ISOLATION, PARTIAL PURIFICATION AND CHARACTERIZATION OF A CELLULOLYTIC ENZYME PRODUCED BY A BACTERIUM ISOLATED FROM THE GUT OF LARVA OF BEETLE, *Oryctes rhinoceros* (L.)

BY

SOWOLE, ADEKUNLE DAMILOLA

B.Sc. Microbiology (Ago-Iwoye)

SCP11/12/H/1858

A THESIS SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF SCIENCE, OBAFEMI AWOLOWO UNIVERSITY, ILE-IFE, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE (M.Sc.)IN MICROBIOLOGY

2015

OBAFEMI AWOLOWO UNIVERSITY

AUTHORIZATION TO COPY

OBAFEMI AWOLOWO UNIVERSITY, ILE-IFE, NIGERIA

HEZEKIAH OLUWASANMI LIBRARY

POSTGRADUATE THESIS

AUTHOR: SOWOLE, ADEKUNLEDAMILOLA (MR)

TITLE: ISOLATION, PARTIAL PURIFICATION AND CHARACTERIZATION OF A CELLULOLYTIC ENZYME PRODUCED BY*Bacillus brevis* ISOLATED FROM THE GUT OF LARVA OF BEETLE, *Oryctes rhinoceros* (L.)

DEGREE: MASTER OF SCIENCE (M.Sc.) IN MICROBIOLOGY

YEAR: 2015

I, SOWOLEADEKUNLEDAMILOLA, hereby authorize the Hezekiah Oluwasanmi Library to copy my thesis in whole or part in response to request from individual researcher or organization for the purpose of private study or research.

…… .....

Signature **Date**

CERTIFICATION

This research project was supervised by us and approved in accordance with the partial fulfilment for the award of Masterof Science degree in Microbiology of the Obafemi Awolowo University, Ile-Ife.

....

Dr. K. O. Awojobi Date

(Supervisor)

....

Dr. F.K. Agboola Date

(Co-Supervisor)

DEDICATION

I dedicate this work to

Almighty God

the Alpha and the Omega

I also dedicate this work to the

memory of my father

Surveyor R.A. Sowole.

ACKNOWLEDGEMENTS

My utmost gratitude goes to the Almighty God, the creator of the universe and the lifter of my head. I thank my amiable supervisor,Dr. K.O. Awojobi, a distinct and a resourceful man, for his unrelentless support and guidance during the course of this programme. My sincere appreciation alsogoes to my co-supervisor, Dr. F. K. Agboola for his fatherlysacrifices, support and for granting me access to his laboratory.

I also appreciate the Head of Department of Microbiology, Dr. D. A. Akinpelu, for his tireless effort and support. I wish to use this opportunity to thank Prof. Olu Odeyemi, for all the provisions he made available to me in his laboratory throughout my M.Sc. programme. God will continue to bless you sir.My profound gratitude also goes to Dr. M.K. Bakare for his fatherly role and moral support in the course of my programme. I am indebted to all my lecturers and the entire member of staff of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, most especially to Prof. G.O Babalola, Dr. (Mrs.) A.O. Oluduro, Dr. A.O. Shitu, Dr.(Mrs.) M.A. Bisi-Johnson,Dr. (Mrs.) S.M. Adeyemo, Dr. (Mrs.) N. Torimiro, Dr. J.O. Omololu-Aso, Mr. O.A. Adesina, Mrs. M.O. Japhet,Mr O.Oyedeji, Mr. Taiwo Fadare, Mr O.O. Omoboye, Mr. Oladipupo Aregbesola, Mrs. Lolade Bamgbade, Mrs. O.T. Awotipe, Mrs. E.O. Oyeyemi and Mrs. A.A. Rafiu. I am grateful to Dr. S.I. Ola and Mr. Akin Babatunde of the Department of Animal Science, Obafemi Awolowo University, Ile-Ife for helping me in some of my laboratory work.

I thank Mrs. C. Fakorede, Mrs. YetundeFeruke-Bello and all my friends: Niran Olagoke, Lekan Okunlola, Joseph Odewade, Albert Fasogbon, Bisayo Anifowose, Bola Raji, Titi Olutola, Ajayi Peju, Folake Popoola, Abiola Elizebeth, Abimbola Fabunmi, Wale Adekunle, Bayo Adebambo, Progress Emoitologa, Chukwudi Osuji, Ojo Omolara, Ojo Tomi,

Osunde Michael, Abayomi Akintola, Lekan Bello, Nelson Ademakinwa, Segun Badiora, Dare Agunbiade and Titi Odewole.

My heart felt gratitude goes to my mother, my financier, my counsellor and my well wisher, Mrs. S.O. Sowole, for her moral and financial support. My graitude will not be completed without appreciating my siblings Mr. and Mrs. Bisi Sowole, Mr. and Mrs. Tayo Sowole, Mr. and Mrs. Paul Kuyoro, Wumi Sowole and to my Oluwademilade Ibiwoye.

Adekunle Damilola Sowole

2015.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

List of Abbreviations

bp- Base Pair

BSA- Bovine Serum Albumin

CMC- Carboxymethyl Cellulose

DNSA- Dinitrosalicylic Acid

dNTP- Deoxyribonucleotide 5′-triphosphate (N= A,T,G,C)

O.D. - Optical density

PCR- PolymeraseChainReaction

rpm –Revolution per minute

SDS- Sodium DodecylSulphate

SDS- PAGE – Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Taq -*Thermus aquaticus*

TBE- Tris-boric acid-EDTA

ABSTRACT

The study isolatedand optimized the production of a cellulolytic bacterial organismfrom the gut of the larva of beetleand characterize the cellulase produced. This was with a view to obtaining a bacterial candidate for cellulase production for industrial and biotechnological uses.

Twenty samples of the larva of beetle were collected at Eleweran in Ile-Ife, Osun State, Nigeria. The larva of beetle were surface sterilized with 70% ethanol and dissected under aseptic condition. One gram of the gut of larva of beetlewas then macerated in 10 ml of phosphate buffered saline and ten-fold serial dilution was carried out. Serially diluted samples were plated on nutrient agar in order to isolate the bacteria which were then screened on carboxymethyl cellulose (CMC) agar for cellulase activity. The optimal conditions for maximal cellulase production by the bacterium with the highest cellulase activity were determined by varying the incubation time, inoculum size, carbon, nitrogen sources, percentage CMC, ammonium sulphate, temperature and pH. The enzyme was partially purified by ammonium sulphate precipitation, dialysis and ion-exchange chromatography on CM-Sephadex C-50. The kinetic parameters (K_m and V_{max}) of the purified enzyme and the application of the purified enzyme to some cellulosic materials were studied using standard procedures.

Fourteen bacteria were isolated while the one with highest cellulase activitywas presumptively identified by morphological and biochemical tests as *Bacillus* sp. The result of 16S rRNA sequence analysis identified the isolate as *Bacillus brevis*. The peak of cellulase production was at 38 h of incubation (14.45 µg/ml/min). Carboxymethyl cellulose (CMC) and ammonium sulphate were found to be the best carbon and nitrogen sources for cellulase production respectively. The optimum temperature and pH for the production of cellulase was

observed to be 37°C and 6.0 respectively. The specific activity of the partially purified cellulase from *Bacillus brevis* was determined to be 2.4595 U/mg protein with purification fold of 1.35. The cellulase had a K_m of 0.18 mg/ml for CMC and V_{max} of 1.67 µg/ml/min. The enzyme was also able to degrade rice husk, maize corb, micro-crystalline cellulose and sugarcane baggase with enzyme activities of 11.43 µg/ml/min, 14.89 µg/ml/min, 8.13 µg/ml/min and 16.98 µg/ml/min respectively.

The study concluded that the gut of larva of beetle was a good source for cellulolytic bacteria, which could produce cellulase with desirable charactaristics for industrial uses.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Cellulose is the most abundant biomass on earth and the most abundant renewable bioresource produced in the biosphere (Jarvis, 2003; Zhang and Lynd, 2004; Sethi *et al*., 2013). Cellulose, a polymer of glucose residues connected by beta – 1, 4 linkages, being the primary structural material of plant cell wall, is the most abundant carbohydrate in nature. Therefore, it has become of considerable economic interest to develop processes for effective treatment and utilization of cellulosic waste as inexpensive carbon sources (Nishida *et al*., 2007). The potential of cellulose as an alternative energy source has stimulated research into bioconversion processes which hydrolyze cellulose to soluble sugars (Coughan, 1990). Enzymatic hydrolysis of cellulose gives a relatively pure product with the consumption of less energy during the process (Fennigton *et al*., 1982). Cellulose is commonly degraded by an enzyme called cellulase.

Cellulase is the enzyme that hydrolyses the beta – 1, 4 glycosidic bonds in the polymer to release glucose units (Saha *et al*., 2006). Cellulase can be divided into three types: endoglucanase, exoglucanase and β – glucosidase (Li *et al*., 2006; Gao *et al*., 2008; Ahmed, 2009). Cellulase yields appear to depend upon a complex relationship involving a variety of factors like inoculums size, pH value, temperature, presence of inducers, medium additives, aeration and growth time (Immanuel *et al*., 2006).

Cellulase is produced by several microorganisms, commonly by bacteria and fungi (Magwelli and Forchiassin, 1999; Immanuel *et al*., 2006). Bacteria are being aggressively

pursued to provide new enzymes that are highly thermostable depending on the environment of the native organism (Brennan, 1996). Bacteria which have high growth rate as compared to fungi have good potential to be used in cellulase production. Bacteria, due to their high natural diversity, faster growth have the capability to produce highly thermostable enzyme and may serve as highly potent sources of industrially important enzymes (Deka *et al*., 2011; Sethi *et al*., 2013). The cellulolytic property of some bacterial genera such as *Cellulomonas, Cellovibiro, Pseudomonas, Bacillus* and *Micrococcus* sp has been reported (Nakamura and Kppamura, 1982).

Oryctes rhinoceros, so called because of its resemblance to the rhino is primarily a pest of coconut in most part of the world, especially in Southern Asia and Africa, Nigeria in particular; it lives and feeds mostly on oil and raphia palms (Okaraonye and Ikewuchi, 2009). While the adults attacks the palm tree, the larvae are harmless, feeding only on decaying organic matter such as decaying palm logs, manure and rubbish dumps (Delaibera *et al*., 2005). Usually, when left to decay, old stems of coconut, raphia and oil palms, provide excellent breeding sites or grounds for the larva of beetle. Some species of the larva of beetle play beneficial ecological roles, such as assisting in nutrient cycling (Delalibera *et al*., 2005; Okaraonye and Ikewuchi, 2009). Several insect cellulases have been purified and characterized. A diverse assemblage of protozoan, fungal and bacterial species living in the guts of insects produces cellulases (Gental *et al*., 2003). Many aerobes and facultative anerobes that degrade cellulose have been isolated from termites (Breznak and Brune, 1994; Varma *et al*.,1994).

The cellulose-degrading enzyme can be used in the formation of washing powders, extraction of fruit and vegetable juices and starch processing (Kuhad *et al*., 2011). Cellulases are used in the textile industry for cotton softening and finishing, in laundry detergents, for colour care and cleaning, in the food industry for mashing; in the pulp and paper, fibre modification; generation of bioethanol and they are even used for pharmaceutical applications (Singh *et al*.,

2007). The cellulase enzymes are commonly used in many industrial applications and the demands for more stable, highly active and specific enzymes will also grow rapidly, cellulase enzyme will be the most stirring technology in future.

1.2 Statement of Research Problem

There is rising demand for cellulase with desirable characteristics. Microbial cellulase is the the best option for the production of cellulase and one of the sources of such microorganisms is bacteria in the gut of the larva of beetles. This study therefore intends to isolate and characterise cellulase produced by a bacterium isolated from the gut of the larva of beetle.

1.3 Specific Objectives of Research

The specific objectives of this research are to:

a) isolate, screen and identify cellulolytic bacteria from the gut of larva of beetle;
b) study the effect of physiological and nutritional factors on the production of cellulase;
c) partially purify the cellulase and investigate the characteristics of the enzyme; and
d) test the partially purified cellulase on some cellulolytic waste materials such as crystalline cellulose, sugar cane baggase, maize cob and rice husk

1.4 Expected Contribution to Knowledge

This study will provide information on a symbiotic bacterium in the gut of the larva of beetle capable of producing cellulolytic enzyme.

For more information, please **contact ir-help@oauife.edu.ng**