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Screening Antimicrobial Activity of Endophytic Fungi from *Eucommia ulmoides* (Eucommiaceae) Against Phytopathogenic Fungi

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Research into plant-derived endophytic fungi had grown in recent decades. Endophytic fungi still had enormous potential to inspire and influence modern agriculture. The aim of the present study was to screen for antimicrobial activity of endophytic fungi isolated from surface sterilized leaves, stems and fruits of *Eucommia ulmoides* oliv. to Gibberella zeae. The fermentation broths from 32 isolated fungi were tested for antimicrobial activity by the mycelium growth rate method. The results showed that fermentation broths from the majority of isolates exhibited antifungal activity and the strongest antimicrobial activity was exhibited by strain DZGS01 against Gibberella zeae, showing 66.47 % inhibition at 1 mg/mL , compared to 62.36 % for 12 μg/mL carbendazim. Moreover, compared to water control , the control efficacy of the fermentation broth of the DZGS01 was 63.16 % by pot experiment. And the endophytic fungus DZGS01 was belonged to Myrothecium based on rDNA sequencing of ITS region .The results indicate that the endophytic fungus DZGS01 from the plant *Eucommia ulmoides* oliv. was a promising source of novel bioactive inhibitors against Gibberella zeae.

Key words: Antimicrobial activity, Endophytic fungi, *Eucommia ulmoides* (Eucommiaceae), Phytopathogenic fungi.

Endophytes were the micro-organisms that colonize healthy plant tissue, without causing any apparent symptoms or diseases in the host plants (Arnold *et al*. 2003). They were quite ubiquitous and have been found in all plant species examined to date (Arnold *et al.* 2000). Endophytes played an important role in plant community health by providing resistance to hosts against different biotic and abiotic stresses (Kharwar *et al*. 2008; Gond *et al*. 2010). On one hand, endophytes could mediate interactions between host plants and herbivores and pathogens. on the other hand, most endophytes were capable of synthesizing bioactive compounds that mayed provide plants with a defense against pathogens (Owen and Hundley 2004). Hence, the endophytic fungi were regarded as a potential source of new natural bioactive products.

Fusarium head blight (FHB) epidemics was caused by *Gibberella zeae* (anamorph *Fusarium graminearum*) in wheat and barley and ear rot in corn, and it was regarded as one of the destructive diseases due to its extensive damage in yield and quality to wheat and barley around the world (Cuthbert *et al*. 2007; Liu and Anderson 2003; Shi and Wang 1999). At present, chemical fungicides were still the major measure to control *Fusarium* head blight. But the use of chemical fungicides not only polluted environment but also

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enhanced the tendency of pathogens to develop resistance to chemicals (Bajwa *et al*. 2003).Therefore, there was an interest in technologies that reduced dependency on synthetic chemical pesticides. Biological control of plant diseases had received attention as an alternative to the intensive use of chemically synthesized products. This alternative was perceived to be safer and to have a minimal environmental impact (Brimmer and Boland 2003). *Eucommia ulmoides* oliv. was a medicinal plant in southern China, which was known to host several metabolites having medicinal property (Hussain *et al*. 2003; Wang *et al*. 1994; Li and Yan 1996). In the last years, many studies on it had been carried out in terms of some products of secondary metabolism. However, reports on the anti-phytopathogen activity of the endophytic community of this plant were relatively few. Keeping in view the medicinal values of this host, the objectives of the work reported in this article were to isolate the endophytic fungi from living symptomless tissues of leaves, stems and fruits of *Eucommia ulmoides* oliv., and to evaluate their potential as biocontrol agents against *Gibberella zeae*. This work could be of great help as selecting an environmentally friendly disease biocontrol agents based on the endophytic fungi from *Eucommia ulmoides* oliv..

MATERIALS AND METHODS

Collection of Plant Material

The healthy leaves, stems and fruits of *Eucommia ulmoides* oliv. were collected during June 2011–November 2011 from Anhui Agricultural University (31°86' N and 117°25' E), Anhui Province, China. The samples were processed within 24 h following collection, and plant parts were washed with tap water and processed for isolation of endophytic fungi.

Isolation, identification and phylogenetic analyses of endophytic fungi

Isolation of the endophytic fungi was performed as follows (Xu *et al*. 2008): The cleaned samples were surface-sterilized by immersing in 75 % ethyl alcohol for 1 min, then soaked in 0.05 $g.mL^{-1}$ sodium hypochlorite (5 % available chlorine) for 3 min and finally the samples were rinsed three times in sterile distilled water and surface-dried with sterile filter paper. To confirm that the disinfection process was successful, aliquots of the sterile distilled water used in the final rinse were also plated onto the surface of potato dextrose agar (PDA) plates supplemented with streptomycin (30 $\mu g.mL^{-1}$), and the plates were examined for growth after incubation at 25 °C for 3 days. After surface sterilization, the samples were cut into small pieces approximately 3 mm^2 and placed on the surface of potato dextrose agar (PDA) medium supplemented with streptomycin (30 $\mu g.mL^{-1}$) for incubation at 25 °C with controlled temperature and light conditions (normal daily cycle of 12 hours of light followed by 12 hours of darkness). in an incubator , and checked every other day for 3–15 days for the development of fungal colonies growing out from the segments. Hyphal tips of the developing fungal colonies were transferred to fresh potato dextrose agar plates to get pure culture.

The endophytic fungal isolates were identified using molecular tools. A pair of primers ITS1 (sequence: 5' -TCCGATGGTGAACCTGCGG-3') and ITS2 (5' -TCCTCGTTATTGATATGC-3') were used to amplify the 5.8S and flanking ITS regions of the orphospecies (Phongpaichit *et al*. 2006). The DNA fragment was amplified and sequenced according to previously described methods (Guo *et al*. 2000). The sequence datas from this study had been submitted to GenBank under accession Nos. JX179222"JX179253. Multiple sequence alignments were performed using the CLUSTAL X program (Thompson *et al*. 1994). Molecular evolutionary analyses were conducted using MEGA Version 4.0 (Kumar *et al*. 2008). The Kimura (1980)'s two-parameter model was used to estimate evolutionary distance. The phylogenetic tree was constructed using the neighbor-joining (NJ) algorithm (Naruya and Masatoshi, 1987)and maximum-parsimony (MP) analyses, with bootstrap values calculated from 1000 replicate runs using the software routines included in the MEGA software (Li *et al*. 2008).

Fermentation and treatment of the fermentation broth of endophytic fungi

The endophytic fungi were added to 200 mL potato dextrose liquid medium in Erlenmeyer flasks and incubated at 25 °C and 160 rpm with normal daily light and dark periods for ten days. The mycelia of the isolates were separated by

centrifugation (5000 r. min^{-1}, 10 min), so that the fermentation broth of isolates were obtained. Finally, samples were dried in a freeze-drying system.

Microorganisms used

The test microorganism *Fusarium graminearum* (anamorph of *Gibberella zeae*) was obtained from the School of Plant Protection, Anhui Agricultural University, Anhui Province, Southeast China. Prior to testing, indicator organism was cultured in PDA medium at 25 °C.

Evaluation of antagonistic activity of fermentation broth of endophytic fungi in vitro

The antagonistic effect of fermentation broth of endophytic fungi were evaluated against *Fusarium graminearum* (anamorph of *Gibberella zeae*) by the method of mycelial growth. The freeze-dried fermentation broth were dissolved in sterilized water at a concentration of 1 $mg.mL^{-1}$ and the solution sterilized by filtration with 0.22 µm Millipore filters, then 100 µL samples were poured on sterile Petri dishes containing 10 mL PDA, followed by adequate mixing. A negative control was prepared using 100 µL non-inoculated sterile fermentation broth. 12 $\mu g.mL^{-1}$ 50.% carbendazim wettable powder was used 100 µL for the positive control.

A 6 mm diameter plug of the actively growing mycelium of *Fusarium graminearum* was placed in the center of the plate. The plates were incubated at 25 °C with controlled light conditions (normal daily cycle of 12 hours of light followed by 12 hours of darkness) in an incubator (5 plates per treatment). The diameters of the inhibition zones were measured by vernier caliper. According to the growth rate of the pathogen, colony diameter data was taken after 7 days,when the negative control covered dish. The inhibitory activity of each treatment was carried out using the following formula,

$$\text{Growth inhibition (\%)} = \frac{DC - DT}{DC} \times 100\%$$

where DC = diameter of control and DT = diameter of fungal colony with treatment. The experiments were repeated twice and the data presented here are the averages of two experiments.

Antagonistic activity of the selected DZGS01 isolate in pot experiment

The disease-suppression effects of DZGS01 isolate against *Gibberella zeae* were measured using wheat seeds cultivars of Sámán (moderately susceptible, MS) cultured in polypropylene pots (7×19×14 cm) in the glass greenhousewith normal daily cycle of 12 hours of light followed by 12 hours of darkness and normal temperature.Each pot had five seeds containing sandy pre-pasteurized soil. Pathogen inoculation was performed with *Fusarium graminearum* (anamorph of *Gibberella zeae*). It was recovered from stored inoculum (silicagel 4 °C) and grown on PDA plates for 10 days, then added the sterile water to the plates and scraped the conidia from the agar surface to obtain the conidia suspension of *Fusarium graminearum* at concentration of 1.0×10^{6} $cfu.mL^{-1}$. The freeze-dried fermentation broth of the DZGS01was dissolved in sterilized water at a concentration of 1 $mg.mL^{-1}$ and the solution sterilized by filtration with 0.22 µm Millipore filters.

The anti-pathogen activity of DZGS01 was researched in the blooming period of the wheat, and the test was carried out as follows: At the beginning of the assay, the 10 mL conidia suspension of *Fusarium graminearum* at concentration of 1.0×106 $cfu.mL^{-1}$ was sprayed to the wheatear of plants , and the infected tissues were cultured with moisture using bagging for 24 h, then the fermentation broth of DZGS01 were sprayed on the same point of wheat at the concentration of 1 $mg.mL^{-1}$ (5 plants per treatment). the negative control and positive control were sterilized water and 12 $\mu g.mL^{-1}$ 50 % carbendazim wettable powder respectively.

Disease severity was monitored at 2–3 day intervals, and disease severity of negative control began on the ninth day, while the disease severity of positive control and the treatment were observed on the fourteenth day. The disease severity was scored based on a symptom severity scale (Khan *et al.* 2001). In each disease assessment, the mean of disease severity per pot was calculated. Disease index and control efficacy of each treatment were carried out using the following formula,

$$\text{Disease index} = \frac{\sum(hi \times i)}{H \times 5}$$

$$\text{Control efficacy (\%)} = \frac{IC - IT}{IC} \times 100\%$$

where hi = the number of diseased ears, i = illness severity level, H = total number of

investigated ears, IC = disease index of control and IT = disease index of treatment. The experiments were repeated twice and the data presented here were the averages of two experiments.

RESULTS AND DISCUSSION

Isolation and identification of endophytic fungi

A total of thirty-two morphologically distinct fungal isolates from *Eucommia ulmoides* oliv. were isolated and identified. The quantity, population and distribution of the endophytic fungi varied in different positions of *Eucommia ulmoides* oliv.. Among them, 14 were isolated from leaves, 9 from stems and 9 from fruits. These endophytic fungi were identified by ITS sequence analysis and they were classified into 9 genera (Table 1). The common species found in three tissue segments were *Nigrospora*, *Phomopsis* and *Colletotrichum*, while *Pestalotiopsis* and *Hypocreales* were isolated only from leave

Table 1. Isolation and identification of endophytic fungi from *Eucommia ulmoides* and effects of the fermentation broth of endophytic fungi on growth of *Gibberella zeae* in vitro

Host site	Strain no.	Taxa	GenBank accession No.	Activity against *Gibberella zeae* (% Growth inhibition)
Leaves	DZY01	*Nigrospora* sp. 1	JX179222	5.81^{l}
	DZY02	*Pestalotiopsis* sp.	JX179223	4.39^{m}
	DZY03	*Alternaria* sp. 1	JX179224	12.55^{i}
	DZY04	*Nigrospora* sp. 2	JX179225	-5.27^{q}
	DZY05	*Myrothecium* sp. 1	JX179226	-1.83^{op}
	DZY06	*Nigrospora* sp. 3	JX179227	23.17^{f}
	DZY07	*Hypocreales* sp.	JX179228	14.22^{h}
	DZY08	*Phomopsis* sp. 1	JX179229	9.21^{j}
	DZY09	*Colletotrichum* sp. 1	JX179230	-2.14^{op}
	DZY10	*Nigrospora* sp. 4	JX179231	6.30^{l}
	DZY11	*Nigrospora* sp. 5	JX179232	19.23^{g}
	DZY12	*Colletotrichum* sp. 2	JX179233	-2.72^{p}
	DZY13	*Colletotrichum* sp. 3	JX179234	33.61^{d}
	DZY14	*Colletotrichum* sp. 4	JX179235	11.14^{i}
Stems	DZJ01	*Alternaria* sp. 2	JX179236	23.11^{f}
	DZJ02	*Nigrospora* sp. 6	JX179237	31.95^{e}
	DZJ03	*Colletotrichum* sp. 5	JX179238	-1.04^{o}
	DZJ04	*Alternaria* sp. 3	JX179239	-4.09^{q}
	DZJ05	*Phomopsis* sp. 2	JX179240	3.40^{mn}
	DZJ06	*Colletotrichum* sp. 6	JX179241	11.13^{i}
	DZJ07	*Colletotrichum* sp. 7	JX179242	23.11^{f}
	DZJ08	*Colletotrichum* sp. 8	JX179243	-5.30^{q}
	DZJ09	*Colletotrichum* sp. 9	JX179244	33.10^{de}
Fruits	DZGS01	*Myrothecium* sp. 2	JX179245	66.47^{a}
	DZGS02	*Nigrospora* sp. 7	JX179246	-2.34^{op}
	DZGS03	*Colletotrichum* sp. 10	JX179247	-1.42^{op}
	DZGS04	*Phomopsis* sp. 3	JX179248	2.15^{n}
	DZGS05	*Nigrospora* sp. 8	JX179249	12.59^{i}
	DZGS06	*Colletotrichum* sp. 11	JX179250	23.69^{f}
	DZGS07	*Colletotrichum* sp. 12	JX179251	38.38^{c}
	DZGS08	*Thielavia* sp.	JX179252	-8.19^{r}
	DZGS09	*Botryosphaeria* sp.	JX179253	7.77^{k}
	Positive control^{A}			62.36^{b}

Statistical analysis of the data was performed with the JMP software (DPS, 2000) using the Duncan HSD test . the letter designations that appear as superscripts in the far-right column were represented significant difference between treatments at the P = 0.05 level of significance.^{A}carbendazim (120μg.disc^{-1}).

Table 2. Inhibition effect of the fermentation broth from DZGS01 against *Gibberella zeae* in wheat spikelet test in the greenhouse (artificial infection)

Treatment	Disease index	Control efficacy(%)
Check of water	92.89[a]	—
50 % carbendazim wettable powder (12 μg.mL^{-1})	26.04[c]	68.42[a]
Fermentation broth of DZGS01 (1 mg.mL^{-1})	32.92[b]	63.16[a]

Statistical analysis of the data was performed with the JMP software (DPS, 2000) using the Duncan HSD test. The letter designations that appear as superscripts in the far-right column were represented significant difference between treatments at the P = 0.05 level of significance.

segments, *Thielavia* and *Botryosphaeria* were only found colonizing fruits. What's more, among the 32 isolates, *Colletotrichum* was the predominant genus with about 37.5 % of strains belonging to it.

Effects of the fermentation broths of endophytic fungi on growth of *Gibberella zeae* in vitro

The fermentation broths of the thirty-two endophytic fungi isolates from *Eucommia ulmoides* oliv. were screened for antimicrobial activity against *Gibberella zeae* by the method of mycelial growth. The results showed that the antifungal activities of the fermentation broths of the isolates were significantly different (Table 1). Among the thirty-two endophytic fungi, the fermentation broths of twenty-two strains showed antifungal activity against *Gibberella zeae*, the inhibition of which was 2.15–66.47 %. And DZGS01 showed higher growth inhibitory effects, where the freeze-dried fermentation broth at 1 mg/mL caused 66.47 % inhibition, compared to 62.36 % for 12 μg/mL carbendazim used as the positive control (Fig. 1). Whereas, there were 10 strains such as DZY04, DZY05, DZY09, DZY12, DZJ03, DZJ04, DZJ08, DZGS02, DZGS03, DZGS08 showed no inhibitory effects. The results indicated that fungi isolated from *Eucommia ulmoides* oliv. were a good source of natural antimicrobial compounds. Furthermore, the constructed NJ phylogenetic tree of DZGS01 showed that DZGS01 had similar genetic relationship with *Myrothecium* sp.Z16 (Fig. 2).

Effect of DZGS01 on test pathogen in pot experiments

The disease-suppression effects of DZGS01 isolate against *Gibberella zeae* was measured using wheat plants cultured in polypropylene pots (7×19×14 cm) in the glass greenhouse with normal daily cycle and normal temperature

After spraying the 10 mL conidia suspension of *Fusarium graminearum* at concentration of 1.0×106 cfu.mL^{-1}, the onset of

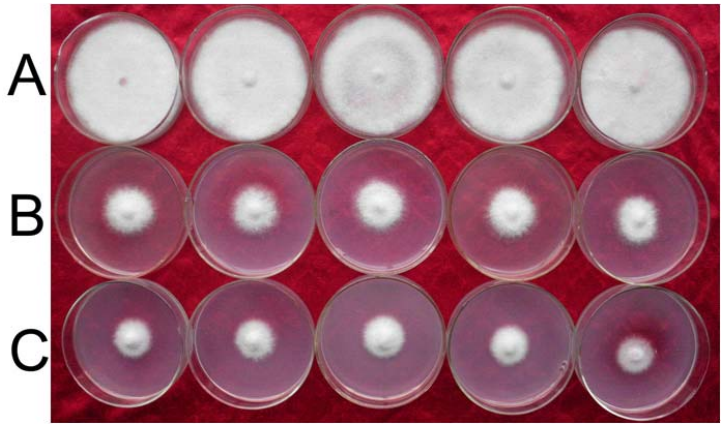


Fig. 1. A was the colony diameter of *Fusarium graminearum* (anamorph of *Gibberella zeae*) on PDA medium mixing negative control(non-inoculated sterile fermentation broth), B was the colony diameter of *Fusarium graminearum* on PDA medium mixing positive control(12 μg.mL^{-1} 50.% carbendazim wettable powder), C was the colony diameter of *Fusarium graminearum* on PDA medium mixing treatment(the fermentation broth of isolate DZGS01)

symptoms of negative control appeared five days earlier than that of positive control and the treatment, and the symptoms of negative control appeared in ninth day, while the symptoms of positive control and the treatment were observed in fourteenth day. The symptoms at the end of assays indicated that the treatment of fermentation broth of DZGS01 had significant suppressive effects on *Gibberella zeae* in comparison with the negative control, which showed the highest values of severity (Fig. 3). Disease index which expresses disease level showed that the fermentation broth of DZGS01 sharply reduced the disease severity caused by *Gibberella zeae* by disease index 32.92 %, when it was introduced into the wheat during blooming stage in greenhouse conditions (Table 2), and nearly equaled to that observed in naturally suppressive 12 µg/mL carbendazim which was used as the positive control (Fig. 3). Moreover, the control efficacy of DZGS01 against *Gibberella zeae* was 63.16 %, which had no significant difference with the 12 $\mu g.mL^{-1}$ fungicide carbendazim (68.42 %) (Table 2). From this data, it is clear that DZGS01 has a good potentiality of controlling *Gibberella zeae*.

Exploitation of novel classes of antimicrobial metabolites was increasingly noticeable over recent years. Endophytic fungi isolated from various medicinal plants had been recognized as a repository of novel secondary metabolites, some of which had beneficial biological activities (Vaz *et al*. 2009). Souwalak *et al*. obtained 65 crude extracts from 51 endophytic fungi isolated from Garcinia plants and assessed these extracts

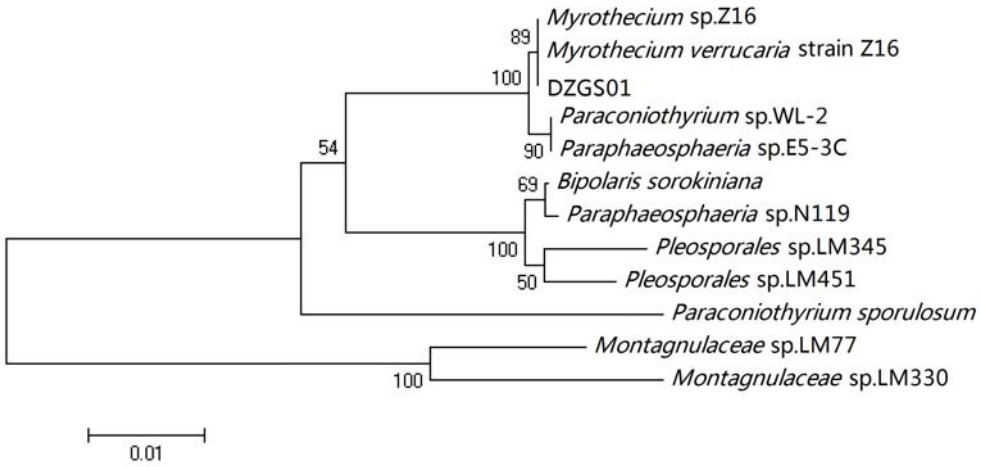


Fig. 2. Phylogenetic tree of ITS1-5.8S-ITS2 rDNA sequences of the isolate DZGS01, compared with sequences obtained from public databases. NJ-tree was constructed using ITS1-5. 8S-ITS2 rDNA sequences. Bootstrap values were calculated using 1,000 replications

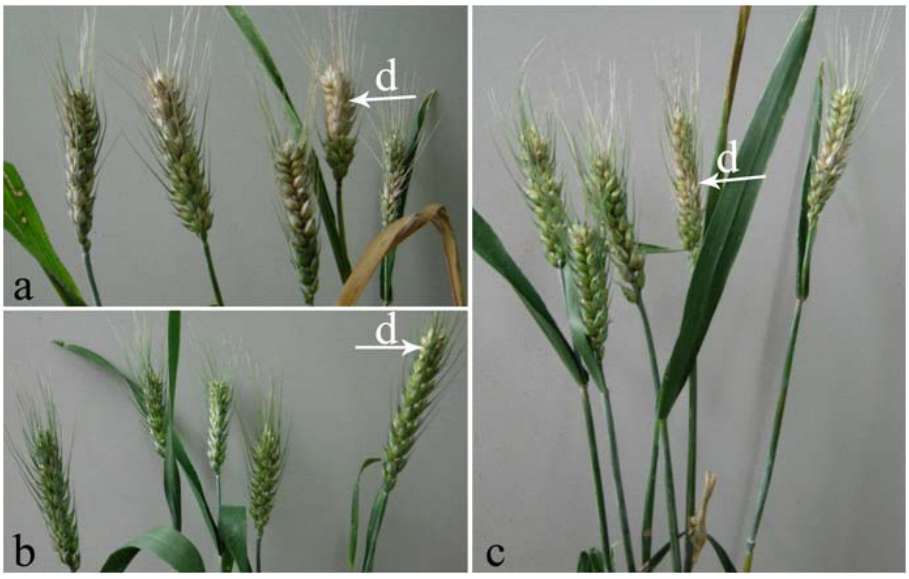
for various bioactivities (Souwalak *et al*. 2007). Huang *et al*. isolated 172 endophytic fungi from three medicinal plants and tested their fermentation broths for cytotoxicity (Huang *et al*. 2001). The preliminary results of screening endophytic fungi from *Eucommia ulmoides* oliv. indicated their potential source of bioactive metabolites for novel antibiotic discovery.

The endophytic fungi were identified to belong to 9 taxa (Table 1). Some endophytic genera such as *Pestalotiopsis*, *Thielavia* and *Botryosphaeria* which were known to produce various bioactive products (Sajeewa *et al*. 2011; Kumar *et al*. 2010; Kumar and Kaushik 2012) were only dominated in the leaves or fruits of *Eucommia ulmoides* oliv. due to their host specificity, and they mayed be of potential practical value. The most common and predominant genera isolated in this study was *Colletotrichum*. *Colletotrichum* had worldwide occurrence as plant pathogen causing disease in several plants (Begum *et al*. 2008). Despite the pathogenic nature of the fungus, it had been reported as a biocontrol agent against several disease (Boyette *et al*. 2007). Some earlier researchers had also isolated many antimicrobial compounds, such as colletotric acid from

Colletotrichum sp. (Zou *et al*. 2000), which indicatesd the genera *Colletotrichum* mayed act as a source of antifungal compounds.

Currently, there was demanded to a search for new antimicrobial agents because of the development of pathogen resistance to available synthetic fungicides. In the present study, the antifungal activity of endophytic fungi from *Eucommia ulmoides* oliv. was screened against *Gibberella zeae,* as an indication of their capability to produce secondary metabolites of potential therapeutic interest. Different isolates exhibited different antifungal spectra (Table 2). The results in vitro experiment revealed the fermentation broths of 10 strains didn't show any activity against *Fusarium graminearum*, the fermentation broths of 13 strains showed mild activity against *Fusarium graminearum* (percent inhibition 2.15 %–19.23 %), the fermentation broths of 8 strains were found active against the test pathogen (percent inhibition 23.11 %–38.38 %), and the fermentation broths of DZGS01 strain had the highest activity of isolates against *Fusarium graminearum* (percent inhibition 66.47 %) in vitro. Moreover, the control efficacy of DZGS01 against *Gibberella zeae* was 63.16 % in pot experiments in glass greenhouse conditions , compared to water control. Xue *et al.* reported that *Clonostachys rosea* strain ACM941 reduced mycelial growth of the *Fusarium graminearum* by 52.6 %, and when strain ACM941 was sprayed onto wheat heads 2 days prior to inoculation with *Gibberella zeae*, it significantly reduced infected spikelets by 64 % (Xue *et al*. 2009). Doohan *et al.* Found, under the greenhouse conditions, treatment with *Pseudomonas ûuorescens* strain MKB 158 reduced the severity of FHB symptom development on wheat by 48 % (Doohan and Khan 2009). Musyimi *et al.* have reported four promising biocontrol agents (*Trichoderma*, *Epicoccum*, *Alternaria* and *Penicillium*) which suppressed *Fusarium graminearum* growth by up to 53 % in vitro (Musyimi *et al*. 2012). Compared with these dates, it was possible for DZGS01 to act as a potential source of bioactive antifungal agents, and thiswas the first report using endophytic fungi to control FHB disease in this study.

DZGS01 was identiûed by rDNA sequencing of ITS region and constructed the phylogenetic tree, which indicated DZGS01



Note:
a. The biocontrol efficacy of sterilized water on *Fusarium graminearum* ;
b. The biocontrol efficacy of carbendazim (12 μg.mL^{-1}) on *Fusarium graminearum*;
c. The biocontrol efficacy of the fermentation broth (1 mg.mL^{-1}) of strain DZGS01 on *Fusarium graminearum*;
d. The disease symptoms of wheatear.

Fig. 3. The biocontrol efficacy of the fermentation broth of DZGS01 in Pot experiment under greenhouse conditions on *Fusarium graminearum* (anamorph of *Gibberella zeae*)

belongs to *Myrothecium*. *Myrothecium* had been reported as endophyte from several host plant such as *Hevea brasiliensis*, *Holarrhena antidysenterica*, *Crataeva magna* (Anderson *et al*. 2011; Kanika *et al*. 2012; Monnanda *et al*. 2005) and so on. Some studies had showed the secondary metabolites of *Myrothecium* had antimicrobial activity. Such as, the fungi *Myrothecium* sp. JS9 has successfully managed the *Sclerotinia sclerotiorum*, a disease of plants and vegetables (Xie *et al*. 2008). In addition, *Myrothecium* sp. could produce various bioactive products such as acid and antifungal trichothecenes (Liu *et al*. 2006), which mayed account for the inhibitory effects of DZGS01 on *Gibberella zeae*.

The try out to In summary, the results presented in this work showed that the fermentation broths of endophytic fungi of *Eucommia ulmoides* oliv. mayed have antimicrobial potential, and the fermentation broths of the DZGS01 strain belonging to *Myrothecium* had high activity against *Gibberella zeae*. Therefore, the next goal is to speculate the antifungal mechanism of the DZGS01 strain, to find out bioactive compounds that the DZGS01 strain may produce. In this way, we hoped to lay a sound foundation for development and application of the DZGS01 strain.

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REFERENCES

1. Anderson CS, Rocha DG, Ana PT *et al.*, Foliar endophytic fungi from *Hevea brasiliensis* and their antagonism on *Microcyclus ulei*. *Fungal Diversity* 2011; **47**: 75–84.
2. Arnold AE, Maynard Z, Gilbert GS, Coley PD, Kursat TA., Are tropical fungal endophytes hyperdiverse. *Ecology Lett* 2000; **3**: 267–274.
3. Arnold AE, Mejia LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA., Fungal endophyte limit pathogen damage in a tropical tree. *Proc Natl Acad Sci USA* 2003; **100**: 15649-15654.
4. Bajwa R, Khalid A and Cheema TS., Antifungal activity of allelopathic plant extracts III: Growth response of some pathogenic fungi to aqueous extract of Parthenium hysterophorus. *Plant Pathol J* 2003; **2**: 145–156.
5. Begum MM, Sariah M, Puteh AB, Abidin MAZ., Pathogenicity of *Colletotrichum truncatum* and its influence on soybean seed quality. *Intl J Agric Biol* 2008; **10**: 393–398.
6. Boyette CD, Jackson MA, Bryson CT, Hoagland RE, Connick WJ Jr, Daigle DJ., *Sesbania exaltata* biocontrol with *Colletotrichum truncatum* microsclerotia formulated in pesta granules. *Biocontrol* 2007; **52**: 413–426.
7. Brimmer T and Boland G., A review of the non-target effects of fungi used to biologically control plant diseases. *Agric Ecosyst Environ* 2003; **100**: 3-16.
8. Cuthbert PA, Somers DJ and Brulé-Babel A., Mapping of Fhb2 on chromosome 6BS: a gene controlling Fusarium head blight ûeld resistance in bread wheat (Triticum aestivum L.). *Theoretical and Applied Genetics* 2007; **114**: 429–437.
9. Doohan FM and Khan MR., Comparison of the efûcacy of chitosan with that of a *ûuorescent pseudomonad* for the control of *Fusarium* head blight disease of cereals and associated mycotoxin contamination of grain. *Biological Control* 2009; **48**: 48-54.
10. Gond SK, Verma VC, Mishra A, Kumar A, Kharwar RN., Role of fungal endophytes in plant protection. In: Arya A, Perello AE(eds) Management of Fungal plant pathogens. CAB International, Wallingford, 2010; 183–197.
11. Guo LD, Hyde KD and Liew ECY., Identification of endophytic fungi from Livistona chinensis based on morphology and rDNA sequences. *New Phytol* 2000; **147**: 617–630.
12. Huang YJ, Wang JF, Li GL, Zheng ZH, Su WJ., Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants *Taxus mairei*, *Cephalataxus fortunei* and *Torreya grandis*. *Immunology and Medical Microbiology* 2001; **31**: 163–167.
13. Hussain M, Hussain MT, Rama NH *et al.*, Synthesis and antimicrobial activities of some isocoumarin and dihydroisocoum arin derivatives. *Nat Prod Res* 2003; **17**(3): 207–214.
14. Kanika Chowdhary, Nutan Kaushik, Azucena Gonzalez Coloma *et al.*, Endophytic fungi and their metabolites isolated from Indian medicinal plant. *Phytochem Rev* 2012; **11**: 467–485.
15. Khan NI, Schisler DA, Boehm MJ *et al.*, Selection and evaluation of microorganisms for biocontrol of *Fusarium* head blight of wheat incited by *Gibberella zeae*. *Plant Dis* 2001; **85**(12): 1253–

1258.
16. Kharwar RN, Verma VC, Strobel G, Ezra D., The endophytic fungal complex of Catharanthus roseus (L.) *G. Don. Curr Sci* 2008; **95**: 228–233.
17. Kumar S, Dudley J, Nei M, Tamura K., MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Brief Bioinform* 2008; **9**(4): 299–306.
18. Kumar S and Kaushik N., Metabolites of endophytic fungi as novel source of biofungicide: a review. *Phytochem Rev* 2012; **11**: 507–522.
19. Kumar S, Kaushik N, Ebel R *et al*., Isolation, characterization, and bioactivity of endophytic fungi of *Tylophora indica. World J Microbiol Biotechnol* 2010; **27**: 571–577.
20. Li J, Zhao GZ, Chen HH, Wang HB, Qin S, Zhu WY, Jiang CL, Li WJ., Antitumour and antimicrobial activities of endophytic streptomycetes from pharmaceutical plants in rainfrest. *Lett Appl Microbiol* 2008; **47**(6): 574–580.
21. Li JS, Yan YN., A preliminary analysis on leaves and cortexs of Eucommia ulmoides (Chinese). *Bull Chin Trad Med* 1996; **11**: 4–5.
22. Liu JY, Huang LL, Ye YH *et al.*, Antifungal and new metabolites of *Myrothecium* sp. Z16, a fungus associated with white croaker *Argyrosomus argentatus*. *Journal of Applied Microbiology* 2006; **100**(1): 195–202.
23. Liu S and Anderson JA., Targeted molecular mapping of a major wheat QTL for Fusarium head blight resistance using wheat ESTs and synteny with rice. *Genome* 2003; **46**: 817–823.
24. Monnanda S Nalini, Basavanna Mahesh, Mysore V Tejesvi *et al.*, Fungal endophytes from the three-leaved caper, *Crataeva magna* (Lour.) DC. (Capparidaceae). Mycopathologia 2005; **159**: 245–249.
25. Musyimi SL, Muthomi JW, Narla RD, Wagacha JM., Efficacy of biological control and cultivar resistance on *Fusarium* head blight and T-2 toxin contamination in wheat. *American Journal of Plant Sciences* 2012; **3**: 599–607.
26. Naruya S and Masatoshi N., The Neighbor-joining method: a new method for reconstructing phylogenetic tree. *Mol Biol Evol* 1987; **4**(4): 406–425.
27. Owen NL and Hundley N., Endophytes-The chemical synthesizers inside plants. *Sci Prog* 2004; **87**: 79–99.
28. Phongpaichit S, Rungjindamai N, Rukachaisirikul N, Sakayaroj J., Antimicrobial activity in cultures of endophytic fungi isolated from Garcinia species. *Immunol Med Microbiol* 2006; **48**(3): 367–372.
29. Sajeewa SN, Guo LD, Ekachai C *et al.*, *Pestalotiopsis* morphology, phylogeny, biochemistry and diversity. *Fungal Diversity* 2011; **50**: 167-187.
30. Shi JR and Wang YZ., The occurrence and control of wheat scab and sharpeye spot in Jiangsu in 1998. *Plant Protection* 1999; **2**: 17–19.
31. Souwalak P, Jaru N, Nattawut R *et al*., Biological activities of extracts from endophytic fungi isolated from Garcinia plants. *Immunology and Medical Microbiology* 2007; **51**(3): 517–525.
32. Thompson JD, Higgins DG, Gibson TJ., CLUSTAL W: improving the sensitivity of progressive multiple alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 1994; **22**(22): 4673–4680.
33. Vaz AB, Mota RC, Bomfim MR *et al.*, Antimicrobial activity of endophytic fungi associated with Orchidaceae in Brazil. *Canadian Journal of Microbiology* 2009; **55**: 1381–1391.
34. Wang JL, Chen PL, Zhu BC., Amino acid in Eucommia ulmoides (Chinese). *Hebei Univ J* 1994; **14**: 80–82.
35. Xie LW, Jiang SM, Zhu HH et al., Potential inhibitors against *Sclerotinia sclerotiorum*, produced by the fungus *Myrothecium* sp. associated with the marine sponge *Axinella* sp. *European Journal of Plant Pathology* 2008; **122**(4): 571–578.
36. Xue AG, Voldeng HD, Savard ME *et al.*, Biological control of *Fusarium* head blight of wheat with *Clonostachys rosea* strain ACM941. *Can J Plant Pathol* 2009; **31**: 169–179.
37. Xu L, Zhou L, Zhao J, Li J, Li X, Wang J., Fungal endophytes from Dioscorea zingiberensis rhizomes and their antibacterial activity. *Lett Appl Microbiol* 2008; **46**(1): 68–72.
38. Zou WX, Meng JC, Lu H, Chen GX, Shi GX, Zhang TY, Tan RX., Metabolites of *Colletotrichum gloeosporioides*, an endophytic fungus in *Artemisia mongolica*. *Nat J Prod* 2000; **63**:1529–1530.