Induction and purification of extracellular chitinases from insect pathogenic fungi, *Beauveria bassiana*

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**Abstract:** This research aims to purify extracellular chitinase produced from insect pathogenic fungi, *Beauveria bassiana* for insect pest control. Induction of extracellular chitinases was investigated by comparing two different substrates. Mycelia of *B. bassiana* were cultured in synthetic media containing ball milled chitin or colloidal chitin. It was found that the production of extracellular chitinases was remarkably induced in the colloidal chitin media. The enzymes were then purified by ultrafiltration followed by anion exchange chromatography. Two activity peaks, chitinase I and II were identified. Further investigations involving purification, characterization and N-terminal sequencing of these two isolated peaks are in progress.

**Methodology:**

1) **Culturing conditions:** Fungi were grown on PDA plates at 28°C for 14 days. Spores were harvested and then inoculated in 200 ml YMB. The culture was incubated at 28°C for 4 days with shaking at 100 rpm. Mycelium were isolated, washed with 20 mM sodium phosphate buffer pH 6.2 and then cultured in 200 ml synthetic media (0.1% KH₂PO₄, 0.05% MgSO₄, 0.0002% FeSO₄, 0.0001% ZnSO₄, 0.000002% NaMoO₄, 0.000002% CuSO₄, 0.000002% MnCl₂) containing 1% ball milled chitin or 1% colloidal chitin. It was found that the production of extracellular chitinases was remarkably induced in the colloidal chitin media. The enzymes were then purified by ultrafiltration followed by anion exchange chromatography. Two activity peaks, chitinase I and II were identified. Further investigations involving purification, characterization and N-terminal sequencing of these two isolated peaks are in progress.

2) **Ultrafiltration:** The culture medium was filtered through membrane (molecular weight cut off 3 kD) and concentrated to one-tenth volume. Protein content and enzyme activity were determined.

3) **Ion exchange chromatography:** The concentrated culture medium was applied to a 1 ml MonoQ HR 5/5 column previously equilibrated with buffer A (20 mM sodium phosphate pH 6.5). The column was washed with the same buffer and the proteins were eluted at 1 ml/min by an increasing linear gradient of NaCl from 0 to 1 M in buffer A. Fractions of 1 ml were collected and the chitinase activity was measured.

**Results and Discussion:** The production of *B. bassiana* extracellular chitinases was induced in the synthetic medium. By comparing two different substrates, it was found that the chitinases were remarkably induced in the media containing colloidal chitin with 24.10 unit/mg specific activity. This current optimum condition has been proved to significantly improve the production of chitinases (9.90 unit/mg) from ball milled chitin. In addition, colloidal chitin may be easily degraded by chitinases due to its smaller than ball milled chitin. The enzymes were then purified and concentrated to one-tenth volume by ultrafiltration. The concentrates showed the increasing of chitinase specific activity to 30.86 unit/mg indicating the removal of some impurity proteins. The concentrates were then applied to anion exchange column. Two peaks of chitinase activity, chitinase I and II were determined. These results were also observed in parasitic fungi, *Isaria japonica* extracellular chitinases (2). However, this study is still under investigation concerning further purification, characterization and N-terminal sequencing. By discovering the N-terminal sequence, oligonucleotide primers could then be deduced and used to isolate the genes by PCR. This would then allow such gene to be cloned and transformed into plants to construct the insect resistant transgenic plants.

**References:**


**Keywords:** chitinase, *Beauveria bassiana*, enzyme purification, pest control