



**CHARACTERIZATION OF MICROORGANISMS AND EXTRACELLULAR  
ENZYMES ASSOCIATED WITH THE EFFECTIVENESS OF A POST-  
HARVEST STORAGE SYSTEM FOR TOMATO (*Lycopersicon esculentum*  
Mill.) FRUITS**

**BY**

**OBAFEMI, YEMISI DORCAS**

**(15PCQ01221)**

**A RESEARCH THESIS SUBMITTED TO THE DEPARTMENT OF  
BIOLOGICAL SCIENCES (MICROBIOLOGY UNIT), COLLEGE OF  
SCIENCE AND TECHNOLOGY, COVENANT UNIVERSITY, OTA  
NIGERIA**

**IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD  
OF THE DEGREE OF MASTER OF SCIENCE (M.Sc.) HONOURS IN  
MICROBIOLOGY.**

**JULY, 2017.**

### CERTIFICATION

This is to certify that **OBAFEMI, Yemisi Dorcas**, a student of the Department of Biological Sciences (Microbiology Unit), Covenant University, Ota, Ogun State with matriculation number 15PCQ01221 has successfully completed this research work supervised by Prof. A. A Ajayi, and submitted to the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun State.

Prof A. A Ajayi	.....	.....
(Supervisor's Name)	Signature	Date

Prof A. A Ajayi	.....	.....
(Head of Department)	Signature	Date

Prof A.I Sanni	.....	.....
(External Examiner)	Signature	Date

**DECLARATION**

I hereby declared that I, **OBAFEMI, YEMISI DORCAS**, is the sole author of this research work and that it has not been presented by previous application for award of Masters' in Science Degree. This project is based on my original study and the views of other researchers have been duly expressed and acknowledged. I hereby authorize Covenant University to lend it to other institutions or individuals for the purpose of their research work.

.....

**OBAFEMI, Yemisi Dorcas**  
July, 2017

## **DEDICATION**

This work is dedicated to the glory of the Almighty God who in His infinite mercies and grace has kept me thus far. He is my all sufficiency, ever dependable, ever reliable creator.

## ACKNOWLEDGEMENTS

First and foremost I want to acknowledge the name of the Most High God. This research will not be a reality without the support, strength and divine provisions. Thank you my Creator.

The entire Management team of the Covenant University Community is also recognized for the training during this programme.

I further acknowledge my project supervisor Prof A. A Ajayi whom I have been privileged to get guidance from. I bless the name of the Lord for this wisdom upon her life and the knowledge she has imparted me with. Thanks for all her immense contribution and efforts in making this project a success and reality. I pray that God will continue to honour and keep her. I also acknowledge her as the Head of Department of Biological Sciences. I appreciate her motherly love, support and advice throughout my Programme in the Department. God will continue to bless and keep her.

I acknowledge Prof K. I. T Eniola and his family for their constant academic advice, financial assistance and fatherly support. May God bless you sir.

I express my gratitude to the Head of Microbiology Unit, Dr. O. O Ayepola, for her support and continuous encouragement, which has contributed immensely to the success of this research work.

I acknowledge Dr G. I Olasehinde for her immense support and advice on this research work. God will continue to bless and keep her.

To other Faculty and Staff of the Department of Biological Sciences, Prof S. U Oranusi, Prof L. O Egwari, Dr. O. C Nwinyi, Dr. A. Eni, Mr. E. A Omonigbehin, Mrs. B. T Adekeye, Miss Magaret Oniha, Mr. O. S Taiwo, Mr S. J Olorunsola, Mr. J. I Ojo, Mrs. A. O Awotoye, Miss A. E Onibokun, Miss Selina Anosike and Mrs Bunmi Atolagbe. I bless God for their lives, and for the knowledge received from them all. Thanks for all the contributions added to my life throughout my years of this study, I pray God will continually keep and bless them abundantly.

I appreciate my parents Elder J. O Obafemi and late Deaconess A. O Obafemi who have supported me morally and in prayers towards my program. I love you. I

appreciate my brothers and sisters' Mrs F. T Olorunjuwon of blessed memory, Mrs G. F Medubi, Mr J. K Obafemi, Mr L. N Obafemi, Mrs O. H Johnson, Mrs M. R Ochim and Miss O. I Obafemi.

My profound gratitude also goes to my darling husband Adeniyi for his support and understanding. Love to my beautiful children, Adeola and Adebola. I appreciate all my in-laws for being sources of inspiration and encouragement to me.

I also acknowledge my friends and colleagues please keep up the good work.

## TABLE OF CONTENTS

CERTIFICATION .....	ii
DECLARATION .....	iii
DEDICATION .....	iv
ACKNOWLEDGEMENTS .....	v
TABLE OF CONTENTS .....	vii
LIST OF TABLES .....	xi
LIST OF FIGURES .....	xii
LIST OF PLATES .....	xiii
ABSTRACT .....	xiv
CHAPTER ONE .....	1
1.0. Introduction .....	1
1.1 Background of Study .....	1
1.2 Statement of Research Problem .....	2
1.3 Justification for Research .....	2
1.4 Aims of research .....	3
1.5 Objectives of Research .....	3
CHAPTER TWO .....	4
2.0 Literature Review .....	4
2.1 The Tomato Fruits .....	4
2.2 Varieties of Tomato Fruits .....	5
2.2.1 Round Tomato Fruits .....	5
2.2.2 Plum Tomato Fruits .....	5
2.2.3 Cherry Tomato Fruits .....	5
2.3 Microbial Enzymes .....	6
2.3.1. Characteristics of Enzymes .....	6
2.3.2. Classification of Enzymes .....	7
2.3.3. Cellwall Degrading Enzymes .....	8
2.4 Role of Cellwall Degrading Enzymes in Pathogenicity .....	10
2.5 Spoilage of Tomato Fruits .....	10
2.6 Factors affecting the Growth of Microorganisms in Fresh Tomato Fruits..	12
2.6.1 Environmental Factors associated with the Tomato Spoilage .....	12
2.6.2 Processing of Tomato Fruits .....	12
2.6.3 Transportation and Storage of Tomato Fruits .....	13

2.7	Microorganisms on Fresh Tomato Fruits .....	13
2.7.1	Contamination from Water Used in Washing Fresh Tomato Fruits .....	14
2.8	Changes in Quality of Refrigerated Tomato Fruits.....	15
2.9	Post-harvest Storage System for Fresh Tomato Fruits.....	16
CHAPTER THREE .....		17
MATERIALS AND METHODS.....		17
3.0	Materials.....	17
3.0.1	Substrates.....	17
3.0.2	Sterilization of Materials .....	18
3.1	Methodology .....	18
3.1.1	Collection of Samples .....	18
3.1.2	Physical Observation and pH of Tomato Fruits .....	18
3.1.3	Preparation of Tomato Fruit Sample.....	18
3.2	Isolation of Microorganisms from Tomato Fruits.....	18
3.2.1	Selection of Stock Cultures from Tomato Fruits.....	19
3.2.2	Identification of Bacterial Isolates .....	19
3.2.3	Identification of Fungal Isolates.....	21
3.3	Screening of Isolated Bacteria for Production of Extracellular Enzymes...22	
3.3.1	Screening and Selection of Amyolytic Bacteria .....	22
3.3.2	Screening and Selection of Cellulolytic Bacteria.....	22
3.3.3	Screening and Selection of Polygalacturonase Producing Bacteria.....	22
3.3.4	Screening of Bacterial Isolates for Production of Tannase .....	22
3.4	Preparation of Standard Inoculum for Production of Enzymes .....	23
3.4.1	Preparation of Standard Bacteria Inoculum for Production of Enzymes ....	23
3.4.2.	Preparation of Fungal Spore Suspension for Production of Enzymes .....	23
3.5	Growth of Isolated Fungi in Basal Salt Medium Containing Soluble Starch, CMC and Pectin as Source of Carbon.....	23
3.5.1	Growth of Isolated Fungi in Basal Salt Medium Containing Starch.....	23
3.5.2	Growth of Isolated Fungi in Basal Salt Medium Containing CMC .....	23
3.5.3	Growth of isolated fungi in basal salt medium containing pectin.....	24
3.6	Crude Enzyme Extraction by Filtration.....	24
3.7	Determination of Enzyme Activity .....	25
3.7.1	Amylase Assay .....	25
3.7.2	Cellulase Assay .....	25



3.7.3	Polygalacturonase Assay .....	26
3.8	Partial Purification of Enzymes.....	26
3.8.1	Ammonium Sulphate Precipitation .....	26
3.8.2	Characterization of Enzymes.....	27
3.9	Extraction of DNA from Isolated Bacteria.....	29
3.9.1	PCR Amplification of the 16S rRNA Gene (27F and 1492R).....	29
3.9.2	Molecular Characterization .....	30
3.10	Statistical Analysis .....	30
CHAPTER FOUR.....		31
RESULTS .....		31
4.1	Physical Examination of Tomato Fruits.....	31
4.2	pH Analysis of Tomato Fruits.....	31
4.3	Enumeration of Bacterial Population .....	31
4.4	Enumeration of Fungal Population.....	31
4.5	Identification of Bacterial Isolates .....	37
4.6	Identification of Fungal Isolates.....	37
4.7	Screening of Bacterial Isolates for Production of Enzymes.....	37
4.7.1	Growth of Bacterial Isolates on Starch Agar.....	37
4.7.2	Growth of Bacterial Isolates of Carboxymethylcellulose (CMC) Agar.....	37
4.7.3	Growth of Bacterial Isolates on Pectin Agar.....	37
4.7.4	Growth of Bacterial Isolates of Tannic Acid.....	38
4.7.5	Fungal Spore Suspension for Enzymes Production .....	38
4.8	Production of Enzymes .....	38
4.8.1	Production of Amylase.....	38
4.8.2	Production of Cellulase .....	38
4.8.3	Production of Polygalacturonase.....	38
4.9	Characterization of Enzymes.....	51
4.9.1.1	Effect of Temperature on Amylase AMY B5 and AMY F2.....	51
4.9.1.2	Effect of Temperature on Cellulase CMC B18 .....	51
4.9.1.3	Effect of Temperature on Polygalacturonase PEC B5 .....	51
4.9.2.1	Effect of pH on Amylase AMY B5 and AMY F2 .....	51
4.9.2.2	Effect of pH on Cellulase CMC B18.....	51
4.9.3.1	Effect of Substrate Concentration on Amylase AMY B5 and AMY F2.....	52
4.9.3.2	Effect of Substrate Concentration on Cellulase CMC B18.....	52

4.9.3.3	Effect of Substrate Concentration on Polygalacturonase PEC B5 .....	52
4.9.4.1	Effect of Heat on Amylase AMY B5 and AMY F2.....	52
4.9.4.2	Effect of Heat on Cellulase CMC B18 .....	52
4.9.4.3	Effect of Heat on Polygalacturonase PEC B5 .....	53
4.10.1	PCR Amplification of Extracted DNA .....	53
4.10.2	Molecular Characterization of Bacterial Isolates .....	53
CHAPTER FIVE .....		74
DISCUSSION .....		74
CONCLUSION AND RECOMMENDATIONS .....		79
REFERENCES .....		80
APPENDIX I .....		93
APPENDIX II .....		95
APPENDIX III.....		97

## LIST OF TABLES

Table 1: Physical Examination of Tomato Fruits .....	32
Table 2: pH Analysis of Tomato Fruits .....	34
Table 3: Enumeration of Bacterial Population.....	35
Table 4: Enumeration of Fungal Population.....	36
Table 6: Morphological And Biochemical Characteristics of Isolated Bacterial strains .....	43
Table 7: Identification of Isolated Fungal Strains.....	44
Table 8: Diameter of Zones of Hydrolysis of Amylolytic Bacteria.....	45
Table 9: Diameter of Zones of Hydrolysis of Cellulolytic Bacteria.....	46
Table 10: Diameter of Zones of Hydrolysis of Pectinolytic Bacteria.....	47
Table 11: Diameter of Zones of Hydrolysis of Tanninolytic Bacteria .....	48
Table 12: Spore Count of Fungal Isolates.....	49
Table 13: Partial Purification of Amylase Obtained from Microorganisms Isolated from Tomato ( <i>Lycopersicon esculentum</i> Mill.).....	50

## LIST OF FIGURES

Figure 1: Effect of Temperature on Partially purified Amylase AMY B5 .....	54
Figure 2: Effect of Temperature on Partially Purified Amylase AMY F2 .....	55
Figure 3: Effect of Temperature on Partially Purified Cellulase CMC B18.....	56
Figure 4: Effect of Temperature on Partially Purified Polygalacturonase PEC B5.....	57
Figure 5: Effect of pH on Partially Purified Amylase AMY B5 .....	58
Figure 6: Effect of pH on partially Purified Amylase AMY F2 .....	59
Figure 7: Effect of pH on Partially Purified Cellulase CMC B18 .....	60
Figure 8: Effect of pH on Partially Purified Polygalacturonase PEC B5 .....	61
Figure 9: Effect of Starch Concentration on Partially Purified Amylase AMY B5 ....	62
Figure 10: Effect of Starch Concentration on Partially Purified Amylase AMY F2...	63
Figure 11: Effect of CMC Concentration on Partially Purified Cellulase CMC B18 .	64
Figure 12: Effect of Pectin Concentration on Partially Purified Polygalacturonase PEC B5 .....	65
Figure 13: Effect of Heat (80°C) on Partially Purified Amylase AMY B5 .....	66
Figure 14: Effect of Heat (80°C) on Partially Purified Amylase AMY F2.....	67
Figure 15: Effect of heat (80°C) on Partially Purified Cellulase CMC B18.....	68
Figure 16: Effect of Heat (80°C) on Partially Purified Polygalacturonase PEC B5 ....	69
Figure 17: 16S rRNA sequence of Isolate B5 isolated from Tomato ( <i>Lycopersicon esculentum</i> Mill.) Fruits .....	71
Figure 18: 16S rRNA sequence of Isolate B18 isolated from Tomato ( <i>Lycopersicon esculentum</i> Mill.) Fruits .....	72
Figure 19: 16S rRNA sequence of Isolate B27 isolated from Tomato ( <i>Lycopersicon esculentum</i> Mill.) Fruits .....	73

## LIST OF PLATES

Plate 1: PCR Electrophoretic Gel of Isolated bacterial strains using 27F and 1492R primer pair .....	70
Plate 2: The Post-Harvest Storage System.....	99
Plate 3: Loaded tomatoes in Post-Harvest Storage System .....	100
Plate 4: Back View of the Post-Harvest Storage System.....	101
Plate 5: Physical Observation of Tomato Fruits on Day 5.....	102
Plate 6: Zone of Hydrolysis on Pectin Agar .....	103
Plate 7: Zone of Hydrolysis on Starch Agar .....	104
Plate 8: Zone of Hydrolysis on CMC Agar .....	105
Plate 9: Yeasts Isolated from tomato Fruits ( <i>Lycopersicon esculentum</i> Mill.).....	106
Plate 10: <i>Aspergillus flavus</i> isolated from tomato Fruits ( <i>Lycopersicon esculentum</i> Mill.) .....	107
Plate 11: <i>Aspergillus niger</i> isolated from tomato fruits ( <i>Lycopersicon esculentum</i> Mill.).....	108

## ABSTRACT

Amylase (EC 3.2.1.1), Cellulase (EC 3.2.1.4), Polygalacturonase (EC 3.2.1.15) and Tannase (EC 3.1.1.20) are cellwall degrading enzymes which promote pathogenicity in microorganisms associated with spoilage of tomato fruits. Potential use of these enzymes has made it very important to optimize production process to achieve maximum yields. One hundred and eighty tomato fruits each of average weight were sorted, washed and stored in the locally made post-harvest storage system, refrigerator and at ambient temperature for a period of fourteen days. Pure bacterial and fungal isolates were screened for amylase, cellulase, polygalacturonase and tannase production based on their ability to produce clear zones of hydrolysis on the starch, carboxymethylcellulose (CMC), pectin and tannic acid supplemented agar plates respectively. The isolates were characterized by biochemical and molecular tests and the activities of the enzymes were determined. Partial purification of the crude enzymes was carried out by ammonium sulphate precipitation. The enzymes were characterized using the parameters of temperature, pH, substrate concentration and effect of heating time. Five bacterial isolates and two fungal isolates were able to produce the cellwall degrading enzymes in tomato fruits. Isolate B5 and F2 exhibited highest amylase activity; Isolate B18 and F3 exhibited highest cellulase activity, and Isolate B5 and F3 had highest polygalacturonase activity. None of the isolate was able to produce tannase. Optimum conditions were ascertained at pH 6.0, and 5.5; temperature 30°C, and 35°C; substrate concentration of 0.25mg/ml, and 0.3mg/ml, heating for 5min and 10min for Amylase AMY F2 and AMY B5 respectively. Cellulase CMC B18 had an optimum pH of 4.5, optimum temperature of 35°C substrate concentration of 0.4mg/ml and heating for 5min while the optimum pH of 4.5, optimum temperature of 35°C, substrate concentration of 0.3mg/ml and heating for 5min was obtained for Polygalacturonase PEC B5. 16S rRNA revealed isolate code B5, B18 and B27 as *Enterobacter tabaci*, *Enterobacter aerogenes* and *Citrobacter freundii* respectively while the fungi isolate code F2 and F3 were identified as *Aspergillus niger* and *Rhizopus stolonifer*. This research established the efficiency of the post-harvest storage system for the storage of tomato fruits.

**Keywords:** Storage system, Extracellular Enzymes, Amylase, Cellulase, Polygalacturonase, Post-Harvest

## CHAPTER ONE

### 1.0. Introduction

#### 1.1 Background of Study

Fruits are important part of our food products in Nigeria because they play powerful roles in microbial, animals and human diets. Common fruits in Nigeria include crops such as tomatoes, vegetables and pepper which are normally eaten fresh, cooked or processed in industries into products such as paste and juice. The tomato (*Lycopersicon esculentum* Mill.) is a seasonal fruit, which has a very high nutritional constituent due to its composition and water content (Ajayi *et al.*, 2015). This includes the soluble solids, simple sugars, dry matter, organic acids, pigments, citric acids, amino acids and wide range of substances which makes up more than 400 compounds contributing to its taste and aroma (Oranusi *et al.*, 2015).

Economically, tomatoes are one of the important edible vegetables in Nigeria and in most parts of the world. Tomatoes are source of balanced diet because of their rich content in sugars, vitamins, essential amino acids, minerals and dietary fibers. They also have high content of vitamin A and they contain lycopene, which is an antioxidant that helps to protect against carcinogenic substances (Agbabiaka *et al.*, 2015).

The quality of tomatoes at harvest depends on the cultural practices, genotype and maturity. These also depend on the time and method of harvest, postharvest handling practices, storage and transportation (Charles *et al.*, 2016). Ripening in fresh fruits including tomato is normally associated with collection of sugars and utilization of the organic acids (Chen *et al.*, 2012). The harvest of tomato fruits is seasonal and this results in certain periods of scarcity and when available, they tend to be very expensive.

Tomato plants also grow well with humus soil at pH 5 and 7. Normally, the ripe tomato fruits can be harvested and stored at ambient temperature ( $28\pm 1^{\circ}\text{C}$ ) and thus can stay without getting spoilt for five (5) days. However, some tomato fruits known as the 'iron tomatoes' can be stored for 10 days if kept well in a good environment where the storage conditions are favorable (Agbabiaka *et al.*, 2015)