

Diversity of fungal communities and changes in antibacterial activity of artificially culturable fungal secondary metabolites from diseased and healthy fruits of *Cinnamomum burmannii*

Wei Wang

South China Agricultural University

Teng Cai

Huazhong University of Science and Technology

Yuting Yang

South China Agricultural University

Hui Guo

Zhejiang University of Technology

Zhuo Shang

Shandong University

Hamza Shahid

South China Agricultural University

Yirong Zhang

South China Agricultural University

Sirun Qiu

South China Agricultural University

Xinnian Zeng

South China Agricultural University

Xiaoli Xu

South China Agricultural University

Yi Liu

South China Agricultural University

Ping Fang

South China Agricultural University

Ping Ding

Guangzhou University of Chinese Medicine

Ziling Mao

South China Agricultural University

Tijiang Shan (✉ tjshanscau@163.com)

South China Agricultural University

Research Article

Keywords: Fungal communities, Symbiotic fungi, *Cinnamomum burmannii*, Secondary metabolites, Antibacterial activity

Posted Date: November 14th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-2246960/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Abstract

Background

Studies on the function of endophytic fungal communities in healthy plant tissues are not new, while little is known about the functional changes of fungal communities in pathogenic plant tissues. Healthy fruits of *Cinnamomum burmannii* are an important medicinal resource, infection by phytopathogenic fungi causes changes in volatile components, with the corresponding, the functional characteristics and potential value of fungal communities in diseased fruits have not been reported. Consequently, characterization in the composition structure and antibacterial activity of fungal communities from healthy and diseased fruits of *C. burmannii* was investigated, the secondary metabolites in the fungus *Medicopsis romeroi* were reported for the first time.

Results

The fungal community in diseased fruits differed from that of healthy fruits at the Phylum, Class, Order or Genus levels, with important changes in the species and relative abundance of the dominant flora. Forty-one different strains (11 from healthy fruits and 30 from diseased fruits) were successfully identified by morphological and molecular biological methods which were classified into 8 groups and 23 genera using phylogenetic tree analysis, with Pleosporales, Glomerellales and Hypocreales were the dominant group at the phylum level and *Colletotrichum* was the dominant group at the genus level. The secondary metabolites of all strains had different degrees of antibacterial activity, while the secondary metabolites of diseased fruit symbiotic fungi were generally stronger than those of healthy fruits, with the active secondary metabolites dominated by small and medium polar compounds. Both the up-regulated and down-regulated flora in diseased fruit had strong antibacterial activity. Two new compounds, 5, 6-Dimethoxy-[1',1:4,1"-terphenyl]-2-ol (**1**), 5-(methoxycarbonyl)-2-methylbenzo[d][1, 3]dioxole-2-carboxylic acid (**2**) and three known compounds (**3**, **4**, **5**) were isolated and identified for the first time from the symbiotic fungus *Medicopsis romeroi*.

Conclusion

Although the diversity of fungal communities decreases after plant fruit disease, the antibacterial activity capacity of the fungi among them is generally enhanced, and the development of secondary metabolites of active strains from diseased fruits holds great promise. This study is significant for understanding the functional variation of bioactivity in fungal communities and developing a broader range of bioactive resources.

Introduction

Plant secondary metabolites and their derivatives are considered an important source for the development of new natural active pharmaceuticals and are essential alternatives to synthetic chemicals [1–2]. However, the yield of secondary metabolites extracted from plants is low, and a large quantity of plant materials is required in order to obtain larger amounts of chemical components. This extensive research may directly harm the ecosystem [3]. Plant symbiotic fungi are an underexploited resource with strong scientific novelty and potentially challenging active molecules [4]. In symbiotic fungi, endophytic fungi are organisms that reside in plant tissues without causing disease symptoms and have a significant impact on the host plant as they can increase plant resistance by producing a wide range of active macromolecules [5–7]. Symbiotic fungi such as *Aspergillus fumigatus*, *Rhizopycnis vagum*, *Diaporthe maritima*, and *Phialophora mustea* have been reported extensively to produce antimicrobial active compounds [8]. In addition, symbiotic fungi produce similar biological active secondary metabolites as the host plant [9]. The study of active secondary metabolites of plant symbiotic fungi is essential for the conservation of plant diversity and the environment. However, as the limitations of endophytic fungal secondary metabolites developed from traditional plant parts, increasing numbers of researchers are turning their attention to the development of promising endophytic microorganisms from specific environments or less studied plant parts [10].

Plant characteristics can have a significant impact on the diversity and community composition of symbiotic fungi [11–12]. The secretion of active secondary metabolites of symbiotic fungi is also altered with the aim of facilitating plant responses to these biotic or abiotic-like changes, such as pathogens, drought conditions and salinity [13]. A previous study showed different endophytic assemblages on yellowing and healthy leaves on citrus due to the possible effect of leaf yellowing on endophytic growth [14]. It indicates that the relationship between plants and symbiotic fungi is not static [15]. *Cinnamomum burmannii* (Nees and Nees) Blume is a small tree of the Lauraceae family called Indonesian cassia with a wide distribution in Vietnam, Indonesia, Philippines, Myanmar, India and China, can be used as a raw material for spices and is also considered a traditional herb in folklore for the treatment of several health disorders [16–18]. *C. burmannii* extracts have been demonstrated to possess antioxidant [19], anti-inflammatory [20], antibacterial [21], and anticancer activities [22]. The phytochemical composition of *C. burmannii* is diverse and rich in flavonoids and alkaloids [23]. The main constituents reported in the study are cinnamylalcohol, coumarin, cinnamic acid, cinnamaldehyde, anthocyanin, essential oils and other components. Prior studies have mostly developed active secondary metabolites from endophytic fungi in healthy plant tissues [24], ignoring the bioactive capacity of symbiotic fungal secondary metabolites in diseased tissues. Studies have suggested that while plant tissues are susceptible to invasion by other pathogens after disease, the endophytic flora of plants also undergo important functional changes, such as detoxification, biofilm formation and activation of microbiome-related signaling pathways [25], implying that symbiotic fungi in diseased plants may also be enriched with bioactive secondary metabolites. Studies on the pathogenic bacteria of powdery fruit have been discussed in previous studies, there were significant differences in the volatile components in diseased and healthy fruits due to the influence of pathogenic bacteria [26]. However, changes in microorganisms and their secondary metabolites in healthy and diseased fruit are not clearly evident.

In this study, we used healthy and diseased fruits of *C. burmannii* as subjects, firstly characterized the composition and structure of the endophytic fungal community in them by high-throughput sequencing, then isolated and identified 41 symbiotic fungi from healthy and diseased fruits, compared the antimicrobial activity capacity of secondary metabolites of symbiotic fungi from healthy and diseased fruits, combined with the results of phylogenetic tree analysis and fungal community change characterization, one active strain (Esf-14 *Medicopsis romeroi*) was screened, followed by two new compounds (containing a triptolide) and three known compounds were isolated and identified from it. The investigation of fungal community variation, culturable symbiotic fungal communities, and secondary metabolites from healthy and diseased plant fruits is important for fungal community function or to develop a wider spectrum of bioactive resources in the future.

Materials And Methods

Plant material

The plant material for this study was from the Huolu Mountain (23°19' N, 113°39' E, 600 ha, 321.8 m above sea level) in Guangzhou, Guangdong Province, southern region of China, which is located in a subtropical monsoon climate zone with an average annual temperature of 24°C and an average annual humidity of 72%-90%. Healthy and diseased *Cinnamomum burmannii* fruits were collected in the Huolu Mountain in 2016 (Figure S1). Three biological replicates each, for a total of six samples, were immediately placed in well-labeled sterile plastic bags, transported back to the laboratory in ice boxes, then stored at -80°C until used for DNA extraction. The isolated material of endophytic fungi was also collected by the same method described above, but with the difference that the strains were isolated immediately after shipment back to the laboratory.

DNA extraction and amplification sequencing

The preserved plant material was removed from -80°C, melted at room temperature, rinsed with tap water for 15 min, transferred to a sterile bench, treated with 70% anhydrous ethanol for 5 min, soaked in 2.5% NaClO solution (0.1% Tween 80) for 5 min, then treated with 70% anhydrous ethanol for 3 min, and finally washed three times with sterile water for 5 min each. After drying on sterile filter paper, the DNA of fruits was extracted using CTAB method [27]. PCR amplification of the ITS1-ITS2 regions of obtained DNA groups was performed using the fungal universal primers ITS1F: CTTGGTCATTTAGAGGAAGTAA and ITS2R: GCTGCGTTCTTCATCGATGC. The PCR amplification conditions were: pre-denaturation at 94°C for 30 s, annealing at 52°C for 45 s, extension at 72°C for 1 min, 35 cycles, extension at 72°C for 10 min, and storage at 4°C. The amplified products were detected by 2% agarose gel electrophoresis, and the qualified PCR products were further purified and quantified to obtain the sequencing library, which was sequenced by Shenzhen HENGCHUANG Gene Technology Co., Ltd. (China).

Bioinformatics analysis

Raw data obtained by the Illumina MiSeq/HiSeq sequencing platform needs to be pre-processed to filter low quality data. Firstly, the raw data were split using the Per script, and then the reads of each sample were spliced using FLASH (V1.2.7, <http://ccb.jhu.edu/software/FLASH/>) to obtain the raw Tags data (Raw Tags), and the raw data were processed using Quantitative Insights into Microbial Ecology (Qiime V1.9.0, <http://qiime.org/index.html>) to truncate Raw Tags from the first low quality site where the number of consecutive low quality values (default quality threshold ≤ 3) bases reaches a set length (default length value of 3). Further filter out the tags whose length of consecutive high quality bases is less than 75% of the Tags length. The Clean Tags sequence obtained after these processes is compared with the database (Gold database, http://drive5.com/uchime/uchime_download.html) (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html) to detect chimeric sequences, and after removing the chimeras, effective tags were finally obtained. The valid sequences of all samples were computed using Uparse software (Uparse v7.0.1001, (<http://drive5.com/uparse/>)) to classify operable classification units at 97% threshold to form OTUs. Representative OTUs were classified by Qiime software combined with RDP Classifier method. The representative OTUs were compared with GreenGene, RDP, Silva and Unite databases to obtain species annotation information (the confidence threshold was 0.8 or higher by default). The proportion of annotations and the relative abundance of species at each taxonomic level of OTUs were counted using the R software. The effective sequences of all samples were computed using Uparse software (Uparse v7.0.1001, (<http://drive5.com/uparse/>)) at 97% threshold to classify operable taxonomic units to form OTUs. representative OTUs were compared by Qiime software combined with RDP Classifier method. The representative OTUs were compared with GreenGene, RDP, Silva and Unite databases to obtain species annotation information (the confidence threshold was 0.8 or higher by default). The proportion of annotations and the relative abundance of species at each taxonomic level of OTUs were counted using the R software. Phylogenetic analysis of OTU was performed using KRONA software, QIIME software, and Perl scripts. Heat map of relative abundance at the genus level using R software, along with inter-sample and inter-species clustering analysis. QIIME software was used to calculate four diversity indices and their Rarefaction Curve (Observed species, Chao, Shannon and PD whole tree), among which the PD Whole Tree index needed OTU's phylogenetic Tree data. Rank Abundance was also established with QIIME software at the same time. Three kinds of beta diversity distances (Bray Curtis, Weighted Unifrac, Unweighted Unifrac) were calculated by QIIME software, and heat mapping of beta diversity distance was completed by using native Perl and SVG software. PCA and NMDS were analyzed with R software. Cluster tree of samples was established by using QIIME software with the method of UPGMA. Differences in alpha diversity indices between healthy and diseased fruits were determined using Student's *t*-test.

Isolation and culture of the endophytic fungi

The isolation of symbiotic fungi from the healthy and diseased fruit of *C. burmannii* was carried out according to the method used by Wang [28] with some modifications. The healthy and diseased fruit of *C. burmannii* was washed with running water, treated with 70% ethanol for 30 s and 0.2% mercury chloride for 20 min, later, washed with sterile water for 5 min and dried on sterile filter paper. The fruit was cut into pieces about 0.3 cm × 0.3 cm × 0.3 cm, and placed on PDA medium (streptomycin sulfate 500 µg/mL), 3

pieces for one dish and incubated on a constant temperature incubator at 25 °C for 30 days. Some mycelia were selected from the edge of the colony in the petri dish where mycelia grow vigorously, and then inoculated on PDA medium, repeated purification was carried out until the colony morphology was stable and consistent without major changes in this way. The purified strains were stored at 4 °C for further use.

Molecular biology identification of endophytic fungi

DNA sequences of isolates were extracted according to the fungal genomic DNA extraction kit method. ITS-rDNA was amplified by polymerase chain reaction (PCR) and the primers used for sequencing were common primers ITS4 (5'-TCCTCCGCTTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3'). The reaction system of PCR was as follows, 1 µL ITS4, 1 µL ITS5, 3 µL DNA extract, 25 µL PCR Master Mix and 20 µL sterile deionized water. The PCR amplification conditions was denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 1 min, it was extended at 72°C for 8 min and stored at 4°C finally. Later, the PCR products were sequenced and purified by the company (Shanghai Biological Engineering Co., China). DNAMAN software was used for complementation, primer sequences were added to both ends of complementary sequences of forward and reverse primers to form a complete sequence. In order to obtain the accession numbers, the sequences of ITS-rDNA were submitted to the Gen Bank database (National Center for Biotechnology Information website [<http://www.ncbi.nlm.nih.gov>]) and analyzed by the BLAST program. After MAFFT (version 7.0) processing, all sequences were analyzed by MEGA7 software with the maximum likelihood method.

Fungal material and preparation EtOAc extracts

Different crude extracts of isolates were prepared according to the previous report [29]. First of all, the selected isolates were inoculated into triangular bottles (50 mL) containing 20 mL potato glucose liquid medium (PDB), each fungus culture for two bottles. After that, all cultures were added into sterilized rice culture medium at 25°C for 60 days. Secondly, the fermentation product was soaked in methanol and extracted three times for seven days each time. Finally, the fermentation liquid was filtered and dried by vacuum with reduced pressure to prepare crude extract. The next step was to prepare ethyl acetate extract, the crude extracts were dissolved with water, extracted with equal volume of ethyl acetate for 3–5 times, and then decompressed and concentrated again to obtain crude extract of ethyl acetate, which was collected in a clean bottle and stored at 4–5°C for further used.

Antibacterial activity of EtOAc crude extracts

The antibacterial activity of ethyl acetate extracts of 30 endophytic fungi from diseased fruits and 11 endophytic fungi from healthy fruits was determined using the TLC-MTT bioautography method described by Ouyang et al. [29]. The formula $R_f = D_1/D_2$ was used to characterize the polar size of the active substance, while the diameter size of the inhibitory spots was measured [28].

Multigene identification of the endophytic fungus Esf-14

The endophytic fungus Esf-14 was identified as *Medicopsis* sp. according to the ITS-based phylogenetic tree analysis of 41 strains, further species identification of the strain was performed using ITS and LSU (LROR (5'-ACCCGCTGAACTTAAGC-3') and LR5 (5'-TCCTGA GGGAACTTCG-3')) genes. The sequences of ITS and LSU (*Medicopsis* sp.) were blasted separately in the NCBI nucleotide database and downloaded, other species of the same genus or similar species sequences were used as reference sequences, the combinatorial matrix-based comparison was then constructed using MAFFT version 7 (<https://mafft.cbrc.jp/alignment/software/algorithms/algorithms.html>) after merging with the sequenced sequences. The best tree building model was found using MEGA 7.0 (GTR + G + I), bootstrap method was used with 1000 bootstrap replicates.

Fermentation and extraction of endophytic fungus Esf-14

The crude extract of endophytic fungus Esf-14 was prepared and adopted the liquid shaking method. Firstly, some mycelia of endophytic fungus Esf-14 were selected from cryopreserved tube stored at 4°C and inoculated on PDA medium plate for activation culture for 5–7 days. After 4–5 days of subculture, their mycelia were inoculated in triangular flask (500 mL) with 200 mL PDB medium, a total of 100 L, shaken cultured for 7 days at 25°C and 150 rpm, and then stood for 21 days. After one month, the liquid and mycelium were separated, the liquid was extracted with ethyl acetate for three times to obtain the extract of liquid. While in order to obtain the mycelium extract, the mycelium was treated three times at -20°C, and then was extracted with ethyl acetate for three times (seven days each time). The liquid and mycelium extracts were dried and stored at 4°C for further use.

Isolation of secondary metabolites produced by endophytic fungus Esf-14

The EtOAc extract (12.37 g) was subjected to column chromatography (CC) over silica gel (200–300 mesh) eluted with a gradient of PE-EtOAc (100:0–0:100) to obtain ten main fractions (F1-8). F2 (1.26 g) was chromatographed over Sephadex LH-20 (eluted with CH₂Cl₂-MeOH, 1:1) to afford six subfractions, and subfraction F2-3 (38.9 mg) was further purified by semi-preparative HPLC (MeOH-H₂O, 60:40, 4 min/L) to yield compound **2** (1 mg, 19.537 min), and subfraction F2-5 (36 mg) was further purified by semi-preparative HPLC (MeOH-H₂O, 60:40, 4 min/L) to afford compound **1** (6.2 mg, 61.820 min). Similarly, F3 (145.9 mg) was fractionated by column chromatography over Sephadex LH-20 (eluted with CH₂Cl₂-MeOH, 1:1), followed by semi-preparative HPLC (MeOH-H₂O, 35:65, 4 min/L) to give compound **4** (4.1 mg, 32.815 min). F4 (408.6 mg) was separated by chromatography using a Sephadex LH-20 (eluted with CH₂Cl₂-MeOH, 1:1) to yield six subfractions. F4-6 (78.8 mg) was directly purified by semi-preparative HPLC (MeOH-H₂O, 50:50, 4 min/L) to afford compound **3** (37.2 mg, 37.083 min). F7 (260.9 mg) was fractionated by column chromatography over Sephadex LH-20 (eluted with CH₂Cl₂-MeOH, 1:1) twice, followed by semi-preparative HPLC (MeOH-H₂O, 10:90, 4 min/L) to yield Esf-36-5 compound **5** (2.7 mg, 5.495 min).

5, 6-dimethoxy-[1',1:4,1"-terphenyl]-2-ol (**1**): 14 – 9: Colorless waxy solid; HR-ESI-MS m/z 307.1331 $[M + H]^+$ (calcd for $C_{20}H_{19}O_3$, 307.1331), 329.1132 $[M + Na]^+$ (calcd for $C_{23}H_{26}O_7Na$, 329.1132); ^1H NMR (600 MHz, $CDCl_3$): δ_H 7.59 (m, 2H, H-2", 6"), 7.53 (m, 2H, H-3', 5'), 7.44 (m, 5H, H-2', 4', 6', 3", 5"), 7.36 (m, 1H, H-4"), 6.78 (s, 1H, H-6), 3.68 (s, 3H, OCH_3 -3), 3.60 (s, 3H, OCH_3 -4); ^{13}C NMR (151 MHz, $CDCl_3$): δ_C 151.44 (C-3), 149.13 (C-1), 144.56 (C-4), 138.10 (C-1"), 136.41 (C-5), 132.62 (C-1'), 130.58 and 128.39 (C-2', 6', 3", 5"), 129.46 (C-3', 5'), 129.34 (C-2", 6"), 128.50 (C-4'), 127.53 (C-4"), 122.45 (C-2), 112.07 (C-6), 61.17 (3- OCH_3), 61.04 (4- OCH_3).

5-(methoxycarbonyl)-2-methylbenzo[d][1, 3]dioxole-2-carboxylic acid (**2**): 14 – 10: δ_H 7.55 (dd, J = 1.8, 8.4 Hz, 1H, H-6), 7.34 (d, J = 1.2 Hz, 1H, H-4), 6.99 (d, J = 7.8 Hz, 1H, H-7), 3.80 (s, 3H, H-9), 1.80 (s, 3H, H-11). δ_C 167.36 (C-10), 165.56 (C-8), 151.30 (C-1), 147.49 (C-3), 124.83 (C-6), 123.28 (C-5), 113.57 (C-2), 108.25 (C-4), 107.97 (C-7), 52.01 (C-9), 21.85 (C-11).

3,7-dihydroxy-1,9-dimethyldibenzofuran (**3**) 14 – 4: white crystal; ^1H NMR (600 MHz, Acetone- d_6): δ_H 8.51 (s, 2H, OH-3, 7), 6.80 (s, 2H, H-4, 6), 6.64 (s, 1H, H-3), 2.80 (s, 6H, CH_3 -1, 9); ^{13}C NMR (151 MHz, Acetone- d_6): δ_C 158.71 (4a, 5a-C), 156.82 (3, 7-C), 132.81 (1, 9-C), 117.30 (9a, 9b-C), 114.90 (2, 8-C), 96.55 (4, 6-C), 24.98 (1, 9- CH_3). The ^1H NMR and ^{13}C NMR data were consistent with the literature [30].

Methyl 3-hydroxybenzoate (**4**) 14 – 7: Colorless waxy solid; ^1H NMR (600 MHz, DMSO): δ_H 9.82 (s, 1H, OH-1), 7.38 (dt, J = 1.8, 6.0 Hz, 1H, H-6), 7.34 (dd, J = 1.8, 2.4 Hz, 1H, H-2), 7.31 (t, J = 7.8 Hz, 1H, H-5), 7.02 (m, H-4), 3.82 (s, 3H, CH_3 -8); ^{13}C NMR (151 MHz, DMSO): δ_C 166.21 (C-7), 157.50 (C-1), 130.82 (C-3), 129.82 (C-5), 120.28 (C-4), 119.76 (C-6), 115.55 (C-2), 52.05 (C-8). The ^1H NMR and ^{13}C NMR data were consistent with the literature [31].

Uracil (**5**) 14 – 5: ^1H NMR (600 MHz, DMSO- d_6): δ_H 11.00 (br s, H-3), 10.80 (br s, H, H-1), 7.38 (d, 1H, H-6), 5.44 (dd, 1H, H-5); ^{13}C NMR (151 MHz, DMSO- d_6): δ_C 164.31 (C-4), 151.49 (C-2), 142.17 (C-6), 100.20 (C-5). The ^1H NMR and ^{13}C NMR data were consistent with the literature [32].

Results

Analysis of the results of Illumina sequencing data

A total of 212,165 effective sequences were obtained by high-throughput sequencing of six samples of diseased and healthy fruit of *C. burmannii* (Figure S2). The OTU numbers of healthy fruit and diseased fruit groups were 114 and 597, respectively, with an average value of 35361 effective tags, final effective data with an average number of bases of 8554061 bp and effective sequence lengths of 234–258, whose average length was 241.55. The clustering was performed at 97% similarity level, yielding an average number of OTUs of 90. The quality control data results showed that Q20 was above 79% and Q30 was above 75%. The rarefaction curves were constructed by randomly selecting sequence numbers and their

corresponding diversity indices (Figure S3). The observed species index and Shannon index dilution curves for both individual and intergroup samples tended to be flat, indicating that the sequencing data are reasonably reliable and accurately reflect the microbial information in the samples.

Community composition of healthy and diseased fruits

Based on the species annotation results, a total of 5 phyla, 19 classes, 39 orders, 57 families and 116 genera were identified. The relative abundance of phylum and genus level species in each sample was calculated based on OTU absolute abundance and annotation information (Fig. 1). The results of the relative abundance plots indicated that the fungal communities in healthy and diseased fruits were similar in composition at the phylum level, with Basidiomycota (HF-7.67%, DF-53.48%), Ascomycota (HF-58.10%, DF-42.51%) and Chytridiomycota (HF-0.20%, DF- 0.26%) were the dominant groups common to both healthy and diseased fruits, however, the relative abundance of the dominant phylum Basidiomycota (HF-7.67%, DF-53.48%) was obviously different. A similar situation was observed at the class level, where Eurotiomycetes (HF-6.50%, DF-13.99%) and Sordariomycetes (HF-8.30%, DF-15.64%) were the dominant groups common to both healthy and diseased fruits, with Dothideomycetes (33.32%) being the dominant group specific to healthy fruits and Exobasidiomycetes (42.45%) in diseased fruits. 33.32% and Exobasidiomycetes (42.45%) in diseased fruits. At the order level, there were no identical dominant colonies in healthy and diseased fruits, and the relative abundance of dominant colonies in both healthy fruits was less than 20%, with Capnodiales (18.81%), Pleosporales (11.66%) and Chaetothyriales (4.37%) dominant in healthy fruits and Exobasidiales (42.45%), Eurotiales (13.63%) and Cystofilobasidiales (6.80%). Interestingly, at the genus level, *Clinoconidium* (HF-2.97%, DF-42.45%) was the dominant colony in both healthy and diseased fruits, while *Aspergillus* (12.57%), and *Guehomyces* (5.30%) were the dominant colonies specific to diseased fruits, while the relative abundance of all colonies in healthy fruits was less than 3%.

Based on the species annotation and abundance information of all samples at order and genus levels, heat map clustering analysis was performed on 35 orders or genera with higher abundance in six samples, respectively, as a way to analyze the characteristics of fungal community changes in diseased and healthy fruits (Fig. 2). The fungal community composition of the six samples was clustered into two large branches, the three samples of diseased fruit and three samples of healthy fruit each had one branch. The fungal community compositions of diseased and healthy fruits were significantly different. Moreover, there were significant differences between DF1 and other samples in the diseased fruit group. Fungal communities in DF and HF groups were significantly altered. At the Order level, the DF group Exobasidiales, Eurotiales and Cystofilobasidiales were upregulated as the dominant groups, while the original dominant groups Capnodiales, Pleosporales and Chaetothyriales were downregulated in the healthy fruit. At the Genus level, compared to the HF group, the dominant genera in the DF group, *Clinoconidium*, *Aspergillus* and *Guehomyces*, were all up-regulated, in addition to *Mrakia*, *Madurella*, *Paraconiothyrium*, *Fusarium*, *Eurotium*, *Coprinus*, *Villosiclava*, *Gibellulopsis*, *Hansfordia*, *Candida*, *Rosenscheldia*, *Kluyveromyces*, *Pseudallescheria*, *Monographella*, *Ascochyta*, *Chaetomium* and *Sporobolomyces*, while the genera *Strelitziana*, *Septobasidium*, *Elsinoe*, *Corynespora*, *Pseudorobillarda*,

Pseudocercospora, *Ramichloridium*, *Aureobasidium*, *Torula*, *Pestalotiopsis*, *Stagonospora*, *Mycosphaerella*, *Devriesia* and *Cladosporium* underwent a more pronounced down-regulation. All these results demonstrated a replacement of the fungal community and composition between healthy and diseased fruits

Biodiversity analysis of fungal communities in healthy and diseased fruits

The observed species, Chao1, Shannon, and PD Whole Tree indices were used to figure out the rich and varied the sample species. Among them, observed species index and Chao 1 index measure the sample richness, while Shannon and PD index reflect the diversity of sample species. The higher these alpha diversity indices are, the more complex the diversity of the sample is. From the results of alpha diversity index violin distribution (Fig. 3-a) and Fig. 3-b, it can be seen that the diversity index of endophytic fungal communities in *C. burmannii* fruits in HF group was significantly higher than that in DF group except for Shannon index in Chao1, observed species and PD whole tree. The (Fig. 4) rank abundance curves showed that the abundance and evenness of the three samples in the HF group were stronger than those in the DF group, with the species in HF2 and DF2 having the highest abundance and evenness of distribution in both the HF and DF groups. All these observations may indicate that the species diversity of healthy fruits is more complex than that of diseased fruits.

Beta diversity index can be used to evaluate fungal community differences between different samples. Bray Curtis, Weighted UniFrac and Unweighted UniFrac distance can be calculated according to OTU abundance information of samples. As the Beta diversity distance, they are indicators to measure the dissimilarity coefficient between two samples, with smaller values, the smaller the difference in species diversity between the two samples.

Weighted UniFrac distance was concerned with the composition differences of species and numbers between samples. The mean values of Bray Curtis, Weighted UniFrac and Unweighted UniFrac distance between different samples are shown in Fig. 5-a. The mean distance between HF group was 0.462 ~ 0.529 (Weighted uniFrac), 0.333 ~ 0.470 (Bray Curtis), and 0.373 ~ 0.485 (Unweighted uniFrac). The mean distance between DF group was 0.466 ~ 0.546 (Weighted uniFrac), 0.388 ~ 1.146 (Bray Curtis), and 0.675 ~ 0.805 (Unweighted uniFrac). The mean distance between HF and DF groups was 0.670 ~ 0.784 (Weighted uniFrac), 1.033 ~ 1.159 (Bray Curtis), and 0.890 ~ 0.956 (Unweighted uniFrac). It may be noticed that the average distance between the HF and DF groups is greater than the distance within the groups. UPGMA clustering tree (Weighted uniFrac) of the species composition of the fungal groups in healthy and diseased fruits of *C. burmannii* (Fig. 5-b) was performed and the results were in general agreement with the PCA results. At a genetic distance of 0.02, two branches were generated, the similarity between the species composition of healthy and diseased fruits was low and the difference was significant. At a genetic distance of 0.2, there were two branches in diseased fruits, DF2 and DF3 clustered into one category with high similarity and insignificant difference, and DF1 produced one branch with low similarity. Principal component analysis (PCA, Fig. 5-c) shows that the first principal

component (PC1) and the second principal component (PC2) explained 78.8% and 18.6% of the microflora of diseased fruit and healthy fruit from *C. burmannii*, respectively. The distance between HF samples and DF samples was far, indicating that the community composition of healthy fruit was quite different from diseased fruit. Within the group, the community composition of the three samples in HF group was similar, while the community composition of the samples in DF group was relatively different. As shown in Fig. 4-d, the results of non-metric multidimensional scaling analysis (NMDS) showed good discrimination between the two groups, indicating that the fungal community composition of HF group was different from that of DF group. In addition, the composition of fungal community in DF group was also different.

Identification and phylogenetic relationship analysis of culturable symbiotic fungi

A total of 171 fungal isolates were isolated from healthy and diseased fruits of *C. burmannii*. Among them, 122 strains were isolated from diseased fruit and 49 strains were isolated from healthy fruit. According to molecular identification results, 41 strains were successfully identified as shown in Table 1 and Fig. 7, among which 30 were from diseased fruit and 11 were from healthy fruit. All the symbiotic fungi were assigned to 23 genera: *Acremonium*, *Allophoma*, *Alternaria*, *Aspergillus*, *Campylocarpon*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Diaporthe*, *Endomelanconiopsis*, *Fusarium*, *Medicopsis*, *Paraconiothyrium*, *Penicillium*, *Periconia*, *Phaeosphaeria*, *Phaeosphaeriopsis*, *Pseudocercospora*, *Pseudopithomyces*, *Setophoma*, *Teichospora*, *Trichoderma* and *Xenoacremonium*. For symbiotic fungi of diseased and healthy fruits, the dominant genus was *Colletotrichum* sp.. Their ITS4-5.8S-ITS5 partial sequences were submitted to GenBank, the accession numbers (OK242732 - OK242772) and closest related species with more than 97% similarity were obtained.

Table 1
Identification of symbiotic fungi isolated from healthy and diseased fruits of *C. burmannii*

Strains	Accession number	Macro- and microscopic identification	Similarity (%)	Closest related species
Cbf-1	OK242732	<i>Cladosporium</i> sp.	100.00	MF473111.1
Cbf-2	OK242733	<i>Acremonium</i> sp.	100.00	KT878351.1
Cbf-3	OK242734	<i>Colletotrichum</i> sp.	99.67	LC488852.1
Cbf-4	OK242735	<i>Periconia</i> sp.	99.65	MF435088.1
Cbf-5	OK242736	<i>Alternaria</i> sp.	100.00	MN955458.1
Cbf-6	OK242737	<i>Allophoma</i> sp.	100.00	MW036299.1
Cbf-7	OK242738	<i>Colletotrichum</i> sp.	100.00	KX197393.1
Cbf-8	OK242739	<i>Colletotrichum</i> sp.	100.00	MH930414.1
Cbf-9	OK242740	<i>Alternaria</i> sp.	100.00	MT453271.1
Cbf-10	OK242741	<i>Cladosporium</i> sp.	100.00	MF422154.1
Cbf-11	OK242742	<i>Diaporthe</i> sp.	100.00	MK942683.1
Esf-1	OK242743	<i>Fusarium</i> sp.	100.00	MK355724.1
Esf-2	OK242744	<i>Colletotrichum</i> sp.	100.00	MK211267.1
Esf-3	OK242745	<i>Fusarium</i> sp.	99.66	MN216215.1
Esf-4	OK242746	<i>Teichospora</i> sp.	97.89	MW764308.1
Esf-5	OK242747	<i>Endomelanconiopsis</i> sp.	100.00	GQ469968.1
Esf-6	OK242748	<i>Pseudopithomyces</i> sp.	100.00	MT186145.1
Esf-7	OK242749	<i>Alternaria</i> sp.	100.00	MN955458.1
Esf-8	OK242750	<i>Curvularia</i> sp.	100.00	JN116704.1
Esf-9	OK242751	<i>Colletotrichum</i> sp.	100.00	MG830360.1
Esf-10	OK242752	<i>Diaporthe</i> sp.	99.00	MH930433.1
Esf-11	OK242753	<i>Trichoderma</i> sp.	99.84	HQ608121.1
Esf-12	OK242754	<i>Setophoma</i> sp.	98.04	NR168153.1
Esf-13	OK242755	<i>Aspergillus</i> sp.	100.00	MN650839.1
Esf-14	OK242756	<i>Medicopsis</i> sp.	100.00	KM246269.1
Esf-15	OK242757	<i>Colletotrichum</i> sp.	100.00	KU642471.1

Strains	Accession number	Macro- and microscopic identification	Similarity (%)	Closest related species
Esf-16	OK242758	<i>Periconia</i> sp.	99.50	KC954157.1
Esf-17	OK242759	<i>Fusarium</i> sp.	99.66	MT466521.1
Esf-18	OK242760	<i>Diaporthe</i> sp.	96.86	MH930427.1
Esf-19	OK242761	<i>Colletotrichum</i> sp.	100.00	AY266394.1
Esf-20	OK242762	<i>Campylocarpon</i> sp.	99.84	MK211263.1
Esf-21	OK242763	<i>Phaeosphaeria</i> sp.	98.47	MG827187.1
Esf-22	OK242764	<i>Phaeosphaeriopsis</i> sp.	99.46	GU017524.1
Esf-23	OK242765	<i>Alternaria</i> sp.	100.00	MK972909.1
Esf-24	OK242766	<i>Xenoacremonium</i> sp.	99.31	KM231833.1
Esf-25	OK242767	<i>Paraconiothyrium</i> sp.	99.32	EU715661.1
Esf-26	OK242768	<i>Pseudocercospora</i> sp.	99.82	KP896029.1
Esf-27	OK242769	<i>Phaeosphaeriopsis</i> sp.	99.07	KJ780762.1
Esf-28	OK242770	<i>Penicillium</i> sp.	100.00	MN788102.1
Esf-29	OK242771	<i>Phaeosphaeria</i> sp.	98.47	MG827187.1
Esf-30	OK242772	<i>Phaeosphaeriopsis</i> sp.	99.82	JQ936272.1

All symbiotic fungi were divided into 8 groups according to the result of phylogenetic tree, Botryosphaeriales, Cladosporiales, Diaporthales, Eurotiales, Glomerellales, Hypocreales, Mycosphaerellales and Pleosporales. As is shown in Fig. 7, the number of symbiotic fungi in Pleosporales is the largest, followed by Glomerellales and Hypocreales. Diseased fruit may produce a greater variety of symbiotic fungi than healthy fruit. It could find that endophytic fungi from healthy fruit were grouped into 5 groups, while diseased fruit symbiotic fungi were classed as 7 groups. Botryosphaeriales, Eurotiales and Mycosphaerellales were unique to diseased fruit, while Cladosporiales was unique to healthy fruit. Forty-one symbiotic fungi were divided into 23 different genera and 8 groups based on ITS sequence, which reflected the diversity of symbiotic fungi in diseased and healthy fruits of the plant. In addition, the results also indicated the changes of symbiotic fungi in diseased and healthy fruits.

Antimicrobial activity screening of the EtOAc extracts from the endophytic fungi

The antibacterial activity of the crude extracts of symbiotic fungi from 41 strains were determined by TLC-bioautography assay. The results of anti-bacterial activity of 41 symbiotic fungi are shown in Table 1. As it can be seen from the Table 2, the crude extracts of 41 isolates showed certain inhibitory

ability to different tested bacteria. Specifically, the antibacterial activity of crude extracts of isolates in diseased fruit was stronger than that in healthy fruit. Among the symbiotic fungi of diseased fruit of *C. burmannii*, except Esf-2, Esf-4, Esf-10, Esf-12, Esf-25, Esf-26, Esf-27 and Esf-28, all the other symbiotic fungi showed certain antibacterial activity against all the tested bacteria, especially Esf-3 showed strong antibacterial activity against all the tested bacteria, the diameter of antibacterial plaque is more than 10 mm. On the other hand, about half of the isolates from healthy fruit suggested certain antibacterial activity against all tested bacteria, among which, Cbf-6, Cbf-7 and Cbf-10 showed stronger antibacterial activity against all tested bacteria, the diameter of antibacterial plaque ranged from 5 mm to 10 mm. There was no significant difference in inhibitory activity against Gram-positive and gram-negative bacteria. The range of R_f value could reflect the polarity of the active component in different crude extracts. It was also observed that R_f values with antibacterial activity in different crude extracts mostly ranged from 0.00 to 0.50. The results indicated that the substances with antibacterial activity in the crude extracts of isolates from the diseased and healthy fruit were mainly compounds with low or moderate polarity.

Table 2
Antibacterial activities for symbiotic fungi secondary metabolites of *C. burmannii*

Strains	<i>R_f</i> value (Inhibition spot diameter)					
	<i>E. coli</i>	<i>P. lachrymans</i>	<i>X. vesicatoria</i>	<i>B. subtilis</i>	<i>R. solanacearum</i>	<i>A. tumefaciens</i>
Esf-1	0.00-0.47 ⁺⁺⁺	0.00-0.15 ⁺⁺ ; 0.32–0.40 ⁺⁺	0.00-0.12 ⁺⁺ ; 0.25–0.37 ⁺⁺	0.00-0.42 ⁺⁺⁺	0.00-0.40 ⁺⁺	0.00-0.13 ⁺⁺ ; 0.20–0.27 ⁺ ; 0.33–0.37 ⁺⁺
Esf-2	—	0.00-0.13 ⁺ ; 0.17–0.27 ⁺⁺ ; 0.30–0.50 ⁺⁺	0.00-0.28 ⁺⁺	—	0.00-0.48 ⁺⁺	0.00-0.13 ⁺⁺ ; 0.17–0.25 ⁺ ; 0.42–0.50 ⁺
Esf-3	0.00-0.43 ⁺⁺⁺	0.00-0.25 ⁺⁺⁺	0.00-0.40 ⁺⁺⁺	0.00-0.37 ⁺⁺⁺	0.00-0.38 ⁺⁺⁺	0.02–0.47 ⁺⁺⁺
Esf-4	0.00-0.38 ⁺⁺	0.20–0.23 ⁺	0.20–0.23 ⁺	0.18–0.45 ⁺⁺⁺	0.00-0.48 ⁺⁺	-
Esf-5	0.00-0.50 ⁺⁺	0.00-0.25 ⁺⁺ ; 0.32–0.47 ⁺⁺	0.00-0.43 ⁺⁺	0.00-0.43 ⁺⁺	0.00-0.42 ⁺⁺	0.00-0.05 ⁺ ; 0.32–0.42 ⁺
Esf-6	0.03–0.08 ⁺ 0.22–0.30 ⁺⁺	0.00-0.52 ⁺⁺	0.00-0.37 ⁺⁺	0.00-0.12 ⁺ ; 0.18–0.25 ⁺⁺ ; 0.35–0.40 ⁺	0.00-0.50 ⁺⁺	0.20–0.25 ⁺
Esf-7	0.00-0.10 ⁺ ; 0.25–0.27 ⁺	0.00-0.30 ⁺⁺ ; 0.43–0.47 ⁺	0.00-0.17 ⁺⁺ ; 0.22–0.27 ⁺	0.00-0.12 ⁺⁺ ; 0.18–0.23 ⁺	0.00-0.28 ⁺⁺ ; 0.43–0.50 ⁺⁺	0.00-0.17 ⁺

—: Inhibition spot was not observed; +: Maximum inhibition spot diameter d 5 mm ++: Maximum inhibition spot diameter 5 mm ≤ d 10 mm; +++: Maximum inhibition spot diameter d ≥ 10 mm; The positive control streptomycin sulfate was only sampled on TLC plate.

Strains	<i>R_f</i> value (Inhibition spot diameter)					
	<i>E. coli</i>	<i>P. lachrymans</i>	<i>X. vesicatoria</i>	<i>B. subtilis</i>	<i>R. solanacearum</i>	<i>A. tumefaciens</i>
Esf-8	0.00-0.55 ⁺⁺	0.00-0.22 ⁺⁺ ; 0.25-0.33 ⁺⁺ ; 0.50-0.55 ⁺⁺	0.00-0.60 ⁺⁺⁺	0.00-0.52 ⁺⁺⁺	0.00-0.43 ⁺⁺	0.00-0.08 ⁺ ; 0.23-0.28 ⁺ ; 0.37-0.47 ⁺⁺
Esf-9	0.00-0.50 ⁺⁺⁺	0.00-0.33 ⁺⁺ ; 0.38-0.45 ⁺⁺ ; 0.47-0.57 ⁺	0.00-0.60 ⁺⁺⁺	0.00-0.48 ⁺⁺⁺	0.00-0.38 ⁺⁺⁺	0.00-0.17 ⁺ ; 0.22-0.25 ⁺ ; 0.33-0.45 ⁺⁺
Esf-10	—	—	0.00-0.18 ⁺⁺ ; 0.23-0.35 ⁺⁺	0.37-0.42 ⁺	—	0.00-0.07 ⁺ ; 0.23-0.27 ⁺ ; 0.42-0.50 ⁺
Esf-11	0.00-0.08 ⁺⁺ ; 0.33-0.42 ⁺	0.00-0.12 ⁺⁺	0.00-0.30 ⁺⁺ ; 0.37-0.50 ⁺⁺	0.00-0.13 ⁺⁺ ; 0.38-0.45 ⁺⁺	0.00-0.13 ⁺⁺ ; 0.42-0.45 ⁺	0.00-0.18 ⁺⁺ ; 0.22-0.25 ⁺⁺ ; 0.32-0.50 ⁺⁺
Esf-12	0.08-0.15 ⁺	—	0.00-0.20 ⁺⁺ ; 0.23-0.32 ⁺⁺ ; 0.33-0.47 ⁺	0.00-0.05 ⁺ ; 0.10-0.17 ⁺	0.00-0.07 ⁺ ; 0.45-0.48 ⁺	0.00-0.17 ⁺⁺ ; 0.22-0.25 ⁺ ; 0.35-0.45 ⁺
Esf-13	0.00-0.05 ⁺	0.22-0.27 ⁺	0.00-0.13 ⁺⁺ ; 0.20-0.37 ⁺⁺ ; 0.45-0.48 ⁺	0.00-0.07 ⁺ ; 0.23-0.28 ⁺	0.23-0.30 ⁺	0.18-0.27 ⁺⁺ ; 0.33-0.42 ⁺

—: Inhibition spot was not observed; +: Maximum inhibition spot diameter d 5 mm ++: Maximum inhibition spot diameter 5 mm≤d 10 mm; +++: Maximum inhibition spot diameter d ≥10 mm; The positive control streptomycin sulfate was only sampled on TLC plate.

Strains	<i>R_f</i> value (Inhibition spot diameter)					
	<i>E. coli</i>	<i>P. lachrymans</i>	<i>X. vesicatoria</i>	<i>B. subtilis</i>	<i>R. solanacearum</i>	<i>A. tumefaciens</i>
Esf-14	0.00-0.03 ⁺ ; 0.17 – 0.05 ⁺ ; 0.40 – 0.43 ⁺	0.00-0.08 ⁺ ; 0.17 – 0.28 ⁺⁺ ; 0.42 – 0.50 ⁺⁺	0.00-0.13 ⁺ ; 0.17 – 0.37 ⁺⁺ ; 0.38 – 0.55 ⁺⁺	0.00-0.53 ⁺⁺	0.00-0.10 ⁺ ; 0.12 – 0.30 ⁺⁺ ; 0.33 – 0.48 ⁺⁺	0.02 – 0.05 ⁺ ; 0.07 – 0.13 ⁺ ; 0.18 – 0.37 ⁺
Esf-15	0.00-0.17 ⁺⁺ ; 0.22 – 0.28 ⁺⁺ ; 0.30 – 0.35 ⁺ ; 0.43 – 0.48 ⁺	0.00-0.13 ⁺⁺ ; 0.25 – 0.30 ⁺⁺	0.00-0.57 ⁺⁺	0.00-0.50 ⁺⁺	0.00-0.40 ⁺⁺	0.00-0.10 ⁺⁺⁺
Esf-16	0.00-0.03 ⁺	0.00-0.10 ⁺⁺ ; 0.20 – 0.25 ⁺ ; 0.38 – 0.47 ⁺⁺	0.00-0.55 ⁺⁺	0.00-0.53 ⁺⁺⁺	0.00-0.30 ⁺⁺ ; 0.32 – 0.52 ⁺⁺	0.35 – 0.38 ⁺
Esf-17	0.00-0.07 ⁺⁺	0.00-0.10 ⁺⁺ ; 0.22 – 0.23 ⁺ ; 0.38 – 0.42 ⁺	0.00-0.20 ⁺⁺ ; 0.22 – 0.30 ⁺ ; 0.38 – 0.50 ⁺⁺	0.00-0.47 ⁺⁺⁺	0.00-0.13 ⁺⁺ ; 0.18 – 0.27 ⁺ ; 0.35 – 0.47 ⁺⁺	0.08 – 0.15 ⁺⁺
Esf-18	0.00-0.13 ⁺⁺ ; 0.20 – 0.33 ⁺	0.00-0.37 ⁺⁺	0.00-0.43 ⁺⁺ ; 0.47 – 0.53 ⁺⁺	0.00-0.48 ⁺⁺⁺ ; 0.50 – 0.53 ⁺	0.00-0.43 ⁺⁺ ; 0.45 – 0.50 ⁺⁺	0.37 – 0.42 ⁺

—: Inhibition spot was not observed; +: Maximum inhibition spot diameter d 5 mm ++: Maximum inhibition spot diameter 5 mm ≤ d 10 mm; +++: Maximum inhibition spot diameter d ≥ 10 mm; The positive control streptomycin sulfate was only sampled on TLC plate.

Strains	<i>R_f</i> value (Inhibition spot diameter)					
	<i>E. coli</i>	<i>P. lachrymans</i>	<i>X. vesicatoria</i>	<i>B. subtilis</i>	<i>R. solanacearum</i>	<i>A. tumefaciens</i>
Esf-19	0.00-0.13 ⁺⁺ ; 0.18–0.30 ⁺⁺ ; 0.37–0.50 ⁺⁺	0.00-0.40 ⁺⁺	0.00-0.57 ⁺⁺	0.00-0.52 ⁺⁺⁺	0.00-0.53 ⁺⁺⁺	0.00-0.10 ⁺⁺
Esf-20	0.00-0.07 ⁺	0.00-0.17 ⁺⁺ ; 0.22–0.27 ⁺ ; 0.37–0.42 ⁺⁺	0.00-0.08 ⁺ ; 0.13–0.17 ⁺ ; 0.35–0.48 ⁺	0.00-0.47 ⁺⁺	0.00-0.17 ⁺⁺ ; 0.22–0.28 ⁺ ; 0.33–0.43 ⁺⁺	0.20–0.25 ⁺ ; 0.35–0.40 ⁺
Esf-21	0.00-0.28 ⁺⁺	0.00-0.08 ⁺⁺ ; 0.17–0.23 ⁺⁺ ; 0.35–0.40 ⁺	0.00-0.08 ⁺⁺ ; 0.17–0.27 ⁺⁺ ; 0.40–0.45 ⁺⁺	0.00-0.12 ⁺⁺ ; 0.18–0.47 ⁺⁺	0.00-0.12 ⁺⁺ ; 0.22–0.33 ⁺⁺ ; 0.37–0.53 ⁺⁺	0.00-0.07 ⁺⁺ ; 0.17–0.23 ⁺⁺ ; 0.38–0.42 ⁺
Esf-22	0.00-0.05 ⁺⁺	0.02–0.50 ⁺⁺⁺	0.00-0.55 ⁺⁺⁺	0.00-0.50 ⁺⁺⁺	0.00-0.38 ⁺⁺⁺	0.00-0.10 ⁺ ; 0.23–0.33 ⁺⁺
Esf-23	0.00-0.17 ⁺⁺	0.00-0.17 ⁺⁺ ; 0.22–2.67 ⁺ ; 0.38–0.50 ⁺⁺	0.00-0.30 ⁺⁺ ; 0.37–0.52 ⁺⁺	0.00-0.50 ⁺⁺	0.00-0.22 ⁺⁺ ; 0.25–0.38 ⁺⁺	0.00-0.12 ⁺⁺ ; 0.13–0.18 ⁺
Esf-24	0.00-0.05 ⁺	0.00-0.17 ⁺⁺ ; 0.20–0.23 ⁺ ; 0.33–0.52 ⁺⁺	0.00-0.18 ⁺⁺ ; 0.18–0.25 ⁺ ; 0.30–0.52 ⁺⁺⁺	0.00-0.50 ⁺⁺⁺	0.00-0.40 ⁺⁺⁺	0.00-0.08 ⁺⁺

—: Inhibition spot was not observed; +: Maximum inhibition spot diameter d 5 mm ++: Maximum inhibition spot diameter 5 mm≤d 10 mm; +++: Maximum inhibition spot diameter d ≥10 mm; The positive control streptomycin sulfate was only sampled on TLC plate.

Strains	<i>R_f</i> value (Inhibition spot diameter)					
	<i>E. coli</i>	<i>P. lachrymans</i>	<i>X. vesicatoria</i>	<i>B. subtilis</i>	<i>R. solanacearum</i>	<i>A. tumefaciens</i>
Esf-25	—	0.00-0.15 ⁺ ; 0.37– 0.47 ⁺⁺	0.00-0.08 ⁺ ; 0.10– 0.18 ⁺ ; 0.32– 0.47 ⁺⁺	0.00- 0.25 ⁺⁺ ; 0.18– 0.43 ⁺⁺	0.00-0.17 ⁺⁺ ; 0.20–0.37 ⁺⁺	0.00-0.40 ⁺⁺ ; 0.43–0.50 ⁺
Esf-26	—	0.00-0.15 ⁺⁺ ; 0.22–0.25 ⁺ ; 0.35– 0.48 ⁺⁺	0.00- 0.28 ⁺⁺ ; 0.33– 0.48 ⁺⁺⁺	0.00- 0.43 ⁺⁺⁺	0.00-0.12 ⁺⁺ ; 0.13–0.33 ⁺⁺⁺	0.00-0.53 ⁺⁺
Esf-27	—	0.00-0.05 ⁺ ; 0.13– 0.25 ⁺⁺ ; 0.38–0.47 ⁺	0.13– 0.32 ⁺ ; 0.38– 0.52 ⁺⁺	0.00- 0.08 ⁺ ; 0.15– 0.40 ⁺⁺	0.00-0.38 ⁺⁺	0.00-0.20 ⁺⁺ ; 0.30–0.45 ⁺⁺
Esf-28	—	0.00-0.15 ⁺⁺ ; 0.22–0.25 ⁺ ; 0.35– 0.48 ⁺⁺	0.00- 0.28 ⁺⁺ ; 0.33– 0.48 ⁺⁺⁺	0.00- 0.43 ⁺⁺⁺	0.00-0.12 ⁺⁺ ; 0.13–0.33 ⁺⁺⁺	0.00-0.53 ⁺⁺
Esf-29	0.00- 0.08 ⁺ 0.23– 0.28 ⁺ 0.43– 0.48 ⁺	0.00-0.08 ⁺	0.00-0.12 ⁺ 0.25– 0.30 ⁺⁺ 0.43– 0.48 ⁺	0.00- 0.28 ⁺⁺ 0.33– 0.50 ⁺⁺	0.00-0.55 ⁺⁺⁺	0.00-0.10 ⁺ 0.4–0.48 ⁺
Esf-30	0.00- 0.07 ⁺ ; 0.10– 0.17 ⁺⁺	0.00-0.07 ⁺ ; 0.08– 0.15 ⁺⁺	0.00- 0.17 ⁺⁺	0.00- 0.07 ⁺ ; 0.08– 0.17 ⁺⁺	0.00-0.45 ⁺⁺	0.00-0.15 ⁺⁺

—: Inhibition spot was not observed; +: Maximum inhibition spot diameter d 5 mm ++: Maximum inhibition spot diameter 5 mm ≤ d 10 mm; +++: Maximum inhibition spot diameter d ≥ 10 mm; The positive control streptomycin sulfate was only sampled on TLC plate.

Strains	<i>R_f</i> value (Inhibition spot diameter)					
	<i>E. coli</i>	<i>P. lachrymans</i>	<i>X. vesicatoria</i>	<i>B. subtilis</i>	<i>R. solanacearum</i>	<i>A. tumefaciens</i>
Cbf-1	0.00-0.05 ⁺ ; 0.17-0.25 ⁺ ; 0.33-0.50 ⁺⁺	-	0.00-0.25 ⁺⁺ ; 0.40-0.47 ⁺	0.42-0.47 ⁺	0.00-0.25 ⁺⁺ ; 0.32-0.50 ⁺⁺	0.00-0.05 ⁺ ; 0.38-0.42 ⁺
Cbf-2	0.00-0.50 ⁺⁺	0.00-0.15 ⁺⁺ ; 0.18-0.25 ⁺ ; 0.35-0.43 ⁺	0.00-0.52 ⁺⁺	0.00-0.23 ⁺⁺ ; 0.35-0.45 ⁺	0.00-0.25 ⁺⁺ ; 0.33-0.50 ⁺	0.00-0.10 ⁺ ; 0.15-0.22 ⁺ ; 0.32-0.40 ⁺
Cbf-3	0.40-0.48 ⁺	0.00-0.07 ⁺	0.00-0.10 ⁺ ; 0.27-0.28 ⁺ ; 0.48-0.53 ⁺⁺	-	-	0.03-0.08 ⁺
Cbf-4	0.00-0.33 ⁺⁺ ; 0.37-0.50 ⁺	0.10-0.13 ⁺ ; 0.20-0.27 ⁺	0.10-0.13 ⁺ ; 0.20-0.27 ⁺⁺	0.20-0.27 ⁺⁺ ; 0.32-0.40 ⁺⁺	0.00-0.30 ⁺⁺ ; 0.33-0.40 ⁺ ; 0.42-0.50 ⁺⁺	-
Cbf-5	0.00-0.10 ⁺ 0.20-0.23 ⁺	-	0.00-0.10 ⁺	0.40-0.48 ⁺	0.00-0.22 ⁺	0.37-0.45 ⁺
Cbf-6	0.00-0.18 ⁺⁺ ; 0.22-0.28 ⁺ ; 0.37-0.55 ⁺⁺	0.07-0.15 ⁺⁺	0.07-0.13 ⁺⁺	0.02-0.17 ⁺⁺ ; 0.20-0.28 ⁺⁺ ; 0.32-0.5 ⁺⁺	0.00-0.27 ⁺⁺ ; 0.42-0.50 ⁺⁺	0.05-0.12 ⁺ ; 0.37-0.43 ⁺
Cbf-7	0.00-0.15 ⁺ ; 0.25-0.40 ⁺⁺ ; 0.42-0.58 ⁺	0.22-0.25 ⁺ ; 0.37-0.47 ⁺	0.00-0.08 ⁺ ; 0.20-0.25 ⁺	0.00-0.48 ⁺⁺⁺	0.00-0.50 ⁺⁺	0.00-0.10 ⁺

—: Inhibition spot was not observed; +: Maximum inhibition spot diameter d 5 mm ++: Maximum inhibition spot diameter 5 mm ≤ d 10 mm; +++: Maximum inhibition spot diameter d ≥ 10 mm; The positive control streptomycin sulfate was only sampled on TLC plate.

[illegible]

observed that the symbiotic fungi isolated from diseased fruits exhibited strong antibacterial activity not only in the up-regulated genera, such as the well-known *Penicillium* and *Fusarium* [8], but also in the down-regulated genera, suggesting that the down-regulated genera may also contain active secondary metabolites. Even though Pleosporales was down-regulated in diseased fruits, it was still the dominant flora in both healthy and diseased fruits, where strains in Pleosporales also showed a certain antimicrobial activity compared to the up-regulated flora in diseased fruits, pointing out that the down-regulated flora of plant fruits may contain strains with antimicrobial activity or secondary metabolites in response to the invasion of pathogenic bacteria. Therefore, a symbiotic fungal strain Esf-14 in Pleosporales was selected as a study subject to further demonstrate that it is feasible to search for active secondary metabolites in the down-regulated flora of plant diseased fruits.

Table 3
Correlation analysis of microbiome and isolates

Order	HF	DF	Up/Down
Hypocreales	Cbf-2 <i>Acremonium</i> sp. OK242733	Esf-1 <i>Fusarium</i> sp. OK242743	Up
		Esf-3 <i>Fusarium</i> sp. OK242745	
		Esf-11 <i>Trichoderma</i> sp. OK242753	
		Esf-17 <i>Fusarium</i> sp. OK242759	
		Esf-20 <i>Campylocarpon</i> sp. OK242762	
		Esf-24 <i>Xenoacremonium</i> sp. OK242766	
Botryosphaeriales	-	Esf-5 <i>Endomelanconiopsis</i> sp. OK242747	Up
Eurotiales	-	Esf-13 <i>Aspergillus</i> sp. OK242755	Up
		Esf-28 <i>Penicillium</i> sp. OK242770	
Pleosporales	Cbf-4 <i>Periconia</i> sp. OK242735	Esf-4 <i>Teichospora</i> sp. OK242746	Down
	Cbf-5 <i>Alternaria</i> sp. OK242736	Esf-6 <i>Pseudopithomyces</i> sp. OK242748	
	Cbf-6 <i>Allophoma</i> sp. OK242737	Esf-7 <i>Alternaria</i> sp. OK242749	
	Cbf-9 <i>Alternaria</i> sp. OK242740	Esf-8 <i>Curvularia</i> sp. OK242750	
		Esf-12 <i>Setophoma</i> sp. OK242754	
		Esf-14 <i>Medicopsis</i> sp. OK242756	
		Esf-16 <i>Periconia</i> sp. OK242758	
		Esf-21 <i>Phaeosphaeria</i> sp. OK242763	
		Esf-22 <i>Phaeosphaeriopsis</i> sp. OK242764	
		Esf-23 <i>Alternaria</i> sp. OK242765	
		Esf-25 <i>Paraconiothyrium</i> sp. OK242767	
“-” Indicates that the data is not available.			

Order	HF	DF	Up/Down
		Esf-27 <i>Phaeosphaeriopsis</i> sp. OK242769	
		Esf-29 <i>Phaeosphaeria</i> sp. OK242771	
		Esf-30 <i>Phaeosphaeriopsis</i> sp. OK242772	
Diaporthales	Cbf-11 <i>Diaporthe</i> sp. OK242742	Esf-10 <i>Diaporthe</i> sp. OK242752	Down
		Esf-18 <i>Diaporthe</i> sp. OK242760	
Cladosporiales	Cbf-1 <i>Cladosporium</i> sp. OK242732	-	-
	Cbf-10 <i>Cladosporium</i> sp. OK242741		
Glomerellales	Cbf-3 <i>Colletotrichum</i> sp. OK242734	Esf-2 <i>Colletotrichum</i> sp. OK242744	Down
	Cbf-7 <i>Colletotrichum</i> sp. OK242738	Esf-9 <i>Colletotrichum</i> sp. OK242751	
	Cbf-8 <i>Colletotrichum</i> sp. OK242739	Esf-15 <i>Colletotrichum</i> sp. OK242757	
		Esf-19 <i>Colletotrichum</i> sp. OK242761	
Mycosphaerellales	-	Esf-26 <i>Pseudocercospora</i> sp. OK242768	Down
“-” Indicates that the data is not available.			

Species identification results of the endophytic fungus Esf-14

Phylogenetic trees based on ITS or LSU were constructed using strains of *Medicopsis* sp. and other similar strains as outgroups (Fig. 8). It could be seen that strain Esf-14 was consistent among these phylogenetic tree analyses. The ITS-based single gene phylogenetic tree indicated that strain Esf-14 was located in the same evolutionary branch as *Medicopsis romeroi* with a spreading value of 100. The LSU-based single gene phylogenetic tree also showed that Esf-14 was closely related to *Medicopsis romeroi*. The results of the multigene association tree suggested that both Esf-14 and *Medicopsis romeroi* were on the same evolutionary branch, with spreading values of 100 (ITS) and 92 (LSU), respectively. Consequently, strain Esf-14 could be identified as *Medicopsis romeroi*.

Purification and structure elucidation

Five compounds 1–5 were further separated and purified from the ethyl acetate extract of the strain Esf-14 using chromatographic techniques. Of which, compounds 3–5 are known compounds, while 1 and 2 are probably new compounds, the following structural characterization of these compounds was performed (Fig. 9). The chemical structures of compounds 1–5 were elucidated by HR-ESI-MS and 1D and 2D NMR experiments (COSY, HSQC and HMBC).

5, 6-Dimethoxy-[1',1:4,1"-terphenyl]-2-ol (**1**) was isolated as a colorless waxy solid. It showed a prominent pseudomolecular peak at m/z 307.1331 [M + H]⁺ (calcd for C₂₀H₁₉O₃, 307.1329)⁺ and 329.1132 [M + Na]⁺ (calcd for C₂₀H₁₈O₃Na, 329.1148) in the positive high resolution electrospray ionization mass spectrometry (HRESIMS, Figure S5), indicating its molecular formula as C₂₀H₁₈O₃ with twelve degrees of unsaturation. The UV spectrum (Figure S6) showed maximal absorptions at 200, 247 and 256 nm, which were similar to those of 4, 4"-deoxyterphenyllin [33]. The ¹³C NMR (Table 4) and HSQC spectra suggested 20 carbon signals, including three O-substituted aromatic quaternary carbons (δ_C 157.30, 154.40, 153.02 and 145.87), ten methines and two methoxy carbons (δ_C 61.04, 60.84 and 56.43).

Table 4
¹H (600 MHz) and ¹³C (150 MHz) NMR data of **1** (CDCl₃, *J* in Hz)

Position	δ_C , Type	δ_H	HMBC
1	149.13	-	
2	122.45	-	
3	151.44	-	
4	144.56	-	
5	136.41	-	
6	112.07	6.78 (s, 1H)	C-2, C-5, C-1"
1'	132.62	-	
2'/6' 3"/5"	130.58, 128.39	7.44 (m, 4H)	C-1, C-4'
3'/5'	129.46	7.53 (m, 2H)	C-1'
4'	128.50	7.44 (m, 1H)	C-2', C-6'
1"	138.10	-	
2"/6"	129.34	7.59 (m, 2H)	C-4, C-4"
4"	127.53	7.36 (m, 1H)	C-2", C-6"
5-OCH ₃	61.17	3.68 (s, 3H)	C-5
6-OCH ₃	61.04	3.59 (s, 3H)	C-6

5-(methoxycarbonyl)-2-methylbenzo[d][1, 3]dioxole-2-carboxylic acid (**2**): was isolated as colorless waxy solid. Its molecular formula was established as C₁₁H₁₀O₆ (seven degrees of unsaturation), according to the prominent pseudomolecular ion peak at m/z 237.0406 [M - H]⁻ in the HR-ESI-MS spectrum (Fig. S12). The ¹³C NMR (Table 5) and HSQC spectra suggested 11 carbon signals, including six quaternary carbons (δ_C 167.36, 165.56, 151.30, 147.49, 123.28, 113.57), three methines (δ_C 124.83, 108.25, 107.97), one methoxy carbon (δ_C 52.01) and one methyl carbon (δ_C 21.85). The ¹H NMR spectrum (Table x) of **2** showed the presence of one trisubstituted benzene at δ_H 7.55 (dd, *J* = 1.8, 8.4 Hz, 1H, H-6), 7.34 (d, *J* = 1.2 Hz, 1H, H-4) and 6.99 (d, *J* = 7.8 Hz, 1H, H-7). The COSY correlations (Fig. S18) of H-6 and H-7 suggested that they are two adjacent aromatic hydrogens.

Table 5
¹H (600 MHz) and ¹³C (150 MHz) NMR data of **2** (DMSO-*d*₆, *J* in Hz)

Position	δ _C , Type	δ _H	HMBC
1	151.30, C	-	
2	113.57, C	-	
3	147.49, C	-	
4	108.25, CH	7.34 (d, <i>J</i> = 1.2 Hz, 1H)	C-6, C-3, C-1, C-8
5	123.28, C	-	
6	124.83, CH	7.55 (dd, <i>J</i> = 1.8, 8.4 Hz, 1H)	C-7, C-4, C-1, C-8
7	107.97, CH	6.99 (d, <i>J</i> = 7.8 Hz, 1H)	C-5, C-3, C-1
8	165.56, C	-	
9	52.01, CH ₃	3.80 (s, 3H)	C-8
10	167.36, C	-	
11	21.85, CH ₃	1.80 (s, 3H)	C-10, C-2

The known compounds **3–5** were identified according to their spectroscopic data (¹H NMR, ¹³C NMR and 2D NMR) as well as comparison with literature data. The clear UV and NMR spectra of known compounds were also provided in Supplementary Materials to further confirm the structure (Figures. S19–S28).

Discussion

The community composition of plant symbiotic fungi is important for the biodiversity of natural ecosystems [34]. These symbiotic fungi not only enhance plant growth performance [35] and improve plant tolerance to biotic and abiotic factors in the environment [36–37], but also involved in waste

decomposition and nutrient cycling [38]. However, when infested with pathogenic bacteria, the composition of the fungal community in plant tissues changes, which in turn causes changes in various metabolic functions [39]. Therefore, it is necessary to understand the changes in the composition of fungal communities in healthy and powdery diseased fruits of *C. burmannii*.

Previous studies have suggested that symbiotic fungi are associated with environmental factors, tissues, or age of the plant survival [40]. In addition, such variation on leaves has been reported [14], while this study reported for the first time the variation of symbiotic fungal communities between healthy and powdery diseased fruits of *C. burmannii* using high-throughput sequencing analysis. The structure and composition of fungal communities in healthy and diseased fruits of *C. burmannii* were significantly different at the Phylum, Class, Order or Genus levels, fungal species richness was significantly lower in diseased fruits compared to fungal communities in healthy fruits, which indicated a decrease in the number of fungal communities and some differences in fungal community diversity were observed in the diseased fruit group, the reason for the differences may be related to the different time of pathogenic bacteria infestation [41].

The main microbiota in diseased fruits *Clinconidium*, *Cladosporium*, *Mrakia*, *Madurella*, *Paraconiothyrium*, *Fusarium*, *Eurotium*, *Coprinus*, *Villosiclava*, *Gibellulopsis*, *Aspergillus*, *Hansfordia*, *Candida*, *Rosenscheldia*, *Kluyveromyces*, *Guehomyces*, *Pseudallescheria*, *Monographella*, *Ascochyta*, *Chaetomium* and *Sporobolomyces* demonstrated significant up-regulation changes, and the pathogenic group *Clinconidium* had the highest relative abundance, which likewise proved that *Clinconidium* may be the main pathogen of *C. burmannii* fruits [42]. The relative abundance of *Clinconidium* in the microbiota may be related to the regulatory function of plant fungal communities or pathogenesis. In this study, the genus *Clinconidium*, in which the causative agent of *C. burmannii* powdery mildew is located, was found to be present in both healthy and diseased fruits [42], and both were dominant, the only difference being that the abundance of *Clinconidium* in healthy fruit was significantly lower than those in diseased fruit. *Clinconidium* did not show symptoms of disease although it was present in healthy fruit, which may be related to the more abundant fungal community in healthy fruit, as it has been shown that the diversity of fungal communities might plays an important role in reducing the invasion of pathogens [27].

Recent studies have pointed out that volatile chemicals in plants are regulated by microbiota [43]. we previously reported a dramatic change in the volatile composition of healthy and powdery diseased fruits of *C. burmannii* [26]. The major volatile components in healthy fruits were α -caryophyllene (32.94%) and *L*-caryophyllene (17.75%), after fruit pathogenesis, the major volatiles of diseased fruits became β -guaiene (10.01), (-)- β -cadinene (8.64%), *L*-caryophyllene (5.78%), pinene (5.68%) and α -caryophyllene (5.25%), the changes in volatile chemical composition might be related to the turnover of fungal community. Infection of healthy fruit by pathogenic bacteria was originally thought to be possibly disturbed by toxic substances produced by the plant, resulting in changes in the chemical composition of the fruit [44–46], however, the present study could also explain that changes in chemical composition in plants may also be associated with fungal community turnover. All of these illustrated that the fungal

community in the plant fruit will respond to the invasion of pathogenic bacteria when the healthy fruit is infested with pathogenic bacteria, perhaps generating more abundant bioactive resources during this turnover process.

Numerous studies have shown that when plants are stressed by biotic factors such as pathogens, the fungal community in the tissues changes in response to the stimulus [47–48], while the plant symbiotic fungi also secrete a greater variety of bioactive substances to counteract the adverse factors. Fungal groups exhibited a more important role in the plant disease coexistence network than in the healthy network [25], as also demonstrated by our study. The present study also supported the above by measuring the antimicrobial activity of 41 symbiotic fungal secondary metabolites and further found that the fungal populations secreting active secondary metabolites were distributed in both healthy and diseased fruits, in addition to that, the symbiotic fungal secondary metabolites in diseased fruits exhibited stronger antimicrobial activity than those in healthy fruits. Moreover, the finding seems interesting as in previous studies researchers have focused more on healthy plant tissues [24] and less on the bioactive function of fungal secondary metabolites in diseased tissues. All symbiotic fungal strains were classified by phylogenetic tree analysis into 8 orders and 23 genera, which responded to the diversity and exploitability of culturable symbiotic fungi in plant fruits. The correlation analysis of fungal communities, phylogenetic trees and antimicrobial activity revealed that fungi with antimicrobial activity in diseased fruits were distributed in both up-regulated or down-regulated groups, an unexpected finding that seems to lead us to an interesting pathway where not only up-regulated stressed microbiota in plant fruits secrete active secondary metabolites after the plant fruits are subjected to pathogenic invasion pressure, but down-regulated microbiota. The discovery may be useful for understanding the function of microbiota and the study of active secondary metabolites.

Pleosporales is the largest and most diverse order in Dothideomycetes, which includes a variety of symbiotic fungi, and the strains in this group produce abundant secondary metabolites that are often used to develop new antimicrobials, chemical pesticides, or provide biopesticide molecules [49]. Subsequently, an active strain Esf-14 identified as *Medicopsis romeroi* in Pleosporales was screened by phylogenetic tree, fungal community and antimicrobial activity assay, from which two new and three known compounds were isolated, the structures of the new compounds were elucidated for the first time. One of the new compounds 1 (5, 6-Dimethoxy-[1',1:4,1"-terphenyl]-2-ol) is a new *o*-terphenyl analog, which is an aromatic compound consisting of three benzene rings with three isomers: *o*-terphenyl, *m*-terphenyl and *p*-terphenyl, with *p*-terphenyl and *m*-terphenyl predominating in nature. Those compounds often possess good biological activity and play an important role in pharmaceutical and industrial applications [50–51]. The newly discovered compound in this study is of great research value as a triplet biphenyl compound, perhaps equally rich in biological activity.

Conclusions

In this study, we reported for the first time the diversity of fungal communities in diseased and healthy fruits of *C. burmannii*, the antibacterial activity of artificially cultivatable fungal secondary metabolites.

Following pathogen infection, the structure and composition of the fungal community of *C. burmannii* fruit changed, with a significant reduction in the number of fungal populations in diseased fruit compared to healthy fruit. Although fungal communities in diseased fruits were reduced, secondary metabolites produced by the fungi in these fruits appeared to have stronger antibacterial functions. Two new (5, 6-dimethoxy-[1',1:4,1"-terphenyl]-2-ol (**1**) and 5-(methoxycarbonyl)-2-methylbenzo[d][1, 3]dioxole-2-carboxylic acid (**2**)) and three known compounds were obtained for the first time from the active strain Esf-14 (OK242756) *Medicopsis romeroi*, isolated from the diseased fruit of *C. burmannii*. All of these results have an essential significance for understanding the active functions of microbiota and development of novel bioactive resources.

Declarations

Acknowledgements

We thank Dr. Mingxuan Zheng from the College of Forestry and Landscape Architecture, South China Agricultural University, for the taxonomic identification of the plant materials.

Author's Contributions

W.W., Y.L., P.F. and T.S. collected the plant material. W.W., Y.L, Y.Y. and T.S. performed the isolation and identification of the symbiotic fungi. W.W., Y.Y, T.C. and R.H. evaluated the antimicrobial activity. W.W., Y.Z. and Y.Y contributed in the diversity analysis of symbiotic fungi. W.W., T.C and T.S. performed HPLC analysis of the crude extract. W.W., T.C., H.G. and T.S. performed the isolation, structure elucidation and recorded the spectra of the compounds. W.W., Y.Z., Z.S and T.S. performed the bioinformatics-related analysis. W.W., T.C., S.Q. and T.S. contributed in preparing figures and tables. W.W., Z.M. and T.S. designed the research. All the authors contributed in writing, editing and revising the manuscript. All authors have read and agreed to the published version of the manuscript.

Author's Information

College of Forestry and Landscape Architecture, College of Plant Protection, Instrumental Analysis and Research Center of SCAU, South China Agricultural University, Guangzhou, 510642, China

School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, China

College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou 310014, China; tggh635@163.com

School of Pharmaceutical Sciences, Shandong University, Jinan 250012, China; zshang@sdu.edu.cn

Wei Wang, Teng Cai, Yuting Yang, Hui Guo, Zhuo Shang, Hamza Shahid, Yirong Zhang, Sirun Qiu, Xinnian Zeng, Xiaoli Xu, Yi Liu, Ping Fang, Ziling Mao, Tijiang Shan

Funding

This research was co-financed by the National Natural Science Foundation of China (32071766), the Guangdong Provincial Rural Revitalization Strategy Special Project (2021KJ268), the Guangzhou Basic and Applied Basic Research Project (202201010537) and the Forestry Science and Technology Innovation Project of Guangdong Province (No. 2020KJCX004).

Availability of data and materials

The generated nucleotide sequence of the endophytic fungal isolates (isolation number Cbf-1 Cbf-1, Esf-1 Esf-30) can be accessed in GenBank under accession numbers OK242732 to OK242772 (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Healthy and diseased fruits of *Cinnamomum burmannii* (Nees and Nees) Blume were collected in June 2016 from Huolu Mountain, Guangzhou City, Guangdong Province, China. The taxonomic identification of the plant material was performed by Dr. Mingxuan Zheng from the College of Forestry and Landscape Architecture, South China Agricultural University, where the voucher specimens of the plants (SCAULPMH-160905) were deposited. All experiments were approved by the School of Forestry and Landscape Architecture (SCAU) and were critically evaluated in accordance with the IUCN Policy Statement on Research Involving Endangered Species and the Convention on Trade in Endangered Species of Wild Fauna and Flora.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹ College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou, 510642, China

² College of Plant Protection, South China Agricultural University, Guangzhou, 510642, China

³ School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, China

⁴ College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou 310014, China; tggh635@163.com

⁵ School of Pharmaceutical Sciences, Shandong University, Jinan 250012, China; zshang@sdu.edu.cn

⁶ Instrumental Analysis and Research Center of SCAU, South China Agricultural University, Guangzhou, 510642, China

References

1. Geethangili M, Ding S. A review of the phytochemistry and pharmacology of *Phyllanthus urinaria* L. Front Pharmacol. 2018;9:01109.
2. Campolo O, Giunti G, Russo A, Palmeri V, Zappalà L. Essential oils in stored product insect pest control. J Food Qual. 2018;2018:906105.
3. Gupta S, Chaturvedi P, Kulkarni MG, Staden JV. A critical review on exploiting the pharmaceutical potential of plant endophytic fungi. Biotechnol Adv. 2020;39:107462.
4. Gakuubi MM, Munusamy M, Liang Z, Ng SB. Fungal endophytes: a promising frontier for discovery of novel bioactive compounds. J Fungi. 2021;7:786.
5. Cao M, Liu R, Xiao Z, Hashem A, Allah EFA, Alsayed MF, Harsonowati W, Wu Q. 2022. Symbiotic fungi alter the acquisition of phosphorus in *Camellia oleifera* through regulating root architecture, plant phosphate transporter gene expressions and soil phosphatase activities. J Fungi. 2022;8:800.
6. Jia M, Chen L, Xin H, Zheng C, Rahman K, Han T, et al. A friendly relationship between endophytic fungi and medicinal plants: A systematic review. Front Microbiol. 2016;7:1–14.
7. Rodrigo S, Santamaria O, Halecker S, Lledó S, Stadler M. Antagonism between *Byssosclamyces spectabilis* (anamorph *Paecilomyces variotii*) and plant pathogens: involvement of the bioactive compounds produced by the endophyte. Ann Appl Biol. 2017;171:464–476.
8. Deshmukh SK, Gupta MK, Prakash V, Saxena S. Endophytic fungi: A source of potential antifungal compounds. J Fungi. 2018;4:77.
9. Zhao L, Kim JC, Paik MJ, Lee WJ, Hur JS. A multifunctional and possible skin UV protectant, (3*R*)-5-Hydroxymellein, produced by an endolichenic fungus isolated from *Parmotrema austrosinense*. Molecules. 2017;22:26.
10. Gakuubi MM, Ching KC, Munusamy M, Wibowo M, Liang Z, Kanagasundaram Y, et al. Enhancing the discovery of bioactive secondary metabolites from fungal endophytes using chemical elicitation and variation of fermentation media. Front Microbiol. 2022;13:898976.
11. Vincent JB, Weiblen GD, May G. Host associations and beta diversity of fungal endophyte communities in New Guinea rainforest trees. Mol Ecol. 2016;25:825–841.
12. Yan L, Zhu J, Zhao X, Shi J, Jiang C, Shao D. Beneficial effects of endophytic fungi colonization on plants. Appl Microbiol Biotechnol. 2019;103:3327–3340.

13. Kannadan S, Rudgers JA. Endophyte symbiosis benefits a rare grass under low water availability. *Funct Ecol.* 2008;22:706–713.
14. Douanla-Meli C, Langer E, Talontsi Mouafo F. Fungal endophyte diversity and community patterns in healthy and yellowing leaves of *Citrus limon*. *Funct Ecol.* 2013;6:212–222.
15. Carvalho TLG, Ballesteros HGF, Thiebaut F, Ferreira PCG, Hemerly AS. Nice to meet you: genetic, epigenetic and metabolic controls of plant perception of beneficial associative and endophytic diazotrophic bacteria in non-leguminous plants. *Plant Mol Biol.* 2016;90:561–574.
16. Costello RB, Dwyer JT, Saldanha L, Bailey RL, Merkel J, Wambogo E. Do cinnamon supplements have a role in glycemic control in type 2 diabetes? A narrative review. *J Acad Nutr Diet.* 2016;116:1794–1802.
17. Wang Y, Zhang L, Feng Y, Zhang D, Guo S, Pang X, et al. Comparative evaluation of the chemical composition and bioactivities of essential oils from four spice plants (Lauraceae) against stored-product insects. *Ind Crop Prod.* 2019;140:111640.
18. Yang Y, Song Y, Xin P. The chloroplast genome of aromatic plants *Cinnamomum burmannii* (Lauraceae). *Mitochondrial DNA*, 2019;B4:3616–3617.
19. Hariri M, Ghiasvand R. Cinnamon and chronic diseases. *Adv Exp Med Biol.* 2016, 929: 1–24.
20. Tung YT, Chua MT, Wang S, Chang S. Antiinflammation activities of essential oil and its constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) twigs. *Bioresour Technol.* 2008;99:3908–3913.
21. Carmo ES, Lima ED, de Souza EL, de Sousa FB. Effect of *Cinnamomum zeylanicum* essential oil on the growth and morphogenesis of some potentially pathogenic *Aspergillus* species. *Braz J Microbiol.* 2008;39:91–97.
22. Tuzcu Z, Orhan C, Sahin N, Juturu V, Sahin K. Cinnamon polyphenol extract inhibits hyperlipidemia and inflammation by modulation of transcription factors in high-fat diet-fed rat. *Oxidative Med Cell Longev.* 2017;2017:1583098.
23. Ranasinghe P, Galappaththy P, Constantine GR, Jayawardena R, Weeratunga HD, Premakumara S, et al. *Cinnamomum zeylanicum* (Ceylon cinnamon) as a potential pharmaceutical agent for type-2 diabetes mellitus: study protocol for a randomized controlled trial. *Trials*, 2017;18:446.
24. Li S, Zhang X, Wang X, Zhao C. Novel natural compounds from endophytic fungi with anticancer Activity. *Eur J Med Chem.* 2018;156:316-343.
25. Gao M, Xiong C, Gao C, Tsui CKM, Wang M, Zhou X, et al. Disease-induced changes in plant microbiome assembly and functional adaptation. *Microbiome*, 2021;9:187.
26. Shan T, Wu C, Shahid H, Zhang C, Wang J, Ding P, et al. Powdery-fruit disease of *Cinnamomum burmannii* and its influence on fruit essential oil. *Int J Agric Biol.* 2020b;24:1077-1083.
27. Yao H, Sun X, He C, Pulak M, Li XC, Guo LD. Phyllosphere epiphytic and endophytic fungal community and network structures differ in a tropical mangrove ecosystem. *Microbiome*, 2019;7:57.

28. Wang W, Mao Z, Wu C, Shahid H, Wang S, Shan T. Diversity and Antibacterial and Antioxidant Activities of Fungal Endophytes from the Roots of *Eucalyptus deglupta*. Sains Malays. 2022;51:1687-1696.
29. Ouyang J, Mao Z, Guo H, Xie Y, Cui Z, Sun J, et al. Mollicellins O–R, four new depsidones isolated from the endophytic fungus *Chaetomium* sp. Eef-10. Molecules. 2018;23:3218.
30. Tanahashi T, Takenaka Y, Nagakura N, Hamada N. Dibenzofurans from the cultured lichen mycobionts of *Lecanora cinereocarpa*. Phytochemistry. 2001;8:1129–1134.
31. van der Sluis WG, Labadie RP. Secoiridoids and Xanthones in the Genus *Centaurium*. Planta Med. 1981;41:150–160.
32. Salpin J, Latrous L, Haldys V, Lamsabhi AM. Interactions of Dimethyltin(IV) with Uracil as studied in the gas phase. J Phys Chem A. 2018;122:992-1003.
33. Shan T, Wang Y, Wang S, Xie Y, Cui Z, Wu C, et al. A new *p*-terphenyl derivative from the insect-derived fungus *Aspergillus candidus* Bdf-2 and the synergistic effects of terphenyllin. PeerJ. 2020a;8:e8221.
34. Yao Y, Lan F, Qiao Y, Wei J, Huang R, Li L. Endophytic fungi harbored in the root of *Sophora tonkinensis* gapnep: diversity and biocontrol potential against phytopathogens. Microbiology. 2017;6:e00437.
35. Behie SW, Zelisko PM, Bidochka MJ. Endophytic insect-parasitic fungi translocate nitrogen directly from insects to plants. Science. 2012;336:1576–1577.
36. Hartley SE, Gange AC. Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. Annu Rev Entomol. 2009;54:323–342.
37. Yuan ZL, Druzhinina IS, Labbe J, Redman R, Qin Y, Rodriguez R, et al. Specialized microbiome of a halophyte and its role in helping non-host plants to withstand salinity. Sci Rep. 2016;6:32467.
38. Purahong W, Wubet T, Lentendu G, Schlöter M, Pecyna MJ, Kapturska D, et al. Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. Mol Ecol. 2016;25:4059–4074.
39. Zhu L, Liao R, Wu N, Zhu G, Yang C. Heat stress mediates changes in fecal microbiome and functional pathways of laying hens. Appl Microbiol Biotechnol. 2019;103:461–472.
40. Miguel PSB, Delvaux JC, de Oliveira MNV, Moreira BC, Borges AC, Tótol MR, et al. Diversity and distribution of the endophytic fungal community in eucalyptus leaves. Afr J Microbiol Res. 2017;11:92-105.
41. Bulgari D, Casati P, Quaglino F, Bianco PA. Endophytic bacterial community of grapevine leaves influenced by sampling date and phytoplasma infection process. BMC Microbiol. 2014;14:198.
42. Jiang MG, Kirschner R. Unraveling two East Asian species of *Clinoconidium* (Cryptobasidiaceae). Mycoscience. 2016;57:440–447.
43. Yang F, He L, Shen M, Wang F, Chen H, Liu Y. A Correlation between pericarpium *Citri Reticulatae* volatile components and the change of the coexisting microbial population structure caused by environmental factors during aging. Front Microbiol. 2022;13:930845.

44. Shan T, Sun W, Liu H, Gao S, Lu S, Wang M, et al. Determination and analysis of ustiloxins A and B by LC-ESI-MS and HPLC in false smut balls of rice. *Int J Mol Sci.* 2012;13:11275–11287
45. Sun W, Wang A, Xu D, Wang W, Meng J, Dai J, et al. New *Ustilaginoidins* from rice false smut balls caused by *Villosiclava virens* and their phytotoxic and cytotoxic activities. *J Agric Food Chem.* 2017;65:5151–5160
46. Meng J, Gu G, Dang P, Zhang X, Wang W, Dai J, et al. Sorbicillinoids from the fungus *Ustilaginoidea virens* and their phytotoxic, cytotoxic, and antimicrobial activities. *Front Chem.* 2019;7: 435.
47. Tan L, Zeng W, Xiao Y, Li P, Gu S, Wu S, et al. Fungi-bacteria associations in wilt diseased rhizosphere and endosphere by interdomain ecological network analysis. *Front Microbiol.* 2021;12:722626.
48. Yang F, Zhang J, Zhang H, Ji G, Zeng L, Li Y, et al. Bacterial blight induced shifts in endophytic microbiome of rice leaves and the enrichment of specific bacterial strains with pathogen antagonism. *Front Plant Sci.* 2021;11:963.
49. Yu X, Zhang S, Liu J. Morpho-phylogenetic evidence reveals novel Pleosporalean taxa from Sichuan Province, China. *J. Fungi.* 2022;8:720.
50. Xu K, Gao Y, Li YL, Xie F, Zhao ZT, Lou HX. *Cytotoxic p*-terphenyls from the endolichenic fungus *Floricola striata*. *J Nat Prod.* 2018;81:2041-2049.
51. Mir MA, Chat O, Najar MH, Younis M, Dar AA, Rather GM. Solubilization of triphenylamine, triphenylphosphine, triphenylphosphineoxide and triphenylmethanol in single and binary surfactant systems. *J Colloid Interface Sci.* 2011;364:163-169.

Figures

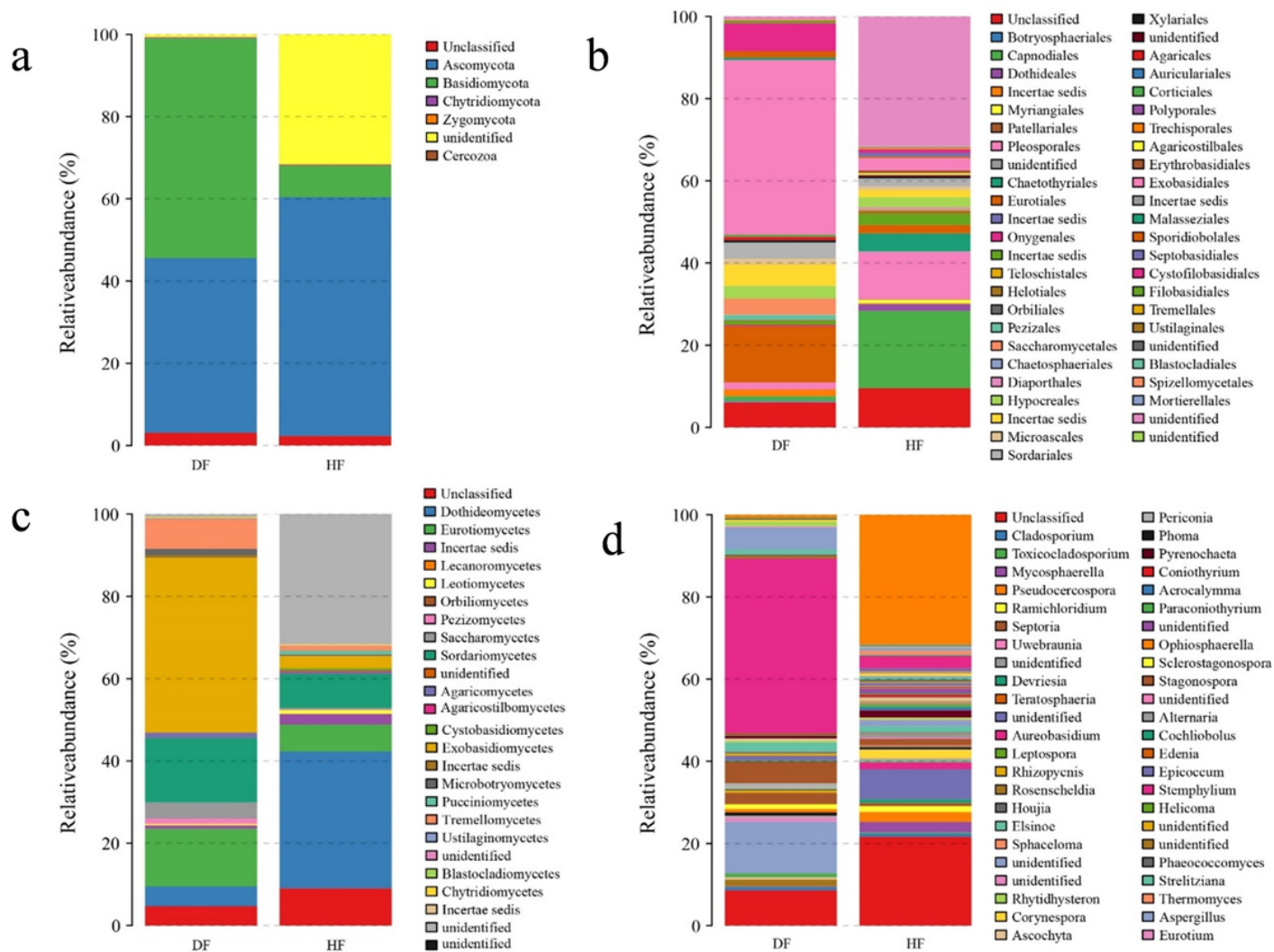


Figure 1

Relative abundance of fungi in healthy and diseased fruit samples of *C. burmannii*.

a, Phylum level; **b**, Class level; **c**, Order level; **d**, Genus level.

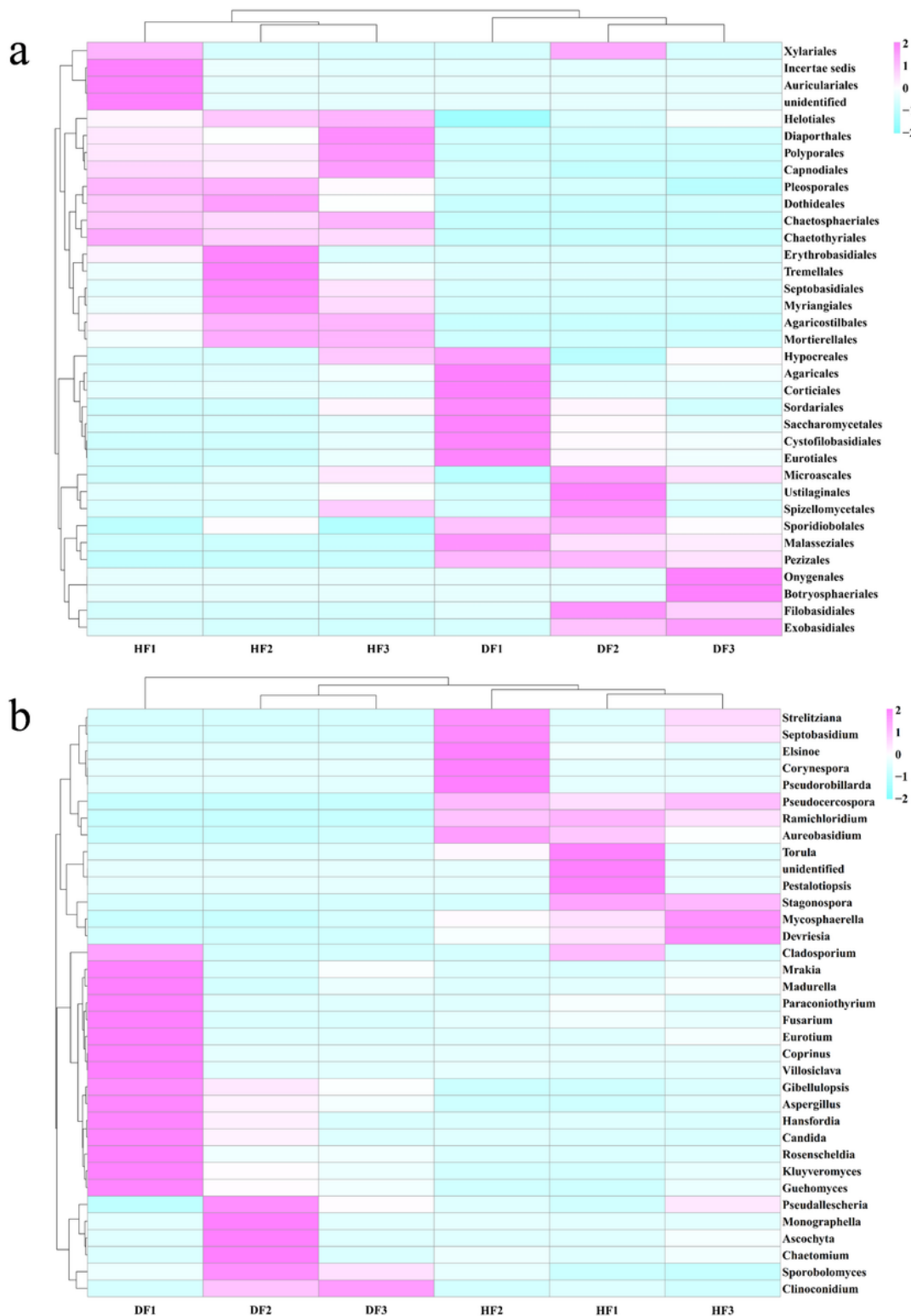


Figure 2

Heat map of fungi in healthy and diseased fruits of *C. burmannii*. **a**, Order level. **b**, Genus level. The value corresponding to the color of each heat map cell is the Z value obtained by normalizing the relative abundance of species in each row (http://en.wikipedia.org/wiki/Standard_score), i.e., the Z value of a sample on a given classification is the difference between the relative abundance of the sample on that

classification and the average relative abundance of all samples on that classification divided by the value obtained by dividing the standard deviation of the samples on that classification.

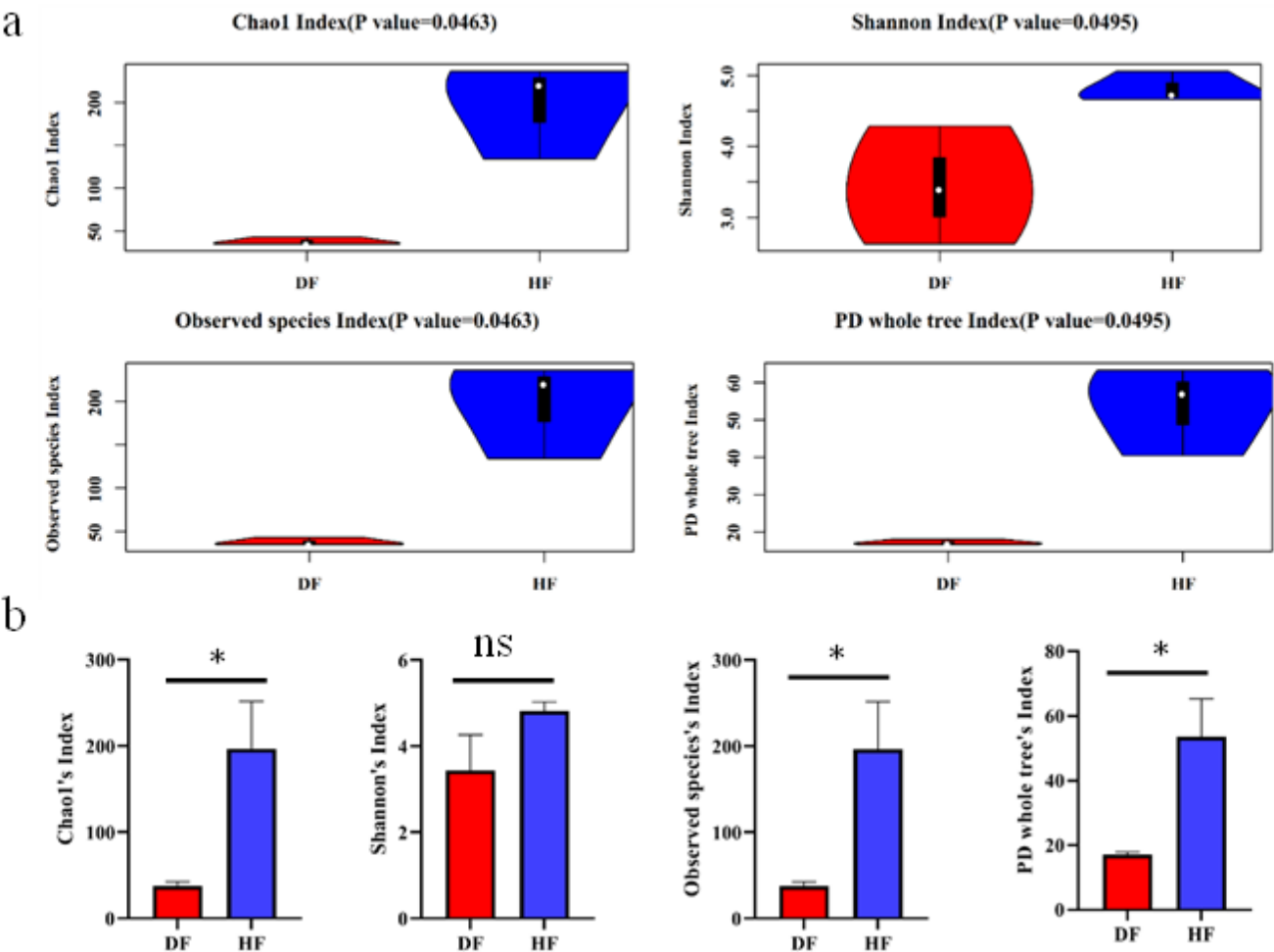


Figure 3

Alpha-diversity analysis of healthy and diseased fruit fungi in *C. burmannii*. **a** is Alpha diversity index violin distribution between sample groups, the violin diagram in the figure is a combination of boxplot and nuclear density diagram, white dot is the median, black box type range is lower quartile to upper quartile, thin black line represents whiskers. The outer shape is the kernel density estimate. The p value at the top of the figure is the p value compared among the three groups, and it is generally believed that less than 0.05 indicates differences between groups. **b**, * indicates significant differences by Student's *t* test (**P* < 0.05), ns indicates no significant difference.

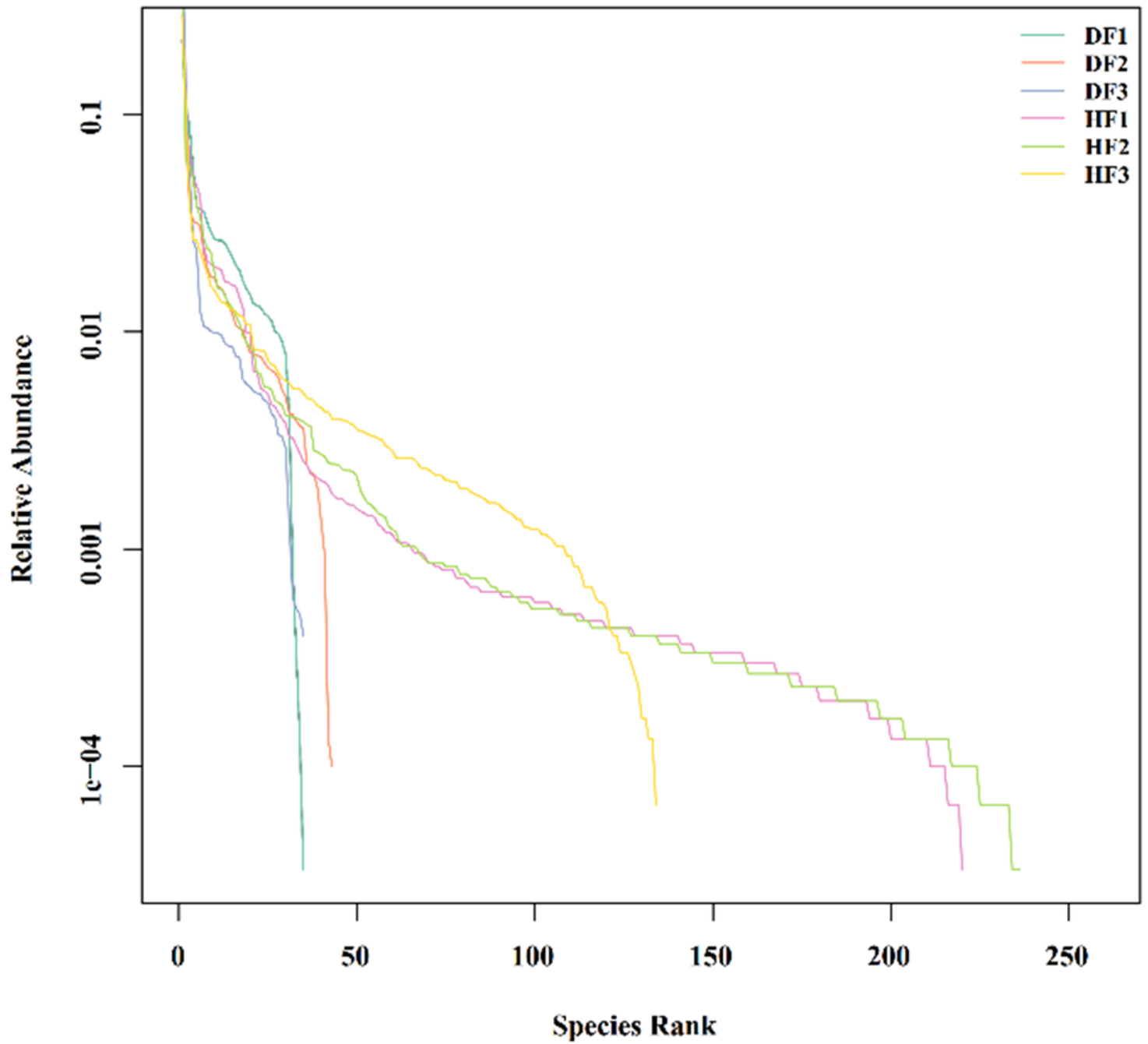


Figure 4

Rank abundance curve of different samples in *C. burmannii*. the abscissa is the serial number sorted by OTU abundance (Rank), and the ordinate is the abundance of corresponding OTU (Abundance). Different samples are represented by curves with different colors and linear lines.

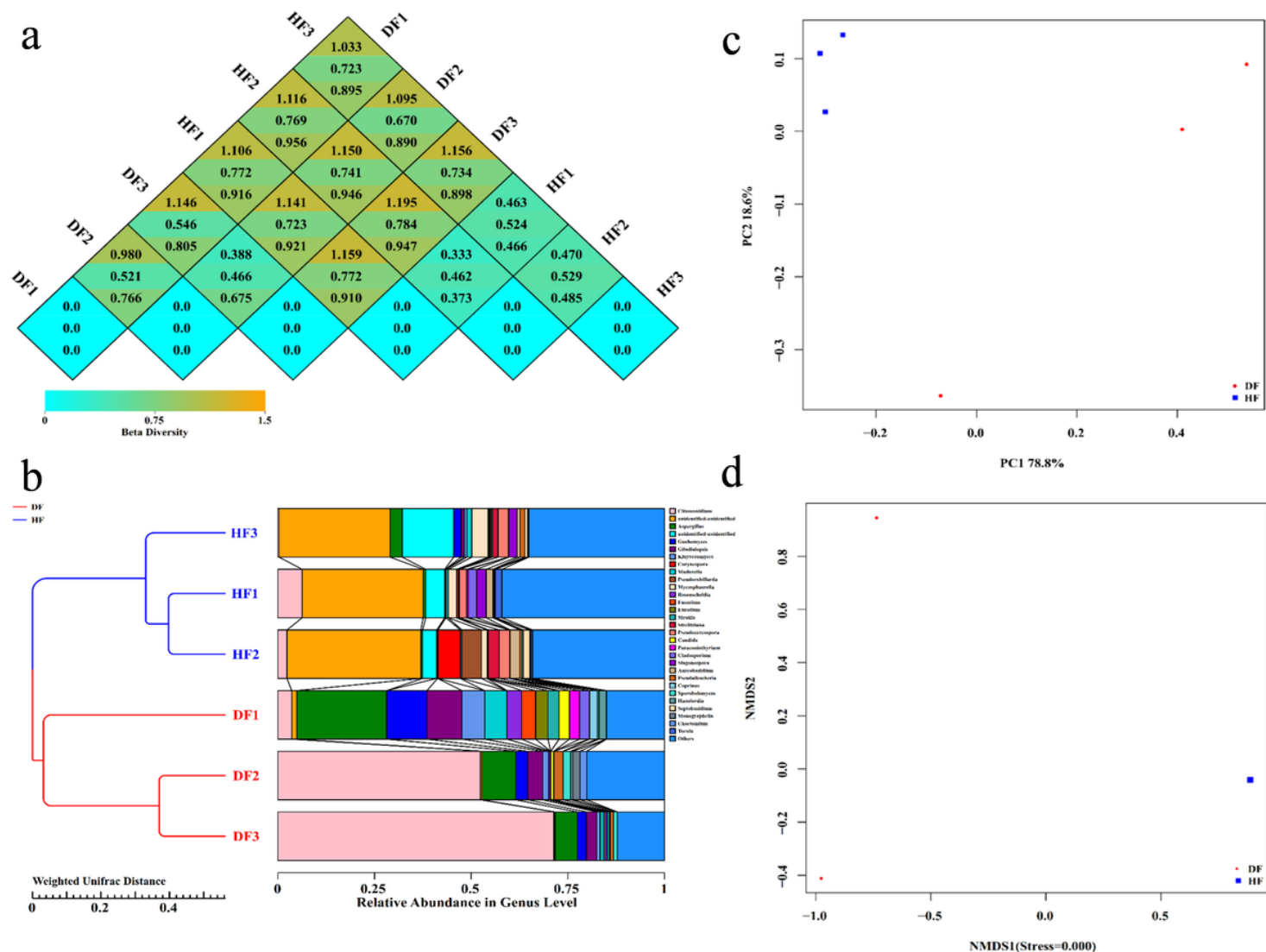


Figure 5

Beta-diversity analysis. **a** is heat map of Beta diversity index, the numbers in the figures' squares are the difference coefficient between two samples. The smaller the differential coefficient is, the smaller the difference of species diversity is. In the same grid, the top, middle and bottom values represent Bray Curtis, Weighted UniFrac and Unweighted UniFrac distance respectively. **b**, UPGMA clustering analysis based on the weighted UniFrac distance on the left, and relative abundance maps at the genus level on the right. **c** is PCA analysis results were presented based on genus level, the abscissa (x) represents the first principal component, while the percentage represents the contribution value of the first principal component to the sample difference. The ordinate (y) represents the second principal component, and the percentage represents the contribution value of the second principal component to the sample difference. Z coordinate represents the third principal component, and percentage represents the contribution value of the third principal component to the sample difference. Each dot in the figure represents a sample, and samples in the same group are indicated in the same color. **d** is NMDS analysis diagram, Points in the figure represent samples, and the distance between points indicates the degree of difference; When Stress is less than 0.2, it indicates that NMDS analysis has certain reliability.

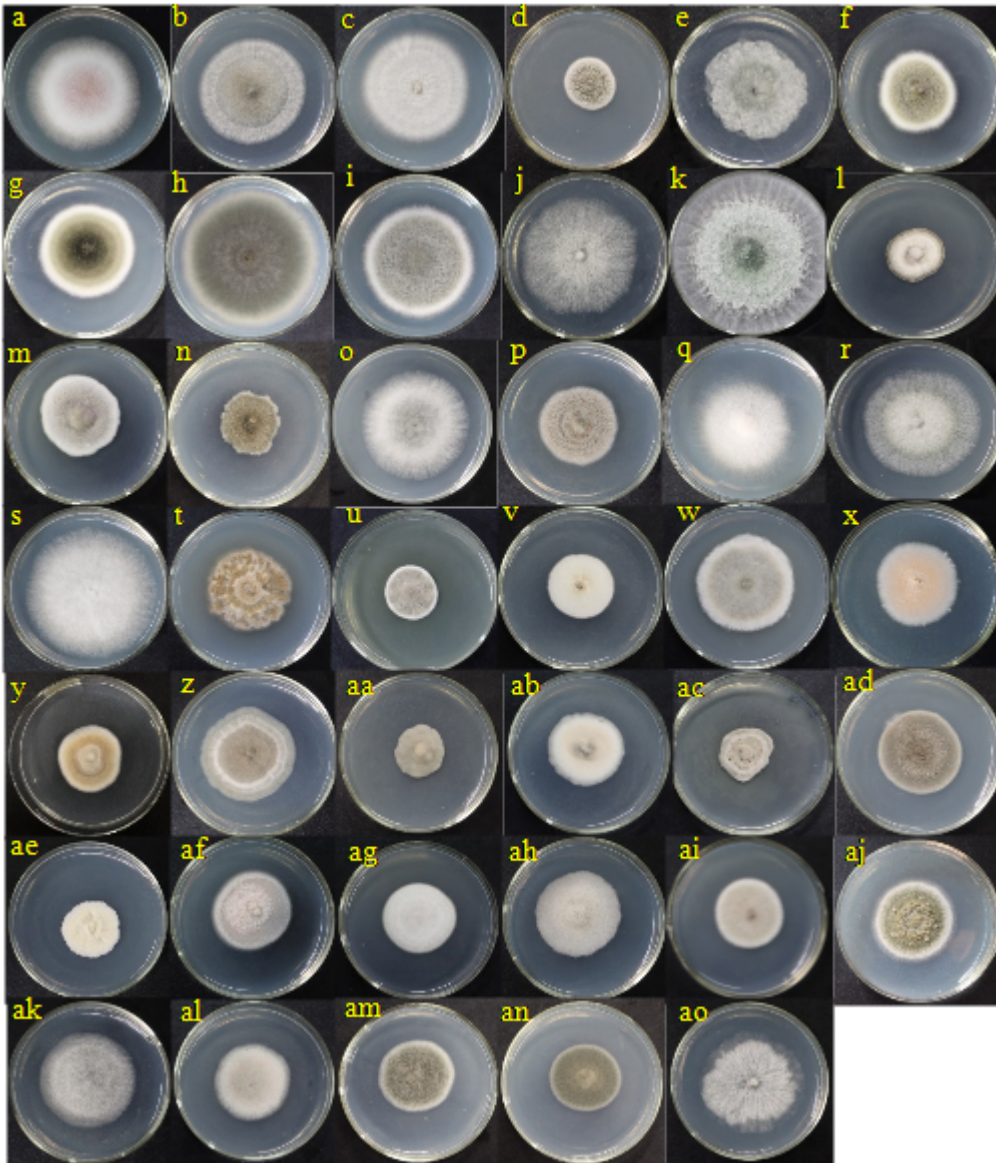


Figure 6

Symbiotic fungi isolated from diseased and healthy fruits of *Cinnamomum burmannii*. a ad were symbiotic fungal isolates Esf-1 Esf-30, respectively. ae ao were symbiotic fungal isolates Cbf-1 Cbf-11, respectively.

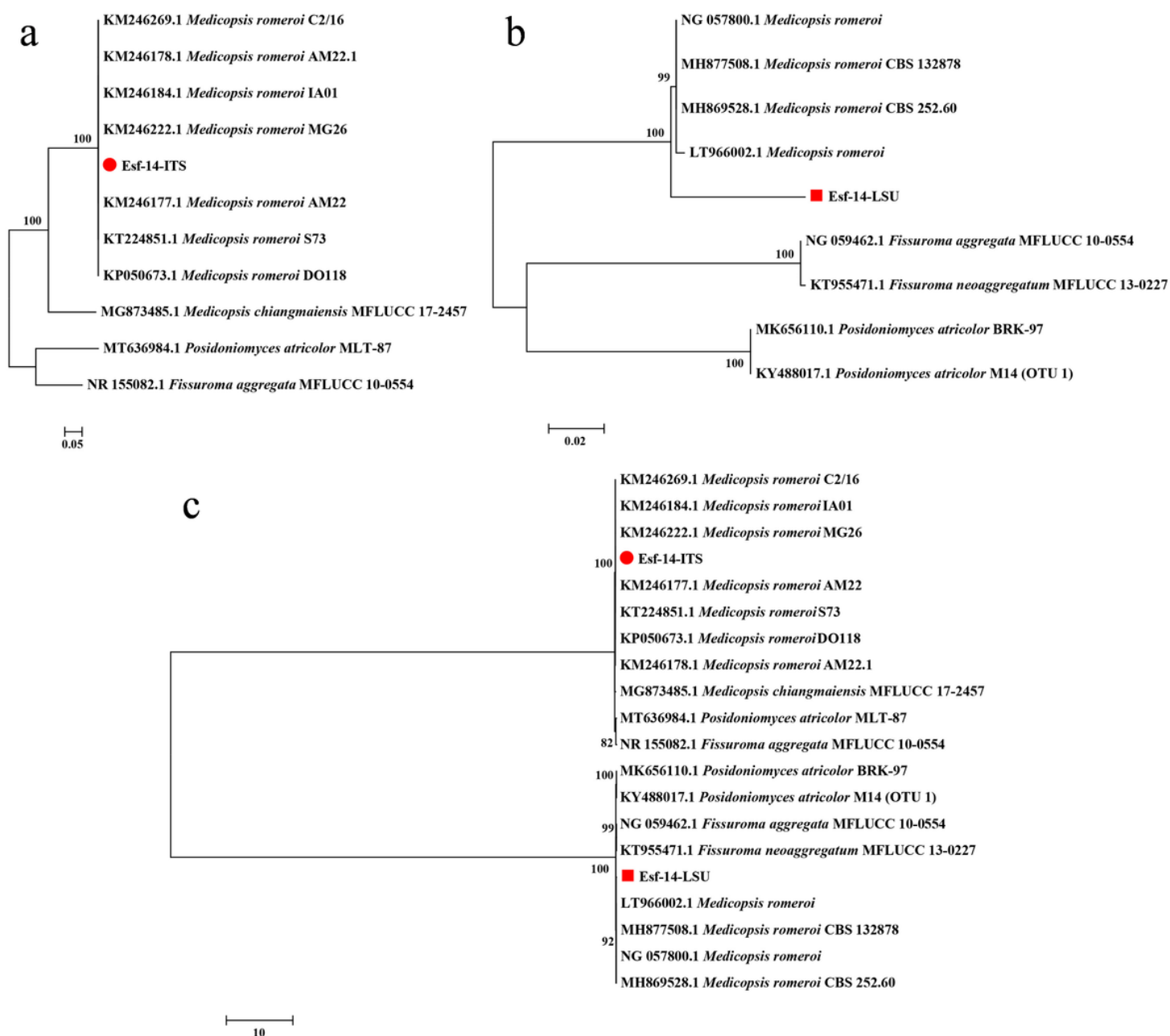


Figure 8

Construction of phylogenetic tree of strain Esf-14 and other similar species based on ITS or LSU gene sequences. **a** is single gene phylogenetic tree (ITS). **b** is single gene phylogenetic tree (LSU). **c** is multi-gene association phylogenetic tree (ITS and LSU).

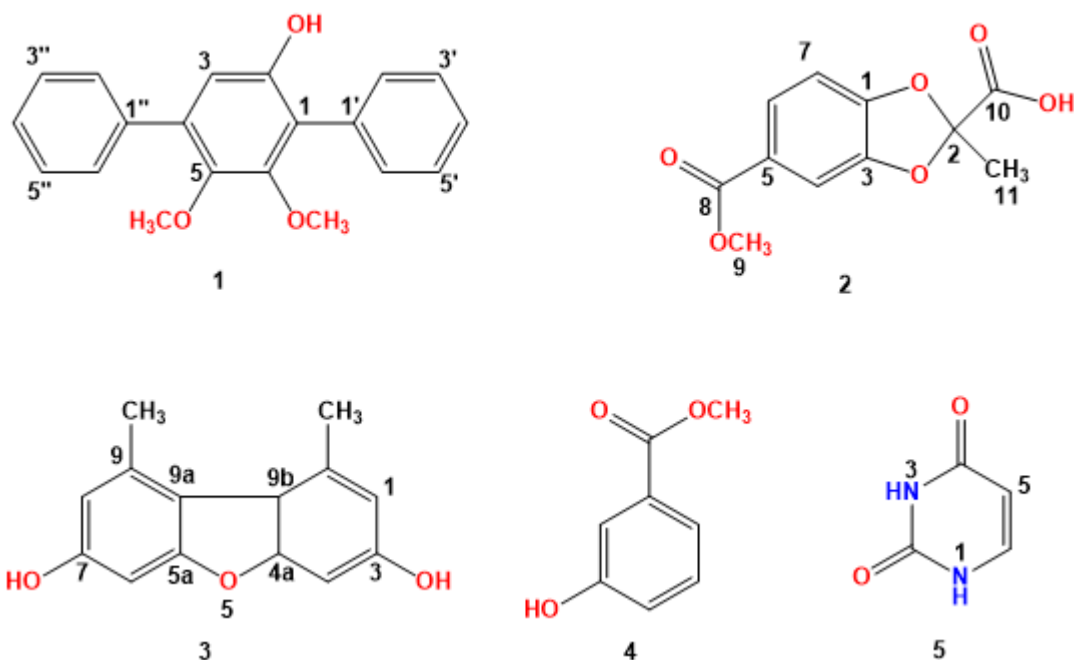


Figure 9

Chemical structures of the isolated compounds 1-5

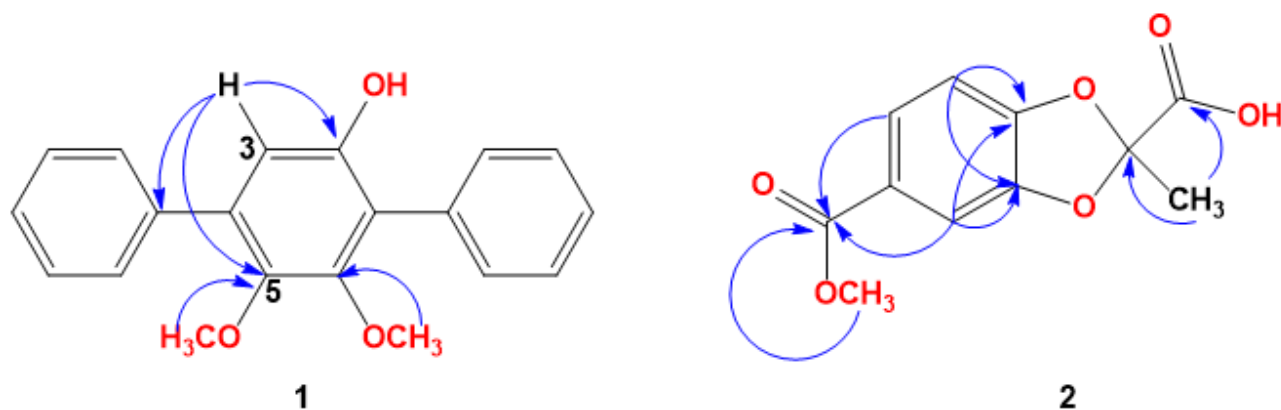


Figure 10

Key HMBC (H-C) correlations of 1 and 2

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryfileSpectrogramsofthecompounds.docx](#)
- [Supplementaryfile.docx](#)