

**Sudan University of Science and Technology**  
**Faculty of Agricultural Studies**  
**Department of Food Science and Technology**

**Production of Amylolytic Enzymes by *Bacillus spp.* and  
*Rhizopus spp.* and their Use in Ethanol  
Production**

**By**

**El Mutaz Nassir Hassan Farag**

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**Supervisor:** Dr. Ibtisam Ismail Mekki

**Co- Supervisor:** Dr. Ahmed El Awad El Faki

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## **Dedication**

*To the soul of my mother  
and  
my family*

# List of Contents

	<b>Page</b>
<b>Acknowledgements</b>	<b>II</b>
<b>Dedication</b>	<b>III</b>
<b>List of Contents</b>	<b>IV</b>
<b>List of Tables</b>	<b>IX</b>
<b>List of Figures</b>	<b>X</b>
<b>List of Plates</b>	<b>XIII</b>
<b>Abstract</b>	<b>XIV</b>
<b>Abstract(Arabic)</b>	<b>XVI</b>
<b>Chapter One: Introduction</b>	<b>1</b>
<b>Chapter Two: Literature Review</b>	<b>5</b>
2.1. Enzymes	5
2.1.1. General.	5
2.1.2. Nomenclature and classification	8
2.2. Starch.	8
2.3. Amylases	9
2.3.1. Cereal amylases	10
2.3.2. Animal amylases	10
2.3.3. Microbial amylases	11
2.3.3.1. Fungal amylases	11
2.3.3.1.1. Isolation	11
2.3.3.1.2. Amylase production	11
2.3.3.1.3. Amylase activity	12
2.3.3.2. Bacterial amylases	13
2.3.3.2.1. Isolation	13

2.3.3.2.2. Amylase production	14
2.3.3.2.3. Amylase activity	16
2.4. Ethanol production	18
<b>Chapter Three: Materials and Methods</b>	<b>20</b>
3.1. Screening for amyloytic microbes	20
3.2. Potential microbes for production of amylase	20
3.3. Identification of bacteria	21
3.3.1. Morphological test	21
3.3.1. 1. Motility test	21
3.3.2. Biochemical test	21
3.3.2.1. Gram stain	21
3.3.2.2. Oxidase test	21
3.3.2.3. Catalyse test	22
3.3.2.4. Oxidation – Fermentation test	22
3.4. Identification of fungi	22
3.5. Enzyme production	22
3.5.1. Chemical analysis of wheat bran.	22
3.5.2. Inoculum preparation	22
2.5.3. Wheat bran culture	23
3.6. Enzyme extraction	23
3.7. Determination of amyloytic activity	23
3.7.1. Buffer	23
3.7.2. DNS solution	23
3.7.3. KI solution	24
3.7.4. Enzyme assays	24
3.8. Optimum condition for enzyme production	24

3.8.1. Incubation time	24
3.8.2. Incubation temperature	25
3.8.3. pH value of culture	25
3.9. Optimum condition for enzyme activity	25
3.9.1. Effect of temperature	25
3.9.2. Effect of pH value	25
3.9.3. Effect of reaction time	25
3.10. Enzyme inactivation	26
3.10.1. Thermal inactivation	26
3.10.2. pH inactivation	26
3.10.3. Storage inactivation	26
3.11. Detection of enzyme products	26
3.12. Ethanol production	27
3.12.1. Yeast isolation	27
3.12.1.1. Collection of samples	27
3.12.1.2. Recovery the yeasts from the samples	27
3.12.1.3. Purification of the yeast isolates	28
3.12.2. Identification of yeast	28
3.12.2.1. Microscopic appearance of non- filamentous vegetative cell	28
3.12.2.2. Microscopic examination for filamentous growth	28
3.12.2.3. Microscopic examination of ascospores	29
3.12.2.4. Utilization of carbohydrate anaerobically	29
3.12.2.5. Utilization of carbohydrate aerobically	29
3.12.2.6. Utilization of nitrogen compounds for aerobic	30

growth	
3.12.2.7. formation of extra-cellular starch – compounds	30
3.12.2.8. Testing growth at high concentration of ethanol	30
3.12.2.9. Starch hydrolysis	30
3.12.3. Conversion and fermentation of grain mashes	31
<b>Chapter Four: Results and Discussion</b>	<b>32</b>
4.1. Screening for amylotic microorganisms	32
4.1.1. Identification of bacterial isolates	32
4.1.2. Identification of fungal isolates	32
4.2. Optimum condition for enzyme production	36
4.2.1. Incubation time	36
4.2.2. Incubation temperature	40
4.2.3. pH value of culture	40
4.3. Optimum condition for enzyme activity	45
4.3.1. Incubation temperature	45
4.3.2. pH value	48
4.3.3. Incubation time	48
4.4. Enzyme inactivation	53
4.4.1. Thermal inactivation	53
4.4.2. pH inactivation	68
4.4.3. Storage inactivation	73
4.5. Identification of the hydrolytic products of soluble starch	79
4.6. Ethanol production.	79
4.6.1. Identification of yeasts.	79
4.6.2. Best combination between two enzymes	86

4.6.3. Sugar production	86
4.6.4. Conversion and fermentation of grain mashes	86
<b>References</b>	<b>89</b>
<b>Appendix</b>	<b>100</b>

## **List of Tables**

<b>Table</b>	<b>Page</b>
(1) Screening for amylotic microorganisms (Bacteria)	33
(2) Screening for amylotic microorganisms (Fungi)	34
(3) Identification of fungi	35
(4) Chemical analysis of wheat bran (%)	37
(5) Yeasts isolated from different samples and their ability to grow at different concentrations of D-glucose (w/v)	82
(6) Some morphological characters of isolated yeast	83
(7) Some biochemical characteristics of isolated yeast	84-85

## List of Figures

Figure	Page
1-(a) and (b) The effect of different incubation times on crude amylase production by different <i>Bacillus</i> strains (B1, B2, B11) and <i>Rhizopus</i> strains (F16, F22, F23)	38 39
2-(a) and (b) The effect of different incubation temperatures on crude amylase production by different <i>Bacillus</i> strains (B1, B2, B11) and <i>Rhizopus</i> strains (F16, F22, F23)	41 42
3-(a) and (b) The effect of different pH levels of culture on crude amylase production by different <i>Bacillus</i> strains (B1, B2, B11) and <i>Rhizopus</i> strains (F16, F22, F23)	43 44
4-(a) and (b) The effect of different temperatures on crude amylase activity by different <i>Bacillus</i> strains (B1, B2, B11) and <i>Rhizopus</i> strains (F16, F22, F23)	46 47
5-(a) and (b) The effect of different pH values on crude amylase activity by different <i>Bacillus</i> strains (B1, B2, B11) and <i>Rhizopus</i> strains (F16, F22, F23)	49 50
6-(a) and (b) The effect of different incubation times on crude amylase activity by different <i>Bacillus</i> strains (B1, B2, B11) and <i>Rhizopus</i> strains (F16, F22, F23)	51 52
7-(a) Thermal inactivation of saccharolytic activity of <i>Rhizopus</i> strain (F16)	54
7-(b) Thermal inactivation of dextrinogenic activity of <i>Rhizopus</i> strain (F16)	55
8-(a) Thermal inactivation of saccharolytic activity of <i>Rhizopus</i> strain (F22)	57

<b>Figure</b>	<b>Page</b>
8-(b) Thermal inactivation of dextrinogenic activity of <i>Rhizopus</i> strain (F22)	58
9-(a) Thermal inactivation of saccharolytic activity of <i>Rhizopus</i> strain (F23)	59
9-(b) Thermal inactivation of dextrinogenic activity of <i>Rhizopus</i> strain (F23)	60
10-(a) Thermal inactivation of saccharolytic activity of <i>Bacillus</i> strain (B1)	61
10-(b) Thermal inactivation of dextrinogenic activity of <i>Bacillus</i> strain (B1)	63
11-(a) Thermal inactivation of saccharolytic activity of <i>Bacillus</i> strain (B2)	64
11-(b) Thermal inactivation of dextrinogenic activity of <i>Bacillus</i> strain (B2)	65
12- (a) Thermal inactivation of saccharolytic activity of <i>Bacillus</i> strain (B11)	66
12- (b) Thermal inactivation of dextrinogenic activity of <i>Bacillus</i> strain (B11)	67
(13) The effect of pH on saccharolytic inactivation by different <i>Rhizopus</i> strains (F16, F22, F23)	69
(14) The effect of pH on saccharolytic inactivation by different <i>Bacillus</i> strains (B1, B2, B11)	70
(15).pH inactivation of dextrinogenic activity of different <i>Bacillus</i> strains (B1, B2, B11) and <i>Rhizopus</i> strains (F16, F22, F23)...	72
(16) Storage inactivation of saccharolytic activity by different	

<b>Figure</b>	<b>Page</b>
<i>Rhizopus</i> strains (F16, F22, F23)	74
(17) Storage inactivation of dextrinogenic activity by different <i>Rhizopus</i> strains (F16, F22, F23)	75
(18) Storage inactivation of saccharolytic activity by different Bacillus strains (B1, B2, B11)	77
(19) Storage inactivation of dextrinogenic activity by different Bacillus strains (B1, B2, B11)	78
(20) Combination between two enzymes	87
(21) Sugar production	88

## List of plates

<b>Plate</b>	<b>Page</b>
(1) Hydrolytic products of soluble starch by <i>Rhizopus</i> enzymes	80
(2) Hydrolytic products of soluble starch by <i>Bacillus</i> enzymes	81

## Abstract

Twenty- eight strains of bacteria and fungi were isolated from soil and soil and bread, respectively. Preliminary screening for amylytic bacteria and fungi was performed on starch media. Out of twenty – eight bacterial and fungal isolates, 61% and 86% of isolates showed positive result with iodine solution, respectively.

Identification of bacteria and fungi was carried out and it found that all the isolates of bacteria belonged to the genus *Bacillus* and nine isolates of fungi belonged to the genus *Aspergillus* and the other isolates belonged to the genus *Rhizopus*.

Three strains of *Bacillus* (B1, B2, and B11) and *Rhizopus* (F16, F22, F23) were chosen for this study according to the amylytic activity.

Optimum conditions for enzyme production were studied, solid state fermentation method was used and wheat bran was used as the main substrate and it was analysed. The incubation time 48 hours and incubation temperatures 25 and 35<sup>0</sup>C were found the best for *Rhizopus* and *Bacillus* strains, respectively except *Rhizopus* F23 with the best at 30<sup>0</sup>C. pH 7.0 was found the best for all the *Rhizopus* and *Bacillus* strains except *Bacillus* B1 with the best at pH 8.0.

Optimum conditions for enzyme activity were studied. Temperatures 40, 50, 60<sup>0</sup>C and pH 6.0 and 7.0 and incubation times of 30, 40, 50 minutes were found the best for activity of *Rhizopus* and *Bacillus* strains enzymes.

Thermal inactivation of enzyme for *Rhizopus* and *Bacillus* strains was also studied. Incubation at low temperatures for enzyme did not show any

decrease in activity and incubation at medium temperatures showed decrease in activity and increased with increase in high incubation temperatures.

pH inactivation of enzyme for *Rhizopus* and *Bacillus* strains was detected. The activity of enzyme for *Rhizopus* and *Bacillus* strains was increased above pH 4.0 and enzymes were inactivated above pH 8.0.

Storage inactivation of enzyme for *Rhizopus* and *Bacillus* strains was detected as well. High activity was observed at the beginning of storage at room temperature and refrigerator and slight decrease in activity was found with increase in time.

Identification of hydrolytic products of soluble starch by *Rhizopus* and *Bacillus* strains enzymes was investigated on paper chromatography. Glucose and maltose were produced by *Rhizopus* strains enzymes and maltose was produced by *Bacillus* strains enzymes.

Different yeasts were isolated from different sources, two isolates of yeast were identified to *Shizosaccharomyces octosporus* and *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* was used in this study for fermentation in ethanol production.

The best combination of *Rhizopus* and *Bacillus* enzymes for high level of activity was found in combination of *Bacillus* B1 and *Rhizopus* F22. Two samples of sorghum and corn starch were used in ethanol production, sorghum starch was found the best for producing sugars. High level of ethanol was produced by sorghum starch (81.13%) compared with corn starch (60.33%).

## ملخص الدراسة

تم عزل ثمانية وعشرون سلالة بكتيرية وفطرية من (التربة) ومن (التربة و الخبز) على التوالي ، و قد أختبرت كل من الباكثيريا والفطريات علي وسط غذائي محتويآ علي النشآ لمعرفة مقدرتها علي إنتاج انزيم الاميليز، و قد وجد في الثمانية والعشرون عزلة لكل من الباكثيريا والفطريات أن ٦١% من الباكثيريا و ٨٦% من الفطريات لها المقدره علي انتاج الانزيم.

قامت الدراسة بتصنيف للسلالات الباكثيرية و الفطرية، و قد وجد أن كل السلالات الباكثيرية تنتمي للجنس *Bacillus* و السلالات الفطرية بها ٩ سلالات تنتمي للجنس *Aspergillus* و ١٩ سلالة تنتمي للجنس *Rhizopus*

تم اختيار ثلاثة من السلالات البكتيرية وهي B1, B2, B١١ و كذلك ثلاثة من السلالات الفطرية F16, F22, F23 للدراسة و ذلك من حيث النشاط الانزيمي.

درست الظروف المثالية لانتاج أنزيم الأميليز حيث استخدمت طريقة التخمير الصلب وكانت الردة هي المادة الخاضعة الرئيسية حيث تم تحليلها لمعرفة مكوناتها الغذائية ومن ثم استخدامها في تنمية الأحياء الدقيقة، وقد وجد أن زمن التحضين ٤٨ ساعة ودرجة حرارة التحضين ٢٥ و ٣٥ م مناسبة لكل من الفطريات والباكتيريا بالتتابع ما عدا الفطر F23 الذي يحتاج الى درجة تحضين ٣٠ م . وقد وجد أن الرقم الهيدروجيني ٧ هو الأفضل لكل من الفطريات والباكتيريا ما عدا الباكثيريا B1 التي تحتاج الى رقم هيدروجيني هو ٨ .

تمت دراسة الظروف المثالية لنشاط أنزيم الأميليز حيث أن درجات الحرارة ٤٠، ٥٠، ٦٠ م وكذلك الرقم الهيدروجيني ٦ و ٧ وزمن التحضين ٣٠، ٤٠، ٥٠ دقيقة هي الافضل لنشاط الانزيم لكل من الفطريات والباكتيريا.

تمت دراسة تحمل انزيمات الفطريات والباكتيريا لدرجات الحرارة، وقد وجد أن درجات الحرارة المنخفضة لا تؤثر في تقليل نشاط الانزيم، بينما يبدأ نشاط الانزيم في الانخفاض بازياد درجات الحرارة.

تمت دراسة تحمل انزيمات الفطريات والباكتيريا لدرجات الحموضة والقلوية، وقد وجد أن نشاط الانزيمات يزيد عند الرقم الهيدروجيني ٤ حتى يصل الى الرقم الهيدروجيني ٨ يبدأ عندها في انخفاض نشاطه.

تمت دراسة تحمل انزيمات الفطريات والباكتيريا للتخزين في درجة حرارة الغرفة والثلاجة، وقد وجد ان نشاط الانزيمات يكون عاليا عند بداية التخزين في كل من درجة حرارة الغرفة والثلاجة، ثم يحدث بعد ذلك أنخفاض في نشاط الانزيمات بازياد زمن التخزين.

نواتج تحلل النشا بواسطة انزيمات الفطريات والباكتيريا عن طريق استخدام الاستشراب الورقي ، قد أوضح ان كل من الجلوكوزوالمالتوز تنتج بواسطة انزيمات الفطريات، كما وجد أن المالتوز ينتج بواسطة انزيمات البكتيريا.

عزل عدد من الخمائر من مصادر مختلفة وقد اختيرت اثنين منها، وقد صنفت الى *Shizosaccharomyces octosporus* and *Saccharomyces cerevisiae* . وقد تم

اختيار الخميرة *Saccharomyces cerevisiae* في هذه الدراسة لعملية التخمير في إنتاج الكحول.

وجد أن أفضل خلطة من انزيمات الفطريات والباكتيريا للحصول على أعلى نشاط للانزيم هي الخلطة المكونة من الانزيم الناتج من البكتيريا B1 والفطر F22. استخدمت عينتان من نشا الذرة و الذرة الشامية لإنتاج الكحول. وجد أن نشأ الذرة ينتج كمية عالية من السكر، وقد وجد أن أعلى معدل من الايثانول ناتج من نشا الذرة (٨١.١٣%) مقارنة مع نشا الذرة الشامى (٦٠.٣٣%).