

شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو

# بسم الله الرحمن الرحيم





HANAA ALY



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكرونيله



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



HANAA ALY



شبكة المعلومات الجامعية التوثيق الإلكترونى والميكروفيلم

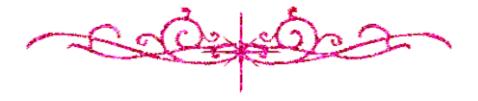
## جامعة عين شمس التوثيق الإلكتروني والميكروفيلم قسم

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



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



HANAA ALY



# Studies on the Production of Bacterial Cellulases

### **A Thesis**

Submitted in Partial Fulfillment of the Requirements for

Master's degree

In Pharmaceutical Sciences (Microbiology and Immunology)

By

### Mery Sameh Waheeb Gabrah Morgan

Bachelor of Pharmaceutical sciences, Faculty of Pharmacy, Ain Shams University, 2013



#### **Studies on the Production of Bacterial Cellulases**

#### **A Thesis**

Submitted in Partial Fulfillment of the Requirements for

#### Master's degree

In Pharmaceutical Sciences (Microbiology and Immunology)

By

#### Mery Sameh Waheeb Gabrah Morgan

Bachelor of Pharmaceutical sciences, Faculty of Pharmacy, Ain Shams University, 2013

Under Supervision of

#### Dr. Nadia Abdel-Halim Hassouna, PhD

Professor of Microbiology and Immunology, Faculty of Pharmacy – Ain Shams University

#### Dr. Mahmoud Abdul-Magead Yassien, PhD

Professor of Microbiology and Immunology, Faculty of Pharmacy – Ain Shams University

#### Dr. Walid Faisal Elkhatib, PhD

Professor of Microbiology and Immunology, Faculty of Pharmacy – Ain Shams University

Vice Dean, Faculty of Pharmacy - Galala University

## Acknowledgment

Praise be to **Allah**, Lord of all creation, for granting me the power to accomplish this work.

I would like to express my deepest thanks to **Prof. Dr.**Nadia Abdel-Halim Hassouna, Professor of Microbiology and Immunology, and founder of the Microbiology and Immunology Department, Faculty of Pharmacy, Ain Shams University, for her constant support and valuable scientific supervision throughout the work.

I am greatly indebted to **Prof. Dr. Mahmoud Abdul-Magead Yassien**, Professor of Microbiology and Immunology, Faculty of Pharmacy, Ain Shams University, for suggesting the point, his utmost support, follow up and scientific guidance throughout the work, kindly supplying laboratory facilities whenever needed, and for his constructive criticism throughout this study. I am also very grateful for the kind and in-depth revision of the thesis.

I am indebted to **Prof. Dr. Walid Faisal Elkhatib**, Professor of Microbiology and Immunology, Faculty of Pharmacy, Ain Shams University, and Vice & Acting Dean, Faculty of Pharmacy, Galala University for providing continuous scientific supervision. I also thank him for his kind revision of the thesis.

I am grateful to **Dr. Nooran Elleboudy**, Lecturer of Microbiology and Immunology, Faculty of Pharmacy, Ain Shams for her great help throughout the work.

I am also thankful to my **dear colleagues** and all the **workers** at the Microbiology and Immunology Department, Faculty of Pharmacy, Ain Shams University for their help and support during this work.

In addition, I would like to express my eternal heartfelt gratitude to **my parents, my brothers, and my sister** for being the greatest support I could ever get to complete this study.

Finally, a very special thanks directed to **my dear** husband for his continuous support and encouragement. His patience and sacrifice will remain my inspiration throughout my life. My thesis acknowledgment would be incomplete without thanking my son, **Daniel**, whose smiling face always made me happy and inspired me. This thesis is dedicated to my family, husband, and son.

**Mery Sameh Waheeb** 

## **Table of Contents**

Fable of Contents		
List of AbbreviationsVIII		
Abstract	1	
Introduction	4	
Literature Review	6	
1. Cellulases	6	
1.1. Classification and mode of action of cellulases	6	
1.2. Substrates for cellulases	7	
1.3. Screening of cellulase-producing bacteria	8	
1.4. Quantitative assays for cellulases	8	
1.5. Applications	9	
1.6. Thermophilic cellulases	4	
1.7. Cellulase production by microorganisms 1	5	
2. The Genus Streptomyces	7	
2.1. Genetic identification of <i>Streptomyces</i> <b>1</b>	8	
2.2. Bioactivity of <i>Streptomyces</i>	8	

3. Optimization of microbial enzymes production	20
3.1. Optimization of process parameters	20
3.2. Optimization by mutation	25
4. Purification of cellulases	26
5. Enzyme immobilization	27
5.1. Choice of supports	27
5.2. Methods of immobilization	29
Materials and Methods	33
1. Soil samples	33
2. Microorganisms	33
3. Chemicals	33
4. Culture media	35
4.1. Ready-to-use culture media	36
4.2. In house formulated culture media	36
5. Buffers, solutions and reagents	38
5.1. Sodium phosphate buffer (50 mM) with carboxymethyl cellulose	
5.2. 3,5 Dinitrosalicylic acid reagent	39
5.3. Citrate buffer	39
5.4. Phosphate buffer (100 mM)	40
5.5. Glycine–NaOH buffer (100 mM)	41
5.6. Protein assay reagents and standard solutions	41

5.7. Reagents for Sodium Dodecyl Sulfate Polyacrylamide
Gel Electrophoresis and zymogram analysis42
6. Devices
Methods 48
7. Recovery of cellulase-producing bacteria from different soil samples
7.1. Collection of soil samples
7.2. Isolation of cellulase-producing bacteria48
8. A confirmatory test for the ability of the collected <i>Streptomyces</i> isolates for cellulase production using Congo red method
9. Quantitative determination of cellulase production by the selected <i>Streptomyces</i> isolates using dinitrosalicylic acid assay
9.1. Preparation of the crude enzyme
9.2. Enzyme assay <b>50</b>
10. Calibration curve for cellulase
11. Dry cell weight determination
12. Preservation of isolates
13. Molecular identification of the selected isolates with highest cellulase productivities
14. Effect of different factors on growth and production of cellulase by the selected <i>Streptomyces</i> isolate in shake flasks
~ ! ***

14.1. Preparation of seed culture
14.2. Preliminary studies on the effect of nutritional and
environmental factors55
14.3. Response Surface Methodology for optimization of
cellulase activity
14.4. Statistical and graphical investigations 61
14.5. Validation of the applied model data <b>61</b>
15. Improvement of cellulase production by genetic manipulation
15.1. Mutagenesis by treatment with gamma radiation <b>61</b>
15.2. Screening of the selected colonies for their cellulase production
16.Purification of cellulase produced from the selected isolate with highest cellulase productivity
16.1. Enzyme production
16.2. Ammonium sulfate precipitation
16.3. DEAE–Sepharose column chromatography 64
16.4. Sephadex G-75 gel chromatography 64
16.5. Determination of protein content using Lowry method
16.6. Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis and zymogram for the purified cellulase
preparations 67

17. Enzyme characterization
17.1. Enzyme activity at different temperatures <b>69</b>
17.2. Enzyme activity at different pH values <b>69</b>
17.3. Effect of temperature on enzyme stability <b>69</b>
18. Immobilization of cellulase enzyme <b>70</b>
18.1. Physical aggregation of cellulase enzyme <b>70</b>
18.2. Preparation of crosslinked cellulase aggregates using glutaraldehyde
18.3. Immobilization of the purified cellulase preparation
18.4. Reusability of crosslinked cellulase aggregates 73  Results
1. Recovery of cellulase-producing bacteria from different soil samples
2. A confirmatory test for the ability of the collected <i>Streptomyces</i> isolates for cellulase production
3. Quantitative determination of cellulase production by the selected <i>Streptomyces</i> isolates
4. Identification of the selected <i>Streptomyces</i> isolates
5. Effect of different factors on growth and production of cellulase by the selected <i>Streptomyces</i> isolate

environmental factors
5.2. Response Surface Methodology Experimental Design
6. Improvement of the enzyme production of <i>S. thermodiastaticus</i> by genetic manipulation
6.1. Evaluation of the cellulase productivity of the obtained mutants using Congo red method
6.2. Quantitative determination of cellulase productivity by the selected mutants using dinitrosalicylic acid assay <b>107</b>
7. Purification of cellulase from the Culture Supernatants <b>110</b>
8. Enzyme Characterization
8.1. Temperatures
8.2. The pH of surrounding medium
8.3. Thermotolerance 114
9. Immobilization of cellulase enzyme
9.1. Cross-linked enzyme aggregates preparation and optimization
9.2. Formation of cross-linked cellulase aggregates using
the partially purified cellulase 120
9.3. Reusability of cross-linked enzyme aggregates <b>121</b>
Discussion
Conclusion150

### **Table of Contents**

Summary	
References	155
الملخص العربي	

## **List of Abbreviations**

Abbreviation	Definition	
CAGR	Compound Annual Growth Rate	
CMC	Carboxy methyl cellulose	
RSM	Response Surface Methodology	
OFAT	One-factor-at-a-time	
BBD	Box-Behnken design	
Gy	Gray	
SDS-PAGE	Sodium Dodecyl Sulfate –	
	Polyacrylamide Gel Electrophoresis	
XCA	Cross-linked cellulase aggregates	
Sp.	Species	
DNS	Dinitrosalicyclic acid	
DOE	Design of experiments	
PBD	Plakett Burman Design	
CDE	Cross-linked dissolve enzymes	
CLEC	Cross-linked crystalline enzymes	
CLSD	Cross-linked sprayed dried enzymes	
CLEA	Cross-linked enzyme aggregates	
DTT	Dithiothreitol	
APS	Ammonium Persulfate	
Н	Hour (s)	
DCW	Dry Cell Weight	
CFCS	Cell free culture supernatant	
CFU	Colony forming unit	
DNA	Deoxyribonucleic acid	
PCR	Polymerase chain reaction	
ANOVA	Analysis of variance	
BLAST	Basic Logic Alignment Search Tool	
CV	Coefficient of variation	
v/v	Volume/Volume	
w/v	Weight/Volume	
Rpm	Round per minute	
UV	Ultraviolet	
DF	Degrees of Freedom	