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Original Article

DNA metabarcoding analysis of fungal community on surface of four root herbs

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ABSTRACT

Objective: *Angelicae Sinensis Radix* (ASR, Danggui in Chinese), *Cistanches Herba* (CH, Roucongrong in Chinese), *Ginseng Radix* et *Rhizoma* (PG, Renshen in Chinese), and *Panacis Quinquefolii Radix* (PQ, Xiyangshen in Chinese), widely used as medicine and dietary supplement around the world, are susceptible to fungal and mycotoxin contamination. In this study, we aim to analyze their fungal community by DNA metabarcoding.

Methods: A total of 12 root samples were collected from three main production areas in China. The samples were divided into four groups based on herb species, including ASR, CH, PG, and PQ groups. The fungal community on the surface of four root groups was investigated through DNA metabarcoding via targeting the internal transcribed spacer 2 region (ITS2).

Results: All the 12 samples were detected with fungal contamination. *Rhizopus* (13.04%–74.03%), *Aspergillus* (1.76%–23.92%), and *Fusarium* (0.26%–15.27%) were the predominant genera. Ten important fungi were identified at the species level, including two potential toxigenic fungi (*Penicillium citrinum* and *P. oxalicum*) and eight human pathogenic fungi (*Alternaria infectoria*, *Candida sake*, *Hyphopichia burtonii*, *Malassezia globosa*, *M. restricta*, *Rhizopus arrhizus*, *Rhodotorula mucilaginosa*, and *Ochroconis tshawytschae*). Fungal community in ASR and CH groups was significantly different from other groups, while fungal community in PG and PQ groups was relatively similar.

Conclusion: DNA metabarcoding revealed the fungal community in four important root herbs. This study provided an important reference for preventing root herbs against fungal and mycotoxin contamination.

1. Introduction

With the numerous advantages, herbs have been known and popular around the world for thousands of years (Yu et al., 2021). Root herbs have been used as medicinal and nutritional materials due to its distinctive characteristics (Sofowora, 1996). Recently, as an increasing number of studies on mycotoxins occurring in herbs, the safety issue of herbs has been paid close attention by the public (Guo, Jiang, Luo, Yang, & Pang, 2018). The content of aflatoxins and ochratoxin A has been strictly regulated by the Chinese Pharmacopeia Commission in *Chinese Pharmacopoeia* (2020 edition). Previous studies pointed out that the main potential toxigenic fungi were *Penicillium*, *Aspergillus*, and *Fusarium* in herbs (Rocha-Miranda & Venâncio, 2019). Fungal contamination could occur during the whole production process of herbs, including planting, harvesting, processing, transporting, and storage (Stevic et al., 2012). As root herbs are direct contacting with the soil, they are prone to be contaminated by harmful materials, including fungi, heavy metals, and pesticides (Stevic et al., 2012). Su et al. (2018) collected 48 root herbs from Chinese market and found 1 844 isolates belonging to 25 genera. The majority of the isolates were from *Penicillium* and *Aspergillus*. Chen et al. (2010) reported that *Penicillium* was the dominant fungal genus in seven root materials, followed by *Fusarium* and *Aspergillus*. Wang et al. (2010) investigated the fungal community in *Ophiopogonis Radix* (Maidong in Chinese), a traditional root product with both food and medical functions. The study noted that the main fungal invasion species during the storage were from *Aspergillus*. Therefore, it is necessary to monitor fungal community of root herbs by using an effective technology.

DNA metabarcoding technology has become a useful tool for analyzing fungal communities in soil, air, and sediment samples (Raclariu, Heinrich, Ichim, & de Boer, 2018). In complex sample, it could identify multiple species with low abundance (Bittinger et al., 2014; Tedersoo et al., 2014). At present, DNA metabarcoding technology has been successfully used in fungal identification of herbs. The internal transcribed spacer (ITS) region has been consid-

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ered as primary DNA barcode to identify fungal species. Guo, Jiang, Yang, Dou, & Pang (2020) investigated the fungal contaminations in *Cassiae Semen* (Juemingzi in Chinese), a traditional herbal medicine and roasted tea in China, through DNA metabarcoding by targeting the ITS2 region. The result showed that Ascomycota and *Aspergillus* were the predominant at the phylum and genus levels. The study detected a total of six potential toxigenic fungi as well. *Myristicae Semen* (Roudoukou in Chinese), as a vital herbal medicine and spice for years, was prone to be contaminated by fungi and mycotoxins under some conditions. Jiang et al. (2020) detected the fungal community of the herb by using DNA metabarcoding via targeting the ITS2 sequence. The study found that *Aspergillus* was the most abundant fungi at the genus level and six potential toxigenic fungi were identified, including *Penicillium capsulatum*, *Penicillium steckii*, and *Aspergillus fumigatus*. The fungal community of *Platycladi Semen* (Baiziren in Chinese) was analyzed by DNA metabarcoding through shooting at the ITS2 region (Yu et al., 2020). The results indicated that Ascomycota and *Aspergillus* were the most abundant phylum and genus, and four potential toxigenic fungi were found, including *Penicillium steckii*, *Aspergillus fumigatus*, and *A. flavus*.

In this study, DNA metabarcoding technology was applied to analyze the fungal community on the surface of four root samples, including *Angelicae Sinensis Radix* (ASR), *Cistanches Herba* (CH), *Ginseng Radix et Rhizoma* (PG), and *Panacis Quinquefolii Radix* (PQ), via targeting the ITS2 sequence. It could provide references for efficiently supervising fungal contamination in herbs to ensure safety and quality of the herbal industry.

2. Materials and methods

2.1. Sampling

A total of 12 batches of ASR, CH, PG, and PQ samples were collected from three main production areas in China, namely Sichuan, Gansu, and Jilin Provinces. They were identified by Prof. Xiaohui Pang. The samples were divided into four groups (ASR, CH, PG and PQ groups) based on herb species. Detailed information of the samples was listed in Table 1.

2.2. DNA extraction and PCR amplification

Approximate 5.0 g root samples were transferred into a 50 mL sterilized centrifuge tube with 20 mL of 1 × PBS buffer (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), then shaken and filtered by double layers of sterilized gauze in the sterile condition. The fungal DNA was extracted by the EZNA® 434 soil DNA kit (Omega Bio-tek., Inc., Norcross, GA, USA) on the basis of the manufacturer's instructions. The total DNA was stored at − 20 °C.

To amplify the ITS2 sequences, the polymerase chain reaction (PCR) was conducted by the primer pairs ITS3 (5′-GCATCGATGAA GAACGCAGC-3′) and ITS4 (5′ -TCCTCCGCTTATTGATATGC-3′) (White, Bruns, Lee, & Taylor, 1990). The conditions of the amplification were as the following steps: the initial denaturation at 95 °C for 5 min, and 37 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 50 s, then elongation at 72 °C for 45 s, the final extension at 72 °C for 10 min. To minimize the PCR bias, the amplification was performed for each sample in triplicate. Then the products were verified and purified respectively by 2% agarose gel and DNA gel extraction kit (Axygen, Union City, CA, USA) for the desired fragment.

2.3. Sequencing and data analysis

The Illumina Miseq PE300 platform (Illumina, San Diego, CA, USA) was applied for sequencing the PCR amplifications. We demultiplexed and checked the raw reads via fastp software (version 0.19.6 https://github.com/OpenGene/fastp) (Chen et al., 2018), then merged the sequences by using the FLASH (version 1.2.11 https://ccb.jhu.edu/software/FLASH/index.shtml) (Magoc & Salzberg, 2011). The ITS2 reads were truncated to obtain an average quality score of < 20 over a 50 bp sliding window. The ambiguous bases or < 10 bp overlapping sequences and chimeric sequences were detected and removed through USEARCH software (version 7.0 http://www.drive5.com/usearch/). The sequences were clustered into OTUs with 97% similarity by Uparse software (version 7.0.1090 http://www.drive5.com/uparse/) (Edgar, 2013). To ensure the 100% accuracy of the taxonomical classification of the OTUs, we manually search the reads via the basic local alignment search tool (BLAST) in the International Nucleotide Sequence Database Collaboration. Based on the UNITE database (version 8.0 https://unite.ut.ee/) (Edgar, 2010), OTUs were denominated at different taxonomical levels, including phylum, class, order, family, genus, and species through bar map, heatmap, and Cricos diagram (Krzywinski et al., 2009). Rarefaction curves were performed by R software to illustrate the normalization to even depths across each sample. Five α-diversity indices were calculated to demonstrate the fungal community by using the MOTHUR software (version 1.30.2 https://www.mothur.org/wiki/Download_mothur) (Amato et al., 2013). Venn analysis was conducted by R software to indicate the distribution of the OTUs in different groups (version 3.3.1). The β-diversity was shown by principal co-ordinates analysis (PCoA) analysis and hierarchical clustering and non-metric multidimensional scaling (NMDS) through Quantitative Insights into Microbial Ecology (version 330 1.9.1 http://qiime.org/install/index.html). Statistical difference analysis was performed by using the Kruskal-Wallis H test through stats in R software and SciPy in Python software. Linear discriminant analysis effect size (LEfSe) analysis was conducted to compare the significant differences of

Table 1
Sample information for 12 root samples.

Estimators No.	Samples	Collection date	Sampling location	Groups
ASR1	*Angelicae Sinensis Radix*	2020.10–2020.11	Sichuan	ASR
ASR2	*Angelicae Sinensis Radix*	2020.10–2020.11	Sichuan	ASR
ASR3	*Angelicae Sinensis Radix*	2020.10–2020.11	Sichuan	ASR
CH1	*Cistanches Herba*	2021.10–2021.11	Gansu	CH
CH2	*Cistanches Herba*	2021.10–2021.11	Gansu	CH
CH3	*Cistanches Herba*	2021.10–2021.11	Gansu	CH
PG1	*Ginseng Radix* et *Rhizoma*	2021.07–2021.08	Jilin	PG
PG2	*Ginseng Radix* et *Rhizoma*	2021.07–2021.08	Jilin	PG
PG3	*Ginseng Radix* et *Rhizoma*	2021.07–2021.08	Jilin	PG
PQ1	*Panacis Quinquefolii Radix*	2021.07–2021.08	Jilin	PQ
PQ2	*Panacis Quinquefolii Radix*	2021.07–2021.08	Jilin	PQ
PQ3	*Panacis Quinquefolii Radix*	2021.07–2021.08	Jilin	PQ

the taxonomical levels, from phylum to genus (http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=lefse_upload). The co-occurrence analysis was conducted by Network X packages in Python (Hagberg et al., 2008).

3. Results

3.1. Fungal diversity in four root herbs

After excluding the chimeric sequences, there were 724 194 high-quality ITS2 sequences detected in 12 herbal samples. The average length of reads was 345 bp. Rarefaction curves indicated that all sobs indices almost reached asymptote, which reflected that the sequences were sufficient to represent the fungal community for each sample (Fig. 1A). These reads were clustered into 226 OTUs. Venn analysis showed a total of 28 shared OTUs based on the types of root herbs (Fig. 1B). There were 152, 105, 60, and 63 OTUs detected in ASR, CH, PQ, and PG groups, respectively.

Five α-diversity indices of 12 root samples were shown in Table 2, namely ACE, Chao1, Shannon, Simpson, and Coverage. The high value of ACE and Chao1 illustrated the high fungal diversity. The highest abundant was in ASR1, and PQ2 had the lowest variation among species. The indices of Shannon and Simpson in ASR3 indicated that the fungal diversity in the samples was the lowest. CH2 had the highest Shannon index and lowest Simpson index, reflecting its highest fungal diversity. The Coverage results showed that the values in 12 samples were over 99.9%, indicating a good overall sampling.

3.2. Fungal composition in four root herbs

The detected 226 OTUs were further clustered into five phyla, 18 classes, 41 orders, 94 families, and 146 genera. There were three phyla with the relative abundance > 1% (Fig. 2A). Mucoromycota were the predominant phylum, accounting for 13.07%–74.07% of the fungal reads. Ascomycota and Basidiomycota were followed, with the relative abundance of 24.98%–81.72% and 0.95%–11.76%. At the class level, Mucoromycetes (13.07%–74.07%) were dominant in all samples collected from four root herbs, followed by Sordariomycetes (6.54%–46.00%) and Eurotiomycetes (3.02%–25.77%) (Fig. 2B). Among the 21 fungal orders with relative abundance > 1%, Mucorales was the most dominant (13.07%–74.07%), followed by Eurotiales (1.98%–25.77%) and Hypocreales (2.90%–28.85%) (Fig. 2C). Further taxonomical classification at the family level indicated that the three most abundant were Rhizopodaceae, Aspergillaceae, and Nectriaceae, with the relative abundance of 13.04%–74.03%, 1.98%–25.77%, and 2.63%–26.21% (Fig. 2D).

There were 36 genera with the relative abundance > 1% in 12 samples at the genus level. *Rhizopus* (13.04%–74.03%), *Aspergillus* (1.76%–23.92%), and *Fusarium* (0.26%–15.27%) were the most common genera detected in 12 root samples. Besides, the relative abundance of *Penicillium* was comparatively higher than 31 genera. The distribution of the top 30 genera was demonstrated in Fig. 3.

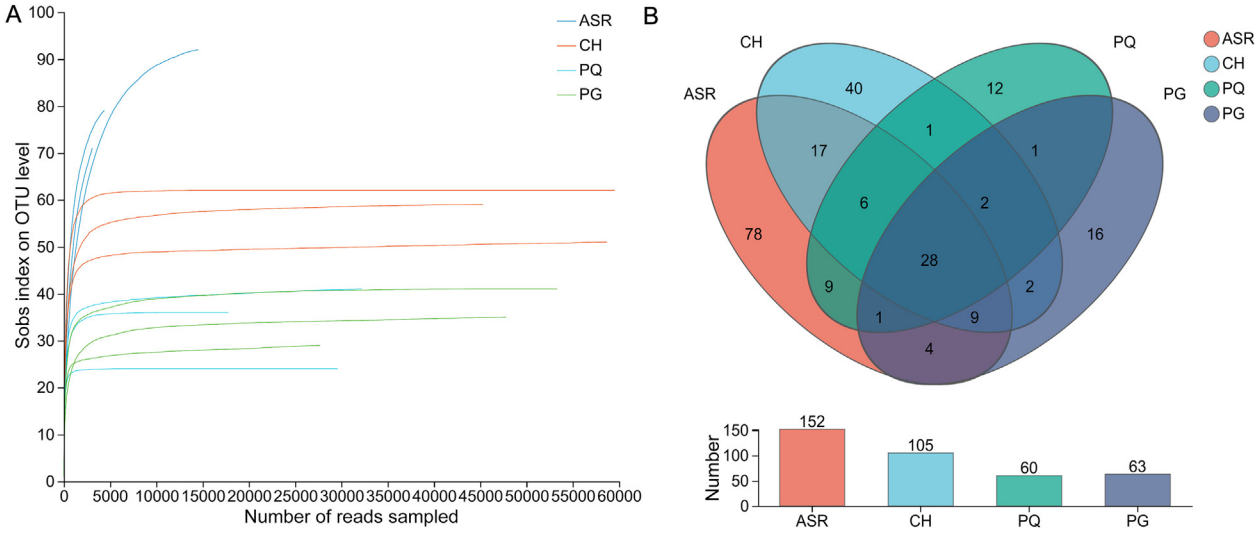

Fig. 1. Fungal diversity of 12 root samples. (A) Rarefaction curves for OTU. (B) Venn diagram of OTUs.

Table 2
Alpha diversity of fungal community in 12 root samples.

Estimators	ACE	Chao1	Shannon	Simpson	Coverage/%
ASR1	106.62	106.27	0.87	0.64	99.99
ASR2	100.18	99.80	0.40	0.87	99.98
ASR3	102.98	99.15	0.36	0.88	99.96
CH1	97.22	96.50	2.44	0.19	100.00
CH2	82.00	82.00	2.76	0.13	100.00
CH3	66.91	67.00	2.61	0.13	100.00
PG1	50.00	50.00	1.84	0.36	100.00
PG2	48.35	47.33	1.55	0.42	100.00
PG3	37.73	36.50	1.81	0.29	100.00
PQ1	54.59	53.50	2.02	0.25	100.00
PQ2	0.00	29.00	2.04	0.23	100.00
PQ3	45.21	45.00	1.54	0.41	100.00

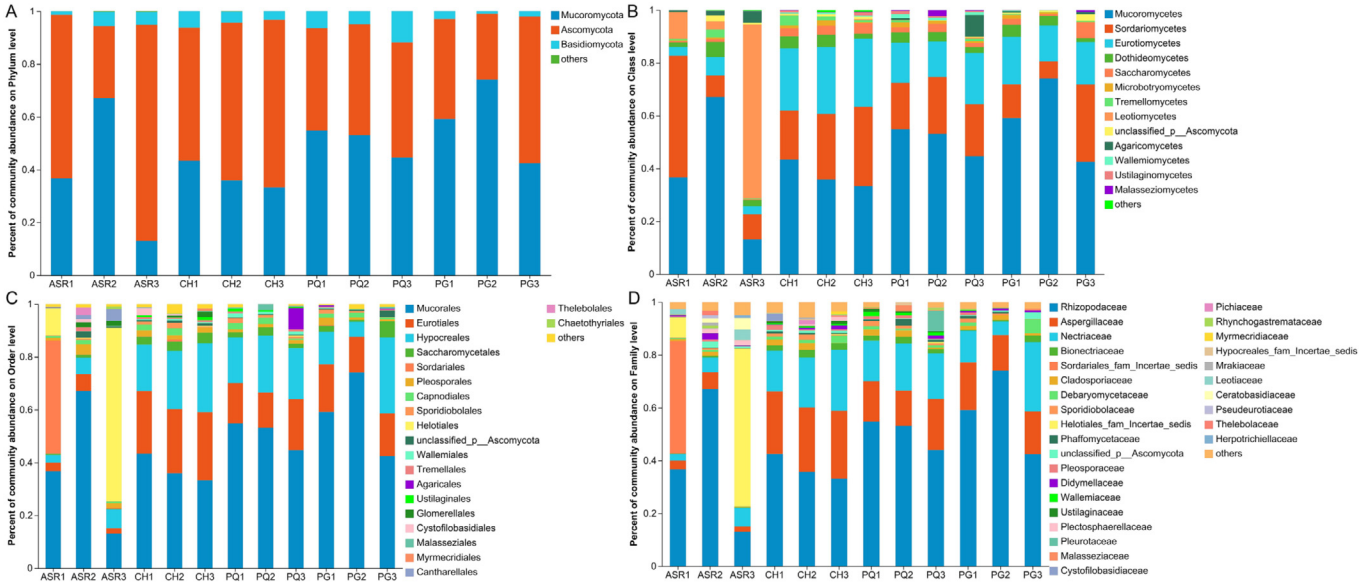

Fig. 2. Percentage of community abundance at phylum (A), class (B), order (C), and family (D) levels in12 root samples.

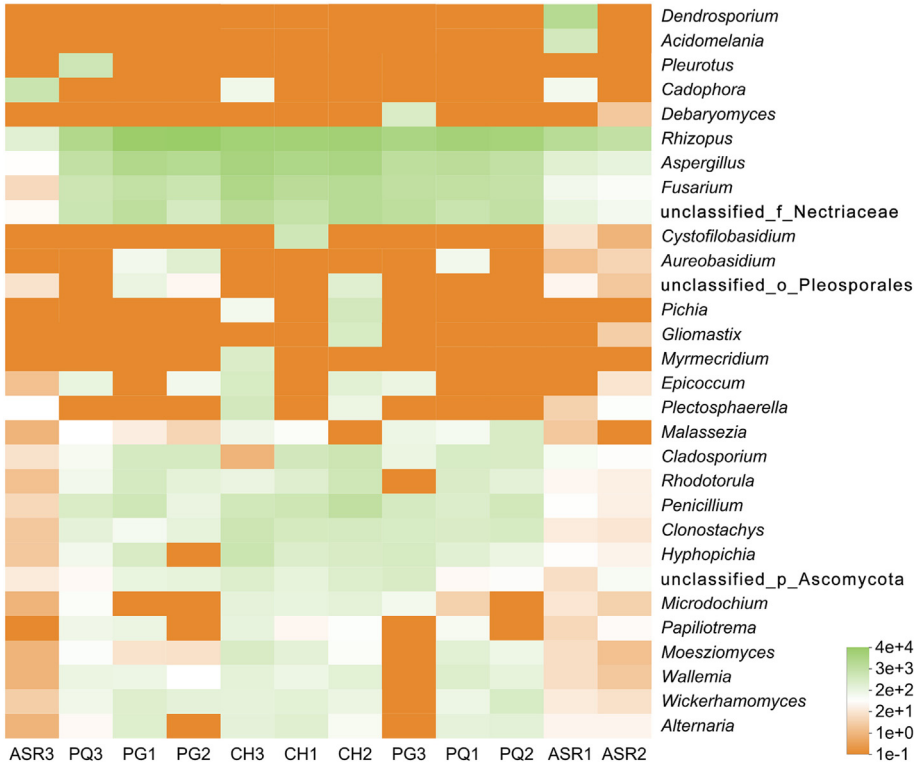

Fig. 3. Heatmap of top 30 abundant genera in root samples.

Rhizopus, *Aspergillus*, *Fusarium*, and *Penicillium* were distributed in all herbal samples. Only ASR1 found the occurrence of *Dendrosporium* and *Acidomelania*, and *Pleurotus* only distributed in PQ3.

Additionally, a total of 44 OTUs could be identified at the species level among the 226 OTUs via manual BLAST search. There were ten important fungi investigated in 12 root samples, including two potential toxigenic fungi (*Penicillium citrinum* and *Penicillium oxalicum*) and eight human pathogenic fungi (*Alternaria infectoria*, *Candida sake*, *Hyphopichia burtonii*, *Malassezia globosa*, *Malassezia restricta*, *Rhizopus arrhizus*, *Rhodotorula mucilaginosa*, and *Ochroconis tshawytschae*).

3.3. Fungal comparison in four root herbs

The ACE index represented that the richness of the ASR group was the highest, followed by CH, PG, and PQ groups (Fig. 4A). The observed species in the CH group were significantly higher than that in the PG group ($P < 0.05$). Moreover, in terms of the

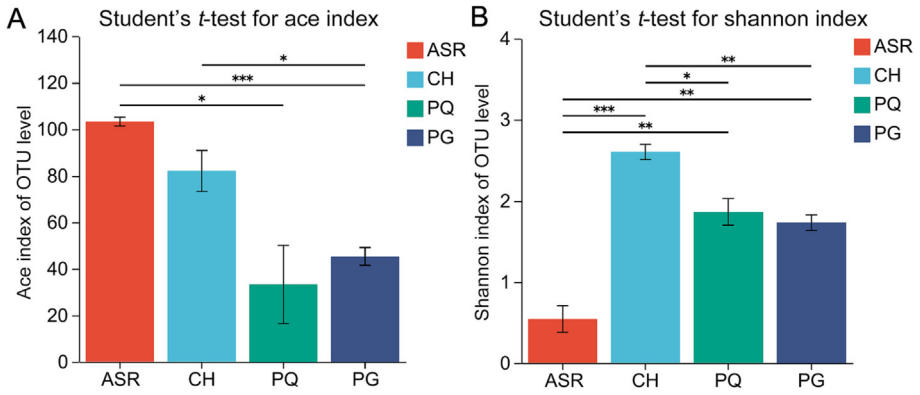

Fig. 4. Differences in ACE (A) and Shannon (B) indices in 12 root samples. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

Shannon index, the fungal diversity of the CH group was the highest (Fig. 4B). The Shannon index of PQ group was much higher than the ASR group ($P < 0.01$). Meanwhile, the diversity in ASR group was significantly lower than that in PG group ($P < 0.01$). The PCoA analysis was conducted to illustrate the beta diversity of four groups at the genus level (Fig. 5A). The result indicated that ASR and CH groups distinguished from other groups, while the differences between PQ and PG groups were small based on the type of root herbs. Meanwhile, a similar result was shown in the NMDS analysis, which was performed at the OTU level (Fig. 5B). ASR and CH groups were distinguishable from the other groups.

The study compared the differences in the fungal structure of four groups from the genus level to the phylum level (Fig. 6A). Leotiomycetes in ASR group had the highest average percentage of community abundance. At the family level, the relative abundances of Piskurozymaceae and Plectosphaerellaceae in ASR group were significantly higher than that in other groups. It was observed that the relative abundance of *Solicoccozyma* was much higher in

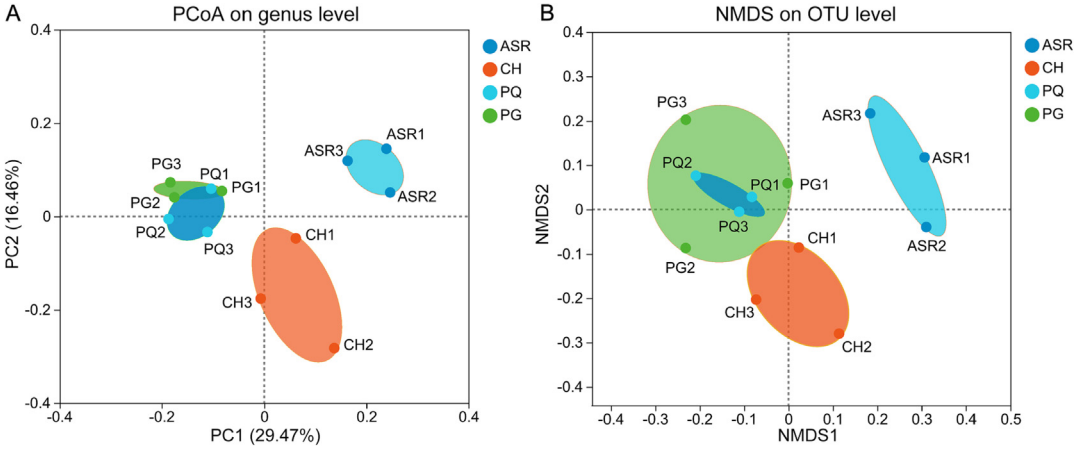

Fig. 5. Comparison of fungal community in different groups. (A) Principal coordinate analysis (PCoA) plots of fungal compositions at the genera level. (B) NMDS diagram estimated at the OTU level.

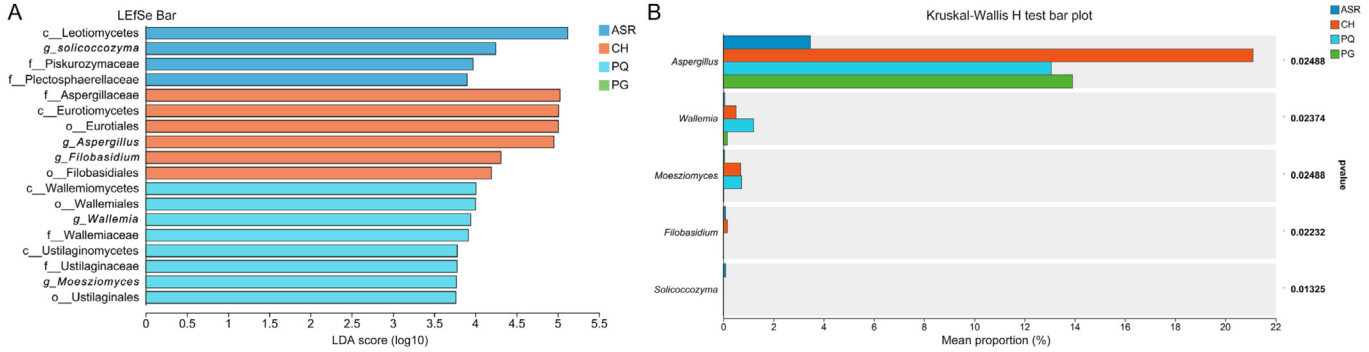

Fig. 6. Significant difference of 12 root samples. (A) Hierarchical clustering based on β-diversity distance matrix analysis. (B) Relative abundances of fungal community at various taxonomic levels in four groups as visualized by LEfSe analysis.

ASR group than in other groups. The relative abundances of Eurotiomycetes, Eurotiales, Filobasidiales, Aspergillaceae, *Aspergillus*, and *Filobasidium* in CH group were significantly higher than that in other groups at the class, order, family, and genus level. Furthermore, the PQ group had the highest abundances of Wallemiomycetes, Ustilaginomycetes, Wallemiales, Ustilaginales, Wallemiaceae, Ustilaginaceae, *Wallemia*, and *Moesziomyces* at the class, order, family, and genus level. The statistical analysis was calculated by the Kruskal-Wallis H test to compare the significant difference in fungal composition of 12 root samples from four types of root herbs (Fig. 6B). The results were similar to the LefSe analysis. The relative abundances of *Wallemia* and *Moesziomyces* were much higher in the PQ group (P = 0.023 74, P = 0.024 88). It was illustrated that CH group had the highest abundances of *Aspergillus* and *Filobasidium* (P = 0.024 88, P = 0.022 32). In addition, *Solicoccozyma* was much higher in ASR group (P = 0.013 25).

3.4. Co-occurrence analysis

The interaction between fungal genera in four groups was analyzed to reveal the community diversity (Fig. 7). The top 20 fungal genera belonged to three phyla, namely Ascomycota, Basidiomycota, and Mucoromycota. There were 57 correlations detected in 12 root samples. As the dominant genus, *Rhizopus* was positively correlated with *Aspergillus*, *Cladosporium*, *Rhodotorula*, and *Wickerhamomyces*. *Penicillium* displayed positive correlation with *Cladosporium*, *Rhodotorula*, *Aspergillus*, *Wallemia*, *Clonostachys*, and *Fusarium*. *Aspergillus* had positive correlation with *Rhodotorula*, *Alternaria*, *Wallemia*, *Clonostachys*, *Moesziomyces*, *Epicoccum*, *Plectosphaerella*, *Fusarium*, and *Hyphopichia*. *Fusarium* exhibited positive correlation with *Wallemia*, *Clonostachys*, *Moesziomyces*, *Epicoccum*, *Plectosphaerella*, and *Hyphopichia*. Moreover, *Alternaria*, belonging to Ascomycota, positively correlated with *Wallemia* and *Moesziomyces* belonging to Basidiomycota.

4. Discussion

4.1. Fungal community in four root herbs

The four root herbs have been used as important edible and medicinal materials in many countries, including China, America, and Korea. With the functions of promoting the body's circulation nourishment and repairing energy, ASR has been applied for the treatment of constipation and rheumatism in Asia (Chinese Pharmacopeia Commission, 2020). Recorded by *Shennong's Classic of Materia Medica* and *Compendium of Materia Medica*, CH could improve the activities of the kidneys, loins, and knees (Xu et al., 2008). Modern pharmacological studies reported that PG and PQ work on promoting healing, antitumor, and antiaging (Wang et al., 2020). Hundreds of researchers around the world considered that the two *Panax* species benefit blood vessels, immune, and central nervous systems (Holden, 2004). The numerous benefits of root herbs have led to a growing demand in both domestic and international markets. Nevertheless, some studies have indicated that fungal contaminations could affect herbal safety and quality (Ting et al., 2013). Chen et al. (2011) reported that 16 fungal species mainly from *Aspergillus*, *Fusarium*, *Penicillium*, and *Mucor* were detected in liquorice root, a medicinal material in China. In 2017, the study conducted by Zheng et al. showed that there were seven fungal species from *Aspergillus*, *Eurotium*, *Penicillium*, and *Fusarium* detected in three ASR samples. Besides, it was revealed that three PQ samples were contaminated by 11 fungal species belonging to *Penicillium* and *Cladosporium* (Zheng et al., 2017). In the present study, the results indicated that Mucoromycota, Mucoromycetes, Mucorales, and Rhizopodaceae were the most abundant at the phylum, class, order, and family levels in 12 root samples. *Rhizopus*, *Aspergillus*, and *Fusarium* were the dominant genera, with the relative abundance of 13.04%–74.03%, 1.76%–23.92%, and 0.26%–15.27%. *Rhizopus*, *Dendrosporium*, and *Cadophora* were the domi-

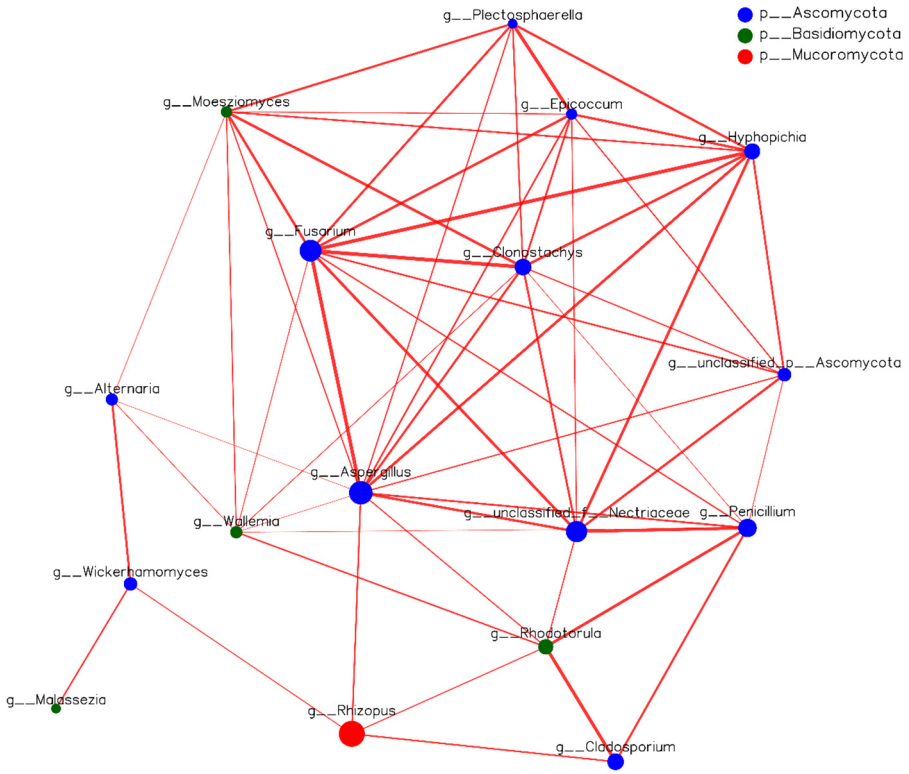

Fig. 7. Co-occurrence analysis of fungal members estimated of top 20 fungal taxa in root samples at genus level. The size and different colors of nodes represent abundance of species and different species, separately.

nant fungal genera in three ASR samples. In PQ, PG, and CH samples, *Rhizopus*, *Aspergillus*, and *Fusarium* were the most predominant genera. The relative abundance of *Rhizopus* was higher than that of other genera in all the four root herbs. A total of two potential toxigenic fungi and eight human pathogenic fungi were identified in the present study. *P. oxalicum* could produce aflatoxin B_1 and secalonic acid D under suitable conditions (Adegoke et al., 1993; Balasubramanian et al., 2000). These mycotoxins, strictly restricted in many countries, could greatly damage human and animal health. *P. citrinum* was the main producer of citrinin that was nephrotoxic and could affect the male reproductive system (Aydin et al., 2021). Moreover, the human pathogenic fungi could influence various important systems and functions of human. For example, *A. infectoria* was harmful for the human skin and immune function (Almeida et al., 2019; Kieselová et al., 2021). *C. sake*, a commonly pathogenic fungus, had a close relationship with the human immunodeficiency virus (HIV) (Hoegl, Schönian, Ollert, & Korting, 1998). *H. burtonii* could cause the peritonitis reported by (Chamroensakchai et al., 2021). *M. globose* and *M. restricta* were the major contributors of dandruff and seborrheic dermatitis (Dawson, 2007; Koga et al., 2020). *R. arrhizus* could induce angioinvasion (Li et al., 2021). *R. mucilaginosa* should be responsible for human chronic renal disease (Jarros et al., 2020). *O. tshawytschae* was verified as the cause of human subcutaneous phaeohyphomycosis (Ge et al., 2012). In addition, there were five plant pathogens identified in 12 root samples, namely *Stemphylium vesicarium*, *Ilyonectria robusta*, *Mycocentrospora acerina*, *Nigrospora oryzae*, and *Podosphaera leucotricha*. These pathogens are mainly related to root and leaf diseases, affecting plant growth and quality, and even leading to plant death. In 2020, investigations in New York and Arequipa considered that *S. vesicarium* was the primary culprit of the foliar disease in onion and *Medicago sativa* that was the local main cultivated crop (Díaz-Valderrama et al., 2020; Sharma, Hay, & Pethybridge, 2020). Zheng et al. (2021) found that *Codonopsis tangshen* Oliv., as a widely medical and agricultural herb, was affected by *I. robusta*, which caused the root rot disease (Zheng et al., 2021). In Spain, a report revealed that *I. robusta* was the main contributor to black foot disease of grapevine (Martínez-Diz et al., 2018). You et al. (2021) indicated that *M. acerina* could cause the leaf spot in *Panax japonicus* C. A. Mey, a medicinal herb (You et al., 2021). Besides, during the storage, carrots were susceptible *M. acerina*, which might lead its rot and even being discarding (Louarn et al., 2012). *N. oryzae* was the main contributor to the leaf spot in Asiatic dayflower and the wilt in Summer Cypress (Anjum et al., 2021; Qiu, Zhu, Niu, & Liu, 2021). The studies in 2020 and 2021 revealed that the powdery mildew in apple and pear trees, resulting large economic loss of commercial orchards, was caused by *P. leucotricha* (Gañán, White, Friesen, Peever, & Amiri, 2020; Gañán-Betancur, Peever, & Amiri, 2021). Therefore, it is necessary to supervise the fungal community in root herbs during the whole production process, so as to guarantee the quality and safety of medicinal materials.

4.2. Prospect of DNA metabarcoding in analyzing fungal community in herbs

DNA metabarcoding technology has been successfully applied in many fields such as food, soil, and environment so far (Chang et al., 2022; Ercolini, 2013; Guo, Jiang, Yang, Dou, & Pang, 2020). Compared with traditional culture-based identification methods, DNA metabarcoding overcomes some restrictions. It could efficiently identify multiple fungal species with low abundances in complex environment (Daniel, 2004). Besides, it exhibited remarkable ability in identifying fungi that fails to grow in traditional isolation culture. In recent years, DNA metabarcoding has been used to monitor the fungal contamination in the whole production chain of herbs. Wei et al. (2020) investigated the fungal community in 12 rhizospheric soil of *Salvia miltiorrhiza* samples through ITS sequencing. The result showed that *Aspergillus* and *Candida* were detected significantly with high relative abundances. Rasmussen et al. (2018) analyzed the roots and root-associated soil from *Plantago lanceolata* plants by targeting ITS sequence. The report indicated that fungal community structures were much similar between roots and root-associated soil, including fungal genera and shared OTUs. Guo, Jiang, Yang, Dou, & Pang, 2020 compared the fungal community between the raw and roasted *Cassiae Semen* (Juemingzi in Chinese) samples through high-throughput sequencing. The relative abundance of *Penicillium* and *Periconia* were higher in roasted samples compared with the raw samples. Wei et al. (2019) reported the fungal diversity in *Magnoliae Officinalis Cortex* (Houpu in Chinese) during the "sweating" process by targeting ITS1 region (Wei et al., 2019). At the beginning of "sweating", the dominant fungi were not obvious. *Candida* and *Aspergillus* were the predominant fungal species during the medium and last stage of "sweating", respectively. Yang et al. (2021) applied high-throughput sequencing to investigate the microbial community in *Citri Reticulatae Pericarpium* (Chenpi in Chinese) during three-year aging process by targeting ITS1 sequence. At the early stages, *Aspergillus* was not detected. The relative abundance of *Aspergillus* gradually increased at the later stage of storage. Meng et al. (2019) detected the fungal community in Pheretima through traditional plate method and high-throughput sequencing. *Wallemia* was the most abundant among 127 detected fungal genera. The differences of fungal community in herbs during the storage could be observed through DNA metabarcoding (Ercolini, 2013). DNA metabarcoding has been demonstrated as an efficient method for detecting fungal contaminations in herbs, feathering broad application prospects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Adegoke, G., Akinnuoye, O., & Akanni, A. (1993). Effect of processing on the mycoflora and aflatoxin B1 level of a cassava-based product. *Plant Foods for Human Nutrition, 43*(3), 191–196.

Almeida, M. C., Antunes, D., Silva, B., Rodrigues, L., Mota, M., Borges, O., & Gonçalves, T. (2019). Early interaction of Alternaria infectoria conidia with macrophages. *Mycopathologia, 184*(3), 383–392.

Amato, K. R., Yeoman, C. J., Kent, A., Righini, N., Carbonero, F., Estrada, A., & Leigh, S. R. (2013). Habitat degradation impacts black howler monkey (Alouatta pigra) gastrointestinal microbiomes. *The ISME Journal, 7*(7), 1344–1353.

Anjum, R., Khan, I. A., Afzal, M., Yousaf, M., Razzaq, K., Kanwal, W., & Ahmad, J. (2021). First report of *Nigrospora oryzae* causing wilt in summer cypress (*Bassia scoparia*) in Pakistan. *Plant Disease, 105*(12), 4167.

Aydin, Y., Orta Yilmaz, B., Yildizbayrak, N., Korkut, A., ArabulKursun, M., Irez, T., & Erkan, M. (2021). Evaluation of citrinin-induced toxic effects on mouse Sertoli cells. *Drug and Chemical Toxicology, 44*(6), 559–565.

Balasubramanian, G., Hanumegowda, U., & Reddy, C. S. (2000). Secalonic acid D alters the nature of and inhibits the binding of the transcription factors to the phorbol 12-*O*-tetradecanoate-13 acetate-response element in the developing murine secondary palate. *Toxicology and Applied Pharmacology, 169*(2), 142–150.

Bittinger, K., Charlson, E. S., Loy, E., Shirley, D. J., Haas, A. R., Laughlin, A., & Bushman, F. D. (2014). Improved characterization of medically relevant fungi in the human respiratory tract using next-generation sequencing. *Genome Biology, 15*(10), 487.

Chamroensakchai, T., Kanjanabuch, T., Saikong, W., Panya, W., Thaweekote, S., Eiam-Ong, S., & Hyde, K. D. (2021). The first human report of Hyphopichia burtonii, initially misdiagnosed as sterile peritonitis in a patient on peritoneal dialysis. *Medical Mycology Case Reports, 33*, 26–29.

Chang, J. R., Yao, X. H., Zhang, X. W., Zhou, Y., Liu, C. J., & Liu, X. (2022). DNA barcoding sequences and cluster analysis of 10 species of Astragalus. *Chinese Traditional and Herbal Drugs, 53*(22), 7201–7206.
Chen, A. J., Huang, L. F., Wang, L. Z., Tang, D., Cai, F., & Gao, W. W. (2011). Occurrence of toxigenic fungi in ochratoxin A contaminated liquorice root. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 28*(8), 1091–1097.
Chen, J., Gao, W., Tang, D., Cai, F., & Yang, M. (2010). Investigation of fungal populations in seven ochratoxin A contaminated root herbs. *China Journal of Chinese Materia Medica, 35*(20), 2647–2651.
Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). Fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics (Oxford, England), 34*(17), i884–i890.
Chinese Pharmacopeia Commission (2020). *Pharmacopoeia of the People's Republic of China*. China Medical Science and Technology Press, Beijing.
Daniel, R. (2004). The soil metagenome–a rich resource for the discovery of novel natural products. *Current Opinion in Biotechnology, 15*(3), 199–204.
Dawson, T. L. Jr., (2007). Malassezia globosa and restricta: Breakthrough understanding of the etiology and treatment of dandruff and seborrheic dermatitis through whole-genome analysis. *Journal of Investigative Dermatology. Symposium Proceedings, 12*(2), 15–19.
Díaz-Valderrama, J. R., Casa-Coila, V. H., Sencia-Torres, V., Macedo-Valdivia, D., Zanabria-Gálvez, J., Baributsa, D., & Woloshuk, C. (2020). First report of Stemphylium leaf spot caused by *Stemphylium vesicarium* on alfalfa (*Medicago sativa*) in Peru. *Plant Disease, 105*(4), 1196.
Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics, 26*(19), 2460–2461.
Edgar, R. C. (2013). UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nature Methods, 10*(10), 996–998.
Ercolini, D. (2013). High-throughput sequencing and metagenomics: Moving forward in the culture-independent analysis of food microbial ecology. *Applied and Environmental Microbiology, 79*(10), 3148–3155.
Gañán, L., White, R. A., 3rd, Friesen, M. L., Peever, T. L., & Amiri, A. (2020). A genome resource for the apple powdery mildew pathogen *Podosphaera leucotricha*. *Phytopathology, 110*(11), 1756–1758.
Gañán-Betancur, L., Peever, T. L., & Amiri, A. (2021). No evidence of resistance to trifloxystrobin, triflumizole, and boscalid in *Podosphaera leucotricha* isolates from U.S. commercial apple orchards. *Plant Disease, 105*(9), 2356.
Ge, Y. P., Lv, G. X., Shen, Y. N., Li, M., Deng, S. W., De Hoog, S., & Liu, W. D. (2012). First report of subcutaneous phaeohyphomycosis caused by *Ochroconis tshawytschae* in an immunocompetent patient. *Medical Mycology, 50*(6), 637–640.
Guo, M. Y., Jiang, W. J., Luo, J. Y., Yang, M. H., & Pang, X. H. (2018). Analysis of the fungal community in Ziziphi Spinosae Semen through high-throughput sequencing. *Toxins, 10*(12), 494.
Hagberg, A., Swart, P., & S Chult, D. (2008). *Exploring network structure, dynamics, and function using NetworkX* (No. LA UR-08-05495; LA-UR-08-5495). Los Alamos National Lab.(LANL), Los Alamos, NM (United States).
Guo, M. Y., Jiang, W. J., Yang, M. H., Dou, X., & Pang, X. H. (2020). Characterizing fungal communities in medicinal and edible Cassiae Semen using high-throughput sequencing. *International Journal of Food Microbiology, 319* 108496.
Hoegl, L., Schönian, G., Ollert, M., & Korting, H. C. (1998). Candida sake: A relevant species in the context of HIV-associated oropharyngeal candidosis? *Journal of Molecular Medicine, 76*(1), 70–73.
Holden, C. (2004). The two faces of ginseng. *Science, 305*(5690), 1560.
Jarros, I. C., Veiga, F. F., Corrêa, J. L., Barros, I., Gadelha, M. C., Voidaleski, M. F., & Svidzinski, T. (2020). Microbiological and virulence aspects of Rhodotorula mucilaginosa. *EXCLI Journal, 19*, 687–704.
Jiang, W., Guo, M., Yang, M., Mantri, N., Chen, X., & Pang, X. (2020). High-throughput analysis of fungal communities in *Myristicae Semen*. *Food Science and Technology, 128*, 109499.
Kieselová, K., Gomes, T., Santiago, F., & Martinha, H. (2021). Emerging cutaneous phaeohyphomycosis caused by Alternaria infectoria. *Acta Medica Portuguesa, 34*(11), 774–778.
Koga, H., Munechika, Y., Matsumoto, H., Nanjoh, Y., Harada, K., Makimura, K., & Tsuboi, R. (2020). Guinea pig seborrheic dermatitis model of *Malassezia restricta* and the utility of luliconazole. *Medical Mycology, 58*(6), 820–826.
Krzywinski, M., Schein, J., Birol, I., Connors, J., Gascoyne, R., Horsman, D., Jones, S. J., & Marra, M. A. (2009). Circos: An information aesthetic for comparative genomics. *Genome Research, 19*(9), 1639–1645.
Li, D. M., Lun, L., Ge, J., Zhang, G. J., Li, X. L., & de Hoog, G. S. (2021). Case report: *Rhizopus arrhizus* rhino-orbital-cerebral mycosis and lethal midline granuloma: Another fungal etiological agent. *Frontiers in Medicine, 8*, 578684.
Louarn, S., Nawrocki, A., Edelenbos, M., Jensen, D. F., Jensen, O. N., Collinge, D. B., & Jensen, B. (2012). The influence of the fungal pathogen *Mycocentrospora acerina* on the proteome and polyacetylenes and 6-methoxymellein in organic and conventionally cultivated carrots (*Daucus carota*) during post harvest storage. *Journal of Proteomics, 75*(3), 962–977.
Magoc, T., & Salzberg, S. L. (2011). FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics (Oxford, England), 27*(21), 2957–2963.
Martínez-Diz, M. P., Díaz-Losada, E., Armengol, J., León, M., Berlanas, C., Andrés-Sodupe, M., & Gramaje, D. (2018). First report of *Ilyonectria robusta* causing black foot disease of grapevine in Spain. *Plant Disease, 102*(11), 2381.
Meng, J. Q., Li, H. X., Luo, X. M., Chen, X. F., Liu, C. S., & Yang, Y. J. (2019). Diversity of fungi on surface of Pheretima. *China Journal of Chinese Materia Medica, 44*(20), 4433–4438.
Qiu, C. D., Zhu, W. H., Niu, T. T., & Liu, Z. Y. (2021). *Nigrospora oryzae* causing leaf spot on Asiatic Dayflower in Chongqing. *China. Plant Disease, 106*(2), 763.
Raclariu, A. C., Heinrich, M., Ichim, M. C., & de Boer, H. (2018). Benefits and limitations of DNA barcoding and metabarcoding in herbal product authentication. *Phytochemical Analysis, 29*(2), 123–128.
Rasmussen, P. U., Hugerth, L. W., Blanchet, F. G., Andersson, A. F., Lindahl, B. D., & Tack, A. (2018). Multiscale patterns and drivers of arbuscular mycorrhizal fungal communities in the roots and root-associated soil of a wild perennial herb. *New Phytologist, 220*(4), 1248–1261.
Rocha-Miranda, F., & Venâncio, A. (2019). Mycotoxigenic fungi in plant-based supplements and medicines. *Current Opinion in Food Science, 30*, 27–31.
Sharma, S., Hay, F. S., & Pethybridge, S. J. (2020). Genome resource for two *Stemphylium vesicarium* isolates causing Stemphylium leaf blight of onion in New York. *Molecular Plant-Microbe Interactions, 33*(4), 562–564.
Sofowora, A. (1996). Research on medicinal plants and traditional medicine in Africa. *Journal of Alternative and Complementary Medicine, 2*(3), 365–372.
Stevic, T., Pavlovic, S., Stankovic, S., & Savikin, K. (2012). Pathogenic microorganisms of medicinal herbal drugs. *Archives of Biological Sciences, 64*(1), 49–58.
Su, C., Hu, Y., Gao, D., Luo, Y. I., Chen, A. J., Jiao, X., & Gao, W. (2018). Occurrence of toxigenic fungi and mycotoxins on root herbs from Chinese markets. *Journal of Food Protection, 81*(5), 754–761.
Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., & Abarenkov, K. (2014). Fungal biogeography. Global diversity and geography of soil fungi. *Science, 346*(6213), 1256688.
Ting, A., Chow, Y., & Tan, W. (2013). Microbial and heavy metal contamination in commonly consumed traditional Chinese herbal medicines. *Journal of Traditional Chinese Medicine, 33*(1), 119–124.
Wang, H., Qi, J., Xu, T., Liu, J. H., Qin, M. J., Zhu, D. N., & Yu, B. Y. (2010). Effects of storage condition factors on fungal invasion of *Radix Ophiopogonis*. *Journal of Agricultural and Food Chemistry, 58*(9), 5432–5437.
Wang, L., Huang, Y., Yin, G., Wang, J., Wang, P., Chen, Z. Y., & Ren, G. (2020). Antimicrobial activities of Asian ginseng, American ginseng, and notoginseng. *Phytotherapy Research, 34*(6), 1226–1236.
Wei, D., Wu, Q. H., Liu, Y. P., Tang, X. M., Ren, C. X., Chen, J., & Pei, J. (2019). Microbial community diversity and its characteristics in *Magnolia Officinalis Cortex* "sweating" process based on high-throughput sequencing. *China Journal of Chinese Materia Medica, 44*(24), 5405–5412.
Wei, X., Cao, P., Wang, G., & Han, J. (2020). Microbial inoculant and garbage enzyme reduced cadmium (Cd) uptake in *Salvia miltiorrhiza* (Bge.) under Cd stress. *Ecotoxicology and Environmental Safety, 192* 110311.
White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). *PCR Protocols*. Academic Press.
Xu, R., Chen, J., Chen, S. L., Liu, T. N., Zhu, W. C., & Xu, J. (2008). *Cistanche deserticola* Ma cultivated as a new crop in China. *Genetic Resources and Crop Evolution, 56*(1), 137–142.
Yang, F., Yang, D., Liu, S., Xu, S., Wang, F., Chen, H., & Liu, Y. (2021). Use of high-throughput sequencing to identify fungal communities on the surface of *Citri Reticulatae Pericarpium* during the 3-year aging process. *Current Microbiology, 78*(8), 3142–3151.
You, J., Tang, T., Wang, F., Guo, J., Duan, Y., & Guo, X. (2021). Occurrence of leaf spot caused by *Mycocentrospora acerina* on Zhujieshen (Panax japonicus C. A. Mey.) in China. *Plant Disease, 106*(6), 1754.
Yu, J., Guo, M., Jiang, W., Yang, M., & Pang, X. (2020). Assessment of the microbiome and potential aflatoxin associated with the medicinal herb *Platycladus orientalis*. *Frontiers in Microbiology, 11*, 582679.
Yu, J., Yang, M., Han, J., & Pang, X. (2021). Fungal and mycotoxin occurrence, affecting factors, and prevention in herbal medicines: A review. *Toxin Reviews*, 1–19.
Zheng, R. S., Wang, W. L., Tan, J., Xu, H., Zhan, R. T., & Chen, W. W. (2017). An investigation of fungal contamination on the surface of medicinal herbs in China. *Chinese Medicine, 12*, 2.
Zheng, Z., Cao, J., Li, Y., Luo, T., Zhu, T., Li, S., Liu, Y., Qiao, T., Yang, C., Qin, G., Jiang, Y., Yi, J., Xiang, L., Chen, X., & Han, S. (2021). Root rot of *Codonopsis tangshen* caused by *Ilyonectria robusta* in Chongqing. *China, Plant Disease, 106*(7), 1989.