

**CHARACTERIZATION, PRODUCTION OPTIMIZATION AND APPLICATION
STUDIES OF LACCASE FROM SOIL FUNGI USING SAWDUST AS SUBSTRATE**

AKINYEMI, OLUWATOBI DOMINION

(19PCQ02060)

OCTOBER, 2021

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STUDIES OF LACCASE FROM SOIL FUNGI USING SAWDUST AS SUBSTRATE**

BY

AKINYEMI, OLUWATOBI DOMINION

(19PCQ02060)

B.Sc Microbiology, Crawford University, Igbesa, Ogun State

**A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF
MASTER OF SCIENCE (M.Sc) DEGREE IN MICROBIOLOGY IN THE
DEPARTMENT OF BIOLOGICAL SCIENCES, COLLEGE OF SCIENCE AND
TECHNOLOGY, COVENANT UNIVERSITY.**

OCTOBER, 2021

ACCEPTANCE

This is to attest that this dissertation is accepted in partial fulfillment of the requirements for the award of the degree of Master of Sciences in Microbiology in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria

Mr. John A. Philip

(Secretary, School of Postgraduate Studies)

Signature and Date

Prof. Akan B. Williams

(Dean, School of Postgraduate Studies)

Signature and Date

DECLARATION

I, **AKINYEMI, OLUWATOBI DOMINION (19PCQ02060)** declare that this research was carried out by me under the supervision of Dr. Eze F. Ahuekwe of the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Nigeria. I attest that the dissertation has not been presented either wholly or partially for the award of any degree elsewhere. All sources of data and scholarly information used in this dissertation are duly acknowledged.

AKINYEMI, OLUWATOBI DOMINION

Signature and Date

CERTIFICATION

We certify that this dissertation titled “**CHARACTERIZATION, PRODUCTION OPTIMIZATION AND APPLICATION STUDIES OF LACCASE FROM SOIL FUNGI USING SAWDUST AS SUBSTRATE**” is an original research work carried out by **AKINYEMI, OLUWATOBI DOMINION (19PCQ02060)** in the Department of Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria under the supervision of Dr. Eze F. Ahuekwe. We have examined and found this work acceptable as part of the requirements for the award of Master of Science in Microbiology.

Dr. Eze F. Ahuekwe

(Supervisor)

Signature and Date

Prof. Solomon U. Oranusi

(Head of Department)

Signature and Date

Prof. Oluwatoyin R. Afolabi

(External Examiner)

Signature and Date

Prof. Akan B. Williams

(Dean, School of Postgraduate Studies)

Signature and Date

DEDICATION

This work is dedicated to GOD who is the Father of all research and the inspiration behind this work.

ACKNOWLEDGEMENT

I would like to acknowledge GOD and all who have played a role or the other in the completion of this research. I acknowledge, especially, the impact of Dr. Ahuekwe F. Eze, my supervisor, for putting me right in gray areas for the completion of this research.

My heartfelt thanks extend to the whole Biological Sciences faculty and staff, as well as of the entire Covenant University, for their valuable impact on my life as a student in this great institution. I deeply appreciate all who have showed concern and helped me throughout this research. My appreciation extends to Mr. Michael, Miss Damilola and Mrs Adekeye from the Microbiology Laboratory as well as Mr. Taiwo from the Biology Laboratory.

I sincerely appreciate my colleagues and coursemates for constant encouragement all through the period of our journey as students and friends. My appreciation, especially, goes to my friend and brother, Dozie for his friendship through thick and thin.

My appreciation goes to my fiancée, Aderonkemi for being the best always. Words will fail me to express my gratitude for the understanding showed to me by this jewel.

And with great honour I appreciate my Parents, Dr. and Mrs. Akinyemi. There is no possibility of quantifying all that they do and represent to me. My gratitude as well as my hope and decision never to disappoint them is all I can offer them for the constant love shown to me.

Above all, I acknowledge once again, GOD, the Father of all research for His help in this phase of life. I pray that He blesses all who have helped me through this phase beyond imaginations and causes this research to significantly advance the frontiers of science.

TABLE OF CONTENTS

Content	Page
COVER PAGE	i
TITLE PAGE	ii
ACCEPTANCE	iii
DECLARATION	iv
CERTIFICATION	v
DEDICATION	vi
ACKNOWLEDGEMENTS	vii
TABLE OF CONTENTS	viii
LIST OF FIGURES	xii
LIST OF TABLES	xiii
LIST OF PLATES	xiv
ABSTRACT	xv
CHAPTER ONE: INTRODUCTION	1
1.1 Background of the study	1
1.2 Statement of problem	2
1.3 Aim of the study	2
1.4 Objectives of the study	2
1.5 Scope of the study	3
1.6 Significance of the study	3
CHAPTER TWO: LITERATURE REVIEW	4
2.1 Laccases	4
2.1.1 Sources of laccase	5
2.1.2 Molecular properties of laccase	13
2.1.3 Mechanism of laccase action	15
2.2 Mode of laccase production in ligninolytic microbes	17
2.3 Fermentation / cultivation method	19
2.3.1 Submerged fermentation	19
2.3.2 Solid state fermentation	20

2.3.3	Factors influencing production	20
2.4	Molecular approaches to laccase production	25
2.4.1	Classical breeding approach	25
2.4.2	Heterologous Expression	25
2.4.3	Homologous expression	29
2.4.4	Expression Regulation	29
2.4.5	Gene modification methods	33
2.5	Use of sawdust as an agro-industrial residue for enzyme production	34
2.6	Recovery/purification of laccases	35
2.7	Applications of laccase	37
2.7.1	Laccase Application in Food Industry	38
2.7.2	Laccase Application in Textile Industry	39
2.7.3	Application of laccase in Pharmaceutical Industry	40
2.7.4	Application of laccase in biodegradation of PAH and other Contaminants	40
2.7.5	Application of laccase in Forest Products Industry	41
2.7.6	Application of laccase in Petroleum Industry	41
2.7.7	Applications of laccase in Biosensors and Enzymatic Biofuels	42
2.8	Current research and research gaps	42
CHAPTER THREE: MATERIALS AND METHODS		44
3.1	Material	44
3.1.1	Equipment	44
3.1.2	Consumables	44
3.1.3	Media, Buffers and Reagents	44
3.2	Source and sampling	44
3.3	Analysis of soil and sawdust carbon, nitrogen and phenolic content	45
3.3.1	Carbon content	45
3.3.2	Nitrogen content	45
3.3.3	Phenolic content	45
3.4	Isolation and Screening of microorganisms	48
3.4.1	Culture of fungi from sawdust-polluted soil	48
3.4.2	Tannic acid screening	48
3.4.3	Molecular screening and identification of isolates	48
3.4.4	DNA isolation	48
3.4.5	DNA quantification	49
3.4.6	Polymerase Chain Reaction (PCR)	49
3.4.7	Detection of degenerate laccase gene	49
3.4.8	Detection of <i>laccase-1</i> gene	50
3.4.9	Gel Electrophoresis	50

3.5	Fermentation Studies	51
3.5.1	Preliminary production/studies for a 14-day period in a minimal salt medium	51
3.5.2	Optimization of laccase production using the central composite design of response surface methodology	51
3.6	Recovery and purification of enzyme	54
3.7	Screening of laccase activity	54
3.8	Characterization of laccase	55
3.8.1	Differential Scanning Colorimetry (DSC)	55
3.8.2	Fourier transform-infrared (FT-IR) spectroscopy	55
3.9	Application studies - degradation of spent engine oil	55
CHAPTER FOUR: RESULTS		56
4.1	General Characteristics of Sample sites	56
4.2	Analysis of Carbon, Nitrogen and Phenolic content	56
4.3	Isolation of Microbes	58
4.4	Screening for Laccase production	62
4.5	Preliminary production/studies for a 14-day period in a minimal salt medium	64
4.6	Optimization of fermentation	68
4.6.1	Plackett Burmann	68
4.6.2	Response Surface Methods	70
4.7	Recovery and Purification of laccase	82
4.8	Characterization of Laccase	82
4.8.1	DSC	82
4.8.2	FTIR	82
4.9	Degradation of Spent Engine oil	82
CHAPTER FIVE: DISCUSSION		92
5.1	Soil and Sawdust carbon, Nitrogen and phenolic content	92
5.2	Isolation of Fungi from Sawdust-polluted Soil	93
5.3	Plackett Burmann design	94
5.4	Response Surface Methodology	95
5.5	Recovery and Purification of Laccase	96
5.6	DSC analysis of laccase	97
5.7	FT-IR analysis of laccase	97
5.8	Application of Laccase in degradation of spent engine oil	98

CHAPTER SIX: CONCLUSION AND RECOMMENDATION	100
6.1 Summary	100
6.2 Conclusion	101
6.3 Contributions to Knowledge	101
6.4 Recommendations	102
REFERENCES	103
APPENDIX	131

LIST OF FIGURES

Figure	Title	Page
2.1	Molecular Structure of Laccase	14
2.2	Catalytic cycle of Laccase	16
2.3	Phenolic subunits of lignin oxidation by laccase and oxidation of non-phenolic lignin compounds by laccase mediator system	16
2.4	Concentration and separation sequence of laccase from a culture liquid	37
2.5	Scheme of applications of laccase	37
3.1	Map showing sample sites	46
3.2	Sample of sawdust and sawdust contaminated soil	47
4.1	The influence of numerous variables on laccase production as depicted in a Pareto chart.	69
4.2	Comparison of observed and predicted values using the RSM model: A narrow line represents zero inaccuracy.	74
4.3	The impact of the interaction of Glucose (A) with (NH ₄) ₂ SO ₄ (B) on a three-dimensional response surface plot	75
4.4	Effect of interaction of Glucose (A) and (NH ₄) ₂ SO ₄ (B)	76
4.5	The impact of the interaction of (NH ₄) ₂ SO ₄ (B) with L/S ratio (C) on a three-dimensional response surface plot	77
4.6	Effect of interaction of Glucose (A) and L/S ratio (C)	78
4.7	The impact of the interaction of Glucose (A) with L/S ratio (C) on a three-dimensional response surface plot	79
4.8	Effect of interaction of (NH ₄) ₂ SO ₄ (B) and L/S ratio (C)	80
4.9	Factors affecting laccase yield	81
4.10	Differential scanning calorimetric (DSC) analysis of ML2 laccase enzyme	83
4.11	Differential scanning calorimetric (DSC) analysis of ML5 laccase enzyme	83
4.12	Differential scanning calorimetric (DSC) analysis of OL2 laccase enzyme	84
4.13	Differential scanning calorimetric (DSC) analysis of IL1 laccase enzyme	84
4.14	Differential scanning calorimetric (DSC) analysis of IL2 laccase enzyme	85
4.15	FT-IR analysis of ML2 laccase	86
4.16	FT-IR analysis of ML5 laccase	86
4.17	FT-IR analysis of OL2 laccase	87
4.18	FT-IR analysis of IL1 laccase	87
4.19	FT-IR analysis of IL2 laccase	88
4.20	Percentage degradation of spent engine oil by laccase from different isolates	91

LIST OF TABLES

Table	Title	Page
2.1	Some Fungal Laccase Producers, their Taxonomic Identification and Redox Potential of Laccase produced	7
2.2	Some Baterial Laccase Producers, their Taxonomic Identification and Redox Potential of Laccase produced	10
2.3	Plant Laccases, Taxonomical Identification and Optimum pH	12
2.4	Laccase producing Insects	13
2.5	Inducers and their effect on lacasse activity in PDA medium and liquid cultures at shaking conditions	24
2.6	Heterologous Expression of Laccase	27
2.7	Heterologous expression of fungal laccases in yeast	28
2.8	Heterologous expression of fungal laccases in filamentous fungi, plants and bacteria	30
3.1	Laccase degenerate and Laccase-1 primers used in the study	49
3.2	Range of different variables studied in the Plackett-Burmann design	53
3.3	Range of levels and variables in CCD	54
4.1	Percentage Carbon, Nitrogen and Phenolic content of soil	57
4.2	Percentage Carbon, Nitrogen and Phenolic content of sawdust	57
4.3	Probable identification of isolates using Cultural and Microscopic characteristics	60
4.4	Laccase-screening of Isolates using Tannic Acid	63
4.5	Laccase activity of Enzymes produced from Isolates	66
4.6	Laccase production experimental values represented in a CCD matrix with three variables	72
4.7	Central composite design (CCD) regression analysis	73
4.8	Ammonium sulphate purification of crude laccase from Isolates	90

LIST OF PLATES

Plate	Title	Page
4.1	Plates containing pure culture of isolates	59
4.2	Positive plate screening for laccase production using tannic acid with brownish black colouration	62
4.3	Picture of preliminary fermentation studies	65
4.4	Tannic Acid screening of laccase activity – (a) negative and (b) positive	67
4.5	Filtration with Whattman 90mm filter paper in purification of laccase	89

ABSTRACT

Challenges of low yield and high production costs remain the major obstacles to laccase production and application. In this study, fungi were isolated from sawdust-polluted soils collected from Morogbo-Agbara, Iju and Ota in Ogun State, Nigeria. The fungal isolates were identified as *Aspergillus niger*, *Penicillium sp*, *Aspergillus flavus*, *Penicillium expansum*, *Rhizopus sp*, *Saccharomyces cerevisiae*, *Fusarium sp*, *Cryptococcus sp* and *Candida sp*. This was done after the physicochemical assessment of the soil and sawdust samples. The isolates were screened for laccase production ability using tannic acid; and specific DNA primers (laccase degenerate and *laccase-I*) to identify laccase genes in the isolates before sequencing. Five of the ten fungal isolates were positive for laccase screening using tannic acid. Using sawdust as a substrate under solid-state fermentation (SSF) conditions, significant medium components were selected via the Plackett-Burman design. Thereafter, the Central Composite Design (CCD) of response surface methodology (RSM) was used to optimize the significant variables – Glucose, $(\text{NH}_4)_2\text{SO}_4$. Sample ML2 showed highest laccase activity of 1.984 U/ml after 14 days, followed by ML5 (1.807 U/ml) and IL2 (1.241 U/ml). Laccase yield was observed to be strongly impacted by glucose, ammonium sulphate ($(\text{NH}_4)_2\text{SO}_4$), and the liquid/solid (L/S) ratio in ML2. The yield was raised by two folds (3.975 U/ml) after optimization. The result of the degradation potential of the produced laccase on spent engine oil showed effective degradation of 78 % and a minimum of 51 %. The findings of this study show sawdust as a useful waste substrate in the optimized production of fungal laccases.

Keywords: laccase, soil, sawdust, fungi, fermentation, optimization