Faculty of Science
Microbiology Dept.

Studies on safe biological control agents synthesis utilizing crustacean wastes and their technological application

A THESIS

Submitted for the degree of Doctor of Philosophy of Science (Ph.D.) in Microbiology

By

Heba Mohammed Shalaby Mohammed

Assistant Researcher in Chemistry of Natural and Microbial Products Department - National Research Center

B.Sc. Microbiology (2003), Al-Azhar University

M.Sc. Microbiology (2011), Al-Azhar University

Under supervision of

Prof. Dr.
Saadia Mohamed H. Easa
Prof. of Microbiology, Faculty of Science, Ain Shams University

Prof. Dr.
Abd El Mohsen S. Ismail
Prof. of Natural and Microbial Products Chemistry, Chemistry of Natural and Microbial Products Department, National Research Center

Prof. Dr.
Salah Abo-El Nassr Abo- Sedera
Prof. of Microbiology,
Agricultural Microbiology Department,
National Research Center

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Supervisors Approval

Prof. Dr. Saadia Mohamed H. Easa
Assistant Prof. of Microbiology, Faculty of Science, Ain Shams University

Prof. Dr. Abd El Mohsen S. Ismail
Prof. of Natural and Microbial Products Chemistry, Chemistry of Natural and Microbial Products Dept., National Research Center

Prof. Dr. Salah Abo- El-Nassr Abo- Sedera
Prof. of Microbiology, Agricultural Microbiology Dept., National Research Center

Examination Committee:

Prof. Dr. Mohamed I. A. Ali
Prof. of Microbiology, Botany and Microbiology Dept., Faculty of Science, Cairo University

Prof. Dr. Gamal Hassan Rabie
Prof. of Microbiology, Botany and Microbiology Dept., Faculty of Science, Zagazig University

Prof. Dr. Saadia Mohamed H. Easa
Assistant Prof. of Microbiology, Faculty of Science, Ain Shams University

Prof. Dr. Abd El Mohsen S. Ismail
Prof. of Natural and Microbial Products Chemistry, Chemistry of Natural and Microbial Products Dept., National Research Center

Declaration

This thesis has not previously submitted for any other universities.

Heba mohammed shalaby mohammed


بسم الله الرحمن الرحيم
﴿قالوا سبحانك لا علم لنا إلا ما علمتنا
إنك أنت العليم الحكيم﴾
صدق الله العظيم
سورة البقرة الآية: ٣٢

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ABSTRACT

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The objective of the present study was to formulate the production medium and to pinpoint the growth proper conditions for the chosen microorganism producing highly active chitinase enzymes. The general properties of the crude and the partially purified enzyme preparation were determined to define the proper conditions for enzyme action. Under the specified conditions, the two forms of the enzyme have capability for antimicrobial and antioxidant activities were decided.

Eighteen recommended microbial strains (6 bacteria, 6 fungi and 6 actinomycetes) were screened for chitinolytic enzymes productivity. Among all the eighteen organisms and based on the enzyme production ability, *Streptomyces halstedii* H2 was the most potent producer and was chosen for chitinase production. The effects of the culture media composition and other fermentation conditions for optimization of chitinase production by the selected organism were studied. The maximum chitinase activity of (49.5 U/mL) was obtained from glucose (6 g/L), ammonium nitrate and urea mixture (0.9 and 0.64 g/L, respectively) at 30°C and pH 9.0. Among the tested substrates in the production medium, chitin powder (5 g/L) was the most favorable and afforded the maximal chitinase activity.

In this respect, many marine chitinaceous wastes were examined for microbial chitinase production comparing with chitin powder. The marine chitinaceous wastes were subjected to different physical, chemical and physico-chemical pretreatments and among all of those, the 0.1M-NaOH pretreated crab (*Liocarcinus vernalis*) shells powder successfully offered high productivity level but still lesser than that with chitin powder with separation difficulties. Regarding the proper C and N sources and their concentrations in the culture medium, glucose (6 g/L) and ammonium nitrate & urea mixture (0.9 & 0.64 g/L, respectively) were decided. In addition $MgSO_4.7H_2O$ and trace elements solution also were required for the perfect enzyme productivity. The important properties of the *Streptomyces halstedii* H2 chitinase were duly pinpointed as follows: optimum enzyme and substrate concentrations were investigated for crude and partially purified enzyme 1.6, 1.6 mg/mL and 1.4, 1.6 % (w/v), respectively, optimum reaction pH and temperature for both forms of the enzyme 7.2 & 45°C, respectively. The crude and the partially purified enzyme preparations were stable for 60 min at pH 7.2 and 30°C and retained 92.6 % of the original. The partially purified chitinase Michaelis constant (K_m) and maximum velocity constant (V_{max}) for colloidal chitin were 1

mg/reaction and 43.48 Umg^{-1} protein, respectively, applying the Woolf plot. Each of glucose, GlcNAc, Mn^{2+}, Cu^{2+}, Fe^{3+} and Ca^{2+} variably activated the partially purified enzyme. Under studied specified conditions the optimized enzyme preparation (crude or PPE) exhibited good biocontrol activity against some plant bacterial and fungal pathogens. At varying concentrations the two forms of the enzyme preparation exhibited considerable antioxidant activity.

Key words: chitin, chitinase enzyme, microorganisms, marine chitinaceous wastes, biological activities, DPPH radical scavenging activity.