Table II

Antimicrobial effects of the fermentation broth of endophytic fungi from *Edgeworthia chrysantha*

	Antimicrobial effect[a]				
Concentration	*E. coli*	*S. aurius*	*C. albicans*	*R. cerealis*	*C. gloeosporioides*
58	-	-	-	-	-
3-11	-	-	-	-	-
F	-	-	-	-	-
5-19	-	++++	+++	-	-
D	++	++	++	-	-
BZ	-	++++	-	-	-
3-8	-	-	-	-	+
4-12	-	-	-	+	-
B	-	-	-	-	-
28	++	-	-	-	-
Ampicillin	++++	++++	-	-	-
Amphotericin B	-	-	++++	++++	++++
DMSO	-	-	-	-	-

[a]Expressed by the diameter of inhibition zones: -, no inhibition; +, <10 mm; ++,10-15 mm; +++, 16–20 mm; ++++, >20 mm

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Isolation and antimicrobial effects of endophytic fungi from *Edgeworthia chrysantha*

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Abstract

Ten fungal strains isolated from *Edgeworthia chrysantha*, one of traditional medicinal plants in China, were evaluated their antimicrobial activities against three human pathogens, *Escherichia coli*, *Staphyloccocus aureus* and *Candida albicans*, and two phytopathogens, *Rhizoctonia cerealis* and *Colletotrichum gloeosporioides*. The results indicated that most ethyl acetate extracts of fermentation broth of these fungal endophytes had stronger antimicrobial activities than their fermentation broth. Among these endophytic strains, both fermentation broth and the ethyl acetate extract of strain D showed the strongest inhibitory effects on all pathogens. Strains 5-19 and BZ also exhibited potent antibacterial activities. However, other strains had weak or no antimicrobial effect. This was the first report on the isolation and antimicrobial effects of endophytic fungi from *E. chrysantha*.

Introduction

Endophyte, an important member of plant microbial community, has been shown to be a prolific producer of bioactive secondary metabolites (Zhang et al., 2006). As we know, medicinal plants have been used for centuries as remedies to treat human diseases. A great number of valuable therapeutic agents from medicinal plants had been isolated and identified from their derived endophytic microbes, such as taxol, camptothecin, vinblastine (Zhang et al., 2014). Therefore, chemical investigation of medicinal plant-derived endophyte not only contributes to the protection of plant resources, but also provides an alternative way to look for natural leading compounds and/or sustainably supply natural medicines.

Edgeworthia chrysantha Lindl. (*E. papyrifera S. et Z., Thymelaeaceae*), widely distributed in eastern Asia, is used to make paper in Korea and Japan. Its alabastrum is the succedaneum of traditional Chinese medicine "*Buddleja officinalis* Maxim (Chinese name: Mi Meng Hua)" used to treat swelling of eye, ophthalmalgia, delacrimation, nephelium of eye and nocturnal emission (Baba et al., 1989; Hashimoto et al., 1991; Baba et al., 1990). Pharmacological study showed that *E. chrysantha* has many biological effects, such as anticoagulated blood, antimicrobial, anti-inflammation and antioxidant activities. However, there is not any report on biology and chemistry of endophytic microbe associated with this plant.

In our continuous investigation of functional endophytic fungi from medicinal plants, ten endophytic strains (numbered as 58, 3-11, F, 5-19, D, BZ, 3-8, 4-12, B, 28) were isolated from *E. chrysantha* and evaluation of their antimicrobial activities was carried out in this work.

Material and Methods

The healthy plant of *E. chrysantha* was obtained from Zhaohui campus of Zhejiang University of Technology (Hangzhou, China) and used for endophyte isolation within 48 hours after harvest. Five testing pathogenic strains, including three human pathogens, *Escherichia coli*, *Staphyloccocus aureus*, *Candida albicans*, and two phytopathogens, *Rhizoctonia cerealis*, *Colletotrichum glo-*

eosporioides, were purchased from China Center for Type Culture Collection. All chemicals used in this project were of analytical grade.

Isolation of endophytic strain: Endophytic fungal strains from *E. chrysantha* were isolated according to our reported procedure (Zhang et al., 2012). All these fungal strains were transferred into potato dextrose agar slants followed by storing at 4°C.

Preparation of fermentation broth and its ethyl acetate extract: Each fungal strain was cultured on potato dextrose agar at 28°C for 7 days. Then a balanced amount of fungal colony was transferred to culture broth in a 500 mL erlenmeyer flask, which contained 200 mL sterilized potato dextrose broth, followed by shaking at 150 rpm for 15 days at 28°C. After fermentation, the mycelium and the culture broth were separated by centrifugation at 4,500 rpm for 15 min at 4°C. About 2 mL fermentation broth of each endophytic fungus was filtered with a bacterial filter (0.22 μm) and preserved at 4°C until antimicrobial assay. The residual fermentation broth was extracted twice with 400 mL ethyl acetate (Merck). Then the upper solvent was evaporated at 25°C in vacuum to yield the ethyl acetate extract, which possibly had antimicrobial secondary metabolites. Each afforded extract was kept in a vacuum drier for 3 days and dissolved in dimethyl sulfoxide (DMSO, Merck) before bioassay. The final concentration of each ethyl acetate extract had two levels, including 1, 10 mg/mL.

Antibacterial test: The antibacterial activity was carried out using disc diffusion method (Taylora et al., 1995). *E. coli* and *S. aureus* were separately transferred into two 500-mL Erlenmeyer flasks, which contained 200 mL sterilized nutrition agar, and incubated at 37°C on a rotary shaker at 150 rpm for 24 hours. Firstly, 5 mL melt water agar medium was evenly poured into petri dishes (Φ = 9 cm). Next, 200 μL seed liquid was added to fresh nutrition agar medium and mix well. Thirdly, the same amount of fresh nutrition agar medium was poured on the solidified water agar medium and the testing plate with double medium for bioassay was prepared. After 5 holes (Φ = 6 mm) were equidistantly drilled on inoculated media, a piece of standard sterile filter paper (Φ = 6 mm) was put in one hole followed by adding 100 μL fermentation broth or ethyl acetate extract of endophytic fungal strain. Ampicillin sodium (30 μg/disk, Amresco) was used as the positive control while DMSO and sterilized water were the negative controls. Then the plates were incubated at 37°C for 48 hours. All tests were performed in triplicate and the anti-bacterial activity was expressed as the average value of inhibition diameters (mm).

Antifungal test: The antifungal assay was also carried out using disc diffusion method. Three fungal blocks of *C. albicans*, *R. cerealis* and *C. gloeosporioides* were separately transferred into three 500-mL Erlenmeyer flasks, each flask had 200 mL sterilized potato dextrose broth. The seed liquid was prepared after incubation at 28°C on a rotary shaker at 150 rpm for 3 days. The testing plate with double medium for bioassay was made using the same approach described above. Amphotericin B (30 μg/disk, Sigma-Aldrich) was used as the positive control and the pure DMSO or sterilized water was the negative control. The diameter of inhibition zone (in mm) was measured to assess anti-fungal activity. All tests were carried out in triplicate.

Results

A total of 10 endophytic fungi were isolated from the healthy *E. chrysantha*, which were numbered as 58, 3-11, F, 5-19, D, BZ, 3-8, 4-12, B, 28. As shown in Tables I and II, the ethyl acetate extracts of endophytic fungi and their fermentation broth had different inhibitory effect on pathogenic microbes, including *E. coli*, *S. aureus*, *C. albicans*, *R. cerealis* and *C. gloeosporioides*. Generally, their antibacterial ability was better than their antifungal. Moreover, the inhibitory effects of their ethyl acetate extracts were stronger than those of fermentation broth. The higher concentration (10 mg/mL) of ethyl acetate extract had more potent antimicrobial activity than the low (1 mg/mL).

Discussion

During the long co-evolution of endophytes and their host plants, endophytes have adapted themselves to their special microenvironments by genetic variation, including uptake of some plant DNA into their own genomes (Germaine et al., 2004). This could have led to the ability of certain endophytic microbes to biosynthesize some 'phytochemicals' originally from their host plants (Zhang et al., 2014). A growing evidence suggests that endophytic microbes are widely distributed in plants on the earth and have abundant biodiversity. Numerous chemical investigations indicate that endophyte is one of rich sources of bioactive natural products. These functional compounds would effectively accelerate new drug discovery and contribute to the development of natural products chemistry.

In the present work, ten fungal strains (coded as 58, 3-11, F, 5-19, D, BZ, 3-8, 4-12, B, 28) were characterized from *E. chrysantha*. Bioassay results showed that both fermentation broth and its ethyl acetate extract of strain D exhibited the strongest inhibitory effects on all test pathogens. Strains 5-19, D, 3-8, B and 28 had inhibitory effects on at least three pathogens. Strains 5-19, D and BZ had the strongest activities against *E. coli* and *S. aureus* at the concentration of 10 mg/mL, which inhibition zones were respectively as much as 20, 20, 22, 25, 33, 30 mm. It also suggested that strains 5-19, D and BZ were the best biocontrol candidates. Preliminary identification showed that 5-19 and D respectively belonged

Table I

Antimicrobial activities of ethyl acetate extracts of endophytic fungi from *Edgeworthia chrysantha*

		Antimicrobial effect[a]				
Strain No.	Concentration	*E. coli*	*S. aurius*	*C. albicans*	*R. cerealis*	*C. gloeosporioides*
58	1	-	++	-	-	-
	10	-	+++	-	-	-
3-11	1	-	+	-	-	-
	10	-	++	-	+	-
F	1	++	-	+	-	-
	10	++++	-	+++	-	-
5-19	1	-	+	-	-	-
	10	+++	++++	+++	+	-
D	1	++	+++	-	++	+++
	10	+++	++++	+	+++	++++
BZ	1	+	-	-	-	-
	10	+++	++++	-	-	-
3-8	1	-	-	-	-	-
	10	+	+	-	-	++
4-12	1	-	-	-	-	-
	10	+	+	-	-	-
B	1	-	+	-	-	-
	10	+	++++	-	-	-
28	1	+	-	-	-	-
	10	++	-	+	++	-
Ampicillin	30 μg/disk	+++	++++	-	-	-
Amphotericin B	30 μg/disk	-	-	++++	++++	++++
DMSO		-	-	-	-	-

[a]Expressed by the diameter of inhibition zones: -, no inhibition; +, <10 mm; ++,10-15 mm; +++, 16–20 mm; ++++, >20 mm

Table II

Antimicrobial effects of the fermentation broth of endophytic fungi from *Edgeworthia chrysantha*

	Antimicrobial effect[a]				
Concentration	*E. coli*	*S. aurius*	*C. albicans*	*R. cerealis*	*C. gloeosporioides*
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3-11	-	-	-	-	-
F	-	-	-	-	-
5-19	-	++++	+++	-	-
D	++	++	++	-	-
BZ	-	++++	-	-	-
3-8	-	-	-	-	+
4-12	-	-	-	+	-
B	-	-	-	-	-
28	++	-	-	-	-
Ampicillin	++++	++++	-	-	-
Amphotericin B	-	-	++++	++++	++++
DMSO	-	-	-	-	-

[a]Expressed by the diameter of inhibition zones: -, no inhibition; +, <10 mm; ++,10-15 mm; +++, 16–20 mm; ++++, >20 mm

to *Fusarium*, *Aspergillus* genus and had potential application in medicine and pesticide industry. This was the first report on isolation and evaluation of antimicrobial effect of endophytic fungi from *E. chrysantha*.

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Conflict of Interest

Authors declare no conflict of interest

References

Baba K, Tabata Y, Taniguti M, Kozawa M. Coumarins from

Baba K, Taniguti M, Yoneda Y, Kozawa M. Coumarin glycosides from *Edgeworthia chrysantha*. Phytochemistry 1990; 29: 247-49.

Germaine K, Keogh E, Garcia-Cabellos G, Borremans B, Lelie D, Barac T, Oeyen L, Vangronsveld J, Moore FP, Moore ERB, Campbell CD, Ryan D, Dowling DN. Colonisation of poplar trees by gfp expressing bacterial endophytes. FEMS Microbiol Ecol. 2004; 48: 109-18.

Hashimoto T, Tori M, Asakawa Y. Piscicidal sterol acylglucosides from *Edgeworthia chrysantha*. Phytochemistry 1991; 30: 2927-31.

Taylora RS, Manandharb NP, Towers GHN. Screening of selected medicinal plants of Nepal for antimicrobial activities. J Ethnopharmacol. 1995; 46: 153-59.

Zhang HW, Bai XL, Wu BX. Evaluation of anti-microbial activities of extracts of endophytic fungi from *Artemisia annua* Linn. Bangladesh J Pharmacol. 2012; 7: 249-57.

Zhang HW, Song YC, Tan RX. Biology and chemistry of endophytes. Nat Prod Rep. 2006; 23: 753-71.

Zhang HW, Ying C, Bai XL. Advancement in endophytic microbes from medicinal plants. Int J Pharm Sci Res. 2014; 5: 1589-600.

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