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

BY

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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.

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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.

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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.

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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, carboxylmethylcellulose (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±1˚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)