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RESEARCH ARTICLE

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Fungal Community Analyses of Endophytic Fungi from Two Oak Species, *Quercus mongolica* and *Quercus serrata*, in Korea

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ABSTRACT

Fungal endophytes have been recorded in various plant species with a richness of diversity, and their presence plays an essential role in host plant protection against biotic and abiotic stresses. This study applied the Illumina MiSeq sequencing platform based on the amplification of fungal ribosomal ITS2 region to analyze fungal endophytic communities of two oak species (*Quercus mongolica* and *Q. serrata*) with different oak wilt disease susceptibilities in Korea. The results showed a total of 230,768 sequencing reads were obtained and clustered at a 97% similarity threshold into 709 operational taxonomic units (OTUs). The OTUs of *Q. serrata* were higher than that of *Q. mongolica* with the number of 617 OTUs and 512 OTUs, respectively. Shannon index also showed that *Q. serrata* had a significantly higher level of fungal diversity than *Q. mongolica*. Total of OTUs were assigned into 5 fungal phyla, 17 classes, 60 orders, 133 families, 195 genera, and 280 species. Ascomycota was the dominant phylum with 75.11% relative abundance, followed by Basidiomycota with 5.28%. *Leptosillia, Aureobasidium* and *Acanthostigma* were the most abundant genera detected in *Q. serrata* with the average relative abundance of 2.85, 2.76, and 2.19%, respectively. On the other hand, *Peltaster*, *Cladosporium* and *Monochaetia* were the most common genera detected in *Q. mongolica* with the average relative abundance of 4.83, 3.03, and 2.87%, respectively. Our results indicated that fungal endophytic communities were significantly different between two oak species and these differences could influence responses of host trees to oak wilt disease caused by *Raffaelea quercus-mongolicae*.

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1. Introduction

Endophytes are microorganisms that colonize internal living tissues of plant, but without causing any immediate over adverse effects or during a part of their life cycle reside inside plant tissue of host trees without doing substantive harm [1,2]. Endophytes may include bacterial and fungal species or others belong to actinomycetes and mycoplasma [1]. In addition, bioactive compounds produced by endophytes play an important role in fitness enhancements for host plants [1,3]. On the other hand, endophytes can improve the ability of plants' nutrient uptake and protect host plants against biotic and abiotic stresses [3,4]. Fungal endophytes have been used to extract antimicrobial compounds and produce antibiotic drugs [1]. Endophytic fungi have been recorded in various plant species with a richness of diversity [1,2]. The most common endophytic fungi that have been identified belong to Ascomycota, followed by Basidiomycota and other fungi [1,2].

It is well known that metagenomics based on next-generation sequencing (NGS) technologies helps scientists to discover and understand the diversity, function, and evolution of the uncultivated microbiology of diverse environments or habitats [5]. Metagenomics is considered as an optimal tool to discover the nucleic acids from uncultivated microbes in different environments [5,6]. Metagenomics is defined as a technical chain rooted in genomics, microbial genetics, microbial ecology, and bioinformatics to analyze directly genomes of microbial community sampled from their natural habitats [5]. It is also applied to recover target genes through sequencing to determine not only the microbial diversity but also their roles in environmental samples [5–7]. Among the NGS technologies, Pyrosequencing (Roche 454) and Solexa

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Supplemental data for this article can be accessed here.

(Illumina) sequencing systems have been extensively used to explore microbial communities in different environments [5]. For instance, results of 454 sequencing indicated that fungal communities in the phyllosphere of *Quercus macrocarpa* were significantly different either between urban and nonurban environments or between different seasons [8,9]. Differences in bacterial and fungal endophytic communities between *Acer campester* and *A. platanoides* leaves were deciphered by using Illumina MiSeq sequencing [10]. Fungal endophytic communities in needles of *Pinus sylvestris* were also analyzed by using Illumina MiSeq sequencing, and the study showed the most abundant fungi was assigned to phylum Ascomycota with 91.2% of the samples [11]. In addition to the two above-mentioned, the Ion Torrent Personal Genome Machine (PGM) sequencing was also applied to analyze endophytic fungal communities in *Eucalyptus grandis* [12] or investigate microbial community dynamic in liquid waste [13].

Quite a few studies about endophytic fungi in Korea have been studied in recent years [14–19]. For instance, *Alternaria* spp., *Cladosporium* spp., and *Penecillium* spp. were the dominant endophytic fungi in several medicinal plants [14]. Species richness and diversity indices of endophytic fungi were different among three Halophytes, namely *Sedum oryzifolium*, *Lysimachia mauritiana*, and *Aster spathulifolius* [17]. The most common genera of endophytic fungi isolated from *Pinus thunbergii* were identified as *Fusarium,* *Penicillium,* and *Trichoderma* [16], while *Lophodermium conigenum* and *Annulohypoxylon turcatum* were dominant in *Pinus densiflora* and *Juniperus rigida*, respectively [18]. However, endophytic fungi obtained from *P. densiflora* and *J. rigida* were influenced by host plants' distribution and had a lower diversity index than that of *Larix kaempferi* [15]. Studies on endophytic fungi of other coniferous trees, namely *Cryptomeria japonica*, *Pinus koraiensis*, *Pinus rigida*, etc. have been also reported, with a total of about 80 taxa belonged to 52 genera [19].

Applying metagenomics to analyze microbial community from various environments was also conducted in Korea [20–26]. Of which, NGS platform of Roche 454 was applied to identify airborne fungal community in Seoul [21], while Illumina MiSeq platform was performed to compare soil higher fungal communities associated with dead and living Korean fir (*Abies koreana*) in Jeju island [26]. However, using metagenomics to analyze endophytic fungal communities in forest trees is still limited. *Quercus serrata* was known as the most susceptible oak species to the oak wilt fungus *Raffaelea quercivora* in Japan [27], but *Quercus mongolica* is highly susceptible to the oak wilt fungus *Raffaelea quercus-mongolicae*, and *Q. serrata* is relatively resistant to this pathogen in Korea [28]. The difference in susceptibility of these two oak species to *R. quercus-mongolicae* could be partially due to differences in their endophytic fungal communities. Hence, our objectives in the present study were to (i) compare species richness and diversity of endophytic fungi between *Q. mongolica* and *Q. serrata* and to (ii) characterize taxonomic structures of endophytic fungi that differ between two oak species through Illumina MiSeq sequencing platform.

2. Materials and methods

2.1. Sampling collections

Samples were collected in triplicate from different organs of healthy trees (leaf, petiole, twig, branch, stem, and root) of two oak species (*Q. mongolica* and *Q. serrata*) in Chuncheon city, Gangwon province, Korea (37°52'12" N, 127°45'58" E) in September 2019. Leaf, petiole, twig, branch, and root samples were collected by pruning shears, while stem samples were collected by increment borer at breast height position on the trees. All samples were then kept in plastic bags with coolers to bring to the laboratory. The root samples were gently washed with tap water to remove soil. Surface disinfection of samples was performed with solutions including 2.0% NaOCl for 3 min, followed by 70% ethanol for 1 min. Then, samples were washed with sterilized water for 15 sec in three times. Finally, all samples were dried using sterilized tissue paper, cut in 0.5×0.5 cm size (leaf), with 0.5 cm length (other tissues), and stored at −86 °C until DNA extraction.

2.2. DNA extraction

Samples were shredded and then grinded using Tube Mill Control (IKA-Werke GmbH & CO. KG, Staufen, Germany) with speed 5000 rpm for 3 min. The DNA was purified by using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, Irvine, CA, USA), following instructions of the manufacturer. The final 20 µl pure DNA eluted from each sample. To prepare for amplification, pure DNA from each sample of three replications was pooled and a total of 12 DNA samples (6 samples for each oak species) was stored at −20 °C until amplification.

2.3. ITS amplification and DNA library construction

The quantification and quality of extracted DNA were determined using Quant-IT PicoGreen

Table 1. Summary of genome assembly of two *Quercus* species by FLASH program per sample.

Samples	Plant species	Tissue	Total bases (raw PE)	Read count (raw tags)	N[a] (%)	GC[b] (%)	Q20[c] (%)	Q30[d] (%)
ML.01	*Q. mongolica*	Leaf	54,126,929	138,342	0	60.96	99.09	95.65
MP.02	*Q. mongolica*	Petiole	41,461,653	106,203	0	60.79	99.09	95.66
MT.03	*Q. mongolica*	Twig	45,367,451	121,913	0	58.14	99.19	96.06
MB.04	*Q. mongolica*	Branch	52,175,663	134,759	0	60.67	99.15	95.90
MS.05	*Q. mongolica*	Stem	48,018,514	123,822	0	59.65	99.05	95.49
MR.06	*Q. mongolica*	Root	31,056,235	79,747	0	59.44	99.00	95.35
SL.07	*Q. serrata*	Leaf	47,729,808	121,134	0	61.53	99.05	95.37
SP.08	*Q. serrata*	Petiole	39,921,750	110,715	0	54.54	99.30	96.63
ST.09	*Q. serrata*	Twig	45,102,554	118,695	0	59.18	99.12	95.68
SB.10	*Q. serrata*	Branch	42,153,555	117,148	0	56.64	99.24	96.29
SS.11	*Q. serrata*	Stem	43,534,842	115,772	0	58.31	99.15	95.97
SR.12	*Q. serrata*	Root	53,601,813	136,099	0	61.45	99.01	95.18

[a]The percentage of ambiguous bases (N) in sequence reads.
[b]The GC content percentage in sequence reads.
[c]The percentage of bases in which the phred quality score (Q score) is above 20.
[d]The percentage of bases in which Q score is above 30.

(Invitrogen, Molecular Probes, Eugene, OR, USA). After performing quality control (QC), qualified samples proceed to library construction. The sequencing libraries are prepared according to the Illumina fungal metagenomic sequencing demonstrated protocol to amplify the fungal ribosomal internal transcribed spacer second (ITS2) region [29]. The universal primer pair with Illumina overhang adapter sequences used for amplicon PCR were as follows: ITS-3F (5′-GCATCGATGAAGAACGCAGC-3′) and ITS-4R (5′-TCCTCCGCTTATTGATATGC-3′). The input gDNA of each sample (5 ng/µl) was amplified with 2x KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Inc., Wilmington, MA, USA) and 500 nM each of the universal forward/reverse PCR primer. The cycle conditions for the first PCR were 3 min at 95 °C for heat activation, and 25 cycles of denaturation for 30 sec at 95 °C, annealing for 30 sec at 55 °C and extension for 30 sec at 72 °C, followed by a 5 min final extension at 72 °C. The first PCR products were analyzed on Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA) using Agilent DNA 1000 Kit (Agilent Technologies, Waldbronn, Germany) and purified then with the AMPure XP beads (Beckman Coulter, Indianapolis, IN, USA). The procedure of analysis and cleanup for the first PCR products were conducted following the manufacturer's instructions. The 5 µl of the first PCR product was used in dual-indexed PCR amplification for final library construction using the Nextera XT Index Kit v2 (Illumina, San Diego, CA, USA). The cycle conditions for the second PCR were same as the first PCR conditions, but only 8 cycles were performed. The AMPure XP beads (Beckman Coulter) were used to clean up the final library before quantification. The library was quantified using a Bioanalyzer DNA 1000 chip (Agilent Technologies, Waldbronn, Germany). The amplicon libraries were then normalized and pooled at equimolar concentrations before sequencing. The paired-end (v3, 2×300 bp) sequencing was performed by the Macrogen Inc. using the Illumina MiSeq platform (Seoul, Korea).

2.4. Sequencing processing and taxonomical assignment

Reads from paired-end sequences were merged using FLASH (Fast Length Adjustment of SHort reads) program [30] with default parameters. Preprocessing and clustering were conducted using CD-HIT-OTU-MiSeq program [31,32] with steps as follows: short reads were filtered out and trimmed to remove bases with a Phred quality score less than 20; filtered reads were then clustered at 100% identify using CD-HIT-DUP to remove duplicates and identify chimeric reads; secondary clusters were recruited into primary clusters; chimeric reads and small clusters with below 140 bp were removed. The remaining reads from nonchimeric clusters were clustered using a greedy algorithm into Operational Taxonomic Unit (OTUs) at a 97% similarity threshold. Representative sequences from each OTU were used to assign taxonomy using UCLUST classifier in QIIME [33] against the UNITE database [34]. Unclassified OTUs and nonfungal OTUs were removed using the UNITE + INSD Fungi Version 8.2 (Feb 2020) to generate only fungal OTU table and taxonomy.

2.5. Statistical analysis and computations

Alpha-diversity indices of fungal community such as observed OTUs, Chao1, Shannon index, and so on were calculated using the alpha_diversity.py and alpha_rarefaction.py, while the beta-diversity distance matrix was computed based on UniFrac analysis, and weighted UniFrac distances were used to construct principal coordinates analysis (PCoA) plots using make_2d_plots.py workflow in QIIME to visualize similarities in the fungal community composition. Differences in alpha diversity between two oak species were compared by the independent

Table 2. Summary of community richness and alpha diversity per sample.

Samples	Sequence reads (clean tags)	OTUs[e]	Chao1[f]	Shannon[g]	Inverse Simpson[h]	Good's coverage[k]
ML.01	7,486	170	205.36	4.70	0.94	0.9940
MP.02	6,694	173	222.04	4.47	0.88	0.9928
MT.03	33,852	272	310.61	4.06	0.83	0.9986
MB.04	11,306	161	184.38	3.33	0.74	0.9970
MS.05	23,184	193	228.65	2.65	0.54	0.9982
MR.06	8,112	98	104.88	4.42	0.92	0.9986
SL.07	2,885	181	224.38	5.73	0.97	0.9834
SP.08	49,059	390	428.08	5.76	0.96	0.9989
ST.09	23,072	320	352.81	5.58	0.94	0.9977
SB.10	39,766	325	353.00	5.13	0.94	0.9988
SS.11	21,321	226	254.50	5.32	0.95	0.9982
SR.12	4,031	92	108.50	4.29	0.90	0.9945

[e]Operational Taxonomic Units.
[f]The Chao1 richness estimate for an OTU definition.
[g]The Shannon index takes into account the number and evenness of species.
[h]The Inverse Simpson index represents the probability that two randomly selected individuals in the habitat will belong to the same species.
[k]Coverage is calculated as $C = 1\text{-}(s/n)$, where s is the number of unique OTUs and n is the number of individuals in the sample. This index gives a relative measure of how well the sample represents the larger environment.

samples *t*-test at 5% probability level using R program [35]. The relative abundances of OTU proportion for data from different taxonomical levels (phyla, class, and genera) were also graphed using R program [35].

3. Results

3.1. Fungal diversity and richness

A De Novo Genome Assembly resulted that the total bases obtained from Illumina sequencer are 544,250,767 bp and 1,424,349 raw tags without ambiguous base (Table 1). The GC content percentage ranged from 54.54 to 61.53% and the percentages of bases with Q20 were more than 99.0%, while the rates of bases with Q30 were above 95.0% (Table 1). These results demonstrated that the data set from Illumina sequencer had good quality.

The results of preprocessing obtained in a total number of 230,768 sequence reads (clean tags) with a minimal and maximal number of 2885 and 49,059 reads, respectively (Table 2). These sequences were clustered into 709 OTUs (ranging from 92 to 390) at a 97% similarity level (Table 2 and Figure 1). The sequencing depth and species richness was described using rarefaction analysis in the Figure 2. The total OTUs of *Q. serrata* were higher than that of *Q. mongolica* with the total number of 617 OTUs and 512 OTUs, respectively (Figure 1); however, the average of OTUs was not significantly different ($p > 0.05$) between two oak species (Table 2 and Figure 3). Although two oak species had no significant differences ($p > 0.05$) in the average Chao1 richness, samples from each plant tissue of *Q. serrata* had relatively higher Chao1 richness than those of *Q. mongolica* (Table 2 and Figure 3). Shannon index also showed that *Q. serrata* had a significantly higher level ($p < 0.05$) of fungal diversity than *Q. mongolica,* and the petiole sample of *Q. serrata* was the most diverse with Shannon index of 5.76, while

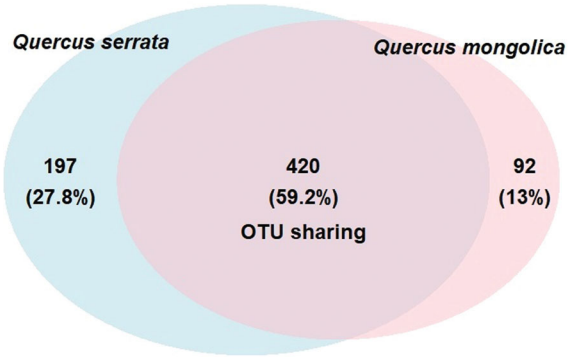

Figure 1. Venn diagram showing the number of fungal OTUs overlapped and nonoverlapped from *Quercus mongolica* and *Quercus serrata*.

the lowest diversity with Shannon index of 2.65 belonged to stem sample of *Q. mongolica* (Table 2 and Figure 3). Inverse Simpson index also indicated that *Q. serrata* had a higher level of fungal diversity than *Q. mongolica*, however, this difference was not significant ($p > 0.05$) between two oak species (Table 2 and Figure 3). Good's coverage index gives a relative measure of how well the sample represents the larger environment. In our study, Good's coverage index of twelve samples was between 98.34 and 99.89% (Table 2).

3.2. Taxonomic assignment of endophytic fungi

Total of OTUs was assigned into five fungal phyla, 17 classes, 60 orders, 133 families, 195 genera, and 280 species (Supplementary Table S1–S5). Fungal endophytes colonized in *Q. mongolica* belonged to five fungal phyla, 16 classes, 52 orders, 110 families, 157 genera, and 220 species, while those of *Q. serrata* included four fungal phyla, 17 classes, 59 orders, 127 families, 180 genera, and 246 species (Supplementary Table S1-S5). Members of Ascomycota were the most common endophytic fungi among all samples with 75.11%, followed by

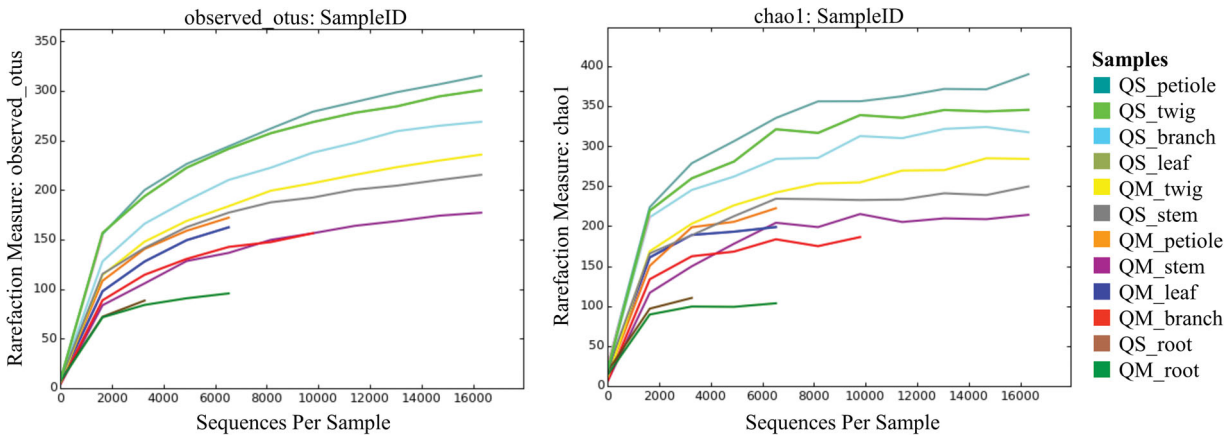

Figure 2. Rarefaction curves described for observed OTUs metric (left) and chao1 metric (right) among all 12 samples (QS: *Quercus serrata;* QM: *Quercus mongolica*).

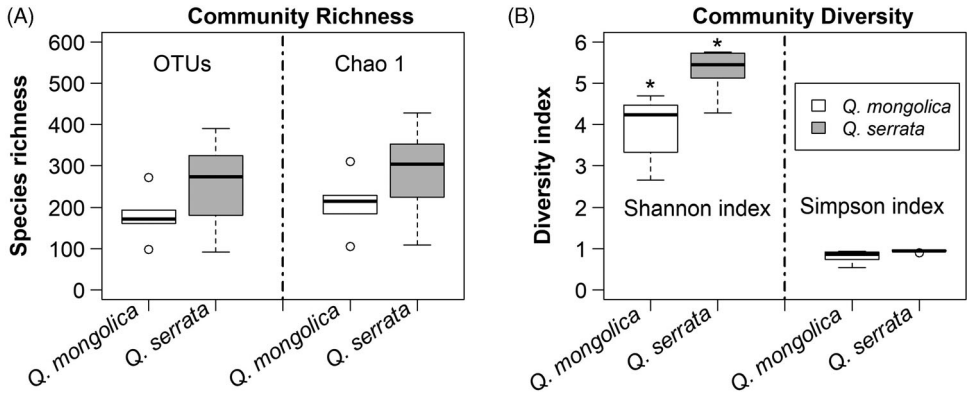

Figure 3. Fungal community richness (A) and diversity; (B) in *Quercus mongolica* and *Quercus serrata* described by boxplot. *Indicate a significant difference ($p < 0.05$) by two sample *t*-test.

unidentified fungi with 19.50% and Basidiomycota with 5.28%, while Mucoromycota, Mortierellomycota, Kickxellomycota and others were found with very low rate of 0.07, 0.02, 0.01, and 0.01%, respectively (Figure 4(A)). The percentage of Ascomycota ranged from 44.88 to 95.28% among samples of *Q. mongolica*, whereas that of *Q. serrata* was between 50.68 and 91.65% (Figure 4(A)). Basidiomycota accounted for 0.63 to 15.15% and 0.88 to 30.22% in *Q. mongolica* and *Q. serrata* respectively (Figure 4(A)). Mucoromycota and Mortierellomycota were mainly detected in root and stem samples in both two oak species; however, Kickxellomycota was only detected in root sample of *Q. mongolica* (Figure 4(A) and Supplementary Table S1). Members of fungal classes also varied in different samples, in which, Dothideomycetes, Eurotiomycetes, Leotiomycetes and Sordariomycetes were dominant within Ascomycota with average relative abundances of 41.91, 11.01, 9.70 and 6.94%, respectively (Figure 4(B) and Supplementary Table S1). Agaricomycetes had the highest proportions within Basidiomycota and this fungal class mainly distributed in root samples of *Q. mongolica* and *Q. serrata* with relative abundances of 14.78 and 29.20% respectively (Figure 4(B) and Supplementary Table S1).

There were 9 and 10 detected genera with average relative abundance of more than 1% in *Q. mongolica* and *Q. serrata*, respectively. In which, three genera of *Aureobasidium, Camptophora* and *Cladosporium* were distributed in both two oak species (Supplementary Table S1). *Leptosillia, Aureobasidium* and *Acanthostigma* were the most abundant genera detected in *Q. serrata* with the average relative abundance of 2.85, 2.76 and 2.19%, respectively. On the other hand, *Peltaster*, *Cladosporium* and *Monochaetia* were the most common genera detected in *Q. mongolica* with the average relative abundance of 4.83, 3.03 and 2.87%, respectively (Supplementary Table S1).

Relative abundance of different fungal genera in various plant tissues between two oak species was described in the Figure 5. The number of fungal genera with relative abundances above 1.0% in leaf, petiole, and twig tissue was higher than that in branch, stem, and root tissue (Figure 5). The most abundant genera in leaf, petiole, and twig of *Q. mongolica* and *Q. serrata* were *Zeloasperisporium*

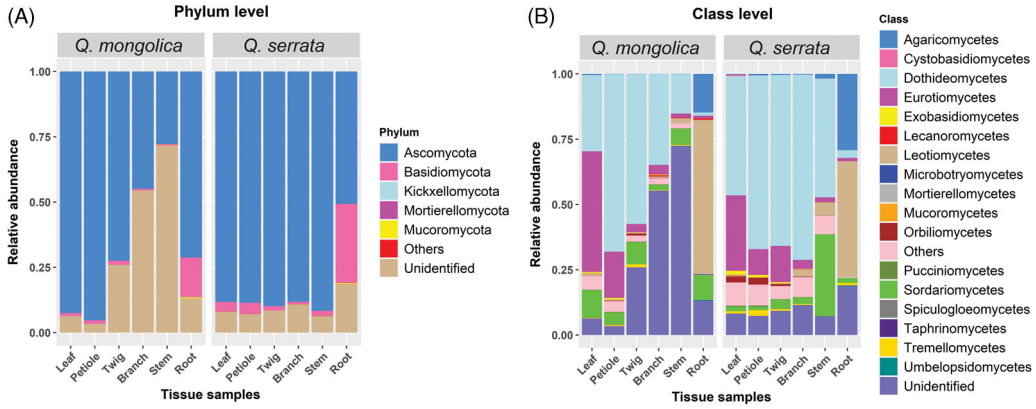

Figure 4. Relative abundance of endophytic fungi at the phylum (A) and class level; (B) in 12 different tissue samples between *Quercus mongolica* and *Quercus serrata*.

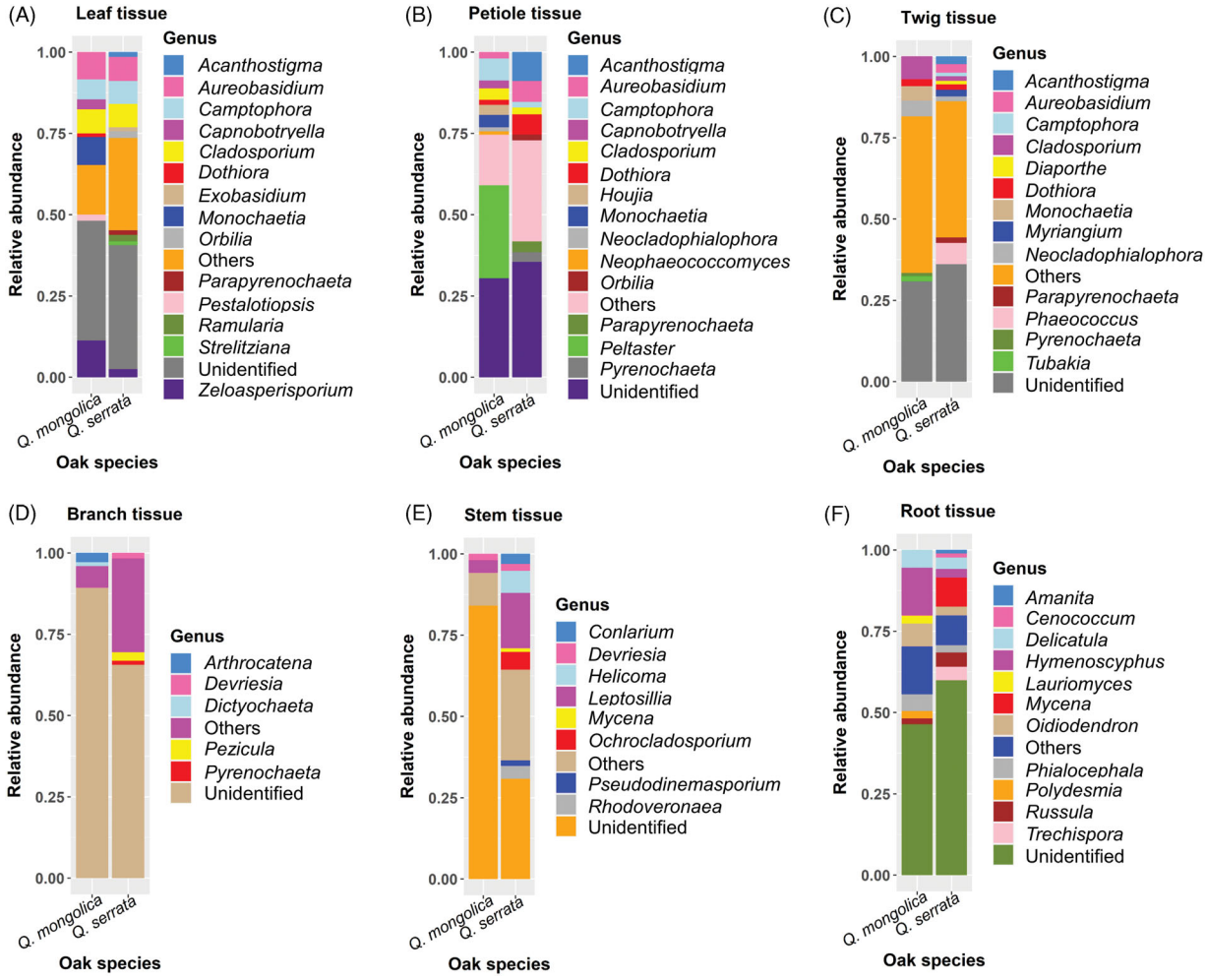

Figure 5. Relative abundance of different fungal genera in various plant tissues between *Quercus mongolica* and *Quercus serrata*. The fungi with a relative abundance below 1% were grouped into others.

(11.29%) and *Aureobasidium* (7.35%), *Peltaster* (28.5%) and *Acanthostigma* (8.87%), *Cladosporium* (7.01%) and *Phaeococcus* (6.57%), respectively (Figure 5). The most common genera presented in both branches and stem of *Q. mongolica* were *Arthrocatena* (2.81%) and *Leptosillia* (3.82%), respectively, while *Pezicula* (2.62%) and *Leptosillia* (17.04%) were the most abundant genera in branch and stem of *Q. serrata*, respectively (Figure 5). The root tissue of both *Q. mongolica* and *Q. serrata* had a difference in the diversity of fungal genera compared to other tissues and this difference was due to the presence of several genera belong to Basidiomycota such as *Delicatula* (5.51%), *Russula*

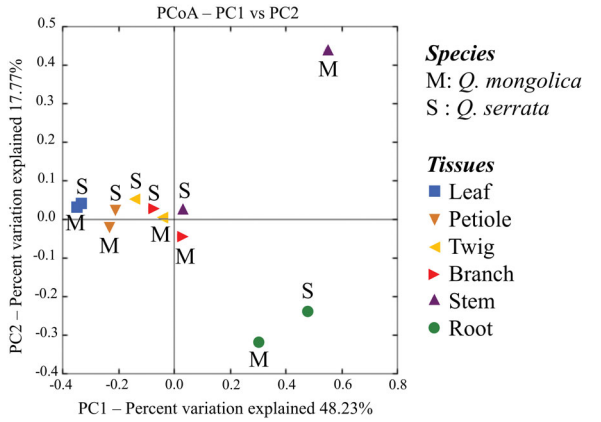

Figure 6. Principal Coordinate Analysis (PCoA) based on the weighted UniFrac distances for fungal communities from plant tissues of two oak species (*Quercus mongolica* and *Quercus serrata*) indicating two distinct groups in the stem tissues between two oak species using two principal coordinates (PC1 and PC2).

(1.84%), *Mycena* (8.93%), and so on (Figures 5 and 6). *Hymenoscyphus* (14.66%) was the most abundant genus in root of *Q. mongolica*, while *Mycena* (8.93%) accounted for the highest relative abundance in root of *Q. serrata* (Figure 5).

4. Discussion

A total of 709 distinct OTUs were generated from two oak species (Figure 1). Among these, there were 59.2% OTUs were distributed in both *Q. serrata* and *Q. mongolica*, while 27.8 and 13.0% OTUs were only detected in *Q. serrata* and *Q. mongolica*, respectively (Figure 1). It was indicated that *Q. serrata* is highly resistant to oak wilt fungus (*R. quercus-mongolicae*), while *Q. mongolica* is highly susceptible to this pathogen [28]. Our results showed that the Shanon index of endophytic fungi in *Q. serrata* was significantly higher than that of *Q. mongolica* (Figure 3). This is consistent with previous findings that either endophytic fungi of disease-resistant species had a higher diversity index compared with susceptible species to disease [36] or fungal endophytic community had a significant difference between pathogen-infected trees and healthy trees [10,37,38]. For instance, endophytic fungi colonized in *Rosa multiflora* had higher diversity than *Rose multiflora* var. *carnea* in several specific developmental stages of plants where *R. multiflora* showed high resistance to powdery mildew disease and *R. multiflora* var. *carnea* was highly susceptible to this pathogen [36]. Two maple trees, *Acer campestre* and *Acer platanoides* had a significantly higher fungal richness and diversity in control leaves compared to leaves infected with pathogens [10], while European beech (*Fagus sylvatica*) showed a significant difference in the fungal species composition between living and decaying leaves [38]. Fungal diversity indices were different among plant tissues of two oak species (Table 2). Principal Coordinate Analysis (PCoA) also showed that the fungal endophytic community had a difference in various plant tissues (leaf, petiole, twig, branch, stem, and root), and the biggest difference between two oak species was indicated in stem samples (Figure 6). Fungal endophytic communities were affected not only by plant varieties, plant tissues but also by management practices, plant's development stages, and hosts' ecological conditions [8,9,36,39]. The diversity of endophytic fungi isolated from plants was also different among parts [40–42] with a higher abundance in leaves compared to other tissues [40,41]. Our study obtained that the highest diversity index of endophytic fungi was indicated in the leaf sample of *Q. mongolica*, while endophytic fungi of *Q. serrata* showed the highest diversity index in the petiole sample (Table 2).

Ascomycota was dominant in both *Q. mongolica* and *Q. serrata*, with the average relative abundances of 67.34 and 82.88%, respectively (Figure 4(A) and Supplementary Table S1). Ascomycota is the most dominated endophytic fungi colonized in many plants that have been indicated in previous studies [11,36,39,43–45]. The most abundant fungal genus in *Q. mongolica* was *Peltaster* with average relative abundances of 4.83% and this genus was mainly colonized in petiole tissue with relative abundances of 28.50%, while *Leptosillia* was the most common endophytic fungi in *Q. serrata* with average relative abundances of 2.85% and it was mainly colonized in stem tissue with relative abundances of 17.04% (Figure 5 and Supplementary Table S1). To date, the information about endophytic fungi identified as *Peltaster* spp. and *Leptosillia* spp. is still limited.

Cladosporium spp. and *Aureobasidium* spp. were dominant endophytic fungi in both *Q. mongolica* and *Q. serrata* (Supplementary Table S1). *Cladosporium* spp. accounted for 3.03 and 1.99% of endophytic fungi in *Q. mongolica* and *Q. serrata* respectively, while *Aureobasidium* spp. had 1.80% in *Q. mongolica* and 2.76% in *Q. serrata* (Supplementary Table S1). *Cladosporium* spp. have been reported as dominant fungal endophyte from various either host trees such as oak, ash, and cinnamon species [46–48] or other hots plants, such as sweet citrus [49], rice [50], and wheat [51]. *Aureobasidium* spp. was one of the most common endophytic fungi colonized in several tree species [52], of which, endophytic fungus *Aureobasidium pullulans* accounted for 24.2% of detected fungi in European ash (*Fraxinus excelsior*) [53] and was the most dominant taxon in European aspen (*Populus*

tremula) [54]. Moreover, *Aureobasidium pullulans* was also one of major endophytic fungi in maize and common bean [55,56]. Studies on bioactive compounds obtained from *Cladosporium* spp. and *Aureobasidium* spp. were also conducted in biological controls of plant pathogens [57–59]. For instance, *Cladosporium* spp. isolated from stem of *Sesbania grandiflora* can inhibit development of bacterial and fungal pathogens in Thailand such as *Staphylococcus aureus, Escherichia coli,* and *Cryptococcus neoformans* [57]; *Cladosporium* sp., an endophytic fungus isolated from a medicinal plant (*Cyclosorus parasiticus*), had antibacterial activity against *Staphylococcus aureus* and *Salmonella enterica* damaging on *C. parasiticus* [60]. In addition, extracellular enzymatic activities of *Cladosporium* spp. isolated from various medicinal plants were also reported [61,62]. On the other hand, *Aureobasidium* spp. isolated from *Posidonia oceanica* can produce antimicrobial compounds, namely hydroxylated decanoic acids against *Candida albicans* and *Staphylococcus aureus*, and aureobasidin which exhibited insecticidal activity against the larval settlement of *Balanus amphitrite* larvae [59]. Among *Aureobasidium* spp., *A. pullulans* has been identified as one of the biocontrol agents to control various fruit postharvest pathogens such as *Monilinia laxa* on plums, peaches, sweet cherries, apricots, and table grapes [63–66]; *Botrytis cinerea* on apples, sweet cherries, tomatoes. and table grapes [63,66–68]; *Penicillium expansum* on apples, lemons [63,67,69]; *Monilinia fructicola, Monilinia polystroma* and *Monilinia fructigena* on sweet cherries, peaches, and apricots [64,65]; *Colletotrichum acutatum* on apples [67]; *Penicillium italicum* and *Penicillium digiatum* on citruses, apples, and lemons [67,69]. *Aureobasidium pullulans* was also used to control plant pathogens, namely *Phytophthora infestans* causing tomato late blight [70], *Rhizoctonia solani* causing damping-off in tomato, bean, and soybean seedlings [71,72], *Fusarium culmorum* causing Fusarium head blight of common wheat (*Triticum aestivum*) [73], and *Neofusicoccum parvum* causing stem canker disease in apple trees [74]. Other phytopathogenic fungi such as *Fusarium oxysporum* and *Alternaria alternata* were also inhibited by *A. pullulans* isolated from healthy grapevines [75]. Moreover, *A. pullulans* was also identified as plant growth promoters for bean and soybean plants [72], while *Aureobasidium* sp. isolated from *Boswellia sacra* not only displayed extracellular enzymatic activities but also produced indole acetic acid (IAA) for promoting growth of *B. sacra* [76]. In brief, *Aureobasidium* spp. had a higher abundance in *Q. serrata* than *Q. mongolica*, and these fungi showed a wide range of bioactive activities, especially antifungal and insecticidal activities. Thus, they could play important roles in pathogen tolerance and repellent of insect vector in oak wilt pathosystem.

Oak wilt disease caused by *R. quercus-mongolicae* has emerged rapidly in Korea since 2004 [28], and *Ceratocystis quercicola*, a novel *Ceratocystis* species can cause a very low level of damage on *Quercus variabilis* [77]. However, these two fungal species were not found in the present study. To the best of our knowledge, this is the first study that was conducted to analyze endophytic fungal community from *Q. mongolica* and *Q. serrata* based on the ITS2 region through Illumina MiSeq. Our results provided a better understanding of differences in diversity of fungal endophyte colonized in these two oak species. These differences could affect the interactions between endophytic fungi and host tree species in producing specific enzymes or volumes of bioactive compounds, therefore, the responses of *Q. mongolica* and *Q. serrata* to oak wilt pathogen are different.

5. Conclusion

Our results indicate that the diversity of endophytic fungi was significantly different between two oak species, and the biggest difference of fungal endophytic community occurred in stem tissues. A total of 709 OTUs were obtained in both two oak species. In which, the OTUs of *Q. serrata* were higher than that of *Q. mongolica* with the number of 617 OTUs and 512 OTUs, respectively. Total of OTUs were assigned into 5 fungal phyla, 17 classes, 60 orders, 133 families, 195 genera, and 280 species. Ascomycota was the dominant phylum with 75.11% relative abundance in all samples, followed by Basidiomycota with 5.28%, while Kickxellomycota, Mortierellomycota, Mucoromycota, and other fungi had very low relative abundance of 0.01, 0.02, 0.07, and 0.01% respectively. In the total, 19.50% of fungal OTUs remained unidentified. *Leptosillia, Aureobasidium* and *Acanthostigma* were the most abundant genera detected in *Q. serrata* with the average relative abundance of 2.8, 2.76, and 2.19%, respectively. On the other hand, *Peltaster*, *Cladosporium* and *Monochaetia* were the most common genera detected in *Q. mongolica* with the average relative abundance of 4.83, 3.03, and 2.87%, respectively.

Disclosure statement

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