ELSEVIER

FEMS Microbiology Letters 252 (2005) 309–313

FEMS MICROBIOLOGY Letters

www.fems-microbiology.org

Antifungal activity against plant pathogenic fungi of chaetoviridins isolated from *Chaetomium globosum*

Joong-Hyeop Park [a], Gyung Ja Choi [a], Kyoung Soo Jang [a], He Kyoung Lim [a], Heung Tae Kim [b], Kwang Yun Cho [a], Jin-Cheol Kim [a,*]

[a] *Biological Function Research Team, Korea Research Institute of Chemical Technology, Yusong P.O. Box 107, Taejon 305-600, Republic of Korea*
[b] *Plant Medicine Division, Chungbuk National University, Cheongju 361-763, Republic of Korea*

Received 12 April 2005; received in revised form 29 August 2005; accepted 7 September 2005

First published online 21 September 2005

Edited by B. Paul

Abstract

The *Chaetomium globosum* strain F0142, which was isolated from barnyard grass, showed potent disease control efficacy against rice blast (*Magnaporthe grisea*) and wheat leaf rust (*Puccinia recondita*). Two antifungal substances were purified from broth from this organism and identified as chaetoviridins A and B. Chaetoviridin A exhibited higher antifungal activity than chaetoviridin B against plant pathogenic fungi both in vitro and in vivo. Treatment with chaetoviridin A at 62.5 μg/mL suppressed the development of rice blast and wheat leaf rust by over 80%. The molecule also exhibited moderate control of tomato late blight, resulting in 50% control following the application of 125 μg/mL chaetoviridin A.

Keywords: *Chaetomium globosum*; Chaetoviridin; *Magnaporthe grisea*; *Puccinia recondita*

1. Introduction

Pathogenic fungi cause plant diseases that result in considerable losses to crop yields. Crop growers generally apply synthetic fungicides as preventive and therapeutic measures to control plant diseases. The indiscriminate and excessive use of a wide range of fungicides has led to environmental pollution and the production of resistant pathogen populations. Therefore, the demand for organic agricultural products cultivated without using any agricultural chemicals or chemical fertilizers is increasing. These events have caused many scientists to conduct research into the integrated control of fungal diseases, including biological controls using antagonistic microorganisms and safer chemicals such as food preservatives and plant-derived products [1–3].

The ascomycete *Chaetomium globosum* is a common colonizer of soil and cellulose-containing substrates [4]. The fungus has been reported to be a potential antagonist of various plant pathogens, most of which are soilborne and seedborne [5–13]. The antagonism of *C. globosum* against these pathogens is exerted through three modes of action: competition, mycoparasitism, and antibiosis. Di Pietro et al. [8] reported that the ability of *C. globosum* strains to produce chaetomin in liquid culture is correlated with their activity against damping-off of sugar beet caused by *Pythium ultimum*. High antifungal metabolite production by *C. globosum* results in potent in vivo antifungal activity against spot blotch (*Cochliobolus sativus*) of wheat under laboratory and glasshouse conditions [5].

* Corresponding author. Tel.: +82 42 8607436; fax: +82 42 8614913.
E-mail address: kjinc@krict.re.kr (J.-C. Kim).

0378-1097/$22.00
doi:10.1016/j.femsle.2005.09.013

During a search for an antagonist of several soilborne and airborne plant pathogenic fungi, we found that liquid cultures of *C. globosum* F0142, which was isolated from barnyard grass, showed potent in vivo antifungal activity against rice blast (*Magnaporthe grisea*) and wheat leaf rust (*Puccinia recondita*), and moderate in vivo antifungal activity against tomato late blight (*Phytophthora infestans*). The production of antifungal substances by this organism is thought to play an important role in its antifungal activity. The objective of this work was to purify and identify the antifungal substances produced by this fungus that are responsible for the suppression of several plant diseases.

2. Materials and methods

2.1. Fungal isolation and identification

C. globosum F0142 was isolated from a healthy stem of barnyard grass. The fungus was deposited with the Korean Collection for Type Cultures at the Genetic Resources Center, Korea Research Institute of Bioscience and Biotechnology, Taejon, Korea under Accession No. KCTC0957BP. The isolate was incubated on potato dextrose agar (PDA; Becton and Dickinson Co., MA, USA) at 28 °C for 15 days with 12 h of illumination daily. Mycelial fragments, ascomata, and ascospores were collected, placed on glass slides, and observed. The isolate was identified to the species level based on the criteria described by Ellis [14], including the presence or absence of aleuriconidia and the shape and size of the ascomata and ascospores.

2.2. Isolation of antifungal metabolites from C. globosum

Erlenmeyer flasks (1 L) containing 200 mL potato dextrose broth (PDB; Becton and Dickinson Co., MA, USA) were autoclaved at 121 °C for 15 min and then inoculated with mycelium plugs from a 5-day-old culture of *C. globosum* F0142 on PDA. The flasks were incubated for 2 weeks on a rotary shaker at 150 rpm and 25 °C. After filtration through Whatman No. 2 filter paper, 8 L of culture filtrate were extracted three times with equal volumes of ethyl acetate, and the ethyl acetate layer was concentrated to dryness. The organic layer showed in vivo antifungal activity against tomato late blight caused by *P. infestans*. The residue (7.3 g) was loaded onto a silica gel column (5 cm i.d. × 60 cm) containing 500 g of Kiesel gel 60 (70–230 mesh; E. Merck, Darmstadt, Germany). The column was successively eluted with 800 mL each of *n*-hexane, dichloromethane, diethyl ether, ethyl acetate, and methanol. The fractions eluted with diethyl ether and ethyl acetate, which were active, were pooled and concentrated to dryness. The active residue (5.1 g) was loaded onto a silica gel column [200 g Kiesel gel 60 (230–400 mesh), 3.6 cm i.d. × 60 cm] that was then eluted with chloroform:methanol (9:1, v/v). The fractions were monitored by TLC (Kiesel gel GF 254, 0.25-mm film thickness; Merck) and reduced to one active fraction, F1 (1.6 g). The F1 fraction was subjected to silica gel column chromatography [150 g Kiesel gel 60 (230–400 mesh), 3.6 cm i.d. × 60 cm, *n*-hexane–ethyl acetate (2:1, 1:1, 1:5, v/v)] to yield 280 mg of compound **1** and 640 mg of compound **2**.

2.3. Identification of metabolites

The structures of the two metabolites isolated from a liquid culture of *C. globosum* F0142 were determined by spectroscopic analysis. Mass spectra were recorded on a double-focusing high-resolution (HR) mass spectrometer (JEOL JMS-DX303; JEOL Ltd., Tokyo, Japan). ^{1}H NMR, ^{13}C NMR, DEPT 135°, ^{1}H–^{13}C COSY, and ^{1}H–^{1}H COSY spectra were recorded in $CDCl_3$ on a Bruker AMX-500 (500 MHz) NMR spectrometer (Bruker Analytische Messtechnik GmbH, Rheinstetten, Germany). ^{1}H- and ^{13}C-NMR spectra were referenced to tetramethylsilane (TMS) and the solvent signal, respectively.

2.4. In vitro antifungal activity

Both antifungal metabolites were dissolved in dimethyl sulfoxide (DMSO) and tested for in vitro activity against nine plant fungi using a modified version of the antimicrobial bioassay of Nair et al. [15]. The microorganisms *Alternaria mali*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *M. grisea*, *Phytophthora capsici*, *P. infestans*, *Pythium ultimum*, and *Rhizoctonia solani* were used to determine the minimum inhibitory concentrations (MICs) of compound **1** (chaetoviridin A) and compound **2** (chaetoviridin B). Clarified V8 juice broth was used as the basal medium for *P. infestans*, and the other test fungi were incubated in potato dextrose broth. Aliquots of medium (1 mL) containing both metabolites at concentrations of 100, 33.3, 11.1, 3.7, or 1.2 μg/mL were pipetted into wells of a 24-well microtiter plate (Cell Wells; Corning Glass Works, Corning, NY) to determine the MICs against the fungal species. A mycelial suspension of *R. solani* and spore suspensions (10^5 spores/mL) of the other fungi were used as inocula in the test. Ten microlitre aliquots of suspension were added to each well. Control wells containing medium mixed with DMSO (10 μl/mL) were prepared. Five replicates of each concentration for each fungus were incubated at 20 °C for *P. infestans* and *B. cinerea* and 25 °C for the rest of the test fungi. The lowest concentrations of the antifungal metabolites that completely inhibited mycelial growth were defined as the MICs.

2.5. In vivo antifungal activity

Liquid cultures of *C. globosum* F0142, fractions obtained during the purification of the active substances, and the purified metabolites were tested for in vivo antifungal activity against six plant pathogenic fungi. *M. grisea*, which causes rice blast; *R. solani*, which causes rice sheath blight; *B. cinerea*, which causes tomato gray mold; *P. infestans*, which causes tomato late blight; *P. recondita*, which causes wheat leaf rust; and *Blumeria graminis* f. sp. *hordei*, which causes barley powdery mildew; were subjected to testing. The in vivo antifungal bioassays were performed as described [16,17]. All experiments were conducted twice, and the six estimates for each treatment were converted into the control percentage (±SD) as compared to the controls.

Table 1
Disease-control activities of *Chaetomium globosum* F0142 liquid culture filtrate against six plant diseases[a]

Dilution	Control value (%)[b]					
	RCB[c]	RSB	TGM	TLB	WLR	BPM
1-fold	96 ± 0	0	14 ± 0	99 ± 0.9	98 ± 2.4	0
3-fold	93 ± 1.2	0	10	38 ± 12	97 ± 1.2	0
9-fold	80 ± 3.2	0	0	0	98 ± 0.4	0
27-fold	30 ± 12	0	0	0	90 ± 1.0	0

[a] Seedlings were inoculated with spores or mycelial suspensions of the test organisms 1 day after various dilutions of liquid culture filtrate from *C. globosum* F0142 were sprayed on the leaves to run-off.

[b] Control value (%) = 100 × {disease severity of untreated plants − disease severity of treated plants}/disease severity of untreated plants. Each value represents the mean of six estimates ± SD.

[c] RCB, rice blast; RSB, rice sheath blight; TGM, tomato gray mold; TLB, tomato late blight; WLR, wheat leaf rust; BPM, barley powdery mildew.

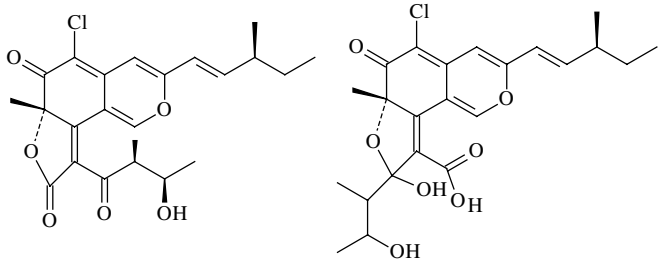
3. Results and discussion

The F0142 isolate was identified as *C. globosum* based on the criteria of Ellis [14]. The mean size of the ascomata was 330 × 221 μm. The ascomata were dark brown to black and globose to subglobose. The terminal hairs were branched, undulate, and flexuous. The asci were clavate and unitunicate, and the ascus walls were evanescent. The mean ascospore dimensions in culture were 10 × 7.6 μm, and the ascospores were dark brown, regular, and lemon-shaped with an apical germ pore and no septum.

A liquid culture of *C. globosum* F0142 showed potent antifungal activity against *M. grisea* and *P. recondita* on rice and wheat plants, respectively (Table 1). The culture suppressed the development of these diseases by more than 80% even when diluted 9-fold, and also exhibited moderate in vivo antifungal activity against *P. infestans* in tomato plants. However, the culture showed little to no activity against the other plant pathogenic fungi tested.

Two antifungal metabolites compound **1** (brown gum) and compound **2** (yellow gum), were purified from the extract of a filtrate (8 L) of a 14-day-old culture. The electron impact (EI) mass spectrum of compound **1** displayed a molecular ion at *m*/*z* 432 and major fragment ions at *m*/*z* values of 389, 345, 332, 316, 289, and 234. The EI mass spectrum of compound **2** exhibited an $[M–H_2O]^+$ ion at *m*/*z* 434 and major fragment ions at *m*/*z* 419, 400, 390, 355, 289, and 263. High-resolution EI mass spectrometry gave the molecular formula $C_{23}H_{25}O_6Cl$ for compound **1** and $C_{23}H_{27}O_6Cl$ for compound **2**. The interpretation of NMR data suggests that compound **1** and compound **2** are identical to chaetoviridins A and B (Fig. 1), respectively, which had been reported previously by Takahashi et al. [18].

Chaetoviridins containing a pyrone-quinone structure are usually called azaphilones because of the affinity of these compounds for ammonia, yielding vinylogous γ-pyridones. Many metabolites of this type, including sclerotiorin, monascorubrin, and monascoflavin, have been characterized from various fungi. Some of these fungal species have been used as colorants in foodstuffs and alcoholic beverages in certain Asian countries. Chaetoviridins were first characterized from *C. globosum* var. *flavo-viridae* [18]. Four chaetoviridins have been reported to date: A, B, C, and D. Little is known



A B

Fig. 1. Chemical structures of chaetoviridins A and B.

about the biological activities of the chaetoviridins. Chaetoviridin A inhibits tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mice [19]. Tomoda et al. [20] found that chaetoviridins A and B inhibit cholesteryl ester transfer protein. In addition, Takahashi et al. [18] have noted that Iwasaki (unpublished data) discovered that chaetoviridin has inhibitory activity against the growth of *Pyricularia oryzae* mycelia (Teleomorph; *M. grisea*).

The chaetoviridins isolated here inhibited, to varying degrees, the growth in amended PDB of mycelia of various plant pathogenic fungi (Table 2). Among the tested fungi, *M. grisea* and *P. ultimum* were the most sensitive to the two substances. Chaetoviridin A inhibited the growth of fungal mycelia more strongly than chaetoviridin B. Chaetoviridin A strongly inhibited the growth of *M. grisea* and *P. ultimum* mycelia at low concentrations in amended PDB, with MIC values of 1.23 μg/mL. Iwasaki (unpublished data), as cited by Takahashi et al. [18], found that chaetoviridin A inhibited the growth of *P. oryzae* mycelia at 2.5 μg/mL. However, to our knowledge, there has been no report of inhibition of the growth of mycelia of other fungi by chaetoviridins.

Table 2
Minimum inhibitory concentrations of chaetoviridins A and B isolated from *Chaetomium globosum* F0142 against the growth of plant pathogenic fungal mycelia in potato dextrose broth containing the individual chemicals

Plant pathogen	MIC value (μg/mL)[a]	
	Chaetoviridin A	Chaetoviridin B
Alternaria mali	33.3	>100
Botrytis cinerea	33.3	>100
Colletotrichum gloeosporioides	33.3	>100
Fusarium oxysporum	33.3	>100
Magnaporthe grisea	1.23	33.3
Phytophthora capsici	33.3	>100
Phytophthora infestans	33.3	>100
Pythium ultimum	1.23	33.3
Rhizoctonia solani	>100	>100

[a] Concentration that completely inhibits mycelial growth.

The in vivo efficacies of chaetoviridins A and B in the control of rice blast, rice sheath blight, tomato gray mold, tomato late blight, wheat leaf rust, and barley powdery mildew were evaluated under growth-chamber conditions (Table 3). Of the six plant diseases, both compounds most effectively inhibited the development of rice blast, caused by *M. grisea*, and wheat leaf rust, caused by *P. recondita*. Both compounds resulted in at least 88% control of rice blast when applied at concentrations greater than 62.5 μg/mL. Chaetoviridin A exhibited higher antifungal activity against wheat leaf rust than chaetoviridin B. Chaetoviridin A was also moderately active against *P. infestans* on tomato plants and weakly active against *B. cinerea* on tomato plants, whereas chaetoviridin B was not. Both compounds were virtually inactive against rice sheath blight, caused by *C. sasaki*, and barley powdery mildew, caused by *B. graminis* f. sp. *hordei*.

The present work shows that chaetoviridins A and B exhibit inhibitory activity against various plant pathogens and strong in vivo antifungal activity against rice blast and wheat leaf rust. The substances were found to be responsible for the in vivo antifungal activity of the liquid *C. globosum* F0142 culture. The production of antibiotics by *C. globosum* has been suggested as being important for the antagonistic activity of the fungus. Di Pietro et al. [8] suggested that chaetomin plays an important role in the biocontrol of *P. ultimum* by *C. globosum*. Aggarwal et al. [5] reported that four strains of *C. globosum* produced inhibition zones in *Cochliobolus sativus* cultures, and that the production of antifungal compounds by the isolates was positively correlated with the antagonism against *C. sativus* on wheat plants. However, the antifungal metabolites responsible were not characterized. A *C. globosum* F0142 ascospore suspension (7×10^5 spores/mL) applied onto foliage 1 day before inoculation with plant pathogens effectively suppressed the development of rice blast and wheat leaf rust (unpublished data), with control values of 95% and 93%, respectively. However, the

Table 3
Disease-control activities of chaetoviridins A and B isolated from *Chaetomium globosum* F0142 against six plant diseases[a]

Compound	Concentration (μg/mL)	Control value (%)[b]					
		RCB[c]	RSB	TGM	TLB	WLR	BPM
Chaetoviridin A	250	99 ± 0.3	20 ± 15	63 ± 9.3	87 ± 2.5	87 ± 2.5	0
	125	94 ± 1.1	20 ± 15	9 ± 17	50 ± 10	97 ± 0.3	0
	62.5	88 ± 5.2	0	0	0	83 ± 4.5	0
Chaetoviridin B	250	96 ± 2.1	0	3	0	91 ± 2.2	0
	125	96 ± 2.5	10 ± 13	20 ± 15	0	65 ± 5.7	0
	62.5	94 ± 1.2	10 ± 13	3 ± 5.7	0	0	0

[a] Seedlings were inoculated with spores or mycelial suspensions of the test organisms 1 day after solutions of each chemical were sprayed onto leaves to run-off.

[b] Control value (%) = 100 × {disease severity of untreated plants − disease severity of treated plants}/disease severity of untreated plants. Each value represents the mean of six estimates ± SD.

[c] RCB, rice blast; RSB, rice sheath blight; TGM, tomato gray mold; TLB, tomato late blight; WLR, wheat leaf rust; BPM, barley powdery mildew.

ascospore suspension was not active against rice sheath blight, tomato gray mold, tomato late blight, or barley powdery mildew. Thus, the in vivo antifungal spectrum of suspensions of *C. globosum* F0142 ascospores is similar to that of the chaetoviridins produced by the fungus. This result suggests that chaetoviridins A and B play an important role in the antagonism of *C. globosum* against several plant pathogens. Further studies will be necessary to obtain evidence for the production of chaetoviridins on foliage and to confirm their role in the activity of *C. globosum* F0142 against *M. grisea* and *P. recondita*.

Acknowledgment

This work was supported by a grant from the Bio-Green 21 Program of the Rural Development Administration of the Republic of Korea.

References

[1] Copping, L.G. and Menn, J.J. (2000) Biopesticides: a review of their action, applications and efficacy. Pest Manag. Sci. 56, 651–767.
[2] Istvan, U. (2002) Transforming natural products into natural pesticides – experience and expectations. Phytoparasitica 30, 439–442.
[3] Simmonds, M.S.J., Evans, H.C. and Blaney, W.M. (1992) Pesticides for the year 2000: mycochemicals and botanicals In: Pest Management and the Environment in 2000 (Kadir, A.S.A. and Barlow, J.S., Eds.), pp. 127–164. CAB International, Wallingford.
[4] Domsch, K.H. and Gams, W. (1972) Fungi in Agricultural Soils. Longman, London.
[5] Aggarwal, R., Tewari, A.K., Srivastava, K.D. and Singh, D.V. (2004) Role of antibiosis in the biological control of spot blotch (*Cochliobolus sativus*) of wheat by *Chaetomium globosum*. Mycopathologia 157, 369–377.
[6] Boudreau, M.A. and Andrews, J.H. (1987) Factors influencing antagonism of *Chaetomium globosum* to *Venturia inaequalis*: a case study in field biocontrol. Phytopathology 77, 1470–1475.
[7] Cullen, D. and Andrews, J.H. (1984) Evidence for the role of antibiosis in the antagonism of *Chaetomium globosum* to the apple scab pathogen, *Venturia inaequalis*. Can. J. Bot. 62, 1819–1823.
[8] Di Poetro, A., Gut-Rella, M., Pachlatko, J.P. and Schwinn, F.J. (1992) Role of antibiotics produced by *Chaetomium globosum* in biocontrol of *Pythium ultimum*, a causal agent of damping-off. Phytopathology 82, 131–135.
[9] Hubbard, J.P., Harman, G.E. and Eckenrode, C.J. (1982) Interaction of a biological control agent, *Chaetomium globosum* with seed coat microflora. Can. J. Microbiol. 29, 431–437.
[10] Kommedahl, T. and Chang Mew, I. (1975) Biocontrol of corn root infection in the field by seed treatment with antagonists. Phytopathology 65, 296–300.
[11] Tveit, M. and Wood, R.K.S. (1955) The control of *Fusarium* blight in oat seedlings with antagonistic species of *Chaetomium*. Ann. Appl. Biol. 43, 538–552.
[12] Vannacci, G. and Harman, G.E. (1987) Biocontrol of seed-borne *Alternaria raphani* and *A. brassicicola*. Can. J. Microbiol. 33, 850–856.
[13] Walter, D. and Gindrat, D. (1988) Biological control of damping-off of sugarbeet and cotton with *Chaetomium globosum* or a fluorescent *Pseudomonas* sp. Can. J. Microbiol. 34, 631–637.
[14] Ellis, M.B. (1971) Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Surrey, England.
[15] Nair, M.G., Mishra, S.K. and Putnam, A.R. (1992) Antifungal anthracycline antibiotics, spartanamicins A and B from *Micromonospora* spp. J. Antibiot. 45, 1738–1745.
[16] 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.
[17] Kim, J.-C., Choi, G.J., Lee, S.-W., Kim, J.-S., Chung, K.Y. and Cho, K.Y. (2004) Screening for antifungal extracts against various plant pathogenic fungi and control of powdery mildew with extracts of *Achyranthes japonica* and *Rumex crispus*. Pest Manag. Sci. 60, 803–808.
[18] Takahashi, M., Koyama, K. and Natori, S. (1990) Four new azaphilones from *Chaetomium globosum* var. *flavo-viridae*. Chem. Pharm. Bull. 38, 625–628.
[19] Yasukawa, K., Takahashi, M., Natori, S., Kawai, K., Yamazaki, M., Takeuchi, M. and Takido, M. (1994) Azaphilones inhibit tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mice. Oncology 51, 108–112.
[20] Tomoda, H., Matsushima, C., Tabata, N., Namatame, I., Tanaka, H., Bamberger, M.J., Arai, H., Fukazawa, M., Inoue, K. and Omura, S. (1999) Structure-specific inhibition of cholesteryl ester transfer protein by azaphilones. J. Antibiotic. 52, 160–170.