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Original Article

DNA metabarcoding uncovers fungal communities in *Zingiberis Rhizoma*

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ABSTRACT

Objective: *Zingiberis Rhizoma* (ZR, Ganjiang in Chinese), also known as dried ginger, is a popular spice and medicinal herb that has been used for several thousand years. However, ZR is easily contaminated by fungi and mycotoxin under suitable conditions, and might be hazardous to the health and safety of consumers, thus concerns about the herb's safety have been raised. The aim of this study was to investigate the fungal community and the effects of collection areas and processing methods on the fungal community in ZR.

Methods: A total of 18 ZR samples were collected from four provinces of China, and the samples were divided into four groups based on collecting sites. Meanwhile, the samples collected in Sichuan Province, China were divided into three groups based on the processing methods. We employed the Illumina MiSeq PE300 platform and targeted the internal transcribed spacer 2 (ITS2) sequences to investigate fungal contamination in ZR samples, and the difference in fungal community among the groups of different collection sites and processing methods.

Results: All 18 samples were contaminated with fungi. Ascomycota was the dominant phyla, accounting for 34.46%–100% of the fungal reads. At the genus level, *Candida*, *Diutina*, and *Aspergillus* were the most dominant genera, with relative abundances of 0–98.37%, 0–99.82%, and 0–79.08%, respectively. Meanwhile, four potential toxigenic fungi and seven human pathogens were found. Furthermore, differences in the community composition of ZR samples from four collecting sites and three processing methods were observed.

Conclusion: DNA metabarcoding provides a novel insight into fungal community diversity in ZR samples, providing references to ensure the sustainable utilization and quality research of ZR.

1. Introduction

Herbs and spices are widely used in cooking because of their distinctive flavors and nutritional value, and their potentially therapeutic roles have been accepted by Canada, Japan, and other developed countries (Chen, 2023). Unfortunately, the quality and safety of herbs and spices are affected by fungi and mycotoxins. For example, Akiko and Mehta (2016) collected 63 samples of herbs and spices in India and showed that only five samples were free of fungal contamination, and 47% of samples exceeded the fungal load set by the World Health Organization. In Vietnam, it was found that fungi were isolated in 174 out of 505 samples, and aflatoxin B_1 (AFB_1) contamination was detected in 24 samples by high performance liquid chromatography (Tang, Loi, Dau, & Le, 2022). Fungal invasion and mycotoxin contamination in herbs and spices may occur in every step of the production chain, including processing, handling, transportation, and storage (Kabak, Dobson, & Var, 2006). Fungal contamination may not only reduce the medicinal and edible value of herbs and spices but may also lead to the production of mycotoxin, posing a potential threat to the health and safety of consumers (Thanushree, Sailendri, Yoha, & Moses, 2019). To date, at least 400 mycotoxins have been identified, of which aflatoxin (AF) and ochratoxin A (OTA) are the most common in spices and herbs (Khodaei, Javanmardi, & Khaneghah, 2021). The Agency for Research on Cancer (IARC) has assessed the carcinogenicity of AFs and OTA and classified them into groups 1 and 2B carcinogens, respectively. Therefore, monitoring fungi and mycotoxins in spices and herbs is necessary.

Zingiberis Rhizoma (ZR, Ganjiang in Chinese) is derived from the dried rhizome of *Zingiber officinale* Rosc. (Chinese Pharmacopeia Commission, 2020). ZR is not only a widely used edible spice but also a traditional herb with a long history of medicinal use. China is a major country in ginger producer and exporter of ginger, where ZR has been used for over 2 500 years (Madan, 2005). During processing, ZR can be converted into two forms of traditional Chinese

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medicine for clinical use: *Zingiberis Rhizoma Praeparatum* (ZRP, Paojiang in Chinese) and *Zingiberis Rhizome Carbonisata* (ZRC, Jiangtan in Chinese). ZRP, which is obtained by roasting ZR with sand until the surface is swollen and brown, is traditionally thought to relieve pain and warm the meridians to arrest bleeding. When stir-fried for extended periods, ZR displays a black surface and brown interior, producing ZRC, which has robust efficacy in arresting bleeding (Zhao, et al., 2010). Moreover, modern pharmacology has demonstrated that ZR possesses biological activities, including anti-inflammatory, immunomodulatory, antitumorigenic, antimicrobial, and antiviral effects (Chrubasik, Pittler, & Roufogalis, 2005). However, fungal invasion and mycotoxin contamination in ZR are frequently reported and might be hazardous to the health and safety of consumers. Jeswal and Kumar (2015) investigated fungal and mycotoxin contamination from Indian spices with LC-MS/MS methods and revealed that 16 out of 36 samples of ZR were contaminated with AF, and eight samples were positive for AFB_1. A literature review of spices contaminated by potentially toxic fungi in Saudi Arabia showed that ginger was the most contaminated sample, which had fungal counts exceeding 5 000 CFU/g (Hashem & Alamri, 2010). In Nigeria, Lippolis et al. (2017) investigated AFs and OTA in ZR, and the content of AFB_1 in 23% of samples exceeded the maximum level (5 μg/kg) recommended by the European Union. Meanwhile, Yang et al. (2017) studied the relationship between mycotoxins and active components in ZR, the results suggested that AFs or OTA accumulation was inversely correlated with the content of the four main active components in ZR infected with *Aspergillus flavus* and *A. carbonarius*, and new unknown components were generated. Therefore, to ensure the security and effectiveness of ZR and reduce economic losses, detecting fungi in ZR is necessary.

Traditionally, fungal identification in herbs relies on morphological characteristics and culturing techniques (Chen et al., 2023). These methods are time-consuming and laborious, many fungi cannot be cultured *in vitro*, and quantifying them accurately is impossible (Lievens, Rep, & Thomma, 2008). In recent years, DNA metabarcoding has emerged as an effective tool for studying fungal diversity in environmental samples (e.g., soil, air, and water) (Banchi, Pallavicini, & Muggia, 2020). DNA metabarcoding refers to the use of high-throughput sequencing to simultaneously identify multiple species present in a complex environmental sample through PCR (Taberlet, et al., 2012, Wang et al., 2023). The nuclear ribosomal nuclear transcribed spacer (ITS) region is a highly variable and informative marker for fungal identification (Lindahl, et al., 2013), and its use in DNA metabarcoding has been extensively validated. Despite the wide popularity of DNA metabarcoding in monitoring and uncovering microbial diversity from diverse environments, few researchers have applied DNA metabarcoding to detect fungal contamination in ZR.

In this study, we investigated fungal communities in ZR samples via DNA metabarcoding and analyzed differences among fungal communities of the ZR samples obtained from different collecting sites and processed through different methods. This study can provide a basis for the quality improvement and safe utilization of the production chain of ZR.

2. Materials and methods

2.1. Sample collection

A total of 18 batches of ZR samples were collected from major ZR production areas, including Yunnan, Guangxi, Shandong and Sichuan, China. Among the 18 ZR samples, 12 ZR samples were divided into four groups by collection areas: ZRYN, ZRGX, ZRSD and ZRSC. Moreover, the samples collected in the Sichuan Province were divided into three groups according to processing methods: ZRD, ZRP, and ZRC. Table 1 provides the details of each ZR sample.

2.2. DNA extraction and ITS2 amplicon sequencing

About 10 g of each ZR sample was placed in a 50 mL centrifuge tube, and 20 mL of phosphate-buffered saline (PBS) (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China) was added. The mixture was vortexed for 10 min and filtered through a single layer of sterile gauze. The filtrate was centrifuged at 12 000 r/min for 20 min. The total DNA extraction of ZR was conducted using EZNA® soil DNA kit (Omega Bio-Tek., Inc., Norcross, GA, USA). The quality and quantity of DNA were evaluated by using a Nanodrop 2 000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA) through 2% agarose gel electrophoresis. DNA of each sample was stored at −20 °C. The ITS2 region of fungi was amplified using the ITS3F (5′−GCATCGATGAAGAACGCAGC−3′) and ITS4R (5′−TCCTCCGCTTATTGATATGC−3′) primer pair (White, et al., 1990). The PCR conditions were as follows: 95 °C denaturation for 3 min, 30 cycles of 95 °C denaturation for 30 s, 54 °C annealing for 30 s, 72 °C extension for 45 s, and a final extension at 72 °C for 10 min. Finally, the sequencing was conducted on an Illumina PE300 platform (Illumina, San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw reads were uploaded to the Sequence Read Archive database of NCBI and the accession numbers of the ZR samples were SAMN34535655–SAMN34535672.

2.3. Statistical analysis

Raw ITS sequence data were demultiplexed and quality filtered using Fastp soft (v.0.19.6, https://github.com/OpenGene/fastp) and merged using FLASH (v.1.2.11, https://ccb.jhu.edu/software/FLASH/index.shtml) (Magoč & Salzberg, 2011) to obtain high-quality sequences. Then, after the removal of chimeras, the sequences were clustered into operational taxonomic units (OTUs) with 97% similarity through UPARSE (v.11, https://www.drive5.com/uparse/) (Edgar, 2010). Referring to the UNITE database (v.8.0) (Nilsson, et al., 2019), we annotated the OTUs at various levels, ranging from phylum level to species level. The alpha diversity of the samples was evaluated using five indicators: Shannon, Chao1, Sob, Simpson, and Good's coverage in MOTHUR (v.1.30.2, https://www.mothur.org/wiki/Download_mothur) (Schloss, et al., 2009). Based on the unweighted-Unifrac distance matrix, PCoA, and NMDS were used to estimate differences in community composition among different groups. Furthermore, we used the LEfSe algorithm (LDA score = 2.0) to compare differences in fungal composition among different ZR samples. To ensure 100% identification accuracy, we performed taxonomic verification of each OTUs via manual BLAST search in the International Nucleotide Sequence Database Collaboration. We used R (v. 3.3.1) to compute Veen and rarefaction curves (Jami, Israel, Kotser, & Mizrahi, 2013). Co-occurrence analysis using the NetworkX Python package revealed the genus-level interactions of the fungal communities among different processing groups (Hagberg, Swart, Chult, 2008).

3. Results

3.1. Analysis of fungal community diversity in ZR samples

For 18 ZR samples, a total of 1 006 431 ITS2 sequences were obtained, and the average sequence length was 314 bp. The sequences were clustered into 633 OTUs based on 97% similarity. Fig. 1A showed the number of different OTUs and shared OTUs in the various collection sites. A total of 54 OTUs were common to

Table 1
Sample information and GenBank accession numbers for ZR samples.

Sample No.	Collection location (China)	Group 1	Group 2	Process methods	Accesion No.
ZRYN1	Yunnan	ZRYN	/	Drying	SAMN34535655
ZRYN2	Yunnan	ZRYN	/	Drying	SAMN34535656
ZRYN3	Yunnan	ZRYN	/	Drying	SAMN34535657
ZRGX1	Guangxi	ZRGX	/	Drying	SAMN34535658
ZRGX2	Guangxi	ZRGX	/	Drying	SAMN34535659
ZRGX3	Guangxi	ZRGX	/	Drying	SAMN34535660
ZRSD1	Shandong	ZRSD	/	Drying	SAMN34535661
ZRSD2	Shandong	ZRSD	/	Drying	SAMN34535662
ZRSD3	Shandong	ZRSD	/	Drying	SAMN34535663
ZRSC1	Sichuan	ZRSC	ZRD	Drying	SAMN34535664
ZRSC2	Sichuan	ZRSC	ZRD	Drying	SAMN34535665
ZRSC3	Sichuan	ZRSC	ZRD	Drying	SAMN34535666
ZRP1	Sichuan	/	ZRP	Roasting	SAMN34535667
ZRP2	Sichuan	/	ZRP	Roasting	SAMN34535668
ZRP3	Sichuan	/	ZRP	Roasting	SAMN34535669
ZRC1	Sichuan	/	ZRC	Charring	SAMN34535670
ZRC2	Sichuan	/	ZRC	Charring	SAMN34535671
ZRC3	Sichuan	/	ZRC	Charring	SAMN34535672

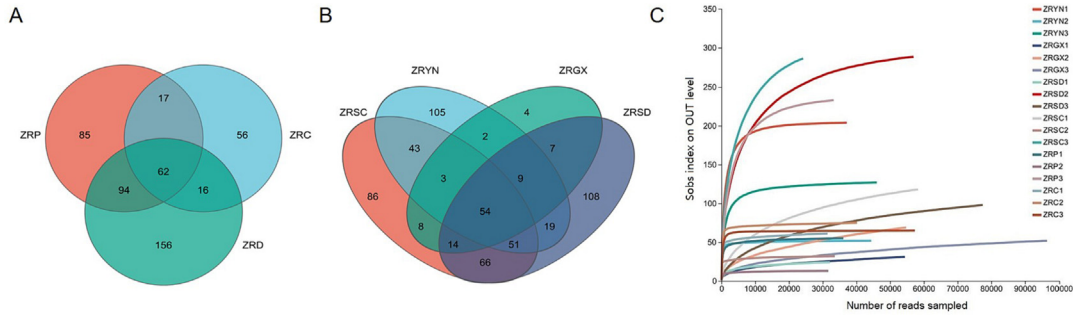

Fig. 1. Diversity analyses of fungal communities in ZR samples. Venn diagram depict different and common OTUs in different collecting sites (A) and processing methods (B). (C) Rarefaction curves of ZR samples.

four collection site groups and 86, 105, 4 and 108 OTUs were unique in the ZRSC, ZRYN, ZRGX and ZRSD groups, respectively. Moreover, 62 shared OTUs were observed in three groups based on processing methods, accounting for 12.76% of the total OTUs. The number of unique OTUs was 85, 56 and 156 for ZRP, ZRC, and ZRD, respectively (Fig. 1B). Alpha diversity can reveal the richness, coverage and diversity of the fungal community in ZR (Table S1). The Good's coverage was over 99%, indicating sufficient sampling depth for all ZR samples. The Sob and Chao1 indices in ZRSD2 were the highest, demonstrating that these samples had the highest fungal community richness. Similarly, the highest Shannon and lowest Simpson indices were observed in ZRC2, indicating that these samples featured the highest fungal community diversity. The rarefaction curve of the sobs indexs nearly reached a plateau, indicating the adequacy of sequencing depth (Fig. 1C).

3.2. Fungal community composition in ZR samples

The detected 633 OTUs were further assigned to eight phyla, 28 classes, 73 orders, 189 families, and 331 genera. At the phylum level, the dominant phylum was Ascomycota with a relative abundance of 34.46%–100%, followed by Basidiomycota (0–65.45%) and Mucoromycota (0–13.72%) (Fig. 2A). In addition, we found that the relative abundance of Ascomycota in the ZRSD1 group was 100%, which was higher than that in the other groups. At the class level, Saccharomycetes was the most predominant (0–99.97%), followed by Eurotiomycetes (0–93.50%), and Dothideomycetes (0–80.85%) (Fig. 2B). The further identification efforts showed that Saccharomycetales-fam-Incertae-sedis was predominant, accounting for 0–95.09 % of the fungal reads (Fig. 2C). A total of 36 genera with relative abundance >3% were found, and their distribution in each sample was demonstrated in Fig. 2D. The predominant fungal genus comprised *Candida*, *Diutina*, and *Aspergillus*, with relative abundances of 0–98.37%, 0–99.82%, and 0–79.08%, respectively. As for the groups based on processing methods, the abundances of *Aspergillus*, *Alternaria*, and *Diutina* were highest at the genus level, accounting for 0–83.56%, 0–49.61%, and 0–57.64% of the fungal reads, respectively (Fig. S1).

In addition, among all the 633 OTUs, 42 fungal taxa were accurately identified at the species level through a manual BLAST search. Four potential toxigenic fungi and seven human pathogenic were found. *Aspergillus fumigatus* was detected in ZRC2, ZRP3, ZRSC1, ZRSC3, and ZRSD2. *Penicillium steckii* was detected in ZRGX1, ZRGX3, ZRP3, ZRSD2 and ZRSD3. *P. paxilli* was detected in ZRSD3 and ZRYN1. *Wallemia sebi* was detected in ZRC2, ZRP3, ZRSC3, ZRSD3, ZRSD3, ZRYN1, ZRYN2, ZRGX1, and ZRGX2. Meanwhile, seven human pathogens, *Schizophyllum commune*, *Candida tropicalis*, *Diutina catenulata*, *Arthrographis kalrae*, *Mucor circinelloides*, *Rhizopus arrhizus*, and *Kodamaea ohmeri* were identified in the ZR samples.

3.3. Comparison of fungal community in ZR samples

We collected 12 batches of ZR samples and grouped them into four groups (ZRSC, ZRYN, ZRGX, and ZRSD) according to the collection site. The species diversity of the ZRYN group was the highest, followed by the ZRSC, ZRSD and ZRGX groups. The Chao1 indices indicated that the species abundance of the ZRSC group was the highest. Beta diversity can reveal the similarities or differences in species diversity among samples. As illustrated in Fig. 3, the

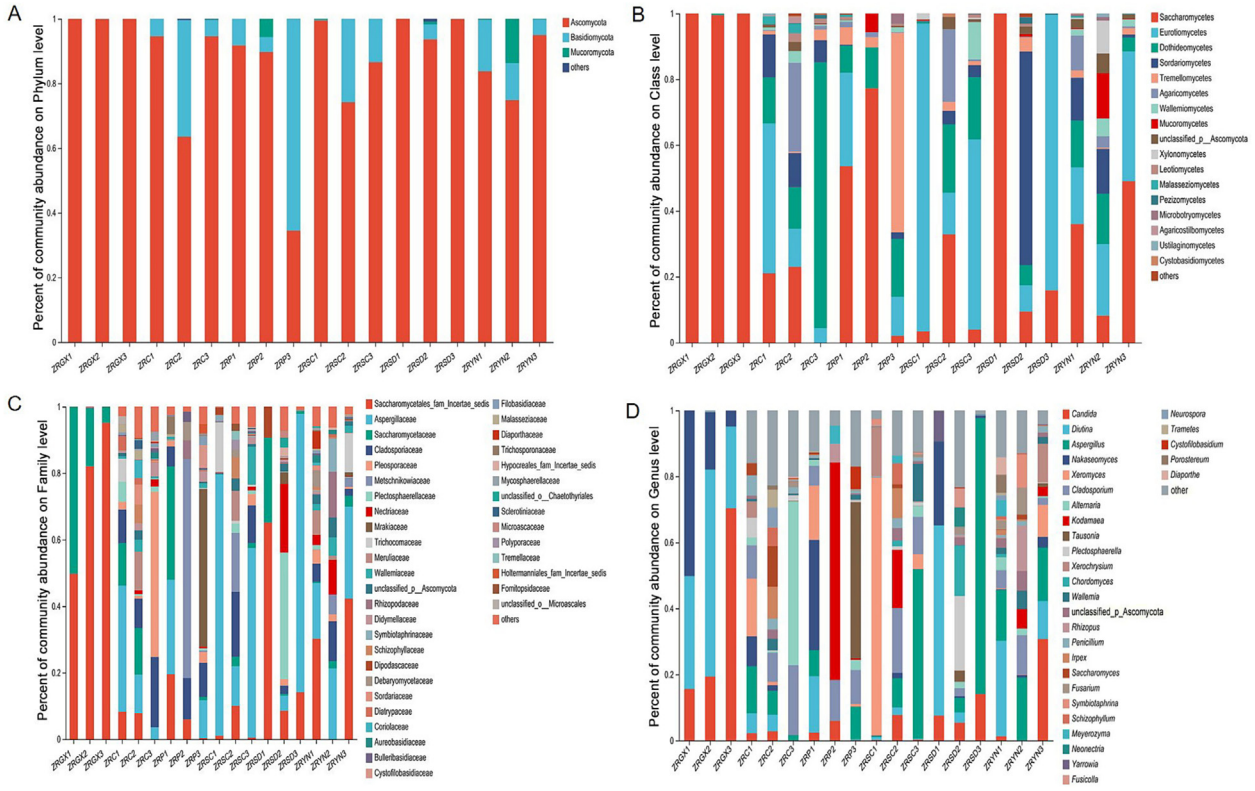

Fig. 2. Fungal community composition in 18 ZR samples at phylum (A), class (B), family (C) and (D) genus levels.

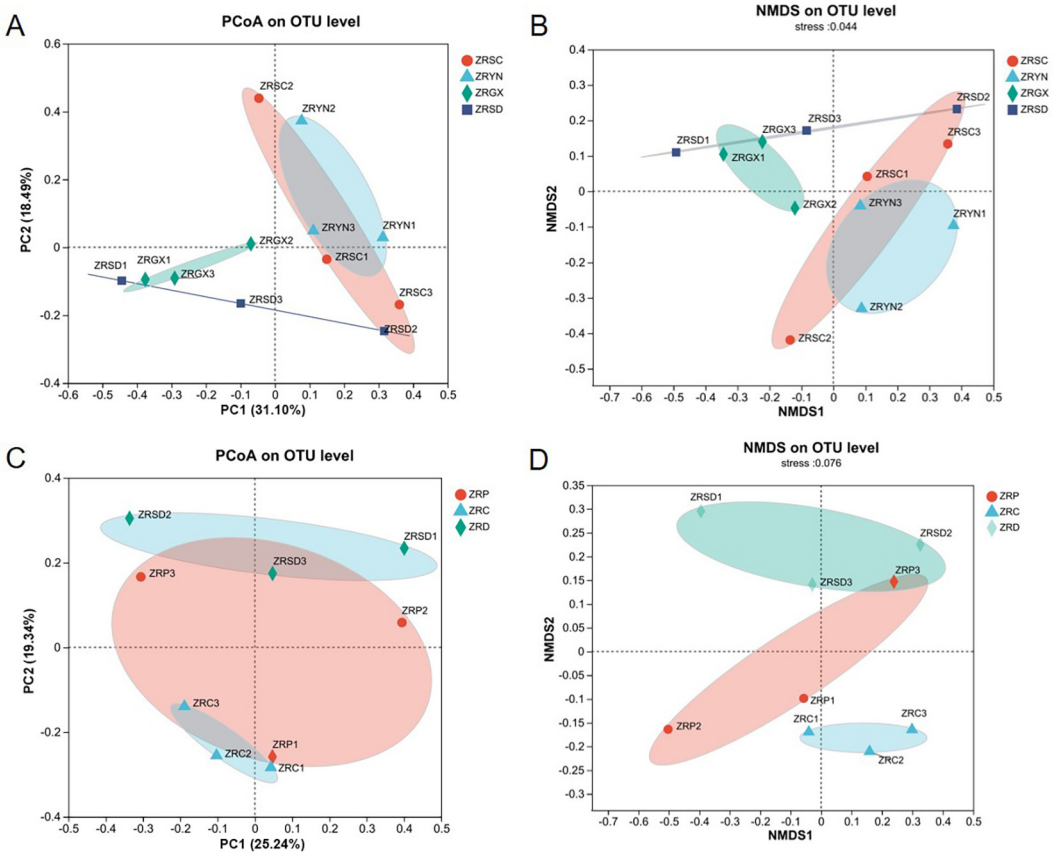

Fig. 3. Beta diversity analyses of fungal community in ZR samples. PCoA (A) and NMDS (B) plots at OTU level in groups based on collection sites. PCoA (C) and NMDS (D) plots at OTU level in groups based on processing methods.

proximity of samples on the PCoA (Fig. 3A) and NMDS (Fig. 3B) plots demonstrated that samples from the same collection site had similar levels of fungal diversity. LEfSe analysis showed no enrichment of fungal taxa in the ZRGX and ZRSD groups. In the ZRSC group, three families (Schizophyllaceae, Erythrobasidiaceae and Tremellaceae) and two genera (*Schizophyllum* and *Erythrobasidium*) were enriched. In the ZRYN group, the fungal taxon enriched was the genus (*Saccharomyces*) (Fig. 4A). Moreover, the Kruskal-Wallis H test indicated that *Schizophyllum* (P = 0.022) and *Erythrobasidium* (P = 0.013) in the ZRSC group were significantly more enriched than those in the other groups. Similarly, *Saccharomyces* in the ZRYN group were significantly more enriched (P = 0.042) than those in the other groups (Fig. 4B).

In the ZR samples from three different processing methods, the Chao1 and Shannon indices of the ZRD and ZRC groups were the highest, respectively. For beta analysis, PCoA and NMDS analysis showed that the ZRP samples were assigned to other groups (Fig. 3C and D), and LEfSe analysis showed no enriched fungal taxon in the ZRP group. The fungal taxa enriched in the ZRC group were the order Auriculariales and the genus *Penicillium*. The genus *Yarrowia* was the only fungal taxon enriched in the ZRD group (Fig. 4C). As shown in Fig. 4D, the relative abundance of *Penicillium*, which may produce toxic secondary metabolites, was significantly higher in the ZRC group than in the ZRD and ZRP groups (P = 0.039). By contrast, *Yarrowia* had significantly higher relative abundance in the ZRD group than in the other groups (P = 0.022).

3.4. Co-occurrence analysis

The relationships among the top 20 fungal genera were compared in ZR samples from different processing methods (Fig. 5). A total of 34 positive and 25 inverse correlations were found in the ZRP group, whereas the ZRC group had 23 positive and 17 inverse correlations. Meanwhile, 45 positive and 12 inverse correlations were recorded in the ZRD group. The *Aspergillus* was positively correlated with *Alternaria* and *Wallemia* and inversely correlated with *Candida* in the ZRP group. *Aspergillus* was positively correlated with *Xeromyces*, *Nakaseomyces*, and *Penicillium*, but negatively correlated with *Cladosporium* in the ZRC group. In the ZRD group, *Aspergillus*, which was the most abundant genus, was inversely correlated with *Yarrowia*. Furthermore, *Nakaseomyces* was inversely correlated with seven genera: *Plectosphaella*, *Dacyonectria*, *Cladosporium*, *Setophoma*, *Alternaria*, *Rhizopus*and *Fusarium*.

4. Discussion

4.1. Importance of detecting fungal contamination in ZR samples

ZR is a common and widely used spice and herb for over 2500 years in China. The pharmacological effects of ZR suggest that the herb can be used as an alternative agent for COVID-19 treatment (Jafarzadeh, Jafarzadeh, & Nemati, 2021; Tillu, Chaturvedi, Chopra, & Patwardhan, 2020). However, fungal contamination has been frequently reported in herbs in recent years. The safety of ZR has attracted extensive attention. Few reports have investigated the fungal contamination of dried ginger, especially by DNA metabarcoding technology. In this study, we assessed fungal communities in 18 ZR samples in China with the DNA metabarcoding technology. The results showed that Ascomycota, Eurotiomycetes, and Saccharomycetales-fam-Incertae-sedis were the most dominant among phylum, class, and family, respectively in 18 ZR samples. *Candida*, *Diutina* and *Aspergillus* were the dominant genera in the ZR samples. Spices and herbs are frequently contaminated by *Aspergillus*. It was found that *Aspergillus* is the dominant

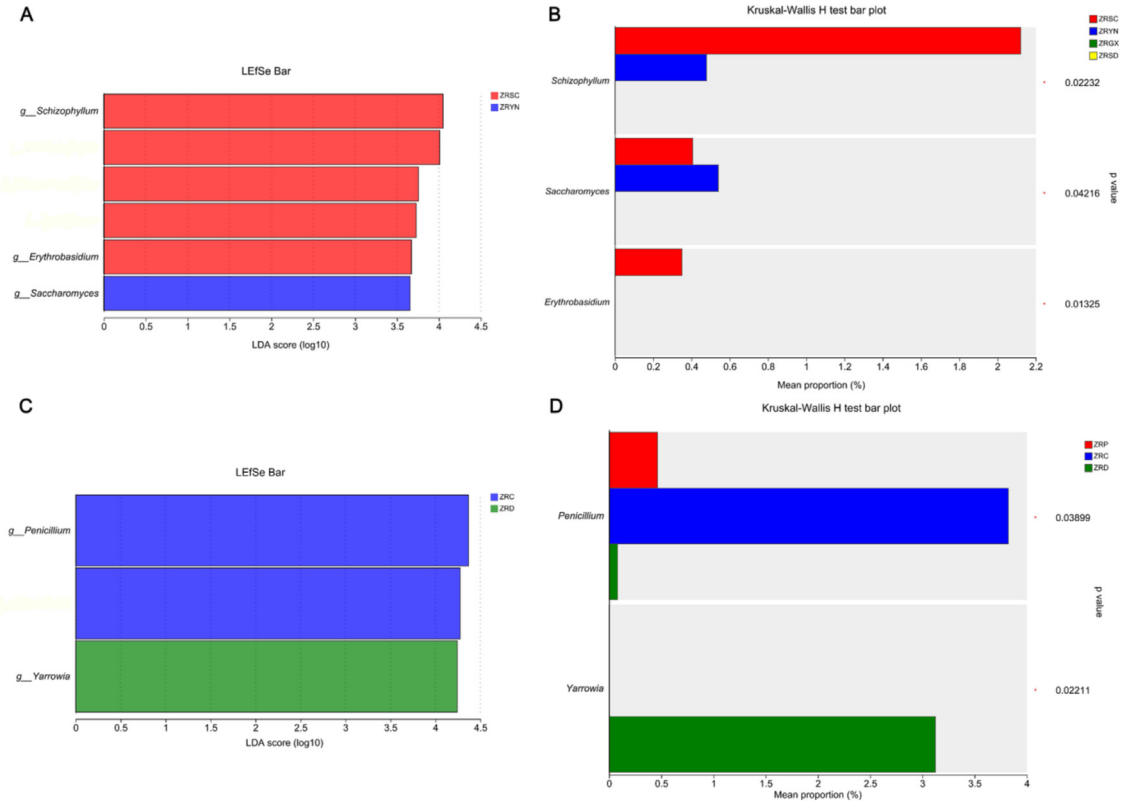

Fig. 4. Differences in relative abundances of fungal communities in different groups of ZR samples. Differentially abundant fungal taxa analysed by LEfSe (A) and significant differences at genus level generated via Kruskal-Wallis H test (B) among four collection sites. Differentially abundant fungal taxa analysed by LEfSe (C) and significant differences at genus level generated via Kruskal-Wallis H test (D) among three processing methods. $^{*}P < 0.05$.

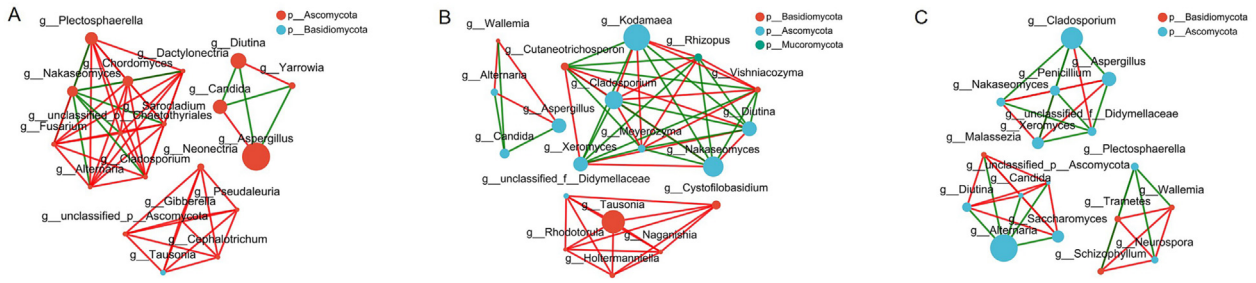

Fig. 5. Network analysis of fungal taxa in ZRD (A), ZRP (B) and ZRC (C) groups. Lines in red and green represent positive and negative correlations, respectively.

contamination fungi in *Polygoni Multiflori Radi* (Heshouwu in Chinese), which is a commonly utilized functional food and herb (Guo, Yu, Dao, Jiang, & Pang, 2020). In West Africa, Gnonlonfn et al. (2013) evaluated fungal contamination in 114 spice samples, and the results showed that the microflora in the spice samples was mainly composed of *Aspergillus*. Bugno et al. (2006) investigated fungal contamination in herbs and found that *Aspergillus* was the predominant microbiome and the detection rate was 90%. In the present research, *Aspergillus* was the common fungus, which was consistent with reports on many spices and herbs (Gnonlonfin, et al., 2013; Hammami, et al., 2014; Mandeel, 2005).

In addition, we detected four potential toxigenic fungi, namely, *A. fumigatus*, *P. paxillin*, *P. steckii* and *Wallemia sebi*. *A. fumigatus*, which is a major cause of invasive aspergillosis (Latgé, 2001), can produce several toxic metabolites, including fumagillin, verruculogen, fumagillin, and helvolic acid (Boudra & Morgavi, 2005). It is a widespread fungus with high adaptation capacity due to its metabolic diversity, thermal tolerance, and ability to spread quickly (Alshareef & Robson, 2014; Guruceaga et al., 2019; Kwon-Chung & Sugui, 2013). Consistent with other studies, *A. fumigatus* was detected in five ZR samples. This species has been detected in many herbs containing fats and other nutrients, such as *Crataegi Fructus* (Shanzha in Chinese) (Yu, Guo, Jiang, Dao, & Pang, 2022), *Cassiae Semen* (Juemingzi in Chinese) (Guo, Jiang, Yang, Dou, & Pang, 2020), and *Myristicae Semen* (Roudoukou in Chinese) (Jiang, et al., 2020). *P. paxillin* and *P. steckii* which produce citrinin were detected in five and two samples, respectively. *Wallemia sebi* grows well in harsh environments and produces the highly toxic metabolite walleminol and walleminone (Jančič, et al., 2015). This fungus was detected in nine ZR samples. Given the damage caused by toxigenic fungi, monitoring and detecting the toxigenic fungi in ZR samples is of great importance for risk assessment.

4.2. Processing methods affecting fungal community structure in spices and herbs

The processing of traditional Chinese medicine is a characteristic of Chinese medicine; this is the standard practice before clinical use. The effects of processing methods on herbal medicinal products were reducing toxicity, changing chemical composition of medicinal products, increasing the solubility of the composition and physically changing the existing form of the composition, affecting excipients (Guo, Brand, & Zhao, 2015). Processing methods also change the fungal community structure of herbs. Different processing methods can be used to produce different types of processed ginger products from ZR, namely ZRP and ZRC. In our study, we compared the species abundance, composition, and diversity of fungi for ZR samples from raw and two different processing methods. The results revealed that the species abundance of the no-processing group was higher than that of the processing group consistent with the research on *Crataegi Fructus* (Yu, Guo, Jiang, Dao, & Pang, 2022) and black tea (Tong, et al., 2021). Moreover, processing methods can affect ZR fungal communities. At the genus level, *Yarrowia* was more common in the ZRD group, whereas *Penicillium* was more prevalent in the ZRC group. Various processing methods can significantly affect fungal growth and mycotoxin production. Zhang et al. (2020) investigated the contamination of toxigenic fungi during postharvest processing in *Polygalae Radix* (Yuanzhi in Chinese). The results showed that *Cladosporium* and *Fusarium* increased during sweating, and *Aspergillus* increased significantly during drying after processing. He et al. (2020) studied AF contamination and fungal community changes during the processing of *Polygalae Radix* and showed that the richness of fungi taxa was decreased, while the relative abundance of the storage fungus *Penicillium* significantly increased after the heart of the roots was discarded. In addition, Wei et al. (2019) analyzed the microbial community diversity in the *Magnoliae Officinalis Cortex* (Houpo in Chinese) "sweating" process and found that the dominant flora varied by stage of "sweating," In the early stage of "sweating", the dominant fungi genera were not obvious. By contrast, in the middle and late stages of "sweating", *Candida* and *Aspergillus* were the dominant fungi, respectively. The fungal communities in *Crataegi Fructus* samples processed by drying, roasting, or charring. The result showed that five genera, including *Alternaria* spp., were more common in the dried group than in the roasted and charred groups (Yu, Guo, Jiang, Dao, & Pang, 2022). Therefore, investigating the relationship between processing methods and fungal communities provides a reference for industrial processors to establish standard processing parameters.

5. Conclusion

In this study, we employed DNA metabarcoding to investigate the diversity and composition of fungal communities in ZR. We discovered that 18 ZR samples analyzed were contaminated with fungi. Among the 42 species identified, four were potential mycotoxin producers, namely *A. fumigatus*, *P. steckii*, *P. paxillin*, and *Wallemia sebi*. *Candida*, *Diutina*, and *Aspergillus* were the top three genera. Meanwhile, significant differences in microbial communities were observed among the different collecting sites and processing methods. The study provides an early warning for the safe use of ZR and lays the foundation for future targeted prevention and control of fungal and mycotoxin contamination in spices.

CRediT authorship contribution statement

Chune Fan: Methodology, Formal analysis, Data curation, Writing – original draft. **Yanan Xu:** Formal analysis. **Yufeng Li:** Formal analysis. **Meihua Yang:** Methodology. **Jianping Han:** Methodology. **Xiaohui Pang:** Conceptualization, Validation, Methodology, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Funding acquisition.

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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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chmed.2023.12.001.

References

Aiko, V., & Mehta, A. (2016). Prevalence of toxigenic fungi in common medicinal herbs and spices in India. *3 Biotech, 6*(2), 159.

Alshareef, F., & Robson, G. D. (2014). Prevalence, persistence, and phenotypic variation of *Aspergillus fumigatus* in the outdoor environment in Manchester, UK, over a 2-year period. *Sabouraudia, 52*(4), 367–375.

Banchi, E., Pallavicini, A., & Muggia, L. (2020). Relevance of plant and fungal DNA metabarcoding in aerobiology. *Aerobiologia, 36*(1), 9–23.

Boudra, H., & Morgavi, D. (2005). Mycotoxin risk evaluation in feeds contaminated by *Aspergillus fumigatus*. *Animal Feed Science and Technology, 120*(1–2), 113–123.

Bugno, A., Almodovar, A. A. B., Pereira, T. C., Pinto, T. d. J. A., & Sabino, M. (2006). Occurrence of toxigenic fungi in herbal drugs. *Brazilian Journal of Microbiology, 37*, 47–51.

Chen, J. S. (2023). Essential role of medicine and food homology in health and wellness. *Chinese Herbal Medicines, 15*(3), 347–348.

Chen, M. Q., Wang, X., Li, J. Y., Tan, Q. W., Li, J. B., Yao, H. X., ... Deng, Z. J. (2023). Study of endophytic fungi diversity of Saxifraga stolonifera based on high-throughput sequencing method and tissue isolation method and their antioxidant activities. *Chinese Herbal Medicines, 54*(6), 1924–1934.

Chrubasik, S., Pittler, M., & Roufogalis, B. (2005). *Zingiberis Rhizoma*: A comprehensive review on the ginger effect and efficacy profiles. *Phytomedicine, 12*(9), 684–701.

Chinese Pharmacopeia Commission, 2020. Pharmacopoeia of the People's Republic of China. Part I. China Medical Science and Technology Press, 15–16.

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics, 26*(19), 2460–2461.

Gnonlonfin, G., Adjovi, Y., Tokpo, A., Agbekponou, E., Ameyapoh, Y., De Souza, C., Brimer, L., & Sanni, A. (2013). Mycobiota and identification of aflatoxin gene cluster in marketed spices in West Africa. *Food Control, 34*(1), 115–120.

Guo, M. Y., Jiang, W. J., Yang, M. H., Dou, X. W., & Pang, X. H. (2020). Characterizing fungal communities in medicinal and edible *Cassiae Semen* using high-throughput sequencing. *International Journal of Food Microbiology, 319*, 108496.

Guo, M. Y., Yu, J. S., Dao, Y. J., Jiang, W. J., & Pang, X. H. (2022). DNA metabarcoding of fungal communities in Heshouwu (*Polygonum multiflorum* Thunb.). *Food Control, 136*, 108850.

Guo, P., Brand, E., & Zhao, Z. Z. (2015). Chinese medicinal processing: A characteristic aspect of the ethnopharmacology of traditional Chinese medicine. *Journal of Ethnopharmacology, 26*, 303–316.

Guruceaga, X., Perez-Cuesta, U., Abad-Diaz de Cerio, A., Gonzalez, O., Alonso, R. M., Hernando, F. L., Ramirez-Garcia, A., & Rementeria, A. (2019). Fumagillin, a mycotoxin of *Aspergillus fumigatus*: Biosynthesis, biological activities, detection, and applications. *Toxins, 12*(1), 7.

Hagberg, A., Swart, P., & S Chult, D. (2008). Exploring network structure, dynamics, and function using NetworkX. In Proceedings of the 7th Python in Science Conference.

Hammami, W., Fiori, S., Al Thani, R., Kali, N. A., Balmas, V., Migheli, Q., & Jaoua, S. (2014). Fungal and aflatoxin contamination of marketed spices. *Food Control, 37*, 177–181.

Hashem, M., & Alamri, S. (2010). Contamination of common spices in Saudi Arabia markets with potential mycotoxin-producing fungi. *Saudi Journal of Biological Sciences, 17*(2), 167–175.

He, L., Bai, L., Shan, L. A., Yu, P. H., Zhang, L., Dou, X. W., & Yang, M. H. (2020). Variations in fungal microbiota and aflatoxin contamination during the processing of Yuanzhi, a traditional Chinese medicine. *Industrial Crops and Products, 152*, 112509.

Jafarzadeh, A., Jafarzadeh, S., & Nemati, M. (2021). Therapeutic potential of ginger against COVID-19: Is there enough evidence? *Journal of Traditional Chinese Medical Sciences, 8*(4), 267–279.

Jami, E., Israel, A., Kotser, A., & Mizrahi, I. (2013). Exploring the bovine rumen bacterial community from birth to adulthood. *The ISME Journal, 7*(6), 1069–1079.

Jančič, S., Nguyen, H. D., Frisvad, J. C., Zalar, P., Schroers, H. J., Seifert, K. A., & Gunde-Cimerman, N. (2015). A taxonomic revision of the *Wallemia sebi* species complex. *PLoS One, 10*(5), e0125933.

Jeswal, P., & Kumar, D. (2015). Mycobiota and natural incidence of aflatoxins, ochratoxin A, and citrinin in Indian spices confirmed by LC-MS/MS. *International Journal of Microbiology, 2015*, 1–8.

Jiang, W. J., Guo, M. Y., Yang, M. H., Mantri, N., Chen, X. Y., & Pang, X. H. (2020). High-throughput analysis of fungal communities in *Myristicae Semen*. *Food Science and Technology, 128*, 109499.

Kabak, B., Dobson, A. D., Var, I., & I. (2006). Strategies to prevent mycotoxin contamination of food and animal feed: A review. *Critical Reviews in Food Science and Nutrition, 46*(8), 593–619.

Khodaei, D., Javanmardi, F., & Khaneghah, A. M. (2021). The global overview of the occurrence of mycotoxins in cereals: A three-year survey. *Current Opinion in Food Science, 39*, 36–42.

Kwon-Chung, K. J., & Sugui, J. A. (2013). *Aspergillus fumigatus*-what makes the species a ubiquitous human fungal pathogen? *PLoS Pathogens, 9*(12), e1003743.

Latgé, J. P. (2001). The pathobiology of *Aspergillus fumigatus*. *Trends in Microbiology, 9*(8), 382–389.

Lievens, B., Rep, M., & Thomma, B. P. (2008). Recent developments in the molecular discrimination of formae speciales of *Fusarium oxysporum*. *Pest Management Science: Formerly Pesticide Science, 64*(8), 781–788.

Lindahl, B. D., Nilsson, R. H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., Kõljalg, U., Pennanen, T., Rosendahl, S., & Stenlid, J. (2013). Fungal community analysis by high-throughput sequencing of amplified markers–a user's guide. *New Phytologist, 199*(1), 288–299.

Lippolis, V., Irurhe, O., Porricelli, A. C. R., Cortese, M., Schena, R., Imafidon, T., Oluwadun, A., & Pascale, M. (2017). Natural co-occurrence of aflatoxins and ochratoxin A in ginger (*Zingiber officinale*) from Nigeria. *Food Control, 73*, 1061–1067.

Madan, M. S. (2005). *Production, marketing, and economics of ginger*. Ginger-The Genus Zingiber. Chemical Rubber Company Presss, 435-468.

Magoč, T., & Salzberg, S. L. (2011). FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics, 27*(21), 2957–2963.

Mandeel, Q. A. (2005). Fungal contamination of some imported spices. *Mycopathologia, 159*, 291–298.

Nilsson, R. H., Larsson, K. H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F. O., & Tedersoo, L. (2019). The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research, 47*(D1), D259–D264.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., & Robinson, C. J. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology, 75*(23), 7537–7541.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology, 21*(8), 2045–2050.

Tang, H. X., Cao, L. B., Dau, H. H., & Le, T. A. (2022). The presence of aflatoxin B1 and fungi in traditional drugs in Vietnam aflatoxin B1 and fungi in traditional drugs. *Acta Medica Iranica, 60*(4), 235–243.

Thanushree, M., Sailendri, D., Yoha, K., Moses, J., & Anandharamakrishnan, C. (2019). Mycotoxin contamination in food: An exposition on spices. *Trends in Food Science & Technology, 93*, 69–80.

Tillu, G., Chaturvedi, S., Chopra, A., & Patwardhan, B. (2020). Public health approach of ayurveda and yoga for COVID-19 prophylaxis. *Journal of Alternative and Complementary Medicine, 26*(5), 360–364.

Tong, W., Yu, J., Wu, Q., Hu, L. C., Tabys, D., Wang, Y. J., Wei, C. L., Ling, T. J., Ali Inayat, M., & Bennetzen, J. L. (2021). Black tea quality is highly affected during processing by its leaf surface microbiome. *Journal of Agricultural and Food Chemistry, 69*(25), 7115–7126.

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.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications, 18*(1), 315–322.

Yang, Z. X., Wang, H. W., Ying, G. Y., Yang, M. Y., Nian, Y. J., Liu, J. J., & Kong, W. J. (2017). Relationship of mycotoxins accumulation and bioactive components variation in ginger after fungal inoculation. *Frontier in Pharmacology, 8*, 331.

Yu, J. S., Guo, M. Y., Jiang, W. J., Dao, Y. J., & Pang, X. H. (2022). Illumina-based analysis yields new insights into the fungal contamination associated with the processed products of *Crataegi Fructus*. *Frontiers in Nutrition, 9*, 883698.

Zhang, X. M., Gao, W. W., Hao, Y. H., Jiao, X. L., Guo, B. L., Xue, Y. L., Bai, J. T., & Lei, Z. H. (2020). Investigation of aflatoxins and toxigenic fungi contamination during post-harvest processing of *Polygalae Radix*. *Chinese Herbal Medicines, 51*(24), 2851–2856.

Zhao, Z. Z., Liang, Z. T., Chan, K., Lu, G. H., Lee, E. L. M., Chen, H. B., & Li, L. (2010). A unique issue in the standardization of Chinese materia medica: Processing. *Planta Medica, 76*(17), 1975–1986.