RESEARCH ARTICLE

Identification of Endophytic Fungi of Medicinal Herbs of Lauraceae and Rutaceae with Antimicrobial Property

Min-Yuan Ho[(1)], Wen-Chuan Chung[(2)], Hung-Chang Huang[(1)] , Wen-Hsia Chung[(3)] and Wen-Hsin Chung[(1*)]

1. Department of Plant Pathology, National Chung Hsing University, 250 Kuo Kuang Rd. Taichung 402, Taiwan.
2. Taiwan Seed Improvement and Propagation Station, No. 46, Hsingchung St., Tanan, Hsinshe, Taichung 42642, Taiwan.
3. Min-Hwei College of Health Care Management, No.1116, Sec. 2, Zhongshan E. Rd., Liuying Township, Tainan 73658, Taiwan.
** Corresponding author. Tel: 866-4-22602783; Fax: 886-4-22877585; Email: wenchung@nchu.edu.tw*

(Manuscript received 16 April 2012; accepted 31 May 2012)

ABSTRACT: This study was conducted to determine taxonomical features and antimicrobial activities of 156 isolates of endophytic fungi collected from twigs of medicinal plants of Lauraceae (67 isolates) and Rutaceae (89 isolates) in central and northern Taiwan. The 156 isolates of fungi were classified into 35 genera in 19 families based on morphological characteristics of mycelia and asexual/sexual spores, as well as molecular phylogenetic analysis of rDNA LSU D1/D2 and ITS regions. The most common endophytes were in the taxa of *Colletotrichum*, *Guignardia*, *Hypoxyl*on, *Nigrospora*, *Phomopsis* and *Xylaria*, and the most common hosts were *Citrus* and *Zanthoxylum* of Rutaceae and *Cinnamomum* of Lauraceae. Molecular phylogenetic analysis showed that xylariaceous isolates could be separated into Xylaria and Hypoxylon groups based on rDNA of LSU D1/D2 and ITS regions. Four isolates of endophytic fungi including *Lasmenia* sp. isolate CB10, *Ophioceras tenuisporum* isolate CI02, *Xylaria cubensis* isolate LA04 and *Cyanodermella* sp. isolate TR09 were tested for antimicrobial activities using a dual culture method and *Lasmenia* sp. isolate CB10 and *Cyanodermella* sp. isolate TR09 showed better antimicrobial activity against 12 plant pathogens including 9 fungi and 3 bacteria. Spraying Chinese cabbage (*Brassica rapa*) plants with culture filtrates of the endophytic fungus *Lasmenia* sp. isolate CB10 significantly reduced severity of anthracnose of Chinese cabbage caused by *Colletotrichum higginsianum* under greenhouse conditions. This study suggests that the *Lasmenia* sp. isolate CB10 may be of potential for management of anthracnose of Chinese cabbage.

KEY WORDS: Antimicrobial activity, endophytic fungi, herb plant, Lauraceae, Rutaceae.

INTRODUCTION

Endophytic fungi are capable of living in host plants without causing any symptoms (Petrini et al., 1992). To date, endophytic fungi have been separated into four classes based on host range, type of tissue(s) colonized, colonization in planta, diversity in planta, transmission and fitness benefits (Rodriguez et al., 2009). Moreover, some endophytic fungi may produce secondary metabolites with potential for antimicrobial or anticancer property (Shu et al., 2005; Xu et al., 2008). For example, the endophytic fungus *Taxomyces andreanae* produced taxol with anticancer activity (Stierle et al., 1993). Other reports showed that certain endophytic fungi produced more than twelve metabolites similar to those produced by host plants with therapeutic function, including alkaloids, steroids, terpenoids, isocoumarin derivatives, flavinoids, quinines, phenylpropanoids, phenylpropanoids and ligans, peptides, phenol and phenolic acid, aliphatic compounds and chlorinated metabolites (Li and Liu, 2004; Shu et al., 2005; Strobel, 2003; Wang et al., 2007).

Many plants and algae have been reported as hosts of fungal endophytes (Davis et al., 2003). Among the host plants, the medicinal herbs are one of the important groups of hosts for endophytic fungi (Huang et al., 2008; Li et al., 2007; Li et al., 2004; Xu et al., 2008; Yan et al., 2007). Previous reports have demonstrated that fungal endophytes from medicinal herbs show efficacy as pharmaceutical and agricultural compounds, especially from Chinese herbs (Kusari et al., 2008; Li et al., 2004; Li et al., 2007; Shentu et al., 2007; Yan et al., 2007; Yi, 2003). Recently, certain isolates of endophytic fungi from Chinese herbs have been used as biocontrol agents for agricultural crops (Backman and Sikora, 2008; Gabler et al., 2010; Kunkel and Grewal, 2003; Maciá-Vicente et al., 2009; Mercier and Jiménez, 2009; Mejía et al., 2008; Redman et al., 1999; Schulz et al., 2002). In this study, endophytic fungi were isolated from two families of medicinal plants, Lauraceae and Rutaceae, collected from natural habitat and arboretum in northern and central Taiwan. Some species of Lauraceae were used for fruits, dyes, medicine and perfume, whereas some species of Rutaceae were used for fruits, medicine, wood and extracted oil. The objectives of this study were to identify endophytic fungi in Lauraceae and Rutaceae in Taiwan and to

臺灣大學學術期刊資料庫

Investigate their biodiversity and antimicrobial activities against fungal and bacterial pathogens of plants.

MATERIALS AND METHODES

Plant material and endophytic fungi

Healthy twigs of medicinal herbs of Lauraceae and Rutaceae were collected from Taiwan Seed Improvement and Propagation Station (TSIPS), Taichung, central Taiwan and Fushan Botanical Garden (FBG), Yilan, northern Taiwan (Table 1), 10 twigs/species. Twigs were washed with reverse osmosis (RO) water, cut into segments (3-5 mm), surface-sterilized followed a procedure described by Bills (1996), and inside tissues were took and placed on cornmeal dextrose agar (CDA, Difico, USA) medium supplemented with streptomycin (50 mg/ml) to suppress the growth of bacteria. After incubation at 25℃ for 3~5 days, fungi growing out from the segments were transferred onto potato dextrose agar (PDA, Difico, USA) in Petri dishes and each fungus was purified by single hyphal tip isolation. Pure cultures were maintained on PDA slants at 4~10℃ for further experiments.

Plant pathogens

Twelve isolates of plant pathogens (9 fungi and 3 bacteria) isolated from diseased plants were used for testing antimicrobial activities of endophytic fungi. They were *Alternaria solani* AS01 from tomato, *Botrytis cinerea* NBC12 from strawberry, *Colletotrichum gloeosporioides* TC01 from yam, *C. higginsianum* PA01 form Chinese cabbage, *Cylindocladiella lageniformis* CL01 from loquat, *Fusarium oxysporum* f. sp. *lilii* Fol-04 from lily, *Monilinia fructicola* TW01 from peach, *Penicillium digitatum* DOB02 from orange, *Pestialotia psidii* TG01 from guava, *Pythium aphanidermatum* P374 from pea, *Erwinia carotovora* subsp. *carotovora* Ec4 and *Xanthomonas campestris* pv. *campestris* Xcc17 from cabbage and *Ralstonia solancearum* PW2 from tomato.

Morphological and molecular identification of endophytic fungi

A total of 156 isolates of endophytic fungi were selected for morphological and molecular identifications. They were grown on PDA at 25°C under 12-h photoperiod for 10-21 days and examined for colony morphology by naked eye and/or characteristics of asexual spores and/or sexual spores under a compound microscope. In molecular identification, fungal genomic DNA was extracted and prepared from each endophytic fungus by the method of Goodwin and Lee (1993). Species identification of endophytic fungi was performed using the primer pairs NL1/NL4 (5'-GCATATCAATAAGCGGAGGAAAAAG-3'/ 5'-CTTGGTCATTTAGAGGAAGTAA-3') for rDNA LSU D1/D2 regions (O'Donnell, 1993) and ITS1/ITS4 (5'-CTTGGTCATTTAGAGGAAGTAA-3'/ 5'-TCCTCCGCTTATTGATATGC-3') for rDNA ITS regions (White et al., 1990). The sequences of individual isolate were blasted with sequence from the GenBank of NCBI/DDBJ/EBML. Amplifications of the D1/D2 and ITS rDNA regions were conducted using 25 µl of PCR reaction mixtures, each containing 1.5 µl total fungal genomic DNA, 0.2 µm of each primer, one unit of Taq DNA polymerase (GeneMark, Taichung, Taiwan) and dNTP mixture (containing 250 µm of each dNTP) and Taq reaction buffer (containing 2 mm $MgCl_2$). For LSU D1/D2 regions amplification, the PCR reaction program was as follows: 95°C for 5 min; 30 cycles of 95°C for 1 min, 55°C for 1 min, 72°C for 2 min; 72°C for 10 min. For amplification of ITS regions, the PCR reaction program was as follows: 95°C for 5 min; 35 cycles of 95°C for 1 min, 53°C for 1 min, 72°C for 1 min; 72°C for 10 min. The PCR products were run in 1.5% agarose gels containing 0.5 g/ml ethidium bromide in TAE (Tris-acetate, EDTA) buffers. PCR products were purified by spin columns (PCR Clean-Up Kit, GeneMark, Taiwan) and DNA sequencing was performed on an ABI PRISM 3100 automated sequencers (Applied Biosystems, Ramsey Inc., USA).

Phylogenetic analysis of endophytic Xylariaceae

Since endophytic fungi belonging to Xylariaceae were most frequently isolated from samples of medicinal plants collected in this study, isolates of Xylariaceae were further compared by the phylogenetic analysis out of both rDNA LSU D1/D2 and ITS regions. The sequences of all xylariaceous isolates were aligned using CLUSTAL X v.1.8 (Thompson et al., 1997), and further visual alignments were done in Sequence Alignment Editor (Se-Al) v.2.0 (Rambaut, 2000). The aligned sequences were analyzed together with outgroup sequences of *Neocosmospora vasinfecta* (AY381155) in D1/D2 analysis and *Pestalotiopsis* sp. (AB440094) in ITS analysis. Moreover, 7 species of *Xylaria*, 2 species of *Hypoxylon* and 6 species of Xylariaceae from GenBank were added in D1/D2 analysis, and 7 species of *Xylaria*, 2 species of *Hypoxylon* and one species of *Nemania* sp. (EF682111) were added in ITS analysis. These species from GenBank were analyzed with the isolates in this study (Table 2). The aligned sequences were analyzed with the kimura 2-parameter distance matrix by pairwise distances with PAUP*4.0 beta 10 (Swofford, 2002). Bootstrap (Felsenstein, 1985) values were generated with 1,000 replicate heuristic searches to estimate support for clade stability of the consensus tree

臺灣大學學術期刊資料庫

using the same program.

Antimicrobial assay of endophytic fungi

Isolates of endophytic fungi from Lauraceae and Rutaceae were tested for antagonistic effects against plant pathogens using dual-culture method. Primary test showed that four isolates, of endophytic fungi, including *Lasmenia* sp. (isolate CB10), *Ophioceras tenuisporum* (isolate CI02), *Xylaria cubensis* (isolate LA04) and *Cyanodermella* sp. (isolate TR09), can inhibit the mycelial growth of *C. gloeosporioides* TC01. These isolates were further tested for the inhibition on nine isolates of plant fungal pathogens and three strains of plant bacterial pathogens by a dual culture method. For the dual culture test, an agar block (0.5 cm in diameter) containing mycelial mates was removed from each endophyte grown on PDA for 7~14 days and inoculated at the center of PDA in a Petri dish (9 cm in diameter). After incubation at 25°C for 7~14 days, each endophyte was tested against plant pathogens by spraying 500 µl of spore suspensions (10^5 spores/ml) of each fungal pathogen or 500 µl of bacterial suspensions (10^7 cfu/ml) of each bacterial pathogen in each Petri dish. After incubation at 25°C for 2 days, the dual culture in each dish was examined for the formation of inhibition zone. The inhibition index is 0 to 3, where 0 = no inhibition zone; 1 = inhibition zone > 0 < 5 mm; 2 = inhibition zone > 5 < 10 mm; and 3 = inhibition zone > 10 mm. The experiment was repeated twice and for each experiment, there were four replicates in each treatment.

Table 1. List of plants of Lauraceae and Rutaceae used for isolation of endophytic fungi

Location*	Family	Genus and species
Fushan	Lauraceae	*Cinnamomum insularimontanum*, *Cin. kanehirae*, *Cin. osmophloeum*, *Lindera aggregata*, *Lin. communis*, *Litsea cubeba*, *Machilus thunbergii*
	Rutaceae	*Phellodendron amurense*, *Tetradium ruticarpum*, *Zanthoxylum wutaiense*
Shinshe	Lauraceae	*Cinnamomum cassia*, *Cin. kanehirae* *Cinnamomum* sp.
	Rutaceae	*Citrus aurantium*, *Cit. kotokan*, *Cit. medica*, *Cit. medica* var. *sarcodactylis*, *Cit. modurensis*, *Cit. sinensis*, *Citrus* sp., *Fortunella japonica*, *Murraya euchrestifolia*, *Toddalia asiatica*, *Zanthoxylum nitidum*

***Plants were collected from the Botanical Garden in Shinshe (central Taiwan) and a natural forestry area in Fushan (northern Taiwan)**

Control of anthracnose of Chinese cabbage by culture filtrates of *Lasmenia* sp. isolate CB10 (Greenhouse Experiment)

Since *Lasmenia* sp. isolate CB10 showed a strong antagonistic effect against *Colletotrichum higginsianum* isolate PA01 in vitro test, this isolate was selected for further testing on control of anthracnose of Chinese cabbage (*Brassica rapa* L.) under greenhouse conditions. Crude filtrates of *Lasmenia* sp. isolate CB10 were collected from 2-week-old, PDB (potato dextrose broth) cultures and diluted to 10- and 100-fold with sterile distilled water. There were two methods of inoculations: 1), 24-day-old plants of Chinese cabbage (cv. San Feng) were sprayed with filtrates of *Lamenia* sp. and then inoculated with conidia suspensions ($2x10^5$ conidia/ml) of *C. higginsianum* PA01 from 7-10 days old PDA cultures four days later and 2), 28-day-old plants of Chinese cabbage were inoculated with conidia suspensions of *C. higginsianum* for 30 minutes and then sprayed with filtrates of *Lasmenia* sp. Inoculated plants were kept in the greenhouse at 24~26°C. Chinese cabbage plants sprayed with sterile distilled water or the pathogen alone were used as controls. After incubation for 7 days, plants were examined for symptoms of foliar necrosis and scored for severity of anthracnose based on rating of 0-4, where 0 = no visible symptom; 1 = 1%~10% of necrotic area on leaf; 2 = 11%~25% of necrotic area on leaf; 3 = 26%~50% of necrotic area on leaf; 4 = >50% of necrotic area on leaf (Lin, 2001). The disease severity index was calculated by the formula (N: total number of leaves; n_0~n_4: number of leaves under each disease index).

For each experiment, there were four replicates for each treatment and three plants per replicate. The experiment was repeated twice.

RESULTS

Diversity of fungal endophytes

A total of 156 isolates of endophytic fungi were isolated from 22 species of medicinal plants in Taiwan. Based on morphological characteristics and rDNA LSU D1/D2 and ITS characteristics, 125 isolates were identified as representatives of 30 genera in 19 families, 7 isolates were identified only to family level, 14 isolates were identified as representatives of 5 genera without

臺灣大學學術期刊資料庫

Table 2. Number and identification of endophytic fungi isolated from medicinal herbs of Rutaceae and Lauraceae in Taiwan

Fungal family	Fungal genus	Plant species (fungal isolate)		Total
		Lauraceae	Rutaceae	
Amphisphaeriaceae	*Discostroma*	—[a]	*Citrus medica* (CM08)	1
	Pestalotiopsis	*Cinnamomum kanehirai* (CK01), *Cin. osmophloeum* (CO01, CO08), *Litsea cubeba* (LC03)	*Phellodendron amurense* (PA01), *Zanthoxylum wutaiense* (ZW01)	6
Apiosporaceae	*Arthrinium*	*Lit. cubeba* (LC13, LC14)	*Fortunella japonica* (FJ05)	3
Botryosphaeriaceae	*Botryosphaeria*	—	*Cit. aurantium* (CA06-07)	2
	Camarosporium	—	*Cit. medica* var. *sarcodactylis* (CB07)	1
	Guignardia	*Cin. cassia* (CC01), *Cin. kanehirai* (CK09)[c], *Cinnamomum* sp. (LCC01)	*Cit. aurantium* (CA02), *Cit. medica* var. *sarcodactylis* (CB02), *Citrus* sp. (CE02-03), *Tetradium ruticarpum* (TR07), *Z. wutaiense* (ZW13)	9
Chaetomiaceae	*Chaetomium*	—	*Cit. sinensis* (CS04)	1
Diaporthaceae	*Diaporthe*	*Cin. cassia* (CC03), *Lit. cubeba* (LC08, LC11)	*Cit. aurantium* (CA05), *Toddalia asiatica* (TA02)	5
	Eutypella	—	*Cit. medica* var. *sarcodactylis* (CB03)	1
Lasiosphaeriaceae	*Podospora*	*Machilus thunbergii* (MT08-09, MT11)	—	3
Leptosphaeriaceae	*Leptosphaeria*	*Cin. kanehirai* (CK13)[c]	—	1
Halosphaeriaceae	*Lanspora*[b]	*Cinnamomum* sp. (WLC02)	*Murraya euchrestifolia* (ME05), *To. asiatica* (TA04), *Z. nitidum* (ZN03)	4
Hypocreaceae	*Fusarium*	*Cin. kanehirai* (CK10)[c]	*Cit. sinensis* (CS05-06)	3
	Myrothecium	*Cin. insularimontanum* (FCI02)	—	1
Magnaporthaceae	*Magnaporthe*[b]	*Cin. insularimontanum* (FCI01)	—	1
	Mycoleptodiscus	—	*Cit. aurantium* (CA08-09)	2
	Ophioceras[b]	*Cin. insularimontanum* (CI02)	—	1
Melanconidaceae	*Pilidiella*[b]	*Lindera aggregata* (LA06)	—	1
Mycosphaerellaceae	*Cercospora*	*Ma. thunbergii* (MT04)	—	1
	Pseudocercospora	—	*F. japonica* (FJ06)	1
Phyllachoraceae	*Colletotrichum*	*Cin. osmophloeum* (CO04, CO10), *Ma. thunbergii* (MT03)	*Cit. sinensis* (CS01-02), *Cit. aurantium* (CA01, CA10), *Cit. medica* var. *sarcodactylis* (CB06), *P. amurense* (PA04), *Z. wutaiense* (ZW03, ZW05, ZW09)	14
Pleosporaceae	*Alternaria*	—	*Cit. kotokan* (CCK01), *Mu. euchrestifolia* (ME01), *Toddalia asiatica* (TA01), *Z. nitidum* (ZN04)	4
	Stemphylium	—	*Cit. aurantium* (CA03), *F. japonica* (FJ03, FJ08)	3
Psathyrellaceae	*Coprinopsis*	—	*Cit. medica* (CM05)	1
Stictidaceae	*Cyanodermella*[b]	—	*T. ruticarpum* (TR09, TR10, TR12, TR13)	4
Trichosphaeriaceae	*Nigrospora*	*Cin. kanehirai* (CK11)[c]	*Cit. medica* var. *sarcodactylis* (CB01), *Citrus* sp. (NS01-02), *P. amurense* (PA02), *To. asiatica* (TA03), *Te. ruticarpum* (TR03, TR06), *Z. wutaiense* (ZW02, ZW04)	10
Valsaceae	*Phomopsis*	*Cin. insularimontanum* (CI03), *Cin. kanehirae* (CK12)[c], *Lin. communis* (LC02)	*Cit. aurantium* (CA04-05), *Cit. medica* (CM01-04), *Cit. sinensis* (CS02), *To. asiatica* (TA05), *Z. nitidum* (ZN01), *Z. wutaiense* (ZW08, ZW11, ZW25)	15
Xylariaceae	*Xylaria*	*Cin. kanehirae* (CK02), *Cin. osmophloeum* (CO03, CO06-07), *Lin. aggregata* (LA04-05), *Lit. cubeba* (LC07-08), *Ma. thunbergii* (MT06)	*F. japonica* (FJ01), *Z. wutaiense* (ZW12, ZW17)	12

臺灣大學學術期刊資料庫

Table 2. Continued

Fungal family	Fungal genus	Plant species (fungal isolate)		Total
		Lauraceae	Rutaceae	
Xylariaceae	*Hypoxylon*	*Cin. osmophloeum* (CO02, CO05, CO09), *Lin. aggregata* (LA01-03), *Lit. cubeba* (LC05-06), *Ma. thunbergii* (MT01, MT05)	*P. amurense* (PA03), *Te. ruticarpum* (TR01), *Z. wutaiense* (ZW06)	13
	Annulophypoxylon	—	*Z. wutaiense* (ZW18)	1
	Unknown	*Cin. osmophloeum* (CO11-12), *Lit. cubeba* (LC09-10, LC12), *Ma. thunbergii* (MT08-09)	—	7
Taxnomical uncertain	*Phomatospora*	*Lin. aggregate* (FLA10-11)	*Mu. euchrestifolia* (ME06), *Z. nitidum* (ZN06-07), *To. asiatica* (TA07), *Z. wutaiense* (ZW23)	7
	Pyrenochaeta	*Lit. cubeba* (LC02)	—	1
	Lasmenia[b]	—	*Cit. medica* var. *sarcodactylis* (CB10-11)	2
	Strelitziana[b]	*Cin. kanehirai* (CK08),	*Z. nitidum* (ZN07), *Z. wutaiense* (ZW24)	3
	Ochroconis[b]	—	*Z. wutaiense* (ZW16)	1
Unknown		*Cin. kanehirai* (CK03-05), *Cinnamomum* sp. (WLC01), *Cin. insularimontanum* (CI05), *Cin. cassia* (CC04), *Ma. thunbergi*i (MT10)	*Citrus* sp. (NS03), *Cit. medica* var. *sarcodactylis* (CB08), *Z. mitidum* (ZN02)	10
Total		**67**	**89**	**156**

[a] **—: no fungal isolate**
[b] **first recorded endophyte**
[c] **isolated from Taiwan Seed Improvement and Propagation Station**

family, and 10 isolates were unknown (Table 2). Among 156 isolates of endophytic fungi, 67 isolates, belonging to 21 genera in 15families, were from Lauraceae and 89 isolates, belonging to 27 genera in 16 families, were from Rutaceae. The most abundant genera were *Colletotrichum*, *Guignardia*, *Hypoxylon*, *Nigrospora*, *Phomopsis* and *Xylaria*, especially *Colletotrichum* spp. with 14 isolates and *Phomopsis* spp. with 15 isolates. In addition, species of *Chaetomium*, *Alternaria*, *Stemphylium*, *Coprinopsis* and *Cyanodermella* were dominant and host specific to Rutaceae, whereas species of *Podospora*, *Leptosphaeria* and *Pilidiella* were only observed in Lauraceae (Table 2). These presented fungal endophytes were preserved in Plant Fungal Parasite and Molecular Diagnosis of Fungicide Resistance Lab, Department of Plant Pathology, National Chung Hsing University.

Phylogenetic analysis of endophytic Xylariaceae

Although *Xylaria* and *Hypoxylon* are the two popular genera in medicinal plants in Taiwan, certain other endophytes of Xylariaceae are still obscure based on the morphology and sequence of nucleotide. According to the results of phylogenic analyses based on rDNA LSU D1/D2 and ITS regions, the 33 isolates of endophytic fungi belonging to Xylariaceae could be divided into two major molecular groups, 19 isolates for *Xylaria* and 13 isolates for *Hypoxylon* (Figs. 1, 2). In the group of *Hypoxylon*, all isolates except isolate LA01, the diversity was 0~0.1% for rDNA LSU D1/D2 sequences and 0~3.1% for rDNA ITS sequences. In the group of *Xylaria*, the diversity was 0~5.5% for rDNA LSU D1/D2 sequences and 0~30.6% for rDNA ITS sequences. For D1/D2 analysis, the bootstrap was 99% for the group of *Hypoxylon* and 97% for the group of *Xylaria*. For ITS analysis, the bootstrap was 90% for the group of *Hypoxylon* and 89% for the group of *Xylaria*. Based on ITS region analysis, the results indicated that the unidentified xylariaceous isolates could be classified as Xylaria. However, certain unidentified xylariaceous isolates still could not be classified by D1/D2. Thus, ITS region could contribute more diversity to classify fungi in Xylariaceae.

Antimicrobial activity assay

The four isolates of endophytic fungi, *Lasmenia* sp. isolate CB10, *Ophioceras tenuisporum* isolate CI02, *Xylaria cubensis* isolate LA04 and *Cyanodermella* sp. isolate FTR09, showed different level of antagonistic effects against 9 fungal isolates and 3 bacterial strains of plant pathogens (Table 3). *Cyanodermella* sp. isolate TR09 from *Tetradium ruticarpum* was highly antagonistic to the fungal pathogens *Cynlidocladiella lageniformis* CL01, *Fusarium oxysporum* f. sp. *lilii* Fol-04 and *Monilinia fructicola* TW01 and the bacterial pathogen *Ralstonia solanacearum* PW2, with an

臺灣大學學術期刊資料庫

Table 3. Antimicrobial activities of endophytic fungi against plant pathogens

Plant pathogen	Inhibition index[1]			
	CB10[2]	CI02	LA04	TR09
Fungus				
Alternaria solani AS01	2	2	1	1
Botrytis cinerea NBC12	1	2	0	1
Colletotrichum gloeosporioides TC01	3	1	1	2
Colletotrihcum higginsianum PA01	3	2	0	1
Cylindrocladiella lageniformis CL01	1	1	0	3
Fusarium oxysporum f. sp. *lilii* Fol-04	2	2	1	3
Monilinia fructicola TW01	3	1	0	3
Penicillium digitatum DOB02	0	2	1	2
Pestalotiopsis psidii TG01	2	1	1	1
Pythium aphanidermatum P374	2	2	0	3
Bacterium				
Erwinia carotovora Ec4	0	0	0	2
Xanthomonas campestris Xcc17	2	2	0	1
Ralstonia solanacearum PW2	0	2	0	3

[1] Inhibition zone index: 0 = No inhibition; 1 = inhibition zone 0.-5 mm; 2 = inhibition zone 5-10 mm; 3 = inhibition zone> 10mm.
[2] CB10: *Lasmenia* sp. isolated from *Citrus medica* var. s*arcodactylis*; CI02 isolate: *Ophioceras* sp. isolated from *Cinnamomum insularimontanum*; LA04 isolate: *Xylaria* sp. isolated from *Lindera aggregate*; TR09 isolate: *Cyanodermella* sp. isolated from *Tetradium ruticarpum*.

inhibition index of 3 (inhibition zone > 10mm). *Lasmenia* sp. isolate CB10 from *Citrus medica* var. s*arcodactylis* was highly antagonistic to the fungal pathogen *Monilinia fructicola* TW01, with an inhibition index of 3; whereas *Xylaria cubensis* isolate LA04 from *Lindera aggregate* showed only weak or none antagonistic effects to the nine isolates of fungal pathogens, with the inhibition index of 1 (inhibition zone < 5 mm) or 0 (no inhibition) and none antagonistic effects to the three isolates of bacterial pathogens (Table 3).

Control of anthracnose of Chinese cabbage by culture filtrates of *Lasmenia* sp. isolate CB10 (Greenhouse experiment)

Spraying Chinese cabbage plants with culture filtrates of *Lasmenia* sp. isolate CB10, diluted by 10- or 100-fold, significantly reduced disease severity of anthracnose of Chinese cabbage compared to the pathogen-inoculated control (disease severity of 50%) (Fig. 3). The disease severity was 14.5 and 12.5% for the treatment of culture filtrates diluted to 10 folds and 100 folds at 4 days prior to inoculation of the pathogen, However, the disease severity was 37.5 and 31.3% for the treatment of culture filtrates diluted to 10 folds and 100 folds at 30 min after inoculation of the pathogen. Meanwhile, no symptoms of phytotoxicity on Chinese cabbage plants were observed by the treatment of 10- and 100-fold dilution of the culture filtrates of *Lasmenia* sp. isolate CB10.

DISCUSSION

Based on morphological studies and molecule analyses, one hundred and forty-six of the 156 isolates of endophytic fungi obtained from medicinal plants of Lauraceae and Rutaceae in Taiwan were assigned to 35 genera in 19 families, with 21 genera from Lauraceae and 27 genera from Rutaceae. The fungal flora of this study showed that fungal isolates identified as Sordariomycetes and Dothideomycetes are most abundant fungal endophytes in tropical or subtropical plants as reported in previous studies (Arnold et al., 2007; Higgins et al., 2007; Rodriguez et al., 2009). In this study, fungal genera of *Coprinopsis* and *Cyanodermella* were rarely reported as endophytes in

臺灣大學學術期刊資料庫

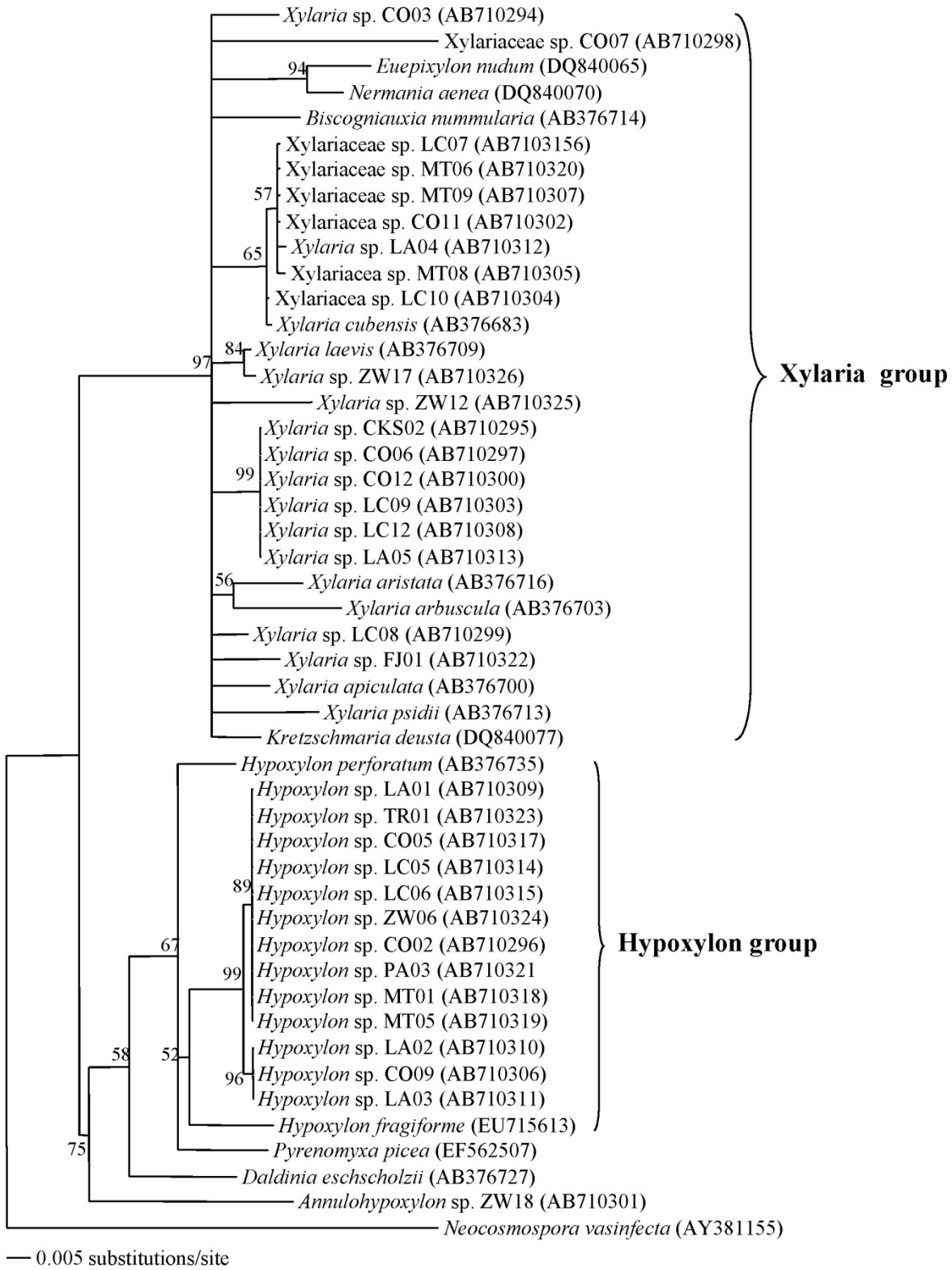


Fig 1. A Neighbor-joining tree based on partial LSU rDNA sequences of D1/D2 unknown isolates of the Xylariales (33 isolates) and 14 reference sequences from Genbank. *Neocosmospora vasinfecta* (AY381155) was used as an outgroup to root the tree. Numbers at branch nodes are bootstrap values, indicating branch support, based on 1000 replication.

臺灣大學學術期刊資料庫

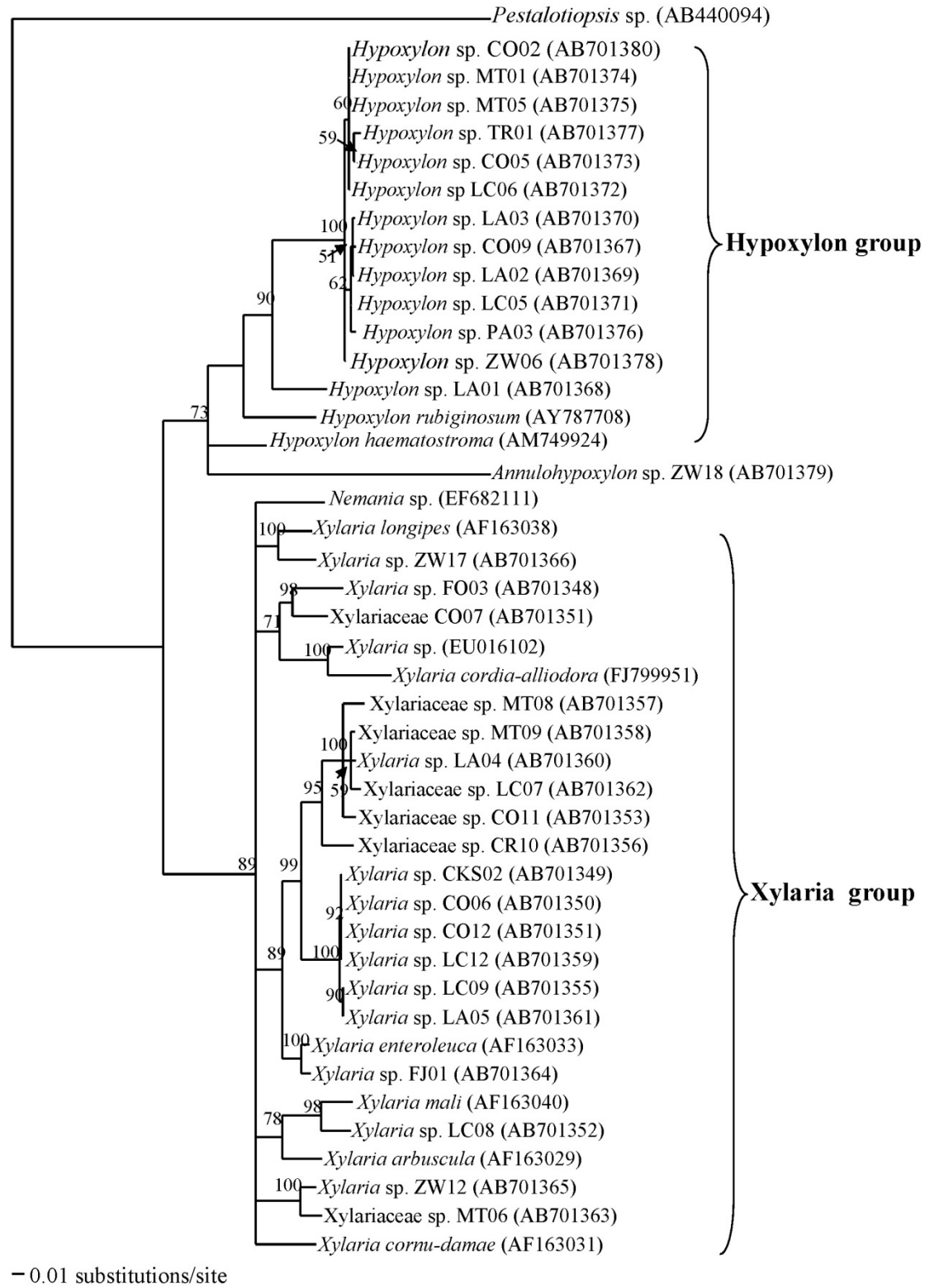


Fig 2. A Neighbor-joining tree based on partial ITS rDNA sequences unknown isolates of the Xylariales (33 isolates) and 9 reference sequences from Genbank. *Pestalotiopsis* sp. (AB440094) was used as an outgroup to root the tree. Numbers at branch nodes are bootstrap values, indicating branch support, based on 1000 replication.

臺灣大學學術期刊資料庫

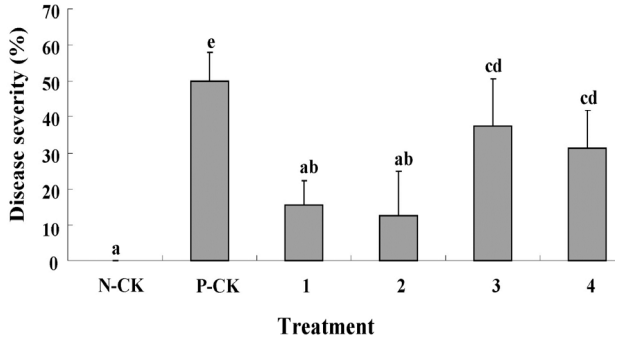


Fig. 3 Effect of 10-fold and 100-fold dilution of culture filtrates of *Lasimenia* sp. isolate CB10 on control of anthracnose of Chinese cabbage under greenhouse conditions. The disease severity was recorded seven days after inoculation. 1: Plants treated with 10-fold dilution of culture filtrates at 4 days prior to inoculation of the pathogen; 2: Plants treated with 100-fold dilution of culture filtrates at 4 days prior to inoculation of the pathogen; 3: Plants treated with 10-fold dilution of culture filtrates at 30 min after inoculation of the pathogen; 4: Plants treated with 100-fold dilution of culture filtrates at 30 min after inoculation of the pathogen . N-CK: negative control; plants treated with sterile distilled water alone; P-CK: positive control; plants treated with *C. higginsianum* alone. Bars with different letters are significantly different ($P < 0.05$) according to Duncan's multiple range test.

Lauraceae and Rutaceae but they were found to occur on these hosts. The new record of *Coprinopsis* isolated from *Citrus medica* belongs to Agaricomycetes of basidiomyces and the *Cyanodermella* isolated from *Zanthoxylum wutaiense* belongs to Lecanoromycetes of ascomyces. In addition, *Citrus* and *Zanthoxylum* are popular hosts of endophytic fungi in this study. The species of *Citrus* and *Zanthoxylum* are popular medicinal herbs, especially the endemic species of *Z. wutaiense* often used by aborigine as medicine in Taiwan. Previous studies indicated that the genera of *Alternaria*, *Cladosporium*, *Colletotrichum*, *Guignardia*, *Nigrospora*, *Nodulisporium*, *Sporormiella* and *Xyalariaceae* could be obtained from *Citrus* spp. (Araujo et al., 2001; Durán et al., 2005) and *Aspergillus*, *Cochliobolus*, *Colletotrichum*, *Penicillium*, *Rhizopus*, *Schizophyllum* and *Trichoderma* could be obtained from *Zanthoxylum* spp. (Maysarah, 2010). In our study, more than 15 genera of endophytic fungi were isolated from *Citrus* spp. and more than 14 genera of endophytic fungi were isolated from *Zanthoxylum* spp. Thus, the fungal endophytes in Citrus and Zanthoxylum are more divertive than other medicinal herbs. Moreover, the results showed that *Citrus* and *Zanthoxylum* are important hosts for fungal endophytes.

The genera *Phomopsis* and *Xylaria* were reported as common endophytes (Bayman et al., 1998; Davis et al., 2003; Huang et al., 2008; Joshee et al., 2009; Nalini et al., 2005; Wang et al., 2008). In this study, *Phomopsis* and *Xylaria* were also common genera in Lauraceae and Rutaceae in Taiwan; however, the genera of *Colletotrichum* and *Hypoxylon* were more common endophytes than *Xylaria* (Table 2). Results of comparing the dominant endophytes between Lauraceae and Rutaceae showed that *Colletotrichum*, *Guignardia*, *Nigrospora* and *Phomopsis* are commonly obtained from Rutaceae and *Hypoxylon* and *Xylaria* are from Lauraceae. Although the xylariaceous fungi are not associated with host species significantly (Okane et al., 2008), the Lauraceae may be one of the plant families showing highly related to Xylariaceae. Moreover, the 6 species of Lauraceae as hosts of *Xylaria* and *Hypoxylon* are growing in the plant collection at Fushan Botanical Garden, Ilan which was located in low elevation and belonged to virgin forests. Previous studies indicated that *Xylaria* and *Hypoxylon* are common in tropical hosts (Roger, 1979) and are dominant fungal endophytes in the forest (Bayman et al., 1998). Thus, the *Xylaria* and *Hypoxylon* are more common in the natural condition than in artificial condition. Presently, *Xylaria* and *Hypoxylon* are considered as common saprophytic genera in wood, and the related information of *Xylaria* and *Hypoxylon* is not clear. Several reports indicated that *Xylaria* and *Hypoxylon* could produce antimicrobial (Liu et al., 2008; Tomsheck et al., 2010) and antitumor (Healy et al., 2004) compounds or phytotoxins (Abate et al., 1997) or cytotoxins (Dagne et al., 1994). Certain endophytic *Neotyphodium* in grasses have been demonstrated that it can produce alkaloid and cause strongly toxic effects on vertebrates (Faeth, 2002). In this study, one isolate of *Xylaria* sp. isolate LA04 showed minor effects on mycelial growth of pathogenic fungi, and the result revealed that metabolites produced by *Xylaria* sp. isolate LA04 have activity to against microbes. It is necessary to analyze the effects of compound produced by *Xylaria* sp. isolate LA04 to vertebrates in the future.

Eight fungal genera are identified as endophytes for the first time in this study, including *Lanspora*, *Magnaporthe*, *Ophioceras*, *Pilidiella* and *Lasmenia* in Sodariomycetes; *Cyanodermella* in Lecanoromycetes, *Strelitziana* in Eurotiomycetes and *Ochroconis* in Ascomycetes. Previous reports indicated that certain genera of fungal endophytes could possibly cause diseases in crops (Chen et al., 2011; Wang et al., 2008) or animal (Rippon 1988). For example, certain species of *Magnaporthe* (Thongkantha et al., 2009) and *Pilidiella* (Van Niekerk et al., 2004) have been reported as pathogens plants and a number of *Ochroconis* species are identified as pathogens of poultry (Rippon, 1988). Therefore, further investigation is warranted to confirm the pathogenicity of the endophytic fungi isolated from medicinal herbs of Lauraceae and Rutaceae in Taiwan.

Although the genera *Xylaria* and *Hypoxylon* are

臺灣大學學術期刊資料庫

common and dominant endophytes in tropical forest (Chen et al., 2011; Davis, 2003; Okane et al., 2008; Joshee et al., 2009), most *Xylaria* species still could not be identified by rDNA of ITS and D1/D2 LSU regions (Okane et al., 2008). In this study, the unknown species identified as Xylariaceae did not have high sequence homology with known species of *Xylaria* in GenBank. The molecular phylogenic analysis demonstrated that the unknown species of Xylariaceae are closely related with the species of *Xylaria*. Moreover, the isolates of *Hypoxylon* showed simple topology based on D1/D2 LSU rDNA regions analysis and showed diverse topology based on ITS rDNA region analysis. Although the D1/D2 sequences of *Hypoxylon* isolates have 97% identity with *H. fragiforme* (EU715613) and *H. perforatum* (AB376735) in GenBank, the topology revealed that the isolates of *Hypoxylon* from FBG in Taiwan are distinct from *H. fragiforme* (EU715613) and *H. perforatum* (AB376735). The molecular analysis demonstrated that the isolates of *Hypoxylon* from FBG are different from other *Hypoxylon* species in GenBank.

Previous reports indicated that some endophytic fungi can produce metabolites with antimicrobial property (Shu et al., 2005; Xu et al., 2008) and withpotential for use as biocontrol agents for control of crop diseases (Chaves et al., 2009; Ezra et al., 2004; Gabler et al., 2010; Mejía et al., 2008; Mercier and Jiménez, 2009; Stewart et al., 1993; Wali et al., 2006) and insects (Omacini et al., 2001; Patterson et al., 1991; Siegel et al., 1990). In this study, three isolates, *Lasmenia* sp. isolate CB10, *O. tenuisporum* isolate CI02 and *Cyanodermella* sp. isolate TR09, showed high antagonistic effects on the growth of plant pathogens. Results of the experiment in greenhouse showed that culture filtrates of *Lasmenia* sp. isolate CB10 significantly reduced the disease severities of Chinese cabbage anthracnose. Therefore, fungal endophytes from medicinal herbs in Taiwan have potential to inhibit the pathogens growth and control crop diseases. Moreover, certain endophytic fungi could produce volatile compounds (Gabler et al., 2010; Mercier and Jiménez, 2009), induce resistance (Chaves et al., 2009; Kunkel and Grewal, 2003) and against stress (Redman et al., 2002; Rodriguez et al., 2008; Schardl and Phillips, 1997). In this study, *Lasmenia* sp. isolate CB10 has been confirmed to produce metabolites showing activity to against plant pathogens. Moreover, the metabolites seem to induce resistance of Chinese cabbage against anthracnose. The mechanism of induced resistance of host plant is related with many signals triggered by aggressors, like chemicals, metabolites extracted from plants and microbes and microorganisms (Pieterse et al., 2009). The nature of metabolites of CB10 involved in induced disease resistance warrants further studies. In addition, *Cit. medica var. sarcodactylis* is a host plant of CB10 and the species of medicinal herb contained the compounds that were active against bacteria and fungi (Dahiya and Kumar, 2008; Matsumoto et al., 2002). Thus, it is also important to analyze chemical nature of metabolites of CB10 that showed activities against plant pathogens.

ACKNOWLEDGEMENT

We sincerely thank Dr. C. J. Chang, Department of Plant Pathologist, University of Georgia, USA, for critical review of this manuscript. Funding to support this research was provided by NSC 99-2313-B-005-015-MY3 National Science and Technology Program for Agricultural Biotechnology, National Science Council, Taiwan, ROC, and in part by the Ministry of Education, Taiwan, ROC, under the ATU plan. Dr. H. C. Huang (Emeritus Principal Research Scientist of Agriculture and Agri-Food Canada, Lethbridge Research Centre, Alberta, Canada) is a visiting Chair Professor at the Department of Plant Pathology, National Chung-Hsing University, Taichung, Taiwan, 2008-2011.

LITERATURE CITED

Abate, D., W. Abraham and H. Meyer. 1997. Cytochalasins and phytotoxins from the fungus *Xylaria obovata*. Phytochemistry **44**: 1443-1448.

Araujo, W. L., Jr. W. Maccheroni, C. I. Aguilar-Vildoso, P. A. V. Barroso, N. O. Saridakis and J. L. Azevedo. 2001. Variability and interactions between endophytic bacteria and fungi isolated from leaf tissues of citrus rootsotck. Can. J. Microbiol. **47**: 229-236.

Arnold, A. E., D. A. Henk, R. A. Eells, F. Lutzoni and R. Vilgalys. 2007. Diversity and phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and environmental PCR. Mycologia **99**: 185-206.

Backman, P. A. and R. A. Sikora. 2008. Endophytes: An emerging tool for biological control. Biol. Control **46**: 1-3.

Bayman, P., P. Angulo-Sandoval, Z. Báez-Ortiz and D. J. Lodge.1998. Distribution and dispersal of *Xylaria* endophytes in two tree species in Puerto Rico. Mycol. Res. **102**: 944-948.

Bills, G. F. 1996. Isolation and analysis of endophytic fungal communities from woody plants. pp. 31-65. In: Redllin, S. C. and M. L. Carris (eds.), Endophytic Fungi in Grasses and Woody Plants: Systematics, Ecology, and Evolution. APS Press, St. Paul, Minnesota, USA.

Chaves, N. P., L. E. Pocasangre, F. Elango, F. E. Rosales and R. Sikora. 2009 Combining endophytic fungi and bacteria for the biocontrol of *Radopholus similes* (Cobb) Thorne and for effects on plant growth. Sci. Hortic. **122**: 472-478.

Chen, X.-Y., Y.-D. Qi, J.-H. Wei, Z. Zhang, D.-L. Wang,

臺灣大學學術期刊資料庫

J.-D. Feng and B.-C. Gan. 2011. Molecular identification of endophytic fungi from medicinal plant *Huperzia serrata* based on rDNA ITS analysis. World J. Microbiol. Biotechnol. **27**: 495-503.

Dagne, E., A. A. L. Gunatilaka, S. Asmellash, D. Abate, D. G. I. Kingston, G. A. Hofmann and R. K. Johnson. 1994. Two new cytotoxic cytochalasins from *Xylaria obovata*. Tetrahedron **50**: 5615-5620.

Dahiya, R. and A. Kumar. 2008. Synthetic and biological studies on a cyclopolypeptide of plant origin. J. Zhejiang Univ. Sci. **B 9**: 391-400.

Davis, E., J. B. Frankilin, A. J. Shaw and R. Vilgalys. 2003. Endophytic *Xylaria* (Xylariaceae) among liverworts and angiosperms: phylogenetics, distribution, and symbiosis. Am. J. Bot. **90**: 1661-1667.

Durán, E. L., L. Daniel-Polper, J. C. Ramallo, R. A. Piccolo-Grandi, A. C. Hupper-Giancoli and J. L. Azevedo. 2005. The foliar fungal endophytes of *Citrus limon* in Argentina. Can. J. Bot. **83**: 350-355.

Ezra, D., W. M. Hess and G. A. Strobel. 2004. New endophytic isolates of *Muscodor albus*, a volatile-antibiotic-producing fungus. Microbiology **150**: 4023-4031.

Faeth, S. H. 2002. Are endophytic fungi defensive plant mutualists? OIKOS **98**: 25-36.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**: 783-791.

Gabler, F. M., J. Mercier, J. I. Jimenez and J. L. Smilanick. 2010. Integration of continuous biofumigation with *Muscodor albus* with pre-cooling fumigation with ozone or sulfur dioxide to control postharvest gray mold of table grapes. Postharvest Biol. Tec. **55**: 78-84.

Goodwin, D. C. and S. B. Lee. 1993. Microwave miniprep of total genomic DNA from fungi, plants, protists and animals for PCR. Biotechniques **15**: 438-444.

Higgins, K. L., A. E. Arnold, J. Miadlikowska, S. D. Sarvate and F. Lutzoni. 2007. Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from three major plant lineages. Mol. Phylogenet. Evol. **42**: 543-555.

Huang, W.-Y., Y.-Z. Cai, K. D. Hyde, H. Corke and M. Sun. 2008. Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. Fungal Divers. **33**: 61-75.

Joshee, S., B. C. Paulus, D. Park and P. R. Johnston. 2009. Diversity and distribution of fungal foliar endophytes in New Zealand Podocarpaceae. Mycol. Res. **113**: 1003-1015.

Healy, P. C., A. Hocking, N. Tran-Dinh, J. I. Pitt, R. G. Shivas, J. K. Mitchell, M. Kotiw and R. A. Davis. 2004. Xanthones from a microfungus of the genus *Xylaria*. Phytochemistry **65**: 2373-2378.

Kunkel, B. A. and P. S. Grewal. 2003. Endophyte infection in perennial ryegrass reduces the susceptibility of black cutworm to an entomopathogenic nematode. Entomol. Exp. Appl. **107**: 95-104.

Kusari, S., M. Lamshöft, S. Zühlke and M. Spiteller. 2008. An endophytic fungus from *Hypericum perforatum* that produces hypericin. J. Nat. Prod. **71**: 159-162.

Li, H.-Y. and L. Liu. 2004. Recent advances on bioactive compounds producing endophytes. Nat. Prod. Res. Dev. **16**: 482-485.

Li, H.-Y., L. Lu, D.-Q. Wei and Z.-W. Zhao. 2007. Isolation and screening for endophytic fungi with antitumor activities from 12 Yunnan medicinal plants. Nat. Prod. Res. Dev. **19**: 765-771. [in Chinese with English abstract]

Li, Z., S. Li, B. Zhou, L. Yang and Y. Chen. 2004. Antifungal activity of endophytic fungi from three pharmaceutical plants. J. Microbiol. **24**: 35-37.

Lin, C.-L. 2001. Biological characteristics and control of the causal agent of cruciferous vegetable anthracnose. Master thesis. National Chung Hsing University, Taichung, Taiwan. pp. 66. [in Chinese with English abstract]

Liu, X., M. Dong, X. Chen, M. Jiang, X. Lv and J. Zhou. 2008. Antimicrobial activity of an endophytic *Xylaria* sp. YX-28 and identification of its antimicrobial compound 7-amino-4-methylcoumarin. Appl. Microbiol. Biotechnol. **78**: 241-247.

Maciá-Vicente, J. G., L. C. Rosso, A. Ciancio, H. B. Jansson and L. V. Lopez-Llorca. 2009. Colonization of barley roots by endophytic *Fusarium equiseti* and *Pochonia chlamydosporia*: Effects on plant growth and disease. Ann. Appl. Biol. **155**: 391-401.

Matsumoto, T., K. Nishimura and K. Takeya. 2002. New cyclic peptides from *Citrus medica* var. *sarcodactylis* Swingle. Chem. Pharm. Bull. **50**: 875-860.

Maysarah. 2010. The isolation and ability of antifungal of endophytic fungi from andaliman (*Zanthoxylum acanthopodium* DC.) to food spoilage fungi. FMIPA Universitas Sumatera Utara, Indonesia. pp. 36.

Mejía, L. C., E. I. Rojas, Z. Maynard, S. Van Bael, A. E. Arnold, P. Hebbar, G. J. Samuels, N. Robbins and E. A. Herre. 2008. Endophytic fungi as biological agents of *Theobroma cacao* pathogens. Biol. Control **46**: 4-14.

Mercier, J. and J. I. Jiménez. 2009. Demonstration of the biofumigation activity of *Muscodor albus* against *Rhizoctonia solani* in soil and potting mix. Biocontrol **54**: 797-805.

Nalini, M. S., B. Mahesh, M. V. Tejesvi, H. S. Prakash, V. Subbaiah, K. R. Kini and H. S. Shetty. 2005. Fungal endophytes from the three-leaved caper, *Crataeva magna* (Lour.) DC. (Capparidaceae). Mycopathologia **159**: 245-249.

O'Donnell, K. 1993. Fusarium and its near relatives. In: Reynolds, D. R. and J. W. Taylor. (eds.), The Holomorph: Mitotic, Meiotic and Pleomorphic Speciation in Fungal Systematics. CAB International, Wallingford, UK. pp. 225-233.

Okane, I., P. Srikitikulchai, K. Toyama, T. Laessoe, S. Sivichai, J. N. Hywel, A. Nakagiri, W. Potacharoen and K. Suzuki. 2008. Study of endophytic Xylariaceae in Thailand: diversity and taxonomy inferred from rDNA sequence analyses with saprobes forming fruit bodies in the field. Mycoscience **49:** 359-372.

Omacini, M., E. J. Chaneton, C. M. Chersa and C. B. Müller. 2001. Symbiotic fungal endophytes control insect host-parasite interaction webs. Nature **409:** 78-81.

Patterson, C. G., D. A. Potter and F. F. Fannin. 1991. Feeding deterrency of alkaloids from endophyte-infected grasses to Japanese beetle grubs. Entomol. Exp. Appl. **61:**

臺灣大學學術期刊資料庫

285-289.
Petrini, O., T. N. Sieber, L. Toti and O. Viret. 1992. Ecology, metabolite production, and substrate utilization in endophytic fungi. Nat. Toxins **1:** 185-196.
Pieterse, C. M. J., A. Leon-Reyes, S. Van der Ent and S. C. M. Van Wees. 2009. Networking by small-molecule hormones in plant immunity. Nat. Chem. Biol. **5:** 308-316.
Rambaut, A. 2000. Sel-Al: Sequence alignment editor. Department of Zoology, University of Oxford, Oxford, UK.
Redman, R. S., S. Freeman, D. R. Clifton, J. Morrel, G. Brown and R. J. Rodriguez. 1999. Biochemical analysis of plant protection afforded by a nonpathogenic endophytic mutant of *Colletotrichum magna*. Plant Physiol. **119:** 795-804.
Redman, R. R., K. B. Sheehan, R. G. Stout, R. J. Rodriguez and J. M. Henson. 2002. Thermotolerance generated by plant/fungal symbiosis. Science **298:** 1581.
Rippon, J. W. 1988. Medical Mycology. 3rd edn. W. B. Saunders Co., Philadelphia, USA. pp. 169-275.
Rodriguez, R. J., J. Henson, V. E. Van, M Hoy, L. Wright, F. Beekwith, Y. O. Kim and R. S. Redman. 2008. Stress tolerance in plants via habitat-adapted symbiosis. The Isme J. **2:** 404-416.
Rodriguez, R. J., J. F. White, A. E. Arnold and R. S. Redman. 2009. Fungal endophytes: diversity and functional roles. New Phytol. **182:** 314-330.
Roger, D. J. 1979. The Xylariaceae: systematic, biological and evolutionary aspect. Mycologia **71:** 1-42.
Schardl, C. L. and T. D. Phillips. 1997. Protective grass endophytes, where are they from and where are they going? Plant Dis. **81:** 430-438.
Schulz, B., C. Boyle, S. Draeger and A. Römmert. 2002. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycol. Res. **106:** 996-1004.
Shentu, X.-P., L.-Z. Chen and X.-P. Yu. 2007. Anti-fungi activities and cultural characteristics of gingko endophytic fungus No. 1028. Acta. Phytophyl. Sin. **34:** 147-152. [in Chinese with English abstract]
Shu, Y., S.-X. Guo, D.-M. Zhang and C.-L. Wang. 2005. Studies on active components from endophytic fungi. Chin. Trad. Herb. Drugs **36:** 772-776. [in Chinese with English abstract]
Siegel, M. R., G. C. M. Latch, L. P. Bush, F. F. Fannin and D. D. Rowan. 1990. Fungal endophyte-infected grasses: alkaloid accumulation and aphid response. J. Chem. Ecol. **16:** 3301-3315.
Stewart, T. M., C. F. Mercep and J. L. Grante. 1993. Development of *Meloidogyne naasi* on endophyte-infected and endophyte-free perennial ryegrass. Aust. Plant Pathol. **22:** 40-41.
Stierle, A., G. Strobel and D. Stierle. 1993. Taxol and taxane production by T*axomyces andreanae*, an endophytic fungus of pacific yew. Science **260:** 214-216.
Strobel, G. .A. 2003. Endophytes as sources of bioactive products. Microbes Infect **5:** 535-544.
Swofford, D. L. 2000. PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods). Version 4. Sinauer Associates, Sunderland, MA.
Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin and D. G. Higgins. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **25:** 4876-4882.
Thongkantha, S., R. Jeewon, D. Vijaykrishna, S. Lumyong, E. H. C. Mekenzie and K. D. Hyde. 2009. Molecular phylogeny of *Magnaporthaceae* (Sordariomycetes) with a new species *Ophioceras chiangdaoense* from *Dracaena loureiroi* in Thailand. Fungal Divers. **34:** 157-173.
Tomsheck, A. R., G. A. Strobel, E. Booth, B. Geary, D. Spakowicz, B. Knighton, C. Floerchinger, J. Sears, O. Liarzi and D. Ezra. 2010. *Hypoxylon* sp., an endophyte of *Persea indica*, producing 1,8-Cineole and other bioactive volatiles with fuel potential. Microb. Ecol. **60:** 903-914.
Van Niekerk, J. M., 'J. Z. Ewald' Groenewald, G. J. M. Verkley, P. H. Fourie, M. J. Wingfield and P. W. Crous. 2004. Systematic reappraisal of *Coniella* and *Pilidiella*, with specific reference to species occurring on *Eucalyptus* and *Vitis* in South Africa. Mycol. Res. **108:** 283-303.
Wali, P. R., M. Helander, O. Nissinen and K. Saikkonen. 2006. Susceptibility of endophyte-infected grasses to winter pathogens (snow molds). Can. J. Bot. **84:** 1043-1051.
Wang, L.-J., X.-S. Yang and X.-S. He.. 2007. Screening of antibacterial strains from endophytic fungi in ginkgo leaves. Sichuan Food Ferment. **4:** 34-36. [in Chinese with English abstract]
Wang, Y.-T., H.-S. Lo and P.-H. Wang. 2008. Endophytic fungi from *Taxus mairei* in Taiwan: first report of *Colletotrichum gloeosporioides* as an endophyte of *Taxus mairei*. Bot. Studies **49**: 39-43.
White, T. J., T. Bruns, S. Lee and J. W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. pp. 315-322. In: Innis, M. A., et al. (eds.). PCR Protocols: A Guide to Methods and Applications. Academic Press: San Diego, USA.
Xu, L.-J., L.-G. Zhou, J.-L. Zhao and W.-B. Jiang. 2008. Recent studies on the antimicrobial compounds produced by plant endophytic fungi. Nat. Prod. Res. Dev. **20:** 731-740. [in Chinese with English abstract]
Yan, Z.-Y., J. Luo, X.-H. Guo, Q.-Q. Zeng. 2007. Screening of ginkgolides-producing endophytic fungi and optimal study on culture condition. Nat. Prod. Res. Dev. **19:** 554-558. [in Chinese with English abstract]
Yi, D.-W. 2003. Study on active components of metabolites from endophytic fungus of *Ginkgo biolba* L. A. Shenyang: Shenyang Phamaceutical University, Master thesis. [in Chinese with English abstract]

臺灣大學學術期刊資料庫

樟科與云香科之中草藥植物內生真菌的鑑定與抗菌特性

何旻遠[(1)]、鍾文全[(2)]、黃鴻章[(1)]、鍾文霞[(3)]、鍾文鑫[(1*)]

1. 國立中興大學植物病理學系，402 臺中市國光路 250 號，臺灣。
2. 行政院農業委員會種苗改良繁殖場，426 臺中市新社區大南里興中街 46 號，臺灣。
3. 敏惠醫護管理專科學校，73658 臺南市柳營區中山東路二段 1116 號，臺灣。
* 通信作者。*Tel: 866-4-22602783; Fax: 886-4-22877585; Email: wenchung@nchu.edu.tw*

(收稿日期：2012 年 4 月 16 日；接受日期：2012 年 5 月 31 日)

摘要：本研究為鑑定分離自臺灣中部與北部中草藥植物之樟科(67株)與云香科(89株)共156株的內生真菌。依形態與分子序列鑑定顯示，156株內生真菌可被分成35屬19科，以*Colletotrichum*、*Guignardia*、*Hypoxylon*、*Nigrospora*、*Phomopsis*及*Xylaria*等屬真菌被分離頻率較高，其中又以Xylariaceae中的*Hypoxylon*與*Xylaria*屬真菌被分離比率最高。此外云香科中的*Citrus*與*Zanthoxylum*屬植物和樟科中的*Cinnamomum*屬植物，則最易分離到內生真菌。進一步利用D1/D2與ITS rDNA序列分析Xylariaceae科內生真菌的分子親緣性，得知所分離Xylariaceae科內生真菌主要可分成Xylaria與Hypoxylon兩個分子群。測試云香科與樟科所分離內生真菌對植物病原之拮抗性，顯示*Lasmenia* sp. CB10菌株、*Ophioceras tenuisporum* CI02菌株、*Xylaria cubensis* LA04菌株及*Cyanodermella* sp. TR09菌株對抑制12種植物病原真菌、3種植物病原細菌及1種卵菌表現較佳。於溫室評估CB10菌株對降低白菜炭疽病之效果，顯示該菌株可明顯降低白菜炭疽病的發生，證明CB10菌株具有防治白菜炭疽病之潛力。

關鍵詞：拮抗能力、內生真菌、中草藥植物、樟科、云香科。

臺灣大學學術期刊資料庫