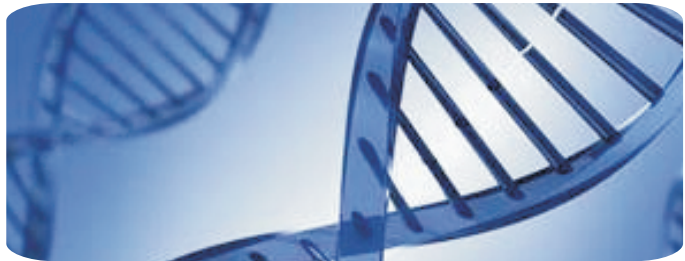


# Recent Advances in Biotechnology

**Oloke Julius Kola et al.**





# Recent Advances in Biotechnology



Edited by Oloke Julius Kola (PhD)

Professor of Microbiology and Biotechnology

Co-authors

Elijah Adebayo (PhD)

Yemisi Adesiji (PhD)

Oke Adefola (PhD)

Iyabo Ola (PhD)

Charles Adetunji (PhD)

Published by  
Science Publishing Group  
548 Fashion Avenue  
New York, NY 10018, U.S.A.  
<http://www.sciencepublishinggroup.com>

ISBN: 978-1-940366-55-5



© Authors 2016.

The book is published with open access by Science Publishing Group and distributed under the terms of the Creative Commons Attribution 3.0 Unported License (<http://creativecommons.org/licenses/by/3.0/>) which permits any use, distribution, and reproduction in any medium, provided that the original author(s) and source are properly credited.

## List of Authors

### **Oloke Julius Kola (Chapters 1, 2, 3, 4 & 5)**

Department of Microbiology and Immunology, All Saints University; St Vincent and Grenadines and Department of Pure & Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.

jkoloke@yahoo.co.uk

### **Elijah Adebayo (Chapter 1)**

Department of Pure & Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

eaadebayo@lautech.edu.ng

### **Iyabo Ola (Chapter 2)**

Department of Pure & Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

olaiyabo@yahoo.com

### **Oke Adefola (Chapter 3)**

Department of Biological Sciences, BOWEN University, Iwo, Nigeria

adefolaames@yahoo.com

### **Adesiji Yemisi (Chapter 4)**

Department of Medical Microbiology, College of Medicine, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

drmradesiji@yahoo.com

**Charles Adetunji (Chapter 5)**

Nigerian Stored Product Research Institute Ilorin and Landmark University,  
Offa, Nigeria

charliguitar@yahoo.com

## Preface

This book is written to exhibit the successes of our team in producing novel strains of different microorganisms for solving different human problems using biotechnology techniques. The book is a compilation of the well defended PhD thesis of my formal five doctoral students. Theses papers belong together as all of them are addressing how different attributes of various microorganisms can be enhanced for solving human problems through biotechnology practice.

Description of methods of enhancing microbial performance like cross-breeding and mutagenesis are often not specific for a particular organism. Research project students and other researchers at industries often face a lot of difficulties in adopting unspecific experimental protocols for their work. In this book specific experimental protocols for enhancement of microbial performance are accompanied with exciting results. Since these protocols can be reproduced; they could be easily adapted by others for similar projects.

The book will be very useful for teaching post graduate and undergraduate students in universities. Both students and university lecturers will find the book useful for teaching and research. Researchers in different Institutes and Industry will also find the book very useful. In addition; while the book will be useful in providing vital information for Entrepreneurs in business set-up; the book will be of immense benefit for farmers in the area of bio-herbicide production.

Although the book is a compilation of defended projects of five prior students, conscious effort has been taken to integrate the content so that it flows together logically as follows:

- a) In chapter one, emphasis is on the use of cross-breeding and mutagenesis to produce better strains of *Pleurotus* (oyster mushroom) with enhanced spawn production, sporophore production, enzyme and protein production. Since *Pleurotus* are known to contain different antioxidants, this chapter

demonstrates an efficient means of producing immunomodulators. The chapter ends by concluding that *Pleurotus* may be a cheap source of immunotherapy.

- b) Tyrosinase is one of the major enzymes produced by *Pleurotus* (oyster mushroom). In chapter two, enhanced production of tyrosinase and its immobilization is discussed. After reading chapter one; one will like to understand how tyrosinase can be immobilized using efficient cheaply available polymers.
- c) In addition to *Pleurotus* been a cheap source of immunomodulators, in chapter three, Protein A from *Staphylococcus aureus* is exhibited as a possible candidate of immunotherapy.
- d) In chapter four, the challenges of *Arcobacter* as an emerging food-borne and zoonotic opportunistic pathogen is discussed. The chapter is concluded by mentioning that immunotherapy is an efficient way of combating the emerging challenges of *Arcobacter*.
- e) In chapter five, discussion is made of the effectiveness of different formulations of bio-herbicide containing mutant strains of *Pseudomonas aeruginosa* and *Lasiodiplodia pseudotheobroma* in the control of target weed with no effect on non-target crop. This is no disconnect from the first four chapters as effective strains of microorganisms are developed for biotechnology processes.

I gratefully acknowledge the thoughtful contributions of my prior doctoral students viz: Dr Adebayo Elijah, Dr Iyabo Ola, Dr Oke A J, Dr Yemisi Adesiji and Dr Adetunji Charles. I am indebted to the management of Ladoke Akintola University of Technology, Ogbomoso, Nigeria for allowing to utilize my accumulated leave at All Saints University during the time materials in this book



were compiled. The financial support provided by the management of All Saints University for the publication of this book is highly appreciated.

The invaluable assistance of my wife and children in making this book a reality is also gratefully acknowledged.

Oloke Julius Kola (PhD)

Professor of Microbiology and Biotechnology

June 2015



# Contents

List of Authors.....	III
Preface .....	V

## **Chapter 1 Efficiency of Cross-Breeding and Mutagenesis in the Development of the New Strains of *Pleurotus Pulmonarius* ..... 1**

1.1 Introduction .....	3
1.2 Oyster Mushroom ( <i>Pleurotus</i> Species).....	3
1.3 Genetics and Breeding of Mushroom.....	7
1.3.1 Breeding Strategies.....	7
1.3.2 Induction of Mutants .....	8
1.3.3 Ultraviolet Light (UV) as Mutagenic Agent.....	8
1.3.4 Cross Breeding .....	9
1.3.5 Protoplast Fusion .....	11
1.3.6 Transgenic Breeding.....	12
1.4 Importance of Mushrooms.....	13
1.5 Nutritional Value of Mushrooms.....	13
1.6 Mushrooms as Medicine.....	14
1.7 Mushroom Nutraceuticals .....	17
1.8 Strain Enhancement.....	19
1.9 Strains Collection .....	20
1.10 Collection of Data with Genbank Accession Numbers for Isolates of <i>Pleurotus</i> Species .....	21
1.11 Mutation and Hybridization Procedure .....	22
1.12 Development of Mutant and Hybrid Strains of <i>P. pulmonarius</i> .....	23
1.13 Effect of Temperature, pH and Light on the Mycelia Yield of Wild, Mutant and Hybrid Strains.....	29
1.14 Statistical Analysis for Mycelia Yield Production .....	30
1.15 The Yield Performance of Wild, Mutant and Hybrid Strains of <i>P. pulmonarius</i> .....	31
1.16 Spawn Production.....	37
1.17 Sporophore/Fruit Body Production (Rice Straw as Substrate).....	39

1.18 Sporophore/Fruit Body Production (Sawdust as Substrate) .....	40
1.19 Genetic Diversity Study .....	43
1.19.1 Genomic DNA Extraction Protocol .....	43
1.19.2 Electrophoretic Separation of Genomic DNA .....	44
1.19.3 Spectral Analysis of Genomic DNA .....	44
1.19.4 Polymerase Chain Reaction (PCR) .....	44
1.19.5 Internal Transcribed Spacer (ITS) DNA Assay .....	44
1.20 Sequencing and Phylogenetic Analysis .....	46
1.20.1 Quantification and Purification of Genomic DNA .....	47
1.20.2 Internal Transcribed Spacer (ITS) Fingerprint of Hybrid and Mutant Strains of <i>P. pulmonarius</i> .....	50
1.20.3 The Phylogenetic Analyses of Mutant and Hybrid Strains of <i>P. pulmonarius</i> Based on Internal Transcribed Spacer (ITS) 5.8s and 28s Ribosomal RNA Gene .....	52
1.21 Conclusion .....	60

## **Chapter 2 Use of Mutagenesis Enhanced Tyrosinase Production from Pleurotus Species and Potential of Natural Polymers in Its Immobilization ..... 71**

2.1 Introduction .....	73
2.2 Objective.....	74
2.3 Fermentation Technology .....	74
2.3.1 Solid Substrate Fermentation (SSF) .....	75
2.3.2 Submerged Liquid Fermentation (SLF).....	75
2.4 Enzymes .....	76
2.4.1 Classification of Enzymes .....	76
2.4.2 Enzyme Kinetics.....	77
2.4.3 Production of Enzymes in Filamentous Fungi.....	78
2.4.4 Immobilization of Enzymes.....	79
2.4.5 Advantages of Immobilized Enzymes .....	79
2.4.6 Applications of Immobilized Enzymes .....	79
2.4.7 Tyrosinase.....	80
2.5 Mushrooms .....	82
2.6 Sources of Natural Polymers .....	85

---

2.6.1	Brachystegia Nigerica.....	85
2.6.2	Detarium Microcarpum (English Tallow Seeds).....	87
2.7	Strain Selection.....	88
2.8	Mutagenesis.....	88
2.9	Selection of Organism for Growth in Fermentation Medium.....	90
2.10	Preparation of Fermentation Medium.....	90
2.11	Extraction of Intracellular Proteins.....	91
2.12	Assay of Fungal Strains for Tyrosinase Activities .....	91
2.13	Tyrosinase Purification.....	92
2.14	Purification of Crude Tyrosinase from Wild-Type and Mutants of <i>P. ostreatus</i> and <i>P. florida</i> Using DEAECellulose-52.....	92
2.15	Gel filtration Purification of Crude Tyrosinases from Wild-Type and Mutants of <i>P. ostreatus</i> and <i>P. florida</i> Using Sephadex G-100.....	95
2.16	Purification of Tyrosinase from <i>P. ostreatus</i> Wild Type (POW) and Mutant (PO90) .....	98
2.17	Purification of Tyrosinase from <i>P. florida</i> Wild Type (PFW) and 30 Minute Mutant (PF30) .....	99
2.18	Electrophoresis .....	102
2.19	Kinetic Study of Tyrosinase .....	105
2.19.1	Effects of Enzyme Concentrations on Activity.....	105
2.19.2	Effect of pH on Tyrosinase Activities.....	105
2.19.3	Effect of Temperature on Tyrosinase Activities .....	106
2.19.4	Effects of Substrate Concentration on Tyrosinase Activity .....	107
2.20	Prttreatment of Natural Polymers .....	111
2.20.1	Deproteinization of Natural Polymers .....	111
2.20.2	Preparation of Crosslinked Natural Polymers.....	112
2.21	Tyrosinase Immobilization Procedure.....	112
2.22	Comparative Characterization of Immobilized Tyrosinase Using <i>Brachystegia Nigerica</i> , <i>Detarium Microcarpum</i> , Silica Gel and Sodium Alginate .....	113
2.23	Conclusions .....	126

**Chapter 3 Potentials of *Staphylococcus Aureus* Protein A  
in Immunotherapy..... 133**

3.1 Introduction .....	135
3.2 Uses and Mode of Action of Immunomodulators .....	136
3.2.1 Immunomodulators for Inflammatory Bowel Diseases.....	136
3.2.2 Immunomodulator as Antibacterial Agents.....	137
3.2.3 Immunomodulators for Viral Infections .....	140
3.2.4 Immunomodulators in Heat and Cold Stresses.....	143
3.2.5 Immunomodulators in the Therapy of Serious Burns Infections ...	143
3.2.6 Immunomodulators in Dermatology.....	145
3.3 Synthetic Immunomodulators.....	148
3.4 Screening for Immunomodulators .....	149
3.5 <i>Staphylococcus Aureus</i> .....	149
3.6 <i>Staphylococcus Aureus</i> Resistance to Antibiotics.....	150
3.7 Physiochemical and Biological Properties of Staphylococcal Protein A .....	152
3.8 Protein A Nature Endowment.....	153
3.9 Mechanism of Binding Staphylococcal Protein A to Immunoglobulin G .....	156
3.10 Immunomodulatory Properties and Mechanism of Action of Staphylococcal Protein A .....	160
3.11 Screening <i>Staphylococcus Aureus</i> for the Possession of Protein A.....	162
3.12 Immunomodulatory Effect of <i>Staphylococcus Aureus</i> Protein an Extract in Rats .....	163
3.13 <i>Staphylococcus Aureus</i> Protein A in Immunotherapy .....	166

**Chapter 4 Arcobacters: Emerging Food-Borne and Zoonotic  
Opportunistic Pathogens ..... 191**

4.1 Introduction .....	193
4.2 The Bacteria.....	193
4.3 History and Taxonomy .....	194
4.4 Cultural Characteristics and Media of Isolation of Arcobacter .....	198
4.5 Morphology .....	201
4.6 Growth Atmosphere .....	201

4.7 Identification of <i>Arcobacter</i> Strains .....	202
4.7.1 Identification Based on Phenotypic Characteristics .....	202
4.7.2 Biochemical Identification .....	202
4.7.3 Serological Identification .....	203
4.7.4 Identification Based on Fatty Acid Composition .....	204
4.7.5 DNA - Based Identification Methods .....	204
4.8 Epidemiological Characterisation of <i>Arcobacter</i> Strains .....	209
4.9 Phylogenetic and Genomic Analysis of <i>Arcobacter</i> .....	210
4.10 Virulence Attributes of <i>Arcobacter</i> .....	215
4.11 <i>Arcobacter</i> and Human .....	218
4.12 Mode of Transmission .....	219
4.13 <i>Arcobacter</i> in Food Animals.....	220
4.14 Water and Milk.....	221
4.15 <i>Arcobacters</i> in Fruits and Vegetables .....	221
4.16 Antibiotic Susceptibility of <i>Arcobacters</i> .....	222
4.17 Epidemiology .....	224
4.18 Pathogenicity and Virulence Properties of <i>Arcobacter</i> .....	224
4.19 <i>Arcobacter</i> in Poultry .....	226
4.20 <i>Arcobacter</i> in Pigs .....	228
4.21 Pathogenicity of <i>Arcobacter</i> .....	228
4.21.1 Agglutination Study .....	229
4.21.2 Cell Culture Study.....	229
4.22 Role of <i>Arcobacter</i> in Infertility .....	230
4.23 Distribution in Human .....	231
4.24 Recommendations, Prevention and Control of <i>Arcobacter</i> .....	231

## **Chapter 5 Advances in Formulation of Multi-Combination Bioherbicides ..... 239**

5.1 Introduction .....	241
5.2 Characterization, Production and Evaluation of Phytotoxic Potential from Bioherbicidal Agents .....	242
5.2.1 Isolation of Bioherbicidal Isolates .....	242
5.2.2 Isolation and Identification of <i>Pseudomonas aeruginosa</i> .....	243
5.3 Isolation and Identification of <i>Lasiodiplodia pseudotheobromae</i> .....	252

5.4	Structural Elucidation of Bioactive Compounds from the <i>Lasiodiplodia pseudotheobromae</i> and <i>Pseudomonas aeruginosa</i> .....	258
5.5	Genetically Improvement of the Bioherbicidal Wild Strain.....	262
5.5.1	Exposure of <i>Lasiodiplodia pseudotheobromae</i> to UV Light to Induce Mutation .....	262
5.5.2	Exposure of <i>Pseudomonas aeruginosa</i> to UV Light to Induce Mutation .....	262
5.6	Multi-Combination Formulation of the Bioherbicides .....	263
5.6.1	Preparation of Pestal Granules .....	263
5.6.2	Various Formulations .....	263
5.7	Performance of the Bioherbicides in the Control of Weed in Maize and Cowpea Cropping System as Well as Their Effects on Crop Performance/Yield.....	264
5.8	Host Range Test .....	264
5.9	Non Target Effects of Various Formulated Bioherbicides on Soil Microorganisms.....	267
5.10	Green House Study.....	271
5.10.1	Procedures for Data Collection in the Green House .....	271
5.10.2	Greenhouse Parameters.....	271
5.11	Field Evaluation of Pre-Emergence of Different Bioherbicides in Weed Control in Maize and Cowpea Cropping System .....	275
5.11.1	Site Description.....	275
5.11.2	Pre-Emergence Effect of the Formulated Bioherbicides on the Field.....	276
5.11.3	Weed Morphological Type .....	282
5.11.4	Weed Control Efficiency .....	282
5.12	Persistence of <i>Lasiodiplodia pseudotheobromae</i> and <i>Pseudomonas aeruginosa</i> in the Soil After Application of the Pestal Granules for the Field Studies .....	286
5.12.1	Recovery and Enumeration of <i>Lasiodiplodia pseudotheobromae</i> from Soil .....	286
5.12.2	Recovery and Enumeration of <i>Pseudomonas aeruginosa</i> from Soil.....	286
5.13	Conclusion.....	291