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Harvesting of the insecticidal chitinase produced from entomopathogenic fungi, *Beauveria bassiana* SFB-205 using Enzyme absorption method

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Objectives

Most insecticidal enzymes involved in pathogenesis are unstable against the thermal-stress on the concept of industrialization. In consequence, it may decrease the expiration time of final products made by supernatant. We harvested a chitinase, main insecticidal enzymes, more practicably from the *B. bassiana* SFB-205 culture broth by novel enzyme adsorption method and we make it in to powder form for keeping it more stable for long times.

Materials and Methods

Fungal strains and preparation of supernatant

The SFB-205 were propagated on Sabouraud dextrose agar medium supplemented with yeast extract at 0.5%(w/w) (SDYA) at 27±1℃for 14~15 days. Liquid culture media were based on Sabouraud dextrose broth medium supplemented with yeast extract at 0.5% (w/v) (SDYB).

Enzyme assay

Chitinase activity of SFB-205 supernatant and its protein pellet or freeze-dried powder were measured by determining the release of p-nitrophenol from p-nitrophenyl β-D-acetyl glucosaminide (PNG) on the basis of the method. 100 ul of enzyme solution was added to 100 ul of 10 mM PNG (Sigma) and 300 ul of 0.1 M citrate-phosphate buffer (pH 6.0). After incubation at 37℃ for 1 h, 500 ul of 1.0 M Na_2CO_3 was added into reaction solution. The kinetic assay was done in a spectrophotometer at 405nm.

Harvesting of enzyme

Another enzyme harvesting method was tried, which was unique point of this paper. Several kinds of enzyme adsorbents (0.5%, w/v), silicagel, cellulose, pyrophillite, skim

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milk, kaoline, cellite, attagel and polyvinyl alcohol were poured into culture filtrates to adsorb soluble chitinase and incubated by shaking for 1 h at room temperature. The adsorbent containing mainly chitinase was precipitated and freeze-dried to powder.

Bioassay against Aphis gossypii

Red hot pepper leaves infected with second instars of *A. gossypii* nymphs were dipped into supernatant and other test samples for 10 second and dried at room temperature for about 20min .The leaves were placed in a 90mm petri dish containing moisturized.

Results

The insecticidal chitinase activity of the supernatant decreased from 4.7 to 0.4m M p-nitrophenol per hour after 2hours of thermal stress at 50℃. However, the chitinase activity of a freeze-dried pellet made by using the ammonium sulfate precipitation method was stable even after same thermal stress. The harvesting efficiency of attagel powder in corn-oil for chitinase was about 88.2%.

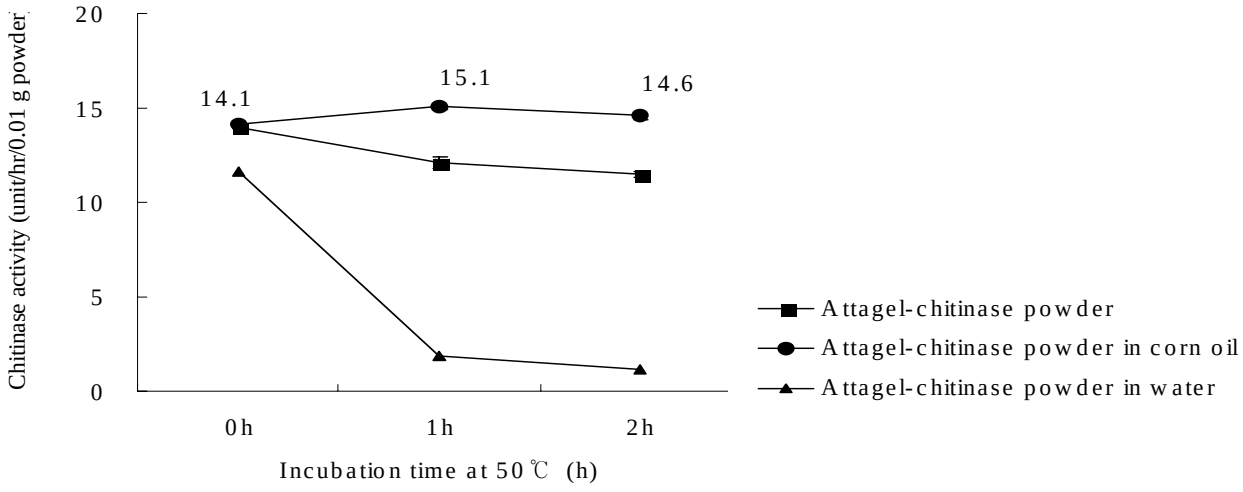

Fig. Thermal stability of SFB-205 attagel-chitinase powder in corn oil.