Mycobiology

Research Article

Enhancing the Thermotolerance of Entomopathogenic *Isaria fumosorosea* SFP-198 Conidial Powder by Controlling the Moisture Content Using Drying and Adjuvants

Jae Su Kim[1,*], Se Jin Lee[1] and Hyang Burm Lee[2]

[1]*Department of Agricultural Biology, College of Agriculture & Life Sciences, Chonbuk National University, Jeonju 561-756, Korea*
[2]*Department of Biological Chemistry, College of Agriculture & Life Sciences, Chonnam National University, Gwangju 500-757, Korea*

Abstract Entomopathogenic fungi are promising pest-control agents but their industrial applicability is limited by their thermosusceptibility. With an aim to increase the thermotolerance of *Isaria fumosorosea* SFP-198, moisture absorbents were added to dried conidial powder, and the relationship between its water potential and thermotolerance was investigated. Mycotized rice grains were dried at 10°C, 20°C, 30°C, and 40°C and the drying effect of each temperature for 24, 48, 96, and 140 hr was determined. Drying for 48 hr at 10°C and 20°C reduced the moisture content to < 5% without any significant loss of conidial thermotolerance, but drying at 30°C and 40°C reduced both moisture content and conidial thermotolerance. To maintain thermotolerance during storage, moisture absorbents, such as calcium chloride, silica gel, magnesium sulfate, white carbon, and sodium sulfate were individually added to previously dried-conidial powder at 10% (w/w). These mixtures was then stored at room temperature for 30 days and subjected to 50°C for 2 hr. The white carbon mixture had the highest conidial thermotolerance, followed by silica gel, magnesium sulfate, and then the other absorbents. A significant correlation between the water potential and conidial thermotolerance was observed in all conidia-absorbent mixtures tested in this study ($r = -0.945$). Conidial thermotolerance in wet conditions was evaluated by adding moisturized white carbon (0~20% H_2O) to conidia to mimic wet conditions. Notably, the conidia still maintained their thermotolerance under these conditions. Thus, it is evident that conidial thermotolerance can be maintained by drying mycotized rice grains at low temperatures and adding a moisture absorbent, such as white carbon.

Keywords *Isaria fumosorosea*, Moisture absorbent, Thermotolerance, Water potential, White carbon

Entomopathogenic fungi are promising biological control agents in integrated pest management against agriculturally harmful pests. Several fungal species including *Beauveria bassiana* (Bals.) Vuillemin, *Metarhizium anisopliae* (Metsch.), Sorokin, and *Isaria fumosorosea* (Wize) have been registered in the United States Environmental Protection Agency and commercialized [1]. These fungal products, based on conidia, constitute only a small percentage of the total insecticide market [2], due to their high variation in control efficacy and short shelf life [3]. In the past decade, efforts have been made to improve their shelf life; biopesticides for successful commercial products require 12~18 months shelf life but most products have only 6~12 months at room temperature [4, 5].

Long-term persistence of conidia in unfavorable environments (e.g., high temperature) can be attained by careful manipulation of solid culture methods [6, 7]. In solid cultures, one approach is the use of modified culture media for producing thermotolerant conidia, by adding constituents based on the correlation of fungal thermotolerance with the endogenous accumulation of polyols and trehalose in fungi [8, 9] or the alteration of cell wall lipid compositions in yeast or bacteria [10].

Drying of conidia is another approach during the downstream culturing process to enable storage without loss of viability in both *Beauveria* and *Metarhizium* [11, 12]. An important step in developing entomopathogenic

Mycobiology 2014 March, **42**(1): 59-65
http://dx.doi.org/10.5941/MYCO.2014.42.1.59
pISSN 1229-8093 • eISSN 2092-9323

*Corresponding author
E-mail: jskim10@jbnu.ac.kr

Received October 1, 2013
Revised December 20, 2013
Accepted January 6, 2014

fungal insecticides is the optimization of post-harvest handling of the conidia. Both pre-drying the conidia and keeping them dry are critical for long term storage at room temperature (25~32°C) [13]. After storage for 98 days at room temperature, ~76% of the conidia pre-dried with silica gel were viable compared with conidia dried without silica gel. Moore *et al.* [14] found that dried conidia of *M. anisopliae* var. *acridum* are prone to damage, upon direct exposure to water. A short equilibration in a saturated atmosphere, prior to immersion in water, was also reported to maintain the viability and infectivity of the conidia. Hong *et al.* [15] demonstrated that slow desiccation enhanced the survival of *M. flavoviride*, which implied that the rate of drying influenced conidial viability. In addition, Magalhães and Boucias [16] reported that pre-dried *M. anisopliae* var. *acridum* (isolate CG423) exhibited higher viability than un-dried conidia.

The shelf life of conidia can be increased by modifying the formulation and the ingredient composition of the media. Addition of an oil-based suspension concentrate results in improved shelf life of the products made with *M. anisopliae* conidia and can maintain their viability for extended periods [17]. A plausible reason behind this improvement is that oil prevents direct exposure to moisture, as direct contact with even a small amount of water can significantly reduce the viability of conidia, thus terminating their dormant state [18]. Isoparaffinic hydrocarbon solvents, such as paraffin and mineral oil have been used as carriers for oil-based formulations. Vegetable oils are also suitable for storing *M. anisopliae* and *B. bassiana* [19]. For example, Naturalis-L (*B. bassiana*) and Green Muscle (*M. acridum*) were formulated using soybean oil as a carrier [3].

Although efforts have been made to commercialize fungi in oil-based formulations, many are still formulated as wettable powders (WPs) due to the high production costs of oil-based formulations. The oil-based formulations require a low temperature homogenization and often need to solve caking, which results in nozzle clogging during spraying. Many WP products have been developed, such as BotaniGard (BioWorks), Mycotrol (Koppert), and Boverin (Biodron) based on *B. bassiana*, Betel (Natural Plant Protection) based on *Beauveria brongniartii*, Vertalec (Koopert) based on *Lecanicillium longisporum*, Biogreen (Becker Underwood) based on *Metarhizium flavoviride*, and PreFeRal (Biobest) and Priority (T. Stanes & Company) based on *I. fumosorosea* [20]. Information on the development and industrialization of WPs using fungi such as *B. bassiana* and *M. anisopliae* is available, while not much is known on the persistence of *I. fumosorosea* conidia in WP formulations [3]. To increase the thermotolerance of *I. fumosorosea* SFP-198 (KCTC 0499BP) [21], which was developed as a mycopesticide by Dongbu Hannong, we investigated the relationship between conidial thermotolerance and water potential using a mixture of conidia and moisture absorbents.

MATERIALS AND METHODS

Solid culture. *I. fumosorosea* SFP-198 was developed as a mycopesticide by Dongbu Hannong in Korea to control greenhouse pests [22]. The fungus was propagated on quarter strength Saubraud dextrose agar (1/4 SDA) (pH 6.0) at 25 ± 1°C in the darkness for 14 days as a seed culture for this study [23]. SFP-198 conidia were produced on rice grains in polyethylene bags using a modified protocol based on previously reported methods [18, 24]. First, 500 g of rice (variety: Chuchung) were placed in a polyethylene bag (60 cm × 30 cm) and then 250 mL of distilled water containing 0.4 mL (50% [w/v] stock solution)/L citric acid was added to the bag. Three bags were prepared and were placed at 90 ± 2°C in a water bath for 1 hr, followed by autoclaving at 121°C for 30 min. After cooling to ambient temperature, each bag was inoculated with a 5-mL aliquot from a liquid culture of the isolate. The inoculum was produced in 75 mL of potato dextrose broth (Difco, Sparks, MD, USA) in a 250-mL baffled flask held on a rotary shaker (180 rpm) at 25 ± 1°C for 3 days. All inoculated bags were shaken for approximately 1 min to ensure complete distribution of the inoculum throughout the rice grains. Bags were held at 25 ± 1°C in the dark for 3 wk. Mycotized rice grains were then harvested, and the number of conidia and their germination rates were determined on 1/4 SDA medium [5].

Drying of conidia. To investigate the effects of the temperature and time of drying on conidial thermotolerance, 5 g mycotized rice grain was placed in a plastic Petri dish (9 cm diam.) without lid, and the dish was placed in an incubator. To assess the effect of different temperatures, the dishes were incubated at each temperature (10°C, 20°C, 30°C, and 40°C) (blowing condition: 0.4 m/sec). Total 15 dishes were incubated at each temperature, and three replicates were maintained for each drying time. All dishes were kept at each temperature for 24, 48, 96, and 140 hr. Mycotized rice grain samples (2 g/sample) were randomly taken and the moisture content was measured using a moisture analyzer (Sartorius-Omnimark, Arvada, CO, USA). The mycotized rice grains, which were not subjected to drying and placed in ambient room conditions (~25°C and ~45% relative humidity [RH] without blowing), served as drying control.

Thermotolerance assay. The conidia are often subjected to unexpected thermal stresses during mass production and hence we used a drying period of 140 hr for this assay, which is much longer than the optimal drying time of 48 hr. Here, to investigate the influence of drying temperature on thermotolerance, mycotized rice grains (2 g/sample) were dried at different temperatures in Petri dishes with lids and placed in an incubator at 50°C for 2 hr. Three mycotized grains were randomly selected and added to 1 mL of 0.02% (v/v) sterile siloxane solution (Silwet L-77;

Loveland Industries Co., Greeley, CO, USA) and vortexed for 30 sec to make a conidial suspension. This was replicated three times. A conidial suspension of 5 μL was dropped on to the 1/4 SDA medium and held at 20 ± 1°C in darkness for 24 hr. Percent germination was assessed by randomly counting 100 conidia under 400× magnification. The presence of a visible germ tube denoted a germinated conidium.

Bioassay against *Trialeurodes vaporariorum*. Following thermal exposure, the control efficacy of conidia against greenhouse whitefly (GWF) nymphs in tomatoes was investigated in glasshouse conditions. Fresh five-leaf stage tomatoes, *Lycopersicon esculentum* M., in a transparent plastic chamber (90 × 30 × 45 cm) with whitefly-proof mesh, were randomly infested with 5 GWF adults which were grown in an insectary per plant. The chambers were held at 25 ± 2°C and a 16 : 8 (L/D) photoperiod with 40 ± 5% RH in a glasshouse. Tomatoes were considered ready to use for virulence tests when second instar GWF nymphs were observed (30~40 nymphs/plant). Before application, the number of second instar per plant was counted, and all adults were removed from the test plants. Conidial suspensions were adjusted to 1×10^6 conidia/mL, and water treatment served as a non-treated control. The suspensions (20 mL/plant) were sprayed on both sides of the leaves with the same number of spray per side. Applications were conducted in a spray booth equipped with a turntable (65 cm diam., 15 rpm) and a hand sprayer (Gardena 864; Gardena Co., Ulm, Germany) adjusted to a constant pressure (12 psi). After spraying, each plant was covered with a whitefly-proof mesh and transferred to a glasshouse (25 ± 2°C and 16 : 8 [L/D] photoperiod with 40 ± 5% RH). Plants were arranged in a completely randomized design. The numbers of live GWF nymphs per plant were counted before application (N_0) and at 7 day post-application (N_t) and % live population was calculated as follows: $(N_t/N_0) \times 100\%$. GWF nymphs were scored for mortality based on their size. The size of the nymphs and the degree of hyphal coverage and/or fungal growth on their exocuticles in the treated plants were also compared with those in the non-treated controls. Each treatment was replicated three times (three plants) within an experimental replicate.

Storage of conidia and moisture absorbents. Moisture absorbents were added to the conidial powder, which was separated from mycotized rice grains dried at 20°C for 48 hr. The mixtures were kept at room temperature (~25°C) for 30 days to investigate their effects on conidial thermotolerance during storage. A 0.9-g of conidial powder was mixed with 0.1 g of each moisture-absorbent as follows: calcium chloride, silica gel, magnesium sulfate, white carbon, and sodium sulfate (all from Daejung Chemical, Goryung, Korea). The mixture was placed in an aluminum bag (10 × 12 cm^2) and sealed for storage. In each moisture absorbent treatment, six bags were prepared by mixing for about one min, and before storage 3 bags were exposed to 50°C for 2 hr, and the conidia were subjected to a germination test as described above. After 30 days of storage at room temperature (25 ± 1°C), the stored bags were subjected to the same thermal exposure to examine conidial germination rates. Conidial powder without moisture absorbent served as a control. Before storage, the mixture of conidia and moisture absorbent was measured using a water activity meter (AquaLITE; Aqualab Ltd., Pullman, WA, USA), and the data were converted to water potential [25]. This measurement was replicated three times for each treatment.

Storage of conidia and white carbon mix. To investigate the moisture-absorbing potential of white carbon, white carbon powder was mixed with various quantities of distilled water before mixing it with conidial powder. The mixtures were then stored at room temperature (~25°C) for 30 days and subsequently exposed to thermal stress. White carbon (10 mL; equivalent to 0.1 g) was individually mixed for 1 min with 0.5, 1, 2, and 4 mL of distilled water in polyethylene bags, and then mixed with 0.9 g conidial powder harvested from mycotized grains (dried at 20°C for 48 hr). Each mixture was placed in an aluminum bag (10 × 12 cm^2) and sealed tightly for storage. After storage at room temperature for 30 days, bags were exposed to 50°C for 1, 2, 3, or 4 hr, and a germination test done was done as described above.

Data analysis. Data on the percentage water content, percentage germination, and water potential were analyzed using a two-way analysis of variance (ANOVA) and/or the general linear model. This was followed by Tukey's honestly significant difference for multiple comparisons. All analyses were conducted using SPSS ver. 17.1 (SPSS Inc., Chicago, IL, USA) at the 0.05 (α) level.

RESULTS

Influence of drying conditions on conidial thermotolerance. Water content of the conidia varied with drying temperatures: the water content reduced fast, when dried at higher temperature ($F_{3,40} = 315.0$, $p < 0.001$). At 48 hr, all grain samples had low moisture (Fig. 1A). After solid culturing, grains had ~53% water content. In the non-drying conditions, the mycotized grains still had ~24% water after 140 hr, but under drying conditions, the grains had < 5% moisture at 140 hr. The speed at which drying occurred was dependent on temperature. Drying at 40°C for 24 hr reduced the moisture content to < 5%, while 48 hr of drying was achieve the same reduction 10°C, 20°C, and 30°C.

When the dried mycotized rice grains were exposed to 50°C for 2 hr, the conidia that were dried at lower temperature conditions exhibited a higher conidial thermotolerance than those dried under higher temperature conditions ($F_{3,16} = 67.3$, $p < 0.001$) (Fig. 1B). Non-dried conidia showed ~96%

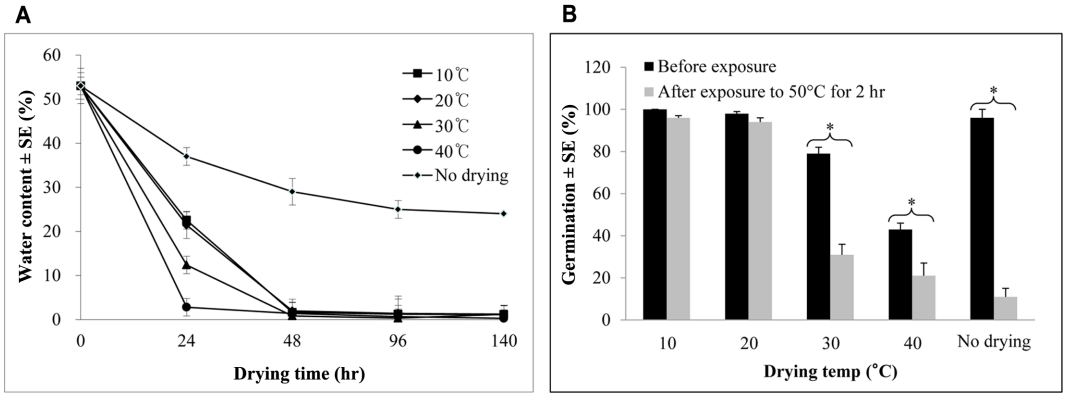

Fig. 1. Drying of mycotized rice grains at different temperatures and conidial thermotolerance. A, Moisture content (%) of mycotized rice grains incubated for 24, 48, 96, and 140 hr at each of 10°C, 20°C, 30°C, and 40°C; B, % Germination of differently dried conidia before and after exposure to 50°C for 2 hr. Asterisks indicate significant differences in the percentage germination data between the two groups, $p < 0.01$.

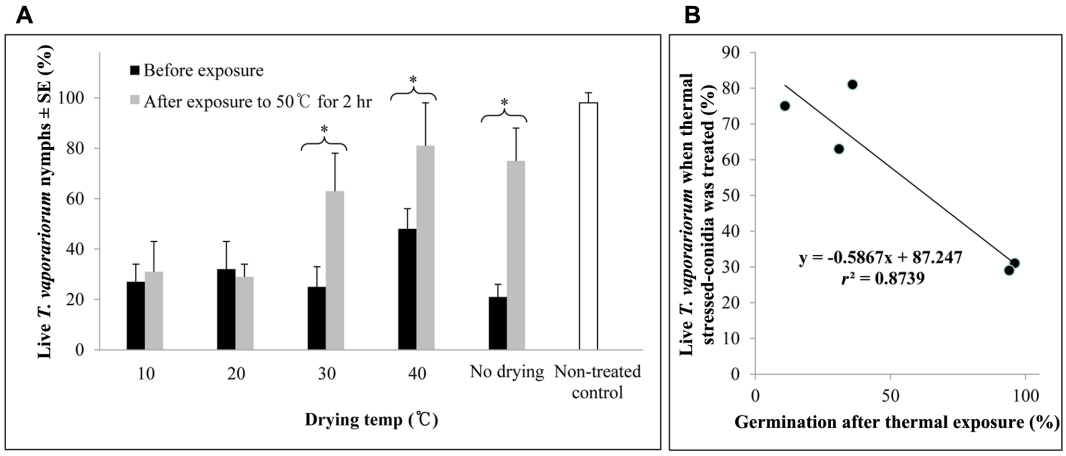

Fig. 2. Virulence against 2nd *Trialeurodes vaporariorum* nymphs of dried conidia before and after thermal exposure in glasshouse conditions, 7 days after the application. (A) Percentage of live *T. vaporariorum* nymphs at different drying temperature treatments before and after thermal stress and (B) correlation between germination of conidia (%) with thermal exposure and live *T. vaporariorum* nymphs (%), when thermally stressed conidia were applied. Asterisks indicate significant differences in germination data (%) between the two groups, $p < 0.01$.

germination, but after thermal exposure the germination levels were significantly lower (11%). Conidia dried at 10 and 20°C retained > 94% germination after thermal stress, but those dried at 30°C and 40°C had significantly reduced germination rates (79% and 43%, respectively), and their germination levels were even lower (31% and 36%, respectively) after thermal exposure.

Virulence of thermal stressed-conidia against *T. vaporariorum*. After thermal exposure (50°C for 2 hr), conidia dried at lower temperatures showed higher virulence against *T. vaporariorum* nymphs than those dried at higher temperatures ($F_{3,16} = 43.1$, $p < 0.001$) (Fig. 2A). In 7 days, dried conidia not exposed to thermal stress showed 68~73% of mortality, except that conidia dried at 40°C showed a lower mortality of 52%. Thus, thermal exposure reduced conidial virulence against the insect. Conidia dried at 10°C and 20°C and exposed to the thermal stress showed 69~71% of mortality, which was similar to the % mortality of conidia not exposed to stress. Conidia dried at 30°C and 40°C and exposed to stress showed 37% and 19% mortality, respectively. After thermal exposure, a significant relationship between % germination of conidia and % live *T. vaporariorum* population was found as follows: $Y_{population} = 0.5867X_{germination} + 87.247$ ($r^2 = 0.8739$) (Fig. 2B).

Effect of moisture absorbents on conidial thermotolerance. White carbon treatment showed the highest conidial thermotolerance, followed by magnesium sulfate, silica gel, calcium chloride, and sodium sulfate ($F_{5,24} = 51.6$, $p < 0.001$) in that order (Table 1). A significant relationship between conidial thermotolerance and water potential of the mixture of conidia and moisture absorbent was found ($r = -0.945$, n = 18, $p < 0.001$) (Fig. 3). The

Table 1. Germination (%) of the conidia-moisture absorbent mixtures (9 : 1, w/w) after exposure to 50°C for 2 hr before and after storage at room temperature for 30 days and water potential of the mixtures before storage

Mixture	Germination of conidia ± SE after exposure to 50°C for 2 hr (%)		Water potential (Mpa ± SE)
	Before storage	After storage	
Conidia + calcium chloride	98.3 ± 2.1 a	81.3 ± 3.7 c	−5.73 ± 0.55 d
+ silica gel	97.1 ± 2.3 a	88.3 ± 2.7 b	−75.91 ± 2.70 c
+ magnesium sulfate	98.5 ± 1.7 a	89.7 ± 2.3 b	−98.55 ± 7.31 b
+ white carbon	99.0 ± 1.5 a	96.1 ± 1.5 a	−217.20 ± 11.05 a
+ sodium sulfate	98.1 ± 2.0 a	78.3 ± 3.1 c	−2.39 ± 0.41 e
+ non-treated control	97.7 ± 1.1 a	81.1 ± 3.1 c	−1.06 ± 0.17 f

Means followed by the same lower case letters in each column are not significantly different according to the Tukey's HSD ($p > 0.05$).

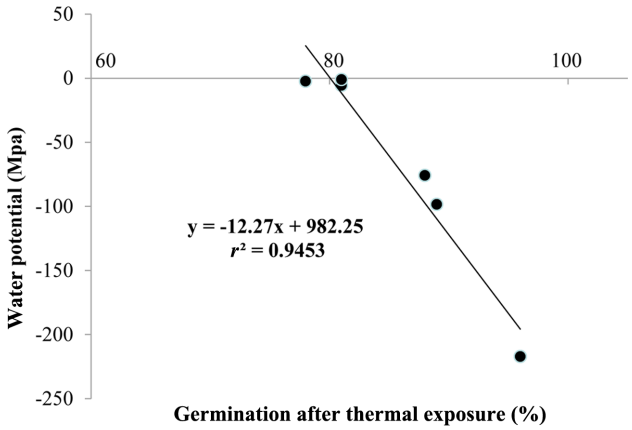

Fig. 3. Correlation between percentage germination of the conidia and adjuvants mixture with thermal exposure and water potential of the mixture.

conidial mixture with white carbon possessed the highest water potential level of was −217.20 Mpa, the highest among the five treatments. Sodium sulfate treatment, which showed the lowest thermotolerance, also had the lowest water potential level of −2.39 Mpa.

Maintenance of thermotolerance by white carbon. When the dried conidial powder was mixed with less than 20% moisturized white carbon under moisturized conditions and stored at room temperature for 30 days and exposed to 50°C for 2 hr, the mixture did not show any significant decrease of conidial thermotolerance ($F_{3,16} = 39.8$, $p < 0.001$) (Table 2). Analysis of the non-moisturized (dried) white carbon treated conidia after thermal stress showed ~99% germination of conidia with no significant reduction in their thermotolerance. Treatments with 5%, 10%, and 20% moisturized white carbon showed no significant decrease in conidial thermotolerance (94~96% of germination); however, in the 40% moisturized white carbon mixture, a significant decrease in conidial thermotolerance was observed (76% germination), compared to thermotolerance before storage (99%).

Table 2. Germination (%) of the conidia-moisturized white carbon (WC) mixtures (9 : 1, w/w) after exposure to 50°C for 2 hr before and after storage at room temperature for 30 days

Mixture	Germination of conidia ± SE after exposure to 50°C for 2 hr (%)	
	Before storage	After storage
Conidia + WC (5% moisturized)	98.1 ± 1.3 a	96.4 ± 1.3 a
+ WC (10% moisturized)	97.3 ± 1.0 a	94.3 ± 1.7 a
+ WC (20% moisturized)	98.3 ± 1.0 a	95.7 ± 1.7 a
+ WC (40% moisturized)	99.1 ± 0.7 a	76.1 ± 2.1 b
+ non-moisturized WC	98.7 ± 1.0 a	99.0 ± 0.3 a

Means followed by the same lower case letters in each column are not significantly different according to the Tukey's HSD ($p > 0.05$).

DISCUSSION

The successful industrialization of insect-killing fungi requires not only high conidial productivity, but also the potential to retain a high cell viability (close to 100%), and consistent biological performance during and after storage [26, 27]. After solid culturing, maintenance of moisture content at a very low level is an important step in the maintenance of conidial thermotolerance during long-term storage. A high moisture content creates a suitable environment for contamination of substrates by other bacteria or fungi. The moisture content of mycotized grains might be related to the type of grains used as substrates in solid cultures.

Herein, we report a significant correlation between the moisture content and conidial thermotolerance. Un-dried micronized rice grains had high levels of moisture, with significantly decreased conidial thermo tolerance after exposure to 50°C compared to the thermo tolerance of conidia dried at low temperature. Our data suggest that among the drying methods, drying at a lower temperature is strongly recommended to maintain conidial thermotolerance, with an optimal time and temperature of 48 hr and 10~20°C, respectively. On the other hand, drying at higher temperatures may result in decreased conidial viability, which also correlates with poor thermotolerance.

Treatment of conidia with silica gel and magnesium sulfate resulted in high levels of conidial thermotolerance.

Silica gel is a granular, vitreous, porous form of silicon dioxide synthesized from sodium silicate [3]. Silica gel is a naturally occurring mineral that is purified and processed into either granular or beaded form. It is tough and hard, and has more solids than other common household gels such as gelatin or agar. As a desiccant, it has an average pore size of 2.4 nm and has a strong affinity for water molecules. The high surface area of silica gel (~800 m^2/g) allows it to absorb water readily, making it useful as a moisture-absorbent. Once saturated with water, the gel can be regenerated by heating at 120°C for 2 hr. Magnesium sulfate (anhydrous) is also used as a drying agent [28], the anhydrous form of which is hygroscopic (readily absorbs water from the air), making it difficult to weigh accurately. Therefore, magnesium sulfate hydrate is often preferred when preparing solutions, for example in medical preparations.

White carbon, commonly referred to as chaoite is the best moisture absorbent for achieving conidial thermotolerance. White carbon occurs in shock-metamorphosed graphite gneisses and ureilite meteorites [29], is dark gray in color, and formed as lamellae (thin laminae producing a lamellar structure) with hardness between talc and gypsum. White carbon has a high ability to absorb liquid materials, which makes it a beneficial ingredient in pesticide formulations to constitute liquid active ingredients into a powder form. White carbon can absorb more than 10 times its volume. Here, we mixed white carbon with un-dried conidial powder (1 : 9, w/w), so that the existing moisture could be fully absorbed.

A significant relationship between conidial thermotolerance and water potential suggests methods to maintain conidial viability during storage. When conidia are placed in potential high moisture conditions, the moisture around the conidia is likely to move to the substrate, such as white carbon, which has high water absorption potential. Thus, white carbon acts as a moisture-absorbent, protecting the conidia from direct contact with moisture, and retaining their thermotolerance. In conidia-adjuvant mixtures with low water absorption potential, there is no significant movement of water or absorption by the substrate, and the conidia are still at risk for direct moisture contact, decreasing their viability.

In this work, white carbon in the conidial mixture held a maximum of 20% of the water, which did not reduce conidial thermotolerance. Even though white carbon meets a small amount of moisture during long-term storage of conidia, conidia have very little chance to contact available water, thus maintaining their thermotolerance. However, 40% of moisturized white carbon treatment may possess reduced ability to absorb moisture, and contact of conidia with the residual moisture may reduce their thermotolerance, upon exposure to thermal stress.

If white carbon is added to a WP formulation, conidial viability is maintained longer than in the white carbon-free WP formulation. Excess amounts of white carbon may physically downgrade the WP. Fully dried conidia should be used in WP formulations, and white carbon should be added as a moisture-absorbent to further increase the storage stability. Further studies are required to determine whether white carbon could also be added to oil-based conidial formulations to reduce the risk of conidial contact with moisture and enhance conidial viability.

In conclusion, we determined that rice grains produced high conidial yields in solid culture, and that the mycotized rice grains should be dried at low temperatures for at least 48 hr to maintain conidial thermotolerance at 50°C. A significant relationship between conidial thermotolerance and water potential of the conidia powder containing moisture absorbents was found. When the dried conidial powder was added to white carbon under moisturized conditions, 20% of moisture in the mixture did not significantly reduce conidial thermotolerance. These results suggest that conidial thermotolerance could be maintained by drying cultured-conidia at low temperatures and by adding a moisture absorbent such as white carbon.

ACKNOWLEDGEMENTS

We are grateful to Dr. Margaret Skinner and Bruce L. Parker (University of Vermont, USA) for their comments and enthusiastic assistance. This research was supported by Advanced Production Technology Development Program, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

REFERENCES

1. United States Environmental Protection Agency. Biopesticide Active Ingredient Fact Sheets [Internet]. Washington, DC: United States Environmental Protection Agency; 2009 [cited 2013 Sep 15]. Available from: http://www.epa.gov/pesticides/biopesticides/ingredients/.
2. Yatin BT, Venkataraman NS, Parija TK, Panneerselvam D, Govindanayagi P, Geetha K. The new biopesticide market. Denver: Business Communications Research; 2006.
3. Burges HD. Formulation of microbial biopesticides: beneficial microorganisms, nematodes and seed treatment. Dordrecht: Kluwer Academic; 1998. p. 131-86.
4. Devi KU, Sridevi V, Mohan ChM, Padmavathi J. Effect of high temperature and water stress on *in vitro* germination and growth in isolate of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuillemin. J Invertebr Pathol 2005;88:181-9.
5. Kim JS, Je YH, Roh JY. Production of thermotolerant entomopathogenic *Isaria fumosorosea* SFP-198 conidia in corn-corn oil mixture. J Ind Microbiol Biotechnol 2010;37:419-23.
6. Rangel DE, Anderson AJ, Roberts DW. Evaluating physical and nutritional stress during mycelia growth as inducers of tolerance to heat and UV-B radiation in *Metarhizium anisopliae* conidia. Mycol Res 2008;112(Pt 11):1362-72.
7. Ying SH, Feng MG. Medium components and culture conditions affect the thermotolerance of aerial conidia of fungal biocontrol agent *Beauveria bassiana*. Lett Appl Microbiol

2006;43:331-5.
8. Hallsworth JE, Magan N. Culture age, temperature, and pH affect the polyol and trehalose contents of fungal propagules. Appl Environ Microbiol 1996;62:2435-42.
9. Tarocco F, Lecuona RE, Couto AS, Arcas JA. Optimization of erythritol and glycerol accumulation in conidia of *Beauveria bassiana* by solid-state fermentation, using response surface methodology. Appl Microbiol Biotechnol 2005;68:481-8.
10. Guerzoni ME, Lanciotti R, Cocconcelli PS. Alteration in cellular fatty acid composition as a response to salt, acid, oxidative and thermal stresses in *Lactobacillus helveticus*. Microbiology 2001;147(Pt 8):2255-64.
11. Hong TD, Edgington S, Ellis RH, de Muro MA, Moore D. Saturated salt solutions for humidity control and the survival of dry powder and oil formulations of *Beauveria bassiana* conidia. J Invertebr Pathol 2005;89:136-43.
12. Horaczek A, Viernstein H. Comparison of three commonly used drying technologies with respect to activity and longevity of aerial conidia of *Beauveria brongniartii* and *Metarhizium anisopliae*. Biol Control 2004;31:65-71.
13. Moore D, Douro-Kpindou OK, Jenkins NE, Lomer CJ. Effects of moisture content and temperature on storage of *Metarhizium flavoviride* conidia. Biocontrol Sci Technol 1996; 6:51-61.
14. Moore D, Langewald J, Obognon F. Effects of rehydration on the conidial viability of *Metarhizium flavoviride* mycopesticide formulations. Biocontrol Sci Technol 1997;7:87-94.
15. Hong TD, Jenkins NE, Ellis RH. The effects of duration of development and drying regime on the longevity of conidia of *Metarhizium flavoviride*. Mycol Res 2000;104:662-5.
16. Magalhães BP, Boucias DG. Effects of drying on the survival of conidiosphores of *Metarhizium anisopliae* var. *acridum* Driver & Milner. J Orthoptera Res 2004;13:155-9.
17. Moore D, Bateman RP, Carey M, Prior C. Long-term storage of *Metarhizium flavoviride* conidia in oil formulation for the control of locusts and grasshoppers. Biocontrol Sci Technol 1995;5:193-9.
18. Jenkins NE, Heviefo G, Langewald J, Cherry AJ, Lomer CJ. Development of mass production technology for aerial conidia for use as mycopesticides. Biocontrol News Inf 1998; 19:21-31.
19. Morley-Davies J, Moore D, Prior C. Screening of *Metarhizium* and *Beauveria* spp. conidia with exposure to stimulated sunlight and a range of temperatures. Mycol Res 1996;100:31-8.
20. De Faria MR, Wraight SP. Mycoinsecticides and Mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biol Control 2007;43:237-56.
21. Lee IK, Shim HJ, Woo SD, Je YH, Yang Z, Kang SK. Variations in growth and pathogenicity of *Beauveria bassiana* and *Paecilomyces fumosoroseus* pathogenic to the pine gall midge, *Thecodiplosis japonensis*. Korean J Appl Microbiol Biotechnol 1999;27:415-8.
22. Kim JS, Je YH, Choi JY. Complementary effect of phloxine B on the insecticidal efficacy of *Isaria fumosorosea* SFP-198 wettable powder against greenhouse whitefly, *Trialeurodes vaporariorum* West. Pest Manag Sci 2010;66:1337-43.
23. Humber RA. Fungi: preservation of cultures. In: Lacey LA, editor. Manual of techniques in insect pathology. San Diego: Academic Press; 1997. p. 269-79.
24. Hua L, Feng MG. Broomcorn millet grain cultures of the entomophthoralean fungus *Zoophthora radicans*: sporulation capacity and infectivity to *Plutella xylostella*. Mycol Res 2005;109(Pt 3):319-25.
25. Ramirez ML, Chulze SN, Magan N. Impact of osmotic and matric water stress on germination, growth, mycelial water potentials and endogenous accumulation of sugars and sugar alcohols in *Fusarium graminearum*. Mycologia 2004;96:470-8.
26. Slininger PJ, Behle RW, Jackson MA, Schisler DA. Discovery and development of biological agents to control crop pests. Neotrop Entomol 2003;32:183-95.
27. Singer MA, Lindquist S. Multiple effects of trehalose on protein folding *in vitro* and *in vivo*. Mol Cell 1998;1:639-48.
28. Schenck FJ, Callery P, Gannett PM, Daft JR, Lehotay SJ. Comparison of magnesium sulfate and sodium sulfate for removal of water from pesticide extracts of foods. J AOAC Int 2002;85:1177-80.
29. Setaka N, Sekikawa Y. Chaoite, a new allotropic form of carbon, produced by shock compression. J Am Ceram Soc 1980;63:238-9.