LIFE SCIENCES RESEARCH AND DEVELOPMENT

RESEARCH ADVANCES IN THE FUNGAL WORLD

Culture, Isolation, Identification, Classification, Characterization, Properties and Kinetics

PANKAJ KUMAR CHAURASIA
SHASHI LATA BHARATI
EDITORS

NOVA

RESEARCH ADVANCES IN THE FUNGAL WORLD

CULTURE, ISOLATION, IDENTIFICATION, CLASSIFICATION, CHARACTERIZATION, PROPERTIES AND KINETICS

LIFE SCIENCES RESEARCH AND DEVELOPMENT

Additional books and e-books in this series can be found on Nova's website under the Series tab.

LIFE SCIENCES RESEARCH AND DEVELOPMENT

RESEARCH ADVANCES IN THE FUNGAL WORLD

CULTURE, ISOLATION, IDENTIFICATION, CLASSIFICATION, CHARACTERIZATION, PROPERTIES AND KINETICS

PANKAJ KUMAR CHAURASIA
AND
SHASHI LATA BHARATI
EDITORS

nova
science publishers
New York

Library of Congress Cataloging-in-Publication Data

Names: Kumar Chaurasia, Pankaj, Assistant Professor, PG Department of Chemistry, L.S. College, Muzaffarpur Bihar (India) (Constituent unit of BRA Bihar University), editor. | Bharati, Shashi Lata, Assistant Professor, Department of Chemistry, North Eastern Regional Institute of Science and Technology, Nirjuli, Itanagar, Arunachal Pradesh (India), editor.
Title: Research Advances in the Fungal World: Culture, Isolation, Identification, Classification, Characterization, Properties and Kinetics
Description: New York: Nova Science Publishers, [2019] | Series: Life Sciences Research and Development | Includes bibliographical references and index.
Identifiers: LCCN 2019957626 (print) | ISBN 9781536171976 (hardcover) |
ISBN 9781536171983 (adobe pdf)

Published by Nova Science Publishers, Inc. † New York

To my father 'Shri Hari Narayan Chaurasia' and my mother 'Smt. Sumitra Devi'
due to their costless contribution in my life.
Dr. Pankaj Kumar Chaurasia

To my loving father 'Shri Om Prakash' and mother 'Smt. Ramanti Devi'.
Dr. Shashi Lata Bharati

CONTENTS

FOREWORD

The book entitled “Research Advances in the Fungal World: Culture, Isolation, Identification, Classification, Characterization, Properties and Kinetics” (Editors: Pankaj Kumar Chaurasia and Shashi Lata Bharati) is relevant for the academicians of graduate and postgraduate as well as researchers working in the field of mycology, life sciences, biochemistry, biotechnology and enzymology etc. This book has an excellent collection of 17 chapters based on the relevant studies of fungi and fungal based enzymes. This book is broadly divided into two sections: Section A and Section B which makes the book more attractive and informative. Section A has total 14 chapters corresponding to the different fungal studies while Section B has 03 chapters corresponding to the studies of fungal enzymes. Chapter 1 is focused on the introductory discussion of fungi and fungal enzymes according to the need of any book that requires an introductory chapter. Chapter 2 is based on novel studies of diversity and distribution of indigenous vesicular arbuscular mycorrhizal fungi isolated from different wheat cultivars of Pratapgarh (Uttar Pradesh) India. Chapter 3 has nice study on the isolation, identification and studying the potential application of tropical fungi in lignocellulolysis. Chapter 4 deals the diversity, distribution and ethnomycology of wild edible mushrooms. Chapter 5 has a nice discussion on ‘rhizospheric fungi’ regarding their diversity and therapeutic potential. Chapter 6 deals the discussion on present and future prospects of lichen diversity and associated fungi. Chapter 7 has excellent scientific information on the potential of secondary metabolites in bio-based products developments. Chapter 8 includes an update of phytopharmacology and medicinal properties of biologically active mushroom *Agaricus bisporus.* Nutraceutical potential of mushrooms found in Nigeria has been demonstrated in Chapter 9. Chapter 10 deals a study on ancient and recent methods of characterization of fungi and its breakthrough in their identification. Role of edible mushrooms in the treatment of cancer has been well discussed in chapter 11. Chapter 12 deals the study of the use of mushroom technology in bioconversion of agro-wastes. Chapter 13 has nice discussions on a leading pathogen of skin and nail infection called

Trichophyton rubrum while Chapter 14 deals the literature study on mycotoxins and their influences on human health. Chapter 15-17 have relevant materials and information regarding fungal enzymes. Chapter 15 has valuable discussion regarding purification, characterization and clinical applications of therapeutic fungal enzymes. Physiological role and biotechnological applications of lignocellulolytic fungal enzymes has been excellently discussed in Chapter 16. Last chapter of this book, Chapter 17 has basic kinetic studies of the fungal laccases valuable from the kinetic points of view.

All the chapters written by the eminent contributors from different countries are highly germane and full of advantageous knowledge on the fungi and fungal enzyme research. For the ease of understanding and proper dissemination of information, whole book has been divided into two sections. In my view, handy nature of the book, excellent scientific collaborations in the form of chapters and easy writing style make this book very fruitful for the students/scholars/researchers of undergraduate, postgraduate, PhD, post doctoral fellows studying and working in the field of life sciences, biochemistry, biotechnology etc.

Ashutosh Mani, PhD
Department of Biotechnology
Motilal Nehru NIT, Allahabad/Prayagraj, Uttar Pradesh, India

PREFACE

Fungi are the omnipresent microbial organism called microbes or microorganisms and studied under the branch of science “Mycology”. They are unable to prepare their own foods and depend basically on dead organic materials for their foods and nutrition. They are also eukaryotic organisms like plants and other animals and consider as separate kingdom “Fungi”. Fungi and plants have some basic similarities like both are eukaryotic organisms, both have cell walls and non-motile properties due to which they were initially grouped together but there are some distinct differences between fungi and plants: (i) Fungi are heterotrophs depend on other organic sources (plants or animals) for their foods while plants are autotrophs having ability to prepare their food by the process of photosynthesis (ii) Cell wall of the fungi are made of chitin while that of plants are made of cellulose. These distinct differences forced the scientific society to consider them as a separate kingdom. Most of the fungi have cryptic life style and are inconspicuous. Due to their dependency on other organisms like plants, animals etc for their food requirements, they show the specific phenomenon known as symbiosis. Due to their worldwide presence and unique properties, fungi had been widely absorbed as interesting research objectives. Instead of creating several problems for human being as pathogens, they have also great significances due to their rich applications in the biotechnological sector. Their momentous role in the field of fermentations, food industries, medicinal areas, genetic engineering, bioremediation of xenobiotics and poisonous metals etc had widely been accepted by the scientific communities. They are also the rich source of various types of industrially valuable extracellular enzymes.

The present book entitled “Research Advances in the Fungal World: Culture, Isolation, Identification, Classification, Characterization, Properties and Kinetics” have been developed to produce and disseminate the novel materials worldwide regarding culture, isolation, identification, classification, characterization, kinetics and other properties of the fungi. Some materials are also on the fungal based enzymes with their properties and kinetics and have been included inside the book to demonstrate the fungal

importance from the enzyme points of view. This book contains total 17 chapters written and contributed by the eminent experts of their subjects from worldwide like India, Brazil, Sri Lanka, Bangladesh, Portugal and Nigeria. All the chapters inside book are very rich in the informative research knowledge on fungi. Its handy nature and advance research materials makes it valuable for the academician and researchers of undergraduate, postgraduate, Ph.D., Post doctoral research levels in the field of biochemistry, environmental chemistry and life sciences.

The whole book is divided into the two major sections. 'Section A' contains the chapters regarding the fungal studies (Chapter 1-14) while 'Section B' contains the chapters regarding the fungal enzymes and their studies (Chapter 15-17).

Chapter 1 written by Chaurasia and Bharati is focused on introductory discussion of fungi and fungal enzymes. Fungus is a type of microbe with worldwide presence. The branch of science under which study of fungi occur is known as 'Mycology'. It is any member of the eukaryotic organism that means they have clear defined nuclei and organelles inside the cell bound by a cell membrane. Fungi are one of the most widely distributed organisms in the world. They are also strong sources for the different types of extracellular lignolytic enzymes like laccases, different types of peroxidases, cellulases, pectinases etc. Their secreted extracellular enzymes are also very important for the various scientific purposes. Such enzymes are highly significant in the field of organic syntheses, biotransformations, medicinal sciences, bioremediation, agriculture, nanosciences etc. This chapter deals the basic study of fungi as their growth, sources, production, and role for the society as well as different types of fungal enzymes secreted from fungi.

Chapter 2 is written by Dwivedi on 'Diversity and Distribution of Indigenous Vesicular Arbuscular Mycorrhizal Fungi Isolated from Different Wheat Cultivars of Pratapgarh (Uttar Pradesh) India'. A study was conducted by him to assess the species diversity of vesicular arbuscular mycorrhizal fungi in wheat cultivated area of Pratapgarh district (U.P.). Nine different wheat cultivars which were frequently grown in the study area were selected and examined for the VAM infection. All the wheat cultivars were found to be infected with vesicular arbuscular mycorrhizae. His study clearly indicates the frequent occurrence of Acaulosporaceae with dominating of *Acaulospora* species and rare occurrence of *Entrophospora* species, in addition to members of Glomaceae and Gigasporaceae.

Chapter 3 deals the study performed by Jayasekara et al. on "Isolation, identification and studying the potential application of tropical fungi in lignocellulolysis". Biodiversity rich tropical countries like Sri Lanka have an enormous number of microorganisms including bacteria and fungi in their natural environments. This microbial diversity is a consequence of unique climatic conditions exists in the area. In this chapter, an account of fungi with the ability to produce lignocellulolytic enzymes which was isolated from central areas of Sri Lanka is presented. Furthermore, the morphological as well as

molecular biological identification of the most efficient cellulase enzyme producing microorganisms have also been reported. The major focus of their study was to characterize these fungal isolates based on their ability to produce total cellulase, xylanase and laccase like lignocellulolytic enzymes.

Chapter 4 is written by Singh and Bhatt on wild edible mushrooms. Mushrooms have long been regarded all over the world as the most delectable, succulent of foods and one of the world's greatest natural resources since they have the ability to transform nutritionally valueless substances into high protein foods. The present study is a part of exploration of wild edible mushrooms and documentation of their use by inhabitants.

Chapter 5 is written by Sahu and Jha on diversity and therapeutic potential of 'Rhizospheric fungi'. In this chapter different groups of fungi associated with rhizospheric region of the plant and their therapeutic potential with possible applications in diverse sectors are discussed by authors.

Chapter 6 written by Devi and Tayung deals the literature study on the present and future prospects of lichen diversity and its associated enzymes. Lichens are symbiotic association between an alga and a fungus. This unique association has evolved as a special adaptation to varied microhabitats withstanding extreme microclimatic conditions. The ecology of lichens is very interesting and can be used for varied purposes. This is the reason that authors have highlighted in this chapter the global and India's lichen diversity emphasizing on their uses and associated fungi particularly on endolichenic fungi as source of bioactive natural products.

Chapter 7 written by Silva et al. on "Secondary Metabolites by Endophytic Fungi: Potential for Bio-based Products Developments". Secondary metabolites of endophytic fungi show their potential for biobased products developments. In this chapter, they discussed the state of the art of secondary metabolites obtained from endophytic fungi to demonstrate its use as an important tool in the development of natural and eco-friendly agrochemicals, which can be less harmful to humans and environment, leading to new opportunities in the development of a bio-based commercial product of great importance for sustainable management of insect pests.

Chapter 8 deals the study on biologically active mushroom *Agaricus bisporus* written by Kulshreshtha and Srivastava. *Agaricus bisporus* (button mushroom) is an edible, widely grown and cultivated species of a fungus consisting of more than 40% of world's total production of mushroom. This chapter deals with the biochemistry, pharmacognosy, pharmacology, and formulations of *Agaricus bisporus* involved in disease cure based on scientific data published online and offline. This chapter could serve as a gateway for newer and beneficiary researches in the field of disease cure.

Chapter 9 deals the study on nutraceutical potential of mushrooms in Nigeria written by Oyetayo and Oyetayo. This chapter has detailed discussion on health promoting properties of some edible and non-edible mushrooms found in Nigeria and practical steps that can be taken to ensure the full exploitation of these mushrooms.

Chapter 10 is based on the study of Sridharan and Krishnaswamy. They performed their study on "Ancient and Recent Methods of Characterization of Fungi and Its Breakthrough in Their Identification". The process of gaining knowledge about the fungal organisms, classification, their genetic makeup, enzyme production and other application are summarized under the chapter.

Chapter 11 deals the discussion on the role of edible mushrooms in cancer treatment written by Uddin Pk et al. The main medicinal applications of edible mushrooms discovered are anti-cancer, anti-oxidant, anti-diabetic, anti-microbial, hypochelesterolemic and immunomodulatory. In this chapter, they showed the recent findings of biologically active compounds with their anticancer activity and mechanism of biological action in order to draw attention of scientists for future investigations and to develop cancer drugs from mushrooms.

Chapter 12 deals the solid discussion on bioconversion of agro-wastes using mushroom technology written by Salami and Bankole. They have investigated the ability of different agro-wastes products to produce mushroom using various mushroom technology. This was with the aim to determine and improve the potentials of various agro-waste products to mushroom production. Their results showed that each substrate responded to the treatment as yields were obtained maximally, although at different rates. In addition, the essential elemental component of Oyster mushroom defined the rationale behind the ability of agro-wastes to respond to mushroom production.

Chapter 13 is written by Anita and deals the study on a leading pathogen of skin and nail infection known as *Trichophyton rubrum.* In recent studies, it has been reported that *Trichophyton rubrum* has become the leading pathogen of skin and nail infections followed by *Trichophyton mentagrophytes*. *Trichophyton rubrum* and *Trichophyton mentagrophytes* are also reported most common pathogens causing dermatophytosis in India. This chapter reviews the isolation, methods of isolation and management of *Trichophyton rubrum* causing dermatomycoses.

Chapter 14 is written by Anita and demonstrates the different types of mycotoxins and their impacts on the health of humans. The effect of mycotoxins on human and animal health depends on the amount of the mycotoxin consumed, the toxicity of the compound and the body weight of the individual. There are many factors which favour mycotoxin production as temperature, climate, moisture content, oxygen levels, preservatives, fungal strain and microbiological ecosystem.

Chapter 15 deals the enzymatic section of this book on purification, characterization and clinical applications of therapeutic fungal enzymes written by Almeida et al. This book chapter very nicely presents an overview of therapeutic fungal enzymes and their developments in biopharmaceuticals for the treatment of several diseases, clinical applications and investigation.

Chapter 16 is written by Paul et al. on fungal lignocellulolytic enzymes like cellulases, hemicellulases, peroxidases and laccase and discussed their physiological

roles and biotechnological applications. They have also discussed application in the field of agriculture, alcohol and brewing industry, paper and pulp industry, textile industry as well as in different medical practices in this chapter.

Chapter 17 is written by Chaurasia and Bharati. The huge level of applications of fungal laccases are due to their strong catalytic efficiency which depends on their binding capability, active sites, pH stability, thermal stability, pH optima, temperature optima, Michaelis-Menten constant (K_m) and catalytic constant (K_{cat}). In this chapter, authors have discussed and reviewed the different kinetic aspects of fungal laccases based on previous studies.

ACKNOWLEDGMENTS

Dr. Pankaj Kumar Chaurasia is thankful to the PG Department of Chemistry, LS College Muzaffarpur, Bihar (India) for providing necessary conditions and facilities during the works. I am also very much thankful to our colleagues Dr. Shashi Kumari Singh (Associate Professor), Dr. Shailendra Kumar Sinha (Associate Professor), Dr. Vijay Kumar (Associate Professor), Dr. Arbind Kumar (Associate Professor) and Dr. S.H. Faizi (Assistant Professor) for their inspiring and supportive suggestions during the works. I am also very much thankful to Dr. Ajay Kumar Chaurasia (Philosophy) for his inspiring and motivational suggestions. I am heartiest thankful to my dear brother Er. Neeraj Kumar Chaurasia and whole family for their moral supports during the work.

Dr. Shashi Lata Bharati is very much thankful to the Department of Chemistry, NERIST, Nirjuli, Arunachal Pradesh (India) for necessary support and facility during the work. I am grateful to our colleagues Prof. H.S. Yadav, Prof. AK Gupta, Prof. PK Tripathy, Dr. J. Bhuyan (Assistant Professor) and N. Ghanshyam (Assistant Professor) for any supports during the work. I am thankful to my elder brother Pradeep Kumar and younger sisters namely Pusp Lata, Sneh Lata and Hem Lata for their moral and inspiring support during the work.

Pankaj Kumar Chaurasia, PhD
PG Department of Chemistry, L.S. College, Muzaffarpur, Bihar, India
Shashi Lata Bharati, PhD
Department of Chemistry, NERIST, Nirjuli, Arunachal Pradesh, India
Editors

SECTION A. FUNGI

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 1

FUNGI AND THEIR ASSOCIATED ENZYMES

Pankaj Kumar Chaurasia[1,*] and Shashi Lata Bharati[2,†]
[1]PG Department of Chemistry, LS College, Muzaffarpur
(Under BRA Bihar University, Muzaffarpur), Bihar, India
[2]Department of Chemistry, North Eastern Regional Institute of
Science and Technology, Nirjuli, Arunachal Pradesh, India

ABSTRACT

Fungi or funguses are microorganisms that include molds, mushrooms and yeasts and are a member of eukaryotic organisms. They are found abundantly in nature. They are generally considered as a separate kingdom as fungi from plants and animals. Branch of the science of fungal studies is to referred as 'mycology'. Initially, they grow as cylindrical thread like 'hyphea'; after hyphal fusion (anastomosis), they appear in the form of mycelium that can be seen by naked eye very easily. Their worldwide presence makes them valuable for scientific studies due to their association with plants as well as animals. They have several positive as well as negative roles for humans with a lot of scientific interest. Their significant uses in the field of therapeutics, food and nutrition, environmental safety and pest control, etc. make them very valuable for new researches. They are also strong sources for different types of extracellular lignolytic enzymes like laccases, different types of peroxidases, cellulases, pectinases, etc. Their secreted extracellular enzymes are also very important for the various scientific purposes. Such enzymes are highly significant in the field of organic syntheses, biotransformations, medicinal sciences, bioremediation, agriculture, nanosciences, etc. This chapter deals the basic study of fungi as their growth, sources, production and role for the society as well as different types of fungal enzymes secreted from fungi.

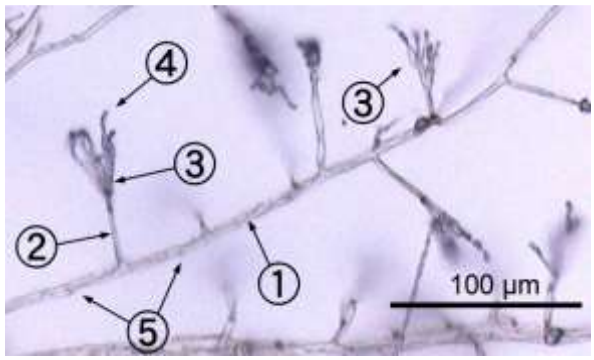
* Corresponding Author's Email: pankaj.chaurasia31@gmail.com; pankaj.chaurasia1987@rediffmail.com.
** Corresponding Author's Email: shashilatachem@gmail.com.
† Corresponding Author's Email: shashilatachem@gmail.com.

Keywords: fungi, habitats, applications, fungal enzymes, laccases, peroxidases, cellulases

INTRODUCTION

Fungus is a type of microbe with worldwide presence. Its plural form is ‘funguses’ or ‘fungi’. The branch of science under which study of fungi occur is known as ‘Mycology’. It is a member of the eukaryotic organisms that means they have clear defined nuclei and organelles inside the cell bound by a cell membrane. Fungi are one of the most widely distributed organisms in the world. They grow in the wide range of habitats. They can even grow in the deserts, high salt fields (Vaupotic et al., 2008) as well as in the deep see sediments (Raghukumar and Raghukumar, 1998). Yeasts, molds, mushrooms, rusts, etc. are a few examples of fungi.

They make up a separate kingdom like plants and animals because there are approximately 144,000 known species of these organisms (https://www.britannica.com/science/fungus). They have some properties which make them differ from plants, bacteria and some protists, one of which is the presence of chitin in their cell walls (https://en.wikipedia.org/wiki/Fungus). Initially, fungi were considered as plants but they can not perform the photosynthesis because they have not chlorophyll like plants. They grow as cylindrical, thread like ‘hyphae’ (grow from tip) and further growth of hyphae and hyphal fusion results in the formation of fungal adult form that is known as ‘mycelium’. Hyphal growth also takes place through branching by growing tips to tips. Branching gives rise to the formation of two parallel hyphae (Harris, 2008). Actually, mycelium is the interconnected network of the hyphae. Hyphae may be septate or coenocytic i.e., a septa or internal cell wall which divides the structure into compartments and each compartment may has one or more nuclei while coenocytic are not divided into different compartments (https://en.wikipedia.org/wiki/Fungus, Deacon, p. 51 Figure 1).



1= Hypha 2= Conidiophore 3=phialide 4= conidia 5= septa

Figure 1. An isolate of Penicillium. https://en.wikipedia.org/wiki/Fungus#/media/File:Penicillium_labeled_cropped.jpg.

Different fungi have different role for the human. They are used for the purpose of medicine preparation. Several fungi produce metabolites which are the major source of the different types of medicinally active drugs. Fungi have also ability to generate such types of compounds which inhibit the growth of harmful viruses (el-Mekkawy et al. 1998; El Dine et al., 2008) and undesirable growth of cells (cancer) (Hetland et al., 2008; Yuen and Gohel, 2005). There are certain mushrooms which are used as therapeutics in folk medicines also called traditional medicine like *Agaricus subrufescens* (Hetland et al., 2008; Firenzuoli et al., 2008), *Ganoderma lucidum* (Paterson, 2006) and *Ophiocordyceps sinensis* (Paterson, 2008). Fleming was the scientist who discovered antibiotics derived from fungi. Several substances have also been isolated playing other types of medicinal significances like control cholesterol and ward off coronary heart diseases. They have also capability to produce vitamins, proteins and enzymes (https://www.britannica.com/science/fungus/Importance-of-fungi). Fungi have also well known for their nutritional significances. Species of *Saccharomyces* is used in the preparation of bread and pizza dough (Kulp, 2000), alcoholic beverages through fermentation (Piskur et al., 2006), etc. Various types of mushrooms are fungi with full of the nutritional values. Fungi are also well known for their negative effects like pathogenic effects on crops, plants, animals as well as human. They are also known for their production of mycotoxins, a substance produced by the fungal species which are toxic or harmful for the livings. In the context of human, mycotoxins produced by molds responsible for the food spoilage and toxic mushrooms are some examples (https://en.wikipedia.org/wiki/Fungus).

Enzymes secreted from fungi have also a lot of significances in the field of industries as well as in the field of research. Fungal based lignolytic enzymes have a lot of applications in several fields of sciences like food, beverages, medicines, organic synthesis, bioconversions, polymerization and depolymerizations, bioremediations, etc. (Chowdhary et al., 2019; Chaurasia et al., 2016, Chaurasia et al., 2015; Placido and Capareda, 2015). Laccases, lignin peroxidases, manganese peroxidases, chloro-peroxidases, proteases, cellulosases, pectinases, phytases, etc. are the biologically valuable enzymes isolated from fungi. The main objective of this chapter is to introduce about fungi and fungal based lignolytic enzymes and discuss their significances for the human beings, environment and other branch of sciences.

HABITATS

Fungi have distinct and worldwide presence. They have unique ability to grow in any conditions and atmosphere. They can grow in the adverse conditions of deserts, most suitable terrestrial lands as well as aquatic system. Soil and dead materials are the habitats for the most of fungi (https://www.ck12.org/biology/fungi-habitat/lesson/Habitats-of-Fungi-Advanced-BIO-ADV/). Figure 2 shows the fungal growth on plants.

Mostly, fungi are inconspicuous due to their very small size and cryptic life style in soil or dead materials (https://en.wikipedia.org/wiki/Fungus). They are symbionts of animals, plants as well as other fungi and parasites. Decomposition of the organic materials is also due to the combined role of fungi with bacteria which is the process of converting organic materials into their nutrients form in soil. Fungi can be found in their best probable habitats like plants and meadow (grazing lands) and more than 80% of the discovered fungi are found linked with trees (https://www.ck12.org/biology/fungi-habitat/lesson/Habitats-of-Fungi-Advanced-BIO-ADV/).

Figure 2. Fungus growth on wood/tree stump.
https://commons.wikimedia.org/wiki/File:Fungus_in_a_Wood.JPG.

Mycorrhiza is a type of symbiotic fungal association with plants (Kirk et al., 2001). This is found in the rhizosphere of plants (Figure 3). Mycorrhizal association is valuable from the points of view of plants nutrition. Mycorrhizal association may be either extracellular as in ectomycorrhizal fungi (fungi colonizes extracellularly in roots of host plants) or intracellular as in arbuscular mycorrhizal fungi (fungi colonizes intracellularly in roots of the host plants) (https://en.wikipedia.org/wiki/Mycorrhiza).

FUNGAL GROWTH AND THEIR MORPHOLOGY

Fungi grow as hyphae on solid waste or as single cell in aquatic systems. They grow in such a way that they could extract the nutrients efficiently (Moss St, 1986). There is excellent adoptability in hyphae to grow on solid and invade the substrates (Penalva and Arst, 2002). Fungi have ability to exert a powerful penetrative mechanical force. *Magnaporthe grisea*, a plant pathogen also called rice blast fungus, forms an appressorium (a specialized cell used to infect the host) that evolved to puncture the plant tissues (Howard et al., 1991). *Paecilomyces lilacinus* (a filamentous fungus) penetrates the nematodes' eggs by using similar structure (Money, 1998).

Fungi are found in the forms of microscopic as well as macroscopic structures. They grow as cylindrical, thread like hyphae (diameter 2-10 μm) (https://en.wikipedia.org/wiki/Fungus). Their further development by hyphal fusion and branching give the macroscopic mycelium. The most of the filamentous fungi grow in the polar way i.e., in one direction *via* hyphal tips (Fischer et al., 2008). In some endophytic fungi, there is another form of growth known as intercalary extension (Christensen et al., 2008). Fungal growth in case of development of mushroom stipe and other large organs is by the volume expansion (Money, 2002). Multicellular growth of fungi consisting of somatic (body cells) as well as reproductive cells have various roles including the development of fruit bodies and biofilms (Daniels et al., 2002; https://en.wikipedia.org/wiki/Fungus). Fruiting bodies are for the purpose of propagation of sexual spores while biofilms are for the colonization of substrate and intercellular communication.

Reproduction in fungi takes place in different ways like asexual, sexual and spore dispersal. In fungi, conidia are responsible for the asexual reproduction. Actually conidia are the vegetative spores of fungi which are non-motile in nature. Conidium is a single non-motile fungal spore. Mycelial fragmentation is another way of asexual reproduction (https://en.wikipedia.org/wiki/Fungus). Sexual reproduction has been observed for all fungal phyla except Glomeromycota (Redecker and Raab, 2006) *via* meiosis cell division (four haploid cells are generated during meiotic cell division, each of them are genetically different from the parent cell). There are two types of species based on their mating style-heterothallic species and homothallic species. In heterothallic species, mating takes place between two species of opposite mating types while in homothallic there is mating between two similar mating species with any other species or itself. Two suitable and more compatible species combine together through tips of hyphae by fusion also called anastomosis which is the urgent process for the initiation of the sexual reproduction in fungi (https://en.wikipedia.org/wiki/Fungus).

MAJOR FUNGAL DIVISIONS

Fungi have been classified on the basis of the properties of sexual reproduction in seven major phyla or divisions (Hibbett et al., 2007): (i) Microsporidia (ii) Chytridiomycota (iii) Blastocladiomycota (iv) Neocallimastigomycota (v) Glomeromycota (vi) Ascomycota (vii) Basidiomycota

Figure 3. Mycorrhizal association of fungi on root of plant (https://en.wikipedia.org/wiki/Mycorrhiza; Nilsson, 2005).

Microsporidia are phylum of highly derived endobiotic fungi i.e., parasite living within the tissues of another species (James et al., 2006; Gill et al., 2006; https://en.wikipedia.org/wiki/Fungus). Actually, microsporidia is a group of spore forming unicellular parasites (https://en.wikipedia.org/wiki/Microsporidia) and they are, now, known as fungi (Hibbett et al., 2007) or sisters group of fungi (Silar philippe, 2016). Chytridiomycota or simply chytrids are fungi with worldwide occurrence and active moving zoospores with a single flagellum. Fungi of the *phylum blastocladiomycota*, also called as blastocladiomycetes are the saprotrophs which participate in organic material's decomposition (https://en.wikipedia.org/wiki/Fungus). Neocallimastigomycota is a small phylum of fungi. They are anaerobic organisms living inside of the digestive system of herbivorous mammals as well as in cellulose enriched terrestrial and aquatic environments (Lockhart et al., 2006). Arbuscular mycorrhizae are formed by fungi of the group Glomeromycota. Arbuscular mycorrhiza is an example of mycorrhizae in which symbiosis takes place between plants and fungi. Symbiont fungi form arbuscules by penetrating the root of the vascular plant. Fungi of this phylum form the arbuscules and vesicles which are the specific characteristics of the arbuscular mycorrhizas (https://en.wikipedia.org/

wiki/Arbuscular_mycorrhiza). Asexual reproduction is generally observed for all Glomeromycota (Redecker and Raab, 2006). Fungi of the phylum Ascomycota are commonly known as sac fungi or ascomycetes. Ascospores also called as meiotic spores are enclosed within a special sac like structure known as 'ascus'. Morels, a few mushrooms, truffles, unicellular yeasts and many filamentous fungi found as saprotrophs, parasites and symbionts (lichens) are studied under this phylum. Some examples are *Saccharomyces, Candida, Aspergillus, Penicillium* and *Fusarium, etc.* Ascomycetes form the largest group of 'Eumycota' (https://en.wikipedia.org/ wiki/Fungus). Fungi of the basidiomycota are also known as 'basidiomycetes' or 'club fungi'. They generate basidiospores on basidia which is a club like stalk. Major pathogenic fungi like rust and smut fungi as well as common mushrooms are generally come in this category. Figure 4 shows the different groups of fungi showing initial spore level properties (https://commons.wikimedia.org/wiki/File:02_01_groups_of_Fungi_(M._Piep enbring).png).

BIOTECHNOLOGICAL APPLICATIONS OF FUNGI

Fungi also have a lot of significances in the life of human. There are varieties of biotechnological applications of fungi as discussed below (https://www.streetdirectory.com/travel_guide/119227/science/potential_applications_of_fungi_a_biotechnological_aproach.html):

(i) In the Field of Food

Mushrooms are well known fungi generally used as highly nutritious food for human. They are source of proteins, fibres and minerals. Mushrooms are important due to their low calorific value. Examples of a few edible mushrooms are *Agaricus compestris*, *Volvariella* (paddy straw mushroom), *Morchella* (temperate zone mushroom), *Pleurotus sp.* (oyster mushroom), *Agaricus bisporus* (white button mushroom).

(ii) In Enhancement of the Food Quality

Many fungi are used for the enhancement of the nutritional and other quality of the foods. Carbohydrates and phosphorus contents are more in smutted corn as well as rusted wheat grains in comparison to the healthy plants.

(iii) As Bioherbicides

There are many fungi which have applicability in the field of destruction of the herbs. Due to such nature of fungi, they may be used as bioherbicides. *Septagloeum gillis, Cercospora ageratinae, Phyllosticta*, etc. are the examples of some fungi acting as 'weed killer'. They are specific and selective towards weeds.

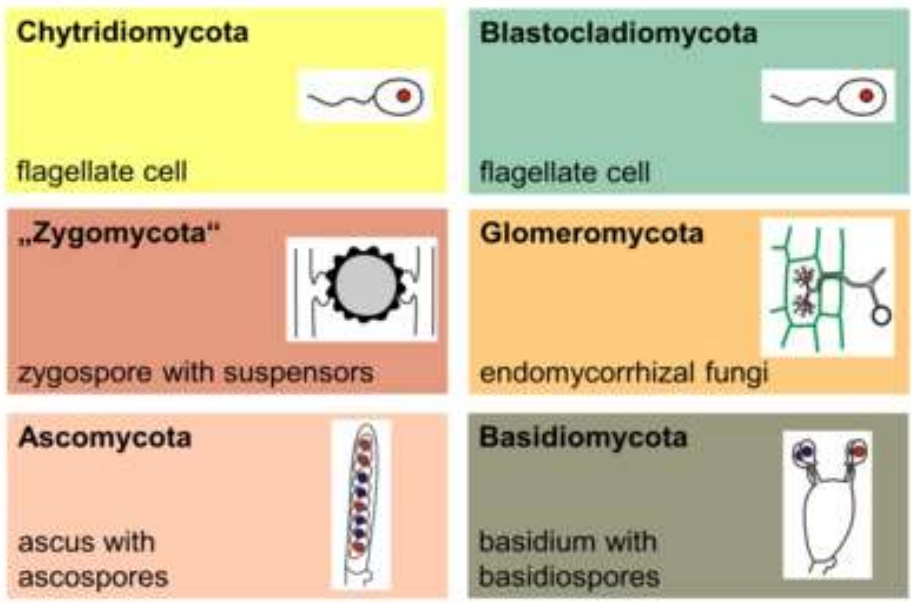


Figure 4. Major phylum of fungi showing spore level properties.

(iv) Use in the Degradation of Cellulose and Lignin and in Paper Industry

Fungal cellulases are responsible for the degradation of cellulose to produce bioenergy. Cellulases from *Fusarium, Trichoderma, Penicillium*, etc. can be used for such purposes. Ability of lignin degradation to low molecular weight petroleum products is another significant application of fungi like white rot fungi e.g., *Coriolus versicolor* and brown rot fungi like *Lenzitis trabea*. These fungi are also used in the field of paper industries for wood softening.

(v) In the Bioremediation/Biodetoxification/Biodegradation

Fungi have role in the field of degradation of toxic materials to less toxic materials or non-toxic forms. White rot fungi have useful role in the detoxification of Dichloro

diphenyl trichloroethane (DDT), dioxin, polychlorinated biphenyl (PCB), pentachlorophenol, benzopyrenes, etc. They have also role inin the degradation of dyes as well as toxic hydrocarbons. Fungal based enzymes are involved in the degradation of many other several hazardous explosive chemicals. Fungi have also application in the field of biomineralisation of several poisonous metals like Mercury (Hg), Cadmium (Cd), Lead (Pb), Nickel (Ni), etc.

(vi) In Pharmaceutical Industries

Mushrooms act as anticancer material. Many fungal metabolites are significantly used in the field of pharmaceutical field. Fungal metabolites show the ability to be used in the cholesterol lowering, antibacterial and antitumour activities, etc.

(v) Other Fungal Applications

Many fungi are the rich source of single cell proteins like Yeast (*S. cerevisae, Aspergillus niger, Penicillium chrysogenum, Fusarium avenacum*, etc). Many are also used in genetic engineering.

FUNGAL BASED ENZYMES AND THEIR APPLICATIONS

There are number of enzymes secreted from different types of fungi (Bharati and Chaurasia, 2018). Fungi secrete their enzymes in the liquid solution either during their catalytic reactions or during isolation processes. Laccases, peroxidases, cellulases, xylanase, pectinases, amylases, etc. There are many more enzymes based on fungi but in this chapter authors a have discussed briefly about only aforementioned enzymes:

Laccase [1.10.3.2]

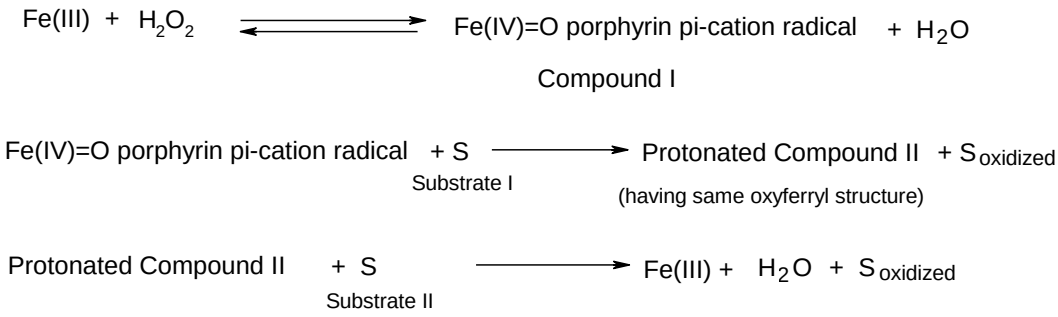
Laccases are the enzyme of oxidoreductase with enzyme classification EC.1.10.3.2. They are multicopper containing oxidase enzymes (Hoegger et al., 2006). They are also known as lignolytic enzymes and obtained by extracellular secretion from fungi. Laccase has been reported in fungi (Baldrian, P., 2006), plants (Yoshida, 1883; Bao, 1973; Huang, 1999), bacteria (Enquita, 2004; Givaudan et al., 1993, Sanchez-Amat, et al., 2001), insects (Thomas et al., 1989) and vasp venum (Parkinson et al., 2001). They have unique

ability to convert molecular oxygen into water as by-product by its four electron reduction. Laccase catalyzed reaction, as reported, can take place in two ways either in the presence of mediator molecule or absent of mediator molecules. In the presence of mediator molecule, laccase is first oxidized by molecular oxygen. This oxidized laccase reacts with mediator and oxidizes the mediator and itself undergoes in its initial state. Now, oxidized mediator reacts with substrate and oxidizes the substrate. In case of the absence of mediator molecule, laccase oxidized by molecular oxygen oxidizes the substrate directly. In general, non-phenolic compounds are oxidized by laccase in the presence of mediator molecule (Chaurasia et al., 2013a; Chaurasia et al., 2013b) while phenolic compounds are oxidized in the absence of mediator molecule. Phenols, substituted phenols, polyphenols, amines and its derivatives and several metal ions are the substrates of this enzymes upon which this enzyme act as one electron oxidation causing crosslinking (https://en.wikipedia.org/wiki/Laccase). In biotechnology, laccases have great meaning in the field of food technology, paper industries, pharmaceutical industries, bioremediation, biosyntheses, bioconversions, depolymerization, etc. (Chaurasia et al., 2016; Chaurasia et al., 2015).

Peroxidase [EC.1.11.1.x]

Peroxidases are enzymes which may contain cofactor haem/heme or not. They are large family of the enzymes found in microbes, plants and animals. They oxidize the different types of substrates using peroxides like hydrogen peroxides or other. Haem containing peroxidases react with hydrogen peroxides or other peroxides and break them in to non-toxic form. In catalytic cycle of the peroxidase reaction, there is involvement of specific types of intermediates (Essays, UK, 2018; Wong 1995). An unstable intermediate compound I is formed from the result of the oxidation of the haem containing enzymes with hydrogen peroxide (H_2O_2). This unstable compound has haem structure containing Fe(IV)=O porphyrin π-cation radical. During the same time, H_2O_2 is reduced into water molecule. Now, this oxidized unstable compound I further oxidizes the substrate and gives compound II which has protonated oxyferryl structure. Now, second substrate molecule reduces compound II and generates another free radical with regeneration of iron (III) (Essays, UK, 2018, Carlos et al., 2004).

Reaction steps of the haem containing enzymes with hydrogen peroxides are as follow (https://en.wikipedia.org/wiki/Haem_peroxidase; Nelson et al, 1994; Poulos and Li, 1994):



In general, peroxidases may be divided in two major classifications i.e., haem containing peroxidases and non-haem peroxidases: Haem containing peroxidases are haem peroxidase, catalase, haloperoxidase, etc. and non-heme peroxidase are manganese peroxidase, vanadium bromo peroxidase, NADH peroxidase, etc. (https://en.wikipedia.org/wiki/Peroxidase).

Cellulases

Cellulases are the enzymes that have the ability to decompose cellulose and some polysaccharides and degrade them into monosaccharides (like D-glucose), oligosaccharides and smaller polysaccharides (cellulolysis). Hydrolysis of cellulose with help of this enzyme generally takes place at 1,4-beta-D-glycosidic linkages. Such hydrolysis also takes place in hemicellulose, lichenin, and cereal beta-D-glucans (https://en.wikipedia.org/wiki/Cellulase). Decomposition of cellulose has one of the great significance in various field such as food industry, chemical industry, etc. Cellulases are named in different ways according to their functions. Beta-1-4-glucanase, endoglucanase D, β-1,4-endoglucan hydrolase, carboxymethylcellulase (CMCase), celludextrinase, cellulase A, alkali cellulose, etc. are the enzymes associated with cellulase as synonyms (https://en.wikipedia.org/wiki/Cellulase). Cellulases can be divided into five major types as: Endocullulases (EC 3.2.1.4), exocellulases or cellobiohydrolases (EC 3.2.1.91), cellobiases or β-glucosidases (EC 3.2.1.21), oxidative cellulases and cellulose phosphorylases. They show synergistic property i.e., they produce more sugar unit when working together in comparison to their individual action. There are also progressive and non-progressive type's cellulase. Progressive type's cellulase will continue to interact with single strand polysaccharide while non-progressive type's cellulase will interact once, then, disengage and engage another polysaccharide strand. (https://en.wikipedia.org/wiki/Cellulase). Cellulases are the industrially valuable enzymes. They are valuable in the fermentation of biomass, into coffee processing, pulp and paper industry, textile industry, pharmaceutical industry. They have capability to degrade the polymicrobial bacterial biofilm by hydrolysing the β-1,4-glycosidic linkage present in

exopolysaccharides of the extracellular polymeric substances (Fleming and Kendra, 2017; Fleming et al., 2017).

Xylanase

Xylanases [EC 3.2.1.8] are the enzymes that have tendency to degrade the linear polysaccharide xylan into xylose (Beg et al., 2001). Thus, they participate in the breakdown process of hemicelluloses present in the plant cell wall. Fungi, bacteria, marine algae, snails, crustaceans, insect, etc. are the producers of xylanases while they are not produced by mammals (Polizeli et al, 2005). Filamentous fungi are the main source of xylanases for the commercial uses (Polizeli et al., 2005). They have wide applications in various fields as in pulp and paper industry, nutritional enhancement in agricultural silage and grain feed, food additive to poultry and baked product (Beg et al., 2001), extraction of coffee, plant oil and starch (https://en.wikipedia.org/wiki/Xylanase). They are also used in the field of fruit juices clarifications and degumming of plant fibres in combination with two other valuable enzymes pectinase and cellulase (https://en.wiki pedia.org/wiki/Xylanase).

Pectinases

These are the enzymes performing the function of breakdown of polysaccharide material pectin present in cell walls. Fungi, plants, bacteria, etc. are the sources of the pectic enzymes producers (Whitaker, 1990; Gummadi and Panda, 2002). They can be divided into three major classes: pectin esterases, depolymerizing enzymes like hydrolases and lyases and protopectinases (Alkorta et al., 1998). Elimination of methoxyl and acetyl residues from pectin are performed by esterase to give polygalacturanic acid, breakdown of α-1,4-glycosidic linkage between galacturonic acid residues are performed by depolymerases like polygalacturonases (hydrolysis), pectin lyases and pectate lyases (transelimination) while protopectinases convert soluble pectin by solubilizing the protopectin (Shet et al., 2018). They have varieties of application in various fields like food industries (the removal of pectin from the fruit juices, maceration of vagetables, winemaking), pretreatment of pectin containing waste water, paper and pulp industry, processing of textile fibres, fermentation and degumming, etc. (Shet et al., 2018).

Fungal Amylases

Amylases are the enzymes found in the saliva of humans as well as other mammals. The main function of this enzyme is the catalysis of the hydrolysis of starch into sugars. They may be further divided into three categories- alpha (α) amylase (EC 3.2.1.1), beta (β) amylase (EC 3.2.1.2) and gamma (γ) amylase (EC 3.2.1.3). All amylases are glycoside hydrolase and act on α-1,4-glycosidic linkage. Fungal amylases are the α-amylases. This enzyme is the calcium containing metalloenzyme having alternative name as glycogenase or 1,4-α-D-glucanohydrolase. This acts randomly on starch chain and break long chain saccharides into maltotriose and maltose from amylose or maltose and glucose from amylopectin. This enzyme works fast and contributes as the major digestive enzyme in animals. They are found in animals, plants, fungi (mainly ascomycetes and basidiomycetes) and bacteria (https://en.wikipedia.org/wiki/Amylase). This is also active in acidic pH and neutral pH. This also shows the good activity in mild alkaline conditions. This enzyme shows active applications in textiles, starch modification, fermentation, food industry, etc. (https://www.americanbiosystems.com/products/enzymees/fungal-amylase/).

CONCLUSION

It is cleared from the above discussions that fungi are omnipresent microorganisms with a lot of structural variations and physical appearances. They can exist in normal to very adverse conditions. They can cause various diseases and problems for human as well as for other living organisms; however, they are also very much useful for the human beings due to their strong applications in varieties of the fields like bioremediation, pharmaceutical applications, genetic engineering, paper and pulp industry, food industry, etc. They are also the rich sources of various types of nutrients. Their importance is also due to the secretion of various biotechnologically valuable extracellular enzymes like laccases, different peroxidases, cellulases, amylases, etc. which have already been discussed with their applications in concise form.

ACKNOWLEDGMENTS

Dr. PK Chaurasia is very much thankful to the Department of Chemistry, LS College, Muzaffarpur, Bihar (India) and Dr. SL Bharati is thankful to the Department of Chemistry, NERIST, Nirjuli, Arunachal Pradesh (India) for providing any facility during the work.

REFERENCES

Alkorta, I., Garbisu, C., Llama, M. J. & Serra, J. L. (1998). Industrial applications of pectic enzymes: a review. *Process Biochemistry*, *33*, 21-28.

Baldrian, P. (2006). Fungal laccases - occurrence and properties, *FEMS Microbiology Reviews*, *30*, 215-242.

Bao, W., Mally, D. M. O., Whetten, R. & Sederoff, R. R. (1973). A laccase associated with lignification of ioblolly pine xylem. *Science*, *260*, 672-674.

Beg, Q. K., Kapoor, M., Mahajan, L. & Hoondal, G. S. (2001). Microbial xylanases and their industrial applications: a review. *Applied Microbiology and Biotechnology*, *56*(3-4), 326–38.

Bharati, S. L. & Chaurasia, P. K. (Eds.) (2018). Research advancements in pharmaceutical, nutritional and industrial enzymology. IGI Global publisher, USA. ISBN: 978-1522552376 (hardcover) | ISBN: 9781522552383 (ebook). Pages: 1-549. Doi: DOI: 10.4018/978-1-5225-5237-6.

Carlos, R., Blanca, E., Garcia, A., Miguel, A. & Duarte, V. (2004). Biotechnological applications of peroxidases. *Phytochemistry Reviews*, *3*, 243-256

Chaurasia, P. K., Bharati, S. L. & Sarma, C. (2016) Laccases in pharmaceutical chemistry: A comprehensive appraisal. *Mini-reviews in Organic Chemistry*, *13*, 430-451. DOI:10-2174/1570193X13666161019124854.

Chaurasia, P. K., Bharati, S. L., Sharma, M., Singh, S. K., Yadav, R. S. S. & Yadava, S. (2015). Fungal laccases and their biotechnological significances in the current perspective: A review. *Current Organic Chemistry*, *19*(19), 1916-1934. DOI: 10.2174/1385272819666150629175237.

Chaurasia, P. K., Bharati, S. L. & Singh, S. K. (2013). Comparative studies on the blue and yellow laccases. *Research in Plant Sciences*, *1*(2), 32-37. DOI: 10.12691/plant-1-2-5.

Chaurasia, P. K., Yadav, R. S. S. & Yadava, S. (2013). A review on mechanism of laccase action, *Research and Reviews in Biosciences*, *7*(2), 66-71.

Chowdhary, P., More, N., Yadav, A. & Bharagava, R. N. (2019). Chapter 12-Ligninolytic Enzymes: An Introduction and Applications in the Food Industry. In: Kuddus, M. (Ed), Enzymes in Food Biotechnology: Production, Applications, and Future Prospects, https://doi.org/10.1016/B978-0-12-813280-7.00012-8.

Christensen, M. J., Bennett, R. J., Ansari, H. A., Koga, H., Johnson, R. D., Bryan, G. T., Simpson, W. R., Koolaard, J. P., Nickless, E. M. & Voisey, C. R. (2008). Epichloë endophytes grow by intercalary hyphal extension in elongating grass leaves. *Fungal Genetics and Biology*, *45*(2), 84-93. doi:10.1016/j.fgb.2007.07.013.

Daniels, K. J., Srikantha, T., Lockhart, S. R., Pujol, C. & Soll, D. R. (2006). Opaque cells signal white cells to form biofilms in Candida albicans. *The EMBO Journal*, *25*(10), 2240-52. doi:10.1038/sj.emboj.7601099.

El Dine, R. S., El Halawany, A. M., Ma, C. M. & Hattori, M. (2008). Anti-HIV-1 protease activity of lanostane triterpenes from the vietnamese mushroom Ganoderma colossum. *Journal of Natural Products*, *71*(6), 1022-6. doi:10.1021/np8001139.

el-Mekkawy, S., Meselhy, M. R., Nakamura, N., Tezuka, Y., Hattori, M., Kakiuchi, N., Shimotohno, K., Kawahata, T. & Otake, T. (1998). Anti-HIV-1 and anti-HIV-1-protease substances from *Ganoderma lucidum*. *Phytochemistry*, *49*(6), 1651-7. doi:10.1016/S0031-9422(98)00254-4.

Enguita, F. J. (2004). Substrate and dioxygen binding to the endospore coat laccase from *Bacillus subtilis. Journal of Biological Chemistry*, *279*, 23472-23476.

Essays, UK. (2018). Uses Of Peroxidases Enzymes. Retrieved from

Firenzuoli, F., Gori, L. & Lombardo, G. (2008). The medicinal mushroom *Agaricus blazei* Murrill: review of literature and pharmaco-toxicological problems. *Evidence-Based Complementary and Alternative Medicine*, *5*(1), 3-15. doi:10.1093/ecam/nem 007.

Fischer, R., Zekert, N. & Takeshita, N. (2008). Polarized growth in fungi--interplay between the cytoskeleton, positional markers and membrane domains. *Molecular Microbiology*, *68*(4), 813-26. doi:10.1111/j.1365-2958.2008.06193.x.

Fleming, D., Chahin, L. & Rumbaugh, K. (2017). Glycoside hydrolases degrade polymicrobial bacterial biofilms in wounds. *Antimicrobial Agents and Chemotherapy*, *61*(2), DOI: 10.1128/AAC.01998-16.

Fleming, D. & Rumbaugh, K. P. (2017). Approaches to dispersing medical biofilms. *Microorganisms, 5* (2), 15. doi: 10.3390/microorganisms5020015.

Gill, E. E. & Fast, N. M. (2006). Assessing the microsporidia-fungi relationship: Combined phylogenetic analysis of eight genes. *Gene*, *375*, 103-9. doi:10.1016/j.ge ne.2006.02.023.

Givaudan, A., Effose, A., Faure, D., Potier, P., Bouillant, M. L. & Bally, R. (1993). Polyphenol oxidase in *Azospirillum lipoferum* isolated from rice rhizosphere: Evidence for laccase activity in non-motile strains of *Azospirillum lipoferum. FEMS Microbiology Letters*, *108* (2), 205-210. https://doi.org/10.1111/j.1574-6968.1993.tb 06100.x.

Gummadi, S. N. & Panda, T. (2002). Purification and biochemical properties of microbial pectinases: a review. *Process Biochemistry*, *38*, 987-996.

Harris, S. D. (2008). Branching of fungal hyphae: regulation, mechanisms and comparison with other branching systems. *Mycologia*, *100*(6), 823-832. doi:10.3852/08-177.

Hetland, G., Johnson, E., Lyberg, T., Bernardshaw, S., Tryggestad, A. M., Grinde, B. (2008). Effects of the medicinal mushroom Agaricus blazei Murill on immunity, infection and cancer. Scandinavian *Journal of Immunology*, *68*(4), 363–70. doi:10.1111/j.1365-3083.2008.02156.x.

Hibbett, D. S., Binder, M., Bischoff, J. F., Blackwell, M., Cannon, P. F., Eriksson, O. E., et al. (2007). A higher-level phylogenetic classification of the Fungi. *Mycological Research*, *111* (Pt 5), 509–47. doi:10.1016/j.mycres.2007.03.004.

Hoegger, P. J., Kilaru, S., James, T. Y., Thacker, J. R. & Kuees, U. (2006). Phylogenetic comparison and classification of laccase and related multicopper oxidase protein sequences, *FEBS Journal*, *273*(10), 2308-2326. DOI:10.1111/j.1742-4658.200 6.0 5247.x.

Howard, R. J., Ferrari, M. A., Roach, D. H. & Money, N. P. (1991). Penetration of hard substrates by a fungus employing enormous turgor pressures. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(24), 11281–4. DOI:10.1073/pnas.88.24.11281.

https://commons.wikimedia.org/wiki/File:Fungus_in_a_Wood.JPG.

https://en.wikipedia.org/wiki/Arbuscular_mycorrhiza.

https://en.wikipedia.org/wiki/Cellulase.

https://en.wikipedia.org/wiki/Fungus.

https://en.wikipedia.org/wiki/Laccase.

https://en.wikipedia.org/wiki/Microsporidia.

https://en.wikipedia.org/wiki/Mycorrhiza.

https://en.wikipedia.org/wiki/Pectinase.

https://en.wikipedia.org/wiki/Peroxidase.

https://en.wikipedia.org/wiki/Xylanase.

https://www.britannica.com/science/fungus).

https://www.britannica.com/science/fungus/Importance-of-fungi.

https://www.ck12.org/biology/fungi-habitat/lesson/Habitats-of-Fungi-Advanced-BIO-ADV/.

https://www.ukessays.com/essays/biology/manufacture-and-commercial-uses-of-peroxidases-enzymes-biology-essay.php?vref=1.

Huang, H. W., Zoppellaro, G. & Sakurai, T. (1999). Spectroscopic and kinetic studies on the oxygen-centered radical formed during the four-electron reduction process of dioxygen by *Rhus vernicifera* laccase, *Journal of Biological Chemistry*, *274*(46), 32718-32724. DOI:10.1074/jbc.274.46.32718.

James, T. Y., Kauff, F., Schoch, C. L., Matheny, P. B., Hofstetter, V., Cox, C. J., et al. (2006). Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature*., *443*, 818–22. doi:10.1038/nature05110.

Kirk, P. M., Cannon, P. F., David, J. C. & Stalpers, J. (2001). Ainsworth and Bisby's Dictionary of the Fungi (9th ed.). Wallingford, UK: CAB International.

Kulp, K. (2000). Handbook of Cereal Science and Technology. CRC Press. ISBN 978-0-8247-8294-8.

Li, H. & Poulos, T. L. (1994). Structural variation in heme enzymes: a comparative analysis of peroxidase and P450 crystal structures. *Structure*, *2*(6), 461–464.

Lockhart, R. J., Van Dyke, M. I., Beadle, I. R., Humphreys, P. & McCarthy, A. J. (2006). Molecular biological detection of anaerobic gut fungi (Neocallimastigales) from landfill sites. *Applied and Environmental Microbiology*, *72*(8), 5659–61. doi:10.1128/AEM.01057-06.

Money, N. P. (1998). Mechanics of invasive fungal growth and the significance of turgor in plant infection. Molecular Genetics of Host-Specific Toxins in Plant Disease: Proceedings of the 3rd Tottori International Symposium on Host-Specific Toxins, Daisen, Tottori, Japan, August 24–29, 1997. Netherlands: Kluwer Academic Publishers. pp. 261–271. ISBN 978-0-7923-4981-5.

Money, N. P. (2002). Mushroom stem cells. *Bio Essays*, *24*(10), 949–52. doi:10.1002/bies.10160.

Moss, S. T. (1986). The Biology of marine fungi. Cambridge, UK: Cambridge University Press. p. 76. ISBN 978-0-521-30899-1.

Nelson, R. E., Fessler, L. I., Takagi, Y., Blumberg, B., Keene, D. R., Olson, P. F., Parker, C. G. & Fessler, J. H. (1994). Peroxidasin: a novel enzyme-matrix protein of Drosophila development. *EMBO Journal*, *13*(15), 3438–3447.

Nilsson, R. H., Kristiansson, E., Ryberg, M. & Larsson, K. H. (2005). Approaching the taxonomic affiliation of unidentified sequences in public databases- an example from the mycorrhizal fungi. *BMC Bioinformatics*, *6*, 178. DOI:10.1186/1471-2105-6-178

Parkinson, N., Smith, I., Weaver, R. & Edwards, J. P. (2001). A new form of arthropod phenol oxidase is abundant in venom of the parasitoid wasp *Pimpla hypochondriaca*. *Insect Biochemistry Molecular Biology*, *31*, 57-63. https://doi.org/10.1016/S0965-1748(00)00105-3.

Paterson, R. R. (2006). Ganoderma - a therapeutic fungal biofactory. *Phytochemistry*, *67*(18), 1985–2001. doi:10.1016/j.phytochem.2006.07.004

Paterson, R. R. (2008). Cordyceps: a traditional Chinese medicine and another fungal therapeutic biofactory?. *Phytochemistry*, *69*(7), 1469–95. doi:10.1016/j.phytochem.2008.01.027.

Peñalva, M. A. & Arst, H. N. (2002). Regulation of gene expression by ambient pH in filamentous fungi and yeasts. *Microbiology and Molecular Biology Reviews*, *66*(3), 426–46. doi:10.1128/MMBR.66.3.426-446.2002.

Piskur, J., Rozpedowska, E., Polakova, S., Merico, A. & Compagno, C. (2006). How did Saccharomyces evolve to become a good brewer? *Trends in Genetics*, *22*(4), 183–6. doi:10.1016/j.tig.2006.02.002.

Placido, J. & Capareda, S. (2015). Ligninolytic enzymes: a biotechnological alternative for bioethanol production. *Bioresources and Bioprocessing*, *2*, 1-12. DOI 10.1186/s40643-015-0049-5.

Polizeli, M. L., Rizzatti, A. C., Monti, R., Terenzi, H. F., Jorge, J. A. & Amorim, D. S. (2005). Xylanases from fungi: properties and industrial applications. *Applied Microbiology and Biotechnology*., *67*(5), 577–91. DOI:10.1007/s00253-005-1904-7.

Raghukumar, C. & Raghukumar, S. (1998). Barotolerance of fungi isolated from deep-sea sediments of the Indian Ocean. *Aquatic Microbial Ecology*, *15*(2), 153–163. doi:10.3354/ame015153.

Redecker, D. & Raab, P. (2006). Phylogeny of the glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia*, *98*(6), 885–95. doi:10.3852/mycologia.98.6.885.

Sanchez-Amat, A., Lucas-Elio, A. P., Fernandez, E., Garcia-Borron, J. C. & Solamo, F. (2001). Molecular cloning and functional characterization of a unique multipotent polyphenol oxidase from *Marinomonas mediterranea*. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, *1547*, 104-116. https://doi.org/10.1016/S0167-4838(01)00174-1.

Shet, A. R., Desai, S. V. & Achappa, S. (2018). Pectinolytic enzymes: classification, production and applications. *Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences*., *4*(3), 337-348. DOI: 10.26479/2018.0403.30.

Silar, P. (2016). Protistes Eucaryotes: Origine, Evolution et Biologie des Microbes Eucaryotes. HAL. p. 462. ISBN 978-2-9555841-0-1.

Thomas, B. R., Yonekura, M., Morgan, T. D., Czapla, T. H., Hopins, T. L. & Kramer, K. J. (1989). A trypsin solubilised laccase from pharate pupal integument of the tobacco hornworm, *Manduca sexta*. *Insect Biochemistry*, *19*, 611-622.

Vaupotic, T., Veranic, P., Jenoe, P. & Plemenitas, A. (2008). Mitochondrial mediation of environmental osmolytes discrimination during osmoadaptation in the extremely halotolerant black yeast Hortaea werneckii. *Fungal Genetics and Biology*, *45*(6), 994–1007. doi:10.1016/j.fgb.2008.01.006.

Whitaker, J. R. (1990). Microbial Pectinolytic enzymes. Microbial enzymes and biotechnology (2ndEdition). Elsevier Science Ltd, England, 1990, pp. 133-176.

Wong, D. W. S. (Ed.) (1995). Food Enzymes: Structure and Mechanism (1st Edn), Chapman and Hall, New York, pp 321-345.

Yoshida, H. (1883). LXIII.—Chemistry of lacquer (Urushi). Part I. Communication from the Chemical Society of Tokio. *Journal of Chemical Society*, *43*, 472-486.

Yuen, J. W. & Gohel, M. D. (2005). Anticancer effects of Ganoderma lucidum: a review of scientific evidence. *Nutrition and Cancer*, *53*(1), 11–7. doi:10.1207/s15327914nc5301_2. hdl:10397/26313.

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 2

SPECIES DIVERSITY AND DISTRIBUTION OF INDIGENOUS VESICULAR ARBUSCULAR MYCORRHIZAL FUNGI ISOLATED FROM DIFFERENT WHEAT CULTIVARS OF PRATAPGARH (UTTAR PRADESH) INDIA

O. P. Dwivedi*
Lab of Mycology and Plant Pathology, P. G. Department of Botany,
S. V. M. Science and Technology P. G. College,
Lalganj, Pratapgarh, (U.P.) India

ABSTRACT

A study was conducted to assess the species diversity of vesicular arbuscular mycorrhizal fungi in wheat cultivated area of Pratapgarh district (U.P.). Nine different wheat cultivars which were frequently grown in the study area were selected and examined for the VAM infection. All the wheat cultivars were found to be infected with vesicular arbuscular mycorrhizae. However, their indigenous population in rhizosphere and root infection varied to a considerable extent from species to species. Present study indicates the frequent occurrence of Acaulosporaceae with dominating of *Acaulospora* species and rare occurrence of *Entrophospora* species, in addition to members of Glomaceae and Gigasporaceae. However, members of Glomaceae recorded the highest species diversity at all the test sites. *Glomus mosseae* (LMSS), *Glomus fasciculatum* (LFSC) and *Glomus hoi* (LHOI) were found as most frequent (above 70%) VAM fungal species in all the four study sites while, *Glomus ambisporum* (LABS) with above 70% frequency occurred in almost all the sites excepting site IV.

* Corresponding Author's Email: dropd@rediffmail.com.

Keywords: vesicular arbuscular mycorrhizal fungi, wheat cultivar, species diversity

INTRODUCTION

The diversity, distribution, abundance and life history of many plants can easily be observed without any specialized or sophisticated techniques. In addition the taxonomy of many plant groups is well established to the species level. This information form a sound basis from which it is easier to pursue experimental studies of their ecology, evolution and role within natural ecosystem. In the case of some organisms, particularly those below ground, the difficulty of the task of discussing their taxonomy, distribution, abundance and life history hinders meaningful experimental investigations of their role in natural ecosystem. One such group of organisms is the zygomycete fungi of the order Glomales, which are commonly known as VAM fungi. Most species of plants are capable to associating with VAM fungi. VAM fungi are extremely successful fungi that form mutualistic symbioses. This symbiotic association of endomycorrhizal fungi and roots of plants are beneficial with the uptake of nutrients between the symbionts and often improves the plant productivity. The main advantage of mycorrhizae to the host plants are increased efficiency of mycorrhizal roots versus non−mycorrhizal roots caused by the active uptake and transport of nutrients especially immobile minerals like P, Zn and Cu (Phiri et al., 2003; Jamal et al., 2002). The AM fungal symbiosis with the roots of plants is beneficial to both partners (Harley and Smith, 1983). The Glomalean fungi associated with the majority of the land plant species are found worldwide in virtually all habitats. Arbuscular mycorrhizas are ubiquitous with approximately ninety-five percent of terrestrial plant species. They form large multinucleate spores in the soil, but growth of hyphal stage is dependent on the association with plant roots. Taxonomists have described three families, six genera and about more than 150 species, most of which are in the genus *Glomus*. This taxonomy is based on morphological characters and especially on features of the spores. A single morphospecies e.g., *Glomus mosseae* may however, has a very wide distribution both ecologically and geographically (Dwivedi, 2013).

The study of VAM fungi has been impeded by their obligate biotrophic nature and by difficulties in the identification of spores, especially in the field material. In this situation, molecular techniques have enormous potential and there have been a number of important resent advances. Both mitochondrial DNA and the multicopy nuclear ribosomal RNA (r-RNA) gene have been used for species identification and phylogenetic analysis in fungi (Bruns et al. 1991). In mycorrhizal symbiosis formed between plant roots and the arbuscular mycorrhizal (AM) fungi, the Glomales is of great interest to botanists because of its potential influence on ecosystem processes, its role in determining plant diversity in natural communities and the ability of these fungi to induce a wide variety of growth responses in coexisting plant species. Little attention, however,

has been paid to the ecological role of diversity of AM fungi. AM fungi have been shown to play a significant role in the floristic diversity and structure of annual and perennial plant communities. However, in most of these ecological investigations, little or no attention has been paid to diversity of AM fungi themselves. This is because experiments conducted in pots have indicated that AM fungi show little host specificity. Consequently it was thought that few selection pressures that it favors extensive divergence arise in mutualistic symbiosis.

The lack of basic information about the VAM fungi stems from a few fundamental difficulties encountered in their identification, culture and taxonomy, coupled with the great difficulty in manipulating AM fungi in natural ecosystem without greatly modifying their environment in other ways. Some of the problems of identification and possibly of taxonomy can be using various techniques. In this investigation we outline briefly as to why the study of VAM fungal diversity warrants greater attention. Understanding the real, rather than the potential significance of VAM fungal diversity in natural communities has posed the greater problem. For a given plant community, we need to know how diverse the VAM fungal community is, which plant roots are colonized by which VAM fungi, whether, there is seasonality in the pattern of colonization, whether any specificity between plant and VAM fungi occurs and what the effect of those VAM fungi will be on plant and the ecosystem. However, the difficulty of identifying VAM fungi in the roots of plants has always been an obstacle to their study in natural communities though the identification of VAM fungi in roots based on morphological observation has been successful in pot experiment, the usefulness of this technique for field investigations appears to be limited because the hyphal morphology of one VAM fungal isolate is likely to vary with host species.

Our interest in diversity of VAM fungi arose from considerations of differences in mycorrhizal efficiency i.e., the variation in nutritional benefits and costs to the plant that are associated with differences in amounts of inorganic nutrients such as P or Zn transferred to the plant in return to organic carbon e.g., Sugar transferred to the fungus. Previously, it can be widely assumed that bi-directional transfer of inorganic nutrients and organic carbon occurs solely across arbuscules. Indeed in their taxonomic revision of the Glomales, Morton and Benny (1990) emphasized on this assumedly unique role of the arbuscules as a fundamental feature of the order.

Diversification of VAM Fungi

The VAM symbiosis is ancient; the fossil records suggest that it occurred in the first land plants. Morphological diversification is low, with only 152 recognized species in six genera (Walker and Trappe, 1993). Law (1985) pointed out that considering the age of the symbiosis this diversification is low, and he suggested that this is typical of

mutualistic symbiosis, as there are few external pressures which would favor the selection of new traits. However, the low divergence of VAM fungi seems at odds with their functional diversity. Information regarding the genetic diversity of VAM fungi can also be obtained using molecular techniques and the results of such investigations can and have helped to construct a phylogenetic classification of VAM fungi (Simon et al. 1993).

Until recently, methods for studying VAM fungal diversity and the ecology of the symbiosis have been wholly reliant on the morphology of the spore phase. Spore of VAM fungi are relatively large, easy to extract from soil and have several morphological characters that allow species identification to be determined by experienced personnel. However, the use of spore data alone for the assessment of ecosystem diversity and ecology has long been recognized as unsatisfactory. The relationship between the morphological diversity of VAM fungi and their genetic and functional diversity has not been established. According to Walker (1992) many described taxa are workable in that they are recognizable and are found repeatedly in different part of the world, albeit in diverse environments. For example, several species of VAM fungi which were reported in a semi-arid site in Australia (Mc Gee, 1989) also occurred in a species rich meadow in the north of England (Sanderes, 1993), both ecosystem comprising completely different soils, vegetation and climate. This indicates that VAM fungi are very plastic in their environmental adaptability. Wheat (*Triticum aestivum* L.) is the world's third produced cereal after maize and rice. It is estimated that the production of this cereal will increase because of the growing needs of the human population for food. To meet this demand, and because of the limited availability of uncultivated land agriculture faces a problem to intensive grain production. Strategies such as the use of more environmentally friendly alternatives, natural processes, and environmental conservation play a vital role in current agricultural production. A large number of microorganisms exert positive effects on the growth and development of plants in the rhizosphere region, and are involved in various activities, including dynamic resources availability to plants and preservation of soil fertility (Priyadharsini & Muthukumar, 2015). Therefore the present study was undertaken to find out the biodiversity of VAM fungi, which is naturally occurred in the rhizosphere soils of wheat at four different study sites of this region.

MATERIALS AND METHODS

The present study represents an attempt to establish the qualitative and quantitative distribution of VAM fungal species in rhizosphere soils of wheat. Study was taken up in four different sites e.g., I- Kunda, II- Lalganj, III- Sadar and IV- Raniganj tahseel of Pratapgarh, (U.P.) during November 20014 to October 2017. The soil properties of study sites were also analyzed. The rhizosphere soil samples from 10-20 cm. depths were

collected from nine cultivars i.e., PBW- 343, PBW- 373, PBW- 502, PBW- 443, PBW- 154, UP- 2338, PBW- 17, HD- 2967 and HD- 2338 of wheat plants.

Isolation and Identification of VAM Spores

The rhizosphere soil samples from 10-20 cm depths were collected from nine cultivars i.e., PBW- 343, PBW- 373, PBW- 502, PBW- 443, PBW- 154, UP- 2338, PBW- 17, HD- 2967 and HD- 2338 of wheat plants. The soil samples were collected then processed by wet sieving and decanting techniques of Gerdemann and Nicolson (1963) to isolate VAM spores. Spores were flushed in to petridishes and counted under a steriozoom dissecting microscope. Only healthy spores were counted. Spores were mounted in polyvinyl alcohol-lactoglycerol (PVLG), examined for their various morphological characters and identified with the help of key provided by Schenck and Perez (1987), Mehrotra (1997), Mehrotra and Baijal (1994), Hall and Fish (1979) and species code of VAM fungi were followed after Perez and Schenck (1990).

Frequency Distribution of VAM Fungi

Frequency of different species of VAM fungi was calculated at four different test sites on the basis of their occurrence with selected wheat cultivars.

$$\%F = \frac{No.\,of\ host\ plants\ having\ VAM\ species}{Total\ No.\,of\ host\ plant\ examined} \times 100$$

RESULTS

Identification and Morphological Description of VAM Species

In the present study, 64 VAM species were isolated and identified from the rhizosphere soil of nine different wheat cultivars from four different study sites of Pratapgarh district (U.P.). The fundamental problem in studies of the distribution of VAM fungi lies in identifying the fungi, because the VAM fungi can neither, be cultured in the absence of living root nor isolated on agar plates by standard microbiological techniques. The taxonomy of VAM, which form VAM, has not been completely clarified. It relies almost entirely on spore morphology, which may change with spore age. Another difficulty in determining the distribution of VAM fungi is that spores of all species are

easy to recover from soil, because they are either very small or are non-randomly dispersed in dense sporocarps of small spores. In present study both qualitative and quantitative morphological characters have been considered for description and identification of VAM fungi. The possible identification was done up to species level.

Frequency and Diversity of VAM Fungi

Population of VAM fungi from four different study sites represented 64 different species in the rhizosphere soils of nine selected wheat cultivars and these belongs to six genera viz. *Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora*.

The genus *Glomus* was most dominant VAM fungi constituted up to 62.50% of the total 64 isolates followed by *Acaulospora* (21.87%) then *Scutellospora* (6.25%), *Sclerocystis* (4.68%), *Gigaspora* (3.12%) and least was *Entrophospora* (1.56%). *Glomus mosseae* (LMSS), *Glomus fasciculatum* (LFSC) and *Glomus hoi* (LHOI) were found as most frequent (above 70%) VAM fungal species in all the four study sites while, *Glomus ambisporum* (LABS) with above 70% frequency occurred in almost all the sites excepting site IV (Figure 1).

In site I, 62 species (96.87%) occurred out of total isolates (64 species) of VAM fungi. The highest percentage of VAM fungi occurred in genus *Glomus* (61.29%) followed by *Acaulospora* (22.58%) then *Scutellospora* (6.45%), *Sclerocystis* (4.83%), *Gigaspora* (3.22%) and lowest percentage occurred in *Entrophospora* (1.61%) (Table 1, Figure 2). The most frequent VAM fungal species (above 70% frequency) occurred with rhizosphere soils of different selected wheat cultivar was *Glomus mosseae* (LMSS) (92.3%); *Glomus fasciculatum* (LFSC), *Glomus hoi* (LHOI) (84.61%); *Glomus ambisporum* (LABS) and *Scutellospora pellucida* (CPLC) (76.92%), while *Glomus pubescens* (LPBS) (7.69%) was least frequent at the site I (Table 2).

Table 1. Diversity of VAM fungi from total (64) isolated spp. in rhizosphere soils of wheat cultivars at different test sites

Study sites	Occurrence of VAMF from total (64) isolated species	Frequency of VAMF (%)						
		From total isolated spp.	*Glomus spp.*	*Acaulospora spp.*	*Scutellospora spp.*	*Sclerocystis spp.*	*Gigaspora spp.*	*Entrophospora spp.*
I	62	96.87	61.29	22.58	6.45	4.83	3.22	1.61
II	58	90.62	60.34	24.13	6.89	3.44	3.44	1.72
III	60	93.75	60.00	23.33	6.66	5.00	3.33	1.66
IV	59	92.18	59.32	23.72	6.77	5.08	3.38	1.69

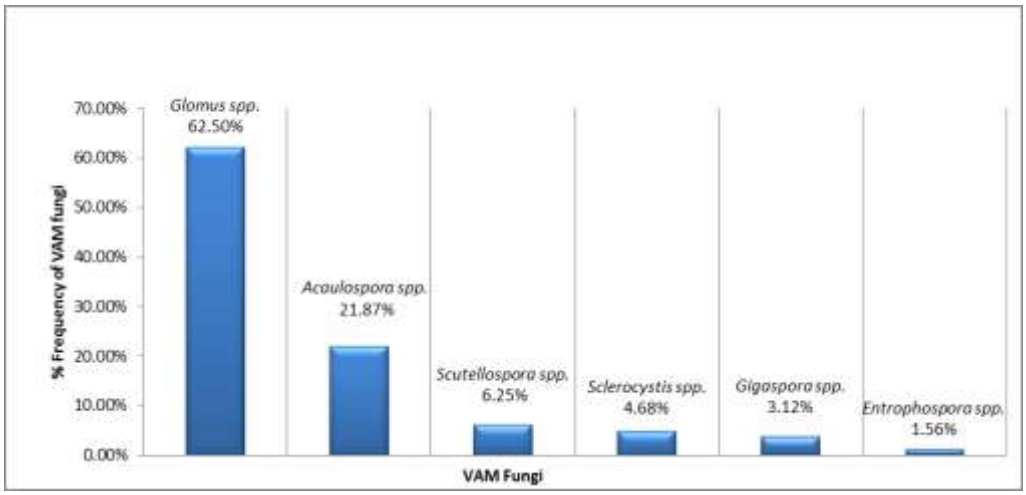


Figure 1. Composition of different VAM Fungal Spores from Total (64) Isolated Species.

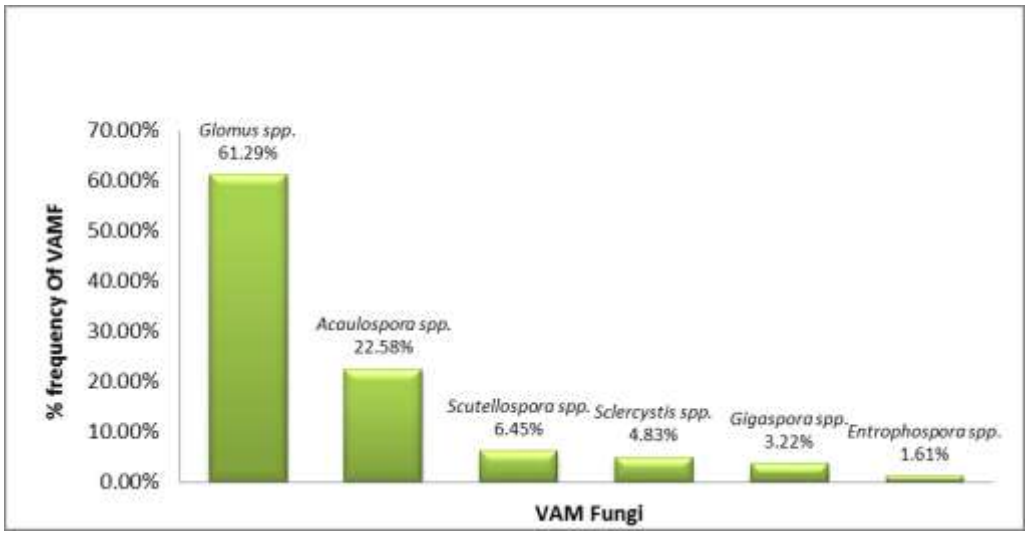


Figure 2. Composition of Different VAMF at site I.

In site II, out of 64 isolates, 58 species of VAM fungi (90.62%) were recorded with the rhizosphere soils of selected wheat cultivar. The highest percentage of VAM fungi occurred in *Glomus* (60.34%) followed by *Acaulospora* (24.13%) then *Scutellospora* (6.89%), *Gigaspora*, *Sclerocystis* (3.44%), and lowest in *Entrophospora* (1.72%) (Table 1, Figure 3). Result shows that the most frequent VAM fungal species (above 70% frequency) was *Glomus fasciculatum* (LFSC) (92.3%), *Glomus ambisporum* (LABS) (84.61%); *Gigaspora albida* (GABD), *Glomus geosporum* (LGSP), *Glomus hoi* (LHOI), *Glomus mosseae* (LMSS) and *Scutellospora calospora* (CCLS) (76.92%). However, *Glomus fulvum* (LFLV) (7.69%) showed least frequency with the rhizosphere soils of selected wheat cultivar (Table 2).

Table 2. Frequency of VAM fungal species in rhizosphere soils of selected wheat cultivars at different study sites

S. No.	VAM fungal species (with species code)	Frequency (%)			
		Study Sites			
		I	II	III	IV
1.	Acaulospora delicate (ADLC)	69.23	46.15	36.36	27.27
2.	Acaulospora denticulate (ADTC)	30.76	30.76	45.45	45.45
3.	Acaulospora dilatata (ADLT)	53.84	46.15	45.45	36.36
4.	Acaulospora foveata (AFVT)	23.07	38.46	27.27	18.18
5.	Acaulospora gerdemannii (AGDM)	61.53	69.23	63.63	63.63
6.	Acaulospora lacunose (ALCN)	69.23	61.53	54.54	36.36
7.	Acaulospora laevis (ALVS)	53.84	46.15	63.63	54.54
8.	Acaulospora mellea (AMLL)	61.53	54.15	63.63	63.63
9.	Acaulospora nicolsonii (ANCS)	38.46	38.46	27.27	36.36
10.	Acaulospora rehmii (ARHM)	46.15	53.84	45.45	36.36
11.	Acaulospora scrobiculata (ASCB)	69.23	61.53	63.63	45.45
12.	Acaulospora spinosa (ASPN)	53.84	30.76	27.27	18.18
13.	Acaulospora sporocarpia (ASPC)	46.15	38.46	36.36	36.36
14.	Acaulospora trappei (ATRP)	46.15	38.46	45.45	45.45
15.	Entrophospora infrequens (EIFQ)	30.76	46.15	36.36	45.45
16.	Gigaspora albida (GABD)	69.23	76.92	72.72	63.63
17.	Gigaspora rosea (GRSA)	30.76	23.07	18.18	9.09
18.	Glomus albidum (LABD)	23.07	38.46	54.54	54.54
19.	Glomus ambisporum (LABS)	76.92	84.61	72.72	63.63
20.	Glomus australe (LAST)	23.07	15.38	-	36.36
21.	Glomus citricolum (LCTC)	46.15	53.84	54.54	63.63
22.	Glomus claroideum (LCRD)	69.23	61.53	54.54	72.72
23.	Glomus clarum (LCLR)	38.46	38.46	27.27	18.18
24.	Glomus delhiense (LDLH)	23.07	23.07	18.18	27.27
25.	Glomus deserticola (LDST)	46.15	46.15	45.45	36.36
26.	Glomus diaphanum (LDPH)	30.76	30.76	36.36	27.27
27.	Glomus dimorphicum (LDMR)	46.15	38.46	63.63	63.63
28.	Glomus etunicatum (LETC)	30.76	46.15	45.45	36.36
29.	Glomus fasciculatum (LFSC)	48.61	92.30	90.90	81.81
30.	Glomus fecundisporum (LFCS)	69.23	61.53	45.45	45.45
31.	Glomus flavisporum (LFLS)	-	46.15	45.45	45.45
32.	Glomus fulvum (LFLV)	23.07	7.69	18.18	-
33.	Glomus geosporum (LGSP)	84.61	76.92	72.72	54.54
34.	Glomus gerdemannii (LGDM)	46.15	38.46	36.36	63.63
35.	Glomus globiferum (LGBF)	15.38	23.07	-	9.09
36.	Glomus heterosporum (LHTS)	69.23	53.84	54.54	45.45
37.	Glomus hoi (LHOI)	84.61	76.92	72.72	72.72
38.	Glomus intraradices (LINR)	30.76	15.38	-	27.27
39.	Glomus invermaium (LIVM)	15.38	23.07	9.09	-
40.	Glomus lacteum (LLCT)	23.07	-	18.18	27.27
41.	Glomus leptotichum (LLPT)	38.46	30.76	45.45	45.45
42.	Glomus macrocarpum (LMCC)	61.53	46.15	54.54	54.54
43.	Glomus maculosum (LMCL)	53.84	53.84	63.63	45.45

S. No.	VAM fungal species (with species code)	Frequency (%)			
		Study Sites			
		I	II	III	IV
44.	Glomus magnicaule (LMGC)	38.46	38.46	27.27	27.27
45.	Glomus melanosporum (LMLS)	30.76	30.76	36.36	36.36
46.	Glomus monosporum (LMNS)	23.07	-	27.27	45.45
47.	Glomus mosseae (LMSS)	92.30	76.92	81.81	72.72
48.	Glomus multicaule (LMTC)	38.46	46.15	45.45	45.45
49.	Glomus multisubstensum (LMST)	-	38.46	36.36	-
50.	Glomus occultum (LOCT)	23.07	30.76	36.36	-
51.	Glomus pallidum (LPLD)	23.07	-	27.27	45.45
52.	Glomus pubescens (LPBS)	69.23	38.46	-	27.27
53.	Glomus pustulatum (LPST)	23.07	38.46	54.54	63.63
54.	Glomus radiatum (LRDT)	30.76	23.07	27.27	-
55.	Glomus reticulatum (LRTC)	23.07	15.38	18.18	18.18
56.	Glomus scintillans (LSTL)	23.07	-	9.09	18.18
57.	Glomus versiforme (LVSF)	46.15	-	36.36	45.45
58.	Sclerocystis pachycaulis (SPCC)	61.53	46.15	63.63	63.63
59.	Sclerocystis pakistanica (SPKS)	23.07	-	9.09	9.09
60.	Sclerocystis rubiformis (SRBF)	38.46	38.46	27.27	27.27
61.	Scutellospora calospora (CCLS)	61.53	76.92	63.63	54.54
62.	Scutellospora fulgida (CFLG)	38.46	46.15	54.54	45.45
63.	Scutellospora minuta (CMNT)	69.23	53.84	63.63	72.72
64.	Scutellospora pellucida (CPLC)	76.92	46.15	45.45	45.45

(- Absent).

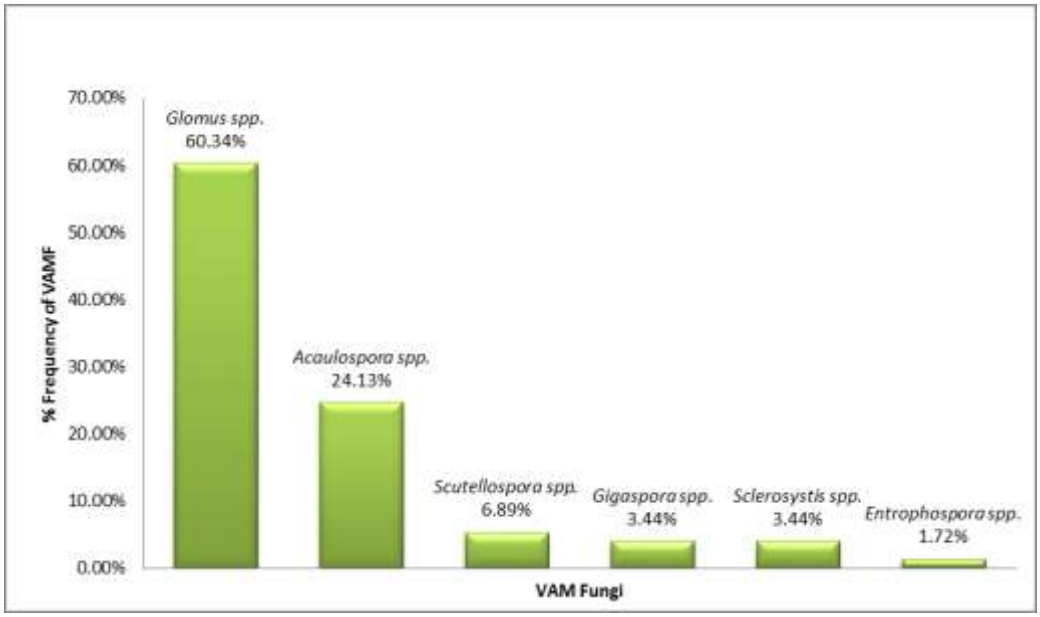


Figure 3. Composition of Different VAMF at site II.

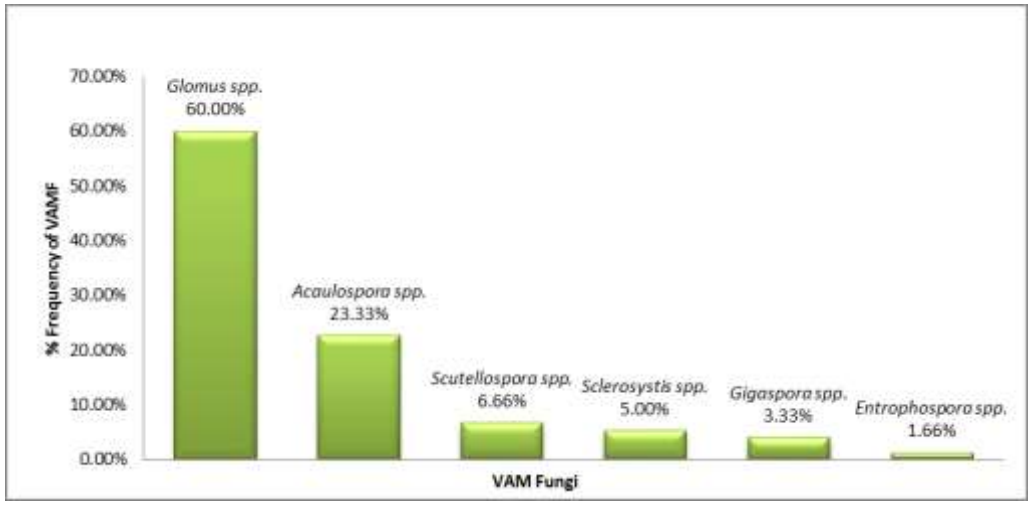


Figure 4. Composition of different VAMF at site III.

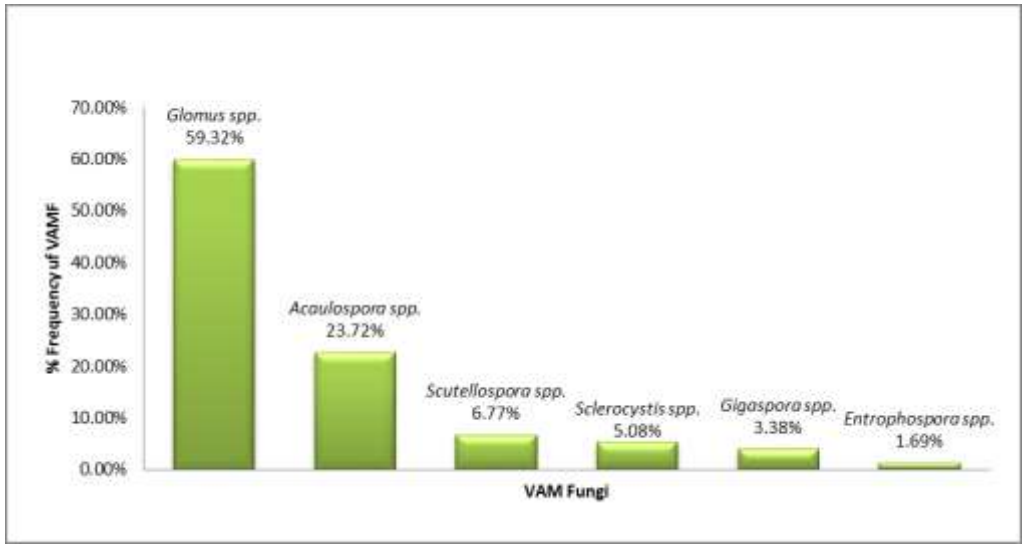


Figure 5. Composition of VAMF at site IV.

In site III, 60 species (93.75%) occurred out of 64 isolates of VAM fungi; in which the highest percent of VAM fungal species occurred in *Glomus* (60.00%) followed by *Acaulospora* (23.33%) then *Scutellospora* (6.66%), *Sclerocystis* (5.00%), *Gigaspora* (3.33%) and least was observed with *Entrophospora* (1.66%) (Table 1, Figure 4). The most frequent VAM fungal species (above 70% frequency) were recorded as *Glomus fasciculatum* (LFSC) (90.9%), *Glomus mosseae* (LMSS) (81.81%), *Gigaspora albida* (GABD), *Glomus geosporum* (LGSP) and *Glomus hoi* (LHOI) (72.72%), while *Glomus invermaium* (LIVM), *Glomus scintillans* (LSTL) and *Sclerocystis pakistanica* (SPKS) (9.09%) showed little frequency with all the selected wheat cultivars (Table 2).

Among all the sites, site IV had 59 (92.18%) different VAM fungal species. The highest VAM fungal species occurred with *Glomus* (59.32%) followed by *Acaulospora* (23.72%) then *Scutellospora* (6.77%), *Sclerocystis* (5.08%), *Gigaspora* (3.38%) and lowest percent of species was recorded with *Entrophospora* (1.69%) (Table 1, Figure 5). In this site the most frequent VAM fungal species (above 70% frequency) were recorded as *Glomus fasciculatum* (LFSC) (81.81%), *Glomus claroideum* (LCRD), *Glomus hoi* (LHOI), *Glomus mosseae* (LMSS) and *Scutellospora minuta* (CMNT) (72.72%). The VAM fungal species *Gigaspora rosea* (GRSA), *Glomus globiferum* (LGBF) and *Sclerocystis pakistanica* (SPKS) (9.01%) showed poor frequency with all the selected wheat cultivars (Table 2).

CONCLUSION

The VAM fungi belong to a very old category of Zygomycetes and have been recently regrouped in a single order, the Glomales (Morton and Benny, 1990), which include all species capable of living in symbiosis with plant. The bulk of known species belong to the family Glomaceae, which include the genera *Glomus* and *Sclerocystis.* The arbuscular mycorrhizal fungi consist actually of approximately 160 species belonging to three family and six genera and have a worldwide distribution. The bulk of known species have been described over the last two decades, which indicates the increased interest in these organisms and also the difficulty inherent in their taxonomic treatment. In fact, the greatest difficulty is that the entire taxonomy of these organisms is currently based on the morphological characters of the spores. They are single-celled structures, of generally globoid shape, with thick walls made up of several layers of different textures, connected to the filamentous network by a suspensor hypha of varied morphology. Since the morphological characters are reduced and often variable depending on the maturity of the spores studied, ultrastructural studies serve to support observations made previously by optic microscopy.

Arbuscular mycorrhizal (AM) fungi are ubiquitous in the terrestrial habitats invading over 80% of the land plants. The structural and functional aspects of this association, as evident from fossil records, appear to be quite concerned through time (Harley and Smith, 1983). This benevolent relationship has not only helped the emergence of first land plants but also supported further successional establishment in widely diversified environments, viz. agricultural soils, mine soils, coal wastes, alkaline soils, desert soils and other habitats. The colonization of VAM fungi varies greatly with various factors such as soil fertility, soil type, species of mycorrhizal fungi and plant cultivars. Before the significance of Mycorrhizal fungi play a significant role in growth and yield of plant (Dwivedi, 2004). Vesicular arbuscular mycorrhizal (VAM) fungi interact at the root–soil interface in a coordinated manner. Their hyphae absorb the water and nutrients from soil

and translocate to plant to increase its growth and development (Priyadharsini & Muthukumar, 2015; Dwivedi, 2015).

The generic diversity of AM fungi in four sites of Pratapgarh district (U.P.) studied here appears to be more elaborated than those reported from any other part or any other types of soils, where species are restricted to Glomaceae and Gigasporaceae, with species in Glomaceae dominating over others. In contrast, this study indicates the frequent occurrence of Acaulosporaceae with dominating of *Acaulospora* species and rare occurrence of *Entrophospora* species, in addition to members of Glomaceae and Gigasporaceae. However, members of Glomaceae recorded the highest species diversity at all the test sites.

As such on the basis of results of present study it may be concluded that AM fungi are not host (different cultivars of wheat) specific rather they formed symbiotic association with any of the cultivars grown here. Though any change in environmental conditions directly influence the morphology of VAM fungi resulting appearance of new species.

REFERENCES

Bruns, T. D., White, T. J. and Taylor, J. W. (1991). Fungal molecules systematic. *Anu. Review Ecol. Systematics*, 22, 525-564.

Dwivedi, O. P. (2004). Effect of VA-Mycorrhiza on Winter Wheat Genotype C-306. *J. of Mycol. and Pt. Pathol*, 34 (2), 623-625.

Dwivedi, O. P. (2013). Distribution of Arbuscular-Mycorrhizal Fungi in Different Cultivars of Wheat. *Indian Phytopathology*, 66, 220-223.

Dwivedi, O. P. (2015). Distribution and Association of Arbuscular Mycorrhizal Fungi in Different Cultivars of Wheat from Lalganj Pratapgarh District of Utter Pradesh, India. *Advances in Bioscience and Biotechnology*, 6, 353- 357. http://dx.doi.org/10.4236/abb.2015.65034.

Gerdemann, J. W. and Nicolson, T. N. (1963). Spores of mycorrhizal *Endogone* sp. extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.*, 46, 235-244.

Harley, J. L. and Smith, S. E. (1983). *Mycorrhizal symbiosis*. Academic Press. London. New York, 483.

Hall, I. R. and Fish, B. J. (1979). A Key to the Endogonaceae. *Trans. Br. Mycol. Soc.*, 73, 261-270.

Jamal, A., N. Ayub, M. Usman and A. G. Khan. (2002). AM Fungi enhance Zinc and Nickle uptake from contaminated soil by soyabean and lentil. *Int. J. Phytoremed.*, 4(3), 203-221.

Law, R. (1985). Evolution in a mutualistic environment. In: *The biology of mutualism: Ecology and evolution*. (Ed.), Boucher D. H. London: Croon Helm, 145-170.

Mc Gee, P. A. (1989). Variation in propagule number of vesicular – arbuscular mycorrhizal fungi in a semi-arid soil. *Mycological Res.*, 92, 28-33.

Mehrotra, V. S. (1997). Problem associated with morphological taxonomy of AM fungi. *Mycorrhiza News*, 9, 1-10.

Mehrotra, V. S. and Baijal, U. (1994). Advances in the taxonomy of vesicular- arbuscular mycorrhizal fungi. *Biotechnology in India*, 227-286.

Morton, J. B. and Benny, G. L. (1990). Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): New orders, Glomales, tow new suborders, Glomineae and Gigasporineae and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. *Mycotaxon*, 37, 471-491.

Perez, Y. and Schenck, N.C. (1990). A Unique code for each species of VA- mycorrhizal fungi. *Mycologia*. 82, 256-260.

Phiri, S., I. M. Rao, E. Barrios, B. R. Singh. (2003). Mycorrhizal association, Nutrient uptake and phosphorus Dynamics in a volcanic-ash. Soil in Colombia as affected by the establishment of Tithonia diverssifolia. *J. Sustainable Agriculture*, 21(3), 41- 59.

Priyadharsini, P., & Muthukumar, T. (2015). Insight into the role of arbuscular mycorrhizal fungi in sustainable agriculture. In: P. Thangavel & G. Sridevi (Eds), *Environmental sustainability: Role of green technologies* (pp. 3-37). New Delhi: Springer. http://dx.doi.org/10.1007/978-81-322-2056-5_1.

Sanderes, I. R. (1993). Temporal infectivity and specificity of vesicular – arbuscular mycorrhizas in co-existing grassland species. *Oecologia*. 93, 389-355.

Schenck, N. C. and Perez, Y. (1987). *Manual for identification of VAM fungi.* Synergistic Pub. Gainesville. Fl., U.S.A.

Simon, L., Bousquet, J., Levesque, C. and Lalone, M. (1993). Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. *Nature*. 363, 67-69.

Walker, C. (1992). Systemics and taxonomy of the arbuscular endomycorrhizal fungi (Glomales) - a possible way forward. *Agronomic*. 12, 887-897.

Walker, C. and Trappe, J. M. (1993). Names and epithets in the Glomales and Endogonales. *Mycological Res*. 97, 339-344.

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 3

ISOLATION, IDENTIFICATION AND STUDYING THE POTENTIAL APPLICATION OF TROPICAL FUNGI IN LIGNOCELLULOLYSIS

S. K. Jayasekara, M. Kathirgamanathan and R. R. Ratnayake*
Bioenergy and Soil Ecosystems Research Project,
National Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka

ABSTRACT

Biodiversity rich tropical countries like Sri Lanka have an enormous number of microorganisms including bacteria and fungi in their natural environments. The microbial diversity is a consequence of unique climatic conditions which exists in the area. The microbial entities partake in numerous biogeochemical reactions in this tropical environment. Lignocellulolysis is one such process which degrades plant biomass. Both bacteria and fungi participate in this lignocellulolytic reaction by producing an array of protein-based enzymes. Especially, fungi are reported to be far more efficient in producing extracellular lignocellulolytic enzymes including cellulases, hemicellulases and ligninases in order to hydrolyze cellulose, hemicellulose and lignin in plant debris respectively. Exploring the natural environment for these fungi is important because their enzymes have attracted a wide range of industrial applications where lignocellulosic biomass could be utilized as an inexpensive raw material. For instance, biofuel production could be emphasized. The objective of this chapter is to discuss the outcomes of a research conducted to isolate fungi from natural environment of different areas of central Sri Lanka, identify them and study their lignocellulolytic enzyme production potential. Moreover, the chapter includes descriptive information about the research methodology, results obtained and a discussion of the lignocellulolytic enzyme production potential of several filamentous as well as basidiomycetes fungal isolates included in genera *Trichoderma* sp., *Aspergillus* sp., *Penicillium* sp., *Talaromyces* sp.,

* Corresponding Author's Email: Renuka.ra@nifs.ac.lk.

and *Earliella* etc. based on their enzyme activities such as total cellulase, xylanase as well as laccase.

Keywords: lignocellulolysis, lignocellulolytic enzymes, cellulolytic fungi, tropical lignocellulolytic fungi, total cellulase activity, xylanase activity, laccase activity

INTRODUCTION

Fungi are a group of diverse eukaryotic microorganisms that play a pivotal role in balancing the environment. Although usually found in moist, dark and cool environments with a supply of decaying organic matter, they surprisingly colonize and easily survive in diverse habitats in the world. Some of them are unbelievably extreme environments such as hot springs, deserts, areas with high salinity, deep sea vents, spots emitting ionizing radiation and ocean hydrothermal areas (Kamburaet al., 2016). For instance, a type of fungus that lives in the hot springs is called gasteromycetes. Very few are also restricted to the arctic areas where the environment is unbearably cold. According to e-learning portal for arctic biology, fungi which grow on arctic seashores, e.g., *Arrhenia salina*, are ecologically bound to that arctic zone. In general, they can even make their home in decaying logs or fallen branches, nooks and corners of home gardens and biologically diverse tropical regions. Most of the fungi grow in terrestrial environments, though several species live partly or solely in aquatic habitats, such as the chytrid fungus *Batrachochytrium dendrobatidis*, a parasite that has been responsible for a worldwide decline in amphibian populations (Brem and Lips, 2008).

A description about fungi is incomplete without an account of their importance to the world. This is explained by tremendous roles played by them. Secretion of hydrolyzing enzymes as well as oxidizing enzymes for degradation of lignocellulosic biomass is one of the most crucial processes carried out by fungi. Lignocellulosic biomass is an abundant complex polysaccharide present on earth. Its application as a raw material in many industrial processes has become feasible due to enzymatic activities of microorganisms including fungi. For instance, biofuel production using plant cell wall polysaccharides could be mentioned. Biofuel is an attractive ecofriendly alternative to fossil fuels. However, in order to convert the complex polysaccharides in plant materials into fuel, it is essential to have a group of efficient microorganisms with the potential to degrade cellulose, hemicellulose and lignin which form lignocellulosic biomass into fermentable sugars. Lignin degrading enzymes *viz*; laccase, manganese peroxidase (MnP), lignin peroxidase (LiP) are oxidizing enzymes which carryout breaking down of lignin component in plant biomass (Pollegioni, Tonin and Rosini 2015). Prior lignin degradation which is called pretreatment allows easy access of hydrolytic enzymes into cellulose and hemicellulose components in the plant biomass. The hydrolytic enzymes

mainly required for the saccharification of plant polysaccharides are cellulases and xylanase (Bischof, Ramoni and Seiboth 2016). Therefore the pretreatment and saccharification are two important processes carried out by lignocellulolytic enzymes secreted by microorganisms.

The mechanisms behind the hydrolysis of lignocellulosic biomass by lignocellulolytic enzymes have been explained. Lignin is an aromatic, complex heteropolymer of phenyl-propanoid units (aromatic alcohols) which is found in plant cell walls. This polymer is playing a major role as it confers structural rigidity to woody plant tissues while protecting them from possible microbial attack (Higuchi, 1990). As previously mentioned, to depolymerize lignin, extracellular enzyme systems including laccases, peroxidases and oxidases producing H_2O_2 are required. The enzymatic composition of the ligninolytic system depends on the fungal species, with laccase being the common component (Hatakka, 1994). Laccase (EC 1.10.3.2) is an enzyme which contains a copper atom in it which is essential for its biological activity. These enzymes are called cuproenzymes. Laccase enzyme oxidizes various types of phenols and similar aromatic compounds, aromatic amines with the reduction of molecular oxygen to water. The lignin, as it is an aromatic complex will be oxidized by laccase.

Cellulases are the enzymes that hydrolyze β -1, 4 linkages in cellulose chains. In nature, complete cellulose hydrolysis is mediated by a combination of three main types of cellulases: (1) endoglucanases, (2) exoglucanases, including cellobiohydrolases (CBHs) and (3) β–glucosidase. Endoglucanase (1, 4-β-d-glucanohydrolase, EC 3.2.1.4) randomly attacks the internal *O*-glycosidic bonds, resulting in glucan chains of different lengths. Exoglucanase (1, 4-β-d-glucan cellobiohydrolase, EC 3.2.1.91) acts on the ends of the cellulose chain and releases β-cellobiose as the end product. β-Glucosidase (β-d-glucoside glucohydrolase, EC 3.2.1.21) is an enzyme that hydrolyzes terminal, nonreducing β-d-glucosyl residues with release of β-d-glucose (Mojsov, 2017). Xylanase (EC 3.2.1.8) is any of a class of enzymes that degrade the linear polysaccharide xylan into xylose thus breaking down hemicellulose, which is one of the major components of plant cell walls (Beg et al., 2001).

The collective action of all these three enzymes is therefore essential for the complete hydrolysis of lignocellulosic biomass. Furthermore, fungal scavenging of plant and animal debris in the environment releases simple inorganic molecules into the soil. Therefore, in natural ecosystems, fungi are the main decomposers which play an indispensable role in nutrient cycling as saprotrophs and symbionts that degrade organic matter into inorganic molecules (Barea et al., 2005).These nutrients facilitate plant growth.

In addition to replenishing the environment with nutrients, fungi interact directly with other organisms in different beneficial as well as disadvantageous ways. These are symbiotic relationships which usually involve a fungus and another organism. The other organisms might be cyanobacteria, algae, plants or animals. The complex mycorrhizal

associations with the roots of plants are one such mutualistic relationship which is beneficial for both members in the relationship. Fungal hyphae which are in contact with soil act as pathways of providing water and minerals into plants. In exchange, the plants supply their photosynthetic byproducts to carry out the metabolic activities of fungus. Some ants farm fungi as a supply of food. Lichens are another example for a symbiotic relationship between a fungus and a photosynthetic organism such as an alga or a cyanobacterium. The photosynthetic counterpart provides energy derived from light and carbohydrates to the fungus, while the fungus supplies minerals and protection to the photosynthetic organism. Some animals that consume fungi help to disseminate spores over long distant areas.

Fungi are also directly important for humans as food. Especially, some of the basidiomycetes generally known as mushrooms are edible. Besides, they take part in producing cheeses, beer and wine, bread, some cakes and some soya bean products. However, some of them are deadly poisonous that should be avoided consumption. Furthermore, fungi produce different metabolic byproducts that could be utilized for various purposes. For instance, many antibiotics are derived from fungi for medical purposes. One of the major examples is Penicillin produced by *Penicillium* sp.

Fungi also become detrimental by causing diseases to humans, animals as well as crop plants. Aspergillosis is an example for a human infectious disease caused by *Aspergillus* which weakens human immune system. Ringworm infection or Dermatomycosis is another fungal infectious disease. It is a parasitic skin disease of man and animals. Damping-off is an example for a fungal disease occurred in crop plants such as tomato. Pathogenic fungi such as *Fusarium oxysporum, Rhizoctonia solani, Phytophthora* and *Pythium* sp. are responsible for causing this disease. Although there are above disadvantages, fungi are extremely necessary for sustainability of the environment and also for human well-being.

Although biodiversity could be simply explained as different types of life on earth, according to National Wildlife Federation of USA, biodiversity can be evaluated in 3 levels. They are Species Diversity (different varieties of living organisms starting from primitive microorganisms to flowering plants and animals), Ecosystem Diversity (from tropical rainforests, deserts, swamps, tundra to all the ecosystems) and Genetic Diversity (variety of genes within a species which creates variations within species that results in their evolution). The diversity among fungi also might be categorized according to these 3 levels of biodiversity. Fungal diversity also depends on the regions they grow. Tropical regions are considered to be rich with a large biological diversity which always draws the attention of many researchers worldwide.

Tropics, the regions of the earth near to the equator are home for large number of flora and fauna including microorganisms like fungi. There are about 100,000 species of tropical fungi that have been described so far, while estimates for the total number of species go as high as 5.1 million and upwards (Aime and Brearley,2012). Sri Lanka is

enlisted as a biodiversity hotspot by United Nations because of its species richness. Fungal diversity is also playing an indispensable role in it. Especially, tropical rain forest ecosystems in Sri Lanka might contain different species of fungi ranging from microscopic yeast and molds to large mushrooms. Apparently, the records on fungal biodiversity of Sri Lanka are rare. However, a large number of researches are conducted on potential applications of different fungal species in agriculture, environment etc. Therefore, it is necessary to conduct more research to explore diverse ecosystems in the island to record available fungal species. Estimation of the number of fungal species is also important. Moreover, there may be undescribed tropical fungal species still remaining to be discovered. It will contribute greatly to fill the gap in knowledge on tropical fungi. Especially the knowledge of the distribution of tropical endophytic fungi, arbuscular mycorrhizal fungi, and soil fungi which are poorly documented in the tropics, await further studies.

In the current chapter, an account of fungi with the ability to produce lignocellulolytic enzymes which was isolated from central areas of Sri Lanka is presented. Furthermore, the morphological as well as molecular biological identification of the most efficient cellulase enzyme producing microorganisms have also been reported. The major focus of the study was to characterize these fungal isolates based on their ability to produce total cellulase, xylanase and laccase like lignocellulolytic enzymes.

METHODS

Isolation of Fungi from Environment

Soil samples were collected from natural environment *viz;* paddy fields, home gardens and forest areas. Samples were also collected from decaying wood and leaf litter. Clean polythene bags were used to collect and transport the soil to the laboratory.The isolation of fungi was done by preparation of soil dilution series. Diluting of soil was essential in order to obtain discrete fungal colonies on agar plates without overgrowth because the soil is a rich source of microorganisms. The initial 10^{-1} dilution was prepared by suspension of 1g of soil in 9ml of sterile distilled water. It was vortexed in order to get a homogenized `suspension. This initial suspension was serially diluted up to 10^{-7}. Fungi were isolated by pour plate technique on Potato Dextrose Agar (PDA).In order to remove bacterial interferences on fungal growth; chloramphenicol antibiotic (50mg/l) was added into the liquid state PDA medium. The plates were incubated at room temperature for 4-7 days and observed daily. Morphologically different fungal growths were sub cultured repeatedly on fresh PDA plates until the pure isolates were obtained. All these procedures were carried out under aseptic conditions. This method was suitable for isolating filamentous fungi.

The isolation of basidiomycetes was different from isolating filamentous fungi or molds. Basidiomycetes were producing this special structure called basidiocarp which is visible to naked eye. The basidiocarps were observed arising from tree trunks, decaying logs, decaying plant litter. Some basidiocarps were found growing on piles of soil in the environment. The basidiocarps were collected for culturing. The isolation of basidiomycetes on to laboratory culture plates was done using the basidiocarps. First, a piece of tissue was removed from the stipe of the basidiocarp by using a sterile scalpel. It was surface-sterilized with 70% (v/v) ethanol for 60 seconds followed by 3% (v/v) H_2O_2 for 30 seconds. It was then washed with sterile distilled water and placed on a plate of PDA with yeast extract (5 g/l), gentamicin (50 mg/l), chloramphenicol (50 mg/l) and Carbendazim (1 mg/l). The plates were incubated at 25°C for up to 14 days and pure growths of basidiomycetes were sub-cultured on to fresh PDA slopes.

After isolation of fungi, they were grown in special broth culture media to facilitate their lignocellulolytic enzyme production and a series of enzyme assays were also conducted to assess their enzyme production potential as mentioned below.

Enzyme Production by Filamentous Fungi

Usually aerobes are producing extracellular enzymes. Submerged fermentation involves the growth of the microorganism as a suspension in a liquid medium in which various nutrients are either dissolved or suspended as particulate solids in many commercial media (Frost and Moss, 1987).The culture filtrate was directly used as the crude enzyme extract. This concept was applied in producing the enzymes from fungi.

Filamentous fungi were grown on PDA slopes for 10 days to allow for sporulation. Spores were harvested by adding sterile 0.1% (v/v) tween 80 solution and the spore concentrations were estimated by using a Neubauer counting chamber and were adjusted to 10^7-10^8spores per ml. The Tween 80 solution was prepared by dissolving 0.1 ml of Tween 80 in 100 ml of distilled water. One hundred microliter of the suspension was inoculated into 100 ml Erlenmeyer flasks containing 20 ml of fungal cellulase production medium described by Mandels and Weber (1969).The Mandels and Weber medium included (per litre): NH_4SO_4 1.4 g; KH_2PO_4 2.0 g; Urea 0.30 g; $CaCl_2$ 0.30 g; $MgSO_4.7H_2O$ 0.30 g; Tween 80 2.00 ml; yeast extract 0.25 g; cellulose 10.0 g; trace metal stock solution 1.00 ml (which was composed of $FeSO_4.7H_2O$ 4.6 g; $MnCl_2.4H_2O$ 0.89 g; ZnSO4. H_2O 1.78 g; and $CoCl_2.5H_2O$ 1.83 g dissolved in 495 ml distilled water and 5 ml of Conc. HCl).The pH of the medium was adjusted to 5.5.The cultures were incubated at 28°C for 7 days with rotary shaking at 100 rpm. Cultures were centrifuged at 4800 rpm for 15 minutes and culture supernatant was used as crude enzyme extracts in enzyme assays.

Enzyme Production by Basidiomycetes

Cellulase and xylanase activities were evaluated in a medium modified from Peláez, Martínez and Martinez (1995). It contained (per litre): cellulose, 10 g; glycerol, 0.5 g; KNa tartrate tetrahydrate, 3 g; $(NH_4)_2HPO_4$, 1 g; yeast extract, 1 g; KCl, 0.5 g; $MgSO_4.7H_2O$, 0.5 g; $CaCl_2.2H_2O$, 0.3 g; $FeSO_4.7H_2O$, 5 mg; trace element solution, 1 ml. Trace element solution contained [per 100 ml]: $ZnSO_4.7H_2O$, 100 mg; $CuSO_4.5H_2O$, 25 mg; $MnSO_4.H_2O$, 100 mg; $CoCl_2.6H_2O$, 100 mg; $(NH_4)_6Mo_7O_{24}.4H_2O$, 10 mg; $Na_2B_4O_7.10H_2O$, 10 mg. pH of the medium was adjusted to 5.6. Laccase, Manganese Peroxidase (MnP) and Lignin Peroxidase (LiP) activities were evaluated in a medium modified from the afore-mentioned medium by replacing cellulose and glycerol with glucose (10 g/l) because it has been demonstrated that glucose like readily utilizable and efficiently metabolized carbon sources by the microorganism results in high levels of laccase activity (Galhaup and Haltrich, 2001). The basidiomycetes were grown on Potato Dextrose Yeast Extract Agar and circular discs of growth (diameter = 5 mm) were cut from near the edge of each colony by using a sterile Cork Borer. Each disc was radially cut into 8 equal pieces with a sterile scalpel, in order to reduce the pellet size of growth under submerged condition. Growth from three discs from each basidiomycete was inoculated to 20 ml of the medium in 100 ml Erlenmeyer flasks and incubated at 28°C in the dark with shaking at 100 rpm. After 7 days, the cultures were filtered with Whatman No.1 filter paper and the filtrates were used for the enzyme assays.

Enzyme Assays

Total cellulase assay was performed using Whatman No.1 filter paper as the substrate (Mandels, Andreotti and Roche, 1976). Reducing sugars formed were measured by using 3, 5-dinitro salicylic acid (DNS) reagent (Miller, 1959) with glucose standards. The total cellulase activity was expressed as filter paper units per ml (FPU/ml) which is the amount of reducing sugar, in micromoles, released by 1 ml of undiluted enzyme per minute.

Endoglucanases (Endo-β-1, 4-glucanase) activities of fungal isolates were measured by a method modified from protocols described by Mandels, Andreotti and Roche (1976). Carboxy Methyl Cellulose (CMC) solution (2% w/v, 20 mg/ml) was prepared in 50 mM citrate buffer (pH 4.8 for fungi).Hundred microliter of enzyme was added into test tubes and they were kept in 50 ˚C water bath. Then the prepared CMC solution was added into each tube and incubated for 30 minutes. After that the reaction was quenched by addition of DNS reagent and released sugar was estimated by the methods described by Sumner(1921) and Miller (1959).

Exoglucanase activities of fungal isolates were determined to understand their potential of breaking down crystalline cellulose. Microcrystalline cellulose suspension

(2%w/v, 20 mg/ml) was prepared in 50 mM citrate buffer (pH 4.8 for fungi).Two hundred microliter of particular enzyme extract was added into 200 µl of the above suspension. The mixture was incubated at 50 ˚C for 2 hours. Glucose standards were added into separate tubes (400 µl of 2.0, 1.0, 0.5 and 0.25 mg/ml).All the test samples as well as standards were kept in boiling water bath for 5 minutes after addition of 600 µl of DNS reagent. The tubes were cooled after that and they were transferred to microcentrifuge and were centrifuged at 10000 g to deposit remaining cellulose. Six hundred microliter of each supernatant was transferred into fresh test tubes and vortexed after adding 3 ml of distilled water. The absorbance of the resulted solution was measured at 540 nm.

Xylanase activity was measured according to methods described by Gottschalk, Oliveira and Bon (2010) with beech wood xylan (Sigma) as the substrate and the reducing sugars formed were measured by using DNS reagent with xylose standards.

Laccase activity was measured by using 2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulphonate (ABTS) as the substrate (Bourbonnais et al., 1995). The assay mixture (3 ml) contained 0.5 mM ABTS, 100 mM sodium acetate (pH 5.0) and 300 µl of culture supernatant. The oxidation of ABTS was followed by measuring the absorbance at 420 nm at one second intervals for 60 seconds at room temperature. The absorbance readings were plotted against time. If the absorbance readings became non-linear within 60 seconds, then the culture supernatant was diluted in distilled water to obtain tenfold dilutions and the diluted supernatant was used for the assay. The enzyme activity of undiluted supernatant was calculated by multiplying the activity of the diluted supernatant by the dilution factor. The amount of oxidized product formed was calculated by using the Beer-Lambert equation $A = \varepsilon cl$, where A= absorbance, ε= molar absorptivity, c = concentration of the analyte and l= path length of the cuvette. Molar absorptivity of 36 mM^{-1} cm^{-1} (Bourbonnais et al., 1995) was used for the calculations.

Manganese Peroxidase activity was measured by using phenol red as the substrate (Peláez, Martínez and Martinez 1995). The reaction mixture (3 ml) contained 0.1 mM H_2O_2, 0.1 mM $MnSO_4$, 0.1 mg/ml phenol red, 100 mM sodium tartrate buffer (pH 5.0) and 1 ml of crude enzyme. The reaction was stopped by the addition of 120 µl of 2 M NaOH after 30 minutes. The increase in absorption at 610 nm was measured against the corresponding enzyme blank (to which 120 µl of 2 M NaOH was added prior to the addition of the reaction mixture). The amount of phenol red oxidized was calculated by using the Beer-Lambert equation and the molar absorptivity $\varepsilon 610 = 4460$ $M^{-1}cm^{-1}$ (Peláez, Martínez and Martinez 1995).

Lignin Peroxidase activity was measured by using methylene blue as the substrate (Magalhães et al., 1996). The assay mixture (3 ml) contained 0.1 ml of 1.2 mM methylene blue, 0.6 ml of 0.5 M sodium tartrate (pH 4.0) and 2.2 ml of enzyme. Reaction was started by the addition of 0.1 ml of 2.7 mM H_2O_2. Conversion of the dye to Azure C was determined by measuring the absorbance at 664 nm immediately after the addition of

H_2O_2 and again after 30 minutes. Enzyme activity was expressed as the decrease in absorbance at 664 nm per minute.

Identification of Filamentous Fungi

The most efficient cellulolytic fungi were morphologically identified using their macroscopic as well as microscopic characteristics. It was essential to obtain fresh fungal cultures on PDA plates in order to clearly observe macroscopic characteristics regarding colony morphology. For instance, the size and shape of the fungal colonies were considered. Although there are unicellular fungi like yeast, here in the current chapter multicellular forms easily seen with the naked eye (e.g., molds) were studied. The molds form large multicellular aggregates of long branching filaments called hyphae. The characteristics of hyphae were considered in identification. Spore size, shape and structure were also used in the identification of fungi. Furthermore, the form (the basic shape of the colony - circular, filamentous, etc.), size (The diameter of the colony), elevation(the side view of a colony), margin/border (the edge of a colony) and the surface (smooth, glistening, rough, wrinkled, or dull),opacity (transparent, clear, opaque, translucent (like looking through frosted glass), etc.) and colour (pigmentation- white, buff, red, purple, etc.) of the fungal colonies and spores were considered as macroscopic morphological characters.

The microscopic characters of the isolates were observed using microscopic slides prepared by sticky tape method. The prepared slides were observed under oil immersion objective (×100) of light microscope (BioBlue. Lab).Types of hyphae (septate or non-septate),mycelium (clear or dark), color of mycelium (colored or colorless), types of sexual spores, types of asexual spores, characteristics of aerial hyphae or spores, sporangia (size, color, shape and location), conidia and their arrangement (chain, budding, single or masses), shape and arrangement of sterigmata or phialides, arrangement of soprangiophores or conidiophores (simple or branched, type of branching), size and shape of collumela at tip of soprangiophore, single conidiophore or bundle of conidiophore and presence of special structures (stolen, rhizoids, apophysis etc.) were some of the characters considered in recording the microscopic characteristics of the fungal isolates. The identification of the isolates was done by referring the identification keys described in literature (Barnett and Barry1998; Dugan 2006).

The most efficient cellulase enzyme producing isolates were identified using molecular biological techniques. Genomic DNA was extracted from the fungi according to methods described by Cenis (1992) with an additional step of freezing and thawing prior to homogenization of the cells. The PCR amplification was performed with ITS1 and ITS4 primers (White et al., 1990). The sequencing of PCR products was done at Macrogen Inc. (South Korea). Consensus sequences were made from the forward and

reverse sequences and were analyzed for similarity with existing nucleotide sequences in the NCBI and ENA databases by using the BLAST tool.

Identification of the Basidiomycetes

The basidiomycetes were identified based on morphological characteristics and/or DNA sequencing of the ITS region. The same method used for molecular biological identification of filamentous fungi was used for the identification of basidiomycetes as well. Morphological characteristics were not always helpful in determination of basidiomycetes genera.

Statistical Analysis of Data

Statistical analyses were carried out by using Minitab®16 statistical software. Comparisons were made by one-way ANOVA with Tukey's method or by two sample t-test ($\alpha = 0.05$).

RESULTS

Isolation, Identification and Screening of Enzyme Activities of Filamentous Fungi (Molds)

Isolation and Identification of Filamentous Fungi

More than150 filamentous fungi were isolated. They were isolated basically from soil samples collected from Kandy and Kegalle districts of Sri Lanka. According to Department of Meteorology Sri Lanka, the climate of the island is characterized as tropical because it is located between 5˚ 55' to 9˚ 5' North latitude and between 79˚ 42' to 81˚ 53' East longitude. Normal temperature of these areas is usually fluctuating from 24-30˚C. The climatic conditions usually shift from dry season to rainy season throughout the year. However, these areas are getting a significant rainfall throughout the year. Therefore, these tropical climatic conditions have a huge impact on fungal diversity of the area.

The fungal isolates were next screened for their total cellulase, endoglucanase, exoglucanase and xylanase activities. The most efficient cellulolytic filamentous fungi were found to be from 3 main genera *viz; Trichoderma, Penicillium* and *Aspergillus* (Figure 1 and Figure 2).

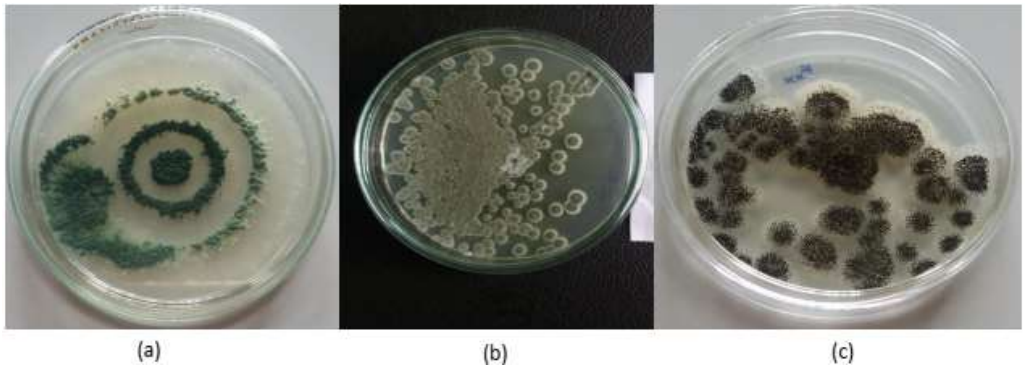


Figure 1. Some of the isolated cellulolytic fungi. The genera which found to be most efficient in cellulolytic enzyme production (figures are not in actual scale). (a) *Trichoderma viridae* (b) *Penicillium* sp. (c) *Aspergillus niger*.

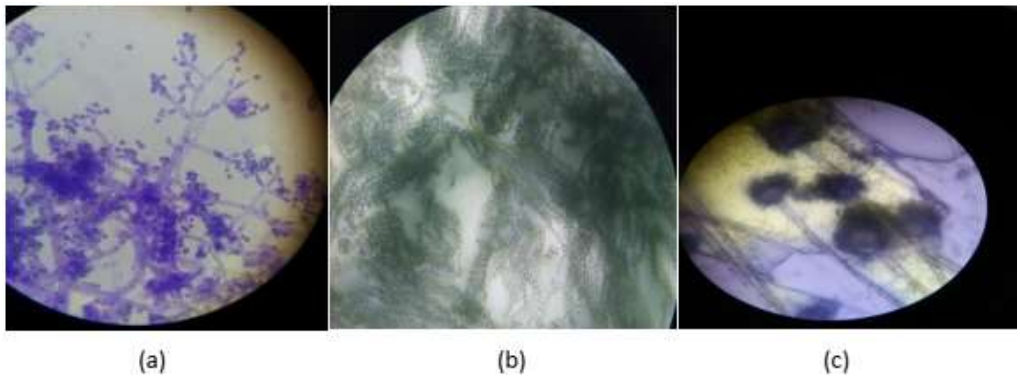


Figure 2. The microscopic observations of main cellulolytic fungal isolates. (a) *Trichoderma viridae* (b) *Penicillium* sp.(c) *Aspergillus niger*.

The morphlogical characteristics of filamentous fungi were very helpful in their identification.The *Trichoderma* isolates showed rapid colony growth.The plate was covered within 4 days. Initially they were hyaline with white filaments appearing gradually.Soon the filaments became whitish to dark green. The middle part of the colony appeareddark green and the edges of the colony appeared white. The colony surface looked rough due to the presence of tufted green conidial areas. Colony margin was filiform. Elevation of the colonies was flat and had a dry texture on the surface.They showed concentric rings of growth on plates.According to microscopic characteristics,conidiophores were hyaline, much branched and were not verticillate. Phialides were single or in groups. Conidia (phialospores) were single-celled hyaline which borne in small terminal clusters. Large conidiophores showed extensive branching.Spores were green in color.Most of the efficient isolates were *T.viridae* isolates (Figure 1.(a)).

The *Aspergillus* isolates which appeared in different colours due to their spore formation (Figure 3) had conidiophores in different shapes (Figure 4) when observed

under microscope. However, the most efficient in cellulolytic activities was the isolates with typical dark brown spores. The spores were observed on the surface of the white mycelium. The surface was flat and dry. Growth was not fast as in *Trichoderma* sp. or no zonation was observed.

The *Penicillium* isolates also were commonly found in soil samples. The frequently observed *Penicillium* colonies were greyish blue green in colour (Figure 1 (b)). The underside was yellowish. Centre of the colony was darker and margin was a white single line. The elevation was flat while entire margins were observed. Almost all the *Penicillium* isolates had the typical conidiophores (Figure 2 (b)). They were elongated, septate, branched conidiophores ending in a group of phialides and then chains of conidia. Light greenishconidiophores arisen from the mycelium singly or less often in synnemata, branched near the apex, penicillate, ending in a group of phialides. Conidia were brightly colored in mass. They were small and 1-celled, globose greenish phialospores (conidia) are present in dry basipetal chains.

Figure 3. The different *Aspergillus* isolates. (a) *Aspergillus fumigatus*. (b)*Aspergillus clavatus* (c) *Aspergillus* yellow mold.

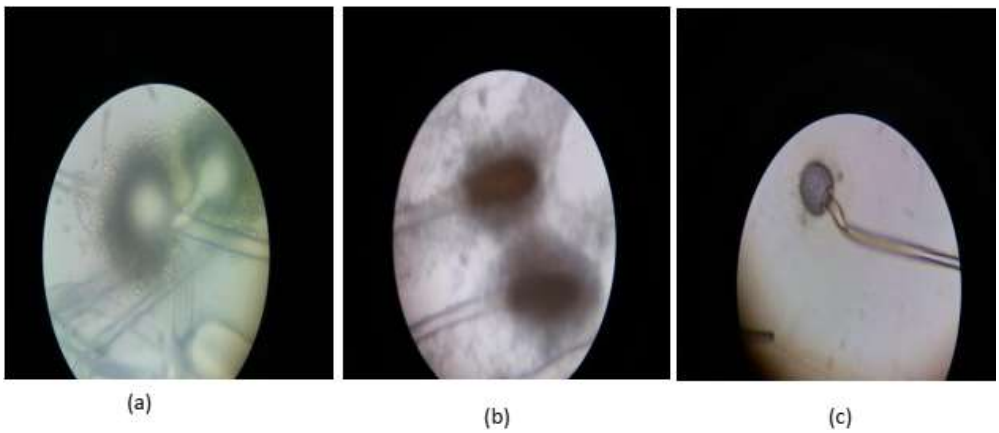


Figure 4. Different conidiophores of *Aspergillus* isolates (a) *Aspergillus fumigatus* conidiophores (b) *Aspergillus clavatus* conidiophores (c) *Aspergillus* yellow mold.

These morphological characteristics are extremely useful in identifying fungal isolates. It was observed that the typical sexual as well as asexual reproductive structures of fungi play a major role in fungal identification. Those typical structures are sometimes capable of predicting the identification up to species level by following the identification keys.

Enzyme Activities of Filamentous Fungi

Filamentous fungi could be considered as preferential sources of industrial enzymes because of their excellent capacity for extracellular protein production. The Figure 5 shows the total cellulase activities of fungal isolates in filter paper units. Most efficient cellulolytic microorganisms recorded were aerobic filamentous fungi. Out of them members of genus *Trcichoderma* were reported as the most efficient cellulase producers. The highest total cellulase activity was observed in *Trichoderma viridae* isolate as 0.937 FPU/ml followed by two other *Trichoderma* isolates giving total cellulase activities 0.775 FPU/ml and 0.74 FPU/ml respectively.

These results gain support from many recent reports on isolating cellulolytic microorganisms from natural environment. One such study identified *Trichoderma* isolates as the most efficient strains in cellulase production out of all the other filamentous fungal genera. However, the highest cellulase activity among them was produced by *T. reesei* (F118) (0.21 FPU/ml) (Mohanan, 2019). In the study reported in the current chapter, the highest cellulase activity was given by a *T. viridae* isolate as 0.937 FPU/ml. It is approximately a more than 4 times increment of cellulase activity than the activity of aforementioned *T. reesei*. This might be resulted by the species variation among the two *Trichoderma* isolates. Different species of the same genus have shown different cellulase activities.

On the other hand, there is proteose peptone 1.0 g/l and Tween 80 2.0 ml/l in Mandels & Weber (1969) medium. In a different study, the effect of Tween 80 and proteose peptone on cellulase production by *Trichoderma* species was studied. Sugar cane pith was used in the medium as carbon source. It was found that Tween 80 increases cellulase production while proteose peptone has influence on enzyme adsorption to the substrate. They also revealed that there is a combined effect of these two ingredientson cellulase production (Hung et al., 1988). The same idea was confirmed in a very recent study as well. According to that study, nitrogen sources had a remarkable effect on enzyme produced by *Trichoderma* sp. The maximum enzyme activities were obtained with yeast extract (1.0 %) which brought about an improvement in all the three cellulase components, including exoglucanase (2.40 U/ml), endoglucanase (2.28 U/ml), and β-glucosidase (1.99 U/ml), where peptone also produced the second most highest cellulose (Gautam et al., 2011).The medium optimized by Mandels contains both yeast extracts and peptone components. Peptone is an efficient nitrogen source for cellulase production.

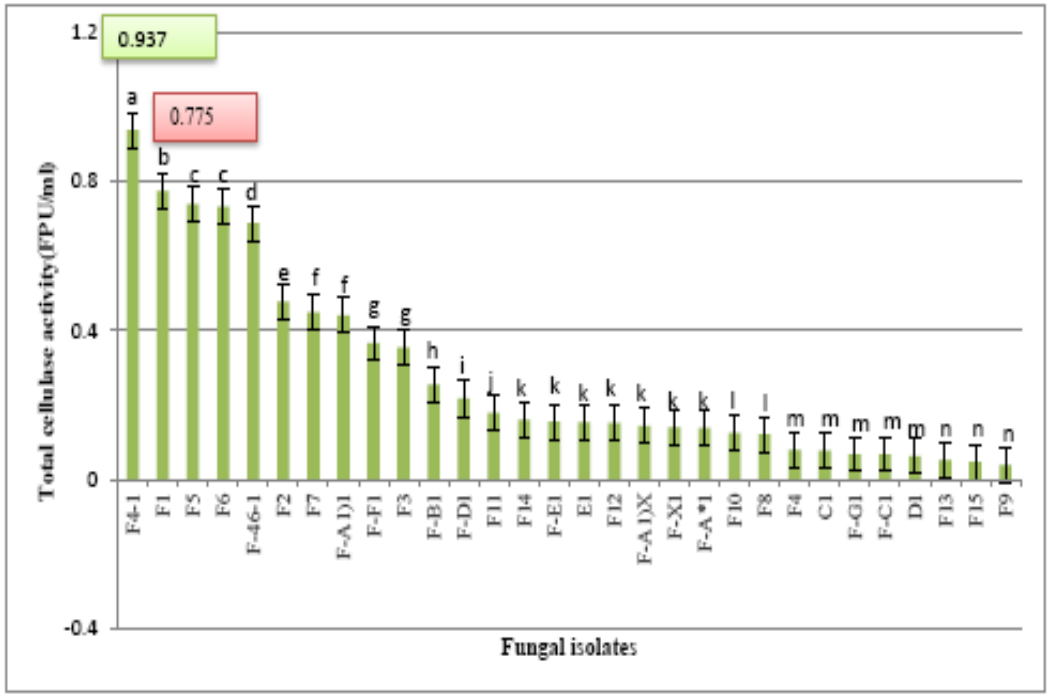


Figure 5. Total cellulase activities of fungal isolates. Error bars indicate the standard errors of the means. Values not sharing the same letter are significantly different ($P < 0.05$).). F4-1, F1, F5 etc. are code names of different fungal isolate.

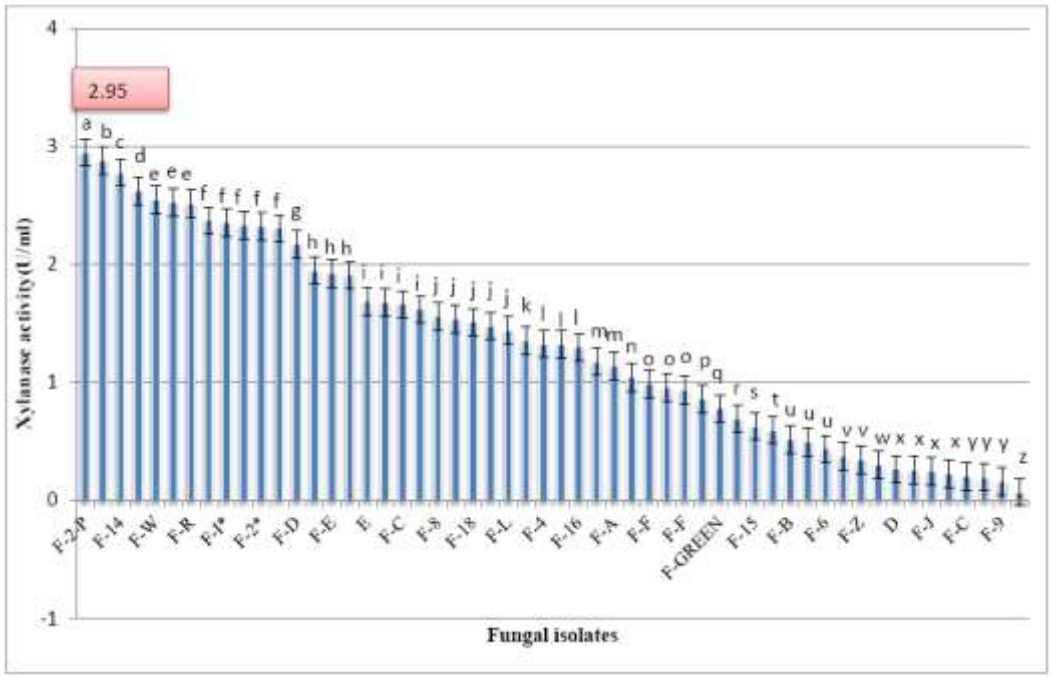


Figure 6. Xylanase activities of fungal isolates. Error bars indicate the standard errors of the means. Values not sharing the same letter are significantly different ($P < 0.05$). F4-1, F1, F5…. etc. are code names of different fungal isolates.

According to results of a study conducted by Pardo (1996), it was revealed that Tween 80 was the best compound for enhancing the production of all of the cellulolytic enzymes of the cellulase complex of *Nectria catalinensis* fungus. It caused an increase of endoglucanase and exoglucanase production in 70% and 72% respectively. It is believed that Tween 80 is a sort of detergent which makes changes in membrane permeability, so that free enzyme in solution is enhanced as a consequence of more excretion(Reese, Lola and Parrish, 1969).But in the above *T. reesei* cellulase production medium there was a slight difference to be noticed. Although tween 80 was present, there is 0.5 g/l of glycerol that has been added to the medium while proteose peptone 1.0 g/l was not added to the medium. Glycerol is an easily utilizable carbon source which could repress the cellulase production (Mandels and Weber, 1969).These reasons might have collectively affected the cellulase activity differences in these *Trichoderma* isolates.

Furthermore, *Aspergillus* as well as *Penicillium* species were recorded to be efficient cellulase producers. Several similar studies have been conducted in India. For example, Sudha and her group conducted a study (2018) about biodiversity of cellulolytic fungi isolated from Kattalagar Kovil, Tamilnadu, India. *Aspergillus niger* was found to produce the highest quantity of cellulase (0.854 ± 0.003 IU/ml). Similarly, Uttamkumar et al., (2014) studied on the fungi isolated from different sources like degrading wood, leaf litter and soil collected from forest nearby Forest Research Institute, Dehradun, India. The microorganisms they identified were *T. viridae, A. niger, A. fumigatus, A. flavus, Fusarium oxysporum* and species of *Trichoderma, Chaetomium, Curvularia, Penicillium, Alternaria* etc. Among these *A. niger* and *T. viridae* were recorded from all samples. However, only two fungi have exhibited considerable activity to degrade the cellulose, highest activity been recorded for *T. viridae* followed by *A. niger*. India is also a tropical country. The studies mentioned above are proof that the same climatic conditions may share the same genera of fungi.

Figure 6 illustrates the xylanase activities of fugal isolates. The highest xylanase activity recorded is 2.95 U/ml which was shown by a *Trichoderma* isolate. Thirty fungal isolates which showed higher xylanase activities above 0.07 U/ml level have been reported here. Most of the isolates which showed higher xylanase activities belong to genus *Trichoderma*. Xylanase is not an enzyme included in the cellulase enzyme complex. It is a hemicellulase which is required to break down hemicellulose counterpart in lignocellulosic plant material. Therefore, the ability of fungal isolates to produce this enzyme is essential in terms of utilizing cellulosic plant biomass as a substrate.

In Figure 7, the endoglucanase activities of fungal isolates are compared. The highest endoglucanase activity was expressed by a *Penicillium* isolate as 1.51 U/ml. This is an enzyme which attacks the amorphous regions of cellulose polymer complex. The 25 fungal isolates with endoglucanase activities above 0.07 U/ml endoglucanase activity level have been illustrated here.

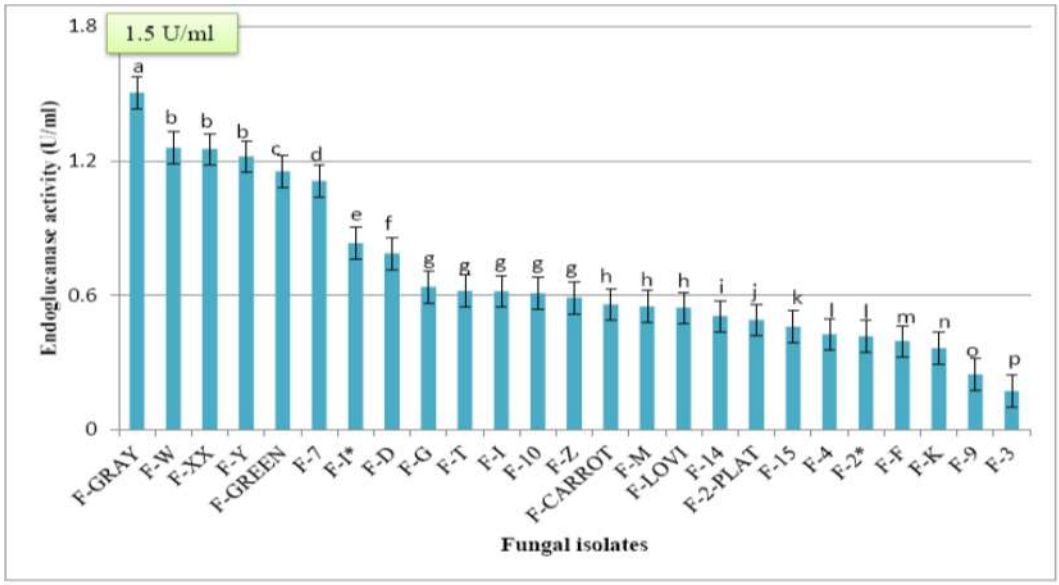


Figure 7. Endoglucanase activity of fungal isolates. Error bars indicate the standard errors of the means. Values not sharing the same letter are significantly different ($P < 0.05$). F4-1, F1, F5…. et care code names of different fungal isolates.

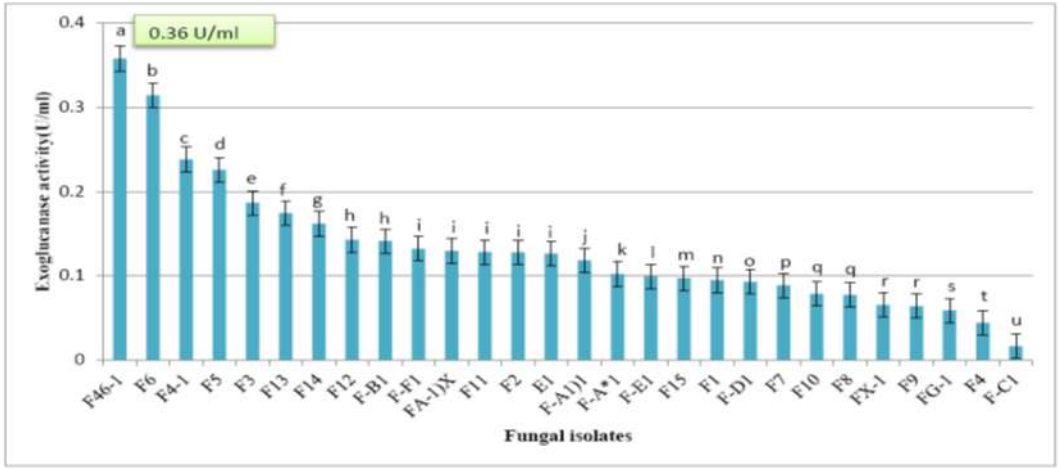


Figure 8. Exoglucanase activities of fungal isolates. Error bars indicate the standard errors of the means. Values not sharing the same letter are significantly different ($P < 0.05$). F4-1, F1, F2, F5…. etc are code names of different fungal isolates.

The Figure 8 illustrates the exoglucanase activities of fungal isolates. The highest activity was observed in a *Trichoderma* isolate as 0.36 U/ml. The 28 fungal isolates which showed exoglucanase activity above 0.015 U/ml have included here. This enzyme is responsible for breaking down the crystalline regions of cellulose.

According to overall results, the *Trichoderma* isolates were the most efficient in producing cellulolytic enzymes. Depending on the species diversity, their enzyme

activities were varying. As complete degradation of cellulose requires the activity of both exoglucanase as well as endoglucanase, the capability of microorganisms to produce these enzymes are essential. However, every individual isolate was not producing all the component enzymes in copious amounts. Moreover, their enzyme production patterns were very diverse. Therefore, it seems to be fairer to select the microbial isolates with highest total cellulase activities as the best isolates in degradation of cellulose because the total cellulase activity of an organisms is a representation of collective cellulase activity of cellulase enzyme complex.

Isolation Identification and Screening of Enzyme Activities of Basidiomycetes

Isolation and Identification of Basidiomycetes

Basidiomycetes are a class in phylum Basidiomycota of kingdom fungi. They are potential sources of lignocellulolytic enzymes. The aim of this section was to provide an account on several basidiomycetes isolated from Central Province, Kandy, Sri Lanka. Those isolates were screened for their lignocellulose degrading enzymes activities, namely cellulase, xylanase, laccase, Manganese peroxidase and lignin peroxidase. The data obtained on enzymatic lignocellulolysis of those basidiomycetes are mainly discussed here.

The isolation of basidiomycetes was challenging and difficult because of two reasons. Firstly, the plates were frequently getting contaminated with filamentous fungi. According to literature, they characteristically grow more slowly in culture than members of other fungal classes such as ascomycetes, zygomycetes and deuteromycetes (Miller, Grand and Tredway, 2011). Most common contaminant was *Trichoderma* species. It rapidly covered the surface of the basidiomycetes inoculated PDA culture plates, completely blocking the basidiomycetes growth. To overcome this problem, a mixture of fungicides was introduced into the medium to suppress the growth of the molds. Secondly, it was noticed that the basidiocarps collected were not live or might be unculturable because they did not produce colonies on culture plates. It was not easy to determine whether they were live or not especially with isolates like *Polyporus* sp. During isolation these information were taken into major concern. Therefore, isolation of basidiomycetes was different from isolating molds.

Identification of wild type basidiomycetes which are generally called mushrooms is basically morphological. The body or thallus of the basidiomycete fungus (the mycelium) is normally hidden within the substrate, and it is generally only the fruiting body or basidiocarp that is visible on the surface. The fruiting body being the most visible part of the basidiomycete with the greatest morphological variation, conventional mycology relies on a number of macroscopic and microscopic features of the fruiting body to distinguish between species (Hood, 2006). This method sometimes leads to

misidentification of the species. Therefore, it is indeed necessary to use molecular approach in order to have accurate and more reliable identification. The morphological characters were first recorded when they were collected from their natural substratum. The recorded information and photos taken were compared with literature to identify the isolates up to genera level. On the culture plates, their growth was mostly limited only to produce a simple white filamentous growth. This might be a thallus of the basidiomycetes. It was not quite different from each isolate. Moreover; basidiomycetes rarely produce sexual structures in culture upon which identification can be based (Miller, Grand and Tredway, 2011).The presence of clamp connections is a special characteristic often used to identify fungal hyphae as a basidiomycete but many basidiomycetes do not form clamp connections (Duncan and Keay, 1990). Therefore, microscopic hyphal characters were inadequate to do a reliable identification. The molecular biological identification with the aid of the DNA sequence of ITS region was essential to confirm the identity of the isolates.

The current chapter specifically includes enzymatic activity data about 18 efficient basidiomycetes in producing lignocellulolytic enzymes (Figure 9). The basidiomycetes and molds that have been described in the chapter are preserved in the Bioenergy Laboratory culture collection of National Institute of Fundamental Studies, Kandy, Sri Lanka. According to their identification, several basidiomycetes isolates belonged to orders Agaricales (*Schizophyllum commune, Coprinopsis cinerea, Marasmius* sp., *Lepiota* sp.), Xylariales *(Annulohypoxylon stygium, Xylaria* sp.*)* and Polyporales (*Trameteshirsuta, Trametes* sp., *Polyporus* sp., *Phlebiopsis* sp., *Pycnoporus* sp., *Microporus* sp., *Fomes* sp., *Ganoderma* sp., *Earliella scabrosa, Lentinus sajor-caju).*

Figure 9. Several basidiomycetes isolates in the culture collection. (a) *E. scabrosa* (M14), (b) *Pycnoporus* sp. (M21), (c) *Trametes hirsuta* (M29), (d) *Schizophyllum commune* (M1).

Some of the isolates including *E. scabrosa, T. hirsuta, L. sajor-caju* and *A. stygium* were collected from the Knuckles Mountain Range (Dumbara Mountain Range) of Sri Lanka which lies in central Sri Lanka, in the districts of Matale and Kandy. This mountain range is famous for collectively representing all the climatic conditions observed in the island. This specific ecosystem harbors a rich biodiversity. Most of the above isolates have been recorded to be wood rotting fungi including white rot fungi as well as brown rot fungi. *Ganoderma* and *Trametes* like isolates are considered to be white rot fungi which are capable of decaying diverse organic compounds besides lignin.

Schizophyllum commune is an example for a brown-rot fungus among these isolates. The brown rot fungi can degrade cellulose and hemicelluloses, but they can only modify lignin, which remains as a polymeric residue in the decaying wood (Arantes and Goodell, 2014).

Enzyme Activities of Basidiomycetes

Enzyme activities observed in basidiomycetes were very diverse (Table1).Total cellulase activity of basidiomycetes was detected from 16 out of the 18 basidiomycetes evaluated. However, xylanase activity was detected in all isolates. *Pycnoporus* sp. produced the highest cellulase activity of 0.23 FPU/ml, which is comparable to the activities produced by the very first wild type filamentous fungus *Trichoderma reesei* QM6a which was isolated and recorded by Mandels (1975).It is the parent strain of mutant strain *Trichoderma reesei* RUT-C30which is currently used for industrial production of cellulases (Peterson and Nevalainen, 2012).Therefore, there is an extensive potential of modifying these basidiomycetes isolates into more efficient cellulase producers. However, according to the results in Table 1, the total cellulase enzyme activities shown by all the basidiomycetes are lower below 0.1 FPU/ml level. As previously mentioned, the highest total cellulase activity recorded by an isolate was 0.937 FPU/ml which was given by a *T. viridae* isolate. In accordance with the calculation, it is approximately more than 4 times higher than the total cellulase activity of above *Pycnoporus* sp. The highest xylanase activity of 5.4 U/ml was produced by *Phlebiopsis* sp. Other isolates that produced high xylanase activities included *Schizophyllum commune* (M1) (5.12 U/ml) and *Pycnoporus* sp. (4.59 U/ml).

Earliella scabrosa, *Polyporus* sp. (M20), *Pycnoporus* sp., *Trametes hirsuta* (M29), *T. hirsuta* (M36) and *T. hirsuta* (M40) produced laccase activities greater than 10 U/l. *Coprinopsis* sp., *E. scabrosa* and *T. hirsuta* (M40)gave Manganese Peroxidase activities above 10 U/l level. The highest laccase (91.2 U/l) and MnP activities (17.5 U/l) were both observed in *E. scabrosa*. However, its total cellulase activity was 0 FPU/ml. Xylanase activity also was very lower when compared with the highest xylanase activity which was recorded in *Phlebiopsis* sp. Lignin Peroxidase activity was not detected from any of the isolates. In a similar study, Peláez, Martínez and Martinez (1995) also found no LiP activity within a larger collection of basidiomycetes. Thus, it appears LiP activity is rare among the basidiomycetes and limited to a few species such as *Phanerochaete chrysosporium* (Januszet al., 2013).These results suggest that basidiomycetes are more efficient in producing laccase like lignin degrading enzymes than the molds. The laccase production by molds was negligible when compared with the basidiomycetes giving negative results by most of the filamentous fungi for laccase, MnP and LiP activity assays. In other words, basidiomycetes are efficient in lignin degradation than other filamentous fungal isolates like molds.

Table 1. A Summary of Enzyme Activities Observed in Basidiomycetes Isolates

Basidiomycete	Cellulase (FPU/ml)	Xylanase (U/ml)	Laccase (U/l)	MnP (U/l)
*Schizophyllum commune** (M1)a	0.025	5.12	0.6	0
Coprinopsis sp.* (M5)	0	0.08	2.7	10.45
Phlebiopsis sp.* (M7)	0.074	5.41	0	0
Marasmius sp. (M12)	0.054	0.7	0.8	0.29
*Earliella scabrosa** (M14)	0	0.16	91.2	17.5
Trametes sp. (M15)	0.082	0.97	0.5	0
Polyporus sp. (M20)	0.024	0.3	79.9	0.91
Pycnoporus sp.* (M21)	0.232	4.59	33.1	0.64
Microporus xanthopus (M25)	0.073	2.6	0	0
Trametes hirsuta (*M29)b	0.082	0.93	60.4	0.58
Annulohypoxylon stygium (*M31)	0.033	3.8	0.5	0
Schizophyllum commune (*M33)a	0.029	1.6	0	0.08
Trametes hirsuta (*M36)b	0.074	1.67	15.1	0
Lentinus sp.* (*M37)	0.018	0.1	0.6	0.16
Polyporus sp. (*M39)*	0.018	0.16	0.2	0.19
*Trametes hirsuta** (M40)	0.059	1.24	69.3	14.87

(*) denotes the code names given to basidiomycetes isolates during initial screening.

According to the summary of enzyme activities, it is understandable that the composition of enzyme complexes produced by the fungal isolates is very diverse. The types of enzymes produced by a certain fungus and the efficiency of its enzymes are likely to depend on the particular fungus itself. These different features reflect the genetic makeup of the particular isolates. The regulation of cellulolytic enzyme production is finely controlled by activation and repression mechanisms that occur in the genes which are responsible for expressing these enzymes. Hence, cellulases are known as inducible enzymes. Only in the presence of the specific substrate these enzymes are induced and repressed whenever easily utilizable sugars are available (Sukumaran, Singhania and Pandey2005). Some isolates produce cellulase that breakdown cellulose whiles some are negative for cellulase production. This might indicate the missing genes of cellulase enzyme in the genome of these isolates. Same phenomenon is observed with their xylanase, laccase, MnP and LiP production as well. Important information that must be considered is the titers of cellulases produced by the fungal isolates. Although cellulolytic activity has been reported in most of the isolates studied here, they have not produced the high titers of cellulases required at industrial scale (Ang et al.,2013).

Moreover, enhancement of lignocellulolytic enzyme production and activities by individual isolates is also very essential. As previously mentioned in the Methods section, all the lignocellulolytic microorganisms described in this chapter have been isolated from natural environment, especially from soil. They were sharing the same environment.

According to enzyme activity results, it is revealed that different microorganisms show unique enzyme production capabilities. Therefore, there is no doubt that these isolates show different efficiencies in degrading lignocellulose. In natural environments also lignocellulolysis is rarely accomplished by the activity of a single microorganism. The process becomes more efficient because of the combined effect of different amounts of enzymes from different microorganisms. It is rather achieved through the sequential order and collective effort of several microorganisms that produce multiple carbohydrate-active enzymes (CAZymes) (Lombard et al., 2013) to degrade the different polymers. Hence, it might be possible to mix lignocellulolytic enzyme extracts from different isolates in different ratios to prepare more efficient lignocellulose deconstructing enzyme mixtures. In other words, to obtain highly efficient enzyme systems, it is possible to formulate enzyme cocktails by mixing the lignocellulolytic enzymes extracted from different microorganisms that are efficient in producing different enzymes. Another approach is to create Genetically Modified Microorganisms (GMM) with genes that express all the essential enzymes to degrade cellulose, hemicellulose and lignin components in lignocellulosic biomass. However, formulation of enzyme cocktails as well as genetic modification of cellulolytic microorganisms to improve their cellulolytic enzyme production has to be done with prior experiments to optimize and stabilize the process. Co-culturing of several cellulolytic fungi together could be utilized as an efficient mode of enhancing cellulase production although mixing the most suitable component fungi is essential in this process (Jayasekara Abayasekara and Ratnayake 2018).

Moreover, cellulolytic enzyme production has been reported to be influenced by various process parameters including pH of the medium, substrate fermentation, and temperature (Sharma and Yazdani, 2016). The lignocellulosic carbon sources utilized for growing the isolates profoundly affect the enzyme production. For instance, a recent study conducted by Bioenergy Laboratory, NIFS found that the laccase activity of *E. scabrosa* isolate to be an average of 764 U/l when it was grown in sugarcane bagasse (Jayasekara, 2019). In another study, the effect of rice bran on laccase production by the same isolate was analyzed. According to the results of that study, laccase production by *E. scabrosa* was significantly higher when rice bran (10 g/l) was used as the main source of carbon. The increase was profound which observed a mean laccase activity of 13060 U/l (Mohanan, 2019). Therefore, it is evident that studying about these fungi and their lignocellulolytic enzyme production potentials is not that simple. However, it is extremely important to isolate, identify and keep records about fungal isolates of this kind from tropical regions. Most importantly, research activities must be expanded to learn about potential applications of enzymes extracted from these isolates in industrial processes. Moreover, it is not good to forget that these fungal isolates are important not only because they are rich sources of lignocellulolytic enzymes but also because they produce wide variety of secondary metabolites for the benefit of mankind. Therefore, collecting data about fungal diversity in diverse climatic regions of the world including

tropical regions is eminently important as it will definitely unravel new microbial species and support to understand evolutionary relationships between those microorganisms.

CONCLUSION

Lignocellulolysis is a natural process which is efficiently taking place in the environment by means of cellulolytic microorganisms. If these microorganisms could be isolated from the environment, cultured and improved under laboratory conditions, there is a huge potential of using them in industrial applications where lignocellulosic material could be utilized as a low-cost raw material. The findings discussed in the current chapter indicate the enormous potential of fungal isolates in lignocellulolysis by means of producing diverse array of enzymes *viz;* cellulase, hemicellulase (xylanase), laccase etc. Both filamentous fungi (molds) *viz; Trichoderma, Penicillium*, *Aspergillus* etc. and basidiomycetes such as *E. scabrosa* have a great potential in industrial applications as they are efficient in producing lignocellulolytic enzymes. *Trichoderma viridae* was found to be the best cellulolytic filamentous fungus among isolates giving 0.937 FPU/mlof total cellulase activity. Among basidiomycetes *E. scabrosa* was the most efficient laccase producer basidiomycete with the highest laccase activity of 91 U/l. *Pycnoporus* sp. produced the highest cellulase activity along with high xylanase activity. The lignocellulolytic potential of these tropical fungal isolates could be further enhanced by means of genetic engineering techniques supported by optimization of their culturing conditions. Preparation of more efficient lignocellulolytic enzyme cocktails by mixing enzyme extracts from different isolates in different ratio could be a more feasible approach of getting more efficient enzymes.

REFERENCES

Aime M. C. and Brearley F. Q. (2012). Tropical fungal diversity: closing the gap between species estimates and species discovery. *Biodiversity and Conservation* 21, 2177–2180. doi 10.1007/s10531-012-0338-7.

Ang, S. K., Shaza, E. M., Adiba, Y., Surini, A. A. and Madihah, M. S., (2013). Production of cellulases and xylanase by Aspergillus fumigatus SK1 using untreated oil palm trunk through solid state fermentation. *Process Biochem*. 48, 1293–1302.

Arantes, V. and Goodell, B. (2014). Current understanding of brown-rot fungal biodegradation mechanisms: a review. In: *Deterioration and protection of sustainable biomaterials. ACS Symposium Series*, Vol. 1158. Washington, DC: American Chemical Society: 3-21. Chapter 1. doi 10.1021/ bk-2014-1158.ch001.

Barea, José-Miguel., Pozo, M. J., Azcón, R., and Azcón-Aguilar, C., (2005). Microbial co-operation in the rhizosphere. *Journal of Experimental Botany*, 56(417), 1761–1778, https://doi.org/10.1093/jxb/eri197.

Barnett, H. L. and Hunter, B. B., (1998). *Illustrated Genera of Imperfect Fungi*, *4th edn.* APS Press, Minnesota.

Beg, Q.K., Kapoor, M., Mahajan, L. and Hoondal, G. S. (2001). Microbial xylanases and their industrial applications: a review. *Applied Microbiology and Biotechnology*. 56 (3–4): 326–38. doi:10.1007/s002530100704.

Bischof, R. H., Ramoni, J. and Seiboth, B. (2016). Cellulases and beyond: the first 70 years of the enzyme producer. *Trichoderma reesei*. *Microbial cell factories* 15, 1-13.

Bourbonnais, R., Paice, M.G., Reid, I.D., Lanthier, P., and Yaguchi, M. (1995). Lignin oxidation by laccase isozymes from Trametes versicolor and role of the mediator 2, 2'-azinobis (3-ethylbenzthiazoline-6-sulfonate) in kraft lignin depolymerization. *Applied and Environmental Microbiology* 61, 1876-1880.

Brem, F. R. and Lips, K. (2008). "*Batrachochytrium dendrobatidis* infection patterns among Panamanian amphibian species, habitats and elevations during epizootic and enzootic stages."*Diseases of Aquatic Organisms*, 81, 189-202.

Cenis, J. L. (1992). Rapid extraction of fungal DNA for PCR amplification. *Nucleic Acids Research* 20: 2380.

Dugan, F. M. (2006). *The Identification of Fungi*. APS Press, Minessota.

Duncan, E. and Keay, S. M. (1990). Aberrant dikaryons in the Lycoperdaceae. *Mycologia*, 82:17-22.

Frost, G. M. and Moss, D. A. (1987) Production of Enzymes by Fermentation. In: Rehm, H. J., Reed, G. (Eds). *Biotechnology, Vol. 7a*, VCH, Weinheim, 65-102.

Galhaup, C. andHaltrich, D. (2001). Enhanced formation of laccase activity by the white-rot fungus *Trametes pubescens* in the presence of copper. *Appl Microbiol Biotechnol*. 56(1-2),225-32.

Gautam, S. P., Bundela, P. S., Pandey, A. K., Khan, J., Awasthi, M. K.and Sarsaiya, S.(2011).Optimization for the Production of Cellulase Enzyme from Municipal Solid Waste Residue by Two Novel Cellulolytic Fungi. *Biotechnology Research International*. https://doi.org/10.4061/2011/810425.

Gottschalk, L. M. F., Oliveira, R. A. and Bon, E. P. S. (2010). Cellulases, xylanases, β-glucosidase and ferulic acid esterase produced by *Trichoderma* and *Aspergillus* act synergistically in the hydrolysis of sugarcane bagasse.*Biochemical Engineering.*

Hatakka, A. (1994). Lignin-modifying enzymes from selected white-rot fungi: production and role in lignin degradation. *FEMS Microbiol. Rev*. 13,125–135.

Higuchi, T. (1990). Lignin biochemistry: biosynthesis and biodegradation. *Wood Sci. Technol*. 24,23–63.

Hood, I. A. (2006). The mycology of the Basidiomycetes. Rimbawanto P.K. and Beadle, C., ed., Heart rot and root rot in tropical Acacia plantations. *Proceedings of a*

workshop held in Yogyakarta, Indonesia, 7–9 February 2006. *ACIAR Proceedings* No. 124.

Hung, B. R., Lara, L., Patrón, M. A., Clappes, S., Ugarova, N. N., and Bechstedt, W. (1988). Tween 80 and proteose peptone effect on cellulase production. *ActaBiotechnologica*, 8(5), 461–464. doi:10.1002/abio.370080515.

Janusz, G., Kucharzyk, K.H., Pawlik, A., Staszczak, M. and Paszczynski, A.J. (2013). Fungal laccase, manganese peroxidase and lignin peroxidase: gene expression and regulation. *Enzyme and Microbial technology* 52: 1-12.

Jayasekara, S.K. (2019). Microbial cellulases: application in biofuel production and other value-added products and processes."M.Phil. thesis".Submitted to Post Graduate Institute of Science, University of Peradeniya, Sri Lanka.

Jayasekara, S.K., Abayasekara, C.L. and Ratnayake, R.R. (2018) Coculturing cellulolytic fungi: A method for enhancing cellulase production. *Proceedings ofWayamba International Conference*, (p.82), Kuliyapitiya: Wayamba University of Sri Lanka.

Kambura, A., Mwirichia, R., Kasili, R., Karanja, E., Makonde, H. and Boga, H.(2016). Diversity of fungi in sediments and water sampled from the hot springs of Lake Magadi and Little Magadi in Kenya. *African Journal of Microbiological Research*. 10. 330-338. 10.5897/AJMR2015.7879.

Kathirgamanathan, M., Abayasekara, C.L., Kulasooriya, S.A., Wanigasekera, A. and Ratnayake, R. R. (2017) Evaluation of 18 isolates of basidiomycetes for Lignocellulose degrading enzymes.*Ceylon Journal of Science*.46 (4), 77-84. doi: http://doi.org/10.4038/cjs.v46i4.7470.

Lombard V, Ramulu H. G., Drula E., CoutinhoP.M.andHenrissat B.(2013) The carbohydrate-active enzymes database (CAZy).*Nucleic Acids Res.* 2014;42:D490–5.

Magalhães, D.B., Carvalho, M. E. A., Bon, E., Neto, J. S. A. and Kling, S. H. (1996).Colorimetric assay for lignin peroxidase activity determination using methylene blue as substrate.*Biotechnology Techniques* 10, 273-276.

Mandels, M. and Weber, J. (1969).The production of cellulases. In: Cellulases and their applications. *Advances in Chemistry Series* 95 (23), 391 – 414.

Mandels, M., Andreotti, R. and Roche, C. (1976).Measurement of saccharifying cellulase.*Biotechnology and Bioengineering Symposium* 6, 21-33.

Miller, G. L., Grand, L. F.andTredway, L. P. (2011). Identification and Distribution of Fungi Associated with Fairy Rings on Golf Putting Greens. *Plant Disease*, 95(9), 1131–1138. doi:10.1094/pdis-11-10-0800.

Miller, G.L. (1959). Use of Dinitrosalicylic acid reagent for determination of reducing sugar.*Analytical Chemistry* 31 (3) 426 – 428.

Mohanan, K. (2019). Isolation, identification and screening of microbial strains and development of co-cultures and biofilms for lignocellulose degradation. "PhD thesis"

Mojsov, K. D. (2016). *Aspergillus Enzymes for Food Industries*, Editor(s): Vijai Kumar Gupta. New and Future Developments in Microbial Biotechnology and

Bioengineering, Elsevier, Chapter 16, 215-222,ISBN 9780444635051, https://doi.org/10.1016/B978-0-444-63505-1.00033-6.

Pardo, A. G. (1996). Effect of surfactants on cellulase production by *Nectria catalinensis*. Current Microbiology, 33(4), 275–278. doi:10.1007/s002849900113.

Peláez, F., Martínez, M.J. and Martinez, A.T. (1995).Screening of 68 species of basidiomycetes for enzymes involved in lignin degradation. *Mycological Research* 99 (1),37-42.

Peterson, R. and Nevalainen, H. (2012).*Trichoderma reesei* RUT-C30–thirty years of strain improvement. *Microbiology* 158: 58-68.

Pollegioni, L., Tonin, F. and Rosini, E. (2015). Lignin-degrading enzymes. *The FEBS journal* 282: 1190-1213.

Reese E.T., Lola J. E. and Parrish F. W. (1969). Modified substrates and modified products as inducers of carbohydrates. *J Bacteriol*.100,1151–1154.

Sharma, S., and Yazdani, S. S. (2016). Diversity of Microbial Cellulase System.New and Future Developments. *Microbial Biotechnology and Bioengineering, 49–64.* doi:10.1016/b978-0-444-63507-5.00006-x.

Sudha, A., Suganya, S.P., Janani, R., Priya, R., Nirmala, M. and Shankar, T. (2018). Biodiversity of cellulolytic fungi isolated from KattalagarKovil,Tamilnadu. *International Journal of Scientific Research and Review* 7(1), 221-234.

Sukumaran, R.K., Singhania, R.R., Pandey, A. (2005). Microbial cellulases; production, applications and challenges. *Journal of Scientific and Industrial Research*, Vol.64, Pp, 832-844.

Sumner, J. B.(1921) Dinitrosalicylic Acid: A reagent for the estimation of sugar in normal and diabetic urine. *The Journal of Biological Chemistry* 47, 5-9.

Uttamkumar, A., Tapwal, A., Kalkal, P., Varghese, S. and Chandra, S. (2014). Isolation and screening of cellulase producing fungi from forest waste. *International Journal of Pharmaceutical and Biological Archive* 5(1), 56-59.

White, T. J., Bruns, T., Lee, S.J.W.T. and Taylor, J.W. (1990).Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (Eds.) *PCR protocols: a guide to methods and applications*, Academic Press, New York. Pp. 315-322.

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 4

WILD EDIBLE MUSHROOMS OF RUDRAPRAYAG DISTRICT, UTTARAKHAND HIMALAYA: DIVERSITY, DISTRIBUTION AND ETHNOMYCOLOGY

Upendra Singh[1,*] and R. P. Bhatt[1]
[1]Department of Botany and Microbiology,
H.N.B. Garhwal University (A Central University),
Srinagar, Garhwal, Uttarakhand, India

ABSTRACT

Mushrooms have long been regarded all over the world as the most delectable, succulent of foods and one of the world's greatest natural resources since they have the ability to transform nutritionally valueless substances into high protein foods. The present study is a part of exploration of wild edible mushrooms and documentation of their use by inhabitants.

As a result, 23 species of wild edible macrofungi were identified. Of these, 13 species were ectomycorrhizal that live in symbiosis with trees of *Quercus, Rhododendron, Pinus, Lyonia, Myrica* and *Abies*. Among the all collected mushroom species, *Boletus indoedulis* constitutes the new regional record from Uttarakhand Himalaya.

Keywords: ectomycorrhiza, edible macrofungi, Northern India

[*] Corresponding Author's Email: upenrana04@gmail.com.

INTRODUCTION

Rudraprayag is one of the hilly districts, situated in Uttarakhand state of northern India. With an area of 1984 km^2 this district is located between 30° 16' to 30° 28' N latitude and 78° 58' to 78° 78' E longitude and has dynamic altitudinal variations ranging from 800 to 8000 m. The region is blessed with a rich forest growth, and the forest cover is about 1125km^2, including reserve forests. On the basis of physiognomy, species composition, structure, habitat and physiography forests of the district are classified into four types viz., temperate forest, temperate mixed forest, subtropical forest and subtropical mixed forest. The district is mostly covered by temperate forests and partially by the sub tropical forest. The forest cover of the Rudraprayag extends over an elevation range of 800 to 3500 m and most of the forests present are dominated by species of *Quercus*, *Rhododendron*, *Pinus*, *Cedrus*, *Cupressus*, *Abies*, *Picea*, *Lyonia* and *Betula*. These forests provide sufficient diversity in vegetation and the favourable environmental conditions to support the growth and development of macrofungi (Joshi et al. 2012; Raturi 2012; State of Forest report 2011).

Wild edible mushrooms have been collected and consumed by people for thousands of years (Boa 2004). The archaeological record reveals edible species associated with people living 13000 years ago in Chile (Rojas and Mansur 1995) but it is in China where the eating of wild fungi is first reliably noted, several hundred years before the birth of Christ (Aaronson 2000). There are historical evidences of mushroom consumption in ancient India (Chopra 1933). In an attempt of macrofungal exploration in Rudraprayag district of Uttarakhand Himalaya, a large number of wild mushroom species were collected. Through critical macro- and micromorphological studies of these species, a total of 23 wild edible mushroom species were identified.

MATERIALS AND METHODS

Numerous field trips were made to four study sites in the Rudraprayag district of Uttarakhand Himalaya. These were Chopta-Baniyakund (2200 – 3200 masl), Chirbatiya (2050 – 2250 masl), Hariyali Devi forest (1600 – 1750 masl) and Jakhdhar (1550 – 1800 masl) (Figure 1). The field trips took place between March and September of 2017 − 2018 and a total of 156 specimens of macrofungi were collected. Information related to the use of wild edible mushrooms by local villagers was collected through verbal interviews and questionnaires. Standard methods were followed for the collection, preservation and macro- and microscopic studies of the specimens (Singer 1986). Photographs of all specimens were obtained using Sony digital DSC–W730 and Cannon Power Shot SX 50 cameras.

Figure 1. Four study sites in the Rudraprayag district of Uttarakhand Himalaya.

Identification of species was based on critical observations of the specimens and perusal of the relevant literature (e.g., Bhatt et al. 2003; Boa 2004; Buyck et al. 2018; Das and Sharma 2005; Hesler and Smith 1979, Heilmann-Clausen et al. 1998; Kibby and Fatto 1990; Yang 1997; Singh and Das 2019). Mycorrhizal association were ascertained by digging the soil and tracing the roots of the tree to the fructifications (Young 1936, 1940; Zak 1971, 1973) and based on relevant literature. The color terminology used is that of the *Methuen Handbook of Colour* (Kornerup and Wanscher 1978).

RESULTS AND DISCUSSION

During this study, 23 species belonging to 16 genera and 14 families of wild edible macrofungi were identified from different selected study sites in Rudraprayag district.Observations on ecological preference of the species revealed that 13 species were ectomycorrhizal that live in symbiosis with trees of *Quercus, Rhododendron, Pinus, Lyonia, Myrica* and *Abies.*

Table 1. List of wild edible macrofungi in Rudraprayag district of Uttarakhand Himalaya

S. No.	Scientific Name	Family	Local Name	Habitat	Growing Period	Altitude (masl)	Ecological preference
1.	*Agaricus campestris* L.	*Agaricaceae*	Unknown	On ground among grasses.	July to September	1920–2170	Saprotrophic
2.	*Amanita caesareoides* Lj.N. Vassiljeva	*Amanitaceae*	Unknown	On ground under *Quercus spp.*	July to September	1750–2550	Ectomycorrhizal
3.	*Amanita hemibapha* (Berk. and Broome) Sacc.	*Amanitaceae*	Haldya-chyun	On ground under *Pinus roxburghii*	July to September	1600–1750	Ectomycorrhizal
4.	*Boletus indoedulis* D. Chakr., K. Das, A. Baghela, S. Adhikari and Halling	*Boletaceae*	Unknown	On ground under *Quercus spp.*	July to September	1900–2600	Ectomycorrhizal
5.	*Cantharellus cibarius* Fr.	*Hydnaceae*	Pirul-chyun	On ground under *Quercus leucotrichophora*, *Rhododendronarboreum*, *Pinusroxburghii*.	July to October	1600–2400	Ectomycorrhizal
6.	*Cantharellus lateritius* (Berk.) Singer	*Hydnaceae*	Unknown	On ground under *Quercus leucotrichophora* and *Pinus roxburghii*	July to September	1600–1650	Ectomycorrhizal
7.	*Craterellus cornucopioides* (L.) Pers.	*Hydnaceae*	Unknown	On ground under *Quercus leucotrichophora*	July to September	1600–2250	Ectomycorrhizal
8.	*Grifola frondosa* (Dicks.) Gray	*Meripilaceae*	Unknown	On the dead or living stem of *Quercus sp.*	July to September	2300–3100	Saprotrophic or Parasitic
9.	*Hericium coralloides* (Scop.) Pers.	*Hericiaceae*	Guccha-Chyun	On dead wood of *Quercus sp*	July to August	2400–3150	Saprotrophic
10.	*Hericium erinaceus* (Scop.) Pers.	*Hericiaceae*	Guccha-Chyun	On dead wood of *Quercus sp.*	July to September	2350–2550	Saprotrophic
11.	*Hericium rajendrae* U. Singh and K. Das	*Hericiaceae*	Lal Guccha-Chyun	On living tree trunk of *Quercus sp.*	July to August	2500–2950	Saprotrophic

S. No.	Scientific Name	Family	Local Name	Habitat	Growing Period	Altitude (masl)	Ecological preference
12.	*Lactarius deliciosus* (L.) Gray	*Russulaceae*	Unknown	On ground under *Abies pindrow*	July to September	2250–3150	Ectomycorrhizal
13.	*Lactarius subindigo* Verbeken and E. Horak	*Russulaceae*	Unknown	On ground under *Quercus Leucotrichophora* and *Rhododendron arboreum*	July to October	1800–1950	Ectomycorrhizal
14.	*Lactifluus hygrophoroides* (Berk. and M.A. Curtis) Kuntze	*Russulaceae*	Dudhi-chyun	On ground under *Quercus leucotrichophora*, *Lyonia ovalifolia*, *Myrica esculenta* and *Rhododendron arboreum*	July to September	1750–2500	Ectomycorrhizal
15.	*Laetiporous sulphureus* (Bull.) Murrill	*Fomitopsitaceae*	Unknown	On dead or living wood of *Quercus sp*	July to August	2450–3150	Saprotrophic
16.	*Macrolepiota procera* (Scop.) Singer	*Agaricaceae*		On ground in forests and grassy area	July to September	1650–2600	Saprotrophic
17.	*Morchella esculenta* (L.) Pers.	*Morchelaceae*	Gucchi	On ground in the forest and along the road sides	March to August	1650–2350	Saprotrophic
18.	*Phaeotremella foliacea* (Pers.) Wedin, J.C. Zamora and Millanes	*Phaeotremellaceae*	Unknown	On the dead log of *Quercus sp.*	July to September	2300–2450	Saprotrophic
19.	*Pleurotus ostreatus* (Jacq.) P. Kumm.	*Pleurotaceae*	Unknown	On dead logs or living trees *Quercus spp.*	July to September	1900–2350	Saprotrophic
20.	*Ramaria sanguinea* (Pers.) Quel.	*Gomphaceae*	Unknown	On ground under *Quercus spp*	July to September	2350–2580	Ectomycorrhizal
21.	*Russula brevipes* Peck	*Russulaceae*	Unknown	On ground under deciduous and coniferous trees	July to September	1750–3150	Ectomycorrhizal
22.	*Russula cyanoxantha* (Schaeff.) Fr.	*Russulaceae*	Unknown	On ground under deciduous and coniferous trees	July to September	1750–2450	Ectomycorrhizal
23.	*Russula virescens* (Schaeff.) Fr.	*Russulaceae*	Unknown	On ground under *Quercus leucotrichophora* and *Pinus roxburghii*	July to September	1620–2100	Ectomycorrhizal

Among the all collected mushroom species, *Boletus indoedulis* constitutes the new regional record from Uttarakhand Himalaya.

From 48 questionnaires and verbal interviews with tribal and local inhabitants, it was concluded that only about 23% of people have knowledge of mushroom consumption. Of the species collected, *Grifola frondosa*, *Hericium coralloides, H. erinaceus, Laetiporus sulphurous, Macrolepiota procera, Morchella esculenta, Pleurotus ostreatus* and *Ramaria sanguinea* were found to be the most significant seasonal food species. Local people (called as 'Garhwali'), Van- gujjar (a tribal caste) and Nepali residing in this area use these wild mushrooms for table purposes. Cooking methods strictly include cleaning of fresh mushrooms, boiling in water and adding spices to diminish the ill effects. Consuption of alcohol is strictly prohibited with mushroom diet.Fruiting bodies are cut open, air-dried and pickled for long time storage and saved in the form of sundried flakes for use after the rainy season. Among the 23 species of edible mushroom recorded, only *Morchella esculenta* is used for trade in local markets.

CONCLUSION

This study is a direct benefit to people as forests of the high altitudes of Himalaya resides variety of mushrooms many of which are edible and can be consumed as food by the inhabitants. During our work of exploration through Rudraprayag we observed that transportation becomes tough during the rains for interior regions of this district. Due to which people (shopkeepers, tribes and people living with their cattles in pastures) suffer to fulfil their daily food requirements. In this prospect wild mushrooms can be a healthy, suitable and easily available option for these people. In addition, as we know mushrooms are treasures of proteins and digestible fibres which make these not worthy to perish unused. Our study helped these people to identify and consume some of wild mushrooms, which are popular and prized in European and American countries. Evidently, there is a great need for extensive exploration of the wild edible mushrooms from this fragile part of the Himalaya. There is also a wide gap between the knowledge of flora and conservation status of mushrooms in India, warranting detailed ecological and systematic investigation.

ACKNOWLEDGMENTS

The authors are grateful to the Head, Department of Botany and Microbiology, HNB Garhwal University, Srinagar Garhwal, for providing the necessary laboratory facilities during the course of present study and also thankful to Govind Ballabh Pant National

Institute of Himalayan Environment and Sustainable Development (GBPNIHESD) Kosi-Katarmal, Almora for the financial assistance received to carry out this study. Dr. Priyanka Uniyal, Dr. Tahir Mehmood and Mr. Aniket Ghosh (HNBGU) are duly acknowledged for their assistance in field.

REFERENCES

Aaronson, S. (2000). Fungi. In K.F. Kiple and K.C. Ornelas, eds. *The Cambridge world history of food*, pp. 313 - 336. Cambridge, UK, Cambridge University Press. 1958 pp.

Bhatt, R. P., Tulloss, R. E., Semwal, K. C., Bhatt, V. K., Moncalvo, J.- M. and Stephenson, S. L. (2003). "Amanitaceae reported from India. A critically annotated checklist". *Mycotaxon*,88, 249 - 270.

Boa, E. (2004) *Wild edible fungi: A global overview of their use and importance to people*. In: Non wood Forest products, Vol. 17. Food and Agriculture Organization, Rome. 147 pp.

Buyck, B., Zoller, S. and Hofstetter, V. (2018) Walking the thin line… ten years later: the dilemma of above- versus below-ground features to support phylogenies in the Russulaceae (Basidiomycota). *Fungal Diversity*,89(1), 267 - 292.

Chopra, R. (1933). *Indigenous drugs of India: their scientific cultivation and manufacture, with numerous suggestions intended for educationists and capitalists*. Academic Publishers, Kolkata.

Das, K. and Sharma, J. R. (2005) *Russulaceae of Kumaon Himalaya*. Botanical Survay of India, 255 pp.

Heilmann-Clausen, J., Verbeken, A. and Vesterholt, J. (1998). *The Genus Lactarius*. The Danish Mycological Society, Denmark, 287 pp.

Hesler, L. R. and Smith, A. H. (1979) *North American Species of Lactarius*. The University of Michigan Press, Ann Arbor.

Joshi, S., Bhatt, R. P. and Stephenson, S.L. (2012). The current status of the family Russulaceae in the Uttarakhand Himalaya, India. *Mycosphere*,3(4), 486 - 501.

Kibby, G. and Fatto, R. (1990) *Keys to the Species of Russula in Northeastern North America*. Kibby Fatto Enterprises, Somerville, New Jersey.

Kornerup, A. and Wanscher, J.H. (1978). *Methuen Handbook of Colour*. 3rd Ed. Eyre Methuen, London.

Raturi, G.P. (2012). Forest Community Structure along an Altitudinal Gradient of District Rudraprayag of Garhwal Himalaya, India. *Ecologia*, 2, 76 - 84.

Rojas, C. and Mansur, E. (1995). Ecuador: Informaciones generals sobre productos non madereros en Ecuador. In: *Memoria, consulta de expertos sobre productos forestales no madereros para America Latina Y el Caribe*. Serie Forestal #1. Santiago, Chile, pp. 208 - 223.

Singh, U. and Das, K. (2019) *Hericium rajendrae* sp. nov. (Hericiaceae, Russulales): an edible mushroom from Indian Himalaya. *Nova hedwigia,* 108 (3-4), 505 – 515.

Sate of Forest report (2011). Envis Centre of Himalayan ecology, GB Pant Institute of Himalayan.

Singer, R. (1986). *The Agaricales in Modern Taxonomy*. 4th Ed. Bishan Singh Mahendra Pal Singh, Dehradun.

Yang, Z. L. (1997). Die *Amanita*-Arten von Südwestchina. Bibliotheca *Mycologica,* 170, 1 - 240.

Young, H. E. (1936). A mycorrhiza forming fungus of *Pinus*. *Journal of the Australian Institute of Agricultural Science*,2, 32 - 34.

Young, H. E. (1940). Mycorrhizae and growth of *Pinus* and *Araucaria*. The influence of different species of mycorrhizal forming fungi on seedling growth. *Journal of the Australian Institute of Agricultural Science*,6, 21 - 25.

Zak, B. (1971). Characterization and identification of Douglas fir mycorrhizae. In: *Mycorrhyzae*, pp 38-53. Ed. Hocekaylo. E. USDA. Misc. Publ. 1189. U.S. Govt. Printing office, Washington, D.C.

Zak, B. (1973). Classification of ectomycorrhizae. In: *Ectomycorrhizae: Ecology and Physiology*, pp 42-78. Edt. Marks, G.C. and Kozlow- ski, T.T. Acad. Press, Inc., NewYork.

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 5

RHIZOSPHERIC FUNGI: DIVERSITY AND THERAPEUTIC POTENTIAL

Mahendra Kumar Sahu and Harit Jha*
Department of Biotechnology, Guru Ghasidas Viswavidyalaya,
Bilaspur, Chhattisgarh, India

ABSTRACT

Fungi are heterotrophic eukaryotic multicellular organisms that are the source of a large number of potent secondary metabolites. These secondary metabolite products are reported for their therapeutic values like antioxidant kojic acid (Pandit et al., 2018), antimicrobial Patulin (Demain and Fang 2000), anticancer 2-morpholinoethylester (Miranda et al., 2010), immunomodulatory drug cyclosporine-A (Hunt et al., 2010) and anti-inflammatory astaxanthin (Barredo et al., 2017). Other useful products derived from fungi are insecticides like Versimide (Meligy et al., 2014), cholesterol-lowering drugs like Lovastatin (Otto et al., 1995), anticoagulants like tacrolimus (Niwa et al., 2003) and Griserofulvin for Dermatomycosis (Taboada and Grooters 2008). These compounds of fungal origin are produced as a response to biotic and abiotic stress and for protection and self-defence from enemies and may also affect other interacting organisms. It is proposed that because the rhizospheric soil is most active region where prokaryotic species like bacteria and eukaryotic like fungi, worms and plants interact, this biome may create conditions conducive to the production of novel and useful secondary products.

Keywords: Secondary metabolite, rhizospheric, antioxidant, anti-proliferative

* Corresponding Author's Email: harit74@yahoo.co.in.

INTRODUCTION

Soil represents the most active region of the world inhabited by a diverse group of microbial community in close relationship with different plant roots and is responsible for the occurrence of a variety of biological and physiological processes. Soil is a platform to provide interaction between inhabiting microorganism and plant roots, thereby contributing to the widespread functions such as soil fertility, plant productivity, heavy metal biosorption, disease mitigation, regulation of atmosphere, nutrient recycling, and overall ecosystem stability. Soil exhibits the most dynamic environment and is responsible for physiological and biogeochemical processes in a particular region termed as 'hotspots'. The soil hotspots can be categorized into four classes such as rhizosphere (root exudates), detritusphere (recalcitrant organic compounds), biopores (processing of recalcitrant organics), and aggregate surfaces (leached out substances from detritusphere) (Kuzyakov and Blagodatskaya 2015). A belowground, root-affected soil portion known as rhizosphere, represents an exclusive ecological niche, surrounded by many range of microbiota such as bacteria, nematodes, actinobacteria and fungi living near the root system for essential ecosystem services. Fungi are traditional vital sources of natural products and they continue to provide new chemical entities with novel biological activities (Baker and Alvi 2004). Natural products and their derivatives are a major source of new anticancer agents. Fungal derived natural products have been an excellent source of pharmacoactive compounds. These chemical entities from fungal secondary metabolites have been observed to be either beneficial (such as a broad range of useful antibiotics and therapeutic activities as well as less desirable immunosuppressant and toxic substances) (Miranda et al., 2010) or harmful to mankind. In this chapter different groups of fungi associated with the rhizospheric region of plants and their therapeutic potential with possible applications in diverse sectors are discussed.

RHIZOSPHERIC REGION

During seed germination and seedling growth, the developing plant interacts with a range of microorganisms present in the surrounding soil. As the seed germinates and roots forms, it discharges organic material that drives the growth of active microbial populations in surrounding soil regions. This phenomenon is referred to as the rhizosphere effect (Nihorimbere et al., 2011). The rhizosphere effect can thus be viewed as the creation of a dynamic environment where microbes can develop and interact. The term “rhizosphere” was coined for the first time by Hiltner in the early twentieth century which represents a hot spot for numerous microorganisms. The rhizosphere is the region of soil nearest to the plant root system. A large number of metabolites from living root

hairs or fibrous root systems are released into the soil environment during the active growth of the plant. The released metabolites act as chemotactic signal molecule leading to the movement of fungi and other microbes towards the root surface, it also represents the main nutrient sources available for support, growth, and persistence in the rhizosphere.

The rhizosphere is a core region of intense biological activity due to the food supply provided by the root exudates. Bacteria, actinomycetes, fungi, protozoa, slime molds, algae, nematodes, enchytraeid worms, earthworms, millipedes, insects, small animals and soil viruses compete constantly for water, food, and space. Soil chemistry and pH can influence the growth and function of microbes in the rhizosphere. Most of the microorganisms present in soils do not interact with plant roots, possibly due to the constant and diverse secretion of antimicrobial root exudates. However, some microorganisms do interact with plants. These interactions can be pathogenic (invade and kill roots and plants), symbiotic (mutual relationship), harmful (arrested plant growth), saprophytic (depend on dead roots and plants) or neutral (zero effect on plants). Relationships beneficial to crops and agriculture involve mycorrhizae, legume nodule formation, and production of antimicrobial compounds that inhibit the growth of pathogens (Abbott and Murphy 2003). These fungi are referred to as plant growth-promoting mychorrhiza.

INTERACTION BETWEEN RHIZOSPHERIC MICROORGANISMS AND PLANT

The soil region associated with plants is occupied by different types of microorganisms. Biotic and abiotic factors are responsible for the structure and composition of the rhizospheric microbial community which reflects in the developmental stage of the host plant, genotype, or cultivar of the associated host plant and plant health. Abiotic factors such as soil properties, nutrient status, and climatic conditions also greatly influence the structural and functional importance of the plant-associated rhizospheric microbial community (Hartmann et al., 2009).

Plant growth-promoting rhizobacteria (PGPR) fulfills important functions for plant growth and health in various manners. Diverse mechanisms are involved in the suppression of plant pathogens, which is often indirectly connected with plant growth. In this environment, very exclusive and intensive interactions take place between the microfauna, plant, and soil. Exchanges of signal molecules and biochemical interactions between plants and soil microbes have been described and reviewed (Pinton et al., 2007). The rhizosphere inhabiting microorganisms compete for water, nutrients, and space and sometimes improve their competitiveness by developing an intimate association with

plants (Hartmann et al., 2009). Figure 1 represents the correlations between biotic factors, abiotic factors and rhizosphere region.

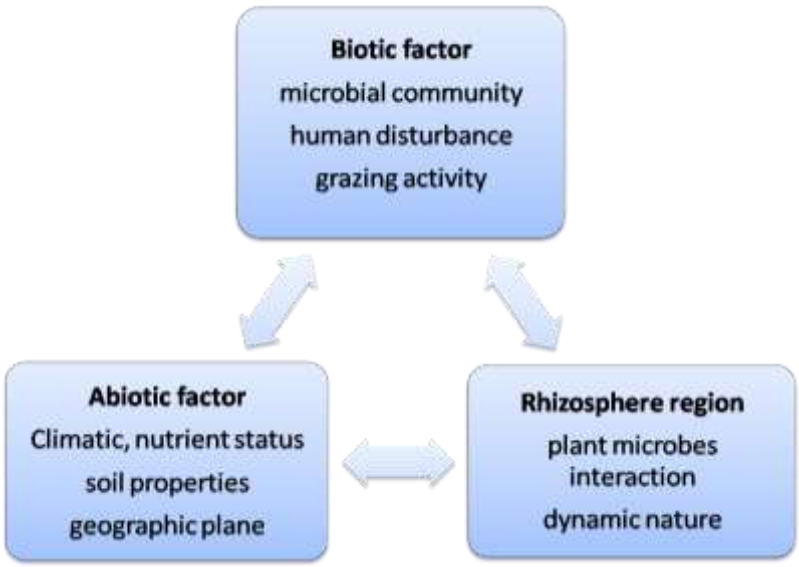


Figure 1. Overview of different factors reversibly associated with rhizosphere region.

COMPOSITION OF RHIZOSPHERIC MICROBIOTA AND ITS IMPORTANCE

Fungus, bacteria, algae and nematodes etc. are notable organisms that readily produce lots of natural products, often called secondary metabolites. Secondary metabolites are not required for primary metabolic processes but these are useful for protecting itself from harmful organisms. In many cases, the benefit of these compounds is unknown. However, interest in these compounds is considerable, as many natural products with a biological activity which may be harmful, such as mycotoxins and phytotoxins, or beneficial, such as antibiotics and other pharmaceuticals (Demain and Fang 2000). The microorganisms of the rhizospheric range produce many secondary metabolites. These metabolites accomplish the pharmacological activity by interacting with the microorganisms, plant cells, and soil components. Secondary metabolites have the ability to regulate the prokaryotic cells, metabolism of other fungus and plant cells. Production of low molecular weight compounds is not necessary for the primary growth of the fungus but may provide several benefits to the organism like self-growth, protection, communication and interaction. Many secondary metabolites are used in industry and medicine (Bennett 2003). The role of fungi in the soil is extremely important and critical to the proper functioning of the soil ecosystem by intricately affecting

nutrient recycling, maintaining plant growth and development, and maintaining environmental sustainability (Shivanna and Vasanthakumari 2011) (Figure 2).

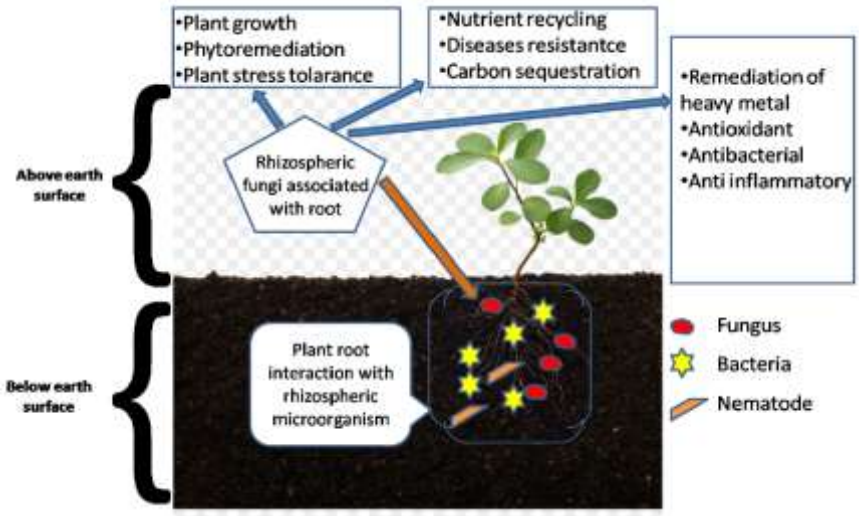


Figure 2. A schematic representation of plant-associated rhizosphere and its associated microbiota. The rhizospheric fungi exhibit widespread applications in the field of agriculture, pharmaceutical and biomedical industries.

RHIZOSPHERIC FUNGI

Some of the microbes that inhabit the rhizospheric area are fungi that are able to persist very efficiently in the roots and rhizosphere soil of crop plants. The idea of "Rhizosphere" exhibits a mutual response between roots and microorganisms. As living plants create a unique habitat around the roots, the microbial population on and around the roots is considerably higher than that of the root-free soil environment and the differences may be both qualitative and quantitative (Lim et. al., 2015). Fungi are important components of biodiversity which has a major role in global ecological processes. Fungi contain unicellular, multinucleate, and multicellular forms. All existing fungi are classified based on their reproductive spores and the nature of their multinucleate or multicellular filaments known as hyphae. They are dependent on the nature of the substrate and temporal region that favors the colonization, growth and substrate possession of the fungi (Rani 2010). The rhizosphere microbes also play a very important role in improving medicinal values of plants. Rhizospheric microbes affect the plant physiology by imparting several useful effects such as nitrogen fixation, nutrient uptake, and production of secondary metabolites in the medicinal and aromatic plants. There is renewed interest in the research of the relation between rhizosphere microbes associated with medicinal plants for the improvement of quality of medicinal plants. A

large variety of fungi and bacteria recognized in the rhizosphere soil of medicinal plants show a significant effect on secondary metabolite alteration and uptake of plant nutrients. There are reports that rhizosphere fungi not only enhanced the growth parameters in plants but also considerably modulated essential oil quality (Table 1) (Shaikh 2018).

THERAPEUTIC POTENTIAL OF RHIZOSPHERIC FUNGUS

Nature has immense potential to provide a broad spectrum of structurally diverse secondary metabolites (Badri et. al., 2009). The distribution of secondary metabolites is also unique and some metabolites are found in a range of related microorganisms, while others are only found in one or a few species. Microorganisms are considered as miniatures of chemical factories. Filamentous microorganisms such as fungi and actinomycetes are the main source of secondary metabolites with antibiotic activity. The filamentous microorganisms freshly isolated from the soil are the best source of secondary metabolites. History of microbial biotechnology reveals that many desirable materials like food, beverages, pesticides and antibiotics were produced using microorganisms. A very well-known fungal secondary metabolite is Penicillin, as an antibiotic that changed the practice of medicine, changed the route of pharmaceutical research, influenced the course of World War II and saved countless lives. With the advent of new screening and isolation techniques, a variety of β-lactam-containing molecules and other types of antibiotics have been identified. Most of the secondary metabolites were used in clinical practice as antibiotics. Some metabolites like mithramycin, bleomycin, daunomycin and adriamycin were used as antitumor compounds (Usha 2011). Secondary metabolites are also reported to have activity as anabolics, anesthetics, anticoagulants, anti-inflammatories, immunosuppressants (cyclosporine-A and tacrolimus), hemolytic, hypocholesterolemics (statin), and vasodilatories (Niwa et al., 2003). In a report, more than 23,000 active metabolites were assessed from which 17,000 antibiotics originated from the microorganisms (Berdy 2005). The basic functions or importance of secondary metabolites for the organisms is usually ecological as they are used to defend against predators, parasites, diseases, interspecies competition and to facilitate the reproductive processes (coloring agents, attractive smells, etc).

Use of Fungal Metabolites against Plant Diseases

Strong mycorrhizal association with plant induce fungus to develop natural products against the plant pathogens for example, Gliotoxin is isolated from *Gliocladium* species, which is active against wood-rotting fungus, *Armillarriab mellea* (Scharf et al., 2012).

Verticellium chlymadosporium showed strong inhibitory effect on *M. phaseolina, R. solani* and *F. solani* both *in vitro* and *in vivo* (Ehteshamul-Haque et al., 1994); *Peniophora gigantean* is used to control infestation caused by *Heterobasidium annosum*, root rot of pines (Asiegbu et al., 2005); *Trichoderma harzianum* produces an antifungal agent, alkyl-pyrone which is active against a wide range of fungi and bacteria (Claydon et al., 1987). *Pisolithus arhizus* produces the compounds hydroxy benzyl formic acid and R-(-)-p-hydroxymendelic acid which is active against *Truncatella hartigii* (Kope and Fortin 1991). Most of the *Trichoderma* sp. produce gliotoxin, active against root pathogenic fungus *Rhizoctonia solani* (Henis et al., 1983).

Fungal Secondary Metabolites against Plant Parasitic Nematodes

Soil contains various nematophagous fungi which are natural enemies to nematodes. A large number of nematophagous fungi with significant nematicidal activity have been discovered so far (Askary et al., 2015). The fungus *Paecilomices lilacinus* was also widely tested for nematode control (Li et al., 2004). *Vericillium chamydosporium* also showed significant activity against the cysts of *Meloidogyne spp*. and *Heterodera* spp. (Kerry et al., 2000).

Fungal Metabolites as Insecticidal Compounds

Aspergillus versicolor produces well-known contact insecticides versimide and methyl-α-(methylsuccinimido) acrylate. Acetylcholine esterase, a neurotransmitter is restricted by the action of acetylene and furanocoumarins. Larvicidal activity was shown by tenuazonic acid and diacetoxyscirpenol produced from *Alternaria tenuis* and *Fusarium lateritium* respectively (Meligy et al., 2014). Various fungi produced scirpene, patulin, rubratoxin, cycloheximide, trichothecin. Thiolutin and actinomycin also had insecticidal activity (Table 2) (Cole and rolinson 1972).

Fungal Metabolites as Antimicrobial Agents

Identification of a structurally novel compound that possesses novel and potentially useful biological activity and the process of new drug discovery is driven largely by the desire to overcome the lacunae of existing drugs. Antibiotics are heterogeneous organic molecules of microbial origin and are deleterious to the growth and metabolic activities of other microorganisms. The most important field in microbial biotechnology is antibiotic research, which is to produce compounds against pathogenic microorganisms.

Table 1. Fungi associated with rhizosphere range of the medicinal plant

S. No.	Plant species	Microorganisms associated with root	References
1	*Azadirachta indica*	*Acaulospora scrobiculata Glomus fasciculatum*	Radhika and Rodrigues (2010)
2	*Atractylodes lancea Dioscorea zingiberensis, Euphorbia pekinensis Ophiopogon platyphyllum, Pinellia ternata.*	*Fusarium sp. Verticillium sp*	Dai et al., (2009)
3	*Bacopa monnieri*	*Glomus mosseae Glomus intraradices*	Khaliel et al.,(2011)
4	*Cassia alata C. occidentalis C. sophera*	*Glomus spp*	Chatterjee et al., (2010)
5	*Panax Ginseng*	*Soil fungi*	Li et al., (2018)
6	*Paeonia suffruticosa*	*Glomus Acaulospora Scutellospora*	Shi et al., (2013)
7	*Sorghum bicolor*	*G. mosseae* *G. intraradices*	Sun and Tang (2013)
8	*Artemisia annua*	*Glomus mosseae G. aggregatum G. fasciculatum G. intraradices*	Awasthi et al., (2011)
9	*Curculigo orchioides*	*G. geosporum* *G. microcarpum*	Sharma et al., (2008)
10	*Curcuma mangga*	*Alternaria brassicicola, Colletotrichum gloeosporioides Fusarium oxysporum, Penicillium digitatum, Sclerotium rolfsii*	Khamna et al., (2009)
11	*Centella asiatica and Ocimum sanctum*	*AM and endophytic fungi*	Sagar and Kumari (2009)
12	*Lycium barbarum*	*Gi. margarita, G. albidum*	Tang et al., (2004)
13	*Magnolia cylindrica*	*Acaulospora Glomus Gigaspora Scutellospora*	Yang et al., (2011)
14	*Echinacea purpurea*	*G. intraradices*	Araim et al., (2009)
15	*Leptadenia reticulata Mitragyna parvifolia Withania coagulans*	*G. constrictum G. fasciculatum G. geosporum G. intraradices G. mosseae G. rubiforme*	Panwar and Tarafdar (2006)
16	*Euptelea pleiosperma*	*G. ambisporum, G. constrictum, G. fasciculatum, G. geosporum, G. hyderabadensis, G. intraradices, S. verrucosa*	Wang et al., (2008)
17	*Arnica montana*	*G. geosporum, G. constrictum, G. intraradices, G. mosseae, G. macrocarpum, G. fasciculatum, G. versiforme*	Jurkiewicz et al., (2010)
18	*Bacopa monnieri*	*Glomus mosseae Glomus intraradices*	Khaliel et al., (2011)
19	*Panax notoginseng*	*G. versiforme, G. monosporum, G. mosseae, G. constrictum, G. claroideum*	Zhang et al., (2011)

Table 2. Therapeutic activity of fungal secondary metabolites

S. No.	Therapeutic value	Fungal products	Source of fungi	Application	Reference
1	Antioxidant	2-hydroxy methylenzoic acid, 2- hydroxy tetradecyl ester	*Talaromyces purpurgenus*	Reducing the reactive oxygen species	Pandit et al., 2018
		kojic acid, maltol	*Aspergillus allahabadii*	Molecule inhance the scavenging properties	Rajamanikyam et al., 2017
2	Antimicrobial	Patulin	*Penicillium, Aspergillus, and Byssochlamys species*	metal transporting agents	Demain and Fang 2000
		polyene	*Aspergillus spp*	their mode of action is disruption of the fungal cell membrane by binding to ergosterol, the main sterol in the membrane	Dowd et al., 2017
3	Anticancer	2-morpholinoethylester	*Penicillium brevicompactum*	associated with gain-of-function mutations in zinc finger-containing transcription factors Pdr1p and Pdr3p.	Miranda et al., 2010
		Fusidienol	*Fusarium griseum*	inhibits farnesyl protein kinase transferase	Kelloff et al., 1997
		Taxol	*Nodulisporium sylviforme and Taxodium distichurn*	promotes polymerization and stabilization of tubulin to microtubules and interferes with the mitotic spindle	Li et al., 1998
4	Immunomodulatory drug	Cyclosporine-A	*Tolypocladium inflatum*	enhances neural precursor cell survival	Sachewsky 2014
5	Anti-inflammatory	Astaxanthin	*Xanthophyllomyces dendrorhous*	by arresting cell cycle progression and promoting apoptosis	Barredo et al., 2017
6	Dermatomycosis	Griserofulvin	*Penicillium griseofulvum*	Tubulin Inhibiting Agent used to treat fungal infections of the skin	Taboada and Grooters 2008
7	Cholestrol lowering drugs	Lovastatin	*Pleurotus ostreatus*	to treat high blood cholesterol and reduce the risk of cardiovascular disease	Otto et al., 1995
8	Immunosuppressive drug	Tacrolimus	*Streptomyces tsukubaensis*	necessary to ensure stable renal function and good tolerability	Melo et al., 2015
9	Antitumor	Pentostatin, peplomycin, and epirubicin	*Aspergillus spp.*	treatment of chronic lymphocytic leukemia and reduced cardiac toxicity	Grever et al., 2003

Table 2. (Continued)

S. No.	Therapeutic value	Fungal products	Source of fungi	Application	Reference
10	Fungiside	Gliotoxin	*Gliocladium species*	active against wood-rotting fungus, *Armillarriab mellea*	Scharf et al., 2012
			Trichoderma sp.	active against root pathogenic fungus *Rhizoctonia solani*	Henis et al., 1983
11	Inhibitory effect		*Verticellium chlymadosporium*	inhibitory effect on *M. phaseolina*, *R. solani* and *F. solani*	Ehteshamul-Haque et al.,1994
12	Infestation		*Peniophora gigantean*	to control infestation caused by *Heterobasidium annosum*, root rot of pines	Asiegbu et al., 2005
13	Antifungal agent	Alkyl-pyrone	*Trichoderma harzianum*	active against wide range of fungi and bacteria	Claydon et al., 1987
		Hydroxy benzyl formic acid and R-(-)-p-hydroxymendelic acid	*Pisolithus arhizus*	active against Truncatella hartigii	Kope and Fortin 1991
14	Cytotoxic activity	Roridins	*Myrothecium sp.*	against colon tumor cell lines	wagennar et al., 2001
15	Insecticide (larvicidal activity)	Versimide and methyl-α-(methylsuccinimido) acrylate	*Aspergillus versicolor*	a novel contact insecticide	Cole and rolinson 1972
		Acetylene and furanocoumarins Tenuazonic acid and diacetoxyscirpenol	*Alternaria tenuis* *Fusarium lateritium*	Act as inhibitors of acetylcholine esterase, a neurotransmitter.	Meligy et al., 2014

Fungi produce various types of metabolites used in current chemotherapy, for example, penicillin, cephalosporin and fusidic acid, which have antibacterial and antifungal activity (Sarker et al., 2007). After the discovery of penicillin in 1928 from *P. notatum*, the modern era in the research of antibiotics started. Antibiotics like 2-hydroxy methylenzoic acid 2 hydroxy tetradecyl ester from *Talaromyces purpurgenus*, (Pandit et al., 2018) kojic acid and maltol from *Aspergillus allahabadii* (Rajamanikyam et al., 2017) are produced and used in medicine. Most of the β-lactam antibiotics like pencillin, cephalosporin and their relatives are produced from *Penicillium* and *Cephalosporium* group whereas polyene antibiotics are produced from *Aspergillus spp.* (Liras, et al., 2005).

Fungal Metabolites as Antitumor Agents

Myrothecium spp. Produce roridins having cytotoxic activity against colon tumor cell lines (Wagennar et al., 2001). *Aspergillus* dimorphicus produces several antitumor agents (Xu et al., 2015). Pentostatin, peplomycin, and epirubicin are the commercialized antitumor agents isolated from fungi (Grever et al., 2003). Fusidienol metabolite is formed by *Fusarium griseum* which inhibits farnesyl protein kinase transferase responsible for cancer (Kelloff et al., 1997). The metabolite of *Nodulisporium sylviforme* and *Taxodium distichurn* also produces taxol (Li et al., 1998). Increased clinical needs, multidrug resistance strains, new emerging pathogens, neoplastic and viral diseases led to the search and discovery of new pharmacophores.

Fungal Metabolites as Antioxidants Agents

Secondary metabolites have the capability to act as antioxidants and antimicrobials (Barapatre et al., 2015; Aadil et al., 2014). Antioxidants provide defense against metal toxicity by trapping free radicals, thus terminating the chain reaction by chelating metal ions and preventing the reaction with reactive oxygen species or by chelating metals and maintaining them in a redox state leading to inability to reduce molecular oxygen (Swaran, 2009). Some molecules have been characterized as DPPH free radical scavengers, such as terrestrols (Chen et al., 2008). The variecolorins (Zhou et al., 2010), compound JBIR-124 (Kawahara et al., 2012) and Sargassopenillines A and E (Zhuravleva et al., 2014) are examples of secondary metabolites that act as antioxidant, isolated from fungi.

Fungal Metabolites as Antiaging Agents

Fusarium oxysporum produces 2, 3-pentanediol compound demonstrated improved antiaging (antioxidant, thermotolerance) properties against *Caenorhabditis elegans* (Tiwari 2014). Multi-functional fungal extract regulating central cellular metabolism and metabolic pathways may serve as an effective anti-aging entrant (Zhao Wei et al., 2017). Slow aging is linked with delayed carcinogenesis, Reports suggest that calorie restriction decelerates aging by neutralizing MTOR (Target of Rapamycin) (Xie et al., 2016). Rapamycin and other similar compounds suppress cellular senescence, slow down aging, and postpone age-related diseases including cancer (Blagosklonny 2012). *In silico* analysis was followed by *in vitro* validation assays using a standard drug, Sirolimus (rapamycin). It is reported in the literature that molecular targets like 1AH8, 2KM1and 2L7E are involved in the cell signalling of the aging process (Carlson et al., 2008). Interestingly, the same signaling molecules are well-known targets for cancer therapy. This information could be potentially employed for anti-aging interventions (given that similar signaling molecules are involved in both cancer and aging) (Blagosklonny 2012).

Fungal Metabolites as Anticancer Agents

The docking of fungal secondary metabolites against specific cell cycle progression and regulators provides useful information on their potential as anticancer agents. The method is thus effectively used for the screening of potent molecules before to experimental validation (Usui 1998).

Fusarisetin A is formed by *Fusarium spp.* FN080326, isolated from a soil sample from Daejeon, Korea (Jang 2011). Fusarisetin A is an acinar morphogenesis inhibitor and powerful cancer migration inhibitor and ex vivo studies show that this compound can inhibit different types of cell migration (Xu 2012). Gliotoxin was isolated from the liquid culture of strains Y90086 (Lee 2001). Gliotoxin inhibits the proliferation of HUVEC cells in a dose-dependent manner with IC50 values of 40 ng mL. The compound displays weaker in vitro growth-inhibitory activity in cancer than in HUVEC cells; in addition, this compound also reduces the migration of HUVEC cells and the formation of HUVEC-related tubes. All these data highlight the potential antiangiogenic effects of gliotoxin (Lee 2001). Tryprostatins A and B are indole alkaloidal fungal products isolated from *Aspergillus fumigatus*, (Cui 1995). Tryprostatin A was first demonstrated to be an inhibitor of the mitogen-activated protein (MAP)-kinase-dependent microtubule assembly disruption of the microtubule.

Other Miscellaneous Therapeutic Function

Apart from antitumor importance, root colonization with AM (arbuscular mycorrhiza) fungi in medicinally important plants also seem to be very important in synthesizing antimicrobial important metabolites with widespread applications. *Penicillium vericulum* produces a vermiculin with antiprotozoal potential (Fuska et al., 1972). The compound As-186 from *Penicillium asperosporium* exihibit acyl-CoA: cholesterol acyltransferase (ACAT) activity. Lovastatin reported from *Aspergilus terrus* is used in the inhibition of cholesterol biosynthesis (Boruta and Bizukojc 2017). Griserofulvin is a metabolic product of *Penicillium nigricans, P. urticae and P. raistrickii*, used as a therapeutic agent in the treatment of dermatomycosis (Taboada and Grooters 2008).

CURRENT TRENDS AND FUTURE PERSPECTIVES

The development of improved industrial material and therapeutic drugs for particular diseases represents a major challenge for the future. Finding of fungal secondary metabolites which may be involved in different therapeutic and industrial applications facilitates discovering new approaches for developing new drugs. Novel fungal secondary metabolites may be useful as anti-cancerous agents capable of recognizing tumors in *in vitro* studies. Detailed findings about nature and the structural and functional group also forms the basis of several new therapeutic approaches. It has been noted that the production of metabolites is often related to fungal growth. Furthermore, the genes required for the biosynthesis of some natural products are clustered, perhaps as a consequence of these factors. Gene clusters contain all or most of the genes required for natural product biosynthesis. These genes will be conserved during evolution only if the natural product confers a specific advantage to the producing organism. This will open up new, wide and exciting fields of applications in which the production of beneficial natural products could be enhanced and the production of those with poisonous effects could be reduced or eliminated.

It is pertinent to the search for bioactive substances produced by rhizospheric fungi, with pharmaceutical potential and industrial importance for a better understanding of this interesting group of organisms. The development of improved industrial material and therapeutic drugs for particular diseases presents a major challenge for the future. Screening for fungal secondary metabolites involved in different therapeutic and industrial applications may be a pragmatic approach towards the new drug discovery. Some compounds might function as virulence factors, or their presence could provide a competitive edge to the producing organism or enhance the survivability of spores. Secondary metabolites may stimulate sporulation and consequently influence the growth

of the producing organism and neighboring members of the same species, and may be involved in improving the fitness of a community of related species (Figure 3).

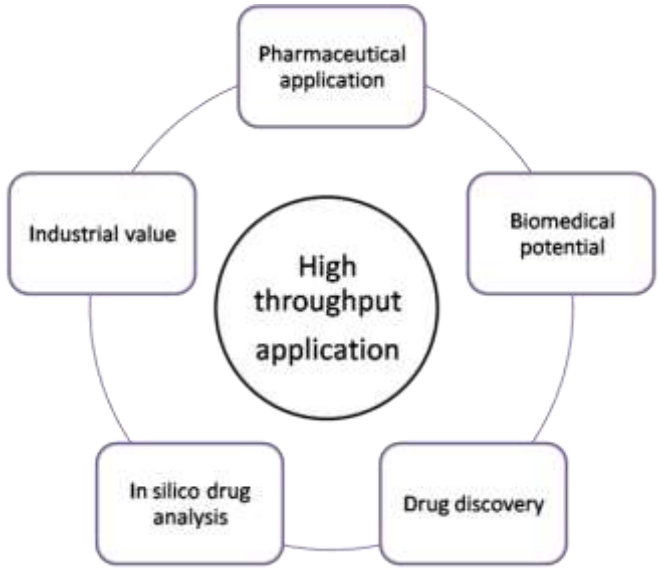


Figure 3. Overview of high-throughput molecular and advanced application to study the molecular identification of plant-associated rhizospheric fungi in relation to the plant root system for their potential application.

CONCLUSION

Rhizospheric region is an exclusive and dynamic hotspot of soil microflora. This region holds a characteristic niche where close interaction has been found between the plant root system and colonizing microbes. Among the wide spectrum of soil microorganisms, rhizospheric fungi occupy a prominent position for widespread biotechnological, pharmaceutical, and industrial applications and for agricultural sustainability. Though rhizospheric fungi have been characterized both morphologically and at molecular level for exploring their potential for the welfare of mankind, majority of rhizospheric fungi are still undefined and present an untapped reservoir for use in a number of avenues. The advent of high-throughput molecular tools, biotechnological applications, metagenomics and metatranscriptomics strategies, genome mining approaches, and metabarcoding strategies may help to define and discover the untapped potential of rhizospheric fungi.

ACKNOWLEDGMENTS

The authors would like to acknowledge the support and resources from Department of Biotechnology, Guru Ghasidas Vishwavidyalaya, Bilaspur Chhattisgarh, India. The authors gratefully acknowledge Dr. Anurag Chauhan, Assistant Professor from the Department of English Guru Ghasidas Vishwavidyalaya, Bilaspur for proofreading and editing of the manuscript.

REFERENCES

Aadil, K. R., Barapatre, A., Sahu, S., Jha, H., & Tiwary, B. N. (2014). Free radical scavenging activity and reducing power of Acacia nilotica wood lignin. *International journal of biological macromolecules*, 67: 220-227.

Abbott, L. K., Murphy, D, V. (2004). Soil Biological Fertility. Book, Springer.

Asiegbu, F. O., Adomas, A., Stenlid, J. (2005). Conifer root and butt rot caused by Heterobasidion annosum (Fr.) *Bref. s.l., molecular plant pathology*, 6: 395–409.

Askary, T. H., Abd-Elgawad, M. M. (2018). Fungal and bacterial nematicides in integrated nematode management strategies. *Egyptian Journal of Biological Pest Control*, 28:74.

Baker, D. D., and Alvi, K. A. (2004). Small-molecule natural products: new structures, new activities. *Current Opinion in Biotechnology*, 15: 576–583.

Badri, D. V., Weir, T. L., Lelie, D., Vivanco, J. M. (2009). Rhizosphere chemical dialogues: plant–microbe interactions. *Curr. Opin. Biotechnol*, *20*: 642-650.

Barapatre, A., Aadil, K. R., Tiwary, B. N., Jha, H. (2015). In vitro antioxidant and antidiabetic activity of biomodified Acacia wood lignin. *Int J BiolMacromol*, 75: 81–89.

Barredo, J. L., Kosalkova, C. K., Barreiro, C. (2017). Biosynthesis of Astaxanthin as a Main Carotenoid in the Heterobasidiomycetous Yeast Xanthophyllomyces dendrorhous, *J. Fungi*, 3: 44.

Bérdy, J. (2005). Bioactive microbial metabolites. *J Antibiot* (Tokyo), 58: 1-26.

Blagosklonny, M. V., 2012. Rapalogs in cancer prevention: anti-aging or anticancer? *Cancer Biol Ther*, 13: 1349-1354.

Boruta, T., Bizukojc, M. (2017) Production of lovastatin and itaconic acid by Aspergillus terreus: a comparative perspective. *World J Microbiol Biotechnol*, 33: 34.

Carlson, M. E., Silva, H. S., & Conboy, I. M. (2008). Aging of signal transduction pathways, and pathology. *Experimental Cell Research*, 314: 1951–1961.

Chen, L., Fang, Y., Zhu, T., Gu, Q., Zhu, W. (2008). Gentisyl alcohol derivatives from the marine-derived fungus Penicillium terrestre. *J Nat Prod*, 71: 66-70.

Claydon, N., Allan, M., Hanson, J. R., Avent, A. G. (1987). Antifungal alkyl pyrones of Trichoderma harzianum. *Transactions of the British Mycological Society*, 88, 503-513.

Cole, M., Rolinson, G. N. (1972). Microbial Metabolites with Insecticidal Properties. *Appl Microbiol*, 24: 660–662.

Cui, C. B., Kakeya, H., Okada, G., Onose, R., Ubukata, M., Takahashi, I., Isono, K., Osada, H. (1995). Tryprostatins A and B, novel mammalian cell cycle inhibitors produced by Aspergillus fumigatus. *J Antibiot* (Tokyo), 48: 1382-4.

Demain, A. L., and Fang, A. (2000). The Natural Functions of Secondary Metabolites. In: Fiechter, A., Ed., History of Modern Biotechnology I, *Advances in Biochemical Engineering/Biotechnology*, 69: 1-39.

Dowd, F. J., Johnson, B. S., Mariotti, A. J., (2017). Pharmacology and Therapeutics for Dentistry. Mosby 7th Edition 864.

Ehteshamul-Haque, S., Zaki, M. J., Abid, M., Ghaffar, A. (2014). Use of Verticillium chlamydosporium in the control of root rot disease of chickpea. *Pakistan Journal of Botan*y, 26: 229-234.

Fuska, J., Nemec, P., Khur, I. (1972). Vermiculine, a new antiprotozoal antibiotic from Penicillium vermiculatum. *The Journal of Antibiotics*, 25: 208–211.

Grever, M. R., Doan, C, A., Kraut, E. H. (2003). Pentostatin in the treatment of hairy-cell leukemia. *Best Pract Res Clin Haematol*, 16: 91-9.

Hartmann, A., Schmid, M., Tuinen, D. V., Berg, G. (2009). Plant-driven selection of microbes. *Plant Soil*, 321: 235–257.

Henis, Y., Lewis, J. A., Papavizas, G. C. (1991). Interactions between Sclerotium rolfsii and Trichoderma spp: Relationship between antagonism and disease control. *Soil Biology and Biochemistry*, 16: 391-395.

Hunt, J., Cheng, A., Hoyles, A., Jervis, E., Morshead, C. M. (2010). Cyclosporin A Has Direct Effects on Adult Neural Precursor Cells. *The Journal of Neuroscience*, 30: 2888 –2896.

Jang, J. H., Asami, Y., Jang, J. P., Kim, S. O., Moon, D. O., Shin, K. S., Hashizume, D., Muroi, M., Saito, T., Oh, H., Kim, B. Y., Osada H., Ahn, J. S. (2011). Fusarisetin A, an acinar morphogenesis inhibitor from a soil fungus, Fusarium sp. FN080326. *J. Am. Chem. Soc*, 133: 6865-7.

Kawahara, T., Takagi, M., Shin-ya K. (2012). JBIR-124: a novel antioxidative agent from a marine sponge-derived fungus Penicillium citrinum SpI080624G1f01. *J Antibiot* (Tokyo), 65: 45-7.

Kelloff, G. J., Lubet, R. A., Fay, J. R., Steele, V. E., Boone, C. W., Crowell, J. A., Sigman, C. C. (1997). Farnesyl Protein Transferase Inhibitors as Potential Cancer Chemopreventives. *Cancer Epidemiology, Biomarkers & Prevention*, 6: 267-282.

Kerry, B. R., Simon, A., Rovira, A. D. (2008). Observations on the introduction of Verticillium chlamydosporium and other parasitic fungi into soil for control of the cereal cyst-nematode Heterodera Avenue. *Annals of Applied Biology*, 105: 509 - 516

Kope, H. H., Fortin, J. A. (1991). Genetic variation in antifungal activity by sibling isolates of the ectomycorrhizal fungus Pisolithus arhizus. *Soil Biology and Biochemistry*, 23: 1047-1051.

Kuzyakov, Y., Blagodatskaya, E. (2015). Microbial hotspots and hot moments in soil: Concept & review. *Soil Biology & Biochemistry*, 83: 184-199.

Lee, H. J., Lee, J. H., Hwang, B. Y., Kim, H. S., Lee, J. J. (2001). Anti-angiogenic activities of gliotoxin and its methylthio-derivative, fungal metabolites *Arch. Pharmacal Res.*, 24: 397.

Li, Z., Fu, J., Zhou, R., Wang, D. (2018). Effects of phenolic acids from ginseng rhizosphere on soil fungi structure, richness and diversity in consecutive monoculturing of ginseng. *Saudi Journal of Biological Sciences*, 25: 1788–1794.

Li, J. Y., Sidhu, R. S., Bollon, A., strobel, G. A. (1998). Stimulation of taxol production in liquid cultures of Pestalotiopsis microspore. Mycological Research, 102: 461-464.

Lim, S. L., Wu, T. Y., Lim, P. N., Shak, K. P. (2015). The use of vermicompost in organic farming: overview, effects on soil and economics. *J Sci Food Agric*, 95: 1143-56.

Liras, P., Martín, J. F., Casqueiro, J. (2005). Secretion systems for secondary metabolites: how producer cells send out messages of intercellular communication. *Curr Opin Microbiol*, 8: 282-93.

Miranda, H., Simão, R., Santos V. P., Salles B. F., Pacheco, M. T., Willardson, J. M. (2010). Exercise order interacts with rest interval during upper-body resistance exercise. *J Strength Cond Res*, 24: 1573-1577.

Meligy, M. E., Zain, M, E., Awaad, A. S., Othman, M. R., Alafeefy, A. M. (2014). Biological activity of fungal secondary metabolites. *International Journal of Chemical and Applied Biological Sciences*, 1: 14-22.

Melo, M. J., Gonçalves, J., Guerra, J. O., Santana, A., Nascimento, C. (2015) Impact of conversion from Advagraf to twice-daily generic tacrolimus in kidney transplant recipients: a single-center study. *Transplant Proc*, 47: 911-3.

Niwa, Y., Terashima, T., Sumi, H. (2003). Topical application of the immunosuppressant tacrolimus accelerates carcinogenesis in mouse skin. *Br J Dermatol*, 149: 960-7.

Nihorimbere, V., Ongena, M., Smargiassi, M., Thonart, P. (2011). Beneficial effect of the rhizosphere microbial community for plant growth and health *Biotechnol. Agron. Soc. Environ*, 15: 327-337.

Otto, J., Ordovas, J. M., Smith, D., Dongen, D. V., Nicolosib, R. J., Schaefer, E. J. (1995). Lovastatin inhibits diet induced atherosclerosis in FlB Golden Syrian hamsters. *Atherosclerosis*, 114: 19-28.

Pandit, S., Puttananjaih, M. H., Harohally, N., Dhale, M. A. (2018). Functional attributes of a new molecule- 2-hydroxymethyl-benzoic acid 2'-hydroxy-tetradecyl ester isolated from Talaromyces purpureogenus CFRM02. *Food Chemistry*, 255: 89-96.

Pinton, R., Varanini, Z., Nannipieri, P. (2007). The Rhizosphere. Biochemistry and Organic Substances at the Soil-Plant Interface, CRC Press.

Rani, C., Paneerselvam, A., (2010). Fungal diversity in the sediments of point calimere, East coast of India. *J. Pure Appl Microbiol*., 4: 1999-2006.

Sachewsky, N., Hunt, J., Cooke, M. J., Azimi, A., Zarin, T., Miu, C., Shoichet, M. S., Morshead, C. M. (2014). Cyclosporin A enhances neural precursor cell survival in mice through a calcineurin-independent pathway. *Disease Models & Mechanisms*, 7: 953-961.

Sarker, S. D., Nahar, L., Kumarasamy, Y. (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. Methods, 42: 321-324.

Scharf, D. H., Heinekamp, T., Remme, N., Hortschansky, P., Brakhage, A. A., Hertweck, C. (2012). Biosynthesis and function of gliotoxin in Aspergillus fumigatus. *Appl Microbiol Biotechnol*, 93: 467-72.

Shaikh, M. N., Mokat, D. N., (2018). Role of Rhizosphere Fungi Associated With Commercially Explored Medicinal and Aromatic Plants: *A Review Current Agriculture Research Journal*, 6: 72-77.

Shivanna, M. B., and Vasanthakumari, M. M. (2011). Temporal and spatial variability of rhizosphere and rhizoplane fungal communities in grasses of the subfamily Chloridoideae in the Lakkavalli region of the Western Ghats in India. *Mycosphere*, 2: 255–271.

Spiroketals from the Alga-Derived Fungi Penicillium thomii and Penicillium lividum. Mar Drugs, 12: 5930–5943.

Swaran, J. S. (2008). Flora Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. *Oxid Med Cell Longev*, 2: 191–206.

Taboada, J., Grooters, A, M. (2008). Systemic antifungal therapy, in *Small Animal Clinical Pharmacology*, (Second Edition).

Tiwari, S., Singh, S., Pandey, P., Saikia, S. K., Negi, A. S., Gupta, S. K., Pandey, R., Banerjee, S. (2014). Isolation, structure determination, and antiaging effects of 2, 3-pentanediol from endophytic fungus of Curcuma amada and docking studies. *Protoplasma*, 251: 1089-98.

Usui, T., Kondoh, M., Cui, C. B., Mayumi T., Osada, H., (1998). Tryprostatin A, a specific and novel inhibitor of microtubule assembly. *Biochem J*, 333543-8.

Xie, J., Wang, X., & Proud, C. G. (2016). mTOR inhibitors in cancer therapy. *F1000Research*, 5: 2078.

Xu, J., Caro-Diaz, E. J., Lacoske, M. H., Hung, C. I., Jamora, C., Theodorakis, E. A. (2012). Fusarisetin A: *Scalable Total Synthesis and Related Studies Chem. Sci.*, 3: 3378-3386.

Xu, R., Xu, G. M., Li, X. M., Li, C. S., Wang, B. G. (2015). Characterization of a Newly Isolated Marine Fungus Aspergillus dimorphicus for Optimized Production of the Anti-Tumor Agent Wentilactones, Mar. *Drugs*, 13: 7040-7054.

Zhou, L. N., Zhu, T. J., Cai, S. X., Gu, Q. Q., Li, D. H. (2010). Three New Indole-Containing Diketopiperazine Alkaloids from a Deep Ocean Sediment Derived Fungus Penicillium griseofulvum. *Helvetica Chimica Acta*, 93: 1758- 1763.

Zhuravleva, O. I., Sobolevskaya, M. P., Afiyatullov, S. S., Kirichuk, N. N., Denisenko, V. A., Dmitrenok, P. S., Yurchenko, E. A., Dyshlovoy, S. A. (2014). Sargassopenillines A–G, 6, 6-Spiroketals from the Alga-Derived Fungi Penicillium thomii and Penicillium lividum. Mar Drugs, 12: 5930–5943.

Zhao, W., Zhenga, H. Z., Zhoua, T., Hongc, X. S., Cui, H. J., Jianga, Z. W., Chena, H. J., Zhoud, Z. J., Liu, X. Z., 2017. CTT1 overexpression increases the replicative lifespan of MMS-sensitive Saccharomyces cerevisiae deficient in KSP1. *Mechanisms of Ageing and Development*, 164: 27–36.

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 6

A REVIEW ON LICHEN DIVERSITY AND ITS ASSOCIATED FUNGI: PRESENT AND FUTURE PROSPECTS

Dipanjali Devi and Kumanand Tayung*
Mycology and Plant Pathology Laboratory, Department of Botany,
Gauhati University, Guwahati, Assam, India

ABSTRACT

Lichens are symbiotic association between an alga and a fungus. This unique association has evolved as a special adaptation to varied microhabitats withstanding extreme microclimatic conditions. Thus, lichens are cosmopolitan in distribution and pioneer colonizers of terrestrial habitats on this planet. There are approximately about 20,000 taxa of lichen species across the world. India is highly rich in lichen biodiversity contributing 15% of total global lichen flora. The south Indian region is represented by maximum number of lichen genera (33.9%) followed by Himalayan region (20%) and central India and Andaman Islands (about 10% each). Along with fungal partner (mycobiont) and algal or cyanobacterial symbionts (photobionts), lichen thalli often consists of associated microfungi. The fungal group mostly comprises of endolichenic and lichenicolous fungi. The endolichenic fungi are almost similar to that of the endophytic fungi of vascular plants in many aspects; they occur internally in the lichens, asymptomatic i.e., do not produce any visible disease symptoms and are transmitted horizontally. Unlike endolichenic fungi, lichenicolous fungi are highly specialized group of organisms that develop on lichens thalli and are mostly parasitic but they also include a wide range saprotrophs, and commensals. The ecology of lichens is very interesting and can be used for varied purposes. Most importantly it is being used as a bioindicator, as medicines, food and plays a key role in bio-weathering. The associated fungi of lichen thalli particularly endolichenic fungi have been recently identified as a repository of

* Corresponding Author's Email: kumanand@gauhati.ac.in.

bioactive natural products. Several interesting metabolites with anticancer, antioxidant, antitumor and antimicrobial properties have been isolated and reported from endolichenic fungi. Thus the present chapter will highlight the global and India's lichen diversity emphasizing on their uses and associated fungi particularly on endolichenic fungi as source of bioactive natural products.

Keywords: lichen diversity, associated microfungi, endolichenic fungi, bioactive metabolites

INTRODUCTION

Earth is flourished with rich biodiversity of flora and fauna. The diversity of flora ranges from the simplest algal form to the complex angiosperms. Among them, Lichens are unique group of plant taxa which are often ignored and unexplored. Lichen is "an ecologically obligate, stable mutualism between an exhabitant fungal partner (the mycobiont) and inhabitant population of extracellularly located unicellular or filamentous algal or cyanobacterial cells (the photobiont)" (Hawksworth and Honegger, 1994). Along with fungal partner (mycobiont) and algal or cyanobacterial symbionts (photobionts), lichen thalli often consists of associated microfungi and bacteria (Girlanda et al., 1997; U'Ren et al., 2012; Aschenbrenner et al., 2014). Lichens are found everywhere, from all terrestrial and even in some aquatic and marine habitats, where they occupy locations which are inhospitable for the vascular plants. Lichens are found to be attached with rocks, barks, on ice, leaves etc. Lichens are very important to the mankind because of its use as a bioindicator, in food and fodder and also have significant medicinal uses. It also plays a key role in bioweathering. With the increase in the extremity of the habitat, lichens usually play the most important ecological role i.e., biomonitoring of pollution. Lichens are prototypical stress-tolerators for natural environmental factors: most species can bear wide temperature fluctuations and complete aridness for prolonged times. Lichens lack specific structures such as roots for water and nutrient acquirement, and their outer layers do not significantly diminish water loss. This limits the time when conditions are good for physiological activity, leading to slow growth since, without physical protection, lichens tolerate environmental extremes through dehydration. The lack of protective structures enables lichens to readily absorb environmental chemicals from the nature. The medicinal use of lichens is also very diverse. The use of lichens in medicine can be traced back from first Egyptian and Chinese civilization. Lichens have been used for various disease and disorders such as in headache, skin diseases, urinary problems, boils, vomiting, diarrhoea, heart trouble, cough, leprosy, and as blood purifier since earlier times (Nayaka et al., 2010**).** Unlike the mycobiont, lichen thallus is also a home to many associated fungi. The associated fungi reside inside the lichen thallus. Lichenicolous fungi, endolichenic fungi and culturable and non-culturable non-

photosynthetic bacteria are the lichen associates (Biosca et al., 2016; Muggia et al., 2016). Lichenicolous fungi are a highly specialized group of organisms that develop on lichens and form with them three or sometimes four to five-membered associations (Lawrey and Diedrich, 2003). Lichenicolous fungi are symptomatic fungi that harbours the lichen thallus causing infections. Unlike lichenicolous fungi, the endolichenic fungi are almost similar to that of the endophytic fungi of vascular plants in many aspects; they occur internally in the lichens asymptomatically i.e., do not produce any visible disease symptoms and are transmitted horizontally (Arnold et al., 2009; Kannangara et al., 2009; U'Ren et al., 2012). Endolichenic fungi is a rich source of bioactive metabolites. Antibacterial, antifungal, anticancerous potentialities had been isolated from endolichenic fungi. Harnessing endolichenic fungi from the lichens thus is very useful in the field of medicine and industries. Thus endolichenic fungi can be a source of novel herbal medicines and studied for further bioprospection.

GLOBAL LICHEN DIVERSITY

There are approximately 19,000 fungi (lichens are named from the fungus) forming lichens, belonging to several distinct lineages contained by the kingdom fungi, particularly the phyla Ascomycota and Basidiomycota.The diversity of lichens is very unique in the sense that lichens can be found in most of the places. The ability of the lichens to adhere in most of the substratum leads to highly diversified lichen diversity.According to Lumbsch et al. (2011), regardless of the conspicuous morphological diversity and well-documented ecological significance, lichens are considered among the least well understood macroscopic organisms. The study on lichens was not flourished to large extent because of less interest in this field of area. However, some works had been recorded enumerating the lichen diversity from different parts of the world. The first record of lichen vegetation forming the soil crust was enumerated from semi-desert area of the Republic of South Africa. Many lichen species were recorded from the area of which thirty five were terricolous lichens and fifty two percent of the lichen species were endemic to the area. Squamulose and crustose lichens were also recorded which were common and most of them contained green algal communities. The study area was unique and were mostly threatened by land management practices and climatic changes (Zedda and Rambold, 2009).The lichenological evidence has also been found from inland rain forest in western North America (Goward and Spiribille, 2005). The area is internationally renowned as the archetypal expression of the temperate rain forest biome. The study on the area found that (1) 40% of oceanic, epiphytic macrolichens found in Pacific coastal rain forests occur also in inland regions; (2) epiphytic species richness decreases with decreasing latitude; (3) the southward decline in lichen diversity is correlated with a parallel decrease in summer precipitation, but the

decline is not associated with mean annual precipitation. Lichen diversity Study conducted by Tripp et al. (2016) across the United States suggested that the diversity of lichen was not affected by urbanization or human population density. The area has large number of lichen diversity and more interesting fact that came out was that the propagules of asexually reproducing species of lichens have wider geographical ranges than propagules from sexually reproducing species, thus it more likely reflects the lichenized nature of asexual spores that disperse both the mycobiont and photobiont versus non-lichenized sexual spores, which disperse only the mycobiont. The lichen diversity in Colombian Carribean dry forest remnants was studied by Lucking et al. (2019). Sampling conducted in two different parts provided 61 species including four new species namely *Fissurina linoana*, *Graphis lurizana*, *G. mokanarum*, *Phaeographis galeanoae*, *Arthonia erupta* and *Coenogonium saepincola*. Such study exemplify occurrence of lichens in different parts of the world which needs to studied and explore further so that it might contribute substaintailly to the global lichen diversity.

There are approximately about 20,000 taxa of lichen species across the world. India is highly rich in lichen biodiversity contributing 15% of total global lichen flora (Singh and Sinha, 1997; Upreti, 1997). It is estimated that a total of about 1750 species under 234 genera and 70 families of which 700 species and 72 genera belongs to microlichens in India. The south Indian region is represented by maximum number of lichen genera (33.9%) followed by Himalayan region (20%) and central India and Andaman Islands (about 10% each). Studies on Indian Lichen flora has been made by some European and Indian workers as enumerated by Awasthi in recent years (Upreti, 1997). The Western Ghats has maximum number of crustose lichens represented by 617 taxa followed by foliose and fruticose with 269 and 62 species respectively. The Nilgiri Biosphere area of Western Ghats in Tamil Nadu has been better explored for lichens while other localities remain under explored. Hence, among different states Tamil Nadu has the highest number of lichens with 657 taxa followed by Karnataka, Kerala and Maharashtra with 336, 277 and 91 taxa respectively. The lichens recorded from Goa are included under Maharashtra as they are few in number, while no records from Gujarat are available. In case of Tamil Nadu most of the lichens are from Nilgiri and Palni Hills. There is an occurrence of 949 taxa of lichens in Western Ghats with high percentage of endemism (26.7%), which is highest for any Lichenogeographic regions in India. Graphidaceous, Parmelioid, Physcioid, Pyrenocarpous and Thelotremataceous lichens dominate the region (Nayaka and Upreti, 2005). Many other works also had been done enumerating lichen diversity in different parts of India. There is an occurrence of about 404 species of microlichens belonging to 105 genera and 39 families known from Arunachal Pradesh, which is a part of Himalaya biodiversity hotspot. 66 species are reported to be new from Arunachal Pradesh in India (Singh et al., 2015). A study of the diversity and distribution of lichens at 10 sites within the Mehao Wildlife Sanctuary in Arunachal Pradesh, India, revealed 177 species, belonging to 71 genera and 35 families. The Sanctuary exhibited

almost all the habit and habitat groups of lichens within its climatically heterogenous and altitudinally (400–2700 m) varied landscape. Among the different habitat groups, obligately corticolous lichens were dominant (133 species), followed by facultatively corticolous lichens (occurring on both rock and bark; 25 species), saxicolous lichens (17 species) and terricolous lichens (2 species). The corticolous habitat group was dominated by crustose species while saxicolous and terricolous groups were made up of mostly fruticose species (Pinokiyo et al., 2007). The Indo-Burma region is fully blessed with the lichen diversity. Thus, a survey was made to evaluate the lichens diversity and distribution in central Part of Murlen National Park (MNP), Mizoram. Based on collected data, the present study enumerated 20 lichen species which belonged to 15 genera and 10 families. The family Parmeliaceae showed the highest number of species. Moreover, the growth form was dominated by crustose compared to foliose and fruticose (Thangjam et al., 2019). Further, Logesh et al. (2015) also initiated work on lichen diversity in Mizoram. A total of about 159 species of lichens were recorded. Some of the lichens reported were found new for Indian Lichen. Among the north eastern states, Sikkim is believed to be extremely rich in lichen diversity. The maximum lichen diversity is found in Sikkim out of all north eastern States. A comparative account on lichen diversity of Sikkim with other north eastern states was carried out by Sinha and Jagdeesh Ram (2006) (Table 1). The diversity of lichen is thus very diverse. However, there is an increasing research been carried out across the world focusing on lichen diversity and factors affecting its diversity. But still many areas are still unexplored to a large extent. There is a great need to explore more and more lichens from many unexplored areas since lichen is very beneficial to the mankind.

Table 1. Comparative data on lichen diversity of North-East India, neighbouring West Bengal and Sikkim

Name of States	Total geographical area (in sq.km)	Actual forest cover (in sq. km)	No. of species recorded so far
Arunachal Pradesh	73,743	67,757	477
Assam	77,437	24,751	141
Manipur	22,327	17,675	291
Meghalaya	22,429	15,775	179
Mizoram	21,071	17,553	02
Nagaland	16,579	14,321	306
Sikkim	7,096	3,033	506
Tripura	10,476	5,535	---
West Bengal	77,752	7,015	510

(Source-Sinha and Jagdeesh Ram, 2006).

THE ECOLOGY OF LICHENS

The ecology of lichens is very interesting in the fact that lichens can be used for varied purposes. Most importantly it is being used as a bioindicator. Apart from being used as a bioindicator, it is also used as medicines, food and plays a key role in bioweathering.

Lichens as Bioindicator

Pollution is a major problem affecting human health, causing respiratory problems. The extent of pollution is known with the help of bioindicator. Lichen is one such bioindicator, in the sense that lichens grows in unpolluted areas. Lichens are considered to be among the most sensitive organisms for several types of pollutants. The distribution of lichens is affected usually by three main sources; rain, dew and humid air. These water sources cause hydration of lichens and activates photosynthesis, they also shape lichens differently (Gauslaa, 2014). Vascular plants usually take nutrients mainly from the soil through roots, but lichens particularly epiphytic take nutrients from plants (Nimis et al., 2002). Lichens are so much sensitive to the change in N deposition and to gaseous NH_3 concentrations in the atmosphere (Sheppard et al., 2009), but different species shows different responses. Oligotrophic species are disappearing from N rich habitats as they are very sensitive to eutrophication (Frati et al., 2007; Pinho et al., 2007; Geiser et al., 2010), while others (termed 'nitrophytic') are favoured by high N levels. For the diversity of lichens climatic variables (specifically average yearly temperature and rainfall), anthropogenic pressures (like harvesting), bark pH and texture and forest structure are relevant (Giordani, 2006, 2007; Giordani and Incerti, 2007).

Lichens Used as Traditional Medicines

Nature is endowed with rich resources which are extremely beneficial to all the living creatures on the world. Those natural resources have medicinal value which is mostly known by ethnobotanic studies. Lichens have many medicinal properties. "A plant could treat a disease it most looked like" is written in the 15th century in "Doctrine of Signatures". This formed the basis of phyto-therapeutics in traditional systems of medicines like Traditional Indian Medicine (TIM) or Ayurveda, Traditional Chinese Medicine (TCM), and Western Medical Herbalism (Bown, 2001). *Usnea* is the most widely used genus as a traditional medicine, apart from it many genera of lichen are also being used in different parts of the world. The folk taxonomy of lichens within a given

culture is not similar with the scientific taxonomy and reflects the cultural value of those lichens and the traditional method of their identification (Stuart, 2015). The medicinal use of lichens was known when *Evernia furfuracea* or (Parmeliaceae) was first used as a drug which can be traced back to the 17th dynasty (Launert, 1981). *Letharia vulpina* (Parmeliaceae) was used by the people of North California in stomach diseases. A novel species of *Dictyonema* was used by the Waorani as hallucinogen (Davis and Yost, 1973). *Usnea diffracta* is used for treating uterine ailments and *Lethariella cashmeriana*, *Thamnolia vermicularis* are used as medicated teas in China (Wang et al., 2001). In the Indian drug chharila three *Parmelia* sp. (*Parmelia chinense*, *P. sancti-angeli* and *P. peforatum* are used, which is used as aphrodisiac (Lal and Upreti, 1995; Kumar and Upreti, 2001). *Parmelia chinense* is used as diuretic and as liniment for headache and powder to help wounds heal in India. *Parmelia sancti-angeli* is used to treat Tinea (ringworm) like disease in Central India, the ash of the lichen is mixed with mustard or linseed oil, is applied to the affected area. *Parmelia peforatum* is also used as medicine in Afghanistan (Chandra and Singh, 1971). Apart from these; many other lichens are also used to treat various diseases.

Lichens Used as Food

In Yunnan Province of China, lichens are used in many ways. Ethnic peoples use five species of lichens as foods (*Lobaria isidiophora, L. kurokawae, L. yoshimurae, Ramalina conduplicans, and R. sinensis*) and five others as health-promoting teas (*Lethariella cashmeriana, L. sernanderi, L. sinensis, Thamnolia vermicularis, and T. subuliformis* (Wang et al., 2001).Lichens can be used as an alternative; seven species have been consumed, especially for making lichen flour at the times of severe lack of products for bread. People considered lichens as non-poisonous “plants”. They were gathered from everywhere, in particular those with bigger biomass and those that are found on the bark of trees. The mostly used species were *Evernia prunastri*, widely spread in these areas, *Pseudevernia furfuraceaa*, *Lobaria pulmonaria*, *Usnea barbata* and *Cetraria islandica* (Redzic et al., 2010). Schwarz, (2018) has developed a range of food products made from lichen, a hardy fungus-like species that grows on plants, tree bark and rocks. Apart from algae and insects, which are already gaining a lots of attention, lichens posses a great deal of potential as a source of nutrition in the future. Lichen can be grown in Mars, thus they are optly called as superfood. The lichen rock tripe (*Lasallia pustulata*) was investigated whether it can be used as food during survival situations and experimented over mice. The growth rate, metabolism and immune functions of female Balb/c mice were studied with the application of 30% lichen. After 3 weeks on this diet, it was observed that the lichen supplementation did not affect the growth rate or the well-being of the animals. The growth rate was seen to be higher in the lichen group when compared to control

mice. Food consumption was similar in both groups, but with a trend towards slightly higher intake (12%) in the lichen group. The body organs were not affected by the lichen. Histological hematoxylin eosin staining showed that all these organs were normal. Plasma glucose levels were unchanged, but plasma urea levels decreased by 24% ($p < 0.05$) with the lichen diet. RBC, WBC and the number of lymphoid cells in the thymus and spleen were normal. The activity of thymocytes and spleen T-lymphocytes were not affected by the lichen diet, but spontaneous cell-mediated cytotoxicity (NK cells) tended (n.s.) to increase and spleen B-lymphocyte activity increased by 40% ($p < 0.05$).The lichen tripe has immune stimulating effect and thus can be used as food in survival situation (Ilbäck and Källman, 1999).

Role of Lichen in Weathering

Lichens and bryophytes play significant role in nitrogen and biotic enhancement of surface weathering rates (Porada, 2014).The interface between lichens and their rock substrates strongly suggests that the weathering of minerals can be accelerated by the growth of at least some lichen species. Lichens plays a key role in the chemical weathering of rocks by the excretion of various organic acids, particularly oxalic acid, which can effectively dissolve minerals and chelate metallic cations. As a result of the weathering induced by lichens, many rock-forming minerals exhibit extensive surface corrosion. The precipitation of poorly ordered iron oxides and amorphous alumino-silica gels, the neoformation of crystalline metal oxalates and secondary clay minerals have been frequently identified in a variety of rocks colonized by lichens in nature (Chen et al., 2000). The presence of calcium, magnesium, manganese and copper oxalate crystals at the rock–lichen interface and in the lichen thalli suggests that oxalic acid, secreted by the mycobiont, is one of the most active agents of chemical alteration. Lichen acids are a group of polyphenolic compounds which acts as bioweathering agents (Adamo, 2000). *Lecidea auriculata* penetrates rock surfaces, detaching, incorporating and expelling flakes of rock. Surface rocks which are exposed are weathering more than those in damp hollows and snow patches (McCarroll and Viles, 1995). The release of oxalic acids and polyphenolic secondary compounds of lichen metabolism “lichen acids” plays key role in lichen weathering and neogenesis. The differences in the bio weathering capability of lichen is related to the physiology of the lichen species (Adamo et al., 2002).

ASSOCIATIVE FUNGI OF LICHENS

Lichen is a symbiotic association between algae (photobiont) and fungi (my cobiont). Apart from this obligate symbiotic association between algae and fungi, lichen is

particularly a home to many fungi. Those fungi which resides within the lichen thallus are called as associative fungi. Associative fungi are different from that of the lichen my cobiont. Lichenicolous fungi and endolichenic fungi are mainly two types of associative fungi which reside within the lichen thallus. Lichenicolous fungi are symptomatic fungi which cause diseased lesions on the lichen thallus while endolichenic fungi are beneficial fungi. Endolichenic fungi are asymptomatic, cryptic fungi that resides inside the lichen thallus. Endolichenic fungi are beneficial to the mankind in the sense that many bioactive metabolites can be isolated from the endolichenic fungi which may have either antibacterial, antifungal, antitumor properties etc.

Lichenicolous Fungi

Lichenicolous fungi represent a highly specialized and successful group of organisms that live exclusively on lichens, most commonly as host-specific parasites, but also as broad-spectrum pathogens, saprotrophs and commensals (Diederich et al., 2017).For developing a connections with the host, the lichenicolous fungus hyphae by forming haustoria reach the algal layer, while some species establish connections with the mycobiont (Rambold and Triebel, 1992; De los Ríos and Grube 2000; De los Ríos et al., 2000).

Diversity of Lichenicolous Fungi

Lichenicolous (lichen-dwelling) fungi have proved to be a major ecological group of fungi, with around 2000 species already described. However, it is assumed that their species diversity is much greater (Hawksworth and Rossman, 1997), the estimated total number of species lying in between 3000 and 4000 (Hawksworth, 2001; Lawrey and Diederich, 2003). Many of the genera consist only of lichenicolous species, and most are restricted to particular lichen hosts, commonly single host genera or single lichenized species. The lichen floras of dolomite, limestone, opoka, clay, sand and gravel quarries, as well the few existing natural dolomite outcrops suitable for lichens in various parts of Lithuania were studied. Two new records of lichenicolous fungi *Didymellopsis pulposi* and *Stigmidium peltideae*, are new for Lithuania (Motiejūnaitė and Skridlaitė, 2017). A new lichenicolous fungi *Tremella cetrariellae* (Tremellales, Basidiomycota, Fungi), on *Cetrariella delisei* has been reported. *Tremella cetrariicola* is shown to be heterogeneous and represents two phylogenetic sister species, which have distinct in the morphology of the galls, basidia and basidiospores, and in their host selection. *Tremella cetrariicola* s. str. is confined to Tuckermannopsis. The new species is known from Finland, Greenland, Norway, Russia, Svalbard, and Sweden (Millanes et al., 2015). *Phoma* Sacc. is a large genus which consists of either saprobes, plant pathogens and endophytes (Boerema et al. 2004). Twenty-four species in this genus, however, are obligately lichenicolous

(Hawksworth, 1971, Hawksworth and Cole, 2004; Diederich et al., 2007; Brackel, 2007; Kondratyuk et al., 2010; Halıcı et al., 2014). First *Phoma* species has been reported from *Candelariella –Phoma candelariellae*. *Phoma candelariellae* was found from the apothecia *Candelariella aurella* in the Central Anatolia region of Turkey. It is different from the reported lichenicolous *Phoma* species in the fact that it consists of clustered conidiomata arising in groups and it's subglobose conidia (Kocakaya et al., 2016). Brackel, (2015) during the field study of central Italy of a total of 177 sites of lichenicolous interest found new species *Abrothallus teloschistis*, *Arthonia rangiformicola*, *Pronectria zhurbenkoi* and *Trimmatostroma glebarum.* A total of 161 species of lichenicolous fungi, four lichenicolous lichens and 21 undescribed or doubtful taxa is provided. Eleven species of lichenicolous fungi are recorded from Bi Doup-Nui Ba National Park in Vietnam and out of these *Clypeococcum rugosisporum* and *Zwackhiomyces diederichii* are reported as new to Asia. New lichenicolous fungal species *Capronia josefhafellneri* and *Septotrapelia triseptata* are also found. *Abrothallus usneae*, *Arthophacopsis* cf. *parmeliarum*, *Lichenoconium erodens*, *Pseudoseptoria usneae* and *Roselliniella cladoniae* are new to Southeast Asia (Zhurbenko et al., 2016). Eighteen species of lichenized and two species of lichenicolous fungi from oak strands of east-central Europe' rural landscape were found. New records of lichenicolous fungi from eastern Europe, Carpathians, Polish Carpathians, Hungary, Romania or Ukraine. For the protection of the lichen diversity of the Carpathians, open canopy oak strands and solitary oaks in wood pastures is important (Czarnota et al., 2017). 29 lichenicolous fungi are reported as new or noteworthy from northern Germany or one of its federal states. *Epicladonia simplex* and *Minutoexcipula tephromelae* are reported new to Germany; *Didymocyrtiscladoniicola*, *D. foliaceiphila*, *Endococcus fusiger*, *Hymenelia ceracea*, *Lichenochora coarctatae*, *Lichenostigmachlaroterae*, *Pyrenochaeta xanthoriae*, *Schismatomma umbrinum* and *Vouauxiella verrucosa* are recorded for the first time from northern Germany (Schiefelbein et al., 2017).

Many molecular biology techniques are also used to characterize the chemicals produced by lichenicolous fungi. Raman spectroscopy is an established method for the characterization of chemicals *in situ*, and this technique is applied to a lichenicolous fungus for the first time. *Xanthoriicolaphysciae* occurs in the apothecia of *Xanthoria parietina*, producing conidia at the hymenium surface. Raman spectroscopy of apothecial sections revealed that parietin and carotenoids were destroyed in infected apothecia. Those compounds protect healthy tissues of the lichen from extreme insolation and their removal may contribute to the deterioration of the apothecia. Scytonemin was also detected, but was most probably derived from associated cyanobacteria (Edwards and Seaward, 2016). Lichenicolous species are widely distributed in the Basidiomycota, sclerotia or bulbils are produce by them with few additional structures to permit taxonomic placement. The Cantharellales includes many of these species and there is a new species that grows over *Cladonia rangiferina* and forms yellow-orange, initially

immersed bulbils similar to *Burgella flavoparmelia*, a familiar species in the order. The new name for the genus Bulbilla is proposed as Adamflankia, as Bulbilla coincides with the technical term 'bulbilla' used in previous descriptions of bulbil-forming species, *Adamflakia applanata* comb. nov. is proposed (Lawrey et al., 2016). Quantitative Real-Time PCR (q PCR) is used to detect and quantify lichenicolous fungi and lichen bionts since the biology of lichenicolous fungi is poorly known, including the intrathalline hyphal distribution patterns and the density of hyphae. The lichenicolous fungi *Plectocarponlichenum* and *Tremella lobariacearum* which inhabit species of the genus *Lobaria* were investigated to determine the intrathalline distribution patterns; material with obvious infection, material next to infection as well as visually plectenchyma from central and marginal thallus parts. Based on the qPCR data, the two lichenicolous fungi occur predominantly in the symptomatic areas and in a certain area around symptomatic areas. Samples derived from lichen thalli without symptoms of infection showed no evidence of the lichenicolous fungi (Bergmann and Werth, 2017). Single-strand conformation polymorphism (SSCP) fingerprinting analyses and sequencing of internal transcribed spacer (ITS) fragments are used to identify lichenicolous fungi. The systematic, stratified sampling strategy is used to identify undiscovered lichenicolous fungi of alpine habitats (Fleischhacker et al., 2015). The phylogenetic placement on molecular data for a number of lichenicolous fungi is missing. Phylogenetic studies of 19 species of lichenicolous fungi was determined using four loci (LSU rDNA, SSU rDNA, ITS rDNA and mtSSU) were done and it revealed that those lichenicolous fungi are widespread across the phylogeny of Lecanaromycetes (Pino-Bodas et al., 2017). An updated checklist of lichenicolous fungi from India has been reported from India (Joshi et al., 2015) (Table 2)

ENDOLICHENIC FUNGI

Fungi occur in different types of ecological habitats which vary from terrestrial to fresh water and marine environments. They behave either as saprobes, pathogens or as symbionts. There is an occurrence of highly diversified numerous, asymptomatic, cryptic microfungi which reside within the internal tissue of the lichen which lives in close association with the photobiont. These are called as Endolichenic fungi (ELF) (Arnold et al., 2009). Endolichenic fungi were discovered when attempts were being done to culture the lichen mycobiont (Crittenden et al., 1995; McDonald et al., 2013; Petrini et al., 1990). These fungi are similar to the endophytic fungi (sometimes also referred to as endophyte-like fungi) (Arnold et al., 2009; U'Ren et al., 2016), which reside within healthy plant tissues and are a phylogenetically and ecologically diversified without causing any disease symptoms (Arnold 2001, 2007; Petrini 1991).

Table 2. Updated checklist of lichenicolous fungi from India along with their host lichens and distribution

S. No.	Lichenicolous Fungi	Host	Distribution
1	*Abrothallus peyritschii* (Stein) I. Kotte	*Vulpicida pinastri*	Himachal Pradesh
2	*Arthonia diorygmae* S. Joshi & Upreti	*Diorygma junghuhnii*	Tamil Nadu
3	*A. molendoi* (Heufl. ex Frauenf.) R. Sant.	*Xanthoria elegans*	Jammu & Kashmir
4	*Biatoropsis usnearum* Räsänen	*Usnea austroindica*	Tamil Nadu
5	*Carbonea vitellinaria* (Nyl.) Hertel	*Candelaria vitellina*	Jammu & Kashmir
6	*Cercidospora macrospora* (Uloth) Hafellner & Nav.-Ros.	*Lecanora* sp.	Jammu & Kashmir
7	*C. melanophthalmae* Nav.-Ros., Calat. & Hafellner	*Rhizoplaca melanophthalma*	Jammu & Kashmir
8	*C. xanthoriae* (Wedd.) R. Sant.	*Xanthoria elegans*	Jammu & Kashmir
9	*Cercidospora* sp.	*Lecanora* sp.	Jammu & Kashmir
10	*Cladosporium licheniphilum* Heuchert & U. Braun	*Xanthoria candelaria*	Jammu & Kashmir
11	*Corticifraga peltigerae* (Fuckel) D. Hawksw. & R. Sant.	*Peltigera elisabethae* & *P.ponojensis*	Jammu & Kashmir
12	*Endococcus incrassatus* Etayo & Breuss	*Endocarpon pusillum*	Jammu & Kashmir
13	*E. rugulosus* (Borrer ex Leight.) Nyl.	*Rhizocarpon disporum* *Aspicilia* sp.	Jammu & Kashmir Jammu & Kashmir
14	*Homostegia hertelii* D. Hawksw., V. Atienza & M.S. Cole	*Flavoparmelia caperata* & *Punctelia rudecta*	Uttarakhand
15	*H. piggotii* (Berk. & Broome) P. Karst.	*Parmelia* sp.	Uttarakhand
16	*Intralichen christiansenii* (D. Hawksw.) D. Hawksw. & M.S. Cole	*Candelariella aurella*	Jammu & Kashmir
17	*Lichenoconium usneae* (Anzi) D. Hawksw.	*Flavoparmelia caperata*	Jammu & Kashmir
18	*L. xanthoriae* M.S. Christ.	*Melanelixia subargentifera*	Jammu & Kashmir
19	*Lichenodiplis lecanorae* (Vouaux) Dyko & D. Hawksw.	*Caloplaca cerina* & *anthoriacandelaria* *Lecanora* sp.	Jammu & Kashmir Uttarakhand
20	*L. lichenicola* Dyko & D. Hawksw.	*Rinodina* sp.	Uttarakhand
21	*Lichenopeltella swaminathaniana* Harih., Mibey & D. Hawksw.	*Porina* sp.	Tamil Nadu
22	. *Lichenostigma alpinum* (R. Sant., Alstrup & D. Hawksw.) Ertz & Diederich	*Pertusaria albescens*	Jammu & Kashmir`
23	*L. cosmopolites* Hafellner & Calat.	*Xanthoparmelia stenophylla*	Jammu & Kashmir
24	*L. cf. elongatum* Nav.-Ros. & Hafellner	*Lecanora* sp. &*Lobothallia Praeradiosa*	Jammu & Kashmir
25	*L.* subgen. *Lichenogramma* sp.	*Seirophora contortuplicata*	Jammu & Kashmir
26	*Marchandiomyces corallinus* (Roberge) Diederich & D. Hawksw.	*Physcia aipolia* &*Xanthoria candelaria*	Jammu & Kashmir
27	*Melaspilea amarkantakensis* S. Joseph & G.P. Sinha	*Pertusaria amarkantakana*	Madhya Pradesh
28	*M. insitiva* Stirt.	*Pertusaria leioplaca*	West Bengal
29	*Monodictys epilepraria* Kukwa & Diederich	*Lepraria* sp.	Jammu & Kashmir
30	*Muellerella erratica* (A. Massal.) Hafellner & Volk. John	*Lecidea lapicida*&*Lecanora* sp.	Jammu & Kashmir
31	*M. pygmaea* (Körb.) D. Hawksw.	*Xanthoria elegans*&*Acarospora* sp.	Jammu & Kashmir
32	*Nectriopsis lecanodes* (Ces.) Diederich & Schroers	*Peltigera elisabethae* & *P. scabrosa*	Jammu & Kashmir`
33	*Opegrapha foreaui* (Moreau) Hafellner & R. Sant.	*Heterodermia leucomelos*	Tamil Nadu
34	*Phoma* sp.	*Xanthoria elegans*	Jammu & Kashmir

S. No.	Lichenicolous Fungi	Host	Distribution
35	*Phyllosticta galligena*Moreau	*Parmotrema perforatum*	Tamil Nadu
36	*Polycoccum clauzadei* Nav.-Ros. & Cl. Roux.	*Xanthoria elegans*	Jammu & Kashmir
37	*P. pulvinatum* (Eitner) R. Sant.	*Physcia dubia*	Jammu & Kashmir
38	*Pronectria subimperspicua* (Speg.) Lowen	*Punctelia borreri*	Jammu & Kashmir
39	*Pyrenidium actinellum* Nyl.	*Peltigera elisabethae* &*P. Praetextata* *Punctelia rudecta*	Jammu & Kashmir Uttarakhand
40	*Rosellinula frustulosae* (Vouaux) R. Sant.	*Lecanora argopholis*	Jammu & Kashmir
41	*Sarcogyne sphaerospora* J. Steiner	*Candelariella* sp.	Jammu & Kashmir
42	*Skyttea fusispora* Sherwood, D. Hawksw. & Coppins	*Ochrolechia trochophora*	Assam
43	*Sphaeropezia* cf. *lecanorae* (Diederich & G. Marson) Baloch & Wedin	*Lecanora muralis*	Jammu & Kashmir
44	*Sphinctrina anglica* Nyl.	*Pertusaria* sp.	Tamil Nadu & Uttar Pradesh
45	*S. tubaeformis* A. Massal.	*Pertusaria* sp.	Assam, Manipur, Tamil Nadu & Uttarakhand
46	*Stigmidium gyrophorarum* (Arnold) D. Hawksw.	*Umbilicaria vellea*	Jammu & Kashmir
47	*S. pumilum* (Lettau) Matzer & Hafellner	*Phaeophyscia ciliate*	Jammu & Kashmir
48	*S. tabacinae* (Arnold) Triebel	*Toninia tristis*	Jammu & Kashmir
49	*Vouauxiella lichenicola* (Linds.) Petr. & Syd.	*Lecanora* sp.	Jammu & Kashmir
50	*Zwackhiomyces coepulonus* (Norman) Grube & R. Sant.	*Xanthoria elegans*	Jammu & Kashmir
51	*Z.* cf. *kiszkianus* D. Hawksw. & Miadl.	*Peltigera elisabethae*	Jammu & Kashmir

(Source-Joshi et al., 2015)

Diversity of Endolichenic Fungi

At the time when the lichens were first reported since then the occurrence of the endolichenic fungi can be traced back. Fossil lichen thalli were traced back from as early as the Lower Devonian, since then it had endolichenic fungal association in them (Honegger et al., 2013), which suggests that the survival within lichen thallus is a successful strategy for endolichenic fungi. They are different from the other fungal associates of lichen, namely the mycobiont (Lutzoni and Miadlikowska, 2009) and the lichenicolous fungi associated with the lichens (Arnold et al., 2009). The endolichenic fungi (ELF) are closely associated with the photobiont of the lichen. According to Arnold et al. (2009), it is suggested that such an association could have led to the evolution of plant endophytes. However, it is observed that endolichenic fungi are distinct from the current endophytic fungi associated in plants (U'Ren et al., 2012; Zhang et al., 2015). According to Chagnon et al. (2016), endolichenic fungi are not strictly host specific and thus it behave as generalists when compared with endophytes. Some endophytic fungi such as *Colletotrichum*, *Pestalotiopsis*, *Phomopsis* and *Xylaria* (Suryanarayanan et al.,

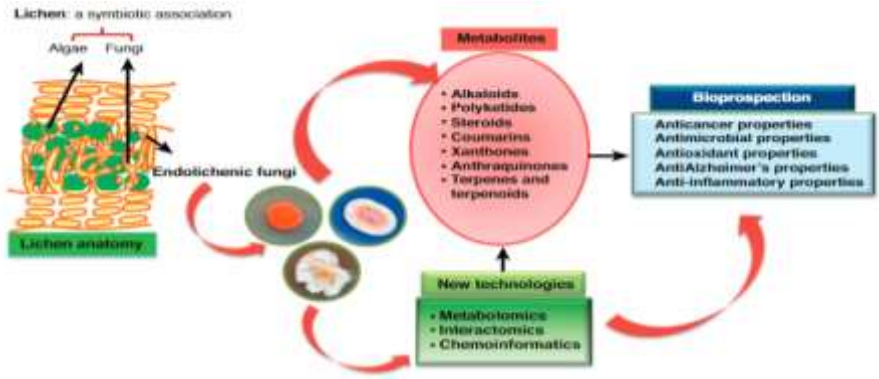
2011; Rajulu et al., 2013; Reddy et al., 2016) also are not host specific and infect taxonomically unrelated plants.

As like many other heterotrophic associates of lichens, endolichenic fungi are also dependent on photobiont (the algal partner) for its nutrition. The photobiont of a lichen species is known to vary (Ruprecht et al., 2014); however, it is not known if such a change in the photobiont partner affects the endolichenic fungal assemblage till yet. This has gained a lot of importance since such a low specificity in the selection of the photobiont by the mycobiont in forming a lichen thallus has widened the ecological amplitude thus enables the lichen to colonise extreme environments also (Muggia et al., 2014) and ultimately to the endolichenic fungal association which is influenced by climate, host lineage and geographic isolation (U'Ren et al., 2012). The first study on endolichenic fungi associated with some corticolous lichens collected from Guindy National Park, Chennai, India (Suryanarayanan et al., 2005).Studies on endolichenic fungi from North east India is still lacking.

ENDOLICHENIC FUNGI AS A SOURCE OF BIOACTIVE METABOLITES

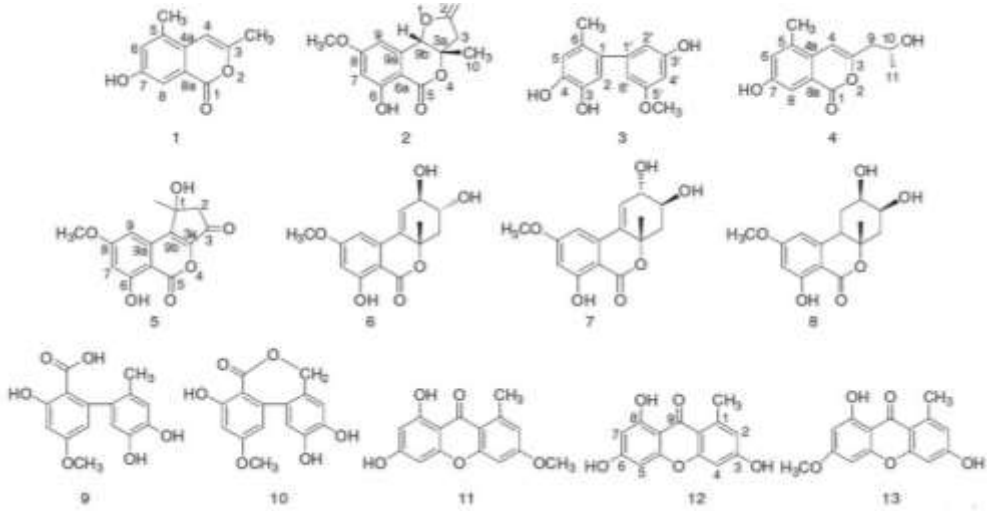
Endolichenic fungi are a rich source of active metabolites that have varied anticancer, antifungal, antibacterial properties, etc. (Figure 1).Many works had been done to extract the secondary metabolites from the endolichenic fungi. Four new cytotoxic Arborinane type triterpenes has been isolated from the endolichenic fungus *Myrothecium inundatum.* The structures of the compounds were elucidated with the help of NMR and MS analyses. Some of the compounds extracted from the cultured endolichenic fungus *Myrothecium inundatum* showed cytotoxic activity against K562 & RKO human cell lines (Basnet et al., 2019). A first report on the effect of endolichenic fungi from *Nephroma laevigatum* was done, 46 isolates were obtained which were identified with the help of DNA barcoding, out of which 6 isolates were tested for antiproliferative and antibiofilm potentialities (Legarde et al., 2019).First report of norlichexanthanone having antioxidant activity produced by cultured endolichenic fungi induced from *Pertusaria laeviganda* have the same level of antioxidant activity as like ascorbic acid (Kawakami et al., 2019). From the culture of endolichenic fungus *Dothideomycetes* sp. ELF003334, two pyrone derivative has been found which shows anticancerous activity and one of the compound also inhibits the protein expression of inducible nitric oxide synthase(Kim, 2018).Compounds were isolated from the endolichenic fungi *Phoma* sp. which were termed as phomalichenones. The structure of the compounds were determined with the help of NMR and MS. Some of the compounds have potent antitumor properties (Kim et al., 2018) Phytotoxic secondary metabolites were isolated from the endolichenic fungus *Myxotrichum* sp. Some of the compounds significantly reduced shoot elongation of *Arabidopsis thaliana* (Yuan et al., 2018). Polyketides like Ophiosphaerellins and

Ophiosphaerekorrius were isolated from the endolichenic fungi *Ophiosphaerella korrae* (Yuelan et al., 2018). Polyketides- Terpene hybrid metabolite from the endolichenic fungi Pestalotiopsis sp. has been isolated. The structures were elucidated by extensive NMR experiments (Yuan et al., 2017). Secondary metabolites were also isolated from the endolichenic fungi *Apiospora montagnei* from the lichen *Cladonia* sp. (Blessing et al., 2017). Heptaketides from an endolichenic fungus *Biatriospora* sp. were isolated. The heptaketides showed antifungal activity against *Candida albicans* (Zhou et al., 2016). Allelopathic polyketides were isolated from an endolichenic fungus *Myxotrichum* sp. were isolated by using OSMAC strategy. Allelopathic test showed that one of the compound significantly retarded root elongation of *Arabidopsis thalliana* seed, indicating that the fungus contributed to the defense of its host lichen (Yuan et al., 2016). A group of anthraquinones 8-O-methylversicolorin A and B isolated from the endolichenic fungus *Aspergillus vesicolor* colonizing the lichen thallus *Lobaria retigeras* showed cytotoxic against PC3 and H460 cell lines (Dou et al., 2014). An another natural compound Diorcinol G has been isolated from an endolichenic fungus *Aspergillus vesicolor* colonizing the lichen thallus *Lobaria quercizans* exhibited cytotoxic activity (Zhao et al, 2014). Conioxepinol B and D, metabolites has been isolated from *Coniochaeta* sp. showed anticancer activities against some selected cell lines (Wang et al., 2010). One of the isolate has also been isolated from endolichenic fungi *Neurospora terricola* were active against HeLa, MCF-7 cell lines (Zhang et al., 2009). Zhang et al. (2012) isolated some metabolites which showed anticancerous activity. Two natural compounds has been isolated from the endolichenic fungus *Coniochaetae* sp. reported to have antibacterial activity against *Enterococcus faecium* and *E. faecalis* (Wang et al., 2010). Ding et al. (2009) have isolated an ambuic acid derivative from *Pestalotiopsis* sp., an endolichenic fungus showed antimicrobial activity against *Staphylococcus aureus*. Nodulisporiviridin A, B, C, D, E, F. G and H has been isolated from Nodulisporium sp. associated with lichen thallus of *Everniastrum* sp. showed inhibitory activity in an anti-Aβ42 aggregation assay (Zhao et al.,2015). Padhi and Tayung (2015) recorded some of the antimicrobial potential of endolichenic fungi isolated from *Parmelia* Lichen against some human pathogens. One compound from *Penicillium* sp. inhabiting the lichen thallus *Parmotrema* span another compound from *Curvularia trifolii* inhabiting *Usnea* sp. are some of the antioxidants metabolites known so far (Jiao et al., 2015). Some of the compounds were isolated from the endolichenic fungi *Ulocladium* sp. were named as 7-hydroxy-3, 5-dimethyl-isochromen-1-one and 6-hydroxy-8-methoxy-3a-methyl-3a,9b-dihydro-3H-furo [3,2-c]isochromene-2,5-dione, along with eleven known compounds, 5′-methoxy-6-methyl-biphenyl-3,4,3′-triol, 7-hydroxy-3-(2-hydroxy-propyl)-5- methyl-isochromen-1-one, rubralactone, isoaltenuene, altenuene, dihydroaltenuenes A, altenusin, alterlactone, 6-O-methylnorlichexanthone, norlichexanthone, and griseoxanthone C (Wang et al., 2011) (Figure 2).



(Source- Singh et al., 2017).

Figure 1. Use of endolichenic fungi as a promising resource for biosprospection.



(Source-Wang et al., 2011).

Figure 2. Some of the compounds isolated from endolichenic fungi. (1) 7-hydroxy-3, 5-dimethyl-isochromen-1-one (2) 6-hydroxy-8-methoxy-3a-methyl-3a,9b-dihydro-3H-furo[3,2-c]isochromene-2,5-dione (3)5′-methoxy-6-methyl-biphenyl-3,4,3′-triol (4) 7-hydroxy-3-(2-hydroxy-propyl)-5- methyl-isochromen-1-one, (5) rubralactone, (6) isoaltenuene, (7) altenuene, (8) dihydroaltenuenes A, (9) altenusin, (10) alterlactone, (11) 6-O-methylnorlichexanthone, (12) norlichexanthone and (13)griseoxanthone C.

CONCLUSION

Nature has endowed with rich diversity of lichens which are beneficial to the mankind in many ways. Out of the total global lichen diversity, only few are documented and studied. Many lichen species are yet to be explored specially from virgin and cryptic habitats. Further, lichen associated fungi particularly endolichenic fungi are still unexplored and understudied. In the recent times, these fungi are considered as repository

of many bioactive metabolites which can be used as antibacterial, antifungal, anticancer agents, etc. Considering the myriad of lichen species in this planet, study of lichen associated fungi such as endolichenic fungi might leads to the discovery of new and novel bioactive metabolites for wide therapeutic applications in the field of pharmacy and medicine. Such molecules could also be an ideal candidature to combat with the deadly and dangerous diseases the world facing at present.

REFERENCES

Adamo, P. (2000). Weathering of rocks and neogenesis of minerals associated with lichen activity. *Applied Clay Science*, 16(5-6), 229–256.

Adamo, P., Vingiani, S. and Violante, P. (2002). *Lichen-rock interactions and bioformation of minerals. Soil Mineral-Organic Matter-Microorganism Interactions and Ecosystem Health*,377–391.

Arnold, A.E. (2001). Fungal endophytes in neotropical trees: abundance, diversity, and ecological implications. InK. N. Ganeshaiah, R.U. Shaanker and K.S. Bawa (Eds.) *Tropical ecosystems: structure, diversity and human welfare*, (pp. 739–743). Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

Arnold, A.E. (2007). Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers.*Fungal Biol Rev*., 21(2–3), 51–66

Arnold, A., Miadlikowska, E., Higgins, J., Sarvate, K.L., Gugger, S.D., Way, P., Hofstetter, A., Kauff, V. & Lutzoni, F. (2009). A phylogenetic estimation of trophic transition networks for ascomycetous fungi: are lichens cradles of symbiotrophic fungal diversification? *Syst Biol.*, 58, 283–297.

Aschenbrenner, I.A., Cardinale, M., Berg, G. and Grube, M. (2014). Microbial cargo: do bacteria on symbiotic propagules reinforce the microbiome of lichens? *Environmental Microbiology*,16(12), 3743–3752.

Asplund, J., Gauslaa, Y. and Merinero, S. (2017). Low synthesis of secondary compounds in the lichen *Lobaria pulmonaria* infected by the lichenicolous fungus *Plectocarpon lichenum*. *New Phytologist*, 217(4), 1397–1400.

Basnet, B.B., Liu, L., Chen, B., Suleimen, Y.M., Yu, H., Guo, S., Bao, L., Ren, J. & Liu, H. (2019). Four New Cytotoxic Arborinane-Type Triterpenes from the Endolichenic Fungus *Myrothecium inundatum*. *Planta Medica*.

Bergmann, T. C. and Werth, S. (2017). Intrathalline distribution of two lichenicolous fungi on *Lobaria* hosts — an analysis based on quantitative Real-Time PCR. *Herzogia*,30 (1), 253–271.

Biosca, E.G., Flores, R., Santander, R.D., Díez-Gil, J.L. & Barreno, E.(2016).Innovative approaches using lichen enriched media to improve isolation and culturability of lichen associated bacteria. *PLoS One*, 11(8), e0160328.

Boerema, G. H., Gruyter, J. de., Noordeloos, M.E. & Hamers, M. E. C.(2004). *Phoma identification manual: Differentiation of Specific and Infra-specific Taxa in culture*. CABI Publishing, Wallingford, UK.

Bown, D. (2001). *Encyclopedia of Herbs and their Uses*. Dorling Kindersley, London.

Brackel, W. von. (2008). *Phoma ficuzzae* sp. nov. and some other lichenicolous fungi from Sicily, Italy. *Sauteria*, 15,103–120.

Brackel, W. von. (2015). Lichenicolous fungi from Central Italy with notes on some remarkable hepaticolous, algicolous and lichenized fungi. *Herzogia*, 28(1), 212–281.

Chandra, S. and Singh, A. (1971).A lichen crude drug (chharila) from India. *Journal of Research in Indian Medicine*, 6, 209-215.

Chen, J., Blume, H. P. and Beyer, L. (2000). Weathering of rocks induced by lichen colonization — a review. *Catena*, 39(2), 121–146.

Crittenden, P.D., David, J.C., Hawksworth, D.L. and Campbell, S.F. (1995).Attempted isolation and success in the culturing of a broad spectrum of lichen-forming and lichenicolous fungi. *New Phytologist*, 130, 267-297.

Czarnota, P., Mayrhofer, H. and Bobiec, A. (2018). Noteworthy lichenized and lichenicolous fungi of open-canopy oak stands in east-central Europe. *Herzogia*, 31, 172–189.

Crawford, S.D. (2015). Lichens used in traditional medicine. In B. Rankovic (Ed.) *Lichen Secondary Metabolites*, (pp. 27-80). Springer International Publishing, Switzerland.

Davis, E.W. and Yost, J. A. (1983). Novel hallucinogens from eastern Ecuador. *Botanical Museum Leaflets*, Harvard University, 29(3), 291–295.

Deduke, C., Halden, N. M. and Piercey-Normore, M.D. (2016).Comparing element composition of rock substratum with lichen communities and the fecundity of *Arcto-parmelia* and *Xanthoparmelia* species. *Botany*, 94, 41-51.

De los Rios, A. Sancho, L.G., Grube, M., Wierzchos, J. and Ascaso, C. (2004). Endolithic growth of two Lecidea lichens in granite from continental Antarctica detected by molecular and microscopy techniques. *New Phytotologist*, 165(1),181–190.

De los Rios, A. & Grube, M. (2000). Host-parasite interface of some lichenicolous fungi in the Dacampiaceae (Dothideales, Ascomycota). *Mycological Research*. 104, 1348–1353.

Diederich. P., Kocourkova, J., Etayo, J. & Zhurbenko, M. (2007).The lichenicolous *Phoma* species (coelomycetes) on *Cladonia*. *Lichenologist*, 39, 153–163.

Diederich, P., D. Ertz, M. Eichler, R. Cezanne, P. van den Boom, E. Fischer, D. Killmann, D. Van den Broeck and E. Sérusiaux, 2012. New or interesting lichens and lichenicolous fungi from Belgium, Luxembourg and northern France. XIV. Bulletin de la Société des naturalistes luxembourgeois 113: 95-115.

Diederich, P., Lawrey, J. D. & Ertz, D. (2018). The 2018 classification and checklist of lichenicolous fungi, with 2000 non-lichenized, obligately lichenicolous taxa. *The Bryologist*, 121(3), 340–425.

Ding, G., Li, Y., Fu, S., Liu, S., Wei, J. and Che, Y. (2009).Ambuic acid and Torreyanic Acid Derivatives from the Endolichenic Fungus *Pestalotiopsis* sp. *Journal of Natural Products*, 72(1), 182–186.

Dou, Y., Wang, X., Jiang, D., Wang, H., Jiao, Y., Lou, H. and Wang, X. (2014). Metabolites from *Aspergillus versicolor*, an endolichenic fungus from the lichen *Lobaria retigera*. *Drug Discovery & Therapeutics*, 8(2), 84–88.

Edwards, H. G. M., Seaward, M. R. D., Preece, T. F., Jorge-Villar, S. E. and Hawksworth, D. L. (2016). Raman spectroscopic analysis of the effect of the lichenicolous fungus *Xanthoriicola physciae* on its lichen host. *Symbiosis*, 71(1), 57–63.

Esslinger, T.L. (1997).A cumulative checklist for the lichen-forming, lichenicolous and allied fungi of the continental United States and Canada. North Dakota State University.

Fleischhacker, A., Grube, M., Frisch, A., Obermayer, W. and Hafellner, J. (2016). *Arthonia parietinaria*- A common but frequently misunderstood lichenicolous fungus on species of the Xanthoria parietina group. *Fungal Biology*, 120(11), 1341–1353.

Fleischhacker, A., Grube, M., Kopun, T., Hafellner, J. and Muggia, L. (2015). Community analyses uncover high diversity of lichenicolous fungi in alpine habitats. *Microbial Ecology*, 70(2), 348–360.

Frati, L., Santoni, S., Nicolardi, V., Gaggi, C., Brunialti, G., Guttova, A., Gaudino, S., Pati, A., Pirintsos, S. A. and Loppi, S. (2007). Lichen biomonitoring of ammonia emission and nitrogen deposition around a pig stockfarm. *Environmental pollution*, 146, 311-6.

Gauslaa, Y. (2014). Rain, dew, and humid air as drivers of morphology, function and spatial distribution in epiphytic lichens. *Lichenologist*, 46(01), 1–16.

Giordani, P. (2006). Variables influencing the distribution of epiphytic lichens in heterogeneous areas: a case-study for Liguria (NW-Italy). *Journal of Vegetation Science*, 17, 195–206.

Giordani, P. (2007). Is the diversity of epiphytic lichens a reliable indicator of air pollution? A case study from Italy. *Environmental Pollution*, 146, 317–323.

Giordani, P. and Incerti, G. (2007). The influence of climate on the distribution of lichens: A case study in a borderline area (Liguria, NW Italy). *Plant Ecology*, 195, 257-272.

Girlanda, M., Isocrono, D., Bianco, C. and Luppi-Mosca, A. M. (1997). Two foliose lichens as microfungal ecological niches. *Mycologia*, 89, 531–536.

Govinda Rajulu, M.B., Thirunavukkarasu, N., Babu, A.G., Aggarwal, A., Suryanarayanan, T.S. and Reddy, M.S. (2013). Endophytic Xylariaceae from the

forests of Western Ghats, southern India: distribution and biological activities. *Mycology*, *4*, 29–37.

Goward, T. and Spribille, T. (2005). Lichenological evidence for the recognition of inland rain forests in western North America. *Journal of Biogeography*, *32*(7), 1209–1219.

Halıcı, M.G., Candan, M., Güllü, M. and Özcan, A. (2014). *Phoma recepii* sp. nov. from the *Caloplaca cerina*. *Mycotaxon*.

Hawksworth, D. L. and Honegger, R. (1994). The lichen thallus: asymbioticphenotype of nutritionally specialized fungi and itsresponse to gall producers. In M. A. J. Williams (Ed.) *Plantgalls: organisms*, *interactions*, *populations*, (pp.77–98). Oxford, UK: Clarendon Press.

Hawksworth, D. L. and Rossman, A.Y. (1997). Where are all the undescribed fungi? *Phytopathology*, 87, 888–891.

Hawksworth, D. L. (1982).Secondary fungi in lichen symbioses: parasites, saprophytes and parasymbionts. *Journal of the Hattori Botanical Laboratory*, 52, 357–366.

Hawksworth, D. L., Halıcı, M. G., Kocakaya, Z. and Kocakaya, M. (2016). *Henfellra muriformisgen.et* sp. nov., a new dictyosporous pycnidial fungus on *Candelariella*, with a Key to the Lichenicolous Fungi Known from that Genus. *Herzogia*, 29(2), 329–336.

Hawksworth, D.L. and Cole, M.S. (2004).*Phoma fuliginosa* sp. nov., from *Caloplaca trachyphylla* in Nebraska, with a key to the known lichenicolous species. *Lichenologist*, 36(1), 7–13.

Honegger, R., Axe, L. and Edwards, D. (2013). Bacterial epibionts andendolichenic actinobacteria and fungi in the lower Devonian lichen *Chlorolichenomycites salopensis*. *Fungal Biolology*, 117, 512–518.

Ilbäck, N. and Källman, S. (1999). The lichen rock tripe (Lasallia pustulata) as survival food: effects on growth, metabolism and immune function in Balb/c mice. *Natural toxins*, 7, 321–9.

Inoue, T., Kudoh, S., Uchida, M., Tanabe, Y., Inoue, M. and Kanda, H. (2014). Effects of substrate differences on water availability for Arctic lichens during the snow-free summers in the High Arctic glacier foreland. *Polar Science*, *8*(4), 397–41.

Jiao, Y., Li, G., Wang, H.Y., Liu, J., Li, X., Zhang, L., Zhao, Z. and Lou, H. (2015).New metabolites fromendolichenic fungus *Pleosporales* sp. *Chemistry and Biodiversity*, 12(7), 1095–1104.

Joshi, Y., Upadhyay, S., Shukla, S., Nayaka, S. and Rawal, R.S.(2015) – New records and an updated checklist of lichenicolous fungi from India. *Mycosphere*, 6(2), 195–200.

Kannangara, B.T.S.D.P., Rajapaksha, R.S.C.G. and Paranagama, P.A. (2009).Nature and bioactivities of endolichenic fungi in *Pseudocyphellaria* sp., *Parmotrema* sp. and *Usnea* sp. at Hakgala montane forest in Sri Lanka. *Lett Appl Microbiol*., 48,203–209.

Kawakami, H., Suzuki, C., Yamaguchi, H., Hara, K., Komine, M. and Yamamoto, Y. (2019). Norlichexanthone produced by cultured endolichenic fungus induced from *Pertusaria laeviganda* and its antioxidant activity, *Bioscience, Biotechnology, and Biochemistry*, 83(6),996-999.

Kim, G. S., Ko, W., Kim, J. W., Jeong, M.H., Ko, S.K., Hur, J.S. & Ahn, J. S. (2018). Bioactive α-Pyrone Derivatives from the Endolichenic Fungus *Dothideomycetes* sp. EL003334. *Journal of Natural Products*, 81(4), 1084–1088.

Kocakaya, Zekiye., Halıcı, Mehmet. & Kocakaya, Mustafa. (2016). *Phoma candelariellae* sp. Nov., a lichenicolous fungus from Turkey. *Mycotaxon*., 130, 1185-1189.

Kondratyuk, S., Karnefelt, I., Goward, T., Galloway, D., Kudratov, I., Lackovičova, A., Lisicka, E. and Guttova, A. (2010). *Diagnoses of new taxa.*, 435–445.

Kumar, K.and Upreti, D. K. (2001). *Parmelia* sp. (lichens) in ancient medicinal plant lore of India. *Economic Botany, 55*(3), 458-459.

Lagarde, A., Millot, M., Pinon, A., Liagre, B., Girardot, M., Imbert, C., Ouk, T. S., Jargeat, P. and Mambu, L. (2019). Antiproliferative and antibiofilm potentials of endolichenic fungi associated with the lichen *Nephroma laevigatum*, *Appl Microbiol*., 126(4), 1044-1058.

Lal, B.M. & Upreti, D.K. (1995). Ethno botanical notes on three Indian lichens. *Lichenologist*, 27(1), 77-99.

Launert, E. (1981). Edible and Medicinal Plants.*Hamlyn.*

Lawrey, J. D., Zimmermann, E., Sikaroodi, M. and Diederich, P. (2016). Phylogenetic diversity soft bulbil-forming lichenicolous fungi in Cantharellales including a new genus and species. *The Bryologist*, 119(4), 341–349.

Lawrey, J. D. and Diederich, P. (2003). Lichenicolous fungi: interactions, evolution, and biodiversity. *Bryologist*, 106, 80–120.

Li, Y.L., Zhu, R.X., Li, G., Wang, N.N., Liu, C.Y., Zhao, Z.T. and Lou, H.X.(2019). Secondary metabolites from the endolichenic fungus *Ophiosphaerella korrae.RSC Advances*, 9(8), 4140–4149.

Logesh, A. R., Chinlampianga, M., Shukla, A. C. and Upreti, D. K. (2015). Studies on Lichens of Mizoram, Northeast India. Proceedings of the National Academy of Sciences, India Section B: *Biological Sciences*, 87(2), 445–457.

Lücking, R., Moncada, B., Martínez-Habibe, María Cristina., Salgado-Negret, Beatriz E., Celis, Marcela., ojas-Zamora, Oscar., Rodríguez, M. Gina., Brokamp, Grischa. and Borsch, T.(2019). Lichen diversity in Colombian Caribbean dry forest remnants. *Caldasia* [online], 41,194-214.

Lutzoni, F. and Miadlikowska, J. (2009). Lichens.quick guide. *Curr Biol*.,19,502-503.

McCarroll, D. and Viles, H. (1995). Rock-weathering by the lichen *Lecisea auriculata*in an Arctic alpine environment.*Earth Surf. Process Landform*,20,199-206.

McDonald, T. R., Gaya, E. and Lutzoni, F. (2013). Twenty-five cultures of lichenizing fungi available for experimental studies on symbiotic systems. *Symbiosis*, 59(3),165–171.

Millanes, A. M., Diederich, P., Westberg, M., Pippola, E. and Wedin, M. (2015). *Tremella cetrariellae* (Tremellales, Basidiomycota, Fungi), a new lichenicolous fungus on *Cetrariella delisei. The Lichenologist*, 47(06), 359–368.

Motiejūnaitė, J. and Skridlaitė, G. (2017). New Records of Lichens and Lichenicolous Fungi in Lithuania, Mainly from Quarries. *Herzogia*, 30(1), 126–137.

Muggia, L., Pérez-Ortega, S., Kopun, T., Zellnig, G.and Grube, M. (2014). Photobiont selectivity leads to ecological tolerance and evolutionary divergence in a polymorphic complex of lichenized fungi. *Ann Bot.*, 114,463–475.

Nayaka. S., Upreti, D.K. and Khare, R. (2010). Medicinal lichens of India. In *Drugs from Plants* (Ed. P.C. Trivedi). Avishkar Publishers, Distributors, Jaipur, India.

Nayaka, S.and Upreti, D.K. (2005). Status of Lichen Diversity in Western Ghats, India.*Sahyadri E-News*., *16*.

Nimis, P., Scheidegger, C. and Wolseley, P. (2002). Monitoring with Lichens–Monitoring Lichens. 10.1639/000.

Padhi, S. and Tayung, K. (2015). *In vitro* antimicrobial potentials of endolichenic fungi isolated from thalli of *Parmelia* lichen against some human pathogens. *Beni-Suef University Journal of Basic and Applied Sciences*, 4(4), 299–306.

Petrini, O., Hake, U. & Dreyfuss, M. (1990). An analysis of fungal communities isolated from fruticose lichens. *Mycologia*., 82,444–451.

Petrini, O. (1991). Fungal Endophytes of Tree Leaves.10.1007/978-1-4612-3168-4_9.

Pino-Bodas, R., Laakso, I. and Stenroos, S. (2017). Genetic variation and factors affecting the genetic structure of the lichenicolous fungus *Heterocephalacria bachmannii (Filobasidiales, Basidiomycota).PLOS ONE*, 12(12), e0189603.

Pino-Bodas, R., Zhurbenko, M. P. and Stenroos, S. (2017). Phylogenetic placement within Lecanoromycetes of lichenicolous fungi associated with Cladonia and some other genera. *Persoonia - Molecular Phylogeny and Evolution of Fungi*, 39(1), 91–117.

Pinokiyo, A., Singh, K.P. and Singh, J.S. (2008). Diversity and distribution of lichens in relation to altitude within a protected biodiversity hot spot, north-east India. *The Lichenologist*., 40(01), 47-62.

Porada, P., Weber, B.,Elbert, W., Pöschl,U. and Kleidon,A.(2014).Estimating impacts of lichens and bryophytes on global biogeochemical cycles. *Global Biogeochemical Cycles*, 28(2), 71-85.

Rambold, G. and Triebel, D.(1992).The inter-lecanoralean associations. *Bibliotheca Lichenologica*,48(1),201.

Redzic, S., Barudanovic, S. and Pilipovic, S. (2010). Wild Mushrooms and Lichens used as Human Food for Survival in War Conditions; Podrinje - Zepa region (Bosnia and Herzegovina, W. Balkan). *Human Ecology Review*.,17, 175-181.

Ruprecht, U., Brunauer, G.and Türk, R. (2014). High photobiont diversity in the common European soil crust lichen *Psora decipiens*. *Biodivers Conserv.*, 23,1771–1785.

Schiefelbein, U., Brackel, W. von, Cezanne, R., Eichler, M., Krzewicka, B., Neumann, P. and Dolnik, C. (2018). Additional Interesting Records of Lichenized and Lichenicolous Fungi from Northern Germany. *Herzogia*, 31(1), 114–132.

Schwarz, J. (2018).Use of lichen to create food for the apocalypse.

Sheppard, L. J., Leith, I. D., Crossley, A., van Dijk, N., Cape, J. N., Fowler, D. and Sutton, M. A. (2009). 'Long-Term Cumulative Exposure Exacerbates the Effects of Atmospheric Ammonia on an Ombrotrophic Bog: Implications for Critical Levels'. In M. A. Sutton, S. Reis, and S. M. H. Baker (eds.). 'AtmosphericAmmonia: Detecting emission changes and environmental impacts'. Dordrecht, Netherlands: Springer.

Singh, B. N., Upreti, D. K., Gupta, V. K., Dai, X.F. and Jiang, Y. (2017).Endolichenic Fungi: A Hidden Reservoir of Next GenerationBiopharmaceuticals. *Trends in Biotechnology*, 35(9), 808–813.

Singh, K.P. and Sinha, G. P. (1997).Lichens. In: Floristic Diversity and Conservation Strategies in India, Vol. *1 Cryptograms and Gymnosperms (V. Mudgal and P.K. Hajra, eds.)*, Botanical Survey of India, New Delhi, India, 195-234.

Singh, P., Singh, K. and Bhatt,A.(2015).Diversity and distribution of microlichens in the state of Arunachal Pradesh, Eastern Himalaya, India, *Check List*, 11(6),1-20 .

Sinha, G. & Jagadeesh Ram, T. A. M.(2019). *Lichen Diversity in Sikkim.*

Sudhakara Reddy, M., Murali, T.S., Suryanarayanan, T.S., Govinda Rajulu, M. B. and Thirunavukkarasu, N.(2016). *Pestalotiopsis* species occur as generalist endophytes in trees of Western Ghats forests of southern India. *Fungal Ecol*., *24*,70–75.

Suija, A., Kaasalainen, U., Kirika, P. M. and Rikkinen, J. (2018). Taitaia, a novel lichenicolous fungus in tropical montane forests in Kenya (East Africa). *The Lichenologist*, 50(02), 173–184.

Suryanarayanan, T.S., Murali, T.S., Thirunavukkarasu, N., Govinda Rajulu, M.B., Venkatesan, G. and Sukumar, R. (2011). Endophytic fungal communities in woody perennials of three tropical forest types of the Western Ghats, southern India. *Biodivers Conserv*., 20,913–928.

Suryanarayanan, T. S., Thirunavukkarasu, N., Hariharan, G.N. and Balaji, P. (2005). Occurrence of non-obligate microfungi inside lichen thalli.*Sydowia*.,57,120–130.

Thangjam, N., Awadhesh,K., Amritesh, C.S. and Upreti, D.K. (2019). Diversity and Distribution of Lichens in Murlen National Park of Mizoram, India. *Environment and Ecology*, 37 (3) : 664—672

Tripp, E. A., Lendemer, J. C., Barberán, A., Dunn, R. R. and Fierer, N. (2016). Biodiversity gradients in obligate symbiotic organisms: exploring the diversity and traits of lichen propagules across the United States. *Journal of Biogeography*, *43*(8), 1667–1678.

U'Ren, J.M., Lutzoni, F., Miadlikowska, J., Laetsch, A.D. & Arnold, A. E. (2012). Host and geographic structure of endophytic and endolichenic fungi at a continental scale.*Am J Bot*.,99,898–914.

Upreti, D.K. (1998). Diversity of lichens in India.In*: Perspectives in Environment (S.K. Agarwal, J.P. Kaushik, K.K. Kaul and A.K. Jain, eds.)*, APH Publishing Corporation, New Delhi, India,71-79.

Van den Boom, P. P. G. (2016). Lichens and lichenicolous fungi of the Azores (Portugal), collected on São Miguel and Terceira with the descriptions of seven new species. *Acta Botanica Hungarica*, 58(1-2), 199–222.

Wang, Y., Niu, S., Liu, S., Guo, L., and Che,Y. 2010). The first naturally occurring thiepinols and thienol from an endolichenic fungus *Coniochaeta* sp. *Org Lett*,12(21),5081–5083.

Wang, Y., Zheng,Z., Liu, S., Zhang, H., Li, E., Guo, L.,and Che, Y. 2010b). Oxepinochromenones, furochromenone, and their putative precursors from the endolichenic fungus *Coniochaeta* sp., *J Nat Prod, 73*(5),920–924.

Wang, H., Umeokoli, B. O., Eze, P., Heering, C., Janiak, C., Müller, W. E. G. and Proksch, P. (2017). Secondary metabolites of the lichen-associated fungus *Apiospora montagnei*. *Tetrahedron Letters*, *58*(17), 1702–1705.

Wang, Li.S.,Narui,T.,Harada, H.,Culberson,F.C. and Culberson, L.W.(2001) Ethnic Uses of Lichens in Yunnan, China.*The Lichenologist*,*104*(3), 345-349.

Won, K., Jong., K., Wonmin., K., Eun., S., Kim, G., Ja Hwang, G., Son, S., Jeong, M., Hur, J., Oh, H., Ko, S., Jang, J. and Seog Ahn, J. (2018). Anti-inflammatory phomalichenones from an endolichenic fungus *Phoma* sp.*The Journal of Antibiotics*.,71. 753–756.

Yuan, C., Guo,Y. H., Wang, H. Y. X. J. M., Jiang,T., Zhao, J. L., Zou, Z. M. and Ding, G. (2016). Allelopathic polyketides from an endolichenic fungus *Myxotrichum* sp. by using OSMAC strategy. *Sci rep.*, 5, 14850.

Yuan, C., Ding, G., Wang, H. Y., Guo, Y. H., Shang, H., Ma, X. J. & Zou, Z. M. (2017). Polyketide-Terpene Hybrid Metabolites from an Endolichenic Fungus *Pestalotiopsis* sp. *BioMed research international*, *6961928*.

Zedda, L. and Rambold, G. (2009). Diversity and ecology of soil lichens in the Knersvlakte (South Africa). *The Bryologist*, 112(1), 19–29.

Zhang, F., Li, L., Niu, S., Si, Y., Guo, L., Jiang, X., & Che, Y. (2012). A Thiopyran-chromenone and Other Chromone Derivatives from an Endolichenic Fungus, *Preussia africana. Journal of Natural Products*, 75(2), 230–237.

Zhang, F., Liu, S., Lu, X., Guo, L., Zhang, H., & Che, Y. (2009). Allenyl and Alkynyl Phenyl Ethers from the Endolichenic Fungus *Neurospora terricola*. *Journal of Natural Products*, 72(10), 1782–1785.

Zhang, T., Wei, X.-L., Zhang, Y.-Q., Liu, H.-Y., & Yu, L.-Y. (2015). Diversity and distribution of lichen-associated fungi in the Ny-Ålesund Region (Svalbard, High Arctic) as revealed by 454 pyrosequencing. *Scientific Reports*, 5, 14850.

Zhao, H., Wang, G.-Q., Tong, X.-P., Chen, G.-D., Huang, Y.-F., Cui, J.-Y., Gao, H. (2014). Diphenyl ethers from *Aspergillus* sp. and their anti-Aβ42 aggregation activities. *Fitoterapia*, 98, 77–83.

Zhao, Q., Chen, G.D., Feng, X. L., et al. (2015a). Nodulisporiviridins A-H, Bioactive Viridins from *Nodulisporium* sp. *J Nat Prod*,78(6),1221–1230

Zhou, Y.-H., Zhang, M., Zhu, R.-X., Zhang, J.-Z., Xie, F., Li, X.-B., … Lou, H.-X. (2016). Heptaketides from an Endolichenic Fungus Biatriospora sp. and Their Antifungal Activity. *Journal of Natural Products*, 79(9), 2149–2157.

Zhurbenko, M. P., Etayo, J., Demidova, A. N. & Zhdanov, I. S. (2016). *Capronia josefhafellnerisp.nov. (Ascomycota, Herpotrichiellaceae)* and some other Lichenicolous Fungi from Vietnam. *Herzogia*, *29*(2), 364–373.

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 7

SECONDARY METABOLITES BY ENDOPHYTIC FUNGI: POTENTIAL FOR BIO-BASED PRODUCTS DEVELOPMENTS

Ana Paula Sant'Anna da Silva[1], Rebeca Xavier da Cunha[1], Jadson Diogo Pereira Bezerra[2], Thaíse Gabriele da Silva Brito[1], Caíque Silveira Martins da Fonseca[1], Irailton Prazeres dos Santos[1] and Vera Lúcia de Menezes Lima[1,*]

[1]Departamento de Bioquímica, Centro de Biociências,
Universidade Federal de Pernambuco, Recife, PE, Brazil
[2]Departamento de Micologia, Prof. Chaves Batista,
Programa de Pós-Graduação em Biologia de Fungos (PPG-BF),
Centro de Biociências, Universidade Federal de Pernambuco, Recife, PE, Brazil

ABSTRACT

Endophytic fungi are microorganisms that colonize healthy plant tissues inter and/or intracellularly without causing any apparent damage to their host. Endophytes, as they are named, are able to produce secondary metabolites that may offer protection against different harmful agents to plants. These microorganisms have developed a wide diversity of microbial adaptations to special and uncommon environments, which have made them of great potential as sources of novel chemicals for medicinal, industrial and agricultural purposes. Secondary metabolites production processes by fungi are complex due to the presence of several chemical groups, such as phenols, terpenes, alkaloids, steroids, among others, that are interesting for agriculture. For example, it has been observed that *Diaporthe eres* [*Phomopsis oblonga*], an endophytic fungus from Elm trees

* Corresponding Author's Email: lima.vera.ufpe@gmail.com.

(*Ulmus* sp.) produces several secondary metabolites, such as nectnapyrone, 4-hydroxyphenylethanol, a novel norsesquiterpene y-lactone with insecticide activity against *Scolytus scolytus*. On the other hand, *Aspergillus flavus* and *Nigrospora oryae* [*Nigrospora sphaerica*], endophytic fungus from *Tectona grandis* produces the secondary metabolites duroquinone, naphthelene and adamantine derivatives, with larvicide activity against *Hyblaea purea*, *Atteva fabriciella* and *Eligma narcissus*. Alkaloid molecules have been strongly associated with insecticidal properties. This chapter discuss the state of the art of secondary metabolites obtained from endophytic fungi to demonstrate its use as an important tool in the development of natural and eco-friendly agrochemicals, which can be less harmful to humans and environment, leading to new opportunities in the development of a bio-based commercial product of great importance for sustainable management of insect pests.

Keywords: agrochemicals, agriculture, biocontrol agentes, endophytes, bioactive metabolites, alkaloids, loline, lolitrem B, pest

INTRODUCTION

Microorganisms are present everywhere and have versatility to adapt to different ecosystems all over the world. Numerous microorganisms are known to be endophytic, colonizing inter- and intracellular spaces of plant tissues without causing apparent damage and appearing to be associated with all plants in natural ecosystems (Lata et al. 2018). Among endophytic microorganisms, fungi have an intimate relationship with host plants and can produce compounds that promote vegetative growth, competitiveness and protection to the host against herbivores and pathogens (Plett and Martin 2018). Endophytic fungi present a wide diversity of microbial adaptations that have been evolved in special and unusual environments, making them a great research source for novel active principles as potential bio-control agents (Saad, Ghareeb, and Saeed 2019).

The discovery of secondary metabolites produced by endophytic fungi and their potential applications has been increasing in the past years. Researchers from around the world have found a number of secondary metabolites belonging to different classes such alkaloids, steroids, phenols, quinones, terpenoids, flavonoids and polyketides. Many of them has a great applicability on medicine, agricultural and industrial areas (Schulz et al. 2002; Gouda et al. 2016; Gao, Li, and Lou 2018; Zhang, Li, and Wang 2016; Nisa et al. 2015; Kusari, Hertweck, and Spiteller 2012; Palanichamy et al. 2018; Bano et al. 2016; Stierle and Stierle 2015; Strobel and Daisy 2003).

The rising costs involved in controlling pathogens or pests that promote plant damage have kept financial pressure on farmers (Savary et al. 2019). Additionally, several products used as protective measures have proved quite toxic to the ecological balance. Insect plant pests are of particular interest, as they bear the potential to cause tremendous impact on crop production and are really difficult to control (Douglas 2017).

Both the perception of the existence of biologically active metabolites within endophytic microorganisms and the environmental and economic problems of pests in agriculture have created the favorable situation for the research and development of biotechnological products from endophytes with the objective of eliminating or minimizing the effects of pests on agriculture (Lugtenberg, Caradus, and Johnson 2016; Raman, Wheatley, and Popay 2012).

The scientific investigation of endophytic fungi as possible agents for controlling insect plant pests apparently begun in the 1980's in a study describing that endophytic fungi were able to control a plant disease transmitted by an insect (Webber 1981). Since then, a number of works has been published on this matter, some of them studying an secondary metabolite isolated from endophytic fungi.

In this chapter, we present the current research on endophytic fungi and their secondary metabolites used as potential bio-based products, less toxic to the environment, for the control of economically important plant insect pests.

ENDOPHYTIC FUNGI

The term endophyte was early described by Bary (1866), as being "organisms occurring within plant tissue". Schulz and Boyle (2005) have narrowed this definition to fungi that colonize a plant tissue without causing any visible disease symptoms at any stage of the plant's life cycle. A more recent definition was proposed by Azevedo (2014), who considered endophytes all cultivable or non-cultivable microorganisms, comprising algae, fungi, bacteria, viruses and insects, which inhabit the internal parts of plant tissues without causing damage to their hosts. However, the term endophytic is mainly used for bacteria and fungi, as they play an important role in their host plants (Hiruma, Kobae, and Toju 2018).

Endophytes are usually transmitted horizontally through root-zonepenetration, although air microorganisms can use natural opening sat the plant's aerial parts (leaves, flowers, fruits, stems and cotyledons), such as stomata and hydathodes (Brader et al. 2017; Li et al. 2017). Moreover, endophytes development is systematic, intercellular and vertically transmitted through seeds (Shearin et al. 2018). Scientists have classified endophytic fungi within two ecological groups: balansiaceous and non-balansiaceous (Mishra et al. 2014). The first group is composed by Ascomycota fungi, which belong to the genera *Epichloe* and *Balansia* (asexual morphs *Neotyphodium* and *Ephelis*). They show particular ecological needs and adaptations from the other endophytic fungi. In contrast, non-balansiaceous endophytes are phylogenetically diverse and have a different life strategy. They are horizontally transmitted and have being isolated from most plants (Schulz and Boyle 2005). The species isolated with more frequency belongs to

Ascomycota phylum, but Basidiomycota and Mucoromycota species are also recognized as endophytes (Rungjindamai et al. 2008; Field et al. 2015).

FUNGI HOST INTERACTION

Although virtually all plant species could be hosts for a diverse number of endophytes, only few of them were studied. Usually, thousands of endophytic species can be isolated from one specimen, but this does not limit the need for investigation of different plants, since the endophyte mechanism of interaction with its host changes according to several factors, including the host itself (Backman and Sikora 2008; Khare, Mishra, and Arora 2018).

Then, the complete understanding of these associations is still on course. Part of the complexity has been justified by the myriad of chemistry and physical barriers, which must be transposed to successfully establish the association. In general, this intimate relationship involves a number of processes, which, in turn, can be influenced by genotype, growth stage, physiological conditions and plant tissue, in addition to environmental conditions and agricultural practices(Bacon, Glenn, and Yates 2008; Plett and Martin 2018).

In this context, researches have been postulated two main hypotheses about the endophytic-host interactions: the mutualistic symbiosis and the opposing balance. The first hypothesis suggests that endophytes co-evolved with their hosts, showing an intimate mutualistic relation, where the endophytic fungi receive nutrients and protection from the host and acquires resistance to stress caused by abiotic factors as pH, temperature, hydric stress, strong winds, salinity, humidity, light, contaminants and by biotic factors such as herbivory decrease, insect attack and control of different phytopathogens (Das and Varma 2009; Lata et al. 2018).

Some endophytes may acquire the ability to produce substances originally synthesized by plants. This capacity could be related to a "genetic recombination" between the endophyte and the host during the co-evolved process, probably caused by gene horizontal transference, which has enabled the receptor to create the same biosynthetic reactions of the host. That ability can reduce the collection of rare plants and the biodiversity conservation. This type of interaction also enables resistance against pathogens, enhance vegetable growth and the induction of the process of seed germination (Arora et al. 2018; Tisserant et al. 2013).

The second hypothesis, the balanced antagonism is explained by an endophyte avoiding host defense activation, which guarantees self-resistance, since host's toxic metabolites are not produced. When fungal virulence and plant defense are balanced, the association remains asymptomatic and enables fungus to grow within the plant. In case that plant defense mechanisms counter attack the fungal virulence factors, the fungus will

die. On the other hand, if plant succumbs to fungus virulence, a plant-pathogen relationship would lead to plant disease. The fact that many endophytes can be latent pathogens turns them influenced by certain intrinsic or environmental conditions to express factors leading to pathogenicity (Schulz and Boyle 2005).

This hypothesis does not exclude the possibility that endophytic fungi may contribute positively to its host. In such interaction, endophytic fungus influences production of plant secondary metabolites with defense function, conferring some advantageous side effects to plants, as resistance to insect attack and production of antimicrobials against phytopathogenic microorganisms (Arora and Mishra 2016). The complexity of these interactions has resulted in products of biotechnological interest, varying according to host, geographical distribution, plant age, environmental and seasonal conditions, including altitude and precipitation levels (Márquez et al. 2007; Lata et al. 2018).

The interaction between endophytic communities and host plant has a significant influence on plant physiological processes, such as activation of silent gene pools, leading to new secondary metabolites synthesis. One well-established question is that both plant and its endophytes can produce a great number of common secondary metabolites from similar precursors. On the other hand, fungi may also develop peculiar biochemical pathways, resulting in specific metabolites. Although the production of these molecules depends of several factors, the secondary metabolism usually generates molecules of low molecular weight and belonging to different chemical classes, such as alkaloids, steroids, terpenoids, isocoumarins, quinones, phenylpropanoids, lignins, phenols and phenolic acids, aliphatic metabolites, lactones, cytochalasins, flavonoids, peptides and xanthones (Aly et al. 2010; H. W. Zhang, Song, and Tan 2006; Schulz and Boyle 2005). The products of these metabolic pathways include important drugs, such as penicillin and statins, or toxic substances, such as aflatoxins (Keller 2019). Thus, plant-isolated endophytic microorganisms represent a rich source of novel natural and bioactive products, since a single endophyte may be capable of producing a variety of bioactive metabolites (Chowdhury et al. 2018; Tidke et al. 2018).

The agrochemical industry has been investing in order to discover new bioactive products for pest control due to biodegradability and low toxicity observed in many substances. The biotechnological potential of fungi, which have a wide spectrum of action, is based on the thousands of isolates already known for their biological activity in other areas, as medicine (Li et al. 2018).

DEFINITION AND CONSEQUENCES OF PLANT INSECT PEST

According to the International Standards for Phytosanitary Measures (ISPM) No. 5 of the year of 2010, plant pest is “any species, strain or biotype of plant, animal or pathogenic agent injurious to plants or plant products”(FAO 2011).Pests can affect crop

plants with a high agricultural value and cause varying levels of damage that can be associated to food insecurity, poverty and losses to farmers and markets. In other words, plant pests bring human, environmental and economic issues that concerns all the world (FAO 2018a).

Other plants also affected by pests are forest trees. In this case, the damage is related to disturbances to local ecosystems, which leads to losses in the biodiversity associated to different environmental changes in soil, water, and in the conversion of carbon dioxide into oxygen. Economic losses are problems to be faced too, as production of wood and non-wood products may be present (FAO 2009).

Another example of the negative effects caused by plant pests have been observed in plant pasture used to feed animals, where they cause losses in livestock production and income generation. Grass species of the *Brachiaria* genus, for example, are frequently affected by Cercopoidea insects, as the spittlebug (Batello et al. 2008).

Pests have often been used as justification for the use of pesticides in attempt to control or to suppress them, which can cause environmental damage, as it affects water sources, soil, air, other plants, animals, and even humans health (Aktar, Sengupta, and Chowdhury 2009). The socioeconomic consequences are the loss of the farmer invested money and, for consequence, to the entire production chain, from losses in the market of agricultural products to people or other animal to food deprivation or insecurity (Collette et al. 2011). When the loss is in relation to native or commercial forests, the consequences come in a reaction chain affecting the whole ecosystem, causing ecological and environmental impacts as well as human social and health problems (Moore 2005).

About 20% of the crop production in destroyed by insect pests. However, less than 0.5% of the known insect species can damage plants (Sallam 2001). These insects could affect leaves, roots, flowers, shell, fruits or stalks. They are usually beetles, caterpillars, grasshoppers, flies, moths, and aphids. They are capable of causing galls, transmit plant pathogens, inhibit plant egg-laying or feeding, or even kill the plant (Douglas and Cowles 2001).

The Centre for Agriculture and Biosciences International (CABI), in their most recent study, covering publications from 2012 to 2016, found that the cotton bollworm (*Helicoverpa armigera*), tobacco whitefly (*Bemisia tabaci*), two-spotted spider mite (*Tetranychus urticae*), diamondback moth (*Plutella xylostella*), taro caterpillar (*Spodoptera litura*), red flour beetle (*Tribolium castaneum*), green peach aphid (*Myzus persicae*), fall armyworm (*Spodoptera frugiperda*), cotton aphid (*Aphis gossypii*), brown planthopper (*Nilaparvata lugens*), beet armyworm (*Spodoptera exigua*), western flower thrips (*Frankliniella occidentalis*), Mediterranean fruit fly (*Ceratitis capitata*), codling moth (*Cydia pomonella*), cowpea weevil (*Callosobruchus maculatus*), cotton leafworm (*Spodoptera littoralis*), pea aphid (*Acyrthosiphon pisum*), Asian citrus psyllid (*Diaphorina citri*), tomato leaf miner (*Tuta absoluta*) and onion thrips (*Thrips tabaci*) are the twenty most worldwide cited insect pests among almost other 1200 arthropod species.

Together, they have a potential to cause US$540 billion per year for agriculture sector (Royal Botanical Gardens - Kew 2017).

The two most discussed insect plant pests, which are concerning FAO, are the desert locust (*Schistocerca gregaria*) and the fall armyworm (*S. frugiperda*) (FAO 2018b; Huis 2014). The desert locust is a member of the family Acrididae, which can increase enormously in number at favourable environmental conditions (gregarious phase), has a high ingestion capacity (one insect eat approximately 2 grams of the plant every day), and can promptly spread over large areas, making a giant devastation (FAO 2018b; Symmons and Cressman 2001). Another insect, the fall armyworm, is originally from America tropical and subtropical areas. At its larvae stage, has a great capacity to feed from leaf plant, so destroying crops, mainly maize, but some other important plants. Another problem in relation to this insect pest is that it can fly and spread relatively fast throughout a region (FAO n.d.).

ENDOPHYTIC FUNGI SECONDARY METABOLITES AGAINST PLANT PEST INSECTS

There are many ways to control or quell a pest; one of them is using natural compounds as biocontrol agents. This way to defend against those pests has the advantage of being less harmful to the environment since this biocontrol agent cohabit with the plant (Arnold and Lewis 2005; Sandhu et al. 2012).

Endophytic fungi and its secondary metabolites hold an important role in the plant defence against biotic (plant pathogens, insects and nematodes) and abiotic (temperatures, salination, drought, extreme pH and nutrient limitation) stresses (Figure 1) that can be explored and used as a method to control insect plant pests (Lugtenberg, Caradus, and Johnson 2016). They are rich sources of products for sustainable protection of crop, pasture, wood extraction and native plants (Kumar et al. 2008; Rohlfs and Churchill 2011; Sumarah and Miller 2009).

As endophytic fungi produce a wide range of metabolites, it is expected they would also show a good variety of pathogen inhibition mechanisms. Those can cause repellence, weight loss, growth and development deficiency, toxin production, death, reduced breeding or even turn the plant unpalatable (Azevedo et al. 2000).

The first reports associating the presence of an endophytic fungus to the protection against an insect pest are from 1980's decade. In 1981, Webber (1981) designed a very interesting study in which they verified that an endophyte – "*Phomopsis* sp."–that colonized elm trees (*Ulmus* sp.) was able to inhibit *Scolytus scolytus* and *Scolytus multistriatus* breeding. These are beetles, vectors of the *Ophiostoma ulmi* (formerly *Ceratocystis ulmi*), which is one of the causative agents of the Dutch elm disease. Some years later, Claydon and colleagues conducted an investigation to demonstrate that

"*Phomopsis oblonga*" produces a large diversity of secondary metabolites (see Table 1), acting as a deterrent of the elm beetle *S. scolytus* wood boring activity (Claydon, Grove, and Pople 1985). These two studies began a series of other researches investigating the intrinsic relationship between endophytic fungi, their secondary metabolites, and its action against plant insect pests.

Phenolic compounds from *Aspergillus niger*, a fungus isolated from *Acacia arabica*, were also described as the causative factors of a number of negative effects in *S. litura*, such as the increase in larvae mortality and developmental delay, decline in feeding, morphological deformities and inhibitory effects on adult emergence, longevity, fecundity and hatchability of its eggs (Kaur et al. 2016).

The most discussed secondary metabolites with anti-insect action are the alkaloids, mainly peramine and lolitrem B, and they are a pyrrolopyrazine alkaloid and a complex indole terpene, respectively, that appears in perennial ryegrass colonized by the endophytic fungus *Acremonium lolii* (Rowan 1993). Peramine is a feeding deterrent for adult Argentine stem weevils (*Listronotusbonariensis*) (Rowan and Gaynor 1986) and lolitrem B, purified from Ellet ryegrass. Although it has not caused feeding deterrent activity when artificially incorporated to a diet, lolitrem B has demonstrated interference in the larvae growth and development, including some degree of mortality (Prestidge and Gallagher 1985).

Another alkaloid secondary metabolites are the lolines, that in a study with *Acremonium coenophialum* colonizing tall fescue (*Festuca arundinacea*), caused antifeedant and toxicity activities against *Rhopalosiphum padi* and *Schizaphis graminum*. Pyrrolizidine alkaloids (N-acetyl and N-formyl loline), the most concentrated constituents of the methanol extract looked to be the main molecules responsible for the biological actions (Johnson et al. 1985).

Later, also Popay, Tapper, and Podmore (2009) used two purified loline alkaloids (N-formyl loline and N-acetyl norloline) in an artificial diet gave to Argentine stem evil eggs (*L. bonariensis*). These two compounds were isolated from *Lolium pratense* infected with *Neotyphodium uncinatum* and *L. Arundinaceum* colonized with *Neotyphodium coenophialum* (AR542), respectively. These compounds affected the growth, development and survival during larvae stage.

Patchett et al. (2011) also observed that loline alkaloids are a common source of naturally occurred compounds with action against pest plant insects. Differently from other works mentioned, this study was conducted with 12 different meadow fescue (*Festuca pratensis*) genotypes colonized by *N. uncinatum* evaluating loline alkaloids content and correlating them with the action against the grass grub (*Costelytra zealandica*) third-instar larvae. In most of the genotypes, high concentrations of total root lolines were associated with a decrease in the root weight consumed by the grubs, as well as with their reduced bodyweight. These authors had also found higher amounts of lolines in roots attacked by the grass grubs, specially the N-formyl loline.

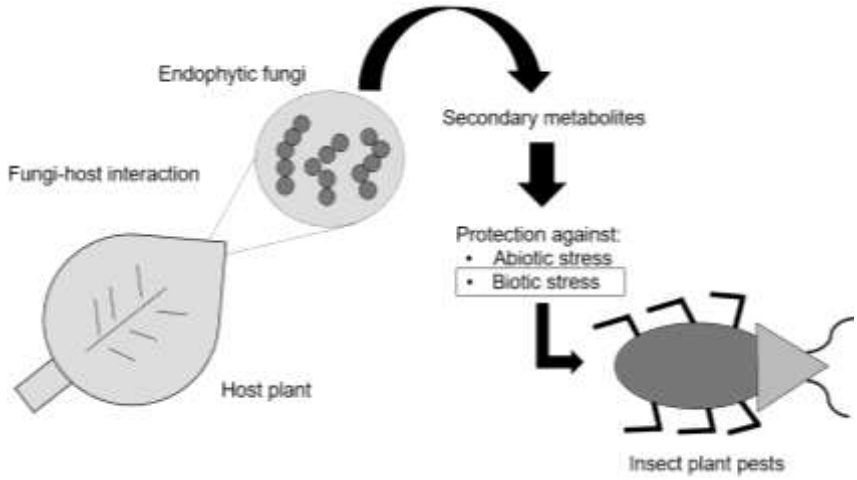


Figure 1. Interrelationship between endophytic fungi, their metabolites and action against insect plant pest.

Recently, Barker, Patchett, and Cameron (2015) found that an artificial diet with a loline isolated from a meadow fescue-perennial (*Festulotium*) colonized with *Epichloë uncinata*, offered to second instar larvae and adults of black beetle (*Heteronychus arator*) was able to cause feeding decrease in a concentration-dependent manner.

Siegel et al. (1990) studied this and other alkaloids, aiming to investigate the relation between different grass species (*Festuca* sp., *Lolium* sp., *Poa* sp., *Bromusanomalus*, *Elymus Canadensis*, *Sitanion longifoliun* and *Agrostis hamalis*) colonizedbydifferent endophytic fungi (*Acremonium* sp., *Epichloetyphina* and *Philophora* sp.) with the production of some alkaloids (N-formyl and N-acetillolines, peramine, lolitrem B and ergovaline) and the action of these alkaloids against two aphid insect pest (*R. padi*, oat-birdcherry and *S. graminum*, greenbug). The results of this study demonstrated that some associations (plant-endophyte) were not able to produce alkaloids. Seven of these associations had produced only peramine, alolitrem, and four different ergovaline molecules, six had produced peramine and ergovaline molecules, one had produced lolines and ergovaline, and one had produced peramine and loline compounds. The *A. coenophialum* infected tall fescue (*F. arundinacea*) and perennial ryegrass (*Loliumperenne*) produced lolines, peramine and ergovaline. The most present alkaloids were peramine and ergovaline and these were related with the presence of *A. coenophialum* and *A. lolii*. In the bioassay against the aphid insect plant pest, loline alkaloids caused mortality for both insects, peramine showed mortality only for *S. graminum*, ergovaline did not affect both, and lolitrem could not be correlated with toxicity to these aphids because it appeared alone in only one association. The lack of a non-infected control does not allow more precise conclusions. Nevertheless, this study contributed to the understanding that the relation among endophytic fungi, their host

plant, their secondary metabolite production, and the action against one insect pest are very complex and dependent of many factors.

In a more recent study, Shymanovich et al. (2019) demonstrated that *Epichloëalsodes* colonizing *Poaalsodes* had caused larval mortality, and *Epichloëschardlii* var. *pennsylvanica*had caused feeding deterrence and affected the development of *S. frugiperda.* Although *E. Alsodes* activity could be explained by the great amount of N-acetylnorlonine produced, in the *E. schardlii* var. *Pennsylvanica* was not detected any of the identified alkaloids, which indicate that some other compound is responsible for its activity.

Another type of alkaloid studied is the janthitrem, this compound has only been found in a strain of *Neotyphodium lolii* (AR37) and was used to infect a perennial ryegrass (*L. perenne*) to evaluate its action against the pasture pest known as porina (*Wiseanacervinata*). Porina larval stage fed with ryegrass colonized by *N. lolii* (AR37) showed defective growth and increased mortality (Jensen and Popay 2004).

Eastern spruce budworm (*Choristoneura fumiferana*), an insect that cause drastic mortality in coniferous forests of eastern North America, is another example of plant pest that already have fungal secondary metabolite with action against it. This metaboliteis called rugulosin, an anthraquinone capable of reduce this budworm growth (Miller, Sumarah, and Adams 2008). For this same pest, also two new diterpenoid (9α-hydroxy-1,8 (14), 15-isopimaratrien-3,7,11-trione and 9α-hydroxy- 1,8(14), 15-isopimaratrien-3,11-dione) showed toxicity. These two diterpenoids were isolated from anunidentified endophytic fungi of balsam fir (*Abies balsamea*) and demonstrated toxicity to cells and larvae of *C. Fumiferana* (Findlay et al. 1995). Another secondary metabolite, cyclodepsipeptideenniatins, was isolated from the same plant from *Fusarium avenaceum,* an endophytic fungus from balsam fir foliage that also showed larvae toxicity to the spruce budworm larvae (Strongman et al. 1988).

Some studies have reported pest control action of different secondary metabolites produced by specific endophytes colonizing a single plant species. This is the case of *Picearubens*, where nine secondary metabolite compounds from three endophytic fungi (*Dwayaancolodena* and two other unidentified endophytes) were found. One of these compounds was a polyketide metabolite, two were biosynthetically related compounds, two others were sesquiterpenes, and four compounds were biosynthetically related maleic anhydrides. When included in a synthetic diet, all of these compounds showed larvae toxicity against *C. fumiferana,* an eastern spruce budworm pest (Sumarah et al. 2010).

Another study also demonstrated the presence of a significant number of secondary metabolitesinanendophytic-entomopathogenic *Aspergillus flavus* and *Nigrospora sphaerica* fungi isolated from *Tectona grandis,* also known as teak. These metabolites were duroquinone, naphthalene, lauric acid, adamantine derivatives and amylmetacresol, and they caused larvae mortality to *Hyblaea purea,* a teak defoliator, and to *Atteva fabriciella* and *Eligma narcissus*, Ailanthus defoliators, in leaf discs of *Ailanthus excelsa*

and *T. grandis* with the fungal extract (Senthilkumar, Murugesan, and Babu 2014).Balansiae fungi tribe also have a large number of secondary metabolites called ergot alkaloids. Among these metabolites, the ergonovine, ergotamine, ergocryptine, lysergol, agroclavine and elymoclavine were tested for insecticidal activity determination though deposition of them in *Zea mays* leaves. Results showed that excluding lysergol, all others alkaloids affected *S. frugiperda* (fall armyworm) feeding larvae and all demonstrated few effects in the larval stage (Clay and Cheplick 1989).

Another study that also evaluated the control of fall armyworm (*S. frugiperda*) found that volatile terpenes secondary metabolites of the endophytic fungus *Trichoderma atroviride*, which was inoculated in *Z. mays*leaves, was capable to reduce the fall armyworm herbivory (Contreras-Cornejo et al. 2018). Another volatile compound identified was the naphthalene from an endophytic fungus (*Muscodorvitigenus*) isolated from *Paulliniapaullionioides* which showed repellence against the insect *Cephuscinctus* (Daisy et al. 2002)

Two new benzofuran compounds [5-hydroxy-2-(1´-oxo-5´-methyl-4´-hexenyl) benzofuran and 5-hydroxy-2-(1´-hydroxy-5´-methyl-4´-hexenyl) benzofuran] obtained from an unidentified endophytic fungus present in the wintergreen, *Gaultheria procumbens*, demonstrated toxicity to cells and one of them also demonstrated toxic capacity to the larvae of spruce budworm *C. fumiferana* (Findlay et al. 1997).

In the recent past year, some researches have explored more specific pathways by which endophytic fungi secondary metabolites can control an insect pest, one of the most prominent candidates has been the alfa glucosidase enzyme, one important digestive insect enzyme. This pathway was first reported when a strain of the endophytic fungus *Exophiala spinifera*, isolated from *Trachyspermum ammi*, showed an inhibitory activity to alfa glucosidase that increased larval mortality and deformities in early adults of the insect pest *S. litura* (Kaur et al. 2018). In another study, a different substance against the same pest was found, chlorogenic acid, an alfa glucosidase inhibitor produced bytheendophytic fungus *Cladosporium velox*, isolated from *Tinosporacordifolia*. This inhibitor has the capacity to delay the development and increase the mortality of larvaeand to increase adult deformities (Singh et al. 2016).

There are entomopathogenic fungi that naturally or artificially behaviour as an endophyte. The importance of these organisms is that they can cause damage to the insect by physical penetration in their tissues or due to production of metabolites, but is less harmful to the host plant (Jaber and Ownley 2018; Vega et al. 2008). An interesting study found that entomopathogenic fungi (*Lecanicilliumlecanii* and *Beauveria bassiana*), which curiously also colonize crop plants are able to increase the mortality, decrease the reproductive period and fecundity and also avoid leaf colonization in choice assay of *A. gossypii*, a cotton insect pest. The authors hypothesized that these effects were due to insect contact with conidia and secondary metabolites of these fungi (Gurulingappa, McGee, and Sword 2011).

Table 1. Secondary metabolites from endophytic fungi with action against plant insect pest

Endophytes	Secondary metabolites	Insect pest	Plant isolated/inoculated	Mechanism of action	References
Phomopisoblonga	A novel norsesquiterpene y-lactone	*Scolytusscolytus*	Elm trees (*Ulmus* sp.)	Boring or feeding deterrent activity	Claydon, Grove, and Pople 1985
	Tiglic esters of two novel 5,6-dihydro-5-hydroxy-2-pyrones				
	Nectnapyrone				
	4-hydroxyphenylethanol				
	5-methylmellein				
	2-furoic				
	Orsellmic				
	3-nitropropanoic acids				
	Mellein-5-carboxylic acid				
	Portensterol				
	Thymine				
Aspergillus niger	Phenolic compounds	*Spodopteralitura*	*Acacia arabica*	Increase of larvae mortality and extension larval development, decline in feeding, morphological deformities and inhibitory effects on adult emergence, longevity, fecundity and hatchability of eggs	Kaur et al. 2016
Acremonium loliae	Lolitrem B	*Listronotusbonariensis*	Ellet ryegrass	Reduce larvae growth and development	Prestidge and Gallagher 1985
	Peramine		*Loliumperenne*	Deterrent feeding	Rowan and Gaynor 1986
Acremonium coenophialum	Loline alkaloids	*Rhopalosiphumpadi*	*Festuca arundinacea*	Deterrent feeding and toxicity activity	Johnson et al. 1985
		Schizaphisgraminum			
Neotyphodium uncinatum		*Listronotusbonariensis*	*Lolium pretense*	Affect the growth, development and survival of larvae stage	Popay, Tapper, and Podmore 2009
Neotyphodiumcoenophialum (AR542)			*Loliumarundinaceum*		
Epichloë uncinatum		*Heteronychusarator*	*Festulotium*	Decrease in the feeding in second instar larvae and adult	Barker, Patchett, and Cameron 2015
Neotyphodium uncinatum		*Costelytrazealandica*	12 different *Festuca pratensis* genotypes	Decrease in the feeding and bodyweight	Patchett et al. 2011

Endophytes	Secondary metabolites	Insect pest	Plant isolated/inoculated	Mechanism of action	References
Epichloëalsodes		*Spodoptera frugiperda*	*Poaalsodes*	Feeding deterrence and impaired development	Shymanovich et al. 2019
*Epichloëschardii*var.*pennsylvanica*				Larval mortality	
Neotyphodiumlolii (AR37)	Janthitrems	*Wiseanacervinata*	*Loliumperenne*	Low survival and growth	Jensen and Popay 2004
Hialocephala spp.	Rugulosin	*Choristoneura fumiferana*	*Piceaglauca*	Growth reduction	Miller, Sumarah, and Adams 2008
Unidentified endophytic fungi	Diterpenoids		*Abiesbalsamea*	Toxic to cells and larvae	Findlay et al. 1995
Fusarium avenaceum	Cyclodepsipeptideenniatins			Larvae toxicity	Strongman et al. 1988
Endophytic fungi (CBS 121942, CBS 121944 and DAOM 239833)	One polyketide metabolite		*Picea Rubens*	Larvae toxicity	Sumarah et al. 2010
	Two biosynthetically related compounds				
	Two sesquiterpenes				
	Four biosynthetically related maleic anhydrides				
Aspergillus flavus and *Nigrosporasphaerica*	Duroquinone	*Hyblaeapurea*, *Attevafabriciella* and *Eligma narcissus*	*Tectonagrandis*	Larvae mortality	Senthilkumar, Murugesan, and Babu 2014
	Naphthelene				
	Lauric acid				
	Adamantine derivatives				
	Amylmetacresol				
Balansiae tribe	Ergonovine	*Spodopterafrugiperda*	*Zea mays*	Larvae feeding	Clay and Cheplick 1989
	Ergotamine				
	Ergocryptine				
	Agroclavine				
	Elymoclavine				
Trichoderma atroviride	Volatile terpenes	*Spodopterafrugiperda*	*Zea mays*	Reduce the feeding	Contreras-Cornejo et al. 2018
Muscodorvitigenus	Naphthalene	*Cephuscinctus*	*Paulliniapaullionioides*	Insect repellent	Daisy et al. 2002
Unidentified	Benzofuran compounds	*Christoneurafumiferana*	*Gaultheria procumbens*	Toxic for cells and larvae	Findlay et al. 1997
Closdoporiumvelox	Chlorogenic acid	*Spodopteralitura*	*Tinosporacordifolia*	Alfa-glicosidase inhibitor that increase the larvae mortality and period of development and promote adults deformities	Singh et al. 2016
Beuveriabassiana and *Metarhiziumbrunneum* strains	Destruxin A	*Bemisiatabaci*	Melon leafs (*Cucumis melo* L. cv. Galia)	Nymphs mortality	Garrido-Jurado et al. 2017
Acremonium vitellinum	Chloramphenicol derivates	*Helicoverpaarmigera*	Unidentified marine red alga	Third instar larvae mortality through action in the detoxification and protective enzymes	Chen et al. 2018

Although plant colonization by entomopathogenic fungi can be persistent, occasionally, this colonization can take place for only a few days after propagules deposition, when occurs a transient colonization. This is the case demonstrated by Garrido-Jurado et al. (2017), which used a conidial suspension from three *B. bassiana* and two *Metarhizium brunneum* strains to immerse melon leaves in order to investigate the ensuing mortality of the nymphs of the sweet potato whitefly *B. tabaci*. In addition to the immersion, authors also performed a spray application of the conidial suspension in leaves infested with the whitefly insect. The experiment of immersion demonstrated significant mortality and the spray application showed a transient colonization that was capable to cause a great mortality among nymphs. The authors attribute such effects to the fungal secondary metabolite named destruxin A, found in significant amount within the nymphs.

Recently, algae has also been associated to endophytic fungus producers of secondary metabolites with insecticidal activity, as demonstrated by Chen et al. (2018). The authors isolated three chloramphenicol derivates from *Acremonium vitellinum*,an endophytic fungus of an unidentified marine red alga, and tested their activity against the third instar larvae of the cotton bollworm, *H.armigera.*The results showed that two of this chloramphenicol derivates had a lower insecticidal activity, while the chloramphenicol containingoxazolidin-2-one, described as a natural compound had the strongest larvicidal activity. It was also investigated the mechanisms through which this compound act in the larval mortality. They found differences in the activity of the glutathione S-tranferase (GST), catalase (CAT), acetylcholinesterase (AChE) enzymes and also the total antioxidant capacity (T-AOC), which could lead to the *H. armigera* mortality observed.

CONCLUSION

In this chapter, we discussed several classes of secondary metabolites obtained from endophytic fungi isolated from different parts of various plant species, focusing on the biotechnological potential to entomological control, highlighting many advances in this growing field. Lately, the agrochemical industry is searching for new alternatives for the already commercialized insecticides; they are searching specifically for products that can be eco-friendly, safe for humans, cheap and in the same time effective and selective. The metabolites produced by endophytes showed here and others yet to be discovered has the great potential to be a source for this kind of insecticide that the agrochemical industries are looking for.

REFERENCES

Aktar, Md Wasim., Dwaipayan, Sengupta.& Ashim, Chowdhury. (2009). "Impact of Pesticides Use in Agriculture: Their Benefits and Hazards." *Interdisciplinary Toxicology, 2* (1), 1–12. https://doi.org/10.2478/v10102-009-0001-7.

Aly, Amal H., Abdessamad, Debbab., Julia, Kjer.& Peter, Proksch. (2010). "Fungal Endophytes from Higher Plants: A Prolific Source of Phytochemicals and Other Bioactive Natural Products." *Fungal Diversity,41,* 1–16. https://doi.org/10.1007/s13225-010-0034-4.

Arnold, A.Elizabeth.& Leslie, C. Lewis. (2005). "Ecology and Evolution of Fungal Endophytes and Their Roles against Insects." In *Insect-Fungal Associations: Ecology and Evolution,* edited by Fernando E. Vega and Meredith Blackwell, 74–96. New York: Oxford University Press.

Arora, Naveen Kumar., Ekta, Khare., Sachin, Singh.& Sakshi, Tewari. (2018). "Phenetic, Genetic Diversity and Symbiotic Compatibility of Rhizobial Strains Nodulating Pigeon Pea in Northern India." *3 Biotech,8* (52), 1–11. https://doi.org/10.1007/s13205-017-1074-1.

Arora, Naveen Kumar.& Jitendra, Mishra. (2016). "Prospecting the Roles of Metabolites and Additives in Future Bioformulations for Sustainable Agriculture." *Applied Soil Ecology,107,* 405–7. https://doi.org/10.1016/j.apsoil.2016.05.020.

Azevedo, João Lúcio. (2014). "Endophytic Fungi from Brazilian Tropical Hosts and Their Biotechnological Applications." In *Microbial Diversity and Biotechnology in Food Security*, edited by R. N. Kharwar, R. S. Upadhyay, N. K. Dubey, and Richa Raghuwanshi, 17–22. New Delhi: Springer India. https://doi.org/10.1007/978-81-322-1801-2.

Azevedo, João Lúcio., Walter, Maccheroni., José, Odair Pereira.& Welington, Luiz De Araújo. (2000). "Endophytic Microorganisms: A Review on Insect Control and Recent Advances on Tropical Plants." *Electronic Journal of Biotechnology,3* (1), 40–65. https://doi.org/10.2225/vol3-issue1-fulltext-4.

Backman, Paul A. & Richard, A. Sikora. (2008). "Endophytes: An Emerging Tool for Biological Control." *Biological Control,46,* 1–3. https://doi.org/10.1016/j.biocontrol.2008.03.009.

Bacon, C. W., Glenn, A. E.& Yates, I. E.(2008). "Fusarium Verticillioides: Managing the Endophytic Association with Maize for Reduced Fumonisins Accumulation." *Toxin Reviews,27,* 411–46. https://doi.org/10.1080/15569540802497889.

Bano, Naseema., Ismat, Fatima Rizvi., Neha, Sharma., Mohd. Haris, Siddiqui., Mohd. Kalim, Ahmad Khan. & Salman, Akhtar. (2016). "Production of Bioactive Secondary Metabolites From Endophytic Fungi." *International Research Journal of Engineering and Technology,3* (6), 1859–66.

Barker, G. M., Patchett, B. J.& Cameron, N. E.(2015). "Epichloë Uncinata Infection and Loline Content Afford Festulolium Grasses Protection from Black Beetle (Heteronychus Arator)." *New Zealand Journal of Agricultural Research*,*58* (1), 35–56. https://doi.org/10.1080/00288233.2014.978480.

Bary, A. de (Anton). (1866). *Morphologie Und Physiologie Der Pilze, Flechten Und Myxomyceten*. Leipzig: W. Engelmann. https://doi.org/https://doi.org/10.5962/bhl.title.120970.

Batello, C., Mannetje, L., Martinez, A.& Suttie, J.(2008). "Plant Genetic Resources of Forage Crops, Pasture and Rangelands." *Thematic Background Study. FAO Report*. Rome. https://doi.org/10.1016/j.electacta.2010.03.081.

Brader, Günter., Stéphane, Compant., Kathryn, Vescio., Birgit, Mitter., Friederike, Trognitz., Li-Jun, Ma.& Angela, Sessitsch. (2017). "Ecology and Genomic Insights into Plant-Pathogenic and Plant-Nonpathogenic Endophytes." *Annual Review of Phytopathology*,*55* (1), 61–83. https://doi.org/10.1146/annurev-phyto-080516-035641.

Chen, Dan., Peng, Zhang., Tong, Liu., Xiu-Fang, Wang., Zhao-Xia, Li., Wei, Li.& Feng-Long, Wang. (2018). "Insecticidal Activities of Chloramphenicol Derivatives Isolated from a Marine Alga-Derived Endophytic Fungus, Acremonium Vitellinum, against the Cotton Bollworm, Helicoverpa Armigera (Hübner) (Lepidoptera: Noctuidae)." *Molecules*,*23* (11), 1–15. https://doi.org/10.3390/molecules23112995.

Chowdhury, Farhana Tasnim., Mohammad, Riazul Islam,. Md. Rakibul, Islam.& Haseena, Khan. (2018). "Diversity of Plant Endophytic Volatile Organic Compound (VOC) and Their Potential Applications." In *Endophytes and Secondary Metabolites*, edited by S. Jha, 1–27. Switzerland: Springer Nature. https://doi.org/10.1007/978-3-319-76900-4_10-1.

Clay, Keith.& Gregory, P. Cheplick. (1989). "Effect of Ergot Alkaloids from Fungal Endophyte-Infected Grasses on Fall Armyworm (Spodoptera Frugiperda)." *Journal of Chemical Ecology*,*15* (1), 169–82. https://doi.org/10.1007/BF02027781.

Claydon, Norman., John, Frederick Grove.& Michael, Pople. (1985). "Elm Bark Beetle Boring and Feeding Deterrents from Phomopsis Oblonga." *Phytochemistry*, *24* (5), 937–43. https://doi.org/10.1016/S0031-9422(00)83157-X.

Collette, L., Hodgkin, T., Kassam, A., Kenmore, P., Lipper, L., Nolte, C., Stamoulis, K.& Steduto, P.(2011). "Save and Grow: A Policymaker's Guide to Sustainable Intensification of Smallholder Crop Production." Rome. http://www.fao.org/3/a-i2215e.pdf.

Contreras-Cornejo, Hexon Angel., Lourdes, Macías-Rodríguez., Ek, Del-Val.& John, Larsen. (2018). "The Root Endophytic Fungus Trichoderma Atroviride Induces Foliar Herbivory Resistance in Maize Plants." *Applied Soil Ecology*,*124*, 45–53. https://doi.org/10.1016/j.apsoil.2017.10.004.

Daisy, Bryn H., Gary, A. Strobel., Uvidelio, Castillo., David, Ezra., Joe, Sears., David, K. Weaver.& Justin, B. Runyon. (2002). "Naphthalene, an Insect Repellent, Is Produced by Muscodor Vitigenus, a Novel Endophytic Fungus." *Microbiology,148* (11), 3737–41. https://doi.org/10.1099/00221287-148-11-3737.

Das, Aparajita.& Varma, Ajit.(2009). "Symbiosis: The Art of Living." In *Symbiotic Fungi. Soil Biology*, edited by A.Varma and A.C. Kharkwal, 18:1–28. Berlim: Springer. https://doi.org/10.1007/978-3-540-95894-9_1.

Douglas, Angela E. (2017). "Strategies for Enhanced Crop Resistance to Insect Pests." *Annual Review of Plant Biology,69* (1), 637–60. https://doi.org/10.1146/annurev-arplant-042817-040248.

Douglas, S. M. & Cowles, R. S.(2001). *Plant Pest Handbook*. New Haven: Connecticut Agricultural Experiment Station. https://portal.ct.gov/CAES/Publications/Publications/Plant-Pest-Handbook--A-Guide.

FAO. n.d. "Plant Pests and Diseases." Accessed September 5, 2018. http://www.fao.org/emergencies/emergency-types/plant-pests-and-diseases/en/.

———. (2009). "Global Review of Forest Pest and Diseases." *FAO Forestry Paper*., Vol. *156*. Rome. http://www.fao.org/3/a-i0640e.pdf.

———. (2011). "Guide to Implementation of Phytosanitary Standards in Forestry." Rome. http://www.fao.org/3/i2080e/i2080e00.htm.

———. (2018a). "Forecasting Threats to the Food Chain Affecting Food Security in Countries and Regions." *Food Chain Crisis: Early Warning Bulletin*., Vol. *28*. Rome. http://www.wipo.int/amc/en/mediation/rules.

———. (2018b). "Forecasting Threats to the Food Chain Affecting Food Security in Countries and Regions." *Food Chain Crisis: Early Warning Bulletin*., Vol. *29*. Rome. http://www.fao.org/3/CA2076EN/ca2076en.PDF.

Field, Katie J., William, R. Rimington., Martin, I. Bidartondo., Kate, E. Allinson., David, J. Beerling., Duncan, D. Cameron., Jeffrey, G. Duckett., Jonathan, R. Leake.& Silvia, Pressel. (2015). "First Evidence of Mutualism between Ancient Plant Lineages (Haplomitriopsida Liverworts) and Mucoromycotina Fungi and Its Response to Simulated Palaeozoic Changes in Atmospheric CO2." *New Phytologist*, *205*, 743–56. https://doi.org/10.1111/nph.13024.

Findlay, J. A., Buthelezi, S., Li, G.& Seveck, M.(1997). "Insect Toxins from an Endophytic Fungus from Wintergreen." *Journal of Natural Products*, *60* (11), 1214–15. https://doi.org/10.1021/np970222j.

Findlay, John A., Guoqiang, Li., Peter, E. Penner.& David Miller, J.(1995). "Novel Diterpenoid Insect Toxins from a Conifer Endophyte." *Journal of Natural Products*,*58* (2), 197–200. https://doi.org/10.1021/np50116a006.

Gao, Han., Gang, Li.& Hong, Xiang Lou. (2018). "Structural Diversity and Biological Activities of Novel Secondary Metabolites from Endophytes." *Molecules*, *23* (3), 1–31. https://doi.org/10.3390/molecules23030646.

Garrido-Jurado, I., Resquín-Romero, G., Amarilla, S. P., Ríos-Moreno, A., Carrasco, L.& Quesada-Moraga, E.(2017). "Transient Endophytic Colonization of Melon Plants by Entomopathogenic Fungi after Foliar Application for the Control of Bemisia Tabaci Gennadius (Hemiptera: Aleyrodidae)." *Journal of Pest Science*,*90* (1), 319–30. https://doi.org/10.1007/s10340-016-0767-2.

Gouda, Sushanto., Gitishree, Das., Sandeep, K. Sen., Han, Seung Shin.& Jayanta, Kumar Patra. (2016). "Endophytes: A Treasure House of Bioactive Compounds of Medicinal Importance." *Frontiers in Microbiology*,*7*, (Article 1538), 1–8. https://doi.org/10.3389/fmicb.2016.01538.

Gurulingappa, Pampapathy., Peter, Allan McGee.& Gregory, Sword. (2011). "Endophytic Lecanicillium Lecanii and Beauveria Bassiana Reduce the Survival and Fecundity of Aphis Gossypii Following Contact with Conidia and Secondary Metabolites." *Crop Protection*, *30* (3), 349–53. https://doi.org/10.1016/j.cropro.2010.11.017.

Hiruma, Kei., Yoshihiro, Kobae.& Hirokazu, Toju. (2018). "Beneficial Associations between Brassicaceae Plants and Fungal Endophytes under Nutrient-Limiting Conditions: Evolutionary Origins and Host–Symbiont Molecular Mechanisms." *Current Opinion in Plant Biology*, *44*, 145–54. https://doi.org/10.1016/j.pbi.2018.04.009.

Huis, A. van. (2014). *The Global Impact of Insects*. Wageningen: Wageningen University. http://edepot.wur.nl/410394.

Jaber, Lara R. & Bonnie, H. Ownley. (2018). "Can We Use Entomopathogenic Fungi as Endophytes for Dual Biological Control of Insect Pests and Plant Pathogens?" *Biological Control*,*116*, 36–45. https://doi.org/10.1016/j.biocontrol.2017.01.018.

Jensen, J. G.& Popay, A. J.(2004). "Perennial Ryegrass Infected with AR37 Endophyte Reduces Survival of Porina Larvae." *New Zealand Plant Protection*, *57*, 323–28.

Johnson, M. C., Dahlman,D. L., Siegel, M. R., Bush, L. P., Latch, G. C. M., Potter, D. A.& Varney, D. R.(1985). "Insect Feeding Deterrents in Endophyte-Infected Tall Fescue." *Applied and Environmental Microbiology*,*49* (3), 568–71. http://aem.asm.org/cgi/content/abstract/49/3/568.

Kaur, Tamanreet., Jasleen, Kaur., Amarjeet, Kaur.& Sanehdeep, Kaur. (2016). "Larvicidal and Growth Inhibitory Effects of Endophytic Aspergillus Niger on a Polyphagous Pest, Spodoptera Litura." *Phytoparasitica*,*44* (4), 465–76. https://doi.org/10.1007/s12600-016-0541-2.

Keller, Nancy P. (2019). "Fungal Secondary Metabolism: Regulation, Function and Drug Discovery." *Nature Reviews Microbiology*, *17* (3), 167–80. https://doi.org/10.1038/s41579-018-0121-1.

Khare, Ekta., Jitendra, Mishra.& Naveen, Kumar Arora. (2018). "Multifaceted Interactions between Endophytes and Plant: Developments and Prospects." *Frontiers in Microbiology*,*9* (2732), 1–12. https://doi.org/10.3389/fmicb.2018.02732.

Kumar, Susheel., Nutan, Kaushik., Ruangelie, Edrada-Ebel., Rainer, Ebel.& Peter, Proksch. (2008). "Endophytic Fungi for Pest and Disease Management." In *Integrated Management of Diseases Caused by Fungi, Phytoplasma and Bacteria*, edited by Aurelio Ciancio and K.G. Mukerji, 365–87. Dordrecht: Springer.

Kusari, Souvik., Christian, Hertweck.& Michael, Spiteller. (2012). "Chemical Ecology of Endophytic Fungi: Origins of Secondary Metabolites." *Chemistry and Biology, 19* (7), 792–98. https://doi.org/10.1016/j.chembiol.2012.06.004.

Lata, R., Chowdhury, S., Gond, S. K.& White, J. F.(2018). "Induction of Abiotic Stress Tolerance in Plants by Endophytic Microbes." *Letters in Applied Microbiology*, *66* (4), 268–76. https://doi.org/10.1111/lam.12855.

Li, Hui., Ying, Guan., Yilun, Dong., Lu, Zhao., Songhao, Rong., Wenqian, Chen., Miaomiao, Lv., et al. (2018). "Isolation and Evaluation of Endophytic Bacillus Tequilensis GYLH001 with Potential Application for Biological Control of Magnaporthe Oryzae." *PLoS ONE, 13* (10), 1–18. https://doi.org/10.1371/journal.pone.0203505.

Li, Xiu Zhang., Mei, Ling Song., Xiang, Yao., Qing, Chai., Wayne, R. Simpson., Chun, Jie Li.& Zhi, Biao Nan. (2017). "The Effect of Seed-Borne Fungi and Epichloë Endophyte on Seed Germination and Biomass of Elymus Sibiricus." *Frontiers in Microbiology*,*8*, 1–8. https://doi.org/10.3389/fmicb.2017.02488.

Lugtenberg, Ben J.J., John, R. Caradus.& Linda, J. Johnson. (2016). "Fungal Endophytes for Sustainable Crop Production." *FEMS Microbiology Ecology*, *92* (12), 1–17. https://doi.org/10.1093/femsec-fiw194.

Márquez, Luis M., Regina, S. Redman., Russell, J. Rodriguez.& Marilyn, J. Roossinck. (2007). "A Virus in a Fungus in a Plant: Three-Way Symbiosis Required for Thermal Tolerance." *Science*,*315*, 513–15. https://doi.org/10.1126/science.1136237.

Miller, J. David., Mark, W. Sumarah. & Gregory, W. Adams. (2008). "Effect of a Rugulosin-Producing Endophyte in Picea Glauca on Choristoneura Fumiferana." *Journal of Chemical Ecology*, *34* (3), 362–68. https://doi.org/10.1007/s10886-007-9412-0.

Mishra, Yash., Abhijeet, Singh., Amla, Batra.& Madan, Mohan Sharma. (2014). "Understanding the Biodiversity and Biological Applications of Endophytic Fungi: A Review." *Journal of Microbial & Biochemical Technology*, *s8* (004), 1–11. https://doi.org/10.4172/1948-5948.s8-004.

Moore, Beverly A. (2005). *Alien Invasive Species: Impacts on Forests and Forestry. A Review*. Rome: Forest Resources Division FAO. http://www.fao.org/3/j6854e/J6854E00.htm.

Nisa, Humeera., Azra, N. Kamili., Irshad, A. Nawchoo., Sana, Shafi., Nowsheen, Shameem.& Suhaib, A. Bandh. (2015). "Fungal Endophytes as Prolific Source of Phytochemicals and Other Bioactive Natural Products: A Review." *Microbial Pathogenesis*,*82*, 50–59. https://doi.org/10.1016/j.micpath.2015.04.001.

Palanichamy, Prabha., Govindan, Krishnamoorthy., Suganya, Kannan.& Murugan, Marudhamuthu. (2018). "Bioactive Potential of Secondary Metabolites Derived from Medicinal Plant Endophytes." *Egyptian Journal of Basic and Applied Sciences*,*5* (4), 303–12. https://doi.org/10.1016/j.ejbas.2018.07.002.

Patchett, B., Gooneratne, R., Chapman, B.& Fletcher, L.(2011). "Effects of Loline-Producing Endophyteinfected Meadow Fescue Ecotypes on New Zealand Grass Grub (Costelytra Zealandica)." *New Zealand Journal of Agricultural Research*,*54* (4), 303–13. https://doi.org/10.1080/00288233.2011.608686.

Plett, Jonathan M. & Francis, M. Martin. (2018). "Know Your Enemy, Embrace Your Friend: Using Omics to Understand How Plants Respond Differently to Pathogenic and Mutualistic Microorganisms." *Plant Journal*, *93*, 729–46.https://doi.org/10.1111/tpj.13802.

Popay, A. J., Tapper, B. A.& Podmore, C.(2009). "Endophyte-Infected Meadow Fescue and Loline Alkaloids Affect Argentine Stem Weevil Larvae." *New Zealand Plant Protection*,*62*, 19–27.

Prestidge, R. A. & Gallagher, R. T.(1985). "Lolitrem B-a Stem Weevil Toxin Isolated from Acremonium-Infected Ryegrass." In *Proceedings of the NZ Weed and Pest Control Conference, 38*, 38–40. New Zealand Plant Protection. https://www.nzpps.org/journal/38/nzpp_380380.pdf.

Raman, Authors A., Wheatley, W.& Popay, A(2012). "Endophytic Fungus — Vascular Plant — Insect Interactions." *Environmental Entomology*,*41* (3), 433–47. https://doi.org/http://dx.doi.org/10.1603/EN11317.

Rohlfs, Marko.& Alice, C.L. Churchill. (2011). "Fungal Secondary Metabolites as Modulators of Interactions with Insects and Other Arthropods." *Fungal Genetics and Biology*,*48* (1), 23–34. https://doi.org/10.1016/j.fgb.2010.08.008.

Rowan, D. D.& Gaynor, D. L.(1986). "Isolation of Feeding Deterrents against Argentine Stem Weevil from Ryegrass Infected with the Endophyte Acremonium Loliae." *Journal of Chemical Ecology*,*12* (3), 647–58.

Rowan, Daryl D. (1993). "Lolitrems, Peramine and Paxilline: Mycotoxins of the Ryegrass/Endophyte Interaction." *Agriculture, Ecosystems and Environment*,*44* (1–4), 103–22. https://doi.org/10.1016/0167-8809(93)90041-M.

Royal Botanical Gardens - Kew. (2017). "Plant Health: State of Research." *State of the World's Plants 2017*. London. https://stateoftheworldsplants.org/2017/report/SOTWP_2017_10_plant_health_state_of_research.pdf.

Rungjindamai, N., Choeyklin, R, Hattori, T.& Jones, E. B. G. (2008). "Molecular Characterization of Basidiomycetous Endophytes Isolated from Leaves, Rachis and Petioles of the Oil Palm, Elaeis Guineensis, in Thailand." *Fungal Diversity*,*33*, 139–61. http://www.ncbi.nlm.nih.gov.

Saad, Mona M. G., Rehab, Y Ghareeb.& Ali, A Saeed. (2019). "The Potential of Endophytic Fungi as Bio-Control Agents against the Cotton Leafworm, Spodoptera

Littoralis (Boisd.) (Lepidoptera: Noctuidae)." *Egyptian Journal of Biological Pest Control,29* (1), 1–7. https://doi.org/10.1186/s41938-019-0108-x.

Sallam, M. N. (2001). "Insect Damage: Post-Harvest Operations." *INPhO-Post-Harvest Compendium.* Rome. http://www.fao.org/3/a-av013e.pdf.

Sandhu, Sardul Singh., Anil, K. Sharma., Vikas, Beniwal., Gunjan, Goel., Priya, Batra., Anil, Kumar., Sundeep, Jaglan., Sharma, A. K.& Sonal, Malhotra. (2012). "Myco-Biocontrol of Insect Pests: Factors Involved, Mechanism, and Regulation." *Journal of Pathogens*, 1–10. https://doi.org/10.1155/2012/126819.

Savary, Serge., Laetitia, Willocquet., Sarah, Jane Pethybridge., Paul, Esker., Neil, McRoberts.& Andy, Nelson. (2019). "The Global Burden of Pathogens and Pests on Major Food Crops." *Nature Ecology & Evolution*, *3* (3),430–39. https://doi.org/https://doi.org/10.1038/s41559-018-0793-y.

Schulz, Barbara.& Christine, Boyle. (2005). "The Endophytic Continuum." *Mycological Research,109* (6), 661–86. https://doi.org/10.1017/S095375620500273X.

Schulz, Barbara., Christine, Boyle., Siegfried, Draeger., Anne-katrin, Rommert.& Karsten, Krohn. (2002). "Endophytic Fungi: A Source of Novel Biologically Active Secondary Metabolites." *Mycological Research,106* (9), 996–1004. https://doi.org/10.1017/S0953756202006342.

Senthilkumar, N., Murugesan, S.& Suresh Babu, D.(2014). "Metabolite Profiling of the Extracts of Endophytic Fungi of Entomopathogenic Significance, Aspergillus Flavus and Nigrospora Sphaerica Isolated from Tropical Tree Species of India, Tectona Grandis L." *Journal of Agriculture and Life Sciences,1* (1), 108–14.

Shearin, Zackery R. C., Matthew, Filipek., Rushvi, Desai., Wesley, A. Bickford., Kurt, P. Kowalski.& Keith, Clay. (2018). "Fungal Endophytes from Seeds of Invasive, Non-Native Phragmites Australis and Their Potential Role in Germination and Seedling Growth." *Plant and Soil,422* (1–2), 183–94. https://doi.org/10.1007/s11104-017-3241-x.

Shymanovich, Tatsiana., Ashleigh, M. Musso., Nadja, B. Cech. & Stanley, H. Faeth. (2019). "Epichloë Endophytes of Poa Alsodes Employ Alternative Mechanisms for Host Defense: Insecticidal versus Deterrence." *Arthropod-Plant Interactions,13* (1), 79–90. https://doi.org/10.1007/s11829-018-9635-8.

Siegel, M. R., Latch, G. C. M., Bush, L. P., Fannin, F. F., Rowan, D. D., Tapper, B. A.& Johnson, M. C.(1990). "Fungal Endophyte-Infected Grasses: Alkaloid Accumulation and Aphid Response." *Journal of Chemical Ecology,16* (12), 3301–15.

Singh, Bahaderjeet., Tamanreet, Kaur., Sanehdeep, Kaur., Rajesh, K. Manhas.& Amarjeet, Kaur. (2016). "Insecticidal Potential of an Endophytic Cladosporium Velox against Spodoptera Litura Mediated through Inhibition of Alpha Glycosidases." *Pesticide Biochemistry and Physiology,131*, 46–52. https://doi.org/10.1016/j.pestbp.2016.01.004.

Stierle, Andrea A. & Donald, B Stierle. (2015). "Bioactive Secondary Metabolites Produced by the Fungal Endophytes of Conifers." *Natural Product Communications, 10* (10), 1671–82. https://doi.org/10.1177/1934578X1501001012.

Strobel, Gary.& Bryn, Daisy. (2003). "Bioprospecting for Microbial Endophytes and Their Natural Products." *Microbiology and Molecular Biology Reviews, 67* (4), 491–502. https://doi.org/10.1128/MMBR.67.4.491–502.2003.

Strongman, Douglas B., George, M. Strunz., Pierre, Giguère., Chao, Mei Yu.& Larry, Calhoun. (1988). "Enniatins from Fusarium Avenaceum Isolated from Balsam Fir Foliage and Their Toxicity to Spruce Budworm Larvae, Choristoneura Fumiferana (Clem.) (Lepidoptera: Tortricidae)." *Journal of Chemical Ecology, 14* (3), 753–64. https://doi.org/10.1007/BF01018770.

Sumarah, Mark W. & David Miller, J.(2009). "Anti-Insect Secondary Metabolites from Fungal Endophytes of Conifer Trees." *Natural Product Communications, 4* (11), 1497–1504. https://doi.org/10.1177/1934578x0900401112.

Sumarah, Mark W., Eva, Puniani., Dan, Sørensen., Barbara, A. Blackwell.& David Miller, J.(2010). "Secondary Metabolites from Anti-Insect Extracts of Endophytic Fungi Isolated from Picea Rubens." *Phytochemistry,71* (7), 760–65. https://doi.org/10.1016/j.phytochem.2010.01.015.

Symmons, P. M. & Cressman, K.(2001). "Desert Locust Guidelines Biology and Behaviour." *Desert Locust Guidelines*. Rome. http://www.fao.org/ag/locusts/common/ecg/347_en_DLG1e.pdf.

Tidke, Shashank A., Kiran, S., Giridhar, P. & Ravishankar, A. Gokare. (2018). "Current Understanding and Future Perspectives of Endophytic Microbes Vis-a-Vis Production of Secondary Metabolites." In *Endophytes and Secondary Metabolites*, edited by S. Jha, 1–16. Switzerland: Springer Nature. https://doi.org/10.1007/978-3-319-76900-4_12-1.

Tisserant, E., Malbreil, M., Kuo, A., Kohler, A., Symeonidi, A., Balestrini, R., Charron, P., et al. (2013). "Genome of an Arbuscular Mycorrhizal Fungus Provides Insight into the Oldest Plant Symbiosis." *Proceedings of the National Academy of Sciences, 110* (50), 20117–22. https://doi.org/10.1073/pnas.1313452110.

Vega, Fernando E., Francisco, Posada., Catherine Aime, M., Monica, Pava-Ripoll., Francisco, Infante. & Stephen, A. Rehner. (2008). "Entomopathogenic Fungal Endophytes." *Biological Control, 46*(1), 72–82. https://doi.org/10.1016/j.biocon=trol. 2 008.01.008.

Webber, Joan. (1981). "A Natural Biological Control of Dutch Elm Disease." *Nature,292* (5822), 449–51. https://doi.org/10.1038/292449a0.

Zhang, Hua Wei., Yong, Chun Song. & Ren, Xiang Tan. (2006). "Biology and Chemistry of Endophytes." *Natural Product Reports, 23* (5), 753–71. https://doi.org/10.1 039/b609472b.

Zhang, Peng., Xin, Li.& Bin, Gui Wang. (2016). "Secondary Metabolites from the Marine Algal-Derived Endophytic Fungi: Chemical Diversity and Biological Activity." *Planta Medica,82* (9–10), 832–42. https://doi.org/10.1055/s-0042-103496.

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 8

A BIOLOGICAL ACTIVE MUSHROOM *AGARICUS BISPORUS*: AN UPDATE OF ITS PHYTOPHARMACOLOGY AND MEDICINAL PROPERTIES WITH RESPECT TO VARIOUS DISEASES

Mayank Kulshreshtha [*] ***and Shristy Srivastava***
Department of Pharmacology, School of Pharmacy, Babu Banarasi Das University, Lucknow (U.P.), India

ABSTRACT

Nature is like a mother to mankind. For centuries, it has been the origin of plenty of herbs and medicinal plants involved in maintaining the health of living creatures. The compounds of natural origin are readily accepted and highly valued due to their minimum side effects. Fungus, a eukaryotic organism sometimes also referred to as lower plants, grows widely in nature and is well known for its curative nature in diseases related to gastrointestinal tract, heart and respiratory disorders. *Agaricus bisporus* (button mushroom) is an edible, widely grown and cultivated species of a fungus which consists of more than 40% of world's total production of mushroom. This chapter deals with the biochemistry, pharmacognosy, pharmacology, and formulations of *Agaricus bisporus* involved in disease cure based on scientific data published online and offline. This chapter could serve as a gateway for newer and beneficiary researches in the field of disease cure.

Keywords: *Agaricus bisporus*, traditional medicine, pharmacognosy, pharmacology, cardiovascular diseases

[*] Corresponding Author's Email: professormayank@gmail.com.

INTRODUCTION

The kingdom fungi consist of about 144,000 known species of fungus characterized into yeasts, rusts, smuts, mildews, molds and mushrooms. Fungi are abundantly found all over the Earth and are essential from both environmental and medicinal perspective. Fungi can exist both as free organisms in soil and water and also as a parasite or in symbiosis with plants and animals, they are abundantly present everywhere in soil, water, air and even on and within plants, animals, food and human beings. The existence of fungi was brought to the attention of humans from baking of leavened bread loaf and formation of grape wine. In ancient Romans, fungi were considered as a disease developed due to the anger of Robigus, god of rust and, in order to please him an annual festival Robigalia was organized. The fungi in collaboration with bacteria work to break down organic matter which is responsible for the release of carbon, oxygen, nitrogen, and phosphorus into the soil and atmosphere. Fungi serve as an essential component in household and industrial procedures like in formation of bread, wine, beer, and cheese. They are not only employed as a necessity of a process in food making but are itself used as food like mushrooms, morels, truffles and mycoproteins (rich protein source extracted from mycelia of certain fungi) (https://www.britannica.com/science/fungus/Importance-of-fungi). The use of fungi as edibles in order to meet human nutritional demands since historical times (Chang ST 2006 and Singha R 2010), besides this, the most prominent antibiotic penicillin is obtained from a fungus *Penicillium*. The introduction of antibiotics in the field of medicine has been a boon to mankind and animals. Fungi also exist as a parasite on caterpillars and this combination is traditionally used as medicines (Thakur MP 2014).

MUSHROOM PRODUCTION AND ECONOMY

The cultivated edible species of mushroom belongs to the class basidiomycetes and has a saprobic lifestyle. The class further consists of primary, secondary and tertiary decomposers (Rahi 2009). Primary decomposers like oyster mushrooms (*Pleurotus spp.*), shiitake (*Lentinula edodes*), Secondary decomposers like button mushroom forms colony over composted material and tertiary decomposers like *Agrocybe spp.* grows on soil where as tertiary decomposers like *Agrocybe spp.* grows on soil, whereas primary decomposers like oyster mushrooms (*Pleurotus spp.*), shiitake (*Lentinula edodes*) do not depend on other plants and their metabolites, instead they decompose lignin, cellulose and other plant materials for their survival.

All these three categories of decomposers have a continuity of their role in the metabolic transformation of lignocellulosic and other organic components to the soil.

Thus, we can think of cultivating mushrooms from various stages in order to compost agricultural waste into the soil but at practical, such large-scale production of mushrooms is a condition that is hard to achieve (Stamets 1993). The mushrooms belonging to the category of primary decomposers could be employed in composting of lignocellulosic materials like straw, cottonseed hulls, corn cobs, peanut shells, coffee, pulp, paper (Sánchez 2010), and leaves (Shah 2004). The traditional method of mushroom cultivation is an outdoor log culture followed by China for millions of years in order to grow shiitake. The technique in the modern world has been replaced by indoor cultivation on artificial logs (plastic bags) provided with nutrient accompanied by sawdust-based substrates. Colonization is followed by unpacking in order to promote fruiting; the sawdust-based substrate is supported by mycelium like glue which holds it from falling apart. Another analogous method is the column culture in which the long plastic bags provided with nutrient accompanied with the sawdust-based substrate is hanged down from the ceiling and waited for the colonization, afterwards they are punched to create holes which then promotes fruiting. *Pleurotus ostreatus* is a primary decomposer specie which produces 50% carbon dioxide, 20% water, 10% mushrooms and 20% residual compost (Spent mushroom substrate) (Stamets 1993) and follows the 2:1 thumb rule of mushroom cultivation which states that ratio of dry residual compost to that of fresh mushroom production must be 2:1. The composition of substrates affects the colonization and fruiting of mushrooms like for example *Pleurotus florida* colonization reduces the dry weight of pea and rice straw to 20% and 12% respectively (Nasehi 2017).

Agaricus bisporus (*A. bisporus*) is categorized under secondary decomposer whereas previously till 1962 its growth was also reported on non-composted substrates such as autoclaved sawdust. Although, button mushroom can grow on self-pasteurized substrates such as corn-cob, primavera tree and Pangola grass (Colmenares-Cruz 2017). Among all the mentioned substrates button mushroom grows best on Pangola grass with a biological efficiency of 52% and yield of 7.6 kg/m^2 which on supplementation can be increased to a biological efficiency of 176% and yield of 26 kg/m^2. However, to date, button mushrooms are grown in fertilizer that has undergone two-stage fermentation and sometimes coated with a peat mulch (Daniel 2018).

Salient Features of *Agaricus* Fungi (http://www.biologydiscussion.com/fungi/salient-features-of-agaricus-fungi/64125)

- The primary mycelium produced by the germination of basidiospores is of short duration. It consists of cells each with a haploid (n) nucleus.
- Soon as a result of hyphal fusions of compatible strains, a binucleate condition established in one of the cells of the primary mycelium.

- The binucleate (dikaryotised) cell by repeated divisions usually without and rarely with clamp connections forms a new mycelium with binucelate (n+n) cells. It is the secondary or dikaryotic mycelium.
- The secondary mycelium is abundant, and long-lived (perennial). It produces mushrooms (basidiocarps) year after year.
- As in the other Basidiomycetes, the mushrooms lack specialised sex organs.
- Plasmogamy which brings the elements of a sexual union together is accomplished by somatogamous copulation (hyphal fusions) of compatible strains.
- Karyogamy or the actual fusion of two nuclei of the dikaryon is delayed and does not occur till the formation of the basidium.
- The plus and minus strain nuclei of dikaryons co-exist in all the cells of the secondary mycelial strands and the fruiting bodies (basidiocarps) which it bears.
- The basidiocarps arise as tiny, white apical swellings on the branches of the underground mycelial strands called rhizomorphs.
- Each enlarges to form a 'button' which comes above ground and rapidly expands into a mushroom.
- The mature mushroom is a massive structure consisting of a stalk-like stipe supporting at its top a broad umbrella-shaped cap, the pileus. More than half way up, the stipe bears a membranous ring, the annulus.
- From the undersurface of the pileus hang down plates of fungal tissue in a vertical position. These are the gills (D).
- The surface of the gill, on both sides, is covered with a fertile layer, the hymenium.
- The hymenium consists of a closely packed palisade-like layer of club-shaped cells, the basidia. Interspersed between the basidia are sterile hyphae, the paraphyses or cystidia.
- The basidium mother cell or protobasidium has two nuclei which function as gametes and fuse. The fusion of the two nuclei (karyogamy) in the protobasidium ends the dikaryophase in the life cycle.
- The probasidium containing the diploid nucleus or synkaryon is called the young basidium. It represents the short-lived diplophase in the life cycle.
- As the young basidium grows in size the synkaryon within undergoes meiosis during which segregation of strains takes place. Of the four haploid nuclei two are of plus strain and the other two of minus strain.
- In the mature basidium the haploid nuclei migrate into the basidiospores through their respective sterigmata.
- With the completion of spore production the basidiocarp collapses and dies.

- The liberated basidiospores which are true meiospores, germinate to give rise to the haploid (primary) mycelia.

Mushroom Culture

The process of mushroom cultivation is not that easy as it seems to be each step is to be taken care of properly. The very first step of cultivation includes a collection of pure mycelium of the desired mushroom strain which can be obtained from its spores, its piece or from various germplasm providers. The inoculum for the process is obtained by cultivating mycelium on cereal grains such as wheat, rye or millet, commonly known as spawn, (Chang and Hayes 1978; Chang and Miles 1989) this step is performed in order to get a rapid colonizing rate on the bulk-growth substrate. The quality of spawn decides the success of mushroom production because spawn has to be prepared in sterile conditions in order to minimize substrate contamination. Regular studies are being performed to discover newer and beneficial techniques for spawn production (Chu and Wang 1977). Poppe (2000) reported 200 types of waste which can be utilized for growing edible mushrooms. The method for substrate preparation, inoculation, incubation, and production varies with mushroom species undertaken (Sánchez 2004).

PHARMACOLOGICAL ACTIVITIES

Cardioprotective Activity

Bhushan 2018, described cardioprotective activity of hydroalcoholic extract of *Agaricus bisporus* (EEAB) *A. bisporus* on Isoproterenol (ISO) induced myocardial infarction (MI) in albino wistar rat. Wistar rats of different sex were randomly split into five groups namely positive control, negative control, standard, test-1 and test-2 and received distilled water, ISO (85 mg/kg), Simvastatin (10 mg/kg/day, oral) and EEAB (200 and 400 mg/kg/day, p.o.) for 30 days, respectively. MI was induced in rats by ISO at an interval of 24 hrs on 31 and 32 day and on the next day, blood was amassed through retro-orbital plexus for the assessment of biochemical markers (cholesterol, low density lipoprotein, high-density lipoprotein, very low-density lipoprotein, triglycerides, alanine aminotransferase and total protein) and finally, the rats were immolated by cervical dislocation. The heart tissue was reaped instantly, cleaned with chilled isotonic saline and clasped in 10% buffered formalin and used for the histopathological analysis. ISO p.o. administration significantly elevated the cholesterol, low density lipoprotein, very low density lipoprotein, triglycerides, alanine aminotransferase and aspartate aminotransfe-

rase levels while it decreases high-density lipoprotein and total protein in plasma and administration of EEAB decreases the level of cholesterol, low-density lipoprotein, very low-density lipoprotein, triglycerides, alanine aminotransferase and aspartate aminotransferase levels while it increases high-density lipoprotein and total protein levels. Pretreatment with EEAB protected the cardiotoxicity induced by ISO. The histopathological findings support the analysis of biochemical parameters, ISO-induced myocardium showed infracted zone with edema, inflammatory cells, lipid droplets, myocardial necrosis and vacuolization of myofibrils which were reduced.

Antioxidants Activity

The antioxidant properties of the ethanolic extract obtained from edible mushroom *Agaricus bisporus* (*A. bisporus*) were calculated both *in-vitro* and *in-vivo*. The *in-vitro* results of the anti-oxidant assay showed strong reducing capacity, superoxide radical, hydroxyl radical and 2,2-diphenyl-1-picrylhydrazyl radical scavenging property and moderate hydrogen peroxide scavenging activity. *In-vivo* study, mice were administered with the ethanolic extract of *A. bisporus* continuously for 30 days and there were increased concentrations of antioxidant enzymes in liver, heart and serum samples of mice (Liu 2013).

Immunomodulatory and Antitumor Properties

The polysaccharides of mushroom are known for their anti-tumor and immunomodulatory activities, but in the case of *A. bisporus* polysaccharides are only known in brief. Size-exclusion chromatography was implied to obtain two polysaccharide fractions (ABP-1 and ABP-2). The calculated molecular weight of ABP-1 was 2000 kDa and for ABP-2 40-70 kDa, sugar composition of both the polysaccharides consist of glucose, mannose, xylose, and fructose. The effect of both fractions of polysaccharides was studied on murine macrophages which showed that both of them triggered the production of nitric oxide, interleukin-6, and tumor necrosis factor-α. The alteration in macrophage function was produced via initiation of nuclear factor-κB leading to the production of p50/105 heterodimers. Both fractions of polysaccharides were capable of inhibiting the growth of human breast cancer MCF-7 cells but showed fewer effects on human colon, prostate, and gastric cancer when evaluated by tetrazolium dye-based assay. A study demonstrated that murine Sarcoma 180 cells after exposure to ABP-1 and ABP-2, when implanted into mice, showed a decrease in growth of the tumor with respect to mice in the control group (Jeong 2012).

Hypoglycemic and Hypolipidemic Activity

In a study, Streptozotacin induced diabetic male Sprague-Dawley rats were fed on *A. bisporus* powder at a dose of 200 mg/kg of body weight for 3 weeks. The outcome demonstrated a considerable decrease in plasma glucose levels (24.7%), triglyceride levels (39.1%), liver enzyme activities, alanine aminotransferase levels (11.7%) and aspartate aminotransferase levels (15.7%). In another model, hypercholesterolemic rats were fed on *A. bisporus* powder for 4 weeks. The outcomes resulted in a noticeable reduction in plasma total cholesterol levels (22.8%) and low-density lipoprotein (33.1%). A similar pattern of reduction was seen in hepatic cholesterol levels (36.2%) and triglyceride levels (20.8%). All these reductions in TC, LDL and TG levels were complemented with a rise in high-density lipoprotein levels (Jeong 2010).

Anti-Inflammatory Activity

The incubation of Caco-2 cells with *A. bisporus* extract lead to falling in the expression of cyclooxygenase-2 and prostaglandin F2α receptor and a rise in expression of nuclear factor (erythroid-derived 2)-like 2 in comparison with LPS- and/or TNF-α initiated cells. A considerable reduction in the levels of interleukin-6 was observed in *A. bisporus* incubated Caco-2 cells along with a significant difference in levels of monounsaturated and polyunsaturated fatty acids in *A. bisporus* incubated and non-incubated Caco-2 cells (Bozena 2018).

Maturation of Bone Marrow-Derived Dendritic Cells

Dendritic cells (DC) establish a link between innate and adaptive immune responses and are the most capable antigen-presenting cells. Dose-dependent supplementation of *A. bisporus* promotes activation of maturation markers CD40, CD80, CD86, and major histocompatibility complex-II. The effect of *A. bisporus* supplementation on DC was studied through functional assay of DC maturation which marked a reduction in DC endocytosis and elevation in intracellular interleukin (IL)-12 levels (Ren 2008).

MUSHROOM-BASED COSMECEUTICAL FORMULATIONS

The ethanolic extracts of *A. bisporus*, *Pleurotus ostreatus* and *Lentinula edodes* were tested for their cosmeceutical potencies in parameters of anti-inflammation, anti-

tyrosinase, antioxidant and anti-bacterial properties. These extracts were loaded into a base cosmetic cream in order to achieve their bioactive purposes. From the results obtained, the final cosmeceutical formulations presented 85%–100% of the phenolic acids and ergosterol levels found in the mushroom extracts, suggesting that there was no significant loss of bioactive compounds. The final cosmeceutical formulation also displayed all the ascribed bioactivities and as such, mushrooms can further be exploited as natural cosmeceutical ingredients. (Oludemi 2016).

POTENTIAL PREBIOTICS

Mushrooms are a rich source of desired dietary nutrients essential for health promotion. They are also rich in non-digestive dietary fibers such as glucan, chitin, and heteropolysaccharides. They are supposed to be beneficiary in the prevention of viral infections due to the fact that they promote the growth of probiotic bacteria in the large intestine. Due to the presence of chitin, hemicelluloses, α and β-glucan, mannans, xylans and galactose they are considered as a good source of prebiotics. Among 2000 known edible species of mushrooms 20 are cultivated on commercial grounds and only 4-5 species are grown at industrial scale. Studies have stated a considerable variation in nutritional values of the stalk and pileus (Bhakta 2013).

BENEFITS OF MUSHROOM

The six major constituents of mushrooms are water, proteins, carbohydrates, fiber, fat, and ash along with minerals and essential amino acids. Mushrooms are a low-cost food and important source of protein in the fight against malnutrition. The antioxidant and antibacterial have the ability to prevent damage which is attributed to free radicals Rhodes, phenolic compounds, etc. They are also considered to be able to reduce cholesterol and reduce stress and certain diseases.

Nutritional Value of *Agaricus bisporus*

In addition to proteins/amino acids, carbohydrates, fats, crude fibers, vitamins, macro and micro elements. An important level of phosphorus is present in *Agaricus* sp. Generally, the percentage of proteins varies from 34% to 44% of the total dried matter of fruiting bodies of *A. bisporus*. Total free amino acids are 77.92 g/kg; the content of monosodium glutamate like components 22.67 g/kg. *A. bisporus* has a complex mix of

unsaturated fatty acids including stearic, oleic, linoleic and palmitic acid. The contents of fructose, mannitol, and other reducing sugars are 26.2, 236.2 and 57 g/kg (dry weight), respectively. Commercially, *A. bisporus* is used in fresh, dried or canned form. The percentage of dry weight *A. bisporus* fruits grown on the wheat straw compost is about 8-10%. Tsai et al. (2007) remarked that content of carbohydrate in its fruits ranged from 38-48%, crude protein was 21-27%, crude fiber 17-23.3, crude ash 8-11.00%, and fat 3-4%based on dry matter. Goyal et al. (2015) referred to crude fibers of *A. bisporus* being a good source of dietary fibers because they help to prevent many of the common diseases like obesity disease due to its low calorie content which up to 30 calories per 100 grams (fresh). *A. bisporus* is a good source of vitamins, such as A, B_1 (thiamine), B_2 (riboflavin), B_3 (niacin acid), B_5 (pantothenic acid), C (ascorbic acid), D and folic acid (Espin JC 1999). It has essential amino acids useful as a food for human health including cystine, methionine, threonine, valine, isoleucine, leucine, lysine, tyrosine and phenylalanine. *A. bisporus* contains K, Fe, Zn, Cu, Na, Se, Co and Mn (Abou‐Heilah AN 1987). Four elements (K, P, Ca and Mg) contribute 97-98% of the total element concentration of *A. bisporus*. Selenium level of *A. bisporus* (2.3–2.7 mg kg–1, dry weight) is a useful Se-supplement. Some studies demonstrate clearly that the Cd and As levels of this species are low enough not to present a toxicological risk (Anderson EE 1942).

Medicinal Value of *Agaricus bisporus*

Medicinal mushrooms like *A. bisporus* have a long history in many traditional therapies. The use of *A. bisporus* extracts for its bioactivity such as antioxidants, antibacterial activity, anticancer and anti-inflammation are increasing with advantages recently demonstrated in the fight against coronary heart diseases, diabetes mellitus, bacterial and fungal infections, immune system disorders and cancers (Zhang JJ 2016). It reported the therapeutic properties of this mushroom and its biomedical applications in human health care and treatment of chronic inflammations and cancers (Blagodastski A 2018). The Canadian Cancer Society selected *A. bisporus* mushroom because of its effectiveness against human diseases. *A. bisporus* has important actions against breast cancer, prostate cancer, and high blood pressure (MC 2007). This mushroom has been considered an important medical source for thousands of years reported (Roberts 2008) that ultraviolet irradiation of fruiting bodies of *A. bisporus* in dosages recommended by Processed Foods Research Unit (PFRU) leads to the accumulation of significant quantities of vitamin D_2 essential for bone health and has potential for enhancing human immunity and defense mechanisms against microbial invasion and tumor development (Ren 2008). The mushroom's Lectin content may be helpful to prevent spread of cancer

epithelial cells, and is increasingly effective against oxidation by selenium and ergothioneine which is not broken by heat or cooking (Halpern 2006).

Agaricus bisporus as Nanoparticles

Owaid MN 2017, developed the methanolic nanoparticles of *A. bisporus* have various advantages to treat cancer, viral, bacterial, fungal diseases, etc., This type of the nanoparticle synthesis by edible and medicinal mushrooms are economic and suitable to apply in nanomedicine due to the huge number of fruiting bodies which are produced in the world. The commercial mushroom production process is usually performed in buildings or tunnels under highly controlled environmental conditions. In nature, the basidiomycete *A. bisporus* has a significant impact on the carbon cycle in terrestrial ecosystems as a saprotrophic decayer of leaf litter (Majumder P 2017).

Table 1. Proximate composition of the mushroom *A. bisporus*

Parameters	**Proximate composition (%) of *A. bisporus***
Moisture	11.29
Crude protein	31.46
Crude fibers	12.43
Ether extract	4.14
Total Ash	11.34
Sand and silica	1.28
Calcium	0.50
Phosphorus	1.07
Salt	1.36
Gross energy	3840 kcal/kg

Diet Preparation with A. bisporus Powder

Diets were formulated with the following branded feed basal ingredients (BI). The fish meal, groundnut oilcake and soybean meal as protein sources, wheat bran as a carbohydrate source, sunflower oil as lipid source, and tapioca flour and egg albumin were used as binding agents. The fishmeal, groundnut oilcake, soybean meal, wheat bran, and tapioca flour were thoroughly mixed, dough was prepared with sterilized water, then it was steam cooked and cooled at room temperature. Then the Sunflower oil and egg albumin were added to the dough and mixed well. *A. bisporus* powder was incorporated with the dough of BI at four different concentrations, 25%, 50%, 75%, and 100% by replacing the right quantity of fishmeal, and in order to prepare isonitric diets, the protein level was maintained by adjusting the groundnut oilcake and soybean meal. Sterilized water was adequately added for maintaining the dough in moist and paste form. Then it

was pelletized in a manual pelletizer (Kolkata, India) fixed with 3 mm diameter mesh. The pellets were dried in a thermostatic oven (M/s Modern Industrial, Mumbai, India) at 40ºC until they reached a constant weight and stored in airtight jars at room temperature. The pelletized feeds were subjected to proximate composition analyses and the results are also presented in Table 2.

Table 2. Ingredients used to formulate isonitric diets, and proximate composition of fishmeal replaced diets with *A. bisporus*

Basal ingredients (g)	**Control**	**Fishmeal replaced diets with *A. bisporus***			
		25%	50%	75%	100%
Fish meal	25	18.75	12.5	6.25	0
Groundnut oil cake	25	29	31	34	35
Soybean meal	25	29	31	34	35
Wheat bran	10	10	10	10	10
Egg albumin	7	7	7	7	7
Tapioca flour	5	5	5	5	5
Sunflower oil	2	2	2	2	2
Vitamin mix*	1	1	1	1	1
A. bisporus	0	6.25	12.5	18.75	25
Total	100	108	112	118	120
Proximate composition (%)					
Moisture	8.87	8.91	8.94	9.01	9.12
Crude protein	42.86	42.82	42.76	42.71	42.62
Crude fibre	1.29	1.26	1.23	1.20	1.18
Crude fat	4.52	4.48	4.45	4.41	4.36
Total Ash	7.72	7.68	7.64	7.61	7.57
Total carbohydrate	34.74	34.85	34.98	35.06	39.73
Gross energy (kcal/kg)	4367	4375	4381	4386	4372

ANTIOXIDANT ACTIVITY OF *A. BISPORUS* MUSHROOMS

Methanolic extracts of cap and stipe of commercially obtained mushrooms *A. bisporus*, *Hypsizygus ulmarius*, and *Calocybe indica* were analyzed for their antioxidant activity in different chemical systems including reducing power, free radical scavenging, ferric reducing antioxidant power (FRAP), superoxide scavenging, peroxide scavenging, metal chelating activities and electrochemical behavior. Scavenging effects on 2,2-diphenyl-1-picrylhydrazyl radicals were moderate (43.5–59.0%) at 1.5 mg/ml. Chelating effects on ferrous ions were moderate to excellent (40.6–96.1%) at 20 mg/ml. At 12 mg/ml, the reducing powers were excellent (2.54–1.71). FRAP results were in the range 2.15–0.98 at 16 mg/ml. The ability to scavenge H2O2 was moderate to excellent (48.9–97.7%) at 1.5 mg/ml. At 10 mg/ml, *Agaricus bisporus* cap proved to be better at

scavenging superoxide radicals than others. Similar electrochemical responses of all extracts suggested similar electroactive chemical composition. The total phenols in the extracts ranged from 14.73–26.72 mg/g. The total flavonoid content ranged from 1.12–2.17 µg/g (Dandamudi RB 2013).

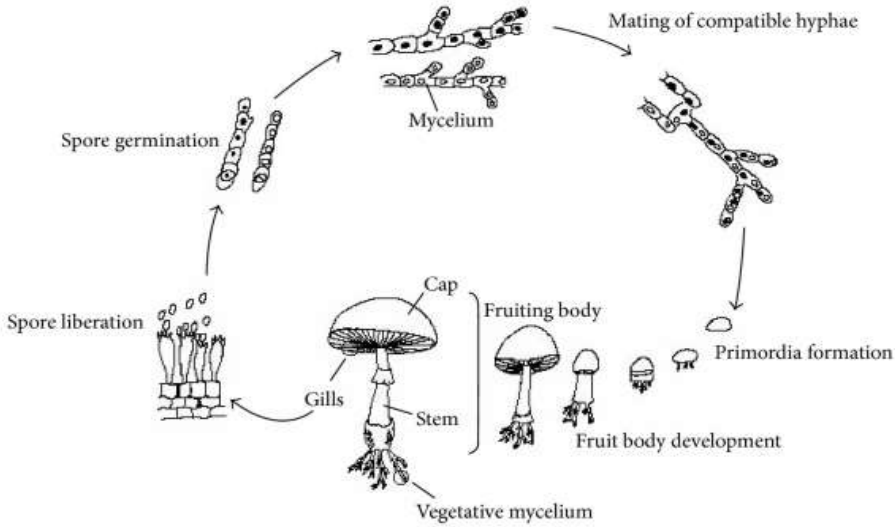


Figure 1. Life cycle of *A. bisporus.*

AGARICUS CLINICAL USES

Agaricus mushroom is a fungus. It originated in Brazil, but is now grown in China, Japan, and Brazil for sale. A solution containing chemicals that are taken from the plant (extract) is used as medicine. *Agaricus* mushroom is used for cancer, type 2 diabetes, high cholesterol, "hardening of the arteries" (atherosclerosis), hepatitis B, digestive problems such as ulcerative colitis and Crohn's disease, and to reduce side effects due to cancer chemotherapy. Other uses include prevention of heart disease, weakened bones (osteoporosis), and stomach ulcers. In Japan, extracts of the *Agaricus* mushroom are approved as a food additive. It is also consumed as food and tea.

How Does It Work

Agaricus mushroom contains chemicals that might improve the body's use of insulin and decrease insulin resistance in people with type 2 diabetes. Some developing research also suggests it might be able to strengthen the immune system, fight tumor development, and work as an antioxidant.

MEDICINAL VALUE

A. bisporus is a good source of trace elements like sodium, potassium, and phosphorus, conjugataed linoleic acid and antioxidants. It can inhibit aromatase, and therefore may be able to lower the estrogen levels in the human body, which might reduce breast cancer susceptibility. In 2009, a case control study of over 2000 women correlated a large decrease of breast cancer incidence in women who consumed mushrooms. Women in the study, who consumed fresh mushrooms daily, were 64% less likely to develop breast cancer, while those that combined a mushroom diet with regular green tea consumption, reduced their risk of breast cancer by nearly 90%. It possesses possible immune system enhancing properties. The most commonly cultivated white mushrooms (*A. bisporus*) are a valuable food in India. They are low in energy, fat, and sodium, while their high water and fiber content makes them a filling and satisfying component of a meal. In the current climate of rising rates of overweight and obesity, they are a useful and versatile ingredient that can easily be added to many dishes without adding much to the energy content. They are a source of numerous vitamins and minerals including some B vitamins, iron, potassium and selenium. In addition, they contain potent antioxidant substances with potential beneficial effects on health. The use of *A. bisporus* with potential therapeutic properties raises global interests from the scientific and clinical community based on two main reasons. First, mushrooms demonstrate their efficiency against numerous diseases and metabolic disturbances as serious as cancer or degenerative diseases. Mushroom compounds would act in combination to influence cell surface receptors, and to trigger various downstream signaling events leading to high pharmacological efficiency and specificity. Secondly, fungal bioactive metabolites can be obtained from many origins either wild or cultivated fruiting bodies or from mycelial biomass and supernatant of submerged cultured using bioreactors. Isolation and purification of natural or hemisynthetic active components (namely polyphenols, polysaccharides, proteinbound polysaccharides, sesquiterpenes, triterpenoids) require common analytical procedures. Since three millennia, traditional uses of medicinal mushrooms have been orally, then via handwritten, passed on to therapists and scientists in Asian countries as China and Japan. The market opened up recently in the USA and Europe to higher fungi providing good health. Hundreds of papers discuss Basidiomycota therapeutic indications mainly antitumor, antidiabetic, antimicrobial, immune-stimulating, anti-inflammatory and antioxidant effects as well as in cardiovascular. Mushroom metabolites defining new generations of pharmacologically active compounds, should definitely help fill some of the weaknesses of current therapeutic arsenal and develop it against present and future therapeutic challenges (Dhamodharan G 2010).

CONCLUSION

Medicinal mushrooms have an established history of use in traditional therapies. Modern clinical practice in Asian countries continues to rely on mushroom derived bioactive compounds. Medicinal effects have been demonstrated for many traditionally used mushrooms. The use of mushroom's extract and their bioactive compounds as antioxidants is becoming increasingly popular and could bring diverse physiological benefits to the consumer, such as protection against human diseases associated with oxidative stress, like coronary heart disease, oxidation associated pathologies, diabetes, infections (fungi, bacteria), immune system disorder and cancer. Recently, considerable attention is focused on anticarcinogenic bioactive compounds particularly those derived from medicinal or edible mushrooms. The aim of the present chapter is to outline the therapeutic characteristics of *Agaricus bisporus* (white button mushroom) medicinal mushrooms, and their applications in human health care. Indeed, metabolites from Basidiomycota demonstrate verified pharmacological activity in major diseases such as chronic inflammation. *Agaricus bisporus* is usually called common mushroom, button mushroom, white mushroom, table mushroom, portobello mushroom, cremini, crimini mushroom, Swiss brown mushrooms, Roman bro`wn mushrooms, Italian brown, Italian mushroom, or cultivated mushroom. It is an edible basidiomycete mushroom native to grasslands in India, Europe and North America. White button mushroom (*Agaricus bisporus*) (WBM) constitute 90% of the total mushroom consumed in the United States, it is one of the most widely cultivated mushroom in the world. The original wild form bore a brownish cap and dark brown gills but more familiar is the current variant with white cap, stalk and flesh and brown gills.

REFERENCES

Aida, F., Shuhaimi, M., Yazid, M. and Maaruf, A.G. (2009). Mushroom as a potential source of prebiotics: a review. *Trends in Food Science and Technology*, 20, 567-575.

Al-Bahadli, A. H. and Al-Zahron, H. H. (1991). *The basics of fungus production (mushroom)*. Dar Al-Hikma for printing and publishing. Baghdad University. Iraq.

Alispahic, A., Sapcanin, A., Salihovic, M., Ramic, E., Dedic, A. and Pazalja, M. (2015). Phenolic content and antioxidant activity of mushroom extracts from Bosnian market. *Bulletin of the Chemists and Technologist of Bosnia and Herzegovina*, 44, 5-8.

Abou‐Heilah, A.N., Kasionalsim, M.Y., Khaliel, A.S. (1987). Chemical composition of the fruiting bodies of *Agaricus bisporus*. *International Journal of Experimental Botany*, 47, 64‐8.

Alkaisi, M.R.M., Hasan, A. A. and Aljuboori, A.W. A. (2016). Evaluation of production efficiency for some cultivated mushroom strains *Agaricus bisporus* which was renovated Mother Culture in multiple methods. *Iraqi Journal of Science*, 57(1B), 383-390.

Andrade, M.C.N., de Jesus, J.P.F., Vieira, F.R., Viana, S.R.F., Spoto, M.H.F. and Minhoni, M.T.A. (2013). Dynamics of the chemical composition and productivity of composts for the cultivation of *Agaricus bisporus* strains. *Brazilian Journal of Microbiology*, 44(4), 1139-1146.

Andrade, M.C.N., Zied, D.C., Minhoni, M.T.A., and Filho, J.K. (2008), Yield of four *Agaricus bisporus* strains in three compost formulations and chemical composition analyses of the mushrooms. *Brazilian Journal of Microbiology*, 39, 593-598.

Anderson, E.E., and Fellers, C.R. (1942). The food value of mushrooms (*Agaricus campestris*). *Journal of the American Society for Horticultural Science*, 41, 301.

Bahl, N. (1983). Medicinal value of edible fungi. In: Proceeding of the International Conference on Science and Cultivation Technology of Edible Fungi. *Indian Mushroom Science* II, 203-209.

Barros, L., Ferreira, M-J., Queiros, B., Ferreira, I.C.F.R., and Baptista P. (2007). Total phenols, ascorbic acid, â-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chemistry*, 103, 314-419.

Baysal, E., Yigitbasi, O.N., Colak, M., Toker, H., Simsek, H., and Yilmaz F. (2007). Cultivation of *Agaricus bisporus* on some compost formulas and locally available casing materials. Part 1: Wheat straw based compost formulas and locally available casing materials. *African Journal of Biotechnoloyg*, 6(1), 2225-2230.

Bernas, E., Jaworska, G., and Kmiecik, W. (2006). Storage and processing of edible mushrooms. *Acta Scientiarum Polonorum Technologia Alimentaria*, 5(2), 5-23.

Beyer, D.M. (2003). *Basic procedures for Agaricus mushroom growing.* Penn State College of Agricultural Sciences Research, Extension, and Resident Education Programs, Pennsylvania, Cheste.

Bhakta, M., and Kumar, P. (2013). Mushroom polysaccharides as a potential prebiotics. *International Journal of Health Sciences and Research*, 3(8), 77-84.

Bhushan, A., and Kulshreshtha, M. (2018). The medicinal mushroom *Agaricus bisporus*: Review of phytopharmacology and potential role in the treatment of various diseases. *Journal of Nature and Science of Medicine*, 1,41-7.

Bonnen, A.M., Anton, L.H., and Orth A.B. (1994). Lignin-degrading enzymes of the commercial button mushroom, *Agaricus bisporus*. *Applied and Environmental Microbiology*, 60(3), 960-965.

Breene, W.M. (1990). Nutritional and medicinal value of specialty mushroom. *Journal of Food Protection*, 53, 883-894.

Bozena, M., Agata, G., Katarzyna, K., and Joanna, G.A. (2018). Anti-Inflammatory Potential of *In Vitro* Cultures of the White Button Mushroom, *Agaricus bisporus*

(Agaricomycetes), in Caco-2 Cells, Anti-Inflammatory Potential of *In Vitro* Cultures of the White Button Mushroom, *Agaricus bisporus* (Agaricomycetes), in Caco-2 Cells. *International Journal of Medicinal Mushrooms*, 20(2), 129-139.

Blagodatski, A., Yatsunskaya, M., Mikhailova, V., Tiasto, V., Kagansky, A., and Katanaev, V.L. (2018). Medicinal mushrooms as an attractive new source of natural compounds for future cancer therapy. *Oncotarget*, 9(49), 29259–29274.

Babu, D.R., and Rao, G.N. (2013). Antioxidant properties and electrochemical behavior of cultivated commercial Indian edible mushrooms. *Journal of food science and technology*, 50(2), 301–308.

Colmenares-Cruz, S., Sánchez, J.E., and Valle-Mora, J. (2017). *Agaricus bisporus* production on substrates pasteurized by self-heating. *AMB Express*, 7, 135.

Chang, S.T. (2006). The world mushroom industry: Trends and technological development. *International Journal of Medicinal Mushriooms*, 8, 297‑314.

Daniel, G., and Han, A.B. (2018). Wösten, Mushroom cultivation in the circular economy. *Applied Microbiology and Biotechnology*, 102(18), 7795–7803.

Dhamodharan, G., and Mirunalini, S. (2010). A Novel Medicinal Characterization of *Agaricus Bisporus* (White Button Mushroom). *Pharmacologyonline*, 2, 456-463.

Espin, J.C., Jolivet, S., and Overeem, A. (1999). Agaritine from *Agaricus bisporus* is capable of preventing melanin formation. *Phytochemistry*, 50, 555‑63.

FAO. (2017). *Aquaculture, the sustainable development goals (SDGs)/Agenda 2030 and FAO's common vision for sustainable food and agriculture. COFI sub-committee on aquaculture*. Ninth session, COFI: AQ/IX/2017/5, 24-27.

Grove, J.F., (1981). Volatile compounds from the mycelium of the mushroom *Agaricus bisporus*. *Phytochemistry*, 20, 2021-2.

Grube, B.J., Eng, E.T., Kao, Y.C., Kwon, A., and Chen, S. (2001). White button mushroom phytochemicals inhibit aromatase activity and breast cancer cell proliferation. *Journal of Nutrition*, 131, 3288-3293.

Have, R.T., Wijngaard, H., and Aries-Kronenburg, N.A.E. (2003). Lignin degradation by *Agaricus bisporus* accounts for a 30% increase in bioavailable holocellulose during cultivation on compost. *Journal of Agricultural and Food Chemistry*, 51, 2242-2255.

Halpern, G.M. (2006). *Healing Mushrooms*. Squareone Publishers. USA.

Hood, P.N., Beets, M.O., and Kimberley, J.F. (2004). Colonisation of podocarp coarse woody debris by decomposer basidiomycete fungi in an indigenous forest in the central North Island of New Zealand. *Forest Ecology and Management*, 196, 311-325.

Jeong, S.C., Jeong, Y.T., Yang, B.K., Islam, R., Koyyalamudi, S.R., Pang, G., Cho, K.Y., and Song, C.H. (2010). White button mushroom (*Agaricus bisporus*) lowers blood glucose and cholesterol levels in diabetic and hypercholesterolemic rats. *Nutrition Research*, 30(1), 49-56.

Jeong., S.C., Koyyalamudi, S.R., Jeong, Y.T., Song, C.H., and Pang, G. (2012). Macrophage immunomodulating and antitumor activities of polysaccharides isolated from *Agaricus bisporus* white button mushrooms. *Journal of Medicinal Food*, 15(1), 58-65.

Karplus, I., Hulata, G., Ovadia, D., and Jaffe, R. (1992). Social control of growth in *Macrobrachium rosenbergii*. III. The role of claws in bull run interactions. *Aquaculture*, 105, 281-296.

Langer, S., Bakhtiyar, Y., and Lakhnotra, R. (2011). Replacement of fishmeal with locally available ingredients in diet composition of *Macrobrachium dayanum*. *African Journal of. Agricultural Research*, 6, 1080-1084.

Liu, J., Jia, L., Kan, J., and Jin, C.H. (2013). *In vitro* and *in vivo* antioxidant activity of ethanolic extract of white button mushroom (*Agaricus bisporus*). *Food and Chemical Toxicology*, 51, 310-6.

Mitra, G., Mukhopodhay, P.K., and Chattopadhyay, D.N. (2005). Nutrition and feeding in freshwater prawn (*Macrobrachium rosenbergii*) farming. *Aqua feeds: Formulation and Beyond*, 2, 17-19.

M.C. (2007). *Mushrooms Canada. Health Sheet.* Published by Data of Mushrooms Canada. Canada, 1-3.

Majumder, P. (2017). Nanoparticle‐assisted herbal synergism an effective therapeutic approach for the targeted treatment of breast cancer: A novel prospective. *Global Journal of Nanomedicine*, 2, 555‐95.

Oludemi, T., Sandrina, A., Heleno, R.C., Calhelha, M.J.A, Lillian, B., Maria, F.B., Ana, M.G.P., and Ferreira, C.F.R. (2016). Development of Mushroom-Based Cosmeceutical Formulations with Anti-Inflammatory, Anti-Tyrosinase, Antioxidant, and Antibacterial Properties. *Molecules*, 21(10), 1372.

Owaid, M.N., and Ibraheem, I.J. (2017). Mycosynthesis of nanoparticles using edible and medicinal mushrooms. *European Journal of Nanomedicine*, 9,5‐23.

Parslew, R., Jones, K.T., Rhodes, J.M., and Sharpe, G.R. (1999). The antiproliferative effect of lectin form the edible mushroom (*Agaricus bisporus*) on human keratinocytes: preliminary studies on its use inpsoriasis. *British Journal of Dermatology*, 140,56-60.

Rahi, D.K., Rahi, S., Pandey, A.K., and Rajak, R.C. (2009). *Enzymes from mushrooms and their industrial application.* International Publishing House Pvt. New Delhi.

Ren, Z., Guo, Z., Meydani, S.N., and Wu, D. (2008). White button mushroom enhances maturation of bone marrow-derived dendritic cells and their antigen presenting function in mice. *Journal of Nutrition*, 138(3), 544-50.

Roberts, J.S., Teichert, A., Mc Hugh, T.H. (2008). Vitamin D_2 formation from post-harvest UV-B treatment of mushrooms (*Agaricus bisporus*) and retention during storage. *Journal of Agricultural and Food Chemistry*, 56, 4541-4544.

Sadler, M. (2003). Nutritional properties of edible fungi. *British Nutrition Foundation Nutrition Bulletin*, 28, 305–308.

Sánchez, C. (2004). Modern aspects of mushroom culture technology. *Applied Microbiology and Biotechnology*, 64(6), 756-62.

Sánchez, C. (2010). Cultivation of Pleurotus ostreatus and other edible mushrooms. *Applied Microbiology and Biotechnology*, 5, 1321–1337.

Shah, Z.A., Ashraf, M., and Ishtiaq, C. (2004). Comparative study on cultivation and yield performance of oyster mushroom (*Pleurotus ostreatus*) on different substrates (wheat straw, leaves, sawdust). *Pakistan Journal of Nutrition*, 3, 158–160.

Stamets, P. (1993). *Growing gourmet and medicinal mushrooms*. Crown Publishing Group. New York.

Singha, R., Bishnoi, D.K., Singh, A. (2010). Cost benefit analysis and marketing of mushroom in Haryana. *Agricultural economics research review*, 23, 165‑71.

Till, O. (1962). Cultivation of mushrooms on sterile substrate and reutilization of spent compost. *Mushroom Science*, 5,127–133.

Thakur, M.P., Singh, H.K. (2014). Advances in the cultivation technology of tropical mushrooms in India. *JNKVV Research Journal*, 48, 120‑35.

Ying, J., Mao, X., Ma, Q., Zong, Y., and Wen, H. (1987). *Icons of medicinal fungi from China*. Science Press. China.

Zhang, J.J., Li, Y., Zhou, T., Xu, D.P., Zhang, P., Li, S., & Li, H.B. (2016). Bioactivities and Health Benefits of Mushrooms Mainly from China. *Molecules* (Basel, Switzerland), 21(7), 938.

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 9

MUSHROOMS IN NIGERIA AND THEIR NUTRACEUTICAL POTENTIALS

Victor Olusegun Oyetayo[1,*] and Folake Lucy Oyetayo[2]
[1]Department of Microbiology, Federal University of Technology, Akure, Nigeria
[2]Department of Biochemistry, Ekiti State University, Ado-Ekiti, Nigeria

ABSTRACT

Mushrooms are macrofungi that possess distinctive fruiting body which can be hypogeous or epigeous and are large enough to be seen with the naked eye and to be picked by hand. In nature, there are edible and non-edible mushrooms. Some of the edible mushrooms have been described as super food because they have high contents of qualitatively good protein, crude fibre, minerals and vitamins. They contain bioactives such as aromatic phenols, fatty acid derivatives, sesterterpenes, polysaccharides, polyketides and many other substances of different origin. The bioactives produced or found in these mushrooms have been described as biological response modifier (BRM). Hence, the presence of these bioactive compounds has conferred on mushrooms several health promoting properties such as anticancer, immunostimulatory, cardiovascular, hypocholesterolaemic, hepatoprotective, antibacterial, antifungal, anti-inflammatory and many other effects that improve the health of human. One major advantage of mushrooms as a source of bioactives that can promote health is the fact that artificial cultivation is possible. Rational preparation and modification of the substrate on which they grow can result to the production of novel bioactive compounds which may be used in alleviating some of the health challenges currently ravaging the human race. Mushrooms found in Nigeria are also known to contain these bioactive compounds and some preliminary studies have shown that these mushrooms if fully exploited can help in promoting the health of the end users. This chapter therefore X-rays the health promoting properties of some edible and non-edible mushrooms found in Nigeria and practical steps that can be taken to ensure the full exploitation of these mushrooms.

* Corresponding Author's Email: ovonew67@gmail.com or vooyetayo@futa.edu.ng.

Keywords: mushroom, cultivation, bioactive, nutraceuticals, potentials, employment, Nigeria

INTRODUCTION

The term "mushroom" and its variations may have been derived from the French word *mousseron* in reference to moss (*mousse*). According to Chang and Miles (1992) mushrooms are macrofungi with distinctive fruiting body, which can be hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand. The use of mushrooms as food and in health promotion has been documented from time immemorial. In terms of food, edible mushrooms have been known as healthy foods rich in proteins and minerals, poor in calories and fat (Oyetayo et al., 2007). In countries where animal protein is expensive and inadequate, edible mushrooms can definitely be a good alternative to animal protein in human nutrition. Moreover, edible mushroom contain all essential amino acids, especially lysine and leucine, in high concentrations (Mdachi et al., 2004; Oyetayo et al., 2007). Records of the health promoting properties of mushrooms have grown over the years. Both edible and non-edible mushrooms are known to possess the following properties; antioxidant, antimicrobial, anticancer, cholesterol lowering, immunostimulatory and many other health promoting effects (Mizuno 1999; Oyetayo et al., 2012; Oyetayo and Ariyo 2013).

The cultivation of mushroom on agricultural residues and other cellulosic waste is a process that converts materials otherwise considered as waste into valuable human food and also source of bioactive compounds with health benefits (Zhang et al., 2002). Artificial cultivation will also help in solving the problem of environmental pollution associated with the current disposal methods. In Nigeria, the art of mushroom cultivation is not yet so popular. Most edible and medicinal mushrooms utilized for one purpose or the other are collected in the wild.

Medicinal mushrooms have been found to be a reservoir of new natural bioactive compounds with potential application in medicine (Oyetayo et al., 2012). This is as a result of the fact that mushrooms produce a wide range of biologically active secondary metabolites with high therapeutic value (Demian 1999). Some biologically active compounds, such as polysaccharides, vitamins, terpenes, steroids, amino acids, and trace elements, have been identified in different species of medicinal mushrooms (Zaidman et al., 2005). These bioactive compounds produced by these mushrooms are referred to as Biological response modifiers (BRM). BRMs are substances that stimulate the body's response to infection and disease. The human body is known to produce some of these BRMs but not in adequate quantities hence an exogenous source of supply is needed. Mushrooms bioactives have been found to be a suitable source of these external supplies. The fruiting bodies and the mycelium of some species of medicinal mushrooms contain

compounds with wide-ranging medicinal properties (Barros et al., 2007). In medicine, biologically active substances are used to immodulate both humoral and cellular immune factors in the body (Wasser 2002). For instance, a polysaccharide isolated from *Lentinus edodes* called **lentinan** has been found to be an immune strengthener (Bohn and Bemiller 1995). These biologically active substances can be produced from the fruiting body and the mycelium of mushroom (Ferreira et al., 2007). Some of these substances are also present in broth used in submerged cultivation. Reshetnikov et al. (2001) reported that there is normally a higher level and number of different polysaccharides extracted from fruit bodies than from the other cultural sources.

Ironically, It has been estimated that the number of mushrooms on earth is about 140,000 yet only 14,000 (10%) are known (Hawksworth 2001). It therefore means that information on the health promoting effect of only 10% mushroom is known while the health benefits of about 90% is still unknown. A large number of the unknown species of mushrooms whose health promoting properties are unknown may be in Africa and probably in Nigeria because there are no data showing that (Oyetayo, 2011). Common data on mushrooms in Nigeria are on their nutritional composition and potentials. Most information on the medicinal potentials is from ethnomycological survey of mushrooms (Ayodele et al., 2009). The reports on ethnomedicinal uses are obtained from local herbalists who in most cases will not totally disclose how their preparations are made.

Some mushrooms whose ethnomedicinal uses have been reported are *Pleurotus tuber-regium, Lentinus squarullosus, Termitomyces microcarpus, Calvatia cyathiformis, Ganoderma lucidum, G. resinaceum, G. applanatum, Schizophyllum commune, Volvariella volvaceae, and Deldinia concentrica*. For instance, P. tuber-regium is used for alleviating headache, stomach pain fever, cold, constipation; L. squarullosus for mumps, heart diseases; T. microcarpus for gonorrhea; C. cyathiformis for leucorrhea, barreness; G. Lucidum for treating arthritis, neoplasia; G. resinaceum is used hyperglycemia, liver diseases (hepatoprotector); G. applanatum used as antioxidant and for diabetes (Oyetayo 2011). In a review, Oyetayo (2011) raised the following questions on the ethnomedicinal properties of mushrooms collected in Nigeria. These questions are listed below.

1. Are the ethnomedicinal properties of mushrooms obtained from survey actually true? There is need to verify these claims.
2. What are the major bioactive constituents in mushrooms found in Nigeria?
3. Are the bioactive substances found in mushrooms indigenous to Nigeria more efficacious or otherwise than those obtained in mushrooms in other parts of the world?

It is obvious that these research questions can only be answered in well structured studies.

MUSHROOMS BIOACTIVES WITH NUTRACEUTICAL PROPERTIES

Nutraceutical properties of edible and medicinal mushrooms have been attributed to the presence of bioactive compounds present in them. These compounds are of diverse chemical nature and functions. Some common bioactives that have been reported to be present in mushrooms are polysaccharides, dietary fibers, lectins and triterpenoids, glycolipids (schizonellin), compounds derived from the shikimic acids (strobilurins and oudemansins), aromatic phenols (drosophilin, armillasirin, amphalone), fatty acid derivatives (filiboletic acid, podoscyphic acid), polyacetylenes (agrocybin, xerulin), polykekides (caloporoside, hericenones A-H), nucleosides (chitocine, nebularines), different sesquiterpenes (protoilludanes, marasmanes, hirsutanes, caryophyllanes, etc.), diterpenes (cyathin, stricital), sesterpenes (aleurodscal), and many other substances of different origin (Lorenzen and Anke, 1998; Wasser and Weis, 1999; Mizuno, 1999).

Emeritus Professor S. T. Chang in 2009 propounded the concept of concert performance for mushroom nutraceuticals. This is because mushroom nutraceutical preparations contain several compounds and possibly other yet to be identified that appear to act in a concert in contributing to health promotion. In essence, they have a multifunctional value which depends on the collaborative effects of the various compounds. The concept of concert performer may lay credence to the claim of traditional herbalist who normally proclaims that their concoction and decoction can cure all manners of diseases under the sun.

Polysaccharides and some other bioactives isolated from mushroom are considered to be biological response modifiers (BRM) which can help to positively modulate various processes in the body (Oyetayo, 2008). BRMs are substances that stimulate the body's response to infection and disease (Kim et al., 2006). Mushrooms are rich sources of 4 critical bioactive compounds important to human health: selenium, vitamin D, L-ergothioneine (Ergo) and glutathione (GSH) (Beelman et al., 2019). These bioactive compounds are important antioxidants thought to mitigate oxidative stress/damage, a key contributor to aging and its related diseases. The specific health promoting properties of some of these bioactive compounds are presented below.

Polysaccharides

Mushrooms are rich in polysaccharides; hence, they represent an unlimited source of polysaccharides. Polysaccharides are polymers containing repetitive structural features of monosaccharides residues linked to each other by glycosidic linkage (Sharon and Lis 1993). Monosaccharides can interconnect at several points to create a wide array of linear and branched molecules that will carry different biological information (Wasser, 2002). The polysaccharides of mushrooms occur mostly as glucans. Some of which are linked

by β-(1-3), (1-6) glycosidic bonds and β-(1-3) glycosidic bonds but many are true heteroglycans. Most often, there is a main chain, which is either β-(1-3), β-(1-4) or mixed β-(1-3), β-(1-4) with β-(1-6) side chains. By this structural diversity, polysaccharides carry enormous amount of biological information.

Mushrooms contain several high molecular weight compounds excreted without digestion and absorption by humans which are called dietary fibre. These compounds belonging to β-glucans, chitin and heteropolysaccharides have been isolated from mushrooms. They are either water soluble or insoluble. They constitute 10 – 50% of the dry matter of mushrooms. A polysaccharide isolated from *Lentinus edodes*, *Lentinan*, had been found to be an effective immunoceutical agent (Chikara, 1992). Polysaccharides as an anticancer agent do not attack cancer cells directly but activate the immune response of host to recognize and fight the invading cancer cells. The mechanism of immunostimulation by polysaccharides involved enhancing immunoreactivity by activation of macrophages, cytotoxic T cells and natural killer (NK) cells (Mizuno, 1999; Ikekawa, 2000; Feng et al., 2001).

Polyphenols

Phenolics are aromatic hydroxylated compounds, possessing one or more aromatic rings with one or more hydroxyl groups. The effectiveness of a natural phenolic antioxidant depends on the involvement of the phenolic hydrogen in radical reactions, the stability of the natural antioxidant radical formed during radical reactions, and the chemical substitutions present on the structure (Ferreira et al., 2009). Combating negative effect of oxidative stress involve the use of Synthetic chemicals such as butylhydroxyanisole (BHA) or butylhydroxytoluene (BHT) (Hinneburg et al., 2006). These synthetic antioxidants are known to elicit negative side effects (Liu et al., 1997). It is therefore expedient that a safe source of natural antioxidant be sourced. Mushrooms have been found to be rich source of these natural antioxidant compounds especially polyphenols and other phenolic compounds.

Tsao (2000) reported that natural phenolic compounds of many fungi accumulate as end-products from the shikimatechorismate pathway and these products can range from relatively simple molecules such as phenolic acids, and phenylpropanoids, to highly polymerized compounds such as melanins and tannins (Ayodel and Okhuoya 2009; Li and Vederas 2009). Some phenolic compounds found in mushrooms are benzoic acid, gallic acid, catechin, tannic acid, caffeic acid, and resveratrol (Kim et al., 2008). These compounds are known to have antioxidant activity and to have anticancer or anticarcinogenic, antiatherosclerotic, antibacterial, antiviral, and anti-inflammatory activities.

A positive relation has been found between this high phenol content and the free radical sequestering ability, as these phenols are the compounds that contribute most to the antioxidant capacity (Dubost, 2007). Among the phenols identified in the common mushroom are tyrosine, catechol, phenolic acids, ρ-hydroxybenzoic acid, tr-cinnamic acid, ρ-coumaric acid and vanillic acid (Dubost, 2007). Antioxidants are needed to combat free radical activities of accelerating ageing processes of tissues and pathologies such as cancer or cardiovascular diseases. Though, there is endogenous supply of antioxidants such as superoxide dismutase, catalase or glutathione peroxidase to protect the cells against excessive levels of free radicals, there is still a need for exogenous supply of antioxidants to combat the effect of excessive free radicals generated as a result of normal cellular metabolism (Oyetayo, 2008).

Terpenes

Terpenes have been found to have a range of medicinal benefits. Specifically, terpenes of mushrooms origins are volatile unsaturated hydrocarbons which are classified as monoterpeniod, sesquiterpenoids, diterpenoids and triterpenoids (Duru and Cayan 2015). The mushroom terpenes have been proved to be associated with multiple health benefits, including antioxidant, antiviral, anticancer, anti-inflammatory, antimalarial and anticholinesterase activities (Gaoxing et al., 2018). The health promoting effects of some of these terpenoids are presented below.

(i) Sesquiterpenes

Sesquiterpenes are C15-terpenoids which have strong odours and are less volatile than other terpenes. Sesquiterpenes can be monocyclic, bicyclic or tricyclic and are a very diverse group (Abraham 2001). They are found in higher plants and in achlorophylous plant (fungi). Sesquiterpenes have been known to possess anti-inflammatory, antitumour, antioxidant and bactericidal properties.

(ii) Diterpenes

Diterpenes are C20 terpenoids. Naturally occurring diterpenes exert several biological activities such as anti-inflammatory action, antimicrobial and antispasmodic activities. Diterpenes had been part of traditional medicine for the treatment of ailments such as anti-cancer, anti-diabetic and various other ailments. Sources of diterpenes ranged from fungal, plant and marine (Liu and Hu 2010). Several diterpenes have been shown to have pronounced cardiovascular effects. These diterpenoids exhibit vasorelaxant action and inhibit the vascular contractility mainly by blocking extracellular Ca(2+) influx.

(iii) Sesterpenes

Sesterpenes are C25 terpenoids. Sesterterpenes and their derivatives known as sesterterpenoids, are ubiquitous secondary metabolites in fungi, marine organisms, and plants. Their structural diversity encompasses carbotricyclic ophiobolanes, polycyclic anthracenones, polycyclic furan-2-ones, polycyclic hydroquinones, among many other carbon skeletons. These groups of terpenoids possess promising biological activities including cytotoxicity and hence, could serve as a good anticancer agent.

(iv) Monoterpenes

Monoterpenes have two (2) isoprene units and ten (10) carbon atoms. They are present in fungi and chlorophylous plants. They are the major odoriferous compounds of many flowers and fruits. Natural monoterpenes and their synthetic derivatives have been reported to be endowed with various pharmacological properties including antifungal, antibacterial, antioxidant, anticancer, antiarrhythmic, anti-aggregating, local anesthetic, antinociceptive, antiinflammatory, antihistaminic and antispasmodic activities (Kozioł et al., 2014). Moreover, monoterpenes also act as regulators of growth, heat, transpiration, tumor inhibitors, inhibitors of oxidative phosphorylation, insect repellants, feline and canine attractants and antidiabetics (Crowell 1999; Kozioł et al., 2014). Monoterpenes are secondary metabolites and the interesting properties they possess have made them of importance in pharmaceutical, food and cosmeceutical industries.

(v) Triterpenes

Triterpenes are C30 terpenoids. They are metabolites of isopentenyl pyrophosphate oligomers and represent the largest group of phytochemicals. Triterpenes are naturally occurring alkenes found in vegetable, animal and also fungi. It has been estimated that more than 20,000 triterpenoids exist in nature (Liby et al., 2007). Triterpenes and their derivatives have been reported to possess the following biological activities; antioxidant, antimicrobial, antiviral, antiallergic, antipruritic, antiangiogenic and spasmolytic activities (Sultana and Ata, 2008; Shah et al., 2009). Anticancer effects of several triterpenoids have been reported against a variety of cancer cells without manifesting any toxicity in normal cells (Bishayee et al., 2011). Structurally, modified triterpenoids have been found to possess potent antiinflammatory and anticarcinogenic.

Proteins and Peptides

Mushrooms are rich in protein which makes up about 19 to 35% dry weight. Proteins and peptides present in mushrooms are known to possess nutraceutical effects such as enhancement of the digestion and absorption of exogenous nutritional ingredient, the modulation of the immune function to help the host in the defense of invasion by

pathogens, and the inhibition of some enzymes (Valverde et al., 2015). Examples of these proteins and peptides in mushrooms are lectins, fungal immunomodulatory proteins (FIPs), ribosome inactivating proteins (RIPs), ribonucleases and laccases (Gaoxing et al., 2018).

Lectins are group of carbohydrates-binding proteins. They play various roles in normal physiological functions such as helping the cells and molecules stick to each other as well as immunomodulatory function. However, at higher level in our diet, lectins can be detrimental at higher concentration by causing digestive issues and long-term health problems.

Polyacetylenes

Polyacetylenes are aliphatic C_{17} compounds. They are known to be highly toxic towards fungi, bacteria, and mammalian cells, and to display neurotoxic, antiinflammatory and antiplatelet-aggregatory effects and to be responsible for allergic skin reactions.

Fatty Acids of Mushrooms

Mushrooms are rich in saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids PUFA). PUFA e.g., oleic acid and others are found to predominate over MUFA and SFA. PUFA have been reported to contribute to the reduction of serum cholesterol. These fatty acids found in mushrooms have been found to have the following health promoting properties; antioxidant, antimicrobial, cardiovascular protection, anti-inflammatory and also decreasing the impact of Alzheimer's disease risk correlating with its inhibition effects against on the acetylcholinesterase(AChE) and butyrylcholinesterase (BChE) (Gaoxing et al., 2018).

MYCONUTRACEUTICAL PROPERTIES OF MUSHROOMS COLLECTED IN NIGERIA

Edible and Medicinal mushrooms have been found to be a reservoir of new natural bioactive compounds with potential application in medicine. Mushrooms collected in Nigeria are not exempted from this fact. The results obtained from the assessment of the nutraceutical properties of some mushrooms collected from Nigeria are hereby presented (Figure 1).

Figure 1. Photographs of some mushrooms collected in Nigeria. A: *Trametes elegans*, B: *Termitomyces clypeatus*, C: *Trametes polyzona*.

Antioxidant Effect

The oxidation process in living organisms' results to the production of free radicals, and uncontrolled production of free radicals is a factor that has been linked with the onset of many diseases and the ageing process. Antioxidants are usually employed to check the activities of reactive oxygen species (ROS) that are produced as a result of oxidation process. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are known to have side effect(s) (Veglioglu et al., 1998)). Extracts of two common *Termitomyces* species, *Termitomyces clypeatus* (TCE) and *Termitomyces robustus* were found to display good antioxidant effect (Oyetayo, 2009). A comparative assessment of ethanol and water extracts of edible mushroom, *Termitomyces clypeatus* (Heim) revealed that the extracts have high and significant ($P < 0.05$) antioxidant and antimicrobial effect (Oyetayo et al., 2009). Comparative assessment of the antioxidant activities of ethanol (ETE) and water extracts (WE) of edible mushroom, *Termitomyces clypeatus* (Heim) revealed that ETE of *T. clypeatus* has a better overall antioxidant property with ability to scavenge free radicals such as superoxide, DPPH and hydroxyl radicals when compared to WE (Oyetayo et al., 2009). ETE has a significantly higher hydroxyl radical activity than the control, BHT. The exhibition of antioxidant properties by ETE and WE from edible mushroom, *T. clypeatus*, might lead to the isolation of safe antioxidant compounds that may serve as promising alternative to the synthetic antioxidants (BHT and BHA) that are known to have side effects.

Other mushrooms collected from Nigeria whose antioxidant properties has been assessed include *Coriolopsis polyzona* (Oyetayo et al., 2013), *Lenzites* species (Ogidi and Oyetayo, 2015; Ogidi, et al., 2018), *Trametes* species (Fagbohungbe and Oyetayo 2014; Awala and Oyetayo 2015a; Awala and Oyetayo 2015b), *Pleurotus* species (Oyetayo and Ariyo 2013). All these mushrooms were found to have significant antioxidant properties when a multimechanistic antioxidant assay was used to assess their efficacy. Some exhibited higher and better antioxidant properties than controls. The antioxidant effects of

extracts from these mushrooms were found to be concentration dependent. The results from above revealed that mushroom, either edible or non-edible sourced from Nigeria will be a good source of bioactive compound that can serve as effective and safe antioxidant agent.

Antimicrobial Properties

The problem of resistance of common disease causing microorganisms to available antibiotics is a well known issue. Antibiotic resistance has been recognized as a global challenge that is growing at an alarming pace. Several factors such as abuse of antibiotics, production of antibiotics with lower active ingredient, self-medication and sales of expired antibiotics have contributed to the rapid spread of antibiotic resistance. It is therefore imperative to search for effective antibiotic that can be used to overcome the emerging problem of antibiotic resistance. Attention has been turned to mushrooms as a possible source of novel bioactive compound that can solve this global problem.

Mushrooms may be producing antibacterial and antifungal compounds to survive in their natural environment (Lindequist et al., 2005). They may therefore be rich source of natural antibiotics. Based on this background information, the antimicrobial properties of several species of mushrooms were investigated. In one of the studies, the antimicrobial potentials of extracts obtained from four wild mushrooms viz; *Termitomyces clypeatus* (TCE), *Termitomyces robustus* (TRE), *Lentinus subnudus* (LSE) and *Lenzites* species (LZE) collected in Nigeria was investigated. The extracts from the wild mushrooms were able to inhibit the growth of all indicator organisms at concentrations between 12.5 mg/mL to 100 mg/mL (Oyetayo 2009). The inhibitory property of ethanolic extracts of two wild macrofungi, *Trametes elegans* and *Coriolopsis* species was assessed against aerobic microflora of meat. The extracts inhibited meat microflora to various degrees. *Trametes elegans* extract (TE) and *Coriolopsis* species extract (CE) exhibited antimicrobial effects against the isolates with inhibitory zones ranging from 2mm to 16 mm at concentrations of 100mg/ml to 400mg/ml (Oyetayo et al., 2010). TE was able to inhibit the growth of *Pseudomonas aeruginosa* which was resistant to the commercial antibiotics, streptomycin and tetracycline. The implication of this result is that extract from these mushrooms may be used to delay or retard microbial spoilage of meat.

Ethanolic extract obtained from *Coriolopsis* sp (CET) has significant inhibitory effect on *E. coli* isolated from some wells in Akure. CET exhibited significant inhibitory effect on *E. coli* from well water at concentration of 100mg/ml. The zones of inhibition range between 1.5 to 13mm. However, ethyl acetate and petroleum extracts (CEA and CPE) do not show any inhibitory effect at concentrations of 12.5mg/ml to 100mg/ml (Oyetayo and Oghumah 2013). Antimicrobial property of several other mushrooms had also been reported (Jonathan and Fasidi 2003; Ezeronye et al., 2005; Ofodile et al., 2008).

Antimicrobial effects of mushroom extracts were also found to be effective against some specific groups of pathogenic microorganisms as presented below.

Antistaphylococcal

Staphylococcus species is a well known human pathogen. It forms one of the major microbiota of the skin and mucous membranes of the humans. It is a common organism associated with nosocomial infections in both hospitalized and healthy people. *S. saprophyticus, S. epidermidis* and *S. aureus* are common species of Staphylococcus which are responsible for wide range of infections such as skin, soft tissue infections, bacteremia, infections of the central nervoussystem, bone and joints skeletal muscles, respiratory and urinary tracts. The antistaphylococcal activity of a wild medicinal macrofungus, *Lenzites quercina* was assessed. Ethanolic extract of *Lenzites quercina* displayed inhibitory effect against *S. saprophyticus, S. epidermidis* and *S. aureus* isolated from clinical samples in two well patronized hospitals in Akure, Nigeria (Abubakar et al., 2016). The zones of inhibition obtained for the extract ranged from 6 mm to 22 mm while commercial antibiotics have zone of inhibition of 8 mm to 24 mm. The control isolates; methycillin resistant *Staphylococcus aureus* (MRSA) and *S. aureus* (ATCC 29213) were also susceptible to the ethanolic extract of *L. quercina* at 50 mg/ml. The result shows that bioactive compounds that are effective against Staphylococcus spp., an organism that has been reported to develop great resistance against most commercial antibiotics, are present in *Lenzites quercina*. The study revealed that ethanolic extract of Lenzites quercina contains bioactive compounds that are efficacious against clinically isolated Staphylococcus aureus, a notorious pathogenic agent that has developed resistance against commonly used antibiotic. Hence, the bioactive compounds from Lenzites quercina may be a good alternative to commercially available antibiotics that are becoming less effective on Staphylococcus aureus.

Anticandidal

Candidiasis, a common fungal infection caused by *Candida albicans* has been reported to be resistant to common fungicidal agents. Candidiasis encompasses infections that range from superficial, such as oral thrush and vaginitis, to systemic and potentially life threatening diseases. Vaginal candidiasis is a common type of gynaecologic disorder with a white discharge, soreness, dyspareunia, irritation and itching. Fidel (2007) estimated that 75% of women will experience at least one episode of candidiasis in their life time, with a projected 50% of all women experiencing multiple episodes. It is

therefore expedient to search for safe and effective anticandidal agents to cure and prevent recurrence of candidiasis.

In a study, 67 *Candida* species were isolated from 132 high vaginal swabs (HVS) collected from pregnant women attending ten selected hospitals in Ondo, Osun and Oyo States, Nigeria. The prevalent *Candida* species were *C. albicans* (49/67, 73.13%), *C. glabrata* (9/67, 13.43%), *C. krusei* (6/67, 8.96%) and *C. tropicalis* (3/67, 4.48%). Zones of inhibition ranging from 5.00 to 30.00 mm, 4.00 to 15.67 mm and 4.33 to 17.67 mm were produced by methanol, acetone and n-hexane extracts respectively against the Candida isolates. Methanol extract of *T. elegans* produced the highest anticandidal activity with an inhibition zone of 30.00 mm against isolate A3 (*C. albicans*). The least inhibition zone (4.00 mm) was recorded with acetone extract of *T. elegans* against isolate A2 (Adeyelu et al., 2017). The anticandidal property of *Trametes lactinea* extract has also been reported against clinically isolated *Candida* species (Adeyelu et al., 2016). The zones of inhibition produced by the macro-fungus extracts against the *Candida* species ranged from 2.33 to 17.00mm, 4.33 to 12.33mm and 3.30 to 17.33mm for methanol, acetone and n-hexane extracts respectively. The highest (17.33mm) and least (2.33mm) inhibitory zone was produced against *Candida albicans* from Mother and child Hospital, Akure, Nigeria by the acetone and n-hexane extract of *T. lactinea* respectively.

Antidermatophyte

The efficacy of mushroom extract of mushrooms collected in Nigeria was also investigated against dermatophytes. Dermatophytes are pathogenic fungi that have the ability to colonize, degrade and paralyse the keratin membrane of the skin. These skin pathogens produce proteolytic enzymes, which it uses to hydrolyze keratin, the main protein constituent of hair, nails, feet and skin. Most dermatophytes have developed resistance against common antifungal agents used for treating them. The resistance of these dermatophytes has come about as a result of the following factors: discontinuous medication, inappropriate self-medication and failure to comply with clinical guideline of the drug. The conditions listed above often result to relapse of the infections. Hence, there is an urgent need to search for effective antidermatophytic agent that can help in the treatment of dermatophytes.

In a study, the inhibitory potential of petroleum ether and ethanolic extracts of three mushrooms viz: *Lenzites quercina*, *Ganoderma lucidum* and *Rigidoporus ulmarius* was assessed against some common dermatophytes. Extracts of *L. quercina*, *G. lucidum* and *R. ulmarius* exhibited wide range of mycelia inhibition at 50 mg/ml on the tested dermatophytes (Ogidi and Oyetayo 2016). The inhibitory property of the mushroom extracts against pathogenic dermatophytes in the present study shows that these mushrooms could be a promising source of antidermatophytic agent.

Table 1. Total plasma cholesterol of rats fed mushroom diet

Treatments	Cholesterol level*
Control (casein diet)	$3.07^{c} \pm 0.01$
Termitomyces clypeatus diet	$2.97^{a} \pm 0.01$
Pleurotus tuberigium diet	$3.03^{b} \pm 0.01$

*Values are mean of three replicates. Values along column with different superscript are significantly different ($P < 0.05$).

Anticholestereamic

It is an established fact that high level of certain types of cholesterol increases the risk of cardiovascular disease, such as heart disease and stroke. This is because it can cause fatty deposits (known as plaques) to build up inside the arteries. This can completely block the blood supply to part of th heart, causing a heart attack and other heart related diseases. Edible mushrooms are healthy foods rich in proteins and minerals, poor in calories and fat. Based on the information above, the potential of edible mushroom to lower cholesterol was studied.

Two common edible mushrooms *Plerotus tuber-regium* and *Termitomyces clypeatus* indigenous to Nigeria had been reported to possess hypolipidemic properties. Male albino rats fed with diet compounded with mushrooms, *Plerotus tuber-regium* and *Termitomyces clypeatus* had lowered and significantly lowered ($P < 0.05$) plasma total cholesterol, low density lipoprotein (LDL) cholesterol, and triglycerides concentrations while high density lipoprotein (HDL) cholesterol were significantly higher ($P < 0.05$) than control (Oyetayo 2006). The result implies that the two tropical edible mushrooms have hypolipidemic properties (Table 1)

Hypoglycemic Effect

The potential of mushroom to reduce blood sugar has been reported. Mohammed et al. (2007) in their study shows the effects of aqueous extract of *Ganoderma lucidum* collected from Zaria, Nigeria on blood glucose levels of normoglycemic and alloxan induced diabetic wistar rats.

Anti-Inflammatory

Inflammation is the reaction of living tissues to injury, infection, or irritation. Inflammatory reactions have been implicated in the pathophysiology of cardiovascular diseases, cancer, colonic adenomas, and Alzheimer's disease. Antiinflammatory agents are therefore needed to combat disorders involving localized increases in the number of

leukocytes and a variety of complex mediator molecules. It is therefore important to prevent inflammatory reactions. The search for safe and efficient pharmacological agents of plant and medicinal mushroom origin against inflammation has increased in the last three decades, since existing synthetic compounds, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and selective COX-2 inhibitors are known to increase the incidence of adverse cardiovascular thrombotic effects.

Ethanolic extracts of three wild medicinal mushrooms, namely *Lenzites betulina* (LET), *Trametes vesicolor* (TET), and *Coriolopsis polyzona* (CET), collected from Akure, southwest Nigeria, were assessed for their lipid peroxidation, anti-inflammatory, and acute toxicity effects. The extracts displayed significant anti-inflammatory effects. Inhibitory effects of the extracts on mice ear edema ranged from 25.21% to 39.58% (Table 1). The inhibition of ear edema in mice by the extract was higher and significantly different ($p < 0.05$) than the control (Oyetayo et al., 2012). Extract obtained from *Coriolopsis polyzona* (CET) had the highest and significantly different ($p < 0.05$) inhibitory effect on ear edema when the three ethanolic extracts were compared. The inhibitory effect of the reference compound, Naproxeno (63.89%), was however higher than the three ethanolic extracts.

Anticancer

Cancer arises from the transformation of normal cells into tumour cells in a multistage process that generally progresses from a pre-cancerous lesion to a malignant tumour. Cancer is the second leading cause of death globally, and is responsible for an estimated 9.6 million deaths in 2018. Globally, about 1 in 6 deaths is due to cancer (WHO 2018). Approximately 70% of deaths from cancer occur in low- and middle-income countries. Chemotherapy, radiotherapy and surgery are the most common treatments for cancers, but several side effects such as fatigue, nausea, vomiting, malaise, diarrhea, mucositis, pain, rashes, infections, headaches occur during treatment. Chemotherapeutic alkylating agents (cyclophosphamide, ifosfamide) and topoisomerase II inhibitors are known to increase the risk of secondary cancer development (acute leukemia). Anthracyclines is also known to induce cardiotoxicity, while cisplatin induces nephrotoxicity; a potential cause of peripheral nerve damage and renal impairment with a decline in glomerular filtration. The failure displayed by some chemotherapy during the treatment of cancers, and the cost of drugs and treatment has become a public health concern.

The search for safe and effective pharmacological substances that can serve as safe and effective anticancer agent had increased of recent. Bioactive compounds obtained from macrofungi maybe the answer to these novel pharmacological agents with safe and effective anticancer property. Macrofungi produce large and diverse variety of secondary metabolites that have pharmacological effect.

In one of our studies, three wild macrofungi, *Lenzites betulina* (Fries), *Trametes vesicolor* (Lloyd) and *Coriolopsis polyzona* (Pers) collected in Akure southwest of Nigeria were assessed for their cytotoxic activities. The extracts were screened *in vitro* against human cancer cell lines: U251 (human glioblastoma), PC-3 (human prostatic adenocarcinoma), K562 (human chronic myelogenous leukemia), HCT-15 (human colorectal adenocarcinoma), MCF-7 (human breast cancer cell line) and SKLU-1 (human lung adenocarcinoma). It was observed that the inhibition of human cancer cell lines varies from one extract to the other. However, ethanolic extract of *Trametes vesicolor* (TET) demonstrated the best cytotoxic activity with 100% inhibition of HCT-5, MCF-7 and SKLU-1 human cancer cell lines (Oyetayo et al., 2012). The study suggests that extracts from these three wild macrofungi contain bioactive compounds that may be exploited as effective antioxidant anticancer agents.

The cytotoxic activity of raw and fermented *Lenzites quercina* extracts was tested against human cervical cancer (HeLa), rhabdomyosarcoma (RD) and African green monkey kidney (VERO) cell lines. A tetrazolium yellow 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay was used to evaluate the reduction in viability of cell cultures with or without the extracts of *Lenzites quercina*. Extracts of *Lenzites quercina* were found to exhibit significant cytotoxic activity (6.0-84.5%) against the following cancer cell lines, HeLa, VERO and RD (Ogidi et al., 2017).

Food Value of Some Edible and Medicinal Mushrooms

The production of protein rich food is required to meet the demand for protein and to overcome malnutrition in the developing parts of the world. Generally, edible mushrooms have been reported to contain between 19–35% of protein on dry weight basis (Oei 1991). Hence, it is a cheap source of protein. The types of amino acids that make up the protein in a food are also of importance. Amino acids are involved in the synthesis of melanin, serotonin, and transportation of ammonia into the liver and kidneys to aid the production of urea (Kayode et al., 2015). Thus, amino acids are the building blocks of proteins as well as enzymes for the normal function during growth and convalescence.

Amino acid analysis shows that edible mushroom, *Pleurotus sajor-caju* is a rich source of nutritionally useful essential amino acids with leucine as the most abundant occurring in highest concentration (mg/g) in the cultivated *Pleurotus sajor-caju* (Cc) (64.8 ± 0.24). Methionine and cysteine concentrations were low as confirmed by their chemical scores (0.29–0.59). There was a significant difference ($P < 0.05$) in the distribution of amino acids in the mushroom varieties and parts. The total essential amino acid (TEAA) in the cultivated mushroom ranged between 42.91–43.69% while in the wild it was between 42.90–43.73% of the total amino acid content (Oyetayo et al., 2007).

Processing methods such as cooking, drying, fermentation and so on, also have effect on protein and amino acids of foods. Fermentation has been widely used in food biotechnology as a method of food processing that involved the conversion of complex organic molecules in substrates into simple byproducts, with the help of various microorganisms. In this context, the chemical components of a substrate are broken down releasing additional bioactive metabolites. In some wild mushrooms, their bioactive substances are protected by a hard sporoderm (Jingjing et al., 2007). Hence, a process that can help in softening the hard sporoderm may help in releasing the food nutrient. In a study, it was observed that the raw and fermented *L. quercina* contain a considerable amount of essential and non-essential amino acids. The raw and fermented *L. quercina* contain essential amino acids such as isoleucine, leucine, lysine, tyrosine, tryptophan and valine which cannot be synthesized in the human body but required to be ingested from external sources (Ogidi et al., 2018). However, fermentation significantly increased the level of the following amino acids viz: alanine, arginine, cysteine, glycine, lysine, isoleucine, serine, tyrosine, tryptophan and valine.

Oleic acid was reported as the predominant fatty acid with values of 38.5% and 37.92% respectively for raw and fermented macrofungus, *Lenzites quercina* (Ogidi et al., 2018). Fermentation significantly increase ($P < 0.05$) the levels of two essential fatty acids, linoleic (12.48%) and linolenic (22.50%). Mushroom may therefore be a source of these essential amino and fatty acids which cannot be synthesized by the body.

CULTIVATION OF MUSHROOMS

The cultivation of mushrooms which is a complex process that requires precision has been described both as an art and science (Chang 2008). The process requires expertise and high level of aseptic procedure to prevent contamination. Practical steps in cultivating mushrooms such as selection of an acceptable mushroom species, secretion of a good-quality fruiting culture, development of robust spawn, preparation of selective substrate/compost, care of mycelia (spawn) running, management of fruiting and mushroom development, and careful harvesting of mushrooms as listed by Chang and Chiu (1992) and Chang (1998) have to be religiously followed.

The cultivated mushrooms as well as the common field mushroom initially forms a minute fruiting body, referred to as the pin stage because of their small size. Slightly expanded, they are called buttons, once again because of the relative size and shape. Once such stages are formed, the mushroom can rapidly pull in water from its mycelium and expand, mainly by inflating preformed cells that took several days to form in the primordia. Mushroom cultivation is a technical process (Beyer 2003). The processes involved in the cultivation of mushrooms can be divided into five phases as listed below (Wuest and Bengtson 1982).

(i) Phase 1: Composting

This is the first phase in the cultivation of mushrooms. This involve bringing the main raw materials (substrate) together and by mixing and wetting the ingredients as they are stacked in a rectangular pile with tight sides and a loose center. The major components of this compost are horse manure, straw, gypsum, chicken manure and water. The straw improves the structure, gypsum ensures the proper acidity and the two manures (horse and chicken manure) are the nutrients. Mushroom compost develops as the chemical nature of the raw ingredients is converted by the activity of microorganisms, heat, and some heat-releasing chemical reactions. These events result in a food source most suited for the growth of the mushroom to the exclusion of other fungi and bacteria.

(ii) Phase 2: Spawning

Fresh compost is pasteurized at 57 to 60^{o}C to eliminate bacteria and other unwanted microorganisms that are potential contaminants. The compost stays in the containers where they have been packed prior to pasteurization, to mature for six days, after which the compost is mixed with spawn (mushroom seed) that will produce the mushrooms: the mycelium. The compost is then kept in another room and the mycelium will spread through the compost. The mycelium grows quickly; after two weeks it might have completely permeated the compost, which means that is has reached the point that it is ready for the growers who in most cases do not produce their own spawn. Spawn makers start the spawn-making process by sterilizing a mixture of millet grain plus water and chalk; rye, wheat, and other small grain may be substituted for millet. At this time the compost looks like light brown peat.

(iii) Phase 3: Casing

This involves the spreading of matured compost on mushroom bed. Casing is a top-dressing applied to the spawn-run compost on which the mushrooms eventually form. The beds are inside special dark rooms called cells. The temperature in the dark room is kept warm and at 23^{o}C. A layer of peat casing material is added on top of the compost to keep the compost moist. Over a period of six days, 20 to 25 litres of water is sprinkled on each m^2 in each cell because more moisture is needed. After this, the fungus has two days to grow through the covering layer of casing soil. Casing does not need nutrients since casing acts as a water reservoir and a place where rhizomorphs form. Rhizomorphs look like thick strings and form when the very fine mycelium fuses

together. Mushroom initials, primordia, or pins form on the rhizomorphs, so without rhizomorphs there will be no mushrooms.

(iv) Phase 4: Pinning

The mycelium starts to form little buds, which will develop into mushrooms. These little white buds are called pins. Pins continue to expand and grow larger through the button stage, and ultimately a button enlarges to a mushroom. Harvestable mushrooms appear 18 to 21 days after casing. At this stage the atmospheric condition must be well monitored. It has been found that low air temperature and low humidity produce more buds, which yield smaller mushrooms. On the other hand, higher air temperature and humidity produce fewer but larger mushrooms.

(v) Phase 5: Cropping

The mushroom is ready for harvesting 3 to 5 days after mycelium starts to form buds called pins. Harvesting can be done when mushrooms are at appropriate size at least two times a day. The temperature is kept steady at 18 degrees Celsius ($18^{O}C$). Mushrooms grow best at this temperature; they will grow to 3 cm (1 inch) in a week, which is the normal size for harvesting. In week 3 the first flush is harvested. Nine days after the first flush, the second flush will be harvested. It has been observed that the second flush often consists of larger, but fewer mushrooms than the first flush. Mushrooms are harvested in a 7- to 10-day cycle, but this may be longer or shorter depending on the temperature, humidity, cultivar, and the stage when they are picked. The terms flush, break, or bloom are names given to the repeating 3- to 5-day harvest periods during the cropping cycle; these are followed by a few days when no mushrooms are available to harvest. This cycle repeats itself in a rhythmic fashion, and harvesting can go on as long as mushrooms continue to mature. Most mushroom farmers harvest for 35 to 42 days, although some harvest a crop for 60 days, and harvest can go on for as long as 150 days.

The possibility of cultivating mushrooms used in the preparation of myconutraceuticals and dietary supplements instead of collecting from the wild has some obvious advantages. Chang (2009) listed the following as some of the advantages associated with nutraceuticals and dietary supplements obtained from cultivated mushrooms.

(i) The overwhelming majority of mushrooms used for production of mushroom dietary supplements or nutraceuticals are cultivated commercially and not

gathered in the wild. This ensures proper identification, pure and unadulterated products. This also guarantees genetic uniformity.

(ii) Mushrooms are easily propagated vegetatively and thus keep to one clone. The mycelium can be stored for a long time.

(iii) Many edible/medicinal mushrooms are capable of growing in the form of mycelia biomass in submerged cultures. This is an area we need to explore since a lot of new bioactive compounds with myconutraceutical properties can be derived from cultural manipulation in submerged cultures

(iv) Edible mushrooms have been used as part of human diet from time immemorial.

FUTURE PERSPECTIVES

The use of mushrooms for food and health promotion is a well established fact. Hence, conscious effort at cultivation and production of nutraceuticals and dietary supplements from mushrooms must be promoted. The mushroom market has been identified as a major source of economic growth and employment of teeming population of able bodied youth. This is because the market for edible and medicinal mushrooms has been growing over the years especially in Asia, Europe and North America. About 10 years ago, it is valued at U.S. $15 billion which represents 10% of the general market of dietary supplements (Wasser 2009). The implication of this is that the mushroom industry and the emerging myconutraceutical market can be a solution to the problem of unemployment presently ravaging several developing nations.

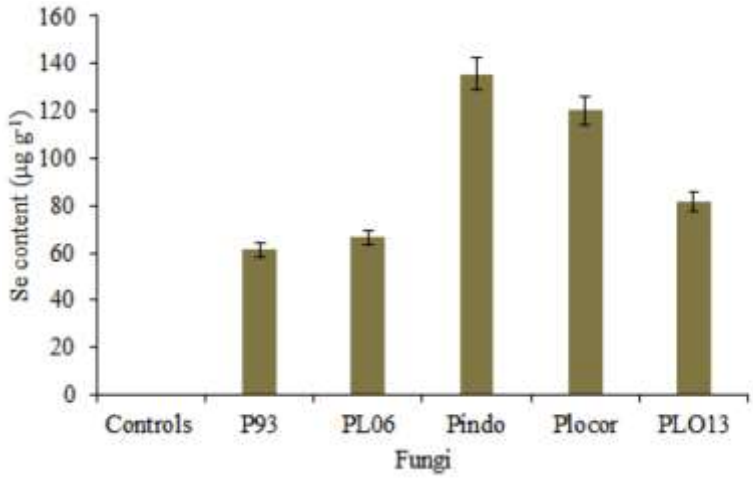


Figure 2. Selenium content (µg/g) absorbed by Pleurotus mushrooms: *P. ostreatus* (P93); *P. ostreatus*; (PL06); *P. pulmonarius* (Pindo); *P. cornucopiae* (Plocor) and P. djamor (PL013) cultivated on U decumbens enriched with Selenium (50mg/kg). Source: Ogidi, Nunes, Oyetayo, Akinyele and Kasuya 2017.

Moreover, the application of molecular farming that can help to modify mushrooms as carrier of known bioactive agent through recombinant DNA technology will also go a long way to enhance the use of mushrooms as safe and healthy super food that will provide all nutrients and also enhance health of consumers. The possibility of using mushroom food and nutraceuticals as means of solving mineral deficiency diseases has also been demonstrated. Mushrooms are known to accumulate a variety of phytoconstituents including carbohydrates, glycocides, phytosterols, phenols, tannins, flavonoids, alkaloids, terpenoids and saponins (Parihar et al., 2015). Edible and medicinal mushrooms have the ability to bioaccumulate minerals from the growth medium into fruiting body (Kalec 2009). Hence, the yield and chemical composition of mushrooms depend on the substrate used for their cultivation (Shashirekha et al., 2005). The bioaccumulation of essential elements such as Zinc, Iron, Selenium and several others has been reported (Milovanoic et al., 2013; Ogidi et al., 2017; 2018; Fasoranti et al., 2019). Essentially, effort should be geared towards using mushroom nutraceuticals in the amelioration of mineral deficiency syndrome. The bioaccumulation of selenium by *Pleurotus* species (Fig. 2) was reported by Ogidi et al. (2017).

CONCLUSION

Bioactive compounds present in mushrooms are important in maintaining sound health. The consumption of food laden with mushrooms can go a long way to help in ameliorating some common ailments such as high blood pressure, obesity, cancer, cardio vascular diseases and so on. Moreover, the market for edible and medicinal mushrooms has been growing over the years especially in Asia, Europe and North America. As at 2019, it was valued at U.S. $50 billion. This means this industry will employ more workers and more income will be generated from myconutraceuticals companies that are springing up. Mushrooms collected in Nigeria have enormous potential as sources of bioactive agents for biopharmaceutical exploitation. However, proper ecological survey, molecular identification and assessment of the medicinal potentials of these mushrooms still need to be done. This will ensure the full and sustainable exploitation of these indigenous mushrooms and the emergence of myconutraceutical industries which will help producing food that will promote health as well as in tackling the problem of unemployment and consequently reduce poverty.

REFERENCES

Abubakar, Z., Ogidi, C. O. & Oyetayo, V. O. (2016). Assessment of Antistaphylococcal Activity of Ethanolic Extract of *Lenzites quercina* on Clinical *Staphylococcus* species. *Clinical Phytoscience*, 2, 8.

Abraham, W. R. (2001). Bioactive Sesquiterpenes produced by fungi: are they useful for humans as well? *Current Medicinal Chemistry*, *8*, 583–606.

Adeyelu, A. T., Oyetayo, V. O. & Awala, S. I. (2016). Evaluation of the anti-candidal property of a wild macrofungus, *Trametes lactinea*, on clinically isolated Candida species. *Annals of Complementary and Alternative Medicine*, *1* (1), 4-8(96).

Adeyelu, A. T., Oyetayo, V. O., Onile, T. A. & Awala, S. I. (2017). Anticandidal Effect of Extracts of Wild Polypore, *Trametes elegans*, on *Candida* Species Isolated from Pregnant Women in Selected Hospitals in Southwest Nigeria. *Microbiology Research Journal International*, *20*(2), 1-10.

Awala, S. I. & Oyetayo, V. O. (2015a). *In Vitro* Antiradical Activities of *Trametes elegans* Collected from Osengere, Ibadan, Nigeria. *International Journal of Biological Chemistry*, *9* (5), 249-259.

Awala, S. I. & Oyetayo, V. O. (2015b). Phytochemicals and Antimicrobial properties of extracts obtained from *Trametes elegans* Collected from Osengere, Ibadan, Nigeria. *Jordan Journal of Biological Sciences*, Volume *8*(4), 289-299.

Ayodele, S. M. & Okhuoya, J. A. (2009). Nutritional and phytochemical evaluation of cultivated *Psathyrella atroumbonata* Pegler, a Nigerian edible mushroom. *South African Journal of Science*, *105*(3–4), 158–160.

Ayodele, S. M., Akpaja, E. O. & Adamu, Y. (2009). Some edible and medicinal mushrooms found in Igala land in Nigeria and their sociocultural and ethnomycological uses. *Proceeding of The 5th International Medicinal Mushroom Conference*, Nantong, China. Pp. 526 – 531.

Barros, L., Calhelha, R. C., Vaz, J. A., Ferreira, I. C. F. R., Baptista, P. & Estevinho, L. M. (2007). Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts. *European Food Research and Technology*, *225*, 151–56.

Beelman, R. B., Kalaras, M. D. & Richie, Jr. J. P. (2019). Micronutrients and Bioactive Compounds in Mushrooms: A Recipe for Healthy Aging? *Nutrition Today*. Volume *54*, Number 1, January/February 2019.

Beyer, D. M. (2003). Basic procedures for *Agaricus* mushroom growing. College of Agricultural Sciences, The Pennsylvania State University, University Park, PA.

Bishayee, A., Ahmed, S., Brankov, N. & Perloff, M. (2011). Triterpenoids as potential agents for the chemoprevention and therapy of breast cancer. *Frontier of Bioscience*, *16*, 980–996

Bohn, A. & Bemiller, J. A. (1995). 1-3 beta D glucans as biological response modifiers: a review of structure functional activity relationships. *Carbohydrate polymers, 28*, 3-14.

Chang, S. T. & Chiu, S. W. (1992). Mushroom production—An economic measure in maintenance of food security. In *Biotechnology: Economic and Social Aspects.* DaSilva, E. J., Ratledge, C., and Sasson, A., editors. New York: Cambridge University Press, pp. 110–141.

Chang, S. T. (1998). Development of novel agroscience industries based on bioconversion technology. In *Frontiers in Biology: The Challenges of Biodiversity.* Chou, C. H. and Shao, K. T., editors. Taipei: Academia Sinica, pp. 217–222.

Chang, S. & Miles, P. G. (2004). *Pleurotus*- A mushroom of broad adaptability in Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact (2nded.) CRC Press. Pp. 315-325.

Chang, S. T. (2008). Overview of mushroom cultivation and utilization as functional foods. In: Mushrooms as Functional Foods, Peter C. K. Cheung (ed) pp. 1-33.

Chikara, G. (1992). Immunopharmacology of lentinan, a polysaccharide isolated from *Lentinus edodes*: Its application as a host defense potentiator. *International Journal of Oriental Medicine, 17*, 55 – 77.

Crowell, P. L. (1999). Prevention and therapy of cancer by dietary monoterpenes. *Journal of Nutrition, 129*, 775S-8S.

Demain, A. L. (1999). Pharmaceutically active secondary metabolites of microorganisms. *Applied Microbiology and Biotechnology, 52*(4), 455–63.

Dubost, J. (2007). The mushrooming health benefits of fungi. *Food Technology, 17*, 61-68.

Duru, M. E. & Cayan, G. T. (2015). Biologically active terpenoids from mushroom original review, *Records of Natural Products, 9*, 456–483.

Ezeronye, O. U., Daba, A. S., Okwujiako, A. I. & Onumajuru, I. C. (2005). Antibacterial of crude polysaccharide extracts from sclerotium and fruitbody (sporophore) of *Pleurotus tuber-regium* (Fried) Singer on some clinical isolates. *International Journal of molecular medicine and advance sciences, 1*(3), 202 – 205.

Fagbohungbe, D. Y. & Oyetayo, V. O. (2014). Phytochemical and Antioxidant Properties of *Trametes* species Collected From Three Districts of Ondo State Nigeria. *Research and Reviews in Biosciences, 9*(10), 345-350.

Fasoranti, O. F., Ogidi, C. O. & Oyetayo, V. O. (2018). Phytochemical constituents and antimicrobial evaluation of ethanolic extracts from *Pleurotus* spp. cultivated on substrate fortified with selenium. *Microbial Biosystems, 3*(2), 29-39.

Fasoranti, O. F., Ogidi, C. O. & Oyetayo, V. O. (2019). Nutrient Contents and Antioxidant Properties of *Pleurotus* spp. Cultivated on Substrate Fortified with Selenium. *Current Research in Environmental and Applied Mycology, 9*(1), 66–76.

Ferreira, I. C. F. R., Baptista, P., Vilas-Boas, M. & Barros, L. (2007). Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: individual cap and stipe activity. *Food Chemistry*, 100, 1511–16.

Ferreira I. C. F. R., Barros, L. & Abreu, R. M. V. (2009). Antioxidants in Wild Mushrooms *Current Medicinal Chemistry*, *16*, 1543-1560.

Feng, W., Nagai, J. & Ikekawa, T. (2001). A clinical pilot study of EEM for advanced cancer treatment with EEM for improvement of cachexia and immune function compared with MPA. *Biotherapy*, *15*, 691-696.

Gaoxing, M., Wenjian, Y., Liyan, Z., Fei, P., Donglu, F. & Qiuhui, H. (2018). A critical review on the health promoting effects of mushroomsnutraceuticals. *Food Science and Human Wellness*, *7*, 125–133.

Hawksworth, D. L. (2001). The magnitude of fungal diversity: The 1.5 million species estimate revisited. *Mycological Research*, *105*, 1422–1432.

Hinneburg, I., Damien Dorman, H. J. & Hiltunen, R. (2006). Antioxidant activities of extracts from selected culinary herbs and spices. *Food Chemistry*, *97*, 122 – 129.

Ikekawa, T. (2000). On biological activity of mushrooms. *Biotherapy*, *14*, 945-951.

Jingjing, M. A., Zhengyi, F. U., Peiyan, M. A., Yanli, S. U. & Qingjie, Z. (2007) Breaking and characteristics of *Ganoderma lucidum* spores by high speed centrifugal shearing pulverizer. *Journal of Wuhan University Technolonogy*, *22*, 617-621.

Jonathan, S. G. & Fasidi, I. O. (2003). Antimicrobial activities of two Nigeria edible macro-fungi: *Lycoperdon pusilum* (Bat. Ex) and *Lycoperdon giganteum* (Pers.), *African Journal of Biomedical Research*, *6*, 85 - 90.

Kalec, P. (2009). Chemical composition and nutritional value of European species of wild growing and edible mushrooms. *Pakistan Journal of Botany*, *38*, 799-804

Kayode, Olakulehin., Adedeji, Ahmed., Aliyu and Badmosi, R. M. O., Olakulehin, T. F., Adedeji, B. S., Ahmed, O., Aliyu, T. H. & Badmos, A. H. A. (2015). Evaluation of amino acid and fatty acid profiles of commercially cultivated oyster mushroom (*Pleurotus sajor-caju*) grown on gmelina wood waste. *Nigerian Food Journal*, *33*, 18-21.

Kim, Y. J., Zitnan, D., Galizia, C. G., Cho, K. H. & Adams, M. E. (2006). A command chemical triggers an innate behavior by sequential activation of multiple peptidergic ensembles. *Current Biology*, *16*(13), 1395-1407.

Kim, M. Y., Seguin, P., Ahn, J. K., Kim, J. J., Chun, S. C., Kim, E. H., Seo, S. H., Kang, E. Y., Kim, S. L., Park, Y. J., Ro, H. M. & Chung, L. M. (2008). Phenolic compound concentration and antioxidant activities of edible and medicinal mushrooms from Korea. *Journal of Agriculture and Food Chemstry*, *56*, 7265-70.

Kozioł, A., Stryjewska, A., Librowski, T., Sałat, K., Gaweł, M., Moniczewski, A. & Lochyński, S. (2014). An Overview of the Pharmacological Properties and Potential

Applications of Natural Monoterpenes. *Mini-Reviews in Medicinal Chemistry*, *14*, 1156-1168.

Liby, K. T., Yore, M. M. & Sporn, M. B. (2007). Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer. *Nature Reviews Cancer*, *7*, 357–369.

Liu, F., Ooi, V. E. C. & Chang, S. T. (1997). Free radical scavenging activities of mushroom polysaccharide extracts. *Life Science*, *60*, 763–771.

Li, J. W. H. & Vederas, J. C. (2009). Drug discovery and natural products: end of an era or an endless frontier. *Science*, *325*, 161–165.

Liu, L. & Hu, C. (2010). Advance in fungal diterpene cyclase—a review, *Wei Sheng Wu Xue Bao*, *50*(11), 1438-45.

Lindequist, U. Niedermeyer, T. H. J. & Julich, W-D. (2005). The pharmacological potential of mushrooms-Review. *E CAM*, *2* (3), 285 – 299.

Lorenzen, K. & Anke T. (1998). Basidiomycetes as a source for new bioactive natural products. Current Organic Chemistry, *2*, 329-64.

Mdachi, S. J. M., Nkunya, M. H. H., Vitas, A., Nyigo, V. A. & Urasa I. T. (2004). Amino acid composition of some Tanzanian wild mushrooms. *Food Chemistry*, *86*, 179–182.

Milovanoic, I., Brcesk, I., Stajic, M., Knezevic, A. & Vukojevic, J. (2013). Potential enrichment of medicinal mushrooms with selenium to obtain new dietary supplement. *International Journal of Medicinal Mushrooms*., *15*(5), 449-455.

Mizuno, T. (1999). The extraction and development of antitumor active polysaccharides from medicinal mushrooms in Japan (Review). *International Journal of Medicinal mushrooms*, *1*, 9 – 30.

Mohammed, A., Adelaiye, A. B., Abubakar, M. S. & Abdurahman, E. M. (2007). Effects of aqueous extract of *Ganoderma lucidum* on blood glucose levels of normoglycemic and alloxan-induced diabetic wistar rats. *Journal of Medicinal Plant Research*, *12*, 034-037.

Ofodile, L. N., Simmons, S. J., Grayer, R. J. & Uma, N. U. (2008). Antimicrobial Activity of Two Species of the Genus *Trametes* Fr. (Aphyllophoromycetideae) from Nigeria. *Journal of Medicinal mushroom*, *10*(3), 265-268.

Oei, P. (1991). Manual on mushroom cultivation. Techniques and opportunity for commercial applications in developing countries, CTA 21–26.

Ogidi, C. O., Nunes, M. D., Oyetayo, V. O., Akinyele, B. J. & Kasuya, M. C. (2016). Mycelial Growth, Biomass Production and Iron Uptake by Mushrooms of *Pleurotus* species Cultivated on *Urochloa decumbens* (L.). *Journal of Food Research*, *5*(3), 13-19.

Ogidi, C. O., Oyetayo, V. O., Akinyele, B. J., Ogbole, O. O., Adeniji, A. & Bolaji, O. (2017). Molecular Identity and cytotoxicity of extracts of a Macrofungus; *Lenzites*

quercina toward Cancer Cell Lines. *Journal of biotechnology, computational biology and bionanotechnology*, Vol. *98*(1), 25-32.

Ogidi, C. O., Nunes, M. D., Oyetayo, V. O., Akinyele, B. J. & Kasuya, M. C. (2017). Growth Rate and Selenium Bioaccumulation in *Pleurotus* species Cultivated on Signal Grass, *Urochloa decumbens* (Stapf) R. D. Webster. *Current Research in Nutrition and Food Science*, Vol. *5*(2), 137-143.

Ogidi, C. O., Oyetayo, V. O. & Akinyele, B. J. (2018). Estimation of total phenol, flavonoid content and free radical scavenging activity of a wild macrofungus, *Lenzites quercina. Current Research in Environmental and Applied Mycology*, *8*(4), 425–437.

Ogidi, C. O., Oyetayo, V. O. Akinyele, B. J., De Carvalho, C. A. & Megumi Kasuya, M. C. (2018). Food Value and Safety Status of Raw (Unfermented) and Fermented Higher Basidiomycetes, *Lenzites quercina* (L) P. Karsten. *Preventive Nutrition and Food Science*, *23*(3), 228-234.

Oyetayo, F. L. (2006). Responses of plasma lipids to edible mushroom diets in albino rats. *African Journal of Biotechnology*, *5*(13), 1263 – 1266.

Oyetayo, F. L., Akindahunsi, A. A. & Oyetayo, V. O. (2007). Chemical profile and amino acids composition of *Pleurotus sajor caju*. *Nutr Health*, 2007, *18*, 383–389.

Oyetayo, V. O. (2009). Free radical scavenging and antimicrobial properties of extracts of wild mushrooms. *Brazilian Journal of Microbiology*, *40*, 380 - 386.

Oyetayo, V. O., Okuo, M. O. & Olatunji, E. K. (2010). Inhibitory potential of ethanolic extracts of two wild polyporales macrofungi on aerobic microbial isolates of meat. *Nigerian Journal of Microbiology*, *24*(1), 2033 – 2037.

Oyetayo V. O. (2011). Medicinal uses of mushrooms in Nigeria: Towards full and sustainable exploitation. *African Journal of Traditional Complementary Alternative Medicine*, *8* (3), 267-274.

Oyetayo, V. O. & Dauda, K. (2012). Antibacterial Properties of Extracts of C*oriolopsis* species against *Staphylococcus aureus* obtained from different clinics in South West Nigeria. *FUTA Journal of Research in Sciences*, *1*(1), 120-123.

Oyetayo, V. O., Nieto-Camacho, A., Baldomero, E. R. & Jimenez, M. (2012). Assessment of Anti-inflammatory, Lipid Peroxidation and Acute Toxicity of Extracts Obtained From Wild Macrofungi Collected From Akure Southwest Nigeria. *International Journal of Medicinal Mushrooms*, Vol. *14*(6), 573–578.

Oyetayo, V. O. & Ariyo, O. O. (2013). Antimicrobial and Antioxidant Properties of *Pleurotus ostreatus* Cultivated on Different Tropical Woody Substrates. *Journal of Waste Conversion Bioprocessing and Biotechnology*, *1*(2), 28-32.

Oyetayo, V. O., Nieto- Camacho, A., Ramırez-Apana, M. T., Baldomero, E. R. & Jimenez, M. (2013). Total Phenol, Antioxidant and Cytotoxic Properties of Wild Macrofungi Collected From Akure Southwest Nigeria. *Jordan Journal of Biological Sciences*, *6*(2), 5 – 10.

Oyetayo, V. O. & Oghumah, M. U. (2013). Antagonistic potential of *Coriolopsis* species extracts on *Escherichia coli* isolated from well water in Akure, Nigeria. *Research and Reviews in Biosciences*, *7*(8), 333 – 337.

Parihar, S., Virani, K. D., Pithawala, E. A., Shukla, M. D., Lahiri, S. K., Jain, N. K. & Modi, H. A. (2015). Phytochemical screening, total phenolic content, antimicrobial and antioxidant activity of wild edible mushroom, *Pleurotus ostreatus*. *International Research Journal of Pharmacy*., *6*(1), 65-69.

Proteins—What For? Where From? How Much? Documentation Center, Corporate Affairs Department, Nestec, Lausanne, 1987.

Reshetnikov, S. V., Wasser, S. P. & Tan, K. K. (2001). Higher Basidiomycetes as a source of antitumour and immunostimulating polysaccharides (Review). *International Journal of Medicinal Mushrooms*, *3*, 361-394.

Shah, B. A., Qazi, G. N. & Taneja, S. C. (2009). Boswellic acids: a group of medicinally important compounds. *Natural Product Reports*, *26*, 72–89.

Shashirekha, M. N., Rajarathnam, S. & Bano, Z. (2005). Effects of supplementing rice straw growth substrate with cotton seeds on the analytical characteristics of the mushroom *Pleurotus florida* (Block &Tsao). *Food Chemistry*, *92*, 255–259.

Sharon, N. & Lis, H. (1993). Carbohydrates in cell recognition. *Sc. Amer*., *268*, 82—89.

Sultana, N. & Ata, A. (2008). Oleanolic acid and related derivatives as medicinally important compounds. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *23*, 739–756.

Tsao, R. (2010). Chemistry and biochemistry of dietary polyphenols. *Nutrients*, *2*, 1231–1246.

Valverde, M. E., Hernández-Pérez, T. & Paredes-López, O. (2015). Edible mushrooms:improving human health and promoting quality life. *International Journal of Microbiology*, Article ID 376387 http://dx.doi.org/10.1155/2015/376387.

Veglioglu, Y. S., Mazza, G., Gao, L. & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agriculture and Food Chemistry*, *46*, 4113 - 4117.

Wasser. S. P. (2002). Medicinal mushrooms as a source of antitumour and immunostimulating polysaccharides. *Applied Microbiology and Biotechnology*, *60*, 258 – 274.

Wasser, S. P. & Weis, A. L. (1999). Therapeutic effects of substances occurring in higher basidiomycetes mushrooms a modern perspective. *Critical review of immunology*, *19* (1), 65-96.

Wuest, P. J. & Bengtson, G. D. (Eds.). (1982). Penn State Handbook for Commercial Mushroom Growers. College of Agricultural Sciences, The Pennsylvania State University, University Park, PA.

WHO (2018). WHO Bulletin on Cancer. http://www.who.int/news-room/fact-sheets/detail/cancer. 12th September 2018.

Zhang, R., Li, X. & Fadel, J. G. (2002). Oyster mushroom cultivation with rice and wheat straw. *Bioresource Technology*, *82*, 277–284.

Zaidman, B. Z., Yassin, M., Mahajna, J. & Wasser, S. P. (2005). Medicinal mushroom modulators of molecular targets as cancer therapeutics. *Applied Microbiology and Biotechnology*, *67*, 453–68.

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 10

ANCIENT AND RECENT METHODS OF CHARACTERIZATION OF FUNGI AND ITS BREAKTHROUGH IN THEIR IDENTIFICATION

Rajalakshmi Sridharan and Veenagayathri Krishnaswamy*
Department of Biotechnology, Stella Maris College (Autonomous), India

ABSTRACT

Fungi are a group of non-photosynthetic organisms that depends on the host for nutrition. It occupies a major place in the five-kingdom classification. Fungi are of various types, forms and function. The fungal kingdom also includes moulds, mushrooms, yeasts and some like slime moulds and oomycetes mimics to appear like fungi but they are not. The major sources of nutrition for fungi are the rotten organic matter or matter rich in moisture. The term Mycology holds a major aspect which includes fungal organisms, its sources, its life cycle, its beneficial and pathogenic effect, enzyme production, bioremediation, bio film formation, etc. The microscopic examination of fungi from various sources explains the structure, pathogenicity and provides information regarding the type of fungi and its function. The fungi present in the sources can also be studied using metagenomics approaches, which aids in the utilization of the fungal species. The fungi are also known for its ability to produce enzymes such as cellulase, pectinase, lipase, proteases, laccase, phytases, etc. which are highly required for remediation purposes and other applications. The fungal organisms also act as a substrate for immobilization for enhancing the activity of biomaterials, nanoparticles which are studied exclusively. Thus, the process of gaining knowledge about the fungal organisms, classification, their genetic makeup, enzyme production and other application are summarized under the chapter entitled "Mycology and its breakthrough in the environment".

* Corresponding Author's Email: veenagayathri2018@gmail.com.

Keywords: taxonomy, phylogeny, metagenomics, bioremediation, laccase, pectinase, cellulase

FUNGI - AN INTRODUCTION

Fungi are a group of microorganisms that are eukaryotic, non-chlorophilic, spore-producing, heterotrophic surrounded by a chitinous cell wall (Sharma, 1989). It is known that fungi are present on the earth for over 550 million years. Fungi are found in soil, water, plants, animals (commonly in moist places). They are both beneficial and harmful. They cause diseases in plants, animals and even in human beings. They are also called decomposers as they grow on the dead organic matters, nourish and develop by feeding by absorption. There are about 144000 species of fungi identified and classified under the kingdom fungi. The study of fungi is called mycology and it has its origin in botany. At the early period of classification, fungi were classified under plants and later due to its unique characteristics it was classified into a separate kingdom. The general structure of fungi consists of thread-like structures called hyphae which are collectively called mycelium. The fungi under favourable conditions produce fruiting bodies which produce spores (a reproductive form of fungi). The hyphae are made of chitinous cell wall which is divided into partitions called the septum. The plasma membrane is a phospholipid bilayer which plays a major role in the transportation of organic substances. The fungi lacking septum are called coenocytic fungi. The fungi reproduce by both sexual (diploid) and asexual method (haploid). The sexual method of the reproduction will lead to the evolution of fungal characteristics whereas asexual reproduction does not help in evolution which occurs often. The identification of mode of fungal reproduction is difficult except for the fungi such as mushrooms which contain fruiting bodies which are the most required reproductive organ. In other fungi, vegetative reproduction occurs using hyphae (Jennifer Viegas and Margaux Baum, 2017). Table 1 shows the significance of each organelle.

The growth of fungi is aided by the addition of glucose, inorganic ions, salts and other growth components. The nutrients supplements required for the fungal growth are classified into macro and micronutrients. The elements such as C, N, O, S, P, K and Mg constitute Macronutrients while Ca, Cu, Fe, Zn and Mn constitutes micronutrients for enrichment. The production of enzymes requires specific elements to stimulate and carryout the metabolic pathway. The elements such as vitamins, proteins, fatty acids play a vital role in the growth and enzyme production. The fungi are cultured in the laboratory using media such as Potato dextrose agar, Sabroud dextrose agar and malt extract. The substances such as corn steep liquor, molasses and malt broth are added as supplementation to the culture media for the production of agricultural benefits. The permeability of the food, ions and other elements in and out of fungi are interrupted by

the presence of capsule, cell membrane and periplasm. The porous structure of the fungal cell wall is permeable for molecules of 300 Da and does not permit molecules greater than 700 Da (Kevin Kavanagh, 2005). The mode of nutrient transport in and out of the cell of fungi was summarized in Table 2.

Table 1. Functional significance of organelles (Kevin Kavanagh., 2005)

S. No	Organelle or cellular structure	Function
1.	Cell envelope	Plasma membrane -selectively permeable barrier for transport of hydrophilic molecules in and out of fungal cells; Periplasm-proteins and enzymes unable to permeate the cell wall; Cell wall - protection and shape and is involved in cell–cell interactions, signal reception and specialized enzyme activities; Fimbriae-sexual conjugation; capsules to protect cells from dehydration and immune cell attack
2.	Nucleus	Relatively small, contains chromosomes (DNA–protein complexes), which pass genetic information to daughter cells at cell division, Nucleolus-site of ribosomal RNA transcription and processing
3.	Mitochondria	Site of respiratory metabolism under aerobic conditions and, under anaerobic conditions, for fatty acid, sterol and amino-acid metabolism
4.	Endoplasmic reticulum	Ribosomes on the rough ER are the sites of protein biosynthesis
5.	Proteasome	Multi-subunit protease complexes involved in regulating protein turnover
6.	Golgi apparatus and vesicles	Secretory system for import (endocytosis) and export (exocytosis) of proteins
7.	Vacuole	Intracellular reservoir (amino acids, polyphosphate, metal ions); proteolysis; protein trafficking; control of cellular pH. In filamentous fungi, tubular vacuoles transport materials bi-directionally along hyphae
8.	Peroxisome	Oxidative utilization of specific carbon and nitrogen sources (contain catalase, oxidases). Glyoxysomes contain enzymes of the glyoxylate cycle

Table 2. Types of transport of nutrients in fungi (Kevin Kavanagh., 2005)

Type of nutrient transport	Description
Free diffusion	Passive penetration of lipid-soluble solutes through the plasma membrane from a high extracellular concentration to a lower intracellular concentration
Facilitated diffusion	Translocate solutes down a transmembrane concentration gradient in an enzyme- (permease) mediated manner. As with passive diffusion, nutrient translocation continues until the intracellular concentrations equal to that of the extracellular medium
Diffusion channels	These operate as voltage-dependent ‘gates’ to transiently move certain nutrient ions down concentrationgradients. They are normally closed atthe negative membrane potential of resting yeast cells but open when the membrane potential becomes positive
Active transport	The driving force is the membrane potential and the transmembrane electrochemical proton gradient generated by the plasma membrane eH+-ATPase. The latter extrudes protons using the free energy of ATP hydrolysis that enables nutrients to either enter with influxed protons, as in ‘symport’ mechanisms, or against effluxed protons, as in ‘antiport’ mechanisms

BIOSYNTHETIC PATHWAYS

The fungal organisms are known for the synthesis of secondary metabolites that have its wide range of application as anti-cancerous, antimicrobial and antifungal products (Selim et al., 2012, Jae-Hyuk Yuand Nancy Keller, 2005). The metabolites (toxin) produced by the pathogenic fungi are called mycotoxins (Meinwald, 2011). The classification of secondary metabolites is based on the pathways involving the enzymes for the synthesis of secondary metabolites. The MVA (mevalonic acid) or MEP (methylerythritol phosphate) pathway which is majorly involved the synthesis of terpenoids. The terpenoids are made of blocks of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) isomer. The animals and fungal organism are only involved in the MEP/MVA pathway while the bacteria and plants utilise the products of the pathway as shown in Figure 1 (Grawert et al., 2011, David Hansson, 2013).

The plants and microorganisms are also involved in the synthesis of aromatic secondary metabolites such as L-phenylalanine, L-tyrosine and L-tryptophan following the shikimic acid pathway. The formation of certain aromatic compounds involves the formation of Chorismic acid as an intermediate which finally results in the formation of Phenylalanine by Claisen rearrangement reaction, decarboxylation, loss of hydroxyl ion and aromatisation of the intermediates as in Figure 2 and Figure 3 (Hansson, 2013).

The polyketides, important secondary metabolite derivates produced by fungi possessing biological activities. The condensation of acetyl-CoA and malonyl-CoA results in the formation of poly-ù-keto chains. The synthesis of the polyketides follows Acetate pathway (Hansson, 2013). Figure 4 explains the synthesis of polyketides. The common aromatic compounds synthesised by the microorganisms are carbohydrates, amino acids, alkaloids and fatty acids are tabulated in Table 3.

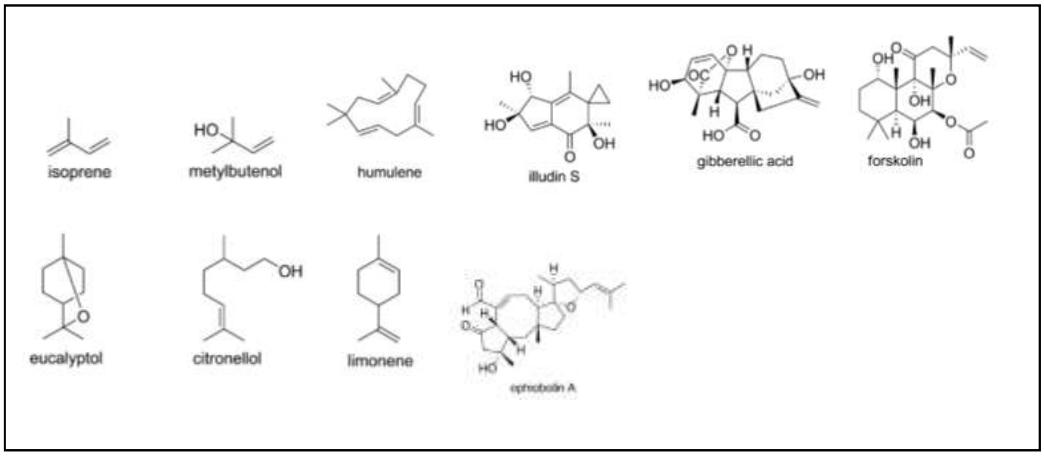


Figure 1. Example of terpenoids (David Hansson., 2013).

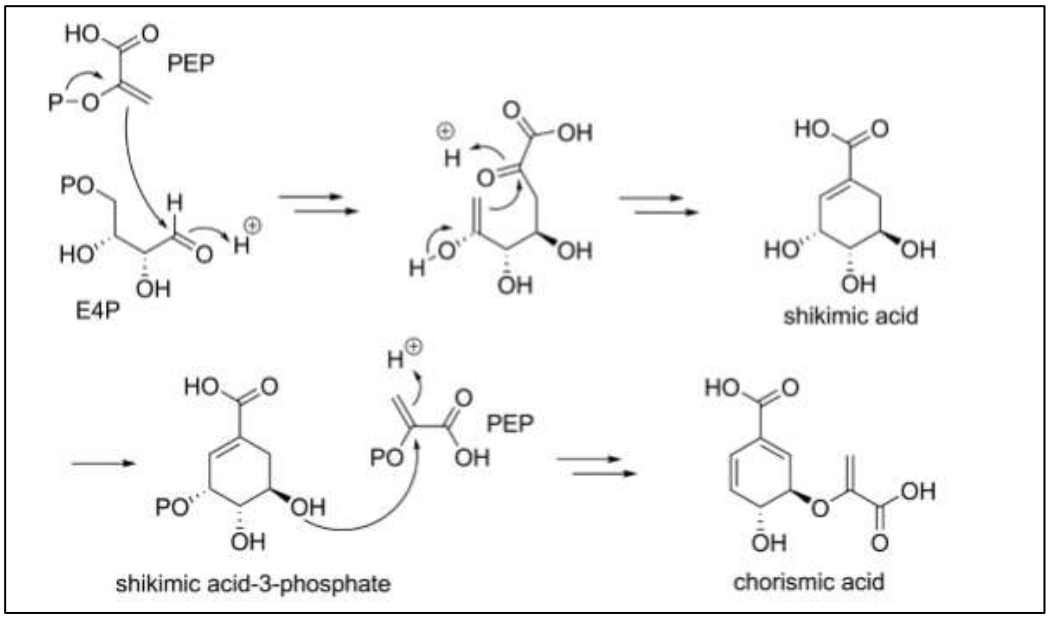


Figure 2. Shikimic acid pathway - Chorismic acid formation (David Hansson., 2013).

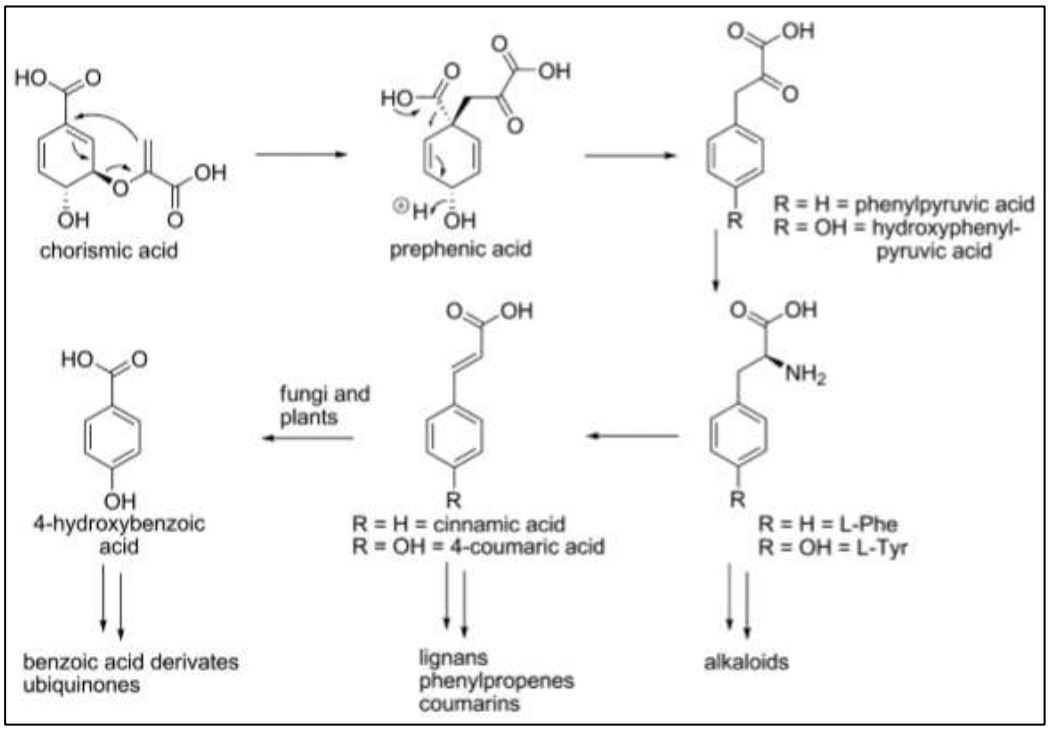


Figure 3. Chorismic acid in Formation of Carbon derivates (David Hansson., 2013).

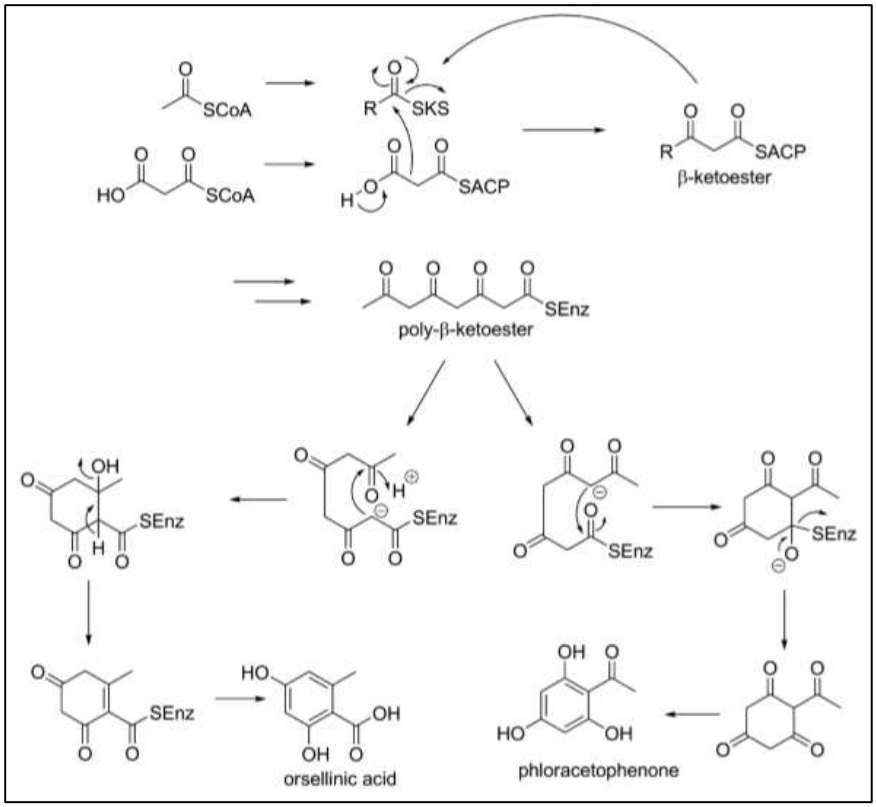


Figure 4. Synthesis of Polyketides (David Hansson., 2013).

Table 3. Aromatic compounds and its Pathways

Aromatic compounds produced	Pathway	Reference
Alkaloids	MAV/MEP	Dewick, 2009
Carbohydrates	Shikimic Acid pathway and Acetate Pathway	Bury et al., 2013
Fatty acids	Acetate Pathway	Clericuzio et al., 2004
Peptides/Proteins	Acetate Pathway	Dewick, 2009

Need for Fungal Classification?

Fungi, one of the largest group of microorganisms present in the world has its role in the biogeochemical cycle. These organisms exist mutualistic, pathogenic and saprophytic behaviour (McLaughlin and Spatafora, 2014). Because many fungi are unculturable and seldom produce visible sexual structures, molecular techniques have become widely used for taxonomic detection of species to understand shifts in their richness and composition along environmental gradients (Peršoh 2015, Balint et al., 2016, Tedersoo and Nilsson,

2016). Accurate taxonomic identification to species, genera and higher taxonomic levels is a key for reliable assignment of ecological and functional traits to taxa for further ecophysiological and biodiversity analyses (Kõljalg et al., 2013, Jeewon and Hyde, 2016, Nguyen et al., 2016, Edgar 2017, Tedersoo and Smith, 2017).

The fungal organisms comprise a major population of microorganisms with different life cycles, morphology and growing patterns. Fungal species richness is estimated to be anywhere between 1.5 and 7.1 million species, with the list of living species continually expanding as new fungal species are identified from around the world (Blackwell, 2011, Hawksworth, 2001, Hibbett et al., 2007, Tedersoo and Smith, 2017). While incredible, this staggering richness has provided a monumental challenge to fungal systematics and classification (Hibbett and Taylor, 2013, Wang et al., 2016). The simple morphology of many fungi and lack of a detailed fossil record have caused difficulties in fungal classification and systematics (Bard, 2008, Botstein, 1997, Davis, 2000, Hibbett et al., 2007, Nicholas Money, 2016, Stajich et al., 2009). While the simple and easily manipulated bauplan of many fungi has been highly advantageous for studies of developmental or cell biology, fungal phylogenetics and subsequent phylogenomic initiatives have made tremendous strides toward a robust fungal Tree of Life. Thus, this creates a need to classify the fungal organisms into phylum, class, order, family, genus and species.

It was studied that around 1.5 million species of fungal organisms exist around which only 5% were classified. Because many fungi are unculturable and seldom produce visible sexual structures, molecular techniques have become widely used for taxonomic detection of species to understand shifts in their richness and composition along environmental gradients (Peršoh 2015, Balint et al., 2016, Tedersoo and Nilsson, 2016). Accurate taxonomic identification to species, genera and higher taxonomic levels is a key for reliable assignment of ecological and functional traits to taxa for further ecophysiological and biodiversity analyses (Kõljalg et al., 2013, Jeewon and Hyde, 2016, Nguyen et al., 2016, Edgar, 2017, Tedersoo and Smith, 2017). The Kingdom Fungi represents an extraordinary amount of eukaryotic morphological and ecological variation (Stajich et al., 2009). Fungal species richness is estimated to be anywhere between 1.5 and 7.1 million species, with the list of living species continually expanding as new fungal species are identified from around the world (Blackwell, 2011, Hawksworth, 2001, Hibbett, 2007, Rachel Martin et al., 2015, Schoch et al., 2012, Leho Tedersoo et al., 2017). While incredible, this staggering richness has provided a monumental challenge to fungal systematics and classification (Hibbett and Taylor, 2013, Wang et al., 2016). The simple morphology of many fungi and lack of a detailed fossil record have caused difficulties in fungal classification and systematics (Bard, 2008, Botstein, 1997, Davis, 2000, Hibbett et al., 2007, Francois Lutzoni et al., 2004, Nicholas Money, 2016, Stajich et al., 2009). While the simple and easily manipulated bauplan of many fungi has been highly advantageous for studies of developmental or cell biology, fungal

phylogenetics and subsequent phylogenomic initiatives have made tremendous strides toward a robust fungal Tree of Life.

The basic classification of fungi was given in the Figure (5 (a), (b)) which was the earlier classification of fungi based on the morphology and reproduction. The Figure (6) shows the Classification of fungi based on Alexopolous and Mims, 1979. The fungal classification is done based on the phenotype, but other characteristics are unstable (temperature, pH, enzyme production, etc.). Nylander, 1866, Rolland, 1887, suggested classification of fungi based on the colour reactions using chemical stains. This method was used to classify lichens and ascomycetes. Later, the biochemical and physiological methods (growth, nutrition, temperature, etc.) were used for systemic classification of fungi. The interrelationship between the fungal organisms was identified using the secondary metabolites, fatty acids, cell wall components and proteins (Ning Jhang et al., 2017).

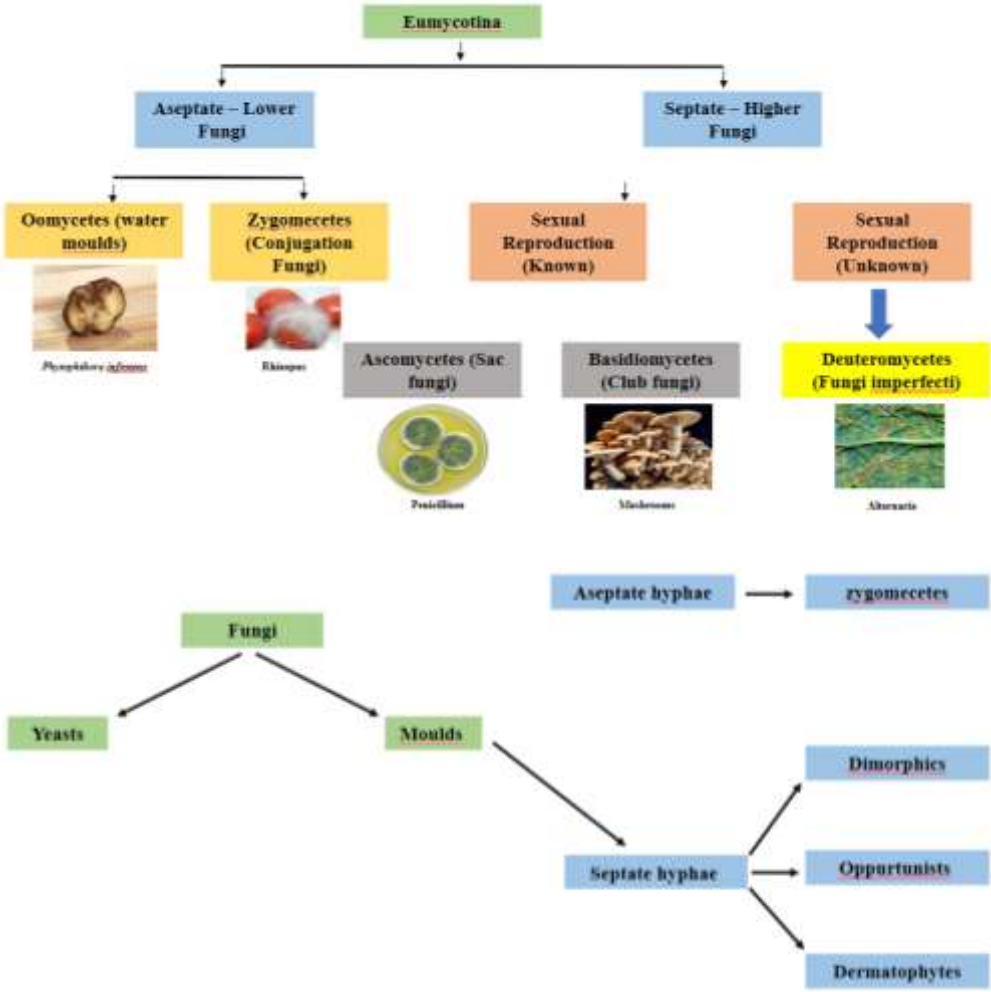


Figure 5. Basic classification fungi.

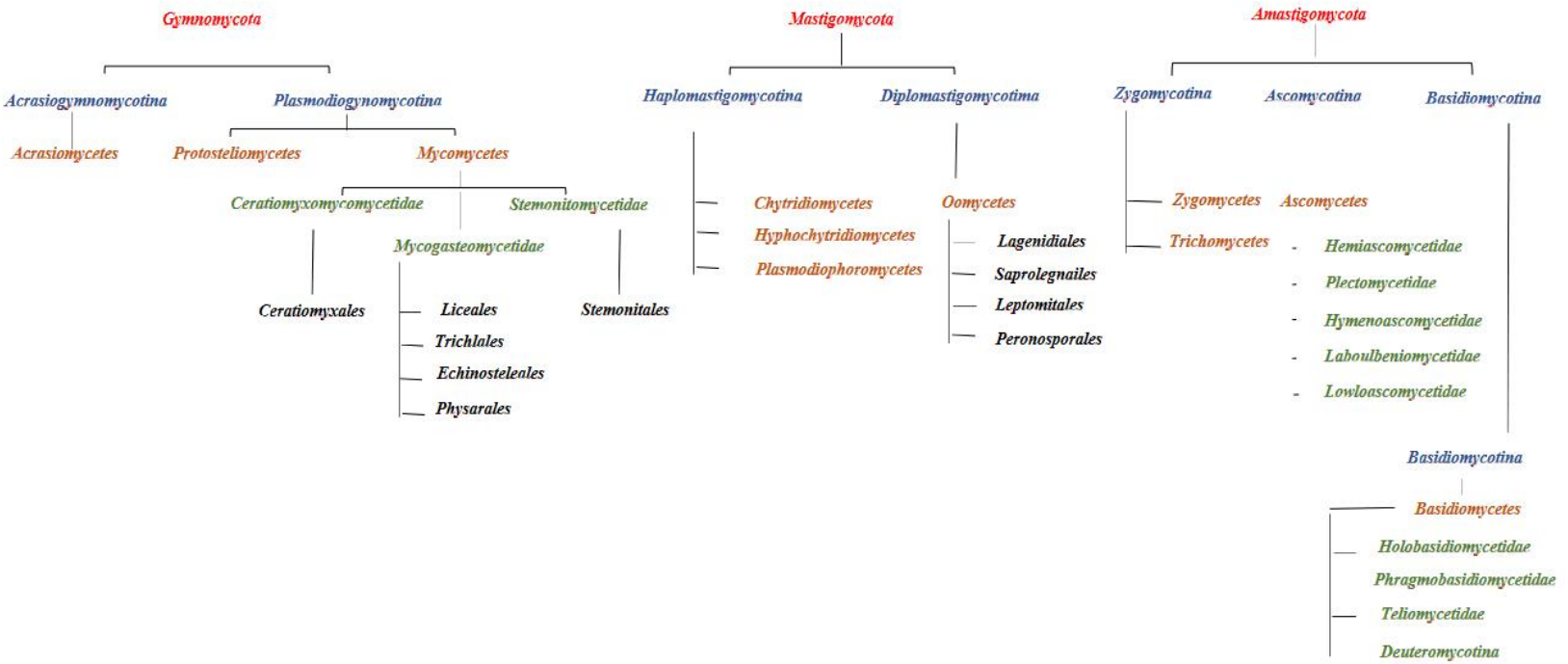


Figure 6. Classification of fungi (Alexopolous and Mims, 1979).

In earlier days, the classification was based on the morphological (microscopic and macroscopic). Due to an increase in the pathogenic fungi and development of new and evolved fungal species, it was difficult to identify and classify based on the traditional method. This gave rise to the molecular technique in the 1990s for the identification and classification of fungal organisms. The first molecular method used was PCR using amplified RNA genes. The sequences of 18s rRNA were used to phylogenetically classify the fungi based on the relationship with other living organisms. The molecular techniques used for the identification of fungal and other microorganisms include

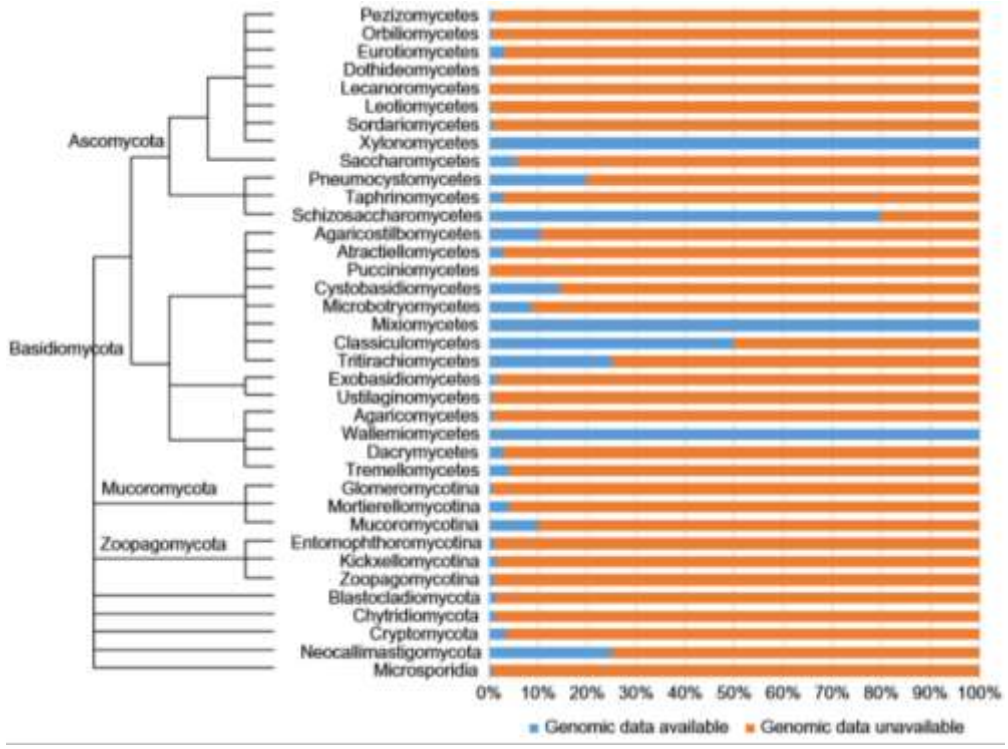
- **PCR**
  - **Conventional PCR** – This method is used to synthesis specific part of DNA using standard PCR protocol which is mainly used in the detection of bacterial and viral diseases (Fang and Ramasamy, 2015).
  - **Nested PCR** – In this method, a single pair of primer is used to amplify the sequences of DNA (larger sequences) which is used as a target for using two internal primers. This method prosses a high risk of contamination as the process of amplification and the target occurs in separate tubes. This method is observed to be most sensitive with high specificity (Rahman et al., 2013).
  - **Multiplex PCR** – This method is used to amplify different regions of DNA simultaneously using multiple pairs of primers which is time and cost-effective process. The pathogenic fungal identification is carried out using the Padlock Probe (PLPs) for amplification of sequences (Dasmahapatra and Mallet 2006, Cho et al., 2016).
  - **Real time PCR (RT PCR)** - This PCR method is used to identify the viable cells as mRNA of the dead cells gets degraded. The mRNA was then reverse transcribed to cDNA and amplified using standard PCR method. This method is mainly used for the identification of infection-causing viruses (Capote et al., 2012).
  - **Real Time PCR (q PCR)** - This method is used to quantify the amplifying DNA using dye called SYBR Green I or sequence-specific fluorescent-labelled probes which shows the increase in signal during amplification (Badali and Nabili, 2012).
  - **Serial analysis of gene expression (SAGE)** - this method is a quantitative gene expression profiling technique in which base pairs long than 15bp length can be quantified. It forms concatemers as the primers are removed (Velculescu et al., 1995, Dawei and Peng, 2014).
  - **DNA Barcoding** - This method is a diagnostic process in which small sequences are used in the identification of the species of the organisms using 500 –800 bp using markers with wide taxonomic range (Krishnamurthy and Francis, 2012).

- **DNA/RNA PROBE method**
  - Northern Blotting
  - In situhybridisation
  - Fluorescence *in Situ* Hybridization (FISH)
- **Post amplification method**
  - Microarray
  - Macroarray
- **Isothermal amplification-based method**
  - Rolling Circle Amplification (RCA)
  - Loop-Mediated Isothermal Amplification (LAMP)
  - Nucleic Acid Sequence Based Amplification (NASBA)
- **Next-Generation Sequencing** (Sidra Aslam et al., 2017)

The development of new techniques in understanding the phylogeny of the fungal species. But these new techniques are useful for culturable fungi rather than non-culturable species. The drawback of this method is that it is difficult or unable to sequence the fungi if contaminated (Ning Zhang et al., 2017). The Figure (7 (a)) shows the details of identified and non-identified genome and Figure 7 (b) explains the percentage of identified fungal genomes at the taxonomic level.

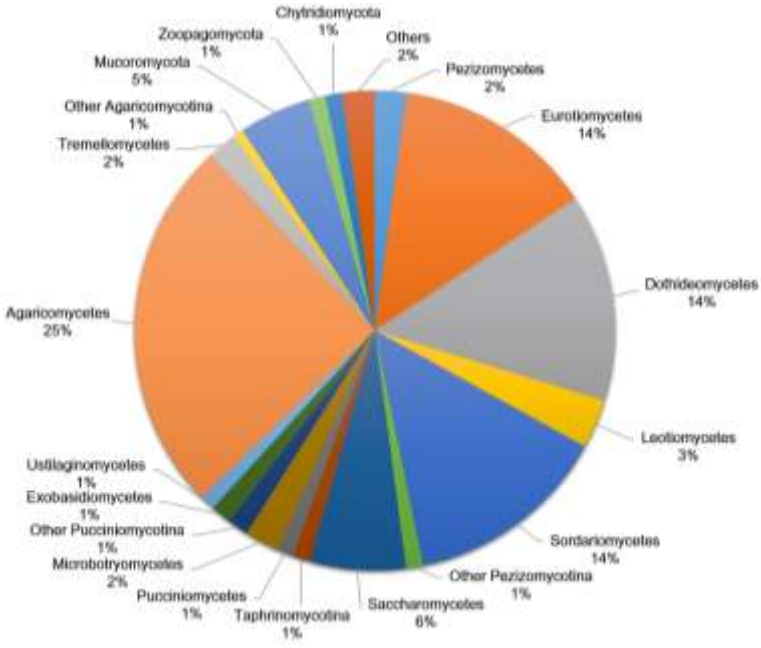
Metagenomic Analysis – Fungi Identification

The method of identification at the genomic level without culturing microorganisms using omics protocol. This process enables to identify the diversity of microorganisms in complex samples and complex places. The system 454 GS FLX is commonly used in the identification of pathogenic microbial strains. The process to ease the culturing and identification of fungal species, Next Generation Sequencing (NGS) was assessed and explored. The identification of microorganisms is performed using the conserved rRNA gene sequences. The target 16S rRNA are amplified by combining it with a binding site of primer and this makes easier identification of microorganisms. The fungal species are identified using pyrosequencing in which 18S rRNA gene sequences are used for fungal typing. The highly conserved 18S, 5.8S, and 28S ribosomal genes in the rRNA gene complex, external transcribed spacer regions 1 and 2 and internal transcribed spacer (ITS1 and 2) are used for identification of fungi (Gelsomino et al., 2011).



(a)

Figure 7.(a) Details of identified and non-identified genome.



(b)

Figure 7. (b)The percentage of identified fungal genomes at the taxonomic level.

DNA Pyrosequencing

This method is also known as sequencing by synthesis by nucleotides addition to the primed template and the sequence of the new DNA strand was identified by incorporating various nucleotides complementary to the template DNA. The nucleotides are added to the primer using DNA polymerase and the process utilises enzymes for detection of inorganic pyrophosphate (PPi) produced. Thus, the ATP sulfurylase converts PPi to ATP. This provides energy for the generation of oxyluciferin from luciferin using lusiferase enzyme (Ronaghi and Elahi, 2002).

There are two sequence-based approaches for the identification of fungal species, PCR Amplification and Short gun method.

PCR Amplification Sequencing

The commonly used DNA markers in the metagenomic analysis are internal transcribed spacer1 (ITS1) region in the rRNA cistron (Illumina, 2018). The workflow of fungal metagenomics is shown in Figure 8 and 9.

The Metagenomics protocol involves two subsequent steps.

1. Modification of known primer pair of ITS1 region and analysis of modified primers
2. The genomic DNA templates are amplified using the modified forward and reverse primers that are complementary to the upstream and downstream sequences of ITS1 region (with overhangs as adapters).

Short Gun Sequencing

The short gun method uses tools namely BLAST, USEARCH and UBLAST, GhostX, and DIAMOND (Altschul et al., 1990, Paulet al., 2018). The similar sequences in the metagenome are read by these tools while the lowest common ancestor (LCA) was read using algorithms such as KAIJU and Kraken. The pipelines are constructed for metagenome analysis such that it reads all the DNA sequences and omits the non-DNA sequences. Paul et al., (2018) constructed a metagenomic pipeline without the use of rDNA amplicon.

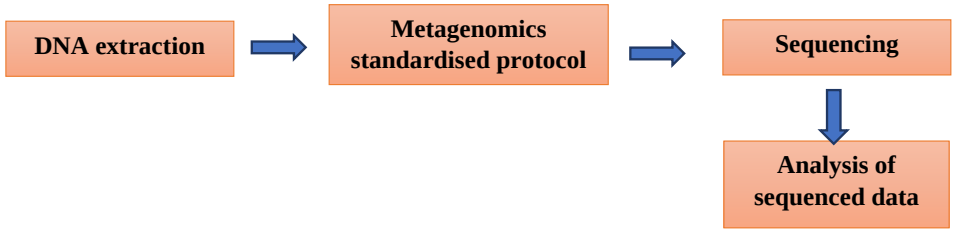


Figure 8. Work flow of fungal metagenomics.

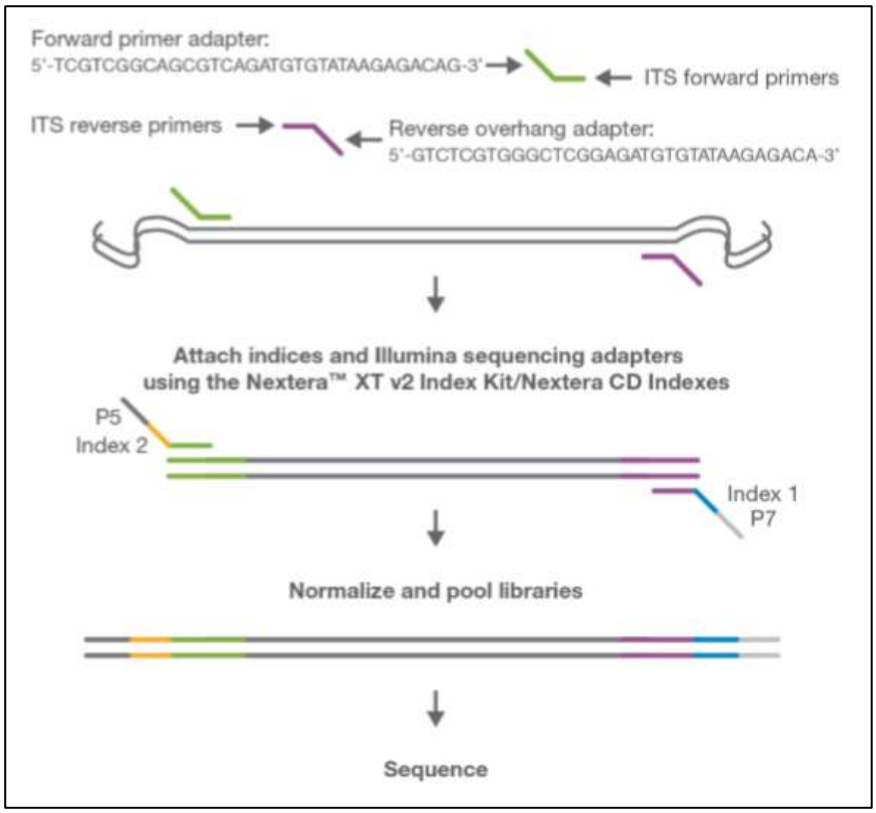


Figure 9. ITS1 Amplicon (Illumina., 2018).

Identification of Pathogenic Fungi

The process of Next Generation Sequencing (NGS) allows detection of pathogens which has led to the identification of novel and undiscovered pathogens. Thus, the identification of pathogen aids in quicker and easier response to the public which improves the personalised and targeted treatment. The detection of the pathogen causing disease may not be exactly identified but it differentiates the infection or diseases caused by fungi, bacteria or virus. This method clinically helps in determining the host-expression response profiling (Steven et al., 2016).

The pathogenicity of various isolates of *Aspergillus flavus* was identified by Mchl and Cotty, (2010). The *Aspergillus flavus* is a well-known food contaminating fungi as it produces mycotoxin named aflatoxin. The *Aspergillus flavus* group of fungi are divided into L, S and T strains which are differentiated by their morphology of sclerotia and other characteristics. The groups are further subdivided into vegetative compatibility groups (VCGs) using heterokaryon incompatibility system. *Candida* isolates clinical (n = 51) and commercial (n = 9) identified by fungal culturing and biochemical properties which were compared with DNA pyrosequencing. More than 60 *Candida* species such *as C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. guilliermondii*, *C. krusei*, *C. lusitaniae*, *C. parapsilosis*, and *C. Tropicalis* using DNA pyrosequencing (Boyanton et al., 2008).

The databases available helps in identification of fungal organisms as shown in Figure 10 with the gene sequences, polyphasic characters and other metadata that are already obtained. The databases have to be accessed as a first step followed by searching particular research. Then, performing search and data retrieval for utilising the available databases. Online fungal databases such as *Aspergillus* Genome Database (AspGD), *Aspergillus* and Aspergillosis Website, BOLD, Broad Institute databases, CBS-KNAW, *Candida* Genome Database (CGD), Doctor Fungus, FungiDB, *Fusarium* Database, *Fusarium* MLST (MLST, multilocus sequence typing), Index Fungorum, Institut Pasteur-Fungi Bank, International Society for Human and Animal Mycology-Internal Transcribed Spacer (ISHAM-ITS), ISHAM-MLST, Mycology Online, MycoBank, NCBI GenBank, NCBI RefSeq, and UNITE. All these databases provide information on nomenclature, taxonomical information, identification and genotyping of pathogenic fungal organisms. The method of DNA barcoding helps in the identification of fungi at species level which constitute 500-800 bp conserved regions which are responsible for species-specific diversity. Other molecular approaches namely MLST (Multi Locus Sequence Typing) aides in the identification of epidemic fungal organisms by utilising four or more gene loci as shown in Figure 10 (Peralam et al., 2017).

The other databases used in the identification of pathogenic fungi are

1. Clinical, biochemical, morphology, and taxonomy-based databases
2. Gene sequence-based databases
3. Fungal strain genotyping databases
4. Genome-based databases (Peralam et al., 2017).

The species of fungi have been classified based on various concepts of mycologists such as

- The phenetic or phylogenetic concept – based on morphological characteristics
- The polythetic concept – a combination of characteristics (may not be present in all fungi)

- The ecological concept - based on the adaptation of fungi to a specific habitat
- The biological concept - based on the modern analysis (molecular method) (Abisheck Katoch and Pooja Kapoor, 2014)

The phylogenetic relationship between the fungal organisms is yet to be explored as there many evolved fungal genomes that will pave way for finding the accuracy of relationships. In 1996, the full genome of *Saccharomyces cerevisiae* was reported which was followed by *Schizosaccharomyces pombe*, *Neurospora crassa* (Goffeau et al., 1996, Wood et al., 2002, Galagan et al.,2003*).* The analysis of the fungal genome using the technique named Next Generation Sequencing (NGS) which involves various sequencing methods such as Roche 454, AB SOLiD, Illumina GA/HiSeq System, and PacBio RS. This method performs simultaneous analysis with high throughput technique in a lesser period (Goodwin et al., 2016, Grigoriev et al., 2011). This newly developed method helped in identifying 14% of *Eurotiomycetes*, *Dothideomycetes and Sordariomycete*s, 25% of *Agariocomycetes* (http://genome.jgi.doe.gov/programs/fungi/index.jsf, Ning Zhang et al., 2017).

The phylogenetic analysis construction helps in understanding the fungal evolution. In early days, the phylogenetic analysis was performed based on the morphology, biochemical characteristics (Alexopoulos, 1962), SSU rDNA (Tehler et al., 2000).

Mycoremediation

The process of remediation of xenobiotics using fungal organisms is termed as mycoremediation. The commonly occurring degradation process in the environment such as degradation of wood, textiles, leather and other materials are degraded by fungi (Harbhajan Singh, 2006). These organisms use the pollutants in the environment as a sole carbon source which nourishes the fungal organisms and transform the pollutants into a soluble form (Krishna Kumar et al., 2008). The biomass of fungi is used as a biosorption of pollutants, which is a cost-effective process (Maurya et al., 2006). Fungi are organisms which can oxidise wide of pollutants due to their tolerance to survive in a highly polluted environment. The process of mycoremediation was first observed in the white-rot fungi *Phanerochaetechrysosporium* and *Trametesversicolor* in mid of the 1980s as they degraded a wide range of xenobiotic pollutants in the environment (Canet et al., 2001, Trejo-Hernandez et al., 2001, Ghani et al., 1996). The fungi are known for its production of various enzymes which plays a key role in the removal of pollutants (Evans et al., 2001). The application of mycoremediation in the environment is shown in Figure 11.

Hutchinson, (1957), developed a Hutchinson niche concept describing the survival of fungal organisms in a highly polluted environment is the major reason for the evolution of fungal populations. The biodiversity of fungi is a key role which provides insurance

for the fungal population to survive under stress of multiple pollutants. The insurance hypothesis states that the biodiversity of fungal organisms is the backbone in the persistence of fungal population in spite of its redundant taxonomy (Loreau, 2000). The process of mycoremediation occurs commonly via co-metabolism process while some fungi represent a specific metabolic pathway in xenobiotic degradation by assimilation of pollutants (Harms et al., 2011). The coexistence of bacteria and fungi in degradation and detoxification of the xenobiotics claims to be the most potent microorganisms. The nature of the recalcitrant compounds, in turn, alters the metabolism, reproduction, spore production and its degradation ability (Ceci et al., 2019). Figure 12 below describes the interaction of microorganisms with the recalcitrant compounds.

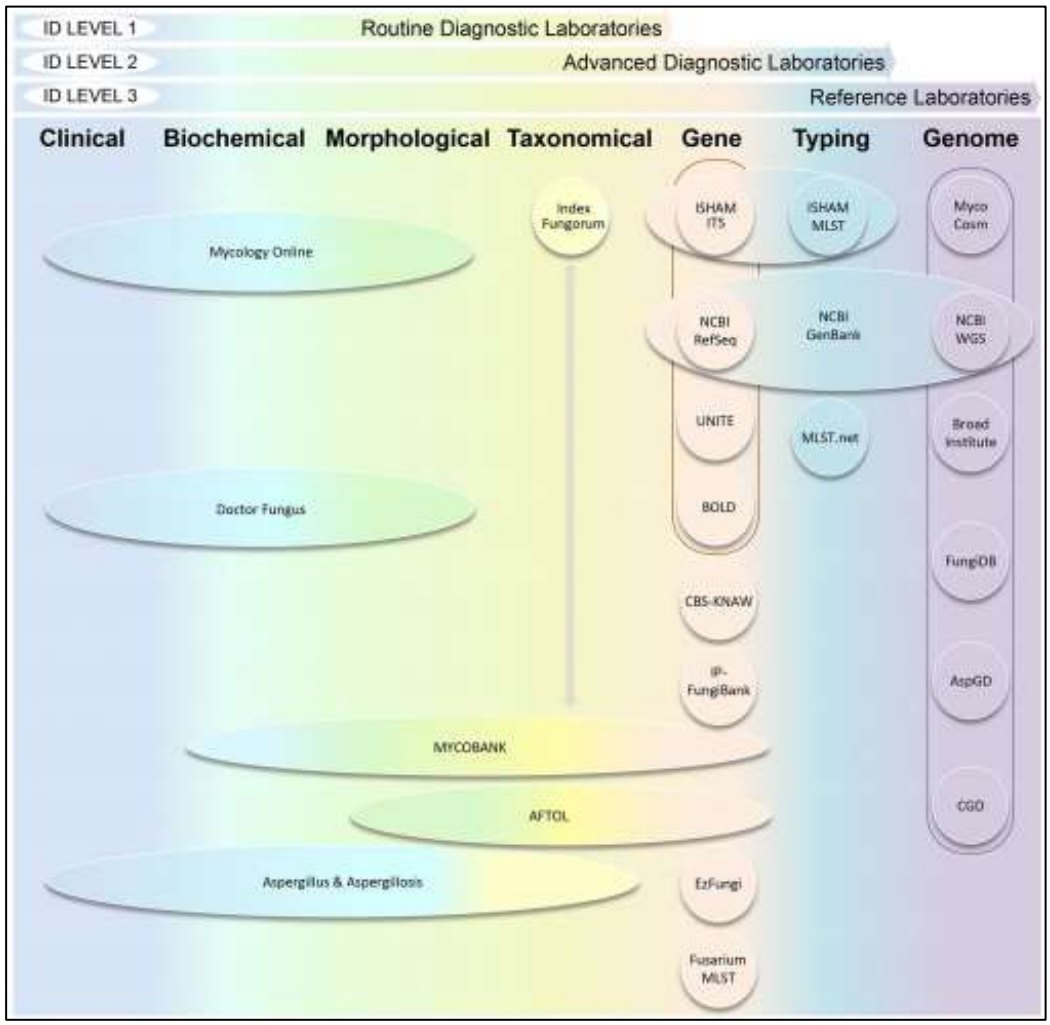


Figure 10. Illustration of online databases for pathogenic fungi (Peralam Yegneswaran Prakash et al., 2017).

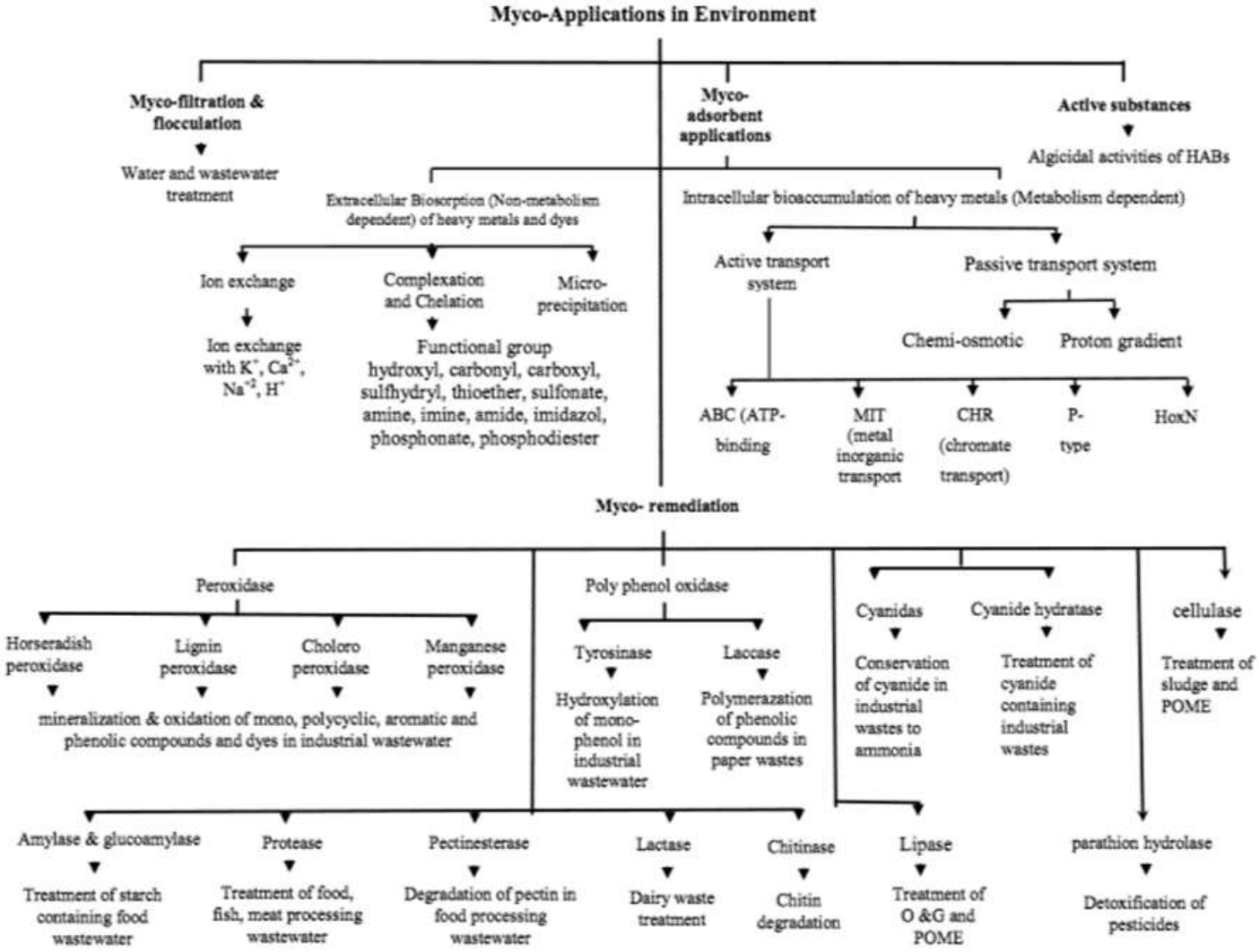


Figure 11. The application of mycoremediation in the environment. (Noman et al., 2019).

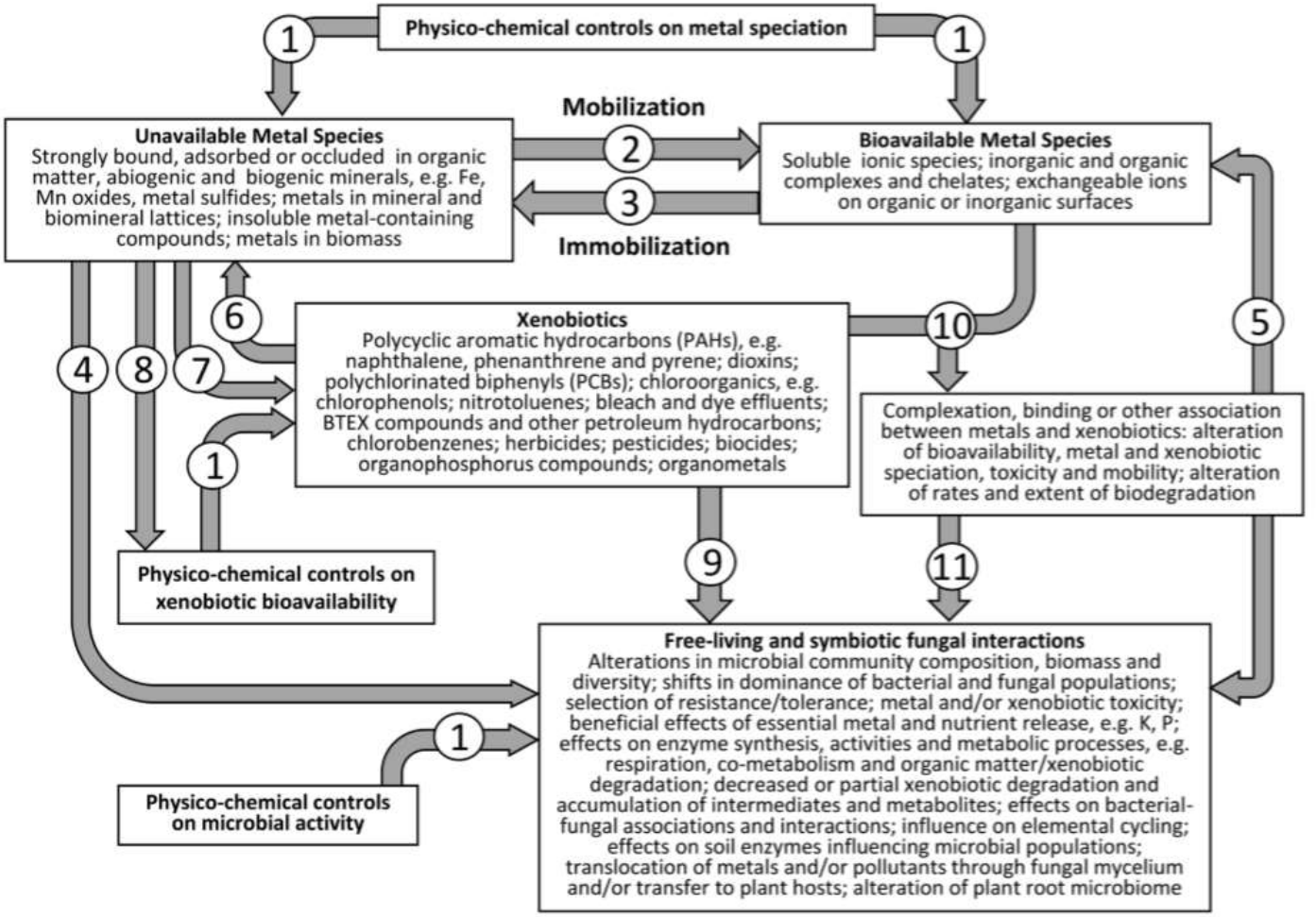


Figure 12. The interaction of microorganisms with the recalcitrant compounds (Ceci et al., 2019).

The white-rot fungi are considered as highly interactable fungal organisms which are susceptible to degrade a wide range of contaminants. *Phanerochae techrysosporium* is considered as an efficient microorganism which can degrade insoluble recalcitrant compounds by both oxidation and reduction mechanisms. *Pleurotusostreatus*, *Trametes versicolor*, *Bjerkanderaadusta*, *Lentinula edodes*, *Irpexlacteus*, *Agaricusbisporus*, *Pleurotustuberregium*, *Pleurotuspulmonarius* are well known fungal organisms involved in the degradation of xenobiotic compounds (Singh, 2006). The bioremediation of recalcitrant compounds was shown in Figure 13 explains the process of fungal bioremediation.

Fungi – Enzyme Produced

The enzymes produced by the fungal organisms are mainly lignolytic enzymes which include Laccase, Cellulases and peroxidases (Manganese and Lignin). The enzymes involved in the hydrolysis of polymeric compounds such as cellulose, protein, starch and lipid. These enzymes are also involved in the remediation of toxic pollutants in the environment (Radhika Deshmukh et al., 2016). Table 4 summarises the sources of fungal enzymes produced by different fungal species.

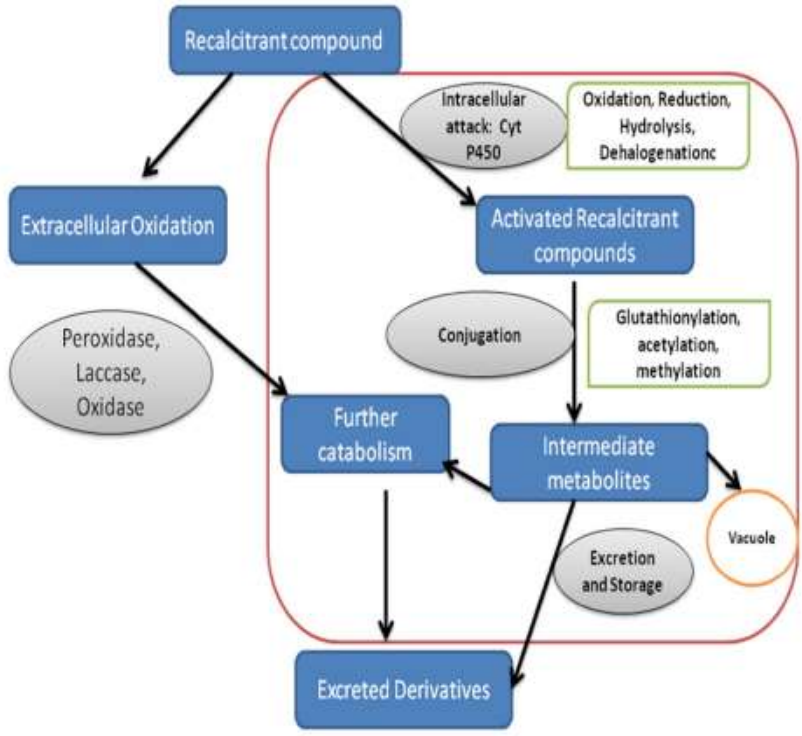


Figure 13. Bioremediation of recalcitrant compounds by fungi (Radhika et al., 2016).

Table 4. Enzymes and its sources (Duran and Esposito, 2000)

Enzymes	**Sources**
Peroxidase	*Artromyces ramosus*
Chloroperoxidase	*Caldariomyces funago*
Lignin peroxidase	*Phanerochaete chrysosporium* *Chrysonilia sitophila*
Manganese peroxidase	*Phanerochaete chrysopsorium* *Nematolona frowardi e Phebia radiate*
Tyrosinase	*Agaricus bisporus*
Laccase	*Trametes hispida* *Pyricularia oryzae* *Trametes versicolor*
Catechol dioxygenase	*Comamonas testosteroni*

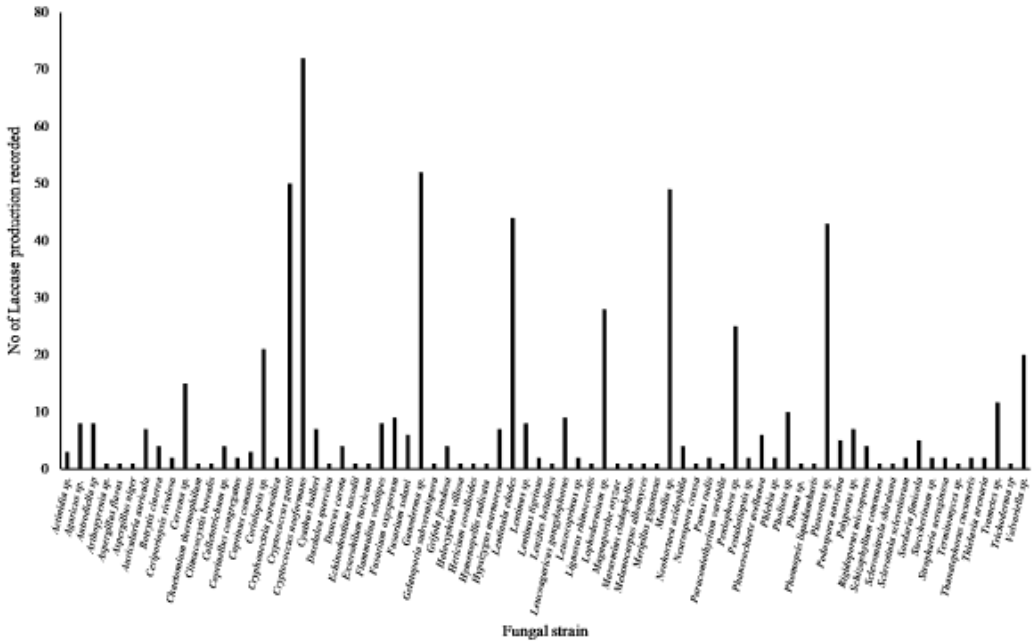


Figure 14. The fungal strains producing a laccase enzyme (http://www.cazy.org/).

The laccase and peroxidases enzymes are mostly involved in lignin degradation. The total of 74 fungal strains was observed to produced laccase enzyme according to Carbohydrate-Active enzymes Database. Figure 14 shows the fungal strains producing a laccase enzyme.

The laccase enzymes are studied in detoxification as it acts as enzymatic biosensors. The laccase enzymes are well-known enzymes for degradation of xenobiotic by producing free radicals, water and by utilising oxygen as a co-substrate (Oberdörster and Cheek, 2001). The decolourisation and detoxification of Reactive blue 4 by laccase enzyme produced by marine fungi (Vishwanath et al., 2014). The peroxidises, lignin peroxidase (LiP), manganese peroxidase (MnP) have broad substrate specificity and

oxidises both phenolic and non-phenolic compounds. The peroxidase produced by *B. adusta* cleaves the phthalocyaninic ring in phthalocyanine dyes by breaking the azo bond. The enzyme catalase acts as a defence mechanism of Reactive Oxygen Species (ROS). The pollutants such as heavy metals inhibit the catalase enzyme which in turn affects the growth the fungi. Thus, the heavy metal tolerance by catalyse producing fungi was exclusively studied. *Aspergillus spp*. tolerance to oxidative stresses induced by Zn was studied by Mitra et al., (2014). The exposure of fungi P. *Chrysosporium* to cadmium resulted in inhibition of catalase and peroxidases and increase on cytochrome P450 was observed (Zhang et al., 2015).

CONCLUSION

Thus, the fungi are termed as a vital and key organism in the environment. The fungal organisms are involved in a wide range of activities such as the production of fermented food, bioremediation of pollutants in the environment, synthesis of antibiotics, antimicrobial and anticancerous compounds which has its benefits in pharmaceutical industries. The fungi have its disadvantages by the production of mycotoxins which causes diseases in plants, animals and human beings. The identification of such pathogenic fungi using developed molecular techniques such as NGS (metagenomic analysis) which aids in the identification of fungal pathogen at the species level. Hence, the process of utilising the developed molecular techniques helps in the development of targeted treatment in both the environment and health care.

REFERENCES

Abhishek, Katoch. & Pooja, Kapoor. (2014). Recent Concepts in Fungal Taxonomy: A Review. *Research and Reviews: Journal of Agriculture and Allied Sciences*, *3*(2), 23-35.

Alexopoulos, C. J. (1962). *Introductory mycology*. John Wiley & Sons.

Alexopoulos, C. J. & Mims, C. W. (1979). *Introductory Mycology*. John Wiley and Sons, 1-613.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol*, *215*, 403–410. [PubMed: 2231712].

Badali, H. & Nabili, M. (2012). *Molecular tools in medical mycology; where we are! Jundishapur J Microbiol*, *6*(1), 1–3. doi: 10.5812/jjm.8566.

Balint, M., Bahram, M., Eren, A. M., Faust, K., Fuhrman, J. A., Lindahl, B., O'Hara, R. B., Öpik, M., Sogin, M. L., Unterseher, M. & Tedersoo, L. (2016). Millions of reads,

thousands of taxa: microbial community structure and associations analyzed via marker genes. *FEMS Microbiol Rev*, *40*, 686–700.

Bard. (2008). Anatomical ontologies of model organisms: the fungi and animals. In A. Burger, D. Davidson & R. Baldocs (Eds). Anatomy ontology of bioinformatics Computational Biology 6 London Springer.

Bard. (2008). Anatomical ontologies of model organisms: the fungi and animals. Anatomy ontology of bioinformatics Computational Biology 6 London Springer.

Blackwell, M. (2011). The fungi: 1, 2, 3 ... 5.1 million species? *Am J Bot*, *98*(3), 426-38.

Botstein. (1997). Genetics: Yeast as a model organism. *Science*, *277* (5330), 1259-1260.

Boyanton, B. L., Luna, R. A., Fasciano, L. R., Menne, K. G. & Versalovic, J. (2008). DNA pyrosequencing-based identification of pathogenic Candida species by using the internal transcribed spacer 2 region. *Arch Pathol Lab Med*, *132*(4), 667-74.

Canet, R., Birnstingl, J. G., Malcolm, D. G., Lopez-Real, J. M. & Beck, A. J. (2001). Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by native microflora and combinations of white-rot fungi in a coal-tar contaminated soil. *Biores Technol*, *76*, 113 – 117.

Capote, N., Aguado, A., Pastrana, A. M. & Sánchez-Torres, P. (2012). Molecular tools for detection of plant pathogenic fungi and fungicide resistance. Valencia: INTECH Open Access Publisher.

Ceci, A., Pinzari, F., Russo, F., Persiani, A. M. &Gadd, G. M. (2019). Roles of saprotrophic fungi in biodegradation or transformation of organic and inorganic pollutants in co-contaminated sites. *Appl Microbiol Biotechnol*,*103*(1), 53-68.

Cho, H. J., Hong, S. W., Kim, H. J. & Kwak, Y. S. (2016). Development of a multiplex PCR method to detect fungal pathogens for quarantine on exported cacti. *Plant Pathol J*, *32*(1), 53–54. doi: 10.5423/PPJ.NT.09.2015.0184.

Dasmahapatra, K. & Mallet, J. (2006). DNA barcodes: recent successes and future prospects. *Heredity*, *97*(4), 254–255. doi: 10.1038/sj.hdy.6800858.

David, Hansson. (2013). Structure and Biosynthesis of Fungal Secondary Metabolites. Swedish University of Agricultural Sciences.

Davis. (2000). Neurospora: Contributions of a model organism. UK Oxford University Press.

Davis. (2000). Neurospora: Contributions of a model organism. UK Oxford University Press.

Dawei, W. & Peng, Y. (2014). Assessing the impact of dominant sequencing-base gene expression profiling techniques (SGEPTs) on phytopathogenic fungi. *Chiang Mai J Sci*, *41*, 922–9444.

Durán, N. & Esposito, E. (2000). Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: a review. *Appl. Catal. B. Enzym*, *28*, 83-99.

Edgar, R. C. (2017). SINAPS: prediction of microbial traits from marker gene sequences. *BioRxiv*, *124156*.

Evans, C. S. & Hedger, J. N. (2001). Degradation of plant cell wall polymers. In Fungi in Bioremediation. Cambridge University Press: Cambridge, UK, 1–26.

Fang, Y. & Ramasamy, R. P. (2015). Current and prospective methods for plant disease detection. *Biosensors*, *5*(3), 537–561. doi: 10.3390/bios5030537.

Francois, Lutzoni., et al. (2004). Assembling the fungal tree of life: Progress, classification, and evolution of subcellular traits. *American Journal of Botany*, *91*(10), 1446–1480.

Galagan, J. E., Calvo, S. E., Borkovich, K. A., Selker, E. U., Read, N. D., Jaffe, D., et al. (2003). The genome sequence of the filamentous fungus Neurospora crassa. *Nature*, *422*, 859–868.

Gelsomino, G., Faedda, R., Rizza1, C., Petrone, G. & Cacciola, S. O. (2011). New platforms for the diagnosis and identification of fungal and bacterial pathogens. Science against microbial pathogens: communicating current research and technological advances.

Ghani, A., Wardle, D. A. & Lauren, D. R. (1996). Interactions between C14- labelled atrazine and the soil microbial biomass in relation to herbicide degradation. *Biol. Fertil. Soil*, *21*, 17–22.

Goffeau, A., Barrell, B. G., Bussey, H., Davis, R. W., Dujon, B., Feldmann, H., et al. (1996). Life with 6000 genes. *Science*, *274*, 546–567.

Goodwin, S., McPherson, J. D. & McCombie, W. R. (2016). Coming of age: Ten years of next-generation sequencing technologies. *Nature Reviews Genetics*, *17*, 333–351.

Grawert, T., Groll, M., Rohdich, F., Bacher, A. & Eisenreich, W. (2011). Biochemistry of the non-mevalonate isoprenoid pathway. *Cellular and Molecular Life Sciences*, *68*(23), 3797-3814.

Grigoriev, I. V., Cullen, D., Goodwin, S. B., Hibbett, D., Jeffries, T. W., Kubicek, C. P., et al. (2011). Fueling the future with fungal genomics. *Mycology*, *2*, 192–209.

Harbhajan, Singh. (2006). Mycoremediation: Fungal Bioremediation. Wiley online library.

Harms, H., Schlosser, D. & Wick, L. Y. (2011). Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nat Rev Microbiol*, *9*(3), 177–192.

Hawksworth, D. L. (2001). The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research*, *105*, 1422–1432.

Hibbett, D. S. & Taylor, J. W. (2013). Fungal Systematics: Is a New Age of Enlightenment at Hand? *Nature Reviews Microbiology*, *11*, 129-133.

Hibbett, D. S., Binder, M., Bischoff, J. F., et al. (2007). A higher-level phylogenetic classification of the Fungi. *Mycological Research*, *111*, 509-547. http://genome. jgi.doe.gov/programs/fungi/index.jsf.

Hutchinson, G. E. (1957). Concluding remarks. population studies: animal ecology and demography. *Cold Spring Harbor Symposia on Quantitative Biology*, *22*, 415–427.

Illumina. (2018). Fungal sequencing and classification with the ITS Metagenomics Protocol. Prepare Library.

Jae-Hyuk, Yu. & Nancy, Keller. (2005). Regulation of Secondary metabolism in filamentaous fungi. *Annu. Rev. Phytopathol*, *43*, 437–58.

Jeewon, R. & Hyde, K. D. (2016). Establishing species boundaries and new taxa among fungi: recommendations to resolve taxonomic ambiguities. *Mycosphere*, *7*, 1669–1677.

Jennifer, Viegas. & Margaux, Baum. (2017). Fungi and Molds. The Rosen Publishing Group, Inc.

Kevin, Kavanagh. (2005). Fungi Biology and Application. John Wiley and Sons.

Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., Bahram, M., et al. (2013). Towards a unified paradigm for sequence-based identification of Fungi. *Mol Ecol*, *22*, 5271–5277.

Krishna, Kumar Yadav., Neha, Gupta., Vinit, Kumar. & Jitendra, Kumar Singh. (2017). Bioremediation of Heavy Metals From Contaminated Sites Using Potential Species: A Review. *IJEP*, *37* (1), 65-84.

Krishnamurthy, P. K. & Francis, R. A. (2012). A critical review on the utility of DNA barcoding in biodiversity conservation. *Biodivers Conserv*, *21*(8), 1901–1919. doi: 10.1007/s10531-012-0306-2.

Lalitha, Gade., Steven, Hurst., Arunmozhi Balajee, S., Shawn, R. Lockhart. & Anastasia, P. Litvintseva. (2017). Detection of mucormycetes and other pathogenic fungi in formalin fixed paraffin embedded and fresh tissues using the extended region of 28S rDNA. *Medical Mycology*, *55*, 385–395.

Leho, Tedersoo., Mohammad, Bahram., Rasmus, Puusepp., Henrik, Nilsson R. & Timothy, Y. James. (2017). Novel soil-inhabiting clades fill gaps in the fungal tree of life. *Microbiome*, *5*, 42.

Loreau, M. (2000). *Biodiversity and ecosystem functioning: recent theoretical advances*, *Oikos*, *91*, 3, 17. (doi:10. 1034/j.1600-0706.2000.910101.x).

McLaughlin, D. J. & Spatafora, J. W. (2014). The Mycota, Systematics and Evolution part A. Springer, Heidelberg.

Mehl, H. L. & Cotty, P. J. (2010). Variation in competitive ability among isolates of Aspergillus flavus from different vegetative compatibility groups during maize infection. *Phytopathology.*, *100*(2), 150-9.

Meinwald, J. (2011). Natural products as molecular messengers. *Journal of Natural Products*, *74*(3), 305-309.

Mitra, A., Roy, D., Roy, P., Bor, A. M. & Sarkar Mitra, A. K. (2014). Sustainability of Aspergillus spp. in metal-enriched substrate aiming towards increasing biore-mediation potential. *World J Pharm Pharm Sci*, *3*, 864–878 113.

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., Schilling, J. S. & Kennedy, P. G. (2016). FUNGuild: an open annotation tool for parsing fungal community data sets by ecological guild. *Fung Ecol*, *20*, 241–248.

Nicholas, P. Money. (2016). Fungi: A very short introduction. Oxford University Press.

Ning Zhang, JingLuo. & Debashish, Bhattacharya. (2017). *Advances in Fungal Phylogenomics and Their Impact on Fungal Systematics*, *Chapter 8. Advances in Genetics*, *100*, 309-328.

Nylander, W. (1866). Hypochlorite of lime and hydrate of potash, two new criteria in study of lichens. *Botanica Journal if Linnean Society*, *9*, 358-365.

Oberdörster, Cheek. (2001). Gender benders at the beach: Endocrine disruption in marine and estuarine organisms. *Environmental Toxicology and Chemistry*, *20*(1), 23-36. DOI: 10.1897/1551-5028(2001)020<0023:GBATBE>2.0.CO;2.

Paul, D. Donovan., Gabriel, Gonzalez., Desmond, G. Higgins., Geraldine, Butler. & Kimihito, Itto. (2018). Identification of fungi in shotgun metagenomics datasets. *Plos one.*

Peralam, Yegneswaran Prakash., Laszlo, Irinyi., Catriona, Halliday., Sharon, Chen., Vincent, Robert. & Wieland, Meyer. (2017). Online Databases for Taxonomy and Identification of Pathogenic Fungi and Proposal for a Cloud-Based Dynamic Data Network Platform. *Journal of clinical Microbiology*.

Peršoh, D. (2015) Plant-associated fungal communities in the light of metabolomics. *Fungi Divers*, *75*, 1–25.

Rachael, Martin., Romina, Gazis., Demetra, Skaltsas., Priscila, Chaverri. & David, Hibbett. (2015). Unexpected diversity of basidiomycetous endophytes in sapwood and leaves of Hevea. *Mycologia*, *107*(2).

Radhika, Deshmukh., Anshuman, khardenavis. & Hemant, Purohit. (2016). *Diverse Metabolic Capacities of Fungi for Bioremediation Indian Journal of Microbiology*, *56*(3), DOI: 10.1007/s12088-016-0584-6.

Rahman, M. T., Uddin, M. S., Sultana, R., Moue, A. & Setu, M. (2013). Polymerase chain reaction (PCR): a short review. *Anwer Khan Mod Med Coll J*, *4*(1), 30–36.

Rolland, L. (1887). De la coloration en bleu developpee par l'iode sur divers champignons. *Bulletin de la Soci ete Mycologique de France*, *3*, 134–137.

Ronaghi, M. & Elahi, E. (2002). Pyrosequencing for microbial typing. *J Chromatogr B Analyt Technol Biomed Life Sci*, *782*(1-2).

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A. &Chen, W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc Natl Acad Sci,109*(16), 6241-6.

Selim, K. A., El-Beih, A. A., Abd El-Rahman, T. M. & El-Diwany, A. I. (2012). Biology of Endophytic Fungi. *Current Research in Environmental & Applied Mycology*.

Sharma, O. P. (1989). Text Book of Fungi. Tata McGraw-Hill Education.

Sidra, Aslam., Aisha, Tahir., Muhammad, Farhan Aslam., Muhammad, Waqar Alam., Arshad, Ali Shedayi. & Sehrish, Sadia. (2017). Recent advances in molecular techniques for the identification of phytopathogenic fungi – a mini review. *Journal of Plant Interactions, 12*(1) 493-504.

Singh, H. (2006). Mycoremediation, Fungal Bioremediation, New Jersey, USA: John Wiley and Sons, pp. 1-28, 29-75.

Stajich, et al., (2009). The Fungi. *Primer*, *19*(18), 840-845.

Tedersoo, L. & Nilsson, R. H. (2016). Molecular identification of fungi. In: Martin F (ed) Molecular mycorrhizal symbiosis. *Wiley, Hoboken*, 301–322.

Tedersoo, L. & Smith, M. E. (2017). Ectomycorrhizal fungal lineages: detection of four new groups and notes on consistent recognition of ectomycorrhizal taxa in high-throughput sequencing studies. *Ecol Stud, 230*, 125–142.

Tehler, A., Farris, J. S., Lipscomb, D. L. & Keallersje, O. M. (2000). Phylogenetic analyses of fungi based on large rDNA data sets. *Mycologia, 92*, 459–474.

Trejo-Hernandez, M., Lopez-Munguia, A. & Ramirez, R. (2001). Residual compost of Agaricus bisporus as a source of crude laccase for enzymic oxidation of phenolic compounds. *Process Biochemistry, 36*, 635-639.

Velculescu, V. E., et al., (1995). Serial analysis of gene expression. *Science, 270*, 484–487. doi: 10.1126/science.270.5235.484.

Vishwanath, B., Rajesh, B., Janardhan, A., Kumar, A. P. & Narasimha, G. (2014). Fungal laccases and their applications in bioremediation. *Enzyme Res*, doi:10.1155/2014/ 163242.

Wang, Henrik Nilsson. & James, Dai Townsend. (2016). Future perspectives and challenges of fungal systematics in the age of big data. *Biology of microfungi*, 25-46.

Wang, Henrik Nilsson. & James, Dai Townsend. (2016). Future perspectives and challenges of fungal systematics in the age of big data. *Biology of microfungi*, 25-46.

Wood, V., Gwilliam, R., Rajandream, M. A., Lyne, M., Lyne, R., Stewart, A., et al., (2002). The genome sequence of Schizosaccharomyces pombe. *Nature*, *415*, 871–880.

Zhang, Q., Zeng, G., Chen, G., Yan, M., Chen, A., Du, J., et al., (2015). The effect of heavy metal-induced oxidative stress on the enzymes in white-rot fungus *Phanerochaete chrysosporium*. *Appl Biochem Biotechnol*, *175*, 1281–1293. doi:10.1007/s12010- 014-1298-z.

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 11

THE ROLE OF EDIBLE MUSHROOMS IN CANCER TREATMENT

Md. Moyen Uddin Pk[1,*], Rumana Pervin[2] and Md. Matiar Rahman[2]

[1]Institute of Biological Science, Rajshahi University, Rajshahi,Bangladesh
[2]Department of Biochemistry and Molecular Biology,
Rajshahi University, Rajshahi,Bangladesh

ABSTRACT

Since ancient times, edible mushrooms have been used as a folk medicine in the management and treatment of various diseases. Pieces of evidence from various research activities regarding anticancer uses of mushrooms have made it prime research interest across the world. The main medicinal applications of edible mushrooms discovered are anti-cancer, anti-oxidant, anti-diabetic, anti-microbial, hypocholesterolemic, and immunomodulatory. Mushrooms belonging to the genusGanoderma, Pleurotus, Lentina, Agaricus, Phellinus, Lactarius, andAntrodiahave been assessed as potential anticancer agent form against cancer cell line growthin vitro/in vivo. The anticancer compounds belong to polysaccharides, polyphenols, lectins, enzymes, lipids, and steroids play a vital role in the inhibition of cancer cell proliferation through mitotic kinase inhibitor, topoisomerase inhibitor, induce apoptosis and angiogenesis inhibitor. In this chapter, we show the recent findings of biologically active compounds with their anticancer activity and mechanism of biological action to draw the attention of Scientists for future investigations and to develop cancer drugs from mushrooms.

*Corresponding Author's Email: biomoyen@gmail.com.

Keywords: polysaccharides, medicinal mushrooms, apoptosis, breast cancer, immunomodulating, oxidative stress, reactive oxygen species

INTRODUCTION

Currently, cancer is a major public health problem in the world. In 2018, about 9.6 million deaths have been estimated worldwide due to cancer and about 70 of 100 deaths have been found in low-and middle-income countries (WHO 2019). Breast cancer, lung and bronchus cancer, prostate cancer, colon and rectum cancer, skin melanoma, bladder cancer, non-Hodgkin lymphoma, pelvic cancer of the kidney and renal, endometrial cancer, leukemia, pancreatic cancer, thyroid cancer, and liver cancer are the most common cancers(Cancer statistic 2015). Most desirous is primary cancer prevention, although it remains difficult. Suitable planning is of paramount importance for its secondary and tertiary prevention. This review focus on discussing the anticancer effects of edible mushrooms.

Ganoderma lucidum

Ganoderma lucidum, known as Reishi in Japan, is a well-known mushroom. It has been used for the treatment of multiple human conditions, such as allergy, arthritis and bronchitis, gastric ulcers, hepatitis, immunological disorders, and cancer(Sliva D. et al. 2004). A wide variety of bioactive compounds like polysaccharides, triterpenoids, glycoproteins, and alkaloids(Baby S. et al.2015). Polysaccharides and triterpenes are the main bioactive compounds found in *Ganoderma lucidum.* Previous studies have been explained that the antitumor activities of *G. Lucidum* are mainlyattributed to its polysaccharides and triterpenes(Chen H.-S. et al.2004; Wu G.-S. et al.2013). Various biologically based compounds, some of them linked in the possibility of therapeutic effects, were extracted from mycelia and the fruiting bodies or spores of *Ganoderma Lucidum* (Table 1).*Ganoderma lucidum* polysaccharide shows the antitumor actions through anti-proliferative, anti-metastatic, anti-angiogenic, anti-oxidant and immuno-modulatory effects (Figure 1).

Anticancer Effects of *Ganoderma lucidum* Polysaccharides

Ljubica M. Harhaji Trajkovic investigated the anticancer activities of *Ganoderma lucidum* extracts against melanoma cells.

Table 1. Biological effects of bioactive compounds from *Ganoderma lucidum*

Bioactive compounds	**Biological effects**
Polysaccharides (Gao Y. etal. 2005)	
[(1→3)-β-D-glucans]	Inhibition of growth of sarcoma S 180 tumor in mice.PS-G, protein-bound polysaccharides (95% polysaccharides and 5% peptides), Activation of immune response, stimulation of the IL-1β, IL-6, TNF-α, and IFN-γ production by macrophages and T lymphocytes.
Triterpenes (Wu G.-S. et al..2913)	Inhibition of neutrophil apoptosis, Induction of neutrophil phagocytosis.
Ganoderic acid (Weng C.-J. et al.2010; Lin Z. et al.2004)	Cytotoxic for hepatoma cells. Inhibition of farnesyl protein transferase Cytotoxic for sarcoma and lung carcinoma cells

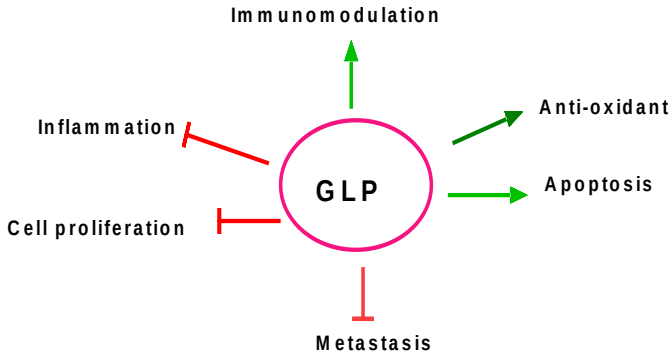


Figure 1. Antitumor role of GLP. Arrows (Green) show activation of process and bars (Red) show inhibition of process.

Here, the antitumor activity comprised cell proliferation inhibition and induction of apoptotic cell death (Ljubica M. Harhaji Trajkovic et al. 2019). Recent evidence suggests that more than 100 triterpene and polysaccharides have been elucidated from mushroom's fruiting bodies and spore/mycelia (Zhou X et al. 2007). Sliva D shows that thses mushroom-derived compounds have exhibited antitumor activites through different modes of action, polysaccharides show anticancer activities via stimulation of anticancer immune response while triterpenoids suppress tumor growth (Sliva D. et al. 2006). Anticancer immune response of isolated polysaccharides is showed via increases the level of IL-2, IFN- γ, and through NK cells & CTL stimulation, whereas anticancer effects of triterpenoids are exerted via the inhibition of tumor cells proliferation(Won S. J. et al. 2007) and cell apoptosis is carried out by the release of cytosolic cytochrome c, caspase-3 activation and mitochondrial membrane disruption(Tang W. et al. 2006). It has been investigated that tumor cells metastasis and invasion are inhibited by triterpenoids from this mushroom(Pk MMU et al. 2019;Kimura Y. et al. 2002).In the study by Didem Sohretoglu et al. (2018), it has been investigated that polysaccharides from mushroom

inhibits proliferation of B16F10(Sohretoglu D. et al. 2018). Previous evidence has ascribed that Ganoderma lucidum polysaccharides show anticancer activity through stimulation of immune response and direct cytotoxic effects on tumor cells (Figure 2)(Yuan Y. et al. 2017).

Pleurotus ostreatus

Pleurotus ostreatus, oyster mushroom, is a common edible mushroom.

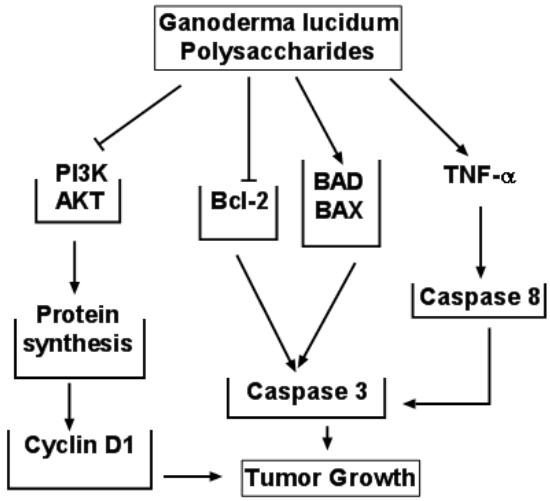


Figure 2. Molecular mechanism of anti-proliferative effects of Ganoderma lucidum polysaccharides on tumor cells. Arrows represent activation, whereas bars represent inhibition.

Due to their medicinal properties like antitumor activities, anti-inflammatory and stimulation of immune system, it has attracted great interest to scientist(Satitmanwiwat S. et al. 2012). Previous studies have investigated that the extracts from the fruiting bodies(Yoshioka Y. et al. 1985), mycelial biomass and the culture broth of the genus Pleurotus have been investigated for the antitumor properties(Wolff E. R. et al. 2019).It has been developed an aqueous polysaccharide extract from *Pleurotus ostreatus* shows antitumor effects on HT-29 colon cancer cells(Lavi I. et al. 2006). Polysaccharide was isolated from the fruiting bodies of *Pleurotus ostreatus*, which showeda dose-dependent cytotoxicity against HeLa tumor cell in vitro(Tong H. et al. 2009). In 2007, Wong et al. investigated the antitumor activiities of polysaccharides from fruiting body and mycelium of *Pleurotus ostreatus*. This polysaccharides-rich extract showed the strongest cytotoxicity against HL-60 (S. M. Wong et al. 2007). In this work, we found that both polysaccharides persuade programmed cell death in HL-60 cells by increasing the Bax/Bcl-2 ratio. Polysaccharides from mycelium extract caused G2/M arrest in HL-60 cells by reducing the expression of Cdk1, while the fruiting body showed S arrest in HL-

60 cells through decreasing of Cdk2 and through increasing of Cyclin E expression. Pk et al. (2019) showed that antioxidant polysaccharide-rich fraction from *Pleurotus ostreatus* induces anti-proliferative activity against Murine Lymphoid Cancer Cells. In this we demonstrated that the quadratic equations were fitted with experimental data using multiple regression studies, the ideal conditions were as follows: the proportion of water/crude material, 26.04mL/g; the extraction time was 62.08 minutes; the extraction temperatures were 70.5°C. The polysaccharide output was 5.32 ± 0.12 percent under such circumstances with the expected output. A dose($p < 0.1$)-and-time($p<0.001$) of dependent cytotoxic potential of ehrlich ascites carcinoma cell line was shown to be excellent scavenger activity against radical DPPH ($p < 0.001$, EC_{50}= 1036.38μg/mL, $R_2 = 0.8313$) and radical ABTS ($p < 0.001$, $EC_{50} = 824.37$μg/mL, $R_2 = 0.8224$). This has shown that polysaccharides (POP) have certain exercises in cancer prevention agents(Pk MMU et al. 2019).

Lentinus edode

Lentinula edodes, Shiitake mushroom, form polysaccharide, β-glucan which inhibit cell proliferation. *L. edodes* decreased time-and dose-dependent cell proliferation and apoptosis caused by carcinoma cells and did not affect non-tumorigenic cells (C50). The assessment of the cell cycle showed the extract caused the transient arrest G(1) without any modifications in non-tumorigenic cells(Gu Y.-H. et al.2005). Hui Xu et al. (Xu H. et al.2017) proved that the *Lentinus edode* β-glucan(LNT) had a deep inhibition ratio of ~53% to the MCF-7 tumor growth (ER+) in nude mice, comparable to the positive cisplatin control. Immunohistochemistry pictures have demonstrated that LNT suppresses the proliferation of cells and promotes apoptosis in tumor tissue from MCF-7. The Western blotting analysis stated that LNT up-regulated protein concentrations of the tumor suppressor p52, extracellular phosphorylated signals regulated kinase 1/2 (p-ERK1/2), cleaved caspase 3 (ADP (ribose)) and polymerase 1 (PARP 1). It decreased the expression of the mouse double minute 2 (MDM2). Also, phosphatidylinositol 3 kinase (PI3 K), protein phosphorylated kinase B (p-Akt) and mammalian rapamycin (mTOR) targets were considerably suppressed by LNT. LNT was suggested to inhibit MCF-7 tumor development, potentially through several mechanisms like PI3K/Akt/mTOR, ERK-ERα-, PI3K-ERα-and p53-dependent pathways, through the suppression of cell proliferation or improving apoptosis. Interestingly, LNT has proposed that cell viability assay, siRNA transfection, west blotting and cytometric flow assessment will only suppress p53/ERα cell proliferation via cell cycle arrest in G2/M without in-vitro academic apoptosis. The large distinction between in vitro and in vivo data showed that the polysaccharide's immune responses are primarily supported by the apoptotic impact.

CONCLUSION

The abundance of bioactive compounds present in medicinal mushrooms makes it an increasing part of today's pharmaceutical sector. Although they have a lengthy history of application in various societies, sensible scientific research supports them now. For viable growth, it is necessary to preserve and clone therapeutic mushrooms. Research on novel anti-cancer and immune stimulator compounds should be conducted in isolation, purification, and structural study. Several compounds and the fundamental mechanism have been recognized to date in studies. Research is, however, necessary to clarify the distinct functions and pathways of various active compounds. These findings and information may provide fresh insights into the potential therapeutic use of champagne and useful suggestions for the development of anti-tumor medicines from champagne for cancer-fighting.

REFERENCES

Baby, S., Johnson, A. J., Govindan, B. (2015). Secondary metabolites from *Ganoderma*. *Phytochemistry*, 114, 66 - 101.

Cancer Statistics (2015). National Cancer Institute. Available from: https://www.cancer.gov/about-cancer/understanding/statistics.

Chen, H.-S., Tsai, Y.-F., Lin, S., Lin, C.-C., Khoo, K.-H. and Lin, C.-H. (2004). Studies on the immuno-modulating and anti-tumor activities of *Ganoderma lucidum* (Reishi) polysaccharides. *Bioorg. Med. Chem.*, 12(21), 5595 - 601.

Gao, Y., Tang, W., Dai, X., Gao, H., Chen, G. and Ye, J. (2005). Effects of water-soluble *Ganodermalucidum* polysaccharides on the immune functions of patients with advanced lung cancer. *J. Med. Food*, 8(2), 159 - 68.

Gu, Y.-H., Belury, M. A. (2005). Selective induction of apoptosis in murine skin carcinoma cells (CH72) by an ethanol extract of *Lentinula edodes*. *Cancer Lett*., 220(1):21 - 8.

Harhaji Trajković, L. M., Mijatović, S. A., Maksimović-Ivanić, D.D., Stojanović, I.D., Momcilović, M. B., Tufegdzić, S. J., Maksimović, V. M., Marjanović, Z. S., Stosić-Grujicić, S. D. (2009). Anticancer Properties of *Ganoderma Lucidum* Methanol Extracts In Vitro and In Vivo: *Nutrition and Cancer*, 61(5), 696-707.

Kimura, Y., Taniguchi, M., Baba, K. (2002). Antitumor and antimetastatic effects on liver of triterpenoid fractions of *Ganoderma lucidum*: mechanism of action and isolation of an active substance. *Anticancer Res*., 22(6A), 3309 - 18.

Lin, Z., Zhang, H. (2004). Anti-tumor and immunoregulatory activities of *Ganoderma lucidum* and its possible mechanisms. *Acta Pharmacol. Sin*., 25(11), 1387 - 95.

Lavi, I., Friesem, D., Geresh, S., Hadar, Y., Schwartz, B. (2006). An aqueous polysaccharide extract from the edible mushroom *Pleurotus ostreatus* induces anti-proliferative and pro-apoptotic effects on HT-29 colon cancer cells. *Cancer Lett*., 244(1), 61 - 70.

Pk, MMU, Islam, M. S., Pervin, R., Dutta, S., Talukder, R. I., Rahman, M. (2019). Optimization of extraction of antioxidant polysaccharide from *Pleurotus ostreatus* (Jacq.) P. Kumm and its cytotoxic activity against murine lymphoid cancer cell line. *PloS one*, 14(1):e0209371.

Sliva, D. (2004). Cellular and physiological effects of *Ganoderma lucidum* (Reishi). *Mini Rev. Med. Chem.*, 4(8), 873 - 9.

Sliva, D. (2006). *Ganoderma lucidum* in cancer research. *Leuk. Res*., 30(7), 767 - 8.

Sohretoglu, D., Huang, S. (2018). *Ganoderma lucidum* Polysaccharides as an anti-cancer agent. *Anticancer Agents Med. Chem.*, 18(5):667 - 74.

Satitmanwiwat, S., Ratanakhanokchai, K., Laohakunjit, N., Pason, P., Tachaapaikoon, C., Kyu, K. L. (2012) . Purification and Partial Characterization of an Acidic α-Glucan–Protein Complex from the Fruiting Body of *Pleurotus sajor-caju* and Its Effect on Macrophage Activation. *Bioscience, Biotechnology and Biochemistry*,76(10), 1884 - 90.

Tang, W., Liu, J.-W., Zhao, W.-M., Wei, D.-Z., Zhong, J.-J. (2006). Ganoderic acid T from *Ganoderma lucidum* mycelia induces mitochondria mediated apoptosis in lung cancer cells. *Life Sci.*, 80(3), 205 - 11.

Tong, H., Xia, F., Feng, K., Sun, G., Gao, X., Sun, L. (2009). Structural characterization and in vitro antitumor activity of a novel polysaccharide isolated from the fruiting bodies of *Pleurotus ostreatus*. *Bioresour. Technol*., 100(4), 1682 - 6.

WHO (2019). *Breast cancer*. https://www.who.int/cancer/prevention/diagnosis-screening/breast-cancer/en/.

Wu, G.-S., Guo, J.-J., Bao, J.-L., Li, X.-W., Chen, X.-P., Lu, J.-J. (2013). Anti-cancer properties of triterpenoids isolated from *Ganoderma lucidum* - a review. *Expert Opin. Investig. Drugs*, 22(8), 981 - 92.

Weng, C.-J., Yen, G.-C. (2010). The in vitro and in vivo experimental evidences disclose the chemopreventive effects of *Ganoderma lucidum* on cancer invasion and metastasis. *Clin. Exp. Metastasis*, 27(5), 361 - 9.

Won, S. J., Lin, M. T., Wu, W. L. (1992). *Ganoderma tsugae* mycelium enhances splenic natural killer cell activity and serum interferon production in mice. *Jpn. J. Pharmacol*., 59(2), 171 - 6.

Wong, S. M., K. K. Wong, Lawrence Chiu and Peter C. K. Cheung (2007). Non-starch polysaccharides from different developmental stages of *Pleurotus tuber-regium* inhibited the growth of human acute promyelocytic leukemia HL-60 cells by cell-cycle arrest and/or apoptotic induction. *Carbohydrate Polymers*,68(2), 206-217.

Wolff, E. R.,Wisbeck, E.,Silveira, M. L.,Gern, R. M.,Pinho, M. S.,Furlan, S. A. (2008). Antimicrobial and antineoplasic activity of *Pleurotus ostreatus*. *Appl. Biochem. Biotechnol*.,151(2-3), 402-12.

Xu, H., Zou, S., Xu, X. (2017). The β-glucan from *Lentinus edodes* suppresses cell proliferation and promotes apoptosis in estrogen receptor positive breast cancers. *Oncotarget*, 8(49), 86693 - 709.

Yuan, Y., Liu, Y., Liu, M., Chen, Q., Jiao, Y., Liu, Y. (2017). Optimization extraction and bioactivities of polysaccharide from wild Russula griseocarnosa. *Saudi Pharm. J.*,25(4), 523 - 30.

Yoshioka, Y., Tabeta, R., Saitô, H., Uehara, N., Fukuoka, F. (1985). Antitumor polysaccharides from *P. ostreatus* (Fr.) Quél.: isolation and structure of a beta-glucan. *Carbohydr. Res*., 140(1), 93 - 100.

Yuan, Y., Liu, Y., Liu, M., Chen, Q., Jiao, Y., Liu, Y. (2017). Optimization extraction and bioactivities of polysaccharide from wild *Russula griseocarnosa*. *Saudi Pharm. J.*, 25(4), 523 - 30.

Yoshioka, Y., Tabeta, R., Saitô, H., Uehara, N., Fukuoka, F. (1985). Antitumor polysaccharides from *P. ostreatus* (Fr.) Quél.: isolation and structure of a beta-glucan. *Carbohydr. Res.*, 140(1), 93 - 100.

Zhou, X., Lin, J., Yin, Y., Zhao, J., Sun, X., Tang, K. (2007). Ganodermataceae: natural products and their related pharmacological functions. *Am. J. Chin. Med.*, 35(4), 559 - 74.

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 12

BIO-CONVERSION OF AGRO-WASTES USING MUSHROOM TECHNOLOGY

Abiodun Olusola Salami** and Faith Ayobami Bankole
Department of Crop Production and Protection,
Obafemi Awolowo University, Ile-Ife, Nigeria

ABSTRACT

The ability of different agro-wastes products to produce mushroom using various mushroom technology have been investigated. This was with the aim to determine and improve the potentials of various agro-waste products to mushroom production. Most agro-waste products have not been really used for the cultivation and this may be due to lack of knowledge of the elemental composition of these wastes and how they can enhance cultivation of Oyster mushroom. The commercial production of mushrooms is largely determined by the availability and utilization of cheap and locally organic materials of which are agricultural wastes (Salami and Bankole 2018). The cultivation of mushrooms which uses biotechnological procedure of bio-conversion of different agro-wastes to edible mushrooms of high nourishing value as well as recycling the agro-wastes into useful agricultural input is gaining credence. Also, the residual substrate (spent mushroom compost) obtained from mushroom cultivation can be used as soil conditioner, natural fertilizer, or food for animals, thereby, recycling the raw materials (Chang and Miles 1997), which today is called "zeri" technology (Chang 2003). The rate at which agro-wastes are produced often exceeds the rate of their use. This leads to wastage of cellular carbon rather than been integrated into the carbon cycle within the biomass present at various trophic levels. This study investigated the potentials and response of different Agro-wastes on mushroom production using various implicit mushroom technologies. These different wastes include: Dry banana leaves, Sugarcane bagasse, Maize straw, Oil palm spadix, Corncobs + Soybean shell (CCSYB), Corncobs + Rice bran (CCRB) and Corncobs + Groundnut shell (CCGS) were used as substrates while

* Corresponding Author's Email: sola1salami@yahoo.com.

soybean shell (SYB), rice bran (RB) and groundnut shell (GS) composted as additives. Spawns were made by weighing 180 g of corncob powder treated with 2% $CaSO_4$ and 3% $CaCO_3$ respectively and mixed adequately with sterile water and were transferred into mayonnaise cream bottles, autoclaved at 121^{o}C for 30 minutes, allowed to cool and then inoculated. Three hundred grams of the single substrates were used and the substrate-additive compost was weighed in ratio 80:20, 70:30 and 60:40 respectively. These were thoroughly mixed and transferred into transparent nylons and subjected to the same treatment for spawn production above, inoculated with spawns and were kept in dark chamber until full ramification. Data were collected on days taken for complete ramification of substrates, days to pinhead formation, number of fruiting bodies, weight of harvested fruiting bodies, length of stipe of matured fruiting body, diameter of the Cap, diameter of the stipe and biological efficiency. The data collected were subjected to analysis of variance and the significant means were separated using LSD at 0.05 level of probability. The results showed that each substrate responded to the treatment as yields were obtained maximally, although at different rates. In addition, the essential elemental component of Oyster mushroom defined the rationale behind the ability of agro-wastes to respond to mushroom production. It is recommended that different agro-wastes from farm should be used for mushroom production.

Keywords: bio-conversion, agro-wastes, mushroom, technology, substrates, autoclave, fruiting bodies

INTRODUCTION

Mushrooms are known as macro fungi with distinctive fruiting bodies, which can be either epigenous or hypogenous. Mushrooms are the fleshy, spore-producing fruiting bodies of a fungus called “Basidiocarp” typically produced above ground on the soil or on its food source. In a narrow sense, mushroom only refer to the fruiting body. They belong to the kingdom fungi, division basidiomycota, class agaricomycetes and order agaricales; moreover, mushrooms have been part of the fungal diversity for around 300 million years ago. They are macro fungi which have fruiting bodies large enough to be seen with the naked eye and to be picked up by hand. Mushrooms are saprophytes, lack chlorophyll and unlike other green plants, cannot get their food from the sun through photosynthesis. Instead, during their vegetative growth stage, mushroom mycelia secrete enzymes that break down compounds such as cellulose and lignin present in the substrate. The mycelium is the vegetative part of the fungal organism. In nature, mycelium is seldom seen, as it will dehydrate if exposed to sun or wind. Mushroom cultivation has two phases, viz; spawn running phase and the fruiting phase which are both dependent on temperature and humidity. They can be grown anywhere as long as the conditions for their growth and cultivation are provided. Its cultivation is affected by environmental factors like temperature, oxygen, carbon dioxide, humidity, light, moisture and pH have been reported to affect mycelia growth in the spawn preparation (Nwanze et al. 2005). Edible mushrooms are highly nutritious and can be compared with eggs, milk and meat

(Oei 2003; Salami et al. 2016). Moore and Chi (2005) confirmed that edible mushrooms have high nutritional attributes and potential applications in industries. One of the values of commercial cultivation of mushrooms, especially in a developing economy like Nigeria, is the availability of large quantities of several agro-industrial wastes, which can serve as substrate for the cultivation of mushrooms (Banjo et al., 2004). Fungi has unlimited importance in the terrestrial ecosystem and consequently in man's life. In nature, the fungi do not only participate in the role of providing food source for humans and other animals; they also play an important role in the cycling of carbon and other elements, by breaking the lignocellulosic residues and animal wastes which serve as a substrate for saprophytic fungi. This way, these decomposing agents play a very important environmental role along with other organisms, complementing the cycling of plants and animals. Simultaneously, they produce multiple enzymes that degrade complex substances that allow the absorption of soluble substances used for their own nutrition (Chang 1993; Salami and Elum 2010; Sławińska and Kalbarczyk 2011). Mushroom is a fleshy, spore-producing fruiting body of some higher fungi. They are saprophytes and they include some members of the Basidiomycota and some members of the Ascomycota (Jebapriya et al. 2013). Mushrooms are thought to be special and supernatural in origin (Bhatti 2007). They belong to class Basidiomycetes, subclass Hollobasidiomycetidae, order Agricale. They are fungi, which feed by secreting enzymes thereby digesting food externally and absorb the nutrients in net like chain called hypha. The hypha, when exposed to stimuli in their ecological niche act as a conscious intellect and respond to stimuli (Jebapriya et al. 2013). It grows wild in the forests and is cultivated in temperate and subtropical regions of the world (Shah et al. 2004). They lack chlorophyll, they cannot, like green plants get their energy from the sun through photosynthesis (Viziteu 2000). Instead, during their vegetative growth stage, mushroom mycelia secrete enzymes that break down compounds such as cellulose and lignin present in the substrate.

The degraded compounds are then absorbed by the hyphae and the mycelium enlarges (Viziteu 2000). The cultivation of Mushroom has two phases, viz; spawn running phase (this is the period of mycelia growth) and fructification phase (this is the growth of the fruiting bodies) which are both dependent on temperature and humidity. They are of different types which include: *Agaricus bisporus* (European or white button mushroom), *Pleurotus spp*. (Oyster mushrooms), *Volvariella volvacea* (Chinese or paddy straw mushroom), *Lentinus edodes* (shiitake mushrooms) and *Auricularia* (black ear mushroom) (Bhatti 2007). *Pleurotus* species are characterized by a white spore pint attached to recurrent gills, often with an eccentric (off center) stipe, or no stipe at all. The common name "oyster mushroom" comes from the white shell-like appearance of the fruiting body (Stanley et al. 2011). The lignocellulosic materials (agro-wastes) are rich in sugar and starch (carbon compounds), which are readily available carbohydrates sources. This speed up colonization and the consequent degradation of the substrate, thereby

reducing the time of fruiting since the mycelium easily converts these carbohydrates in reserve for the fructification, increasing productivity (Przybylowicz and Donoghue 1990). Generally, some carbon rich agro-wastes are low in protein content and are thus, insufficient for the cultivation of mushrooms. They therefore require additional nitrogen, phosphate and potassium for proper use as growth medium for mushroom cultivation (Sales-Campos et al. 2011). The supplements contain a mixture of protein, carbohydrate and fat, where the protein is the main source of nitrogen. They contain minerals and vitamins that also influence the growth of the fungus. The addition of these supplements is to increase the levels of nitrogen and carbohydrates available (Sales-Campos et al. 2011). A wide range of agro-wastes have been reported to include sawdust, paddy straw, sugarcane bagasse, corn stalk, corn cobs, waste cotton, leaves and pseudo stem of banana, water hyacinth, duck weed, rice straw etc. All these do not require expensive processing methods neither enrichment material (Modal et al. 2010). Sugars and starch which are readily available carbohydrates, speed up colonization and the consequent degradation of the substrate. These tend to reduce the time of fruiting since the mycelium easily converts these carbohydrates in reserve for the fructification and increasing productivity (Przybylowicz and Donoghue 1990). Supplements like limestone (CaCO3) or gypsum (CaSO4) is added to the cultivation medium, in order to obtain the right pH condition that is favourable for the growth of the fungus. Various substrates have different effects on the growth, yield and quality of mushrooms (Sarker et al. 2008). The commercial production of mushrooms is largely determined by the availability and utilization of cheap and locally organic materials of which are agricultural wastes (Stanley et al. 2011). The cultivation of edible mushrooms has become an increasingly important practice in modern society due to the biotechnological process of bioconversion of various residues in edible mushrooms or in dietary supplements of high nutritional value, enabling a more efficient utilization of materials, besides, it can reduce the volume of waste or accelerate the decomposition process. This way, the residual substrate obtained from the cultivation of edible mushrooms can also be used as soil conditioner, natural fertilizer, or food for animals, closing the exploitation cycle of raw materials (Chang and Miles 1997), which today is called “zeri” technology, trying to get the maximum use of such material, eliminating the residue of the residue (Chang 2003). In developing countries mushrooms is an important crop that can fetch farmers a substantial income to alleviate poverty and provide employment opportunities (Olumide 2007). Man has constantly realized the nutritional value of mushrooms, as well as their healthy properties compared to other foods, such as red meat. Mushrooms are more advantageous and important as they are great sources of carbohydrates, proteins, mineral salts, vitamins and essential amino acids, which can help to maintain a good nutritional balance (Garcia et al. 1993). Nutritional analyses have shown the importance of mushrooms. They contain more protein than conventional vegetables. Sources of protein such as meat, chicken, have a high level of cholesterol and fat, which are known to cause increase in weight and

cardiovascular diseases. For this reason, the proteins from other sources became more popular in recent years, such as proteins from fungi, algae, bacteria and yeast (Urben *et al*; 2003). Throughout the Eastern and Southern African region, mushrooms have become a popular vegetable due to its protein and vitamin content as well as culinary appeal. Edible mushrooms are highly nutritious and can be compared with eggs, milk and meat (Oei 2003). The content of essential amino acids in mushroom is high and close to the need of the human body. Mushroom is easily digestible and it has no cholesterol content. Sales-Campos et al. (2011) indicated that approximately 200g of mushrooms (dry weight) are sufficient to feed a normal human being weighing approximately 70Kg, providing a good nutritional balance. Nutritionally, these macro fungi are a good food source. A substrate is any substance that can facilitate mycelia growth. High concentrations of carbohydrate and nitrogen sources are usually needed in order to achieve a high yield (dry weight) of mycelium. Mushroom is not grown directly on soil as other crops but on organic substrate either raw or composted. These substrates are mostly agro-wastes products from farm, plantations or factories. During an investigation of the cultivation of mushroom on agricultural residues, it was found that rice husk sorghum stover, saw dust, cotton waste, cocoa bean shell, and sawdust - Gliricidia mixture are suitable substrates for the cultivation of edible mushroom (Belewu and Lawal 2003), while, rice straw, water lilly and banana leaves are equally implicated (Oei 2003). Efficiency of protein production and flavour formation vary with different carbon and Nitrogen sources. The range of carbon: nitrogen (C:N) ratio is important. This also influences the yield and efficiency of the production of mushroom mycelium from agro-wastes. Although some experiments have been carried out using agro-wastes as substrates, yet the full utilization of cheaply available wastes like organic nitrogen supplements have not been certainly used for mushroom production. Some studies show that supplementation with agro-wastes like nitrogen source increase the biomass and mushroom's productivity. Agro-wastes are rich in carbon compounds, which are readily available sources of carbohydrates. This accelerates colonization and the subsequent breakdown of the substrate, which decreases the time of fruiting since the mycelium effortlessly utilizes these simple sugars for the fruiting thus, increasing productivity (Bankole, 2017). Some carbon rich agro-wastes are low in protein and cannot sufficiently support the growth and development of mushrooms. Such agro-waste therefore needs additional mineral nutrient such as nitrogen, phosphate, potassium and vitamins to augment the deficiencies in the main substrates for cultivation (Breene, 1990). Several authors have reported the extensive use of agro-wastes such as sawdust, paddy straw, sugarcane bagasse, corn stalk, corn cobs, waste cotton, leaves and pseudo stem of banana and supplementation with agro-wastes.

Sugar and starch which are readily available in these substrates tend to reduce the time of fruiting since the mycelium easily converts these carbohydrates in reserve for the fructification (Bankole 2017). Supplements like limestone ($CaCO_3$) or gypsum ($CaSO_4$)

is added to the cultivation medium, in order to obtain the right pH condition that is favourable for the growth of the fungus. Various substrates have different effects on the growth, yield and quality of mushrooms (Salami et al. 2018). The commercial production of mushrooms is largely determined by the availability and utilization of cheap and locally organic materials of which are agricultural wastes (Salami and Bankole, 2018). The cultivation of mushrooms which uses biotechnological procedure of bio-conversion of different agro-wastes to edible mushrooms of high nourishing value as well as recycling the agro-wastes into useful agricultural input is gaining credence. Also, the residual substrate (spent mushroom compost) obtained from mushroom cultivation can be used as soil conditioner, natural fertilizer, or food for animals, thereby, recycling the raw materials (Chang and Miles, 1997), which today is called "zeri" technology (Chang, 2003). The rate at which agro-wastes are produced often exceeds the rate of their use. This leads to wastage of cellular carbon rather than been integrated into the carbon cycle within the biomass present at various trophic levels. This study investigated the potentials and response of different Agro-wastes on mushroom production using various implicit mushroom technologies.

MATERIALS AND METHODS

Scope of the Study

Location of the Study

The research was carried out in the Mycology Laboratory of the Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife. Cultured mycelia of Oyster Mushroom for this experiment were obtained from the mushroom collection culture centre of the same Mycology unit. Two centimeters of the mushroom's cap was washed in 1% parazone for five minutes, these little portions from the parazone solution were then transferred on to a pair of filter paper to drain the water droplets from the mushroom. Aseptically in the laminar flow, these tissues on the filter were transferred unto Potato Dextrose Agar (PDA) Plate and incubated for 3 days at 28^{o}C and stored in the slant bottles for future use. Corn cobs, sugarcane bagasse, rice bran and groundnut shell were the agro-wastes used in this study (where Corn cobs, sugarcane bagasse only were used for the tissue culture). A (4 x 3 x 4) factorial experiment laid out in a randomized complete block design was used in this study.

The substrates used for the investigation include; Dry banana leaves, Sugarcane bagasse, Maize straw, Oil palm spadix, Corncobs and soybean shell (CCSYB), Corncobs and Rice bran (CCRB) and Corncobs and groundnut shell (CCGS). Each of the substrate combinations above were in ratio 80:20, 70:30 and 60:40 respectively. The spawns of Oyster mushroom (*Pleurotus florida*) used as case study was collected from the

Mycology laboratory of the Department of Crop Production and Protection, Obafemi Awolowo University, Ile-Ife, and were grown on different substrates named above with three (3) replications which will give a total of 21 plastic plates of cultivated mushroom

Production of Spawn

Spawn of the mushroom was produced using sorghum grains for Dry banana leaves, Sugarcane bagasse, Maize straw, Oil palm spadix, while a bottle of fully ramified grain spawn of *Pleurotus florida* was multiplied using ground corncobs powder for the CCGS, CCSYB and CCRB combined substrates. One hundred and eighty grams of the ground corncobs were soaked in sterile water and $CaSO_4$ and $CaCO_3$ were added in the ratio of 2:3% respectively to regulate the pH of the ground corncob powder (Bankole and Salami 2017, 2018). Sterilized mayonnaise cream bottles were filled with sorghum grains and ground corncobs to about ¾ of the size of the bottles used. The bottles were corked with aluminum foil and autoclaved at 121°C for about 30 minutes after which the containers were allowed to cool for a day at room temperature. After 24 hours of cooling, grain to grain spawning was done to multiply the corncob powder spawn. The sorghum grains for Dry banana leaves, Sugarcane bagasse, Maize straw, Oil palm spadix, substrates were washed and parboiled for about 30 minutes after which it was decanted and allowed to cool. Calcium carbonate was added to regulate the pH of the grains. Labelled, sterilized jam bottles were filled with these grains up to about ¾ of the size of the bottle. The bottles were corked with cotton wool and aluminum foil paper and later autoclaved at 121°C for 20 minutes after which the bottles were allowed to cool at room temperature (Salami and Bankole, 2018).

Grain to Grain Spawning

An axenic environmental state was maintained to lessen contamination and this was achieved with the use of a sterile Laminar flow hood. Hand gloves and nose masks were worn to avoid contamination. The grain spawn obtained from the Mycology section of Department of Crop Production and Protection was lifted out of the bottle into a sterilized bowl with the aid of spatula; small quantity of the grain spawn was introduced into each of the bottles containing the pasteurized ground corncobs and the sorghum grains and covered immediately with foil paper and the foil paper was made firm on the bottles with rubber band. The bottles of the newly spawned grains were incubated at room temperature in complete darkness for 9 days to allow mycelia colonization of the corncob powder spawns (Bankole and Salami 2017).

Preparation of Substrate

Dry banana leaves, Maize straw, Sugarcane bagasse, Oil palm spadix and Corncobs with their additives were steamed with hot water. The banana leaves were shredded into bits of about 2 cm and soaked in hot water. The substrates were soaked with calcium

carbonate ($CaCO_3$) to neutralize the acidity of their acidity and adjust the pH to a range of 5-9 for optimum mycelia colonization. The substrates were then packed into transparent plastic plates.

Similarly, the Corncobs and each of the soybean shell and groundnut shell were shredded into bits of about 2 cm long and the dry weight of each was measured and recorded. The corncobs as well as the supplements (groundnut shell, soybean shell and the rice bran) were poured into separate clean bowls and about 2% $Ca(OH)_2$ and 3% $CaCO_3$ were added to each bowl for optimization and to adjust the pH for optimum mycelia colonization. The corncobs were submerged in hot water as well as the supplements and were allowed to soak for 15 minutes. They were drained and allowed to cool. Three hundred grams of corncobs and the additives were weighed according to the ratio 80:20, 70:30 and 60:40 of the main substrate to each of the additives (groundnut shell, soybean shell and rice bran) respectively. These were thoroughly mixed and packed into transparent nylons and were properly labeled for easy identification. The substrates were sterilized using the autoclave at 121^{o}C for 30 minutes.

Substrate Inoculation

The ramified spawn of *Pleurotus florida* was inoculated into the transparent nylons containing the prepared substrates. The inoculated bags of substrates were transferred into the dark chamber for incubation and were monitored until full mycelia colonization of the substrate. The fully ramified bags were brought out from the dark chamber to the fruiting body chamber for pinhead formation. During this stage, the fully ramified substrates were exposed to light source and wet on a daily basis with clean water. These were set in the mushroom house and close observation was made for pinhead's formation leading to fruiting bodies. The pH of the substrates was maintained at a range of 6.30-7.10. Harvest was done with hand and knife after maturity.

Data Collection

The resulting parameters were collected as the growth occurred: days taken for complete mycelia ramification of substrates (DTCSR), days to pinhead formation (DPHF), number of fruiting bodies (NOFB), weight (g) of harvested fruiting bodies (WTFB) using an electronic weighing balance, length of stipe (LOS) of harvested fruiting bodies using a meter rule, diameter of the Cap (DOC) and diameter of the stipe (DOS) using a thread and a meter rule respectively.

Yield and Biological Efficiency

Total weight of all the fruiting bodies harvested from all the replications were measured as total yield of mushroom. The biological efficiency was calculated by the formula below according to (Salami et al. 2016)

$$\text{B.E (\%)} = \frac{yield\ of\ mushroom}{weight\ of\ substrate} x100$$

Statistical Analysis

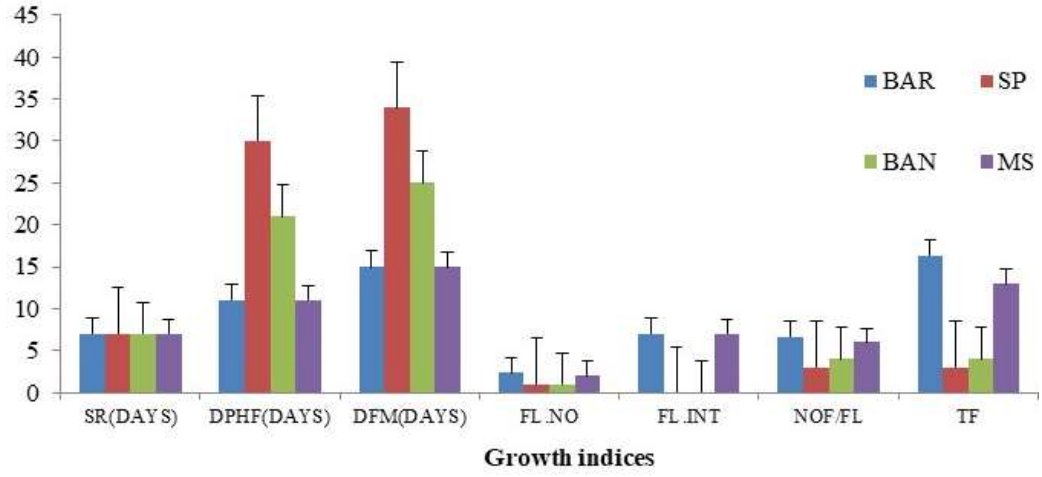
The data obtained were subjected to ANOVA using Statistical Analysis Software (SAS) version 9.1 and significant means were separated using Fischer's least significant difference at 0.05 level of probability. Microsoft excel 2013 was also used to plot the graphs with standard error bar.

RESULTS AND DISCUSSION

Agro-wastes were used in this study because of its manifold availability, and the ability of (*P. florida*) to easily breakdown its polysaccharides for growth than other substrates used for cultivation (Table 3). The possibility of converting the 'wastes' from the farm into potential raw materials for efficient use within the agro-system also informed the choice of agro-wastes for Oyster mushroom (*P. florida*) spawn production in this study. This conforms to the submission of Shah et al. 2004. Oei (2003) reported that mushroom can be grown on agricultural and industrial wastes, and more than half of produce from the land remain unused as wastes. These wastes can be recycled into food and the environment will be friendlier in terms of pollution. The substrates used in this experiment supported the spawn production of *P. florida* significantly although at diverse levels. The perceived differences can be due to the differences in the chemical and elemental composition of these agricultural waste (Chandravadana et al. 2005). The fact remains that the growth media contained the needed substances that is required for growth and may take time for the process to be completed. Thus, increasing the time it will take the organism to fill the Plates. This is similar to (Banjo et al. 2004) who reported that waste cobs from variously processed maize (*Zea mays*) were used as raw materials to prepare growth agar media for fungi and all the formulated media supported the growth of the microorganisms tested. This further asserts the fact that *Pleurotus* species have extensive enzyme systems capable of utilizing complex organic compounds that occur as agricultural wastes and industrial by- products (Salami et al. 2018).

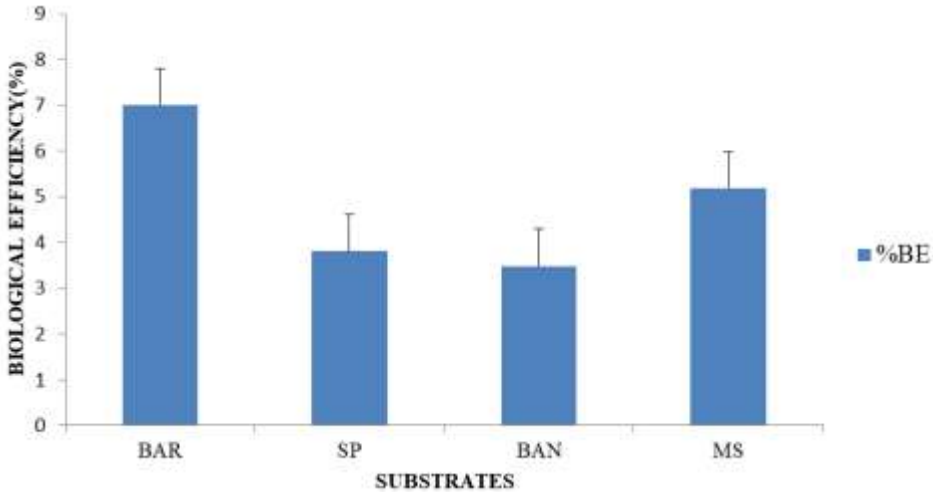
Full ramification of the substrates was observed 14-16 days after inoculation of the substrates with the spawn of *P. florida*. Sequel to the full ramification of the substrates was pinhead formation (Figure 3) which later developed into matured fruiting body (Figure 4). The pin heads began to emerge between 11-30 days after inoculation which got matured 4-5 days later. These matured fruiting bodies were harvested by removal from the substrates. Average growth length was calculated by adding the shortest growth rate and the highest growth rate divided by two which gave an average of 24.5 days (Figure 1). The fresh weight of the mushroom was taken and mushrooms from sugarcane

bagasse substrate had the highest weight (14.02 g) which was significantly different from that of oil palm spadix substrate and banana leaves substrate but not significantly different from maize straw substrate (Table 2). Figure 2 shows the biological efficiency of each substrate (where the weight of the substrates was worked against the average yield of the mushroom) with their standard error bar. It was also observed that the number of days to complete substrate ramification was not significantly different for the substrate combinations (CCSYB and CCGS) as it took *P. florida* 16 days to completely ramify the substrates (CCSYB and CCGS) but was significant for CCRB which was fully ramified by the organism 14 days after inoculation. number of days taken for pin head formation on the substrates were significantly different from each other as pinheads were observed on the substrates (CCSYB, CCGS, and CCRB) after 22, 21, and 20 days respectively of inoculation with completely ramified spawns of *P. florida* (Table 1). The differences in the growth parameters can be alluded to the different nutrient composition of the substrates used in this study (Salami et al. 2018). The highest weight of matured mushroom from the combined substrates was observed on the CCSYB substrate which recorded 29.28 g, this was followed by CCGS and CCRB with corresponding values of 17.19 g and 14.70 g. It was apparent that the recorded weights of the matured fruiting bodies of the oyster mushroom were significantly different from each other at probability levels of 0.05. The average number of matured fruiting bodies of *P. florida* harvested on CCSYB, CCGS and CCRB substrates were recorded respectively and are significantly different from each other at 0.05 level of probability.



SR = Spawn running, DPHF = Days to Pin head formation, DFM = Days to Fruit maturity, FW = Fresh weight, FL.NO = flush number, FL.INT = Flush interval, NOF/FL = Number of fruits per flush, TF = total flush.

Figure 1. Indices of Mushroom growth and the average of growth parameters of *P. florida.*



BAR = bagasse, SP = spadix, BAN = banana leaves, MS = maize straw.

Figure 2. Biological efficiency of oyster mushroom.

Table 1. Effect of different combinations of organic nitrogen supplements on the growth parameters of *P. florida*

Combination	DTCSR (DAYS	DPHF (DAYS	WTFB (g)	NOFB	LOS (cm)	DOS (cm)	DOS (cm)	BE (%)
CCSyB	16.00	22.00	29.28	3.00	4.82	4.12	6.35	9.76
CCGS	16.00	21.00	17.19	3.17	3.85	4.14	5.80	5.73
CCRB	14.00	20.00	14.70	3.25	3.99	2.91	4.50	4.90
LSD 0.05	0.28	0.20	7.62	2.04	0.71	0.59	1.34	2.54

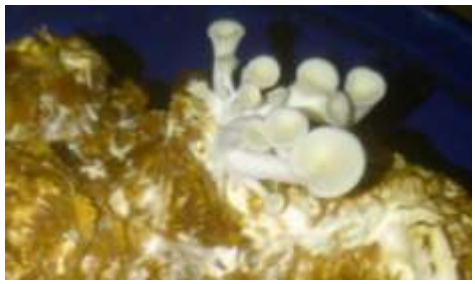
DTCSR: Days to complete substrate ramification, DPHF: Days to pinhead formation, WTFB: Total weight of fruiting body, NOFB: Number of fruiting body, LOS: length of stipe, DOS: Diameter of stipe, DOC: Diameter of cap, BE: Biological efficiency, CC: corncobs, SB: sugarcane bagasse, GS: groundnut shell, CCSYB: corncobs + soybean shell, CCGS: corncobs +Groundnut shell, CCRB: corncobs + rice bran.

The highest BE of *P. florida* was observed on CCSYB substrate which recorded 9.76% followed by bagasse and CCGS whose values were 7.01% and 5.73%. The average length of the stipe of *P. florida* harvested from the CCSYB substrate was 4.82 cm and was obvious to be longer than the length of stipe of *P. florida* harvested from other substrates which was found to be 3.85 cm and 3.99 cm for CCGS and CCRB respectively. The diameter of the stipe of *P. florida* harvested from CCRB was found to be significantly lower than the diameter of stipe of *P. florida* harvested from other substrates used for this investigation. This is expected since the nature of the substrates varies across the substrates used. The fresh weight of the mushrooms was taken as seen below had the highest weight which was significantly different from other substrates but not significantly different from maize straw substrate (Tables 1 and 2).

Table 2. Fresh weight from the substrates

Substrate	Fresh weight	Biological Efficiency
Bagasse	14.02	7.01
Spadix	7.616	3.808
Banana leaves	6.98	3.49
Maize straw	10.38	5.19

All the agro-wastes used in this experiment was found to contain some essential elements which aid the growth and development of Oyster mushroom. These elements were found present in varied amount in the agro-wastes used in this study.



Source: Mycology Laboratory, CPP.

Figure 3. Pinheads of *Pleurotus florida.*

Source: Mycology Laboratory, CPP.

Figure 4. Mature fruiting bodies of *Pleurotus florida.*

Table 3. Elemental composition of the substrates

Substrates	Nitrogen (%)	Phosphorus (ppm)	Potassium (CMol/Kg)	Carbon (%)
Bagasse	0.63	1,704.45	5.25	30
Spadix	0.63	1731.81	14.25	39
Banana leaves	0.63	1804.73	14.25	45.6
Maize Straw	0.56	1713.58	11.25	58

CONCLUSION

It can be concluded from the experiment, that different substrates supported the growth of *Pleurotus florida*. For the fruiting body production, the different substrates have significant effect on improving the production of *Pleurotus florida*. The substrates were observed to ramify faster although at different days. This investigation revealed that Agro-wastes are useful raw materials that could be utilized in mushroom production rather than constituting a challenge to waste management problem encountered in the agricultural industry. This research recommends that agro-waste conservation should be enhanced and supported in that nothing within the cycle of agricultural production should be wasted. Also, that mushroom should be incorporated into our diets more frequently in order to improve the quality of our diet, as well as our overall health and general well-being as mushroom have been discovered to contain with high protein which can be a substitute for expensive fish and meat. Also, the knowledge of mushroom production should be conveyed from our laboratories and universities to the outside world. This is to enable people become enlightened on how to produce mushroom in homes as mushrooms have been established to be substitute for egg and meat. This should be done because the world population is currently increasing at a faster rate and shortage of food and the diminishing quality of human health has been growing concerns due to increase in urbanization and a concomitant reduction in arable land. Arousing people's interest to venturing into mushroom production will arrest the problem of food insecurity and unemployment as they are liable to becoming entrepreneurs.

REFERENCES

Banjo N. O., Abikoye, E. T. and Kukoye, A. O. (2004). Comparison of three nutrient supplements used as additives to saw dust during the cultivation of Oyster mushroom (*Pleurotus pulmonaris*). *Niger J Microbiology.* 18: 335-336.

Bankole, F. A. (2017). Improved production of Oyster mushroom (*Pleurotus florida*) using augmented agro- wastes 63, Unpublished master's thesis.

Bankole, F. A., Salami, A. O. (2017). Use of Agro-Wastes for Tissue Culture Process and Spawn Production of Oyster Mushroom (*Pleurotus florida*). *JALSI.* 14: 1-9.

Belewu, M. A., Lawal, R. A. (2003). Studies of the cultivation of edible mushroom (*Pleurotuspulmonarium*) on cocoa bean shell and sawdust - Gliricidia mixture. *Res Comm Microbiol.* 1: 68-71.

Breene, W. (1990). Nutritional and medicinal value of speciality mushrooms. *Journal of Food Production.* 53: 883-894.

Chandravadana, M. V., Vekateshwarlu, G., Bujji-Babu, C. S. (2005). Volatile flavour components of dry milky mushrooms (*Calocybe indica*). *Flavour Fragr* J 20: 715-717.

Chang, S. T. (1999). Global impact of edible and medicinal mushrooms on human welfare in the 21st centurynon green revolution. *Int J Med Mushrooms* 1: 1-8.

Chang, S. T., Miles, P. G. (1997) Historical record of the early cultivation of *Lentinus* in China. Mushroom. *Journal for the Tropics*. 1997:7:31-37.

Chang, S. T. (1993). Mushroom biology: The impact on mushroom production and mushroom products, In: *Mushroom Biology and Mushroom Products, Proceeding of the first international conference on mushroom biology and mushroom products,* Chang, S. T.; Buswell, J. A.; Chiu, S. W. (Eds), 3-20, The Chinese University Press, ISBN: 962-201-610-3, Hong Kong.

Chang, S. T. O. (2003). *Mushroom Cultivation using the "Zeri" Principle: Potential for Application in Brazil*, Proceeding of First International Symposium on Mushroom in food, health, technology and the environment in Brazil, ISBN: 0102-011.

Garcia, H. S., Khanna, P. K. and Soni, G. L. (1993). Nutritional importance of the mushrooms, In: *Mushroom Biology and Mushroom Products, Proceeding of the first international conference on mushroom biology and mushroom products,* Chang, S. T.; Buswell, J. A. & Chiu, S. W. (Eds), 227-236, The Chinese University Press, ISBN: 962-201- 610-3, Hong Kong.

Jebapriya, G. R., Gnanasalomi, V. D., and Gnanadoss, J. J. (2013). Application of Mushroom Fungi in Solid Waste Management. *International Journal of Computing Algorithm*. 2:279-285.

Modal, S. R., Rehana, M. J., Noman, M. S. and Adhikary, S. K. (2010). Comparative study on growth and yield performance of oyster mushroom (*Pleurotus florida*) on different substrates. *Journal of Bangladesh Agril Univ*. 8(2):213 - 220.

Moore, D., Chi, S. W. (2005). Fungi products as food. In: (Hyde K. O. eds.) Bio-Explotation of filamentous fungi. *Fungi Diversity Res.* 6: 223-251.

Nwanze, P. I., Khan, A. U., Ameh, J. B. (2005). The effect of the interaction of various spawn grains with different culture mediums on carpophores dry weights and stipe and pileus diameters of *Lentinus squarrosulus* (Mon.) singer. *Afr J Biotechnol.* 4: 615- 61.

Oei, P. (2003). Mushroom cultivation, appropriate technology for mushroom growers. Backhugs Publishers, Leiden, the Netherlands.

Olumide, O. J. (2007). Economic analysis of mushroom marketing as a copping strategy for poverty reduction in Ondo State, Nigeria. *Afr. Crop Sci. Conf. Proc.* 1255-1260.

Przybylowicz, P., Donoghue, J. (1990). *Shiitake growers' handbook: The art and science of mushroom cultivation*, Kendall/Hunt Publishing Company, ISBN: 0-8403-4962-9. Dubuque, Iowa.

Salami, A. O. and Bankole F. A. (2018). Don't waste the 'wastes', they are ways to wealth. *EC Microbiology*. 14(8):1-16.

Salami, A. O., Bankole, F. A., Olawole, O. I. (2016). Effect of different substrates on the Growth and Protein content of oyster mushroom (*Pleurotus florida*). *Int J Biol Chem Sci* 10: 475-485.

Salami, A. O. and Elum, E. A. (2010). Bioremediation of a Crude Oil Polluted Soil with *Pleurotus pulmonarius* and *Glomus mosseae* using *Amaranthus hybridus* as a Test Plant. *J. Bioremed Biodegrad*. 1 (3):113.

Salami, A. O., Bankole F. A., Andrew O. C. (2018). Effect of Organic Nitrogen Supplements on the Yield and Nutrient Content of Oyster mushroom (*Pleurotus florida*) Cultivated on Corncobs. *SciFed journal of Mycology*. 1(1):1-9.

Sales-Campos, Ceci, Bazilio, Frasco Vianez and Raimunda, Liege Souza de Abreu (2011). Productivity and Nutritional Composition of Lentinus strigosus (Schwinitz) Fries Mushroom from the Amazon Region Cultivated in Sawdust Supplemented with Soy Bran, Recent Trends for Enhancing the Diversity and Quality of Soybean Products, Prof. Dora Krezhova (Ed.), ISBN: 978-953-307-533-4, In*: Tech*, Available from: http://www.intechopen.com/books/recent-trends-for-enhancing-the-diversity-and-quality-of-soybeanproducts/productivity-and-nutritional-composition-of-lentinus-strigosus-schwinitz-fries-mushroom.

Sarker, N. C., Hossain, M. M., Sultana, N., Mian, I. H., Karim, A. J. M. S. and Amin, S. M. R. (2008). Relationship between Nutrient Content in substrates and Economic Yield of Oyster Mushroom (*Pleurotus ostreatus*). *Bangladesh J. Mushroom*. 2(1):27-33.

Shah, Z. A., Ashraf, M. and Ishtiaq Ch. M. (2004). Comparative Study on Cultivation and Yield Performance of Oyster Mushroom (*Pleurotus ostreatus*) on Different Substrates (Wheat Straw, Leaves, Saw Dust). *Pakistan Journal of Nutrition*. 3(3):158-160.

Sławińska, A. and Kalbarczyk, J. (2011). Evaluation of Enzymatic Activity of *Pleurotus ostreatus*. Regarding Stages of Mycelium Development. *Acta Sci. Pol., Hortorum Cultus.* 10(2):195-202.

Stanley, H. O., Umolo, E. A. and Stanley, C. N. (2011). Cultivation of oyster mushroom (*Pleurotus pulmonarius*) on amended corncob substrate. *Agriculture and Biology Journal of North America*. 2(8):1239 – 1243.

Urben A. F., Uriartt, A. H., Amazonas, M. A. L., Oliveira, H. C. B. Corream. J. Vieira, V. (2003). Cultivo de cogumelos comestíveis e medicinais (apostila do curso). *EMBRAPA*. p169 Brasília, DF.

Viziteu, G. (2000). Substrate-cereal straw and Corn Cobs. In: *Mushroom Growers' Handbook 1*, Gush, R. (Ed.). P and F publishers, USA. ISBN-10: 0932551068:86-90.

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 13

TRICHOPHYTON RUBRUM: A LEADING PATHOGEN OF SKIN AND NAIL INFECTION

K. Anita*
Department of Botany, M.G.P.G. College,
Gorakhpur, Uttar Pradesh, India

ABSTRACT

Dermatophytes are closely interrelated fungi, causing superficial fungal infections of the skin, nails and hairs. The fungal nature of dermatomycoses was recognised by Gruby (1843). He described *Microsporum audouinii* from a case of human capitis. According to WHO (World Health Organization) 1986, the dermatophytes are defined as a group of moulds that form three genera *Epidermophyton, Trichophyton* and *Microsporum*. They comprise about forty five different species. Among them *Epidermophyton floccossum*, *Microsporum audouinii* and *Trichophyton schoeleinii* were the major pathogens causing dermatophytoses. In recent studies, it has been reported that *Trichophyton rubrum* has become the leading pathogen of skin and nail infections followed by *Trichophyton mentagrophytes*. *Trichophyton rubrum* and *Trichophyton mentagrophytes* are also reported most common pathogens causing dermatophytosis in India. Dermatophytes can survive solely on outer cornified layers of the skin. The ability of certain fungi to adhere to particular host arises from numerous mechanisms and host factors, including the ability to adapt to the human body. The natural infection is acquired by the deposition of viable arthrospores or hyphae on the surface of the susceptible individual followed by the stages of adherence and penetration. Natural defenses against dermatophytes depend on both immunological and non-immunological mechanisms. This chapter reviews the isolation, methods of isolation and management of *Trichophyton rubrum* causing dermatomycoses.

* Corresponding Author's Email: anitamaurya913@gmail.com.

Keyword: *Trichophyton,* dermatophytes, dermatomycoses, pathogens, tinea, infections, keratin, antifungal, antidermatophyic activity

INTRODUCTION

The first infectious agent of human diseases to be described was fungus (Schoenlein, 1839). Dermatomycoses are infections of the skin, hair and nails, which are caused in most cases by dermatophytes, and in rare cases by yeasts and moulds. Dermatophytes are closely related filamentous fungi which cause superficial fungal infections of keratinised tissue such as epidermis, hairs and nails. According to the site of infection, dermatophytes are termed as tinea capitis (ringworm of scalp), tinea barbae (ringworm of beard), tinea favosa (ringworm of scalp and skin), tinea corporis (ringworm of body), tinea embricata (ringworm of body characterized by concentric rings of overlapping scales), tinea cruris (ringworm of groin), tinea pedis (ringworm of feet), tinea manuum (ringworm of palm and interdigital area of hand) and tinea unguium (ringworm of nail) (Del Palacio et al., 2000).The fungal nature of dermatomycoses was recognised by Gruby (1843). He described *Microsporum audouinii* from a case of human capitis. Accordingg to Ajello (1962) and George (1960), dermatophytes are divided into anthropophilic, zoophilic and geophilic species, according to their transmission route and main occurrence. Anthropophilic dermatophytes prefer humans as the primary host; therefore transmission from person to person occurs frequently. Around 70% of dermatomycoses in humans are caused by anthropophilic species. Zoophilic dermatophytes are transmitted by close contact particularly with pets. They often cause strong inflammatory reactions in humans. Geophilic dermatophytes cause disease less frequently in humans. According to WHO (World Health Organization), the dermatophytes are defined as a group of moulds that form three genera: *Epidermophyton, Trichophyton* and *Microsporum.* They comprise about fourty five different species. Among them *Epidermophyton floccossum, Microsporum* audouinii and *Trichophyton schoeleinii* were the major pathogens causing dermatophytoses. In recent studies, it has been reported that *Trichophyton rubrum* has become the leading pathogen of skin and nail infections followed by *Trichophyton mentagrophytes*.

The genus *Trichophyton* is characterised morphologically by the development of both smooth walled macro- and micro-conidia. Macroconidia are mostly borne laterally directly on the hyphae or on short pedicels, and are thin or thick-walled, clavate to fusiform and range from 4-8 x 8-50 µm in size. Macroconidia are few or absent in many species. Microconidia are spherical, pyriform to clavate or of irregular shape and range from 2-3 x2-4 µm in size. The presence of microconodia differentiates this genus from *Epidermophyton,* and the smooth walled, mostly sessile macroconidia differentiates it from *Lophophyton, Microsporum, Nannizzia and Pareaphyton.* Present chapter deals the

recent studies on *Trichophyton rubrum*. *Trichophyton rubrum* is most prominent anthrophilic species of dermatophytes, belongs to the phylum Ascommycota. It colonizes the upper layers of dead skin, and is the most common cause of athlete's foot, fungal infection of nail, jock itch and ringworm worldwide. *Trichophyton rubrum* was first described by Malmsten in 1845 and is currently considered to be a complex species that comprises multiple, geographically patterned morphotypes, several of which have been formally described as distinct taxa, including *T. raubitschekki, T. gourvilli, T. megnninii and T. soundanense.*

WORK DONE ON ISOLATION OF *TRICHOPHYTON RUBRUM*

Makendorf (1952) mentioned about the outbreak due to infection of *Trichophyton rubrum* in South Australia. Cremer (1953) reported granulomatous form of mycosis on lower legs of human beings caused by *Trichophyton rubrum.* Gip and Martin (1954) isolated different species of *Trichophyton* viz. *Trichophyton mentagrophytes* and *Trichophyton rubrum* from dogs. An outbreak of the infection of *Trichophyton rubrum* in a genetic hospital has been reported by Peachey and English (1974).

Fungal infections of the skin are the most frequently occurring infectious diseases, with a worldwide prevalence of 20% to 25% and high and growing relapse rates. Although dermatophytoses does not cause mortality, it does cause morbidity and poses a major health problem (Emmons and Binford, 1974) especially in tropical countries like India due to hot and humid climate. Alter as and Lehrer (1977) made a critical survey of 1000 cases of dermatophytes in Tel Aviv area during 1970-1975. They expressed their opinion that, the dermatophytic flora of Israel compromise 10 dermatophytes. *Trichophyton rubrum* being the predominant agent of infection. The other agents they found in their survey were *T. Mentagrophytes, T. Violaceum, T. schoenieinii, T. tonsurans, Epidermophyton floccoslum, Microsporum sp. M. canis and M. gypseum.* Sinski et al., (1977) performed a quantitative study using skin scales infected with *Trichophyton mentagrophytes* and *Trichophyton rubrum* using special dermatophytic isolation media.

Arora et al., (1979) recorded an outbreak of dermatomycoses in pigs. The causative agent identified by them were *Trichophyton rubrum* (27 cases), *T. mentagrophytes* (2 cases) and *T. tonsurans* (1case). Mcleer (1981) worked on fungal infection of nails in Western Australia between 1963 to 1972 and in 1986. *Trichophyton rubrum, T. mentagrophytes, Candida albicans* and *Aspergillus sp* were isolated. Tadaro et al., (1983) reported a case of an epidemic by Tinea cruris and Tinea pedis from Italy. They observed noticeable spread of the causative agents i.e., *Trichophyton beigelli, Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton floccossum, Candida parapsilosis* and *Geotrichum candidum,* throughout environment.

Lopez and Rivera Lona (1984) investigated the dermatophytes in healthy skin of human body. In this study the skin of 200 patients of both sexes, in between 5-56 years age was observed in order to understand the frequency of dermatophytes. From each person, sample of scale from scalp, face, trunk, groin, and feet were taken. The dermatophytes isolated were *Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton tonsurans, Epidermophyton floccosum, Microsporum canis* and *M. gypseum.* They also discussed pathogenicity and epidemiology of the dermatophytes. Lopez (1984) also worked on ecology of the dermatophytes in the human skin. The most frequent species were *Trichophyton rubrum and Trichophyton mentagrophytes.*

Shukla et al., (1985) observed the effect of temperature on growth and survival of dermatophytes. During this study significant increase in the number of colonies at 35°C and 40°C was recorded. Viability remained up to 30 days in experimental specimens, and up to 40 days in clinical specimens. Most favourable temperature for the survival of dermatophytes was 30°C. Maximum growth and keratin degradation was recorded at 30°C. The dermatophytes examined were *Trichophyton rubrum, Microsporum gypseum* and *Epidermophyton floccosum.*

Dermatophytes have worldwide distribution (De Vroey C, 1985), no race in any geographical location is totally free from dermatophytoses (Rippon, 1988). Imwidthaya and Thianprasit (1988) during January to December 1986 observed a total of 719 cases of dermatophytes in the division of dermatology, Department of Medicine, Sriraj Hospital, Mahidol University, Bangkok, Thailand. Infection caused by fungus compromised 12 percent while the incidence of dermatophytoses was 5.5 percent. They isolated *Trichophyton rubrum, Trichophyton mentagrophytes, Epidermophyton floccosum* and *Microsporum gypseum* from skin disorders.

Kamalam and Thambiah (1989) reported the results of the study of Tinea capitis in out patient clinic. Skin Department, Government General Hospital, Madras. Findings suggests that Tinea capitis is endemic in South India and male children were more commonly affected than the female children. The pathogens were *Trichophyton violaceum, T. tonsurans, T. rubrum, T. mentagrophytes and T. simii.*

Okuda et al., (1991) observed the invasion of human hair by *Trichophyton rubrum.* Weitzman and Summerbell (1995) reviewed the etiological agent, epidemiology and ecology and clinical manifestations of dermatophytes. Anstey et al.,(1996) reviewed the literature on Tinea capitis caused by *Trichophyton rubrum*. Sentamilselvi et al.,(1998) reported that Tinea cruris and Tinea corporis were the most common clinical types and Tinea pedis was the least common type observed in the Mycology Section of Department of Dermatology, Govt. Central Hospital, Madras. The most frequent isolate was *Trichophyton rubrum* followed by *Trichophyton mentagrophytes* and *Trichophyton violaceum.* They also reported that patients with immuno- compromised status, diabetic mellitus, atopy and intake of a corticosteroid a are also predisposed to chronic dermatophytosis.

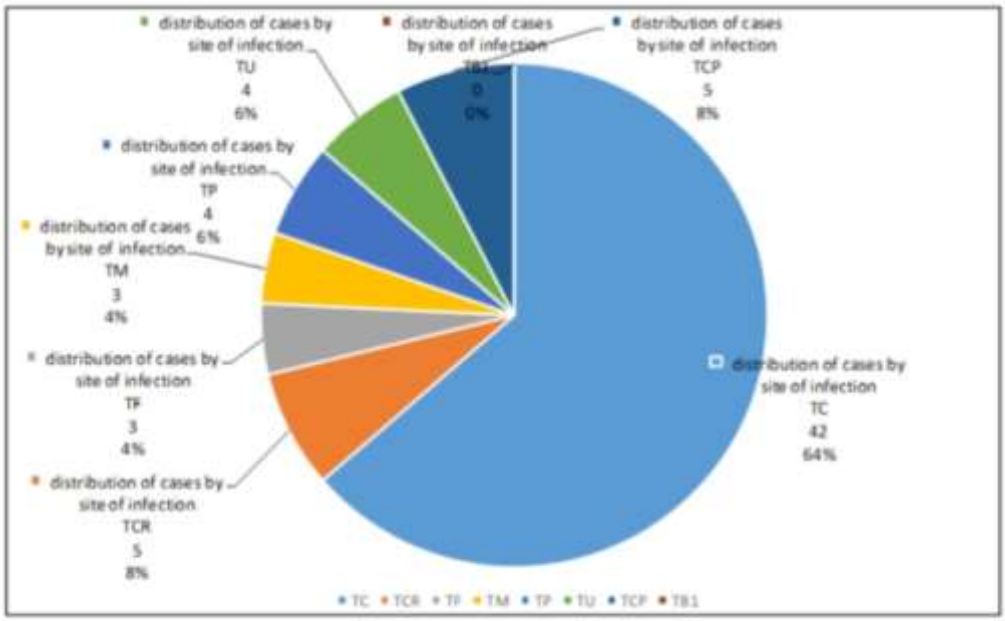
Paz et al., (2003) presented a case report of deep infection by *Trichophyton rubrum* in an immunocompromised patient. They diagnosed the infection by histopathology, immunohistochemistry and culture. The causal fungus was identified by morphological characteristics and confirmed by PCR. Basina et al., (2004) reported/isolated *Trichophyton rubrum* in the external auditory muscle of a 28 years old immunocompetent male. Rad et al., (2005) reported single strains of *Trichophyton rubrum* from skin specimens from 10 patients with Tinea pedis, suggesting a monotypic aetiology of tinea pedis. Tambekar et al., (2007) isolated and identified the dermatophytes and other keratinolytic fungi in the soil of Amrawati, India. Venkatesan et al.,(2007) reported that *Trichophyton rubrum* was the predominant species responsible for the dermatophytoses, especially Tinea corporis in chennai, Tamilnadu, India. Had also obseved that *Trichophyton rubrum* was most predominant species responsible for the chronic dermatophytoses (18.8%).

Sumit Kumar et al., (2012) observed that out of total 250 clinical isolates *Trichophyton rubrum* was the commenest isolates and Tinea corporis is the common clinical types. They also reported that males were more frequently affected by dermatophytoses. Bhatia and Sharma (2014) reported that the climatic conditions of Himachal Pradesh, India favour dermatophytoses in the population. Tinea corporis was the most frequently encountered clinical condition followed by tinea cruiris. They also noted that *Trichophyton mentagrophytes* was the predominating dermatophyte (63.5%) followed by *Trichophyton rubrum* and *Microsporum gypseum.* Male to female ratio of the positive case was recorded as 63:11 and the most effected age group was 21-50 years (64.9) followed by 1-20 years (28.4%) and above 50% (6.8%).

Naglot et al., (2015) studied the prevalence of dermatophytoses and their etiologic agent in Assam, India. He reported that *Trichophyton rubrum* was the prominent causative agent, isolated mostly from tinea corporis, tinea unguium and tinea cruris. In his research paper, he also mentioned the published studies of dermatophytoses in different regions of India. Kaur et al., (2015) studied on 351 cases from Department of Microbiology, Mauulana Azad Medical College, New Delhi over a period of two years (2012-2013). They observed dermatomycoses in 251 cases out of 351(61.2%). Most common isolates were non-dermatophyte Mold (36.1%), followed by dermatophytes (13.8%) and yeast (8.6%). Among the dermatophytes *Trichophyton rubrum* was the most common followed by *Trichophyton verrucossum*. Lee et al., (2015) investigated the clinical and epidemiological characteristics of *Trichophyton rubrum* infections in the Korea. Investigating patient records from the Catholic Skin Disease Clinic from 1979 to 2013, they found that the annual incidence of patients with *Trichophyton rubrum* infection had been increasing during the period and of 131,122 patients with dermatophytosis, 115,846 patients (88.35%) had *Trichophyton rubrum* infection. Disease was most prevalent among patients in their twenties in the 1970s and 1980; in their thirties in the 1990s; in their fourties in the 2000s; and in their fifties in the 2010s. The

sex ratio was 1.5:1. *T. rubrum* infection was most commonly reported in summer and was found predominantly in patients living in the urban areas. Sharma et al., (2015) compiled recent information concerning the nature of keratinolytic moulds and their distribution and prevalence in developing countries including India. Jayanthi et al.,(2016) worked on incidence and culture characteristics of dermatophytoses isolated at Tertiary Care Hospital, Hyderabad, India. They studied upon 60 patients comprising 44 males and 16 females. The distribution of the cases by site are shown in Figure 1.They reported that the predominant infection observed in the study was tinea corporis and the predominant fungus isolated was *Trichophyton rubrum.*

Ramraj et al.,(2016) visited Department of Dermatology, SRMRI, Chennai, India from the period April 2011- March 2014 and observed 210 patients having lesions typical of dermatophytic infection, who . They reported that out of 210 sample received tinea corporis was the predominant clinical site which way followed by tinea cruris. A total of 143 dermatophytes were isolated from clinical samples. Trichophyton rubrum was the predominant etiological agent with 70/143 isolates and *Trichophyton mentagrophytes* was the second common with 64/143 isolates. In their study they found male are more infected than females in the ratio of 4:3.



T.C –tinea corporis, T.CR –tinea cruris, T.F- tinea facei, T.M- tinea manum, T.U –tinea unguim, T.CP- tinea capititis, T.B- tinea barbae

Source: Jayanthi et al., 2016.

Figure 1. Distribution of cases by site of infection.

Ismail and Al- Kafri (2016) did epidemiological survey of dermatophytes in Damasus, Syria from 2008-2016. They found that *Trichophyton rubrum* was the most prevalent fungal pathogen especially in toe nail onychomycosis. Kaliyamoorthi (2018) reported that *Trichophyton rubrum* was the most common cause for dermatophytic infection. He also observed that there was a high prevalence of *Trichophyton rubrum* in diabetic patient than non- diabetic patients.

WORK DONE ON METHODS FOR THE ISOLATION OF *TRICHOPHYTON RUBRUM*

Weitzman and Summerbell (1995) reported laboratory diagnosis of dermatophytes by Direct Microscopic Examination and Culture. He also discussed identification character and diagnostic media of dermatophytes in brief. Simpanya (2000) discussed current concepts of teleomorphic and teliomorphic states of dermatophytes, sampling techniques (eg: Keratin-baiting technique, Hairbrush technique), techniques of mating studies, ecological groupings and sources of infection. In his article, he reviewed pathogenecity of dermatophytes with enphasis on proteolytic enzymes including its biochemical assays, characterization and molecular weight size.

Paz et al., (2003) diagnosed *Trichophyton rubrum* by histopathology, immunohistochemistry and culture in an immunocompromised patient. The causal fungus was identified by morphological characteristics and confirmed by PCR. Baeza et al., (2006) reported strain differentiation of *Trichophyton rubrum* by randomly amplified polymorphic DNA and analysis of r DNA non-transcribed spacer. Santos et al., (2006) established a method of inoculum preparation for susceptibility testing of *Trichophyton rubrum* and *Trichophyton mentagrophytes.*

Tambekar et al., (2007) used hair bait technique to isolate keratinophillic fungi. He cultured the isolated fungi on Sabouraud's dextrose agar medium supplemented with chloramphenicol (50 mg/ l) and cycoheximide (500 mg/ l) and identified on the basis of spore morphology, cultural characteristics and pigment formation on the reverse of slant. He also estimated the keratinolytic potential of fungal isolated by the method described by Lowry et al., (1951) by using Bovine serum albumin as a standard and as reagent. Lee et al., (2015) diagnosed the isolated samples by direct microscopic examination method. Kaur (2015) studied the isolated sample by direct microscopic examination method and culturing them on SDA. The isolates were further identified by studying the culture characteristics, pigment production and microscopic examination of the lactophenol cotton blue (LPCB) mounts and slide culture.

Ismail and Al-Kafri (2016) analyze the isolated sample by direct microscopic examination and cultured on Sabouraud Dextrose Agar medium for further identification.

Ramraj V. et al., (2016) diagnosed the isolated sample by direct microscopic examination, culture technique on SDA medium and slide culture technique and by polymerase chain reaction- Restriction fragment length polymorphism (PCR- RFLP) technique. Monad and Mehul (2019) summarized different tools for the diagnosis of onychomycosis and rapid identification of the pathogen. These methods are - Direct Mycological Examination Method, Culture Method, PCR with Specific Primers, PCR with Pan- Fungal Primers/Sequencing Method, Mass Spectrometry (MRM), Western Blotting Method, ELISA and Immunological Methods. In this review they also discuss the mechanism of nail invasion and resistance of onychomycoses to standard fragments.

Naglot et al., (2015) assessed the collected samples from Assam, by Direct Microscopic Examination Method and identification of cultures was done by macro and microscopic morphology, hair perforation test, urease and cornmeal agar tests. Further species level identification of the isolates was confirmed by ITS rDNA.

WORK DONE ON THE PATHOMECHANISM AND MANAGEMENT OF *TRICHOPHYTON RUBRUM*

Trichophyton rubrum is the leading pathogen of skin and nail infections. Typically, itsinfections are restricted to the upper layers of the dead skin, and are the most common cause of athlete's foot, fungal infection of nail, jock itch and ringworm worldwide. The management of dermatomycosis begin with topical agent. The first therapeutic agent against mycoses was potassium iodide, the use of which is limited to sporotrichosis (Conant et al., 1971). The studies on management of dermatophytoses caused by *Trichophyton rubrum* are briefly discussed. Weitzman and Summerbell (1995) explained the physiology, genetics, molecular biology and histopathology of dermatophytes. He also reviewed the established and recent trends in the therapy of the dermatophytoses.

TOPICAL AND SYSTEMIC ANTIFUNGALS

Ferna'ndez-torres et al.,(2001) reported terbinafine (TF), clotrimazole (CTZ), new triazole UR-9825, voriconazole (VCZ), and itraconazole (ITZ) were the most active drugs against *T. rubrum*; their geometric mean MICs were 0.01, 0.04, 0.09, 0.06, and 0.09 mg/ml, respectively. In contrast, fluconazole (FCZ) and G-1 were the least active. Li et al., (2008) reported that acetylenic acid (e.g., 6-nonadecynoic acid), with low *in vitro* and *in vivo* toxicity profiles, are highly superior to undecylenic acid in terms of their *in vitro* antifungal potencies against *Trichophyton mentagrophytes* and *Trichophyton rubrum.* Their strong fungicidal activities against *Trichophyton mentagrophytes* and

Trichophyton rubrum indicates that they may quickly eradicate these pathogens and this may reduce the chance of developing drug resistance in the treatment of topical infections.

Peres et al., (2010) summarized some proteins identified in dermatophytes involved in virulence. They discussed different aspects of the biology of dermatophytes, with emphasis on the dermatophyte-host interaction and the mechanisms of antifungal resistance. They also explained the mechanism of action, possible mechanisms of drug resistance, and gene expression pattern of *Trichophyton rubrum* against antifungal agents e.g., Terbinafine, Fluconazole, Imazalil, Itraconazole, Cetoconazole, Tioconazole, Amphotericin B, Griseofulvin, Acriflavine, Undecanoic acid, Benomyl, Ethidium bromide, 4NQO and PHS11A.

Hryncewicz-Gwozdz et al., (2013) reported an increase in resistance to fluconazole and itraconazole in *Trichophyton rubrum* by sequential passages *In vitro* under drug pressure. Dogra and Uprety (2016) explain various possible factor for the chronic/ recurrent dermatophytic infection and emergence of antifungal drug resistance in superficial mycoses. Kumar et al., (2017) mentioned that the most common agent for the treatment of dermatophytosis caused by *Trichophyton rubrum* are azoles (e.g: clotrimazole, miconazole, econazole, oxiconazole, tioconazole) and allylamines (e.g: terbinafine and naftifine). Morpholine derivates such as amorolfine and butenafine have been also used.

Poojary (2017) reviewed the entire spectrum of topical antifungals, formulations and their role in management of dermatophytoses. She also discussed the mechanism of action of antifungals, additional action of topical antifungals (e.g:, anti- inflammatory action and antibacterial action), methods to enhance topical drug delivery (e.g., mechanical methods, chemical methods and physical methods) and newer topical antifungals (e.g: efinacozole, tavaborole and ME 1111).

Sahni et al.,(2018) summarized the classification and mechanism of action of antifungal antibiotics. They also reviewed the newer formulations of older antifungal drugs (e.g., amphotericin B in lipid based gel formulations) photodynamic therapy and lasers therapy for the management of onychomycosis. Monad and Mehul (2019) observed that *Trichophyton* developed resistance to terbinafine treatment, due to a missense mutation in the sequalene epoxidase enzymes targeted by the drug, is an emerging problem. They mentioned to switch to azole- based treatment to cure onychomycosis.

PLANT PRODUCTS

There are many plant products as plant extract, essential oils etc. posses antidermatophytic activity. These phytoproduts are very useful in management of dermatophytoses caused by *Trichophyton rubrum*. Essential oils of *Apium graveolens,*

Atalant monophylla, Citrus auranticum, Lantana aculeate, L. indica, Leucas aspera, Osimum Conan, Plyalthia longifolia (Rao and Joseph, 1971), *Psoralea drupacea* (Band Cirenko et al., 1972), *Allium cepa, A. sativum, Azadirachta indica, Coriandrum sativum, Rita graveolens* (Thind and Dahiya, 1976), *Oenanthe javanica* (Sharma and Singh, 1979 a), *Eupatorium avapora* (Sharma and Singh, 1979 b), *Buddleia Asiatics* (Garg and Oswal,1981), *Arachis hypogaea, Chenopodium album, Cuminum cyminum* (Deshmukh and Chile, 1982), *Cymbopogan martini, Eucalyptus golbules, Thuja Orientalist* (Deshmukhet al., 1982), Chloroxylon swietenia (Garg and Bhatia, 1982), *Hyptis suaveolens* (Pandeyet al., 1982b), *Citrus aurantifolia C. zedoaria, Eucalyptus heyneana, Lantana indica, Leucas aspera, Polyalthia longifolia* (Rao and Rao, 1982), *Anethum graveolens, Cymbopogan fexusus* (Dikshit and Husain, 1984), *Carum capticum, Cuminum Cyminum, Germanium SP., Cymbopogan flexuous Cinnamomum zeylanicum* (Saxena, 1984), *Anaphalis contorts* (Saxena et al.,1984), *Juniperus verginiana* (Mall et al.,1985) *Cyperus scarious* (Deshmukh et al.,1986), *Schinus molle* (Dixit et al.,1986), *Eupatorium copillifolium, E. cannabinum* (Mall, 1987), *Capillipedium foetidum, Tagetus erecta* (Garg and Dengree, 1988), *Artemisia nelagrica, Casesula axillaries, Chenopodium ambroisiodes, Cymbopogan citrates, Mentha arvensis* (Kishore et al.,1993), *Pycnoporellus fulgens* (Steinmetz et al., 1995), *Malaleusa alternifolia* (Nensoffet al.,1996), *Trachyspermum ammi* (Shahiet al.,1996), *Cymbopogan citrates* (Wannissorn et al., 1996), *Cymbopogan pendulus* (Pandey et al., 1997), *Trachyspermum ammo, Cymbopogan flexuous* (Shahi, 1997), *Eucalyptus spp* (Shahiet al.,1997 a) *Citrus sinensis* (Shahiet al., 1997b), *Bixa orellana, Hoslundia opposite,Hyptis lanceolata, H. suaveolens, Ocimum basilicum* (Amvam Zolla et al., 1998), *Melaeuca alternifolia* (Concha et al. 1998), *Cymbopogan flexuous* (Shahi et al.,1999), *Eucalyptus laveopenia, Eucalyptus cotriodora, Eucalyptus dalrampleana,* (Shahi et al., 1999), *Foeniculum vulgate* (Patra and Shahi, 2000), *Eucalyptus pauciflora* (Shahiet al., 2000), *Maraleuca alternifolia* (D' Auriaet al.,2000), *Artemisia nilagirica* (Kishore et al., 2001), *Eugenia caryophyllata* (Patra et al.,2001), *Rabdosia millisoides* (Shahi et al.,2001), *Foeniculum vulgate* (Patra et al.,2002), *Melaleuca alternifolia* (Benger et al.,2004), *Osimum gratissimum* (Silva et al., 2005), *Baccharis grisebachiihieron* (Hadad et al.,2005), *Cinnamomum zeylanicum, Eugenia aromatics, Germanium maculatu!, Lavandula angustifolia, Cymbopogan citrates, Cymbopogan martini, Origanum vulgate, Palmarosa, Thymus vulgaris* (Inouye et al.,2007), *Leptospermum petersonii, Syzygium aromaticum* (Parket al., 2007), *Eucalyptus citriodora* (Luqman et al., 2008), *Thymus vulgaris, Mentha spicata, Mentha piperata* (Sokovic et al.,2009), plant extract of *Areca catechu* (Lalita et al.,1964), *Aesculuc hippocasttanum* (Kahl et al.,1966), *Curcuma zeodaria, Brassica sp* (Gupta and Banerjee, 1970), *Thiyopsis dolabrata* (Heijtmankova et al.,1973), *Bachharis glutinosa* (Disalvo,1974), bulb of *Allium sativum* (Tansey and Appleton, 1975), *Allium sativum* (Amer et al.,1980), *Polygonam ariculare* (Kim and Kwang, 1980), *Vinca rosea* (Chileet al.,1981), *Lutinus edodes* (Takazawa et al., 1982), *Casia alata* (Iyenger et al.,

1995), *Everniastrum cirrhatum* (Shahi et al., 2000 a), *Cetraria pallescens, Cladonia furcata, Leptogeum trichophorum, Lobaria retigera* (Shahi et al., 2000 b), *Peltigera paratextala* (Shahi et al., 2001), *Everniastrum cirrihatum* (Shahi et al., 2002 a), *Usnea longisimma* (Shahi et al., 2002 b), *Casia alata* (Villa Senor et al., 2002) found effective against *Trichophyton rubrum.*

Kumar et al., (2017) summariesd the antidermatiphytic activity of various commercially available and conventionally available herbals from different plants and their major role in prevention of *Trichophyton*. The mode of action of dermatophytes causing dermatophytosis as well as mode and mechanism of action of various antifungal agents (herbal extracts, essential oils, suspensions and commercially available compounds) have also discussed in their review.

PHYSICAL METHODS

Smijs et al., (2007) investigated several factors that may affect the PDT (photodynamic treatment) efficacy of sylsens B and DP mme towards the dermatophyte *Trichophyton rubrum* when applied in different growth stages. The efficacy of sylsens B was pH dependent for every tested growth stages with an optimum at 5.2. In case of DP mme. hardly any effect was observed at acidic pH values and the maximum PDT effectiveness was observed at pH 7.4 at 17 h after spore inoculation. For both photosensitizers, a shorter growth stage resulted in a stronger PDT effect.

Vural et al., (2007) studied the effect of laser irradiation on *Trichophyton rubrum* growth. They had reported significant inhibitory effect upon the fungal colony *in vitro*. Cronin et al., (2014) studied the photodynamic effect of Rose Bengal on *Trichophyton rubrum.* They found that Rose Bengal photosensitization using a green laser provides a potential novel treatment for *T. rubrum* infection. Huang et al., (2017) investigated the inhibitory effect of 420 nm intense pulse light on *Trichophyton rubrum* growth. They observed that intense pulse light irradiation can induce oxidative stress in *Trichophyton rubrum* to lead fungal injuries and health.

CONCLUSION

The purpose of this study was to review the recent studies about the isolation, methods of isolation and management of *Trichophyton rubrum* causing dermatomycoses. Direct Mycological Examination Method, Culture Method, PCR with Specific Primers, PCR with Pan- Fungal Primers/Sequencing Method, Mass Spectrometry (MRM), Western Blotting Method, ELISA and Immunological Methods are different tools for the

diagnosis and rapid identification of the pathogen (Monod and Mehul, 2019). Many antifungal agents obtained from plant (e.g: herbal extracts, essential oils etc.) and commercially available compounds (e.g: Terbinafine, Fluconazole, Itraconazole, Cetoconazole, Tioconazole, Amphotericin B, Griseofulvin, Acriflavine etc.) were studied for the control and management of *Trichophyton rubrum.* Complete understanding of Pathomechanism of the dermatophytic infection with special reference to *Trichophyton rubrum* is still less studied. Further study is also needed to explain the molecular mechanism of the antifungal drug resistance and the genetic factors of host that cause more susceptibility to recurrent dermatophytoses.

ACKNOWLEDGMENTS

Author is thankful to Dr. Nisha Misra, Retd. Prof. and Head, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur for her guidance and valuable suggestions.

REFERENCES

Ajello, L. (1962). Present day concepts in the dermatophytes. *Mycopathologia et mycologia Applicata.* 17: 315-324.

Alteras, I. and Lehrer, N. (1977). Critical survey of 1000 cases of dermatophytes in Tel Aviv area during 1970-75. *Mycopathologia,* 95(1): 37-40.

Amer, M., Toha, M. and Tosson, Z. (1980). The effect of aqueous garlic extract on the growth of dermatophytes. *International Journal of Dermatology,* 19: 285-287.

Amvam Zolla, P.H., Biyiti, L., Tchoumbongnang, F., Menut, C., Lamaty, G. and Bouchet, P.H., (1998). Aromatic plants of tropical Central Africa, Part xxxii. Chemical composition and antifungal activity of thirteen essential oil from aromatic plants of Cameron. *Flavour and Fragrance Journal.* **13**: 107-114.

Anstey, A., Lucke, T. W. and Philpot, C.(1996). Tinea capitis caused by *Trichophyton rubrum. British Journal of Dermatology*, 35(1): 113-115.

Arora, B. M., Dad, S. C. and Patgiri, G.P. (1979). Dermatomycosis in pigs (Rubromycosis). *Indian Veterinary Journal.* 56: 791-793.

Baeza, L. C., Matsumoto, M.T., Almeida, A. M. F. and Mendesgiannini, M.J.S. (2006). Strain differentiation of *Trichophyton rubrum* by Randomly Amplified Polymorphic DNA and analysis of r DNA non- transcribed spacer. *Journal of Medicasl Microbiology.* 55:429-436.

Band Cirenko, A. S., Aizanman, B. Y.., Prykhod, Ko. Vo., Mashcherya, A. O., Skorobanat, Ko. TI. and Mishenkova, Y. L. (1972). Antibiotic properties of *Psoralea drupacea* Bye essential oil. Mikrobiol ZH (KYYJV). 34: 612-616.

Benger, S, Townsend, S., Ashford, R. L. and Lambert, P. (2004). An *in vitro* study to determine the minimum inhibitory concentration of Melaleuca alternifolia against the dermatophyte Trichophyton rubrum. *The Foot*. 14: 86-91.

Bhatia, V. K. and Sharma, P. C.(2014). Epidemiological studies on dermatophytosis in human patients in Himachal Pradesh, India. *Springerplus*.3:134.

Buzina, W. D., Lang- Loidolt and Ginter- Hanselmayer, G. (2004). Case report- *Trichophyton rubrum* in the external auditory meatus. *Mycoses*. 47(1-2): 85.

Chile,S.K., Saraf,M. and Barde, A.K. (1981). Efficacy of *Vinca rosea* extract against human pathogenic strains of *Trichophyton rubrum*. *Indian Drugs Pharmaceutical Industries*. 16: 31-33.

Concha, J.M., Moore, L.S. and Hollway, W.J. (1998). Antifungal activity of *Melaleuca altrernifolia* against various pathogenic organisms. *Journal of Americans Podiatric Medical Association* 88: 489- 92.

Conant, N.F., Smith, D. T., Baker, R. D. and Callaway, J. L. (1971). Manual of clinicalmycology 3rd ed. W.B. Sauders Co. Philadelphia.

Cremer, G. (1953). A special granulomatous form of mycosis on lower legs caused by *Trichophyton rubrum*. *Dermatologica*. 107:28–37.

Cronin, L., Moffitt, M., Mawad, D., Morton, O.C., Lauto, A. and Stack, C. (2014). An *in vitro* study of the photodynamic effect of rose bengal on *Trichophyton rubrum*. *Journal of Biophotonics*. 7(6): 410-417.

D' Auria, F.D., Laino, L., Strippoli,V., Tecca, M., Salvatore, G. and Baltinelli, L. (2001). *In vitro* activit of tea tree oil against *Candida albicans* mycelial conversion and other pathogenic fungi. *Chemotherapy* 13: 377-383.

De Vroey, C. (1985). Epidemiology of ringworm (dermatophytosis). *Semin Dermatology*. 4:185-200.

Deshmukh, S.K. and Chile, S.K. (1982). Sensitivity of some dermatophytes. *Indian Drugs*.19: 404-405.

Deshmukh, S. K., Gupta, D. K. and Tiwari, O. P. (1982). Antifungal activity of some essential oils. *Indian Drugs*. 19: 370-371.

Deshmukh, S. K., Jain, P.C. and Agarwal, S.C. (1986). A note on mycotoxicity of some essential oils. *Fitoterapia*. **57**: 295-297.

Del Palacio, A., Garau, M., Gonzalez- Escalada, A. and Teresa Calvo, M. (2000). Trends in the treatment of dermatophytosis. *Revista Iberoamericana de Micologia*.pp 148-158.

Dikshit, A. and Husain, A.(1984). Antifungal action of some essential oil against animal pathogen. *Fitoterapia*.55: 171-176.

Disalvo, A. F. (1974). Antifungal properties of a plant extract: Source and spectrum of antimicrobial activity. *Mycopathologia et mycologia Applicata.*54: 215-220.

Dixit, K., Shukla, H. S. and Dubey, P. (1986). Fungitoxic properties of some seedling extracts. *National Academy of Sciences Letters*, 9.

Dogra, S. and Uprety, S. (2016). The menace of chronic and recurrent dermatophytosis in India. Is the problem deeper than we perceive? *Indian Dermatology Online Journal.* 7: 73-76.

Emmons, C. W., Binford, C. H., Utz, J. P. and Kwon-chung, K. J. (1974). *Medical Mycology*. 3rd Ed. Philadelphia, Lea and Febiges, pp. 115-118.

Ferna´ndez-torres, B., Casrrillo, A. J., Marti'n, E., Del Palacio, A., Moore, M. K., Valverde, A., Serrano, M. and Guarro, J.(2001). *In vitro* activities of 10 antifungal drugs against 508 dermatophyte strain. *Antimicrobial Agents and Chemotherapy.* 45(9): 2524-2528.

Garg, S.C. and Dengre, S.I. (1988). Antifungal efficacy of some essential oils. *Pharmaziec.*43: 141-142.

Garg, S. C. and Oswal,V. B. (1981). *In vitro* antifungal activity of the essential oil of *Chloroxylon swietenia* Roxb. Coram. *Indian Drugs***19**: 189-190.

Garg, S. C. and Oswal, V. B. (1982). *In vitro* antifungal activity of the essential oil of *Buddleia Asiatics. Lour Rev. Ital. Eppos.* 63: 365.

George, L. K. (1960). Epidemiology of the dermatophytoses: sources of infection, modes of transmission and epidemicity. *Annals of the New York Academy of Sciences.* 89: 69-77.

Gip, L. and Martin, B. (1964). Isolation of *Trichophyton terrestre*, *T. mentagrophytes var. asteroids* and *T. rubrum* from dogs. *Acta Dermato-Venereologica* (Stockholm) 44(4): 248-250.

Gruby, M. (1843). Researches sir LA nature, le siege at least development du porrigo decalvans out phyloalopecie.*Comptes rendus de l'Académie des Sciences.* 17: 301-303.

Gupta, S. and Banerjee, A. B. (1970). A rapid method of screening antifungal antibiotic producing plants. *Indian Journal of Experimental Biology*.8: 148-149.

Hadad, M., Julio, A. Z., Lima, B., Derita, M., Feresin, G. E., Zacchino, S. A. and Tapia, A. (2007). Chemical composition and antimicrobial activity of essential oil from *Baccharis grisebachii* hieron (asteraceae). *Journal of the Chilean Chemical Society.* 52(2): 1186-1189.

Heijtmankova N., Leifertova, I. and Santavy, F. (1973). The antifungal effects of some Cupressaceae. *Acta. Univ. Palacki, Glomuc. Fact. Med.*, 66:15-20.

Hryncewicz-Gwozdz, A., Kalinowska, K., Plomer-Niezgoda, E., Bielecki, J. and Jagielski, T. (2013). Increase in resistance to fluconazole and itraconazole in *Trichophyton rubrum* clinical isolates by sequential passages *In Vitro* under drug pressure. *Mycopathologia*. 176:49-55.

Huang, H.,(2017). Effect of intense pulsed light on *Trichophyton rubrum* growth *in vitro*. *Journal of Southern Medical University*. 37(6): 853-857.

Imwidthaya, S. and Thianprasit, M. (1988). A study of dermatomycoses in Bangkok (Thailand). *Mycopathologia,* 102: 13-16.

Inouye, S., Nishiyama, Y., Uchida, K., Yamaguchi,Y. H. H., Abe,S.,(2007). The vapor activity of oregano, perilla, tea tea tree, lavender, clove, and geranium oils against a *Trichophyton mentagrophytes* in a closed box. *Journal of Infection and Chemotherapy*. 12:6, 349.

Ismail, M.T. and Al- Kafri, A. (2016). Epidemiological survey of dermatophytosis in Damascus, Syria, from 2008-2016. *Current Medical Mycology*. 2(3): 32-36.

Iyengar,M.A., RamaRao,M.P. and Rao,G., (1995). Antifungal activity of *Cassia alata* leaf extract. *Indian Drugs*. 32: 230-231.

Jayanthi, R. S., Nagamani, R., Lavanya, M. and Rao, R. (2016). Incidence and culture characteristics of dermatophytes isolated at a Tertiary Care Hospital in the Outskirts of Hyderabad, India. *InternationalJournal of Current Microbiology and Applied Sciences.* 5(8):372-389.

Kahl,W., Kurzlik-Jochym,K. and Starzyk, J. (1966). Microbiological investigations of the components of Horse-chestnut (*Aesculus hippocastanum L.*) seeds part I. Mycostatic action of the Raw extract from the horse-chestnut seeds and of the saponin glycoside aescin. Acta Biologica Cracoviensa Ser Botanica 9: 115-124.

Kaliyamoorthi, D. (2018). Prevalence of dermatophytic infection among diabetic and non- diabetic patients in a Tertiary Level Hospital in Chennai, India. *International Journal of Current Microbiology and Applied Sciences.* 7(6): 2516-2536.

Kamalam and Thambiah (1989) Tinea capitis: an endemic disease in Madras. *Mycopathologia,* 71: 45-51.

Kaur, R., Panda, P. S., Sardana, K. and Khan, S. (2015). Mycological pattern of dermatomycoses in a Tertiary Care Hospital. *Journal of Tropical Medicine.* 5.

Kim,H. and Kwang,H. (1980). A study of antifungal activity with *Polygonum ariculare*. *Korean Journal of Mycology*. **8**: 1-6.

Kishore, N., Mishra, A. K. and Chansouria, J. P. N. (1993). Fungitoxicity of essential oils against dermatophytes. *Mycoses*. 36: 211-215.

Kishore, N., Dubey, N.K. and Chansouria, J.P.N. (2001). Antimycotic activity of the essential oil of *Artemisia nilagirica*. *Flavour and Fragrance Journal*. Vol. 16(1): 61-63.

Kumar, R., Shukla, S.K., Pandey, A., Pandey, H., Pathak, A. and Dikshit, A. (2017). Dermatophytosis: Infection and prevention- A review. *International Journal of Pharmaceutical Sciences and Research.* 7(8):3218-25.

Lalita, K. H., Sirsi, M. and Govindrajan, V. S (1964). Antibacterial and antifungal activities of *Areca catechu* L. *Indian Journal of Experimental Biology.* 3: 66-67.

Lee, W. J., Kim, S. L., Hang, Y. H., Lee, S., Kim, D. W., Bang, Y. J. and Jun, J. B. (2015). Increasing prevalence of *Trichophyton rubrum* identified through an analysis of 115,846 cases over the last 37 years. *Journal of Korean Medical Sciences.* 30: 639-643.

Li, X., Jcob, M. R., Khan, S. I., Ashfaq, M. K., Babu, K. S., Agarwal, A. K., Elsohly, H. N., Manly, S. P. and Clark A. M. (2008). Potent *in vitro* antifungal activities of natural occurring acetylenic acid. *Antimicrobial Agents and Chemotherapy*. 52(7): 2442-2448.

Lopez, M.R. and Rivera Lona, M. (1984). Investigation of dermatophytes in healthy skin from different parts of human body. *Revista Latinoamericana de Microbiología*. 26(4): 365-370.

Lopez, M. R. (1984). Some observations on the ecology of the dermatophytes in human skin. *Boletin de la Sociedad Mexicana de Micologia.* 6 (18): 21-28.

Luqman, S., Dwivedi, G. R., Darokar, M.P., Kalra, A. and Khanuja, S.P.S. (2008). Antimicrobial activity of *Eucalyptus citriodora* essential oil. *International Journal of Essential Oil Therapeutics.* 2(2):69-75 (7).

Makendorf, A. J. (1952). An outbreak of *Trichophyton rubrum* infection in South Australia. *Australian Journal of Dermatology.* 1: 208-212.

Mall, H. V., (1987). Evaluation of some green plants against ringworm fungi. *Ph.D. Thesis,* University of Gorakhpur, Gorakhpur, India.

Mall, H. V., Asthana, A., Dubey, N. K. and Dixit, S. N., (1985). Toxicity of Cedar wood oil against some dermatophytes. *Indian Drugs*. 22: 296-298.

Mcaleer, R. (1981), Fungal infection of the nails in Western Australia. *Mycopathologia.* 23 (3): 51-56.

Monad, M. and Mehul, B. (2019). Recent findings in onychomycosis and their application for appropriate treatment. *Journal of Fungi.5, 20.*

Naglot, A., Shrimali, D. D., Nath, B. K., Gogoi, H. K., Vijay Veer, Chandler, J. and Tewari, R. (2015). Recent trends of dermatophytosis in northeast India (Assam) and interpretation with published studies. *InternationalJournal of Current Microbiology and Applied Sciences.* 4(11): 111-120.

Nenoff,P., Haustein,U.F. and Brandt,W., (1996). Antifungal activity of essential oil *Melaleuca altrernifolia* against pathogenic fungi *in vitro*. *Skin Pharmacology*. 9: 338-394.

Nir-Paz R., Elinav, H., Pierard, G. E., Walker, D., Maly, A., Shapiro, M. Barton, R.. C. and Polacheck, I. (2003). Deep infection by *Trichophyton rubrum* in an immunocomprised patient. *Journal of Clinical Microbiology.* 41(11): 5098-5301.

Okuda, C. M., Ito and Sato, Y. (1991).*Trichophyton rubrum* invasion of human hair apparatus in tinea capitis and tinia barbae. Light microscopic and electron microscopic study. *Archives of dermatological research.* 283(4):233-9.

Panday, D. K., Tripathi, N. N., Tripathi, R. D. and Dixit, S. N. (1982). Fungitoxic and phytotoxic properties of the essential oil of *Caesulia axillaries Roxb. Angewandte Botunik*, 56: 259-267.

Pandey, M. C., Shahi, S. K., Shukla, A. C. and Dikshit, A. (1997). Antidermatophytic bioassy of *Cymbopogon pendulus* oil at defferent pH levels. *Proceedings of National Symposium on Biodiversity Conservation and Evolution of Plants. Allahabad (abs)*: 78.

Park, M.J., Gwak, K.S., Yang,I., Choi,W.S., Jo, H.J., Chang, J.W., Jeung, E.B. and Choi, I.G., (2007). Antifungal activities of the essential oils in Syzygium aromaticum (L.) Merr. Et Perry and *Leptospermum petersonii* Bailey and their constituents against various dermatophytes. *Journal of Microbiology*. 45(5):460-5.

Patra, M. and Shahi, S. K. (2000). Utilization of essential oil as natural antifungal against nail infective fungi. *Proceedings of 23rd All India Botanical Conference. Meerut (abs)*: 53.

Patra, M., Shahi, S.K. and Dikshit, A. (2001).Antifungal evaluation of clove oil for the development of natural antifungal against onychomycosis. *Mycoses*,Germany.

Patra, M., Shahi, S. K., Midgely, G. and Dikshit, A. (2002). Utilization of essential oil as natural antifungal against nail infective fungi. *Flavor and Fragrance J.*17: 91-94.

Peachey, R. D. G. and English, M.P. (1974). An outbreak of *T. rubrum* infection in a Friatric Hospital. *British Journal of Dermatology.* 91: 389-397.

Peres, N. T. A., Maranhao, F. C. A., Rossi, A. and Martinez-Rossi, N. M. (2010). *Anais Brasileiros de Dermatologia* 85(5): 657-667.

Poojary, S. A. (2017). Topical antifungal: A review and their role in current management of dermatophytoses. *Clinical Dermatology Reviews.* 1: S24-S29.

Rad, M. M., Jackson, C., Barton R. and Evans E.G.V.(2005). Single strains of *Trichophyton rubrum* in cases of tinea pedis. *Journal of Medical Microbiology*. 54: 725-726.

Ramraj, V., Vijayaraman, S. R., Rangarajan, S. and Kindo, A. J.(2016). Incidence and prevalence of dermatophytosis in and around Chennai, Tamilnadu, India. *International Journal of Research in Medical Sciences.* 4(3): 695-700.

Rao, B. G. V. N. and Joseph, P. L. (1971). Activity of some essential oils towards phytopathologic fungi. *Riechest Aroma Koer* 21: 405-410.

Rao, N. B. G. V..and Rao, S. P. (1982). The efficacy of some essential oils on pathogenic fungi. *Flavour Industry*. 3: 368.

Rippon, J. W.(1988).*Medical Mycology*, the pathogenic fungi and pathogenic actinomycetes, 3rd ed., WB Saunders, *Philadelphia.*

Sahni K, Singh S, Dogra S. (2018). Newer topical treatments in skin and nail dermatophyte infections. *Indian Dermatology Online Journal.* 9:149-58.

Santos, D. A., Barros, M.E. and Hamdan, J. S. (2006). Establishing a method of inoculum preparation for susceptibility testing of *Trichophyton rubrum* and *Trichophyton mentagrophytes. Journal of Clinical Microbiology.* 44: 98-101.

Saxena,N.K., 1984. Comparative evaluation of some essential oils for their antifungal activity against some dermatophytes. *Indian Perfumer* 28: 35-37.

Saxena,V.K., Sahai,A. and Snadya,G., (1984). Studies on antimicrobial efficacy of essential oil of the leaves of *Anaphalis contorta*. *Indian Perfumer* 28: 177-178.

Schoenlein, J.L. (1839). Zur pathogenic impetigines. *In: Müller J, ed. Archiv für Anatomie, Physiologie und wissenschaftliche Medicin, 1st ed. Berlin.* 82.

Sentamilselvi, G., Kamalam, A., Ajithadas, K., Janaki, C. and Thambiah, A. S.(1998). Scenario of chronic dermatophytosis: An Indian study. *Mycopathologia.* 140: 129-135.

Smijs, T. G. M., Bouwstra, J. K., Talebi, M. and Pavel, S. (2007). Investigation of conditions involved in the susceptibility of the dermatophyte *Trichophyton rubrum* to photodynamic treatment. *Journal of Antimicrobial Chemotherapy*. DOI: 10.1093/jac/dkm304.

Sinski, J.I., Kelley, M.L., Flynt, P.M. and Miegel J. (1977). Dermatophyte isolation media: Quantitative appraisal using skin scales infected with. *Trichophyton mentagrophytes* and *T. rubrum. Journal of Clinical microbiology*. 5: 34-38.

Shahi, S. K. (1997). Antimycotic studies of some plants (control of dermatophytosis in human beings). *D. Phil Thesis*, Allahabad University, Allahabad.

Shahi,S.K., Shukla,A.C. and Pandey M. C.(1996). *In vitro* and *in vivo* studies of *Trachyspermum ammi* against human pathogenic. Fungi. *Proceedings Nineteenth All India Botanical Conference.*Haridwar(abs): 75.

Shahi, S. K., Shukla, A. C., Bajaj, A. K. and Dikshit, A., (1997a). *Cymbopogon flexuosus* vis-a-vis dermatophytoses in human beings. *Proceedings of 84th Indian Science Congress. New Delhi. IV (abs)*: 68.

Shahi, S. K., Shukla, A. C. and Dikshit, A. (1997b). Increased toxicity of the essential oil of some *Eucalyptus* Spp. at different pH levels. *Proceedings of Seminar on Herbal and pesticide. Varanasi. (abs)*: 3.

Shahi, S. K., Shukla, A. C., Bajaj, A. K., Medgely, G., and Dikshit, A. (1999). Broad spectrum antimycotic drug for the control of fungal infection in human being. *Current Science.*76,836-839.

Shahi, S. K., Shukla, A. C. Bajaj, A. K., Midgely, G., Banerjee, U., and Dikshit, A. (2000). Broad spectrum herbal therapy against superficial fungal infections. *Skin Pharmacology and Applied Physiology*. 13: 60-64.

Shahi, S. K., Shukla, A. C., Bajaj, A. K., Midgely, G., Benerjee, U., Rimek, D. and Dikshit, A., **(**2000a). Broad spectrum herbal therapy against superficial fungal infections. *Skin Pharmacology and Applied Skin Physiology .13: 60-64.*

Shahi, S.K., Shukla, A. C., Uperti, D. and Dikshit, A., **(**2000b). Use of lichens as antifungal drugs against superficial fungal infections. *Proceedings National Seminar on the Frontiers of Research and Development in Medicinal Plants. (eds): 22/4A, 23/1A: 169-172.*

Shahi, S. K., Shukla, A. C., Singh, R., Mishra, R. C., Pandey, K.P., Patra, M., Dutta, S. Bajaj, A. K., and Dikshit, A. (2001). Botanical antifungal therapy against fungal infection in human beings. *Proceedings of 27th Annual Meeting of Mycological Society of India, Gorakhpur*. (Abs): 91-92.

Shahi,S.K., Patra,M., Dikshit,A. and Uperti,D.K., (2002a). Botanical antifungal drug from lichen metabolites fight fungal infections. *7th International mycological congress (abs)*. Norway.

Shahi,S.K., Patra,M., Dikshit,A. and Upreti,D.K., (2002b). Utilization of lichen as natural antifungal drug against superficial fungal infection in humans. *Proceedings of 89th Session of Indian Science Congress Association, Lucknow (abs)*. 84.

Sharma, V., Kumawat, T. K., Sharma, A., Seth, R. and Chandra, S. (2015). Distribution and prevalence of dermatophytes in semi- arid region of India. *Scientific Research*. 05(2).

Sharma,S.K. and Singh,V.P., (1979a). Antifungal study of the essential oil of *Oenanthe javanica* Blume DC. *Indian Drugs*16: 289-291.

Sharma,S.K. and Singh,V.P. (1979b). Antifungal activity of some essential oils. *Indian Drugs* 14: 3-6.

Shukla, N.P., Agarwal, G.P. and Gupta D.K. (1985). Effect of temperature on growth and survival of dermatophytes. *Indian Journal of Medical Research.* 79(May): 617-623.

Silva, M. R. R., Oliveira, J. G.Jr., Fernandes, O. F. L., Passos, X. S., Costa, C. R., Souza, L.K.H., Lemos, J. A. and Paula, J. R., (2005). Antifungal activity of *Ocimum gratissimum* towards dermatophytes. *Mycoses*48(3):172 -175.

Simpanya, M. F. (2000). Dermatophytes : Their taxonomy, ecology and pathogenicity. *Revista Iberoamericana de Micologya,* 1-12.

Sokovic,M.D., Vukojevic,J., Marin,P.D., Brkic,D.D. and Vajs,V.(2009). Chemical Composition of Essential Oils of *Thymus* and *Mentha* Species and Their Antifungal Activities, *Molecules,* 14:238-249.

Steinmetz,M.D., Rascol,J.P., Regli,P., Gargadennec,A.and Andary,C. (1995). *In vitro* antifungal activity of polyporaceae against yeasts and dermatophytes. *Mycoses*38: 305-309.

Sumit Kumar, Shrikara Mallya, P. and Shalini Shenoy, M. (2012). *Trichophyton rubrum*: The common isolate from dermatophytosis. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 3(1): 101-107.

Tadaro, F., Germano, D. and Criseo, G. (1983). An outbreak of tinea pedis and tinea cruris in a tyre factory in Messina, Italy. *Mycopathologia.* 83(1): 25-7.

Takazawa,H, Tajima,F and Miyashita,C (1982). An antifungal compound from Shiitake (*Lentinus edodes.*). *Yakugaku Zasshi* .102: 489-491.

Tambekar, D. H., Mendhe, S. N. and Gulhane, S. R. (2007). Incidence of dermatophytes and other keratinolytic fungi in the soil of Amrawati (India).*Trends in Applied Research*. 2(6): 545-548.

Tansey,M.R. and Appleton,J.A., (1975). Inhibition of fungal growth by garlic extract. *Mycologia*67: 409-413.

Thind,T.S. and Dahiya,M.S. (1976). Inhibitory effect of essential oils of some medicinal plants against soil inhabiting dermatophytes. *Indian Drugs*14: 17-20.

Venkatesan, G., Singh, A. J. A. R., Murugesan, A. G., Janaki, C. and Gokul Shankar, S. (2007). *Trichophyton rubrum*- the predominant etiological agent in human dermatophytoses in Chennai, India. *Afrrican Journal of Microbiological Research.* pp. 009- 012.

Villasenor,I.M., Canlas,A.P., Pascua,M.P.I., Sabando,M.N. and Soliven,L.A.P.(2002). Bioactivity studies on *Cassia alata* Linn. Leaf extracts. *Phytotherapy Research*16: 93- 96.

Viral, E., Winfield, H.L., Shingleton, A. W., Horn, T. D. and Shafirstein, G.(2007).The effect of laser irradiation on *Trichophyton rubrum* growth. *Lasers in Medical Science.* 23(4):349-353.

Wannissorn,B., Jarikasem,S. and Soontorntanasart,T. (1996). Antifungal activity of lemon grass oil and lemmon grass oil cream. *Phytoherapy Research.* 10: 551-554.

Weitzman, I. and Summerbell, R.C. (1995). The Dermatophytes.*Clinical Microbiology Reviews.* 8(2):240-259.

WHO, (1986). Guidelines for the diagnosis, prevention and control of dermatophytosis in man and animals. In: Mackenzie D. W. R., Loeffler, W., Mantavoni, A., Fujikura, T. (EDS) CDS/VPH.

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 14

MYCOTOXINS AND THEIR IMPACTS ON HUMAN HEALTH

K. Anita*
Department of Botany, M.G.P.G. College, Gorakhpur, Uttar Pradesh, India

ABSTRACT

Mycotoxins are low molecular weight compounds. These are secondary metabolites, secreted by a variety of fungi. They are ubiquitous and may be present in cereals, cereal products and foods, feeds, animal products and soil. Consumption of such contaminated food with mycotoxin is a serious threat to human health. The effect of mycotoxins on human and animal health depends on the amount of the mycotoxin consumed, the toxicity of the compound and the body weight of the individual. Mycotoxins are reported as carcinogenic, mutagenic, teratogenic, estrogenic and immunosuppressive compounds. They are heat stable, thus, can not be not destroyed by cooking and any industrial processes. There are many factors which favour mycotoxin production as temperature, climate, moisture content, oxygen levels, preservatives, fungal strain and microbiological ecosystem. Determination of mycotoxin level in food sample can be accomplished by different techniques as chromatographic technique, immunochemical technique, molecular technique, biosensor technique etc. This chapter explains the nature of mycotoxins and its impact on human health.

Keyword: mycotoxins, mycotoxicoses, secondary metabolites, toxicity, fungi, immunological techniques, chromatographic techniques, biosensor techniques

* Corresponding Author's Email: anitamaurya913@gmail.com.

INTRODUCTION

Mycotoxins are low molecular weight, highly toxic, secondary metabolites, produced by a variety of fungi. Favourable environmental conditions for mycotoxin production include various factors such as high temperature, relative humidity, heavy rains, poor hygienic practices during transportation, improper storage and processing (Assefa and Geremew, 2018)). The main mycotoxin producing genera in foods are *Aspergillus, Fusarium and Penicillium. Alternaria, Claviseps* and *Stachybotrys* are also considered as important mycotoxin producers. They are very common and may be present in cereals, cereal products, nuts, dried fruit, fruit juice, spices, feeds, animal products and soil. Approximately twenty five percent of the world's crops are affected by moulds or filamentous fungi (Mannon and Johnson, 1985). Consumption of contaminated food with mycotoxin is a serious threat to human and animal's health. Mycotoxicoses are diseases caused by the toxic effect of mycotoxins. Acute mycotoxicoses can cause serious and sometimes fatal diseases. The symptoms of a mycotoxicosis depend on the type of mycotoxin, the amount and duration of the exposure, the age, health, sex of the exposed people, and many poorly understood synegistic effects involving genetics, dietry status and interaction with other toxins. The severity of mycotoxins poisoning can be compounded by factors such as vitamin deficiency, caloric deprivation, alcohol abuse and infectious disease status. In turn, mycotoxicoses can heighten vulnerability to microbial diseases, worsen the effect of malnutrition and interact synergistically with other toxins (Bennett and Klich, 2003).

MAIN MYCOTOXINS

Presently, about 300 mycotoxins are identified. The most important and highly toxic mycotoxins include: aflatoxin, fumonisins, ochratoxins, citrinin, ergot alkaloid, patulin, trichothecenes and zearalenone.

Aflatoxin

Aflatoxins are a group of closely related secondary metabolites produced by *Aspergilus flavus* and *A. parasiticus.* They are first known mycotoxin; identified and characterised chemically in 1960, after the death of more than 100,000 turkey poults due to consumption of contaminated peanut's feed (Blount, 1961). The main components of aflatoxins are called aflatoxin B_1, B_2, G_1, G_2 (based on their florescence under UV light and relative chromatographic mobility during thin- layer chromatography) M_1 and M_2

(produced in milk and dairy products). Aflatoxins are difuranocoumarine derivatives produced by polyketide pathway by many strains of *Aspergillus flavus* and *A. parasiticus.* Aflatoxin contamination has been linked to increased mortality in farm animals and thus, significantly lowers the value of grains as an animal feed and as an export commodity. Milk products can also serve as an indirect source of aflatoxin. When cow consume aflatoxin contaminated feeds, they metabolically bio transform aflatoxin B_1 into a hydroxylated form called aflatoxin M_1. Aflatoxin is associated with both toxicity and carcinogenicity in human and animal population. Acute aflatoxicosis results in death while chronic aflatoxicosis results in cancer, immune suppression and other slow pathological conditions (Zain, 2011).

Fumonisins

Fumonisins were first described and characterised in 1998. They are produced by a number of *Fusarium* species, notably *Fusarium verticillioides* (formerly *Fusarium moniliforme*), *Fusarium proliferatum* and *Fusarium nygamai*, as well as *Alternaria alternata* f. sp. *Lycopersici* (Bennett and Klich, 2003). There are fifteen types of known fumonisins. The most important are FB_1, FB_2 and FB_3 out of these FB_1 is most toxic (Assefa and Geremew, 2018). Being hydrosoluble, fumonisins become more dangerous to human health, as they can remain undetectable most of the time.

Ochratoxins

Ochratoxins are a group of mycotoxins. Members of the Ochratoxin family have been found as metabolites of many different species of *Aspergillus* including *A. ochraceus, A. glaucus, A. alliaceus, A. auricomus, A. melleus, A. niger* and *A. carbonarius* and *Penicillium* species, especially *P. verrucosum* (Bennett and Klich, 2003). Ochratoxin A is the most prevalent and relevant fungal toxin of the group, while ochratoxin B and C are of lesser importance.

Ochratoxin A is known to occur in commodities such as cereals, coffee, dried fruit, milk and red wine. It was discovered as metabolites of *Aspergillus ochraceus* in 1965. Thereafter, it was isolated from a commercial corn sample in the United States and recognised as nephrotoxin. In addition to being a nephrotoxin, it is also reported as a liver toxin, an immune suppressant, a potent teratogen and a carcinogen.

Citrinin

Citrinin is also considered as a nephrotoxic mycotoxin and was isolated initially from *Penicillium citrinium* in 1931(Hetherington and Raistrick, 1931). It is also produced by other species of *Penicillium* (e.g., *Penicillium camamberti*), *Aspergillus* (e.g., *Aspergillus terreus, A. niveus* and *A. oryzae*) and *Monoascus* (e.g., *M. river* and *M. purpureus).* Citrinin is a compound derived from phenol. It can contaminate many commodities, especially, cereals, such as barley, corn, rice, oats and wheat. Citrinin is usually found in association with another nephrotoxic mycotoxin, Ochratoxin A. Citrinin is also embryocidal and fetotoxic and together with ochratoxin A, is believed to be responsible for the etiology of nephropathy. It is also supposed that when citrinin and ochratoxin A occur in combination in grains their effects may be exacerbated due to the similarity of the both toxins (Freire and Rocha, 2016).

Ergot Alkaloid

Sclerotia of fungi belonging to the genus *Claviceps* produce ergot alkaloids. The species which produce these alkaloids include *C. puepurea, C. paspali, C. fusiformis, C. gigantea* and *Sphacelia sorghi*. Apart from *Claviceps,* ergot alkaloids are also produced as secondary metabolites by fungal species belonging to *Penicillium, Aspergillus* and *Rhizopus* (Bhat et al, 2010).The ergot alkaloids isolated from *Claviceps* sclerotia are structurally related to the hallucinogenic drug known as lysergic acid diethyl amide (LSD) and are usually divided into three groups: derivatives of lysergic acid (e.g., ergotamine and ergocristine), derivatives of isolysergic acid (e.g., ergotaminine) and derivatives of dimethylergoline (e.g., agroclavine)(Freire and Rocha, 2016).

Patulin

Patulin was first isolated in 1940s from *Penicillium patulum*. It is mycotoxin that forms smallest group of toxic metabolites referred to as plolyketide and is reported to be produced by other fungi such as *Aspergilllus spp.* (*A. Giganteus and A. terreus*), *Penicillium expansum, Paecilomyces* and *Byssochlamys spp.* (*B. nivea and B. fulva*). Patulin was also isolated from other species and given the name clavivin, claviformin, mycoine c and penicidin. Patulin is considered to be the most dangerous mycotoxin in fruits, particularly apples, pears and their products (Freire and Rocha, 2016).

Trichothecenes

Trichothecenes are mycotoxin produced mostly by the members of the *Fusarium* genus, although other genera (e.g., *Trichoderma*, *Trichothecium*, *Myrothecium* and *Stachybotrys*) are also known to produce these compounds. Total 150 trichothecenes have been isolated, but only a few have been found to contaminate food and feed. The most frequent contaminants are deoxynivalenol (DON), nivalenol (NIV), diacetoxycsirpenol (DAS), while T-2 toxin is rarer (Peraica et al, 1999). According to functional groups, the trichothecenes are divided into four groups: Type A includes HT-2 toxin and T-2 toxin, Type B includes DON, NIV and FUS-X. The type C and D trichothecenes are characterised by a second epoxide or an ester-linked macrocycle, respectively (Freire and Rocha, 2016).

Zearalenone

Zearalenone is a non-steroidal estrogenic mycotoxin. It is a secondary metabolite produced principally by *Fusarium graminearum.* Some other species as *F. culmorum, F. equisetii, F. crookwellense* and *F. sporothichiodes* also reported to produce zearalenone. It shows hormonal activity in many animal species which make it genotoxic, carcinogenic or both at the same time, and it can impose changes in the reproductive organs and systems of many laboratory animals such as mice and rats and different domestic animals such as horses. Thus, zearalenone is capable of causing reproductive organ cancer in both man and animal (Ahmed Adam et al., 2017). Among, the human population, children are the most affected due to consumption of zearalenone contaminated cereal- based food product e.g., grains, corn, barley and wheat grains (Freire and Rocha, 2016).

TOXICITY OF THE MYCOTOXIN

A broad range of commodities can be contaminated with mycotoxins. They are highly toxic and induce different effects on humans. Some are carcinogenic (aflatoxin, ochratoxins and fumonisins), mutagenic (aflatoxin and sterigmatocystin), teratogenic (ochratoxins), estrogenic (zearalenone), hemorrhagic (trichothecenes), immunotoxic (aflatoxin and ochratoxins), nephrotoxic (ochratoxins), hepatotoxic (aflatoxin and phomopsins), dermatotoxic (trichothecenes) and neurotoxic (ergotoxins) (Bueno et al. 2014).

Table 1. IARC Monographs evaluations of carcinogenic hazards of mycotoxins to humans (IARC, 2012)

Mycotoxin	Overall evaluation of carcinogenicity to humans[a]
Aflatoxin, naturally occurring mixture of	Group 1
Aflatoxin B1, B2, G1, G2	Group 1
Aflatoxin M1, Ochratoxins, Fumonisins	Group 2B
Patulin, Citrinin, T-2	Group 3

a-Group 1: carcinogenic to humans;
Group 2B: possibly carcinogenic to humans; Group 3: not classifiable as to its carcinogenicity to humans;

IMPACT OF MYCTOXIN ON HUMANS

Shephard (2008) studied the impact of mycotoxin on human health in developing countries. He reported that aflatoxin plays significant role in liver cancer in developing countries. Bhat et al., 2010, in their review article, discussed various types of mycotoxins in food and feed associated with risks to human and livestock as well as legislation put forth by various authorities. Zain (2010) summarized the negative effects of mycotoxin on human, ruminants (e.g., cattle, sheep, white-tailed deer), non-ruminants (e.g., pigs, poultry, dog, cats, rat and mice) in his article. He also explained the factors, affecting production, contamination of foods and feeds, and toxicity of mycotoxin. He also discussed the different methods for regulation of mycotoxin in foods and feeds. Freire and Rocha (2016) explained the different types of mycotoxins and their adverse effects on human health. They discussed in brief the legislation for mycotoxin in food and feed in different continent e.g., Africa, Asia, Europe, North America and Latin America. Jahanian (2016) updated a mini-review on the current concept on mycotoxin-induced toxicity. Patho-physiological mechanism responsible for cis-platin nephrotoxicity are discussedby him. Mycotoxin contamination induced oxidative stress and apoptosis; consequently, they involved in the regulation of gene expression and finally, cause many health problems such as genotoxicity, immunotoxicity, hepatotoxicity, neurotoxicity etc. Ahmed Adam et al. (2017) reviewed the chemical and physical nature of mycotoxins and the action of mycotoxins on the cellular genome. Many mycotoxins such as aflatoxin B1, citrinin, fumonisins, patulin, trichothecenes and zearalenone have the ability of causing major changes to the human genome which may result in the development of different types of cancer related to different organs as liver, kidney, oesophageal, lung, renal, breast, testicular, stomach and reproductive organ cancer. Chhonker et al. (2018), in their review article, are discussed the different human diseases (Akakabio-byo/red mold disease, alimentary toxic aleukia, balkan nephropathy, cardiac beriberi, celery harvester's disease, ergotism, hepatocarcinoma, kwashiorkor, neural tube defects, oesophageal tumors, onylalai, Reye's syndrome and stachybotryotoxicosis) caused by mycotoxins

contamination. In their article, aflatoxin B1 is reported as a potent hepatocarcinogenic substance. Ratnaseelan and Theoharides (2018) reviewed about 150 articles and reported the neuropsychiatric effect from mold exposure on adults and children. He found that individuals exposed to mold were impaired on a variety of cognitive measures, including verbal learning and memory, psychomotor speed and emotional functioning. But some contradictory reports were also found. They have also interpreted that some neuropshychiatric symptoms may be associated with socioeconomic factors present in individuals living and moldy dwellings.

MAJOR OUTBREAK IN WORLD DUE TO MYCOTOXINS

Outbreaks of ELEM (Equine leukoencephalomalacia) in many countries such as Egypt, South Africa and USA. ELEM is a fatal neurological disease of horses, characterised by liquefactive necrosis of the white matter of the brain (Steyn, 1995). Peraica (1999) reviews the major outbreaks of mycotoxicoses. They also discussed the epidemiological, clinical and histological findings in outbreaks of mycotoxicoses resulting from exposure to aflatoxins, ergot, trichothecenes, Ochratoxins, 3-nitropropionic acid, zearalenone and fumonisins. Reddy and Raghavender (2007) reviewed the disease outbreaks of aflatoxicosis in India, due to the ingestion of contaminated food and feed with aflatoxin.

MYCOTOXIN ANALYSIS TECHNIQUES

The basic techniques used and the problems encountered in isolation of mycotoxins from naturally contaminated commodities and fungal cultures are discussed by Cole (1978). He also discussed the basic factors to be considered in selecting a bioassay organism, methods of preparing formulations for mycotoxin administration, modes of administration, methods available for purification and problem encountered during purification. Bhat et al. (2010) summarized the recent methods developed for the detection of mycotoxins from different food commodities. They have also provided a brief summary of recent and modern pre- and postharvest methods adapted for the removal of mycotoxins in human food, by different researchers. These methods include chemical treatment, radiation treatment, alkaline treatment, thermal treatment, through fermentation, by the use of bentonite and modified bentonite and many more methods. Fernandez-Cruz et al. (2010) reviewed the toxicity of major mycotoxins, their natural occurrence in fruits, dried fruits, juices, wines and other processed products, the analytical methods available for their determination and the strategies for their control.

Table 2. Major outbreaks in world due to mycotoxins

Year	Country	Disease/Clinical symptoms	Subject/organism	Fungus	Types of Mycotoxin	Commodity	References
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Middle Age	Europe	St. Anthony's fire	Human	*Claviceps purpurea*	Ergot alkaloids	Wheat	Van Dongen and de Groot (1995)
1932-47	USSR	Alimentary toxic aleukia	Human	*Fusarium poae, F.sporotrichoides*	Trichothecenes	Grains	Yagen and Joffe (1976)
1956	Japan	Scabby grain toxicosis' nausea, vomiting, drowsiness	-	*Fusarium roseum, F. nivele*	-	Rice	Ueno (1971)
1960	England	Turkey 'X' disease	Turkey	*Aspergillus*	Aflatoxin	Groundnut	Blount (1961)
1970	Uganda	Abdominal pain, oedema of legs, palpable liver, right bundle branch block	Human		Aflatoxin	Cassava	Serck-Hanssen. (1970)
1971	Japan	yellow rice syndrome	human	*P. citrinum*	Citrinine	grains	Saito, et al., (1971)
1974, 1975	India	Aflatoxicosis	Human, children, dog	*Aspergillus flavus A. parasiticus*	Aflatoxin B1 Aflatoxin B2	Maize Groundnut	Krishnamachari et al.,(1975)
1975	India	Ergotism	Human	*Claviceps fusiformis*	Clavine alkaloids	-	Krishnamachari and Bhat (1976)
1975-81	lal People's Democratic Republic	"Yellow Rain"	Human	Aircraft spray leaf	Nivalenol Deoxynivalenol T2	-	Mirocha et al., (1983)
1977	India	Fever, vomiting, jaundice, ascites, oedema of leg, hepatomegaly, splenomegly	Human	-	Aflatoxin B1	Maize	Tandon et al., (1976)
1977-1978	Ethiopia	Gangrenous Ergotism	Human	*Claviceps purpurea*	Ergotamine-Ergocristine alkalod	Barley and Oat	King (1979)
1978-81	Combodia	Vomiting, diarrhoea, haemorrhage, breathing difficulty.	-	Water, Yellow- green powder	Zearalenone Deoxynivalenol Diiaceyoxy-scipenol, T2	-	Watson et al., (1984)
1980	USA	Non- pruritic macular rash, nausea, headache	Human	-	Purified Aflatoxin B1	-	Willis (1980)

Year	Country	Disease/Clinical symptoms	Subject/organism	Fungus	Types of Mycotoxin	Commodity	References
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1981-86	USA	'Sick building syndrome' recurring maladies, cold and flu like symptoms, sore throats, diarrhoea, headache, fatigue, dermatitis	Human	-	Trichothecene	-	Croft et al., (1986)
1982	Kenya	Acute hepatitis	Human	*Aspergillus*	Aflatoxin B1 Aflatoxin B2	Maize	Ngindu et al., (1982)
1985-2003	China	'Scabby grain toxicosis' nausea, vomiting, abdominal pain, diarrhoea, dizziness, headache	Human	*Fusarium sp.*	Deoxy-nivalenol, Zearalenone	wheat Corn	Jianbo et al., (2019)
1987	India	Mild to moderate abdominal pain, feeling of fullness, irritation of throat, diarrhoea, blood in stool	Human	*Fusarium sp.* *Aspergillus flavus*	Nivalenol Deoxynivalenol T2, Acetyldeoxy-nivalenol,	wheat	Bhat et al., (1989)
1987	India	Alimentary toxic aleukia	Human	*Fusarium*	Trichothecene	wheat	Reddy and Raghavender, (2008).
2004	Kenya	Jaundice Aflatoxicoses	Human	*Aspergillus flavus* *A. parasiticus*	Aflatoxin	maize	CDC (2004)
2005, 2006	Kenya	Jaundice Aflatoxicoses	Human	*Aspergillus flavus* *A. parasiticus*	Aflatoxin	maize	Schmale and Munkvold (2009)
2005	United States	Liver problem	Dogs		Aflatoxin	Pet food	Schmale and Munkvold (2009)

Bueno et al. (2014) also discussed different methods to detect mycotoxins. These are screening techniques viz. immunoassay, biosensors, separation techniques viz. thin layer chromatography (TLC), gas chromatography (GC), liquid chromatography (LC), capillary electrophoresis and electroanalytical methods viz. potentiometry, coulometry and voltammetry. Maximum accepted limits, in different countries, for aflatoxin, ochratoxins A, deoxynivalenol, patulin, fumonisins and zearalenone are also reported in their article. Assefa and Geremew (2018) discussed the different techniques for analysis of mycotoxin as various chromatographic techniques (e.g., TLC, HPLC, HPLC-FLD, HPLC-MS/ MS, Luminex's xMAP), immunochemical techniques (e.g., enzyme linked immunosorbent assay-ELISA, florescence polarization immunoassay, biosensor technology) and molecular techniques (e.g., DNA and aptamer based biosensor, Molecular imprinting polymers). They have also explained the prevention and control strategies for mycotoxin production. Carballo et al. (2018) described an analytical method for simultaneous determination of mycotoxins residues in ready-to-eat food samples using "Quick Easy Cheap Rough and Safe" (QuEChERS) extraction and chromatographic methods coupled to mass spectrometry in tandem. Mycotoxins included in this survey were aflatoxins B_1, B_2, G_1, G_2), enniatins (A, A_1, B, B_1), beauvericin (BEA), fumonisins (FB_1, FB_2), sterigmatocystin (STG), deoxynivalenol (DON), 2-acetyl-deoxynivalenol (3-ADON), 15-acetyl-deoxynivalenol (15-ADON), nivalenol (NIV), neosolaniol (NEO), diacetoxyscirpenol (DAS), fusarenon-X (FUS-X), zearalenone (ZEA), α-zearalanol (α-ZAL), β -zearalenone (β -ZAL), α-zearalenol (α-ZOL), β -zearalenol (β-ZOL), T2 and HT-2 toxin. Fallahi et al. (2019) reported isolation, molecular identification and mycotoxin profile of *Fusarium.* From 182 samples of maize kernels, 551 strains were isolated and identified as belonging to *Fusarium* genus. The stains were identified at species level by translation elongation factor sequences.

CONCLUSION

Mycotoxin contamination of food has many adverse effects on human health. Prevention of mycotoxin contamination of crops is the primitive and best strategy for the control of harmful impact of mycotoxicoses. Assefa and Geremew (2018) illustrated different mycotoxin control strategies as physical control, chemical control (mycotoxin detoxification) and biological control (microbial strategies, biotechnology for mycotoxin elimination, molecular breeding approaches etc.). Chkuaseli et al., (2016) demonstrated that the application of Askangel, locally produced bentonite clay of aluminicilicat origin, for the detoxification of mycotoxins in poultry feed is highly effective in scientific and practical terms.

Most of the studies conducted on the effect of mycotoxins on the biological system and their carcinogenecity- toxicity, were done on animals model and the action of

mycotoxins in human biological systems were simply hypothesised and no solid data were generated for many mycotoxins (Ahmed Adam et al. 2017). Mycotoxin may cause major changes to the human genome which results in development of different diseases. Further researches are necessary to study the effect of mycotoxins on human biological system. Considering the harmful impacts of mycotoxins on human health, further investigation need to be conducted to develop easy and quick methods to detect the mycotoxins in food so that contaminated food can be avoided.

ACKNOWLEDGMENT

Author is thankful to Dr. Nisha Misra, Retd. Prof. and Head, Department of Botany, D.D.U. Gorakhpur University, Gorakhpur for her supervision and valuable suggestions in all phases of the preparation of this article.

REFERENCES

Ahmed Adam, M. A., Tabana Y. M., Musa, K. B. and Sandai, D. A. (2017). Effects of different mycotoxins on humans, cell genome and their involvement in cancer. *Oncology Reports*, 37: 1321-1336.

Assefa, T. and Geremew, T. (2018). Major mycotoxins occurrence, prevention and control approaches. *Biotechnology and Molecular Biology*. 12(1), 1-11.

Bennett, J. W. and Klich, M. (2003). Mycotoxin. *Clinical Microbiology Reviews*, 16 (3), 497–516 DOI: 10.1128/CMR.16.3.497–516.

Blount, W. P. (1961). Turkey X disease. *Turkeys* 9:52,55-58,61,77.

Bhat, R., Rai, R. V. and Karim, A. A. (2010). Mycotoxins in Food and Feed: Present Status and Future Concerns. *Comprehensive Reviews in Food Science and Food Safety*, 9, 57-81.

Bhat, R., Ramakrishna, Y. Beedu, S. R. and Munshi, K. L. (1989). Outbreak trichothecenes mycotoxicoses associated with consumption of mould-damaged wheat products in kashmir valley, india, *Lancet*, 333 (8628), 35-37.

Bueno, D., Munoz, R. and Marty, J. L. (2014). Common methods to detect mycotoxins: A review with particular emphasis on electrochemical detection. *Sensing in Electro analysis*. 8 (K. Kalcher, I. Svancara, K. Vytras; Eds.), 85-114.

Carballo, D., Font, G., Ferrer, E. and Berrada, H. (2018). Evaluation of mycotoxin residues on ready-to-eat food by chromatographic methods coupled to mass spectrometry in tandem. *Toxins*. 10, 243. DOI: 10.3390/toxins10060243.

Centre for Disease Control and Prevention (CDC) (2012). Outbreak of aflatoxin poisoining—eastern and central provinces, Kenya. MMWR Morb Mortal Weekly Report. 53(34), 790-793.

Chhonker, S. k., Rawat, D., Naik, R. A. and Koiri, R. K. (2018). An overview of mycotoxin in human health with emphasis on development and progression of liver cancer. *Clinics in Oncology;* 3:1408.

Chkuaseli, A., Khutsishvili- Maisuradze, M., Chagelishvili, A., Natsvaladze, K., Lashkarashvili, T., Chagelishvili, G. and Maisuradze, N. (2016). Application of new mycotoxin absorbent-bentonite clay "Askangel" in poultry feed. *Annals of Agrarian Science*; 14: 295-298.

Cole, R. J. (1978). Basic techniques in studying mycotoxins: Isolation of mycotoxins. *Journal of Food Protection*. 41 (2), 138-140.

Croft, W. A., Jarvis, B. B. and Yatawara, C.S. (1986). Airborne outbreak of trichothecene toxicosis. *Atmospheric Environment.* 20 (3), 549-552.

Fallahi, M., Saremi, H., Javan-Nikkhah, M., Somma, S., Haidukowski, M., Logrieco, A. F. and Moretti, A. (2019). Isolation, molecular identification and mycotoxin profile of *Fusarium* species isolated from maize kernels in Iran. *Toxins.* 11; 297.

Freire F.D.C.O., da Rocha M.E.B. (2016) Impact of Mycotoxins on Human Health. In: Mérillon JM., Ramawat K. (eds.) *Fungal Metabolites.* Reference Series in Phytochemistry. Springer, Cham.

Geetanjli and Simranpreet (2018). Aflatoxins and Safety OF Food, Animal and Human health - An Issue of Global Concern. *An International Journal of Advance Research in Science and Engineering*, 7, (06).

Hetherington, A. C. and Raistrick, H. (1931). On the Production and Chemical Constitution of a New Yellow Colouring Matter, Citrinin, Produced from Glucose by *Penicillium citrinum* Thom. *Studies in the Biochemistry of Micro-Organisms,* 220, 269-295.

IARC (2012). Mycotoxin and human health. *International Research on Cancer (IARC) Publication.* 87-104.

Jahanian, E. (2016). Mycotoxin- induced toxicity; an updated mini- review on the current concepts. *Immune pathologic Persa.* 2(2); 11.

Jianbo, Q. Jianhong, X. and Jianrong, S. (2019) Fusarium Toxins in Chinese Wheat since the 1980s. *Toxins*, 11(5), 248.

King, B. (1979). Outbreak of ergotism in Wollo, Ethiopia. *Lancet.* 313(8131), 1411.

Krishnamachari, K. A., Bhat, R. V., Nagarajan, V., Tilak, T.B. (1975). Hepatitis due to aflatoxicosis. An outbreak in Western India.*Lancet* May 10; 1(7915):1061-3.

Krishnamachari, K. A. V. R., Bhat, R. V. (1976). Poisoning by ergoty bajra (pearl millet) in man. *Indian Journal of Medical Research*, 64(11), 1624-1628.

Mannon, J. and Johnson, E. (1985). Fungi down on the farm. *New Science*.; 105: 12-16.

Mirocha, C. J., Pawlosky, R. A., Chatterjee, K., Watson, S. and Hayes W. (1983). Analysis for Fusarium toxins in various samples implicated in biological warfare in Southeast Asia. *Journal of the Association of Official Analytical Chemists.* 66(6):1485-99.

Ngindu, A., Kenya, P. R., Ocheng, D. M., Omondi, T. M., Ngare, W., Gatei, D., Johnson, B.K., Ngira, J. A., Nandwa, H., Jansen, A. J., Kaviti, J. N. and Siongok, T. A. (1982). Outbreak of acute hepatitis caused by aflatoxin poisoning in Kenya. *Lancet*; 1:13461348.

Peraica, M., Radic, B., Lucic, A. and Pavlovic, M. (1999). Toxic effects of mycotoxins in humans.*Bulletin of the World Health Organization.* 77(9): 754–766.

Reddy, B. N. (2016). *Journal of Plant Pathology and Microbiology,* 7:5 (Suppl) http://dx.doi.org/10.4172/2157-7471.C1.001

Reddy, B. N. and Raghavender, C. R. (2007). Outbreaks of aflatoxicoses in India. *African journal of Food Agriculture Nutrition and Development.* Vol. 7 (5).

Reddy, B. N. and Raghavender, C. R. (2008). Outbreaks of Fusarial -Toxicoses in India. *Cereal Research Communications Supplement: 3rd International Symposium On Fusarium Head Blight.* 36, 321-325.

Saito, M., Enomoto, M., and Tatsuno, T. (1971) Yellowed rice toxins. Luteoskyrin and related compounds, chlorinecontaining compounds, and citrinin, in: *Microbial Toxins,* 6, Academic Press, New York, 293-380.

Serck-Hanssen, A. (1970). Aflatoxin-Induced Fatal Hepatitis? A case report from Uganda. *Archives of Environmental Health.* 20(6):729-31.

Schmale, D. G. And Munkvold, G. P. (2009). Mycotoxins in crops: A threat to human and domestic animal health. *The Plant Health Instructor, American Phytopathological society*. doi: 10.1094/PHI-I-2009-0715-01.

Shephard, G. S. (2008). Impact of mycotoxin on human health in developing countries. *Food Additives and Contaminants*; 25(2): 146-151.

Smoragiewicz, W., Cossette, B., Boutard, A. and Krzystyniak, K. (1993). Trichothecene mycotoxins in the dust of ventilation systems in office buildings.International Archives of Occupational and Environmental Health; 65(2):113-7.

Steyn, P. S. (1995). Mycotoxins, general view, chemistry and structure. *Toxicology Letters*. 82/83: 843-851.

Tandon, B. N., Krishnamurthy, L., Koshy, A., Tandon, H. D., Ramalingaswami, V., Bhandari, J. R., Mathur, M. M. and Mathur, P. D.. (1977). Study of an epidemic of jaundice presumably due to toxic hepatitis in North West India. *Gastroenterology*. 72: 488-494.

Ueno Y. (1971) Toxicological and Biological Properties of Fusarenon-X, A Cytotoxic Mycotoxin of Fusarium Nivale Fn-2B. In: Purchase I.F.H. (eds.) Symposium on Mycotoxins in Human Health. Palgrave Macmillan, London.

Umar, S, Munir, M. T., Shah, M. A. A., Shahzad, M., Sohoo, M-U-R., Khan, R. A., Khan, A. U., Ameen, K., Rafia-Munir, A., Saleem, F. (2015) Outbreak of aflatoxicosis in local cattle farm in Pakistan. *Veterinaria*; 3(1): 13-17.

Van Dongen, P. W.J. and De Groot, A. N. J. A. (1995). History of ergot alkaloids from ergotism to ergometrine.*European Journal of Obstetrics &* Gynecology *and Reproductive Biology*. 60: 109-116.

Watson, A. Mirocha, C. J. and Hayes, A.W. (1984). Analysis for trichothecenes in samples from Southeast Asia associated with "Yellow Rain." *Fundamental and Applied Toxicology*. 4 (5), 700-717.

Willis, R. M., Mulvihill, J. J., Hoofnagle, J. H. (1980). Attempted suicide with purified aflatoxin. *Lancet*. 1198-99.

Yagen, B. and Joffe, A. Z. (1976). Screening of toxic isolates of *Fusarium poae* and *Fusarium sporotrichioides* involved in causing Alimentary Toxic Aleukia. *Applied and Environmental Microbiology*. 32(3), 423-427.

Zain, M. E. (2011). Impact of mycotoxins on humans and animals. *Journal of Saudi Chemical Society*. 15; 129-144.

SECTION B. FUNGAL ENZYMES

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 15

PURIFICATION, CHARACTERIZATIONAND CLINICAL APPLICATIONS OF THERAPEUTIC FUNGAL ENZYMES

Mafalda R. Almeida, Ana M. Ferreira,
João C. F. Nunes and Ana P. M. Tavares*
CICECO - Aveiro Institute of Materials, Department of Chemistry,
University of Aveiro, Aveiro, Portugal

ABSTRACT

This book chapter presents an overview of therapeutic fungal enzymes and their developments in biopharmaceuticals for the treatment of several diseases, clinical applications and investigation. Enzymes are biocatalysts of many reactions with widespread use in the pharmaceutical industry and medicine. Due to their high specificity, greater affinity, and high catalytic efficiency, enzymes have been widely used for therapeutic purposes. More specifically, therapeutic enzymes are being used in the treatment of several diseases, such as leukemia, cancer, pancreatic disorders, etc.For instance, L-asparaginase, which presents antineoplastic properties, has been used for the treatment of leukemia, namely acute lymphoblastic leukemia. Nowadays, more than 50% of the enzymes are produced by fungal sources,including the therapeutic enzymes, due to the advantages of being an economically feasible and consistent process, since it has high yield and is easy for modification and optimization of new therapeutic products.In this book chapter, readers from academies, research institutes and industries will gain useful information and in-deep knowledge on the emerging therapeutic fungal enzymes, their purification processes, characterization and medical applications.

*Corresponding Author's Email: aptavares@ua.pt.

Keywords: therapeutic fungal enzymes, (bio)pharmaceuticals, drugs, biologics, extraction, purification processes, enzyme, clinical applications, diagnostic, pharmaceutical industry

INTRODUCTION

In this book chapter, a review about thetherapeutic use of fungalenzymes over the past decades is explored. Enzymes as biopharmaceuticals have uniquecharacteristics, such as selectivity to their substrates, that distinguish them from other types of drugs(Mane and Tale 2015). These properties make enzymes specific and potent biological witha therapeutic potential.These features have resulted in the development of many therapeutic enzymes for a wide range of diseases (Gurung et al. 2013).In recent years, the potential use of microorganisms as biotechnological sources of industrially relevant enzymes has stimulated interest in the exploration of therapeutic fungal enzymes. Fungal enzymesincluding, glucose oxidase, L-asparaginase, proteases, amylases, cytosine deaminases, laccases, lipases, and chitinases, which areinvolved in pharmaceutical applications, have gain more attention. The potential applications of these enzymes are determined by the ability to screen new and improved enzymes, their fermentation and purification steps in large scale, and the formulations of enzymes. In this scenario, different methods have been established for enzyme purification. For specific pharmaceutical industrial applications, chromatography is still widely preferred due to its robustness, selectivity (high level of the enzyme purity), high clearance of impurities and most importantly, easy validation compared to other purification processes(Aehle 2007). Overall, traditional purification strategies are considered time-consuming with lower yields, and the trends are moving towards precipitation, crystallization and aqueous two-phase systems(Gurung et al. 2013). Furthermore, the characterization of purified fungal therapeutic enzymes has been addressed. Investigations on the pH, temperature and metal ions effect on the enzyme activity have been performedby several authors and described in this book chapter.

THERAPEUTIC FUNGAL ENZYMES

Enzymes are biological macromolecules, produced by a living organism, which acts as a highly selective biocatalyst in a specific biochemical reactionrequired to sustain life(Smith 1997). Enzymes are known to catalyse about 4,000 biochemical reactions accelerating both the rate and specificity of these metabolic reactions (Bairoch 2000). Each enzymeisconstituted by a long and linear chain of amino acids that fold to produce a

specific and unique three-dimensional structure with specific properties (Gurung et al. 2013). During the last decades, due to the intensive research in enzymology,the development of fermentation processes, recombinant DNA technology and protein engineering for enzymes production with specific strains allowed its large-scale production and their introduction into the industrial field (Gurung et al. 2013), with many significant and vital roles in the pharmaceutical industries (Mane and Tale 2015).

Table 1. Some examples of therapeutic applications of fungal enzymes

Enzyme	Therapeutic applications	Fungus	References
α-Amylase	Digestive disorders Pancreatic insufficiency	*Aspergillus* sp.	(Gupta et al. 2003; Somaraju and Solis-Moya 2014)
Chitinases	Treatment of infections Anti-cancer	*Saccharomyces cerevisiae* *Candida albicans*	(Nagpure, Choudhary and Gupta 2014; Roopavathi, Vigneshwari and Jayapradha 2015; Karthik et al. 2014)
L-Asparaginase	Acute lymphocytic leukemia	*Aspergillus terreus*	(De-Angeli et al. 1970)
Cytosine deaminases	Tumour therapy Antimicrobial drug design Gene therapy applications	*Bacillus subtilis* *Yeast**	(Gaded and Anand 2018; Ko et al. 2003; Kievit et al. 1999)
Proteases	Acne or psoriasis Human callus Dermatophytosis Scar removal Epithelia regeneration Acceleration of healing processes	*Trichoderma pseudokoningii* *Meloidogyne incognita* *Metarhiziumanisopliae* *Beauveria bassiana*	(Brandelli, Daroit and Riffel 2010; Vignardet et al. 2001; Chao et al. 2007; Souza et al. 2015; Yike 2011)
Lipases	Reduction of cholesterol Tumour therapy Pancreatic insufficiency	*Candida rugosa*	(Yang et al. 1997; Gurung et al. 2013; Takasu et al. 2012)
Glucose oxidase	Tumour therapy	*Penicillium notatum*	(Fu et al. 2018; Zhao, Hu, and Gao 2017; Sabir et al. 2007; Javed et al. 2013; Bhatti, Haq Nawaz Saleem 2009)
Laccases	Deactivation of HIV-1 reverse transcriptase Hepatitis C inhibition	*Pleurotuscornucopiae* *P. ostreatus*	(Wong et al. 2010; El-Fakharany et al. 2010).

*Name of the species is not available.

Enzymes with therapeutic properties are proteins that themselvesare the therapeutic agent. Theyhave many advantages over non-enzymatic drug products due to the highly specificity towards a target, reduced immunogenicity(most common cause for drug failure), which improve the clinical efficacy (Lutz, Williams and Muthu 2017). Therapeutic enzymes can be used, either alone or in combination with other therapies, for treating a variety of diseases. In general,enzymeas biopharmaceutical are usually used by injection due to their size and sensitivity to denaturation(Vellard 2003).However, the delivery of thisbiotherapeutic depends on the type of disease and the location of the enzyme target. For example, enzymes for digestive aids have been used as an oral

formulation(Vellard 2003). Therapeutic enzymes are being employed in diagnosis, biochemical investigation, monitoring and treatment of several diseases, such as leukemia, skin ulcers, Pompe's disease, cardiovascular diseases, celiac disease, Parkinson's disease, Fabry's disease, inflammation, digestive disorders, pancreatic disorders (Mane and Tale 2015). In specific, enzymes act as oncolytics, anticoagulants, thrombolytics, and replacements for metabolic deficiencies (digestive aids and metabolic storage disorders, among others).

Microbial enzymes display many advantages, such as stability, great yields, financial viability, easy product optimization, steady supply, and fast microbes growth on low-cost media (Gurung et al. 2013). In fact, manymedically important enzymes are obtained from a limited number of fungi, yeast and bacteria. These organisms are also considered when a new enzyme is required (Teal 1991). Medically important enzymes are required in very less quantity as compared to the industrially important enzymes, but with a high degree of purity and specificity. The sources of these kinds of enzymes should be selected with great care and precautions to prevent any possibility of undesirable contamination by incompatible material and also to enable ready purification.A summary of different applications of therapeutic fungal enzymes for diverse health problems ispresented in Table 1.

α-Amylases

α-Amylases(EC 3.2.1.1)areglycoside hydrolase enzymes that catalyse starch into low molecular weight sugars and dextrins, being present in the digestion of carbohydrates. Different species of fungi are able to produce α-amylases, being *Aspergillus* the most common microorganism (Saranraj and Stella 2013). The commercialization of amylases started in 1984, as a pharmaceutical support for thetreatment of digestive disorders. Moreover, amylases find applications in the pharmaceutical and finechemical industries, and in medical diagnosis(Gupta et al. 2003). For instance, blood serum amylase may be measured, and a normalconcentration is between 23-85IU/L(Hardwicke et al. 2010). A higher concentration indicates medical abnormal conditions, including acute inflammation of the pancreas, perforated peptic ulcer, torsion of an ovarian cyst, among others.In fact, α-amylase activity levels in human body fluids are extremely important in pancreatitis, diabetes and cancer research (Das et al. 2011; Gurung et al. 2013).As a therapeutic, α-amylases can be applied in the treatment of cancer, infection, and wound healing, some being approved by the US Food and Drug Administration (FDA), and others are in advanced stages of development(Azzopardi et al. 2016). These α-amylases offer promising solutions for drug delivery and combined diagnostic-therapeutic applications(Azzopardi et al. 2016). As an example, α-amylase can be used as a component in several pharmaceutical enzyme-replacement preparations for the treatment

of pancreatic insufficiency(Somaraju and Solis-Moya 2014).Furthermore, glucose, theproduct of α-amylase catalysis, has been shown to inhibit the production of the toxins responsible for the onset and progression of gangrene, lending some antibacterial efficacy(Méndez et al. 2012).

Chitinases

Chitinases (EC 3.2.1.14) are glycosyl hydrolases that hydrolyse the β-1,4-glycosidic bonds of chitin(Bhattacharya, Nagpure and Gupta 2007). Fungal chitinases belong to the glycoside hydrolases family presenting a similar amino acid sequence.Chitinases can be divided into two main classes: i) endo-chitinases, which cleave chitin randomly at internal sites, generating soluble low molecular oligomers of N-acetylglucosamine, and ii) exo-chitinases, which catalyse the progressive release of di-acetylchitobiose and cleave the oligomeric products of endochitinases and chitobiosidases generating monomers of N-acetylglucosamine(Novotná, Fliegerová and Šimůnek 2008). Chitin is the component of cell wall of many pathogenic organisms, including fungi, protozoa, and helminths and is a good target for antimicrobials (Fusetti et al. 2002). These enzymes have antimicrobial properties andcan be used in the treatment of several infections and also show activity against new drug-resistant bacterial strains (Nagpure, Choudhary and Gupta 2014). Recently, it has also been proved that mammalian chitinase can be used against dermatopathogenic fungi and against *Trichomonas vaginalis*, a protozoan parasite(Chen, Shen and Wu 2009; Loiseau, Bories and Sanon 2002). In the same way, amino oligosaccharide hydrolysates, the product of the hydrolysis of chitin, has an important role in regulating the life metabolism, presenting anti-inflammatory actions and has therapeutic effects on intestine and gastrointestinal ulcers besides improving immunity and anti-tumour activity (Nagpure, Choudhary and Gupta 2014). In summary, the results from the literature indicate that chitinase enzymes can be applied as new drug therapies for human healthcare.

L-Asparaginase

L-asparaginase (LA), (EC3.5.1.1; l-asparagine aminohydrolase), is widely distributed in nature, being found not only in plants and tissues, but also in fungi. In fact, microorganismsare a better source of LA than animals or plants, due to theireasyfermentation production since they grow in simple and inexpensive substrates (Lopes et al. 2017). Different fungi can produceLAwith potential in cancer treatment, more specifically for leukaemia, acute lymphocytic leukemia, with improved therapeutic results(Souza et al. 2017).Thetumour cells lack aspartate-ammonia ligase activity,

responsible for the non-essential amino acid LA synthesis. Normal cells remain unaffected, since they are able to synthesize LA for their need, while generating a free exogenous LA concentration decline, which triggers, in the tumor cells, a state of fatal starvation. Nevertheless, LA intravenous administration effectiveness depend if the blood levels of asparagine are extremely low (Gurung et al. 2013; Mane and Tale 2015).There are two different types of LA, type I and type II, which differ in their affinities for L-asparagine substrate. The type I is a cytoplasmatic enzyme that shows low affinity to asparagine, while type II is in the periplasmic space with a high affinity to substrate. Thus, only type II can be applied as therapeutic drug, due to the enzyme's antitumor activity(Yun et al. 2007). LA is mainly produced by bacteria (Bacelar et al. 2016). However, the production process is very expensive beside the side effects of LA from bacteria. For instance, LA from fungi such as *Penicillium* sp.and*fusarium* sp. are an alternative since extracellular activity is easier topurify than the intracellular LA produced by bacteria(Bacelar et al. 2016). LA from *Aspergillus terreus*exhibited abetter anti-tumour effect then LA from bacteria (De-Angeli et al. 1970). Polyethylene glycol modified LA from *A. terreus* showed effectiveness against proliferationof two leukemic cell lines(Battiston Loureiro et al. 2012). Beside this fact, marketable LA isnot being produced by fungi, LA is already industrialized, being commercialized as: Crastinin®, Elspar®, Ki-drolase®, Leunase®, Asparaginase medac™, Erwinase®, Spectrila®(Souza et al. 2017).

Cytosine Deaminases

Cytosine deaminases (EC 3.5.4.1) are nucleoside-metabolizingenzymes catalysing the hydrolytic deamination of cytosine to uracil and ammonia. Originally, these enzymes are only found in fungi and prokaryotes. In addition to cytosine, cytosine deaminases convert the 5-fluorocytosine (enzyme substrate) to the chemotherapeutic drug5-fluorouracil. This compound is a very potent inhibitor of thymidine synthase, disrupting de novo production of thymidine monophosphate, which makes this enzyme a highly promising antitumor biological. The cytosine deaminase/5-fluorocytosine method is the most studied suicide gene (gene-directed enzyme prodrug) therapy approach(Asadi-Moghaddam and Chiocca 2006). Due to the promising therapeutic action of cytosine deaminases mediated 5-fluorouracil deamination in cancer cells, a hard effort has been carried out to develop new approaches for advanced tumour therapy. For instance, cytosine deaminases have been studied for the treatment of different types of cancer such as endometrial, colon, prostate, breast and gliomas(Yi et al. 2011; Nyati et al. 2002; Miyagi et al. 2003; Li et al. 1997; Wang et al. 1998; Kievit et al. 1999; Kievit et al. 2000).5-fluorouracilcan also be used as an antifungal drug, which is generally used to treat fungal infections in humans (Waldorf and Polak 1983).

Proteases

Proteases (EC3.4.21-24, peptidases or proteolytic enzymes)hydrolyse the peptide bonds of proteins into other proteins, peptides and amino acids, being found in all living organisms(Souza et al. 2015). There are eight types of proteaseswhich are based on their enzymatic catalysis and onthe nature of the functional group at the active site: asparagine, aspartic, cysteine, glutamic, metallo, serine, threonine (Yike 2011). Proteases can be obtainedbymany fungal cultures since they are extracellular enzymes (Monod et al. 2002). These enzymes can be produced by fungi such as *Trichoderma pseudokoningii, Meloidogyne incognita, M.anisopliae*and*Beauveria bassiana*(Yike 2011). In the therapeutic field, proteases are a promisingand well-recognized growing classof biologics due to improved clinical applicationssuch as keratin elimination in acne or psoriasis, human callus elimination and keratinized skin degradation, vaccine preparation for dermatophytosis therapy, ungual drug delivery increase, scar removal and epithelia regeneration, and acceleration of healing processes (Brandelli, Daroit and Riffel 2010; Vignardet et al. 2001; Chao et al. 2007; Souza et al. 2015). FDA has approved twelve proteases, and other new proteases are in clinical development (Craik, Page and Madison 2011).The first protease approved by the FDA in 1978 is the drug u-PA (urokinase) used for thrombolytic therapy, which provides an alternative to the surgical removal of emboli (Craik, Page and Madison 2011). Proteases, marketed as Activase® (Genentech), are used to treat heart attacks (myocardial infarction) (Bode et al. 1996). This enzyme was the first haemophilia drug used for an efficient blood clotting and maintenance of normal haemostasis (Howard et al. 2007). Another application includes its use as surgical sealant (thrombin), a constituent of the coagulation cascade, converts fibrinogen into fibrin monomers that then multimerize to form stable blood clots. Plasma serine protease has been studied as a potential drug to alleviate the hypercoagulable state and thus permit the treatment of myriad effects resulting from sepsis, however, the clinical use is limited due to the pleiotropic effects of plasma serine protease (Yan et al. 2001). Proteases as digestive aids have been applied in patients with cystic fibrosis originated from a deficiency in pancreatic enzymes. Pancreatic enzyme replacement involves a defined mixture of proteases, lipases and amylases which can be used as a therapy. The commercial drug, Zenpep® (Eurand), is an approved pancreatic enzyme for cystic fibrosis (Wooldridge et al. 2009). Proteases can also be used to improve the digestion through the combinationof proteases and other digestive enzymes for the treatment of pancreatic insufficiency (Craik, Page and Madison 2011).

Lipases

Lipases are triacylglycerol acyl hydrolases (EC3.1.1.3) catalysing the hydrolysis of fats and oils toyield glycerol and free fatty acids (Singh and Mukhopadhyay 2012). This type of enzymes is involved in catalytic reactions, such as aminolysis, alcoholysis, esterification, interesterification, transesterification, and acidolysis (Singh and Mukhopadhyay 2012). The hydrolysis essentially occurs at the aqueous/organic interface (Sharma and Kanwar 2014). Lipases can be found in nature and have been isolated from various sources. Lipases can feasibly be produced by filamentous fungi and yeasts. Fungal lipases are extracellular in nature, and they can be recoveredwithout difficulty, which significantly reduces its production costs (Gopinath et al. 2013). Extracellular lipases have been produced by a high variety of fungi, such as *Lipomycesstarkeyi*, *Rhizopus* sp., *Geotrichumcandidum*, *Pencillium*sp., *Acremonium strictum*, *Candida rugosa*, *Humicolalanuginosa*, *Cunninghamellaverticillata*, and *Aspergillus*sp. (Sztajer, Maliszewska and Wieczorek 1988; Gopinath et al. 2003; Helena Sztajer and Maliszewska 1989; Okeke and Okolo 1990; Wu, Guo and Sih 1990; Iizumi, Nakamura and Fukase 1990; Gopinath et al. 2002; Gopinath, Hilda and Anbu 2000; Thota et al. 2012). For instance, lipase from *C. rugosa* has been used for the synthesis of drugs, such as lovastatin (reduction of cholesterol), via a regioselective acylation of a diol-lactone precursor with 2-methylbutyric acid (Yang et al. 1997; Gurung et al. 2013). In fat, fungal lipases have gained a great attention as a therapeutic agent and have high potential in medicine due to their substrate specificity and unique properties (Lott and Lu 1991; Gurung et al. 2013). Moreover, lipases are used in cancer treatment since some types of cancer as colorectal and pancreatic, may be influenced by the levels of triglycerides, and consequently, the role of lipases, that catalyse the hydrolysis of plasma triglyceridesis also realized (Takasu et al. 2012). Lipases are also used for the treatment of pancreatic insufficiency, a condition affecting patients with cystic fibrosis and for the treatment of fat malabsorption in patients with human immunodeficiency virus (HIV) (Schibli, Durie and Tullis 2002; Carroccio et al. 2001). This enzyme is commercialised (TheraCLEC Total™) as a mixture of pancreatic enzymes (lipase, amylase and protease mix). In addition to this, lipases are used in the treatment of malignant tumours. Furthermore, lipases can also be appliedin diagnosis, since its presence or high level can be the sign of a specific infection or disease such as pancreatic injury and acute pancreatitis (Lott and Lu 1991; Gurung et al. 2013).

Glucose Oxidase

Glucose oxidase (GOx) (EC 1.1.3.4) is an endogenous oxidase-reductase broadly distributed in living organisms, including fungus such as *Penicillium notatum*, whose

non-toxicity, biocompatibility and particular catalysis against β-D-glucose, enables its use in cancer diagnosis and therapeutics methods (Fu et al. 2018). Particularly, GOx catalyzes the oxidation of glucose into gluconic acid and H_2O_2, which drives reactive oxygen species (ROS) stimulation promoting cancer cell death (Huggett and Nixon 1957; Imlay, Chin and Linn 1988; Fu et al. 2018).Cancer cells demand glucose due to their high energy need for growth, as they experience low adenosine triphosphate-productive anaerobic glycolysis in the absence of oxidative phosphorylation (Warburg 1956; Fu et al. 2018). Therefore, tumor growth and proliferation are inferred from cancer cells glucose levels (Fu et al. 2018). Due to cancer cells high energy need for growth, uncontrolled proliferation and altered metabolic pathways, more glucose is required, whose proliferation can be monitored via its glucose use (Fu et al. 2018). In the presence of oxygen, GOx catalyzes the oxidation of glucose and production of gluconic acid and H_2O_2, multiple types of therapy such as cancer starvation therapy, hypoxia-activated therapy, pH-responsive drug release, and oxidation therapy have been developed (Fu, Qi, Lin and Huang 2018). Tumor microenvironment (TME) acidity enhances too, which helps in the activation of a pH-responsive drug delivery system (pH-responsive drug release) (Fu et al. 2018; Sato, Yoshida, Takahashi and Anzai 2011). However, tumor heterogeneity, diversity and complexity require the development of multimodal synergistic therapies, in which several types of therapies are combined, as is shown below (Fanet al. 2017; Fu et al. 2018). Zhao et al. successfully developed a glucose-responsive nanomedicine of GOx-polymernanogels, which modulates H_2O_2 production for melanoma starving and oxidation therapy via constraining GOx in the tumor. This new therapeutic strategy revealed an high anti-melanoma efficacy, while not revealing systemic toxicity (Zhao et al. 2017). In another work, it was proposed a starvation and hypoxia-activated therapy alliance via the co-administration of liposome-GOx and liposome-AQ4N, a hypoxia-activated prodrug, which achieved effective tumor growth inhibition, without important toxic side effects in the mouse tumor model (Zhang et al. 2018). Li et al.managed to amplify the synergistic effects of long-term cancer starvation therapy, along with photodynamic therapy (PDT), creating a cancer targeted cascade bioreactor, mCGP, by inserting GOx and catalase in the cancer cell membrane-camouflaged porphyrin metal−organic framework (MOF) of a porous coordination network (PCN-224) (Li et al. 2017).Zhou et al.established a tumor-targeted nanoplatform, which takes advantage of both, tumor starvation and low-temperature photothermal therapy (PTT) by packing porous hollow Prussian Blue nanoparticles with GOx, followed by redox-cleavable linkage of hyaluronic acid (HA) to their surface, allowing CD44-overexpressing tumor cells specific bind, enhancing antitumor efficacy (Zhou et al. 2018). Fan et al. developed an unparalleled coefficient cancer starving-like/gas therapy, through the use of hollow mesoporous organosilica nanoparticle (HMON), which co-delivers GOx and L-Arg, allowing L-Arg oxidation into nitric oxide (NO) by generated acidic H_2O_2, enhancing gas therapy with minimal adverse effects (Fanet al. 2017). Li et

al.orchestrated a tumor-based oxidation/chemotherapy treatment by specific activation at tumor sites, based on GOx-loaded polymersome nanoreactors (GOD@PCPT-NR), which are exclusively triggered by tumor acidity to in situ generate H_2O_2 and further cause the fast release of camptothecin (CPT), an anticancer drug (Li et al. 2017). Nevertheless, there are still unexploited potential of enzyme reactions, which can be applied in many medical research areas (Gurung et al. 2013).

Since GOx displays high selectivity and sensitivity towards glucose, this enzyme can also be used for electrochemical cancer and diabetes mellitus diagnosis and as a biosensor. These methodologies are viable since the catalysis of glucose by GOx, using an electrode, induces an electric current in ratio of the glucose concentration (Wang 2008; Fu et al. 2018). GOx-based biosensors show massive potential for diagnosis of cancer, because GOx catalysis reaction allows the amplification of cancer biomarkers signals via specific target ligands which recognize these biomarkers (Fu et al. 2018). GOx-based biosensors can be classified as oxygen-based, pH sensitive, H_2O_2 dependent such as: H_2O_2-based electrochemiluminescence (ECL) biosensors, H_2O_2-based photoelectrochemical (PEC) immunosensors, H_2O_2 regulates metal-based biosensors, and GOx-based electrochemical biosensors (Fu et al. 2018). Hereby, oxygen consumption analysis using a specific probe by oxygen-based biosensors is followed by glucose levels extrapolation of tumor cells; medium pH decrease due to glucose oxidation to gluconic acid can be detected by a pH-sensitive transducer, which converts pH changes into an electrical signal, allowing single cancer cell glucose concentration calculation (Fu et al. 2018). Furthermore, H_2O_2-based ECL biosensors allow DNA target detection through sensitive ECL signal-change of the $Ru(bpy)_3^{2+}$–tripropylamine (TPrA) system due to H_2O_2 concentration changes, taking advantage H_2O_2 is an ECL quencher for $Ru(bpy)_3^{2+}$. H_2O_2 designed as an ultrasensitive PEC immunosensor for cancer biomarkers detection, since H_2O_2 is able of photocurrent amplifying. H_2O_2 -induced growth of small-sized metal nanoparticles are applied in biosensors development, when in reaction to a biorecognition event, there is a shift in their size, aggregation, and localized surface plasmon resonance (LSPR) (Fu et al. 2018). Additionally, GOx specificity and unique reactivity of manganese dioxide (MnO_2) nanosheets allow glucose detection through trimodal self-indication method, namely fluorescence, ultraviolet-absorbance and magnetic resonance signals (Chen et al. 2017; Fu et al. 2018).

Laccases

Laccases (EC 1.10.3.2) (*p*-diphenol: dioxygen oxidoreductases;benzenediol dioxygen oxidoreductases) are multicopper oxidases catalyzing both phenolic and non-phenolic compounds (Giardina et al. 2010). This type of enzyme only uses molecular oxygenasthe electron acceptorand the substrate to initiate catalysis, i.e., electrons areremoved from the

reducing substrate molecules and transferredto oxygen to produce water (Giardina et al. 2010). Laccase is an extracellular enzyme secreted by various fungiduring their secondary metabolism. Among fungi, ascomycetes, basidiomycetes, and deuteromycetes can produce laccase, and white-rot basidiomycetesare the bestlaccase producers(Arora and Sharma 2010). Laccaseproduction can be achieved by submerged orsolid-state fermentation processes.Laccase has received greatattention from both academia and industry due to these simplerequirements and ability to degrade a diversity of substrates (Chaurasia, Bharati and Sarma 2017). Recently, laccase has a high potential application in the therapeutic field, principally against cancer (Guest and Rashid 2016). Laccases have shown anti-proliferative activities, primarily against breast cancer and liver carcinoma cell lines (Guest and Rashid 2016). Laccase from *Pleurotuscornucopiae* was evaluated for the deactivation of HIV-1reverse transcriptase and the enzyme showed HIV-1 inhibitory activity (Wong et al. 2010). In another study, laccase from *P. ostreatus* was able to inhibit hepatitis C virus entry into peripheral blood cells and hepatoma cells (El-Fakharany et al. 2010).

PURIFICATION PROCESSES OF THERAPEUTIC FUNGAL ENZYMES

As part of the production of therapeutic fungal enzymes, there are three core technologies areas, namely production, purification and the biological activity of the purified enzymes (Figure 1), being the purification the critical process to apply these enzymes in the pharmaceutical industry. In fact, the high-cost production of biopharmaceuticals is usually associated with the purification steps (downstream process). Thus, it has become crucial to investigate how to replace traditional methods with efficient and cost-effective alternative techniques for recovery and purification of fungal enzymes from the fermentation medium. One of the major challenges of the production of therapeutic fungal enzymes is closely related tothe reduction of the purification steps in a way to obtain one single-step process. In fact, different purification techniques having different conditions become suitable for one but not for other enzymes, i.e., a slight change in pH above or below the optimum value may change the activity of the enzyme, which can be a reason for a variation in the percentage yield of the same enzymes using different purification strategies (Polizeli, Jorge and Terenzi 1991). Thus, after the purification process of the enzyme is a pre-requisite to study their structure-function relationships and biochemical properties (Gupta et al. 2003). Moreover, after the purification process, the purity of the enzyme and its molecular weight is usually checked using SDS-PAGE (molecular weight is determined by running the marker and purified enzyme) (Patil 2010).

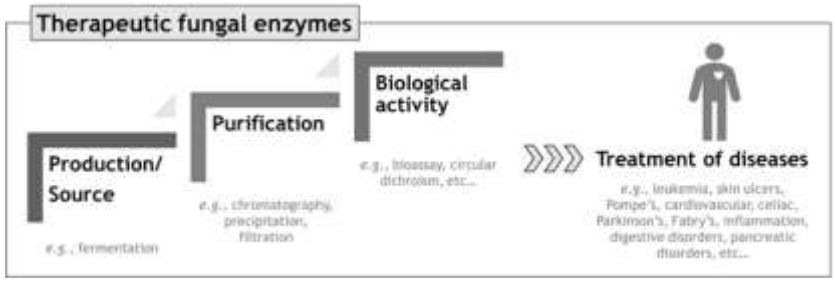


Figure 1. Core technology areas for the production of therapeutic fungal enzymes.

Traditional Processes to Purify Fungal Enzymes

Different processes are used to purify fungal enzymes, since in the pharmaceutical industry a high purity level is required. The purification of fungal enzymes usually includes a first step regarding the concentration of proteins from the crude enzyme extract, by precipitation with organic solvents (ethanol, acetone, among others) or ammonium sulphate (Table 2). Subsequently, a sequence of various steps has been applied, including dialysis and chromatography, e.g., ion exchange, hydrophobic, and gel filtration chromatography (Table 3).

Table 2. Principal techniques used in the purification of enzymes (adapted from Kiiskinen et al. 2004)

Property	Method	Scale
Size or mass	Centrifugation	Large or small
	Dialysis, Ultrafiltration	Mainly small
	Gel filtration chromatography (GFC)	Mainly small
Polarity		
(a) Charge	Ion-exchange chromatography (IEC)	Large or small
	Electrophoresis	Mainly small
(b) Hydrophobic character	Hydrophobic chromatography (HIC)	Mainly small
Solubility / Precipitation	Change in pH	Mainly large
	Change in ionic strength	Large or small

Usually, ammonium sulphate is used to precipitate the enzymes. This phenomenon is related to the 'salting out' effect, i.e., the addition of salt in excess in the aqueous extract leads to the "competition" of hydrophilic solutes (salt and enzyme) for the water molecules, with the formation of hydration complexes between the salt and the water. Consequently, the enzymes stay without water and occurs their precipitation. The quantity of salt required for the precipitation of specific enzyme is directly dependent on its molecular weight. Most of the authors have tried 30–80% (w/v) ammonium sulphate concentration for fungal enzyme extraction (Table 3). Although salt precipitation process brings about conformational changes in the protein, it does not denature them. In fact, these protocols are usually performed at lower temperatures up to 4°C.

Table 3. Summary of enzyme purification from fungi using sequential multi-step purification processes

Enzyme		Fungus	Purification method	Specific activity (U/mg)	Purification fold	Yield of protein (%)	Ref.
Chitinases	---	*Trichoderma viride*	IEC + GFC	1.7	14.3	11.7	(Omumasaba, Yoshida and Ogawa 2001)
	Type I	*Stachybotryselegans*	Precipitation + IEC + HIC	5.6	13.5	8	(Duo-Chuan, Chen and Jing 2005)
	Type II			4.3	9	6.1	
	Type I	*Penicillium aculeatum NRRL 2129*	IEC + GFC	8	94.1	29.1	(Binod et al. 2005)
	Type II			0.7	7.6	9.6	
	Type III			5.3	62.8	31.4	
	Type IV			3.7	43.7	29.9	
	---	*T. lanuginosus*	Precipitation + IEC + GFC	35.5	10.6	1.4	(Guo et al. 2008)
	---	*G. catenulatum HL-1-1*	Precipitation + chromatography + electrophoresis	12.2	10.1	3.2	(Gui-Zhen Ma 2012)
	---	*Rhizopus oryzae*	IEC + GFC	165.2	4.3	19.7	(Nagpure and Gupta 2013)
	---	*Aspergillus terreus*	Precipitation + GFC + IEC	182.1	5.2	12	(Farag et al. 2016)
L-asparaginase		*F. velutipes*	Ultrafiltration + GFC	n.d.	n.d.	n.d.	(Eisele et al. 2011)
		Aspergillus aculeatus	Precipitation + dialysis + GFC	207	267.8	0.5	(Dange and Peshwe 2011)
		Aspergillus aculeatus	Precipitation + GFC + IEC + filtration	29.6	38.2	7.9	(Dange and Peshwe 2011)
		Cladosporium sp.	Precipitation + IEC + GFC	83.3	867.7	n.d.	(Kumar and Manonmani 2013)
		R. miehei	Nickel-iminodiacetic acid column	1984.8	2.6	48.8	(Huang et al. 2014)
		Aspergillus flavus	Precipitation + GFC + IEC	176.5	7.8	25	(Patro et al. 2014)
		Aspergillus fumigatus WL002	Ultrafiltration + precipitation + GFC	355	232	n.d.	(Ghosh and Pramanik 2015)
		Aspergillus sp. *ALAA-2000*	Precipitation + GFC	n.d.	8.3	43.6	(Ahmed 2015)
		Fusarium culmorum ASP-87	Precipitation + IEC + GFC	16.7	14	2.6	(Janakiraman 2015)
		Penicillium cyclopium	Precipitation + GFC	39480	52.3	4.5	(Shafei et al. 2015)
		Streptomyces brollosae NEAE-115	Precipitation + IEC	76.7	7.8	7.3	(El-Naggar et al. 2018)

Table 3. (Continued)

Enzyme		Fungus	Purification method	Specific activity (U/mg)	Purification fold	Yield of protein (%)	Ref.
Cytosine Deaminases		*Saccharomyces cerevisiae*	Precipitation + chromatography + GFC	56.3	21.7	60	(Hayden et al. 1998)
Proteases		*Aspergillus parasiticus*	Precipitation + GFC + IEC	3530	200	17	(Tunga, Shrivastava and Banerjee 2003)
		Engyodontium album BTMFS10	Precipitation + IEC	3148	16	0.6	(Chellappan et al. 2011)
		Aspergillus clavatus ES1	Precipitation + precipitation + GFC + IEC	37600	7.5	29	(Hajji et al. 2007)
		H. rhossiliensis	Precipitation + GFC + IEC	123.1	16	7.1	(Wang, Wu and Liu 2007)
	---	*G. putredinis*	Precipitation + GFC	14.9	8.6	36.5	(Savitha et al. 2011)
	---	*T. harzianum*	Precipitation + GFC	14.5	11.5	29.4	(Savitha et al. 2011)
	---	*Beauveria* sp.	Precipitation + IEC	60.4	10	38.6	(Savitha et al. 2011)
	---	*Botrytis cinerea*	Dialysis + IEC + GFC	58216	19	5.6	(Abidi et al. 2011)
	---	*Aspergillus parasiticus*	Precipitation + dialysis + IEC	106232	2.2	2.5	(Anitha and Palanivelu 2013)
Laccase		*Aspergillus nidulans*	IEC + GFC + IEC	892.7	557.3	9	(Scherer and Fischer 1998)
		M. albomyces	Ultrafiltration + IEC + HIC + GFC	1136	292	17	(Kiiskinen et al. 2004)
		M. grisea	Precipitation + IEC + GFC	225.9	282	11.9	(Iyer and Chattoo 2003)
		*Mauginiella*sp.	Precipitation + IEC + HIC	1449	100	40	(Palonen et al. 2003)
		M. albomyces	HIC + IEC + GFC	560	11	40	(Kiiskinen et al. 2004)
		Trametessanguinea MU-2	Dialysis + IEC + GFC	689	n.d.	73	(Han et al. 2005)
		Trametes versicolor CCT 452	Precipitation + IEC + GFC	101	34.8	38.4	(Minussi et al. 2002)
		P. sajor-caju MTCC 141	Precipitation + ultrafiltration + GFC	n.d.	10.7	3.5	(Sahay, Yadav and Yadav 2008)
		Ganoderma sp. *MK05*	Precipitation + IEC	2.3	3.1	13.6	(Khammuang and Sarnthima 2009)
		*Pleurotus*sp.	Precipitation + IEC + GFC	2600	72.2	22.4	(More et al. 2011)

Enzyme		Fungus	Purification method	Specific activity (U/mg)	Purification fold	Yield of protein (%)	Ref.
		*Marasmius*sp. *BBKAV79*	Dialysis + GFC + IEC	n.d.	376.7	13.5	(Vantamuri and Kaliwal 2016)
		*Pestalotiopsis*sp. *CDBT-F-G1*	Precipitation + Precipitation	31700	14	84.0*	(Yadav et al. 2019)

*partial purification.

n.d.- not determined.

In the chromatographic methods, the selection of the appropriate method among the variety of chromatographic methods is dependent upon the type of enzyme, impurities, charge, size of the molecules and purity of the extract. Hydrophobic interaction chromatography (HIC), ion exchange chromatography (IEC), size exclusion chromatography (SEC) and gel filtration chromatography (GFC) are the chromatographic methods more used for the purification of fungal enzymes from various sources as mentioned in Table 3. The combination of more than one chromatographic operation is usually employed to improve the purification fold. Kiiskinen et al. (2004) used HIC, IEC, as well as GFC for purification of laccase from *Trichoderma reesei* increasing the purification fold from 2 to 11. However, laccase from *Pleurotus* sp. was purified up to 72.2 fold using IEC and GFC, but only after the precipitation with ammonium sulphate (More et al. 2011). Thus, pre-treatment of the crude extract is essential to achieve an efficient purification with chromatography, with many authors applying extraction methods such as, ammonium sulphate precipitation, before adopting chromatography (Table 3), as mentioned before. The application of chromatography seems to be very efficient to obtain a high enzyme purity. More recently, a different chromatography was reported, i.e., affinity chromatography using a nickel-iminodiacetic acid column (More et al. 2011).

Among the selected studies summarized in Table 3, the purification of fungal enzyme employs at least three 3 steps, (1) precipitation, (2) GFC and/or (3) IEC, to obtain a high purity. However, these protocols involve several chromatographic steps, make the process costly and time-consuming (Martínez-Aragón et al. 2009). A solution to suppress these and other shortcomings related with the chromatographic methods, can be the synergism between different unit operations involving easier and cheaper techniques that can be scaled in an industrial context (Dux et al. 2006). More specifically, other low-resolution separation methods have been studied, precipitation and aqueous two-phase systems (ATPS).

ALTERNATIVE PROCESSES TO PURIFY FUNGAL ENZYMES

Precipitation

Besides the addition of the salts, the organic solvents such as acetone and ethanol are used to precipitate the proteins, as mentioned before. The solvent percentage change can also be used for the separation of different type of proteins. Kumarevel et al. (2005) reported a stepwise purification strategy for fungal lipases from *C. verticillata*, using precipitation with 50% acetone with a gradual increment of 5% acetone as the important step to minimize the impurities as much as possible, avoiding many chromatographic purification steps. Moreover, Yadav et al. (2019) could also partially purify laccase from *Pestalotiopsis*using precipitation method, through two steps: first with a mixture of ammonium sulfate (13-fold purification) and then with acetone (14-fold purification). However, in both studies presented here, the enzyme obtained was only partially purified, demonstrating therefore, the need to associate other techniques to obtain a pure enzyme.

Phase Separation

Aqueous Two-Phase Separation

Liquid-liquid extraction (LLE) seems to be more viable than traditional methods since several features of the early processing steps can be combined into a single operation. LLE consists in the transference of certain components from one phase to another when immiscible or partially soluble liquid phases are brought into contact with each other. Aiming to avoid the use of organic solvents in LLE, in 1958, Albertson introduced the ATPSconcept for the separation of (bio)molecules by their partitioning between two liquid aqueous phases(Albertsson 1958). An ATPS consists of two immiscible aqueous-rich phases based on polymer/polymer, polymer/salt or salt/salt combinations.

The practical strategies for the design of an appropriate recovery process using ATPS can be divided into four stages, namely the initial physicochemical characterization of the feedstock, selection of the type of ATPS, selection of the system parameters, and evaluation of the influence of the process parameters upon the product recovery/purity (Benavides and Rito-palomares 2008) (Figure 2). More specifically, different physico-chemical properties affect the partition of the biomolecules in the two-phase systems, like surface hydrophobicity, molar mass, isoelectric point and components of the system and some other factors that influence partitioning are concentration of polymer or surfactant, salt addition and pH.

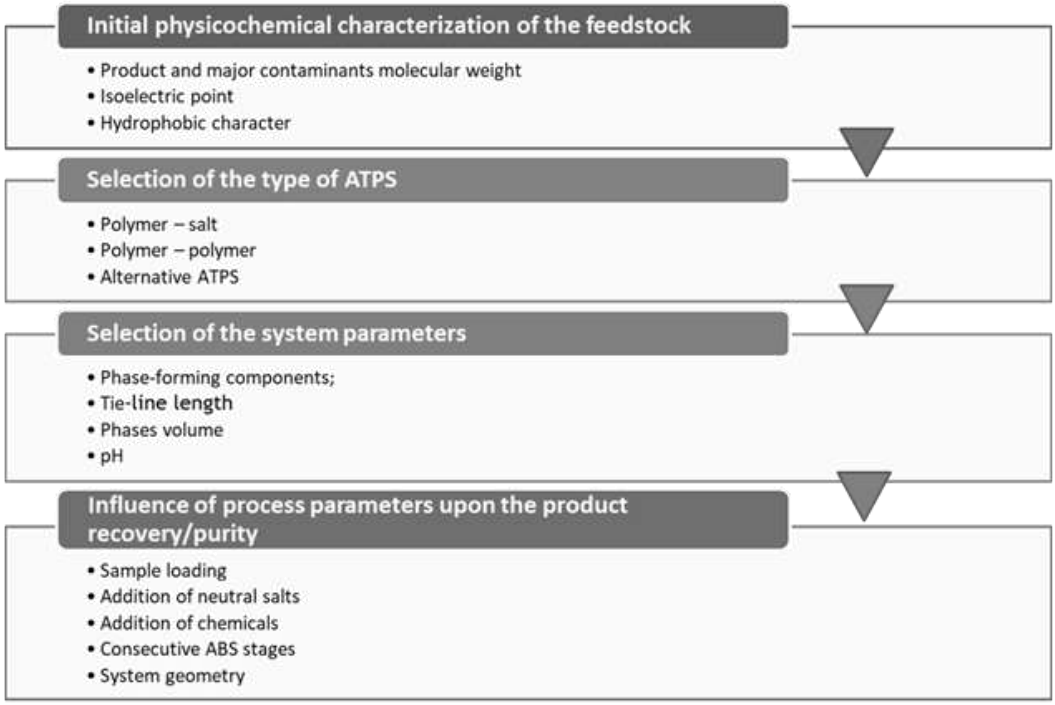


Figure 2. Simplified representation of the strategies for the design of the recovery of biological products using aqueous biphasic systems (ABS) (adapted from Benavides and Rito-palomares 2008).

ATPS are favourable for the extraction of enzymes due to the high amount of water present in the phases (Freire et al. 2012). Moreover, these systems are of low-cost when compared with chromatographic strategies, more environmentally benign since the use of volatile organic compounds is avoided, allow the scale-up and lead to high extraction performance and purity levels. For instance, a comparison between a purification process using IEC, with a previous acetone fractionation, and an ATPS extraction, demonstrated superior overall yield of the enzyme α-galactosidase in ATPS (11.5 vs 87.6%, respectively) (Naganagouda and Mulimani 2008). Other widely used technique for the purification of enzymes, as mentioned before, consists on the precipitation of the target molecule with ammonium sulphate. A comparison between the two methods was already performed and ATPS exceeded the precipitation method, achieving a greater recovery yield (184% vs 53%) and purification factor (7.2 vs 4.8) of laccase (Schwienheer et al. 2015). Thus, it is clear that ATPS constitutes an interesting alternative method over other conventional separation processes (Figure 3), and in particular for enzymes, and so, these systems have been subject of increased attention and research.

For the fungal enzyme extraction by ATPS, most of the authors have used polymer-salt based ATPS as mentioned in Table 4. These systems are mainly composed of biodegradable organic salts, such as sodium citrate (Table 4). The maximum of protein yield (130%) using the conventional ATPS was observed using PEG 10000 and buffer

citrate salt concentration of 15-20% and 8-15%, respectively (Porto et al. 2008). Moreover, Alhelli et al. (2016)have used ATS composed of PEG, a sodium citrate salt and added a third component, sodium chloride to successfully purify protease from *Penicillium candidum* in the salt-rich phase, increasing the purification factor. The authors observed that the sodium chloride concentrations can be a factor that display a significant influence on the purification factor(Alhelli et al. 2016).

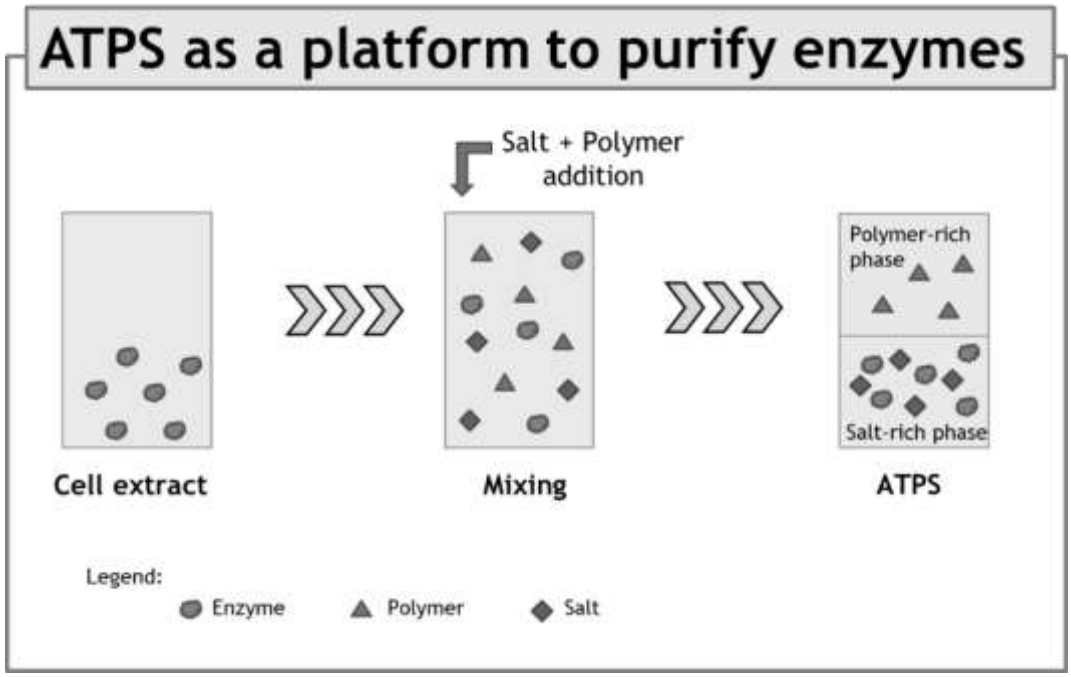


Figure 3. A proposed strategyfor the purification of enzymes from fermentation broth.

Polymer-polymer ABS have been also investigated for the purification of fungal enzymes, for instance chitinases (Chen and Lee 1995). However, these systems display high viscosities at the coexisting phases (Martínez-Aragón, Goetheer and de Haan 2009). Furthermore, dextran is too expensive as a phase-forming component to scale-up the extraction process (Liu et al. 2012). To overcome these drawbacks, most works in literature describe the use of polymer–salt systems (Table 4) thereby decreasing the viscosity of the coexisting phases, providing a higher density difference, and thus faster separation rates, as well as by providing lower cost systems and their scale-up (Martínez-Aragón, Goetheer and de Haan 2009). These systems are mainly composed of inorganic salts, especially phosphate-based, and some biodegradable organic salts, such as sodium citrate (Table 4).

Table 4. Summary of enzyme purification from fungi using ATPS as alternative purification processes

Enzyme	Microorganism	Purification		Purification factor	Yield of protein (%)	Ref
		Type of ATPS	Additive			
Chitinases	*Neurospora crassa*	PEG 6000 22.0% + K_2HPO 10.0%	---	38.0	88.0	(Teotia, Lata and Gupta 2004)
Proteases	*Penicillium roqueforti*	PEG 4000 15.5% + Sodium Phosphate 20.0% - pH 7.5	---	3.5	n.d.	(Pericin, Madjarev-Popovic and Vastag 2008)
	R. mucilaginosa L7	PEG 6000 15.5% + Sodium Tartrate 11.5%	---	2.5	81.1	(Lario et al. 2016)
	Penicillium candidum	PEG 8000 9.0% + Sodium Citrate 15.9%	Sodium chloride	6.8	93.0	(Alhelli et al. 2016)
	Mucor subtilissimus UCP1262	PEG 6000 30.0% + Sodium Citrate 13.2 wt%	---	10.0	100.0	(Nascimento et al. 2016)
Laccase	*A. bisporus*	PEG 1000 18.2% + Buffer Phosphate 15.0% - pH 7	---	2.5	95.0	(Mayolo-Deloisa, Trejo-Hernández and Rito-Palomares 2009)
	L. polychrous	PEG 4000 12.0% + Phosphate salt 16.0%	---	3.0	99.1	(Ratanapongleka and Phetsom 2011)
	P. sapidus	PEG 3000 13.3% + Phosphate salt 6.3%	---	1.7	92.0	(Prinz et al. 2014)
	Trametes versicolor	PEG 3000 13.3% + Phosphate salt 6.3%	---	1.9	90.0	(Prinz et al. 2014)

n.d.- not determined.

One of the most used polymers in ATPS is PEG (Table 4). Polymers offer some degree of design, for instance, by varying the length of the polymeric chains, *i.e.*, by changing their average molecular weight, or by changing the structure of the monomer unit. PEG also displays some attractive properties, such as biodegradability, low toxicity, low volatility, low melting points, high water solubility and low cost(Ferreira et al. 2016). However, the hydrophilic nature of PEG limits the polarity range between the coexisting phases in the ATPS. To overcome this limitation, recent works have introduced ionic liquids to tune the properties of PEG through the modification of its chemical structure and thus increasing the extraction yield. The use of ILs in ATPS leads to the possibility of controlling the phases' polarities by an adequate choice of the constituting ions, and so, this high tunability makes them a desirable class of extraction solvents in liquid-liquid extraction processes. In addition, it was already shown that ionic liquids could be used as adjuvants to tailor the selectivity and extraction aptitude for target biomolecules. In summary, it is clear that low amounts of ionic liquids in the formulation of ATPS are

enough to trigger complete extractions of target compounds in a single step. ATPS composed of PEG, salts and ILs (as adjuvants) are a promising alternative and more efficient method for the purification of biopharmaceuticals. Additionally, we believe that there is a requirement to study further ATPS made up of ionic liquids for the purification of fungal enzymes which appears to be a predominantly promising substitute. However, the commercial purification of fungal enzymes using ATPS still requires more exploration for its implementation. Santos et al.(2018) demonstrated that high purification performance, usually required in pharmaceutical industry, was achieved through the design of an integrated process comprising the steps production, cell disruption, and purification with an ammonium sulphate precipitation followed by the application of ATPS with ionic liquid as adjuvant, and culminating in the L-asparaginase isolation and reuse of the various phases. Additionally, the study of enzyme recuperation from phase, as well as the recycling nature of the ATPS used needs to be more explored in the future. Additional investigations regarding the effects of the phase-forming components through the protein stability and activity are also required.

Three-Phase Partitioning

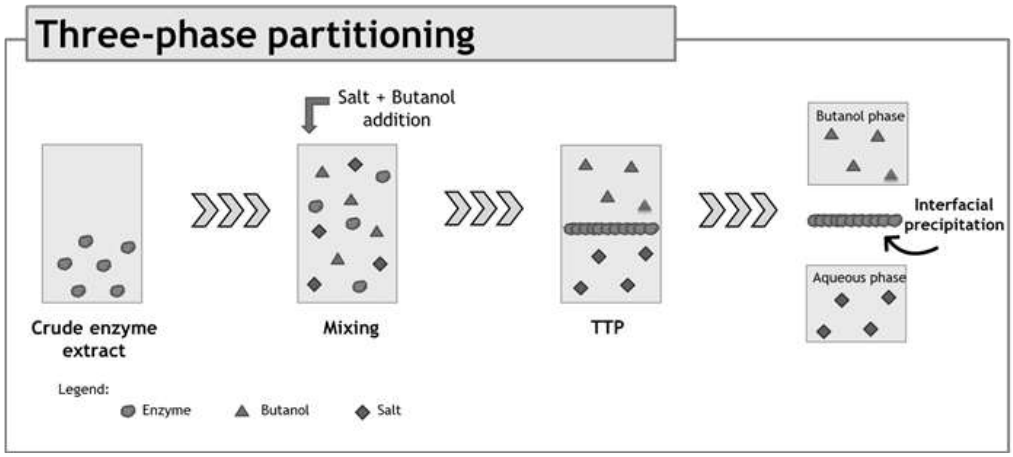


Figure 4. Scheme of three phase partitioning recovery experiment from crude enzyme extract to three distinct separated phases.

Three-phase partitioning (TPP) is an upcoming bio-separation technique developed for the extraction of proteins, especially enzymes from multi-component systems, due to their ability to concentrate proteins from crude broths with higher purification than conventional concentration methods (Gagaoua and Hafid 2016). The principle of this emerging tool consists in mixing the crude protein extract with solid salt (mostly ammonium sulphate) and an organic solvent, usually butanol in order to obtain three phases, i.e., involves the accumulation of the target enzyme at the liquid–liquid interface while the contaminants mostly partition to t-butanol (top phase) and to the aqueous phase

(bottom phase) (Figure 4) (Ketnawa, Rungraeng and Rawdkuen 2017). Kumar et al. (2011) revealed that butanol provided the purity (7.2-fold) and recovery (184%)of laccase from *Pleurotusostreatus*. However, the main drawback of TPP is that the use of a volatile organic solvent such as t-butanol may limit the large-scale use of this technique(Alvarez-Guerra et al. 2014); without forgetting that, some enzymes may lose their activity in the presence of high amount of t-butanol(Ketnawa, Rungraeng and Rawdkuen 2017).

CHARACTERIZATION OF THERAPEUTIC FUNGAL ENZYMES

The activity of each enzyme depends on several parameters, such as pH, temperature, substrate, among others (Figure 5). The enzymes structure influences the parameters in which its activity is optimum, and therefore a deep knowledgeonthe characterization of enzymes is required. Specifically, for therapeutic fungal enzymes, its characterization is even more important, since the efficiency of a therapy depends on the knowledge of the target and the therapeutic.

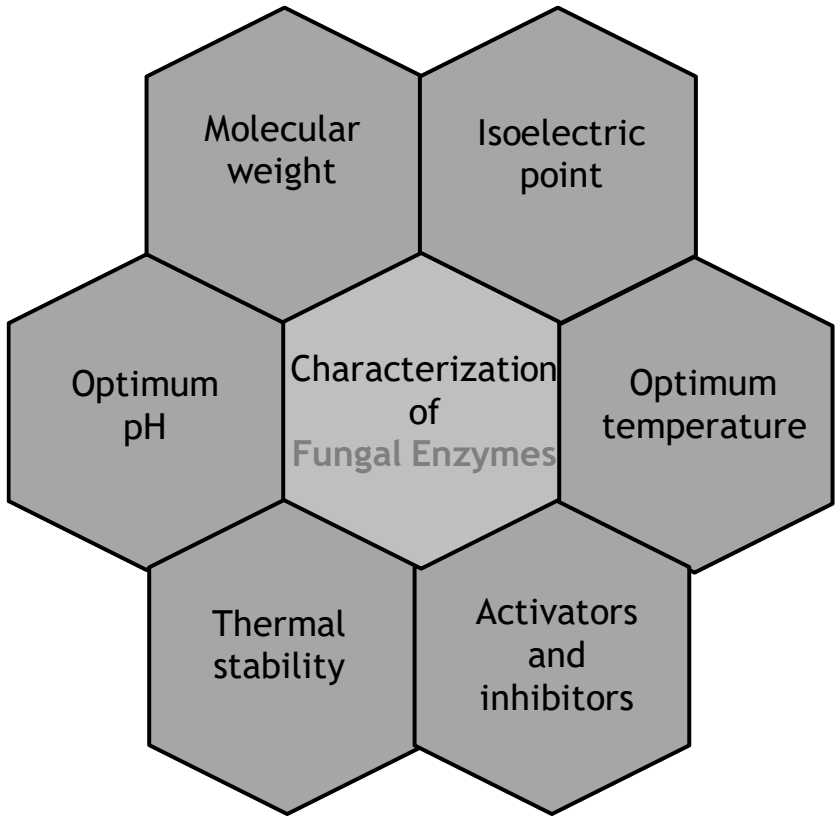


Figure 5. Parameters influencing enzyme activity.

α-Amylases

The properties of α-amylasesare highly dependent on the microorganism where the enzyme is expressed. Regarding to the molecular weight, α-amylase from*A. oryzae* was estimated with 51kDa by the combined use of high-pressure silica gel chromatography and the low angle laser light scattering technique(Patel et al. 2005). The α-amylasefrom halophilic *Engyodontiumalbum* was found to have a single band with relative molecular mass of50 kDa(Ali et al. 2014). On the other hand, α-amylase from *T.lanuginosus* reveal by electrophoreticexperiments a highermolecular weight of 61 kDa(Nguyen et al. 2002).Other fungal α-amylases described, for instance from *Cryptococcus flavus* presented an apparent molecularmass of 75 and 32.5 kDa(Wanderley et al. 2004; Balkan and Ertan 2010). Electro focusing ofα-amylase of *A.niveus*revealed anisoelectric point of 6.6(Silva et al. 2013), incontrast, the α-amylase from *A. flavus*presented anisoelectric point of 3.5(Khoo et al. 1994).

Substrate Specificity and Effect of Substrate on α-Amylases Activity

As holds true for the other enzymes, the substrate specificity of α-amylase varies from microorganism to microorganism. In general, α-amylases display highest specificity towards starch followed by amylose, amylopectin, cyclodextrin, glycogen and maltotriose(Saranraj and Stella 2013). The α-amylase activity from *A. niveus* against various substrates, such as, soluble starch, amylose, amylopectin,and glycogen was investigated by Silva et al. (Silva et al. 2013). The enzyme preferentially hydrolyzed maltopentose, maltotriose, maltotetraose, and malto-oligosaccharide (G10), but sucrose, trehalose, α-cyclodextrin, β-cyclodextrin, and p-nitrophenyl α-D-glucopyranoside were not hydrolyzed(Silva et al. 2013). α-Amylase produced by *A. oryzae* reveal a maximum activity of 36.13U/mg with 1% starch as the substrate concentration(Patel et al. 2005).

Effect of pH on α-Amylases Activity

Optimum pH is required for maximum enzyme activity (Patel et al. 2005). The pH optima of α-amylases vary from 2 to 12. α-amylases from most bacteria andfungi have pH optima from the acidic to neutral range(Saranraj and Stella 2013), since in their catalytic mechanism, an oxidation-reduction reaction is involved and for this particular reaction, the H^+ concentration should be optimum for the proper catalysis (Patel et al. 2005).The optimum pH of an extracellular amylase secreted by *A.niveus*was 6.0(Silva et al. 2013), while α -amylase produced by *A.oryzae*showed that the maximum specific activity was obtained at pH 5 (Patel et al. 2005). Optimum α-amylase from *T.lanuginosus*activity wasfound in the pH rangebetween 4.6and6.6 with changes less than 10%(Nguyen et al. 2002). Enzyme activity decreased drastically at pH below 4.0 orabove 7.0(Nguyen et al. 2002).

In contrast, α-amylase obtained from halophilic *E. album*showed that this enzyme was able to work betterin neutral and alkaline pH ranges(Ali et al. 2014). A steady increase in enzyme activity was observed from pH 5 to 9, with the highest enzyme activityobserved at pH 9.0(Ali et al. 2014).

Effect of Temperature on α-Amylases Activity

The optimum temperature and the activity of α-amylase is related to the growth of the microorganism (Saranraj and Stella 2013). The lowest optimum temperaturefor α-amylasesis reported to be 25 to 30°C for *Fusarium oxysporum* amylase (Saranraj and Stella 2013). Theα-amylase produced by *A.oryzae*showed a maximum activity at 50°C(Patel et al. 2005) while, the optimum temperature for α-amylase from *T. lanuginosus*is exhibit at 70ºC (Nguyen et al. 2002). The α-amylase from halophilic *E. album* has been found to have optimum activity at 60ºC and retain more than 85% of its activity at high temperatures of 70-80ºC,which are considered as thermophilic range for enzymes (Ali et al. 2014).

Activators and Inhibitorson α-Amylases Activity

α-amylase is a metalloenzyme, which contains at least one Ca^{2+} ion. Many fungal amylases described in the literature are activatedby metal ions (Saranraj and Stella 2013; Silva et al. 2013). It has been reported that partially purified α-amylase, particularly those of fungal origin, lose activity above 50°C but the activity could be retained in the presence of Ca^{2+} (Patel et al. 2005). In fact, α-amylase from *A. oryzae*has a specific activity of 22.03 U/mg at 50ºC and 20.93, 12.10 and 11.78 at 60, 65 and 70ºC, respectively. However, when the reaction was carried out at 65ºC in the presence of $CaCl_2$10 mM, the enzyme activity was even better than at 50ºC (Patel et al. 2005).

$BaCl_2$, $CaCl_2$, $HgCl_2$ and $MgCl_2$ increased theamylase activityfrom halophilic *E. album*, but not greater than 110% (Ali et al. 2014).In contrast, β-mercaptoethanol, EDTA, $FeCl_2$ and$ZnCl_2$ decreased the enzyme activity. The greatestinhibition occurred in the presence of $ZnCl_2$. Thedecrease in enzyme activity was never less than 60% by the addition of any inhibitor (Ali et al. 2014).

The activities of α-amylase from *T. lanuginosus* decreased significantly by adding 10mM of Zn^{2+} ion toreaction mixture (Nguyen et al. 2002). Moreover, Co^{2+}showed inhibitor and Ca^{2+} and Ba^{2+} activator effects (Nguyen et al. 2002).

Theα-amylase from *A. niveus* showed a slight increase in itsactivity in the presence of many salts (Silva et al. 2013). This enzyme was activated 17, 14, 80, 28, 39, and 61% in presence of 1 mmol/L ofNH_4F, NaBr, $MnCl_2.4H_2O$, $NaH_2PO_4H_2O$, $ZnCl_2$ andβ-mercaptoethanol, respectively. At10 mmol/L, the α-amylase activity was increased in 23, 20,16, 12, and 16%, in the presence of NH_4F, KH_2PO_4, NH_4Cl,NaCl, and $CoCl_2.6.H_2O$, respectively. $HgCl_2$, $AgNO_3$, and $Fe_2(SO_4)_3$ drastically inhibited the enzyme activity (Silva et al. 2013).

Chitinases

Chitinases, glycosyl hydrolases, have sizes ranging from 20 kDa to about 90 kDa (Javed et al. 2013). Different molecular massesranging from 38 to 45 kDa have been reported forfungalchitinases derived from *P. cinnabarinus* (Ohtakara 1988), *T. Harzianum* (Ulhoa and Peberdy 1992), *A.obclavatum* (Gunaratna and Balasubramanian 1994) and *P. Communis* (Masaru Sakurada et al. 1996). De La Cruz et al. (1992) isolated three chitinases from *T. harzianum*with molecular massesranging from 33 to 42 kDa with isoelectric points, determined by chromatofocusing and isoelectrofocusing, between 5.0 and 7.8, depending on the enzyme (Cruz et al. 1992). Cytosolic chitinase from *P. communis* were purified and a molecular mass of 42 kDa and an isoelectric point of 4.9 was estimated (Sakurada et al. 1996).

Substrate Specificity on Chitinases Activity

Activity of chitinase from *Fusarium chlamydosporum* on both colloidal and pure chitins was high (Mathivanan, Kabilan and Murugesan 1998). This is possibly due to the availability of a larger number of active sites or termini for the enzyme in the purified and colloidal chitins than in crude chitin and cell wall fragments. Chitinases from *T.harzianum* were able tohydrolyze colloidal and glycol-chitin, a β-(1-4)-N-acetylglucos-aminepolymer (Cruz et al. 1992).Chitinase with 33 kDawas only active on colloidal and glycol-chitin, and almost inactive, on β-(1-4)-N-acetylglucosamine. Chitinases with 37 and 42kDawere active incolloidal, glycol-chitin and β-(1-4)-N-acetylglucosamine, and less glycol-chitosan, perhaps because chitosan is only partiallydeacetylated (Cruz et al. 1992).

Effect of pH and Temperature in the Chitinases Activity

Chitinase of *F. chlamydosporum* showed an optimum activity at pH 5 and was stable from pH 4 to 6 with more than 80% activity (Mathivanan, Kabilan and Murugesan 1998). The optimum temperature for this chitinase activity was at 40°C and the activity was stable up to 40°C, above which the activity sharply declined. However, chitinasefrom *P.communis* showed maximum activity at 60°C and stability from 40 to 60°C (Sakurada et al. 1996).Chitinasesfrom *T. harzianum* also revealed optimal temperature and heat-inactivation temperaturequite similar at 50-60ºC (Cruz et al. 1992). Cytosolic chitinase from *P. communis* revealed a higher activity at pH 6.2 at 39ºC, with 50% of the chitinase activity maintained between pH 5 and 8 (Sakurada et al. 1996). However, at pH 6.2 the chitinase activity was greatest at 60°C and 50% chitinase activity remained from 40°C to 60°C. At 65ºC, the chitinase activity decreased to 12% of the activity at 60°C (Sakurada et al. 1996).

Effect of Activators and Inhibitors on Chitinases Activity

Chitinase activity from *F.chlamydosporum* was inhibited by metals and other inhibitors to varying degrees, ranging from 5 to 100%, with $HgCl_2$totally inhibiting the enzyme activity. A similar effect of $HgCl_2$ onchitinases of *A. obclavatum* and *P.communis* was alsoreported (Gunaratna and Balasubramanian 1994; Sakurada et al. 1996). Cytosolic chitinase from *P.communis* decreased its activity, with 1 mM of Ag^+ or Hg^{2+}, more than 60% (Sakurada et al. 1996). Its activity was also inhibited by allosamidin, an analogue of N-acetylglucosamine, which has been reported to be a chitinase inhibitor (Sakuda et al. 1987). Sodium dodecyl sulfate at low concentration (1 mM) had no effect on chitinase activity, however at 10 mM inhibited chitinase activity completely. N-Ethylmaleimide, iodoacetic acid, iodoacetamide and p-chloromercuri-benzoic acid at 10 mM also inhibited chitinase activity by approximately 30% (Sakuda et al. 1987).

Fungal Chitinases Activity

The purified chitinase of *F. chlamydosporum* exhibitedstrong antifungal activity by inhibiting the uredosporegermination of *Puccinia arachidis*, with this effect being dependenton the concentration of the enzyme (Mathivanan, Kabilan and Murugesan 1998). The chitinase of*F. chlamydosporum* completely inhibited the germination of uredospores at a concentration of 30 μg/mL. At 10 and 20 μg/mL, the enzyme caused inhibition of 78 and 92%, respectively (Mathivanan, Kabilan and Murugesan 1998). Gunaratna and Balasubramanian also reported the inhibition of uredospore germination of *P.arachidis* by the chitinase of *A.obclavatum* (Gunaratna and Balasubramanian 1994). Theinhibition of uredospore germination might be due to the actionof chitinase on the newly formed chitin in germ tubewalls (Gunaratna and Balasubramanian 1994).

The antifungal activity of *T. harzianum* chitinases was tested using an assay based upon inhibition of hyphal extensionof the phytopathogenic fungi *Rhizoctonia solani*, *F. oxysporum* and *Verticillium nigerensis*, all of which have chitin in their cell walls (Cruz et al. 1992). However, none of the three chitinases caused inhibition of hyphal extension.

L-Asparaginases

LA occurs abundantly in naturefrom prokaryotic microorganisms to vertebrate (Eisele et al. 2011). In fact, LA can be obtained from a variety of sources, including, many mitosporic fungi genera such as *Aspergillus*, *Fusarium* and *Penicillium* (Luhana, Dave and Patel 2013). The variability in LA molecular weight from different organisms may be inferable to its genetic diversities. LA from *Fusarium culmorum* showed homogeneity and the molecular mass was estimated as 90 kDa, by SDS-PAGE analysis (Janakiraman 2015). The molecular weight of LA from *F.culmorum* (Janakiraman 2015)

is similar to LA from *Penicillium brevicompactum* (94 kDa) (Elshafei 2012)and *Trichoderma viride* (99 kDa) (Thakur et al. 2011). On the other hand, LA from *Cladosporium* sp. (Sarquis et al. 2004) and *Aspergillus niger* (Akilandeswari, Kavitha and Vijayalakshmi 2012) has a molecular weight of 117 kDa and 48 kDa, respectively.

Effect of pH on L-Asparaginases Activity

A critical factor for stability and activity of purified enzyme is the pH, as it impacts on the ionic form of the enzyme active site residues. The effect of pH on the activity of purified LA from *F. culmorum* was done over a wide range of pH from 3.0 to 11.0 at 30°C (Janakiraman 2015). The results revealed that LA was active over a broad range of pH, optimum being pH 8.0, and 100% of activity at pH 8.0 up to 24 h of incubation. Similar results were reported by LA from *P. brevicompactum* (Elshafei 2012)and *Streptomyces* sp. (Sabha, Nadia and Tarek 2013). Thakuret al. reported the opposite, with pH 7.0 as the optimum pH for the activity of LA from *T.viride*with 82% of its activity maintained after 24 h of incubation (Thakur et al. 2011). Moreet al. also demonstrate pH 7.0 as the optimum pH for the activity of LA from *Mucorhiemalis*, however, its stability is only retained during 4 h (More et al. 2013).Eisele et al. reported similar results to More et al., 2013, with the optimum pH for LA from *F. velutipes* being pH 7, a high stability over the broad range of pH 3–9 where wasretained at least 85% of its maximum activity after 16 h (Eisele et al. 2011). LA from *A. niger* showed maximum activity at pH 6 and lowest activity at pH 3 (Luhana, Dave and Patel 2013).

Effect of Temperature on L-Asparaginases Activity

Temperature is an important physical parameter which influences the enzyme activity. The optimum temperature for LA purified from*F.culmorum* was 40°C with a high stability during 120 min at 30-40°C and 50% of its activity retained at 60°C for 1h (Janakiraman 2015). However, increasing the temperature, a sharpdecline in the reaction rate is observed. Similar results were reported for LA purified from *Aspergillus nidulans* (Archana rani and Raja rao 2014). Native LA from *F. velutipes* showed an optimum temperature at 40ºC, being the hydrolysis of L-glutamine and L-asparagine optima at 30ºC and 40ºC, respectively (Eisele et al. 2011). After 1 h at 60ºC, native and recombinant LA from *F. velutipes* displayed 39% and 45% of residual activity, compared to their respective values at 37ºC (Eisele et al. 2011). On the other hand, 37ºC was reported as the optimum temperature for the activity of LA in *T. viride* (Thakur et al. 2011), *M. hiemalis* (More et al. 2013) and *P. brevicompactum* (Elshafei 2012), with this last one, being stable up to 1 hat 37°C. LA from *A. niger* also reveal a high activity at 37ºC, but at 4ºC and 50ºC lost its activity (Luhana, Dave and Patel 2013).

Effect of Activators and Inhibitors on L-Asparaginases Activity

Different metal ions have been investigated as enhancers/inhibitors of LA activity. In fact, Mn^{2+}increases the activity of LA from *F. culmorum* by 18%, while Cu^{2+} and Hg^{2+} inhibited its activity by 84% and 80%, respectively (Janakiraman 2015). Metal ions like Ca^{2+} and Mg^{2+} did not have any effect on the LA from *A. nidulans*(Archana and Raja rao 2014), EDTA inhibited the activity of LA from *T.viride*by 88% while β-mercaptoethanoldid not have any effect on the enzyme activity (Thakur et al. 2011). Non-ionic surfactant, such as tween 80 was found to enhance the activity of LA from *F. culmorum* by 16%, whereas, the anionic surfactant, sodium dodecyl sulphate, completely inhibited the enzyme activity (Janakiraman 2015). Kumar and Monica also reported similar results with tween 80 at 2mM inducing the production of LA in *Cladosporium* sp. and *M. hiemalis* (Kumar and Manonmani 2013; Thakuret al. 2013).

Cytosine Deaminases

Cytosine deaminase from *Aspergillus fumigatus* was the first cytosine deaminase to be found in a mould (Yu et al. 1991). The enzyme was a monomer of 32 KDa with an optimum activity at pH 7 and 35˚C. Beside cytosine, the enzyme also hydrolyses 5-methylcytosine and 5-fluorocytosine. The activity of the enzyme in the presence of heavy metal ions, such as, Fe^{2+},Cu^{2+}, Hg^{2+} and Pb^{2+}, is inhibited.

Cytosine deaminase from *A. parasiticus* has an increased activity at pH 7.2 (Zanna et al. 2012). Although at pH 4 and 7 the enzyme activity wasappreciable. Highest cytosine deaminase activity wasverified between 40ºC and 45ºC, with an enzyme activity decrease at 50ºC butstable up to 80ºC. Cytosine deaminase from *A.parasiticus* is strongly inhibited by some metal ions, losing 47% of its activity in the presence of Ca^{2+}, 58% in the presence of Hg^{2+} and 40% in the presence of Co^{2+} and Zn^{2+}. Cu^{2+} and Fe^{2+} at 50 mm completely inhibited the enzyme activity (Zanna et al. 2012). The study on ionizable groups in the active site of *A. parasiticus* cytosine deaminase revealed the presence of groups with enthalpy of ionization of 43.01 KJ/mole, suggesting histidine in or around the active site of the enzyme (Zanna et al. 2012).

Proteases

As already described in this book chapter, a great number of fungal strains have been used to produce proteases belonging to the genera *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, *Humicola*, *Thermoascus*, *Thermomyces*, among others (Souza et al. 2015). Moreover, for each genera, different types of proteases have been reported, namely, acid,

alkaline, neutral, serine, aspartate, among others. Therefore, the proteases produced describe different properties (Souza et al. 2015).

Effect of pH on Proteases Activity

Acid proteases have an optimum activity in a pH range between 3.0 and 5.0 (Souza et al. 2015). Aleksieva and Peeva report an acid protease from *Humicola lutea* with an optimum activity at pH 3.0-3.5 (Aleksievaa and Peeva 2000), while Negi and Banerjee describe an acid protease from *Aspergillus awamori* with an optimum pH at 5.0 (Negi and Banerjee 2009). Aspartate protease also present an optimum and stable activity at pH ranges between 3.0 and 5.5 (Souza et al. 2015).

The majority of alkaline proteases have been reported to have optimum/stable activity in the pH range between 7.0 and 9.0(Souza et al. 2015). However, Chellapan et al. characterized a protease from marine *E. album* with a higher optimum pH between 10.0 and 11.0 (Chellappan et al. 2011). Neutral proteases have an optimum activity at pH 7.0 (Souza et al. 2015). Serine proteases, as alkaline proteases reveal an optimum/stable activity at alkaline pH values (7.0-8.0). Particularly, serine protease from *T. lanuginosus* present an increased activity at pH 5.0 (Li, Yang and Shen 1997).

Effect of Temperature on Protease Activity

Fungal proteases are usually thermolabile and show reduced activities at high temperatures(Souza et al. 2015). Acid proteases reveal a temperature optimal in a wide range between 25 and 70ºC. For instance, Larsen et al. report a protease from *Penicillium roqueforti* with an optimum temperature at 25ºC (Larsen, Kristiansen and Hansen 1998), while Negi and Banerjee describe a protease from *A. awamori* with an optimum temperature at 55ºC (Negi and Banerjee 2009), and Merheb-Dini et al. report a protease from *T. indicae-seudaticae* with an activity increased at 70ºC (Merheb-Dini et al. 2010). Serine proteases like acid proteases have very different optimum temperatures from 28 to 70ºC, with a higher number of proteases more active between 40 and 50ºC. Alkaline proteases are more active at lower temperatures and the major of reported proteases have an optimum temperature between 30-36ºC (Souza et al. 2015). Aspartate proteases have an optimum temperature at 50-55ºC (Souza et al. 2015).

Proteases Inhibitors

Several compounds have been reported in the inhibition of proteases activity. Protein proteases inhibitors are divided in 71 families. Among the 71 families, 27 include members of microbial and fungal origin, with7 families including members exclusively of bacterial origin, and 5 families being exclusively of fungal origin. Inaddition to protein protease inhibitors, other small-molecule inhibitors synthesized in the laboratory have been described (Sabotič and Kos 2012).Protease from the nematode-trapping fungus *A.oligospora* was completely inhibited by the serine protease inhibitor

phenylmethylsulfonyl fluoride (Wang et al. 2006). The amino acid aldehydes chymostatin and antipain with a Phe and Arg residue,respectively, were also inhibitory. Proteases from *Sporotrichumpulverulentum* were almost completely inhibitedby Ag^{+} and Hg^{2+} at 1 mM concentrations whileCu^{2+} at the same concentration was less inhibitory (Eriksson and Pettersson 2005). The inhibition by p-chloromercuribenzoate was almost completely restored for proteases by the addition of stoichiometric amounts of reduced glutathione ordithiothreitol. Partial inhibition was also observed withEDTA and α,α'-dipyridyl (Eriksson and Pettersson 2005). Trypsin-like protease (serine protease) from *T. harzianum* was strongly inhibited by 1 mM phenylmethylsulfonyl fluoride (78% inhibition) (Suarez et al. 2004). Aspartic-peptidase, cysteine-peptidaseand metallo-peptidase inhibitors (0.1 mM pepstatin,1 mM iodoacetamide, and 1 mM EDTA, respectively) hada weak effect on this protease, with less than 11% of inhibition(Suarez et al. 2004).

Lipases

The number of available lipases has increased since the 1980s and their use as an industrial biocatalyst has also increased, due to their properties like biodegradability, high specificity, highcatalytic efficiency, temperature, pH dependency, activity in organic solvents and nontoxic nature (Mehtaand Gupta 2017).

Effect of pH and Temperature on Lipases Activity

Lipases are active in a large range of pH and temperatures (Barriuso et al. 2016). They possess stability from pH 4.0 to 11.0 and temperature optima between 10 to 96°C. The extracellular lipase produced by *A. niger* is particularly active at low pH (Barriuso et al. 2016). Falony et al. reported the influence of various pH on the activity of *A. niger* lipase (Falony et al. 2006). A higher lipase activitywas achieved at pH 6.0, and this enzyme was 100% stable within a pH range from 4.0 to 7.0 during 24 h. Ülkeret al. describe that pH 8.5 was found to be excellent for maximum activity of lipase from *T. harzianum* (Ülker et al. 2011). Lipase activity was declinedby changing the pH above or below the pH optima. Lipases from *A. niger* (Fukumotoand Tsujisaka 1963) and *Rhizopus japonicas* (Aisaka and Terada 1981) are stable at 50°C, and lipase of thermotolerant *H. lanuginosa* is stable at 60°C (Mehta and Gupta 2017).

Activators and Inhibitors on Lipases Activity

Aspergillus japonicus lipase activity is inhibited by 1 mM of Mn^{2+} and Hg^{2+} while Ca^{2+} was found to be the best for maximum activity after pre-incubation for 1h (Jayaprakash and Ebenezer 2012). *T.harzianum* lipase is stable after pre-incubation for 1h in several metal ions solutions (1 mM) (Ülker et al. 2011). In particular, Ca^{2+} and Mn^{2+}

increased the activity of lipase up to 25% and 15%, respectively, while K^+ and Cr^{3+} inhibited the lipase activity by 22% and 21%. Ca^{2+} also increases the activity of *Rhizopus chinensis* (Yu, Wang and Xu 2009) and *A. oryzae* (Ohnishi et al. 1994) lipases. This might be because the enzyme requires Ca^{2+} as a cofactor for its biological activity.

The activity of *A. oryzae* lipase is inhibited by Cu^{2+}, Fe^{3+},Hg^{2+}, Zn^{2+} and Ag^+ (Toida et al. 1995). Extracellular lipase activity from *C. kikuchii* has increased in the presence of ions like Al^{3+}, Ca^{2+}, Mn^{2+}, Zn^{2+} and Hg^{2+}. Residual lipase activity was increased to 129.3% inpresence of Al^{3+} ion as compared to control (Costa-Silva et al. 2014).

Glucose Oxidases

The most studied and commercialized glucose oxidase (GOx)is obtained from the fungus *A. niger*. The GOx extracted has a high substrate specificity and is stable over a wide range of pH and temperature (Yuivar et al. 2017). The molecular weight of native glucose oxidase from *A. niger* is approximately 160 kDa with two equal subunits (Singh and Verma 2013). The molecular mass of GOx from *P. ostreatus* was found to be 290 kDa consisting in four subunits with a molecular mass of 70 kDa(Shinet al. 1993).

Effect of Temperature on Glucose Oxidases Activity

GOx from *A.niger*has optimal activity at 25°C and exhibited more than 90% of the maximum activitybetween 20-35°C(Singh and Verma 2013). However, above 45°C its activity decreased rapidly. GOxmaintained 90% of its optimum activity at37°C, when compared to optimal activity of this enzyme between 25 and 30°C. On the contrary, GOx from *A.tubingensis*and a recombinant GOx from *Penicillium amagasakiense*presented highest activity at 60°C(Courjean and Mano 2011). The residual activity of purified GOx from*A.niger*remained relatively unchanged over 10 h at 25°C, whereas exhibiting a half-life of approximately 30 min at 50°C(Singh and Verma 2013). The enzyme is stable up to 40ºCbut its stability decreased at highertemperatures. On the other hand, GOxfrom *P.ostreatus* has stability at 70ºC during 120 min(Shinet al. 1993).

Activators and Inhibitors on Glucose Oxidases Activity

The activity of GOxfrom *A. ninger*is highly specific for D-glucose, however, other sugars, such as maltose, fructose, are oxidized at lower rate(Singh and Verma 2013). Similar results have been reported for glucose oxidase from *P. ostreatus*(Shinet al. 1993).

GOx from *A. niger* was inhibited 56.5 and 48% by Cu^{2+} and Ag^{2+}, respectively(Singh and Verma 2013). Similar results were reported for the enzyme from *Phanerochaetechrysosporium*, with the enzyme being inhibited by Ag^{2+} (10 mM) and o-

phthalate (100 mM), but not by Cu^{2+}, NaF, or KCN (10 mM)(Kelley and Reddy 1986). The inhibition of glucose oxidase by Ag^{2+} ions is due to reaction of Ag^{2+} with thiol group of the enzyme, essential for the enzymatic activitywhich is close to the FAD binding region of protein (Singh and Verma 2013).

Laccases

Laccase is currently seen as highly interesting industrial enzymes because of their broad substrate specificity. The molecular weight of most fungal laccases is between 43and 110 kDa (Thurston 1994). The molecular mass of laccase from basidiomycete *Trametes* sp. strain AH28-2A was estimatedto be 62 kDa with an isoelectric point of 4.2 (Xiao et al. 2003). A similar molecular weight was determined for laccase produced by *Mycenapurpureofusca* (Shujing et al. 2013).

Effect of pH and Temperature on Laccases Activity

Purified laccase from *Pleurotus*sp.is a monomer with a molecular mass of 40 kDa, active in a pH range between 3 and 5 with optimum activity at pH 4.5 (More et al. 2011). Similar results were obtained for laccase from basidiomycete *Trametes* sp. strain AH28-2A, stable in a pH range between 4.2 to 8.0, and an optimum pH at 4.5 in citrate-Na_2HPO_4 (Xiao et al. 2003). Laccase from the ascomycete *Thielavia* sp. is highly stable at acidic pH range, with an optimum activity at pH 5.0 and 6.0 (Mtibaà et al. 2018).

Laccase from *Pleurotus* sp. is stable in a temperature range between 35 and 70°C and an optimum temperature at 65°C (More et al. 2011), like laccases from *Sclerotiumrolfsii* (Ryan et al. 2003). Temperature kinetics of this enzyme suggests that the enzyme activity increases sharply from 60 to 65°C followed by a decline after 70°C. The laccase was stable at 60°C during 8 h, while at 75°C was stable up to 30 min, and after 90 min it retained 38% of the activity. *Pleurotus* sp. was stable for 20 days at room temperature and stable for 60 days when stored at −4°C(More et al. 2011). Laccase from basidiomycete *Trametes* sp. strain AH28-2A has an optimum activity at 50ºC and the enzyme is stable at 70ºC for more than 1 h. The activityof laccase is 2.5 times higher at 50ºC than at 20ºC (Xiao et al. 2003).

Activators and Inhibitors on Laccases Activity

Laccase from *Pleurotus* sp. is more inhibited by sodium EDTA(More et al. 2011), similar to laccases from *Chaetomium thermophilum* (Chefetz, Chen and Hadar 1998). The activity of laccase from basidiomycete *Trametes* sp. strain AH28-2A is totally inhibited by 0.1 mM of sodium azide or cyanide, 59.6% inhibited by 25 mM of SDS, and almost unaffected by 25 mM of EDTA(Xiao et al. 2003). Fe^{3+}, Mn^{2+}, Cu^{2+}, Ag^{+}, Ca^{2+}, Ba^{2+} and Zn^{2+}at 0.05M have a slightly stimulating effect on laccase from *M.*

purpureofusca (Shujing et al. 2013). The enzyme activity can be enhanced by 18.7% and 130.5% when Ag^{+} was added to the medium at 0.05 and 0.5 M, respectively. On the contrary, Fe^{2+} strongly inhibited enzyme activity up to 98% at 0.05 and 0.5 mM. Laccasefrom the ascomycete *Thielavia* sp. is inhibited by Hg^{2+} and Fe^{2+}, while the presence of Mn^{2+} at concentrations of 5and 10 mM promoted the enzymatic activity (Mtibaà et al. 2018).

ACKNOWLEDGMENTS

This work was financially supported by the project POCI-01-0145-FEDER-031268 - funded by FEDER, through COMPETE2020 –ProgramaOperacionalCompetitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES. This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, FCT Ref. UID/CTM/50011/2019, financed by national funds through the FCT/MCTES". Ana P. M. Tavares acknowledges FCT for the research contract under the IF (IF/01634/2015) Investigator Programme-2015.

REFERENCES

Abidi, F., Chobert, Jean-M., Haertlé, T. and Marzouki, M. N. (2011). Purification and Biochemical Characterization of Stable Alkaline Protease Prot-2 from *Botrytis-cinerea*. *Process Biochemistry*, 46, 2301–2310.

Aehle, W. (2007). *Enzymes in Industry: Production and Applications.*

Ahmed, A., Nageh, A. D. F., Taher, T.M. and Fareed, H. S. M. (2015). Production, Purification and Characterization of L-Asparaginase from Marine Endophytic *Aspergillus* sp. ALAA-2000 under Submerged and Solid State Fermentation. *Journal of Microbial & Biochemical Technology*, 7, 165–172.

Aisaka, K. and Terada, O. (1981). Purification and Properties of Lipase from *Rhizopus japonicus*. *The Journal of Biochemistry*, 89, 817–822.

Akilandeswari, K., Kavitha, K. and Vijayalakshmi, M. (2012). Production of Bioactive Enzyme L-Asparaginase from Fungal Isolates of Water Sample through Submerged Fermentation. *International Journal of Pharmacy and Pharmaceutical Sciences* 4, 363–366.

Albertsson, P. A. (1958). Partition of Proteins in Liquid Polymer-Polymer Two-Phase Systems. *Nature*, 182, 709–11.

Aleksievaa, P. and Peeva, L. (2000). Investigation of Acid Proteinase Biosynthesis by the Fungus *Humicolalutea* 120-5 in an Airlift Bioreactor. *Enzyme and Microbial Technology*, 26, 402–405.

Alhelli, A.M., Abdul Manap, M.Y., Mohammed, A.S., Mirhosseini, H., Suliman, E., Shad, Z., Mohammed, N.K. and Meor Hussin, A.S. (2016). Response Surface Methodology Modelling of an Aqueous Two-Phase System for Purification of Protease from *Penicillium candidum* (PCA 1/TT031) under Solid State Fermentation and Its Biochemical Characterization. *International Journal of Molecular Sciences*, 17, 1872.

Ali, I., Akbar, A., Anwar,Yanwisetpakdee, M. B., Prasongsuk, S., Lotrakul, P. and Punnapayak, H. (2014). Purification and Characterization of Extracellular, Polyextremophilic α-Amylase Obtained from *Halophilic engyodontium*album. *Iranian Journal of Biotechnology*, 12, 35–40.

Alvarez-Guerra, E., Ventura, S. P. M., Coutinho, J. A. P. and Irabien, A. (2014). Ionic Liquid-Based Three Phase Partitioning (ILTPP) Systems: Ionic Liquid Recovery and Recycling. *Fluid Phase Equilibria*, 371, 67–74.

Anitha, T.S. and Palanivelu, P. (2013). Purification and Characterization of an Extracellular Keratinolytic Protease from a New Isolate of *Aspergillus parasiticus*. *Protein Expression and Purification*, 88, 214–220.

Archana, R. J. and Raja, R. P. (2014). Production, Purification and Characterization of L-Asparaginase from *Aspergillus nidulans* by Solid State Fermentation. *European Journal of Biotechnology and Bioscience*, 2, 5158.

Arora, D. S. and Sharma, R. K. (2010). Ligninolytic Fungal Laccases and Their Biotechnological Applications. *Applied Biochemistry and Biotechnology*, 160, 1760–1788.

Asadi-Moghaddam, K. and Chiocca, E. A. (2006). Prodrug-Activation Gene Therapy. In *Gene Therapy of the Central Nervous System*, 291–301.

Azzopardi, E., Lloyd, C., Teixeira, S. R., Conlan, R. S. and Whitaker, I. S. (2016). Clinical Applications of Amylase, Novel Perspectives. *Surgery*, 160, 26–37.

Bacelar, A., Maia, A. C. F., Rueda, J. A. and Vanzela, A. P. F. C. (2016). Fungal Production of the Anti-Leukemic Enzyme L-Asparaginase: From Screening to Medium Development. *Acta Scientiarum. Biological Sciences*, 38, 387–394.

Bairoch, A. (2000). The Enzyme Database in 2000. *Nucleic Acids Research*, 28, 304–305.

Balkan, B. and Ertan, F. (2010). The production of a new fungal alpha-amylase degraded the raw starch by means of solid-state fermentation. *Preparative Biochemistry and Biotechnology*, 40, 213–28.

Barriuso, J., Vaquero, M. E., Prieto, A. and Martínez, M. J. (2016). Structural Traits and Catalytic Versatility of the Lipases from the *Candida rugosa*-like Family: A Review. *Biotechnology Advances*, 34, 874–885.

Benavides, J. and Rito-palomares, M. (2008). Practical Experiences from the Development of Aqueous Two-Phase Processes for the Recovery of High Value Biological Products. *Journal of Chemical Technology and Biotechnology*, 142, 133–42.

Bhattacharya, D., Nagpure, A. and Gupta, R. K. (2007). Bacterial Chitinases: Properties and Potential. *Critical Reviews in Biotechnology*, 27, 21–28.

Bhatti, H. N.and Saleem, N. (2009). Glucose Oxidase from *Penicillium Notatum*. *Food Technolgy and Biotechnology*, 47, 331–335.

Binod, P., Pusztahelyi, T., Nagy, V., Sandhya, C., Szakács, G., Pócsi, I. and Pandey, A. (2005). Production and Purification of Extracellular Chitinases from *Penicillium aculeatum* NRRL 2129 under Solid-State Fermentation. *Enzyme and Microbial Technology*, 36, 880–87.

Bode, C., Smalling, R. W., Berg, G., Burnett, C., Lorch, G., Kalbfleisch, J. M., Chernoff, R. et al. (1996). Randomized Comparison of Coronary Thrombolysis Achieved With Double-Bolus Reteplase (Recombinant Plasminogen Activator) and Front-Loaded, Accelerated Alteplase (Recombinant Tissue Plasminogen Activator) in Patients With Acute Myocardial Infarction. *Circulation*, 94, 891–898.

Brandelli, A., Daroit, D. J. and Riffel, A. (2010). Biochemical Features of Microbial Keratinases and Their Production and Applications. *Applied Microbiology and Biotechnology*, 85, 1735–1750.

Carroccio, A., Guarino, A., Zuin, G., Verghi, F., Canani, R. B., Fontana, M., Bruzzese, E., Montalto, G. and Notarbartolo, A. (2001). Efficacy of Oral Pancreatic Enzyme Therapy for the Treatment of Fat Malabsorption in HIV-Infected Patients. *Alimentary Pharmacology & Therapeutics*, 15, 1619–1625.

Chao, Y. P., Xie, F. H., Yang, J., Lu, J. L. and Qian, S. J. (2007). Screening for a New *Streptomyces* Strain Capable of Efficient Keratin Degradation. *Journal of Environmental Sciences*, 19, 1125–1128.

Guest, T. C. and Rashid, S. (2016). Anticancer Laccases: A Review. *Journal of Clinical & Experimental Oncology*, 5, 1.

Chefetz, B., Chen, Y. and Hadar, Y. (1998). Purification and Characterization of Laccase from *Chaetomium thermophilium* and its Role in Humification. *Applied and Environmental Microbiology*, 64, 3175–3179.

Chellappan, S., Jasmin, C., Basheer, S. M., Archana Kishore, A., Elyas, K. K., Bhat, S. G. and Chandrasekaran, M. (2011). Characterization of an Extracellular Alkaline Serine Protease from Marine *Engyodontiumalbum* BTMFS10. *Journal of Industrial Microbiology & Biotechnology*, 38, 743–752.

Chen, J. L., Li, L., Wang, S., Sun, X. Y., Xiao, L., Ren, J. S., Di, B. and Gu, N. (2017). A Glucose-Activatable Trimodal Glucometer Self-Assembled from Glucose Oxidase and MnO_2 Nanosheets for Diabetes Monitoring.*Journal of Materials Chemistry B*, 5, 5336–5344.

Chen, J. P. and Lee, M. S. (1995). Enhanced Production of *serratiamarcescens* Chitinase in PEG/Dextran Aqueous Two-Phase Systems. *Enzyme and Microbial Technology*, 17, 1021–1027.

Chen, L., Shen, Z. and Wu, J. (2009). Expression, Purification and in Vitro Antifungal Activity of Acidic Mammalian Chitinase against *Candida albicans, Aspergillus fumigatus* and *Trichophyton rubrum* Strains. *Clinical and Experimental Dermatology*, 34, 55–60.

Costa-Silva, T. A., Souza, C. R. F., Oliveira, W. P. and Said, S. (2014). Characterization and Spray Drying of Lipase Produced by the Endophytic Fungus *cercosporakikuchii*. *Brazilian Journal of Chemical Engineering*, 31, 849–858.

Courjean, O. and Mano, N. (2011). Recombinant Glucose Oxidase from *Penicillium amagasakiense* for Efficient Bioelectrochemical Applications in Physiological Conditions. *Journal of Biotechnology*, 151, 122–129.

Craik, C. S., Page, M. J. and Madison, E. L. (2011). Proteases as Therapeutics. *Biochemical Journal*, 435, 1–16.

Cruz, J., Hidalgo-Gallego, A., Lora, J. M., Benitez, T., Pintor-Toro, J. A. and Llobel, A. (1992). Isolation and Characterization of Three Chitinases from *Trichoderma harzianum*. *European Journal of Biochemistry*, 206, 859–867.

Dange, V. U. and Peshwe, S. A. (2011). Production, Purification and Characterization of Fungal L–Asparaginase. *Bionano Frontier*, 4, 162–167.

Das, S., Singh, S., Sharma, V. and Soni, M. L. (2011). Biotechnological Applications of Industrially Important Amylase Enzyme. *International Journal of Pharma and Bio Sciences*, 2, 486–496.

De-Angeli, L. C., Pocchiri, F., Russi, S., Tonolo, A., Zurita, V. E., Ciaranfi, E. and Perin, A. (1970). Effect of L-Asparaginase from *Aspergillus terreus* on Ascites Sarcoma in the Rat. *Nature*, 225, 549–50.

Duo-Chuan, L.I., Chen, S. and Jing, L.U. (2005). Purification and Partial Characterization of Two Chitinases from the Mycoparasitic Fungus *Talaromyces flavus*. *Mycopathologia*, 159, 223–229.

Dutta, S., Ghosh, S. and Pramanik, S. (2015). L-Asparaginase and L-Glutaminase from*Aspergillus fumigatus* WL002: Production and Some Physicochemical Properties. *Applied Biochemistry and Microbiology*, 51, 425–431.

Dux, M. P., Barent, R., Sinha, J., Gouthro, M., Swanson, T., Barthuli, A., Inan, M. et al. (2006). Purification and Scale-up of a Recombinant Heavy Chain Fragment C of Botulinum Neurotoxin Serotype E in *Pichiapastoris* GS115. *Protein Expression and Purification*, 45, 359–67.

Eisele, N., Linke, D., Bitzer, K., Na'amnieh, S., Nimtz, M. and Berger, R. G. (2011). The First Characterized Asparaginase from a Basidiomycete, *Flammulina velutipes*. *Bioresource Technology*, 102, 3316–3321.

El-Fakharany, E. M., Haroun, B. M., Ng, T. B. and Redwan, E. R. M. (2010). Oyster Mushroom Laccase Inhibits Hepatitis C Virus Entry into Peripheral Blood Cells and Hepatoma Cells. *Protein & Peptide Letters*, 17, 1031–1039.

El-Naggar, N. E. A., Deraz, S. F., El-Ewasy, S. M. and Suddek, G. M. (2018). Purification, Characterization and Immunogenicity Assessment of Glutaminase Free L-Asparaginase from *Streptomyces brollosae* NEAE-115. *BMC Pharmacology and Toxicology*, 19, 51.

Elshafei, A. (2012). Purification, Characterization and Antitumor Activity of L-Asparaginase from *Penicillium brevicompactum* NRC 829. *British Microbiology Research Journal*, 2, 158–174.

Eriksson, K-E. and Pettersson, B. (2005). Purification and Partial Characterization of Two Acidic Proteases from the White-Rot Fungus *Sporotrichum pulverulentum*. *European Journal of Biochemistry*, 124, 635–642.

Falony, G., Armas, J. C., Mendoza, J. C. D. and Hernández, J. L. M. (2006). Production of Extracellular Lipase from *Aspergillus niger* by Solid-State Fermentation. *Food Technolology Biotechnology*, 44, 235–240.

Fan, W., Lu, N., Huang, P., Liu, Y., Yang, Z., Wang, S., Yu, G. et al. (2017). Glucose-Responsive Sequential Generation of Hydrogen Peroxide and Nitric Oxide for Synergistic Cancer Starving-Like/Gas Therapy. *Angewandte Chemie International Edition*, 56, 1229–1233.

Fan, W., Yung, B., Huang, P. and Chen, X. (2017). Nanotechnology for Multimodal Synergistic Cancer Therapy. *Chemical Reviews*, 117, 13566–13638.

Farag, A. M., Hanan, M. A-E., Ibrahim, H. A. H. and El-Shenawy, M. (2016). Purification, Characterization and Antimicrobial Activity of Chitinase from Marine-Derived *Aspergillus terreus*. *The Egyptian Journal of Aquatic Research*, 42, 185–192.

Ferreira, A. M., Faustino, V. F. M., Mondal, D., Coutinho, J. A. P. and Freire, M. G. (2016). Improving the Extraction and Purification of Immunoglobulin G by the Use of Ionic Liquids as Adjuvants in Aqueous Biphasic Systems. *Journal of Biotechnology*, 236, 166–175.

Freire, M. G., Cláudio, A. F. M., Araújo, J. M. M., Coutinho, J. A. P., Marrucho, I. M., Lopes, J. N. C. and Rebelo, L. P. N. (2012). Aqueous Biphasic Systems: A Boost Brought about by Using Ionic Liquids. *Chemical Society Reviews*, 41, 4966–4995.

Fu, L. H., Qi, C., Lin, J. and Huang, P. (2018). Catalytic Chemistry of Glucose Oxidase in Cancer Diagnosis and Treatment. *Chemical Society Reviews*, 47, 6454–6472.

Fukumoto, J., Lwai, M. and Tsujisaka, Y. (1963). Studies on Lipase, I. Purification and Crystallization ofa Lipase Secreted by *Aspergillus niger*. *The Journal of General and Applied Microbiology*, 9, 353–361.

Fusetti, F., Moeller, H. V., Houston, D., Rozeboom, H. J., Dijkstra, B. W., Boot, R. G., Johannes Aerts, J.M. F. G. and Aalten, D. M. F. V. (2002). Structure of Human Chitotriosidase. *Journal of Biological Chemistry*, 277, 25537–25544.

Gaded, V. and Anand, R. (2018). Nucleobase Deaminases: A Potential Enzyme System for New Therapies. *RSC Advances*, 8, 23567–23577.

Gagaoua, M. and Hafid, K. (2016). Three Phase Partitioning System, an Emerging Non-Chromatographic Tool for Proteolytic Enzymes Recovery and Purification. *Biosensors Journal*, 5.

Giardina, P., Faraco, V., Pezzella, C., Piscitelli, A., Vanhulle, S. and Sannia, G. (2010). Laccases: A Never-Ending Story. *Cellular and Molecular Life Sciences*, 67, 369–385.

Gopinath, S. C. B., Hilda, A., Priya, T. L. and Annadurai, G. (2002). Purification of Lipase from *Cunninghamella verticillata* and Optimization of Enzyme Activity Using Response Surface Methodology. *World Journal of Microbiology and Biotechnology*, 18, 449–458.

Gopinath, S., Hilda, A. and Anbu, P. (2000). Screening Methods for Detecting Lipolytic Enzymes by *Aspergillus* species. *Acta Botanica Indica*, 28, 41–44.

Gopinath, S. C. B., Hilda, A., Priya, T. L., Annadurai, G. and Anbu, P. (2003). Purification of Lipase from *Geotrichum candidum*: Conditions Optimized for Enzyme Production Using Box–Behnken Design. *World Journal of Microbiology and Biotechnology*, 19, 681–689.

Gopinath, S. C.B., Anbu, P., Lakshmipriya, T. and Hilda, A. (2013). Strategies to Characterize Fungal Lipases for Applications in Medicine and Dairy Industry. *BioMed Research International*, 2013, 1–10.

Gunaratna, K. R. and Balasubramanian, R. (1994). Partial Purification and Properties of Extracellular Chitinase Produced by *Acremonium obclavatum*, an Antagonist to the Groundnut Rust, Puccinia Arachidis. *World Journal of Microbiology & Biotechnology*, 10, 342–345.

Guo, R. F., Shi, B. S., Li, D. C., Ma, W. and Wei, Q. (2008). Purification and Characterization of a Novel Thermostable Chitinase from *Thermomyces lanuginosus* SY2 and Cloning of its Encoding Gene. *Agricultural Sciences in China*, 7, 1458–1465.

Gupta, R., Gigras, P., Mohapatra, H., Goswami, V. K.and Chauhan, B. (2003). Microbial α-Amylases: A Biotechnological Perspective. *Process Biochemistry*, 38, 1599–1616.

Gurung, N., Ray, S., Bose, S. and Rai, V. (2013). A Broader View: Microbial Enzymes and Their Relevance in Industries, Medicine, and Beyond. *BioMed Research International*, 2013, 1–18.

Hajji, M., Kanoun, S., Nasri, M. and Gharsallah, N. (2007). Purification and Characterization of an Alkaline Serine-Protease Produced by a New Isolated *Aspergillus clavatus* ES1. *Process Biochemistry*, 42, 791–797.

Han, M. J., Choi, H. T. and Song, H. G. (2005). Purification and Characterization of Laccase from the White Rot Fungus *Trametes versicolor*. *Journal of Microbiology*, 43, 555–560.

Hardwicke, J., Moseley, R., Stephens, P., Harding, K., Duncan, R. and Thomas, D. W. (2010). Bioresponsive Dextrin−rhEGF Conjugates: In Vitro Evaluation in Models Relevant to its Proposed Use as a Treatment for Chronic Wounds. *Molecular Pharmaceutics*, 7, 699–707.

Hayden, M. S., Linsley, P. S., Wallace, A. R., Marquardt, H. and Kerr, D. E. (1998). Cloning, Overexpression, and Purification of Cytosine Deaminase from *Saccharomyces cerevisiae*. *Protein Expression and Purification*, 12, 173–184.

Howard, E. L., Becker, K. C. D., Rusconi, C. P. and Becker, R. C. (2007). Factor IXa Inhibitors as Novel Anticoagulants. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 27, 722–727.

Huang, L., Liu, Y., Sun, Y., Yan, Q. and Jiang, Z. (2014). Biochemical Characterization of a Novel L-Asparaginase with Low Glutaminase Activity from *Rhizomucor miehei* and Its Application in Food Safety and Leukemia Treatment. *Applied and Environmental Microbiology*, 80, 1561–1569.

Huggett, A.St.G. and Nixon, D. A. (1957). Use of Glucose Oxidadese, Peroxidase, and O-Dianisidine in Determination of Blood and Urinary Glucose. *The Lancet*, 270, 368–370.

Iizumi, T., Nakamura, K. and Fukase, T. (1990). Purification and Characterization of a Thermostable Lipase from Newly Isolated *Pseudomonas sp*. KWI-56. *Agricultural and Biological Chemistry*, 54, 1253–1258.

Imlay, J. A., Chin, S. M. and Linn, S. (1988). Toxic DNA Damage by Hydrogen Peroxide through the Fenton Reaction in Vivo and in Vitro. *Science*, 240, 640–642.

Iyer, G. and Chattoo, B. B. (2003). Purification and Characterization of Laccase from the Rice Blast Fungus, *Magnaporthe grisea*. *FEMS Microbiology Letters*, 227, 121–126.

Janakiraman, S. (2015). Purification and Characterization of Therapeutic Enzyme L-Asparaginase from a Tropical Soil Fungal Isolate *Fusarium culmorum* ASP-87. *MOJ Proteomics & Bioinformatics*, 2.

Javed, S., Ahmad, M. M., Abdin, M., Hamid, R., Khan, M. and Musarrat, J. (2013). Chitinases: an Update. *Journal of Pharmacy and Bioallied Sciences*, 5, 21–29.

Jayaprakash, A. and Ebenezer, P. S. (2012). Purification and Characterization of *Aspergillus japonicus* Lipase from a Pig Fat Production Medium. *Journal of Academia and Industrial Research*,1, 1–7.

Chaurasia, P. K., Bharati, S. L. and Sarma, C. (2017). Laccases in Pharmaceutical Chemistry: A Comprehensive Appraisal. *Mini-Reviews in Organic Chemistry*, 13, 430–451.

Karthik, N., Akanksha, K., Binod, P. and Pandey, A. (2014). Production, Purification and Properties of Fungal Chitinases-A Review. *Indian Journal of Experimental Biology*, 52, 1025–1035.

Kelley, R. L. and Reddy, C. A. (1986). Purification and Characterization of Glucose Oxidase from Ligninolytic Cultures of *Phanerochaete chrysosporium*. *Journal of Bacteriology*, 166, 269–274.

Ketnawa, S., Rungraeng, N. and Rawdkuen, S. (2017). Phase Partitioning for Enzyme Separation: An Overview and Recent Applications. *International Food Research Journal*, 24, 1–24.

Khammuang, S. and Sarnthima, R. (2009). Laccase Activity from Fresh Fruiting Bodies of *Ganoderma sp*. MK05: Purification and Remazol Brilliant Blue R Decolorization. *Journal of Biological Sciences*, 9, 83–87.

Khoo, S. L., Amirul, A. A., Kamaruzaman, M., Nazalan, N. and Azizan, M. N. (1994). Purification and Characterization of Alpha-Amylase from *Aspergillus flavus*. *Folia Microbiologica*, 39, 392–398.

Kievit, E., Bershad, E., Ng, E., Sethna, P., Dev, I., Lawrence, T. S. and Rehemtulla, A. (1999). Superiority of Yeast over Bacterial Cytosine Deaminase for Enzyme/Prodrug Gene Therapy in Colon Cancer Xenografts. *Cancer Research*, 59, 1417–1421.

Kievit, E., Nyati, M. K., Ng, E., Stegman, L. D., Parsels, J., Ross, B. D., Rehemtulla, A. and Lawrence, T. S. (2000). Yeast Cytosine Deaminase Improves Radiosensitization and Bystander Effect by 5-Fluorocytosine of Human Colorectal Cancer Xenografts 1. *Cancer Research*, 60, 6649–6655.

Kiiskinen, L. L., Kruus, K., Bailey, M., Ylösmäki, E., Siika-Aho, M. and Saloheimo, M. (2004). Expression of Melanocarpus Albomyces Laccase in *Trichoderma reesei* and Characterization of the Purified Enzyme. *Microbiology*, 150, 3065–3074.

Ko, T., Lin, J. J., Hu, C. Y., Hsu, Y. H., Wang, A. H. J. and Liaw, S. H. (2003). Crystal Structure of Yeast Cytosine Deaminase. Insights into Enzyme Mechanism and Evolution. *The Journal of Biological Chemistry*, 278, 19111–19117.

Kumar, V. V., Sathyaselvabala, V., Kirupha, S. D., Murugesan, A., Vidyadevi, T. and Sivanesan, S. (2011). Application of Response Surface Methodology to Optimize Three Phase Partitioning for Purification of Laccase from *Pleurotus ostreatus*. *Separation Science and Technology*, 46, 1922–1930.

Kumar, M. N. S. and Manonmani, H. K. (2013). Purification, Characterization and Kinetic Properties of Extracellular l-Asparaginase Produced by *Cladosporium sp*. *World Journal of Microbiology and Biotechnology*, 29, 577–587.

Kumarevel, T. S., Gopinath, S. C. B., Hilda, A., Gautham, N. and Ponnusamy, M. N. (2005). Purification of Lipase from *Cunninghamella verticillata* by Stepwise Precipitation and Optimized Conditions for Crystallization. *World Journal of Microbiology and Biotechnology*, 21, 23–26.

Lario, L. D., Malpiedi, L. P., Pereira, J. F. B., Sette, L. D. and Pessoa-Junior, A. (2016). Liquid-Liquid Extraction of Protease from Cold-Adapted Yeast *Rhodotorula Mucilaginosa* L7 Using Biocompatible and Biodegradable Aqueous Two-Phase Systems. *Separation Science and Technology*, 51, 57–67.

Larsen, M. D., Kristiansen, K. R. and Hansen, T. K. (1998). Characterization of the Proteolytic Activity of Starter Cultures of *Penicillium roqueforti* for Production of Blue Veined Cheeses. *International Journal of Food Microbiology*, 43, 215–221.

Li, D. C., Yang, Y. J. and Shen, C. Y. (1997). Protease Production by the Thermophilic Fungus *Thermomyces lanuginosus.Mycological Research*, 101, 18–22.

Li, J., Li, Y., Wang, Y., Ke, W., Chen, W., Wang, W. and Ge, Z. (2017). Polymer Prodrug-Based Nanoreactors Activated by Tumor Acidity for Orchestrated Oxidation/Chemotherapy. *Nano Letters*, 17, 6983–6990.

Li, S. Y., Cheng, H., Xie, B. R., Qiu, W. X., Zeng, J. Y., Li, C. X., Wan, S. S., Zhang, L., Liu, W. L. and Zhang, X. Z. (2017). Cancer Cell Membrane Camouflaged Cascade Bioreactor for Cancer Targeted Starvation and Photodynamic Therapy. *ACS Nano*, 11, 7006–7018.

Li, Z., Shanmugam, N., Katayose, D., Huber, B., Srivastava, S., Cowan, K. and Seth, P. (1997). Enzyme/Prodrug Gene Therapy Approach for Breast Cancer Using a Recombinant Adenovirus Expressing *Escherichia coli* Cytosine Deaminase. *Cancer Gene Therapy*, 4, 113–117.

Liu, Y., Wu, Z., Zhang, Y. and Yuan, H. (2012). Partitioning of Biomolecules in Aqueous Two-Phase Systems of Polyethylene Glycol and Nonionic Surfactant. *Biochemical Engineering Journal*, 69, 93–99.

Loiseau, P. M., Bories, C. and Sanon, A. (2002). The Chitinase System from *Trichomonas vaginalis* as a Potential Target for Antimicrobial Therapy of Urogenital Trichomoniasis. *Biomedicine & Pharmacotherapie*, 56, 503–510.

Lopes, A. M., Oliveira-Nascimento, L., Ribeiro, A., Tairum, C. A., Breyer, C. A., Oliveira, M. A., Monteiro, G. et al. (2017). Therapeutic L-Asparaginase: Upstream, Downstream and Beyond. *Critical Reviews in Biotechnology*, 37, 82–99.

Lott, J. A. and Lu, C. J. (1991). Lipase Isoforms and Amylase Isoenzymes: Assays and Application in the Diagnosis of Acute Pancreatitis. *Clinical Chemistry*, 37, 361–368.

Loureiro, C. B., Borges, K. S., Andrade, A. F., Tone, L. G. and Said, S. (2012). Purification and Biochemical Characterization of Native and Pegylated Form of L-Asparaginase from *Aspergillus terreus* and Evaluation of its Antiproliferative Activity. *Advances in Microbiology*, 2, 138–45.

Luhana, K., Dave, A. and Patel, U. (2013). Production, Purification & Characterization of Extracellular L-Asparaginase (Anti Cancerous Enzyme) from *Aspergillus niger*. *International Journal of Chemtech Applications*, 2, 14–25.

Lutz, S., Williams, E. and Muthu, P. (2017). Engineering Therapeutic Enzymes. In *Directed Enzyme Evolution: Advances and Applications* (pp. 17–67). Cham: Springer International Publishing.

Mane, P. and Tale, V. (2015). Overview of Microbial Therapeutic Enzymes. *International Journal of Current Microbiology and Applied Sciences*, 4, 17–26.

Martínez-Aragón, M., Burghoff, S., Goetheer, E.L.V.and De Haan, A.B. (2009). Guidelines for Solvent Selection for Carrier Mediated Extraction of Proteins. *Separation and Purification Technology*, 65, 65–72.

Martínez-Aragón, M., Goetheer,E.L.V. and de Haan, A. B. (2009). Host–Guest Extraction of Immunoglobulin G Using Calix[6]arenas. *Separation and Purification Technology*, 65: 73–78.

Mathivanan, N., Kabilan, V. and Murugesan, K. (1998). Purification, Characterization, and Antifungal Activity of Chitinase from *Fusarium chlamydosporum*, a Mycoparasite to Groundnut Rust, Puccinia Arachidis. *Canadian Journal of Microbiology*, 44, 646–651.

Mayolo-Deloisa, K., Trejo-Hernández, M. R. and Rito-Palomares, M. (2009). Recovery of Laccase from the Residual Compost of *Agaricus bisporus* in Aqueous Two-Phase Systems. *Process Biochemistry*, 44, 435–439.

Mehta, A., Bodh, U. and Gupta, R. (2017). Fungal Lipases: A Review. *Journal of Biotech Research*, 8, 58–77.

Méndez, M.B., Goñi, A., Ramirez, W. and Grau, R. R. (2012). Sugar Inhibits the Production of the Toxins That Trigger Clostridial Gas Gangrene. *Microbial Pathogenesis*, 52, 85–91.

Merheb-Dini, C., Gomes, E., Boscolo, M. and Silva, R. (2010). Production and Characterization of a Milk-Clotting Protease in the Crude Enzymatic Extract from the Newly Isolated *Thermomucor indicae-seudaticae* N31. *Food Chemistry*, 120, 87–93.

Minussi, R. C., Miranda, M. A., Silva, J. A., Ferreira, C. V., Aoyama, H., Marangoni, S., Rotilio, D., Pastore, G. M. and Durán, N. (2002). Purification, Characterization and Application of Laccase from *Trametes versicolor* for Colour and Phenolic Removal of Olive Mill Wastewater in the Presence of 1- Hydroxybenzotriazole. *African Journal of Biotechnology*, 6.

Miyagi, T., Koshida, K., Hori, O., Konaka, H., Katoh, H., Kitagawa, Y., Mizokami, A. et al. (2003). Gene Therapy for Prostate Cancer Using the Cytosine Deaminase/Uracil Phosphoribosyl transferase Suicide System. *The Journal of Gene Medicine*, 5, 30–37.

Monod, M., Capoccia, S., Léchenne, B., Zaugg, C., Holdom, M. and Jousson, O. (2002). Secreted Proteases from Pathogenic Fungi. *International Journal of Medical Microbiology*, 292, 405–419.

More, S. S., Renuka, P. S., Pruthvi, K., Swetha, M., Malini, S. and Veena, S. M. (2011). Isolation, Purification, and Characterization of Fungal Laccase from *Pleurotus sp*. *Enzyme Research*, 2011, 248735.

Mtibaà, R., Barriuso, J., Eugenio, L., Aranda, E., Belbahri, L., Nasri, M., Martínez, M. J. and Mechichi, T. (2018). Purification and Characterization of a Fungal Laccase from the Ascomycete *Thielavia sp*. and Its Role in the Decolorization of a Recalcitrant Dye. *International Journal of Biological Macromolecules*, 120, 1744–1751.

Naganagouda, K. and Mulimani, V. H. (2008). Aqueous Two-Phase Extraction (ATPE): An Attractive and Economically Viable Technology for Downstream Processing of A*spergillus oryzae* α-Galactosidase. *Process Biochemistry*, 43, 1293–1299.

Nagpure, A., Choudhary, B. and Gupta, R. K. (2014). Chitinases: In Agriculture and Human Healthcare. *Critical Reviews in Biotechnology*, 34, 215–232.

Nagpure, A. and Gupta, R. K. (2013). Purification and Characterization of an Extracellular Chitinase from Antagonistic *Streptomyces violaceusniger*. *Journal of Basic Microbiology*, 53, 429–439.

Nascimento, T. P., Sales, A. E., Porto, C. S., Brandão, R. M. P., Campos-Takaki, G. M., Teixeira, J. A. C., Porto, T. S., Porto, A. L. F. and Converti, A. (2016). Purification of a Fibrinolytic Protease from *Mucor subtilissimus* UCP 1262 by Aqueous Two-Phase Systems (PEG/Sulfate). *Journal of Chromatography B*, 1025, 16–24.

Negi, S. and Banerjee, R. (2009). Characterization of Amylase and Protease Produced by *Aspergillus awamori* in a Single Bioreactor. *Food Research International*, 42, 443–448.

Nguyen, Q. D., Rezessy-Szabó, J. M., Claeyssens, M., Stals, I. and Hoschke, A. (2002). Purification and Characterisation of Amylolytic Enzymes from Thermophilic Fungus *Thermomyces lanuginosus* Strain ATCC 34626. *Enzyme and Microbial Technology*, 31, 345–352.

Novotná, Z., Fliegerová, K. and Šimůnek, J. (2008). Characterization of Chitinases of Polycentric Anaerobic Rumen Fungi. *Folia Microbiologica*, 53, 241–245.

Nyati, M. K., Symon, Z., Kievit, E., Dornfeld, K. J., Rynkiewicz, S. D., Ross, B. D., Rehemtulla, A. and Lawrence, T. S. (2002). The Potential of 5-Fluorocytosine/Cytosine Deaminase Enzyme Prodrug Gene Therapy in an Intrahepatic Colon Cancer Model. *Gene Therapy*, 9, 844–849.

Ohnishi, K., Yoshida, Y., Toita, J. and Sekiguchi, J. (1994). Purification and Characterization of a Novel Lipolytic Enzyme from *Aspergillus oryzae*. *Journal of Fermentation and Bioengineering*, 78, 413–419.

Ohtakara, A. (1988). Chitinase and β-N-Acetylhexosaminidase from *Pycnoporus cinnabarinus*. *Methods in Enzymology*, 161, 462–470.

Okeke, C. N. and Okolo, B. N. (1990). The Effect of Cultural Conditions on the Production of Lipase by *Acremonium strictum*. *Biotechnology Letters*, 12, 747–750.

Omumasaba, C. A., Yoshida, N. and Ogawa, K. (2001). Purification and Characterization of a Chitinase from *Trichoderma viride*. *The Journal of General and Applied Microbiology*, 47, 53–61.

Palonen, H., Saloheimo, M., Viikari, L. and Kruus, K. (2003). Purification, Characterization and Sequence Analysis of a Laccase from the Ascomycete *Mauginiella* sp. *Enzyme and Microbial Technology*, 33, 854–862.

Patel, A. K., Nampoothiri, K. M., Ramachandran, S., Szakacs, G. and Pandey, A. (2005). Partial Purification and Characterization of Alfa-Amylase Produced by *Aspergillus oryzae* Using Spent-Brewing Grains. *Indian Journal of Biotechnology*, 4, 336–341.

Patil, N. P. and Chaudhari, B. L. (2010). Production and Purification of Pectinase by Soil Isolate *Penicillium sp.* and Search for Better Agro-Residue for Its SSF. *Recent Research in Science and Technology*, 2, 36–42.

Patro, K. R., Basak, U.C., Mohapatra, A.K.and Gupta, N. (2014). Development of New Medium Composition for Enhanced Production of L-Asparaginase by *Aspergillus flavus*. *J. Environ. Biol./Acad. Environ. Biol. India*, 35, 295–300.

Pericin, D., Madjarev-Popovic, S. and Vastag, Z. (2008). Partitioning of Acid Protease from *Penicillium roqueforti* in Aqueous Two-Phase System Polyethylene Glicol/Phosphate. *Acta Periodica Technologica*,39, 171–180.

Polizeli, M. D. L. T. M., Jorge, J. A. and Terenzi, H. F. (1991). Pectinase Production by *Neurospora crassa*: Purification and Biochemical Characterization of Extracellular Polygalacturonase Activity. *Journal of General Microbiology*, 137, 1815–1823.

Porto, T. S., Medeiros e Silva, G. M., Porto, C. S., Cavalcanti, M. T. H., Neto, B. B., Lima-Filho, J. L., Converti, A., Porto, A. L. F. and Pessoa, A. (2008). Liquid–Liquid Extraction of Proteases from Fermented Broth by PEG/Citrate Aqueous Two-Phase System. *Chemical Engineering and Processing: Process Intensification*, 47, 716–721.

Prinz, A., Hönig, J., Schüttmann, I., Zorn, H. and Zeiner, T. (2014). Separation and Purification of Laccases from Two Different Fungi Using Aqueous Two-Phase Extraction. *Process Biochemistry*, 49, 335–346.

Ratanapongleka, K. and Phetsom, J. (2011). Extraction in Two-Phase Systems and Some Properties of Laccase from *Lentinus polychrous*. *International Journal of Chemical and Molecular Engineering*, 9, 808–811.

Roopavathi, A. S., Vigneshwari, R. and Jayapradha R. (2015). Chitinase: Production and Applications. *Available Online www.jocpr.com Journal of Chemical and Pharmaceutical Research*, 7, 924–931.

Ryan, S., Schnitzhofer, W., Tzanov, T., Cavaco-Paulo, A. and Gübitz, G. M. (2003). An Acid-Stable Laccase from *Sclerotium rolfsii* with Potential for Wool Dye Decolourization. *Enzyme and Microbial Technology*, 33, 766–774.

Thakur, M., Lincoln, L., Niyonzima, F. N.and More, S. S. (2013). Isolation, Purification and Characterization of Fungal Extracellular L-Asparaginase from *Mucor Hiemalis.Journal of Biocatalysis & Biotransformation*, 2, 1-9.

Sabha, M. E. S., Nadia, H. E. B. and Tarek, A. S. (2013). L-Asparaginase Produced by *Streptomyces* Strain Isolated from Egyptian Soil: Purification, Characterization and

Evaluation of Its Anti-Tumor. *African Journal of Microbiology Research*, 7, 5677–5686.

Sabir, S., Bhatti, H. N., Zia, M. A., Munir Ahmad Sheikh, M. A. (2007). Enhanced Production of Glucose Oxidase Using *Penicillium notatum* and Rice Polish. *Food Technology and Biotechnology*, 45, 443–446.

Sabotič, J. and Kos, J. (2012). Microbial and Fungal Protease Inhibitors—Current and Potential Applications. *Applied Microbiology and Biotechnology*, 93, 1351–1375.

Sahay, R., Yadav, R.S.S.and Yadav, K.D.S. (2008). Purification and Characterization of Extracellular Laccase Secreted by *Pleurotus sajor-caju* MTCC 141. *Chinese Journal of Biotechnology*, 24, 2068–2073.

Sakuda, S., Isogai, A., Matsumoto, S. and Suzuki, A. (1987). Search for Microbial Insect Growth Regulators. II Allosamidin, a Novel Insect Chitinase Inhibitor. *The Journal of Antibiotics*, 40, 296–300.

Sakurada, M., Morgavi, D. P., Komatani, K., Tomita, Y. and Onodera, R. (1996). Purification and Characteristics of Cytosolic Chitinase from *Piromyces communis* OTS1. *FEMS Microbiology Letters*, 137, 75–78.

Sakurada, M., Morgavi, D. P., Komatani, K., Tomita, Y. and Onodera, R. (1996). Purification and Characteristics of Cytosolic Chitinase from *Piromyces Communis* OTS1. *FEMS Microbiology Letters*, 137, 75–78.

Santos, J. H. P. M, Flores-Santos, J. C., Meneguetti, G. P., Rangel-Yagui, C. O., Coutinho, J. A. P., Vitolo, M., Ventura, S. P. M. and Pessoa, A. (2018). *In Situ* Purification of Periplasmatic L-Asparaginase by Aqueous Two Phase Systems with Ionic Liquids (ILs) as Adjuvants. *Journal of Chemical Technology & Biotechnology*, 93, 1871–1880.

Saranraj, P. and Stella, D. (2013). Fungal Amylase - A Review. *International Journal of Microbiological Research*, 4, 203–211.

Sarquis, M. I. M., Oliveira, E. M. M., Santos, A. S. and Costa, G. L. (2004). Production of L-Asparaginase by Filamentous Fungi. *Memórias Do Instituto Oswaldo Cruz*, 99, 489–492.

Savitha, S., Sadhasivam, S., Swaminathan, K. and Lin, F. H. (2011). Fungal Protease: Production, Purification and Compatibility with Laundry Detergents and Their Wash Performance. *Journal of the Taiwan Institute of Chemical Engineers*, 42, 298–304.

Scherer, M. and Fischer, R. (1998). Purification and Characterization of Laccase II of *Aspergillus nidulans*. *Archives of Microbiology*, 170, 78–84.

Schibli, S., Durie, P. R.and Tullis, E. D. (2002). Proper Usage of Pancreatic Enzymes. *Current Opinion in Pulmonary Medicine*, 8, 542–546.

Schwienheer, C., Prinz, A., Zeiner, T. and Merz, J. (2015). Separation of Active Laccases from *Pleurotus sapidus* Culture Supernatant Using Aqueous Two-Phase Systems in Centrifugal Partition Chromatography. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, 1002, 1-7.

Shafei, M. S., El-Refai, H. A., Mostafa, H. M., El-Refai, A. M. H., El-beih, F. M., Easa, S. M. H. and Gomaa, S. K. (2015). Purification, Characterization and Kinetic Properties of *Penicillium cyclopium* L-Asparaginase: Impact of L-Asparaginase on Acrylamide Content in Potato Products and its Cytotoxic Activity. *Current Trends in Biotechnology and Pharmacy*, 9, 132–140.

Sharma, S. and Kanwar, S. S. (2014). Organic Solvent Tolerant Lipases and Applications. *The Scientific World Journal*, 2014, 2014, 1–15.

Shin, K. S., Youn, H. D., Han, Y. H., Kang, S. O. and Hah, Y. C. (1993). Purification and Characterisation of D-Glucose Oxidase from White-Rot Fungus *Pleurotus ostreatus*. *European Journal of Biochemistry*, 215, 747–752.

Shujing, S., Yonghui, Z., Youxiong, Q., Bixian, L., Kaihui, H. and Xu, L. (2013). Purification and Characterization of Fungal Laccase from *Mycena purpureofusca*. *Chiang Mai Journal of Science*, 40, 151–160.

Silva, T. M., Damásio, A. R. L., Maller, A., Michelin, M., Squina, F. M., Jorge, J. A. and Polizeli, M. L. T. M. (2013). Purification, Partial Characterization, and Covalent Immobilization–Stabilization of an Extracellular α-Amylase from *Aspergillus niveus*. *Folia Microbiologica*, 58, 495–502.

Singh, A. K. and Mukhopadhyay, M.(2012). Overview of Fungal Lipase: A Review. *Applied Biochemistry and Biotechnology*, 166, 486–520.

Singh, J. and Verma, N. S. (2013). Glucose Oxidase from *Aspergillus niger*:Production, Characterization and Immobilization for Glucose Oxidation. *Advances in Applied Science Research*, 4, 250–57.

Smith, A. D. (1997). Oxford Dictionary of Biochemistry and Molecular Biology. *Oxford University Press*, 41.

Somaraju, U. R. and Solis-Moya, A. (2014). Pancreatic Enzyme Replacement Therapy for People with Cystic Fibrosis. In *Cochrane Database of Systematic Reviews*, ed. Usha Rani Somaraju. Chichester, UK: John Wiley & Sons, Ltd.

Souza, P. M., Bittencourt, M. L. A., Caprara, C. C., Freitas, M., Almeida, R. P. C., Silveira, D., Fonseca, Y. M., Filho, E. X. F., Junior, A. P.and Magalhães, P. O. (2015). A Biotechnology Perspective of Fungal Proteases. *Brazilian Journal of Microbiology*, 46, 337–346.

Souza, P. M., Freitas, M. M., Cardoso, S. L., Pessoa, A., Guerra, E. N. S. and Magalhães, P. O. (2017). Optimization and Purification of L-Asparaginase from Fungi: A Systematic Review. *Critical Reviews in Oncology/Hematology*, 120, 194–202.

Suarez, B., Rey, M., Castillo, P., Monte, E. and Llobell, A. (2004). Isolation and Characterization of PRA1, a Trypsin-like Protease from the Biocontrol Agent *Trichoderma harzianum* CECT 2413 Displaying Nematicidal Activity. *Applied Microbiology and Biotechnology*, 65, 46–55.

Sztajer, H., Maliszewska, I. and Wieczorek, J. (1988). Production of Exogenous Lipases by Bacteria, Fungi, and Actinomycetes. *Enzyme and Microbial Technology*, 10, 492–497.

Sztajer, H. and Maliszewska, I. (1989). The Effect of Culture Conditions on Lipolytic Productivity of *Penicillium citrinum*. *Biotechnology Letters*, 11, 895–898.

Takasu, S., Mutoh, M., Takahashi, M. and Nakagama, H. (2012). Lipoprotein Lipase as a Candidate Target for Cancer Prevention/Therapy. *Biochemistry Research International*, 2012, 1–8.

Teal, A. (1991). *Enzymes and Their Role in Biotechnology*. London: Biochemical Society.

Teotia, S., Lata, R. and Gupta, M. N. (2004). Chitosan as a Macroaffinity Ligand: Purification of Chitinases by Affinity Precipitation and Aqueous Two-Phase Extractions. *Journal of Chromatography A*, 1052, (1–2), 85–91.

Thakur, M., Lincoln, L., Niyonzima, F. N., More, S. S., Niyonzima, F. N. and More, S. S. (2011). Purification and Properties of a Fungal L-Asparaginase from *Trichoderma viride* Pers: SF Grey. *Journal of Microbiology, Biotechnology and Food Sciences*, 8, 310–316.

Thota, P., Bhogavalli, P. K., Vallem, P. R. and Sreerangam, V. (2012). Biochemical Characterization of an Extracellular Lipase from New Strain of *rhizopus*sp. Isolated from Oil Contaminated Soil. *International Journal of Plant, Animal and Environmental Sciences*, 2, 41–45.

Thurston, C. F. (1994). The Structure and Function of Fungal Laccases. *Microbiology*, 140, 19–26.

Toida, J., Kondoh, K., Fukuzawa, M., Ohnishi, K. and Sekiguchi, J. (1995). Purification and Characterization of a Lipase from *Aspergillus oryzae*. *Bioscience, Biotechnology, and Biochemistry*, 59, 1199–1203.

Tunga, R., Shrivastava, B. and Banerjee, R. (2003). Purification and Characterization of a Protease from Solid State Cultures of *Aspergillus parasiticus*. *Process Biochemistry*, 38, 1553–1558.

Ulhoa, C. J. and Peberdy, J. F. (1992). Purification and Some Properties of the Extracellular Chitinase Produced by *Trichoderma harzianum*. *Enzyme and Microbial Technology*, 14, 236–240.

Ülker, S., Özel, A., Çolak, A. B. and Karaoğlu, S. A. (2011). Isolation, Production, and Characterization of an Extracellular Lipase from *Trichoderma harzianum* Isolated from Soil. *Turkish Journal of Biology*, 35, 543–550.

Vantamuri, A. B. and Kaliwal, B. B. (2016). Purification and Characterization of Laccase from *Marasmius species* BBKAV79 and Effective Decolorization of Selected Textile Dyes. *3 Biotech*, 6, 189.

Vellard, M. (2003). The Enzyme as Drug: Application of Enzymes as Pharmaceuticals. *Current Opinion in Biotechnology*, 14, 444–450.

Vignardet, C., Guillaume, Y. C., Michel, L., Friedrich, J. and Millet, J. (2001). Comparison of Two Hard Keratinous Substrates Submitted to the Action of a Keratinase Using an Experimental Design. *International Journal of Pharmaceutics*, 224, 115–122.

Waldorf, A. R. and Polak, A. (1983). Mechanisms of Action of 5-Fluorocytosine. *Antimicrobial Agents and Chemotherapy*, 23, 79–85.

Wanderley, K. J., Torres, F. A., Moraes, L. M. and Ulhoa, C. J. (2004). Biochemical Characterization of Alpha-Amylase from the Yeast *Cryptococcus flavus*. *FEMS Microbiology Letters*, 231, 165–169.

Wang, B., Wu, W. and Liu, X. (2007). Purification and Characterization of a Neutral Serine Protease with Nematicidal Activity from *Hirsutella rhossiliensis. Mycopathologia*, 163, 169–176.

Wang, J. (2008). Electrochemical Glucose Biosensors. In *Electrochemical Sensors, Biosensors and Their Biomedical Applications*, 57–69.

Wang, R.B., Yang, J. K., Lin, C., Zhang, Y. and Zhang, K. Q. (2006). Purification and Characterization of an Extracellular Serine Protease from the Nematode-Trapping Fungus *Dactylella Shizishanna.Letters in Applied Microbiology*, 42, 589-594.

Wang, Z. H., Samuels, S., Sosa, M. A. G. and Kolodny, E. H. (1998). 5-Fluorocytosine-Mediated Apoptosis and DNA Damage in Glioma Cells Engineered to Express Cytosine Deaminase and Their Enhancement with Interferon. *Journal of Neuro-Oncology*, 36, 219–229.

Warburg, O. (1956). On the Origin of Cancer Cells. *Science*, 123, 309–314.

Wong, J. H., Ng, T. B., Jiang, Y., Liu, F., Sze, S. C. W. and Zhang, K. Y. (2010). Purification and Characterization of a Laccase with Inhibitory Activity Toward HIV-1 Reverse Transcriptase and Tumor Cells from an Edible Mushroom (*Pleurotus cornucopiae*). *Protein & Peptide Letters*, 17, 1040–1047.

Wooldridge, J. L., Heubi, J. E., Amaro-Galvez, R., Boas, S. R., Blake, K. V., Nasr, S. Z., Chatfield, B. et al. (2009). EUR-1008 Pancreatic Enzyme Replacement Is Safe and Effective in Patients with Cystic Fibrosis and Pancreatic Insufficiency. *Journal of Cystic Fibrosis*, 8, 405–417.

Wu, S. H., Guo, Z. W.and Sih, C. J. (1990). Enhancing the Enantioselectivity of *Candida* Lipase-Catalyzed Ester Hydrolysis via Noncovalent Enzyme Modification. *Journal of the American Chemical Society*, 112, 1990–1995.

Xiao, Y., Tu, X.,Wang, J., Zhang, M.,Cheng, Q., Zeng, W. and Shi, Y. (2003). Purification, Molecular Characterization and Reactivity with Aromatic Compounds of a Laccase from Basidiomycete *trametessp*. Strain AH28-2. *Applied Microbiology and Biotechnology*, 60, 700–707.

Yadav, M., Bista, G., Maharjan, R., Poudyal, P., Mainali, M., Sreerama, L., Joshi, J. et al. (2019). Secretory Laccase from *Pestalotiopsis* species CDBT-F-G1 Fungal Strain

Isolated from High Altitude: Optimization of Its Production and Characterization. *Applied Sciences*, 9, 340.

Yan, S. B., Helterbrand, J. D., Hartman, D. L., Wright, T. J. and Bernard, G. R. (2001). Low Levels of Protein C are Associated with Poor Outcome in Severe Sepsis. *Chest*, 120, 915–922.

Yang, F., Weber, T. W., Gainer, J. L. and Carta, G. (1997). Synthesis of Lovastatin with immobilized*candida rugosa* Lipase in Organic Solvents: Effects of Reaction Conditions on Initial Rates. *Biotechnology and Bioengineering*, 56, 671–680.

Yi, B. R., Kang, N. H., Hwang, K. A., Kim, S. U., Jeung, E. B. and Choi, K. C. (2011). Antitumor Therapeutic Effects of Cytosine Deaminase and Interferon-β against Endometrial Cancer Cells Using Genetically Engineered Stem Cells in Vitro. *Anticancer Research*, 31, 2853–2861.

Yike, I. (2011). Fungal Proteases and Their Pathophysiological Effects. *Mycopathologia*, 171, 299–323.

Yu, T. S., Kim, J. K., Katsuragi, T., Sakai, T. and Tonomura, K. (1991). Purification and Properties of Cytosine Deaminase from *Aspergillus Fumigatus*. *Journal of Fermentation and Bioengineering*, 72, 266–269.

Yu, X. W., Wang, L. L. and Xu, Y. (2009). Rhizopus Chinensis Lipase: Gene Cloning, Expression in *Pichia Pastoris* and Properties. *Journal of Molecular Catalysis B: Enzymatic*, 57, 304–311.

Yuivar, Y., Barahona, S., Alcaíno, J., Cifuentes, V. and Baeza, M. (2017). Biochemical and Thermodynamical Characterization of Glucose Oxidase, Invertase, and Alkaline Phosphatase Secreted by Antarctic Yeasts. *Frontiers in Molecular Biosciences*, 4, 86.

Yun, M. K., Nourse, A., White, S. W., Rock, C. O. and Heath, R. J. (2007). Crystal Structure and Allosteric Regulation of the Cytoplasmic *Escherichia coli* L-Asparaginase I. *Journal of Molecular Biology*, 369, 794–811.

Zanna, H., Nok, A. J., Ibrahim, S., Inuwa, H. M., Zanna, H., Nok, A. J., Ibrahim, S. and Inuwa, H. M. (2012). Purification and characterization of *Aspergillus parasiticus* cytosine deaminase for possible deployment in suicide gene therapy. *Advances in Biological Chemistry*, 2, 152–159.

Zhang, R., Feng, L., Dong, Z., Wang, L., Liang, C., Chen, J., Ma, Q. et al. (2018). Glucose & Oxygen Exhausting Liposomes for Combined Cancer Starvation and Hypoxia-Activated Therapy. *Biomaterials*, 162, 123–131.

Zhao, W., Hu, J. and Gao, W. (2017). Glucose Oxidase-Polymer Nanogels for Synergistic Cancer-Starving and Oxidation Therapy. *ACS Applied Materials and Interfaces*, 9, 23528–23535.

Zhou, J., Li, M., Hou, Y., Luo, Z., Chen, Q., Cao, H., Huo, R. et al. (2018). Engineering of a Nanosized Biocatalyst for Combined Tumor Starvation and Low-Temperature Photothermal Therapy. *ACS Nano*, 12, 2858–2872.

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 16

FUNGAL LIGNOCELLULOLYTIC ENZYMES: PHYSIOLOGICAL ROLES AND BIOTECHNOLOGICAL APPLICATIONS

Chandana Paul[1,2], Madhumita Maitra[2] and Nirmalendu Das[1,*]
[1]Post Graduate Department of Botany, Barasat Govt. College, Barasat, Kolkata, India
[2]Department of Microbiology, St. Xavier's College, Park Street, Kolkata, India

ABSTRACT

Lignocellulose, a complex of cellulose and hemicelluloses, encrusted with lignin is one of the major components of biomass. Their abundant presence in agricultural and forest residues very often is considered as wastes and is destroyed by burning which creates environmental pollution. The structural integrity of lignocellulosic materials is generally resistant to most of the microorganisms except some white rot fungi which produce different lignocellulolytic enzymes mainly to collect the resources for their growth and development. The lignocellulolytic enzymes comprised of a number of enzymes which includes cellulases, hemicellulases, peroxidases and laccases. In addition to mycelial growth, they may help in different physiological processes in producer organisms *viz*. fruiting body development, sporulation, melanin formation, pathogenesis etc. Based on lignocellulosic degradation, a number of biotechnological applications of these enzymes are suggested including fermentation, food and fodder production, biobleaching, biopolishing, bioremediation etc. The application of various lignocellulolytic enzymes in agriculture, alcohol and brewing industry, paper and pulp industry, textile industry as well as in different medical practices are also discussed in this chapter.

*Corresponding Author's Email: nirmalendus@yahoo.co.uk.

Keywords: biobleaching, bioconversion, biodegradation, bioremediation, cellulase, cellulose, hemicellulase, hemicellulose, laccase, lignin, ligninase, lignocellulose, peroxidase, phenol oxidase, white rot fungi

INTRODUCTION

Lignocelluloses are an abundant biopolymer available on earth and make up about half of the matter produced by photosynthesis. They are inexhaustible or renewable natural resources and known as the major structural component of not only woody plants but also non-woody plants such as grasses (Kuhad et al. 2007). It contains complex polysaccharide microfibers most often formed by cellulose, hemicelluloses and lignin in the average weight ratio of 4:3:3. Cellulose is present in the form of parallelly arranged cellulose micro-fibrils (Gardner and Blackwell 1974). The hemicellulose and lignin components are situated between the cellulose micro-fibrils thus protecting the cell from microbial degradation. The organisms which grow on agro-residues are capable of producing lingocellulolytic enzymes. The ligninolytic enzymes degrade lignin first, so that cellulose and hemicelluloses become available to the hydrolytic enzymes (cellulase and hemicellulase) and then glucose or other monosaccharides are available to them for proper growth and development (Hatti-Kaul and Ibrahim 2013).

The major component of lignocellulosic biomass is cellulose along with hemicellulose and lignin (Figure 1). Cellulose and hemicellulose are macromolecules from different sugars; whereas lignin is an aromatic polymer synthesized from phenylpropanoid precursors. The composition and percentages of these polymers vary from one plant species to another (Table 1). Moreover, the composition within a single plant varies with geographical location, age, stage of growth, and other conditions (Jeffries 1994). The structure of these components is very complex and generally resistant to enzymatic hydrolysis. In softwood, coniferyl alcohol is the principal constituent of lignin whereas the hardwood is composed of guaiacyl and syringyl units. Grass lignin contains guaiacyl, syringyl, and *p*-hydroxyphenyl units. The lignin content is generally higher in softwood than hardwood but hemicellulose content of hardwood is higher than softwood (Kuhad and Singh 2007).

Fungi play a major role for degradation of lignocellulosic biomass. Lignocellulolytic fungi are often divided into three groups namely white rot, brown rot, and soft rot. White rots break down lignin and cellulose and commonly cause rotted wood to feel moist, soft, spongy, or stringy and appear white or yellow; brown rots primarily decay the cellulose and hemicellulose (carbohydrates) in wood, leaving behind the brownish lignin. They decay cellulose, hemicellulose and lignin but only in areas directly adjacent to their growth. Soft rots grow more slowly than brown and white rots and usually do not cause extensive structural damage to wood of living trees (Hickman and Perry 2010).

Lignocellulolytic enzymes are biocatalysts that are responsible for degradation of lignin and cellulosic materials. Ligninolytic enzymes catalyze the breakdown of lignin model compounds; and they fall in two main groups: peroxidases and phenol oxidases (Kuhad and Singh 2007; Shrestha et al. 2009). Peroxidases are enzymes which use hydrogen peroxidase (H_2O_2) as co-substrate. Phenol oxidases, within which laccase is the most important, are responsible for oxidative conversions of organic compounds and metals (Yang et al. 2017). Hydrolases are enzymes that catalyze hydrolysis of chemical bonds, and are classified based on the bonds they act upon. Cellulases are hydrolases that catalyze cellulolytic reactions. Hemicellulases are enzymes that act on hemicellulose, a polymer of pentose sugars. Other types of hydrolases (which are not under purview of present discussion) include pectinases, esterases, chitinases, nucleases, phosphor-diesterases, lipases, phosphatases, DNA glycosylases, glycoside hydrolases, proteases/ peptidases, helicases, GTPases etc. (Muthuvelayudham and Viruthagiri, 2006).

The huge amount of agricultural & forest residues are left without care and generally are considered as waste & destroyed by burning but these residues contain large amount of lignocellulosic biomass. Chemical properties of lignocellulosic components make them substrates of various biotechnological values. They can be potentially converted into various value-added products such as biogas, biofuels, and cheap carbon sources for fermentation, improved animal feeds and human nutrients by lignocellulose degrading microorganisms. In the last few years many processes related to lignocellulose biotechnology has been improved (Streffer 2014; Maciel et al. 2010; Martani et al. 2017; Prajapati and Minocheherhomji 2018).

Fungal lignocellulolytic enzymes are important bio-products that can degrade the inflexible lignocellulosic mass. In the present review various lignocellulosic enzymes from diverse fungal sources are discussed with special emphasis into their physiological functions and biotechnological implications (Arora and Sharma 2010; Majid et al. 2017; Patel et al. 2019).

Cellulose is a linear homopolymer of D-glucose and is the most abundant organic material in nature (Brown and Malcolm, 1996). It comprises approximately 45% of dry wood weight. The linear polymeric chains of cellulose consist of about 8000-12000 ß-1, 4 glycosidic linkages between D-glucose units. In its crystalline form, the individual chains are packed together by hydrogen bonds to form highly insoluble structures, called microfibrils. In addition to the crystalline structure, cellulose also contains amorphous regions within the microfibrils which is non-crystalline structure (de Vries and Visser 2001). The physical structure and morphology of native celluloses are complex and fine structural details are difficult to determine experimentally (O'Sullivan 1997). Although chemically simple, the extensive intermolecular bonding pattern of cellulose generates a crystalline structure that together with hemicellulose and lignin, results in very complex morphologies. Crystalline cellulose is highly resistant to microbial attack and enzymatic

hydrolysis, but amorphous cellulose is degraded at a much higher rate (Eriksson et al. 1990).

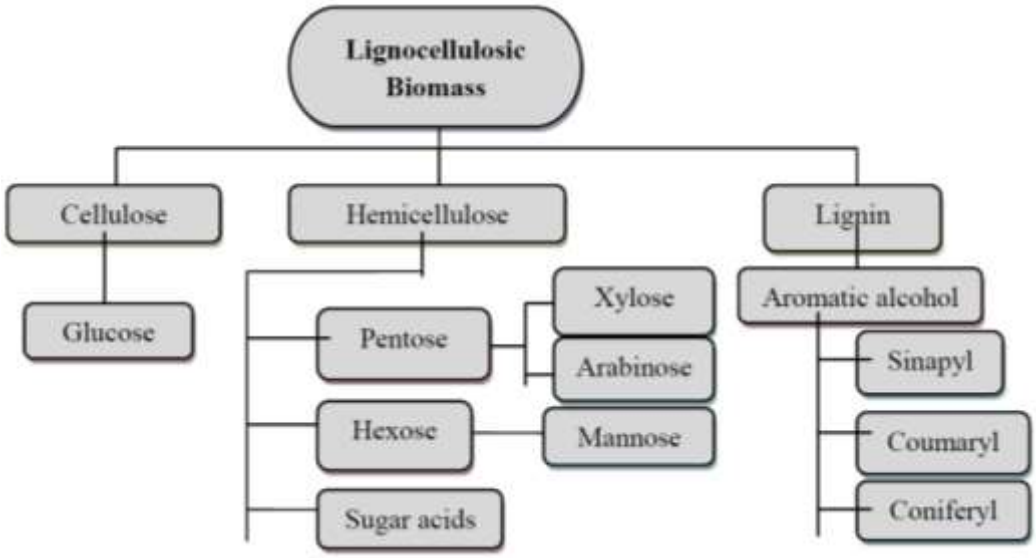


Figure 1. Schematic representation showing the contents present in the lignocelluloses.

Hemicellulose, the second most abundant polysaccharide in plant cell wall is heterogeneous in nature and consisted by different units of sugars. It comprised about 20-30% of dry wood weight. In contrast to cellulose, hemicellulose is structurally more complex. It is usually classified according to the main residues of sugars present in the backbone of the structural polymer. It is found in both secondary and primary walls and the composition varies substantially among species. The biosynthesis of hemicellulose is distinct from cellulose. Hemicellulose is easily hydrolyzed into short chain of branched heteropolysaccharide which is composed of both pentoses and hexoses (Whistler et al. 1970).The arabinoglucuronoxylan and the glucuronoxylan, referred to as xylans, represent approximately 7–12% and 15–30% of softwood and hardwood, respectively (Smook 1982; Sjostrom 1981). Xylan is the major constituent of the secondary wall of dicots, and primary cell wall of monocots (Singh et al. 2017). Xylan is composed by ß-1,4-linked D-xylose units in the main backbone, and can be substituted by different side groups such as D-galactose, L-arabinose, glucuronic acid, acetyl, feruloyl, and *p*-coumaroyl residues (de Vries and Visser, 2001). Xylan may play a significant role in the structural integrity of cell wall by both covalent and non-covalent interaction (Saha 2003). Another two major hemicelluloses in plant cell wall are galacto (gluco) mannans, which consist of a backbone of ß-1,4-linked D-mannose (mannans) and D-glucose (glucomannans) residues with D-galactose side chains, and xyloglucans that consist of a ß-1,4-linked D-glucose backbone substituted by D-xylose (de Vries and Visser 2001). Moreover, in xyloglucan polymer, L-arabinose and D-galactose residues can be attached

to the xylose residues and L-fucose can be attached to galactose residues. The high structural complexity and variability of xyloglucans depend on the diversity of the side groups that may be attached to the main backbone (Aro et al. 2005).

Table 1. Percent composition of lignocellulose components in various lignocellulosic materials

Lignocellulosic biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	References
Bagasse	25.0 – 45.0	28.0 – 32.0	15.0 – 25.0	Putro et al. (2016)
Banana peels	9.90	41.38	8.90	Kabenge et al. (2018)
Barley hull	33.6	37.2	19.3	Kim et al. (2008)
Corn cobs	33.7 – 41.2	31.9 – 36	6.1 – 15.9	Isikgora & Becer (2015)
Corn fiber	14.3	16.8	8.4	Mosier et a. (2005)
Corn pericarp	22.5	23.7	4.7	Kim et al. (2017
Corn stover	37.0	22.7	18.6	Kim et al. (2016)
Grasses	25.0 – 40.0	25.0 – 50.0	10.0 – 30.0	Kumar and Sharma (2017)
Newspaper	40 - 55	25 – 40	18 – 30	Howard et al. (2003)
Nut shells	25 - 30	25 – 30	30 – 40	Abbasi & Abbasi. (2010)
Olive tree pruning	25.0	11.1	16.2	Cara et al. (2008)
Paddy straw	34.8 – 36.4	23.65 – 30.3	10.4 – 11.0	Syazwanee et al. (2018)
Pinewood	40.0	28.5	27.7	Du et al. (2008)
Poplar	43.8	14.8	29.1	Kumar et al. (2009)
Rapeseed	51.3	17.3	44	Pei et al. (2016
Red maple	41.0	15.0	29.1	Ximenes et al. (2013)
Rice husks	25.0 – 35.0	18.0 – 21.0	26.0 – 31.0	Wikee et al. (2017)
Rye straw	30.9	21.5	22.1	García et al.(2009)
Soya straws	44.2	5.9	19.2	Kim (2018)
Sponge gourd fibers	66.59	17.44	15.46	Guimarães et al. (2009)
Spruce	43.8	6.3	28.3	Shafiei et al. (2010)
Sugarcane bagasse	43.1	31.1	11.4	Martín et al. (2007)
Sweet sorghum bagasse	27.3	13.1	14.3	Li et al. (2010)
Switchgrass	39.5	20.3	17.8	Li et al. (2010)
Wheat straw	49.7	14.8	23.5	Santos et al. (2015)

Lignins are amorphous, highly complex, mainly aromatic, non-water soluble and optically inactive molecule (Tuomela et al. 2000). The three-dimensional polymer is made up of C-O-C and C-C linkages. Lignins are consisting of phenyl-propane-based monomeric units linked together by different types of bonds, including alkyl-aryl, alkyl-alkyl, and aryl-aryl ether bonds. The molecular weight of lignins may be ranging from ten thousands Daltons to infinite (Janshekar et al. 1982). Lignins are racemic and therefore optically inactive. The racemic nature of lignins might arise from the fact that its polymerization is a non-enzymatic process (Ralph, 2006). The precursors of lignin biosynthesis are *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Schutyser et al. 2018).

The bulk of the lignin is in the thick secondary cell walls, but the highest amount of lignin is in the middle lamellae i.e., intercellular regions where the lignin cements the

plant cells together thereby providing rigidity, strength for the plant and also resistant against microbial attack and oxidative stress. Lignin content also varies depending on the developmental stage of a plant (Boerjan et al. 2003).

LIGNOCELLULOLYTIC ENZYMES

A large number of microorganisms play an important role in the degradation of lignocellulosic components by producing lignocellulolytic enzymes. Among a large number of lignocellulolytic microorganisms only a few have been studied extensively. Lignocellulolytic enzymes include two groups; the ligninolytic enzymes which contain peroxidases and oxidases, and the cellulolytic enzymes such as cellulases, hemicellulases. Fungi are pointed as the most important microorganisms concerning the biomass degradation (Isroi et al. 2011; Janusz et al. 2017). Table 2 shows the major lignocellulose degrading enzymes and their fungal producers.

van den Brink and de Vries (2011) described that fungal enzymes related to plant biomass degradation consist of at least 35 glycoside hydrolase (GH) families, three carbohydrate esterase (CE) families, and six polysaccharide lyase (PL) families. In Figure 2 major lignocellulolytic enzymes have been shown. This section discusses the fungal enzymatic machineries responsible for lignocellulose degradation.

Ligninolytic Enzymes and Their Producer Organisms

Ligninolytic enzymes degrade the complex polymer of lignin as long carbon chain. They involve mainly oxidative enzymes *viz.* laccase, lignin peroxidase (LiP), and manganese peroxidase (MnP). Lignin is degraded by different microorganisms, of which wood-rotting fungi mainly white rots are the most effective (Eriksson et al. 1990).

Soft-rot fungi efficiently degrade wood polysaccharides but degrade lignin slowly and incompletely (Janshekar et al. 1983). They have been commonly observed on hardwoods than on softwoods. Some lignin degrading soft-rot fungi are *Daldinia concentrica, Lecythophora hoffmannii, Chaetomium globosum, Petrillidium boydii* and *Pialophora mutabilis.* Brown-rot fungi prefer coniferous wood; degrade extensive amount of polysaccharides and limited amount of lignin. Some brown-rot fungi able to degrade lignin are *Gleophyllum trabeum, Fomitopsis pinicola, Poria placenta, Pholiota adipose, Lentinus lepideus* and *Tyromyces palustris* (Kuhad et al. 1997). Most effective wood-rotting fungi are the white-rot fungi, predominanly degrade wood not only from deciduous trees but also coniferous wood (Eriksson et al.1990). Most of them degrade wood by a simultaneous attack on the lignin, cellulose and hemicelluloses but a few are specific lignin degraders (Blanchette et al. 1992). There are a number of white-rot fungi

studied which have ligninolytic activity, some of them are *Cyathus bulleri*, *Coriolus versicolor*, *Ganoderma lucidum*, *Phlebia radiata*, *Pleurotus ostreatus*, *P. eryngii*, *Pycnoporus sanguineus*, *P. cinnabarinus*, *Cereporiopsis subvermispora*, *Phanerochaete chrysosporium*, *Trametes versicolor*, *Bjerkandera adusta*, *Podoscypha elegans* etc. (Khushal et al. 2010; Osma et al. 2011; Kuhara & Papinutti 2014; Kuuskeri et al. 2015; Mali et al. 2017; Kadri et al. 2017; Shaheena et al. 2017; Bouacem et al. 2018). The detailed list of the producer fungi is presented in Table 2. The major ligninolytic enzymes are laccases, lignin peroxidases and manganese peroxidases.

Laccases

Laccases (EC 1.10.3.2) are glycosylated multicopper polyphenol oxydases first isolated from latex of Japaneese lacquer tree *Rhus vernicifera*, presently named as *Toxicodendron vernicifluum* (Mukherjee and Das 2009; Punelli et al. 2009; Couto 2018). The enzyme is characteristically produced as multiple isoenzymes (Leonowicz et al. 2001; Mayer & Staples 2002; Das et al. 2011). The enzymes are purified and several laccase encoding genes have been isolated and studied by different workers (Palmieri et al. 2000; Das et al. 2001; Piscitelli et al. 2011; Ohm et al. 2014).

Although the direct activity of laccases are limited mainly to phenolic substrates but in the presence of small molecules (named mediators) with electron transfer capability, they can also oxidize non-phenolic lignin model compounds which make up 80%–90% of lignin (Bourbonnais and Paice 1990; Wong 2009). Thus in contrast to most other enzymes, laccases are competent to oxidize a wide range of substrates *viz.* non-phenolic or phenolic compounds aromatic amines, diamines, hydroxyindoles, benzenethiols, heterocyclic compounds, carbohydrates, and inorganic/organic metal compounds etc.(Xu et al. 1996; Call and Mu¨cke, 1997; Baldrian 2006; Giardina et al. 2010; Jeon et al. 2012; Karaki et al. 2016). These blue multicopper oxidases catalyze the oxidation of substrates which is one electron reaction concurrently with the reduction of molecular oxygen to water generating a free radical (Thurston 1994; Claus 2003).

Lignin Peroxidases

Lignin Peroxidase (LiP) [(EC 1.11.1.14)] was generally considered as the primary catalyst in ligninolysis. Kirk and Tien (1983) first demonstrated the presence of LiP in *Phanerochaete chrysosporium.* Many isomeric forms are isolated, purified and characterized from a number of fungal sources (Kang et al. 1993; Hofrichter 2010). Lip catalyses the oxidation of veratryl alcohol to veratraldehyde, in presence of catalytic amount of H_2O_2. It also catalyses the H_2O_2 dependent oxidation of a wide range of non-

phenolic lignin model compounds and aromatic pollutants including synthetic lignin (Harvey et al.1992; Schoemaker and Piontek 1996).

These reactions include benzylic alcohol oxidations, side chain cleavages, ring opening reactions, demethoxylations and oxidatative dechlorinations. All these reactions were consistintent with a mechanism involving the initial one electron oxidation of susceptible aromatic nuclei by an oxidized enzyme intermediate to form an aryl cation radical. The later can undergo a variety of non-enzymatic reactions to yield a wide range of final products. According to Gold et al. (1993) the possible mechanism of LiP is:

$LiP + H_2O_2 \text{-----} > LiPI + H_2O$
$LiPI + Ar \text{-----} > LIPII + Ar^+$
$LIPII + Ar \text{-----} > LiP + Ar.^+ + H_2O$
($Ar.^+$ Aryl cation radical)

Manganese Peroxidase

Manganese peroxidase (MnP) [(EC 1.11.1.13)] was also isolated first from *Phanerochaete chrysosporium* by Kuwahara et al. (1984) and Paszczynski et al. (1986). The enzyme degraded lignin and various lignin model compounds but not the veratryl alcohol. Most of the producer organisms acquire several isoenzymes (Lobos et al. 1994). The enzyme was capable of oxidizing a variety of lignin substructures containing free phenols in presence of H_2O_2. The reactions involve the oxidation of Mn^{2+} to Mn^{3+} to cause the degradation of lignin ((Hofrichter 2002). Mn^{3+} oxidizes the organic substrates (Glenn and Gold 1985; Paszczynski et al. 1986). MnP also oxidizes non-phenolic substrates in presence of a second mediator (Wong 2009). According to Gold et al. (1993) the possible mechanism of MnP is as follows:

$MnP + H_2O_2 \text{-----} > MnPI + H_2O$
$MnPI + MnII \text{-----} > MnPII + MnIII$
$MnPII + MnII \text{-----} > MnP + Mn(III) + H_2O$

Cellulolytic Enzymes and Their Producer Organisms

Cellulolytic enzymes including cellulases, hemicellulases, pectinases, chitinases, amylases, and mannases are regularly produced by a wide range of fungi. Fungal cellulolytic enzymes are a group of hydrolytic enzymes responsible for cellulolytic and xylanolytic activities (Mtui 2012).

Cellulases

Microorganisms produce mainly three types of cellulases - endo-1,4-β-D-glucanase, exo-1,4-β-D-glucanase/cellobiohydrolase, and β-glucosidase either separately or in the form of a complex, for the degradation of cellulose. Most of the cellulolytic microorganisms belong to eubacteria and fungi, although some anaerobic protozoa and slime molds are able to degrade cellulose and a few of them produce all the necessary enzymes for degradation of crystalline cellulose. Fungi are the most studied organisms with respect to degradation of cellulose and production of cellulolytic enzymes (Ng et al. 2010; Metrevelia et al. 2017).

Soft-rot fungi mainly degrade the polysaccharides. The best known of these producing a complete set of cellulases is *Trichoderma viride* (Bisaria et al. 1989). Other well-known cellulose degrading fungi are *Aspergillus niger*, *Chaetomium cellullolyticum*, *Fusarium oxysporium*, *Neurospora crassa*, *Penicillium pinophilum* (Singh and Hayashi, 1995). Brown-rot fungi degrade cellulose rapidly, but the enzyme system seems to operate differently from those of soft-rot fungi and white-rot fungi. *Poria placenta*, *Lanzites trabeum*, *Tyromyces palustris* and *Coniophora puteana* are brown-rot fungi among the most studied for their cellulolytic activities (Eriksson et al. 1990). White-rot group of fungi degrade lignin as well as other lignocellulosic components (Eriksson, 1981). The most studied white-rot fungi were *Phanerochaete chrysosporium* that was first isolated from wood chip piles. Other important white-rot fungi are *thermophile* and *Coriolus versicolor* (Li et al. 2011; Isroi et al. 2011). Rumen fungi produce cellulases, often described as a complex of enzymes that, by acting together, solubilize cellulose efficiently (Wood, 1991). Some fungal species of relevance are *Sphaeromonas communis*, *Neocallimastrix frontalis*, *N. patriciarum* and *Piromyces communis*.

Hemicellulases

The complete hydrolysis of hemicellulose into monosaccharides requires the concerted action of several enzymes. These include β-D-xylanases, β-D-galactanases, β-D-mannanases, as well as glycosidases β-D-xylosidase, β-D-galactosidase, and β-D-mannosidase. Esterases also participate by hydrolysis of acetylated carbohydrates. A large number of filamentous fungi, yeasts and bacteria are reported to produce 1.4-β-D-xylanases. Fungal hemicellulolytic enzymes, mainly xylanases, are produced from a wide range of substrates (Haq et al. 2006; Elisashvili et al. 2006; Dobrev et al. 2007; Mohana et al. 2008). Production of xylanases by *Penicillium echinulatum* using sugarcane bagasse has been studied and appreciable hemicellulolytic enzyme activities were observed (Camassola et al. 2009). From liquid culture of *Daldinia caldariorum* xylanases are achieved maximally at 35°C and pH 5 (Ng et al. 2010).

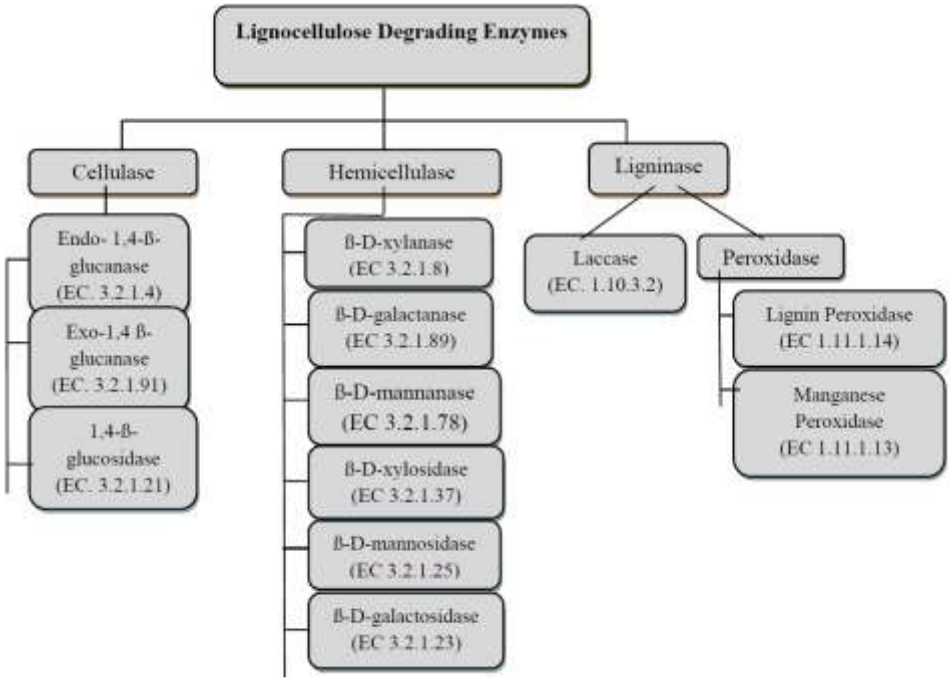


Figure 2. Schematic representation showing the different lignocellulose degrading enzymes.

Table 2. Major lignocellulose degrading enzymes and their fungal producers

Enzyme	Organism	References
MnP, LiP, Laccase	*Aspergillus niger*	Lawal et al. 2010.
LiP MnP, Laccase	*Bjerkandera adusta*	Rodrigues et al. 2008; Tripathi et al. 2011. Bouacem et al. 2018
Laccase, LiP, MnP	*Cerrena unicolor.*	Winquist et al. 2008.
Lip, MnP	*Coriolus versicolor*	Liew et al. 2011;
Laccase, LiP, MnP	*Crepidotus variabilis*	Mtui and Nakamura, 2007, 2008.
Laccase, LiP, MnP	*Flavodon flavus*	Raghukumar et al. 2006; Mtui and Nakamura, 2008.
LiP, MnP, Laccase	*Fomes fomentarius*	Rodrigues et al. 2008; Ramesh et al. 2009.
Lip, MnP, Laccase	*Fusarium oxysporum*	Silva et al. 2009.
Laccase	*Ganoderma lucidum, G. applanatum*	Punnapayak et al. 2009.
Laccase, LiP	*Irpex lacteus*	Xu et al. 2009.
Lip, MnP, Laccase	*Laetioporus sulphureus*	Mtui and Masalu, 2008.
MnP, Aryl oxidases.	*Lentinus squarrosulus*	Tripathi et al. 2011.
LiP, MnP, Laccase	*Lentinus crinitus, L. subnudus*	Niebisch et al. 2010); Jonathan et al. 2010.
MnP, LiP,	*Mucor mucedo*	Lawal et al. 2010.
LiP, MnP, Laccase	*Paecilomyces farinosus*	Sampedro et al. 2009.
LiP, MnP, Laccase	*Phanerochaete chrysosporium*	Lu et al. 2009; Sharma and Arora, 2010, 2011.
Mn, LiP, Laccase	*Phlebia lindtneri, P. brevispora, P. rufa, P. chrysocreus*	Mtui & Nakamura, 2007; Xiao et al. 2011.

Enzyme	Organism	Reference
LiP, Laccase, MnP	*Pleurotus ostreatus, P. pulmonarius, P. ostreatus, P. flabellatus*	Magan et al. 2010; Purnomo et al. 2010; Singh et al. 2011.
LiP, MnP, Laccase	*Pycnoporus coccineus*	Singh et al. 2010
MnP, LiP	*Rhizopus stononifer*	Lawal et al. 2010.
MnP, LiP, Laccase	*Trametes trogii* *T. versicolor*	Rosales et al. 2007; Patrick et al. 2009 Lawal et al. 2010; Singh et al. 2010.
LiP, MnP, Laccase	*Trematosphaeria mangrovei*	Mabrouk et al. 2010.
LiP, MnP, Laccase	*Trichocladium canadense*	Silva et al. 2009.
LiP, MnP, Laccase	*Phanerochaete flavido-alba, Phlebia ochraceofulva, P. tremellosa*	Bugg et al. 2011
LiP, MnP, Laccase	*Phlebia radiata, Pleurotus eryngii, Ceriporiopsis subvermispora, Marasmius quercophilus, Coprinus cinereus, Aspergillus oryzae*	Erden et al. 2009
Laccase, MnP	*Cerrena unicolor*	Elisashvili et al. 2017
Laccase	*Hexagonia hirta*	Kandasamy et al. 2016
Laccase, MnP	*Ganoderma australe*	Chuwech & Nuansri 2015
CMCases, β -glucosidases, Xylanases	*Aspergillus niger, A. japonicus, A. terreus*	Gautam et al. 2011; Jahromi et al. 2011.
β -endoglucanase, β -exoglucanase, β -glucosidase	*Chaetomium sp.*	Ravindran et al. 2010.
β -glucosidase, FPase, Xylanases	*Daldinia caldariorum*	Camassola and Dilon, 2009; Ng et al. 2010.
CMCases, β -glucosidases	*Emericela variecolor*	Herkulano et al. 2011.
Pectinases, Aryl- β -D-glucosidases	*Grifola frondosa*	Zilly et al. 2011.
CMCase, Xylanase	*Lentinus edodes*	Elisashvili et al. 2006, 2008a, 2008b.
β -xylosidases, β -galactosidases	*Macrocybe titans*	Zilly et al. 2011.
Xylanase	*Melanocarpus albomyces*	Biswas et al. 2010.
Endoglucanase, Exoglucanase, β glucosidases	*Neurospora sitophila*	Asad et al. 2006.
Chitinases	*Nigrospora* sp.	El Hadrami et al. 2010.
Cellulases, Xylanases	*Penicillium echinulatum*	Camassola and Dilon 2009.
Xylanase, Carboxymethyl cellulase	*Phanerochaete chrysosporium*	Sharma and Arora, 2011.
Xylanase, Carboxymethyl cellulase	*Phlebia brevispora, P. fascicularia, P. floridensis, P. radiata*	Sharma and Arora, 2011.
Endo-1,4 β- glucanase, Xylanase, Mannanase.	*Piptoporus betulinus*	Valascova and Baldrian, 2006.
Cellulases, xylanases	*Poria subvermispora*	Salvachua et al. 2011.
Endo- and exo-polygalacturonase, (pectinases)	*Rhizopus microsporus* var. *rhizopodiformis*	Silva et al. 2005; Botella et al. 2007; Damasio et al. 2011.
Cellobiohydrolases, CMCases and β -glucosidases	*Rhodotorula glutinis*	Herkulano et al. 2011.
Cellulases, Invertase, Pectinase, Tannase	*Trichoderma viride* *T. reesei*	Rodríguez and Ma, 2005; Gautam et al. 2011.
Cellulase and Xylanase	*Irpex lacteus*	Metrevelia et al. 2017
Xylanase	*Trametes versicolor*	Thiribhuvanamala et al. 2017
Cellulase	*Ganoderma australe*	Chuwech & Nuansri 2015

DEGRADATION OF LIGNOCELLULOSIC BIOMASS

Cellulose Degradation

The endoglucanases cleave cellulose polysaccharide chains internally mainly from the amorphous region, releasing oligosaccharides and new chain ends. The cellobiohydrolases cleave celobiose units (the cellulose-derived disaccahride) from the end of the polysaccharide chains (Aro et al. 2005). Finally, β-glucosidases hydrolyse cellobiose to glucose, the monomeric readily metabolisable carbon source for fungi (Beguin 1990). These three classes of enzymes need to act synergistically and sequentially in order to degrade completely the cellulose matrix. After endo- and exo-cleaving (performed by endoglucanases and cellobiohydrolases, respectively), the β-glucosidase degrade the remaining oligosaccharides to glucose.

T. reesei, typical example with cellulase system is capable of hydrolyzing native cellulose (Reczey et al. 1995; Singhania et al. 2006). *T. reesei* possesses two genes encoding for exoglucanase, eight for endoglucanases and seven for glucosidases (Aro et al. 2005; Sukumaran et al. 2005). According to Murray et al. (2004) *Talaromyces emersonii,* a typical thermophilic fungus capable of producing cellulase, which was active even at 70°C and decomposes the intact cellulose. Li et al. (2011) and Mandels,(1975) were found that *Talaromyces emersonii, Chaetomium thermophilum, Thermoascus aurantiacus*, and *Sporotrichum thermophile* decomposed cellulose very rapidly and grew well by producing thermostable cellulases.

Hemicellulose Degradation

Xylan, a polymer composed by ß-1,4-linked D-xylose units, is degraded through the action of ß-1,4-endoxylanase, which cleaves the xylan backbone into smaller oligosaccharides and then ß-1,4-xylosidase cleaves the oligosaccharides into xylose. ß-Xylosidases are highly specific for small unsubstituted xylose olygosaccharides and they are important for the complete degradation of xylan. Some ß-xylosidases have been shown trans-xylosylation activity, suggesting role for these enzymes in the synthesis of specific oligosaccharides (Shinoyama et al. 1991; Sulistyo et al. 1995). Degradation of xyloglucan requires endoglucanases (xyloglucanases) and ß-glucosidases action. Xyloglucan-active endoglucanases have specific modes of action; a xyloglucanase from *T. reesei* cleaves at branched glucose residues, whereas the xyloglucanase (GH12) from *A. niger* cleaves xylogluco-oligosaccharides containing more than six glucose residues and at least one nonbranched glucose residue (Master et al. 2008; Desmet et al. 2007).

The degradation of mannans, also referred to galacto (gluco) mannans is performed by the action of ß-endomannanases (ß-mannanases) and ß-mannosidases. The ß mannanases cleave the backbone of galacto (gluco) mannans, releasing mannooligosaccharides. Several structural features in the polymer determine the ability of ß-mannanases to hydrolise the mannan backbone, such as the ratio of glucose to mannose and the number and distribution of substituents on the backbone (Mccleary et al. 1991). ß-Mannosidases act on the nonreducing ends of mannooligosaccharides, releasing mannose. ß-Mannosidase is able to completely release terminal mannose residues when one or more adjacent unsubstituted mannose residues are present (Ademark et al. 1999).

Recently a cold active xylanase was isolated from a marine fungus, *Cladosporium* sp. (Del-Cid et al. 2014). The xylanase and endo-xylanase production has been widely studied in many fungi such as *Penicillium thomii* (Palaniswany et al. 2008), *P. pinophilum* (Li et al. 2006), Lee et al. 2011), *A. niger* (Sharma et al. 2015) and *Ceratocystis paradoxa* (Dekker et al. 1975). From an industrial point of view, an alkaline xylanase producing fungi, *A. niger* (Raghukumar et al. 2004) and *P. canescens* (Burtseva et al. 2010) were isolated from marine sources.

Lignin Degradation

The high proportion of ether and polymerizing bonds give lignin its unique structure as a strongly resistant polymer. Though there are involvement of several lignin degrading enzymes like veratryl alcohol oxidase (VAO) (Sannia et al. 1991), glucose oxidase (Eriksson et al. 1986), methanol oxidase (Nishida and Eriksson, 1987) but the main enzymes for lignin degradation are lignin peroxidase (LiP), manganese peroxidase (MnP) and laccases (Hong et al. 2017; Longe et al. 2018). Higuchi (1989) described LiP and laccases as main lignin degrading enzymes in white rot fungi. Side chains and aromatic rings of arylglycerol-β-aryl ethers and diarylpropane 1,3 diols were cleaved via aryl radical cation and phenoxy radical intermediates, in reactions mediated only by lignin peroxidase/ H_2O_2 and laccase/O_2(Sou et al. 2017, Datta et al. 2017).

LiP is generally considered as the primary catalyst in ligninolysis. It was first reported in *Phanerochaete chrysosporium* (kirk and Tien, 1983). The Table 2 enlisted the fungal members producing this enzyme. LiP catalyses the oxidation of veratryl alcohol (3,5 dimethoxy benzylalcohol) to veratraldehyde in presence of H_2O_2 (Tien and Kirk, 1984). LiP also catalyses H_2O_2 dependent oxidation of a wide range of non-phenolic lignin model compounds and aromatic pollutants including synthetic lignin (Gold et al. 1989; Vasudevan and Mahadevan, 1992; Yadav and Reddy, 1993; Barr and Aust, 1994.). These reactions include benzyl alcohol oxidation, side chain cleavage, ring opening reaction, demethoxylation and oxidative dechlorination.

Kuwahara et al. (1984) discover another peroxidase different from LiP in *P. chrysosporium* and identified it as MnP. The enzyme degraded lignin and various lignin model compounds though it did not oxidize veratryl alcohol. The enzyme was capable of oxidizing a variety of lignin substructures containing free phenol in presence of H_2O_2. The reaction involves the oxidation of Mn^{2+} to Mn^{3+} to effect degradation of lignin (Tuor et al. 1992; Hofrichter 2002). Mn^{3+}in turn oxidizes the organic substrates (Glenn and Gold, 1985; Paszczynski et al. 1986).

The enzyme laccase is particularly abundant in many lignin degrading white rot fungi and this has led to speculation that laccases play a role in wood and pulp delignification. Morohasi et al. (1987) found that only one of the three laccases produced by *C. versicolor* depolymerised certain lignin preparations while the other two laccases gave mainly polymerised products. Durrant et al. (1991), directly correlated laccase of *A. bisporus* with the time of disappearance of lignin from the compost substrate and mineralization of labelled lignin to $^{14}CO_2$ and they predicted significant role of laccase in lignin degradation. The ligninolytic activity of *Panus tigrinis* was repoted by Potdnzakova et al. (1994). Singh and Chen (2008) reported the production of diiferent lignin-degrading enzymes in white rot fungus *Phaenerochaete chrysosporium.*

Oxidation of lignin by fungal laccases has been studied intensively since early 1970 (Ishihara Miyazaki 1972). Oxidation of milled wood lignin, demethylation, polymerization, depolymerization and formation of carboxyl groups were observed (Konishi et al.1974; Kim YS 2007; Eriksson et al. 2012; Hilgers et al. 2018). According to Ishihara (1980) and Leonowicz et al. (2001) only phenolic subunits of lignin are attacked, leading to Cα – oxidation, Cα – Cβ cleavage and alkaly – aryl cleavage; often polymerization products are observed when laccase oxidizes phenolic substrates. There are a large number of works on involvement of laccases in biodegradation of different lignin model compounds (Higuchi 1989, Bajpai et al. 2006; Janusz et al. 2017; Hilgers et al. 2018).

The substrate range of laccase can be extended to non-phenolic subunits of lignin by inclusion of a mediator such as ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Bourbonnais et al. 1998, Yao and Ji, 2014). Thus veratryl alcohol is oxidized to veratraldehyde and non-phenolic β-1 and β-0-4 model compounds are cleaved or oxidized at the C- α position. Whatever be the mechanism, it was found that kraft pulp was delignified by the combination of laccase and ABTS but not by laccase alone (Bourbonnais and Paice, 1992). Bourbonnais et al. (1998) showed that in presence of mediator like ABTS, the enzyme prevented and reversed the polymerization of kraft lignin by laccase. Cañ as and Camarero (2010) reported different laccase mediators. Hilgers et al. (2018) reported the effect of different mediators on phenolic lignin sub units also. The mediators HBT (1-Hydroxy benzotriazole) and ABTS differed strongly in respect to their induction on phenolic lignin dimer conversion by laccase. Though the influence of HBT was negligible, but ABTS strongly increased the conversion rate. This

difference in influence between HBT and ABTS can be explained by the much higher oxidation rate of ABTS which is confirmed by measurements of oxygen consumption of both mediators and phenolic lignin dimer with laccase (Hilgers et al. 2018).

The role of laccase in Mn (III) catalysed lignin degradation has also been reported in white rot fungi. In an *in vitro* study using pure laccase enzymes from *Rigidoporous lignosus,* it was observed that laccase and manganese peroxidise acted synergistically to degrade radiolabelled lignin (Galliano et al. 1991). Archibald and Roy (1992) showed that pure laccase from *C. versicolor* produced Mn (III) chelates from Mn (II) in presence of phenolic accessory substrate.

Evans (1985) showed that lignin degradation by *C. versicolor* remained unaffected when laccase activity was inhibited by a specific antibody.Thus, although laccase can (directly or indirectly) cleave a significant proportion of the structures found in lignin, the role of laccase in ligninolysis still remains unsolved. On the contrary, *Trametes* KS-2, a ligninolytic fungus showed the laccase activity but no LiP and MnP activities, indicating that the lignin degradation was specifically dependent on laccase activity (Dong et al. 2019).

PHYSIOLOGICAL ROLE OF LIGNOCELLULOSIC ENZYMES

Different microorganisms particularly the fungi produce different lignocellulosic enzymes. The concentrations of these enzymes vary throughout their life cycle which can be correlated with different physiological functions of the producer organisms. The mutational studies again confirm some physiological roles. Some of these roles are discussed here.

Mycelial Growth

In *P. florida,* there had been a positive relationship between the mycelial growth and one active laccase isoenzyme (Das et al. 1997). The laccase negative mutant strains showed impaired growth and cannot produced fruiting body even after two months growth whereas the revertant mutants regained their normal laccase activities, normal mycelial growth and fruiting pattern. During early stages of growth mainly laccase activity was prevalent which is required to degrade the outer lignin layer, subsequently cellulases are secreted to degrade the cellulose layer and glucose is available for the mycelial growth and development. Tlecuitl-Beristain et al. (2008) also reported the positive correlation between laccase activity and growth of the mushroom *Pleurotus ostreatus.* There was also some correlation between laccase production and mycelial biomass in some species of *Pleurotus.* Irrespective of different laccase isoenzyme pattern,

a growth regulating laccase was universally present in the tested five species of *Pleurotus* viz. *P.florida, P.ostreatus, P. sajorcaju, P. pulmonarius and P. flabellatus* (Das et al. 2011).

Morphogenesis and Fruiting Body Development

In some fungi some well-defined functions of laccases have been demonstrated. Two laccases having different functions have been reported in *Aspergillus nidulans.* The product of the YA gene was a laccase which was involved in formation of the green colour of conidia (Clutterbuck, 1972). The phenol oxidase might be related to fruit body formation as reported by Bu' Lock (1967) and Leatham and Stohman (1981). The formation of rhizomorph was reported to be associated with laccase synthesis in *Armillaria mellea* (Worrel, 1986). Regulation of laccase in relation to fruit body development has been reported in two basidiomycetes, *S. commune* and *A. bisporus* (Wood, 1980; de vries, 1986). In *S. commune*, it was noted that the dikaryotic strains that were able to develop fruiting bodies secreted high levels of laccase but the co-isogenic monokaryotic strains could not do so (Leonard, 1973). Increased laccase activity has also been reported to be associated with the development of fruiting bodies of *Lentinus edodes* (Leatham and Stohman, 1981). According to Bonnen et al. (1994), in *Agaricus bisporus*, peroxidase and laccase activities induced from vegetative stage to early stages of fruiting body formation and fall strongly during maturation of fruiting bodies.

According to Elisashvili et al. (2008) during substrate colonization of *P. ostreatus* the activities of laccase and MnP were high whereas the cellulase and xylanase activities were low. Both cellulase and xylanase activities were increased during primordia development and the activities of these enzymes are optimum at the mature stages of fruiting body. After fruiting, again the laccase and MnP activities were increased and cellulase and xylanase activities were decreased, and the mushroom entered into the vegetative stage.

Sporulation

Chakraborty et al. (2000) have reported the occurrence and accumulation of a natural substrate of laccase during the gill tissue formation in *P. florida* in sporulating fruiting bodies. Though the concentration of this substrate was very low in immature and non-sporulating sporophores, but with the commencement of sporulation there was at least an 8-fold increase in concentration (Chakraborty et al. 2000). They suggested that the predominant presence of this inhibitory compound (regarded as an endogenous laccase substrate) might be responsible for spore formation in *P. florida*. Weaver et al. (1970)

suggested a low-moleucular weight, water-soluble, phenolic compound and a low molecular weight polyphenol oxidase are jointly responsible for sporulation in *A. bisporus*. Chakraborty et al. (2000) had reported that after coming in contact with oxygen, the endogenous laccase inhibitor got oxidized and thereby failed to impart agonistic action on laccase inhibition. Similar reactions might have been preponderant in *P. fossulatus*. In liquid medium, an inhibitor is produced which easily leach out and possibly after oxidation was unable to couple with the active intracellular laccase isoforms, produced de novo, resulting gill-less fruiting bodies in liquid culture (Das et al. 2011). So, there might be a correlation between extracellular laccase activity and gill less sporophore production of *P. fossulatus* in submerged fermenting conditions.

Pathogenesis

Several plant-pathogenic fungi produce cellulases (Carpita and Gibeaut, 1993). A few cellulases of plant-pathogenic fungi have been shown to be involved in pathogenicity (Eshel et al. 2002; Muller et al. 1997; Sexton et al. 2000). It has been proposed that *Macrophomina phaseolina* utilizes an endocellulase for pathogenicity (Wang and Jones, 1995). A cellobiohydrolase is expressed in the initial infection phase of *Claviceps purpurea* on *Secale cereale* (Muller et al. 1997). Transcription of a cellobiohydrolase from *Leptosphaeria maculans* is detectable during infection of *Brassica napus* and *B. juncea* cotyledons and leaves (Sexton et al. 2000). The phytopathogenic fungus *Alternaria alternata* produces one endocellulase which is an important factor in disease development in fruit of *Diospyros kaki* (Eshel et al. 2002). Fungal β-glucanases showed the capability of disease control by degrading cell walls of pathogens (Singh et al. 2007).

There is a large number of evidence demonstrating that laccase produced by pathogenic fungi play a significant role in overcoming host cell defences as well as launching an interaction with the host plant (Leonowicz et al. 2001). Laccases are important virulence factor in some plant pathogenic fungi like *Botrytis cineria*, the pathogen responsible for soft rot infections of carrot and cucumber as well as noble rot and grey rot of grapes. Though the exact mechanism about the part played by laccase in pathogenesis of *B. cinerea* is not clear, still it has been established that laccase somehow represses the biochemical defence system in host plants (Bar-Nun et al. 1988). The chestnut blight fungus *Cryphonectria parasitica* also produced laccase which is responsible for pathogenesis (Mayer and Staples, 2002). In some hypovirulent strains of this fungi, laccase synthesis has been found to be repressed due to prevention of accumulation of laccase mRNA (Ringling, 1991; Powell, 1987). Sun et al. (2014) and Hu et al. (2011) isolated extracellular laccases with antiproliferative activity from the mushrooms *Inonotus baumii* and *Agrocybe cylindrical*, respectively.The pathogen of root and butt rot of conifers, *Heterobasidion annosum*, possess five active laccase isozymes

during pathogenesis. Another example of the foremost pathogenic role played by laccases was found by the interactions between *Ophiostoma ulmi* and *O. novo-ulmi* and chestnut. According to Binz & Canevascini (1996) the secretion of higher levels of laccase by *O. novo-ulmi* in comparison with *O. ulmi* assists the detoxification of tannins and polyphenolics synthesized during plant defence, which augments chances of *O. novo-ulmi* to survive in the bark and infect the host. *Pleurotus eryngii* and *P. ferulae,* two facultative parasitic species synthesize laccases as part of their ligninolytic machineries, play a role in the host–pathogen interaction in the initial step of infection (Punelli et al. 2009). The role of laccase in pathogenesis was also reported in the human pathogenic fungi *Cryptococcus neoformans* which might act as a major virulence factor causing infections in 10% of AIDS patients. (Williamson, 1994; Rigling and Van Alfen, 1993).

Pigment Production

In some fungi like *A. nidulans* pigment production is directly related to laccase activity (Gochev and Krastanov, 2007). Both laccase activity and melanisation as oxygen and phenol scavengers were reported to help the species to adapt in rhizospheric life (Hynes et al. 1988). Melanin production was also reported in *C. neoformans* where laccase production and melanin synthesis were correlated (Rigling and Van Alfen 1993; Williamson 1994). The role of laccase in synthesis of pigment in the basidiospores of *Lentinula edodes* was also reported earlier (Leatham & Stahman 1981). Sapmak et al. (2015) reported a laccase gene for melanin synthesis in conidia of *Talaromyces* (*Penicillium*) *marneffei* wheras Hynes et al. (1988) reported the production of melanin in *Rhizobium leguminosarum* by cryptic plasmids.

Other Functions

Apart from lignin degradation, morphogenesis, plant pathogenesis and pigment formation, several other functions of laccase have been proposed. Laccase has also been reported to relate to the development of sporangiophores of the slime mold *Physarum polycephalum* (Daniel 1963). The bulk of the laccase has been found to be localized in tissuses of wood rotting fungi undergoing active growth even when the tissuses were not markedly pigmented (Harkin 1974). In *Podospora anserina*, appearance of a specific laccase enzyme was found to be associated with autolysis of fungus (Boucherie 1981). Oxidative stress and ageing in the fungus were correlated with `laccase synthesis in this organism. Mutants lacking cytochrome oxidase aa3 or laccase activity showed stable mitochondrial populations and could live eternally (Frese and Stahl 1992). Laccases have

been found to be involved in the initial steps of healing in injured leaves (De Marco and Roubelakis-Angelakis 1997).

BIOTECHNOLOGICAL APPLICATION OF LIGNOCELLULOLYTIC ENZYMES

Degradation of lignocellulosic substances is of great importance for conversion of lignocellulosic materials into biotechnologically important value added products. Lignocellulose degrading enzymes are used in various industries like alcohol, pulp and paper, textile, animal feed and agriculture, pharmaceutical etc. as well as they are used in oil extraction to bioremediation and various other biotechnological purposes. A number of interesting literature are also available (Couto and Toca Herrera 2006; Kuhad and Singh. 2007; Mukherjee and Das. 2009; Kantharaj et al. 2017; Obeng et al. 2017). Some of the important biotechnological applications are discussed here.

Bio-Ethanol Production

The problem of running out of oil, gas and coal and the environmental impacts of fossil fuels are of serious concern (Sun & Cheng 2002). Fermentation of wood hydrolysates is an essential product for biofuel (Rubin 2008). According to Prassad et al. (2007) plant cell walls generally constitute 15–40% cellulose, 10–30% hemicellulose and pectin, and 5–20% lignin; these are the initial substances for microbes to hydrolyze and can be derived to produce bio-ethanol. Recently, many fungi have shown efficiency for use as bio-ethanol production by utilizing wastes. These include *Aspergillus niger* (Izmirlioglu et al. 2016), *Ganoderma lucidum* (Bilal et al. 2015), and *Fusarium* sp., *Spicaria* sp., *Rhizoctonia* sp., and *Paecilomyces* sp. (Dar et al. 2016).

The enzymatic hydrolysis of the hemicellulose and cellulose content of lignocellulosic biomass to their basic monomeric sugars require synergetic action of lignocellulases—cellulases, hemicellulases, ligninases for the production of bio-ethanol and other value-added biochemicals. Recently it is known that for an effective deconstruction activity other lytic polysaccharide mono-oxygenases (LPMO)—are also essential.

Wine and Brewing Industry

In food industry, wine stabilization is the main application of ligninolytic enzymes like laccases (Minussi et al. 2002). In wine production, removal of polyphenols is

necessary because of their unwanted effects and organoleptic features. Many treatments have been used to control this problem, such as enzyme inhibitors, complicated agents, and sulfate compounds to excommunicate discoloration, evaporation and flavour changes. These effects can be prevented and beverage stabilization can be accomplished through use of laccase (Morozova et al. 2007). In wine production, enzymes (for example- cellulase, amylase, amyloglucosidase, pectinase, glucanase, and hemicellulase) improve clarification, colour extraction, filtration and take full responsibility for quality and stability (Singh et al. 2007, Ghorai et al. 2009). The aroma of wines can be improved through modifying glycosylated precursors by β–glucosidases. Macerating enzymes are used to treat grapes for wine fermentation, also strengthen the ability of press, settling and juice yields. Cellulases, hemicellulases, galactomannanase, and pectinases are also used in the coffee industry. Enzymes are used from microbial sources such as *Leuconostoc mesenteroides*, *Saccharomyces marscianus*, *Flavobacterium* spp. and *Fusarium* spp. (Binod et al. 2008). α- and β–amylase, pullulanase, phospholipase, and invertase are used to produce various types of syrups from starch and sucrose (Patel et al. 2016).

Pulp and Paper Industry

Delignification of Lignocelluloses

In paper production it is necessary to separate lignin from cellulose fibers, so chlorine, sulphite and oxygen-based oxidants have been widely used. However, these substances are damaging the environment and should be replaced by biological process. Lignocellulose degrading enzymes should reduce the chemical consumption of this process (Kristensen et al. 2008). Biobleaching, pitch removal and de-inking of paper wastes are possible by the use of some biocatalysts, such as xylanases and laccases (Singh et al. 2016).According to Virk et al. (2012), delignification mechanisms of xylanases and laccases are unlike because xylanases increase the delignification by making pulp more susceptible to attack by bleaching chemicals whereas laccases act directly on lignin and cause its removal from pulps.

Biopulping and Biobleaching

Biopulping is the enzymatic pretreatment of wood chips before chemical methods. Though chemical bleaching is a fast process, but it affects the cellulose in the pulp and the environment is also polluted by various effluents from paper industry. Ligninolytic enzymes particularly laccases are able to delignify and bleach pulp. Although this process is rather slow compared with chemical bleaching, it is an eco-friendly green approach for sustainable environment (Ana et al. 2007). Hakala et al. (2004) chose 86 fungal strains for wood block decay test. Of these, *Physisporinus rivulosus* T241i was the best choice

for softwood bio-pulping application. Behrendt & Blanchette (1997) used *Phlebiopsis gigantean* to treat wood logs and found that the wood pitch content reduction was promoted. Xylanase also helps in biopulping. According to Woolridge (2014) xylanase acts as bleaching aid but not as a true delignification agent, since the enzyme does not directly degrade lignin but disrupting the bonds between xylan and cellulose &/lignin.

Textile Industry

Dye Decolourization

The textile industry has a bad reputation as an environmental polluter. According to Nagaraj and Kumar (2006) textile waste waters contain various dyes that are hardly decolorized by conventional treatment systems and cause high biological oxygen demand (BOD) and chemical oxygen demand (COD). Using biological techniques to decolourize dye is cost effective and has application for a wide range of dyes. For example, ligninolytic enzymes such as peroxidases and laccase, can be used to decolorize dyes in wastewater (Imran et al. 2015, Singh et al. 2015).

Denim Finishing

In the past, fabric was washed with abrasives to produce a worn or faded appearance in denim before it was partially pre-bleached in sodium hypochlorite then neutralized in a rinsing step. This process was a major polluter of the environment. In 1996, Novozyme (Novo Nordisk, Denmark) took the initiative and "DeniLite" became the first industrial laccase and the first bleaching enzyme which acts with the help of a mediator molecule. Research in this field had led to discovering novel microorganism to produce high quality laccase under normal environmental conditions (Sharma et al. 2005).

Cotton Bleaching

Bleaching cotton to reject natural pigments result in flavonoids with white appearance. Hydrogen peroxide was previously used as the bleaching agent, but it damages fibers by decreasing the degree of polymerization. Enzymatic systems not only cause less damage to fibers but also minimize water use and the development of a quality product. Tzanov et al. (2003) found that ligninolytic enzymes work at low concentrations without reducing the quality of the fabric. According to Spina et al. (2016) and Iracheta et al. (2016) laccase from *Trametes pubescens* and *Pycnoporus sanguineus* are used in dye decolorization, cotton bleaching and textile application. Recently, high frequency ultrasound in the range of 850 kHz, 400 W has been applied to enhance the activity of laccase and to improve the whitening effectiveness (Gonçalves et al. 2014).

Food Processing Industry

There are numerous applications for using cellulases in food biotechnology. For example, fruit and vegetable juice production requires cellulases to improve extraction methods, clarification, and stabilization, and also xylanases and pectinases have an important application to increase the yield of juices as part of a macerating enzyme complex (Minussi et al. 2002, Carvalho et al. 2008). Enzyme mixtures containing pectinases, cellulases, and hemicellulases are also used for improved extraction of olive oil. Use of macerating enzymes not only improves the cloud stability and texture of nectars and purees, but also rapidly decreases their viscosity (Grassin & Fauquembergue 1996). Fruits and vegetables thus can be efficiently converted into food commodities by using lignocellulolytic enzymes in the corresponding processing industries. Lingo-cellulolytic enzymes not only accelerate the production rates but also improve product quality in different food processing industries. They also increase the availability of food to diminish worldwide nutritional problems (Toushik et al. 2017).

Animal Feed and Agricultural Industries

Enzymes were first used commercially in animal feed in Finland in the 1980s. Applications of cellulolytic enzymes can improve nutritional value and absorbence (Dhiman et al. 2002). In feed industry, β-glucanases and xylanases are used to hydrolyze non-starch polysaccharides. By improving digestion and absorption of feed materials, cellulase can significantly improve quality of meat and also weight gain in chickens and piglets (Ramesh et al. 2011). Enzyme use in animal nutrition has become essential following the prohibition of using some food supplements and antibiotics in animal feed product in EU countries (Ali et al. 1995). Many producers supplement feed with enzyme additives to improve the efficiency, decrease the volume of manure produced, reduce cost, lower phosphorus and nitrogen excretion, improve consistency and help to maintain gut health (Michael & Gary 2010). Ligninolytic and cellulolytic enzymes have been also tested for enhancing growth of crops and controlling plant pathogens (Ramesh et al. 2011; Bharathiraja et al, 2017).

Oil Extraction

Enzyme treatment is probably the most important in extraction of oil as it digests the complex cell wall of oil seeds, altering permeability favoring oil extraction. Enzymes enhance extraction and separation process, eliminate toxic anti-nutritional factors, catalyse carbohydrate, protein and lipid conversion through their antioxidant and

biocatalytic activities (Kalia et al. 2001). Enzymes are applied in various oil seed materials such as peanut, sunflower, soybean, grape seed, etc. Macerating enzymes during olive oil extraction can increase levels of antioxidants and vitamin E extraction (up to 2 kg oil per 100 kg olives) under cold processing conditions. Enzymes also hold back induction of rancidity and oil content in the waste water (Galante et al. 1998).

Medicinal Importance

Biosensor

Ligninolytic enzyme particularly laccase is used to develop different biosensors, the devices that detect, transmit and record information regarding different physiological or biochemical analysis. Neurotransmitter catecholamine has been detected by laccase electrodes (Lisdat et al. 1997; Leite et al. 2003; Ferry and Leech 2005). A potentiometric immunosensor using laccase without the presence of an electro-chemically active mediator was designed by Ghindilis et al. (1992). Peroxidase and β–galactosidase are commonly used in enzyme immunoassays like alkaline phosphatase (Johnson and Thorpe 1987; Oellerich 1984). A highly sensitive immunoassay based on laccase was developed by Bier et al. (1996). Ray et al. (2010) successfully conjugated laccase with antibody and used as the enzyme-labeled antibody in ELISA. According to Bauer et al. (1999), in drug samples, morphine can be distinguished from codeine by laccase.

Antibiotic-Like Compounds

The oxygen cenred free radicals which are generated during laccase-mediated phenol oxidation produced some dimeric, ologomeric or polymeric compounds of synthetic relevance after coupling reactions. These compounds showed antibiotic like properties against different micro-organisms.

T. versicolor laccase was used for the conversion of 4-methyl-3-hydroxy anthranilic acid to actinomycin like compounds containing a phenoxazinone chromophore (Osiadacz 1999). Synthesis of dimer of Penicillin X (Agematu 1993) has been developed from the phenpomenon of laccase – mediated phenol oxidation mechanism. Laccase can be used in peptide synthesis (Semenov et al. 2003). Laccase mediated oxidation of the steroid hormone 17β-estradiol from *Trametes pubescens* has been done by Nicotra et al. (2004). Florez-Sampedro et al. (2006) showed the anti HIV activity of the enzymatic extract enriched with laccase from *Ganoderma* sp. and *Lentinus* sp. Inhibition of DNA synthesis by the HIV-1 reverse transcriptase was showed by a number of researchers using purified laccase from different fungal species like *P. eryngii, P. cornucopiae, Tricholoma giganteum, T. mongolicum, Ganoderma lucidium, Lentinus edodes, L. tigrinus, Agaricus placomyces, Agrocybe cylindracea* etc. (Florez-Sampedro et al. 2006). Zhu et al. (2016) purified a white laccase from *Lepista nuda* which showed HIV-1 reverse transcriptase

inhibitory activity. A laccase derived from *Pleurotus* sp. inhibited the entrance of Hepatitis C Virus into peripheral blood cell and hepatoma cells (El –Fakharany et al. 2010).

Bioremediation

Cellulose and lignin degrading strains of *T. versicolor* and *P. chrysosporium* showed the capability of degradation of various xenobiotic compounds viz. polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls and various synthetic dyes (Reddy, 2001). According to Eriksson et al. (1990) white-rot fungi have the capability to degrade lignin model compounds. One of the best white-rot fungi is the polypore, *T. versicolor*; secretes enzymes such as phenol oxidase, laccase and perioxidase, which take part in the transformation of aromatic compounds (Lipin et al. 2013). A large spectrum of azo-, triphenylmethane-, anthracene-dyes are also degraded by different white rot fungi having a strong lignocellulolytic enzyme machinaries. They are also used in wastewater treatments as well as biosolid reduction (Singh et al. 2007). *T. villosa* laccase degrades an endocrine disrupting chemical, bisphenol A (Fukuda et al. 2001). Bisphenol A degradation by laccase was also recently reported by Bilal et al. (2019). Alkenes can be efficiently oxidized by *T. hirsutus* laccase (Niku and Vikari 2000). Laccases were able to mediate the coupling of reduced 2, 4, 6-trinitrotolune (TNT) metabolites (Duran and Esposito 2000).

Degradation of Pesticides

Laccase from *P. chrysosporium* and *T. versicolor* modified the active form of herbicide isoxaflutole into inactive benzoic acid analogue in presence of ABTS (Mougin et al. 2002). Oxidized forms of phenylurea herbicides are transformed by purified laccase from *T. versicolor* and *P. cinnabarinus* (Jonas et al. 1998). Hoff et al. (1985) reported the polymerization of 4-chloroaniline, a member of phenylurea group of herbicides by the laccase of *T. versicolor* to pentamers. Degradation of heptachlor and lindane by *P. chrysosporium*, *Pleurotus florida*, *P. eryngi*, and *P. sajor-caju* was reported by Arisoy (1998). Bumpus et al. (1987) reported the degradation of chlorinated pesticide DDT by *P. chrysosporium*, White rot fungi; *Ceriporiopsis subvermispora, Phlebia tremellosa*, and *P. chrysosporium* degraded alachlor (2-chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl)-acetamide) and the related acetanilide herbicides metalochlor and propachlor which are usually considered to be potential carcinogens (Ferrey et al. 1994). The ligninolytic fungus *Dacryopinax elegans* degraded the pesticide diuron in presence of LiP, MnP and laccase (Arakaki et al. 2013). Laccase and peroxidase remove the phenyl hydrazide group under mild conditions (Semenovlrina et al. 1993).

Degradation of Polycyclic Aromatic Hydrocarbons (PAHs)

Laccase from *C. gallica* is involved in the degradation of benzo[a] pyrene, anthracene, 2-methyl anthracene, 9-methyl anthracene, biphenylene, acenaphthene and phenanthrene (Pickard et al. 1999). Hatakka (1994) reported that PAHs degradation was linked with MnP and laccase activation in *P. ostreatus*. Rama et al. (1998) reported that purified laccase isolated from *P. cinnabarinus* degraded benzo[a] pyrene to the corresponding 1,6-, 3,6-, and 6,12- quinine. White rot fungi *G. lucidum* was used for the degradation of PAHs compound phenanthrene and pyrene with 99.65, 99.58% efficiency, respectively (Agrawal et al. 2018). Pyrene is oxidized and formed 4, 5 dihydroxy pyrene by the white rot fungi *Coriolopsis byrsina* (Agrawal and Shahi, 2017). 4,5 dihydroxy pyrene again converted into phenanthrene, phthalic acid and benzoic acid by the action of ligninolytic enzymes laccase, LiP and MnP (Hadibarata and Kristanti, 2013; Khudhair et al. 2015; Agrawal and Shahi, 2017).

Degradation of Chlorinated Aromatic Compounds

Degradation of chlorophenols by crude laccase preparations was first reported by Lyr (1963). Immobilized laccase on montmorillonite could detoxify 2,4-dichlorophenol, a common intermediate of 2,4-D (Ruggiero et al. 1989). Dec & Bollag (1990) found that in presence of laccase various chlorophenols were polymerized to dimers trimers or tetramers). The transformation of trichlorophenol to 2,6–dichloro-1,4-hydroquinol and 2,6-dichloro 1,4-benzoquinone by Mn-peroxidase and laccase of *P. tigrinus* and *C. versicolor* was found by Leontievsky et al. (2000). Ullah et al. (2000) reported removal of pentachlorophenol (PCP) by the purified *T. versicolor* laccase. The degradations of 2,4-dichlorophenol (2,4-DCP), 4-chlorophenol (4-CP) and 2-chlorophenol (2-CP) catalyzed by laccase were reported by Zhang er al (2008). In *T. pubescens* degradation of mixture of 2-chlorophenol, 2,4-dichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol was repotted by Gaitan et al. (2011).

Treatment of Industrial Effluents

Different industries release a number of pollutants which need to be degraded for the sake of our environment. Archiblad et al. (1990) reported the role of laccase in decolorization of bleach plant effluents (BPE) of Paper pulp industries which contain chlorophenol, chlorolignols etc. by *T. versicolor*. Roy and Archiblad (1994) showed that different laccases isozymes of *T. versicolor* dechlorinate a number of polychlorinated phenols. Dechlorination of tetrachloroguaiacol has been catalysed by laccase of *C. versicolor* to tetrachlorocatechol which again is degraded into several other quinines (Imura et al. 1996). Raghukumar (2000) reported that *F. flavus* which produced laccase, MnP and LiP was efficiently decolorized the effluent of BPE. Davis and Burns (1990) proposed that the purified laccase *of C. versicolor* can minimize the pulping effluent color, related with the precipitation and polymerization of phenols. Sahadevan et al.

(2013) reported that alkaline effluents from pulp and paper industry can be treated by ligninolytic enzymes.

CONCLUSION

Lignocellulosic biomass is the most abundant biomaterial on the surface of Earth, though generally considered as waste. Disposal of these materials through burning not only causes environmental pollution by releasing high level of CO_2 but also destroys a large amount of organic materials. The main obstacle is the convoluted structure and composition of lignocellulosics, which are mainly made of lignin, cellulose and hemicellulose. However, nature has equipped many microbes with enzymes that can degrade lignocellulose and release fermentable sugars. The filamentous white rot fungi are one of the best producer group of lignocellulotytic enzymes that can degrade the lignocellulosic materials. After degradation, the released sugars will be taken up by the producer organism and used as energy source, building block of volatile fatty acids, or used for protein synthesis thereby providing them with various nutrients. Thus, the enzymes showed different physiological functions viz. mycelial growth, fruiting body production, sporulation, pathogenesis etc which are directly or indirectly correlated with lignocellulose degradation.

The lignocellulosic materials are enormous source of fermentable sugars, which have a great potentiality in different biotechnological and industrial applications. The isolation and purification of lignocellulose degrading enzymes mainly cellulase, hemicellulase, laccase and peroxidases from white rot fungal members might find applications in wine and brewing, food and fodder, paper pulp, textile, medical diagonostics and other industrial applications.

Lignocellulolytic enzymes are, therefore, critical in processes associated with bioconversion of lignocelluloses. Though they are reported from several bacterial and fungal members but presently the commercially exploited lignocellulases are produced by very few members. The studies can be elaborated in search of new hyper-lignocelluase producing organisms. The production of enzymes needs to be optimized for different biomass. The production of lignocellulases from all microbial sources is still quite expensive. Efforts can be made for cost effective production of these enzymes using high potency enzyme systems with broader range of tolerance that can be active at diverse environmental conditions. Genetic engineering techniques also can be used to construct enzyme systems with desirable characteristics.

REFERENCES

Abbasi, T. and Abbasi, S. A. (2010). Biomass energy and the environmental impacts associated with its production and utilization. *Renewable and Sustainable Energy Reviews*, 14, 919-937.

Ademark, P., Lundqvist, J., Hagglund, P., Tenkanen, M., Torto, N., Tjerneld, F., et al. (1999). Hydrolytic properties of a beta-mannosidase purified from *Aspergillus niger*. *Journal of Biotechnology*, 75, 281-289.

Agematu, H., Tsuchida, T., Kominato, K., Shibamoto, N., Yoshioka, T., Nishida, H., Okamoto, R., Shin, T. and Murao, S. (1993). Enzymatic dimerization of penicillin X. *The Journal of Antibiotics*, 46, 141–148.

Agrawal, N. and Shahi, S.K. (2017). Degradation of polycyclic aromatic hydrocarbon (pyrene) using novel fungal strain *Coriolopsis byrsina* strain APC5. *International Biodeterioration and Biodegradation*, 122, 69–81.

Agrawal, N., Verma, P., Singh, R. S. and Shahi, S. K. (2017). Ligninolytic enzyme production by white rot fungi *Podoscypha elegans* strain FTG4. *International Journal of Current Microbiology and Applied Sciences*, 5, 2757–2764.

Agrawal, N.,Verma P. and Shahi, S. K. (2018). Degradation of polycyclic aromatic hydrocarbons (phenanthrene and pyrene) by the ligninolytic fungi *Ganoderma lucidum* isolated from the hardwood stump. *Bioresources and Bioprocessing*. 5, https://doi.org/10.1186/s40643-018-0197-5.

Ali, S., Hall, J., Soole, K. L., Fontes, C. M. G. A. et al. (1995). Targeted expression of microbial cellulases in transgenic animals. *Progress in Biotechnology*, 10, 279–293.

Ana, M. R. B. X., Ana, P. M. T., Rita, F. and Francisco, A. (2007). *Trametes versicolor* growth and laccase induction with by-products of pulp and paper industry. *Journal of Biotechnology*, 10, 444–451.

Arakaki, R. L., Monteiro, D. A., Boscolo, M., Dasilva, R., Gomes, E. (2013) Halotolerance, ligninase production and herbicide degradation ability of basidiomycetes strains *Brazilian Journal of Microbiology*, 44, 1207-1214.

Archibald, F., Paice, M. G. and Jurasek, L. (1990). Decolorization of kraft bleachery effluent chromophores by *Coriolus versicolor*. *Enzyme and Microbial Technology*, 12, 846-853.

Arisoy, M. (1998). Biodegradation of chlorinated organic compounds by white-rot fungi. *Bulletin of Environmental Contamination and Toxicology*, 60, 872–876.

Aro, N., Pakula, T. and Penttila, M. (2005). Transcriptional regulation of plat cell wall degradation by filamentous fungi. *FEMS Microbiology Reviews*, 29, 719-39.

Asad, M. J., Asgher, M., Sheikh, M. A. and Sultan, J. I. (2006). Production of *Neurospora sitophila* cellulases in solid state cultures. *Journal of the Chemical Society of Pakistan*, 28, 590-595.

Bajpai, P., Anand, A. and Bajpai, P. K. (2006). Bleaching with lignin-oxidizing enzymes. *Biotechnology Annual Review*, 12, 349-378.

Baldrian, P. (2006) Fungal laccases-occurrence and properties. *FEMS Microbiology Reviews*, 30, 215-242.

Bar-Nun, N. and Mayer, A. M. (1990). Cucurbitacins protect cucumber tissue against infection by *Botrytis cinerea*. *Phytochemistry*, 29, 787-791.

Barr, D. P. and Aust, S. D. (1994). Enzyme degradation of lignin. *Reviews of Environmental Contamination and Toxicology*, 138, 49–72.

Bauer, C. G., Kuehn, A., Gajovic, N., Skorobogatko, O., Holt, P. J., Bruce, N. C., Makower, A., Lowe, C. R. and Scheller, F. W. (1999). New enzyme sensors for morphine and codeine based on morphine dehydrogenase and laccase. Fresenius *Journal of Analytical Chemistry*, 364, 179–183.

Beguin, P. (1990). Molecular-biology of cellulose degradation. *Annual Review of Microbiology*, 44, 219-248.

Bharathiraja, S., Suriya, J., Krishnan, M., Manivasagan, P. and Kim, S. K. (2017). Production of enzymes from agricultural wastes and their potential industrial applications. *Advances in Food and Nutrition Research*, 80, 125–148.

Bier, F. F., Ehrentreich-Forster, E., Makower, A. and Scheller, F. W. (1996). An enzymatic amplification cycle for high sensitive immunoassay. *Analytica Chimica Acta*. 328, 27-32.

Bilal, M., Asgher, M. and Ramzan, M. (2015). Purification and biochemical characterization of extracelular manganese peroxidase from *Ganoderma lucidum* IBL-05 and its application. *Scientific Research and Essays*, 10, 456–464.

Bilal, M., Jing, Z., Zhao, Y. and Iqbal, H. 2019. Immobilization of fungal laccase on glutaraldehyde cross-linked chitosan beads and its bio-catalytic potential to degrade bisphenol A. *Biocatalysis and Agricultural Biotechnology*, 19, 101-174.

Binod, P., Singhania, R. R., Soccol, C. R. and Pandey, A. (2008). Industrial enzymes. *Advances in fermentation technology*. Https://books.google.co.th.

Binz, T. and Canevascini, G. (1996). Differential production of extracellular laccase in the dutch elm disease pathogens *Ophiostoma ulmi* and *O. novo-ulmi*. *Mycological Research*, 100, 1060-1064.

Bisaria, V.S. and Mishra, S. (1989). Regulatory aspects of cellulose biosynthesis and secretion. *Critical Reviews in biotechnology*, 9, 61.

Biswas, R., Sahai, V., Mishra, S. and Bisaria, V.S. (2010). Bioprocess strategies for enhanced production of xylanase by *Melanocarpus albomyces* on agro-residual extract. *Journal of Bioscience and Bioengineering*, 111, 702-708.

Blanchette, R.A., Burnes, T.A., Eeerdmans, M.M. and Akhtar, M. (1992). Evaluating isolates of *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora* or use in biological pulping processes. *Holzforschung*, 46, 109.

Boerjan, W., Ralph, J. and Baucher, M. (2003). Lignin biosynthesis. *Annual Review of Plant Biology*, 54, 519–546.

Bonnen, A. M., Anton, L. H. and Orth, A. B. 1994. Lignin-degrading enzymes of the commercial button mushroom, *Agaricus bisporus*. *Applied Environmental Microbiology*, 60, 960-965.

Botella, C., Diaz, A. Ignacio de Ory, I., Webb, C. and Blandino, A. (2007). Xylanase and pectinase production by *Aspergillus awamori* on grape pomace in solid state fermentation. *Process Biochemistry*, 42, 98-101.

Bouacem, K., Rekik, H., Jaouadi, N. Z., Zenati, B. et al. (2018). Purification and characterization of two novel peroxidases from the dye-decolorizing fungus *Bjerkandera adusta* strain CX-9. *International Journal of Biological Macromolecules*, 106, 636–646.

Boucherie, H., Dupont, C.H. and Bernet, J. (1981). Polypeptide synthesis during protoplasmic incompatibility in the fungus *Podosporaanserine*. *Biochimica et Biophysica Acta*, 653, 18-26.

Bourbonnais, R., Leech, D. and Paice, M. G. (1998). Electrochemical analysis of the interactions of laccase mediators with lignin model compounds. *Biohimica et Biophysica Acta*, 1379, 381-390.

Bourbonnais, R. and Paice, M.G. (1990). Oxidation of non-phenolic substrates-an expanded role for laccase in lignin peroxidase. *FEBS Letters*, 267,99-102.

Bourbonnais, R. and Paice, M.G. (1992). Demethylation and delignification of kraft pulp by *Trametes versicolor* laccase in the presence of 2,2´-azinobis-(3-ethylbenzthiozoline-6-sulphonate). *Applied Microbiology and Biotechnology*, 36, 823-827

Brown, Jr. and Malcolm, R. (1996). The biosynthesis of cellulose. *Journal of Macromolecular Science, Part A: Pure and Applied Chemistry*, 33, 1345–1373.

Bugg, T. D. H., Ahmad, M., Hardiman, E. M. and Rahmanpour, R. (2011). Pathways for degradation of lignin in bacteria and fungi. *Natural Product Reports*,28, 1883–1896.

Bumpus, J. A. and Aust, S. D. (1987). Biodegradation of DDT [1, 1, 1-trichloro-2,2-bis (4-chlorophenyl) ethane] by the white-rot fungus *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology*, 53, 2001–2003.

Burtseva, Y.V., Sova, V.V., Pivkin, M.V., Anastyuk, S.D., Gorbach, V.I. and Zvyagintseva, T.N. (2010). Distribution of *o*-glycosylhydrolases in marine fungi of the sea of Japan and the sea of Okhotsk: characterization of exo-cellular N-acetyl-beta-D-glucosaminidase of the marine fungus *Penicillium canescens*. *Applied Biochemistry and Biotechnology*, 46, 648–656.

Call, H. P. Mu¨cke, I. (1997). History, overview and applications of mediated lignolytic systems, specially laccase-mediator systems (Lignozym® -process), *Journal of Biotechnology*, 53, 163e202.).

Camassola, M. and Dillon, A. J. P. (2009). Biological treatment of sugarcane bagasse for the production of cellulase and xylanases by *Penicillium echinulatum*. *Industrial crops and products*, 29, 642-647.

Cañ as, A. I. and Camarero, S. (2010). Laccases and their natural mediators: biotechnological tools for sustainable eco-friendly processes. *Biotechnology Advances*, 28, 694−705.

Cara, C., Ruiz, E., Ballesteros, M., Manzanares, P., Negro, M. J. and Castro, E. (2008). Production of fuel ethanol from steam-explosion pretreated olive tree pruning. *Fuel*, 87, 692–700.

Carpita, N. C. and Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants, consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal*, 3, 1–30.

Carvalho, D. L. M. J., Castro, D. I. M. and Silva, D. C. A. B. (2008). A study of retention of sugars in the process of clarification of pineapple juice (*Ananas comosus*, L. Merril) by micro- and ultra-filtration. *Journal of Food Engineering*, 87, 447–454.

Chakraborty, T. K., Das, N., Sengupta, S. and Mukherjee, M. (2000). Accumulation of a natural substrate of laccase in gills of *Pleurotus florida* during sporulation. *Current Microbiology*, 41, 167-171.

Chuwech, M. and Nuansri, R. (2015). Potential of peanut hulls as substrates for fungal cellulase bioproduction through solid state fermentation. *KKU Research Journal*, 19, 235–243.

Clutterbuck, A. J. (1972). Absence of laccase from yellow- spored mutants *Aspergillus nidulans*. *Journal of General Microbiology*, 70, 423-425.

Couto, S. R. (2018). Solid state fermentation for laccases production and their applications. In *Current developments in Biotechnology and Bioengineering.* (pp 211-234). https://doi.org/10.1016/B978-0-444-63990-5.00011-6.

Couto, S. R., and Toca Herrera, J. L. (2006). Industrial and biotechnological applications of laccases: A review. *Biotechnology Advances*, *24*, 500–513.

Damasio, A.R., Maller, A., Marcio da Silva, T., Jorge, J.A., Terenzi, H.F. and Polizeli, M.T. (2011). Biotecnological potential of alternative carbon sources for production of pectinases by *Rhizopus microsporus* var. *rhizopodiformis*. *Archives of Biology and Technology*, 54, 141-148.

Daniel, J. W. (1963). Metabolism of the phenolic precursor of a Myxomycete spore pigment. *Journal of Cell Biology*, 19, 18-19.

Dar, B., Kyu, T. M., Thein, S. S., Nyunt, S. K. et al. (2016). Preparation of cellulase enzyme and bio-ethanol production from agricultural waste product. Universities' Research Centre Collection. Http://uyr.uy.edu.mm.

Das, N., Chakraborty, T.K. and Mukherjee, M. (2001). Purification and Characterization of a growth regulating laccase from *Pleurotus florida*. *Journal of Basic Microbiology***,** 41, 261-267.

Das, N., Naskar, S., Chowdhury, P., Pasman, B. and Adhikari, D. (2011). Experimental evidence for presence of a growth regulating extracellular laccase in some *Pleurotus* species. *Research Journal of Microbiology*, 6, 496-502.

Das, N., Sengupta, S. and Mukherjee, M. (1997). Importance of laccase in vegetative growth of *Pleurotus florida*. *Applied and Environmental Microbiology*, 63, 4120-4122.

Datta, R., Kelkar, A., Baraniya, D. Molaei, A., Moulick, A., Meena, R.S. and Formanek, P. (2017). Enzymatic degradation of lignin in soil: A Review. *Sustainability*, 9, 1163; doi:10.3390/su9071163.

Davis, S. and Burns, R.G. (1990). Decolourization of phenolic effluents by soluble and immobilized phenol oxidase. *Applied Microbialogy and Biotechnology*, 32, 721-726.

De Marco, A., Roubelakis-Angelakis, K.A., (1997). Laccase activity could contribute to cell-wall reconstitution of regenerating protoplasts. *Phytochemistry*, 46, 421-425.

de Vries, O.M.H., Kooistra, W.H.C.F. and Wessels, J.G.H. (1986). Formation of a extracellular laccase by a *Schizophyllum commune* dikaryon. *Journal of General Microbiology*, 132, 2817-2826.

de Vries, R. and Visser, J. (2001). *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. *Microbiology and Molecular Biology Reviews*, 65, 497-522.

Dec, J. and Bollag, J.M. (1990). Detoxification of substituted phenols by oxidoreductive enzymes through polymerization reactions. *Archives of Environmental Contamination and Toxicology*, 19, 543-550.

Dekker, R.F.H. and Richards, G.N. (1975). Purification, properties and mode of action of hemicellulase I produced by *Ceratocystis paradoxa*. *Carbohydrate Research*, 39, 97–114.

Del-Cid, A., Ubilla, P., Ravanal, M.C., Medina, E., Vaca, I., Levicán, G., Eyzaguirre, J. and Chavez, R. (2014). Cold-active xylanase produced by fungi associated with Antarctic marine sponges. *Applied Biochemistry and Biotechnology*, 172, 524–532.

Desmet, T., Cantaert, T., Gualfetti, P., Nerinckx, W., Gross, L., Mitchinson, C., et al. (2007). An investigation of the substrate specificity of the xyloglucanase Cel74A from *Hypocrea jecorina*. *Federation of European Biochemical Societies*, 274, 356-63.

Dhiman, T.R., Zaman, M.S., Gimenez, R.R., Walters, J.L. and Treacher, R. (2002). Performance of dairy cows fed forage treated with fibrolytic enzymes prior to feeding. *Animal Feed Science and Technology*, 101, 115–125.

Dobrev, G.T., Pishtiyski, I.G., Stanchev, V.S. and Mircheva, R. (2007). Optimization of nutrient medium containing agricultural wastes for xylanase production by *Aspergillus niger* B03 using optimal composite experimental design. *Bioresource Technology*, 98, 2671-2678.

Dong, S. J., Zhang, B.X., Wang, F.L., Xin, L., Gao, Y.F., Ding, W., He, X.M., Liu, D. and Hu, X.M. (2019). Efficient lignin degradation of corn stalk by *Trametes* with high laccase activity and enzymatic stability in salt and ionic liquid. *Bioresources*, 14, 5339-5354.

Du, B., Sharma, L. N., Becker, C., Chen, S. F., Mowery, R. A., van Walsum, G. P. and Chambliss, C. K. (2010). Effect of varying feedstock-pretreatment chemistry combinations on the formation and accumulation of potentially inhibitory degradation products in biomass hydrolysates. *Biotechnology and Bioengineering*, 107, 430–440.

Durán, N. and Esposito, E. (2000). Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: a review. *Applied Catalysis B: Environmental*, 28, 83–99.

El Hadrami, A., Lorne, R.A., El Hadrami, I. and Daayf, F. (2010). Chitosan in plant protection. *Marine Drugs*, 8, 968-987.

El-Fakharany, E.M., Haroun, B.M., Ng, T.B. and Redwan, E.R.M. (2010). Oyster mushroom laccase inhibits Hepatitis C Virus entry into peripheral blood cells and hepatoma cells. *Protein and Peptide Letters*, 17, 1031-1039.

Elisashvili, V., Kachlishvili, E. and Penninckx, M. (2008). Lignocellulolytic enzymes profile during growth and fruiting of *Pleurotus ostreatus* on wheat straw and tree leaves. *Acta Microbiologica et Immunologica Hungarica*, 55, 157–168.

Elisashvili, V., Kachlishvili, E., Asatiani, M.D., Darlington, R. and Kucharzyk, K.H. (2017). Physiological peculiarities of lignin-modifying enzyme production by the white-rot basidiomycete *Coriolopsis gallica* strain BCC 142. *Microorganisms*, 5, 1–73.

Elisashvili, V., Penninckx, M., Kachlishvili, E., Asatiani, M. and Kvesitadze, G. (2006). Use of *Pleurotus dryinus* for lignocellulolytic enzymes production in submerged fermentation of mandarin peels and tree leaves. *Enzyme and Microbial Technology*, 38, 998-1004.

Erden, E., Ucar, M.C., Gezer, T. and Pazarlioglu, N.K. (2009). Screening for ligninolytic enzymes from autochthonous fungi and applications for decolorization of Remazole Marine Blue. *Brazilian Journal of Microbiology*, 40, 346–353.

Eriksson, K.E.L and Krik, T.K. (1985). Biopulping, biobleaching and treatment of kraft bleaching effluents with white rot fungi. In C.L. Cooney and A.E Humphrey (Eds.) *Comprehensive Biotechnology* (pp. 271-294). Oxford: Pergamon Press.

Eriksson, K.E.L., Pettersson, B., Vole, J., and Musilek, V. (1986). Formation and partial characterization of glucose-2-oxidase, a H_2O_2 producing enzyme in *Phanerochaete chrysosporium. Appied Microbiology and Biotechnology,* **23**, 257–262.

Eriksson, K.E.L. (1981). Fungal degradation of wood components. *Pure and Applied Chemistry*, 53, 33.

Eriksson, K. E.L., Blanchette, R.A. and Ander, P. (1990). *Microbial and enzymatic degradation of wood and wood components*. Berlin: Springer.

Eriksson, K. E. L., Blanchette, Ander, P. (2012). Microbial and enzymatic degradation of wood and wood components. Springer-Verlag Berlin Heidelberg. Doi. 10.1007/978-3-642-46687-8.

Eshel, D., Miyara, I., Ailing, T., Dinoor, A. and Prusky, D. (2002). pH regulates endoglucanase expression and virulence of *Alternaria alternata* in persimmon fruit. *Molecular Plant–Microbe Interactions*,15, 774–779.

Faure, D., Bouillant, M.L. and Bally R. (1994). Isolation of *Azospirillum lipoferum* 4T Tn5 mutant affected in melanization and laccase activity. *Applied and Environmental Microbiology*, 60, 3413-3415.

Ferrey, M.L., Koskinen, W. C., Blanchette, R. A. and Burnes, T. A. (1994). Mineralization of alachlor by lignin-degrading fungi. *Canadian Journal of Microbiology*, 40, 795–798.

Ferry, Y. and Leech, D. (2005). Amperometric detection of catecholamine neurotransmitters using electrocatalytic substrate recycling at a laccase electrode. *Electroanalysis*, 17, 2113–2119.

Florez-Sampedro, L., Zapata, W., Orozco, L.P., Mejia, A., Arboleda, C. and Rugeles, M.T. (2016). In Vitro anti HIV-1 activity of the enzymatic extract enriched with laccase produced by the fungi *Ganoderma* sp. and *Lentinus* sp. *Vitae, Revista De La Facultad De Ciencias Farmaceuticas y Alimentarias*, 23, 109-118.

Frese, D. and Stahl, U. (1992). Oxidative stress and aging in the fungus *Podospora anserina*. *Mechanism of Ageing and Development*, 65, 277-288.

Fukuda, T., Uchida, H., Takashima, Y., Uwajima, T., Kawabata, T.and Suzuki, M. (2001). Degradation of bisphenol A by purified laccase from *Trametes villosa. Biochemical and Biophysical Research Communities*, 284, 704– 706.

Gaitan, G.I., Medina, S. C., Rodríguez, A. et al. (2011) Evaluation of toxicity and degradation of a chlorophenol mixture by the laccase produced by *Trametes pubescens. Bioresource Technology, 102, 3632-3635.*

Galante, Y.M., Conti, D.A. andMonteverdi, R. (1998). Application of *Trichoderma* enzymes in food and feed industries. In Harman, G.F. and Kubicek, C.P. (Eds.) *Trichoderma and Gliocladium. Enzymes, Biological Control and Commercial Applications* (pp. 311-326). London: CRC Press.

García-Cubero, M.A., González-Benito, G., Indacoechea, I., Coca, M. and Bolado, S. (2009). Effect of ozonolysis pretreatment on enzymatic digestibility of sugarcane bagasse. *Agricultural Engineering International: CIGR Journal*, 100, 1608–1613.

Gardner, K.H. and Blackwell, J. (1974). The hydrogen bonding in native cellulose. *Biochimica and Biophysica Acta*, 343, 232-237.

Gautam, S.P., Bundela, P.S., Pandey, A. K., Khan, J., Awasthi, M.K. and Sarsaiya, S. (2011). Optimization for the production of cellulase enzyme from municipal solid

waste residue by two novel cellulolytic fungi. *Biotechnology Research International*, p. 8. doi:10.4061/2011/810425.

Ghindilis, A.L., Skorobogat'ko, O.V., Gavrilova, V.P. and Yaropolov, A.I. (1992). A new approach to the construction of potentiometric immunosensors. *Biosensors and Bioelectronics*, 7, 301-304.

Ghorai, S., Banik, S.P., Verma, D., Chowdhury, S. et al. (2009). Review fungal biotechnology in food and feed processing. *Food Research International*, 42, 577–587.

Giardina, P., Faraco, V., Pezzella, C., Piscitelli, A., Vanhulle, S. and Sannia, G. (2010). Laccases: a never-ending story, *Cellular and Molecular Life Sciences*, 67, 369–385.

Glenn, J. K. and. Gold, M. H. (1985). Purification and characterization of an extracellular Mn(II)-dependent peroxidase from the lignin-degrading basidiomycete, *Phanerochaete chrysosporium*. *Archives of Biochemistry and Biophysics*, 242, 329–341.

Gochev, V.K. and Krastanov, A.I. (2007). Fungal laccases. *Bulgarian Journal of Agricultural Science*, 13, 75-83.

Gold M. H. andAlic M. (1993). Molecular Biology of the Lignin-Degrading *Phanerochaete chrysosporium Microbiological Reviews*, 57: 605-622.

Gold, M. H., Wariishi, H. and Valli, K. (1989). Extracellular peroxidases involved in lignin degradation by the white rot basidiomycete *Phanerochaete chrysosporium*. *ACS Symposium Series*, 389, 127–140.

Gonçalves, I., Martins, M., Loureiro, A., Gomes, A. et al. (2014). Sonochemical and hydrodynamic cavitation reactors for laccase/hydrogen peroxide cotton bleaching. *Ultrasonics Sonochemistry*, 21, 774–781.

Grassin, C. and Fauquembergue, P. (1996). Fruit juices. *Industrial Enzymology*, 2, 225–264.

Guimarães, J.L., Frollina, E., Da Silva, C.G., Wypych, F. and Satyanarayana, K.G. (2009). Characterization of banana, sugarcane bagasse and sponge gourd fibers of Brazil. *Industrial Crops and Products*, 30, 407-415.

Hadibarata, T., Kristanti, R.A. (2012) Fate and cometabolic degradation of benzo[a]pyrene by white rot fungus *Armillaria* sp. F022. *Bioresource Technology*, 107, 314–318.

Hakala, T.K., Maijala, P., Konn, J. and Hatakka, A. (2004). Evaluation of novel wood rotting polypores and corticioid fungi for the decay and bio-pulping of Norway spruce (*Picea abies*) wood source. *Enzyme Microbial Technology*, 34, 255–263.

Haq, I.U., Javed, M. and Khan, T.S. (2006). An innovative approach for hyperproduction of cellulolytic and hemicellulolytic enzymes by consortium of *Aspergillus niger* MSK-7 and *Trichoderma viride* MSK-10. *African Journal of Biotechnology*, 5, 609-614.

Harkin, J.M., Larsen, M.J. and Obst, J.R. (1974). Use of syringaldazine for detection of laccase in sporophores of wood rotting fungi. *Mycologia*, 66, 469-476.

Harvey, P.J., Floris, R., Lundell, T., et al. (1992). Catalytic mechanisms and regulation of lignin peroxidase. *Biochemical Society Transactions*, 20, 345–349.

Hatakka, A. (1994). Lignin modification enzymes from selected white-rot fungi: Production and role in lignin degradation. *FEMS Microbiology Reviews*, 13, 125-135.

Hatti-Kaul, R., Ibrahim, V. (2013) Lignin-Degrading Enzymes: An Overview. Eds Yang, S. T., El-Enshasy, H.A., Thongchul, N. *Bioprocessing Technologies in Biorefinery for Sustainable Production of Fuels, Chemicals, and Polymers.* https://doi.org/10.1002/9781118642047.ch10.

Herkulano, P.N., Lima, D.M. M., Fernandes, M.J.S., Neves, R.P., Souza-Motta, C.M. and Porto, A.L.F. (2011). Isolation of cellulolytic fungi from waste of castor (*Ricinus communis* L.). *Current Microbiology*, 62, 1416-1422.

Hickman, G.W. and Perry, E.J. (2010). Wood decay fungi in landscape trees. University of California. UC ANR Publication 74109.

Hilgers, R., Vincken, J. P., Gruppen, H. and Kabel, M.A. (2018). Laccase/Mediator Systems: Their Reactivity toward Phenolic Lignin Structures. *ACS Sustainable Chemical Eng*ineering, 6, 2037−2046.

Hoff, T., Liu, S.Y. and Bollag, J.M. (1985). Transformation of halogen, alkyl and alkoxy-substituted anilines by a laccase of *Trametes versicolor*. *Applied and Environmental Microbiology*, 49,1040-1045.

Hofrichter, M., Ullrich, R., Pecyna, M.J., et al. (2010). New and classic families of secreted fungal heme peroxidases. *Applied Microbiology and Biotechnology*, 87, 87 1–897.

Hofrichter, M. (2002). Review: lignin conversion by manganese peroxidase (MnP). *Enzyme Microbiology and Technology*, 30,13.

Howard, R.L., Abosti, E., Jansen van Rensburg, E.L. and Howard, S. (2003). Lignocellulose biotechnology: Issue of bioconversion and enzyme production. *African Journal of Biotechnology*, 2, 602-619.

Hu, D., Zhang, R., Zhang, G., Wang, H., Ng, T.B. (2011). A laccase with antiproliferative activity against tumor cells from an edible mushroom, white common *Agrocybe cylindracea*. *Phytomedicine*, 18, 374–379.

Hynes, M.F., Brucksch, K. and Priefer, U. (1988). Melanin production encoded by a cryptic plasmid in a *Rhizobium leguminosarum* strain. *Archives of Microbiology*, 150, 326-332.

Imran, M., Crowley, D.E., Khalid, A., Hussain, S. et al. (2015). Microbial technology for decolorization of textile waste waters. *Reviews in Environmental Science and Biotechnology*, 14, 73–92.

Imura, Y., Hartikainen, P. and Tatsumi, K. (1996). Dechlorination of tetrachloroguaiacol by laccase of white-rot basidiomycete *Coriolus versicolor*. *Applied Microbiology and Biotechnology*, 45, 434-439.

Iracheta, C.M.M., Rocha, P.M.A., Galán, W.L.J., Arévalo, N.K. and Tovar, H.O.E. (2016). A *Pycnoporus sanguineus* laccase for denim bleaching and its comparison with an enzymatic commercial formulation. *Journal of Environmental Management*, 177, 93–100.

Isikgora, F.H. and Becer, C.R. (2015). Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. *Polymer Chemistry*, 6, 4497–4559.

Isroi., Millati, R., Syamsiah, S., Niklasson, C., Cahyanto, M.N., Lundquist, K. and Taherzadeh, M. J. (2011). Biological pretreatment of lignocelluloses with white rot fungi and its implications: A review. *BioResources*, 6(4), 5224-5259.

Izmirlioglu, G. and Demirci, A. (2016). Strain selection and medium optimization for glucoamylase production from industrial potato waste by *Aspergillus niger*. *Journal of the Science of Food and Agriculture*, 96, 2788–2795.

Jahromi, M.F., Liang, J.B., Rosfarizan, M., Goh, Y.M., Shokryazdan, P. and Ho, Y.W. (2011). Efficiency of rice straw lignocelluloses degradability by *Aspergillus terreus* ATCC 74135 in solid-state fermentation. *African Journal of Biotechnology*, 10, 4428-4435.

Janshekar, H. and Fiechter, A. (1983). Lignin: biosynthesis, application, and biodegradation. *Advances in Biochemical Engineering/Biotechnology*, 27, 119-178.

Janshekar, H., Haltmeier, T. and Brown, C. (1982). Fungal degradation of pine and straw alkali lignins. *European Journalof Applied Microbiology and Biotechnology*,14, 174–181.

Janusz, G., Pawlik, P., Sulej, J., Świderska-Burek, U., Jarosz-Wilkołazka, A. and Paszczyński, A. (2017). Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution**.** *FEMS Microbiology Reviews*, 41: 941–962,

Jeffries, T.W. (1994). Biodegradation of lignin and hemicelluloses. In C. Ratledge (Ed.) *Biochemistry of Microbial Degradation* (pp. 233–277). Dordrecht: Springer.

Jeon, J. R., Baldrian, P., Murugesan, K. and Chang, Y. S. (2012). Laccase catalysed oxidations of naturally occurring phenols: from in vivo biosynthetic pathways to green synthetic applications, *Microbial Biotechnology*, 5, 318–332.

Jonas, U., Hammer, E., Schauer, F. and Bollag, J.M. (1998). Transformation of 2-hydroxydibenzofuran by laccases of the white rot fungi *Trametes versicolor* and *Pycnoporus cinnabarinus* and characterization of oligomerization products. *Biodegradation*, 9, 60-65.

Jonathan, S.G., Akinfemi, A. and Adenipekun, C.O. (2010). Biodegradation and *in vitro* digestibility of maize husks treated with edible fungi *Pleurotus tuber-regium* and *Lentinus subnudus* from Nigeria. *Electronic Journal of Environmental Agriculture and Food Chemistry*, 9, 742-750.

Kabenge, I., Omulo, G., Banadda, N., Seay, J. et al. (2018). Characterization of banana peels wastes as potential slow pyrolysis feedstock. *Journal of Sustainable Development*, 11, 14–24.

Kadri, T., Rouissi, T., Brar, S.K., Cledon, M. et al. (2017). Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: a review. *Journal of Environmental Sciences*, 51, 52–74.

Kalia, V.C., Rashmi, Sadhana, L. and Gupta M. N. (2001). Using enzymes for oil recovery for edible seeds. *Journal of Scientific and Industrial Research*, 60, 298–310.

Kandasamy, S., Muniraj, I. K., Purushothaman, N., Sekar, A. et al. (2016). High level secretion of laccase (LccH) from a newly isolated white-rot Basidiomycete, *Hexagonia hirta* MSF2. *Frontiers in Microbiology*, 7, 1–12.

Kang S. O., Shin K. S., Han Y. H., youn H. D., Han Y. C. (1993). Purification and characterization of an extracellular peroxidase fromwhite rot fungi *Pleurotus ostreatus*. Biochimica et Biophysica Acta, 1163:158-164.

Kantharaj, P., Boobalan, B., Sooriamuthu, S., Mani, R. (2017). Lignocellulose degrading enzymes from fungi and their industrial applications. *International Journal of current Research and Review*, 9, DOI: 10.7324/IJCRR.2017.9211.

Karaki, N., Aljawish, A., Humeau, C., Muniglia, L. and Jasniewski, J. (2016). Enzymatic modification of polysaccharides: mechanisms, properties, and potential applications: a review, *Enzyme Microbial Technology*, 90, 1–18.

Khudhair, A.B., Hadibarata, T., Yusof, A. R. M., The, Z.C., Adnan, L.A., Kamya, H. (2015) Pyrene metabolism by new species isolated from soil *Rhizoctonia zeae* SOL3. *Water Air and Soil Pollution*, 226,186.

Khushal, B., Anne, R. and Praveen, V.V. (2010). Fungal laccases: production, function, and applications in food processing. *Enzyme Research*, 10, 10.

Kim, D., Orrego, D., Ximenes, E.A. and Ladisch, M. R. (2017). Cellulose conversion of corn pericarp without pretreatment. *Bioresource Technology*, 245, 511–517.

Kim, D., Ximenes, E.A., Nichols, N.N., Cao, G. et al. (2016). Maleic acid treatment of biologically detoxified corn stover liquor. *Bioresource Technology*, 216, 437–445.

Kim, S. (2018). Evaluation of alkali-pretreated soybean straw for lignocellulosic bioethanol production. *International Journal of Polymer Science*, doi: 10.1155/2018/ 52417488.

Kim, T.H., Taylor, F. and Hicks, K.B.(2008). Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment. *Bioresource Technology*, 99, 5694–5702.

Kim, Y.S. (2007). Polyoxometalate (POM) Oxidation of Milled Wood Lignin (MWL).*Journal of Wood Chemistry and Technology*, 27, (3-4): 225-241.

Kirk, T.K. and Tien, M. (1983) Biochemistry of lignin degradation by *Phanerochaete crysosporium*: investigations with nonphenolic model compounds. In T. Higuchi,

H.M. Chang. And T.K. Kirk (Eds) *Recent advances in lignin biodegradation research* (pp 233–245). Tokyo: UNI Publishers.

Kristensen, J.B., Thygesen, L.G., Felby, C., Jorgensen, H. and Elder, T. (2008). Cellwall structural changes in wheat straw pretreated for bioethanol production. *Biotechnology for Biofuels*, 1, 1–9.

Kuhad, R.C. and Singh, A. (2007). *Lignocellulose biotechnology future prospects*, I.K: International Publishing House Pvt. Ltd.

Kuhad, R.C., Gupta, R., and Singh, A. (2011). Microbial Cellulases and Their Industrial Applications. *Enzyme Research*, 1–10. DOI: 10.4061/2011/280696.

Kuhad, R.C., Kuhar, S., Kapoor, M., Sharma, K.K. and Singh, A. (2007). Lignocellulolytic microorganisms, their enzymes and possible biotechnologies based on lignocellulolytic microorganisms and their enzymes. In R.C. Kuhad and A. Singh (Eds.) *Lignocellulose biotechnology: future prospects* (pp. 345-358). New Delhi: I.K. International Publishing House.

Kuhad, R. C., Singh, A. and Eriksson, K. E.L. (1997). Microorganisms and enzymes involved in the degradation of plant fibre cell walls. *Advances in Biochemical Engineering/Biotechnology*, 57, 45-125.

Kuhara, F. and Papinutti, L. (2014). Optimization of laccase production by two strains of *Ganoderma lucidum* using phenolic and metallic inducers. *Revista Argentina de Microbiologia*, 46, 144–149.

Kumar, A.K. and Sharma, S. (2017). Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. *Bioresources and Bioprocessing*, 4, 1–19.

Kumar, R., Mago, G., Balan, V. and Wyman, C.E. (2009). Physical and chemical characterizations of corn stover and poplar solids resulting from leading pretreatment technologies. *BioresourceTechnology*, 100, 3948–3962.

Kuuskeri, J., Mäkelä, M.R., Isotalo, J., Oksanen, I. and Lundell, T. (2015). Lignocellulose-converting enzyme activity profiles correlate with molecular systematics and phylogeny grouping in the incoherent genus *Phlebia* (Polyporales, Basidiomycota). *BMCMicrobiology*, 15, 1–18.

Kuwahara, M., J. K. Glenn, M. A. Morgan, and M. H. Gold. 1984. Separation and characterization of two extracellular H_2O_2-dependent oxidases from lignolytic cultures of *Phanerochaete chrysosporium*. *FEBS Letters*, 169, 247–250.

Lawal, T.E., Iyayi, E.A., Adeniyi, B.A. and Adaramoye, O.A. (2010). Biodegradation of palm kernel oil cake by multienzyme complexes from fungi and its feeding value for broilers. *International Journal of Poultry Science*, 9, 695-701.

Leatham, G.F. and Stahmann, M.A. (1981). Studies on the laccase EC-1.10.3.2 of *Lentinus edodes:* Specificity, localization, and association with the developments of fruiting-bodies. *Journal of General microbiology*,125, 147-158.

Lee, J., Jang, Y., Lee, H., Lee, S., Kim, G.H. and Kim, J.J. (2011). Screening for xylanase and β-xylosidase production from wood-inhabiting *Penicillium* strains for potential use in biotechnological applications. *Holzforschung*, 66, 267–271.

Leite, O.D., Lupetti, K.O., Fatibello-Filho, O., Vieira, I.C. and de Barbosa, A.M. (2003). Synergic effect studies of the bi-enzymatic system laccase peroxidase in a voltammetric biosensor for catecholamines. *Talanta*. 59, 889–896.

Leonard, T.J. and Phillips, L.E.(1973). Studies of phenoloxidase activity during the reproductive cycle in *Schizophyllum commune*. *Journal of Bacteriology*, 114, 7-10.

Leonowicz, A., Cho, N.S., Luterek, J., Wilkolazka, et al. (2001). Fungal laccase: properties and activity on lignin. *Journal of Basic Microbiology*, 41,185-227.

Leontievsky, A.A., Myasoedova, N.M., Baskunov, B.P., Evans, C.S. and Golovleva, L.A. (2000). Transformation of 2,4,6-trichlorophenol by the white rot fungi *Panus tigrinus* and *Coriolus versicolor*. *Biodegradation*, 11, 331–340.

Lequart, C., Kurek, B., Debeire, P. and, Monties, B. (1998). MnO_2 and Oxalate: An abiotic route for the oxidation of aromatic components in wheat Straw. *Journal of Agricultural and Food Chemistry*,*46*, 3868-3874.

Li, B.Z., Balan, V., Yuan, Y.J. and Dale, B.E. (2010). Process optimization to convert forage and sweet sorghum bagasse to ethanol based on ammonia fiber expansion (AFEX) pretreatment. *Bioresource Technology*, 101, 1285–1292.

Li, C., Knierim, B., Manisseri, C., Arora, R., Scheller, H.V., Auer, M., Vogel, K.P., Simmons, B.A. and Singh, S. (2010). Comparison of dilute acid and ionic liquid pretreatment of switchgrass: Biomass recalcitrance, delignification and enzymatic saccharification. *BioresourceTechnology*, 101, 4900–4906.

Li, D.C., Li, A.N. and Papageorgiou, A.C. (2011). Cellulases from thermophilic fungi: recent insights and biotechnological potential. *Enzyme Research*, doi: 10.406/ 2011/ 308730.

Li, Y., Liu, Z., Cui, F., Xu, Y. and Zhao, H. (2006). Production of xylanase from a newly isolated *Penicillium* sp ZH-30. *World Journal of Microbiology and Biotechnology*, 23, 837–843.

Liew, C.Y., Husaini, A., Hussain, H., Muid, S., Liew, K.C. and Roslan, H.A. (2011). Lignin biodegradation and lignin enzyme studies during biopulping of *Acacia mangium* wood chips by tropical white rot fungi. *World Journal of Microbiology and Biotechnology*, 27, 1457-1458.

Lipin, D., Mundur, S., Chandra, S.M. and Thankamani, V. (2013). Ligninolytic enzymes for application in treatment of effluent from pulp and paper industries. *Journal of Environmental Research and Technology*, 1, 14–26.

Lisdat, F., Wollenberger, U., Makower, A., Hortnagl, H., Pfeiffer, D. And Scheller, F.W. (1997). Catecholamine detection using enzymatic amplification. *Biosensors and Bioelectronics*, 12, 1199–1211.

Lobos, S., Larram, J., Salas, L., Cullen, D. and Vicuna, R. (1994). Isoenzymes of manganese dependent peroxidase and laccase produced by the lignin degrading basidiomycete, *Ceriporiopsis subvermispora*. *Microbiology*, 14, 2691–2698.

Longe. L.F., Couvreur, J., Grandchamp, M.L., Garnier, G., Allais, F., Saito, K. (2018). Importance of mediators for lignin degradation by fungal laccase. *ACS Sustainable Chemical Engineering.* 68, 10097-10107.

Lu, W., Yan, L., Wang, Y., Zhou, S., Fu, J. and Zhang, J. (2009). Biodegradation of phenolic compounds from coking wastewater by immobilized white-rot fungus *Phanerochaete chrysosporium*. *Journal of Hazardous Materials*, 165, 1091-1097.

Lyr, H. (1963). Enzymatische detoxification chlorierter phenole. *Phytopathologische Zeitschrift*, 73-83.

Mabrouk, A.M., Kheiralla, Z. H., Hamed, E.R., Youssry, A.A. and Abd El Aty, A.A. (2010). Screening of some marine derived fungal isolates for lignin degrading enzymes production. *Agriculture and Biology Journal of North America*, pp. 2151-7525. (http://www.scihub.org/ABJA).

Maciel, M.J., Silva, A.C. and Ribeiro, H.C.T. (2010). Industrial and biotechnological applications of ligninolytic enzymes of the basidiomycota: A review. *Electronic Journal of Biotechnology*, 13, 6.

Magan, N., Fragoeiro, S. and Bastos, C. (2010). Environmental factors and bioremediation of xenobiotics using white-rot fungi. *Microbiology*, 38, 238-248.

Majid, D., Gholamreza B. K., Amir A. M. and Seyed J.D. (2017) Biotechnological and Industrial Applications of Laccase: A Review, *Journal of Applied Biotechnology Reports*, 4(4), 675-679.

Mali, T., Kuuskeri, J., Shah, F. and Lundell, T.K. (2017). Interactions affect hyphal growth and enzyme profiles in combinations of coniferous wood-decaying fungi of Agaricomycetes. *PLoS ONE*, 12, 1–21.

Mandels, M. (1975). Microbial sources of cellulase. *Biotechnology and Bioengineering Symposium*, 5, 81-105.

Martani, F., Beltrametti, F., Porro, D., Branduardi, P. and Lotti, M. (2017). The importance of fermentative conditions for the biotechnological production of lignin modifying enzymes from white-rot fungi. *FEMS Microbiology*, 364 doi:10.10 93/femsle/fnx134.

Martín, C., Klinke, H.B. and Thomsen, A.B. (2007). Wet oxidation as a pretreatment method for enhancing the enzymatic convertibility of sugarcane bagasse. *Enzyme and Microbial Technology*, 40, 426–432.

Master, E.R., Zheng, Y., Storms, R., Tsang, A. and Powlowski, J. (2008) A xyloglucan-specific family 12 glycosyl hydrolase from *Aspergillus niger*: recombinant expression, purification and characterization. *Biochemical Journal*, 411, 161-70.

Mayer, A.M. and Staples, R.C. (2002). Laccase: new functions of an old enzyme. *Phytochemistry*, 60, 551-555.

Mccleary, B.V. (1991). Comparison of endolytic hydrolases that depolymerize 1,4-Beta-D Mannan, 1,5-Alpha-L-Arabinan, and 1,4-Beta-D-Galactan. *Acs Symosium Series*, 460, 437-49.

Metrevelia, E., Kachlishvilia, E., Singer, S.W. and Elisashvili, V. (2017). Alteration of white-rot basidiomycetes cellulase and xylanase activities in the submerged co-cultivation and optimization of enzyme production by *Irpex lacteus* and *Schizo-phyllum commune*. *BioresourceTechnology*, 241, 652–660.

Michael, R.B. and Gary, G.P. (2010). Enzyme in farm animal nutrition 2nd ed. Https://www.pdfdrive.com.

Minussi, R.C., Pastore, G.M. and Duran, N. (2002). Potential applications of laccase in the food industry. *Trends in Food Science and Technology*, 13, 205–216.

Mohana, S., Shah, A., Divecha, J. and Madamwar, D. (2008). Xylanase production by *Burkholderia* sp. DMAX strain under solid state fermentation using distillery spent wash. *Bioresource Technology*, 99, 7553-7564.

Morozova, O.V., Shumakovich, G.P., Gorbacheva, M.A., Shleev, S.V. and Yaropolov, A.I. (2007). "Blue" laccases. *Biochemistry (Moscow)*, 72, 1136–1150.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y.Y., Holtzapple, M. and Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology*, 96, 673–686.

Mougin, C., Boyer, F. D., Caminade, E. and Rama, R. (2002). Cleavage of the diketonitrite, derivative of the herbicide isoxaflutole by extracellular fungal oxidase. *Journal of Agricultural and Food Chemistry*, 48, 4529-4534.

Mtui, G. and Masalu, R. (2008). Extracellular enzymes from Brown-rot fungus *Laetioporus sulphureus* isolated from mangrove forests of coastal Tanzania. *Scientific Research and Essays*, 3, 154-161.

Mtui, G. and Nakamura, Y. (2008). Lignocellulosic enzymes from *Flavodon flavus*, a fungus isolated from Western Indian Ocean off the Coast of Dar es Salaam, Tanzania. *African Journal of Biotechnology*, 7, 3066-3072.

Mtui, G.Y.S. (2012). Lignocellulolytic enzymes from tropical fungi: Types, substrates and applications. *Scientific Research and Essays*, 7, 1544–1555.

Mukherjee, M. and Das N. (2009). Fungal laccase: a biotechnologically potential Enzyme. In *Biotechnology Applications* ed.: C.S.K. Mishra I.K.International**.** (pp.70-108) New Delhi.

Muller, U., Tenberge, K.B., Oeser, B. and Tudzynski, P. (1997). Cel1, probably encoding a cellobiohydrolase lacking the substrate binding domain, is expressed in the initial infection phase of *Claviceps purpurea* on *Secale cereale*. *Molecular Plant–MicrobeInteractions*,10, 268–279.

Murray, P., Aro, N., Collins, C., Grassick, A., Penttilä, M., Saloheimo, M. and Tuohy, M. (2004). Expression in *Trichoderma reesei* and characterisation of a thermostable

family 3 β-glucosidase from the moderately thermophilic fungus *Talaromyces emersonii*. *Protein Expression and Purification*, 38, 248-257.

Muthuvelayudham, R. and Viruthagiri, T. (2006). Fermentative production and kinetics of cellulase protein on *Trichoderma reesei* using sugarcane bagasse and rice straw. *African Journal of Biotechnology*, 5, 1873-1881.

Nagaraj, C.M. and Kumar, A. (2006). Distillery waste water treatment and disposal. https://www.environmental-expert.com/resulteacharticle4.asp (accessed 14 April 2018).

Ng, I.S., Chen, P.T., Ju, Y.M. and Tsai, S.W. (2010). Novel cellulase screening and optimal production from wood decaying Xylariaceae: *Daldinia* Species. *Applied Biochemistry and Biotechnology*, 1-11, Doi: 10.1007/s12010-010-9102-1.

Nicotra, S., Cramarosa, M.R., Mucci, A. et al. (2004). Biotransformation of resveratrol: synthesis of trans-dehydrodimers catalyzed by laccases from *Myceliophtora thermophyla* and from *Trametespubescens*. *Tetrahedron*, 60, 595–600.

Niebisch, C.H., Malinowski, A.K., Schadeck, R., Mitchel, D.A., Kava-Kordeiro, V. and Paba, J. (2010). Decolorization and biodegradation of reactive blue 220 textile dye by *Lentinus crinitus* extracellular extract. *Journal of Hazardous Materials*, 180, 316-320.

Niku, P.M.L. and Viikari, L. (2000). Enzymatic oxidation of alkenes. *Journal of Molecular Catalysis B Enzymatic*, 10, 435–444.

Nishida, A. and Eriksson, K.E. (1987). Formation, purification and partial characterization of methanol oxidase, a H_2O_2-producing enzyme in *Phanerochaete chrysosporium*. *Biotechnology and Applied Biochemistry*, 9, 325–338.

O'sullivan, A. C. (1997). Cellulose: The structure slowly unravels. *Cellulose*, 4, 173–207.

Obeng, E.M., Adam, S.N.N., Budiman, C., Ongkudon, C.M., Maas, R. and Jose, J. (2017) Lignocellulases: a review of emerging and developing enzymes, systems, and practices. *Bioresources and Bioprocessing*, 4, DOI 10.1186/s40643-017-0146-8.

Oellerich, M. (1984). Enzyme immunoassay: A review. *Journal of Clinical Chemistry and Clinical Biochemistry*, 22, 895-904.

Ohm, R.A., Riley, R., Salamov, A., et al. (2014). Genomics of wood-degrading fungi. *Fungal Genetics and Biology*, 72, 82–90.

Osiadacz, J., Al-Adhami, A.J.H., Bajraszewska, D. et al. (1999). On the use of *Trametes versicolor* laccase for the conversion of 4-methyl-3-hydroxyanthranilic acid to actinocin chromophore. *Journal of Biotechnology*, 72, 141–149.

Osma, J.F., Moilanen, U., Toca-Herrera, J.L. and Couto, S.R. (2011). Morphology and laccase production of white-rot fungi grown on wheat bran flakes under semi-solid-state fermentation conditions. *FEMS Microbiology Letters*, 318, 27–34.

Palaniswany, M., Pradeep, B.V., Sathya, R. and Angayarkanni, J. (2008). Isolation, identification and screening of potential xylanolytic enzyme from little degrading fungi. *African Journal of Biotechnology*, 7, 1978–1982.

Palmieri, G., Giardina, P., Bianco, C., Fontallella, B. and Sannina, G. (2000). Copper induction of laccase isoenzymes in the lignolytic fungus *Pleurotus ostreatus. Applied Microbiology and Biotechnology*, 66, 920-924.

Paszczynski, A., V. B. Huynh, and R. L. Crawford. 1986. Comparison of ligninase-I and peroxidase-M2 from the white rot-fungus *Phanerochaete chrysosporium*. *Archives of Biochemistry and Biophysics*, 244, 750–765.

Patel, H., Divecha, J. and Shah, A. (2016). Optimization of ethanol production from enzymatic hydrolysate of maize stover. *Advances in Recycling and Waste Management*, 1, 2475–7675.

Patel, N., Shahane, S., Shivam, Majumdar, R., Mishra, U. (2019). Mode of Action, Properties, Production, and Application of Laccase: A Review. *Recent Patents on Biotechnology*, 13(1), 19-32.

Patrick, F., Mtui, G., Mshandete, A., Johansson, G. and Kivaisi, A. (2009). Purification and characterization of a laccase from the basidiomycete *Funalia trogii* (Berk) isolated in Tanzania. *African Journal of Biochemistry Research*, 3, 250-258.

Pei, Y., Li, Y., Zhang, Y., Yu, C. et al. (2016). G-lignin and hemicellulosic monosaccharides distinctively affect biomass digestibility in rapeseed. *Bioresource Technology*, 203, 325–333.

Pickard, M. A., Roman, R., Tinoco, R. and V`azuez-Duhalt, R. (1999). Polycyclic aromatic hydrocarbon metabolism by white rot fungi and oxidation by *Coriolopsis gallica* UAMH 8260 laccase. *Applied and Environmental Microbiology*, 65, 3805-3809.

Piscitelli, A., Giardina, P., Lettera., V et al.(2011).Induction and transcriptional regulation of laccases in fungi. *Current Genomics*, 12, 104–12.

Powell,W. A., and Van Alfen, N. K. (1987). Differential accumulation of poly(A)+ RNA between virulent and double stranded RNA-induced hypovirulent strains of *Cryphonectria* (*Endothia*) *parasitica*. *Molecular and Cellular Biology*, 7, 3688-3693.

Prajapati, H.V., and Minocheherhomji, F. P. (2018). Laccase - a Wonder Molecule: A Review of its Properties and Applications, *International journal of Pure Application Bioscience*, 6(1),766-773.

Prassad, S., Singh, A. and Joshi, H. C. (2007). Ethanol as an alternative fuel from agricultural, industrial and urban residues. *Resources, Conservation and Recycling*, 50, 1–39.

Punelli, F., Reverberia, M., Porrettab, D., Nogarottoa, S., Fabbria, A. A., Fanellia, C. and Urbanelli, S. (2009).Molecular characterization and enzymatic activity of laccases in two *Pleurotus* spp. with different pathogenic behavior. *Mycological Research*, 113:381-387.

Punnapayak, H., Prasongsuk, S., Messner, K., Danmek, K. and Lotrakul, A. (2009). Polycyclic aromatic hydrocarbons (PAHs) degradation by laccase from a tropical

white-rot frungus *Ganoderma lucidum*. *African Journal ofBiotechnology*, 8, 5897-59000.

Purnomo, A. S., Mori, T., Kamei, I., Nishii, T. and Kondo, R. (2010). Application of mushroom waste medium from *Pleurotus ostreatus* for bioremediation of DDT-containing contaminated soil. *International Biodeterioration and Biodegradation*, 64, 397-402.

Putro, J.N., Soetaredjo, .E., Lin, S.Y., Ju, Y. H. and Ismadji, S. (2016). Pretreatment and conversion of lignocellulose biomass into valuable chemicals. *RSC Advances*, 6, 46834–46852.

Raghukumar, C. (2000). Fungi from marine habitats: an application in bioremediation. *Mycological Research*, 104, 1222–1226.

Raghukumar, C. and D'Souza-Ticlo Verma, A.K. (2006). Treatment of colored effluents with lignin-degrading enzymes: An emerging role of marine derived fungi. *Critical Reviews in Microbiology*, 32, 189-206.

Raghukumar, C., Muraleedharan, U., Gaud, V. R. and Mishra, R. (2004). Xylanases of marine fungi of potential use for biobleaching of paper pulp. *Journalof Industrial MicrobiologyandBiotechnology*, 31, 433–441.

Ralph, J. (2006). What makes a good monolignol substitute? In T. Hayashi (Ed.) *The Science and Lore of the Plant Cell Wall Biosynthesis, Structure and Function* (pp.285–293). USA: Brown Walker Press.

Rama, R., Mougin, C., Boyer, F. D., Kollmann, A., Malosse, C. and Sigoillot, J. C. (1998). Biotransformation of benzo[a]pyrene in bench scale reactor using laccase of *Pycnoporus cinnabarinus*. *Biotechnology Letters*, 20, 1101-1104.

Ravindran, C., Naveenan, T. and Varatharajan, G.R. (2010). Optimization of alkaline cellulase production by the marine derived fungus *Chaetomium* sp. using agricultural and industrial wastes as substrates. *Botanica Marina*, 53, 275-282.

Ray, S., Chowdhury, O., Das, N. and Bishayi, B. (2010). Development of an efficient and simple method for conjugation of laccase to immunoglobulin and its characterization by enzyme immunoassay. *Journal of Immunoassy and immunochemistry*, 31, 217-232.

Reczey, K., Szengyel, Z., Eklund, R. and Zacchi, G. (1996). Cellulase production by *Trichoderma reesei*. *Bioresource Technology*, 57, 25-30.

Reddy, C. A. and Mathew, Z. (2001). Bioremediation potential of white rot fungi. In G.M. Gadd (Ed) *Fungi in bioremediation* (pp. 52-78) Cambridge: Cambridge University press.

Rigling, D., and Van Alfen, N. K. (1991). Regulation of laccase biosynthesis in the plant-pathogenic fungus *Cryphonectria parasitica* by double stranded RNA. *Journal of Bacteriology*, 173, 8000-8003.

Rigling, D., and Van Alfen, N.K. (1993). Extra and intracellular laccases of the chestnut blight fungus *Cryphonectria parasitica*. *Applied and Environmental Microbiology*, 59, 3634-3639.

Rodrígues, M. A. M., Pinto, P., Bezerra, R. M. F., Dias, A. A., Guedes, C. V. M., Cardoso, V. M. G., Cone, J. W., Ferreira, L. M. M., Colaco, J. and Sequeira, C. A. (2008). Effects of enzyme extracts isolated from white-rot fungi on chemical composition and *in vitro* digestibility of wheat straw. *Animal feed Science and Technology*, 141, 326-338.

Rodríguez, C. S. and Ma, A. S. (2005). Application of solid-state fermentation to ligninolytic enzyme production. *Biochemical Engineering Journal*, 22, 211-219.

Rosales, E., Couto, S. R. and Sanromán, M. A. (2007). Increased laccase production by *Trametes hirsuta* grown on ground orange peelings. *Enzyme and Microbial Technology*, 40, 1286-1290.

Rowel, R. M. (2013). Hand book of wood chemistry and wood composites. In R. M. Rowell, R. Pettersen. and M. A. Tshabalala (Eds) *Cell wall chemistry* (pp. 33-72). New York: CRC Press.

Roy, B.P. and Archibald, F.S. (1994). An indirect free radical based assay for the enzyme cellobiose: Quinone *oxi*doreductase. *Analytical Biochemistry*, 216, 291-298.

Rubin, E.M. (2008) Genomics of cellulosic biofuels. *Nature*, 454, 841-845.

Ruggiero, P., Sarkar, J. M. and Bollag, J. M. (1989). Detoxification of 2,4-dichlorophenol by a laccase immobilized on soil or clay. *Soil Science*, 147, 361-370.

Saha, B.C. (2003). Hemicellulose bioconversion. *Journal of Industrial Microbiology and Biotechnology*, 30, 279-291.

Sahadevan L. D. M., Misra C. S., Thankamani V. (2013). Ligninolytic enzymes for application in treatment of effluent from pulp and paper industries. *Universal Journal of Environmental Research and Technology*, 3: 14-26.

Salvachua, D., Prieto, A., Lopez-Abelairas, M., Ku-Chau, T., Martinez, A. T. and Martinez, A. J. (2011). Fungal pretreatment: An alternative in second generation ethanol from wheat straw. *Bioresource Technology*, 102, 7500-7506.

Sampedro I., Catjhaml T., Marinari S., Petruccioli M., Grego S., D'Annibale A. (2009). Organic matter transformation and detoxification in dry olive mill residue by saprophytic fungus *Paecilomyces farinosus*. *Process Biochemistry,* 44, 216-225.

Sannia, G., Limongi, P., Cocca, E., Buonocore, F., Nitti, G., and Giardina, P. (1991). Purification and characterization of a veratryl alcohol oxidase enzyme from the lignin degrading basidiomycete *Pleurotus ostreatus. Biochimica et Biophysica Acta, 1073*, 114–119.

Santos, J. I., Sampedro, R. M., Fillat, U., Oliva, J. M. et al. (2015). Evaluating lignin-rich residues from biochemical ethanol production of wheat straw and olive tree pruning by FTIR and 2D-NMR. *International Journal of Polymer Science*, doi: 10.1155/2015/314891, 1–11.

Sapmak, A., Boyce, K.J., Andrianopoulos, A. and Vanittanakom, N.(2015) The pbrB gene encodes a laccase required for DHN melanin synthesis in conidia of *Talaromyces (Penicillium) marneffei*. *PLoS One*, 10, e0122728.

Schoemaker, H.E. and Piontek, K. (1996). On the interaction of lignin peroxidase with lignin. *Pure and Applied Chemistry*, 68, 2089–2096.

Schutyser, W., Renders,T., Van den Bosch, S., Koelewijn, S. F., Beckham, G. T. and B. F. Sels (2018). Chemicals from lignin: an interplay of lignocellulose fractionation, depolymerisation, and upgrading, ***Chemical Society Review***, ***47,***852-908.

Semenovlrina, A.N., Vasilly, V.L., Mikhail, I.B. and Titov, I. (1993). Peroxidase and laccase as catalysts for removal of the phenylhydrazide protecting group under mild conditions. *Biotechnology and Bioengineering*, 42, 1137–1141.

Sexton, A.C., Paulsen, M., Woestemeyer, J. and Howlett, B.J. (2000). Cloning, characterization and chromosomal location of three genes encoding host-cell-wall degrading enzymes in *Leptosphaeria maculans*, a fungal pathogen of *Brassica* spp. *Gene*, 248, 89–97.

Shafiei, M., Karimi, K. and Taherzadeh, M.J. (2010). Pretreatment of spruce and oak by N-methylmorpholine-N-oxide (NMMO) for efficient conversion of their cellulose to ethanol. *Bioresource Technology*, 101, 4914–4918.

Shaheena, R., Asgher, M., Hussaina, F. and Bhatti, H.N. (2017). Immobilized lignin peroxidase from *Ganoderma lucidum* IBL-05 with improved dye decolorization and cytotoxicity reduction properties. *International Journal of Biological Macromolecules*, 103, 57–64.

Sharma, H.S.S., Whiteside, L. and Kernaghan, K. (2005). Enzymatic treatment of flax fiber at the roving stage for production of wet spun yarn. *Enzyme and Microbial Technology*, 37, 386–394.

Sharma, K.B. and Arora, D.S. (2011). Biodegradation of paddy straw obtained from different geographical locations by means of *Phlebia* sp. for animal feed. *Biodegradation*, 22, 143-152.

Sharma, R.K. and Arora, D.S. (2010). Changes in biochemical constituents of paddy straw during degradation by white rot fungi and its impact on *in vitro* digestibility. *Journal of Applied Microbiology*,109, 679-686.

Sharma, S., Vaid, S. and Bajaj, B.K. (2015). Screening of thermo-alkali stable fungal xylanases for potential industrial applications. *Current Research in Microbiology and Biotechnology*, 3, 536-541.

Shinoyama, H., Ando, A. Fujii, T. and Yasui, T. (1991). The possibility of enzymatic synthesis of a variety of -xylosides using the transfer reaction of *Aspergillus niger* xylosidase. *Agricultural and Biological Chemistry*, 55, 849–50.

Shrestha, P., Khanal, S.K., Pometto, A.L. and van Leeuwen, J.H. (2009). Enzyme production by wood-rot and soft-rot fungi cultivated on corn fiber followed by

simultaneous saccharification and fermentation. *Journal of Agricultural and Food Chemistry*, 57, 4156-4161.

Silva, E.M., Machuca, A. and Milagres, A.M.F. (2005). Effect of cereal brans on *Lentinula edodes* growth and enzyme activities during cultivation on forestry waste. *Letters in Applied Microbiology*, 40, 283-288.

Silva, I.S., Grossman, M. and Durrant, L.R. (2009). Degradation of polycyclic aromatic hydrocarbons under microaerobic and very low oxygen conditions by soil fungi. *International Biodeterioration and Biodegradation*, 63, 224-229.

Singh Arora, D. and Sharma, R.K. (2010). Ligninolytic fungal laccases and their biotechnological applications, *Applied Biochemistry and Biotechnology*, 160,1760–1788.

Singh, A. and Hayashi, K. (1995). Microbial cellulases: protein architecture, molecular properties and biosynthesis. *Advances in Applied Microbiology*, 40, 1-44.

Singh, A., Kuhad, R.C. and Ward, O.P. (2007). Industrial application of microbial cellulases. In R.C. Kuhad and A. Singh (Eds.) *Lignocellulose biotechnology: future prospects* (pp.345-358). New Delhi: I.K. International Publishing House.

Singh, D. and Chen, S. (2008). The white rot fungus *Phaenerochaete chrysosporium:* conditions for the production of lignin-degrading enzymes. *Applied Microbiology and Biotechnology*, 81, 399-417.

Singh, G., Capalash, N., Kaur, K., Puri, S., Sharma, P., 2016. Enzymes: Applications in pulp and paper industry. In: Dhillon, G. Singh, Kaur, S. (Eds.), *Agro-Industrial Wastes as Feedstock for Enzyme Production: Apply and Exploit the Emerging and Valuable Use Options of Waste Biomass*. Academic Press, 157–172.

Singh, J.K., Vyas, P., Dubey, A., Upadhyaya, C.P., Kothari, R., Tyagi, V.V. and KumarA. (2018). Assessment of different pretreatment technologies for efficient bioconversion of lignocellulose to ethanol. *Frontiers in Bioscience*, 10, 350-371.

Singh, L.R., Singh, K.P. and Singh, P.R. (2015). Enzymatic decolorization and degradation of azo dyes − a review. *International Biodeterioration & Biodegradation*, 104, 10−1016.

Singh, P., Sulaiman, O., Hashim, R., Rupani, P.F. and Peng, L.C. (2010). Biopulping of lignocellulosic material using fungal species: A review. *Reviws in Environmental Science and Biotechnology*, 9, 141-151.

Singh, R., Rani, A., Kumar, P., Singh, C., Shukla, G. and Kumar, A. (2017) Hemicellulolytic activity in the crop residues. *International Journal of Pharmacy Research and Technology*, 1, 18-20.

Singhania, R.R., Sukumaran, R.K., Pillai, A., Prema, P., Szakacs, G., et al. (2006). Solid-state fermentation of lignocellulosic substrates for cellulase production by *Trichoderma reesei* NRRL 11460. *Indian journal of Biotechnology*, 5, 332-336.

Sjostrom, E. (1981). *Wood Chemistry, fundamental and applications*. New York: Academic Press.

Smook, G.A. (1982). *Handbook for pulp and paper technologists.* Joint textbook committee of the paper industry, Atlanta: TAPPI.

Sou, H.D., Ryoo, R., Ka, K.H. and Park, H. (2017). The mycelial growth and ligninolytic enzyme activity of cauliflower mushroom (*Sparassis latifolia*). *Forest Science and Technology*, 13, 158-163.

Spina, F., Junghanns, C., Donelli, I., Nair, R., et al. (2016). Stimulation of laccases from *Trametes pubescens*: use in dye decolorization and cotton bleaching. *Preparative Biochemistry and Biotechnology*, 46, 639–47.

Streffer, F. (2014). Lignocellulose to biogas and other products. *JSM Biotechnology and Biomedical Engineering*, 2, 1023.

Sukumaran, R.K., Singhania, R.R. and Pandey, A. (2005). Microbial cellulases production, applications and challenges. *Journal of Scientific and Industrial Research*, 64, 832.

Sulistyo, J., Kamiyama, Y. and Yasui, T. (1995). Purification and some properties of *Aspergillus pulverulentus* beta-xylosidase with transxylosylation capacity. *Journal of Fermentation and Bioengineering*, 79, 17-22.

Sun, J., Chen, Q. J., Zhu, M. J., Wang, H. X. and Zhang, G. Q. (2014). An extracellular laccase with antiproliferative activity from the sanghuang mushroom *Inonotus baumii*. *Journal of Molecular Catalysis B: Enzymatic*, 99, 20–25.

Sun, Y. and Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*, 83, 1–11.

Syazwanee, M.G.M.F., Shaziera, A. G. N., Izzati, M. Z. N. A., Azwady, A. A. N. and Muskhazli, A. (2018). Improvement of delignification, desilication and cellulosic content availability in paddy straw via physico-chemical pretreatments. *Annual Research & Review in Biology*, 6, 1–11.

Thiribhuvanamala, G., Kalaiselvi, G., Parthasarathy, S. Madhavan, S. and Prakasam, V. (2017). Extracellular secretion of lignocellulolytic enzymes by diverse white rot basidiomycetes fungi. *Annals of Phytomedicine*, 1, 20–29.

Thurston, C. F., (1994). The structure and function of fungal laccases. *Microbioloy*,140, 19-26.

Tien, M. and Kirk, T. K. (1984). Lignin-degrading enzyme from *Phanerochaete chrysosporium:* Purification, characterization, and catalytic properties of a unique H_2O_2-requiring oxygenase. Proceedings of the National Academy of Sciences, USA. 81:2280-2284.

Tlecuitl-Beristain, S., Sanchez, C., Loera, O., Robson, G. D., Diaz-Godinez, G. (2008). Laccases of *Pleurotus ostreatus* observed at different phases of its growth in submerged fermentation: production of novel laccase isoforms. *Mycological Research*,112,1080-1084.

Toushik, S. H., Lee, K. T., Lee, J. S. and Kim, K. S. (2017). Functional applications of lignocellulolytic enzymes in the fruit and vegetable processing industries. *Journal of Food Science*, 82,585-593.

Tripathi, A., Upadhyay, R. C. and Singh, S. (2011). Mineralization of mono-nitrophenols by *Bjerkandera adusta* and *Lentinus squarrosulus* and their extracellular ligninolytic enzymes. *Journal of Basic Microbiology*, 51, 1-15.

Tuomela, M., Vikman, M., Hatakka, A. and Itavaara, M. (2000). Biodegradation of lignin in a compost environment: A review. *Bioresource Technology*, 72, 169–183.

Tuor, U., Wariishi, H., Schoemaker, H. E. and Gold. M. H. (1992). Oxidation of phenolic arylglycerol-b-aryl ether lignin model compounds by manganese peroxidase from *Phanerochaete chrysosporium*: oxidative cleavage of an alpha-carbonyl model compound. *Biochemistry*, 31, 4986–4995.

Tzanov, T., Basto, C., Gubitz, G. M. and Cavaco, P. A. (2003). Laccases to improve the whiteness in a conventional bleaching of cotton. *Journal of Macromolecular Material Engineering*, 288, 807–810.

Ullah, M.A., Bedford, C.T. and Evans, C.S. (2000). Reaction of pentachlorophenol with laccase from *Coriolus versicolor*. *Applied Microbiology and Biotechnology*, 53, 230-234.

Valascova, V. and Baldrian, P. (2006). Degradation of cellulose and hemicellulose by the brown-rot fungus *Piptoporus betulinus* – production of extracellular enzymes and characterization of major cellulases. *Microbiology*, 152, 3613-3622.

van den Brink, J. and de Vries, R.P. (2011). Fungal enzyme sets for plant polysaccharide degradation. *Applied Microbiology and Biotechnology*, 91, 1477-92.

Vasudevan, N. and Mahadevan, A. (1992). Degradation of nonphenolic beta-o-4 lignin substructure model compounds by *Acinetobacter* sp. *Research in Microbiology*,143, 333–339.

Virk, A.P., Sharma, P. and Capalash, N. (2012). Use of laccase in pulp and paper industry. *Biotechnology Progress*, 28, 21–32.

Wang, H. and Jones, R.W. (1995). A unique endoglucanase-encoding gene cloned from the phytopathogenic fungus *Macrophomina phaseolina*. *Applied and Environmental Microbiology*, 61, 2004–2006.

Weaver, R.F., Rajagopalon, K.V., Handler, P., Jeffs, P., Byrne, W.L. and Rosenthal, D. (1970). Isolation of v-L glutaminyl 3,4-benzquinone: a natural sulphohydryl reagent from sporulating gill tissue of the mushroom *Agaricus bisporus. Proceedings of the National Academy of Sciences*, 67, 1050-1056.

Whistler, R.L. and Richards, E.L. (1970). Hemicelluloses. In W. Pigman and D. Horton (Eds). *The carbohydrates*.(pp. 447-469). NewYork: Academic press.

Wikee, S., Chumnunti, P., Kanghae, A., Chukeatirote, A., et al. (2017). Lignocellulolytic capability of endophytic *Phyllosticta* sp. *Journal of Bacteriology and Mycology*, 4, 1–7.

Williamson, P.R. (1994). Biochemical and molecular characterization of the diphenol oxidase of *Cryptococcus neoformans*: identification as a laccase. *Journal of Bacteriology*, 176, 656-664.

Winquist, E., Moilanen, U., Mettala, A., Leisona, M. and Hattaka, A. (2008). Production of lignin modifying enzymes on industrial wastes by solid-state cultivation of fungi. *Biochemical Engineering Journal*, 42, 128-132.

Wong, D.W. (2009). Structure and action mechanism of ligninolytic enzymes. *Applied Biochemistry and Biotechnology*,157,174–209.

Wood, D.A. (1980). Production, purification and properties of extracellular laccase of *Agaricus bisporus*. *Journal of General Microbiology*, 117, 327-338.

Wood, T.M. (1991). Fungal cellulases. In C.H. Haigler and P.J. Weimer (Eds.) *Biosynthesis and biodegradation of cellulose* (pp. 491-534). New York: Marcel Dekker.

Woolridge, E.M. (2014). Mixed enzyme systems for delignification of lignocellulosic biomass. *Catalysts*, 4, 1–35.

Worral, J.J., Chet, I. and Huttermann, A. (1986). Association of rhizomorph formation with laccase activity in *Armillaria* sp. *Journal of General Microbiology*, 132, 2527-2533.

Xiao, P., Mori, T. and Kondo, R. (2011). Biotransformation of the organochloride pesticide *trans*-chlordane by food rot fungi. *New Biotechnology*. doi:10.1016/j. nbt.2011.06.013.

Ximenes, E., Kim, Y. and Ladisch, M.R. (2013). Biological conversion of plants to fuels and chemicals and the effects of inhibitors. In C.E. Wyman (Ed) *Aqueous Pretreatment Plant Biomass Biological Chemical Conversion Fuels Chemicals* (pp. 39-60). Chichester:John Wiley & Sons.

Xu, C., Ma, F. and Zhang, X. (2009). Lignin degradation and enzyme production by *Irpex lacteus* CD2 during solid state fermentation of corn stover. *Journal of Bioscience and Bioengineering*, 5, 372-375.

Xu, F. (1996). Oxidation of phenols, anilines and benzenethiols by fungal laccases: Correlation between activity and redox potentials as well as halide inhibiton. *Biochemistry*, 35, 7608-7614.

Yadav, J. S. and Reddy, C. A. (1993). Degradation of benzene, toluene, ethylbenzene, and xylenes (BTEX) by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. *Applied and Environmental Microbiology*, 59, 756-762.

Yang, J., Li, W., Ng, T.B., Deng, X., Lin, J. and Ye, X. (2017). Laccases: production, expression, regulation, and applications in pharmaceutical biodegradation. *Frontiers in Microbiology*, 8:832, doi: 10.3389/fmicb.2017.00832.

Yao, B. and Ji, Y. (2014). Lignin biodegradation with laccase-mediator systems. *Froniers in. Energy Research*, 2, 12.

Zhang, J., Liu, X., Xu, Z. D. et al. (2008). Degradation of chlorophenols catalyzed by laccase. International *Biodeterioration & Biodegradation*, 61, 351-356.

Zhu, M., Zhang, G., Meng, L., Wang, H., Gao, K. and Ng, T. (2016). Purification and characterization of a white laccase with pronounced dye decolorizing ability and HIV-1 reverse transcriptase inhibitory activity from *Lepista nuda*. *Molecules*, 21, 415.

Zilly, A., Bazanella, G.C.D. and Helm, C.V. (2011). Solid state bioconversion of passion fruit waste by white rot fungi for production of oxidative and hydrolytic enzymes. *Food and Bioprocess Technology*, P. 8. (Published on line; Doi 10.1007/s11947-011-0532-8).

In: Research Advances in the Fungal World ISBN: 978-1-53617-197-6
Editors: P. Kumar Chaurasia and S. Lata Bharati

Chapter 17

INTRODUCTORY APPROACH TO THE KINETIC PROPERTIES OF FUNGAL LACCASES

Pankaj Kumar Chaurasia[1,*] and Shashi Lata Bharati[2,†]
[1]PG Department of Chemistry, LS College,
Muzaffarpur (Under BRA Bihar University), Bihar, India
[2]Department of Chemistry, North Eastern Regional Institute
of Science and Technology, Nirjul, Itanagar,
Arunachal Pradesh, India

ABSTRACT

Laccases are oxidoreductase enzymes [EC 1.10.3.2] also known as multicopper polyphenol oxidases due to the presence of four coppers. They have been reported in fungi, plants bacteria as well as insects. The most study of laccases was performed on the fungal laccases.

This is the enzyme of the great significance in the various fields of research and industries like paper and pulp industry, food and beverages, bioremediation, detoxification of xenobiotics, chemical industries, pharmaceutical chemistry, genetic engineering, biosensor technology, polymerization, carbohydrates chemistry, bio-conversions etc.

The huge level of applications of fungal laccases are due to their strong catalytic efficiency which depends on their binding capability, active sites, pH stability, thermal stability, pH optima, temperature optima, Michaelis-Menten constant (K_m) and catalytic constant (K_{cat}). In this chapter authors have discussed and reviewed the different kinetic aspects of fungal laccases based on previous studies.

* Corresponding Author's Email: pankaj.chaurasia31@gmail.com.
† Corresponding Author's Email: shashilatachem@gmail.com.

Keywords: Laccase, Oxidoreductase, Fungi, Kinetics, Michaelis-Menten constant, Catalytic constant

INTRODUCTION

Laccases are one of the most important enzymes of the oxidoreductase group with multicopper active centres.

They are also known as multicopper polyphenol oxidases (Chaurasia et al. 2013a; Chaurasia et al. 2013b; Dwivedi et al. 2011; Riva 2006) and reported in fungi (Baldrian 2006; Chaurasia et al. 2015a; Giardina et al. 1995; Mayer and Staples 2002; Messerschmidt and Huber 1990), bacteria (Bao et al. 1973; Huang et al. 1999) and insects (Parkinson et al. 2001; Thomas et al. 1989). The most widely studied laccases are the fungal laccases also known as lignolytic enzymes. They occur in the fungal systems in abundant (Messerschmidt and Huber 1990). Almost all white rot fungi are involved in the laccase secretion (Messerschmidt and Huber 1990; Mayer and Staples 2002). They contain four coppers divided into three copper centres- Type 1 (T1), Type 2 (T 2) and Type 3 (T3) (Quinatanar et al. 2005). Each of the T1 and T2 is composed of single copper while T3 is composed of two coppers. T1 copper shows an intense charge transfer near 600 nm which is responsible for the blue colour of the laccases. T2 or normal copper site lacks any strong absorption band in visible region. T3 or two copper containing centre has intense charge transfer band near 330 nm. A trinuclear cluster is formed by the association of the T2 and T3 (Riva 2006).

Phenols, phenolic derivatives, amines as well as many non-phenolic compounds are act as substrates for the laccase. Inorganic ions are also act as substrate for the laccases. They have a large number of applications in the field of paper industries, food beverages, bioconversions, chemical syntheses, carbohydrates chemistry, polymer chemistry, environmental chemistry etc (Chaurasia et al. 2015a; Chaurasia et al. 2015b; Chaurasia et al. 2015c; Mogharabi and Faramarzi 2014). The catalytic activity of the laccase are due to the presence of copper centre but this depends upon their various kinetic properties like pH stability, thermal stability, pH optima, temperature optima, Michaelis-Menten constant (K_m) and catalytic constant (K_{cat}). It is well known fact that enzymes are pH and temperature specific. Performances of laccases directly depend on Michaelis-Menten constant (K_m). Lower the value of K_m higher will be the efficiency of enzyme. This is due to the fact that for the lower value of K_m, enzyme-substrate complex dissociation will be difficult. Thus, lesser ease of dissociation makes the enzyme-substrate complex more stable. Thus, lower the value of K_m of laccase indicates the high efficiency of the enzymes. For further detailed studies readers may consult the book of Bharati and Chaurasia, 2018. This chapter covers the introductory studies on the kinetic of the fungal laccases.

KINETICS OF FUNGAL LACCASES

During the study of laccases, their kinetic study is an important step. Kinetic study of the laccases tells about the efficiency of laccases and its binding capability to the substrates. Laccases have different affinity for the different substrates and due to which their rate of reaction also varies with the types of the substrates. Which substrate would be the best suitable for the reaction with enzymes can be determined from the Michaelis-Menten constant (K_m). Before the determination of the K_m values for the substrates, pH optima, pH stabilities, temperature optima and thermal stabilities should also be determined.

Optimization of pH and Temperature

The pH (other conditions are constant like temperature, concentration etc) and temperature (other conditions are constant like pH, concentration etc) at which laccase shows maximum activity for the selective substrates are called as pH optimum and temperature optimum, respectively. As for example, if pH optimum and temperature optimum of a purified laccase has been reported at 5.0 and 45°C for any feasible substrate, it means that this specific laccase enzyme showed maximum activity at the pH 5.0 and temperature 45°C for that specific substrate. During the optimization processes activity has to be checked on varied pH and varied temperatures having same concentration of the substrate. Laccase purified by Chaurasia et al. (Chaurasia et al. 2013d) from *Xylaria polymorpha* MTCC 1100 has pH optimum 4.0 (Figure 1a) and temperature optimum 40°C (Figure 2a) for the substrate DMP (2,6- dimethoxyphenol). They used 1mL reaction solution that contained 1.0mM DMP in 100mM sodium malonate buffer with varying pH (3.0-6.0) and laccase for pH optimum determination; and for temperature optimum determination, reaction solution contained 1.0 mM DMP in 100mM sodium malonate buffer pH 4.5 with varying temperature (30°C to 70°C). Enzyme assay for the use of DMP as substrate was used (Coll et al. 1993). Laccase purified from *Hexagonia tenuis* MTCC-1119 has pH optimum at 3.5 and temperature optimum at 45°C (Chaurasia et al. 2015d).There are large numbers of laccases isolated from varieties of sources for which pH optimum and temperature optima studied.

The pH Stability, Thermal Stability, Rate Constant and Activation Energy

The pH and thermal stability is determined by taking specified reaction solution of substrate, buffer and enzyme.

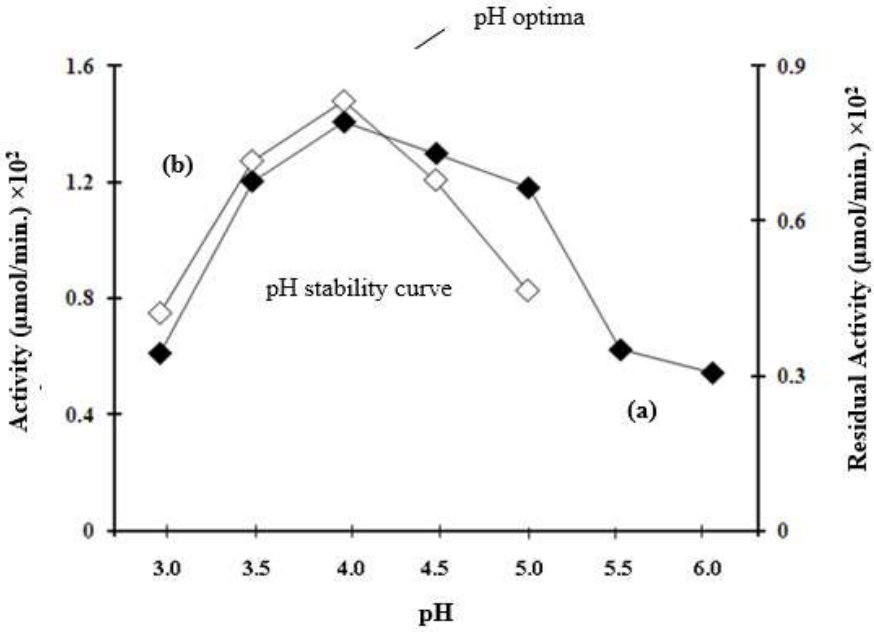


Figure 1. Representation of pH optima and pH stability curve for the purified laccas from X. polymorpha MTCC-1100 (Chaurasia et al. 2013d).

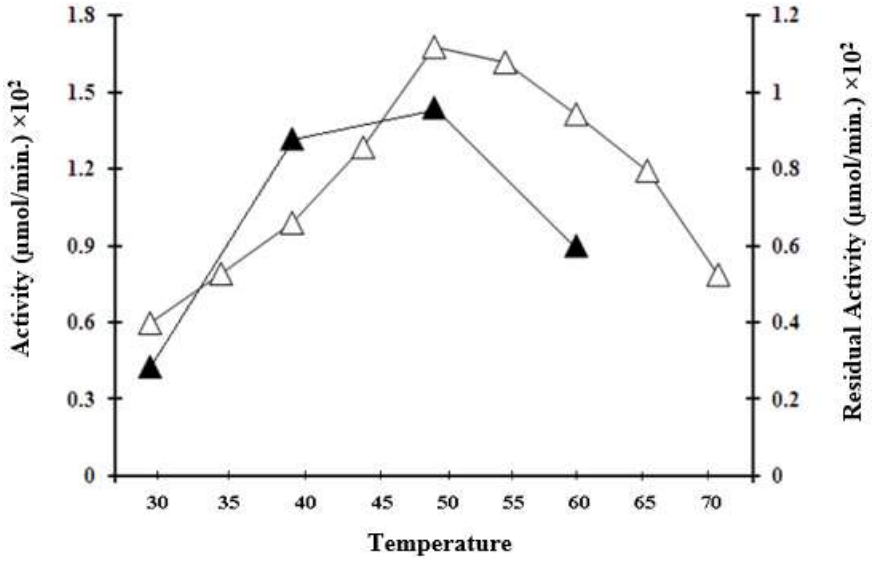


Figure 2. Representation of temperature optima and thermal stability curve for the purified laccass from X. polymorpha MTCC-1100 (Chaurasia et al. 2013).

This solution is now subjected to the test at particular pH (in case for pH stability determination) or particular temperature (in case of thermal stability determination) for one hour (or as researcher select according to the enzyme's activity). Now, residual activities obtained at varying pH or varying temperature is plotted against the different pHs or different temperatures. The purpose behind the determination of pH stability and

temperature stability is to know about the pH and temperature at which particular laccase is most stable with retention of the maximum activity for specific substrate. Using the plot of thermal stability, the rate constants (k) can be determined easily from calculated $t_{1/2}$ values. Activation energy can also be determined using Arrhenius plot.

Let us consider that if any laccase has pH stability at 4.0 and thermal stability at 40°C, it mean this specific laccase has retained its maximum activity for the fixed time at given pH (in case of the determination of pH stability) or given temperature (in case of the determination of thermal stability). Laccase purified from *Xylaria polymorpha* MTCC-1100 has pH stability at 4.0 (Figure 1b) and thermal stability at 50°C (Figure 2b) (Chaurasia et al. 2013d). This study was based on enzyme treatment for one hour. The pH and thermal stability of purified laccase has been studied at varying pHs (The 01 mL of the reaction solution for pH stability determination contained 1.0 mM DMP in 100 mM sodium malonate buffer with varying pH (3.0 - 5.0) and laccase) or varying temperatures (the 01mL of the reaction solution for thermal stability determination contained 1.0 mM DMP in 100 mM sodium malonate buffer (pH 4.5) with varying temperature (30°C - 60°C) and laccase). Now, by plotting residual activity *versus* pH or temperature, Chaurasia et al. (Chaurasia et al. 2013d) found that this laccase was most stable at aforementioned pH and temperature i.e., retained maximum activities for the specific substrate within one hour of the study period. Activation energy for the thermal denaturation was 15.26×10^3J/mol/K for the purified laccase. Similarly, laccase from *Hexagonia tenuis* MTCC-1119 has maximum stability at pH 3.5 exposed for one hour. Activation energy for thermal denaturation was 73.45 J/mol/K for this purified laccase (Chaurasia et al. 2015d).

Michaelis-Menten Constant (K_m)

Enzyme catalysed reactions generally follows the first order reaction kinetics. K_m of any enzyme catalyzed reaction can be determined by plotting the activity of enzymes (rate of reaction) for specific substrate *versus* concentrations. During the study, reaction solution contains a range of concentrations of a specific substrate, buffer solution with fixed pH and temperature and fixed amount of laccase. Activities calculated for each concentration selected between the specified ranges is plotted against different concentrations taken for activities determinations. In this way, K_m can easily be calculated using Michaelis-Menten curve and double reciprocal plots. Double reciprocal plots (Line Weaver-Burk Plot) can also be used for the determination of Michaelis-Menten constant. In this case 1/V is plotted *versus* 1/[S] *(*Lineweaver and Burk 1934; https://en.wikipedia.org/wiki/Lineweaver%E2%80%93Burk_plot*)*. All the enzyme substrates have individual binding ability with enzyme to form enzyme-substrate

complex at different rates. A generalized representation of the plots for the determination of K_m is shown in Figure 3.

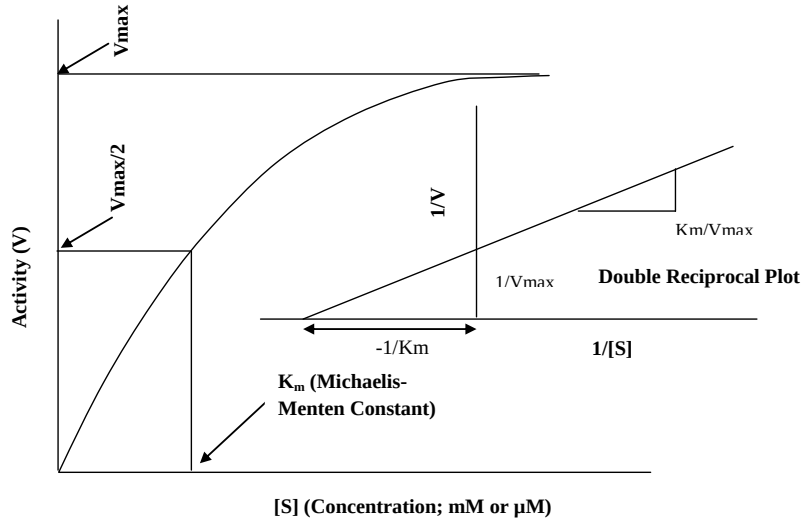


Figure 3. General schematic representation for the determination of K_m (Michaelis-Menten plot and Reciprocal plot).

In the plot, V_{max} and $V_{max}/2$ are the maximum activity and half of the maximum activity of the laccase (or enzyme) while [S] is the concentration of the substrate. K_m is actually the concentration of the substrate at which enzyme has half ($V_{max}/2$) of its maximum activity (V_{max}). Another important term is the catalytic constant (k_{cat}) also known as catalytic rate constant. The k_{cat}/K_m shows the catalytic efficiency of the laccase (or any enzymes). Catalytic efficiency is the ability of enzyme to convert substrate into product. In 1913, Micahelis and Menten proposed a mathematical model of kinetics for an enzyme catalysed reaction (Michaelis and Menten, 1913). Equation for a Michaelis-Menten constant for a typical reaction of enzyme can be written as (https://en.wikipedia.org/wiki/Michaelis%E2%80%93Menten_kinetics) -

$$E + S \underset{k_r}{\overset{k_f}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

$$v = \frac{V_{max}[S]}{K_m + [S]} = k_{cat}[E]_0 \frac{[S]}{K_m + [S]}$$

where, E = Enzyme; S = Substrate; ES = Enzyme-substrate complex; P = Product; k_f = Rate constant for forward reaction; k_f = Rate constant for forward reaction; k_{cat} = Catalytic rate constant; v = Rate of reaction for the formation of product (d[P]/dt);

V_{max} = Maximum activity/rate K_m = Michaelis-Menten constant; [S] = Concentration of substrate; k_{cat} = Catalytic rate constant; $[E]_0$ = Initial concentration of enzyme.

Table 1. Kinetic parameters for laccases isolated from different sources

S. N.	Fungal source of laccase	pH optima	Temp. optima (°C)	K_{cat}	Michaelis-Menten constant (K_m)	Ref.
1	*Pestalotiopsis* Species CDBT-F-G1	6.0	60	-	0.10 mM, (ABTS)	Yadav et al., 2019
2	Cerrena sp. RSD1	3.0 (ABTS)	65 (ABTS, Guaiacol)	52 515 s-1 (ABTS)	36 µM (ABTS)	Wu et al., 2018
3	fungal strain FBV40 (Lac1)	3.0 (ABTS)	60 (ABTS)	-	0.3 µM (ABTS)	Chu et al., 2017
4	*Marasmius* sp. BBKAV79	5.5	40	-	3.03 mM (Guaicol)	Vantamuri and Kaliwal, 2016
5	*Daedalea flavida* MTCC-145	4.5	50	6.45 s^{-1} (DMP)	440 µM (DMP); 366 µM(ABTS)	Sharma et al., 2016
6	*Hexagonia tenuis* MTCC-1119	3.5	45	2.54 s^{-1} (DMP)	80 µM (DMP); 36 µM (ABTS); 87 µM (Syringaldazine)	Chaurasia et al., 2015d
7	*Trametes hirsuta* MTCC-1171	4.5	60	13.04 s^{-1} (DMP)	420 (DMP)	Chaurasia et al., 2014
8	*Pleurotus ostreatus* HP-1	4.5 (ABTS)	50	244.32 s^{-1} (ABTS); 208.33 s^{-1} (DMP); 208.33 s^{-1} (Guaicol)	46.51 mM (ABTS); 400 mM (DMP); 100 mM (Guaicol)	Patel et al., 2014
9	*Cerrena* sp. HYB07	3.0 (ABTS and 2,6-DMP)	45°C (ABTS) 50°C (2,6-DMP)	2468.0 s^{-1} (ABTS)	93.4 µM (ABTS)	Yang et al., 2014
10	*Coriolopsis floccosa* MTCC-1177	5.0	40	5.16 s^{-1} (DMP)	112.5 µM (DMP); 58 µM (ABTS); 100 µM (syringaldazine)	Chaurasia et al., 2013e
11	*Phellinus linteus* MTCC-1175	5.0	45	6.85 s–1 (DMP)	160 µM (DMP); 42 µM (ABTS)	Chaurasia et al., 2013b
12	*Trametes versicolor* IBL-04	5.0	40	-	73µM (ABTS)	Asgher et al., 2012
13	*Pleurotus* sp.	4.5	65	-	250 (ABTS)	More et al., 2011
14	*Fomitella fraxinea*	3 (ABTS)	70 (ABTS)	208.0 s^{-1} (ABTS); 103.2 s^{-1} (2,6-DMP)	270 µM (ABTS); 426 µM (2,6-DMP)	Park and Park, 2008
15	*Magnaporthe grisea*	6.0	30	-	0.118 mM	Iyer and Chattoo, 2003
16	*Trametes sanguinea* M85-2	5.0	60	-	-	Nishizawa et al., 1995

The pH optima, temperature optima, K_m and K_{cat} for the different laccases purified from different sources have been given in table 1.

CONCLUSION

Aforementioned discussion demonstrates about the kinetic parameters for the purified laccases. In this chapter, different kinetic parameters have been discussed in very simpler way with the help of figures. The discussed kinetic is the fundamental study of the enzymatic kinetics that is essential part of any enzymatic studies. This chapter will help the readers in order to start the kinetic studies of any enzymes.

ACKNOWLEDGMENTS

Dr. PK Chaurasia is very much thankful to the Department of Chemistry, LS College, Muzaffarpur, Bihar (India) and Dr. SL Bharati is thankful to the Department of Chemistry, NERIST, Arunachal Pradesh (India) for providing the necessary facility during the work.

REFERENCES

Asgher, M., Iqbal, H. M. N., Asad, M. J., Asad, M. J. (2012). Kinetic characterization of purified laccase produced from Trametes versicolor IBL-04 in solid state bio-processing of corncobs. *BioResources*, 7(1), 1171 - 1188.

Baldrian, P. (2006). Fungal laccases-occurrence and properties. *FEMS Microbiology and Review*, 30, 215 - 242.

Bao, W., Mally, D. M. O., Whetten, R. and Sederoff, R. R. (1973). A laccase associated with lignification of ioblolly pine xylem. *Science,* 260. 672 - 674.

Bharati, S. L. and Chaurasia, P. K. (Eds.) (2018). Research advancements in pharmaceutical, nutritional and industrial enzymology. IGI Global publisher, USA. ISBN: 978-1522552376 (hardcover) | ISBN: 9781522552383 (ebook). Pages: 1 - 549.Doi: DOI: 10.4018/978-1-5225-5237-6.

Chaurasia, P. K., Bharati, S. L., Sharma, M., Singh, S. K., Yadav, R. S. S. and Yadava, S. (2015a). Fungal Laccases and their Biotechnological Significances in the Current Perspective: A Review. *Current Organic Chemistry,* 19, 1916 - 1934.

Chaurasia, P. K., Bharati, S. L. and Singh, S. K. (2013a). Comparative studies on the blue and yellow laccases. *Research in Plant Sciences*, 1(2), 32 - 37. doi: 10.12691/plant-1-2-5.

Chaurasia, P. K., Bharati, S. L., Sunil, S. K. and Yadava, S. (2015b). Amination of *p*-hydroquinones by laccase of *Xylaria polymorpha* MTCC-1100. *Russian Journal General Chemistry*, 85(3), 683 - 685. doi: 10.1134/S1070363215030263.

Chaurasia, P. K., Bharati, S. L., Sunil, S. K. and Yadava, S. (2015c). Synthetic applications of purified laccase from *Pleurotus sajor caju* MTCC-141. *Russian Journal of General Chemistry*, 85(1), 173 - 175.doi: 10.1134/S1070363215010302.

Chaurasia, P. K., Bharati, S. L., Yadava, S. and Yadav, R. S. S. (2015d). Purification, characterization and synthetic application of a thermally stable laccase from *Hexagonia tenuis* MTCC-1119. *Indian Journal of Biochemistry Biophysics*, 52(1), 60 - 67.

Chaurasia, P. K., Yadav, A. and Yadav, R. S. S. and Yadava, S. (2013b). Purification and Characterization of Laccase Secreted by *Phellinus linteus* MTCC-1175 and its Role in the selective Oxidation of aromatic Methyl group. *Applied Biochemistry Microbiology*, 49(6), 592 - 599. doi: 10.1134/S0003683813060069.

Chaurasia, P. K., Yadav, R. S. S., Yadava, S. (2013c). A review on mechanism of laccase action, *Research and Reviews in Biosciences*, 2013, 7(2), 66 - 71.

Chaurasia, P. K., Yadav, R. S. S. and Yadava, S. (2013d). Purification, Characterization and Steady State Enzyme Kinetics of Laccase from *Xylaria polymorpha* MTCC-1100. *International Journal of Research in Chemistry and Environment*, 3(2), 93 - 101.

Chaurasia, P. K., Yadav, R. S. S., Yadava, S. (2014). Purification and characterization of yellow laccase from *Trametes hirsuta* MTCC-1171 and its application in synthesis of aromatic aldehydes, *Process Biochemistry*, 2014, 49, 1647 - 1655. DOI: http://dx.doi.org/10.1016/j.procbio.2014.06.016.

Chaurasia, P. K., Yadav, R. S. S., Yadava, S. (2013e). Purification and Characterization of Laccase from *Coriolopsis floccosa* MTCC-1177 and its use in the selective oxidation of aromatic methyl group to aldehyde without mediators. *Journal of Chemical Sciences*, 125(6), 1395 - 1403.

Chu, P. K. H., Van, N. H. and Ha, D. T. C. (2017). Purification and characterization of laccase involved in the decolorization of synthetic dyes and 2,3,7,8-TCDD congener degradation by the white rot fungus isolated from Bavi Forest of Vietnam. *Vietnam Journal of Science and Technology*, 55(4C), 180 - 185.

Coll, M. P., Fernandez-Abalos, J. M., Villomueva, J. R., Somtamaria, R. and Perez, P. (1993). Purification and characterization of a phenoloxidase (laccase) from the lignindegrading Basidiomycete PM 1 (CECT2971). *Applied and Environmental Microbiology*, 59, 2607 - 2613.

Dwivedi, U. N., Singh, P., Pandey, V. P. and Kumar, A. (2011). Structure – function relationship among bacteria fungal and plant laccases: a review. *Journal of Molecular Catalysis B. Enzymatic*, 68, 117 - 128. https://doi.org/10.1016/j.molcatb.2010.11.002.

Giardina, P., Cannio, R., Martirani, L., Marzullo, L., Palmieri, G. and Sannia, G. (1995). Cloning and sequencing of a laccase gene from the lignin degrading basidiomycete *Pleurotus ostreotus*. *Applied and Environmental Microbiology*, 61, 2408 - 2413. https://en.wikipedia.org/wiki/Michaelis%E2%80%93Menten_kinetics.

Huang, H. W., Zoppellero, G. and Sakurai, T. (1999). Spectroscopic and kinetic studies on oxygen-centered radical formed during the four-electron reduction process of dioxygen by *Rhus Vernicifera* laccase. *Journal of Biological Chemistry*, 274, 32718 - 32724. doi:10/1074/jbc.274.46.32718.

Iyer, G. and Chattoo, B. B. (2003). Purification and characterization of laccase from the rice blast fungus, *Magnaporthe grisea*. *FEMS Microbiology Letters*, 227(1), 121 - 126. https://doi.org/10.1016/S0378-1097(03)00658-X.

Lineweaver, H., Burk, D. (1934). The determination of enzyme dissociation constants. Journal of the American Chemical Society. 56 (3): 658–666. doi:10.1021/ja01318a036

"Lineweaver–Burk Plot." Wikipedia. Wikimedia Foundation, January 1, 2020. https://en.wikipedia.org/wiki/Lineweaver–Burk_plot.

Mayer, A. M. and Staples, R. C. (2002). Laccase: new functions for an old enzyme. *Phytochemistry*, 60, 551 - 565. doi:10.1016/s0031-9422(02)00171-1.

Messerschmidt, A. and Huber, R. (1990). The blue oxidases, ascorbate oxidase, laccase and ceruloplasmin-Modelling and structural relationships, *Europian Journal of Biochemistry*, 187(2), 341 - 352.

Michaelis, L., Menten, M. L. (1913). Die Kinetik der Invertinwirkung [*The kinetics of inverting action*]. Biochem Z. 49, 333–369.

"Michaelis–Menten Kinetics." Wikipedia. Wikimedia Foundation, January 9, 2020. https://en.wikipedia.org/wiki/Michaelis–Menten_kinetics.

Mogharabi, M. And Faramarzi, M. S. 2014. Laccase and laccase-mediated systems in the synthesis of organic compounds. *Advanced Synthetis and Catalysis*, 356, 897 - 927.

More, S. S., Renuka P. S., Pruthvi, K., Swetha, M., Malini, S. and Veena, S. M. (2011). Isolation, Purification, and Characterization of Fungal Laccase from *Pleurotus sp*. *Enzyme Research*, Volume 2011, Article ID 248735, 7 pages; http://dx.doi.org/10.4061/2011/248735.

Nishizawa, Y., Nakabayashi, K. and Shinagawa, E. (1995). Purification and characterization of laccase from white rot fungus *Trametes sanguinea* M85-2. *Journal of Fermentation and Bioengineering*, 80(1), 91 - 93. https://doi.org/10.1016/0922-338X(95)98183-L.

Park, K. M. and Park, S. S. (2008). Purification and Characterization of Laccase from Basidiomycete *Fomitella fraxinea*, *Journal of Microbiology and Biotechnology*, 18(4), 670 - 675.

Parkinson, N., Smith, I., Weaver, R. and Edwards, J. P. (2001). A new form of arthropod phenol oxidase is abundant in venom of the parasitoid wasp *Pimpla hypochondriaca. Insect Biochemistry and Molecular Biology*, 31, 57 - 63.

Patel, H., Gupte, S., Gahlout, M. and Gupte, A. (2014). Purification and characterization of an extracellular laccase from solid-state culture of *Pleurotus ostreatus* HP-1. *3 Biotech.*, 4(1), 77 - 84. doi: 10.1007/s13205-013-0129-1.

Quinatanar, L., Yoon, J., Aznar, C. P., Palmer, A. E., Andersson, K. K., Britt, R. D. and Solomon, E. I. (2005). Spectroscopic and electronic structure studies of the trinuclear Cu cluster active site of the multicopper oxidase laccase: nature of its co-ordination unsaturation, *Journal of American Chemical Society*, 127, 13832 - 13845. https://doi.org/10.1021/ja0421405.

Riva, S. (2006). Laccase: blue enzymes for green chemistry. *Trends in Biotechnology*, 24(5), 219 - 26. doi:10.1016/j.tibtech.2006.03.006.

Sharma, M., Chaurasia, P. K., Yadav, A., Yadav, R. S. S., Yadava, S. and Yadav, K. D. S. (2016). Purification and characterization of a thermally stable yellow laccase from *Daedalea flavida* MTCC-145 with higher catalytic performance towards selective synthesis of substituted benzaldehydes. *Russian Journal of Bioorganic Chemistry*, 42(1), 59 - 68. doi: 10.1134/S1068162016010143.

Thomas, B. R., Yonekura, M., Morgan, T. D., Czapla, T. H., Hopins, T. L. and Kramer, K. J. (1989). A trypsin solubilized laccase from pharate pupal integument of the tobacco hornworm, *Manduca sexta. Insect Biochemistry*, 197, 611 - 622. Doi: 10.1016/0020-1790(89)90095-4.

Vantamuri, A. B. and Kaliwa, B. B. (2016). Purification and characterization of laccase from *Marasmius* species BBKAV79 and effective decolorization of selected textile dyes. *3 Biotech*, 6(2), 189. doi: 10.1007/s13205-016-0504-9.

Wu, M. H., Lee, C. C., Hsiao, A. S., Yu, S. M., Wang, A. H. and Ho, T. D. (2018). Kinetic analysis and structural studies of a high-efficiency laccase from *Cerrena* sp. RSD1. *FEBS Open Bio*, 8(8), 1230 - 1246. doi: 10.1002/2211-5463.12459.

Yadav, Y., Bista, G., Maharjan, R., Poudyal, P., Mainali, M., Sreerama, L. and Joshi, J. (2019). Secretory Laccase from Pestalotiopsis Species CDBT-F-G1 Fungal Strain Isolated from High Altitude: Optimization of Its Production and Characterization. *Applied Sciences*, 9(2), 340. https://doi.org/10.3390/app9020340.

Yang, J., Lin, Q., Ng, T. B., Ye, X., Lin, J. (2014). Purification and Characterization of a Novel Laccase from *Cerrena* sp. HYB07 with Dye Decolorizing Ability. *PLOS ONE*, 2014, 9(10), e110834. https://doi.org/10.1371/journal.pone.0110834.

ABOUT THE EDITORS

Pankaj Kumar Chaurasia

Dr. Pankaj Kumar Chaurasia is Assistant Professor in the PG Department of Chemistry, LS College, Muzaffarpur (A constituent unit of BRA Bihar University, Muzaffarpur). He has a strong academic as well as research career. In academic career, he has throughout first division with average 74% of marks. He has qualified national level eligibility test in 2009 as CSIR-JRF (NET). He was awarded with SRF-NET in 2012. He was awarded by 'Ph.D. degree' in 2014 by DDU Gorakhpur University Gorakhpur (India). He also worked as 'Guest Faculty' during session 2016-2017 in Department of Chemistry, University of Allahabad, Prayagraj (A Central University of India). He was awarded with Research Associateship by CSIR-New Delhi (India) in 2017 and worked as CSIR-RA in Motilal Nehru NIT, Allahabad/Prayagraaj (India). He has approximately 25 research journal papers, 07 book chapters and 02 books. He has worked

in different area of chemistry and has specializations in the field of bioorganic chemistry, biological chemistry, biochemistry, biotechnology, microbiology, enzymology, organometallic chemistry and environmental chemistry.

Shashi Lata Bharati

Dr. Shashi Lata Bharati is working as 'Assistant Professor' in the 'Department of Chemistry, North Eastern Regional Institute of Science and Technology, Nirjuli, Arunachal Pradesh (India). She has good academic as well as research career. She was awarded by UGC-DSA fellowships for the meritorious students during her Ph.D. program. She obtained her Ph.D. degree in 2012 in 'Chemistry'. She has also taught in DVNPG College, Gorakhpur as 'Lecturer/Assistant Professor' on contract basis for more than one year. She was awarded by UGC-post doctoral fellowship for women in 2013 and worked as 'Post Doctoral Fellow' in the 'Department of Chemistry' of DDU Gorakhpur University, Gorakhpur (India). She has published about 18 research journal publications, 03 book chapters and two books in national and international journals/publishers of repute. She has expertise in the field of inorganic chemistry, organometallic chemistry, biological chemistry and enzymology.

INDEX

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

ß

S

T

U

V

W

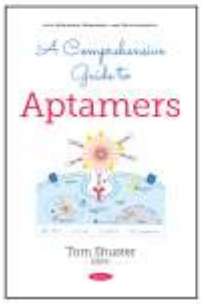
X

Y

Z

Related Nova Publications

A Comprehensive Guide to Aptamers



Editor: Tom Shuster

Series: Life Sciences Research and Development

Book Description: This collection opens with a focus on recent advancements on the development of nucleic acid aptamers as alternative delivery systems for therapeutic oligonucleotides. Additionally, key examples of targeted delivery of the most common nucleic acid therapeutics, including small interfering RNAs, short hairpin RNAs, microRNAs and antisense oligonucleotides for a number of disorders are discussed.

Softcover ISBN: 978-1-53616-293-6
Retail Price: $95

Innovations in Life Science Research

Editors: Rajeshwar P. Sinha, Shashi Pandey-Rei, and Nandita Ghoshal

Series: Life Sciences Research and Development

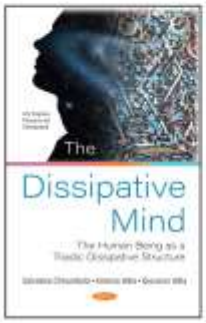
Book Description: This book is an attempt to keep abreast with the recent innovations made in various fields of life science research. The book encompasses topics ranging from prokaryotic microscopic organisms to eukaryotic higher plants, distributed in fifteen chapters for the benefit of graduate and postgraduate students as well as young researchers, scientists and professionals.

Hardcover ISBN: 978-1-53615-868-7
Retail Price: $230

To see a complete list of Nova publications, please visit our website at www.novapublishers.com

Related Nova Publications

The Dissipative Mind: The Human Being as a Triadic Dissipative Structure



Authors: Salvatore Chirumbolo, Antonio Vella, and Giovanni Vella

Series: Life Sciences Research and Development

Book Description: In this book, we attempted to build a new epistemological model of life and the human mind taking into account the intriguing model of Prigogine's dissipative structure.

Hardcover ISBN: 978-1-53616-793-1
Retail Price: $230

To see a complete list of Nova publications, please visit our website at www.novapublishers.com