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Relation of Aphicidal Activity with Cuticular Degradation by *Beauveria bassiana* SFB-205 Supernatant Incorporated with Polyoxyethylene-(3)-Isotridecyl Ether

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The application of *Beauveria bassiana* SFB-205 supernatant incorporated with polyoxyethylene-(3)-isotridecyl ether (TDE-3) significantly reduced the population of two species of aphids including cotton aphid, *Aphis gossypii*, and green peach aphid, *Myzus persicae*, much higher in cotton aphid, compared with supernatant incorporated with Tween 80, which allows the relationship of aphicidal activity with the degradation of aphid cuticles to be determined. Overall, the degradation of the cuticles induced by the supernatant was more remarkable in conjunction with TDE-3 than Tween 80, and this phenomenon was more observable in cotton aphid through SDS–polyacrylamide gel electrophoresis, revealing high correlation with their aphicidal activities.

Keywords: *Aphis gossypii*, *Beauveria bassiana*, *Myzus persicae*, polyoxyethylene-(3)-isotridecyl ether, supernatant

A wetting agent is essential to allow entomopathogenic fungal supernatant including chitinase (E.C. 3.2.1.14), Pr1 (E.C. 3.4.21), and Pr2 (E.C. 3.4.21) proteases, reported to play an important role in penetrating insect cuticles [10, 12, 14, 17, 18, 19, 21, 25, 26], to wet all over the hydrophobic surfaces of insect cuticles [8]. Various non-ionic and non-toxic surfactants have been used as wetting agents: polyoxyethylene–sorbitan monostearate (Tween 80®), –sorbitan monolaurate (Tween 20), –isooctylphenyl ether (Triton X-100), and polysiloxane polyether (siloxane) (Silwet) [4, 5, 8, 9, 11, 20, 24]. Tween 80, so far, is a popular wetting agent and can be used in aqueous liquid formulations because of its hydrolytic stability [8].

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Recently, we selected polyoxyethylene-(3)-isotridecyl ether (TDE-3) (CAS 24938-91-8), non-ionic with hydrolytic stability, as a wetting agent for the application of *Beauveria bassiana* (Balsamo) Vuillemin (*Ascomycota*: Hypocreales) SFB-205 (KCCM 10892P) [22] supernatant to control cotton aphid (*Aphis gossypii* Glover) (Homoptera: Aphididae) and green peach aphid (*Myzus persicae* Sulzer) (Homoptera: Aphididae). This paper reports our interpretative analysis about the relationship of aphicidal activity with the degradation of the insect cuticles by SFB-205 supernatant incorporated with TDE-3 or Tween 80.

B. bassiana SFB-205 was grown on Sabouraud dextrose agar medium supplemented with 0.5% (w/v) yeast extract (SDAY, pH 6.0) in Petri dishes at 27±1°C for 14 days. The harvested conidia were inoculated to SDY broth (pH 6.0), ending up to 1×10^6 conidia/ml in 200 ml of the broth. It was incubated at 27±1°C and 16:8 (L/D) photoperiods with shaking at 150 rpm for 3 days. Supernatant was prepared by centrifugation of the cultured broth at 16,000 ×*g* at 4°C for 10 min and subsequent filtration using a 0.2-mm pore-sized filter (Falcon, CA, U.S.A.).

Greenhouse tests were conducted to investigate the aphicidal activities of the supernatants incorporated with TDE-3 or Tween80 against the two species of aphids in hot peppers (*Capsicum annuum* L. var. *grossum*). Each plant at the 10-leaf stage was infested with 200–220 of cotton aphid or green peach aphid adults, which were obtained from the insectary of the Dongbu Advanced Research Institute at 1-days pre-application (three plants/treatment). The supernatants, incorporated with or without 1.0% (v/v) of the wetting agents, were diluted to 100× solutions (pH 6.0) and sprayed on both sides of the leaves at 20 ml per plant using a hand sprayer (Gardena 864, Germany) with constant pressure in a spray booth equipped with a turn table (diameter 65 cm, 0.25 rotation/s). The wetting agents

(10,000×) or imidacloprid 10% suspension concentrate (SC) (2,000×) served as control treatments in conjunction with non-treated control. Each plant was held in an acryl cage (20×20×45 cm) with a mesh, independently in the greenhouse (24±2°C and 40±5% of relative humidity). The aphicidal activities were evaluated by counting the total living number of aphid per plant at 2 days post-application and exhibited as control efficacy using Abbott's formula [1] based on the percent of aphid population, assuming the initial population as 100%. Each treatment was replicated three times within an experimental replicate, and the entire experiment was repeated twice. The data on the percent of aphid population were analyzed by a general linear model followed by Duncan's MRT ($p<0.05$) and 2-sample t-test using SPSS ver. 17.0 (SPSS Inc., IL, U.S.A.).

The supernatants+TDE-3 treatments were superior to the supernatant+Tween 80 treatments in aphicidal activity over the two species of aphids, and this phenomenon was more remarkable in cotton aphid than green peach aphid (Fig. 1). The supernatant+TDE-3 treatments showed 90.1% and 64.3% of control efficacy against cotton aphid and green peach aphid, whereas the supernatant+Tween 80 treatments exhibited 69.1% and 19.1% of control efficacy against them. The supernatant or the two wetting agent solutions displayed <12% of control efficacy with no significant differences among them ($P>0.05$). Preliminarily, the culture medium that was diluted to 100× or 200× with 0.01% of Tween 80 solution showed less than <10% of control efficacy against the two species of aphids.

Subsequently, the aphid adults were washed with 1.0% of sodium hypochlorite for 1 min, followed with sterile water for 1 min to surface-sterilize the bodies before the investigation of the degradation of the aphid proteins induced by the supernatant incorporated with the wetting agents. Ten adults in each species were held in 1 ml of the supernatant including 1% of the wetting agents (100×, pH 6.0) or 1 ml of the 1% wetting agent solutions (100×, pH 6.0) with three replications at 25±1°C for 12 h. After the adults were removed from the solutions, the remaining solutions were centrifuged at 16,000 ×g at 4°C for 10 min to precipitate pellets. The pellets were gently washed once with excess phosphate-buffered saline (PBS, pH 7.4; AMRESCO) and subjected to 12% SDS–polyacrylamide gel electrophoresis (PAGE) followed by Coomassie Blue R-250 staining [23]. The gels, scanned using a color image scanner, HP Scanjet 8270, were analyzed using ImageMaster VDS 3.0 software (Phamacia Biotech, NJ, U.S.A.). The averaged band intensity of each lane was normalized to relative density value (RDV), compared with the highest value that was given an arbitrary value of 1.00. The Pearson's correlation test (two-tailed, $p<0.05$) of the value with the percent of aphid population was conducted using the program.

The RDV of the aphid+supernatant+TDE-3 treatments was higher than that of the aphid+supernatant+Tween80 treatments over the two species of aphids, and the bands were more observable in cotton aphid treatments than green peach aphid treatments (Fig. 2). No observable protein band was detected in the aphid+TDE-3 or +Tween 80 treatments over the two species of aphids, although some bands with a little quantity of intensity were observed in the green peach aphid+TDE-3 treatments (RDV=0.06). Overall, the RDV correlated highly with the percent of the

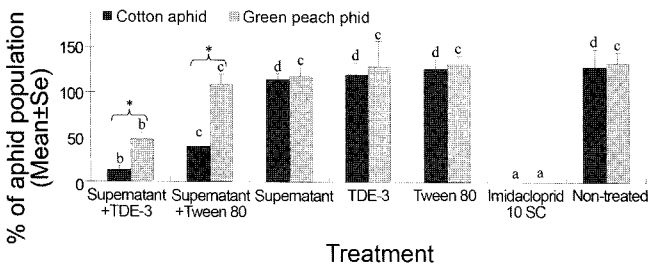

Fig. 1. Percent of aphid population (mean±SE) in the treatments applied with *B. bassiana* SFB-205 supernatant incorporated with TDE-3 or Tween80 in the greenhouse at 2 days post-application.
The same pattern of the bars followed with the same lowercase letters are not significantly different ($p<0.05$), and the two bars in the same treatment followed with an asterisk mark (*) are significantly different ($p<0.05$).

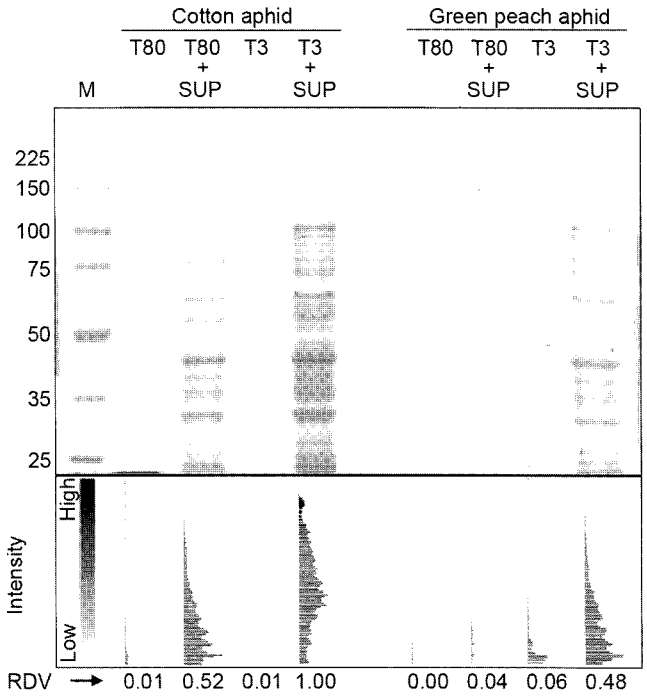

Fig. 2. SDS–polyacrylamide gel electrophoresis of SFB-205 supernatant precipitates after the incubation with cotton aphid or green peach aphid adults in conjunction with or without TDE-3 or Tween 80.
M: protein molecular weight marker; T80: Tween 80; T3: TDE3; SUP: *B. bassiana* SFB-205 supernatant. The averaged relative density value (RDV) of each lane is displayed at the bottom of the densitometric analysis.

aphid population (Coefficient=−0.935**, N=72, $P<0.001$) at the 0.01 level. The molecular mass of the proteins observed in the gel was <100 kDa, which corresponds to previous reports [2, 3, 6, 15, 16]. Given this result and the nature of entomopathogenic fungal enzymes, most of the proteins detected probably had constituted the cuticles of the aphids.

The improvement by TDE-3 may be explained by the greater hydrophile–lipophile balance (HLB) value (representing a surfactant activity) of TDE-3 (HLB=9.0) than Tween 80 (HLB=15.0); a lower HLB value means more lipophilic and more penetrable into lipids compounds, the main components of the most outer part of insect cuticles. Consequently, TDE-3 might serve as a cuticle destroyer, comparable to appressoria or hypha working in fungal pathogenesis, to express the aphicidal activity of the supernatant, which mainly has a function of enzymatic hydrolysis, responsible for the degradation of cuticle proteins, chitin-binding proteins, and chitins (usually located in epicuticle and procuticles) to facilitate hyphal penetration, which is carried out by Pr1-, Pr2 proteases, and chitinase, *etc* [7, 9, 12, 13].

These findings provide evidence that the degradation of the aphid cuticles induced by the supernatant can be accelerated by utilizing TDE-3 and the degradation highly correlates with the aphicidal activity. This methodological approach may be extended to the application of entomopathogenic fungal conidia to accelerate their pathogenesis.

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References

1. Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **18:** 265–267.
2. Alavo, T. B. C. and M. Accodji. 2004. The entomopathogenic fungus *Verticillium lecanii* (Deuteromycetes, Moniliaceae), the proteins hydrophobins and the biological control of aphids (Homoptera, Aphididae): Literature review. *Arch. Phytopathol. Plant Protec.* **37:** 201–204.
3. Andersen, S. O., P. Hojrup, and P. Roepstorff. 1995. Insect cuticular proteins. *Insect Biochem. Mol. Biol.* **25:** 153–176.
4. Bateman, R. P. and R. T. Alves. 2000. Delivery systems for mycoinsecticides using oil-based formulations. *Asp. Appl. Biol.* **57:** 163–170.
5. Bernhard, K., P. J. Holloway, and H. D. Burges. 1998. A catalogue of formulation additives: Function, nomenclature, properties and suppliers, pp. 333–365. *In* H. D. Burges (ed.). *Formulation of Microbial Biopesticides: Beneficial Microorganisms, Nematodes and Seed Treatment*. Kluwer Academic Publishing, Dordrecht.
6. Borror, D. J., C. A. Triplehorn, and N. F. Johnson. 1989. *An Introduction to the Study of Insects*, pp. 336–338. 6th Ed. Saunders College Publishing, Philadelphia.
7. Boucias, D. G. and J. C. Pendland. 1991. Attachment of mycopathogens to cuticle: The initial event of mycoses in arthropod hosts, pp. 101–127. *In* G. T. Cole and H. C. Hock (eds.). *The Fungal Spore and Disease Initiation in Plants and Animals*. Plenum Press, New York.
8. Burges, H. D. 1998. Formulation of mycopesticides, pp. 131–186. *In* H. D. Burges (ed.). *Formulation of Microbial Biopesticides: Beneficial Microorganisms, Nematodes and Seed Treatment*. Kluwer Academic, Dordrecht.
9. Butt, T. M. and M. S. Goettel. 2000. Bioassay of entomogenous fungi, pp. 141–196. *In* A. Navon and K. R. S. Ascher (eds.). *Bioassays of Entomopathogenic Microbes and Nematodes*. CABI Publishing, Wallingford.
10. Campos, R. A., W. Arruda, J. T. Boldo, M. V. Silvia, N. M. Barros, J. L. Azedevo, A. Schrank, and M. H. Vainstein. 2005. *Boophilus microplus* infection by *Beauveria amorpha* and *Beauveria bassiana*: SEM analysis and regulation of subtilisin-like proteases and chitinases. *Curr. Microbiol.* **50:** 257–261.
11. Casanova, H., C. Ortíz, A. Vallejo, and P. Araque. 2004. Nicotine oleate dispersions as botanical insecticides. *Progr. Colloid Polymer Sci.* **128:** 187–192.
12. Charnley, A. K. 2003. Fungal pathogens of insects: Cuticle degrading enzymes and toxins. *Adv. Bot. Res.* **40:** 241–321.
13. Clarkson, J. M. and A. K. Charnley. 1996. New insights into the mechanisms of fungal pathogenesis in insects. *Trends Microbiol.* **4:** 197–203.
14. Cliquet, S. and M. A. Jackson. 2005. Impact of carbon and nitrogen nutrition on the quality, yield and composition of blastospores of the bioinsecticidal fungus *Paecilomyces fumosoroseus*. *J. Ind. Microbiol. Biotechnol.* **32:** 204–210.
15. Dombrovsky, A., H. Huet, H. Zhang, N. Chejanovsky, and B. Raccah. 2003. Comparison of newly isolated cuticular protein genes from six aphid species. *Insect Biochem. Mol. Biol.* **33:** 709–715.
16. Dombrovsky, A., I. Sobolev, N. Chejanovsky, and B. Raccah. 2007. Characterization of RR-1 and RR-2 cuticular proteins from *Myzus persicae*. *Insect Biochem. Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **146:** 256–264.
17. Donatti, A. C., L. Furlaneto-Maia, M. H. Fungaro, and M. C. Furlaneto. 2008. Production and regulation of cuticle-degrading proteases from *Beauveria bassiana* in the presence of *Rhammatocerus schistocercoides* cuticle. *Curr. Microbiol.* **56:** 256–260.
18. Fan, Y., W. Fang, S. Guo, X. Pei, Y. Zhang, Y. Xiao, *et al.* 2007. Increased insect virulence in *Beauveria bassiana* strains overexpressing an engineered chitinase. *Appl. Environ. Microbiol.* **73:** 295–302.
19. Jackson, M. A., A. R. Payne, and D. A. Odelson. 2004. Liquid-culture production of blastospores of the bioinsecticidal fungus *Paecilomyces fumosoroseus* using portable fermentation equipment. *J. Ind. Microbiol. Biotechnol.* **31:** 149–154.
20. Jin, X., K. E. Grigas, C. A. Johnson, P. Perry, and D. W. Miller. 1999. Method for storing fungal conidia. US Patent 5989898.
21. Kaur, G. and V. Padmaja. 2008. Relationships among activities of extracellular enzyme production and virulence against

Helicoverpa armigera in *Beauveria bassiana*. *J. Basic Microbiol.* **48:** 1–10.
22. Kim, J. S., J. Y. Roh, J. Y. Choi, S. C. Shin, M. J. Jeon, and Y. H. Je. 2008. Identification of an entomopathogenic fungus, *Beauveria bassiana* SFB-205 toxic to the green peach aphid, *Myzus persicae*. *Int. J. Ind. Entomol.* **17:** 211–215.
23. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.
24. Luz, C. and I. Batagin. 2005. Potential of oil-based formulations of *Beauveria bassiana* to control *Triatoma infestans*. *Mycopathologia* **160:** 51–62.
25. St. Leger, R. J., L. Joshi, M. J. Bidochka, and D. W. Roberts. 1996. Construction of an improved mycoinsecticide overexpressing a toxic protease. *Proc. Natl. Acad. Sci. U.S.A.* **93:** 6349–6354.
26. Ypsilos, I. K. and N. Magan. 2004. Impact of water-stress and washing treatments on production, synthesis and retention of endogenous sugar alcohols and germinability of *Metarhizium anisopliae* blastospores. *Mycol. Res.* **108:** 1337–1345.