Mycobiology

Research Article

Biological Control of Aphid Using Fungal Culture and Culture Filtrates of *Beauveria bassiana*

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Abstract Aphids are one of the most destructive pests in crop production such as pepper, cucumber, and eggplants. The importance of entomopathogenic fungi as alternative pest control agents is increasing. Conidia of entomopathogenic fungi are influenced by environmental conditions, such as temperature and relative humidity, and cause slow and fluctuating mortality. These factors have prevented wider application and use of biocontrol agents. For investigation of means of mitigation of such problems, we conducted bioassays with 47 fungal culture filtrates in order to evaluate the potential of secondary metabolites produced by entomopathogenic fungi for use in aphid control. Among 47 culture filtrates cultured potato dextrose broth, filtrate of *Beauveria bassiana* Bb08 showed the highest mortality (78%) against green peach aphid three days after treatments. Filtrate of Bb08 cultured in Adamek's medium showed higher toxicity as 100% to third instar nymphs of the aphid compared with seven other filtrates cultured in different broths amended with colloidal chitin or oil. The culture filtrates and fungal cultures from media amended with colloidal chitin or oil had lower control efficacies than filtrates without these additives in three different media. These results indicate that the fungal culture fluid or culture filtrate of *B. bassiana* Bb08 cultured in Adamek's medium has potential for development as a mycopesticide for aphid control.

Keywords *Beauveria bassiana*, Culture filtrate, Green peach aphid, Microbial control, *Myzus persicae*

Aphids are one of the most destructive pests in crop production such as pepper, cucumber, and eggplant [1]. The importance of entomopathogenic fungi as alternative pest control agents is increasing. Twenty eight mycopesticides using seven species of entompathogenic fungi, such as *Beauveria bassiana* and *Lecanicillium* spp., are commercially registered in several countries, including the United Kingdom and the United States, for control of aphids [2, 3]. These mycopesticides mainly use propagules such as conidia, blastospores, or hyphae. These propagules have advantages of direct killing of the target pest as well as secondary infection by horizontal transmission of spores from mycosised cadavers [4]. Conidia of entomopathogenic fungi are influenced by environmental conditions, such as temperature and relative humidity, and are slow in causing mortality. These factors have prevented wider application and use of these biocontrol agents.

Many entomopathogenic fungi produce metabolic compounds that may be toxic to insects [5]. Production of metabolites and their control efficacy against mosquitoes differed among fungal isolates and culture media or media composition [6]. Culture filtrates of entomopathogenic fungi such as *Lecanicillium lecanii* and *B. bassiana* reduce aphid survival rates [7-9] and deter feeding by whitefly and larva of *Spodoptera littoralis* [10, 11]. These culture filtrates may include various enzymes, such as protease, chitinase, and lipase, which are important in the process of infection by conidia. These enzymes can be induced by additives such as colloidal chitin in culture media [7]. Filtrate from cultures in broth amended with chitin or colloidal chitin showed higher toxicity to insects due to induced chitinase [6, 12, 13]. However, insecticidal activity of culture extracts of an isolate of entomopathogenic fungus (*Metarhizium anisopliae*) was not influenced by media composition [10]. Production of toxin, particularly destruxin, differs according to fungal isolate, culture composition, and pH [14]. Consequently, culture extracts or filtrates may contain secondary metabolites or compounds having different insecticidal activity.

The type and concentration of a compound may vary according to fungal isolate, composition of culture media, and culture condition. Because both spores and compounds are killing factors, whole fungal cultures that include spores

Mycobiology 2013 December, **41**(4): 221-224
http://dx.doi.org/10.5941/MYCO.2013.41.4.221
pISSN 1229-8093 • eISSN 2092-9323

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Received November 5, 2013
Revised November 18, 2013
Accepted November 22, 2013

may have higher efficacy and consistency. Therefore, use of culture filtrates or fungal culture including both spores and secondary metabolites may increase the speed of killing compared to conidia or spores only. In this study, we conducted bioassays in order to select an isolate that produces compounds or metabolites that are toxic to the green peach aphid (*Myzus persicae* Sulzer) and to find culture media that can maximize such an effect.

MATERIALS AND METHODS

We cultured 47 entomopathogenic fungal isolates (three isolates of *Isaria* spp., three isolates of *Lecanicillium* spp., 20 isolates of *Beauveria bassiana s.l.*, and 21 isolates of *Cordeceps* spp.) originating from various insect species or soil samples in South Korea. A culture plug (1 cm) of each isolate cultured for 10 days on potato dextrose agar was inoculated into 50 mL potato dextrose broth (PDB) in 150 mL Erlenmeyer flasks and cultured at 25 ± 1°C and 200 rpm for 10 days. During the culture period, 10 mL of culture fluid was collected on the third, fifth, seventh, and tenth day and filtered through a Whatman No. 2 filter paper and a syringe filter (0.2 μm, Cellulose acetate units; Advantec MFS, Dublin, CA, USA) for removal of spores from each culture period. Culture filtrates were kept in the refrigerator until bioassay.

Groups of 20 green peach aphids (*M. persicae*) were dipped into 1 mL culture filtrate in a microtube for 15 sec, then poured onto filter paper for removal of excess solution and transferred onto a Chinese cabbage leaf disc (5 cm diameter) in a 6 cm Petri dish containing a damp filter paper on the bottom. Mortality was recorded daily for five days. This bioassay was conducted twice.

For comparison of aphid control efficacy between culture filtrate and fungal culture cultured in different media, *B. bassiana* Bb08, which demonstrated high mortality with culture extract against green peach aphid, was cultured for three days in eight different culture media: PDB (pH 5.20), Adamek's media (AD, g/L; 40 g glucose, 40 g yeast extract, 30 g corn steep liquor, pH 5.06), Adamek's media plus 1% colloidal chitin (AD + 1% Chi, pH 5.07), SU media (g/L; 30 g corn steep powder, 20 g corn meal, 20 g rice bran, pH 4.74), SU plus 1% colloidal chitin (SU + 1% Chi, pH 4.74), SU plus 5% colloidal chitin (SU + 5% Chi, pH 4.74), chitin broth (CB, g/L; 1 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4$, 0.01 g $FeSO_4$, 10 g colloidal chitin, 0.02 g yeast extract, pH 7.22), and CB plus 1% corn oil (CB + 1% corn oil, pH 7.24). Culture conditions were the same as described above. Fifty milliliters of fungal culture was filtered through sterilized cheese cloths (thereafter called "fungal culture"). Half of

Table 1. Cumulative mortality of green peach aphids three days after treatments with culture filtrates and 50% lethal time of 47 entomopathogenic fungi, including three isolates of *Isaria* spp. (Pf), three isolates of *Lecanicillium* spp. (L), 20 isolates of *Beauveria bassiana* (Bb), and 21 isolates of *Cordeceps* spp. (C)

Isolates	Cumulative mortality at 3rd day (%)	LT_{50} (days)	Isolates	Cumulative mortality at 3rd day (%)	LT_{50} (days)
Control	11.6 ± 3.2 bc	-	C41756	16.6 ± 4.2 bc	6.1 ± 1.6 ab
Pf04	42.5 ± 13.0 abc	3.8 ± 0.9 b	C43314	26.3 ± 6.4 bc	3.2 ± 0.2 b
Pf59	45.0 ± 1.0 abc	3.1 ± 0.0 b	C43318	8.7 ± 5.5 c	4.1 ± 1.0 b
Pf109	57.8 ± 7.6 ab	2.8 ± 0.1 b	C43321	13.7 ± 3.9 bc	3.8 ± 0.7 b
L14	15.9 ± 9.7 bc	3.0 ± 1.1 b	C43322	18.2 ± 7.5 bc	4.4 ± 1.3 b
L18	26.3 ± 11.5 bc	4.1 ± 0.8 b	C43324	20.6 ± 7.1 bc	7.5 ± 3.4 ab
L625	22.3 ± 4.5 bc	4.0 ± 0.8 b	C43330	24.0 ± 7.1 bc	5.1 ± 1.4 b
Bb04	51.9 ± 8.2 abc	2.9 ± 0.1 b	C43331	18.3 ± 6.8 bc	3.2 ± 0.3 b
Bb05	28.0 ± 6.3 bc	9.3 ± 6.1 ab	C43333	41.5 ± 17.7 abc	5.1 ± 1.4 b
Bb07	37.3 ± 10.1 abc	3.5 ± 0.6 b	C50001	17.7 ± 2.9 bc	4.5 ± 0.8 b
Bb08	78.6 ± 10.7 a	2.7 ± 0.3 b	C50064	16.5 ± 8.8 bc	11.7 ± 5.5 ab
Bb11	51.0 ± 6.9 abc	3.0 ± 0.3 b	C50171	17.4 ± 3.5 bc	4.6 ± 1.5 b
Bb12	24.2 ± 10.3 bc	5.0 ± 1.4 b	C50810	17.0 ± 9.1 bc	17.5 ± 6.6 a
Bb14	14.5 ± 6.8 bc	4.4 ± 0.9 b	C51070	13.8 ± 9.0 bc	4.3 ± 0.9 b
Bb16	17.8 ± 4.9 bc	4.8 ± 1.2 b	C51923	11.1 ± 6.7 bc	4.9 ± 0.8 b
Bb17	24.4 ± 1.2 bc	4.1 ± 0.9 b	C51978	22.2 ± 8.2 bc	3.5 ± 0.7 b
Bb18	35.4 ± 9.8 abc	5.9 ± 1.2 ab	C51979	15.8 ± 6.3 bc	3.5 ± 1.0 b
Bb21	14.6 ± 0.8 bc	9.8 ± 6.4 ab	C51994	12.2 ± 4.0 bc	4.8 ± 0.9 b
Bb23	16.7 ± 10.2 bc	4.0 ± 1.0 b	C52193	24.7 ± 13.8 bc	6.7 ± 3.6 ab
Bb24	28.3 ± 3.3 bc	3.6 ± 0.3 b	C52231	17.4 ± 9.0 bc	4.1 ± 0.7 b
Bb25	35.4 ± 10.0 abc	3.6 ± 0.6 b	C52254	29.7 ± 10.9 bc	4.2 ± 0.6 b
Bb26	41.9 ± 10.9 abc	4.2 ± 0.9 b	Bb34	22.2 ± 6.2 bc	3.5 ± 0.5 b
Bb27	35.3 ± 4.8 abc	4.3 ± 0.1 b	Bb35	22.0 ± 4.2 bc	3.4 ± 0.4 b
Bb33	23.6 ± 4.4 bc	3.9 ± 0.6 b	Bb36	48.7 ± 13.1 abc	4.6 ± 0.7 b

The fungi were cultured in potato dextrose broth and aphids were dipped in 1 mL culture filtrate for 15 sec.

[a]Different letters in the same column are significantly different ($p < 0.05$, Tukey's honestly significant difference [HSD] test).

the filtrate (25 mL) was kept in a refrigerator for less than 24 hours for bioassay. The other half was further filtered through a Whatman No. 2 filter paper and a syringe filter for removal of spores and hyphae (thereafter called "culture filtrate"). The fungal culture and culture filtrate were used for bioassay within 24 hr after filtration. For the bioassay, leaf discs infested with 20 third instar nymphs of green peach aphid were sprayed with the filtrates or cultures. The 1 mL solution was applied to each side of the leaf disc using a Plexiglas spray box fitted with a polyvinyl acetal cone nozzle and connected to a vacuum pump fixed at 100 kPA. Mortality was recorded daily for three days. This bioassay was conducted three times and three leaves were treated during each assay.

Two-way analysis of variance (PROC GLM, SAS OnlineDoc® 9.1.3.; SAS Institute Inc., Cary, NC, USA) [15] was used for comparison of mortality among fungal culture filtrates and the control efficacy of fungal culture and culture filtrate in different media. Median lethal time (LT_{50}) was estimated using the LIFEREG procedure and data were fitted to a Weibull distribution (SAS OnlineDoc® 9.1.3.). Differences in mortality in each fungal culture filtrate and in control efficacy of fungal culture and culture filtrate were compared using Tukey's studentized range (honestly siginificant difference [HSD]) test ($\alpha \leq 0.05$).

RESULTS

Culture extracts based on different entomogenous fungal isolates showed different control efficacy to green peach aphids. Among 47 culture filtrates from 47 entomogenous fungal isolates cultured in PDB, *B. bassiana* Bb08 filtrate showed the highest mortality of 79% in green peach aphids three days after treatment ($F = 3.08$; $df = 47, 96$; $p < 0.0001$) (Table 1). *Isaria* isolates showed comparatively higher mortality at 42~58% than *Cordyceps* at 8.7% to 41.7%. Fifty percent lethal times (LT_{50}) were also lower in isolate Bb08 (2.7 days) compared to Bb04 (2.9 days) and C50810 (15.5 days) ($F = 1.53$; $df = 46, 94$; $p = 0.0421$). Mortality with fungal culture of AD was significantly higher at 100% three days after treatment compared with 80% in SU media and 20% in CB media ($F = 19.29$; $df = 8, 17$; $p < 0.0001$) (Fig. 1A). Mortality with culture filtrate was also higher in AD three days after treatment compared with other culture filtrates ($F = 341.41$; $df = 8, 17$; $p < 0.0001$) (Fig. 1B). Mortality with fungal culture cultured in AD medium and AD + 1% Chi medium was much higher at 91.7% and 91.7% on the second day after treatment compared with 35.8% and 23.3% with culture filtrate that spores were removed, respectively. Control efficacies by fungal culture in other media were also slightly higher than those by culture filtrates.

Mortalities with filtrates from cultures amended with colloidal chitin or oil were lower than those for filtrates from cultures without addition of colloidal chitin or oil. Mortality in AD medium was 99% compared to 88% in AD + 1% Chi broth. Control effect with filtrate of SU medium was 48% compared with 33% in SU + 1% Chi and 23% in SU + 5% Chi. After cultivation in each media for three days, pH in fungal cultures and culture filtrates did not show much of a change, such as 5.53 and 5.77 in PDB, 5.31 and 5.27 in AD, 5.16 and 5.06 in AD + 1% Chi, 4.80 and 4.76 in SU, 4.76 and 4.76 in SU + 1% Chi, 4.78 and 4.68 in SU + 5% Chi, 7.49 and 7.56 in CB, and 6.53 and 6.80 in CB + 1% corn oil, respectively.

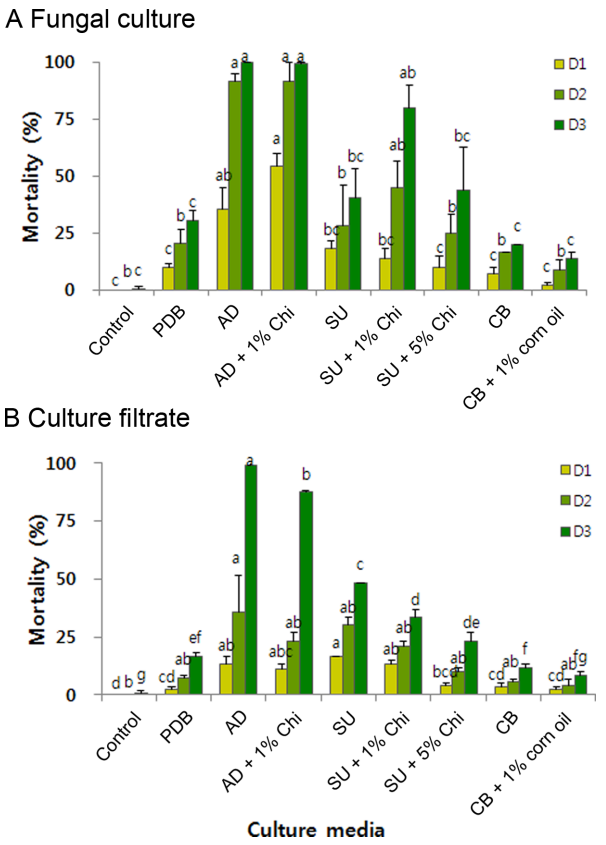

Fig. 1. Comparison of control efficacy of third instar nymphs of green peach aphids between fungal culture filtered through sterilized cheese cloths (A) and culture filtrate filtered through cheese cloths, Whatman No. 2 filter paper, and syringe filter (B) of *Beauveria bassiana* Bb08 cultured in different media. Columns with different letters for the same day are significantly different ($p < 0.05$, Tukey's studentized range [honestly significant difference, HSD] test). PDB, potato dextrose broth; AD, Adamek's media; Chi, colloidal chitin; CB, chitin broth.

DISCUSSION

Among 47 culture filtrates cultured in PDB, filtrates of *B. bassiana* Bb08 showed the highest mortality against the green peach aphid. Culture filtrates from AD had higher toxicity to third instar nymphs of the aphid compared with seven other filtrates with/without colloidal chitin or oil. The culture filtrates from media containing added colloidal chitin or oil had lower control efficacy than filtrates without

these additives in three different media. Fungal culture, including spores, also showed the same trends of aphid control efficacy as those of the filtrates.

Culture filtrates or extracts of entomogenous fungi have shown various effects toward insect pests, such as insecticidal or feeding deterrence. Crude protein extracts from 25 fungal isolates, including *M. anisopliae* and *B. bassiana* cultured in AD exhibited different toxicity to moth larvae and lost its activity after exposure to high temperature and protease [10]. Crude toxin extracted from *L. lecanii* killed eggs, nymphs, and adults of sweet potato whitefly and also showed antifeedant activity [11]. The supernatant of an isolate of *B. bassiana* contained chitinase and degraded haemocoel of cotton aphids [7]. Toxicity of culture filtrates or extracts of entomopathogenic fungi (*M. anisopliae, Chrysosporium lobatum*) to insect pests differed depending on composition of culture media or additives [6, 12]. Filtrates of fungal isolates showed different enzyme activities, such as chitinase, protease and lipase, and the enzyme activities showed correlation with their aphicidal effects [9]. When *M. anisopliae*, *C. labatum*, and *Trichoderma harzianum* were cultured in broth amended with chitin or colloidal chitin, the culture filtrate exhibited high toxicity to insects such as mosquitos and cotton bollworm [6, 12, 13], suggesting that the filtrate might contain chitinase induced by chitin. However, the culture filtrate of *B. bassiana* Bb08 did not show increased mortality in filtrates cultured in colloidal chitin amended broths, which may suggest that this isolate did not induce chitinase into the chitin amended broth during culture for three days and did not influence the mortality. Differences in mortality between fungal culture and culture filtrate one and two days after treatment may be caused by the presence or absence of spores in the culture fluid. Similar mortality between culture filtrate and fungal culture three days after treatment suggests that the isolate may produce toxic compounds as major aphicidal agents instead of enzymes like chitinase. We will determine which compound in the filtrate can influence mortality, as well as the reason for low control efficacy in the culture filtrate from media amended with chitin or oil. We are considering use of the *B. bassiana* Bb08 isolate for development of microbial or biochemical pesticide for control of green peach aphid.

ACKNOWLEDGEMENTS

This study was supported by the research grant (PJ008980012013) of the Rural Development Administration (RDA) of Korea. We would like to thank Dr. Mark Goettel, formerly of the Lethbridge Research Centre, AAFC, Canada, for his critical review of the manuscript.

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