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Antimicrobial and antitumor activity and diversity of endophytic fungi from traditional Chinese medicinal plant *Cephalotaxus hainanensis* Li

Y.-H. Liu*, X.-P. Hu*, W. Li, X.-Y. Cao, H.-R. Yang, S.-T. Lin, C.-B. Xu, S.-X. Liu and C.-F. Li

College of Food Science and Technology, Hainan University, Haikou, Hainan, China

*These authors contributed equally to the study.
Corresponding authors: C.-F. Li / S.-X. Liu
E-mail: congfa@vip.163.com / sixinliu@126.com

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ABSTRACT. Endophytes from *Cephalotaxus hainanensis* Li, an important source of anti-leukemia drugs, have not been widely explored. In this study, 265 endophytic fungal isolates from *C. hainanensis* Li were screened for antimicrobial activities against tilapia, banana, rice, and rape and for antitumor activities against human leukemia cell lines (K562, NB4, and HL-60). Diversity was also analyzed. The results showed that 17.7% of the endophytic fungi had antimicrobial activities against at least three different test microbes, and activity against *Fusarium oxysporum* RKY102 was the highest at 15.8%. Cytotoxicity against at least one tumor cell line tested was observed in 18.5% of the endophytic fungi; with the highest value of 10.6% against K562. The endophytic fungal strains also showed relatively high activities against K562, NB4, and HL-60 while relatively fewer strains were cytotoxic against the human hepatic Hep-G2 and colon LoVo cancer cell lines.

Thirty endophytic fungal strains showed both high antimicrobial and antitumor activities. Moreover, the analyses of the diversity of the 30 highly active strains showed they belonged to 20 species from 14 genera, and this is the first report of endophytic fungi *Albonectria rigidiuscula*, *Colletotrichum magnisporum*, and *Nemania diffusa* being isolated from *Cephalotaxus* plants. These findings suggest that natural antibacterial products for humans and tilapia; antifungal compounds for rice, rape, and banana; and antitumor compounds for leukemia therapy could be isolated from fungal strains derived from *C. hainanensis* Li.

Key words: *Cephalotaxus hainanensis* Li; Endophytic fungi; Diversity; Antimicrobial activity; Cytotoxic activity

INTRODUCTION

Endophytic fungi refer to microorganisms that live in healthy plant tissues at certain or all stages of their life-cycle without affecting the plant significantly (Zhang et al., 2012). While existing in an evolutionary environment such as plant tissues, some endophytic fungi can produce the same or similar secondary metabolites as their hosts as well as other biologically active ingredients simultaneously, including new compounds (Stierle et al., 1993). Recent studies have suggested that novel natural products from endophytes displayed antibiotic effects against plant pathogens (Talontsi et al., 2013) and human tumor cell lines (Wu et al., 2013a). Therefore, they have numerous potential applications in agriculture and modern medicine, and screening for a variety of active substances from endophytic fungi has become the focus of relevant studies.

Cephalotaxus hainanensis Li is an evergreen medicinal plant belonging to the conifer family Cephalotaxaceae (Qiao et al., 2014). It produces active anticancer substances such as alkaloids (Lu et al., 2009), of which harringtonine (HT) and homoharringtonine (HHT) have been developed into clinical drugs for the treatment of leukemia (Itokawa et al., 2005; Pan et al., 2010). Previous studies have demonstrated that the secondary metabolites of some endophytic fungi from *C. hainanensis* Li showed satisfactory antimicrobial effects (Yang et al., 2015a) and mild antitumor activity against the human SMMC-7721 hepatoma cell line and mouse S180 sarcoma cell line (Chen et al., 2010). Moreover, our laboratory has isolated and identified endophytic fungi from *C. hainanensis* Li and investigated the physiological activities of their metabolites.

This present study investigated the antimicrobial and antitumor activities of 265 endophytic fungal strains from *C. hainanensis* Li sampled from three areas in Hainan Province, and the diversity of 30 highly active strains was analyzed. This study aims to lay the foundation for the further study of endophytic fungi from *C. hainanensis* Li to determine their medicinal potentials.

MATERIAL AND METHODS

Endophytic fungi from *C. hainanensis* Li

A total of 265 endophytic fungal strains were isolated from the bark tissues of *C. hainanensis* Li from Hainan Tropical Botanical Garden (code A and B), Hainan Jianfengling Nature Reserve (code C, D, and E), and Bawangling Nature Reserve (code F). The tissue samples were preserved in the laboratory.

Microorganisms for antimicrobial test

The Gram-negative *Escherichia coli* ATCC25922 and Gram-positive *Staphylococcus aureus* ATCC25923 and *Bacillus subtilis* ATCC6633 bacterial strains were provided by the Laboratory of Microbiology, College of Food Science, Hainan University. The animal pathogenic bacterial strain *Streptococcus agalactiae* HYXY08 (Gram-positive bacterium) was isolated from an infected tilapia at the Marine Biology Laboratory, Ocean College of Hainan University. The plant pathogenic fungi *Rhizoctonia solani* ZLL101, *Sclerotinia sclerotiorum* XJS01, and *Fusarium oxysporum* RKY102 were provided by the Institute of Banana and Plantain, Chinese Academy of Tropical Agriculture Sciences.

Cell lines for antitumor testing

The human K562 chronic myeloid leukemia and NB4 acute promyelocytic leukemia cell lines were provided by the Cell Research Laboratory of the College of Materials and Chemical Engineering, Hainan University and the China Center for Type Culture Collection respectively. The human HL-60 acute myeloid leukemia, Hep-G-2 hepatic, and colon LoVo cancer cell lines were purchased from the Cell Bank of Shanghai Institute of Life Sciences, Chinese Academy of Sciences.

Culture media

The culture media used were potato dextrose agar (PDA) and potato dextrose broth (PDB). The K562 and NB4 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 (Gibco, USA) medium with 10% fetal bovine serum (FBS, Hyclone, USA). The HL-60, HepG-2, and LoVo cells were cultured in Iscove's modified Dulbecco's medium (IMDM, Gibco) with 20% FBS, Dulbecco's modiðed Eagle's medium (DMEM, Gibco) supplemented with high glucose medium with 10% FBS and F12K (Sigma, USA) medium with 10% FBS, respectively.

Antimicrobial activity test of endophytic fungi strains

The method of Su et al. (2005) was used with slight modifications. Briefly, under aseptic conditions, 2 mL of microbial suspension (indicator microbes, 1 x 10^6 CFU/mL) was added to 100 mL culture media at approximately 50°C, mixed rapidly, and then loaded onto Petri dishes to prepare the bioassay plates. The fungal plugs were prepared by punching holes into the edges of the endophytic fungal colonies, which had been cultured for 11 days, using a 0.6-cm diameter hole puncher. Then, the plugs were transferred to the prepared Petri dish with the testing microorganism media. The bacterial and fungal plates were incubated at 37°C for 24 h and 28°C for 48 h, respectively. The sizes of the inhibition zones were measured, and the test was repeated thrice on each endophytic fungal strain while a culture plate with no endophytic fungal inoculation was used as the negative control.

Endophytic fungi secondary metabolite extraction

The activated endophytic fungi from *C. hainanensis* Li were inoculated into the PDA medium and incubated at 28°C for 4-10 days, and then the endophytic fungi were inoculated into 1-L Erlenmeyer flasks containing 400 mL PDB medium. Each strain was inoculated into

three flasks, which were incubated at 28°C shaking at 160 rpm. After dynamic fermentation for 11 days, the cultures were taken out and filtered to obtain the fermentation broth, which was extracted thrice with ethyl acetate and ultrasound, vacuum concentrated to obtain the crude extract of the metabolites, and then subsequently stored at 4°C (Wang et al., 2012).

Antitumor activity detection of metabolites

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Mosmann, 1983; Burton, 2005) was used with slight modifications to determine the cytotoxic activity *in vitro*. The cell numbers were counted using an automated cell counter (Bio-Rad, USA), and then the cells were inoculated onto a 96-well cell culture plate with 90 μL of the cell suspension in each well at a density of 1 x 10^5/mL. The plates were incubated in a carbon dioxide (CO_2) incubator (SANYO, Japan) at 37°C with 5% CO_2 and 90% relative humidity. After a 24-h incubation, 10 μL of the prepared crude extracts from the metabolites were added to the wells with five wells for each metabolite. The plates were cultured for a further 48 h, and then 20 μL of a 5 mg/mL MTT solution (Amresco, USA) was added to each well and reacted at 37°C for 4 h. Finally, 100 μL of the "triplex solution" [10% sodium dodecyl sulfate (SDS), 5% isobutanol, 0.012 M HCl] was added, mixed completely, and then incubated overnight. Then, the next day, a microplate reader (Bio-Tek, USA) was used to measure the optical density (OD) of each well at 570 nm. The HHT standard (Aladdin, China) was used as a positive control. All test samples were repeated five times, and the average numbers were used for calculation. The half-maximal inhibitory concentration (IC_{50}) values were calculated using the statistical package for the social sciences (SPSS) software.

Classification of endophytic fungi strains

The 30 endophytic fungi strains with antimicrobial activity against at least three tested pathogens, which also showed antitumor activity against at least one test human tumor cell line, which were called highly active strains. The classification of the highly active endophytic fungi was carried based on their morphological and biological characteristics (Wei, 1979; Shao et al., 1984). The strains were cultured using the dynamic fermentation method. When the mycelia were distributed evenly, the fermentation broth was filtered to obtain the mycelia, which were washed for 3-4 min with 25% ethanol (v/v) and rinsed twice with sterile deionized water. The washed mycelia were centrifuged (4000 rpm) to remove the supernatant. After freeze-drying, a fungal genome extraction kit (Omega, USA) was used to extract the total DNA.

The universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Shanghai Yingjun Biotechnology Co., China) were used to amplify the ITS-rDNA using polymerase chain reaction (PCR, Biometra, Germany). The reaction system was 50 μL while the conditions were 94°C for 3 min for the initial denaturation, followed by 30 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 2 min, and the final extension was at 72°C for 10 min. The PCR fragments were recovered and purified using a gel purification kit (Omega, USA), and the purified PCR products were sequenced. The sequence data of the ITS region of endophytic fungi strains were analyzed using basic local alignment search tool (BLAST) at the National Center for Biotechnology Information (NCBI) website. Using the neighbor-joining algorithm, a phylogenetic tree was constructed (Tamura et al., 2007), and the bootstrap value was 1000 (Cui et al., 2011).

RESULTS

Antimicrobial activity test

Among the 265 strains examined, 77 and 65 showed antibacterial and antifungal activities against at least one test bacterium and one fungus, respectively. Overall, 91 strains showed varying degrees of antimicrobial activities. Moreover, 47 strains had antimicrobial activities against at least three different microbial test stains. The partial results are shown in Table 1.

Table 1. Antimicrobial activity of 30 highly active endophytic fungi strains from *Cephalotaxus hainanensis* Li.

Strains No.	Test microorganisms						
	Escherichia coli	*Staphylococcus aureus*	*Bacillus subtilis*	*Streptococcus agalactiae*	*Fusarium oxysporum*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*
A8	-	-	-	++	+	++	-
A9	-	++	+	-	-	+++	-
A21	-	-	+	+	-	++	-
A24	-	++	+	-	-	+++	++
A25	-	++	-	-	++	+++	++
A29	-	+	++	++++	+++	-	-
A31	-	-	++	-	-	++	++
B6	-	++	++	++	++	+++	++
B14	+++	-	++	++	+++	-	-
B18	-	+++	++	+	++	-	+++
B21	+++	++	+++	-	++	++++	++
B29	-	-	+	-	-	+++	++
B33	+++	-	+++	-	++	+++	+++
C2	-	-	++	+	-	++++	+++
C7A	-	++	+	-	+++	+	++
C14	++	++	+	-	+++	+++	-
D1	-	-	-	++	+	++	-
D4	-	-	++	+	+++	++	-
E2	++	+++	+	-	-	++	-
E4	-	-	++	++	-	-	++
F7	++	++	+++	-	+++	-	-
F26	++	-	-	++	++	++	-
F31	++	-	+	++	-	-	-
F32	++	-	-	-	++	-	++
F45	-	++	+	++	-	++++	-
F52	++	-	-	-	++	++	++
F75	-	++	+	-	-	-	+
F91	-	-	++	+	++	-	-
F103	++	-	++	++	-	-	-
F125	+	-	+	+	++	++	+
CK	-	-	-	-	-	-	-

-, no antimicrobial activity; zones of inhibition: +, 6 mm < Φ < 10 mm; + +, 10 mm < Φ < 16 mm; + + +, 16 mm < Φ < 26 mm; + + + +, Φ > 26 mm.

Metabolites anti-tumor activity test

The *in vitro* antitumor activity test using the ethyl acetate crude extracts of the 265 strains showed that 49 strains had cytotoxic activity against at least one tumor cell line while 32 strains had tumor inhibitory effects on at least two tumor cell lines. The partial results are shown in Table 2.

Classification of highly active endophytic fungi strains

Morphological classification

According to the colony characteristics, 30 endophytic fungi strains with high antimicrobial and antitumor activities were preliminarily divided into 18 morphological types (Figure 1).

Table 2. Antitumor activity of 30 highly active endophytic fungi strains from *Cephalotaxus hainanensis* Li.

Strain No.	Tumor cell lines				
	K562	NB4	HL-60	Hep G2	LoVo
A8	60.11	-	90.69	-	-
A9	-	-	40.58	-	-
A21	-	69.75	53.11	-	-
A24	88.34	-	-	-	-
A25	34.42	49.59	-	-	-
A29	5.53	21.31	0.18	90.14	68.07
A31	-	-	-	62.32	70.22
B6	11.28	36.73	-	-	-
B14	40.14	61.58	-	-	-
B18	-	-	40.69	-	-
B21	12.35	47.97	-	-	-
B29	79.61	-	92.71	-	-
B33	-	47.26	29.75	-	-
C2	89.34	70.32	66.32	-	-
C7A	25.78	38.51	35.07	37.97	-
C14	99.63	-	-	-	-
D1	40.14	-	76.33	-	-
D4	-	40.15	-	-	24.91
E2	-	61.23	20.2	-	-
E4	-	-	67.17	-	-
F7	49.63	57.64	45.27	-	-
F26	55.11	-	-	-	-
F31	-	-	85.41	-	98.13
F32	33.24	80.16	-	-	-
F45	75.91	39.57	48.85	-	-
F52	-	89.79	45.92	92.15	-
F75	12.36	47.97	-	-	-
F91	11.29	36.73	-	-	-
F103	33.63	-	-	94.21	-
F125	20.23	66.56	-	-	-
HHT	<0.001	<0.001	<0.001	24.87	30.56

IC_{50}, half-maximal inhibitory concentration (mg/mL); homoharringtonine (HHT) was positive control; -, IC_{50} >100 mg/mL.

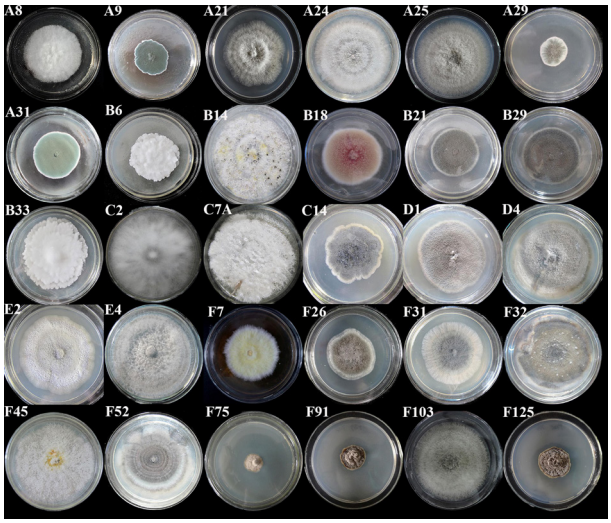

Figure 1. Diagram of morphology of colonies of 30 highly active endophytic fungal strains.

Molecular biological classification

The molecular biological classification analysis revealed the 30 strains belonged to 14 genera, consisting of five, six, four, two, and three, strains, as well as two other strains belonging to two *Diaporthe* spp, three *Phomopsis* spp, four *Colletotrichum* spp, one *Corynespora* sp, three *Penicillium* spp, and one *Nemania* sp, respectively. In addition, one strain from each of the following genera was identified: *Albonectria*, *Trametes*, *Guignardia*, *Clonostachys*, *Periconia*, *Neonectria*, *Alternaria*, and *Leptostroma*. The E2, C7A, and F31 strains were not identified at the species level (Table 3 and Figure 2). These results indicate that the endophytic fungi population of *C. hainanensis* Li bark was rather diverse.

Table 3. Molecular biological classification of 30 highly active endophytic fungal strains.

Strains No.	Morphological identification	Sequence length (bp)	Related strain (BLAST)	Access No. of related strain	Overlap (bp)	Similarity (%)
B18	*Albonectria* sp	556	*Albonectria rigidiuscula*	HM054147	545	99
D1	*Alternaria* sp	520	*Alternaria porri*	HM204456	523	97
C2	*Colletotrichum* sp	551	*Colletotrichum karstii*	KC425666	553	99
D4	*Colletotrichum* sp	539	*Colletotrichum tropicicola*	JN050240	579	99
E4	*Colletotrichum* sp	530	*Colletotrichum thailandicum*	JN050243	523	100
F32	*Colletotrichum* sp	533	*Colletotrichum magnisporum*	NR132056	534	99
B21	*Corynespora* sp	516	*Corynespora cassiicola*	KJ612076	516	100
B29	*Corynespora* sp	516	*Corynespora cassiicola*	KJ612076	516	100
F7	*Clonostachys* sp	555	*Clonostachys agraualii*	AF358241	481	98
B14	*Diaporthe* sp	571	*Diaporthe actinidiae*	FN668392	554	98
F45	*Diaporthe* sp	571	*Diaporthe actinidiae*	FN668392	554	98
A21	*Diaporthe* sp	567	*Diaporthe kyushuensis*	AF230749	465	98
A24	*Diaporthe* sp	527	*Diaporthe kyushuensis*	AF230749	465	98
A25	*Diaporthe* sp	566	*Diaporthe kyushuensis*	AF230749	465	98
C14	*Guignardia* sp	636	*Guignardia mangiferae*	KF920707	757	100
E2	*Leptostroma* sp	622	*Leptostroma* sp	KC354586	545	100
F52	*Neonectria* sp	987	*Neonectria macroconidialis*	AY295327	544	100
B6	*Nemania* sp	520	*Nemania diffusa*	KF881789	584	100
B33	*Nemania* sp	520	*Nemania diffusa*	KF881789	584	99
F26	*Phomopsis* sp	561	*Phomopsis asparagi*	JQ070364	572	97
F103	*Phomopsis* sp	560	*Phomopsis vaccinii*	JQ676190	568	98
F75	*Phomopsis* sp	530	*Phomopsis fukushii*	JQ807429	453	99
F91	*Phomopsis* sp	533	*Phomopsis fukushii*	JQ807429	453	99
F125	*Phomopsis* sp	565	*Phomopsis fukushii*	JQ807429	453	99
F31	*Periconia* sp	549	*Periconia* sp	KJ667742	549	100
A9	*Penicillium* sp	561	*Penicillium citrinum*	JN942858	509	100
A29	*Penicillium* sp	594	*Penicillium copticola*	JN617685	805	100
A31	*Penicillium* sp	578	*Penicillium soppii*	DQ267918	492	100
A8	*Trametes* sp	619	*Trametes polyzona*	KC923291	755	100

BLAST = basic local alignment search tool.

DISCUSSION

Since the first case of an infection caused by penicillin-resistant staphylococci (Ashley and Brindle, 1960) emerged, resistance to traditional antimicrobial therapies has reduced the effectiveness of these drugs, leading to increased morbidity, mortality, and health care expenditure (Smith and Coast, 2002). Therefore, it is imperative to discover novel antimicrobial agents from natural products and endophytes have become a useful source.

In the current investigation, among the 265 endophytic fungi strains investigated, a higher number showed activity against Gram-positive than they did against Gram-negative bacteria with a relatively strong efficacy, and the largest inhibition zone was 20 mm. This result suggests there is a high possibility of obtaining highly effective antibacterial substances from the strains that inhibited the Gram-positive bacteria.

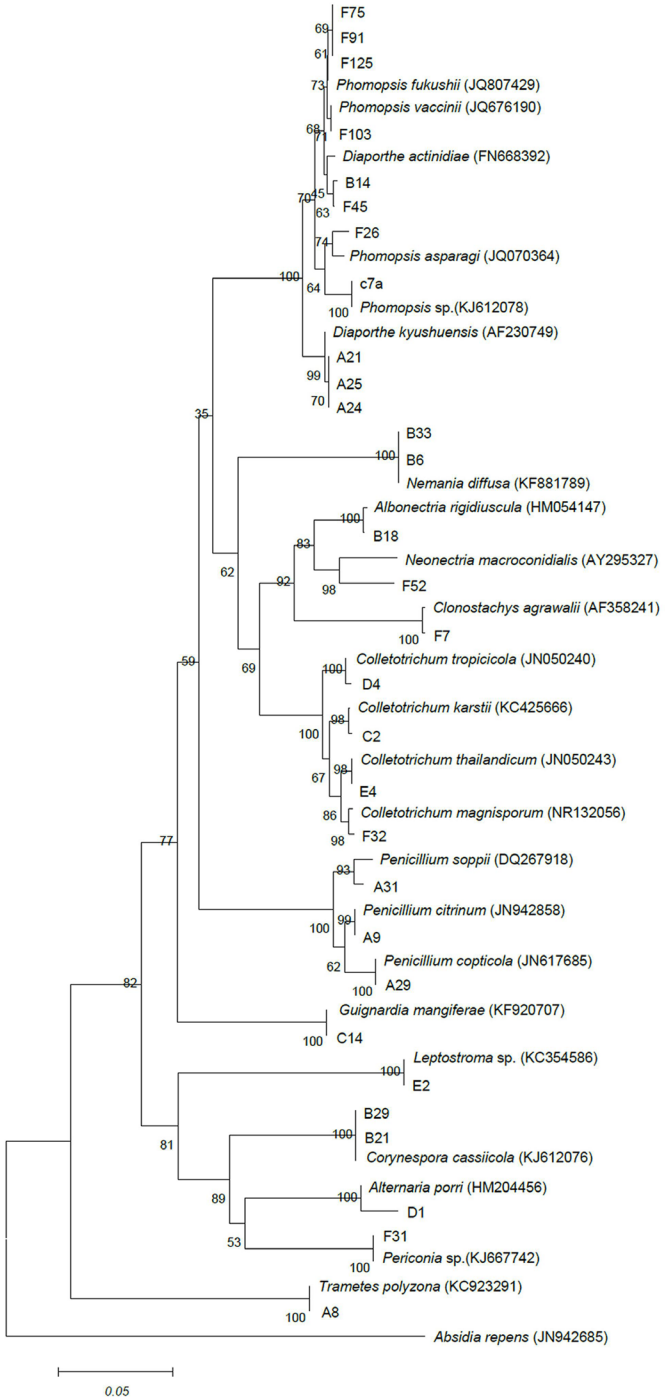

Figure 2. Phylogenetic tree of 30 highly active endophytic fungal strains.

S. agalactiae causes diseases in fish and is the main pathogenic bacterium that infects *Tilapia mossambica*, which leads to great losses to the aquaculture industry every year worldwide. Currently, no effective drugs are available that can suppress this pathogen. In this study, 32 strains showed activity against *S. agalactiae* HYXY08, and particularly, strain A29 showed the largest zone of inhibition (27 mm). It was previously reported that 11 strains from *C. hainanensis* Li showed activity against *S. agalactiae* HYXY08, and the largest zone of inhibition observed was 16 mm (Yang et al., 2015b), which is slightly smaller than that obtained in the current study. These results imply that some endophytic fungi strains from *C. hainanensis* Li could be possible sources of microorganisms that could produce effective drugs to inhibit this pathogen.

R. solani, *S. sclerotiorum*, and *F. oxysporum* are the most destructive plant pathogens that cause huge losses to the agricultural industry every year. In a previous study, 25 endophytic fungi strains from *Ginkgo biloba* L. displayed considerable activity against *R. solani* with zones of inhibition larger than 16 mm (Guo et al., 2005). In the current study, strains B33, C2, and F45 demonstrated the largest zones of inhibition against *S. sclerotiorum, F. oxysporum*, and *R. solani,* with zone sizes of 21, 29, and 28 mm, respectively.

Recent studies have paid considerable attention to the potential anticancer components in medicinal plants and their endophytes. Zhou et al. (2009) isolated 11 compounds from the medicinal plant *Cephalotaxus fortunei,* and the exhibited moderate cytotoxicity against K562 and a human lung cancer cell line (A549). In addition, previous studies on the endophytic fungi from *C. hainanensis* Li have reported their antitumor activities against the human SGC-7901 gastric (Huang et al., 2010), HepG-2 (Zeng, 2011), HL-60, and prostatic carcinoma (Lu et al., 2012) cell lines. Although *C. hainanensis* Li has been widely used to produce drugs that are clinically used to treat leukemia, the literature reports on the antitumor activities of its endophytic fungi against leukemia are very few. The present study focused on the antiproliferative activities of the strains isolated from *C. hainanensis* Li against leukemia cell lines (K562, NB4, and HL-60), human LoVo colon cancer, and human Hep-G-2 hepatoma cell lines. More endophytic fungi exhibited high activities against the K562, NB4, and HL-60, while fewer strains showed cytotoxicity against the LoVo and HepG-2 cells. This may be related to the anti-leukemic activity of the host *C. hainanensis* Li (Efferth et al., 2003). It is worth emphasizing that strain A29 demonstrated the highest activities against K562, NB4, and HL-60 with IC_{50} values of 5.53, 21.31, and 0.18 mg/mL, respectively. These results suggest that A29 possesses considerable cytotoxicity against the leukemia cell lines and may provide new resources for developing natural antitumor ingredients. In addition, strains D4 and C7A showed relatively high cytotoxicity against the LoVo and HepG-2 cells with IC_{50} values of 24.91 and 37.97 mg/mL, respectively.

Regarding the classification of the endophytic fungi from *C. hainanensis* Li, a previous study showed that *Colletotrichum* and *Fusarium* spp were dominant species (Chen et al., 2008). Moreover, Yang et al. (2015a) isolated 21 endophytic fungi strains, which belonged to 14 genera including *Diaporthe*, *Phomopsis*, *Colletotrichum*, *Corynespora*, *Fusarium*, and *Penicillium*. Furthermore, Dai et al. (2009) reported strains with strong antimicrobial and antitumor activities belong to *Colletotrichum*, *Fusarium*, and *Botrytis* spp. In contrast, in the present study, four strains belonged to the *Colletotrichum* sp, while none belonged to *Fusarium* and *Botrytis* spp. Furthermore, it is worth noting that this is the first report of isolation of endophytic fungi such as *A. rigidiuscula* B18, *C. magnisporum* F32, and *N. diffusa* B6 and B33 were isolated from *Cephalotaxus* plants.

Studies on the *A. rigidiuscula* (Li et al., 2009) and *N. diffusa* (Wu et al., 2013b) have reported cytotoxicity against human HeLa cervical cancer cells as well as MCF-7 breast cancer cells and strong antimicrobial activities against *S. aureus*, respectively. Nevertheless, there is no report on the antitumor or antimicrobial activities of *C. magnisporum*. This study found that *A. rigidiuscula* showed strong antibacterial activity against the three Gram-positive bacteria and moderate cytotoxicity against the HL-60 cells. In addition, *N. diffusa* exhibited moderate antimicrobial and cytotoxic activities against the four test microorganism strains and NB4 or HL-60 cells, respectively. Furthermore, *C. magnisporum* exhibited moderate antimicrobial and medium cytotoxic activities against *E. coli*, *S. sclerotiorum,* as well as *F. oxysporum*, and K562 or NB4 cells, respectively.

Finally, strain A29, which showed strong cytotoxic and antimicrobial activity was classified as *Penicillium copticola*. A recent study discovered that one *P. copticola* strain from *Cannabis sativa* L. showed relatively strong activity against the host plant-specific pathogens *Trichothecium roseum* and *Botrytis cinerea* (Kusari et al., 2013). In addition, three compounds from one *P. copticola* soil strain exhibited relatively strong cytotoxicity against the human oral KB epithelial carcinoma and MCF-7 breast cancer cell lines, as well as the African Green monkey Vero kidney cells (Daengrot et al., 2015). Therefore, it is obvious that the metabolites of *P. copticola* have strong antimicrobial and cytotoxic activities. The A29 strain from this study is currently under further investigation.

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REFERENCES

Ashley DJ and Brindle MJ (1960). Penicillin resistance in staphylococci isolated in a casualty department. *J. Clin. Pathol.* 13: 336-338. http://dx.doi.org/10.1136/jcp.13.4.336

Burton JD (2005). Chemosensitivity. In: The MTT assay to evaluate chemosensitivity (Blumenthal RD, ed.). Humana Press, New Jersey, 69-78.

Cui JL, Guo SX and Xiao PG (2011). Antitumor and antimicrobial activities of endophytic fungi from medicinal parts of *Aquilaria sinensis*. *J. Zhejiang Univ. Sci. B* 12: 385-392. http://dx.doi.org/10.1631/jzus.B1000330

Chen CW, Sun QG and Zhu J (2010). Isolation and screening for endophyte with antitumor activities from three medicinal plants. *Microbiol. China* 37: 1462-1466.

Chen P, Dai HF and Xie XC (2008). Isolation and primary identification of endophytic fungi from *Cephalotaxus hainanensis* L. *Microbiology* 35: 1455-1460.

Daengrot C, Rukachaisirikul V, Tansakul C, Thongpanchang T, et al. (2015). Eremophilane sesquiterpenes and diphenyl thioethers from the soil fungus *Penicillium copticola* PSU-RSPG138. *J. Nat. Prod.* 78: 615-622. http://dx.doi.org/10.1021/np5005328

Dai WJ, Dai HF, Chen P, Wu J, et al. (2009). Screening of antitumor and antimicrobial activities of the endophytic fungi isolated from *Cephalotaxus hainanensis* Li. *Microbiology* 36: 1217-1221.

Efferth T, Sauerbrey A, Halatsch ME, Ross DD, et al. (2003). Molecular modes of action of cephalotaxine and homoharringtonine from the coniferous tree *Cephalotaxus hainanensis* in human tumor cell lines. *Naunyn Schmiedebergs Arch. Pharmacol.* 367: 56-67. http://dx.doi.org/10.1007/s00210-002-0632-0

Guo JX, Sun GY, Zhang R, Li Q, et al. (2005). Isolation and screening of antifungal isolates from endophytic fungi in *Ginkgo biloba* L. *Acta Agric. Boreali-Occident Sin.* 14: 14-17.

Huang JL, Dai HF, Wang H, Yu L, et al. (2010). Cytotoxic active metabolites from endophytic fungus S15 from Hainan

Plum-Yew (*Cephalotaxus hainanensis*). *J. Microbiol.* 3: 10-14.
Itokawa H, Wang X and Lee KH (2005). Anticancer agents from natural products. In: Homoharringtonine and related compounds (Cragg GM, eds.). CRC Press, New York, 65-94.
Kusari P, Kusari S, Spiteller M and Kayser O (2013). Endophytic fungi harbored in *Cannabis sativa* L.: diversity and potential as biocontrol agents against host plant-specific phytopathogens. *Fungal Divers.* 60: 137-151. http://dx.doi.org/10.1007/s13225-012-0216-3
Li J, Liu S, Niu S, Zhuang W, et al. (2009). Pyrrolidinones from the ascomycete fungus *Albonectria rigidiuscula*. *J. Nat. Prod.* 72: 2184-2187. http://dx.doi.org/10.1021/np900619z
Lu X, Xu N, Dai HF, Mei WL, et al. (2009). Three new compounds from endophytic fungus L10 of *Cephalotaxus hainanensis*. *J. Asian Nat. Prod. Res.* 11: 397-400. http://dx.doi.org/10.1080/10286020902819947
Lu X, Chen G, Hua H, Dai H, et al. (2012). Aromatic compounds from endophytic fungus *Colletotrichum* sp. L10 of *Cephalotaxus hainanensis* Li. *Fitoterapia* 83: 737-741. http://dx.doi.org/10.1016/j.fitote.2012.02.012
Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65: 55-63. http://dx.doi.org/10.1016/0022-1759(83)90303-4
Pan L, Chai H and Kinghorn AD (2010). The continuing search for antitumor agents from higher plants. *Phytochem. Lett.* 3: 1-8. http://dx.doi.org/10.1016/j.phytol.2009.11.005
Qiao F, Cong H, Jiang X, Wang R, et al. (2014). *De Novo* characterization of a *Cephalotaxus hainanensis* transcriptome and genes related to paclitaxel biosynthesis. *PLoS One* 9: e106900. http://dx.doi.org/10.1371/journal.pone.0106900
Stierle A, Strobel G and Stierle D (1993). Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of *Pacific yew*. *Science* 260: 214-216. http://dx.doi.org/10.1126/science.8097061
Smith RD and Coast J (2002). Antimicrobial resistance: a global response. *Bull. World Health Organ.* 80: 126-133.
Su YQ, Zhu HW, Ma XH, Ma YM, et al. (2005). Screening of antibacterial activities of endophytic fungi isolated from *Eucommiau lmoides*. *Acta Bot. Boreal.* 25: 1153-1157.
Shao LP, Shen RX and Zhang SX (1984). Fungi taxonomy. China Forestry Press, Beijing.
Talontsi FM, Dittrich B, Schüffler A, Sun H, et al. (2013). Epicoccolides: Antimicrobial and antifungal polyketides from an endophytic fungus *Epicoccum* sp. associated with *Theobroma cacao*. *Eur. J. Org. Chem.* 15: 3174-3180. http://dx.doi.org/10.1002/ejoc.201300146
Tamura K, Dudley J, Nei M and Kumar S (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.* 24: 1596-1599. http://dx.doi.org/10.1093/molbev/msm092
Wang Y, Xu L, Ren W, Zhao D, et al. (2012). Bioactive metabolites from *Chaetomium globosum* L18, an endophytic fungus in the medicinal plant *Curcuma wenyujin*. *Phytomedicine* 19: 364-368. http://dx.doi.org/10.1016/j.phymed.2011.10.011
Wei JC (1979). Fungal identification manual. Shanghai science and Technology Press, Shanghai.
Wu JG, Peng W, Zeng PY, Wu YB, et al. (2013b). Antimicrobial activity and cytotoxicity of endophytes from *Scapania verrucosa* Heeg. *Genet. Mol. Res.* 12: 916-924. http://dx.doi.org/10.4238/2013.April.2.8
Wu LS, Hu CL, Han T, Zheng CJ, et al. (2013a). Cytotoxic metabolites from *Perenniporia tephropora*, an endophytic fungus from *Taxus chinensis* var. *mairei*. *Appl. Microbiol. Biotechnol.* 97: 305-315. http://dx.doi.org/10.1007/s00253-012-4189-7
Yang HR, Hu XP, Jiang CJ, Qi J, et al. (2015a). Diversity and antimicrobial activity of endophytic fungi isolated from *Cephalotaxus hainanensis* Li, a well-known medicinal plant in China. *Lett. Appl. Microbiol.* 61: 484-490. http://dx.doi.org/10.1111/lam.12483
Yang HR, Liu SX, Li W, Wu YC, et al. (2015b). Antimicrobial activity of endophytic fungi from *Cephalotaxus hainanensis* Li to *Streptococcus agalactiae* isolated from Tilapia. *Chin. J. Trop. Crops* 36: 1856-1860.
Zeng L (2011). An investigation on the bioactive properties of the chemical components and endophytic fungi of *Cephalotaxus oliveri* Mast. Doctoral thesis, City University of Hong Kong, Hong Kong.
Zhang HC, Ma YM, Liu R and Zhou F (2012). Endophytic fungus *Aspergillus tamarii* from *Ficus carica* L., a new source of indolyl diketopiperazines. *Biochem. Syst. Ecol.* 45: 31-33. http://dx.doi.org/10.1016/j.bse.2012.07.020
Zhou M, Ma L, Hao XJ and Yang XS (2009). Antitumor activities of chemical constituents of *Cephalotaxus fortunei* in Guizhou province. *J. Chin. Pharm. Univ* 40: 209-212.