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Isolation and evaluation of endophytic fungi with antimicrobial ability from *Phyllostachys edulis*

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Abstract

Endophytic fungi (30) isolates from bamboo branches were categorized into 12 genera, based on the blast analyses of ITS nrDNA sequence in GenBank and microscopic examination. The aim of this work was to investigate the antibacterial and antifungal activities of endophytic fungi. Inhibitory effects against clinical pathogens and phytopathogens have been screened for all the isolates preliminarily and strains tentatively identified as *Cladosporium sphaerospermum* (PE106), *Simplicillium lanosoniveum* (PE120), *Curvularia* sp. (PE127), *Didymella* sp. (PE128) and *Penicillium* cf. *raistrickii* (PE130) presented bioactivity against at least four tested pathogens using the agar diffusion method. Crude extracts of PE106, PE120, PE127 and PE130 displayed broad-spectrum activity against plant pathogenic fungi by mycelial radial growth test. All of the four isolates were found to have high bioactivity against the frequent plant pathogenic fungus *Botryotinia fuckeliana*, and two of the isolates (PE120 and PE130) also inhibited the growth of phytopathogen *Thanatephorus cucumeris* noteworthily. This study is the first report on the antimicrobial activity of endophytic fungi associated with branches of *Ph. edulis*.

Introduction

Endophytic fungi are common and diverse, and living asymptomatically within plant tissues or organs. The relationship between the endophytic fungi and their host plant is complicated (Saikkonen et al., 1998; Azevedo et al., 2000). They traditionally have been considered plant mutualists, and may provide some advantage to their hosts, including against array of biotic and abiotic stresses (Carroll, 1988; 1995; Wilson, 2000; Faeth and Fagan, 2002). Many endophytic fungi are capable of synthesizing bioactive compounds that can be used by the plant for defense against pathogenic fungi and bacteria, and producing a wide range of novel metabolites of pharmaceutical and agricultural importance. Since the paclitaxel (taxol®) in the endophytic fungus *Taxomyces andreanae* was firstly detected and extracted (Stierle et al., 1993; 1995), this group of microorganisms have obtained more and more attention. It is reported that many bioactive compounds, such as alkaloids, peptides, steroids, terpenoids, quinones, flavonoids, aliphatic compounds, and phenols, have been isolated from endophytic fungi in decades (Yu et al., 2010). Although there have been a broad-spectrum of biologically active metabolites from soil fungi, it is necessary to search for novel natural products from other fungi, especially interesting endophytic fungi, for resistant pathogenic strains and new diseases.

Bamboos, large perennial grass distributed widely from tropical and subtropical zones, belonging to the family Poaceae, are popularly known as the main food of the endangered giant panda (*Ailuropoda melanoleuca*). In Asian countries, different species and different organs of bamboos have been used for building material,

handicraft article, food material and traditional medicine. For example, "Xian Zhu Li Kou Fu Ye" (as drug approved by SFDA, China) an extract of bamboo, is used as an active element to inhibit inflammation in the throat. Some biologically active components in bamboo and their potential health benefits have been widely studied (Lu et al., 2005; 2006; Keski-Saari et al., 2008; Panee et al., 2008; Mejia et al., 2009; Halvorson et al., 2011; Koide et al., 2011). *Phyllostachys edulis* (Carr.) H. De Lehaie (Bambusoideae, Poaceae) is mainly distributed in subtropic zones of South China. Because of high production, various purposes and wide distribution, it has long been considered the most important economic bamboo species in China.

In the last years, few studies have been conducted on the antipathogenic activity of the endophytic community of this plant (Umali et al., 1999). The aim of the present study was to explore the diversity of culturable endophytes from *Ph. edulis*, and to assess a promising source of bioactive compounds.

Materials and Methods

Collection of plant material and isolation of endophytic fungi

Branches of *Ph. edulis* collected from Yunle, Jingde County of the Anhui Province, in China. Twenty of the branches were chosen and each cut into 25 fragments less than 1 cm. The fragments were surface sterilized by being placed in 75% ethanol for 30 sec, 5% NaOCl for 10 min, and rinsing in sterile water. The small segments were cultured in Potato Dextrose Agar at 20°C without light. The dominant fungal isolates were classified by colony and hyphal characters. Cultures were deposited at China Forestry Culture Collection Center (CFCC).

DNA Extraction, amplification, sequencing and molecular identification

Fungal mycelium was scraped from growing purified colonies and frozen at -20°C over one night. The total genomic DNA was extracted using E.Z.N.A.™ Fungal DNA MiniKit (Omega Biotech, Inc.) according to manufacturers' protocols. The primer pairs ITS1-F/ITS4 and their PCR programs were followed White et al. (1990). The products were sequenced by Invitrogen Biotechnology Co. Ltd. (Beijing, China). To identify the isolates, sequences were subjected to the BLAST search with the NCBI database (http://www.ncbi.nlm.nih.gov/). Only matches of sequences published in journals were used. Priority was given to sequences derived from authoritative material, such as ex-type or ex-epitype cultures. Our sequences were also deposited at GenBank.

Fungal culture and crude preparation

Endophytic fungi isolates were cultured in PDA solid media: PDA, containing (g/L): Potato 200 and dextrose 20; pH 6.0. The fresh mycelia of different endophytic fungi were grown on plates at 25°C for more than 7 d. 5 plugs (6 mm of diameter) of growing culture plus the adhering mycelium were subsequently added to 250 mL Erlenmeyer flasks containing 100 mL of PDA liquid media. All liquid cultures were kept at 25°C for 10 d with shaking (150 rpm).

The fermentation of each fungus was filtered to separate the mycelium from the filtrates. The mycelium was extracted with ethyl acetate (EtOAc) in order to obtain bioactive extracts (Hormazabal and Piontelli, 2009).

Agar diffusion assay

The endophytic fungi were screened using the agar diffusion method, as a rapid and qualitative selection of the bioactive microorganisms. Endophytic fungi were cultivated on the PDA solid media at 25°C over 7 d. The agars (6 mm of diameter) of growing culture plus the adhering mycelium were subsequently added to LB and PDA solid media, supplemented with 0.5% olive oil previously spread with bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Salmonella bacteria*), yeasts (*Rhodotorula rubra*, *Saccharomyces cerevisiae*, *Candida albicans*), and filamentous fungi (*Curvularia eragrostidis*, *Pleospora herbarum*, *Arthrinium sacchari*, *Arthrinium phaeospermum* and *Phoma herbarum*). These cultures of bacteria and fungi were also deposited at CFCC. The mycelia of filamentous fungi were fragmented with pestle and mortar. Plates were incubated at 37°C for 24 hours for the bacteria and 28°C for 2-7 d for the fungi. The inhibition zones around the agars were measured, to confirm the antimicrobial activity (de Siqueira et al., 2011).

Mycelial radial growth test

The antifungal activities of fungal extracts were tested in a number of pathogenic fungi: *Botryotinia fuckeliana*, *Alternaria alternate*, *Thanatephorus cucumeris*, *Gibberella avenacea*, and *Colletotrichum lagenarium*. These cultures fungi were all deposited at CFCC. The bioactive extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/mL. From this solution, 0.3 ml samples were then poured on sterile Petri dishes containing 15 mL PDA to make 200 µg/mL concentration. To check the growth inhibition whether is due to DMSO, the negative control was prepared using 0.3 mL DMSO. PDA solid media were poured in three plates. Plant pathogenic fungi were inoculated in the centre of plates and the diameters of the inhibition zones were measured until the negative control plates full of mycelium. The percent of growth inhibition of extract was calculated as the following formula:

PGI = ((ND-ED)/ND)*100%

Where, ND = diameter of plant pathogenic fungi in

plate with negative control; ED = diameter of plant pathogenic fungi in plate with extracts from mycelium of endophytic fungi. All the experiments were repeated three times. PGI for the each replicate was calculated and analysis of variance of the PGI was conducted by SSPS 18.0.

Results

All of fungal isolates were obtained from the branches of *Phyllostachys edulis*. Among them, over 150 distinct isolates were categorized into different morphotypes on the basis of colony distinction and hyphal characters. The nr ITS DNA sequence analyses were conducted to confirm the identification of these endophytic fungi. 30 representatives were identified belonging to 12 genera, namely *Cladosporium*, *Shiraia*, *Colletotrichum/Glomerella*, *Microdochium*, *Arthrinium*, *Penicillium*, *Aureobasidium*, *Simplicillium*, *Phoma*, *Curvularia* and *Didymella* (Table I). For molecular identification of *Colletotrichum* species, the sequences of ex-types were used (Hyde et al., 2009). Due to lacking of ex-type or ex-epitype of other species, the relevant sequences published in journals were also cited. The list of their respective accession number were obtained by GenBank as well as the accession number of ITS sequences from cultural endophytic fungi for the

Table I

Culturable endophytic fungi isolated from *Phyllostachys edulis* branches

Strain (PE)	%Isolation frquency	Numbers of isolates	ITS (No.)[a]	Most closely related species	% Similarity	Reference ITS (No.)[b]
101	2.7	4	JX875918	*Cladosporium oxysporum*	99	Hyde et al., 2009 EF136374
102	1.3	2	JX875919	*Shiraia* sp. Slf14	99	Zhu et al., 2010 GQ355934
103	3.3	5	JX875920	*Cladosporium* sp. P31E1	99	Loro et al., 2012 JN207316
104	2.7	4	JX875921	*Cladosporium cladosporioides* strain D10	99	Bukovska et al., 2010 GU566222
105	1.3	2	JX875922	*Cladosporium cladosporioides* strain LPSC1088	99	Llorente et al., 2012 JF949719
106	0.7	1	JX875923	*Cladosporium sphaerospermum* isolate wb311	99	Buzina et al., 2003 AF455481
107	1.3	2	JX875924	*Cladosporium* sp. 7306	99	Yarden et al., 2007 EF120415
108	1.3	2	JX875925	*Cladosporium colombiae* strain CBS 274.80B	99	Schubert et al., 2009 FJ936159
109	1.3	2	JX875926	*Colletotrichum dematium*	97	Hyde et al., 2002 AJ301954
110	1.3	2	JX875927	*Microdochium* sp. 5/97-31	99	Ernst et al., 2011 AM502258
111	0.7	1	JX875928	*Arthrinium sacchari* isolate A09	99	Gorfer et al., 2011 HQ115646
112	0.7	1	JX875929	*Shiraia* sp. JP185	95	Morakotkarn et al., 2006 AB354994
113	0.7	1	JX875930	*Shiraia* sp. JP232	97	Morakotkarn et al., 2006 AB255303
114	1.3	2	JX875931	*Penicillium citrinum* strain P-1637	99	Alborch et al., 2012 JQ316514
115	3.3	5	JX875932	*Colletotrichum* sp. JP9	98	Morakotkarn et al., 2006 AB255243
116	0.7	1	JX875933	*Shiraia* sp. slf14	99	Zhu et al., 2010 GQ355934
117	0.7	1	JX875934	*Aureobasidium pullulans* strain ZD-3D	98	Zhang et al., 2011 JF422784
118	0.7	1	JX875935	*Colletotrichum* sp. JP9	99	Morakotkarn et al., 2006 AB255243
119	0.7	1	JX875936	*Cladosporium cladosporioides* isolate wb146	99	Buzina et al., 2003 AF455535

[a]ITS nrDNA sequences of cultural endophytic fungi were deposited at GenBank; [b]Matches of ITS nrDNA sequences published in journals were also from GenBank

Table I

Continued

Strain (PE)	%Isolation frquency	Numbers of isolates	ITS (No.)[a]	Most closely related species	% Similarity	Reference ITS (No.)[b]
120	0.7	1	JX875937	*Simplicillium lanosoniveum strain CBS 962.72*	97	Zare et al., 2008 EF641862
121	0.7	1	JX875938	*Shiraia* sp. slf14	99	Zhu et al., 2010 GQ355934
122	0.7	1	JX875939	*Arthrinium sacchari* strain FBC.045	100	Shrestha et al., 2011 EF076711
123	0.7	1	JX875940	*Phoma* sp. P48E2	99	Loro et al., 2012 JN207349
124	0.7	1	JX875941	*Colletotrichum* sp. JP9	99	Morakotkarn et al., 2006 AB255243
125	0.7	1	JX875942	*Penicillium sclerotiorum* isolate M9	99	Yuan et al., 2011 HM595498
126	0.7	1	JX875943	*Shiraia* sp. slf14	99	Zhu et al., 2010 GQ355934
127	0.7	1	JX875944	*Curvularia* sp. M5	99	Akita et al., 2011 HM371207
128	0.6	1	JX875945	*Didymella exitialis* strain CBS 446.82	95	Simon et al., 2000 EU167564
129	0.6	1	JX875946	*Colletotrichum* sp. JP9	98	Morakotkarn et al., 2006 AB255243
130	0.6	1	JX875947	*Penicillium raistrickii* strain FRR 1044	99	Haugland et al., 2004 AY373927

[a]ITS nrDNA sequences of cultural endophytic fungi were deposited at GenBank; [b]Matches of ITS nrDNA sequences published in journals were also from GenBank (Cont.)

present study and the analyzed and categorized information about 30 strains involved in the study are given in Table I.

A total of 30 fungal endophytes were isolated to evaluate their antimicrobial activities against *S. aureus*, *B. subtilis*, *L. monocytogenes*, *S. bacteria*, *C. albicans*, *R. rubra*, *S. cerevisiae*, *C. eragrostidis*, *Pleospora herbarum*, *A. sacchari*, *A. phaeospermum* and *Phoma herbarum* by diffusion agar assay (Table II and III). The preliminary screening revealed that, of 30 isolates, five endophytic fungi (PE106, PE120, PE127, PE128 and PE130) inhibited the growth of at least two or more human pathogenic bacteria, which showed higher activity than others significantly (Table II). These isolates were also all active against human pathogenic fungi *C. albicans*, but PE127 and PE128 had less effect. There were two strains, PE130 and PE120, both having strongest activity against *S. aureus*, *B. subtilis* and *C. albicans*. In the anti-bacterial assay, PE130 displayed the widest spectrum of anti-activity, and also inhibited *C. albicans* significantly. PE120 exhibited more antifungal activity against *R. rubra*, less bacterial activity against *L. monocytogenes* and *S. bacteria*.

To test anti-plant pathogenic fungi activity of the endophytic fungi, five bambusicolous pathogenic fungi (*C. eragrostidis*, *Pleospora herbarum*, *A. sacchari*, *A. phaeospermum* and *Phoma herbarum*) were selected for further research (Table III). None of the cultivable isolates were found effective against *C. eragrostidis* and *Phoma herbarum*, and five strains (PE106, PE120, PE127, PE128, PE130) had bioactivity against *Pleospora herbarum*. PE120 and PE130 were found most effective against two pathogenic fungi *A. sacchari* and *A. phaeospermum*, and PE106 presented the highest activity against *Pleospora herbarum*.

Of 30 isolates, five strains (PE106, PE120, PE127, PE128 and PE130) presented bioactivity against at least four tested pathogenic micro-organisms, and they were selected to continuation of searching bioactive compounds from fermentation broth. But unfortunately, strain PE128 could only grow on the solid media and it was not suitable for the growth of PE128 in submerged fermentation, so the bioactivities of extracts were also not calculated. Active extracts from mycelium of the other four endophytic fungi (PE106, PE120, PE127 and PE130) were submitted to the percent of growth inhibition (PGI), which is an indication of the efficacy of antifungal activity, and PGI of ethyl acetate extracts were tested at 200 µg/mL against pathogens. From the calculation of PGI, ethyl acetate extracts of four strains had the best effect against typical plant pathogenic fungi *B. fuckeliana* (Table IV), and they also displayed the same board-spectrum of bioactivity against *T. cucumeris*, but extracts of PE120 and PE130 inhibited the growth of the pathogen more weakly. Especially extracts of PE106 had low activity against *T. cucumeris*. In the mycelial radial growth test, no endophytic fungi

Table II

Antimicrobial activity of fungal isolates from *Phyllostachys edulis* against

Isolate No. (PE)	*Staphylococcus aureus*	*Bacillus subtilis*	*Listeria monocytogenes*	*Salmonella bacteria*	*Candida albicans*	*Rhodotorula rubra*	*Saccharomyces cerevisiae*
101	-	-	-	-	-	-	-
102	-	-	-	-	-	-	-
103	-	-	-	-	-	-	-
104	-	-	-	-	-	-	-
105	-	-	-	-	-	-	-
106	+	+	-	-	++	-	-
107	-	-	-	-	-	-	-
108	-	-	-	-	-	-	-
109	-	-	-	-	-	-	-
110	-	-	-	-	-	-	-
111	-	-	-	-	-	-	-
112	-	-	-	-	-	-	-
113	-	-	-	-	-	-	-
114	-	-	-	-	-	-	-
115	-	-	-	-	-	-	-
116	-	-	-	-	-	-	-
117	-	-	-	-	-	-	-
118	-	-	-	-	-	-	-
119	-	-	-	-	-	-	-
120	+++	+++	+	-	+++	++	-
121	-	-	-	-	-	-	-
122	-	-	-	-	-	-	-
123	-	-	-	-	-	-	-
124	-	-	-	-	-	-	-
125	-	-	-	-	-	-	-
126	-	-	-	-	-	-	-
127	-	+	+	+	+	-	-
128	+	+	-	-	+	+	-
129	-	-	-	-	-	-	-
130	+++	+++	++	++	+++	+	-

- : No activity (<10 mm); +: Activity (10-15 mm); ++: Good activity (15-20 mm); +++: Very good activity (>20 mm)

exhibit antifungal activity against *A. alternate* and *G. avenacea* significantly.

With bioactivity against *B. fuckeliana*, PGI of extracts from PE120 and PE127 were higher than PE106 significantly under the same condition. Ethyl acetate extracts of PE120 were found to exhibit most bioactive against *A. alternate* and *C. lagenarium*. PE127 and PE130 showed higher activity against *G. avenacea* than the other strains significantly, and the most prominent activity against *T. cucumeris* was shown from extracts of PE120 and PE127.

Discussion

The present study is the first to investigate the diversity and antimicrobial activity of endophytic fungi from *Ph. edulis*. The results have shown that the fungal isolates were diverse both in morphology and molecular data. All of the cultivable isolates were affiliated with 12 genera by ITS data. The fungal community is common on terrestrial and submerged bamboos pathogen, saprophyte or endophytes (Hyde et al., 2001; Cai et al., 2003; Morakotkarn et al., 2007). The present results showed that the fungi isolated from bamboo branches are extremely frequent, and some have already been reported as pathogens or endophytes on bamboos.

Table III

Antimicrobial activity of fungal isolates from *Phyllostachys edulis* against plant pathogens

Isolate No. (PE)	Curvularia eragrostidis	*Pleospora herbarum*	*Arthrinium sacchari*	*Arthrinium phaeospermum*	*Phoma herbarum*
101	-	-	-	-	-
102	-	-	-	-	-
103	-	-	-	-	-
104	-	-	-	-	-
105	-	-	-	-	-
106	-	+++	++	++	-
107	-	-	-	-	-
108	-	-	-	-	-
109	-	-	-	-	-
110	-	-	-	-	-
111	-	-	-	-	-
112	-	-	-	-	-
113	-	-	-	-	-
114	-	-	-	-	-
115	-	-	-	-	-
116	-	-	-	-	-
117	-	-	-	-	-
118	-	-	-	-	-
119	-	-	-	-	-
120	-	++	+++	+++	-
121	-	-	-	-	-
122	-	-	-	-	-
123	-	-	-	-	-
124	-	-	-	-	-
125	-	-	-	-	-
126	-	-	-	-	-
127	-	+	+	+	-
128	-	+	-	-	-
129	-	-	-	-	-
130	-	++	+++	+++	-

-: No activity (<8 mm); +: Mild activity (8-10 mm); ++: Good activity (12-15 mm); +++: Very good activity (>15 mm)

Strain PE120 was obviously close to *Shiraia* sp., which was proved to be different from *S. bambusicola* and other *Shiraia*-like fungi (Morakotkarn et al., 2008). The *Shiraia* sp. was common pathogenic and endophytic fungus of bamboos, but had not yet been reported on *Ph. edulis* (Li et al., 2009). The fruit-body of *S. bambusicola* Henn., has been used as an orally taken folk medicine in China over hundred years (Liu, 1978; Li et al., 2009), for its antitumor activity and antiangiogenesis (Mazzini et al., 2001; Tong et al., 2004; Chen et al., 2005). Hypocrellins, as dominant compounds, were extracted from *S. bambusicola* (Wan and Chen, 1981), and have attracted a great deal of attention because of their light-induced antifungal, antiviral and antitumor activity, especially against the human immunodeficiency virus (HIV) (Kocisova et al., 1999; Wang et al., 1999; Mirossay et al., 2000; Xu et al., 2001; Yang et al., 2001; Ali and Olivo, 2002; Deininger et al., 2002; Zhou et al., 2003; Chin et al., 2004). However, its efficient antimicrobial activity was only against clinical pathogens and there have been few reports of the antimicrobial activity against plant pathogens (Ma et al., 2004; Su et al., 2009). In the present study, PE120 exhibited broad-spectrum and effective antimicrobial activity, especially showed high bioactivity against human pathogens (*S. aureus*, *B. subtilis*, *C. albicans* and *R. rubra*) and phytopathogens (*A. Sacchari*, *A. Phaeospermum*, *B. fuckeliana* and *T. cucumeris*) in antagonistic test, which would be used as biocontrol

Table IV

Antifungal activity of ethyl acetate extracts of the mycelium of endophytic fungi from *Phyllostachys edulis* branches calculated by mycelial radial growth test

Isolate No (PE)	The percent of growth inhibition %[a]				
	Botryotinia fuckeliana	*Alternaria alternata*	*Thanatephorus cucumeris*	*Gibberella avenacea*	*Colletotrichum lagenarium*
106	67.4 ± 5.58^{h}	4.0 ± 0.6^{b}	20.6 ± 6.7^{de}	5.4 ± 4.3^{bc}	26.8 ± 0.6^{ef}
120	81.0 ± 2.4^{i}	26.2 ± 3.3^{ef}	68.0 ± 5.5^{h}	15.0 ± 2.7^{cd}	41.5 ± 5.1^{g}
127	80.7 ± 0.8^{i}	12.9 ± 4.6^{bcd}	75.9 ± 4.9^{hi}	28.1 ± 7.9^{ef}	14.2 ± 2.9^{bcd}
128	NE	NE	NE	NE	NE
130	74.4 ± 4.0^{hi}	4.0 ± 0.7^{b}	45.8 ± 4.7^{g}	33.3 ± 3.3^{f}	13.9 ± 4.6^{bcd}

[a]Value are average of three replicates ± standard deviation. Statistical analysis of the data was performed with SSPS 18.0 using Student-Newman-Keuls test for determining significant difference (α = 0.05). ^{bc}no activity, ^{de}low activity, ^{fg}moderate activity, ^{hi}high activity

agent after further studies.

As newly bambusicolous fungus, strain PE130 was closely related to *Penicillium* spp., isolated from plants as endophytes. It has proved *Penicillium* spp. to be a good source for the production of bioactive compounds, where *Penicillium striatisporum*, *Penicillium canescens* and *Penicillium janczewskii* all have antifungal activity against typical pathogens (Nicoletti et al., 2007; Ma et al., 2008). In our study, PE130 was also found active against the most of the test pathogens, and displayed the best inhibitory halos against *S. aureus*, *B. subtilis*, *L. monocytogenes*, *S. bacteria*, *C. albicans*, *A. Phaeospermum* and *G. avenacea* respectively. This isolates may also be useful source of effective antifungal agents to improve clinical study and plant defense.

Strain PE106 is a strain of *Cladosporium* sp. belonging to Capnodiales, which had high similarity to *Cladosporium sphaerospermum*, is a kind of relatively common Hyphomycetes, and is widespread mostly as saprophyte of different substrata including soil, grain, fruits, and grass litter (Braun et al., 2003; Park et al., 2008). Despite the pathogenic nature of the fungus, *Cladosporium* sp. has also been reported to display antiviral, antifungal and antitumor activity (Seto et al., 2005; Wang et al., 2007; Hamayun et al., 2009; Zhang et al., 2009; de Medeiros et al., 2011). The present strain PE106, isolated from *Ph. edulis* branches for the first time, had the best effect on the test fungi *Pleospora herbarum*, and was also found bioactive against *C. albicans* and *B. fuckeliana* significantly. Further investigations are required to make use of it as a biocontrol agent.

Strain PE127 was similar to *Curvularia* sp., which was first isolated from *Ph. edulis*. As a plant pathogenic fungus, the distribution of *Curvularia* sp. was widespread among plants, including some bamboo species but without *Ph. edulis* (Mohanan, 2002). Another interesting aspect of *Curvularia* sp. was to produce several bioactive compounds, which included Benzopyrans (Teles et al., 2005), cell cycle inhibitor (Honda et al., 2001), laccase and cellobiase (Banerjee, 1990; Banerjee and Vohra, 1991), Multiplolides A and B (Boonphong et al., 2001), phenylacetic acid derivatives and 4-epiradicinol (Varma et al., 2006), curvulapyrone, curvulalide and curvulalic acid (Trisuwan et al., 2011). Our observations suggest that fungus PE127 have broad -spectrum but mild activity against human pathogens (*B. subtilis*, *L. monocytogenes*, *S. bacteria* and *C. albicans*), and high activity against only two plant pathogens (*B. fuckeliana* and *T. cucumeris*). Further study is in progress, to identify new and useful bioactive agents.

Isolate PE128 from genus of *Didymella*, exhibited different strength of biological activities. PE128 was found antibacterial against *S. aureus* and *B. subtilis* by agar diffusion array, and antifungal against *C. albicans*, *R. rubra* and *Pleospora herbarum*. But with the normal growth on PDA solid media, there was no suited submerged fermentation method for PE128. The extracts of fermentation broth were not evaluated by mycelial radial growth test, and the data of PGI were not calculated. Future research should optimize the submerged fermentation media of PE128 and evaluate the bioactive compounds of mycelium.

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Conflict of Interest

Authors declare no conflict of interest

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