



بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ



# شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



شبكة المعلومات الجامعية

# جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

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Thesis Entitled  
**Biochemical Studies on Some Enzymes  
Used in Industry**

Submitted by

**Samia Abdel-Aziz Ahmed**

(M.Sc. Agric. Biochemistry )  
(Cairo University)

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For the Degree of

**Ph. D. of Agriculture Science  
(Biochemistry)**

Supervised by

**Prof. Dr. Abdel-monem M. Nageb**  
Prof. of Biochemistry  
Dept. of Biochemistry Faculty  
of Agriculture  
Cairo University

**Prof. Dr. Mohamed A. Abdel-Naby**  
Prof. of Biochemistry  
Dept. of Natural and  
Microbial Products  
National Research Centre

Cairo University  
Faculty of Agriculture  
Arab Republic of Egypt  
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Name of candidate: Samia Abdel-Aziz Ahmed Degree: Ph. D. of Agriculture  
Science Biochemistry

Title of thesis : Biochemical studies on some enzymes used in industry

Supervisors : Prof. Dr. Abdel-monem Mohamed nageb

Prof. Dr. Mohamed Ahamed Abdel-naby

Department : Biochemistry

Branch : Biochemistry

Approval : 31/5/2003

### ABSTRACT

The present investigation deals with physiological and biochemical studies on bacterial milk-clotting enzyme. It includes studies aiming at searching for a potent bacterial strain producing active milk-clotting enzyme.

The studies also included the production of the enzyme in batch and continuous cultures, by the free and immobilized cells of *B. circulans* 25.

The studies also include the immobilization of enzyme on different carries. Separation, fractionation, purification, and properties of the purified enzyme were also investigated.

*Bacillus circulans* 25 (at 24 h) showed the highest ratio of MCA/ caseinase activity (5.9).

The effect of some cultural condition (carbon basis, nitrogen sources,  $\text{KH}_2\text{PO}_4$  concentration,...etc) on the productivity of MCE by *B. circulans* 25 was investigated.

Different matrices were investigated for the production of MCE by the immobilized cells of *B. circulans* 25. Of all preparations, the immobilized cells of *B. circulans* 25 on wool (after 24h), showed the highest MCA (1.0 U/ml with MCA/caseinase activity ratio of 11.11) and the highest specific productivity (5.2 U/g wet cells/h).

Continuous production of MCE by the immobilized cells of *B. circulans* 25 was investigated in repeated batch process.

Continuous production of MCE by the immobilized cells of *B. circulans* 25 in packed-bed bioreactor was investigated.

The property of crude MCE produced by *B. circulans* 25 was investigated. Fractional precipitation with ethanol, ammonium sulphate and tannic acid did not affect enzyme purification.

*B. circulans* 25 milk-clotting enzyme was immobilized on different carriers by using different techniques for enzyme immobilization.

The immobilized *B. circulans* 25 MCE on chitosan retained about 77% of the original specific activity. The property of immobilized MCE produced by *B. circulans* 25 was investigated.

The operational stability of the immobilized *B. circulans* 25 MCE was evaluated in repeated batch process.

Purification of *B. circulans* 25 MCE was achieved by using ion exchange chromatography on Ecteola cellulose by batch wise elution.

The active protein of fraction 1 (F1) was subjected to gel filtration through Sephadex G-100.

The purity of this enzyme was judged by polyacrylamide gel electrophoresis

The molecular weight of the pure *B. circulans* 25 MCE was estimated to be 38000 the pure enzyme proved to be a glycoprotein and containing 25.1% carbohydrate.

The property of pure MCE produced by *B. circulans* 25 was investigated.

Approval Sheet

*Biochemical studies on some  
enzymes used in industry*

By

*Samia Abdel-Aziz Ahmed*

*M.Sc. of Agric. Biochemistry*

*Cairo University*

supervised by

*Prof. Dr. Abdel-monem M. nageb*

*Prof. of Biochemistry*

*Biochemistry Dept. Faculty*

*of agriculture*

*Cairo University*

*Prof. Dr. Mohamed A. Abdel-naby*

*Prof. of Biochemistry*

*Dept. of Natural and*

*Microbial products*

*National research centre*

*Approved by*

*عبدالمونيم م. ناجب*  
*محمد أ. عبد النابى*  
*Samia*

*Committee in charge*

*Date:*

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