J. Microbiol. Biotechnol. (2010), **20**(9), 1348–1350
doi: 10.4014/jmb.1005.05023
First published online 20 July 2010

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Plant Oils for Improving Thermotolerance of *Beauveria bassiana*

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Received: May 13, 2010 / Revised: June 15, 2010 / Accepted: June 23, 2010

Conidia of *Beauveria bassiana* ARSEF-7060, produced in millet amended with plant oils such as sunflower, corn, or cotton seed oil, were exposed to 45°C of wet heat for 90 min. Conidia from millet+corn oil medium had the highest thermotolerance [LT_{50} (median survival time): 45.7 min]. The mycotized millet grains were coated with each of the same plant oils as a granular formulation and subjected to 50°C of dry heat for 8 h. Corn oil coating (LT_{50}: 8.68 h) was superior to sunflower and cotton seed oil coatings, suggesting the feasibility of using corn oil to increase conidial thermotolerance.

Keywords: *Beauveria bassiana*, millet, corn oil, thermotolerance, fungal coatings

The susceptibility of Hypocreale entomopathogenic fungi to high temperatures has been an attractive subject in biopesticides development since the fungi were considered effective control agents against sucking insects [5, 6, 12, 13]. This susceptibility can be overcome by careful manipulation of solid cultures [14, 20], as indicated by the correlation of fungal thermotolerance with the endogenous accumulation of polyols and trehalose [9, 10, 22], and also the changes in lipid concentrations in the cell walls of other microorganisms [7, 8, 21, 23]. Another solution is to process fungal conidia in a concentrated liquid suspension or wettable powder formulations [16, 18].

The research herein presents data on the thermotolerance of conidia produced on millet [15] with sunflower, corn, or cotton seed oils added. It demonstrates that adding these plant oils to conidia, as a coating following production, does also increase their thermotolerance.

Beauveria bassiana ARSEF-7060 (also coded ERL1170) was obtained from the USDA, ARS Collection of Entomopathogenic Fungi (Ithaca, NY, U.S.A.) [6]. A 100-g millet (*Panicum miliaceum* L.) grain was incorporated with 1 ml of sunflower, corn, or cotton seed oils. The treated grains were held in polyethylene bags, inoculated with the fungus, and held at room temperature (~21°C) for 3 weeks. First, millet grains (100 g; intact) incorporated with a plant oil (1 ml) were soaked in 50 ml of distilled water containing citric acid (0.4 ml/l), placed in a polyethylene bag (30×20 cm), and boiled at 90°C for 1 h, followed by autoclaving at 121°C for 30 min. The bag was then inoculated with 5-ml aliquots of *B. bassiana* ARSEF-7060 from a 3-day-old liquid culture in Sabouraud dextrose yeast extract broth (SDY, pH 6) [11] and incubated at 25±1°C and a 16:8 (light/dark) photoperiod for 3 weeks. Mycotized millet grains were dried for 2 days in a small drying room with a dehumidifier until <5% moisture content was obtained. The number of conidia per gram of dried mycotized millet grains was counted using a hemacytometer. Each treatment was replicated three times (three bags) in an experimental replicate, and the entire experiment was repeated twice.

A thermotolerance test was conducted by exposing suspensions of conidia from the mycotized millet grains to 45°C for 0 (unexposed) to 90 min, followed by germination tests. Ten mycotized millet grains from each bag were placed in 1 ml of 0.02% (v/v) polysiloxane polyether copolymer (Silwet L-77, Loveland Inc.) solution, filtered using a sterile mesh with square pores (150×150 μm), and adjusted to ca. 1×10^7 conidia/ml in an Eppendorf tube. Three tubes per treatment were held in a 45°C water bath. Prior to and after exposure at intervals of 30 min, 20 μl of suspension from each tube was placed on quarter-strength (¼) SDAY medium and incubated at 20±1°C for 24 h. Percentage germination was determined by examining 100 conidia microscopically (400×) [1], and the data were analyzed by a general linear model, followed by Tukey's honestly significant difference (HSD) using the SPSS ver. 17.0 (SPSS Inc. 2009, IL, U.S.A.) at the 0.05 (α) level. Median survival time (LT_{50}; time for conidia to lose 50% viability) of conidia was estimated by a Probit analysis using the aforementioned program.

Millet grain+corn oil medium was superior in producing the most thermotolerant conidia (Table 1). The LT_{50} of millet

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Table 1. Median survival time (LT_{50}) of *B. bassiana* ARSEF-7060 conidia produced in millet grains amended with a plant oil using polyethylene bags after exposure to 45°C of wet heat for 90 min and the number of conidia (mean±SE) per gram of dried mycotized millet grains.

Substrate	LT_{50} (min)	95% Confidence level	No. of conidia/g substrate (mean±SE)
Millet grains + sunflower oil	32.1 b	30.5–33.7	$9.2\times10^8\pm1.8\times10^8$ a
+ corn oil	45.7 a	44.0–47.3	$9.8\times10^8\pm1.5\times10^8$ a
+ cotton seed oil	31.8 b	30.2–33.3	$8.7\times10^8\pm1.4\times10^8$ a
+ none	25.7 c	24.4–27.0	$8.2\times10^8\pm1.8\times10^8$ a

Values followed by the same lower-case letter in a column do not significantly differ by the Tukey's HSD test (p=0.05).

+corn oil treatment was 45.7 min, which was significantly higher than that of a millet treatment without plant oil (25.7 min) (substrate: $F_{3,96}$=425.2, P<0.001; exposure time: $F_{3,96}$=12,469.3, P<0.001). No significant difference in the yield of conidia was observed among the treatments (P> 0.05). Unsaturated fatty acids, the main components of corn oil, can be involved in the production of such thermotolerant conidia [14], given their roles in protecting cells from thermal stress [8]. The different levels of conidial thermotolerance among the three plant oil treatments may be explained by the percentages of unsaturated fatty acids in the oils; the percentage in corn oil is higher than in the other oils. Corn, sunflower, and cotton seed oils consist of ca. 87% (28% monounsaturated and 59% polyunsaturated), 84% (10% monounsaturated and 74% polyunsaturated), and 70% (18% monounsaturated and 52% polyunsaturated) unsaturated fatty acids, respectively [2, 17, 19], depending on the varieties of source plants.

The mycotized millet grains produced using millet grains+corn oil were coated with each of the above three plant oils to further increase conidial thermotolerance. A 3-g quantity of mycotized millet grains from each polyethylene bag was mixed with 0.8 ml of plant oil in a plastic tray (6 cm diam.). Coated grains in trays were held at 50°C in an incubator for 0 (unexposed) to 8 h. Prior to and 2, 4, and 8 h after the exposure, 10 grains from each tray were placed in 1 ml of 2% (v/v) Tween 80 solution, and then the above procedures were followed for a germination test. Tween 80 was alternatively used to detach conidia from millet grains and to make a conidial suspension. No harmful effect of the surfactant was observed. Conidial suspensions (20 μl) were placed on ¼SDAY medium and incubated at 20±1°C for 24 h. The same analytical methods as above were applied to the data on germination (%).

Coating of mycotized millet grains with corn oil was superior to the other treatments in increasing conidial thermotolerance (Table 2). The LT_{50} of the corn oil treatment was 8.68 h, which was significantly higher than the no-coating treatment (2.13 h) (coating: $F_{3,96}$=1828.5, P<0.001; exposure time: $F_{3,96}$=4496.6, P<0.001). Conidia coated with corn oil can be protected from unfavorable high temperatures and possibly from excess drying caused by these temperatures. This novel approach can be a valuable strategy to increase conidial thermotolerance, given the survey on the commercialization of fungal formulations [3, 4]. Millet grains coated with corn oil are somewhat sticky, and it is hypothesized that this can be eliminated by adding mineral materials; however, in this research, minerals were not used because they might interfere with the determination of conidial germination.

To confirm the activity of plant oils as thermal protectants, dried conidia (0.01 g) produced in millet grains+corn oil were suspended in each of the three plant oils (1 ml), with three replications, and exposed to 50°C for 0 (unexposed) to 8 h, followed by using the methods described above. The corn oil treatment had a higher LT_{50} (7.13 h) than the other plant oil treatments, followed by the water control (1.16 h) (suspension carrier: $F_{3,96}$=2556.4, P<0.001; exposure time: $F_{3,96}$=6886.9, P<0.001) (Table 3).

These results suggest that corn oil can be used in multiple ways to increase the thermotolerance of *B.*

Table 2. Median survival time (LT_{50}) of *B. bassiana* ARSEF-7060 conidia on mycotized millet grains, produced using millet grains+corn oil, coated with a plant oil after exposure to 50°C of dry heat for 8 h.

Coating oil	LT_{50} (h)	95% Confidence level
Sunflower oil	5.87 b	5.67–6.07
Corn oil	8.68 a	8.28–9.14
Cotton seed oil	5.38 c	5.20–5.58
None	2.13 d	2.03–2.23

LT_{50} values followed by the same lower-case letter do not significantly differ by the Tukey's HSD test (p=0.05).

Table 3. Median survival time (LT_{50}) of *B. bassiana* ARSEF-7060 conidia, produced in millet grains+corn oil, suspended in a plant oil after exposure to 50°C of wet heat for 8 h.

Oil carrier	LT_{50} (h)	95% Confidence level
Sunflower oil	3.82 b	3.67–3.96
Corn oil	7.13 a	6.89–7.40
Cotton seed oil	2.30 c	2.19–2.41
None	1.16 d	1.09–1.22

LT_{50} values followed by the same lower-case letter do not significantly differ by the Tukey's HSD test (p=0.05).

bassiana ARSEF-7060 conidia. It can be used as a substrate additive to millet grains and as a coating agent of mycotized millet grains. This approach will provide an increased shelf life for entomopathogenic fungi and a greater tolerance for unfavorable environmental factors such as high temperatures.

Acknowledgments

This work was supported by grants from the Northeast IPM Competitive Grants Program (Award No. 2008-34103-18956), USDA Agriculture Research Service (Project No. 1907-22410-003-10S), Hatch (VT-HO1408, SARE Project No. S-1024), and the Organic Farming Research Foundation.

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