سامية محمد مصطفى

شبكة المعلومات الجامعية

بسم الله الرحمن الرحيم

سامية محمد مصطفى

شبكة المعلومات الجامعية

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

سامية محمد مصطفى

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

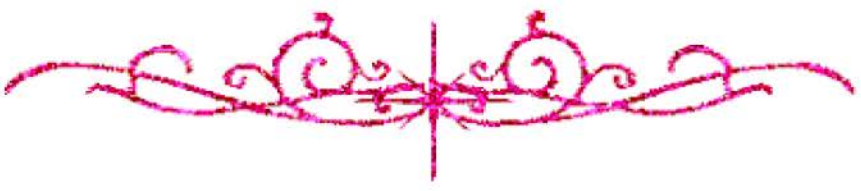
يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

سامية محمد مصطفى

شبكة المعلومات الجامعية

بعض الوثائق الأصلية تالفة



سامية محمد مصطفى

شبكة المعلومات الجامعية

بالرسالة صفحات لم ترد بالأصل

Studies on

Microbial Keratinases

By

Mohammad Hamed Ahmad Ibrahim

(B.Sc. Agric., Soil and Water Sci., 1999)
(Cairo University, El-Fayum Branch)

Thesis submitted for
The partial fulfillment of the requirements for
The degree of
M. Sc. In Agricultural Science
(Microbiology)

To
Department of Microbiology
Faculty of Agriculture
Cairo University
2004

Cairo University
Faculty of Agriculture
Department of Microbiology

Supervision committee

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ABSTRACT

A feather-degrading bacterium was isolated from local hen house. The isolate FH9, genotypically identified as a member of the species of *Bacillus pumilus*, was shown to degrade feather (1.5% w/v) completely. After 48 h of growth, feather degradation led to an increase in free amino acids. Moreover, nutritionally essential amino acids were also produced as microbial metabolites. *B. pumilus* FH9 was immobilized on different carriers, from which the immobilized cells on louf showed the highest specific productivity. Immobilized cells in repeated batch system were not able to keep producing significant level of keratinase. The enzyme was thermostabilized by covalent coupling to $NaIO_4$-oxidized polysaccharides. Glycosylated enzyme with pectin retained the highest activity and stability. The modified enzyme exhibited a higher optimal temperature and pH. It displayed higher level of heat stability. The calculated half-life ($T_{1/2}$) values of heat inactivation at 50, 60, 70, and 80°C were 623, 452, 188, and 143 min, respectively. Whereas, at these temperatures the native enzyme was less stable ($T_{1/2}$ of 102, 74, 30, and 8 min, in the same order). The behaviour of the modified enzyme in the presence of different metal ions and the chelating agent, EDTA, differed from that of the native form. These improved stabilities, which the glycosylated keratinase possess, increasing its potential for use in numerous applications. Enzyme purification was conducted by gel filtration through Sephadex G-100 followed by anion exchange chromatography on DEAE-Cellulose E11 yielding an active protein showing 11.76-fold purification. The purified enzyme was electrophoritecally homogeneous with a molecular mass of 55 kDa. The pure enzyme was optimally active at pH 9.0 and 60°C. Moreover, it showed significant stability in alkaline pH's and temperatures. *B. pumilus* FH9 keratinase showed higher proteolytic activity on casein > BSA > collagen > gelatin > feather > horn > wool. The enzyme was metalloprotease, activated with Ca^{2+} and Mg^{2+} and significantly inhibited by Zn^{2+}, EDTA, Co^{2+} and Hg^{2+}.

M. K. Zahra

DEDICATION