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ORIGINAL ARTICLE

Some fungal endophytes from vegetable crops and their anti-oomycete activities against tomato late blight

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Abstract

Aims: To isolate endophytic fungi from vegetable plants and examine their *in vivo* anti-oomycete activity against *Phytophthora infestans* in tomato plants.
Methods and Results: Endophytic fungi were isolated from surface-sterilized plant tissues and anti-oomycete activity was measured by *in vivo* assay using tomato seedlings. Endophytic fungi showing potent anti-oomycete activity were identified by morphological characteristics and nuclear ribosomal ITS1-5.8S-ITS2 sequence analysis. A total of 152 isolates were obtained from 66 healthy tissue samples of cucumber, red pepper, tomato, pumpkin and Chinese cabbage and the fermentation broths of 23 isolates showed potent *in vivo* anti-oomycete activity against tomato late blight with control values over 90%. The *Fusarium oxysporum* strain EF119, which was isolated from roots of red pepper, showed the most potent disease control efficacy against tomato late blight. In dual-culture tests, it inhibited the growth of *Pythium ultimum*, *P. infestans* and *Phytophthora capsici*.
Conclusions: Among endophytic fungi isolated from healthy tissues of vegetable plants, *F. oxysporum* EF119 showed the most potent *in vivo* anti-oomycete activity against tomato late blight and *in vitro* anti-oomycete activity against several oomycete pathogens.
Significance and Impact of the Study: Endophytic fungi showing anti-oomycete activity *in vitro* and *in vivo* may be used as biocontrol agents particularly of tomato late blight.

Introduction

Endophytic fungi are defined as fungi colonizing healthy plant tissue without causing overt symptoms in or apparent injury to the host (Bills 1996). In contrast to epiphytes, endophytes complete their entire life cycle within their host plant (Strobel and Long 1998). They are presumably ubiquitous in the plant kingdom (Armond *et al.* 2000). Endophytic fungi within plants have been known to produce plant-growth regulatory, antimicrobial, antiviral or insecticidal substances to enhance the growth and competitiveness of the host in nature (Carroll 1988; Wiyakrutta *et al.* 2004). They have been recognized as a repository of novel metabolites of agricultural and/or pharmaceutical importance (Stierle *et al.* 1993; Strobel and Long 1998; Tan and Zou 2001). Thus, endophytic fungi are expected to be potential sources of new bioactive agents and to be useful as agents of biocontrol against plant diseases.

Late blight is caused by the fungus-like micro-organism *Phytophthora infestans*. This pseudofungus is thought to be phylogenetically different from the true fungi and belongs to the phylum Oomycota of the kingdom Chromista (Gunderson *et al.* 1987; Kirk *et al.* 2001). *Phytophthora infestans* is a foliar pathogen and causes serious losses of potato and tomato crops worldwide. It is

probably the most important pathogen of these plants today. Besides potato and tomato, *P. infestans* can infect only a few other, closely related plants.

Late blight, caused by *P. infestans*, is difficult to control by chemical means, because of the high virulence of the pathogen and increasing resistance to fungicide (Griffith *et al.* 1992). Although metalaxyl provides excellent control of oomycete pathogens, metalaxyl-resistant *Phytophthora* isolates have developed rapidly and become widespread worldwide (Schwinn and Staub 1995). The emergence of metalaxyl-resistant *Phytophthora* sp. in potato fields has resulted in devastating late blight problems because of the failure to control the disease in many, if not most areas of production (Davidse *et al.* 1981; Cohen and Reuven 1983; Hwang and Kim 1995). In addition, the demand for organic foods is rapidly increasing. Biocontrol is regarded as an 'environmentally friendly' alternative to synthetic fungicides for protection of the tomato and potato against late blight.

The objectives of this study were to isolate endophytic fungi and to examine their *in vivo* anti-oomycete activity against *P. infestans* in tomato plants as biocontrol agents. The *in vitro* anti-oomycete activity of one selected isolate, *Fusarium oxysporum* EF119, to the three oomycete pathogens *Pythium ultimum*, *P. infestans* and *Phytophthora capsici* was also evaluated.

Materials and methods

Isolation of fungal endophytes

Healthy leaves, stems and roots were sampled from the five vegetable plant species red pepper (*Capsicum annuum* L.), cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum* L.), pumpkin (*Cucurbita pepo* L.) and Chinese cabbage (*Brassica campestris* var. *pekinensis* Makino) which were collected from field plots in various locations of Chungbuk Province, Korea. Samples were randomly collected from three to five healthy plants per site. The plant samples were placed in zip-lock bags, stored in a refrigerator and then used for isolation of endophytic fungi within 72 h after sampling.

Samples were cleaned under running tap water and then air-dried. The samples were surface sterilized by immersion in 70% ethanol for 1 min, 5% sodium hypochlorite solution for 5 min, and then the surface-sterilized samples were washed in sterile water three times to remove the surface sterilization agents (Wiyakrutta *et al.* 2004). The surface-sterilized samples were cut into small pieces and then placed on a malt extract agar medium [20 g malt extract (Becton and Dickinson Co., Sparks, MD, USA), 20 g agar and 1·0 l distilled water], supplemented with 50 μg ml^{-1} chloramphenicol (Sigma Co., St Louis, MO, USA) and incubated at 25°C. Thirty-two fragments processed from each plant were placed onto a total of eight plates (four fragments per plate). The plates were incubated for a period of up to 3 weeks. Individual fungal strains were transferred to potato dextrose agar (PDA, Bacto Potato Dextrose Dehydrated; Becton and Dickinson Co.) and further incubated at 25°C for at least 2 weeks. After checking for purity, each fungal culture was transferred to another agar plate. A total of 152 fungi were isolated from healthy plant leaves, stems and roots. Fungal identification methods were based on the morphology of the fungal culture, the characteristics of the spores (Domsch *et al.* 1980; Hawksworth *et al.* 1983; Barnett and Hunter 1987), and nuclear ribosomal ITS1-5.8S-ITS2 sequence analysis (Park *et al.* 2005). The intra- and inter-specific DNA homologies of the active isolates were compared with those of best matching taxa in the GenBank and analysed by using the National Center for Biotechnology Information.

Fermentation and treatment of the fermentation broth

Erlenmeyer flasks (500 ml) containing 200 ml of potato dextrose broth (PDB; Becton and Dickinson Co.) were autoclaved at 121°C for 15 min and then inoculated with mycelium plugs from the margins of actively growing cultures on PDA. The flasks were incubated for 2 weeks on a rotary shaker at 150 rev min^{-1} and 25°C. Crude fermentation broths were blended thoroughly for 2 min and then added with Tween 20 (Samchun Pure Chemical Co., Pyongtaek, Korea) at a concentration of 250 μg ml^{-1}.

In vivo anti-oomycete and antifungal activity

The blended broths were tested for their *in vivo* anti-oomycete activity against *P. infestans*, a causal agent of tomato late blight. In addition, the fermentation broth of *F. oxysporum* EF119, which was shown to be the most active to *P. infestans* in tomato plants, was tested for *in vivo* antifungal activity against the three additional plant diseases such as red pepper anthracnose (*Colletotrichum coccodes*), tomato grey mould (*Botrytis cinerea*) and barley powdery mildew (*Blumeria graminis* f. sp. *hordei*). The *in vivo* anti-oomycete and antifungal bioassays were performed as previously described by Kim *et al.* (2001). The three estimates for each treatment were converted into the control percentage when compared with the controls. Values were expressed as a percentage of the control (±standard deviation).

Dual-culture tests of *Fusarium oxysporum* EF119

As the results of the *in vivo* anti-oomycete tests, EF119 exhibited the most potent anti-oomycete activity against

P. infestans in tomato plants. In order to examine its anti-oomycete spectrum, dual–culture tests were performed by using a procedure previously described by Cherif and Benhamou (1990). In brief, 8-mm mycelial disks from the margins of actively growing cultures of EF119 and *P. ultimum* (a causal agent of cucumber damping-off), *P. infestans*, or *P. capsici* (a causal agent of red pepper blight) were placed 3 cm apart on the surface of PDA in the case of *P. ultimum* and *P. capsici*, and V-8 juice agar for *P. infestans*, and allowed to grow at 25°C.

Results

A total of 152 endophytic fungi were isolated from 66 healthy plant tissue samples of five vegetable plant species (Table 1). In general, the number of endophytic fungi isolated from roots was higher than those from leaves and stems. The fermentation broths of 152 endophytic fungi were tested for *in vivo* anti-oomycete activity against tomato late blight. Table 1 shows the numbers and percentages of the fermentation broths that controlled the development of tomato late blight by greater than 90%. Among the 152 fungal strains tested, 23 (15%) exhibited potent *in vivo* anti-oomycete activity against *P. infestans* in tomato plants. The endophytic fungi isolated from roots displayed more potent anti-oomycete activity than those isolated from leaves and stems; the percentages of the fermentation broths exhibiting potent anti-oomycete activity were 24% for endophytic fungi from roots, 3·4% for those from leaves and 4·8% for those from stems.

Based on morphological characteristics and nuclear ribosomal ITS1-5.8S-ITS2 sequence analysis, 11 of 23 isolates were classified as *F. oxysporum*, four as *Fusarium* sp., two as *Chaetomium* sp., two as *Penicillium* sp., one as *Coniochaeta* cf. *ligniaria*, one as *Colletotrichum* sp., one as *Talaromyces* sp. and one was not determinable.

The fermentation broths of 23 endophytic fungi with potent anti-oomycete activity were diluted threefold, ninefold and 50-fold with distilled water and then tested for disease-controlling efficacy against tomato late blight (Table 2). The fermentation broth of *F. oxysporum* EF119 was the most active; it suppressed the development of tomato late blight by 90% even when diluted 50-fold. It controlled tomato late blight by 57% and 25% at 100- and 200-fold dilution levels, respectively. The liquid culture of EF119 was also effective against red pepper anthracnose; it suppressed the development of red pepper anthracnose by 96%, 73% and 54% at one-, three- and ninefold dilution levels, respectively. However, it was virtually inactive against tomato grey mould and barley powdery mildew.

In dual cultures, *F. oxysporum* EF119 substantially reduced the growth of the three oomycetes *P. ultimum*, *P. infestans* and *P. capsici* (Fig. 1) and haloes were visible around the three oomycete colonies. This indicates EF119 produced some fungal inhibitors. Overgrowth of EF119 on oomycete strains was not observed. In single cultures, the three oomycete strains grew actively.

Discussion

In this study, a total of 152 isolates of endophytic fungi were isolated from surface-sterilized healthy tissues of vegetable plants. Although the host plants looked healthy, a possibility that the fungi may be merely quiescent could

Table 1 Endophytic fungi isolated from vegetable plants that were collected in various fields in Korea, and screening of fermentation broths with *in vivo* anti-oomycete activity against *Phytophthora infestans* on tomato plants

Plant	Part	No. of samples	No. of strains	No. of active strains (%)*
Capsicum annuum L. (Red pepper)	Leaf	8	27	0 (0)
	Stem	8	17	1 (5·9)
	Root	8	47	9 (19)
Cucumis sativus L. (Cucumber)	Leaf	4	5	0 (0)
	Stem	4	3	0 (0)
	Root	4	11	5 (46)
Solanum lycopersicum L. (Tomato)	Leaf	5	6	0 (0)
	Stem	5	2	0 (0)
	Root	5	7	2 (29)
Cucurbita pepo L. (Pumpkin)	Leaf	1	3	1 (33)
	Stem	1	3	0 (0)
	Root	1	5	1 (20)
Brassica campestris var. *pekinensis*	Leaf	4	1	1 (100)
Makino (Chinese cabbage)	Stem	4	4	0 (0)
	Root	4	12	3 (33)
Total		66	152	23 (15)

*Strains exhibiting greater than 90% *in vivo* anti-oomycete activity against *P. infestans* in tomato plants.

Table 2 Endophytic fungi exhibiting potent *in vivo* anti-oomycete activity against *Phytophthora infestans* in tomato plants*

Strain	Species	Source (plant/part)	Control value (%)†			
			Onefold	Threefold	Ninefold	50-fold
EF001	*Coniochaeta* cf. *ligniaria*	Cs/r	93 ± 1·6	0	0	0
EF017	*Chaetomium* sp.	Ca/r	98 ± 0	0	0	0
EF020	*Fusarium oxysporum*	Ca/r	95 ± 1·6	78 ± 0·8	6 ± 4·9	0
EF021	*Chaetomium* sp.	Ca/r	94 ± 0	0	0	0
EF037	ND	Ca/r	94 ± 0·9	0	0	0
EF038	*F. oxysporum*	Ca/r	96 ± 0	69 ± 1·6	50 ± 4·1	0
EF039	*F. oxysporum*	Ca/r	96 ± 3·3	69 ± 1·6	0	0
EF052	*F. oxysporum*	Ca/r	95 ± 0·8	84 ± 2·4	44 ± 3·3	36 ± 4·9
EF055	*Penicillium* sp.	Ca/r	99 ± 0·9	75 ±0·8	0	0
EF061	*F. oxysporum*	Sl/r	94 ± 0·5	84 2·4	78 ± 0·8	29 ± 3·3
EF088	*F. oxysporum*	Cs/r	95 ± 1·6	44 ± 12	0	0
EF090	*Fusarium* sp.	Cs/r	95 ± 1·6	89 1·6	13 ±2·5	0
EF099	*F. oxysporum*	Sl/r	95 ± 0	19 ± 11	0	0
EF109	*F. oxysporum*	Cp/r	95 ± 0	89 ± 0·8	6 ± 4·9	0
EF117	*Fusarium* sp.	Cp/l	95 ± 0	50 ± 4·1	31 ± 4·2	0
EF119	*F. oxysporum*	RP/r	96 ± 0	96 ± 0	93 ± 2·5	90 ± 1·2
EF129	*Fusarium* sp.	Bc/l	99 ± 0·9	88 ± 0·8	78 ± 0·8	0
EF136	*F. oxysporum*	Bc/r	99 ± 0	93 ± 1·6	78 ± 0·8	7 ± 12
EF147	*F. oxysporum*	Cs/r	96 ± 0	93 ± 0	89 ± 1·6	43 ± 3·3
EF148	*Talaromyces* sp.	Cs/r	99 ± 0	100	98 ± 0	0
EF149	*Fusarium* sp.	Bc/r	94 ± 1·6	95 ± 0·8	78 ± 0·8	0
EF150	*Colletotrichum* sp.	Bc/r	96 ± 1·6	96 ± 0	88 ± 6·5	7 ± 5·2
EF152	*Penicillium* sp.	Ca/s	100	88 ± 1·6	75 ± 0·8	0

ND, not determinable; Cs, *Cucumis sativus*; Ca, *Capsicum annuum*; Sl, *Solanum lycopersicum*; Cp, *Cucurbita pepo*; Bc, *Brassica campestris* var. *pekinensis*; r, root; l, leaf; s, stem.

*Tomato seedlings were inoculated with a zoospore suspension of *P. infestans* 1 day after dilutions of the fermentation broths of the fungi were sprayed on the leaves to run-off.

†Control value (%) = 100 × {disease severity of untreated plants – disease severity of treated plants}÷ disease severity of untreated plants. Each value represents the mean of three estimates ± the standard deviation.

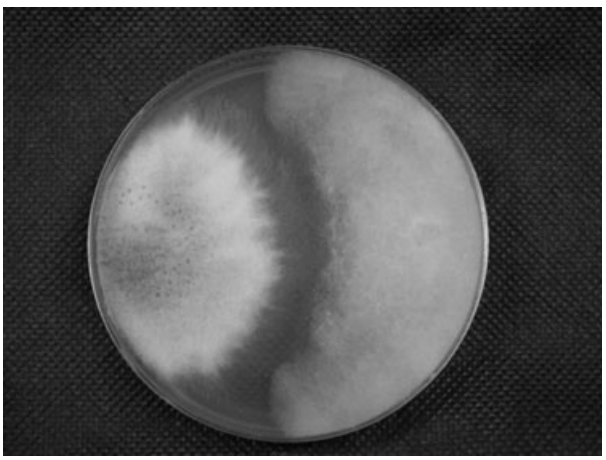

Figure 1 Inhibition of the growth of *Phytophthora capsici* (Pc) by *Fusarium oxysporum* (Fo) EF119 on agar medium.

not be ruled out. This is because many pathogens frequently undergo asymptomatic growth from initial colonization and then quiescent infection before disease symptoms appear.

Among 152 isolates, the fermentation broths of 23 isolates effectively suppressed the development of tomato late blight. Huang *et al.* (2001) reported that 52·3% of the fermentation broths of endophytic fungi isolated from pharmaceutical plants such as *Taxus mairei*, *Cephalataxus tortunei* and *Torreya grandis* displayed growth inhibition on at least one pathogenic fungus. Out of the endophytic fungi isolated from 12 Chinese traditional medicinal plants, 30% exhibited *in vitro* anti-oomycete or antifungal activity against at least one of seven phytopathogenic fungi (Li *et al.* 2005) and 6·9% inhibited the mycelial growth of *Phytophthora nicotianae*, which belongs to Oomycetes. In our previous report (Park *et al.* 2005), nine (4·8%) out of the fermentation broths of 187 endophytic fungi isolated from mainly woody plants were highly active to *P. infestans* in tomato plants. Taken together, the endophytic fungi isolated from vegetable plants in this study exhibited higher anti-oomycete activity than those described in the previous reports.

The majority of 23 active isolates were classified as *Fusarium* sp. and the others were *Chaetomium* sp., *Penicillium* sp., *Coniochaeta* cf. *ligniaria*, *Colletotrichum* sp., or

Talaromyces sp. *Fusarium* spp. including *F. oxysporum*, are commonly found in soil throughout the world. Certain strains of *F. oxysporum*, known as formae speciales, are pathogenic and are responsible for wilt in various plant species (Gordon and Martyn 1997), while other strains are nonpathogenic, living in healthy plants without any disease symptoms (Vu *et al.* 2004; Rubini *et al.* 2005). Several nonpathogenic strains of *F. oxysporum* have been selected as potential biological control agents (Benhamou and Garand 2001; Freeman *et al.* 2002; Shishido *et al.* 2005; Rodriguez *et al.* 2006). For example, Fo47 and Fo-B2 have reduced the disease severity of Fusarium wilt in a variety of crops (Vu *et al.* 2004; Rubini *et al.* 2005). Benhamou *et al.* (2002) reported that Fo47 also protects cucumber plants from *P. ultimum* infection. To our knowledge, this is the first report on the *in vivo* anti-oomycete activity of *F. oxysporum* against tomato late blight.

Lodge *et al.* (1996) reported the most dominant endophytic fungi in leaves of *Manilkara bidentata* to be *Xylaria* spp., and a further 20 fungal species such as *Colletotrichum crassipes*, *Glomerella cingulata* (anamorph: *Colletotrichum gloeosporioides*), *Chaetomium sphaerale*, *Penicillium glabrum*, etc. have been isolated. *Chaetomium globosum* was found to be the most dominant endophyte in coastal sand dune legumes *Canavalia cathartica* and *Canavalia maritama* (Seena and Sridhar 2004). *Chaetomium* spp., *Penicillium* sp. and *Coniochaeta* cf. *ligniaria*, together with *Alternaria*, *Aureobasidium*, *Cladosporium*, *Ulocladium*, etc. were recovered as quiescent filamentous fungi from nonsymptomatic grape berries and dormant (Dugan *et al.* 2002). The anamorph of *Talaromyces* is *Penicillium* subgenus *Biverticillium*. Thus, the anti-oomycete endophytic fungi isolated in this study have already been reported as endophytic or quiescent fungi in other plant species.

In dual cultures, *F. oxysporum* EF119 inhibited the growth of three oomycetes *P. ultimum*, *P. infestans* and *P. capsici*. Nonpathogenic *F. oxysporum* Fo47 was reported to cause the reduction of *P. ultimum* growth by production of certain fungal inhibitors (Benhamou *et al.* 2002). Some strains of *F. oxysporum* have also been reported to suppress the growth of *Phytophthora erythroseptica* and *Screlotinia sclerotiorum* (Park 1963; Zazzerini and Tosi 1985). However, little characterized are the antifungal or anti-oomycete metabolites produced by nonpathogenic *F. oxysporum* (Fravel *et al.* 2003). Recently, cyclosporine A was characterized as a major antifungal substance against *S. sclerotiorum* from the fermentation broth of *F. oxysporum* S6 (Rodriguez *et al.* 2006). Studies are in progress on the isolation and identification of anti-oomycete substances produced by *F. oxsyporum* EF119. During the course of the study, we found two anti-oomycete substances in the fermentation broth of EF119 (data not shown).

In order to examine the pathogenicity of *F. oxysporum* EF119, the spores of the fungus were inoculated to tomato, cucumber, pumpkin, Chinese cabbage and sesame (*Sesamum indicum* L.) as well as red pepper, from which the fungus has been initially isolated. However, the plants showed normal growth of shoot length and no symptoms of disease (data not shown). This indicates that *F. oxysporum* EF119 may be nonpathogenic. The fungus also showed potent *in vivo* anti-oomycete activity against tomato late blight even at a 100-fold dilution of the fermentation broth with a control value of 57%. Therefore, *F. oxysporum* EF119 is considered to be a potential biocontrol agent. Field trials with *F. oxysporum* EF119 against tomato late blight would be valuable to develop the strain as biofungicide.

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References

Armond, A.E., Maynard, Z., Gilbert, G.S., Coley, P.D. and Kursat, T.A. (2000) Are tropical fungal endophytes hyperdiverse? *Ecol Lett* **3,** 267–274.

Barnett, H.L. and Hunter, B.B. (1987) *Illustrated Genera of Imperfect Fungi*. New York: Macmillan Publishing Company.

Benhamou, N. and Garand, C. (2001) Cytological analysis of defense-related mechanisms induced in pea root tissues in response to colonization by the non-pathogenic *Fusarium oxysporum*, strain Fo47. *Phytopathology* **91**, 730–740.

Benhamou, N., Garand, C. and Goulet, A. (2002) Ability of nonpathogenic *Fusarium oxysporum* strain Fo47 to induce resistance against *Pythium ultimum* infection in cucumber. *Appl Environ Microbiol* **68**, 4044–4060.

Bills, G.F. (1996) Isolation and analysis of endophytic fungal communities from woody plants. In *Systematics, Ecology and Evolution of Endophytic Fungi in Grasses and Woody Plants* ed. Redlin, S. and Carris, L.M. pp. 31–65. St Paul, MN: APS Press.

Carroll, G.C. (1988) Fungal endophytes in stems and leaves: from latent pathogen to mutualistic symbiont. *Ecology* **69**, 2–9.

Cherif, M. and Benhamou, N. (1990) Cytochemical aspects of chitin breakdown during the parasitic action of a *Trichoderma* sp. on *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Phytopathology* **80**, 1232–1244.

Cohen, Y. and Reuven, M. (1983) Occurrence of metalaxyl-resistant isolates of *Phytophthora infestans* in potato fields in Ireland. *Phytopathology* **73**, 925–927.

Davidse, L.C., Looijen, D., Turkensteen, L.J. and Vander Wal, D. (1981) Occurrence of metalaxyl-resistant strains of *Phytophthora infestans* in Dutch potato fields. *Neth J Plant Pathol* **87**, 65–68.

Domsch, K.H., Gams, W. and Anderson, T. (1980) *Compendium of Soil Fungi*. London: Academic Press.

Dugan, F.M., Lupien, S.L. and Grove, G.G. (2002) Incidence, aggressiveness and in planta interactions of *Botrytis cinerea* and other filamentous fungi quiescent in grape berries and dormant buds in central Washington state. *J Phytopathol* **150**, 375–381.

Fravel, D., Olivalin, C. and Alabouvette, C. (2003) *Fusarium oxysporum* and its biocontrol. Research review. *New Phytol* **157**, 493–502.

Freeman, S., Zveibil, A., Vintal, H. and Maymon, M. (2002) Isolation of nonpathogenic mutants of *Fusarium oxysporum* f. sp. *melonis* for biological control of Fusarium wilt in cucurbits. *Phytopathology* **92**, 164–168.

Gordon, T.R. and Martyn, R.D. (1997) The evolutionary biology of *Fusarium oxysporum*. *Annu Rev Phytopathol* **35**, 111–128.

Griffith, J.M., Davis, A.J. and Grant, B.R. (1992) Target sites of fungicides to control oomycetes. In *Target Sites of Fungicide Action* ed. Koller, W. pp. 69–100. London: CRC Press.

Gunderson, J.H., Elwood, H., Ingold, A., Kindle, K. and Sogin, M.I. (1987) Phylogenic relationships between chlorophytes, chrysophytes, and oomycetes. *Proc Natl Acad Sci USA* **84**, 5823–5827.

Hawksworth, D.L., Sutton, B.C. and Ainsworth, G.C. (1983) *Dictionary of the Fungi*. Surrey: Common Wealth Mycological Institute.

Huang, Y., Wang, J., Li, G., Zheng, Z. and Su, W. (2001) Antitumor and antifungal activities in endophytic fungi isolated from pharmaceutical plants. *FEMS Immunol Med Microbiol* **31**, 163–167.

Hwang, B.K. and Kim, C.H. (1995) *Phytophthora* blight of pepper and its control in Korea. *Plant Dis* **79**, 221–227.

Kim, J.-C., Choi, G.J., Park, J.-H., Kim, H.T. and Cho, K.Y. (2001) Activity against plant pathogenic fungi of phomalactone isolated from *Nigrospora sphaerica*. *Pest Manag Sci* **57**, 554–559.

Kirk, P.M., Cannon, P.F., David, J.C. and Stalpers, J.A. (2001) *Dictionary of the Fungi*, 9th edn. Oxon: CAB International.

Li, H., Qing, C., Zhang, Y. and Zhao, Z. (2005) Screening for endophytic fungi with antitumor and antifungal activities from Chinese medicinal plants. *World J Microbiol Biotechnol* **21**, 1515–1519.

Lodge, D.J., Fisher, P.J. and Sutton, B.C. (1996) Endophytic fungi of *Manilkara bidentata* leaves in Puerto Rico. *Mycologia* **88**, 733–738.

Park, D. (1963) Evidence for a common fungal growth regulator. *Trans Br Mycol Soc* **46**, 541–548.

Park, J.-H., Choi, G.J., Lee, H.B., Kim, K.M., Jung, H.S., Lee, S.-W., Jang, K.S., Cho, K.Y. *et al.* (2005) Griseofulvin from *Xylaria* sp. strain F0010, an endophytic fungus of *Abies holophylla* and its antifungal activity against plant pathogenic fungi. *J Microbiol Biotechnol* **15**, 112–117.

Rodriguez, M.A., Cabrera, G. and Godeas, A. (2006) Cyclosporine A from a nonpathogenic *Fusarium oxysporum* suppressing *Sclerotinia sclerotiorum*. *J Appl Microbiol* **100**, 575–586.

Rubini, M.R., Silva-Ribeiro, R.T., Pomella, A.W.V., Maki, C.S., Araujo, W.L., dos Santos, D.R. and Azevedo, J.L. (2005) Diversity of endophytic fungal community of cacao (*Theobroma cacao* L.) and biological control of *Crinipellis perniciosa*, causal agent of Witches' broom disease. *Int J Biol Sci* **1**, 24–33.

Schwinn, F. and Staub, T. (1995) Oomycete fungicides. In *Modern Selective Fungicides, Properties, Applications, Mechanisms of Action* ed. Lyr, H. pp. 323–346. New York: Gustav Fischer Verlag.

Seena, S. and Sridhar, K.R. (2004) Endophytic fungal diversity of 2 sand dune wild legumes from the southwest coast of India. *Can J Microbiol* **50**, 1015–1021.

Shishido, M., Miwa, C., Usami, T., Amemiya, Y. and Johnson, K.B. (2005) Biological control efficiency of Fusarium wilt of tomato by nonpathogenic *Fusarium oxysporum* Fo-B2 in different environments. *Phytopathology* **95**, 1072–1080.

Stierle, A., Strobel, G.A. and Stierle, D. (1993) Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungi of Pacific yew. *Science* **260**, 214–216.

Strobel, G.A. and Long, D.M. (1998) Endophytic microbes embody pharmaceutical potential. *ASM News* **64**, 263–268.

Tan, R.X. and Zou, W.X. (2001) Endophytes: a rich source of functional metabolites. *Nat Prod Rep* **18**, 448–459.

Vu, T.T., Sikora, R.A. and Hauschild, R. (2004) Effects of endophytic *Fusarium oxysporum* towards *Radopholus similes* activity in absence of banana. *Commun Agri Appl Biol Sci* **69**, 381–385.

Wiyakrutta, S., Sriubolmas, N., Panphut, W., Thongon, N., Danwisetkanjana, K., Ruangrungsi, N. and Meevootisom, V. (2004) Endophytic fungi with anti-microbial, anti-cancer and anti-malarial activities isolated from Thai medicinal plants. *World J Microbiol Biotechnol* **20**, 265–272.

Zazzerini, A. and Tosi, L. (1985) Antagonistic activity of fungi isolated from sclerotinia of *Sclerotinia sclerotiorum*. *Plant Pathol* **34**, 415–421.