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Contributed Paper

Medicinal Plant Endophytes Produce Analogous Bioactive Compounds

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ABSTRACT

Endophytic fungi from medicinal plants are potential sources of the bioactive medicinal compounds. We isolated 193 endophytic isolates from 360 tissue fragments from the Chinese medicinal plant species *Camptotheca cuminata, Gastrodia elata* and *Pinellia ternata*. Forty-two taxa were identified based on morphological traits and internal transcribed spacer (ITS) rDNA sequence analysis and comprised 33 Ascomycota, four Basidiomycota and five Mucoromycotina. All the fungal strains were screened for bioactive-compound production by submerged culture. Three taxa isolated from *Camptotheca cuminata* produced camptothecin with relatively high yields. *Colletotrichum gloeosporioides* (strain XSXY05) from *Camptotheca cuminata* produced 10-hydroxycamptothecin with yields of 24 μg per gram of myceliium. Three taxa isolated from *Gastrodia elata* produced gastrodin with yields of 57, 89, 184 μg per gram of myceliium, respectively, while three taxa from *Pinellia ternata* produced ephedrine hydrochloride, though in relatively low amounts. Both gastrodin and ephedrine hydrochloride are potentially valuable for pharmacological utilization. All bioactive-compound were located in the ground hypha of the isolates and not in the culture broth.

Keywords: medicinal plant, fungal communities, diversity, phylogeny, alkaloids, phenolic glycoside

1. INTRODUCTION

Camptotheca cuminata Decne. (*Camptotheca*, *Nyssaceae*) is a native species to southern China. It is a source of camptothecin, an indole alkaloid with anti-cancer activity, that was first isolated from this plant ([1]Wall and Wani 1966). 10-hydroxycamptothecin is a natural derivative of camptothecin and mainly exists in fruits and roots of *C. cuminata* ([2]Lorence et al. 2004). *Gastrodia elata* Blume. (*Gastrodia*, *Orchidaceae*) has been considered as one of the most important herbal medicines in China and is used for the treatment of headaches,

migraines, dizziness and other neuralgic and nervous disorders in oriental traditional medicine ([3]Bensky and Gamble 1986; [4]Tang and Eisenbrand 1992). Gastrodin from *G. elata* is the dominant active ingredient and has many pharmacological effects ([5]Hsieh et al. 1997). *Pinellia ternata* (Thunb.) Makino (*Pinellia, Araceae*) is a perennial natural herbal medicine that grows in eastern Asia and mainly in China. It is widely used in Traditional Chinese Medicine for its anti-emetic, anti-coughing analgesic, and sedative effects ([6]Luo *et al*. 2000).

Endophytic fungi colonize internal tissues of healthy plant without causing visible disease symptoms[13]. They are found in virtually all plant species such as *Calamus kerrianus* and *Wallichia caryotoides* (*Arecaceae*) [14]. Some endophytic fungi are known to produce bioactive compounds[9], and these may be similar to those produced by the host plants[10]. For example, a paclitaxol producing endophyte, *Taxomyces andreanae*, was successfully discovered from the Pacific yew, *Taxus brevifolia*[11]. A recent study using a paclitaxel producing endophytic isolate of *Chaetomium sp.* reported a potentially industrial yield of paclitaxel of 1124.34 μg/L via optimized fermentation[12]. Also, other reports refer to endophytic fungi capable of producing compounds of their host plant, including camptothecin and structural analogs [7,14,15].

As fungi can be easily cultured and fermented they have the potential to provide a novel source for producing useful medicinal compounds[16]. The numbers of endophytic fungi in nature are thought to be enormous and have become a research focus as sources of new bioactive compounds[17].

The purpose of the present study was 1 (to establish the endophytic fungi diversity in the medicinal plants *Camptotheca cuminata, Gastrodia elata* and *Pinellia ternata*, and 2) to screen the endophytic fungi to establish if they produced camptothecin, 10-hydroxycamptothicin, gaqstrodin, and ephedrine HCl.

2. MATERIALS AND METHODS

2.1 Sampling

Healthy plants(without apparent disease) tissues (leaves, twigs, roots) of *Camptotheca cuminata* from the campus of Guizhou University were sampled by Cao J.J. in March, June, September and December 2008. *Gastrodia elata* tissues (leaves, twigs, roots, flowers) was collected by Mo L. from Guizhou, Sichuan, Shanxi and Yunnan provinces of China and *Pinellia ternata* tissues (leaves, twigs, roots, flowers) was collected Su H. from Guizhou, Sichuan, Jiangsu, Yunnan, Shanxi and Guangxi provinces of China from June to October, 2008. Samples were enclosed and stored in the refrigerator under -80°C for Endophytic fungi isolation.

2.2 Endophyte Isolation and Morphological Characterization

Samples including leaves, twigs, roots from the medicinal plants were in 70% ethanol for surface sterilization, immersed in 10% NaClO for 5-10min and flushed by sterile water 3 times. The leaves were cut into segments (5 × 5 mm), and twigs and roots were cut into small pieces of 10 mm lengths. The surface sterilized pieces were placed on Petri plates containing potato dextrose agar medium supplemented with 20μg/ml streptomycin (50mg/ml) and 25μg/ml penicillin G(100mg/ml), and incubated for 7-15 days at 25±1°C in an incubator. Actively growing myceliium tips immerging from plant tissues were subcultured in PDA Petri plates for identification and enumeration. The pure endophytic fungal strains were preserved in the the Engineering and Research

Center for Southwest Bio-Pharmaceutical Resources of National Education Ministry of China, Guizhou University. The morphological identification of endophytic fungi was based on fungal morphology of colony, hyphae, spores, and reproductive structures[17]. For inducing sporulation, each of the fungal isolates was separately inoculated on potato carrot agar, or water agar in Petri dishes. Measurements of all fungal characters were made in water mounts, and the slides were subsequently mounted in lactophenol.

2.3 Mycelial Culture Suspension and Bioactive Compound Extraction

A 250 ml Erlenmeyer flask containing 100 ml of liquid PD medium was inoculated with four agar plugs taken from cultures of endophytic isolates purified in PDA. All flasks were incubated at 120 rev min^{-1} on a rotary shaker at 27°C for 7 days. After incubation, mycelia was separated from the broth by filter paper. Culture filtrates were extracted three times with an equal volume of n-butanol. The remaining mycelia was dried and ground with a pestle and mortar, followed by extraction with 100 ml of anhydrous formaldehyde two times for isolates from *Camptotheca cuminata* and *Gastrodia elata*, 0.1% hydrochloric acid solution for isolates from *Pinellia ternata*. Then get the supernatant after centrifugal separation.

HPLC was employed to detect bioactive compounds produced by the endophytic fungi. The standard samples used in this study were camptothecin, 10-hydroxycamptothecin, gastrodin and ephedrine hydrochloride, and all standard materials were provided by National Institutes for Food and Drug Control. Each standard solution were prepared with different gradient: (1) camptothecin: 0.004mg/ml, 0.005 mg/ml, 0.006 mg/ml, 0.007 mg/ml, 0.008 mg/ml; (2) 10-hydroxycamptothecin: 0.0025 mg/ml, 0.003 mg/ml, 0.0035 mg/ml, 0.004 mg/ml, 0.0045 mg/ml; (3) gastrodin: 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml, 0.0312 mg/ml, 0.0156 mg/ml; (4) ephedrine hydrochloride: 0.001mg/ml, 0.002 mg/ml, 0.004 mg/ml, 0.008 mg/ml, 0.016 mg/ml. According to the HPLC test results of standard substances followed by the reaction condition (Table. 1), we construct a linear regression equation for each standard solution: $Y = a + bX$, where Y is the peak area of HPLC chromatogram, X is the concentration of sample injected in each HPLC test, a is the intercept and b is the slop of equation. r for the correlation coefficient is also calculated. Camptothecin was: $Y = 1.9058X - 0.4913$ ($r = 0.9998$); hydroxycamptothecine was: $Y = 3.5357X + 0.1441$ ($r = 0.9997$); gastrodin was: $Y = 1.1681X - 1.1569$ ($r = 0.9998$); ephedrine hydrochloride was: $Y = 10.0293X + 0.2032$ ($r = 0.9999$).

Table 1. HPLC analysis for the Alkaloids and Phenolic glycoside used in this study.

BC	CT	WL(nm)	FR(ml/min)	IV(μl)	℃	MP(V/V)
Camptothecin	150 mm × 4 mm column C18	360	0.8	10	55	CH_3OH/H_2O(40/60)
10-hydroxycamptothecin	150mm×4mm column C18	360	0.8	10	55	CH_3OH/H_2O(40/60)
Gastrodin	250mm×4.6mm column C18	270	2	10	25	CH_3OH/H_2O(8/92)
Ephedrine hydrochloride	150mm×4mm column C18	210	1.0	10	40	CH_3CN/H_2O(3/97)

BC = bioactive compound, *CT* = type of column, *WL* = wavelength, *FR* = flowing, *IV* = volume of injected sample, °C = column temperature, *MP* = ratio of mobile phase

2.4 DNA Extraction, Amplification and Sequencing

Genomic DNA was isolated from fungal mycelia collected from the plates using the isolation protocol of Lee and Taylor [18]. Template DNA (20ng) was amplified in a 25 ml PCR reaction mixture consisting of 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl (pH 8.8), 6 mM MgCl2, and 500 mM each of dATP, dCTP, dGTP, and dTTP, with 60 pmols ITS4 and ITS5 primers[19], and 2.5 units Biotaq DNA polymerase. All the reaction reagents were provided by GenScript Corporation of China. The reaction was set up as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 7 min in a Bio-RAD Gene CyclerTM PCR. A negative control using water instead of template DNA was set up for each experiment. PCR products were analyzed by electrophoresis at 120 V for 30 min in a 1.0% (w/v) agarose gel in 0.5×TAE buffer (0.4M Tris, 0.05M NaAc, and 0.01M EDTA, pH 7.85) and visualized under UV light in a transluminator following ethidium bromide staining. PCR products were purified via a PCR Product Purification Kit according to the manufacturer's protocol. DNA sequencing was performed using primers ITS4 or ITS5 on an ABI 3100 automated sequencer following the manufacturer's instructions.

The ITS rDNA of each taxon was identified using a Blast search in the nucleotide database in GenBank. Nucleotide sequences of ITS rDNA the isolates of this study and their allies retrieved from GenBank as well as the outgroup were assembled using Tex-Edit Plus respectively. The alignment of the sequence files was conducted using the CLUSTAL W software [20]. Phylogenetic analyses were performed with PAUP version 4.0b10[21]. The most parsimonious trees (MPT) were determined from the data sets using the heuristic search options with 1000 random sequence input orders with MULPARS on and TBR branch swapping for the exact solution. The unconstrained topologies of the equally parsimonious trees were compared using the Kishino-Hasegawa test of PAUP. The best topology was selected as the most parsimonious tree topology. Parsimony bootstrap with 1000 replicates in PAUP was applied to the tree to evaluate the stability. Where possible the ITS sequence data was compared with types and epitypes of species matched in GenBank to confirm the identification.

3. RESULTS

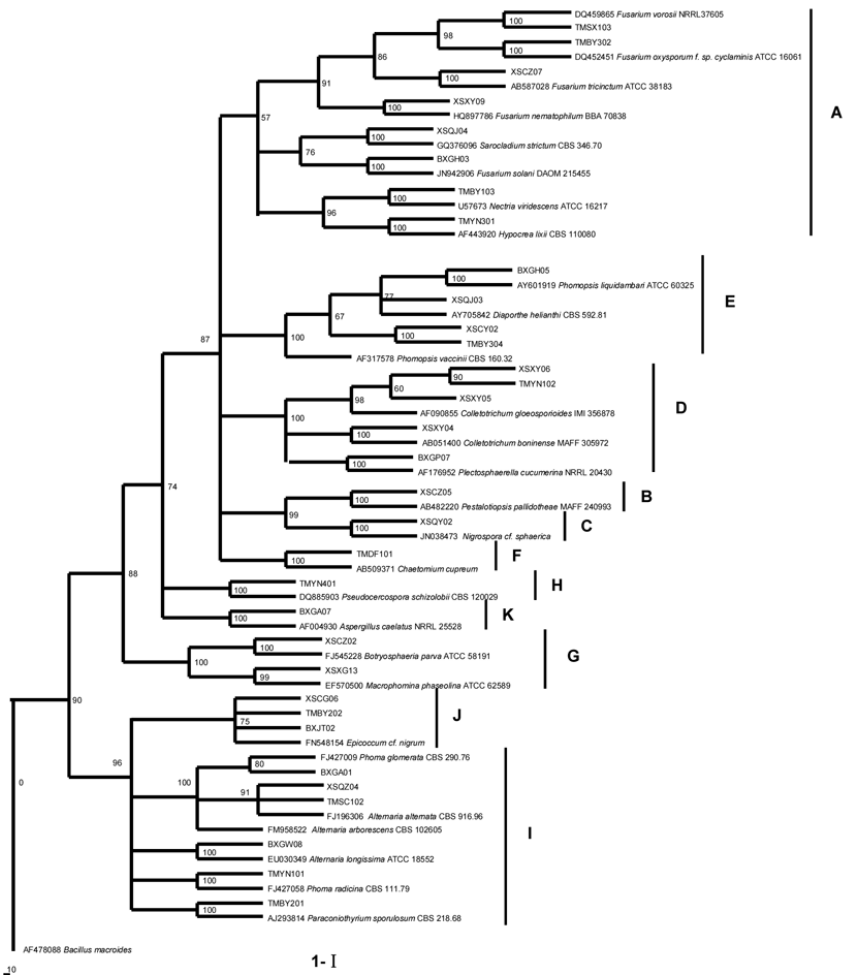
3.1 Phylogenetic Analysis and Molecular Identification

In this study, 40 samples from each tissue (leaf, twig, root) of the three medicinal plant species, and in total 360 tissue segments were used for isolation of endophytes. In total, 193 endophytic isolates were recovered and classified as 42 morphotypes according to their distinct morphological characters. The ITS

rDNA of each morphotype was sequenced and identified by Blast searches with the nucleotide database in GenBank. As a result, 42 representative ITS rDNA genotypes were obtained. Those epitypes or types of ITS rDNA sequences genes (Table 1) from GenBank which had high similarity with the isolates in this study were selected in the phylogenetic analysis to verify their identity. The sequences generated in this study also published on GenBank with accession numbers from JQ676174 to JQ676215.

3.2 Phylogenetic Taxa Composition

The endophytic isolates grouped in Ascomycota, subphylum Pezizomycotina within three classes: Sordariomycetes, Dothideomycetes, Eurotiomycetes; in Basidiomycota, subphylum Agaricomycotina in class Agaricomycetes and in subphylum Mucoromycotina (Figure 1). The most abundant class was Sordariomycetes (56.99%) represented by orders *Hypocreales*, *Xylariales*, *Trichosphaeriales*, *Sordariales*, *Glomerellales* and *Diaporthales*. Class Dothideomycetes (32.12%) was represented by orders *Botryosphaeriales*, *Capnodiales* and *Pleosporales*. *Mucorales* and *Mortierellales* corresponded to Mucoromycotina. Eurotiomycetes represented by orders *Eurotiales*. The least abundant class was Agaricomycetes within the order *Agaricales*.



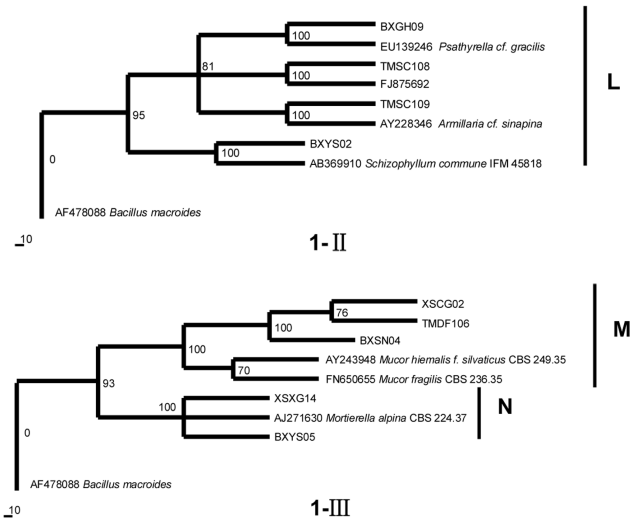


Figure 1. Phylogenetic trees based on MP analysis of ITS rDNA data for 42 endophytic taxa in three groups: 1-I Ascomycota; 1-II Basidiomycota; 1-III Mucoromycotina. The trees were rooted with Bacillus macroides.

The most abundant order was *Hypocreales* (33.16%), which was also the predominant group in Sordariomycetes, followed by *Pleosporales* (27.98%) of Dothideomycetes. On the contrary, *Trichosphaeriales* of Sordariomycetes and *Capnodiales* of Dothideomycetes was least common.

Ko Ko et al.[22] has shown that endophytic isolates cannot be accurately identified by using closest sequence similarity with names in GenBank as it has been shown that a large percentage of names in GenBank have been wrongly applied. For example 86% of *Colletotrichum gloeosporioides* names and 85% of *C. graminicola* names were shown to be wrongly applied[23]. A similar status lies in *Pestalotiopsis*[24], *Phomopsis*[25] and other complex genera *Fusarium* [26]. It is therefore essential to use type of epitype sequences in such analyses. We have therefore blasted our sequences in GenBank for closest matches and then compared these idenitifications with types or epitype of this sequences if available.

Endophytes were isolated from *Camptotheca cuminata* (70 strains, 36.27% of isolates in the study), *Gastrodia elata* (62 strains, 32.12%) and *Pinellia ternata* (61 strains, 31.61%) (Table 2). Twenty-nine fungal genera were discovered, with 23 being Ascomycetes, four Basidiomycetes and two Mucoromycotina. Thirteen genera were identified from *Camptotheca cuminata* distributed in seven orders of Ascomycota and two orders of Mucoromycotina. The 14 genera identified from *Gastrodia elata* were distributed in eight orders of Ascomycota, Basidiomycota and Mucoromycotina, eleven genera were isolated from *Pinellia ternata*. Five taxa occurred in all host plants, while five were unique to *Camptotheca cuminata*, eight to *Gastrodia elata* and four to *Pinellia ternata*. The common endophytes in each host differed (Table 1).

Table 2. Taxa isolated and their GenBank accession number generated in this study.

Isolate code	Closest GenBank taxa	Max ident (%)	ITS	Name given(accession number)
	Sordariomycetes_Hypocreales(A)			
TMBY103	*Nectria viridescens* ATCC 16217	97	U57673	*Nectria viridescens* (JQ676175)
TMSX103	*Fusarium vorosii* NRRL 37605 (type)	99	DQ459865	*Fusarium vorosii* (JQ676176)
TMBY302	*Fusarium oxysporum f. sp. cyclaminis* ATCC 16061 (holotype)	99	DQ452451	*Fusarium oxysporum f. sp. cyclaminis* (JQ676177)
BXGH03	*Fusarium solani* DAOM 215455 (holotype)	99	JN942906	*Fusarium solani* (JQ676178)
XSXY09	*Fusarium nematophilum* BBA 70838	98	HQ897786	*Fusarium nematophilum* (JQ676179)
XSCZ07	*Fusarium tricinctum* ATCC 38183	100	AB587028	*Fusarium tricinctum* (JQ676180)
TMYN301	*Hypocrea lixii* CBS 110080 (epitype)	99	AF443920	*Hypocrea lixii* (JQ676181)
XSQJ04	*Sarocladium strictum* CBS 346.70 (holotype)	100	GQ376096	*Sarocladium strictum* (JQ676174)
	Sordariomycetes_Xylariales(B)			
XSCZ05	*Pestalotiopsis pallidotheae* MAFF 240993 (holotype)	99	AB482220	*Pestalotiopsis pallidotheae* (JQ676182)
	Sordariomycetes_Trichosphaeriales(C)			
XSQY02	*Nigrospora cf. sphaerica*	99	JN038473	*Nigrospora cf. sphaerica* (JQ676183)
	Sordariomycetes_Glomerellales(D)			
XSXY04	*Colletotrichum boninense* MAFF 305972 (holotype)	99	AB051400	*Colletotrichum boninense* (JQ676184)
XSXY05	*Colletotrichum gloeosporioides* IMI 356878 (epitype)	99	AF090855	*Colletotrichum gloeosporioides* (JQ676185)
XSXY06		99		*Colletotrichum gloeosporioides* (JQ676186)
TMYN102		99		*Colletotrichum gloeosporioides* (JQ676187)
BXGP07	*Plectosphaerella cucumerina* NRRL 20430	97	AF176952	*Plectosphaerella cucumerina* (JQ676188)
	Sordariomycetes_Diaporthales(E)			
TMBY304	*Phomopsis vaccinii* CBS 160.32 (holotype)	97	AF317578	*Phomopsis vaccinii* (JQ676189)
XSCY02		97		*Phomopsis vaccinii* (JQ676190)
BXGH05	*Phomopsis liquidambari* ATCC 60325 (holotype)	96	AY601919	*Phomopsis liquidambari* (JQ676191)
XSQJ03	*Diaporthe helianthi* CBS 592.81 (paratype)	96	AY705842	*Diaporthe helianthi* (JQ676194)
	Sordariomycetes_ Sordariales(F)			
TMDF101	*Chaetomium cupreum*	97	AB509371	*Chaetomium cupreum* (JQ676206)
	Dothideomycetes_Botryosphaeriales(G)			
XSCZ02	*Botryosphaeria parva* ATCC 58191(holotype)	99	FJ545228	*Botryosphaeria parva* (JQ676192)
XSXG13	*Macrophomina phaseolina* ATCC 62589	100	EF570500	*Macrophomina phaseolina* (JQ676193)
	Dothideomycetes_Capnodiales(H)			
TMYN401	*Pseudocercospora schizolobii* CBS 120029 (holotype)	99	DQ885903	*Pseudocercospora schizolobii* (JQ676195)
	Dothideomycetes_Pleosporales(I)			
XSQZ04	*Alternaria alternata* CBS 916.96 (epitype)	100	FJ196306	*Alternaria alternata* (JQ676196)
TMSC102	*Alternaria arborescens* CBS 102605 (holotype)	99	FM958522	*Alternaria arborescens* (JQ676197)
BXGW08	*Alternaria longissima* ATCC 18552	99	EU030349	*Alternaria longissima* (JQ676198)
TMBY201	*Paraconiothyrium sporulosum* CBS 218.68 (isotype)	100	AJ293814	*Paraconiothyrium sporulosum* ()
TMYN101	*Phoma radicina* CBS 111.79 (epitype)	99	FJ427058	*Phoma radicina* (JQ676199)
BXGA01	*Phoma glomerata* CBS 290.76 (epitype)	100	FJ427009	*Phoma glomerata* (JQ676201)
	Dothideomycetes_ Epicoccum (J)			
XSCG06	*Epicoccum cf. nigrum*	100	FN548154	*Epicoccum cf. nigrum* (JQ676202)
TMBY202		99		*Epicoccum cf. nigrum* (JQ676203)
BXJT02		99		*Epicoccum cf. nigrum* (JQ676204)
	Eurotiomycetes_Eurotiales(K)			
BXGA07	*Aspergillus caelatus* NRRL 25528 (holotype)	99	AF004930	*Aspergillus caelatus* (JQ676205)

Table 2. (continued)

Isolate code	Closest GenBank taxa	Max ident (%)	ITS	Name given(accession number)
	Agaricomycetes_Agaricales(L)			
TMSC108	*Armillaria cf. sinapina*	99	AY228346	*Armillaria cf. sinapina* (JQ676207)
TMSC109	*Mycena cf. purpureofusca*	99	JN021065	*Mycena cf. purpureofusca* (JQ676208)
BXGH09	*Psathyrella cf. gracilis*	98	EU139246	*Psathyrella cf. gracilis* (JQ676209)
BXYS02	*Schizophyllum commune* IFM 45818	99	AB369910	*Schizophyllum commune* (JQ676210)
	Mucoromycotina_Mucorales(M)			
XSCG02	*Mucor fragilis* CBS 236.35	86	FN650655	*Mucor sp.* (JQ676211)
TMDF106		86		*Mucor sp.* (JQ676212)
BXSN04	*Mucor hiemalis f. silvaticus* CBS 249.35	87	AY243948	*Mucor sp.* (JQ676213)
	Mucoromycotina_Mortierellales(N)			
XSXG14	*Mortierella alpina* CBS 224.37	99	AJ271630	*Mortierella alpina* (JQ676214)
BXYS05		99		*Mortierella alpina* (JQ676215)

The data of similarity obtained from the blastn results between the isolates and GenBank. *ATCC* American Type Culture Collection, Manassas, VA, USA; *NRRL* Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, US Department of Agriculture, Peoria, IL, USA; *DAOM* Plant Research Institute, Department of Agriculture (Mycology), Ottawa, Canada; *CBS* Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; *MAFF* Ministry of Agticulture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan; *IMI* CABI Bioscience, Eggham, UK; *BBA* Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Virology, Microbiology and Biosafety, Braunschweig; *IFM* Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba, Japan.

3.3 Bioactive Compound Detection

In several cases the fungal extracts peaked in the HPLC chromatograms with similar retention times to those of the standards; thus confirming the presence of these bioactive component (Figures 2-5). Camptothecin was detected in ground mycelium (dry weight) of *Fusarium nematophilum* strain XSXY09 at 37 μg/g, *Alternaria alternata* strain XSQZ04 at 29 μg/g and *Phomopsis vaccinii* strain XSCY02 at 24 μg/g. 10-hydroxycamptothecin was detected in mycelia of *Colletotrichum gloeosporioides* strain XSXY05 at 17 μg/g. Gastrodin was detected in the mycelia of *Chaetomium cupreum* strain TMDF101 at 57 μg/g, *Fusarium oxysporum f. sp. cyclaminis* strain TMBY302 at 89 μg/g and *Armillaria cf. sinapina* strain TMSC108 at 184 μg/g. Ephedrine hydrochloride was detected in the mycelia of *Schizophyllum commune* strain BXYS02 at 7 μg/g, *Psathyrella cf. gracilis* strain BXGH09 at 2 μg/g and *Plectosphaerella cucumerina* strain BXGP07 at 1.6 μg/g.

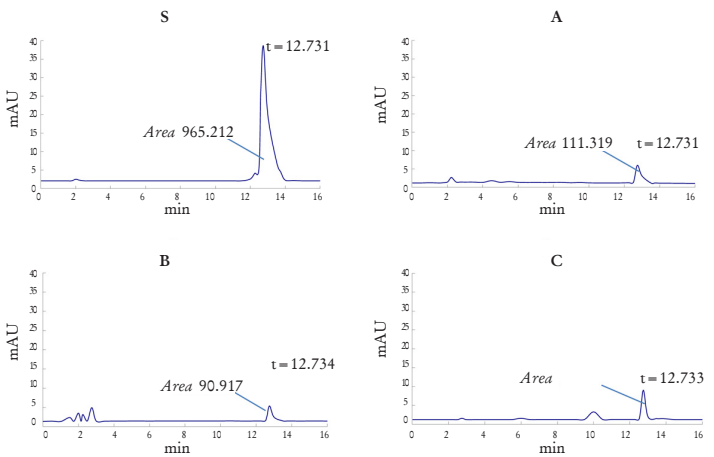


Figure 2. HPLC chromatogram for camptothecin in strains XSQZ04 *Alternaria alternata* (A), XSCY02 *Phomopsis vaccinii* (B), XSXY09 *Fusarium nematophilum* (C); S for standard substance of Camptothecin, t for retention time.

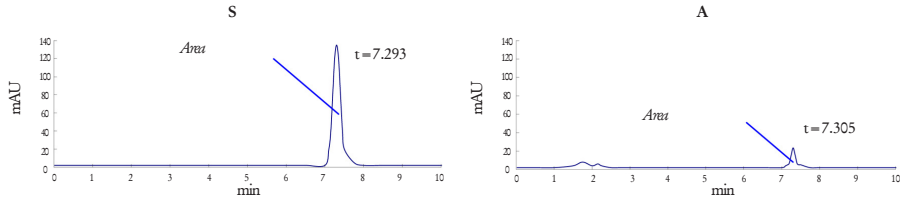


Figure 3. HPLC chromatogram of 10-hydoxycamptothecin in strain XSXY05 *Colletotrichum gloeosporioides* (A); S for standard substance of 10-Hydroxycamptothecin, t for retention time.

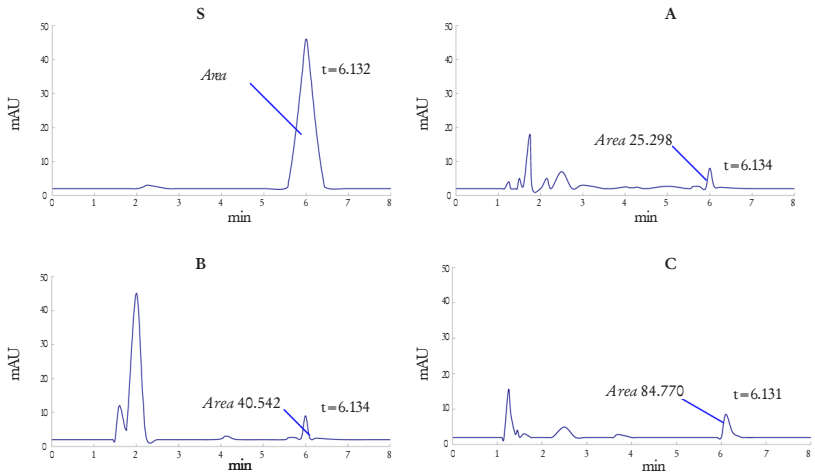


Figure 4. HPLC chromatogram of gastrodin in strains TMDF101 *Chaetomium cupreum* (A), TMBY302 *Fusarium oxysporum f. sp. cyclaminis* (B), TMSC108 *Armillaria cf. sinapina* (C); S for standard substance of Gastrodin, t for retention time.

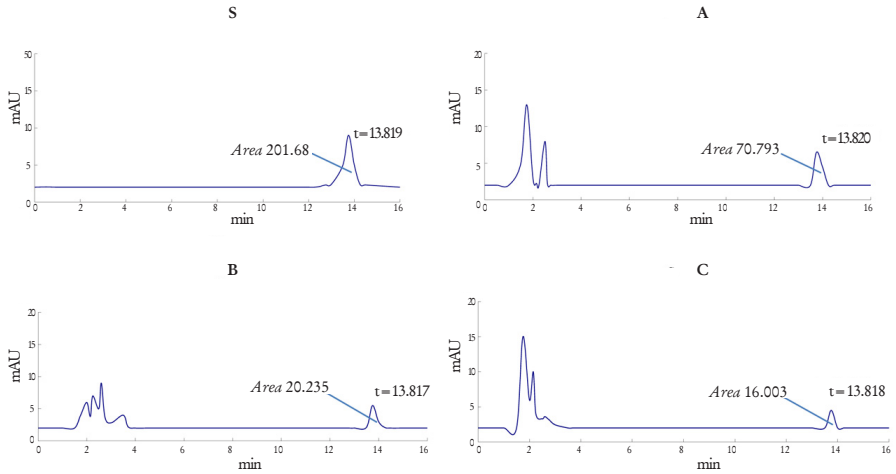


Figure 5. HPLC chromatogram of ephedrine hydrochloride in strains BXYS02 *Schizophyllum commune* (A); BXGH09 *Psathyrella cf. gracilis* (B); BXGP07 *Plectosphaerella cucumerina* (C); S for standard substance of Ephedrine hydrochloride, t for retention time.

4. DISCUSSION

In this study, three plants studied harbored endophytic fungi with different species composition. The endophytic mycota comprised taxa including genera commonly isolated from plants in the previous research, such as species of *Colletotrichum*, *Phomopsis*, *Fusarium*, *Pestalotiopsis* and *Alternaria* [24, 25, 26, 27, 28, 29, 30, 31]. Previous research on endophytic fungi has mostly focused on their diversity and relationships with their host plants. Since paclitaxel was found to be produced by the endophyte *Pestalotiopsis microspora* isolated from *Taxus wallachiana* [32], there has been a trend to search for natural bioactive compounds from endophytic fungi and many of these studies have revealed bioactive compounds[9, 10]. Endophytic fungi from plants are therefore believed to be potential sources for the production of useful medicinal components of the plants [33]. The endophytic *Penicillium brasilianum*, found in root bark of *Melia azedarach*, promoted the biosynthesis of phenylpropanoid amides[34].

There has been considerable research to obtain endophytic fungi that produce camptothecin and its functional analogs [27]. A strain of *Phomopsis* isolated from *Camptotheca acuminata* produced camptothecin in mycelia following liquid culture and yielded 23.45μg/g [36]. A recent study on paclitaxel producing endophytic fungi reported that isolate *Chaetomium sp.* gave a high yield paclitaxel via optimized fermentation[12], which demonstrates the industrial potential value of endophytic fungi as resources for bioactive compounds. In this study, ten strains have the potential to produce bioactive compounds of which three strains produced high yields of camptothecin, three strains produced high yields of gastrodin and ephedrine hydrochloride was produced by three strains but in relatively low yields. The bioactive compound production capacity of the strains in this study may be increased via optimizing fermentation conditions as has been achieved for the paclitaxel producing endophytic fungi. The amounts of these bioactive compounds produced by the isolates can be increased to a scale for industrial microbial fermentation production via optimizing the fermentation condition and other measures[37]. All the three bioactive components were detected in dried mycelia of strains following liquid fermentation; the compounds were not detected in the fermentation broth. Other potential bioactive compounds produced by endophytic fungi found in this study need more work to confirm in future.

Many researchers have reported the industrial use of endophytes for its low and unstable production, which has led to misplaced prejudice about the true prospect of endophytes producing secondary compounds associated with their host plants[35]. But take the economic and other drawbacks in attempting to use endophytes into account, these prejudice conclusions would be changed. Certainly, how to increase the yield of the desired compounds still a formidable problem should be solved. Now, more and more innovative chemical techniques and biotechnological strategies is continuing emerge to tackle the challenges in the process of industrial producing of bioactive compounds by endophytes[37]. Different or new feasible molecular biology tools would be used to explore the genetic basis of natural product biogenesis, whether exclusively or simultaneously with their associated plants, induced by endophytes. Although the feasibility of industrial production of associated plant natural bioactive products by endophytes still not

be confirmed, further deepen research should be taken to comprehensively reveal the endophyte-plant relationship with relate to biochemical, molecular and ecological backgrounds in order to establish, restore and sustain *in vitro* biosynthetic potential of endophytes ought to allow industrial scale endophytic production of the desired products (38).

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