PURIFICATION AND CHARACTERIZATION OF CELLULASE FROM *AEGERITA WEBBERI* ISOLATED FROM DECAYING ORANGE FRUITS

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POSTGRADUATE THESIS

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This is to certify that this research work was carried out by Ese Louisa OKODUGHA in the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

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DEDICATION

This work is dedicated to God Almighty for His unfailing love over me at all times.

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LIST OF ABBREVIATIONS

Abbreviation	Meaning
µg	Microgram
ANF	Anti-Nutritional Factors
C	Carbon
CBM	carbohydrate binding module
CBP	consolidated bioprocessing
cm	Centimeter
CMC	Carboxymethylcellulose
EG	Endoglucanases
EDTA	Ethylene diamine tetraacetic acid
g	Gram
GHG	Green House Gas
h	Hour
M	Molar
mg	Milligram
mins	Minutes
ml	Milliliter
mm	Millimeter
mM	Milli-Molar
nm	Nanometer
^{o}C	Degree Centigrade
OD	Optical Density
Psi	Pascal
rpm	Revolution per minute
RSM	Response surface methodology
SDA	Saboraud Dextrose Agar

SHF	Separate hydrolysis and fermentation
SmF	Submerged fermentation
SSCF fermentation	Simultaneous saccharification and co-
SSF	Simultaneous saccharification and /Solid State Fermentation
UV	Ultra violet

ABSTRACT

The objectives of the study was to isolate, screen and identify cellulase producing fungi from decaying orange fruits; determine the optimum condition of the best cellulase producing isolates to obtain crude cellulase; produce and partially purify the crude cellulase by precipitation and gel filtration; investigate the characteristics of the partially purified cellulase; and apply the partially purified cellulase to cellulose material.

Six fungal strains were isolated fromdecaying orange fruit. They were screened for their ability to degrade cellulose by standard rapid plate screening assay. Cellulolytic fungi were evaluated after 5 days for the production of cellulolytic enzymes by staining with 1% Congo red and *Aegerita webberi* was selected being novel in the production of cellulase. Optimization for cellulase production was done using parameters such as carbon sources, pH, temperature, substrate concentration, nitrogen sources (inorganic and organic) and inoculum size of *A. webberi.*The enzyme produced was partially purified using a combination of ammonium sulphate precipitation and gel filtration on bio-gel P-100. The cellulase was characterized to determine the kinetic properties.

The peak of cellulase production was on the fourth day of incubation (162.36Units/ml).The optimum temperature for the activity of cellulase produced by the fungal strain *A. webberi*was 30^{o}C with the activity of 39.2 Units/ml while the optimum pH was attained at pH 5.5 with an activity of 112.2 Units/ml. Casein was the best nitrogen source with an enzyme activity of 239.4 Units/ml while carboxyl methylcellulose was the best carbon source with an enzyme activity of 121.9 Units/ml. The partially purified cellulase specific activity on bio-gel P-100 had a specific activity of 3.06 Units/mg/ml and the Vmax and Km was 0.26 Unit/ml and

1.184 Unit/ml respectively. The optimum temperature for the partially purified enzyme was 60°C while the enzyme was stable to heat for 30 mins at 70°C before noticeable decrease in activity. Of the metal ions investigated, EDTA and $HgCl_2$resulted in reduced activity of the purified cellulase while NaCl, KCl, $CaCl_2$,$MgCl_2$ and$AlCl_3$ resulted in an increase in the activity of the enzyme. On hydrolysis of raw substrates (yam powder and maize cob), yam powder had a stable higher activity compared to corn cob.

The thermostable cellulase from *Aegerita webberi* isolated from decaying orange fruits could be of great importance in biofuel industry for the saccharification of lignocellulolytic materials into economically useful monosaccharides.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

In 2010, 81% of the world's primary energy demand was met with non-renewable resources: coal, oil and natural gas (IEA, 2012). Increasing energy demand and our great dependence on fossil resources are considered problematic both from environmental and societal aspects. Combustion of non-renewable resources generates greenhouse gas (GHG) emissions that contribute to global warming.Global warming has raised serious environmental concerns due its great impact on ecosystems all over the world. For example, climate change is predicted to lead to the extinction of numerous species (Thomas *et al*., 2004). Furthermore, uneven geographical distribution of fossil energy reserves in the world is a societal risk for countries that are highly dependent on imported oil, coal and natural gas. Improvements in energy efficiency and increased utilization of renewable energy resources, such as plant biomass, are key measures to alleviate the concerns arising from our current dependence on fossil fuels (IEA, 2012). Today the transport sector consumes more than half of the annually produced oil and only 2% of the global fuel demand is met by refining renewable feedstocks into transportation fuels (IEA, 2012). The European Union, the United States and Brazil have binding regulations for blending biomassbased fuel compounds with gasoline or diesel(Solomon, 2010). However, the currently exploited "first generation" biofuel feed-stocks include sugar cane, corn starch and palm oil that may also be used in food production. Violation of the food chain threatens food security and may increase food prices (Solomon, 2010). Furthermore, the GHG emissions

arising from "first generation" biofuel production might be significantly underestimated by some widely used life cycle assessment methodologies (Soimakallio and Koponen, 2011). Lignocellulose is the most abundant renewable biomass resource on Earth and for the past 80 years it has been acknowledged as a potential feedstock for the production of fuels and chemicals (Himmel *et al*., 2007). The majority of plant biomass, including stems and leavesare composed of lignocellulose. Lignocellulose is called "the second generation" feedstock for fuel and chemical productionto emphasize the difference to the edible "first generation" feedstocks. Lignocellulose is a complex and tightly organized matrix of three main polymers, cellulose,hemicellulose and lignin. Historically, lignocellulose recalcitrance has hindered its utilization as a feedstock in fuel and chemical production; however, the current drivers as well as technological development have renewed interest in lignocellulose (Himmel *et al*., 2007). Lignocellulose processing is envisioned to occur analogously to oil refining, meaning that the feedstock is efficiently utilized for the production of fuels, chemicals and energy in a concept called bio-refining (Foust *et al*., 2008). As at April 2013, a database of the International Energy Agency lists thirteen commercial-scale factories that use lignocellulose as a feedstock for liquid fuel production(Foust *et al*., 2008). The thirteenfacilities are either operational, under construction or planned. The biochemical processing route of lignocellulosic biomass aims at enzymatic depolymerisation of cellulose and hemicellulose to monomeric sugars that may be further converted to various desired chemical products, such as ethanol, butanol and alkanes by exploiting microbial metabolism (Fortman *et al*., 2008) or chemical conversion. Pretreatment based on heat, chemicals or mechanical grinding is a prerequisite for enzymatic depolymerisation of the cell wall carbohydrates in lignocellulosic biomass. Different types of

steam pretreatments and treatments with dilute acids or bases are widely exploited in opening up the tightly packed structure of lignocellulose (Mosier *et al*., 2005). Several process configurations have been suggested for the saccharification of lignocellulosic polysaccharides and subsequent fermentation of the monosaccharides to desired chemicals(Mosier *et al*., 2005). The different process configurations, such as separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF) and consolidated bioprocessing (CBP) differ in their degree of integration (Lynd *et al*., 1999). Lignocellulose may also be processed by thermochemical means, such as pyrolysis and gasification. Wright and Brown (2007) conducted an economical comparison of biochemical and thermochemical conversion routes for lignocellulose and concluded that bothapproaches were equally viable with the present state of technology.

1.2 Justification of the study

The search for a source of cellulase that is less expensive with robust hydrolytic and catalytic properties is on-going and micro-organisms (especially fungi) are seen as cheap sources for this enzyme because they are ubiquitous and can be easily manipulated hence this research.

1.3 Objective of the study

To purify and characterize cellulase from Aegerita webberi isolated from decaying orange fruits.

1.4 Specific objectives of the study

The specific objectives of the research are to

a. isolate, screen and identify cellulase producing fungi from decaying orange fruits

b. determine the optimum condition of the best cellulase producing isolates to obtain crude cellulase

c. partially purify the crude cellulase by precipitation and gel filtration

d. investigate the characteristics of the partially purified cellulaseand