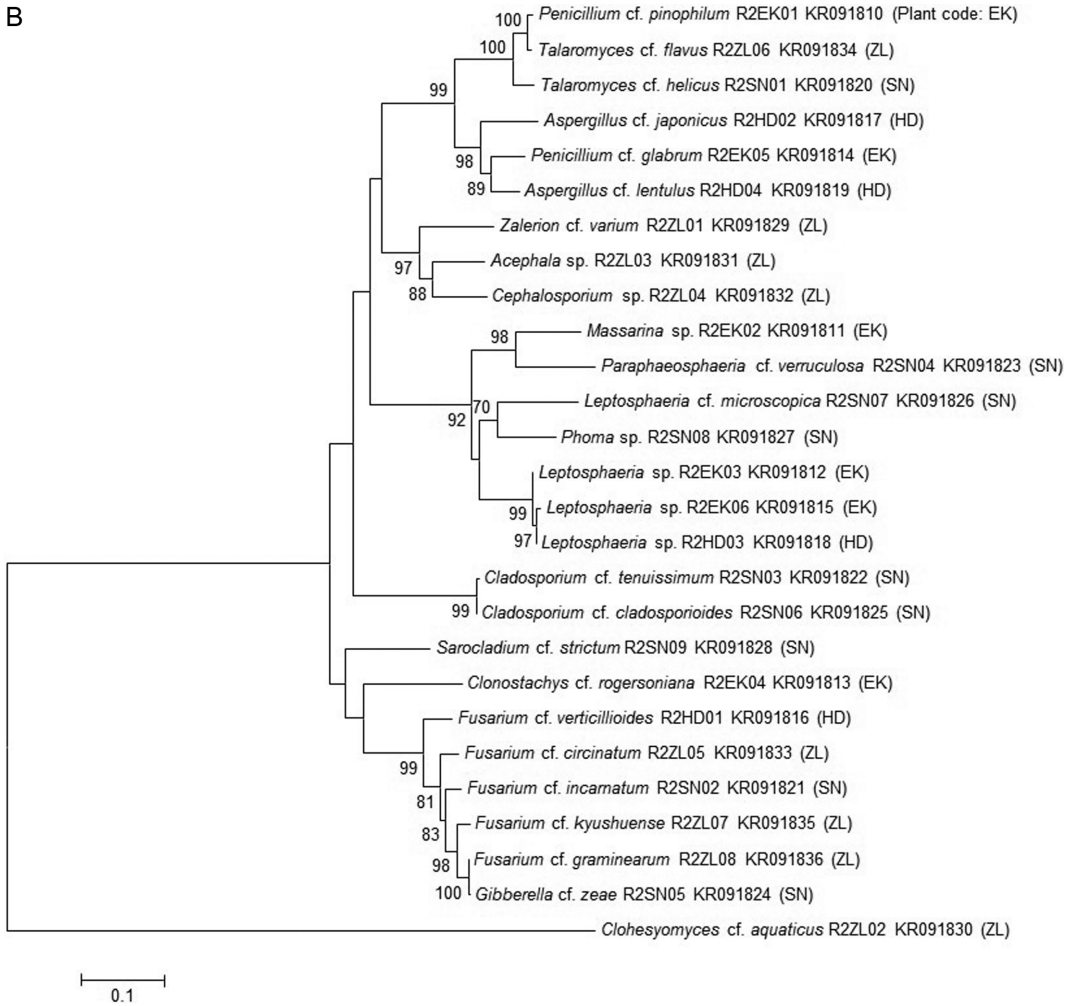

Fig. 1. Continued.

However, these researches have not been vigorously conducted. This study provides endophytic diversity as basic data from these wetland environments and on aquatic plant-endophyte interactions that will be valuable for further study.

Inland wetlands suffer from water stress that is very unfavorable to mesophytic growth [18]. For this reason, aquatic plants adapted to unique environmental features dominate. Differences in the thicknesses of a root epidermis or root cap between each aquatic plants species can lead to distinctive types of fungal biota. Furthermore, these plants have evolved with independent morphological characteristics, even if the species diversity of aquatic plants is less than that of mesophytic plants [18]. Therefore, microbial distribution and diversity may differ from one another [11]. Up to now, endosymbiotic microorganisms of aquatic plants have not been studied well, so this study can serve as a starting point for further research.

The 4 aquatic plants used in this study can be categorized by taxonomical criteria. All plants are tracheophytes. *E. kuroguwai*, *H. dubia*, and *Z. latifolia* form flowers and fruit and are perennial plants. In contrast, *S. natans*, a pteridophyte, is an annual plant that does not form flowers and fruit and that thrives by sporulation [2, 18]. Generally, pteridophytes are more primeval type of vascular plants than others, and they hold a key position in the evolution of vascular plants [19]. Pteridophytes adapted to the drastic environmental changes of primitive ages, and sporulation allows for an explosion of the population under favorable conditions [2, 18]. *S. natans* can eliminate nitrogen and phosphorus from eutrophied water, and a very effective due to their strong propagation ability. Because of this, *S. natans* is a highly valued bio-resource that can remediate contaminated natural environments. Therefore, research on how endophytes interact with *S. natans* has become an important field [19]. However, *S. natans* can also cover the surface of a wetland with blooms and decrease the dissolved oxygen concentration in the water [6, 18]. In conclusion, endophytes may play a major role in growth modulation of *S. natans*, an outstanding eutrophication controller. However, the interaction between the water purifying *S. natans* and their endophytes has not been studied well. In this study, *S.*

Table 4. Diversity index of endophytic fungi isolated from the aquatic plants in wetlands

Fungal taxnomic	Daepyeong wetland				Jilnal wetland			
	EK[a]	HD[a]	SN[a]	ZL[a]	EK[b]	HD[b]	SN[b]	ZL[b]
Acephala	-	-	-	-	-	-	-	1
Acremonium	-	-	-	1	-	-	-	-
Alternaria	-	-	-	1	-	-	-	-
Aspergillus	-	-	1	-	-	2	-	-
Cephalosporium	-	-	-	-	-	-	-	1
Cladosporium	2	1	1	-	-	-	2	-
Clohesyomyces	-	-	-	-	-	-	-	1
Clonostachys	1	-	-	-	1	-	-	-
Diaporhte	-	-	1	-	-	-	-	-
Fusarium	2	3	2	2	-	1	1	3
Gibberella	-	-	-	-	-	-	1	-
Leptosphaeria	1	-	-	2	2	1	1	-
Massarina	-	-	-	-	1	-	-	-
Mucor	-	1	-	-	-	-	-	-
Myxotrichum	-	1	-	-	-	-	-	-
Paraphaeosphaeria	-	-	-	-	-	-	1	-
Penicillium	-	1	-	2	2	-	-	-
Pestalotiopsis	1	1	1	-	-	-	-	-
Phoma	-	-	-	-	-	-	1	-
Plectosphaerella	1	-	1	-	-	-	-	-
Pseudocercosporella	-	-	1	-	-	-	-	-
Sarocladium	-	-	-	-	-	-	1	-
Talaromyces	-	2	-	3	-	-	1	1
Trichoderma	-	1	-	-	-	-	-	-
Zalerion	-	-	-	-	-	-	-	1
Margalef's index (Dmg)	2.404	2.919	2.885	2.085	1.674	1.443	3.186	2.404
Mehinick's index (Dmn)	2.121	2.412	2.475	1.809	1.633	1.500	2.667	2.121

EK, *Eleocharis kuroguwai* Ohwi; HD, *Hydrocharis dubia* Backer; SN, *Salvinia natans* All.; ZL, *Zizania latifolia* Turcz.
[a]Aquatic plants code collected from the Daepyeong wetland.
[b]Aquatic plants code collected from the Jilnal wetland.

natans showed the highest fungal diversity value of the plants surveyed (Table 4). This may have resulted from a prolonged period of endosymbiosis with pteridophytes that appeared at an early stage of tracheophyte evolution. Researches that screen the biological activity (ISR, plang growth promoting) from endophytes secured in this study and application of promising endophytes to *S. natans* to promote (control) their growth or increase the efficiency of water purification activity in eutrophied water, have to be done. This study compared the distribution of endophytic fungi from aquatic plants native to two representative wetlands in Korea for the purpose of discovering diverse and effective microorganisms. This study provides basic information about microbial resources of wetland aquatic plants.

ACKNOWLEDGEMENTS

This work was carried out with the support of "Research Program for Agriculture Science & Technology Development (Project title: Diagnosis of horticultural and herbal crops diseases and insect pests, Project No. PJ01136802)" Rural Development Administration, Republic of Korea.

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