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RESEARCH ARTICLE

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Investigation of Fungal Strains Composition in Fruit Pollens for Artificial Pollination

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ABSTRACT

Plants pollination are conducted through various pollinators such as wind, animals, and insects. Recently, the necessity for artificial pollination is drawing attention as the proportion of natural pollinators involved is decreasing over the years. Likewise, the trade in pollen for artificial pollination is also increasing worldwide. Through these imported pollens, many unknown microorganisms can flow from foreign countries. Among them, spores of various fungi present in the particles of pollen can be dispersed throughout the orchard. Therefore, in this study, the composition of fungal communities in imported pollen was revealed, and potential ecological characteristics of the fungi were investigated in four types of imported pollen. Top 10 operational taxonomic unit (OTU) of fungi were ranked among the following groups: *Alternaria* sp., *Cladosporium* sp., and *Didymella glomerata* which belong to many pathogenic species. Through FUNGuild analysis, the proportion of OTUs, which is assumed to be potentially plant pathogens, was higher than 50%, except for apple pollen in 2018. Based on this study of fungal structure, this information can suggest the direction of the pollen quarantine process and contribute to fungal biology in pollen

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1. Introduction

Pollination is essential for plants to inherit their descendant. This process involves the migration of pollen formed in stamen to the stigma. It is conducted by diverse pollinators depending on plant types or environmental factors [1,2]. There are varied biological factors, including mammals, insects, birds, and lizards, and non-biological factors like wind and water can also be a pollinator [3]. Among them, pollination mediated by insects is known as most common in nature [4,5]. However, recent studies are warning that the density of these insects is decreasing worldwide due to climate change and the usage of chemical pesticides [6,7]. If pollination is not carried out faithfully, fruits will not be properly formed, which will directly affect the yield [8]. Accordingly, artificial pollination with human hands is gradually increasing [9]. The artificial pollination also has the advantage of being able to directly select superior properties for fruit quality [10,11].

Since pollen particles are easily dispersed by wind or water, previous studies of allergies mediated by pollen have been actively reported [12]. In addition, artificial pollination process also allows different microbial communities present in pollen to be migrated to another region. *Aspergillus* spp. and *Penicillium* spp. are representative of pollen-related fungi that are easily dispersed by wind or water [13]. Additionally, *Alternaria* spp., *Botrytis* spp., *Cladosporium* spp., and *Colletotrichum* spp. were reported as pollen-associated fungi in the preceding studies [13]. Among these pollen-associated fungi, toxins such as naptho-γ-pyrones and tetracyclic compounds secreted by *Aspergillus* genus and meleagrin, 3-methoxy-viridicatin, verrucosidin secreted by *Penicillium* genus are reported to cause allergies [14]. Similarly, *Alternaria* genus is known as an allergic fungus by producing alternariol and alternariol monomethyl ether toxin [14], and *Cladosporium* genus is also reported to secret these types of toxin [15,16]. These fungi are known to contain not only allergies based on their toxicity but also pathogen genera in wide range of plant hosts [2,17]. Pollen-associated fungi need special attention because particles can spread to a wide range by wind, water, or pollinators [18]. Due to the imported pollen it poses a potential risk by unknown pathogens [19]. Thus, it is necessary to investigate what microbial clusters

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Supplemental data for this article can be accessed here.

exist in the pollen being imported, however insufficient information has been reported so far. With only limited pathogens being censored for the imported pollen during quarantine process [20,21]. In-depth investigation of fungal composition in the imported pollens well contribute to prevent the unexpected inflow of foreign pathogens and biosecurity. In addition, this study, which has uncovered the composition of fungi present in pollen used in artificial pollination, is thought to be informative in the field of pure microbiology.

2. Materials and methods

2.1. Pollen materials

To dissect the fungal composition of pollen sample, four kinds of fruit pollen imported over two years (2018to 2019) from China were collected. Four kinds of fruit pollen, consisting of apple (*Malus* spp.), kiwifruit (*Actinidia* spp.), peach (*Prunus* spp.), and pear (*Pyrus* spp.) were collected. For apple, peach, and pear pollen, were collected through Wellplus Co., (Daejeon, Republic of Korea) and kiwifruit pollen was collected from Jeju-Biotech Co., (Jeju, Republic of Korea). The collected pollen samples were separated into two 50 mL tubes. The pollen for isolating culturable living fungal stock was stored at 4 °C, and the pollen used for DNA extraction for metagenome was frozen at −80 °C.

2.2. Culturable fungi population

To isolate the culturable fungi, pollen samples diluted from 10^{-2} to 10^{-4}-fold in sterile water were spread onto potato dextrose medium (BD Difco, Franklin Lakes, NJ; 10 g potato dextrose, 10 g peptone, agar 20 g per L) and incubated at 28 °C for four days. The number of colonies were counted for measuring colony forming unit (CFU) value. The calculated CFU values were visualized as bar graphs *via* the ggplot2 (version 3.2.0) package [22] of the R program (version 3.4.4). Statistical analysis was measured by analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test ($p < 0.05$).

2.3. Refining pollen DNA and trimming up fungi polymerase chain reaction (PCR) amplicon

A total of 48 samples consisting of four kinds of fruit trees (three repetitions) were amplified to internal transcribed spacer (ITS) region. Pollen DNA were extracted through the FastDNA^{TM} SPIN Kit (MP Biomedicals, Santa Ana, CA) and peptide nucleic acid (PNA)-mediated PCR [23] was conducted. ITS1 and ITS4 primers were used to amplify ITS region (Table S1). PNA clamping probe (Panagene, Daejeon, Republic of Korea) was used simultaneously for the ITS PCR. The ITS PCR reaction was performed with 100 ng of pollen DNA, 10 µM each ITS1 forward primer and ITS4 reverse primer, 12.5 µL of Kapa HiFi HotStart ReadyMix (Roche, Basel, Swiss), 7.5 µM of each pPNA, mPNA primer, and sterile water to a final volume of 25 µL. The PCR program included an initial denaturation at 98 °C for 3 min, 24 cycles at 98 °C for 10 s, 78 °C for 10 s (annealing temp. of the PNA blocker), 50 °C for 30 s (annealing temp. of primers), 72 °C for 1 min, and a final extension at 72 °C for 5 min. After confirming the size of the PCR product through 1% agarose gel (600 ~ 700 bp for the ITS amplicon), gel elution was performed through Expin^{TM} Gel SV (GeneAll, Seoul, Republic of Korea). Subsequently, qualities of the amplified product (100 ng) were confirmed for constructing library. Quality and quantity of DNA was measured using NanoDrop2000C spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The genomic domain for library constructor was determined ITS2 region of ITS with primer sets of ITS3 forward primer and ITS4 reverse primer. The fungal ITS gene fragments were sequenced through Illumina Miseq v2 flow cell (Macrogen, Seoul, Republic of Korea).

2.4. Analysis of fungal community composition

The error rates were measured through DADA2 package in R program. Calculated error values were expressed through error frequency plot using DADA2 package [24]. To annotating confirmed base, the UNITE database was used [25]. Each specie accumulation of clustered operational taxonomic units (OTUs) was visualized through rarefaction curve [26]. iNterpolation and EXTrapolation (iNEXT, version 2.0.19) package [27] was used to visualize and arrange rarefaction curves of each pollen sample. In addition, each sample's curves were put on a clip using gridExtra (version 2.3) package [28]. Phyloseq (version 1.30.0) package [29] was used to visualize alpha diversity plots for graphically showing complex phylogenetic sequence data. Additionally, the microbiome (version 1.8.0.) [30] package was used to support the Phyloseq data collection. Indexes of Richness, Simpson, and Shannon were used to assess measuring alpha diversity. The Kruskal-Wallis rank-sum test was used to confirm statistically significant variations for the samples [31]. Significant differences of other samples were verified with ANOVA. The cutoff of *p*value was defined at 0.05. To represent beta diversity, vegan (version 2.5-6) package [32] was used to evaluate ecological data, and schematic packages of grid (version 3.6.2), The gridExtra were used for coordinate formation and graphic creation. Non-metric multi-

dimensional scaling (NMDS) plot depicted the beta diversity (Bray-curtis distance) and the cutoff of *p*value was set to 0.05 for verify a significant difference in distance between coordinates. In addition, devtools (version 2.2.1) [33] package was used to develop the function of R program. Local contribution to beta diversity (LCBD) values were measured to demonstrate an ecological uniqueness. The source code of R for data analyses in this study available on GitHub at https://github.com/gmldlfz/Pollen

2.5. Investigation of ecological characteristics of fungal communities

The physiological characteristics of the fungi identified in pollen samples were classified through a bioinformatic tool called FUNGuild (unique identifier: OMICS_30610, version 1.1) [34]. The analyzed FUNGuild data as types of nutrition acquisition were presented as pie charts by ggplot2 package. Also, the ratio of fungi classified as plant pathogens was shown in bar charts through ggplot2 package. Statistical analysis was calculated by ANOVA followed by Tukey's HSD test ($p < 0.05$).

3. Results

3.1. Isolating culturable fungal resources in imported pollen

Culturable fungi population densities from 2018 pollen samples of apple, kiwifruit, peach, and pear, the LogCFU values were measured from the range of 5.0 to 5.5 (Figure S1). No significant differences (Pr (>F) = 0.261) between pollen samples were shown. For pollen samples imported in 2019, the LogCFU value was estimated from the range 5.2 to 5.9. The significant difference between kiwifruit and peach samples was identified in the 2019 samples (P_{adj} = 0.0202817). All replications were analyzed by ANOVA followed by Tukey's HSD test ($p = 0.05$) for mean separation (Figure S1).

3.2. Fungal composition of imported fruit pollen

ITS was amplified using primer set of ITS1, ITS4 (Figure S2) with PNA blocking probes. Refined amplicon was trimmed to library construction through Illumina platform using primer set of ITS3, IT4 (Table S1). Raw data of sequences were analyzed by the DADA2 package of the R program. Error frequency plots were confirmed that each of the bases identified by the Illumina platform was classified as proper bases (Figure S3). Clustered OTUs were verified to retain a consistent number of OTUs *via* rarefaction curves, during which read numbers were evaluated forward to actual reading (Figure 1).

Based on the assigned OTUs, the similarities of fungal composition of each pollen were confirmed through the coordination on the NMDS plot. Comparing the fungal community by fruit tree species regardless of the imported years, peach pollen

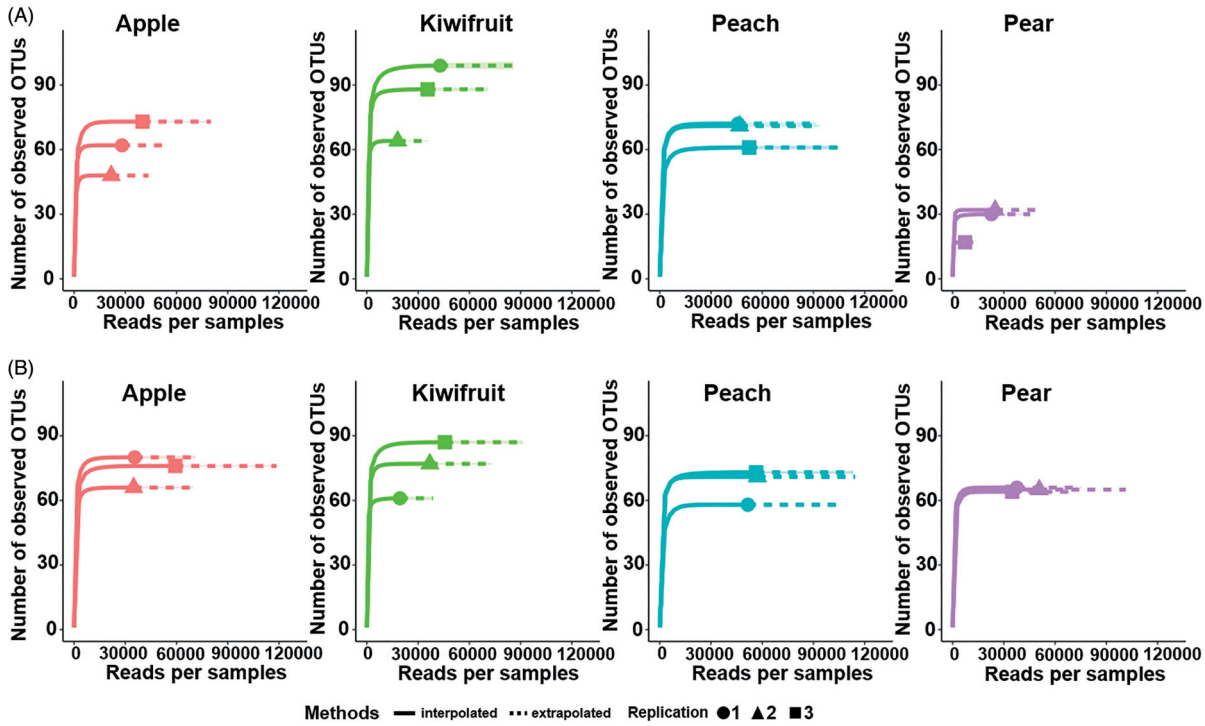

Figure 1. Rarefaction curves for pollen samples showing fungal operational taxonomic unit (OTU). (A) 2018 samples; (B) 2019 samples. Bold lines indicate actual value of reads per sample and dotted lines represent estimated value after the bold line. In rarefaction curve, the number of OTUs was confirmed to be maintained even assuming that the read numbers were read more than the actual read numbers. Pollen samples by year and fruit trees were analyzed in three repetition.

was found to have different types of fungal composition, showing significant differences among all other fruit trees with p values of apple-peach (1.69e-06), kiwifruit-peach (4.64e-06), and peach-pear (6.96e-07). In addition, it was confirmed that no significant difference occurred between the same fruit when the significant difference in the formed coordinates was separated from the imported year (Table 1, Figure 2).

The alpha diversity of fungal communities was found to have the lowest species abundance in 2018 pear through the index of Richness ($p = 0.0025$). It was confirmed that no significant difference was shown in 2019 pollen due to the large variation among the pollen samples. In terms of species evenness, low value of Shannon in 2018 peach and Simpson in 2019 peach that indicated a high dominance value of a particular species (Figure 3).

As a result of taxonomic annotation at family level, abundance bar graph showed that Aspergillaceae was confirmed to account the highest proportion in the 2018 apple, and the ratio of Cladosporiaceae increased in 2019 apple (Figure 4). In contrast, Cladosporiaceae accounted for the highest abundance in kiwifruit pollen in both two years. Additionally, compared to the kiwifruit pollen imported in 2018, the average abundance of Aspergillaceae was increased in 2019 kiwifruit. Peach samples shared the highest percentage of Aureobasidiaceae in both years. In pear sample Didymellaceae showed the highest abundance in 2018 sample (Figure 4). According to top 10 OTUs (Table 2), Aspergillaceae was ranked the first OTU with 32.31% in 2018 apples, in 2019, Cladosporiaceae was ranked the first OTU with 22.46%. In addition, Didymellaceae was ranked the second OTU in both years with 13.63 and 17.62% each year, and Aureobasidiceae also accounted for 8.46 and 14.12%, the third-highest percentage in both years. In 2018 apples, Erysiphaceae, which did not identify in the top 10 OTUs of other samples, was ranked eighth OTU with 3.32%. In Kiwifruit pollen, Cladosporiaceae was ranked the first OTU,

Table 1. Significant differences of fungal composition among the pollens in NMDS.

Beta-dispersion	p value	Significant label
Apple-peach	1.69e-06	***
Kiwifruit-peach	4.64e-06	***
Peach-pear	6.96e-07	***
2018 apple-2019 peach	0.02442923	*
2018 kiwifruit-2018 peach	0.006244778	**
2018 kiwifruit-2019 peach	0.002340998	**
2018 pear-2019 peach	0.0427196	*
2019 kiwifruit-2019 peach	0.026484384	*
2019 peach-2019 pear	0.022754687	*

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

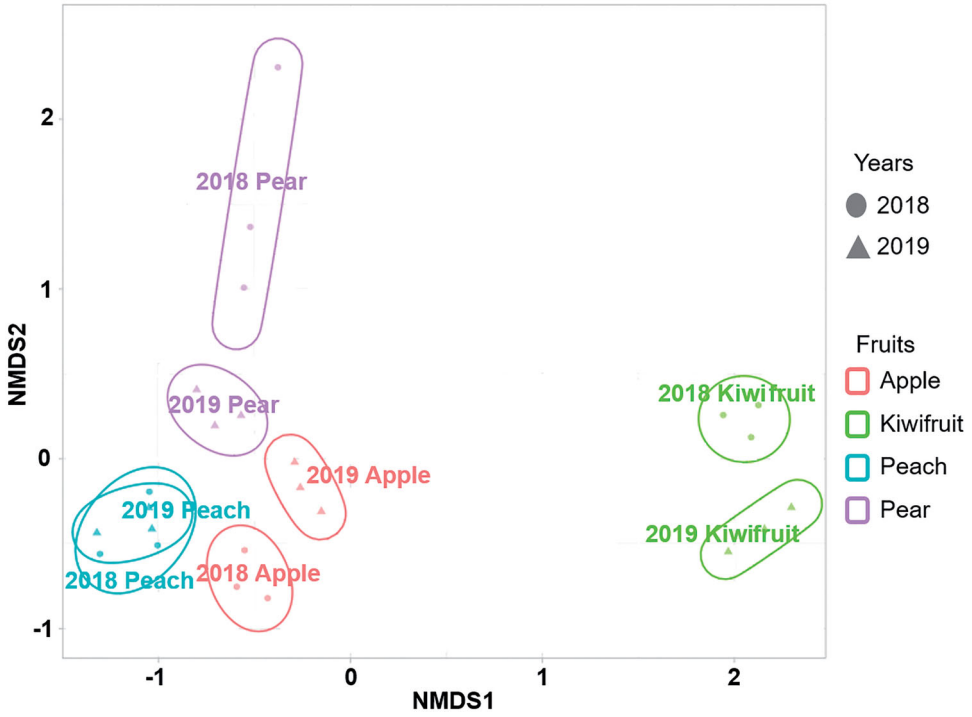

Figure 2. Non-metric multi-dimensional scaling (NMDS) plot for fungal communities. The NMDS plots were interpreted as condensed information through two-dimensional coordinates by multidimensional data, including multiple variables and species, OTUs. Pollen microbial structure with significant differences were formed by the separation of coordinates as beta-dispersion ($p < 0.05$). Points represent each pollen sample. The red, green, blue, and purple circles mean co-ordinates of the points representing the samples by each apple, kiwifruit, peach, and pear trees. Based on a similarity matrix, sample repetition over two years were arranged ordination for each fruit tree. Bray-Curtis dissimilarity was used as an asymmetrical measure for NMDS plot.

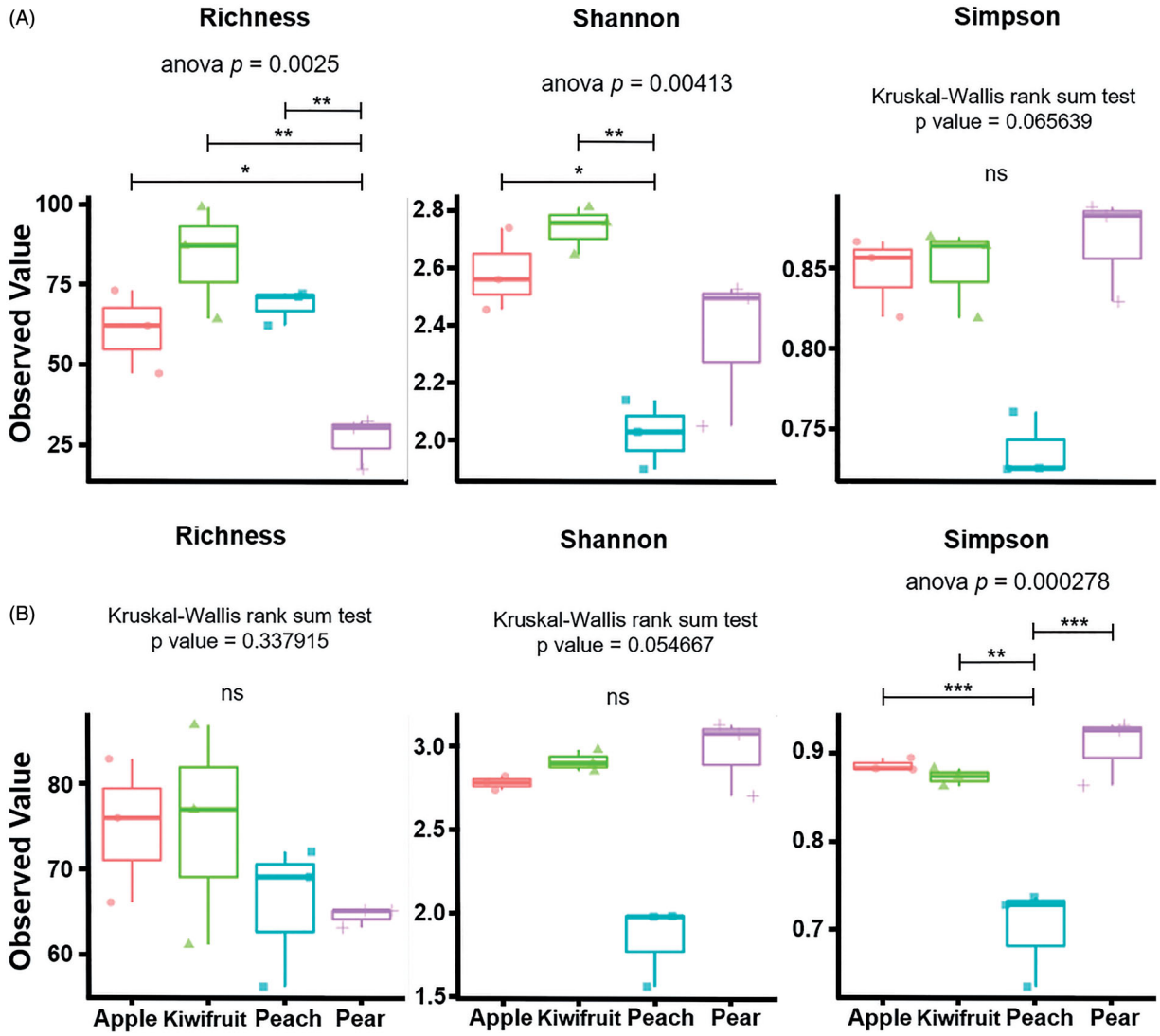

Figure 3. Alpha diversity measure with indexes of Richness, Shannon, and Simpson for fungi. (A) 2018 samples; (B) 2019 samples. Richness index indicates the abundance of OTUs in pollen sample. Boxes indicate the interquartile range representing the variance of between 25 and 75% in each pollen sample. The horizontal line in the box means the second quartiles (median).

accounting for 34.59 and 30.89%. However, in 2018 kiwifruit, Mycosphaerellaceae and Dothideaceae were ranked the second and the third OTU with 8.57 and 6.05% respectively, and Botryosphaeraceae was ranked the fourth OTU with 5.89%, and in 2019 kiwifruit, Aspergilaceae ranked from second to fourth OTU. In kiwifruit, Teichosporaceae, which was not included in the top 10 of other fruit pollen, was ranked the seventh OTU with 3.72%. In both years of peach pollen, Aureobasidiaceae was ranked the first OTU with a high percentage of 43.46 and 47.14%, and Didymellaceae was also ranked the second OTU with 25.72 and 25.97% per each year. Filobasidiaceae, which was not identified in other fruit pollen, ranked the fourth OTU with 3.78% in 2018 peach and the third OTU with 5.49% in 2019 peach. In addition, in 2019 peach pollen, Taphrinaceae, which was not identified in other fruit pollen, was ranked the seventh OTU with 0.91%. In 2018 pear, the OTU assigned as Didymellaceae accounted for 21.24 and 15.63% to take the first and the third order, while in 2019 pear, Plesosporaceae, which was not identified in the top 10 of other fruit pollen, ranked the third OTU with 10.67% (Table 2).

3.3. Predicted trophic mode of identified fungal OTUs

According to the trophic mode of the fungal communities through FUNGuild (Figure S5), the highest percentage in the 2018 apple pollen was classified as acquiring nutrition through saprotroph (range of 39.72–59.41%), but in the 2019 apple pollen, the highest percentage was classified as having three of pathotroph-saprotroph-symbiotroph mode (46.37–49.21%). This pathotroph-saprotroph-symbiotroph mode also common in kiwifruit and peach of 2018 samples (52.02–61.45% for kiwifruit, 47.12–54.21% for peach). In 2018 pear, the ratio of those classified as types with two characteristics of pathotroph-saprotroph was the highest (41.04–44.25%). In 2019, kiwifruit and

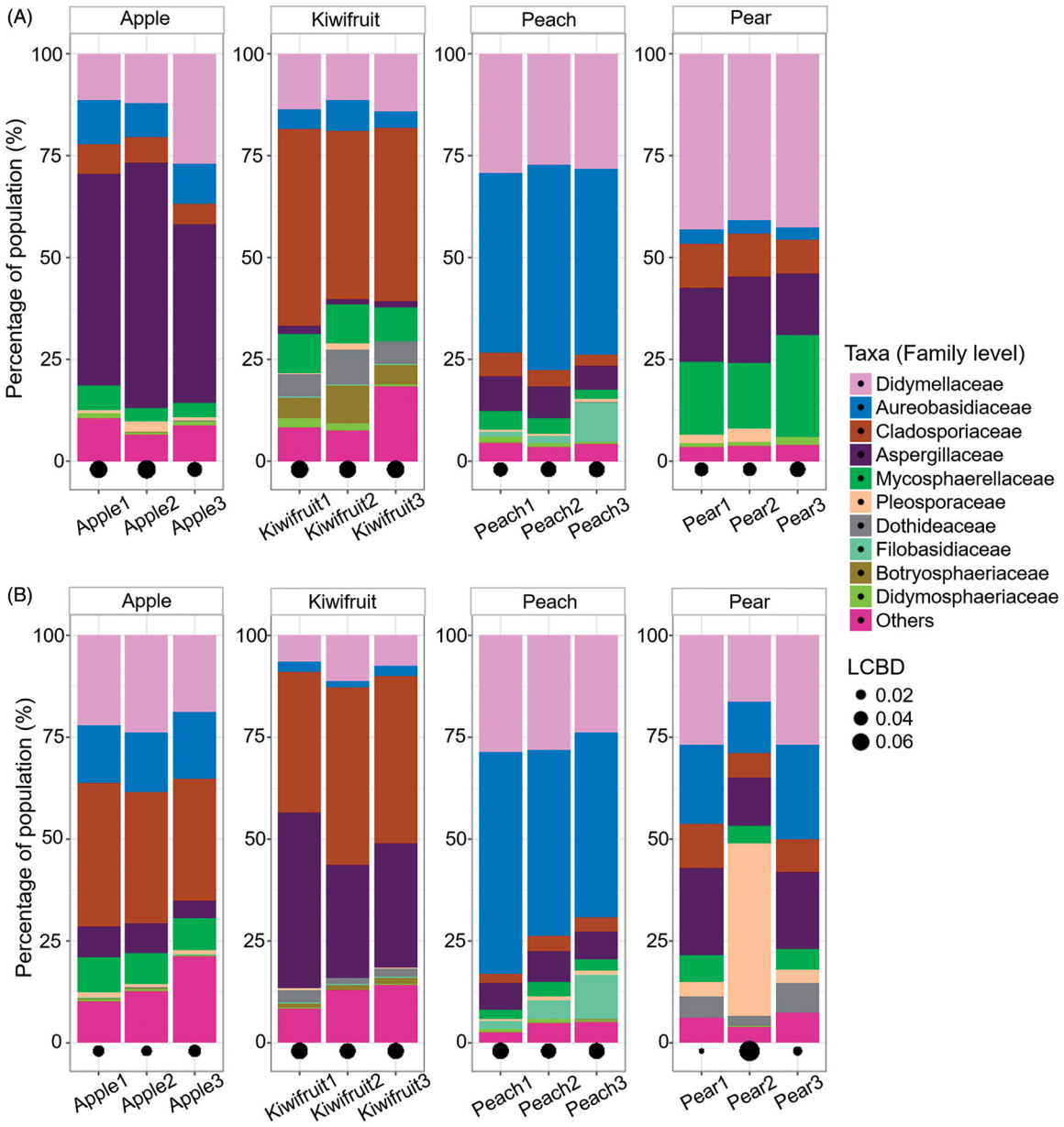

Figure 4. Abundance bar graph of fungal composition at the family level. (A) 2018 samples; (B) 2019 samples. Stacked bar chart of beta diversity represents spatial differentiation and the variation in pollen associated fungi. The fungal composition was transformed in Hellinger transformation that is ecologically necessary method before raw biomass data is calculated. Additionally, local contribution to beta diversity (LCBD) value was used to confirm the total variation in each sample. The LCBD values are an index of the uniqueness of microbial structures among each sample. The UNITE database was used for annotation.

peach samples were also confirmed to the highest proportion to have all three trophic modes simultaneously (range of 42.14–49.56% for kiwifruit, 49.95–56.69% for peach), but in 2019 kiwifruit, characteristics of only saprotroph were also accounted for range of 35.36–45.64% (Figure S5).

3.4. The ratio of fungal-OTUs estimated to have plant pathogenicity

Based on FUNGuild data, putative plant pathogenic fungal OTUs were predicted that the 2018 apple sample accounted for a lower rate than other fruits, with a range of 35.61–42.92%, and no significant difference between kiwifruit, peach, and pear was verified. However, over 70% of all 2018 three fruits were composed to be putative plant pathogenic fungi. In the case of samples in 2019, the number of plant pathogens in apple samples increased at range of 75.58–81.83%. There was no significant difference between apple and peach samples (77.28–87.99%), two of the highest rates of fungi estimated to be plant pathogens among the 2019 samples, and no significant difference was also found between kiwifruit and pear samples (Figure S4).

4. Discussion

Pollen is an essential element for plants to process to the next generation. Pollinators involved in the

Table 2. Top 10 fungal operational taxonomic unit (OTU) of each pollen sample.

	Apple			Kiwifruit			Peach			Pear		
	Taxonomy	AR (%)[a]	OTUs	Taxonomy	AR (%)	OTUs	Taxonomy	AR (%)	OTUs	Taxonomy	AR (%)	OTUs
2018	Aspergillaceae	32.31	OTU4	Cladosporiaceae	34.59	OTU3	Aureobasidiaceae	43.36	OTU1	Didymellaceae	21.24	OTU11
	Didymellaceae	13.63	OTU2	Mycosphaerellaceae	8.57	OTU5	Didymellaceae	25.72	OTU2	Mycosphaerellaceae	18.99	OTU5
	Aureobasidiaceae	8.46	OTU1	Dothideaceae	6.05	OTU18	Aspergillaceae	4.41	OTU4	Didymellaceae	15.63	OTU6
	Not assigned	5.75	OTU14	Botryosphaeriaceae	5.98	OTU20	Filobasidiaceae	3.78	OTU10	Aspergillaceae	12.26	OTU13
	Cladosporiaceae	4.16	OTU3	Aureobasidiaceae	4.14	OTU1	Mycosphaerellaceae	3.06	OTU5	Cladosporiaceae	6.32	OTU7
	Aspergillaceae	3.97	OTU12	Didymellaceae	4.01	OTU2	Cladosporiaceae	2.59	OTU3	Didymellaceae	3.66	OTU2
	Mycosphaerellaceae	3.87	OTU5	Teichosporaceae	3.72	OTU30	Not assigned	1.46	OTU16	Aureobasidiaceae	3.19	OTU1
	Erysiphaceae	3.32	OTU23	Cladosporiaceae	2.93	OTU28	Cladosporiaceae	1.38	OTU7	Cladosporiaceae	3.09	OTU3
	Aspergillaceae	3.26	OTU29	Didymellaceae	2.56	OTU21	Didymosphaeriaceae	1.02	OTU26	Sclerotiniaceae	1.78	OTU33
	Aspergillaceae	2.13	OTU36	Didymellaceae	2.03	OTU6	Aureobasidiaceae	0.87	OTU55	Aspergillaceae	1.75	OTU4
2019	Cladosporiaceae	22.46	OTU3	Cladosporiaceae	30.89	OTU3	Aureobasidiaceae	47.14	OTU1	Not assigned	13.45	OTU8
	Didymellaceae	17.62	OTU2	Aspergillaceae	13.44	OTU4	Didymellaceae	25.97	OTU2	Didymellaceae	11.83	OTU2
	Aureobasidiaceae	14.12	OTU1	Aspergillaceae	5.86	OTU19	Filobasidiaceae	5.49	OTU10	Pleosporaceae	10.67	OTU9
	Mycosphaerellaceae	7.76	OTU5	Aspergillaceae	3.53	OTU24	Aspergillaceae	4.67	OTU4	Aureobasidiaceae	9.87	OTU1
	Aspergillaceae	3.57	OTU4	Didymellaceae	3.22	OTU21	Mycosphaerellaceae	2.69	OTU5	Aspergillaceae	6.53	OTU4
	Cladosporiaceae	3.37	OTU7	Didymellaceae	3.15	OTU6	Cladosporiaceae	2.25	OTU3	Not assigned	5.57	OTU17
	Saccharomycetales	2.88	OTU25	Cladosporiaceae	2.59	OTU7	Taphrinaceae	0.91	OTU37	Not assigned	4.96	OTU16
	Didymellaceae	2.28	OTU6	Cladosporiaceae	2.39	OTU15	Aspergillaceae	0.83	OTU12	Mycosphaerellaceae	3.58	OTU5
	Cladosporiaceae	2.22	OTU15	Aureobasidiaceae	2.24	OTU1	Cladosporiaceae	0.78	OTU7	Cladosporiaceae	3.27	OTU3
	Saccharomycetales	1.98	OTU35	Dothideaceae	1.99	OTU18	Didymosphaeriaceae	0.74	OTU26	Dothideaceae	2.80	OTU32

[a]Abundance ratio (%).

migration of pollen include wind, insects, and animals. Insects account for the largest portion of pollinators [3]. As these pollinators decrease, there is an increasing number of cases of artificial pollination that is directly pollinated by human hands [6]. Currently, large amounts of fruit pollen used in pollination have been traded into abroad worldwide. Unknown pathogens in the imported pollen may be a major potential risk factor in the fruit production as well as in biosecurity. Therefore, understanding and evaluation of pollen-associated microbial composition is necessary step to use uncontaminated pollen in the orchards. However, there is limited research on the microbial structure of pollen has been reported. Currently, only certain pathogens are detected with diagnostic propose [20,21].

Fungi spores presented in pollen can easily disperse along with pollen particles [30]. Therefore, this study investigated fungi composition in the imported pollens with metagenomes approach. The results have been found that the fungal composition of pollen exhibit similar community pattern depending on the species of fruit, even if it was imported from different years (Table 1 and Figure 2). Aspergillaceae (Eurotiomycetesand Ascomycota) was found to be ranked in the top 10 OTUs of all fruit trees except for the kiwifruit in 2018 sample (Table 2). According to prior studies, the ratio of the two genera was known to account for 42.5% of *Aspergillus* and 51.6% of *Penicillium* in the Aspergilaceae family. Among them, *Aspergillus fumigatus* and *A. flavus* are reported to human allergens as well as plant pathogens [35]. Also, since *Penicillium* genera also includes *P. expansum*, and *P. italicum*, which are known as plant pathogenic species, the existence of Aspergilaceae in all fruit pollens are thought to require caution in pollen trade. Additionally, *P. digitatum*, especially known as postharvest pathogen, also belongs to this family [36]. Pollens of 2018 apple and 2019 kiwifruit, Aspergilaceae accounted as a relatively high abundant fungi based on the result of beta diversity. Consistent with this, the FUNGuild result that saprotroph accounted for the largest proportion in 2018 apple and 2019 kiwifruit, which is expected to imply the presence of stored pathogens such as *P. digitatum* [54]. Similarly, Didymellaceae (Ascomycota, Pezizomycotina, and Dothideomycetes), ranked the top 10 OTUs list, has a large number of plant pathogenic species that has a wide range of hosts and distributed the worldwide. A prior study found that some of them contain fungi that require quarantine management [37].

In apple pollen, Didymellaceae ranked the second abundant OTU with 25.72 and 25.97% in both years, and the first and the third ranks were also identified in the 2018 pear sample as Didymellaceae, which had a high proportion of both 21.24 and 15.63%. Furthermore, since the fungi ranked the top 10 OTUs in other fruit pollens, quarantine management may be requested as a potential plant pathogen [37]. Erysiphaceae, which was assigned only in the top 10 OTUs in 2018 apples, belongs to several species that cause a powdery mildew disease, and it is thought that using contaminated pollen such as these pathogens would cause direct damage to apple orchards [38,39].

In peach, the Aureobasidiaceae was detected a higher than 40%dominant abundance in both years, which included *Aureobasidium pullulans*, known for antagonistic properties against postharvest pathogens [40]. Another yeast-like fungi, Filobasidiceae was only assigned with the top 10 OTU list in peach pollen. Filobasidiceae is known that this includes a total of four species, of which shown strong lipase activity, the potential for resources as biofuels such as *F. floriforme* has been revealed by prior research [41]. According to these results, peach pollen

samples, which were analyzed to have significant differences fungi community compare to other fruit pollens in the NMDS plot, are suspected to have more beneficial fungi community than other fruit pollens.

This study on the fungal composition of the imported pollens is expected to be meaningful in strengthening biosecurity by blocking putative plant pathogenic fungi. Our findings provide basic information in terms of pure microbiology in tree pollens, and these results of dissecting the overall fungal structure will provide basic data on not only plant pathogens but also characteristics of communities of the fungal clusters.

Disclosure statement

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