سامية محمد مصطفى



شبكة المعلومات الحامعية

بسم الله الرحمن الرحيم



-Caro-

سامية محمد مصطفي



شبكة العلومات الحامعية



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم





سامية محمد مصطفى

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسو

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



يجب أن

تحفظ هذه الأقراص المدمجة يعيدا عن الغيار



سامية محمد مصطفي



شبكة المعلومات الجامعية



المسلمة عين شعور المسلمة عين شعور المسلمة عين شعور المسلمة عين شعور المسلمة ا

سامية محمد مصطفى

شبكة المعلومات الحامعية



بالرسالة صفحات لم ترد بالأصل



BIOTECHNOLOGICAL STUDIES ON THE BIODEGRADATION OF SOME KERATIN -CONTAINING MATERIALS.

Thesis Submitted in partial fulfillment of the requirements for Master Of Science

Bioscience and Technology



By

Amira Mohamed Embaby Mahmoud

(B.Sc. Microbiology special, 1994) Faculty of Science, University of Alexandria

Department of Bioscience and Technology Institute of Graduate Studies and Research University of Alexandria

B (CE99

2004

APPROVED

BIOTECHNOLOGICAL STUDIES ON THE BIODEGRADATION OF SOME KERATIN-CONTAINING MATERIALS.

Referees:

1- W A. Mashhoer
2- Jaghus
3- Ellahan

Place of Discussion:

Institute of Graduate Studies and Research University of Alexandria Alexandria, Egypt

Date: / / 2004

SUPERVISORS

Dr. Taha Ibrahim Zaghloul

Professor of Microbiology and Molecular Biology

Department of Bioscience and Technology

Institute of Graduate Studies and Research

University of Alexandria

Dr. Hesham Mahmoud Saeed

Lecturer of Biochemistry

Department of Bioscience and Technology

Institute of Graduate Studies and Research

University of Alexandria

TO MARINS

Acknowledgment

I am very grateful to Dr.Taha I. Zaghloul, Professor of Microbiology and Molecular Biology, Department of Bioscience and Technology, Institute of Graduate Studies and Research, University of Alexandria for his excellent supervision, continuous guidance, valuable advice, support and help throughout the whole work.

I would like to express my appreciation to Dr. Hesham Mahmoud Saeed, Lecturer of Biochemistry, Department of Bioscience and Technology, Institute of Graduate Studies and Research, University of Alexandria for his help, valuable advices, and continuous supervision.

I am very grateful also Dr. Hesham I. Zaki, Professor of Environmental Studies department, Institute of Graduate Studies and Research, University of Alexandria for his well appreciated helps presented to me throughout carrying out the practical part of this work.

I would like to express my appreciation to Dr. Ahmed Rafeek El Mahdy, Professor of Biochemistry, Department of Food Science and Technology, Faculty of Agriculture, University of Alexandria, for his valuable help.

I would like to express my great thankfulness to Dr. Ahmed I. Khalil, Lecturer of Environmental Studies department, Institute of Graduate Studies, University of Alexandria for his help.

I am also very grateful to all members in the laboratory of Biotechnology who helped me in the completion of this work.

I am very grateful to Dr. Mohamed Helmy, Lecturer of Biochemistry, Department of Bioscience and Technology, Institute of Graduate Studies and Research, University of Alexandria for his help.

Contents

I.	Con	tents	Pa
II.	List	of tables	
III.	List	of figures	
IV.		of photos	1
1.		oduction	
2.		of the work	
3.		ew of Literature	
	3.1	Keratin and keratin - containing materials	
	3.2	Keratin – degrading microorganisms: an outlook.	
	3.3	Habitats of feather - degrading microorganisms	
	3.4	Chicken feathers	
	3.5	Biodegradation of chicken feathers	
		3.5.1. Biodegradation by whole microbial cells	
		3.5.2. Biodegradation of chicken feathers by the culture filtrate	
	3.6	Microbial keratinases: an outlook.	
		3.6.1 Keratinases of the genus <i>Bacillus</i>	
		3.6.2 Classification of microbial keratinases	
		3.6.3 Microbial keratinases: enzyme assay and purification	
		3.6.4 A possible mechanism for keratin degradation	
		3.6.5 Uses of keratinases and possible applications	
4.	Mate	erials and Methods	4
••	4.1	Bacterial strain and plasmid	4
	4.2	Media	4
	4.3	Preparation of the culture filtrate	4
	4.4	Preparation and pretreatment of chicken feathers	4
	4.5	Alkaline protease activity	4
	4.6	Monitoring soluble proteins and NH ₂ – free amino groups in	4
		the feather/ culture filtrate reactions	
	4.7	Determination of soluble proteins	
	4.8	Determination of NH ₂ - free amino groups	4
	4.9	Biodegradability of different keratin - containing materials	4
		using the culture filtrate	
	4.10	Optimization of the feather/ culture filtrate reaction	4
	4.11	Analysis of the produced amino acids	4
	4.12	Purification of recombinant <i>Bacillus subtilis</i> alkaline protease	4
		enzyme	-
	4.13	SDS- polyacrylamide electrophoresis	
	4.14	Preparation of dialysis tubing	4
	4.15	Extraction of keratin from feathers and wool	
	4.16	Keratin agarose plate assay	4

4.17 Substrate gel electrophoresis (Zymogram technique)
A.19 Effect of EDTA and PMSF
4.20 Determination of the keratinolytic / proteolytic ratio of the alkaline protease. 5. Results and Discussion 5.1 Degradation of some keratin- containing materials by the B. subtilis DB100 (pS1) culture filtrate: a pilot experiment. 5.2. Optimizing the degradation of chicken feathers by the B. subtilis DB100 (pS1) culture filtrate. 5.2.1 Effect of the reaction's pH. 71 5.2.2 Effect of different enzymatic units on the degradation of chicken feathers. 5.2.3 Effect of the percentage of chicken feathers. 80 5.2.4 Effect of adding additional enzymatic units 85 5.2.5 Effect of incubation temperature. 88 5.2.6 In vitro feather degradation requires feather pretreatment. 5.2.6.1 Effect of the culture filtrate on native chicken feathers. 94 5.2.6.2 Effect of autoclaving 99 5.2.6.3 Effect of the culture filtrate source on feather degradation reaction directed by culture filtrate. 119 5.3 Analysis of amino acids released from feathers degradation reaction directed by culture filtrate. 119 5.5 The digestibility of commercial feather meal is improved via culture filtrate. 124 5.6 B. subtilis alkaline protease shows high level of keratinolytic activity: a general outlook.
4.20 Determination of the keratinolytic / proteolytic ratio of the alkaline protease
alkaline protease. 5. Results and Discussion
5.1 Degradation of some keratin- containing materials by the B. subtilis DB100 (pS1) culture filtrate: a pilot experiment. 5.2. Optimizing the degradation of chicken feathers by the B. subtilis DB100 (pS1) culture filtrate. 5.2.1 Effect of the reaction's pH
5.1 Degradation of some keratin- containing materials by the B. subtilis DB100 (pS1) culture filtrate: a pilot experiment. 5.2. Optimizing the degradation of chicken feathers by the B. subtilis DB100 (pS1) culture filtrate. 5.2.1 Effect of the reaction 's pH
5.2. Optimizing the degradation of chicken feathers by the B. subtilis DB100 (pS1) culture filtrate
5.2.1 Effect of the reaction 's pH
5.2.1 Effect of the reaction 's pH
5.2.1 Effect of the reaction 's pH
5.2.2 Effect of different enzymatic units on the degradation of chicken feathers. 5.2.3 Effect of the percentage of chicken feathers. 5.2.4 Effect of adding additional enzymatic units. 5.2.5 Effect of incubation temperature. 88 5.2.6 In vitro feather degradation requires feather pretreatment. 5.2.6.1 Effect of the culture filtrate on native chicken feathers. 94 5.2.6.2 Effect of autoclaving. 99 5.2.6.3 Effect of using some chemical reducing agents. 103 5.2.7 Effect of the culture filtrate source on feather degradation reaction directed by culture filtrate. 5.4 Concentration of the bacterial culture filtrate. 5.5 The digestibility of commercial feather meal is improved via culture filtrate. 5.6 B. subtilis alkaline protease shows high level of keratinolytic activity: a general outlook.
chicken feathers. 5.2.3 Effect of the percentage of chicken feathers. 5.2.4 Effect of adding additional enzymatic units. 5.2.5 Effect of incubation temperature. 88 5.2.6 In vitro feather degradation requires feather pretreatment. 5.2.6.1 Effect of the culture filtrate on native chicken feathers. 5.2.6.2 Effect of autoclaving. 5.2.6.3 Effect of using some chemical reducing agents. 5.2.7 Effect of the culture filtrate source on feather degradation reaction directed by culture filtrate. 5.4 Concentration of the bacterial culture filtrate. 5.5 The digestibility of commercial feather meal is improved via culture filtrate. 5.6 B. subtilis alkaline protease shows high level of keratinolytic activity: a general outlook.
5.2.3 Effect of the percentage of chicken feathers. 5.2.4 Effect of adding additional enzymatic units. 5.2.5 Effect of incubation temperature. 5.2.6 In vitro feather degradation requires feather pretreatment. 5.2.6.1 Effect of the culture filtrate on native chicken feathers. 5.2.6.2 Effect of autoclaving. 5.2.6.3 Effect of using some chemical reducing agents. 5.2.7 Effect of the culture filtrate source on feather degradation reaction directed by culture filtrate. 5.4 Concentration of the bacterial culture filtrate. 5.5 The digestibility of commercial feather meal is improved via culture filtrate. 5.6 B. subtilis alkaline protease shows high level of keratinolytic activity: a general outlook.
5.2.4 Effect of adding additional enzymatic units
5.2.5 Effect of incubation temperature
5.2.6 In vitro feather degradation requires feather pretreatment
5.2.6.1 Effect of the culture filtrate on native chicken feathers 94 5.2.6.2 Effect of autoclaving
5.2.6.1 Effect of the culture filtrate on native chicken feathers 94 5.2.6.2 Effect of autoclaving
5.2.6.2 Effect of autoclaving
5.2.6.3 Effect of using some chemical reducing agents. 5.2.7 Effect of the culture filtrate source on feather degradation 109 5.3 Analysis of amino acids released from feathers degradation reaction directed by culture filtrate. 5.4 Concentration of the bacterial culture filtrate. 5.5 The digestibility of commercial feather meal is improved via culture filtrate. 5.6 B. subtilis alkaline protease shows high level of keratinolytic activity: a general outlook.
5.2.7 Effect of the culture filtrate source on feather degradation 109 5.3 Analysis of amino acids released from feathers degradation reaction directed by culture filtrate. 5.4 Concentration of the bacterial culture filtrate. 5.5 The digestibility of commercial feather meal is improved via culture filtrate. 5.6 B. subtilis alkaline protease shows high level of keratinolytic activity: a general outlook.
5.3 Analysis of amino acids released from feathers degradation reaction directed by culture filtrate. 5.4 Concentration of the bacterial culture filtrate. 5.5 The digestibility of commercial feather meal is improved via culture filtrate. 5.6 B. subtilis alkaline protease shows high level of keratinolytic activity: a general outlook.
reaction directed by culture filtrate
reaction directed by culture filtrate
5.4 Concentration of the bacterial culture filtrate
5.5 The digestibility of commercial feather meal is improved via 121 culture filtrate
5.6 B. subtilis alkaline protease shows high level of keratinolytic 124 activity: a general outlook
activity: a general outlook
5.6.1 Purification of alkaline protease
5.6.2 The release of soluble materials by crude and purified 132
alkaline protease enzyme
5.6.3 Keratin agarose plate assay
5.6.4 Detection of keratinolytic activity in a zymogram
5.6.5 Alkaline protease enzyme is inhibited by the commonly 143
used keratinase inhibitors
5.6.6 Alkaline protease shows keratinolytic activity even when 146
cells grown on non – keratin based medium
5.6.7 Keratinolytic / protealytic ratio of the alkaline protease 148
versus proteinase k
6. Summary

7.	References	
8.	Arabic Summary	157

List of tables

1.	Feather-degrading isolates and their origins.	Pag 10
2.3.	Quantitative amino acid composition of native chicken feathers The nature of some keratinolytic proteases produced by keratinolytic bacteria	15 26
4.	The nature of some keratinolytic proteases produced by keratinolytic fungi.	27
5.	The nature of some keratinolytic proteases produced by keratinolytic actinomycetes.	28
6.	The nature of some keratinolytic proteases derived from of the genus Bacillus	33
7.	Monitoring the level of soluble proteins resleased from the biodegradation of some keratinous materials due to the action of <i>B. subtilis</i> DB100 (pS1) culture filtrate	64
8.	Monitoring the level of NH ₂ – free amino groups released from the biodegradation of some keratinous materials due to the action of <i>B. subtilis</i> DB100 (pS1) culture filtrate	66
9.	Monitoring the level of soluble proteins and NH ₂ – free amino groups released from the feather / culture filtrate reactions due to the action of culture filtrate of <i>B. subtilis</i> DB100 (pS1) cells at different pHs	72
10.	Monitoring the level of soluble proteins and NH ₂ – free amino groups released from the feather / culture filtrate reaction containing different enzymatic units (caseinolytic units)	77
11.	Monitoring the level of soluble proteins released from the feather / culture filtrate reactions using different feather percent	81
12.	Monitoring the level of NH ₂ – free amino groups released from the feather / culture filtrate reaction using different feather percent	83
13.	Monitoring the level of soluble proteins and NH ₂ – free amino groups released from the feather / culture filtrate reaction after addition of more proteolytic units (caseinolytic units)	86
14.	Monitoring the level of soluble proteins and NH ₂ – free amino groups released from the feather / culture filtrate reactions carried out at 37°C, 50°C, and 55°C	89
15.	Monitoring the level of soluble proteins and NH ₂ – free amino groups released from the feather / culture filtrate reaction using native feathers and feathers that have been pretreated with DTT or autoclaving.	95
16.	Monitoring the level of soluble proteins and NH ₂ – free amino groups released from the biodegradation of one and two times autoclaved feathers due to the action of the culture filtrate	100

17.	Monitoring the level of soluble proteins and NH ₂ – free amino groups released from the feather / culture filtrate reactions using preautoclaved feather that has been treated with some reducing agents.	104
18.	Effect of some reducing agents on the proteolytic activity of the culture filtrate	107
19.	Monitoring the level of soluble proteins and NH ₂ – free amino groups resulted from the feather / culture filtrate reaction using culture	110
20.	filtrate derived from two different sources	114
21.	Recovery percent of alkaline protease enzymatic units in the culture filtrate after concentration with the sucrose embedded method and ammonium sulfate precipitation method.	120
22.	Monitoring the level of soluble proteins and NH ₂ – free amino groups released from the biodegradation of commercial feather meal due to	122
23.	Protein content and proteolytic activity of the alkaline protease enzyme samples after S-Sepharose ion exchanger	126
24.	Purification table for the alkaline protease enzyme produced by B. subtilis DB100 (pS1) cells upon growing on sporulation medium	128
25.	(1XSG)	133
26.	Effect of PMSF and EDTA on the keratinolytic activity of the culture filtrate.	144
27.	Keratinolytic and proteolytic activity of culture filtrate derived from the recombinant <i>B. subtilis</i> cells grown on non-keratin based medium	147
28.	for 24 hours	149
29.	Keratinolytic / proteolytic ratio determined for the recombinant B. subtilis culture filtrate and proteinase K using synthetic substrates.	150

•