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Allelopathic potential of root endophytic fungal metabolites of *Casuarina equisetifolia*

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ABSTRACT

In order to explore whether endophytic fungi of *Casuarina equisetifolia* L. are involved in allelopathy, we investigated their diversity and composition of their metabolites. By Illumina Miseq high-throughput sequencing platform, a total of 318,972 sequences were obtained from 9 root samples of *C. equisetifolia* of different ages, which included 11 phyla, 32 families and 32 genera. Four fungi were isolated from the roots by the traditional method and identified by biolog microbial automatic analysis system as *Aspergillus aculeatus* lzuka, *Penicillium melinii* Thom, *Neosartorya fisccheri* (Wehmer) Malloch & Cain BGA and *Penicillium solitum* Westling BGB. Pyrogallic acid, dodecanoic acid, palmitic acid and stearic acid, 2,4-Di-tert-butylphenol, 1,2,3,4-butanetetrol and globulol and other organic compounds were identified in the fermentation broths of four endophytic fungi by GC-MS analysis. Among them, 2, 4-Ditert-butylphenol, 1,2,3,4-butanetetrol and globulol were also found in soil, root and litter extract. These results imply that root-endophytic fungi are involved in the synthesis of substances that affect the host plants. In bioassays, the fermentation broth of the four root-endophytic fungi significantly inhibited the seed germination of *Thespesia lampas* L. and *Calophyllum inophyllum* L.

Key words: Allelopathic potential, *Casuarina equisetifolia*, endophytic fungi diversity, metabolites, seed germination.

INTRODUCTION

Casuarina equisetifolia is a tree used for shelter belts in Hainan Province (2,15,34). In pure forests, the simple community structure and poor ecological adaptability leads to the forest decline and this is presumed to be due to allelopathy. We have previously identified the chemical components of *C. equisetifolia* root, litter and soil extracts by GC-MS (28) and investigated their allelopathic effects on photosynthesis and antioxidant enzymes of *V. mangachapoi* seedlings (22,23).

Plant endophytes are diverse group of microorganisms, including endophytic bacteria, fungi, actinomycetes (6, 16), which are important components of the plant micro-ecosystems. Metabolites produced by Endophytic fungi are similar to those produced by the host plant (26) and are a source of allelochemicals. *C. equisetifolia* is a symbiotic plant with endophytic fungi (31) and these endophytic fungi may be involved in the synthesis of secondary metabolites. However, there are no studies on allelopathy of endophytic fungi of *C. equisetifolia***.**

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In this study, we investigated the endophytic fungal diversity of *C. equisetifolia* by Illumina Miseq high-throughput method. Further we have isolated, purified and cultured four endophytic fungi. Their fermentation broths have been analysed by GC-MS and investigated their effects on *Thespesia lampas* and *Calophyllum inophyllum* seed germination.

MATERIALS AND METHODS

In September 2016 using "S" shape 7-point sampling method (33), 10-15 mm diameter root samples of *C. equisetifolia* were collected from forests of 3-ages (Young forest: 5-8 years; Middle-aged forest: 15-20 years; Mature forest: 30 years and above) on the coast of Guilinyang, Haikou, China (N20°01'02"， E110°31'20", Mean annual temperature: 24.1°C, mean annual rainfall: 1760 mm). These samples were separated from the soil residues, washed with sterile water and then cut into 10mm long pieces and stored at -80 °C for analyzing endophytic fungi diversity and at -20 °C for separating and purifying the culturable endophytic fungi, respectively.

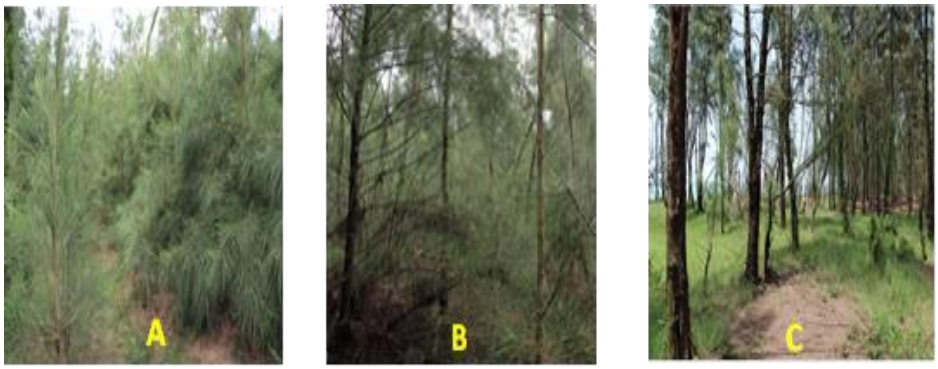

Figure1. The sampling sites of *Casuarina equisetifolia*.
A: Young forest; B: Middle-aged forest; C: Mature forest)

1.1 Illumina Miseq High throughput sequencing

Root samples 200 g, stored at -80℃ were cut into 0.5 × 0.5 cm size, rinsed with 75% ethanol for 1 min and soaked in 1% NaClO for 5-8 min (20). The RNA in root samples was then extracted and amplified with fungal universal primers (ITS) both SSU0817F (5'-TTAGCATGGAATAATRRAATAGGA-3') and SSU1196R (5'-TCTGGAC CTGGTGAGTTTCC-3') and the sequencing work was completed by the Shanghai Majorbio Bio-pharm Technology Co., Ltd. The data were analyzed on the free online platform of Majorbio I-Sanger Cloud Platform (www.i-sanger.com). The RDP (Ribosomal Database Project) classifier Bayesian algorithm was used to conduct taxonomic analysis of 97 % similar OUT (Operational Taxonomic Units) representative sequences and the community composition of each sample was statistically calculated for each level.

1.2 Separation and identification endophytic fungi by culture

The root samples stored at -20 °C were cut into 0.5 × 0.5 cms, rinsed with 75% ethanol for 1 min and soaked in 1% NaClO for 5-8 min (20), then placed on Potato Dextrose agar (PDA) plates (4 pieces per plate) containing streptomycin sulfate (100U/ml) and ampicillin (100 U/ml) at 28 °C for 4 -7days (17,19). Fungal colonies which appeared were selected based on morphology and transferred to fresh PDA and purified by repeated transfer on fresh media. The pure cultures were stored at 4 °C (4). For identification of pure cultures, they were inoculated into the Biolog-FF plates and identified by using the Biolog Filamentous Fungal Database and ITS (SAGENE company) (30).

1.3 GC-MS Analysis of fermentation broths of endophytic fungi

The purified fungal strains were transferred to 500 ml Potato Dextrose Medium (Potato 200g, Dextrose 20g, distilled water 1L) and incubated on a rotary shaker (150 rpm) at 28℃, for 30h (25). Then the fermentation broth was centrifuged at 4000 rpm for 10 min at room temperature, passed through membrane filter (pore size 0.22 μm) and then divided into two parts, one for bioassay and the other for GC-MS analysis.

The fermentation broth for GC-MS analysis (200 ml) was concentrated by rotavapor (55^0C, 40 min)**.** The resulting samples were divided into two and extracted by 1ml **n-hexane** and 1ml **methanol** separately (11) and then analysed by the GC-MS.

The n-hexane and methanol extracts were analyzed using a Thermo Quest TRACE GC/MS system equipped with a programmable split injector (port temperature of 250^{o}C throughout the run) and the Xcalibur analysis software. As described earlier (28).

1.4 Seed germination

Seeds of *Thespesia lampas* and *Calophyllum inophyllum* were collected from the coast of Dongzhaigang, Hainan Island, China. Fifty seeds per treatment were first treated with 0.5 % KMnO4 solution for 2 h and then were placed in Petri dishes (15 cm dia) lined with two sheets of filter paper at room temperature (25 ^{o}C). Then 6 ml of endophytic fungal fermentation broth was added to each treatment. Distilled water and sterile culture medium were used as control (CK). All treatments had three replicates. Germination (%) was determined daily until no new seeds germinated:

Germination (%) = Number of germinating seeds / Number of seeds tested × 100%

Absolute germination potential = Total number of seeds germinated before maximum germination day / Number of seeds tested × 100%

The extent of inhibition was represented by the RI: Reaction index as under:

$$RI=1-C/T$$

Where, T: Treatment value, C: Control value. If RI > 0: Indicates Stimulation, RI <0: Indicates Inhibition (1).

1.5 Statistical analysis

Statistical analyses of Data were performed using SPSS statistical software programme and LSD multiple comparisons with the significance level α= 0.05. The results

were plotted by Microsoft Excel software. All analyses were done in 3 replicates. Statistical significance was determined by SPSS (16.0) and LSD (least significant difference method) test. Difference were considered statistically significant when $P < 0.05$ (15).

RESULTS AND DISCUSSION

The diversity of endophytic fungal communities in root samples

A total of 318,972 sequences were obtained from 9 root samples of three *C. equisetifolia* plantations by using Miseq 250PE. The community composition at the phylum, family and genus was obtained from 97% similar OTU representative sequences. The results were shown in Fig.2.

Further, the abundance of endophytic fungal community was highest in middle-age forest, followed by mature-age forest and lowest in young-age forest. Xu *et al.* (27) also found that with the increase of forest age, bacteria in the soil of *C.equisetifolia* forest showed a tendency of increasing first and then decreasing. The composition of endophytic fungi community of young-age forest was similar to middle-age forest but different from mature-age forest.

(i). Phylum : The proportions of endophytic fungi were slightly different. At the phylum level, root endophytic fungi belonged to 11 phyla. Ascomycota accounted for 68.58 % as the main phylum. The other fungi were Basidiomycota (29.09 %), Glomeromycota (1.79 %) and Zygomycota (0.49 %). In young forest, middle-aged forest and mature forest the proportion of Ascomycota was 70.66 %, 61.05 % and 69.28 %, respectively. The proportion of Basidiomycota was 27.72 %, 30.01 % and 26 %, respectively. This indicated that the endophytic fungi of *C. equisetifolia* were dominated by Ascomycota and Basidiomycota (Fig. 2A).

(ii). Family : At the family level, the endophytic fungi belonged to 32 families. In Ascomycota, the percentage of the dominant families in young forest, middle age forest and mature forest were *Trichocomaceae* (30.41 %, 30.11 %, 20.69 %), unclassified family of Dothideomycetes (19.74 %, 9.87 %, 1.86 %), unclassified family of *Agaricomycetes* of Basidiomycota (24.95%, 18.80%, 15.18%), respectively. This shows that with the increase in forest age, the percentage of these fungi decreased gradually.

(iii). Genus : At the generic level, 32 genera were identified. The dominant genera were those of unclassified genera in *Trichocomaceae*, *Agaricomycetes* and *Dothideomycetes*, with relative abundances of 27.07 %, 19.64 % and 10.49 % respectively (Fig. 2C).

The *Trichocomaceae* belongs to Ascomycota. Members are parasites with aggressive colonization strategies and adaptable to extreme environmental conditions (8). *Agaricomycetes* are usually known as mushrooms, toadstools, bracket fungi, coralloid fungi etc. *Agaricomycetes* function as decayers, pathogens, parasites and mutualistic symbionts of both plants and animals. They make their broadest ecological impacts

through their activities as wood-decayers and ectomycorrhizal symbionts of forest trees (7).

These results imply that the fungal abundance is related to the growth time of *C. equisetifolia*. In young forest, these fungi may enter the plant from the soil and form symbiosis with plants, helping the plant to colonize and grow in poor soils. As the plant grows, their function diminishes and is replaced by other types of fungi.

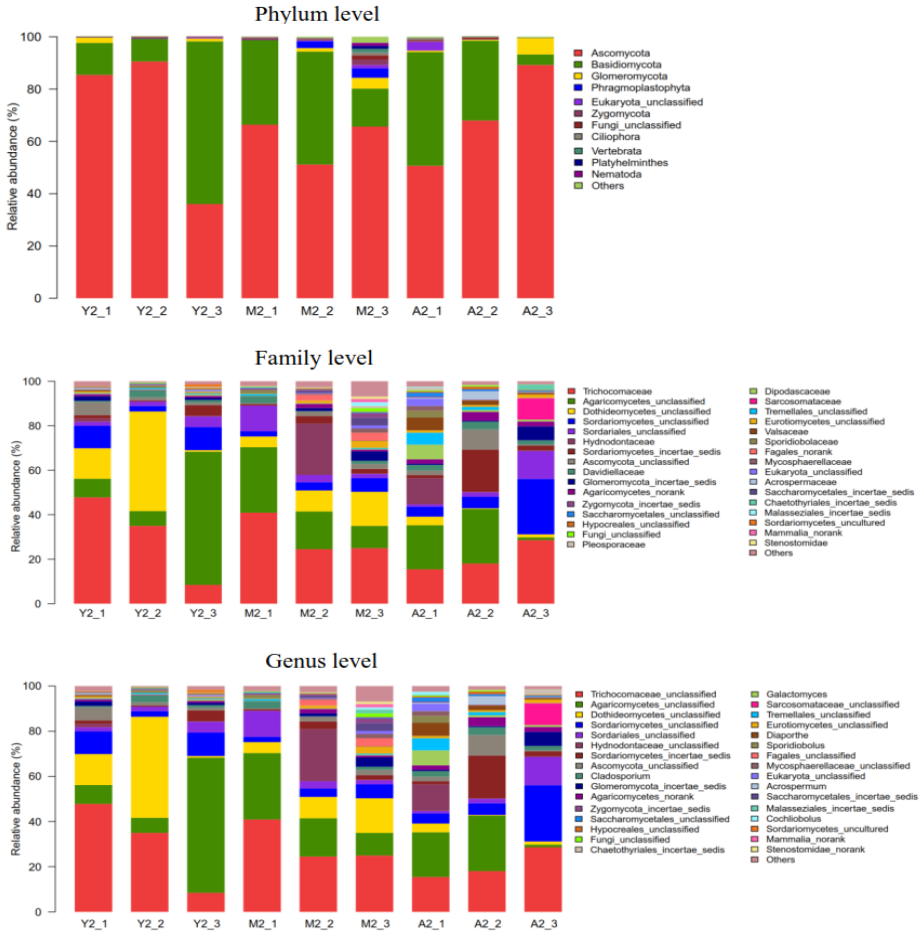

Figure 2. Profiling bar of species at different taxonomical level of root samples of various ages (young- aged forest：Y2-1,Y2-2,Y2-3；middle-aged forest：M2-1,M2-2,M2-3；mature- aged forest: A2-1,A2-2,A2-3)

In addition, Fig. 2 showed that there are large number of endophytic fungi from different age groups that are unclassified, which suggests that there are yet many unknown

species and our information about the endophytic fungi in the root of *C. equisetifolia* is still not complete. Further work on the isolation and identification of these fungi, their source and their role in allelopathy of *C. equisetifolia* needs to be done.

Isolation and identification of endophytic fungi

Four endophytic fungi were isolated from 9 root samples of *C. equisetifolia* plantations of different ages. The pure cultures in the young and middle-aged samples were *Aspergillus aculeatus* lzuka (from No.1 and No. 3 samples) and *Penicillium melinii* Thom (from No. 4, No. 5 and No. 6 samples), respectively and the fungi in mature samples were *Neosartorya fisccheri* (Wehmer) Malloch & Cain BGA (from No. 7 and No. 9 samples) and *Penicillium solitum* Westling (BGB) (from No. 8 and No. 9 samples). *A. aculeatus*, belongs to the family *Trichocomaceae* and is ubiquitous specie that can be isolated from rotting fruits and soil. It has been implicated as a causative agent in some plant diseases (14). Using the traditional methods, we could isolate four fungi, while high throughput sequencing showed that C. *equisetifolia* root has abundant endophytic fungi. It is likely that most of them were unculturable and only very small fraction could grow on synthetic growth media.

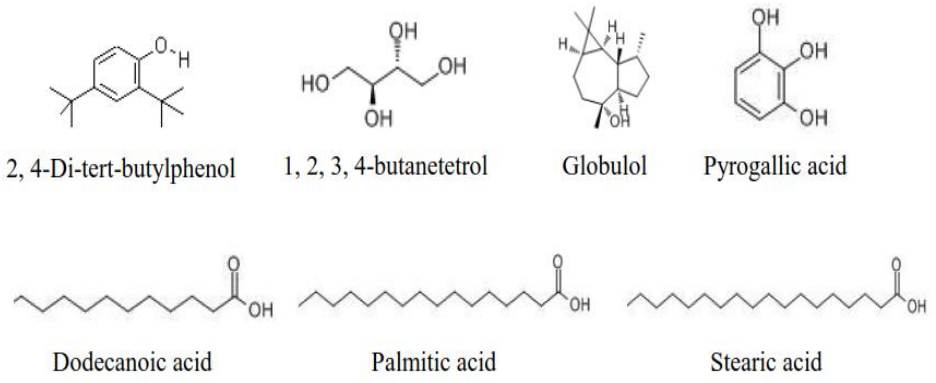

Figure 3. The structural formulas of the common substances and specific substances in endophytic fungal fermentation broth, soil, root and litter extracts at different ages.
(from http://cheman.chemnet.com/dict/zd.html)

GC-MS Analysis

The representative GC-MS chromatograms of four endophytic fungal fermentation broths are shown in Fig. 4 and Fig. 5. Tables 1-2, list the representative chemical components with relatively high content. These components include 33 esters, 17 phenols, 21 organic acids, 40 ketones, 13 aldehydes and other substances (such as ethers, alcohols, pyridines). The number of the metabolites extracted by methanol was more than that extracted from n-hexane.

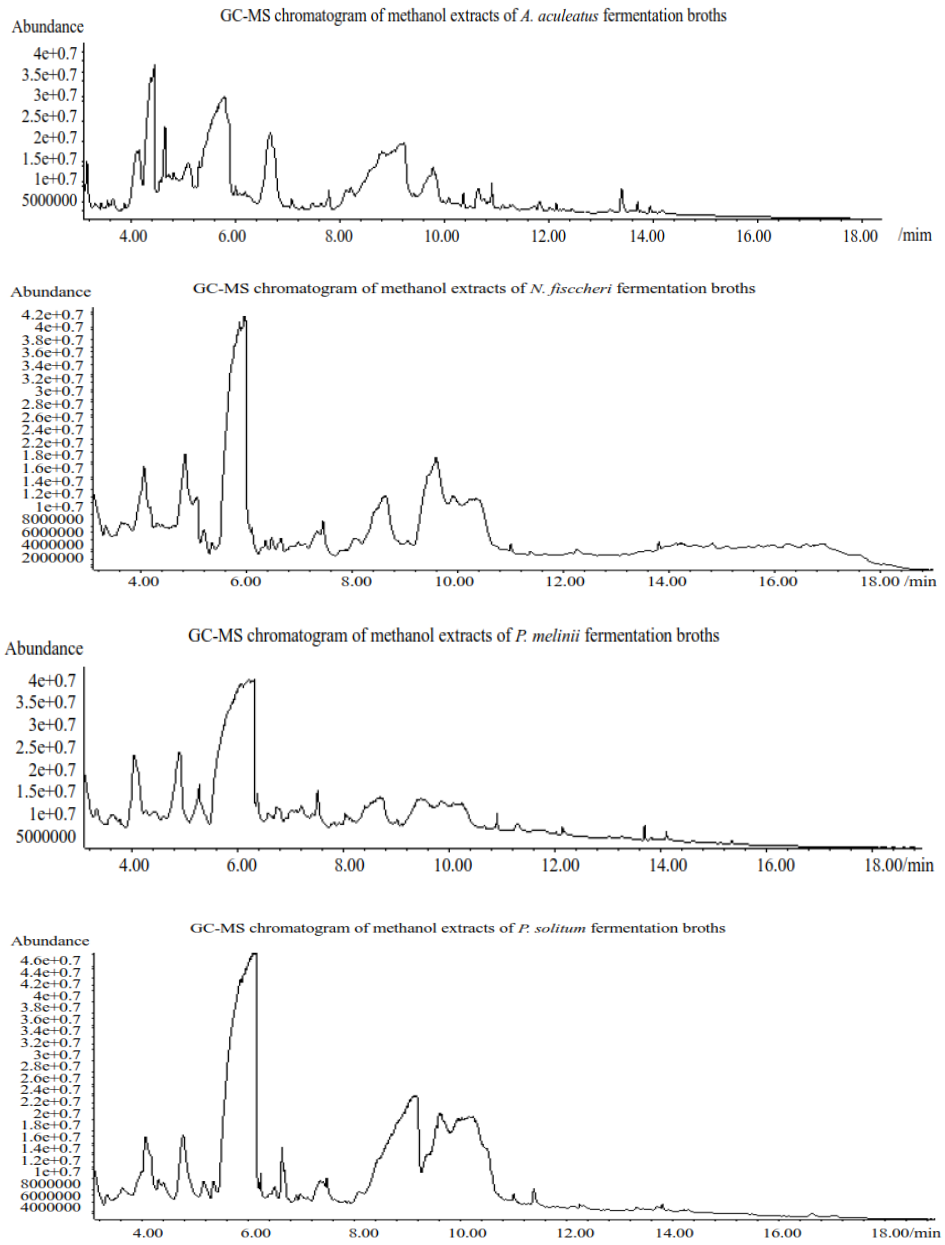

Figure 4. GC-MS chromatogram of methanol extracts of endophytic fungi fermentation broths

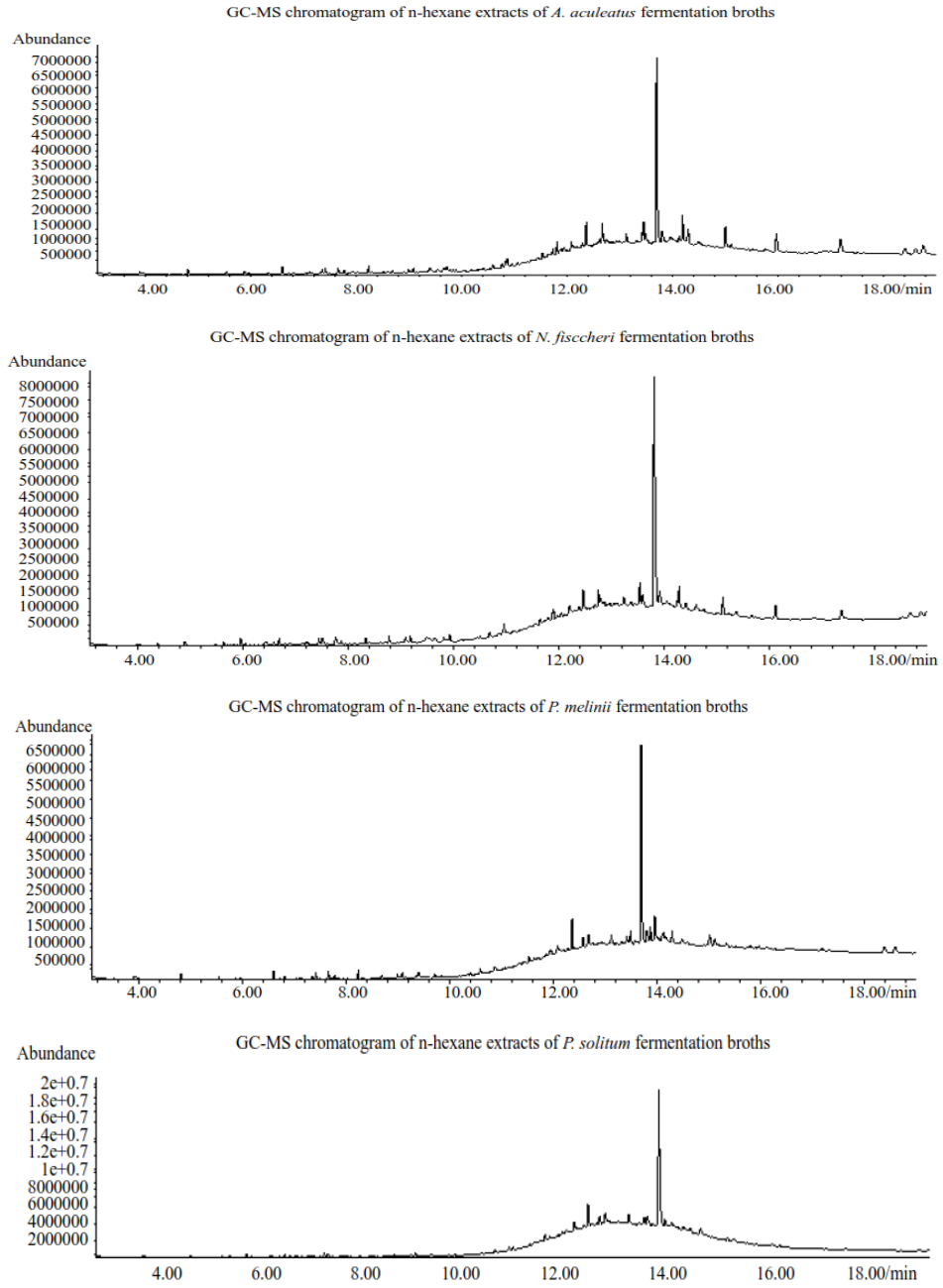

Figure 5. GC-MS chromatograms of n-hexane extracts of endophytic fungal fermentation broths

Table 1. The chemical components of methanol extracts of endophytic fungal fermentation broth

Compounds	Relative content (%)**			
	A. aculeatus	*N. fisccheri*	*P. melinii*	*P. solitum*
Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	0.165	0.635	0.224	0.134
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	-	17.852	26.194	16.771
dodecanoic acid	0.323	1.756	-	-
Pyrogallic acid	0.322	-	-	0.544
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	1.446	5.359	5.865	3.268
4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	-	0.638	2.9	-
Palmitic acid	0.632	-	0.763	0.431
Octanoic Acid	1.346	-	0.363	-
4,5-Diamino-6-hydroxypyrimidine	0.856	-	1.188	-
Stearic acid	0.173	-	-	0.117
5-Acetoxymethyl-2-furaldehyde	1.059	-	-	0.482
Glycerin	2.044	1.598	-	-
1-Nitro-2-acetamido-1,2-dideoxy-d-glucitol	-	0.225	-	0.238
.beta.-D-Glucopyranose, 1,6-anhydro-	1.694	5.355	1.961	5.871
.beta.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl-	-	0.665	-	0.083
p-Menthan-3-one, semicarbazone	-	7.618	-	3.554
D-Allose	2.916	-	-	2.606
Levoglucosenone	4.269	-	-	-
2-Furanmethanol	5.658	-	-	-
2-Furancarboxylic acid, hydrazide	2.502	-	-	-
Diglycerol	4.054	-	-	-
Thiophene, 2-methoxy-5-methyl-	7.551	-	-	-
1,6-Anhydro-.beta.-D-glucofuranose	5.46	-	-	-
1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	11.345	-	-	-
Dodecanoic acid, 1,2,3-propanetriyl ester	-	1.756	-	-
1,2,3,4-Butanetetrol	-	0.57	-	-
3-Deoxy-d-mannoic lactone	-	2.855	-	-
1,3-Dimethylimidazole-2(3H)-thione	-	3.607	-	-
Pyrimidine-2,4(1H,3H)-dione, 6-hydroxy-5-methyliminomethyl-	-	3.808	-	-
Pyrimidine, 2,4,5-triamino-	-	1	-	-
Globulol	-	-	0.156	-
Butanoic acid, 3-oxo-, 1-methylethyl ester	-	-	1.666	-
3-Pentanone, dimethylhydrazone	-	-	1.166	-
1H-Cyclopenta[c]thiophene, hexahydro-, cis-	-	-	4.896	-
2-Propanol, 1-(propylthio)-	-	-	1.012	-
3-Methyl-2-thiophenecarboxaldehyde	-	-	1.04	-
[1,1'-Biphenyl]-3-amine	-	-	2.911	-
Pyridine, 4-(phenylmethyl)-	-	-	2.671	-
.alpha.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl-	-	-	1.165	-
2R,3S-9-[1,3,4-Trihydroxy-2-butoxymethyl]guanine	-	-	1.032	-
Phenol, 4-methoxy-2-nitro-	-	-	-	2.278
1-Propanone, 1-(2-furanyl)-	-	-	-	2.723

2-Thiophenecarboxylic acid, 5-tert-butyl-	-	-	-	1.95
1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol	-	-	-	1.814
1-Deoxy-d-altritol	-	-	-	1.066
D-glycero-D-manno-Heptitol	-	-	-	1.338
1,2,5,6-Di-O-isopropylidene-3-O-methanesulfonyl glucofuranose	-	-	-	1.154

**The relative content percentage determined by normalization method of area

Table 2. The chemical components of n-hexane extracts of endophytic fungal fermentation broth

Compounds	Relative content (%)**			
	A. aculeatus	*N. fisccheri*	*P. melinii*	*P. solitum*
2,4-Di-tert-butylphenol	0.46	-	1.288	-
Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	13.898	13.687	27.565-	8.14
11,13-Dimethyl-12-tetradecen-1-ol acetate	3.072	3.473	1.512	3.886
Decahydro-8a-ethyl-1,1,4a,6-tetramethylnaphthalene	2.034	-	2.511	4.096
Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)-	2.908	3.247	-	0.929
Pentadec-7-ene, 7-bromomethyl-	1.987	-	-	2.963
17-Pentatriacontene	-	1.513	-	0.173
9-Hexacosene	-	-	1.886	2.88
(E,Z)-N-(2,7-Dimethyl-2,7-octadien-1-yl)-2,7-dimethyl-2,7-octadien-1-amine	-	-	1.169	1.984
1-Hexacosene	3.105	-	2.234	-
Methyl 2-octylcyclopropene-1-octanoate	-	1.991	-	2.413
2-Dodecen-1-yl(-)succinic anhydride	-	3.65	1.157	-
Bicyclo[3.1.1]heptan-3-one, 2-(but-3-enyl)-6,6-dimethyl-	-	-	2.565	1.068
1,2-Benzenedicarboxylic acid, diisooctyl ester	3.884	-	-	-
9-Tricosene, (Z)-	3.28	-	-	-
Androst-16-ene-17-carboxylic acid, (5.alpha.)-	3.639	-	-	-
2-(4-Fluoro-phenyl)-4-(3-methyl-benzylidene)-4H-oxazol-5-one	2.924	-	-	-
Triacontyl heptafluorobutyrate	-	2.603	-	-
11,12-Dibromo-tetradecan-1-ol acetate	-	1.872	-	-
5.beta.-Cholest-23-ene, (Z)-	-	1.189	-	-
Phenol, 2,4-bis(1-phenylethyl)-	-	-	3.879	-
2-Ethylbutyric acid, heptadecyl ester	-	-	8.418	-
10-Heneicosene (c,t)	-	-	3.851	-
Naphthalene, 1,2,3,4-tetrahydro-1-methoxy-	-	-	2.875	-
Propionamide, 2-acetylamino-3-(1H-indol-3-yl)-N-thiophen-2-ylmethyl-	-	-	2.488	-
Octanamide, N,N-dimethyl-	-	-	2.744	-
Thiophene-2-carbonitrile, 5-tert-butyl-3-(4-chlorobenzylidenamino)-	-	-	3.638	-
Benzo[h]quinoline, 2,4-dimethyl-	-	-	1.865	-
4,5-2H-Oxazole-5-one, 4-[3,5-di-t-butyl-4-methoxyphenyl]methylene-2-phenyl-	-	-	2.009	-
Octatriacontyl pentafluoropropionate	-	-	-	1.733
Octacosyl heptafluorobutyrate	-	-	-	2.328
Fumaric acid, dodecyl 2-methylallyl ester	-	-	-	1.865

6-Octen-1-ol, 3,7-dimethyl-, acetate	-	-	-	1.899
1-Nonadecene	-	-	-	2.831
Naphthalene, decahydro-1,6-dimethyl-4-(1-methylethyl)-	-	-	-	1.102
Cyclopropane carboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)-	-	-	-	1.928

**The relative content percentage determined by normalization method of area

Comparison of the present GC-MS spectral results with our previous results of GC-MS analysis of root, soil and litter extracts at different ages of *C. equisetifolia* (28), showed that 3 chemicals (2,4-Di-tert-butylphenol, 1,2,3,4-butanetetrol and globulol (The structural formulas were shown in Fig. 3)) were common components present in the endophytic fungal fermentation broth and also in the soil, roots and litter extracts of different-aged *C. equisetifolia*. The relative content of 2,4-Di-tert-butylphenol in fermentation broth of *A. aculeatus* and *P. melinii* was 0.46 % and 1.288 %, respectively. Our previous study (28) showed this substance exists in the soil, root and litter extracts and the relative content in soil and root was higher than in the endophytic fungal broth. The results indicated that 2,4-Di-tert-butylphenol produced by endophytic fungi and host plants, is then released into the soil, where it accumulated.

The relative content of 1,2,3,4-butanetetrol detected in *N. fisccher*i fermentation broths was 0.57 % and this was also found in the soil extract (0.019%) and in the soil bacterium *Mycobacterium hassiacum* (11). This suggested that this substance may be produced directly by root endophytic fungi or by soil microorganisms and enters the soil.

Globulol was detected in the *P. melinii* fermentation broths and the litter extracts of medium-aged forests and its relative content was 0.156 % and 0.187 %, respectively. However, it was not found in the root and soil extracts, indicating that globulol was perhaps either produced directly by the root endophytic fungus (*P melinii*) or in the tissues of *C. equisetifolia* (25). It is known that 2,4-Di-tert-butylphenol, 1,2,3,4-butanetetrol and globulol are synthesized by plants and they are allelochemicals. Cheng (3) found that 2,4-Di-tert-butylphenol is the main allelochemical in lily root exudates. Qin and Zhou (18, 32) detected 2,4-Di-tert-butylphenol in different plants and showed it to have allelopathic effects. Liu (13) reported that 1,2,3,4-butanetetrol in *Phragmites australis*, which is an allelochemical and Li (12) found globulol in the volatile oil of *Merrenia boisiana*. We have found that 2,4-Di-tert-butylphenol, 1,2,3,4-butanetetrol and globulol in *C. equisetifolia* as well as in the fermentation broth of its endophytic fungi. This suggested that perhaps a cooperative mechanism exist between the plant and the endophytic fungi leading to the synthesis of these chemicals.

In GC-MS analysis, we found pyrogallic acid, dodecanoic acid, palmitic acid and stearic acid (The structural formulas were shown in Fig. 3) only in the endophytic fungal fermentation broth. These substance are allelochemicals and inhibits the growth of the some plants (24,29). Thus the endophytic fungi are perhaps involved in the synthesis of allelochemicals.

Allelopathic effects of endophytic fungal fermentation broths on *T. Lampas and C. inophyllum* seed germination

The effects of endophytic fungal fermentation broths on seeds germination of *T. Lampas and C. inophyllum* was shown in Fig. 6. The maximum germination rate of *T. Lampas* seeds was on the 9th day, one day later than the control group. The maximum germination of *C. inophyllum* seeds was on the 10th day, which was delayed by two day than control group. These results suggested a slight delay in seed germination in the presence of fermentation broths.

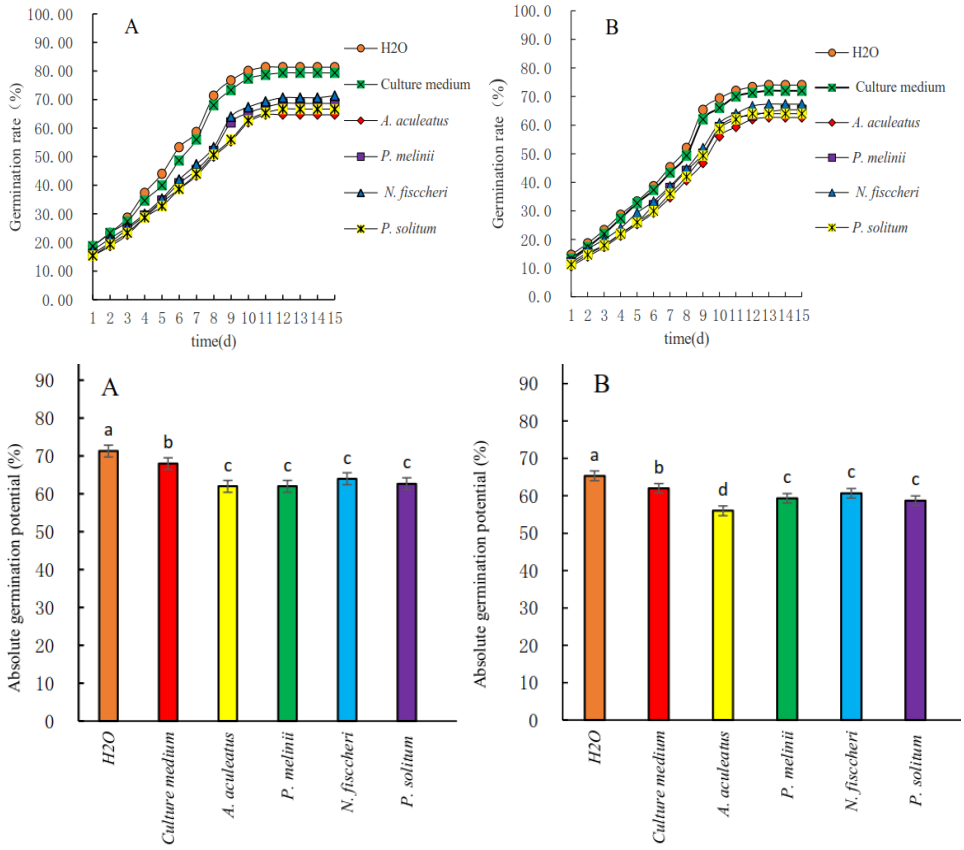

Figure 6. Effects of endophytic fungi fermentation broth on seed germination
(A: *T. lampas* , B: *C. inophyllum*.)

Compared with the control, the fermentation broths of root-endophytic fungi inhibited the germination of two native tree species (*T. Lampas and C. inophyllum*), but there were no significant differences between treatment groups (Fig. 6-2).

The allelopathic effects indices of different-aged root endophytic fungi fermentation broths on seeds of two native tree species are shown in Figure 7**.** It can be seen that the fermentation broths of the four fungi produce different allelopathic effects on the seeds of *T. Lampas and C. inophyllum.* The allelopathy effect index of *T. Lampas and C. inophyllum* was the strongest when treated with *A. aculeatus* fermentation broth, which was -0.26 and -0.17, respectively. This showed that the allelopathy potential of different strains is different.

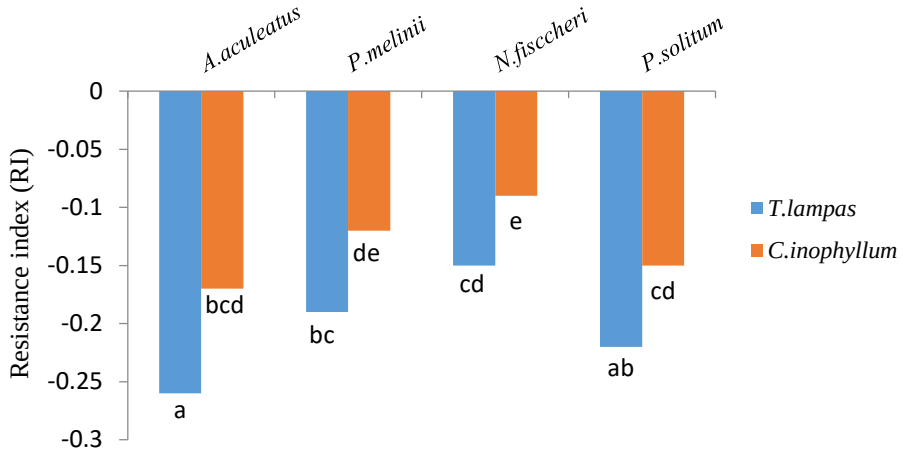

Figure 7. The allelopathy index (Resistance index) of endophytic fungi fermentation broth on seeds germination Values marked with different letters are significantly at P<0.05.

Also, with the same fungal fermentation broth, the allelopathic effect indices on *T. lampas* were stronger than on *C. inophyllum*. This suggested that *C. inophyllum* was less susceptible to allelopathy, which is consistent with earlier findings (11). Among the fungal fermentation broths, the allelopathic effect index of the same seed was strongest with *A. aculeatus* fermentation broth and this was significantly different from the effects of *P. melinii*, *N. fisccheri* and *P. solitum* fermentation broths. This showed that that the allelopathic potential of *A. aculeatus* was strongest.

CONCLUSIONS

C. equisetifolia root has abundant endophytic fungi dominated by Ascomycota and Basidiomycota. The fermentation broths of the endophytic fungi isolated from the root of *C. equisetifolia* had allelopathic effects on the seeds germination of *T. lampas and C. inophyllum.* Among them, the fermentation broth had stronger allelopathy effects on *T. lampas* seed germination than on *C. inophyllum*. Compared with other endophytic fungi, *A. aculeatus* has the strongest allelopathic potential.

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