SUSTAINABLE UTILIZATION OF FUNGI IN AGRICULTURE AND INDUSTRY

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Sustainable Utilization of Fungi in Agriculture and Industry

(Volume 4)

Mycology: Current and Future Developments

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Mycology: Current and Future Developments

Volume # 4

Sustainable Utilization of Fungi in Agriculture and Industry

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FOREWORD

The discipline of biology that is devoted to studying fungi is termed mycology. These organisms are classified in a kingdom, characterized by heterotrophy meaning absorption of nutrients typically by secreting digesting enzymes into their environment. Fungi are abundant worldwide, and the diversity has been estimated at 5.1 to 12 million species by mycologists; among them, a minor fraction of about 1,40,000 species have been characterized and identified. A major part of the extant fungi on Earth is yet to be characterized. Non-culturable fungi in the environmental samples are characterized by constructing their genomes from metagenomes and sequencing internal transcribed spacers.

Fungi occur in normal as well as extreme environments. They perform an essential role in the decomposition of organic matter and have fundamental roles in nutrient cycling and exchange in the environment. Such functions are played by the consortia of fungi and other microbes which are referred to as microbiomes. They are free living or symbionts/parasites on plants and animals. The arrival of next-generation sequencing technology allows fungal genomes to be sequenced for phylogenetic studies up to the species level. Fungal and yeast genome sequencing projects have been launched to sequence 1000 fungal and budding yeast genomes.

Fungi have served a crucial role as model organisms for biological inquiry, such as brewer's yeast, *Saccharomyces cerevisiae*; and pink bread mould, *Neurosporacrassa*. Major insights like the nature of the gene, autophagy, control of cell cycle, and how telomeres function have been made using morphologically simple organisms with complex cellular machinery similar to human cells. Because of their typically small genome sizes and life cycle stages with free-living haploid states, fungi have served as models for genome evolution and reconstruction of phylogenetic relationships using genome scale data.

Although fungi are relatively understudied, they are an essential, fascinating and biotechnologically useful group of organisms with an incredible biotechnological potential for industrial exploitation. Hyde and co-workers (Fungal Diversity, 2019, 97: 1-136) have recently detailed 50 ways in which fungi can be exploited. As compared to other biological systems (*e.g.* plants), fungi have the great advantage that they can be grown in large bioreactors on an industrial scale.

The laudable attempt of editing a book entitled '**Sustainable Utilization of Fungi in Agriculture and industry**' is a welcome step by Dr. S. Ajmera and others. This book comprises 22 chapters, on various aspects of fungi, contributed by those engaged in teaching mycology/microbiology/biotechnology in various academic institutions. I wish to place on record my appreciation for the editors of the book. I sincerely hope and wish that graduate and post-graduate students, scholars and teachers in broad areas of botany, microbiology, biotechnology and Life Sciences will find this book useful.

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PREFACE

Fungi and their application in Agricultural or Industrial Microbiology are being utilized for food security, providing a solution to produce bioenergy, chemicals, and other bioproducts from renewable resources. The fungi that are employed in sustainable agriculture and industrial microbiology are able to secrete compounds that are useful for plant growth promotion, thereby producing organic acids, hydrolytic enzymes, functional bioactive compounds and biocatalytic agents with promising applications in various economically important fields such as environment, agriculture, food and dietary, biotechnology, medicine, pharmaceuticals, and associated sectors.

The present book, "Sustainable Utilization of Fungi in Agriculture and Industry" covers the investigations of different fungal microorganisms (filamentous fungi and yeast) and their potential application in the production of industrial enzymes, organic acids (citric acid, lactic acid, *etc*), biofuel (ethanol, H_2 gas) and bioactive compounds for diverse processes targeted at agriculture, bioremediation, industries, therapeutics, and diagnostics.

I strongly feel that the leading researchers with extensive, in-depth experience and expertise in fungal microbial biotechnology took the time and efforts to develop the outstanding book chapters. Each chapter is well written and explained so that the reader is given an up-to-date and detailed account of knowledge on fungal microbes and their sustainable utilization in agriculture, white biotechnology, and other innumerable industrial applications. I am very much sure that this book will be of great interest to the students, scientists, Ph.D. and postdoc researchers of life sciences especially, microbiologists, biochemists, microbial and fungal biotechnologists who are involved in investigations on fungal diversity and its sustainable utilization in agriculture and industry.

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I am pleased to acknowledge Prof. Tulasi Satyanarayana, Professor Emeritus Netaji Subhas University of Technology, New Delhi, for his expert direction and foreword message for this book. I am extremely thankful for his incredible support.

I thank all authors for their great association and contribution that helped in this herculean task of bringing information in different aspects of mycology.

Sincere regards

Chief Editor
Shanthipriya Ajmera

DEDICATION

We all have dreams, but making dreams a reality takes a lot of determination, commitment, self-discipline, and effort. This book is dedicated to my parents & family for always loving and supporting me.

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CHAPTER 1

The Versatile Potential of Fungi in Human Life and Ecosystem

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Abstract: Fungi play a major role in the well-being of human life as they are involved in health and nutrition processes on a large scale and are a major component of the global economy. Furthermore, they are the natural nutrient recyclers in the environment and thus balance the ecosystem. Also, through mycorhizal relationships, the fungi help in enhancing soil fertility by increasing the surface area for absorption of nutrients such as phosphorous, nitrogen, sulfur, etc., and other minerals such as zinc and copper. As fungi interact with various plant pathogens affecting crop production, they can be used as Microbial biological control agents or biopesticides and can be replaced for the usage of hazardous chemical pesticides for controlling plant pathogens. Here, we tried to explain the fungal importance to mankind and the ecosystem by listing its various applications in human life.

Keywords: Agriculture, Antibiotics, Biopesticides, Biofertilizers, Enzymes, Fungi, Nutrients, Pharmaceutical industries, Prominent source, Vitamins.

INTRODUCTION

Fungi are either single-celled (yeast) or multi-cellular (hyphae) eukaryotic heterotrophs. They help in balancing the ecosystems by acting as decomposers in a wide variety of habitats. On the other hand, fungi are responsible for diseases as they directly interact with other organisms, mostly plants and bacteria [1].

Fungi frequently grow in a dark and damp environment rich in decaying debris from plants and animals. Fungi release elements such as nitrogen and phosphorus from decaying organic matter because of their mode of nutrition (*i.e.*, produce enzymes to digest the matter and then after ingestion). Many habitats have these

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elements in low amounts but are required in large amounts for other living organisms to live and are mostly supplied by the fungi [1, 2].

Fungi are known for their utilization in the production of various foods and beverages. Fungi are found to involve in many industrial fermentative processes such as the production of single-cell proteins (SCP), antibiotics, enzymes, vitamins, etc., and have a major impact on the global industry, mostly in the area of health and nutrition [3].

In this chapter, we tried to elucidate the importance of Fungi in Human Life by listing out the various applications of fungi.

IMPORTANCE OF FUNGI IN HUMAN LIFE

The fungi are in usage for food, preservation, or other purposes by humans in various ways and are listed below.

As a Food

Industries utilize fungi in various processes for manufacturing large varieties of food useful for mankind.

Fungi are involved in the fermentation of grains to produce beer and fruits to produce wine, where they ferment sugars into ethanol and produce carbon dioxide under anaerobic conditions. For example, *Saccharomyces cerevisiae*, a single-cell fungus also known as baker's yeast/brewer's yeast, is an important ingredient for the production of wine, beer, and bread along and other wheat-based products like pizza, along with many applications in medical research. Another example is *Aspergillus oryzae*, involved in the production of a Japanese beverage called “Sake” by the fermentation of rice. The fungal species like *Aspergillus oryzae*, *Pediococcus soyae*, *Saccharomyces rouxii* (Fig. **1**) are used in soy sauce production [4].

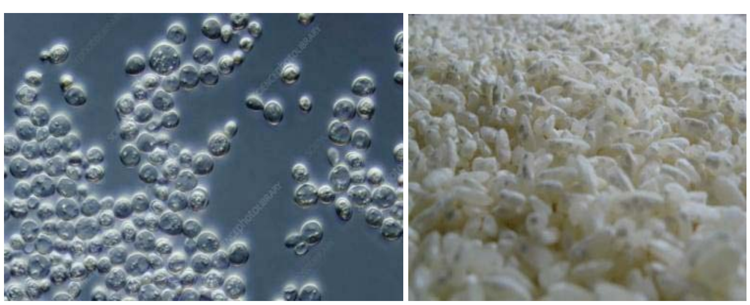

Fig. (1). i. *Saccharomyces cerevisiae,* ii. *Aspergillus oryzae* (Image source: en.wikipedia.org).

Several species, such as the *Agaricus bisporus* and the Portobello, produce button mushrooms for consumption, and other species such as *Pleurotus*, *Lentinus edodes,* and *Auricularia* (Fig. **2**) are produced dominantly. Many other mushroom species such as morels, chanterelles, truffles, Milk mushrooms, porcini mushrooms, and black trumpets all demand a high price on the market due to their high protein and low calorific value [5].

Fig. (2). i. *Agaricus bisporus*, ii. Pleurotus ostreatus, *iii. Lentinus edodes, iv. Auriculariaauricula-judae* (Image source: en.wikipedia.org).

For certain types of cheeses, fungal spores are added to impart a unique flavor and textures to the cheese, for example; the blue color in cheeses (Fig. **3**) such as Stilton and Roquefort is imparted by *Penicillium roquefortii*. Other examples of colored cheese are Gorgonzola, Stilton, and Danish Blue cheese [6].

Fig. (3). Bleu de Gex, a creamy, semi-soft blue cheese made in the Jura region of France (source: en.wikipedia.org https://en.wikipedia.org/wiki/Blue_cheese).

As a Prominent Source of SCP

The SCP or microbial proteins are the biomass or protein extracts from pure or mixed cultures of microorganisms such as algae, yeasts, fungi, or bacteria that may be used as a substitute for protein-rich foods. These are edible unicellular microorganisms suitable for human consumption or as animal feeds [7].

In recent years fungi have been utilized as rich sources of SCP and are now available commercially as human food. The SCP produced from fungi are advantageous over other microorganisms due to their low nucleic acid content, cholesterol, and fat since it contains no animal ingredients. As the fungal mycelium can be processed to give an appearance and 'mouth-feel of meat, it has the advantage of being suitable for vegetarians and those on low-calorie diets. In

CHAPTER 2

Mycobiota - Role in Soil Health and as Biocontrol Agent

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Abstract: Soil health or soil quality is governed by a continuous, functional interplay between the soil and its microbiota, plants and animals. Soil quality is crucial for sustainable agriculture production and for nurturing the health of all living organisms. It is therefore in the best interest of society to prioritize sustainable soil management practices for future generations. Microbes play a vital role in maintaining ecosystems by coordinating with plants to facilitate nutrient and organic matter cycling. A consortium of fungi plays a critical role in degrading and transforming dead organic matter into suitable forms that can be reused by other organisms. As ecosystem regulators, fungi enhance the structure of soil formation and regulate physiological processes within the soil, making it a supportive habitat for other living organisms. They also help in controlling plant diseases and pest infestations by acting as biocontrol agents. Understanding the roles of fungi and soil enzymes in the earth's biogeochemical cycles can facilitate improved agricultural productivity and sustainability. For example, increasing the diversity of beneficial fungi in a habitat improves soil fertility, supporting sustainable production of plant based products while mitigating the application of undesirable chemicals as pest control agents.

Keywords: Biocontrol agents, Ecosystem regulators, Fungi, Soil enzymes.

INTRODUCTION

Sustainable agriculture production is dependent on soil quality and health. Soil is fragile, finite and precious, which is why it is necessary to raise awareness with special attention for its protection, both by its users and consumers. Soil fertility is the ability of soil to assist plant growth with a fruitful outcome in terms of sustainable and measurable yields with improved quality [1].

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Processes Contributing to Soil Fertility

Healthy soil supports healthy food production. The majority of diversified microbial species have the potential to cleave a variety of bonds in chemicals. This reflects their ability to govern the important soil properties and functions [2].

A sequence of processes involving continuous cycling between the organic and inorganic forms of nutrients helps in the development of sustainable, healthy, and fertile soil. This involves a network of processes including mineralization, immobilization and cation exchange:

1. Mineralization- Decomposition of plant debris and animal wastes by microbes liberates nutrients into the soil in its inorganic form by a process called mineralization.
2. Immobilization- Microorganisms transform these available micronutrients like phosphorus, nitrogen or potassium by associating with the microbial biomass through a mechanism often referred to as immobilization.
3. The existence of an equilibrium between the above two processes depends on the accessibility and balanced availability of the major nutrients along with the soil carbon in its organic form to the microorganisms [3, 4].
4. Occurrence of natural unwelcomed phenomena like lightning strikes allows fixation of atmospheric nitrogen in its nitrite form. Sometimes, the presence of denitrifying bacteria under anaerobic conditions like flooding can reduce these nitrogen derivatives.
5. Cation exchange- Micronutrients that are cations like potassium can form electrostatic bonds with the negatively charged components in the soil.

Our planet is a diverse habitat for living organisms and is associated with an intricate food web, facilitated by healthy soil. Healthy soil is comprised of living and non-living matter. The living matter is characterized by rich and abundant microbiota, and the non- living counterparts consisting of organic nutrients. Healthy, fertile soils are resistant to outbreaks of soil-borne infestation [5]. For example fertile soil has reduced and prevented the damage caused by pests as observed in maize stem borers [6], and healthy soil enriched with organic matter can enhance crop productivity.

Healthy soil does not contaminate our environment; rather, it alleviates changes in climate. Growing plants on healthy soil can remove atmospheric carbon dioxide; that is, it reduces greenhouse gas emissions and keeps the carbon underground. Also, healthy soil absorbs and stores water underground that could prevent flooding. The development of healthy soil is dependent on soil structure. It regulates water holding capacity and root depth. Plants uptake nutrients in a water-soluble form and follow biological, chemical, and physical processes for nutrient modifications and exchange it with nature. Plants with the help of microbes like bacteria and fungi acquire their essential nutrients from the soil, like fixing atmospheric nitrogen through root nodules in leguminous plants and mycorrhizae, a symbiotic association between fungi and plant roots.

To keep the soil as a healthy living system and thereby enhancing crop production, factors like improved soil structure with better nutrient and water holding capacity, a symbiotic association of microbes with plant roots to recycle nutrients, the existence of various communities of microbiota to reduce soil-borne infections plays a vital role [7].

Soil Microbiota

About 2-4 billion years ago, ancient microorganisms must have developed within Earth's oceans. They utilized atmospheric nitrogen, increased in number and slowly liberated oxygen [8, 9]. This new environment was a starting material for more diversified microorganisms to grow and develop [10, 11]. These principal investors are now the key contributors in the construction of soil structures, which make them a healthy and fertile resource for other living organisms. Soil is a reservoir of microorganisms like bacteria, actinomycetes, fungi, algae and protozoa and their functions have a direct effect on the properties and functions of soil [12].

Among all the microbes, fungi are also plentiful in soil. Some of them are beneficial as they have a symbiotic relation with plants and are helpful in soil health. Organic materials in soils are utilized by fungi for their nutrition and growth. Fungi can grow in extreme conditions like acidic regions, dry and arid soils and also places that are high in moisture [13].

Etymology

Fungi are a member of the eukaryotic organisms that include both microscopic yeast and molds and macroscopic structures like mushrooms [14]. A fungus or *Eumycota* in Greek (*eu* means true and *mykes*- fungus) [15] has been directly adopted from the Latin word meaning Mushroom [16]. A characteristic that differentiates fungi from other organisms is the presence of glucan and chitin in their cell wall [17, 18]. As heterotrophs, they have absorptive nutrition. They absorb dissolved nutrients by secreting extracellular digestive enzymes into the environment, and this is caused by the absence of chlorophyll. They are spore-forming organisms and have both sexual and asexual types of reproduction. They are of special interest as they are principal decomposers in different ecosystems. A scientist working on fungi is a mycologist and the discipline of biology

CHAPTER 3

Fungi Role in Soil Fertility

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Abstract: Fungi are an important component of microbial ecology. These are a community of eukaryotic species and food sources, organic acids, alcohol, antibiotics, growth-enhancing compounds, enzymes, and amino acids. Fungi contain many microorganisms under the expanse, which include molds, yeasts, and mushrooms. The function of fungi primarily includes the biodegradation of organic waste material and the action on cellulose, lignins, gums, and other organic compounds. Fungi are present everywhere, *i.e.*, they are cosmopolitan, surviving the wide range of pH and temperature. Fungi play a key role in numerous biological cycles, including mineral and water absorption, chemical transition, stomatal activity, and biosynthesis of substances, including biostimulants, auxins, lignan, and ethylene, increasing the functionality of plant species to identify and cope with adaptive challenges, such as drought, salinity, humidity, cold and significant metals. The interaction of fungi with plants can be represented by three terms, namely, mutualism (mycorrhizal association), pathogenicity (parasitic fungi), and nutrient cycling (saprophytic fungi). The application of cultivating fungi as a soil quality booster has been highlighted as it produces various extracellular enzymes, which are integral components of nutrient cycling within natural and disturbed ecosystems.

Keywords: Assimilation, Mobilization, Mycorrhizae, Nutrients.

INTRODUCTION

Generally, fungi are multi-celled organisms that grow quickly, favour the acidic range of conditions, and live in low disturbance regions of the soil profile. These features allow fungi to grow in the soil to uptake nutrients from relatively distant areas and transfer them back to the plants [1]. In India, the slightly acidic conditions favouring the soil are loamy soils with pH 5.2 and 5.9 [2]. The soil pH promotes fungal growth and improves production, thus contributing to plant

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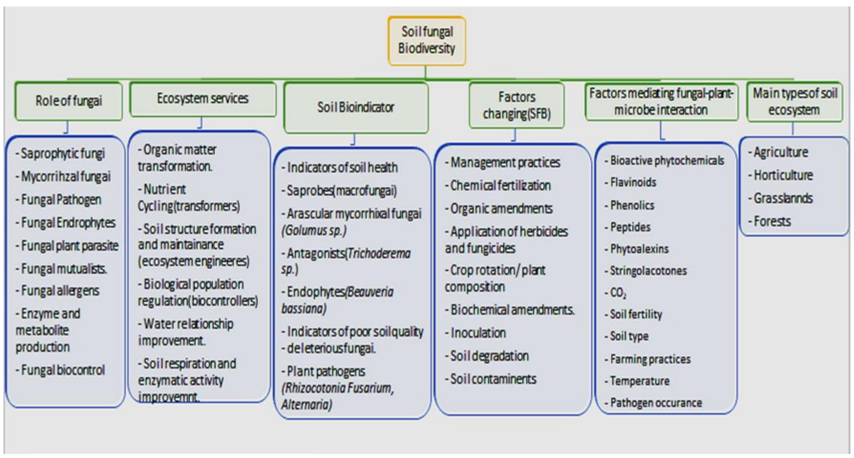
nutrition and soil recovery. Fungus improves the fertility of the soil and enriches the soil with nutrients.

A map using GIS and remote sensing is shown in Fig. (**1**), where loamy soil data is collected. Regarding India's soil coverage, 1-10000 units represent the soil enrichment in the following area.



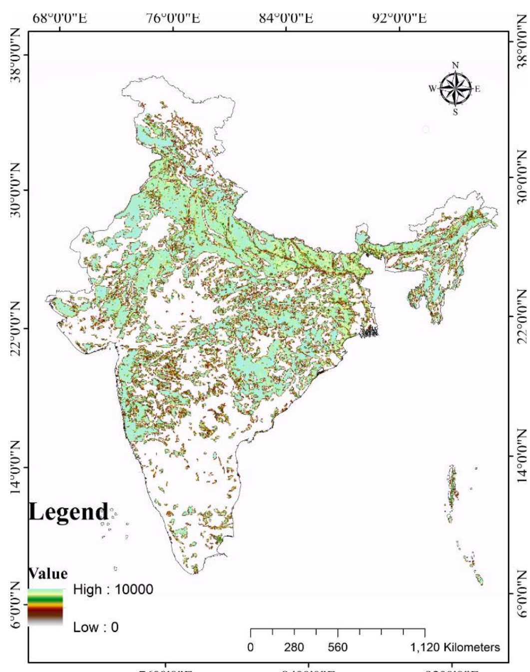


Fig. (1). Loamy soil data of India.

Link of Fungi with Soil Fertility

Despite the fact that fungi cannot fix atmospheric nitrogen alone, they provide fundamental supplements, like carbon and phosphorous, to microbes who perform the function of nitrogen fixation. Particularly, in moderately degraded soil, arbuscular mycorrhiza parasites provide the phosphate needed by the nitrogenase enzyme for nitrogen fixation [1].

Soil microbes, including fungi, are significant ecosystems because of their biochemical activity and nutrient cycling engagement [2].

The soil fungi are primarily classified into three functional classes, namely a) biologic controllers, b) regulators of the environment, and c) species interested in the decomposition and transformation of organic matter.

Ecosystem regulators are majorly concerned with soil composition. Monitoring pathogens and parasites and the development of other species is done *via* biological controllers. Moreover, fungi participate in nitrogen fixation, hormone production, biological control against root pathogens, and protection against drought [2].

In the preservation of soil, the decomposition of organic matter and the residues is done *via* fungi. Ascomycetes predominate in agricultural soils, while basidiomycetes play an important role in the decomposition of dead and organic matter in grassland areas. Human interactions and their practices can also improve soil fungal biodiversity; however, long-term fertilization and other treatments are major pitfalls [2]. Fungi play a significant role in optimizing soil health by increasing the organic matter that enhances the production of N, P, K, Fe, and other nutrients in the soil [5 - 12]. Organic acids are also produced to mobilize nutrients and promote their absorption from the rhizosphere. The role of fungi in enhancing the bioavailability of nutrients is represented in Table **1** [1].

Table 1. Role of fungi in nutrient bioavailability and aggregate formation.

S. No.	Nutrient/Structure	Fungi	References
1.	Nitrogen (N)	Fungi do not fix N but provide growth-limiting nutrients (*i.e.*, carbon and P) to N fixation bacteria. Also, in mycelium, fungi provide shelter to the bacterial enzyme system from O_2 to fix N	[3 - 5]
2.	Phosphorous (P)	Increases P bioavailability through mineralization in soil and mycelia transport. Enhances P solubilization by siderophores, N assimilation, and CO_2 release.	[6, 7]

CHAPTER 4

The Symbiotic Relationship Between Fungi and Plants

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Abstract: There are many non-saprophytic fungi that are involved in symbiotic relationships with higher plants, which include both mutualism and parasitism. The most common mutualistic relationship involving fungi belonging to *Ascomycota, Basidiomycota,* or *Zygomycota,* and roots of 90% of all vascular land plants is termed mycorrhiza. In this association, the fungus grows on ectomycorrhiza or in endomycorrhiza, the roots of most terrestrial plants. The mycorrhizal fungus benefits from the carbohydrates that the host plant provides, whereas the latter is benefitted from the extensive fungal mycelia that have a greater surface area and penetrate not only deep down but also go distantly within the soil to procure water and water-soluble essential nutrients, especially nitrogen and phosphorus, for the host. Also, the mycorrhizal fungi offer increased protection to their host plants against some phytopathogens. As mycorrhizal associations are found in early fossil records, it is believed that they allowed the early terrestrial plants to colonize and survive. Another fungus–plant mutualism involves the endophytic fungi that usually dwell inside the tissues of the host plant, release toxins to repel the herbivores, and also impart resistance to the plant against environmental stresses. On the other hand, fungi parasitic on host plants live in or on them, use specialised structures called haustoria to procure nutrients from them, and produce necrosis-promoting enzymes. This may lead to some of the most devastating diseases in the crop plants, eventually killing them and severely affecting the country's economy.

Keywords: Endophytic fungi, Higher plants, Mutualism, Mycorrhiza, Parasitism.

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Shanthipriya Ajmera, B. Bhima, Krishnappa M. and Ramchander Merugu (Eds.)

INTRODUCTION

Commonly, the term ‘symbiosis’ is applied to denote a close ecological association between any two phylogenetically distant species that live together and are consequently mutually benefitted. In such associations of obligatory nature, neither of the partners can survive independently if the two are somehow separated [1].

Plant-fungi Mutualism

When both the partners of a symbiotic association benefit, the relationship is called ‘mutualism’. Not all fungi are saprophytic, *i.e.*, feed on dead organisms. Many of these fungi form mutualism with cyanobacteria, algae, plants, and animals. Fungi are heterotrophic organisms, and can easily absorb macroelements, in particular, phosphorus and nitrogen. Plants, on the other hand, are autotrophs, which are capable of photosynthesis [1, 2].

Plant-fungi mutualism is classified into two principal types, mycorrhiza and endophytes.

MYCORRHIZA

Definition

Many plants form associations with fungi, which are called ‘mycorrhizae’ (Greek ‘*myco*’ means fungus and ‘*rhizo*’ means root) (Fig. **1**), which helps most of the earth’s terrestrial plants to survive against all odds [1, 2].

Fig. (1). *Mycorrhizal* fungi on plant roots (Photograph Courtesy: Paula Flynn, Iowa State University Extension).

Exceptions

Although the literal meaning of the term *'mycorrhizal'* is 'root fungus,' but in some cases, the interaction may also occur between fungi and plants without roots, such as *Psilotum*, liverworts and mosses.

Conditions Promoting Mycorrhization

Under natural conditions, the soil sometimes lacks the essential macroelements needed by plants. Also, very often, the plants cannot obtain and absorb the available nitrogen and phosphorus. In addition, when there is a rapid soil solution uptake, low diffusion rate, low soil moisture, or low nutrient concentration, a nutrient depletion zone may develop around a plant root system in the soil. Under all these conditions, mycorrhization is promoted in a land plant.

Conversely, in the agricultural soil, which is nutrient rich, plants sometimes may even reject the mycorrhizal fungus, as they usually do not require it for better growth.

Co-evolution of Land Plants and Mycorrhizae

Around 80-90% of all vascular land plants on earth today live in association with mycorrhizal fungi [3]. It is believed that the early colonization of land by terrestrial plants was much dependent on their symbiosis with fungi for a better nutrient uptake opportunity. According to the scientists, fungi had been the key role players in the process of evolution of the plant root system and majorly contributed to the successful colonization of land by angiosperms [3]. Gathered fossil records indicate that bryophytes like mosses and liverworts were the most primitive plants and could maintain their survival on drylands only because of their association with the *mycorrhizal* fungi, which actually preceded terrestrial plants [3]. One of the early associations between fungi and phototrophs included the moss-like plants and the endophytes. According to the fossil records, it is this intimate mutualism that has allowed early land plants like *Aglaophyton major,* a primitive non-vascular terrestrial plant, to establish themselves successfully on the land (Fig. **2**) [3].

Researchers have established that many species of the earliest groups of land plants are symbiotic with fungi which are the members of Mucoromycota, and not Glomeromycota, as initially proposed [4]. It was believed since the 1970s that Glomeromycota was the oldest known endomycorrhizal fungi [4]. This was established from the observation that vascular plant fossils of the early Devonian age (400 Ma) show arbuscule-like structures [4]. Later, however, it was found that

CHAPTER 5

Fungi and their Importance in Sustainable Agriculture

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Abstract: Soil fungi constitute a key component of agroecosystems because they provide green services that influence food and bio-product production. Entophytic fungi play a major role in growth promotion and relieve biotic and abiotic stresses in plants. Phyto-hormones, antimicrobial agents, agrochemical bioactive metabolites produced by fungal endophytes enhance sustainable agriculture. Because of soaring cost and pollution-related issues, interest has shifted towards various methods like the use of biofertilizers and bioinsecticides to control pests. Biofertilizers and bioinsecticides are safe and cost-effective alternatives to chemical pesticides and are considered high potential fertilizers because of their wide range of plant growth-promoting activities. The present chapter describes the role of endophytic fungi in the agriculture sector.

Keywords: Fungal Endophytes, Phyto-hormones, Plant growth Promotion, Sustainable Agriculture.

INTRODUCTION

As a result of forest clearance, high agricultural inputs, and continued usage of land resources for several unsustainable purposes, most of the world's habitats are proclaiming deforestation, low fertility, and productivity, poor quality of water, *etc*.

Sustainable farming, however, produces high yields without causing vulnerability or harm to the natural ecosystems and assets on which yield depends. This method of farming uses a special cultivation technique in which it is possible to completely utilize natural resources and at the same time ensure that no damage has been caused. The output approach is environmentally friendly and ensures

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safe and stable farm crops and goods. The status of microbial soil communities benefits vital processes, which impel agricultural systems to concentrate on fertility, productivity, and stability. Several studies are aimed at improving awareness of the diversity, function, potentials, and importance of soil microbial communities and their beneficial and collaborative activities in the fertilizing soils and the productivity of crops. There is growing evidence showing the ability of various microorganisms in agricultural systems that enhance plant growth and productivity. Knowing the potential of soil-beneficial microorganisms requires an understanding of the activity of microorganisms in growth enhancement, especially in terms of nutrient supplement and disease resistance mechanisms and the difficulties of plant growth-promoting (PGP) microorganisms in the implementation and commercialization. Soil microorganisms have a similar relationship to plants. The majority of them are abundant in the soil biota and are responsible for promoting cycling of nutrients and organic matter, soil fertilization as well as regeneration, plant healthiness and development, and primary production of ecosystems. Among various microbes, two of them include mycorrhizae and rhizobia. Mycorrhizae are considered to be very host-specific fungi that establish symbiotic associations with host plant roots. Some of the fungi may not cause the root surface area to be colonized by the deleterious fungi and are useful as biocontrol agents.

The microbial ecology is an important aspect of fungi. The lignin is hard-to-digest soil organic matter and is decomposed by the majority of fungi, although some of the fungi eat simple sugars. In low pH or lightly acidic soils, fungi dominate where soils appear to be undisturbed [1]. This relationship of being beneficial to each other is called a network of mycorrhizae [2].

Hyphae interact in a filamentous body, which supports soil nutrient forage with soil particles, roots, and rocks. Most of them are either yellow or pure white and are sometimes misidentified [3] as plant hair roots. The rhizosphere is an area dominated by soil microbes next to the root, where many chemical and biochemical processes take place. There are usually fewer individual fungi than bacteria, but because of their greater size in healthy soil, fungi dominate the overall biomass. Soil fungal biomass weighs around 1,100 to 11,000 pounds in healthy soil, which is equivalent to two to six cows [4].

Fungi lodge in slightly acidic habitats, soils having low disturbances, plants with perennial material, internal plant nutrient sources, and very stable types of high carbon-nitrogen organic residue (C: N) values and a slow recycling period. In mostly disrupted environments having rapid nutrient recycling, low C: N values, annual trees, and external plant nutrient additions, bacteria dominate.

This helps soil fungi to bridge gaps to carry nutrients back to the plants at relatively far distances [5].

At least 70,000 different fungal species have been reported, but it is evaluated that 1.5 million species could exist worldwide [6, 7]. Genetically, a trillion years ago, fungi grew in close contact with plants and animals. Fungi have 80 percent or more of the same genes as humans [8, 9]. Four main soil fungal classes exist; Ascomycota, Basidiomycota, Deuteromycota, and Zygomycota. Basidiomycetes comprise fungi, toadstools, and puffballs, while lichens and mycorrhizal fungus are found in Deuteromycota [10]. Heterotrophs are known as fungi, so the origin of carbon stems from the decomposition of organic compounds or residues [11].

SOIL FUNGI

Soil fungi are microscopic plant-like cells that form the structures or hyphae of a long thread which forms a mass called mycelium. The mycelium consumes nutrients, surface organic matter, or soil from the roots it has colonized. Special hyphae that generate the reproductive spores are generated. Some fungi are single-celled (yeast). There are several structures for fungi, but they can function in similar ways and are thus not as plant-determined as other soil bacteria such as Rhizobia. Three functional classes of fungi exist, decomposers, mutualists, fungi mycorrhiza.

Decomposers

Decomposers or saprophytic fungi turn dead organic matter into organic acids, carbon dioxide, and fungal biomass (*i.e.*, their bodies). They play a vital role in immobilizing and preserving nutrients in the soil by eating nutrients in organic matter. The organic acids they manufacture as products help generate degradation-resistant organic matter. Some fungi are willing to degrade cellulose, lignin, and protein, and are highly resistant to breakdowns.

Mutualist

These fungi establish connections with plants that are mutually beneficial. They colonize the roots of plants, where they assist the plant to get nutrients from the soil, such as phosphorus.

CHAPTER 6

Plant Growth-promoting Fungi: Mechanisms and Applications

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Abstract: Soil is one of the main habitats of fungi and bacteria, and their interactions with the host plant help and promote plant growth and productivity in agriculture. Agronomists and environmentalists are focussing on sustainably managing the agroecosystem by using plant growth-promoting microbes (PGPM). They include bacteria called plant growth-promoting rhizobacteria (PGPR) and fungi referred to as plant growth-promoting fungi (PGPF). Plant growth-promoting fungi (PGPF) are nonpathogenic soil-borne fungi that establish a positive interaction with plants in the rhizosphere. PGPF can improve crop productivity by improving seed germination vigour root and shoot morphogenesis, and flowering through direct or indirect mechanisms, which include solubilizing nutrients, regulating hormones, producing enzymes, organic compounds, resistance to abiotic stress, and suppressing phytopathogens. The utilization of PGPF can be considered an eco-friendly method of improving crop production. The potential effective microbes may also be added in the form of biopesticides and biofertilizers promoting the growth of plants. The use of new biotechnological tools to aid genetic engineering of the PGPF has resulted in genetic transformation and overexpression of synergistic action of one or more traits, which could enhance plant growth, confer improved crop benefits, and produce sustainable yield. The chapter describes the diversity of PGPF found in the soil and their role in promoting plant growth and yield. The chapter also discusses the various mechanisms of PGPF interactions with plants and their beneficial roles in promoting crop productivity leading to sustainable agriculture.

Keywords: Biofertilizers, Crop Yield and Productivity, Soil Fungi, Sustainable Agriculture.

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INTRODUCTION

Soil is associated with air, water, rocks, and organisms and is a niche for many different activities carried out in natural ecosystems. Soils play a major role in improving the quality and composition of air and water, regulation of temperature, biogeochemical cycling of carbon and other elements, waste decomposition and remediation, recycling of nutrients, and functioning all ecosystems in the world. Soil is one of the most composite and highly changeable habitats on earth and comprises an enormous diversity of organisms, including soil fauna such as microscopic and macroscopic animals and soil microorganisms like bacteria, fungi, *etc*. Many physical and chemical properties of the soil, such as moisture, temperature, pH, *etc.*, are important determinants that influence the survival, replication, and functions of the soil biota. Along with soil variability at different locations and changes occurring with time, organisms living in the soil have to adapt themselves to the frequently changing conditions. Based on body width, soil biota can be classified into 4 types: i) macrofauna (500 µm–50 mm, *e.g.*, earthworms, termites), ii) mesofauna (80 µm–2 mm, *e.g.*, acari, collembolan), iii) microfauna (5– 120 µm, *e.g.*, protozoa, nematodes), iv) and microflora (1–100 µm, *e.g.*, bacteria, fungi) [1].

Soil biota plays a key role in the liberation of plant-available nutrients through continual and interrelated food webs. Plant roots serve as a hotspot of ecological richness and host a vast range of microorganisms in the rhizosphere region [2]. The soil food chain is a complex interaction among a wide variety of organisms that significantly impacts all facets of the soil environment. The organic matter decomposed by soil biota liberates the nutrients bounded up in organic matter and helps in the recycling of nutrients. They interact with each other, the plant roots, and the environment. The effects of this process are not simple because the nutrients liberated are also available for uptake by bacteria, fungi, protozoa, nematodes, and microarthropods living on or in the vicinity of roots. All of these organisms compete with roots for the uptake of these mineral nutrients. A self-renewing ecosystem capable of sustaining and improving plant growth and yield can be conserved by maintaining a healthy soil ecosystem. Hence, soil biotic communities and soil health are to be focused on enhancing soil function and long-term sustainability.

Ecosystems are the basis of the existence of all organisms' activities. Among various ecosystems, agroecosystems are the most intensively managed natural ecosystem that has been adapted for the production of food and fiber. To fulfill the growing demands for food, agricultural practices extensively use large amounts of chemical-based fertilizers and pesticides, which ultimately lead to land degradation and biodiversity loss. Agronomists and environmentalists are focusing on sustainably managing the agroecosystem by using plant growth-promoting microbes (PGPM). The plant growth-promoting microbes are defined as a heterogeneous group of beneficial microorganisms that can be found in the rhizosphere, on the root surface and that are capable of protecting against phytopathogens and abiotic stresses and also enhancing the plant growth [3 - 5]. Plants partially live with microbes and allow them to penetrate all of their tissues, and the resulting assemblage of microbes is mutually known as the plant-microbiome or phytomicrobiome [6, 7]. The root organization is important for plant development because plant roots encourage the absorption of water and nutrients, provide anchorage of the plant body, and provide overall growth of the plants. Also, a variety of compounds secreted by plant roots act as chemical attractants for various types of soil microorganisms. The zones of activity created around the root, *i.e.*, the soil region, where processes mediated by microorganisms are specifically influenced by the root system is called the rhizosphere. In this zone, plant growth or toxic substances can be produced, but most of these organisms are beneficial. Roots function as the connecting link between the plants and soil microbes. The application of several microbes in agricultural practices has shown to be beneficial for plant growth and development, and suppression of plant pathogens without environmental contamination. They include bacteria called plant growth-promoting rhizobacteria (PGPR) and fungi referred to as plant growth-promoting fungi (PGPF).

PLANT GROWTH PROMOTING FUNGI (PGPF)

Plants are associated with diverse types of microorganisms, especially in the rhizospheric region. These microbes play multifaceted roles in transforming, mobilizing, and solubilizing soil nutrients, which are vital for plants' growth and development and bring about changes in plant metabolism and provide protection from phytopathogens. Amongst the various types of microorganisms present in the rhizosphere region of plants, plant growth-promoting fungi (PGPF) are gaining curiosity and attention in recent times. PGPF exert many positive effects on plants and the environment, thus making them employable in organic farming and cultivation. The group of rhizosphere fungi that colonize plant roots and help in plant growth is known as plant growth-promoting fungi (PGPF). PGPF is a heterogeneous group of nonpathogenic soil-borne fungi that live in the rhizosphere or colonize on/in the root surface and establish a positive interaction with host plants and facilitate the increase in seed germination, seedling vigor, flowering, growth, and yield of plants [8]. They are heterogeneous, non-pathogenic saprophytic fungi that can be endophytic and epiphytic; they maintain a non–obligate mutualism with a wide range of host plants of the diverse taxonomic group. They are mostly involved in multiplex interactions with plants

CHAPTER 7

Mycorrhizae as Bioinoculants and their Molecular Studies

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Abstract: Several studies on bioinoculants have been conducted in order to address the most serious threat to our nation's security, namely food security. This problem can be solved by re-evaluating our practises and skills in order to increase food production through the use of sustainable farming methods. Several microorganisms can be found in rhizospheric soils in nature. These organisms solubilize nutrients and facilitate nutrient uptake by plant roots, promoting plant growth through natural processes such as nutrient cycling and absorption, stress tolerance, immunity induction, and so on. Microbes include mycorrhizae, cyanobacteria, bacteria, actinomycetes, and others. Plant growth-promoting microorganisms rely heavily on mycorrhizae (PGPM). So far, seven different types of mycorrhizae have been identified. The arbuscular and ectomycorrhizal fungi are the most numerous and widespread mycorrhizae. Arbuscular mycorrhizae (AM) fungi are important in improving soil physicochemical properties and interacting with other PGPM such as phosphate solubilizing bacteria, nitrogen fixers, and so on. Chemical fertilisers used in agricultural fields reduce AM and its potency. Because these organisms cannot be cultured, PCR techniques can be used to obtain sufficient amounts of DNA from them. The focus of this chapter has been on arbuscular mycorrhizae (AM) fungi. An attempt was made to study AM fungi colonisation using PCR in order to better understand its efficiency. Such research contributes to the goal of transforming food insecurity into food security.

Keywords: Arbuscular mycorrhizae, Bioinoculants, PCR, PGPM, Sustainable farming.

INTRODUCTION

Mycorrhiza literally means “fungus root.” In nature, there are various types of mycorrhizal associations. The most common and widely distributed plant kingdom type is vesicular-arbuscular (VA). It produces fungal structures called vesicles and arbuscules in the cortex region of the plant root, giving rise to the

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name Vesicular-Arbuscular mycorrhiza (VAM). Previously, it was simply known as Arbuscular mycorrhiza (AM) [1].

Many researchers were initially unaware of mycorrhizae, but later findings by Baylis, Gerdemann, and Mosse on the significance of VAM in boosting phosphorus uptake by plants brought VAM into the spotlight. Biological control of root infections, hormone synthesis, and drought resistance are all part of their involvement in plant growth promotion. As a result, researchers working to expand and direct the impacts of mycorrhizae in agriculture have focused on AM.

Because mycorrhizae are obligate symbionts, they can't be cultured in their purest form in the lab, and large-scale replication is impossible. Only a few researchers have attempted to cultivate these creatures on specialised media. They have, however, had limited success [2, 3]. As many as 250,000 different plant species can produce arbuscular mycorrhizal (AM) symbioses. Only 150–200 AM fungus species have been identified based on morphology, but DNA-based studies show that the true diversity of AM symbionts could be substantially higher [4, 5]. Arbuscules, highly branching fungal structures that grow intracellularly without breaching the host plasmalemma, characterise the relationship.

The ability of many mycorrhizal fungi to mobilise nutrients from complex organic sources is a necessary step in the development of nutrient cycling models, particularly in ecosystems with low nutrient availability [6]. Enzymatic activities of mycorrhizal mycelia and wider recognition of the ability of many mycorrhizal fungi to mobilise nutrients from complex organic sources are reviewed. Fungal hyphae are also significantly thinner than roots, allowing them to penetrate tiny pores [7]. Carbohydrates and mineral nutrients are then exchanged inside the roots as the plant and the fungus come into contact. The root cortex is colonised solely by AM fungal hyphae, which create highly branched structures inside the cells called arbuscules, which are thought to be the functional location of nutrition exchange [8]. AM fungus can have a direct impact on the environment by improving soil structure and aggregation [9 - 12] and driving plant community structure and productivity [13]. Recent research has looked into the impact of AM symbiosis on greenhouse gas (GHG) emissions [14, 15]. A brief summary of various types of mycorrhizal association is given in Table **1**.

Table 1. Brief summary of characteristics of various mycorrhizal associations.

Type/ Name of Association	Partner		Intracellular Colonization	Fungal Sheath	Hartigs Net	Vesicles
	Fungi	Plant				
Ecto	Basidiomycotina Ascomycotina	GymnospermsAngiosperms	Is not formed	Found	Present	Absent

(Table 1) cont.....

Type/ Name of Association	Partner		Intracellular Colonization	Fungal Sheath	Hartigs Net	Vesicles
	Fungi	Plant				
Ectendo	Basidiomycotina	GymnospermsAngiosperms	Formed	Present/ Absent	Present	Absent
VAM	Zygomycotina	Bryophytes to angiosperms	Formed	Absent	Absent	Present /Absent
Arbutoid	Basidiomycotina	Ericales	Formed	Present/ Absent	Present	Absent
Monotropoid	Basidiomycotina	Achlorophyllous angiosperms	Formed	Found	Present	Absent
Ericoid	Basidiomycetous Hymenoschyphus	Ericales	Formed	Absent	Absent	Absent
Orchidoid	Basidiomycetous	Orchidaceae	Formed	Absent	Absent	Absent

ARBUSCULAR MYCORRHIZA INOCULA AND ITS MOLECULAR STUDIES

In order to achieve sustainable agriculture, AM as a bioinoculant is urgently needed, as proper management of them might reduce the usage of agrochemicals. Inoculation of AMF propagules (inoculums) into a target soil is the major approach used to attain this goal.

Different types of AM inocula preparation exist. Because it contains colonised root pieces, spores, and fungal hyphae, soil from the root zone of a plant that hosts AM can be employed as inoculums. Spores taken from soil can be utilized as starters in the creation of crude inoculums. After an isolate of AM and a host plant are cultured in an inert medium designed for AM proliferation, crude inoculums can be obtained. The most typical application of this sort of inoculums is for large-scale crop inoculation [16].

Several techniques like wet sieving, decanting technique, sucrose centrifugation technique are used to isolate the spores. Wet Sieving and Decanting technique, is the most popular and often employed method of Gerdemann and Nicolson [17]. In this technique, 100g of rhizosphere soil is added to 1000ml of sterile distilled water and the soil mixture is subjected to vortex which frees fungal spores adhering to soil particles. Later the water is left undisturbed for 12 hours to overnight. Then, the supernatant is allowed to pass through the standard sieves (35 mesh, 100 mesh, 250 mesh and 270 mesh). The material retained on the fine mesh (sieve) is washed and later passed through the filter paper.

CHAPTER 8

Mushroom Cultivation Using Agricultural Wastes

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Abstract: Mushrooms are protein-rich, highly nutritive, popular food around the world. The cultivation of mushrooms has been in practice for decades. Studies have been conducted to utilize the agricultural waste, and agro-forest remains as the substrate for mushroom production. Agri wastes such as wheat straw, paddy straw, bran, coffee grounds, banana leaves, sawdust, *etc.*, are highly used, and the availability of such substrates is not common among the cultivating areas. Besides the development of new protocols and the invention of a perfect combination of the substrate and supplements is an ongoing interest in the mushroom production industry. In Karnataka, agricultural remains such as areca husk, sugar cane bagasse, paddy straw, and maize stem are available to use for mushroom cultivation. Also, agro-forest residues like *Cassia tora* are a substantial material to turn waste into the substrate. This chapter is an approach towards such substrate utilization to improve the economy of the farmer community and a promotion towards sustainable mushroom production.

Keywords: Agricultural waste, Areca husk, Forest residues, Spawn, Substrate.

INTRODUCTION

Macrofungi is a term used to represent the group of fungi having observable fruiting bodies containing reproductive structures, hence distinguishable from microfungi. The majority of these mysterious creatures are terrestrial, saprophytic, and some are mycorrhizal symbionts. A group of macrofungi is invariably called mushrooms. Mushroom and toadstool are the terms used precisely to represent the edible and poisonous species, respectively. They are seasonal or ephemeral and last for only a few days or weeks, though the mycelium remains in the substrate, such as soil and wood, for most of the year.

The process of mushroom cultivation has been improved over the centuries from caves to homes with the advent of simple, cost-effective methods of cultivation. The cultivation of mushrooms dates back to the 17th century. A melon grower

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from Paris thought to cultivate this exotic delicacy commercially, and then a French gardener thought of growing them in caves having essential moisture and humid conditions for mushroom farming [1]. *Agaricus bisporus* (J.E. Lange) Imbach, called button mushroom, was first described by French botanist De Tournefort as *Fungus sativus equinus,* indicating that it could be seeded with horse dung. The first successful production of sterile

spawn was done by French mycologists Constatin and Matruchot in 1894 [2]. This was the most important discovery in the field of industrial-scale cultivation. Later, the evolution of composting processes and domestication of wild edible mushrooms by understanding the nutritional and nutraceutical values has been in practice by scientists around the world.

Mushroom cultivation has become popular throughout the world with the development in the process of cultivation and dispersion of knowledge among the people about the nutritional values and medicinal properties of mushrooms. The production of mushrooms has met about a 9-fold increase in the last 30 years, from 1.76 million tonnes in 1988 to 8.99 million tonnes in 2018 (FAO Stat. 2018). China, being a leading producer of mushrooms, produces approximately 70% of the total world production, 6,664,606 tonnes to be exact (FAO Stat. 2018) (Fig. **1**). United States of America, Spain, Canada, United Kingdom, France, and other countries come after China.

The United States of America is the world's largest mushroom consumer, as their import of canned mushrooms dominated compared to their export [3]. China, India, and Indonesia are the major exporters of mushrooms from Asia to US since 2003. In China, the mushroom industries follow traditional technology to scale up the production, still, this method is superior to any other method in large-scale production of edible and medicinally important mushrooms. The Chinese mushroom industry includes thousands of small families employing family labour. Government industries contribute maximum to this agri-based business.

Mushrooms need to find a place as a vegetable among Indian consumers. Even though there is the availability of favorable agro-climatic conditions, abundant agricultural waste, fungal diversity richness, and relatively low cost of labour [4], the Indian mushroom production scenario has been maintained linearity for decades (Fig. **1**) and India stands at 15th position in the world for the production of mushrooms with 60,733 tonnes of annual production (FAO Stat. 2018). But, nowadays due to the advancement in research; there has been an improvement in the knowledge of mushroom cultivation and the introduction of new strains of nutritionally important mushrooms [5] with numerous medicinal values showing a vision to increase the production status in future days.

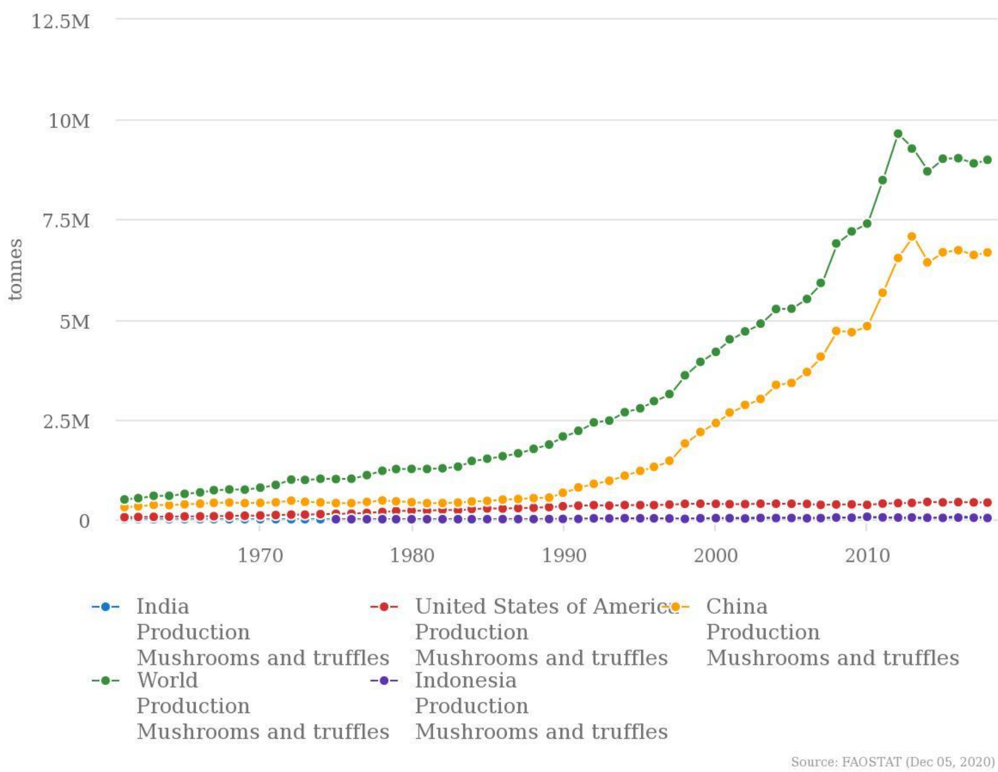

Fig. (1). Comparison of mushroom production by major countries with total production.

Mushrooms are an important source of food; they are cholesterol-free, with a less percentage of fat, carbohydrates, and sodium. Besides, mushrooms also contain a few major elements like vitamin D, *etc.* As long as they are known as food sources, they are also sources of medicinally important chemicals. The research in this area shows that the production of mushrooms and the extraction of bioactive compounds through biotechnological approaches is a key feature for a wide range of metabolites that are used to prevent or treat several diseases. Such as Parkinson's, Alzheimer's, Hypertension, and a High Risk of Stroke [6]. Common edible mushrooms from the genus *Agaricus* are known for their considerable immune stimulation and their extracts are used to prevent the faster development of malignant cells in breast cancer [7], anti-mutagenic, anti-carcinogenic, and immunomodulatory properties by *A. blazei* [8 - 10]. *Lentinula edodes* are known to include potential compounds to alleviate the common cold and they are the source of antioxidant and anti-cancerous compounds [11, 12].

CHAPTER 9

Fungi: An Environment-friendly Approach for the Growth of Plants

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Abstract: The use of chemical fertilizers improves plant growth growth and causes pollution, affecting human health. The use of microorganisms to increase the plant yield has many advantages *viz.*, eco-friendly, economical, and easy. The fungi produce many secondary metabolites and can be used to promote plant growth. This will be a sustainable approach and also avoid pollution. The fungi produce plant growth promoting substances, act as strong biocontrol agents, and also show antimicrobial effects against the harmful pathogens, thus preventing the diseases of the plants. This chapter focuses on the importance of fungi in agriculture, which will help farmers. The solid-state fermentation using *Aspergillus* sp. for plant growth-promoting substances is also mentioned in the chapter. The chapter is significant as it highlights the use of the biological process to improve plant growth, which is the need of the hour.

Keywords: Biopesticide, Chitinase, Eco-friendly, Pollution, Siderophores, Spores.

INTRODUCTION

The farmers make use of chemical fertilizers, pesticides, and various other chemicals to enhance the biomass and yield of plants and crops. But these chemical pesticides are very toxic, cause pollution, and are costly. Also, their use creates a hazardous effect on the surroundings and negatively affects human health [1]. The use of beneficial microorganisms (*i.e.*, bacteria, actinobacteria, and fungi) to increase crop growth and yield has many advantages over the use of pesticides and chemicals, *viz.*, fast, cheap as well as being eco-friendly [2]. The biological process will be a sustainable approach to improve the growth of several plants and crops. The fungi play a significant role in this sustainable approach to agriculture. The fungi are filamentous and produce spores with different colors,

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mycelium, and hyphae [3]. This chapter focuses on the role of fungi in sustainable agriculture.

Fungi

The different types of fungi are *Aspergillus*, *Trichoderma*, *Penicillium*, *Rhizopus*, *Mucor*, *Alternaria*, *Fusarium*, *Cladosporium* sp., *etc.* The total number of species of fungi is about 1.5 million [4]. The fungi produce various metabolites, *viz.*, antibiotics and enzymes. They also act as biological control agents and help control the growth of pathogens [5]. The fungi are also reported to produce 'siderophores' which help in the chelation of metal, *i.e.*, iron [5]. The siderophores help make the iron readily available to the plants. There are many reports on the use

of fungi for sustainable agriculture practice under varying climate conditions [6]. The various metabolites produced by fungi are represented in Fig. (**1**). The fungi convert the complex organic compounds into a very simpler form, which can be made available to the plants in simpler form for their growth.

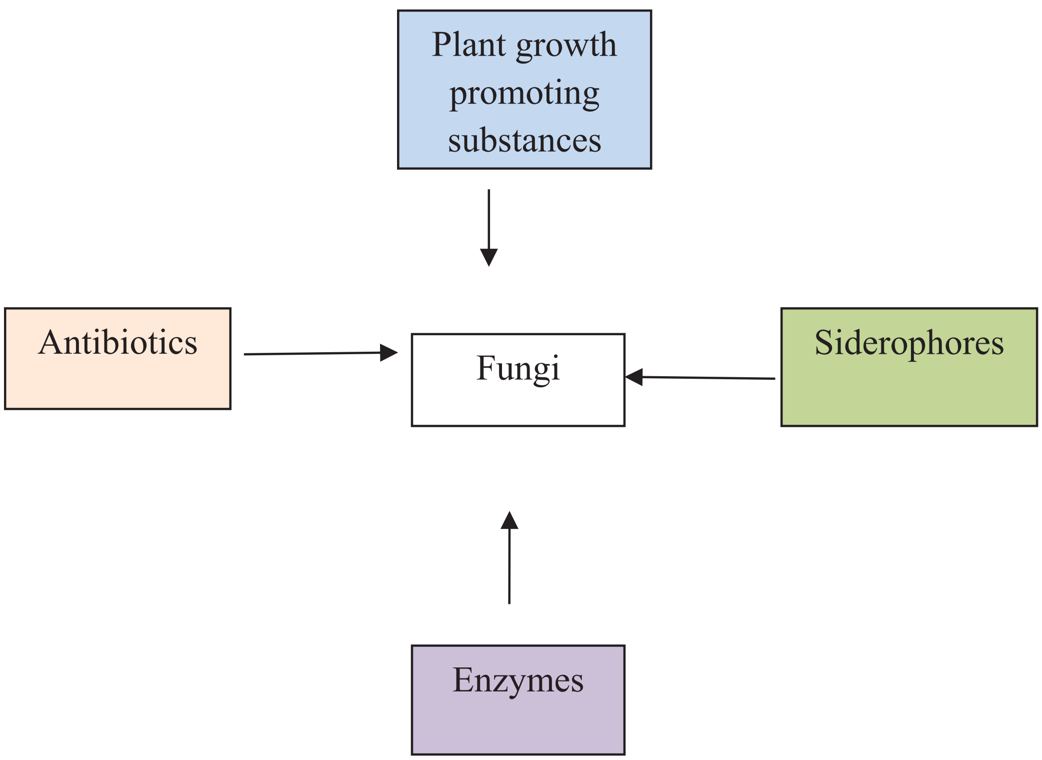

Fig. (1). Various metabolites produced by fungi.

Arbuscular Mycorrhizal Fungi

The Arbuscular Mycorrhiza (AM) fungi interact with the soil and help in uptake of nutrients and water. The AM fungi also enable facilitation of the mobilization of major elements [5]. These fungi are excellent as biofertilizers [7]. AM fungi have increased the growth and yield of many plants and field crops [8]. These fungi can be used as biofertilizers and as a control method for soil erosion [9]. These fungi have been studied to enhance the growth, yield and phosphorus regulation in Gossypium hirsutum L [10]. These fungi can also colonize the plant roots [11]. The ectomycorrhizae increase the tolerance of plants to various stress conditions. AM fungi can also affect plant growth indirectly by improving the soil physico-chemical properties, providing antimicrobial activity against many phytopathogens and also maintain water balance [12]. AM fungi can be used to control the plant diseases caused by many pathogens [9].

Endophytic Fungi

Endophytic fungi are the fungi that live in mutualism with other plants without causing harm to that plant [13 - 15]. Endophytic fungi help to improve the plant growth by various ways *viz.*, solubilize minerals; produce plant growth hormones *viz.*, indole acetic acid (IAA), gibberellins and cytokinins; hydrogen cyanide (HCN); and ammonia (NH_3). The endophytic fungi help the plants to tolerate biotic and abiotic stress [16]. There is a report where Fusarium sp. has been reported to produce siderophore [17]. The diversity of endophytic fungi varies depending on geographic location and hosts.

Plant Growth Promoting Fungi

The plant growth promoting fungi increase the growth and yield of the plants by direct and indirect mechanisms which are very important from the aspect of agriculture.

Fungal-Bioinsecticides / Biopesticides

The fungal bioinsecticides can be used to control the insects and pests on the plants. The fungi produce spores and when these spores are applied, the fungi use the enzymes to break the outer surface of the body of insect, causing the death of the insects [18]. Trichoderma viride and T. harzianum are widely used as biopesticides [19]. The integrated pest management is important for the control of insects and pests.

Fungi as Biofertilizers

The fungi play important role as biofertilizers to increase the plant growth [20].

CHAPTER 10

Fungal Metabolites: Industrial Applications and Challenges

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Abstract: Fungi are the second largest group of eukaryotes and play a vital role in the biological system. These fungi are cell factories that produce a variety of industrially important secondary metabolites (SM) that are beneficial for humans, animals, and the environment. Microorganisms are a rich source of natural compounds with a significant commercial value; on the other hand, they produce low-quality products that may not satisfy industrial standards for commercialization. Numerous fungal species produce key industrial enzymes, antibiotics, anticancer drugs, cholesterol-lowering drugs, and immunosuppressants. Antibiotics belonging to the *β* lactam family are the most often prescribed antibiotics worldwide. Penicillin has a core structure, 6-amino penicillanic acid (6-APA), interwoven with the ß-lactam ring and drives the development of semisynthetic antibiotics. Several dermatophytoses are treated with griseofulvin (GRI), and mycophenolic acid (MPA), and the cholesterol-lowering drugs (lovastatin and compactin) are produced from *Aspergillus* and *Penicillium* species, respectively. The SM is also effective against most of the pandemic novel coronavirus (SARS-CoV2), human immuno virus (HIV), and influenza virus (H1N1) strains protease inhibitors that limit virus propagation. Furthermore, natural antioxidants (amanitin, ferulic acid, resveratrol, and flavonoids) are synthetically conjugated or co-crystallized with active SM for clinical uses. Many biosynthetic gene clusters, comparative genomics, and functional transcripts are encoded in fungal genomes, reigniting interest in discovering new compounds. The strong transcriptional regulation likely clarifies the metabolic routes in the genome. Viable approaches necessitate the availability of genome sequences, molecular tools, and a strategy tailored to alter the genomes of specific strains for industrially significant molecules. Researchers have devised a new strategy for strain generation of various genetic engineering tools, involving recombinant DNA technology, protein engineering, metabolite flux pathways, and synthetic biology approaches, using a basic understanding of genetic makeup.

Keywords: Antibiotics, Anticancer properties, Biotechnological applications, β-lactam antibiotic, Novel molecules, Ssecondary metabolites, Strain development.

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INTRODUCTION

The class of β-lactam antibiotics is a widely used and clinically prescribed drug for treating various infectious diseases and accounts for about half of the global antibiotic market with 65% of the total shares [1]. Penicillin was the first β-lactam antibiotic discovered in 1928. The β-lactam ring is an essential feature for its mode of action against the cell wall synthesis of the bacteria. The side ring attached to a β-lactam ring is classified into penicillins, cephalosporins, carbapenems, and monobactams (Fig. **1**). Penicillin and cephalosporin are mainly produced by *Penicillium chrysogenum and Acremonium chrysogenum*. Griseofulvin (GRI) is another important antifungal antibiotic used to treat the number of dermatophytes produced by *P. griseofulvum.* Griseofulvin (GRI) has attracted significant attention due to its action against viral infections and anticancer properties. Furthermore, mycophenolic acid (MPA), Cellcept (mycophenolate mofetil; Roche), and Myfortic (mycophenolate sodium; Novartis) are active against various infectious bacteria. MPA is (6-(4-hydroxy-6-met-oxy-7-methyl-3-oxophthalanyl)-4-methyl-hexenic acid, C17H20O6) derived from *P. brevicompactum*. Besides, it is mainly used as a frontline therapy in graft rejection in kidney, heart, and liver transplants.

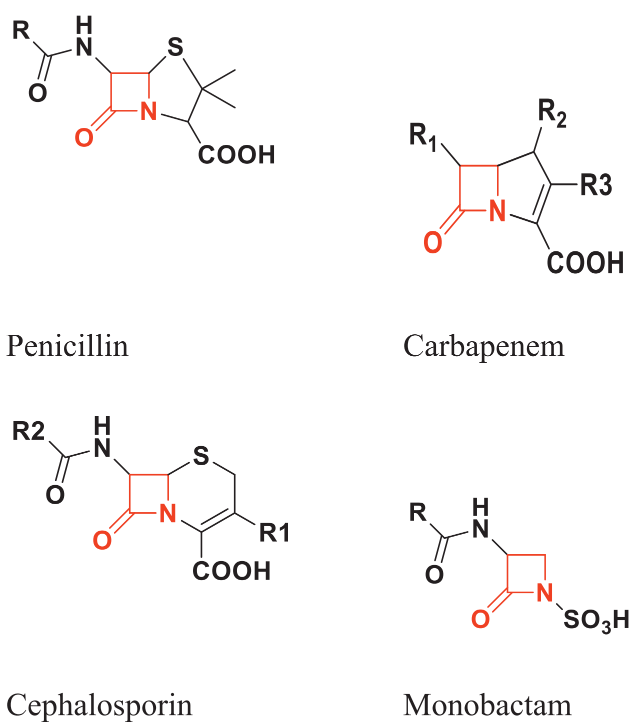

Fig. (1). Classes of β-lactam antibiotic with common β-lactam ring (Red colour).

The elevated low-density lipoprotein (LDL) cholesterol levels that cause atherosclerotic cardiovascular disease (ASCVD) have become overwhelming. Cholesterol has a significant role in body metabolism, precursors for hormone synthesis, membrane transport, and fluidity. Compactin was the first isolated statin molecule that gave a new class of drugs to treat high cholesterol levels. The HDL and LDL are critical for maintaining their optimum level. The imbalance of optimum cholesterol levels leads to various cardiovascular disorders, such as atherosclerosis, hypercholesterolemia, myocardial infarction, atheroma, peripheral vascular diseases. Lovastatin is a polyketide (PKs) metabolite derived from various strains of *Aspergillus and Penicillium.*

Moreover, various fungal-derived metabolites are active against several viruses and their protease. These viruses are explicit, requiring specific host cell receptors to replicate and cause disease. In the current pandemic, due to novel coronaviruses (SARS-CoV-2), it is essential to develop chemotherapy for treatment to stop the progression of the infection. The drugs act as inhibitors of the main protease of SARS-CoV-2, which limits the spread of the virus. To the best of our knowledge, no industrial strains are available for the production of these molecules. The strain improvement programs, genomics, proteomics, metabolomics, metagenomics, transcriptomics (OMICS) tools reduce the import substitutions. We have also summarized a few classical and genetic engineering approaches for developing these strains to produce industrially important molecules.

Penicillins

Benzylpenicillin (Penicillin G) was the first natural penicillin produced industrially by a *P. chrysogenum* active against *Streptococcal* species. Phenoxymethylpenicillin (Penicillin V) was discovered, which has broad activity than penicillin G and has high acid tolerance capacity to be administrated orally. The structural difference between penicillin G and penicillin V is the side-chain precursor, phenylacetic acid (PAA), and phenoxy acetic acid (POA) [2]. However, resistance to natural penicillins has forced the research towards the discovery of new semisynthetic penicillins. The active pharmaceutical ingredient (API), 6-APA, is the core of all semisynthetic penicillins, including natural penicillins, and is used in their production. 6-APA is produced by the hydrolysis of natural penicillins by an enzyme penicillin acylases (Pen G acylase or Pen V acylase). Nowadays, the bulk of penicillins is manufactured and used for the production of 6-APA [3]. Penicillin G acylase and penicillin V acylase are extensively used to produce 6-APA and subsequently in the semisynthetic penicillins, including ampicillin and amoxicillin [4].

CHAPTER 11

Microbiome Perspective: Multisectorial Exploitations of Chitinases

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Abstract: Chitinases are cosmopolitan lytic enzymes secreted by microbiomes that fall under the domain of fungi, yeasts, bacteria and plants. Most fungal plant pathogens, human infectious agents and post-harvest damage caused by pathogens have been a serious threat to the economy and human health. Like fungi, crabs, insects, lobsters, shrimps, and invertebrates all have a hard disintegrating, flexible polymer called chitin that forms the exterior skeleton. It poses a wide-range of environmental problem and a major threat to humans, plants and animals. According to functional genomic research, there is a large diversity of chitinases-producing fungi in nature. They have adapted to a wide range of habitats on Earth including plants, animals and manmade natural and artificial habitats. Chitinases, both native and genetically modified, have been produced and expressed in an expression system such as *Escherichia coli* or *Pichia pastoris* through recombinant DNA technology. This versatile recombinant chitinases can be used for long-term growth and productivity. As a whole, chitinases have a wide range of applications in agriculture, horticulture , plant health, and bio-control of pests, and

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even some fuel processing, genetically engineered molecule-based therapies, polysaccharide hydrolysis, biomedicine, pathogenic/virulence agents, antifungal agents, and as a drug delivery system.

Keywords: Biomedicine, Chitinase, Disease resistance, Renewable bio-homopolymer, Microbiome, N-acetyl D-glucosamine.

INTRODUCTION

Chitin and its derivatives are the un-branched, tough, pliable, widely distributed second natural bio-homo-polymer after cellulose [1].

Chitin is a second natural polymer after cellulose on Mother Earth. The outer skeleton of arthropods, crabs, shrimp and lobsters and cells of invertebrates and fungi are composed of chitin [2]. Chitin has unique properties such as biodegradability, lack of toxicity, biocompatibility, and bio-absorbability [3]. It is the primary source of water-soluble chitosan, which has applications in agriculture, biomedicine, and as a drug and gene delivery vehicle [4]. Chitin has N-acetyl-D-glucosamine (NAG) linked by 1, 4 glycosidic cross-linkage. Naturally, chitin is found in two forms- α-1, 4- chitin and β- 1, 4- chitin. In α-chitin, the chains are anti-parallel and in β-chitin there are parallel [5]. Chitinases are a group of hydrolytic enzymes. Cleaves of α- and β-1, 4- glycosidic cross-linkages contain low molecular weight products [6].

Chitin or colloidal chitin is used as a carbon source by chitinase-producing microorganisms. Chitinases of various types have been discovered in biological sources (Table **1**; refer database- https://www.enzyme-database.org/ & https://www.genome.jp/). EC 3.2.1.14 (Fig. (**1**); source: https://www.ebi.ac.uk/) was assigned to a small number of them cleaves chitin chain at irregular intervals and β-N-acetyl glucosaminidases (EC 3.2.1.52), which cleave non-reducing terminal of the chain and removes N-acetyl glucosamine residues [6, 7]. Different families of glycosyl hydrolases (GH) are classified based on the similarity of the amino acid (AA) in the catalytic domains (CD) [8, 9].

Chitin metabolism is an indispensable part of amino sugar and nucleotide sugar metabolism and is explored in the KEGG reference pathway map. Major enzymes are the part of chitin bioconversion pathway (Fig. **2**). Of the total Fungi, *Fusariumverticillioides* presented in the KEGG database occupies a major position. It has a narrow host range. *F. verticillioides* was reported as a cereal pathogen and contain 37 out of 134 enzymes of standard amino sugar and nucleotide sugar metabolism map. Several genes involved in coding chitinase for biocontrol activity, energy production, degradation of polymeric substances, drug development, biocides, *etc.*, were frequently referred to as molecular markers. The marker genes have played roles in the detection of chitin biosynthesis pathways in genomics analysis.

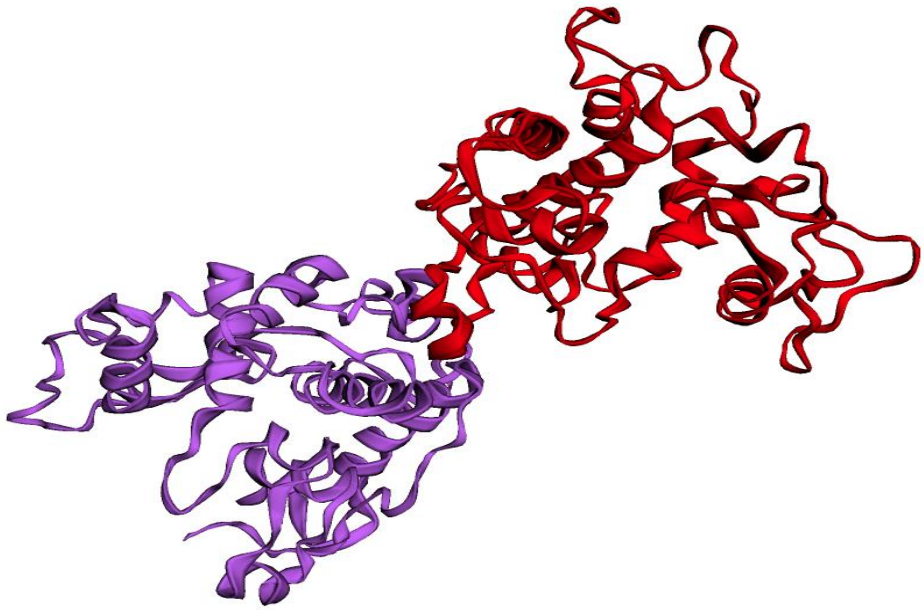

Fig. (1). 3D Molecular structure of Anti-fungal Chitinase (EC 3.2.1.14).

Chitinase producing bacteria and fungi are the important degraders of chitin in the environment. They have contributed to the utilization, assimilation and recycling of organic carbon (C) and nitrogen (N) in the ecosystem [10 - 12]. Microbial chitinases are widely distributed in nature including *Actinomycetes*, *Streptomyces* and yeast and unicellular forms such as *Candida albicans, Kluyveromyces lactis, Saccharomyces cerevisiae* anddimorphic fungi-like *Paracoccidiodes brasilienses*. Chitinases are also produced by filamentous fungi such as *Agaricus*, *Aspergillus, Beauveria, Conidiobolus, Lecanicillium, Metharhizium, Myrothecium, Neurospora, Oenicillium, Stachybotrys, Penicillium, Phoma, Acremonium Cladosporium,* and *Trichoderma* [13]. Also, horticulture and potential chitinases can be employed in sustainable developments (Fig. **3**) such as fuel production, as a biocontrol agent, chitin degradation, polysaccharides hydrolysis, drug development, as virulent factor or agent, recombinant chitinases as an immune enhancer, antifungal agent, in pharmaceutical and medical industries [14 - 17]. This review gives an update on chitinases producing microbiome and uses of chitinases. Chitinases are found to have applications in plant protection, fuel production, polysaccharide hydrolysis, medicine, pharmaceuticals, drug development and antifungal agents.

CHAPTER 12

Fungal Pectinases

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Abstract: Pectin is a heteropolysaccharide found in the primary cell walls of terrestrial plants. It is found along with other cell wall polymers such as cellulose and hemicelluloses. Its degradation is facilitated by about 15 different types of pectinases. Here, the focus is on the basic, applied, and production aspects of pectinases. Pectinases can be isolated from a variety of organisms such as plants, bacteria, and fungi. Of these, fungal pectinases are routinely employed in the food-processing, textile, and paper industries because of their higher activity, specificity, and stability under higher temperatures and extreme pH conditions. Specifically, this review summarizes the fungi that produce pectinases, classification and modes of action of pectinases, the methods for partial purification and characterization of pectinases, and applications of pectinases. In addition, optimization of physical and chemical conditions for improving the production of fungal pectinases in solid state fermentation is described in detail.

Keywords: Pectin, Solid state fermentation.

INTRODUCTION

Pectinases, also known as pectinolytic or pectic enzymes, are a group of related enzymes that cleave glycosidic linkages in pectic substances present in the cell walls of different plants [1]. The cell wall degrading ability of pectinases has been widely exploited for industrial applications. These enzymes are used in food, paper, pulp, and other processing industries [2]. Pectinases are widespread in higher plants, bacteria, fungi, and yeasts. Today, most of the pectinases used in industries are produced by fungi. Although they are produced both in submerged (SmF) and Solid state fermentation (SSF) processes, the SSF process is considered more economical and has more advantages compared to the SmF process: 1) as the cultivating conditions in SSF exist similar to the habitat of filamentous fungi, which allow excreting large quantities of the enzyme [3], 2) SSF process do not need advanced machinery and control system, 3) low volume

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of water is required for extraction of product from the fermented substrate which would reduce the cost of downstream processing, 4) low energy is required, and 5) low risk of contamination results in high product yields [4, 5]. Pectinase production by filamentous fungi depends on the type of strain, medium composition, and the cultivation conditions, including pH, temperature, aeration, agitation, and incubation time [6].

According to several authors [7 - 9] the production of these enzymes on various agricultural residues as solid substrates in SSF, is influenced by culture conditions (including moisture, pH) and the type of bioreactor. In light of this, the present review briefly highlights applications, types, source organisms, culture conditions affecting the SSF process, partial purification and characterization of pectinases, and structural aspects of pectic substances.

Pectinase Producing Microorganism

Pectinases are widespread among plants, fungi, bacteria, and many yeasts [25]. However, molds such as *Trichoderma harzianum*, *Aspergillus niger*, *Rhizopus* sp. are preferred because they secrete 90% of the enzyme into the culture medium [14]. Among fungi, the prominent role is played by the mold *Aspergillus niger* because it produces a fair amount of these enzymes and in addition, it is being recognized as GRAS (generally regarded as safe) microorganisms [26] (Table **2**).

Pectic Substances

Pectic substances are acidic, negatively charged, high molecular weight polysaccharides with complex structures present as the major components of the middle lamella and primary cell wall of higher plants which contribute rigidity and structure to plant tissue [2]. These compounds are acted upon by pectinolytic enzymes and mainly consist of three covalently linked backbones: 1. Homogalcturonan (HG) also called 'smooth region', contains a linear chain of a -(1-4) linked D-galacturonic acid (GalpA/GalA) residues in which some of the carboxyl groups of the GalpA/GalA residues are esterified with methanol or the GalpA/GalA residues may also be esterified with acetic acid at C_2 and C_3 [15, 16] (Fig. **1**). Substituted galacturonans are xylogalacturonan (XGA) and apiogalacturonan (AGA), where D-xylose and D-apiose, respectively are present as saccharide appendants. 2. Rhamnogalacturonan I (RG-I) pectins also called 'hairy region', contain a backbone of repeating disaccharide 4)-a-D-galacturonic acid-(1, 2)-a-L rhamnose - (1 [®4-a-DGalpA/GalA-(®2)-a-1-Rha-(1®]. Branched and linear oligosaccharides composed predominantly of a-L arabinofuranose (Araf) and b-D-galactose (Gal) residues are linked to C_4 of some of the rhamnose (Rha) residues. Some Rha residues may also be O-acetylated at C_2 and/or C_3. Different neutral sugars such as D-galactose (Gal), D-arabinose (Ara), and D-xylose (Xyl) branch off from many rhamnose residues as side chains. These side chains containing neutral sugars are attached through glycosidic bonds to C_3 and C_4 of rhamnose units and C_2 and C_3 of galacturonic acid (GalpA/GalA) units. The type of neutral sugar and its concentration may vary with the origin of pectin [17] (Fig. **2**). 3. Rhamnogalacturonan-II (RG-II) is a branched, complex structure that is less frequent (Fig. **3**). The backbone of RG-II is replaced with heteropolymeric side chains containing different types of sugars [18, 1]. The rhamnogalacturonan-II backbone with four structurally different side chains (A-D) [19] is shown in Fig. (**3**). The pectic substances are abundantly present in apple, orange, mango, lemon, tomato, carrots, beetroot, *etc* [20, 21].

Table 2. List of important pectinase producing fungi.

Organisms	Reference
Aspergillus niger	[27- 30]
Aspergillus fumigatus	[31]
Fusarium oxysporum	[32]
Penicillium viridicatum RFC3	[9]
P. oxalicum	[33]
Moniliella sp.	[34]
Trichoderma harzianum	[35]
Cryptococcus aquaticus – H2	[36]
Cystofilobasidium lari-marini S3B	[36]
Rhizopus stolonifer	[37]
Aureobasiduim pullulans LV10	[38]
Saccharomyces pastorianus	[39]
Saccharomyces cerevisiae IM1-8b	[40]
Neurospora crassa	[41]
Cryptococcus albidus var. albidus	[42]

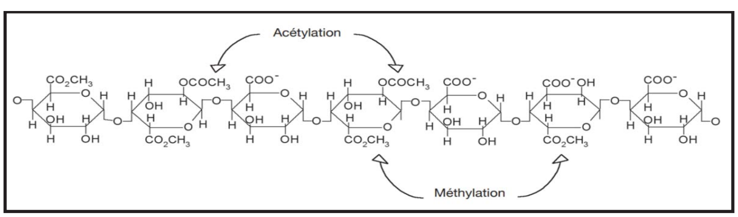

Fig. (1). The primary structure of homogalacturonan (HG). Homogalacturonan is a linear polymer of 1→4 linked α-D-galactopyranosyluronic acid (GalpA/GalA) residues in which some of the carboxy groups of the GalpA/GalA residues are esterified with methanol. The GalpA/GalA residues may also be esterified with acetic acid at C_2 and C_3 [22].

CHAPTER 13

Commercial Fungal Exopolysaccharides: Botryosphaeran, Pullulan, and Scleroglucan

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Abstract: Interest in polymer production by microorganisms has significantly increased due to its widespread use in various sectors. There are three major classes of microbial polysaccharides, namely extracellular, intracellular and structural. Of these, exopolysaccharides are preferred because of their easy isolation and purification and also because of their high yielding potential in a short time period. Exopolysaccharide production is reported in a considerable number of fungi, including higher basidiomycetes, lower filamentous fungi, and yeasts from different ecological niches. Among these, the exopolysaccharides, botryosphaeran, pullulan, and scleroglucan, produced by *Aureobasidium pullulans*, *Botryosphaeria,* and *Sclerotium,* respectively, have been commercially produced and are well known for their applications in diverse fields. Exopolysaccharide production in fungi mainly depends on the fungal strain used, the physical conditions used for fermentation, and the medium components used for the production. Fungal exopolysaccharides are significant primarily because of their properties such as pseudoplasticity, resistance to salt and thermal degradation, high viscosity even at diluted concentrations, and high-temperature tolerance. Fungal polysaccharides have been extensively exploited in various sectors such as petroleum, bioremediation, food, biomedical, cosmetic, textile, and pharmaceutical.

Keywords: Botryosphaeran, Exopolysacharides, Pullulan, Scleroglucan.

INTRODUCTION

Polymers play an important role in our modern society [1]. Polymers from petroleum products have their limitations in that these are limited resources and have an immense environmental impact [1]. Thus, the imperative need for environmentally and biologically friendly polysaccharides has led to the paradigm shift in the exploration of biopolymers [2]. Biopolysaccharides are a group of biopolymers associated with cellular structures and the compositional integrity of cells [2]. For many years, the global market was dominated by algal and plant biopolymers. Interest in polymer production by microorganisms has significantly

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increased as microbial polysaccharides possess novel, unique, and superior properties on account of their unusual molecular structures and peculiar conformations [3, 4]. Microbial biopolymers represent a valuable alternative, whose production is achieved within days and weeks as opposed to plants which take months and may also be prone to geographical limitations and seasonal variations [1, 5]. Microbial sources are also preferred because of their standardized, sustainable, and economical production and extraction at an industrial scale and do not have complicated technological requirements [1, 2]. Furthermore, the yields obtained are high primarily because they are amenable to fully controlled fermentation conditions.

Among the microbial polysaccharides, there are three major classes, namely extracellular, intracellular and structural polysaccharides [6]. The extracellular polysaccharides, also called exopolysaccharides (EPS), are most preferred since they are easy to isolate, purify, and are cost-effective [7]. They are metabolic products produced by selective microorganisms and accumulate on the cell surfaces [8], and are associated with adaptation, survival, and other functionalities [2]. EPS have been reported in diverse groups of microorganisms isolated from different aquatic and terrestrial environments [8].

Although immense attention has been given to the prokaryote producers, many filamentous fungi also produce EPS [9]. For the last two decades, fungal EPS have been recognized as high value biomolecules [4] with only pullulan, scleroglucan, and botryosphaeran (Table **1**) being marketed commercially [4]. These exopolysaccharides find significance in various sectors such as pharmaceutical, food and medicine industries [4]. This chapter provides a short review of these commercially available EPS namely pullulan, scleroglucan, and botryosphaeran, and includes EPS source organism, structure, properties, production and applications.

BOTRYOSPHAERAN

Source Organism

Botryosphaeran is an EPS produced by the ligninolytic fungus belonging to the taxa Botryosphaeriaceae and the genera *Botryosphaeria*. This fungus is pathogenic, infecting woody angiosperms and coniferous trees and causing canker and die-back disease of maple, oak, sycamore, *etc* [10]. These plant pathogens produce enzymes that hydrolyze the carbohydrates in plant cells to liberate fermentable sugars namely galacturonic acid, glucose, and xylose that serve as excellent substrates to produce exocellular botryosphaeran. This botryyospharan, on account of high molecular mass and viscous nature, blocks the vascular tissues of plants ultimately culminating dieback disease of the plant [7].

Table 1. Commercially produced polymers: properties and uses.

Polymer	Source Organism	Properties
Botryosphaeran	*Botryosphaeria* sp.	− Molecular weight of 1.82×10^6 Da − Stable at high temperatures − Insoluble in aqueous solutions
Pullulan	*Aureobasidium pullulans*	− Molecular weight 100–250 KDa − Neutral, odorless and tasteless, non-ionic, non-toxic − Thermo stable, impermeable to oxygen, resistant to oil and grease. − Soluble in water and dilute alkali − Insoluble in alcohol and organic solvents except for dimethylformamide and dimethylsulfoxide − Good mechanical strength and elasticity. Exhibits adhesiveness and film formability
Scleroglucan	*Sclerotium* sp. *Schizophyllum commune* *Botrytiscinerea* sp. *Epicoccumnigrum*sp.	− Tolerance to high temperatures, a broad range of pH, and a variety of electrolytes. − Pseudo plastic

Structure of Botryosphaeran

Botryosphaeran has a backbone of repeatedly (1→3)-linked D-glucosidic residues, with approximately 22% side branching on carbon-6 with glucose and gentiobiose (Fig. **1**). The branch point occurs on every 5th glucose residue of the backbone chain when produced in medium with glucose and sucrose, and one in every 3rd glucose residue when produced in the presence of fructose in the medium. Structure elucidation was facilitated by techniques that include methylation analysis, Smith degradation, FTIR, Gas chromatography-mass spectrometry (GC-MS),^{13}C NMR spectroscopy and Low-angle laser light scattering analysis [11].

Botryosphaeran Properties

Some of the properties of the Botryosphaeran are as mentioned below [7]:

Botryosphaeran has a molecular weight of 1.82×10^6 Da due to the degree of polymerization, chemical nature of the side-branch, constituent chains and their degree of ramification. It is stable at high temperatures and can withstand autoclaving temperatures of 121°C.

CHAPTER 14

Biotechnological Production of Various Fungal Metabolites and their Applications in White Biotechnology

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Abstract: The rapid growth of science and technology resulted in an increase in the production and utilization of chemical-based value-added compounds. Due to the involvement of greenhouse gases and global warming, there is a shift towards alternative strategies to replace chemical-based value-added products. Primary and secondary metabolites produced by various microorganisms could be an effective and environmentally friendly alternative to chemically manufactured value-added products. Metabolites produced from various fungal strains (filamentous fungi and yeast) are of high importance for their widespread applications in the food, agriculture, and pharmaceutical sectors. These value-added bioproducts include biofuel (bioethanol), organic acids (citric acid, lactic acid, succinic acid, and cis, cis-muconic acid), hydrolytic enzymes (cellulases, xylanase, phytase, lipase, ligninolytic enzymes, and proteases), vitamins, amino acids, antibiotics, drug molecules, and other industrial-relevant chemicals. Advances in industrial microbiology and biotechnology by metabolic engineering, protein engineering, systems biology, and synthetic biology led to the analysis and discovery of novel metabolic pathways and successive heterologous expression of metabolites of commercial importance. This chapter highlights the biotechnological production of a few relevant primary and secondary metabolites by both filamentous and unicellular fungi.

Keywords: Bioactive compounds, Biofuels, Fungi, Hydrolytic enzymes, Organic acids, Primary metabolites, Secondary metabolites, White biotechnology, Yeast.

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INTRODUCTION

Biotechnological productions in the industrial sector are categorized by different colour codes; for example, red colour indicates pharmaceuticals, green colour is used for the agricultural sector, and white colour represents industrial microbiology and biotechnology. Generally, white biotechnology encompasses industrial production of value-added bioproducts from living cells such as microbial cells and their biocatalytic components. The bioproducts produced through white biotechnology include organic acids such as lactic acid, citric acid, cis, cis-muconic acid, and industrially relevant hydrolytic enzymes like cellulases, xylanases, laccases, phytases, lipases, and proteases. It also includes the production of different products of fermentation like beer, wine, bread, and products related to paper, pulp, detergent, leather and textile industries [1, 2]. Fungi can be considered as important biocatalysts for the biotechnological production of commercially important bioproducts at an industrial scale, which plays a crucial role in lessening the burden of producing value-added products from non-renewable energy sources such as petrochemicals (Fig. **1**). Nowadays, fungal biology is a major field in the global industry. Generally, fungal cell extracts, enzymes and other organic compounds secreted by them are valuable in white biotechnology, which are mainly categorized into biofuels, and bulk fine chemicals. In addition to their valuable role in white biotechnology, fungi are particularly beneficial in biotransformation processes, which is a prerequisite for fine-chemical industry for drug production [3 - 5].

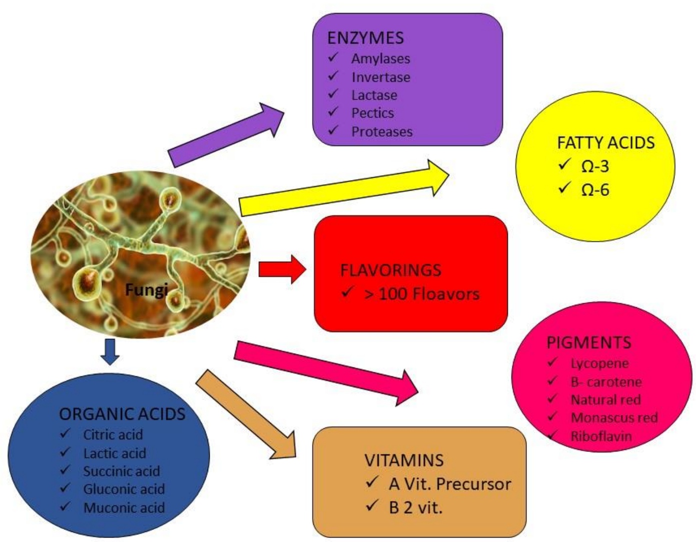

Fig. (1). Value-added products produced by fungi at an industrial scale.

Fungi are either unicellular or multicellular eukaryotic microorganisms, present in great quantity in any type of habitats and have a major contribution to human welfare since a long time for the production of food and beverages. On the basis of cellularity and reproduction, fungi are classified into unicellular, multicellular, and macro filamentous fungi. While, yeast cells are a typical example of unicellular fungi and are widely employed in chemical and food industries to produce ethanol, and bulk and fine chemicals. Filamentous fungi (molds) and yeasts are broadly used in modern industrial fermentation processes for the production of primary (growth, dependent), secondary metabolites (growth independent) and fermented foods (cheese, yogurt, bread, *etc*.). They also play a major role in biocatalytic and biotransformation agents for the industrial production of pharmaceutical and personal care products [6 - 8]. Some fungal species secrete secondary metabolites, for instance, pigments and antibiotics, which have commercial importance in biomedical field. Many filamentous and unicellular fungi such as *Saccharomyces cerevisiae* and *Aspergillus niger* play a major role as microbial cell factories for the bioproduction of value-added compounds at an industrial scale [8 - 10].

Among these fungi, filamentous fungi play a significant role as efficient microbial cell factory for the production of value-added bioproducts. Other than their ability to produce value-added metabolites, physiological features such as high growth rate and biomass formation on a wide range of substrates, and the presence of robust genetic modification tools, rendered them leading industrial producers of both primary and secondary metabolites. Numerous fungal species are presently employed as robust microbial cell factories for the production of organic acids, while industrial production of citric acid by *Aspergillus niger* is considered one of the best examples of large scale industrial biotechnological processes. The other example of fungal strain for the production of organic acid include, lactic acid production by *Rhizopusoryzae* [11 - 13].

Fungal Bioactive Compounds

A group of chemical compounds that promote human health is known as bioactive compounds. Over the past century, these bioactive compounds have proved to enhance the immunity of human health. The major bioactive compounds that improve human health include vitamins, minerals, flavonoids, carotenoids, choline, polyphenols, and antibiotics. Furthermore, functional foods are the important products of nourishment that are high in bioactive compounds like probiotic containing yogurt, prebiotics, and mushrooms. Mushrooms are the most well studied examples of functional and nutritive foods, rich in essential amino acids, fatty acids, minerals, and vitamins which are known to boost immunity in

CHAPTER 15

Marine Fungal Metabolites: Source of Next-generation Antibiotics for Human Microbial Pathogens

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Abstract: Fungi are heterotrophic eukaryotes that feed on dead and decaying matter. The relationship that Fungi hold with their hosts is mutualistic and beneficial for both. Fungi usually grow in a terrestrial place that has moisture content and a carbon source. In marine microorganisms, fungi have great importance due to their significance in pharmacology and ecology. Marine fungi have high chemical and biological properties and are rich in secondary metabolites. They possess antibiotic, antiviral, antifungal, anti-yeast, and stimulant properties. Biological factors are mainly either antibiotic or anticancer. The strains of the genera Penicillium, Emericellopsis, and Simplicillums show high action, whereas the species *Aspergillus oryzae* (G3) and Penicillium chrysogenum (AN12) have high antioxidant properties.

In ecology, the straminipile fungi of mangrove habitats play a very good role in recycling nutrients. The antibacterial property of marine fungi can be determined with the help of a scanning electron microscope. Marine fungi act against parasites at various tropical levels. Auxiliary parasitic metabolites are **assumed to be a** significant part, especially against horrendous infections. The bioactive compounds from fungi are known for their activities against various pathogens, particularly as potential sources for new anti-plasmodial drugs. Studies suggest that the annual number of new fungal metabolites is getting increased.

Keywords: Bioactive compounds, Drugs, Diseases, Habitat, Mangrove.

INTRODUCTION

Fungi are generally present in most habitats. Thousands of fungal species with an abundance of secondary metabolites are varied by their different morphological characteristics. A variety of those specialized metabolites are beneficial, while some are pathogenic to humans. Many of these specialized metabolites exhibit harmful mycotoxins.

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Fungi are known as food spoilers, causing damage to cooked foods and grains, and as plant pathogens, causing various serious diseases. However, fungi favor humanity as manufacturers of antibiotics, food colorants, enzymes, and nutrients. The parasite is thought to be the source of a mix of pig-comparable supplements, catalysts, and over-the-counter medications. There is widespread access to improved production and a new look for fungal metabolites that can give us a secure future against growing microorganisms that are impervious to other feared infections [1].

HISTORY

Mold has a profound effect on our culture and human development. The production of viruses by fungi stimulated the availability of drugs. Although several reports describe the fungal properties of the fungus in the late 1800s and early 1900s, it had inherited prominence in 1929 through the framework of *Penicillium notatum* and penicillin for Sir Fleming's bacteria. But it was not until the early 1940s that the significance of his work did not come true when a gaggle at Oxford University investigated the use of humans as an antibiotic. However, the discovery of metabolites in high quantities led researchers to discover the abundance of marine environments where mold is strong for new biosynthetic diversity. Studies of fungi from the sea have led to the development of 272 new products by incorporating many of the worst carbon offsets by providing evidence that marine fungi can be medicinal [2]. In 1979, Kohlmeyer gave the definition of a binding marine bond as it grows and collapses mainly in the ocean or estuarine, and the strongest marine fungus is that from freshwater or terrestrial or natural marine areas [3].

ROLE OF MARINE FUNGI

The under-sea is an unparalleled area of high hydrostatic pressure and low temperatures, hydrothermal vents with high hydrostatic pressure, high temperatures and concentrations of iron and marine carcasses of other toxic fungi to be tested as sources [4]. The fungus is heterotrophic eukaryotes. They play a critical role in the breakdown of dead plant tissues (cellulose and lignan) and the tissues of small animals, such as keratin and chitin. Molds are often used as various solids that are built biologically and naturally during the diversity of life. Although fungi are well studied, the marine fungal environment is difficult to determine. Decomposition releases nutrients back into the natural environment [2]. According to research, 2225 living organisms from 4196 2as are separated from marine organisms. Here, bioactive compounds were up to 56% of the total. Of the said percentage, 521 compounds make up 13% antibacterial. 14% then represents anti-fungal, anti-pest, and bacterial activity. Since the remaining 16%

compounds can be divided into different functional groups, they are called "another functional group" [5]. Among the many marine organisms, such as sponges, algae, or corals, marine fungi have shown to produce new specialized metabolites (SMs) with different chemical properties and varieties that can hold the key to the latest drug or drug trajectory [6]. Types of marine fungi are powerful regulators of polyketide composed of alkaloids, peptides, terpenes, and mixed biosynthesis that produce chemical groups of secondary metabolites produced by fungi. When marine fungi were isolated, several bioactive metabolites were elevated among them when a new metabolite was separated from the MaG by *Penicillum paxilli K* [5].

Norliquexanthone was isolated from the fungus *Penicillium raistrikki* found in the *Axinella corrugate* sponge. With the well-known chemicals costaclavine, fumgaclavine A, and C, two new indole alkaloids are isolated from the sea fungus Aspergillus fumigatus [5]. The marine environment provides a useful source of the latest natural products that lead to the discovery and development of drugs in the future. Natural products of sea-based fungus are expected to encourage drug users to look for better or newer antitumor agents. Metabolic processes and novel structures gain attention from drug physicians therefore, the biological functions of such chemicals are focused on the developing drug community [5]. Table **1** provides an outline of the uses, Fungi, and their compounds, which are a source of secondary metabolites give out.

Table 1. Marine Fungal Metabolites (compounds) and their Applications.

S. No	Fungi and their compounds as a source (Secondary Metabolites)	Uses
1	*Penicillium notatum*	Penicillin as a drug.
2	*Aspergillus sclerotiorum*	Antifungal and Antibacterial Activity.
3	*Acanthopora spicifera*	Active towards a panel of human tumour cell lines.
4	*Cladosporium sp.*	Antifouling activity towards macro andmicro fouling organisms.
5	*Bugula neritina*	Anti-larval activity.
6	*Ascochyta salicornia*	Anti-plasmodial activity.
7	*Ascosalli pyrrolidinone*	Anti-plasmodial activity.
8	*Colpomenia sinuosa*	Against *Candida Albicans*

CHAPTER 16

Mycosynthesis of Nanoparticles and their Applications

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Abstract: Nanotechnology refers to the creation and application of materials at the nanoscale up to 100 nm in size. Metallic nanoparticles, specifically gold, silver, alloy, *etc*. are being applied for multiple purposes, from medical treatments, industrial production, including solar and oxide fuel batteries for energy storage, to large incorporation into diverse materials of everyday use such as cosmetics or clothes, optical devices, catalytic, bactericidal, electronic, sensor technology, biological labeling and treatment of some cancers. Nanoparticles can be synthesized chemically or biologically. Nature is like a large "bio-laboratory" comprising of plants, algae, fungi, yeast, *etc*., which are constituted of biomolecules. Biogenic synthesis of silver nanoparticles is attractive due to the production of large quantities of proteins, high yields, easy handling, and low toxicity of the residues. Naturally occurring biomolecules have been recognized to play an effective role in the formation of nanoparticles with clear-cut shapes and sizes, thereby acting as driving forces for the designing of greener, safe, and environmentally good protocols for the synthesis of nanoparticles. Mycosynthesis was easy, cheap and eco-friendly, with simple nanoparticle downstream processing. Hence, in this book chapter, an emphasis has been given to mycosynthesis of nanoparticles, discussing the mechanisms and optimization of the synthesis, as well as the applications.

Keywords: Downstream process, Metallic nanoparticles, Mechanism, Nanotechnology.

INTRODUCTION

Nanostructured particles have been known in recent years to create wonderful nano-biotechnology. Bioscience studies have shown that the production and

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design of these particles are caused by natural mechanisms in microbes such as bioaccumulation, biomineralization, precipitation, and biosorption against toxic compounds.

The basic process of microbial detoxification is primarily accountable for nanoparticles synthesis, and this process is called nanobiosynthesis. This is a cheap and effective method according to Siddiqi and Husen [1], whereas chemical and physical synthesis, are still considered risky and expensive. Additionally, their involvement in biomedical functions includes the disadvantages of chemical synthesis procedures. Microbial synthesis is the most favored route because it is undeniably environmentally friendly, beneficial, manipulative, and consistent with various biomedical research [2]. Various extracts are used in microbial processes for nanoparticle synthesis from various species of bacteria, fungi, actinomycetes, algae, *etc* [3]. Fungi are, however, the strongest of all microorganisms, as their ability to secrete higher proteins allows higher productivity [4]. The existence of mycelia also allows them with a wider surface area, and they are both cost-effective and simple to grow and exhibit easy downstream processing. As in the case of certain fungi, NADH-dependent reductase enzymes are observed to be responsible for metal reduction [5 - 8]. Since their wide range of applications in the fields of biology, chemistry and physics, the latest trend in bio-nano science includes particularly metal nanoparticle synthesis. Silver (Ag^{+2}) and gold (Au^{+2}) were shown maximum use in biological applications among various metals that were examined for their significance. They are unbelievably known as antimicrobials, antioxidants, anticancer catalysts, *etc* [9 - 15]. Silver and gold NPs are thus researched extensively over a period of time, contributing to the biosynthesization and considerable discovery of numerous study groups. Extracts of various fungal species such as *Penicillium citrinum* [16], *Aspergillus niger* and *Aspergillus parasiticus* [17], *Aspergillus flavus* [18], *Aspergillus conicus, Penicillium janthinellum* and *Phomopsis* sp [19].]*, Pleurotus* ostreatus [20], *Aspergillus terreus* [21], *Bryophilous rhizoctonia* [22], *Fusarium oxysporum* [23], and *Fusarium semitectum* [24] are used to generate NPs. Similarly, many such fungi are synthesised with AuNPs, namely, *Aspergillus fumigatus* [25] and *Geobacillus* sp [26], of *Aspergillus fischeri*. In addition to fungi, yeasts are important for their easy handling methods and are therefore often chosen for nanoparticle synthesis in the laboratory. The eukaryotic systems, such as *Candida glabrata* and *Schizosaccharomyces pombe*, have been frequently utilized and recorded in several types of literature [27]. The next step is to classify them once the metal particles are synthesised according to the desired properties. Special instruments based on the various principles UV – visible spectrophotometer, X-ray diffractometer (XRD), Dynamic DLS microscope, energy dispersive spectroscopy (EDS) and Fourier transform infrared spectroscopy (FTIR) transformation can be used to determine nanoparticles' characteristics. The spectroscopy was used to analyse particles on the basis of their shape, size, functional groups present on them [28, 29].

Therefore, the analysis aims to show readers an idea of the usual methods and means of biosynthesis of silver and gold nanoparticles that have been carried out in the recent past using various fungal organisms. Characterization techniques help to ensure that the scale, shape, side groups and particle distribution or distribution pattern of bionanoparticles are properly understood.

GREEN SYNTHESIS AND ITS IMPORTANCE

Green synthesis, as the name suggests, includes the use and synthesis of nanoparticles by using environmental and non-toxic methods. Ganesan *et al.*, 2014 [30] explained that these approaches use plants or microbes that are cost effective, simple and safe to handle, unlike some chemical and physical processes. Chemical and physical synthesis processes use elevated temperatures, pressure, harsh reducing agents, organic solvents and capping agents such as sodium borohydride, chloroform and toluene are usually observed. Such systems that cause a hazard to the environment are undesirable. In addition, such deleterious chemicals may have side effects and impair the actual treatment practices as far as biomedical aspects are concerned. This path is also the most effective way to achieve monodispersity and well-defined particle dimensions, in addition to being eco-friendly and biocompatible. The high productivity of relatively superior quality nanoparticles from proteins or enzymes produced by microbes or plants. The finer features allow one to establish unique application characteristics and they demonstrate the definite mode of action that helps to advance various unknown fields of study. To represent different ways of biosynthesizing nanoparticles, particularly microorganisms, many review articles have been published. The literature shows how bacteria, fungi, actinomycetes, and yeasts are biologically blended into diverse groups of metal nanoparticles as well as their alloys [31].

MECHANISM OF SYNTHESIS

There has been very little study of the mechanism behind the green synthesis of nanoparticles. Different forms of microorganisms demonstrate different methods, but the fundamental concept behind all kinds of processes is the reduction reaction. Because of cellular peptides and polysaccharides, both intracellular and extracellular routes are shown to take place, leading to enzymatic oxidation, reduction, sorption, and chelation. Intermembranous transport, resulting in nucleation and nanoparticle growth contribute to the development of extracellular nanoparticles. NADPH-dependent nitrate reductase enzyme eliminates silver ions

CHAPTER 17

Fungal Mediated Synthesis of Nanoparticles: Characterization and Bioapplications

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Abstract: Metal nanoparticles have received a great deal of attention from researchers in recent decades due to their significant impact on a variety of important applications such as biomedicine, agriculture, energy, electronics, and sensing. Biosynthesis is another alternative approach in the synthesis of clean, reliable, cost-effective, and green approaches that interest nano and microbial biotechnologies, in addition to many conventional procedures reported for synthesizing metal nanoparticles. This chapter describes the studies relating to fungal mediated synthesis of metal nanoparticles, as well as their biomedical, catalytic, and agriculture applications. These fungi-based biomolecules undergo highly controlled assembly in the synthesis of suitable metal nanoparticles with different shapes, charges, and sizes. The formation mechanism has not yet been completely explained, in spite of the fact that it is accepted that fungal biomolecules are mostly used in the process. In fact, fungi are efficient biomolecules used in the synthesis of metal nanoparticles that show a wide range of bioapplications such as anti-cancer, anti-bacterial, antifungal, antioxidant, *etc*. This chapter highlights the potential use of fungi as a novel source for the green synthesis of metal nanoparticles with enhanced biomedical applications.

Keywords: Biomolecules, Biomedical applications, Chemical method, Conventional method, Green synthesis, Nanotechnology.

INTRODUCTION

Nanotechnology has become one of the great advancements in every aspect of science and technology. The term “nanotechnology” was authored by the Japanese scientist “Norio Taniguchi in 1974”. Nanotechnology deals with the architecture, identification, fabrication, and execution of the structures and

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devices by manipulating the shape and size at the nanoscale. The extraordinary properties of matter at the nanoscale open up new paradigms that lead to various applications in the scientific field.

Science and engineering are associated with the preparation, characterization, and utility of materials whose smallest functional organization is between 1-100 nm range in at least one dimension [1]. Nanotechnology overlaps the boundaries of several areas, including physics, chemistry, biology, and electronics [2]. The nanoparticles show unique chemical, physical and biological properties compared to their bulk matter [3]. Nanomaterials have a large diversity of applications in medicine, agriculture, cosmetics, food, *etc*., due to the large surface area, small size, high surface energy, and quantum confinement [4]. Metal nanoparticles have attracted attention in many fields, such as catalysis, electronics, sensors, biomedicine, drug delivery, biofuel cells, photonics, cosmetics, *etc* [5] [6].

Since ancient times, metallic nanoparticles have been used, and they have been either prepared by humans or nature. The "Lycurgus Cup" (displayed at the British Museum, London) is a perfect example of this; it is a class of Roman vessels illuminated internally (red) or externally (green), containing gold and silver nanoparticles [7]. Faraday, in 1857 synthesized gold nanoparticles as well as with different metals, for example, Zinc, iron, copper, and sStannum, and studied how they interact with light [8].

Nanoparticles ranging between 0.1 to 100 nm are generally synthesized through top-down and bottom-up methodologies [9]. The top-down method is the breaking up into nanosized structures using physical techniques such as grinding, milling, and crushing [10]. Generally, this approach is not acceptable for synthesizing nano-structured materials of uniform size and shape on a large scale and also produce imperfections in the surface structures that affect the physical properties and surface chemistry of nanomaterials [11]. The bottom-up method is the assembly of material from the bottom, molecule-by-molecule or atom-by-atom, to result in a complex structure with new properties. This is the most accepted approach for the chemical or biological synthesis of nanoparticles [12]. One of the generally utilized bottom-up approaches is the chemical reduction method, where metal salts are reduced using a suitable reducing agent [13]. Three significant classes of chemical compounds needed in this process are solvents, reducing, and stabilizing agents. In both approaches, variation in the morphology of the resultant nanoparticles is common [14]. Nanoparticles can be found in nanotubes, nanoplates, nano prisms, nanorods, nanospheres, nanobelts, and nano tetrapods that can be roughly grouped under icosahedrons, decahedron, face cantered cubic, regular decahedron, and cuboctahedrons [15]. Fig. (**1**) shows the synthesis of nanoparticles using bottom-up and top-down approaches.

This chapter aims to give an overview of the main published articles concerning the use of fungi for the microbial synthesis of nanoparticles, along with their applications in various areas.

VARIOUS APPROACHES FOR THE SYNTHESIS OF METAL NANOPARTICLES

The control over the morphology, size and shape of the nanoparticles forms the basis of materials 'synthesis and the characteristics of nanoparticles depend on them [16, 17]. Hence, the properties of such materials can be studied by controlling these parameters. Metal nanoparticles can be synthesized *via* physical, chemical, and biological processes which are described below.

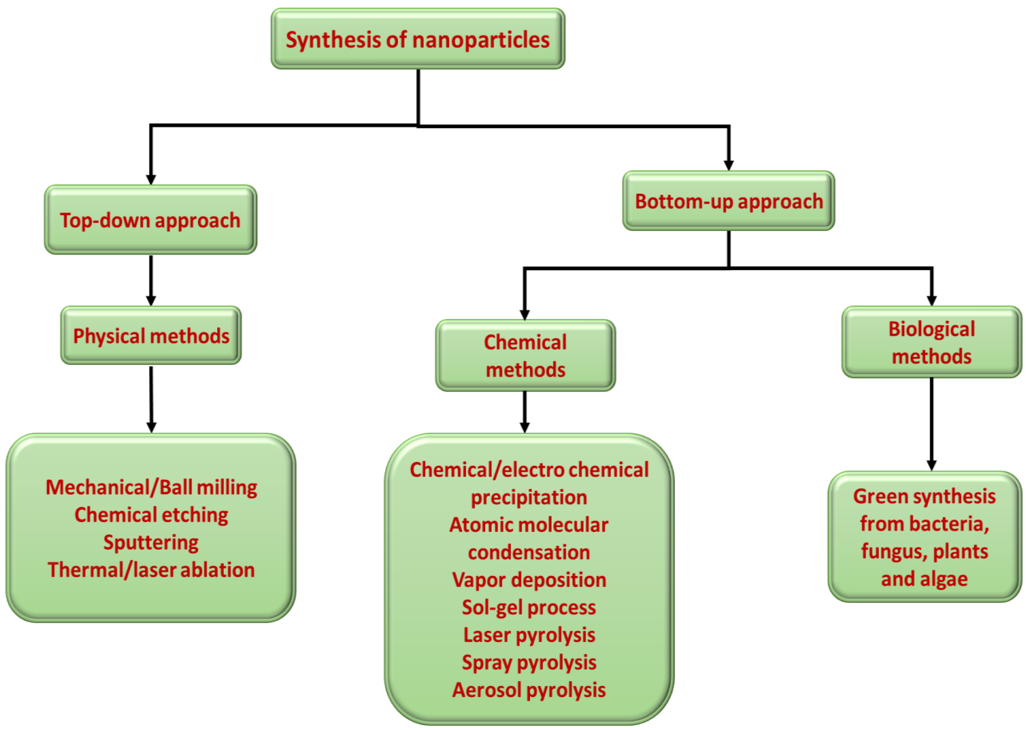

Fig. (1). Illustration of synthesis of nanoparticles using bottom-up and top-down approaches.

Physical Methods

The "physical methods" are considered as top-down approach, for the synthesis of metal nanoparticles, which is accomplished by reducing the size from macro to nanosized by various physical methods [18]. Many physical methods have been

CHAPTER 18

Thermophilic Fungi and their Applications in Biotechnology

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Abstract: Thermophilic fungi survive at a growth temperature above 45°C. Although widely spread in the terrestrial region, they have remained underexplored, diverging from other thermophiles of eubacteria and archaea. Thermophilic fungi elicited a lot of interest due to their ability to produce thermostable and thermotolerant compounds with practical applications in the field of biotechnology. Studies on growth kinetics, physiology, supplement uptake, and protein breakdown rate in thermophilic fungi have provided important information on these fungi. Thermophilic fungi can degrade biomass such as polysaccharides into monomers. The characteristics of their enzymes show contrasts among species, just as among strains of comparative species. Some extracellular compounds separated from thermophilic fungi are commercially produced, and a couple of others have the potential due to financial advantages.

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Thermophilic fungal genes encoding lipase, protease, and various other vital enzymes have been cloned and overexpressed in heterologous fungi for understanding the mechanisms of their natural thermostability and catalysis.

Keywords: Bioethanol, Heat-tolerant enzymes, Hyperthermophiles, Lignocellulo -sic biomass, Metagenomics.

INTRODUCTION

Life has existed on earth for more than 3.7 billion years. The present-day microbial diversity is a very little part of the entire microbial diversity so far investigated. Microorganisms are assumed to have a significant function in the advancement of the biosphere.

Thomas Brock studied thermophilic organisms in Yellowstone National Park (Wyoming, USA) from 1968 to 1978. He found *Thermus aquaticus*, which could survive temperatures over 70°C. Prior to 1970, only a few thermophiles were identified that could grow up to 70°C and many studies were done on strains of *Bacillus stearothermophilus*. Advances in thermophilic research began in 1981 when Karl Stetter and Wolfram Zillig isolated Thermoproteus, a thermo acidophile, from Icelandic natural aquifers. Karl Stetter and his co-workers isolated Pyrodictium, a thermophile, which can grow at 105°C, and Pyrolobus, which could survive temperatures up to 113°C. Numerous thermophiles have been isolated from many habitats, including mine effluents, geothermal regions, underground aquifers, and aqueous ocean vents. Both culture-dependent and culture-independent methodologies have been utilized to understand the diversity of organisms in thermal conditions. Among eukaryotes, only a few organisms can survive in extremophilic conditions. Cooney and Emerson [1] considered thermophilic organisms as those with a survival temperature of 50°C or above and a basic growth temperature of 20°C or above. Correspondingly, thermotolerant organisms are those that grow at temperatures of up to 50°C. In spite of the fact that this grouping is very viable, it is not appropriate for all members of this group. For instance, *Aspergillus fumigatus* can develop at temperatures over 50°C and under 20°C [2]. On the other hand, Maheshwari *et al.* [3] proposed thermophilic microbes as those with an ideal development temperature of 45°C or above. The study of thermophiles in various branches of biology and natural chemistry has expanded drastically in the last few decades.

DIVERSITY, DISTRIBUTION, AND SYSTEMATICS OF THERMOPHILIC MICROORGANISMS

Based on thermotolerance and adaptability, microorganisms are classified as psychrophiles, mesophiles, and thermophiles [6]. The optimal temperature range of each group is unique; the suitable range for one group is not good for the growth of others. The groups were split up into ecological niches that fitted them. Thermophiles are heat-loving organisms occurring in the temperature range of 45°C to 75°C, their favorable temperature range is 50°C to 60°C [4, 5]. These specific characteristics attracted the scientific community's attention and interest in their potential biotechnological applications in standard and adverse environments [5]. Thermophiles are distributed in samples of soil, hot-springs water, and algal mats [4, 5, 7]. They are also found in compost, sewage, profound seafloor stores, and rocks containing hydrocarbons [8]. Although the thermophiles are omnipresent, they are mostly confined to a few places [9]. Few are facultative thermophiles *viz*., *Bacillus coagulans* and *Bacillus circulans* [10]. Commercially, the most significant thermophile is *Bacillus stearothermopilus*. It has the capability to grow in the food industries as some strains develop at 80°C to 85°C [11, 12]. Friedman *et al.* [13] noted the significance of magnesium ions in thermophilic organisms. Temperature is the most important environmental factor, which is crucial for the development and survival of living organisms [7]. Usually, as the temperature rises, the rate of enzymatic reactions usually rises too, but only up to a certain level, above which the nucleic acids, proteins, and other cellular components may be irreversibly denatured. The microbial species which are confined to extreme temperatures and anaerobic conditions are represented in the Archaea group, specifically Euryarchaeota and Crenarchaeota [14]. Euryarchaeota incorporates thermophiles (*Thermococcus* and *Pyrococcus*), methanogens (*Methanopyrus* and *Methanococcus*), and sulfate and iron reducers (*Archaeoglobus*). Thermococcus species occur predominantly in hydrothermal areas. *Crenarchaeota* incorporates thermophilic and hyperthermophilic heterotrophs *viz*., *Hyperthermus, Staphylothermus,* and *Desulfurococcus*. *Pyrolobus fumarii* grows at 113°C and strain 121, a microbe from the group Desulfurococcales, grows at 121°C [15] while Methanopyrus grows at 110°C and 122°C [16]. Barophiles grow at high pressures up to 130 MPa [17]. Species *Methanococcus jannaschii*, isolated at a depth of 2600 m and hyperbaric conditions, grow at 75 MPa. Alain *et al.* [18] first reported the isolation of a thermobarophilic sulfate-reducing bacterium, *Marinito gapiezophila* strain KA3T, from the East-Pacific Rise at 2630 m. Its optimal temperature was 65°C and pressure 40 MPa.

Microorganisms that live in extreme environments utilize their adaptive mechanisms to survive, grow and develop, and this holds good for a greater extent for many extremophiles. Marine extremophiles can thrive and reproduce at extremes of salt concentrations (salinity >1.0 M NaCl), temperature (1–15 °C, >45 °C), pH (>8.0, <5.0), and pressure (average 380 atmosphere, >500–1200 atmosphere and beyond) and in the presence of high radiations, heavy metals, and inhibitors. Extremophiles belonging to the Eubacteria, Archaea, and Eukarya

CHAPTER 19

Biodegradation of Plastic by Fungi

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Abstract: Plastic is considered to be one of the most used polymers in industries, agriculture, packaging, and household matters. The indiscriminate use and extensive accumulation of plastic wastes in our environment due to the unidirectional use of synthetic polymers without their proper degradation has posed a potent global threat to the environment. The environmental contamination has resulted in urgency for developing new strategies for degrading plastics. In recent years, reports on the biodegradation of synthetic plastics by microorganisms or microbial enzymes have sprung up, and these offer a possibility to develop biological treatment technology for plastic wastes. The unique ability of fungi to invade the polymer substrates with the help of enzymes has attracted the attention of researchers. Moreover, fungi produce a chemical substance termed hydrophobin, which helps in the fungal adhesion to the hydrophobic surface, followed by the penetration of the hyphae into the surface of the substrate. Various fungal species comprising *Aspergillusniger, Aspergillusflavus, Fusariumlini*, *Pycnoporuscinnabarinus,* and *Mucorrouxii*are are predominantly used for the process of plastic degradation. This chapter will focus on the contribution of various fungal strains in the degradation of macro and microplastics, including synthetic, biodegradable, and oxo-biodegradable plastics, with a special reference to the analytical techniques used for their assessment.

Keywords: Enzymatic biodegradation, Health hazards, Plastics, Remediation, Wastes.

INTRODUCTION

Petroleum-based plastic is considered to be one of the regularly used synthetic polymers that play an important role in industries, agriculture, packaging, and household matters due to its unique features comprising of resistance to pressure,

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temperature, chemical solvents, and UV light [1]. Recent statistics have shown that in 2015, the production of plastic had reached up to 388 million tons [2].

The continuation of this rate of production of plastics will lead to a doubling in plastic content in the next 20 years. There has been an enormous increase in the use of plastics in various countries that include Europe, United States, and India [3]. The use of low-density polyethylene (LDPE) also showed a marked increase in our regular life. It has also been observed that the use of LDPE is more in comparison to polyethylene (PE) due to various properties that include thermal stability, odourlessness, resistance to degradation by chemicals, as well as various types of biological agents [4]. The cost associated with the production of LDPE is lesser in comparison to PE. This resulted in an overall enhancement in the use of LDPE to a marked extent and is widely used for the manufacturing of bags, containers, bottles, pipes, wrapping films and soil mulch film [5]. In recent years, reports on the biodegradation of synthetic plastics by microorganisms or microbial enzymes have increased, which offers a possibility to develop biological treatment technology for plastic wastes. But the properties of plastics including a high number of aromatic rings, high molecular mass, unusual bonds and halogen substitution made them almost resistant to microbial degradation [6]. The nonstop accumulation of such micro-, meso-, or macro debris, within the ecosystem leads to the development of plastic pollution. It has been also observed that improper disposal of plastic wastes like incineration, landfills and recycling results in the development of toxic gases [7]. The mechanism of improper planning for dumping the plastic wastes has been a threat to plants, animals and human beings. According to a report by the United Nation, only 9% of the total amount of plastic wastes liberated can be recycled and the remaining amounts remain dispersed within the environment.

The proper disposal of plastics including LDPE can be achieved by the degradation by microbial organisms. This process involves the mechanism of converting the carbon atoms that are present within the plastics to some form, which is relatively harmless for the environment. Various studies showed that macrobial and microbial degradation can be considered an efficient process of disposing of plastics [8]. Macro biological degradation of plastics involves various animals like insects and birds, while the microbial degradation of plastics mainly involves bacteria and fungi for the process of plastic degradation [9, 10]. Such biodegradation results in the emission of by-products that are eco-friendly and can be taken up easily by living organisms [11].

This chapter will focus on the role of different fungal strains in the biodegradation of plastic and polyethylene products with special reference to the various strategies they have adopted for converting the non-biodegradable plastics to relatively assimilable and degradable products, relatively harmless to the environment.

MAJOR FUNGI INVOLVED IN THE DEGRADATION OF PLASTICS

Studies have shown that both bacteria and fungi play an important role in the mechanism of plastic degradation [12]. It has been observed that bacteria initially invade the surface of the plastic resulting in colonization thereby allowing fungi and other species to enunciate the mechanism of degradation [13]. Studies have depicted that fungal cells isolated from the agricultural soil samples play a predominant role in the degradation of LDPE as they use the carbon from such plastics as a source of their nutrients. The most common soil-borne fungal species playing a significant role in plastic degradation are *Aspergillus*, *Penicillium* and *Fusarium* [14]. The development of biofilm on the exteriors of the plastics results in a marked reduction of hydrophobicity of the surface [15]. Experimental observation indicates that *Fusarium* sp. possesses the ability to erode the surface of the plastics after initial attachment [16]. It has also been observed that fungal species like *Mucor* and *Aspergillus* are associated with the degradation of polyethylene in the presence of starch.

Aspergillus sp.

One of the most potent fungal species that help in the degradation of polyethylene is *Aspergillus*. Various studies demonstrated that fungal species isolated from the polyethylene polluted sited comprise *Aspergillusniger* and *A. japonica*. It was also observed that the rate of degradation of plastics varies from species to species. Research works showed that *A. niger* possesses the ability to degrade LDPE to about 5.8% whereas *A. japonica* can bring about degradation of 11.11% in incubating for a time duration of one month [17]. It was further observed that the degradation of plastic was enhanced up to 38% by *A. niger* and 31% by *A. flavus,* respectively after 60 days. These fungal cells possess the ability to degrade various types of resistant plastics like LDPE by secreting various types of hydrophobic proteins which help in the attachment and colonization of the fungal cells on the surface [18]. Researchers found that *A. niger* possesses the ability to degrade modified polyethylene and after 30 days of incubation, the weight and tensile strength could be reduced by 3.44% and 61%, respectively.

Penicillium sp.

Various strains of *Penicillium* sp. like *P. chrysogenum* and *P. oxalicum* are involved in the degradation of both LDPE and HDPE by 34.35% and 55.59%, respectively after incubating for 90 days [19]. The mechanism of degradation by

CHAPTER 20

Red Oleaginous Yeast: Powerhouse of Basidiomycetes

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Abstract: Oleaginous red yeast belongs to the group basidiomycetes. The oleaginous yeast(s) of genus *Rhodosporidium*/ *Rhodutorula* is a single-cell mono or di-morphic, teleomorphic, and anamorphic yeast that reproduce sexually and asexually. Numerous species from these "red yeast" generate industrial bioproducts such as lipids, carotenoids, enzymes, biosurfactants, *etc.*. Their potential to grow on various carbon sources, comparatively simple large-scale cultivation, plethora of knowledge, and availability of several genetic tools encourage their use for devising sustainable bio-manufacturing processes. Oleaginous red yeast can accumulate more than 70% of lipids as compared to other fungi. This property makes it attractive to produce 3rd generation biofuel. They accumulate high concentrations of carotenoid pigments that can be employed as nutraceuticals, giving their colonies a distinctive color range of orange, red, or pink. Carotenoids are a set of bioactive compounds that are synthesized by plants and microorganisms in response to different environmental conditions. In general, the chemical synthesis of biofuels generates hazardous wastes; therefore, the microbial production of carotenoids has garnered huge interest due to its benefits, *e.g.*, low-cost substrates, lower production costs, minimal hazardous waste or by-products, *etc.*. Its color and antioxidant properties make carotene remarkably interesting in the food, feed, and pharmaceutical industries. The basidiomycete oleaginous red yeast accumulates and stores hydrocarbon-rich fats, also known as lipids, as primary metabolites during the early log phase and pigments like carotenoids as their secondary metabolite during the late stationary phase of growth from varied carbon sources, thus making it a powerhouse of yeast.

Keywords: Basidiomycetes, Carotenoids, Lipids, Oleaginous, *Rhodutorula*, Yeast.

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INTRODUCTION

Yeasts are mostly single-celled, eukaryotic fungi with approximately 1,500 known species. Most yeasts belong to the phylum Ascomycota while a few belong to Basidiomycota. These thrive mostly in soils and on plant surfaces (animate or inanimate), which are particularly rich in sweetened mediums like flower nectar, fruits, *etc.*.

Among the several varieties of economically important ascomycete yeasts, *Saccharomyces cerevisiae* is generally Regarded As Safe (GRAS). Many varieties of yeasts are usually used commonly to produce bread, beer, wine, and several other fermented foods. Several types of yeasts are also mild to severely hazardous towards humans as well as other animals. These include *Candida albicans*, *Histoplasma*, and *Blastomyces*. The shape of yeasts possesses numerous forms, from spherical to oval as well as filamentous with typically 0.075 mm of diameter. The mode of reproduction is primarily asexual, mainly *via* budding; a tiny bump protrudes from the parent cell, enlarges, matures, and detaches. Fewer yeasts reproduce by fission, a process in which the parent cell divides into two equal numbers of cells.

Red Oleaginous Yeast

The meaning of the word "oleaginous" is "oil-producing" or "rich in oils." These yeasts have gained significant interest as they store excess carbon as TAG (triacylglycerols) instead of polysaccharides [1]. Oleaginous yeasts have been reported to accumulate lipids up to 70% of their dry cell weight (DCW) depending on different growth conditions. The dehydrated cells are then harvested and used as a raw material in the production of second-generation biodiesel. Several species from the genus *Rhodotorula*, *Candida*, *etc.*., can produce glycolipids, known as polyol esters of fatty acids (PEFA), which have a huge significance in the biosurfactants industry. Apart from the oil producers, these yeasts contain "pinkish-red" pigment in their cells. These pigments are the natural compounds called carotenoids that can be extracted from the yeast and can be utilized in different food industries as well as pharmaceutical industries.

History of the Discovery of Oleaginous Yeast Species

Almost 70 oleaginous yeast species have been discovered and identified. Yeast strains intracellularly accumulating oil greater than 20% of their dry weight are considered oleaginous. For years, studies have focused on a small number of commonly available oleaginous species, namely *Yarrowia lipolytica, Lipomyces species, Rhodotorula glutinis, R. graminis, Cryptococcus curvatus, C. albidus,* and *Rhodosporidium toruloides*. *Rhodotorula glutinis* is one of the red oleaginous yeast that falls under the genus *Rhodotorula*, a basidiomycetous genus of pink yeasts, and it contains almost 370 species. Heterogeneity of the genus has rendered its classification cumbersome. These are five identified varieties that have been confirmed; however, until the year 2011, all were considered to represent in a single taxon. The fungus is a common colonist of animals, foods, and environmental materials. Several other oleaginous species have been discovered and studied in detail over the years. A wide range of oleaginous yeast species and strains were selected based on different properties, such as the utilization of substrate, mainly carbon source(s), osmotolerance, pH, and growth inhibitor tolerance. Many studies were done to investigate oleaginous species utilizing sets of randomly selected yeast strains. A survey was conducted in 2011 using unidentified soil and waste palm oil, which resulted in the discovery of 23 oleaginous fungi, introducing one new oleaginous yeast species, *Kodamaea ohmeri* [2]. Random selection of microbes resulted in better chances of oleaginous yeast discovery that increased from 3% to 10% [3]. With time, the selection process of oleaginous yeast strains was switched to their physiological properties, resulting in better success. Certain yeast species that were able to grow in the carbon-free medium and use their own stored lipids as the sole carbon source resulted in the discovery of *Cryptococcus adeliensis*, the other oleaginous yeast strain [4]. The yeasts from enriched soil were able to grow on glycerol as a sole carbon source and resulted in enrichment for oleaginous yeasts, with 13 oleaginous strains out of 40 yeasts examined [5]. Selective cultivation of yeasts from soil was capable of growing on low nitrogen medium, yielding a high proportion of oleaginous yeasts, *i.e.*, 48 strains out of 170 which were screened [6].

Yeast in Biotechnological Research

Yeasts have the potential for research related to the biotechnological field. The main advantages of yeast in biotechnological research are:

1. Yeasts have immense diversity amongst them
2. The exponential growth in yeast is fast; they can divide in every 20 to 90 min
3. They are proficient at exploiting inexpensive substrates
4. They can be easily cultivated on a large scale
5. The spontaneous mutants of yeast strains are easy to isolate
6. Yeasts strains can be genetically manipulated easily.

As compared to other microorganisms, yeast appears as the most modified microorganism for biotechnological research because where bacteria store surplus carbon as polysaccharides and lipids generally in the form of poly-hydroxyl alkanoates or wax ester, yeasts collect excess carbon as glycogen and lipids

CHAPTER 21

Mycoremediation of Synthetic and Xenobiotic Compounds

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Abstract: The speedy rate of industrialization, metropolitanization, and rising population are leading to the contamination of the environment and an increase in health issues. A variety of anthropogenic polymers are introduced into the soil in numerous ways. These compounds may be hazardous chemicals, polythene, pesticides, plastics, *etc.* Accumulation of these synthetic pollutants in the surroundings causes severe illness and can also lead to an ecological threat. Alternative strategies to combat these ever-growing issues have been inaugurated and executed from the microbiological aspect. Myco-remediation is the implementation of fungus or its metabolic derivatives for the detoxification of environmental contaminants. This technology is eco-friendly, more efficient, and very economical. It is a natural technology for the degradation of synthetic polymers. It has additional benefits over other conventional methods. In this chapter, we have highlighted the negative effects of common contaminants on health and the environment and also summarized the contribution of several fungi and enzymes that are able to degrade a variety of widely used synthetic plastics such as agricultural, pharmaceutical wastes, heavy metals, including polycyclic aromatic hydrocarbon. The chapter also explains the strategies which can be used as a future framework to remove or solve the global problem of pollution.

Keywords: Heavy metals, Myco-remediation, Pharmaceutical wastes, Polycyclic aromatic hydrocarbons, Xenobiotics.

INTRODUCTION

The word "mycoremediation" refers to the detoxification of the contaminant site by using numerous fungal species. The fungus is cosmopolitan in nature and represents different forms of groups from various environments [1]. Fungus is a eukaryotic organism. The fungi are saprophytic in nature and secrete a wide range of extracellular enzymes. These enzymes help in the breakdown of natural polymers like chitin, lignin, pectin, keratin, and cellulose. This also plays an

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important role in the degradation of organic molecules [2]. It is a cost-friendly method to remove the contaminants present in the soil. There are various forms of bioremediation depending on the micro-organisms used in the remediation process.

When fungi are implemented for the detoxification of contaminants, it is called myco-remediation. When plants are used for remediation, it is called phytoremediation, and when mycorrhiza is used, it is called myco-rhizoremediation. This article focusses on myco-remediation, *i.e.*, detoxification of contaminants using fungi, forms of myco-remediation, fungi used for detoxification, and advantages of mycoremediation.

The pollution in the environment is mainly due to the contaminants released by industries and the fertilizers used in agriculture. The contaminants that cause pollution are: (a) metal, (b) hydrocarbons, (c) polycyclic aromatic hydrocarbons (PAHs), (d) Polychlorinated biphenyls (PCBs). They also contribute to pesticides and plastics as a softener, paints, and many other products. They are the most harmful anthropogenic substances that lead to pollution. They are mainly by-products of industries, chemical manufacturing and are found in ashes from combustion processes. Dioxins and polychlorinated biphenyls are very recalcitrant substances for biodegradation. They have aromatic structures and high degree of chlorination which makes the biodegradation process slow [3].

The anthropogenic substances like hazardous toxic heavy metals and organic pollutants become a key apprehension in environmental and health problems. These substances lead to soil and water contaminations [4]. Metal pollutants such as Cd, Cu, Hg, Pb, Mn, As, Ni, Zn, *etc.* lead to soil pollution. They are released into the environment from industrial effluent and other human activities [5]. These heavy metal contaminants are not biodegradable and have the potential to enter each trophic level of the food chain *via* bioaccumulation, unlike organic contaminants. Several metals act as a micronutrient. Metals such as Cu, Fe, Mn and Zn are micronutrients and play a vital role in metalloenzymes in most organisms [6]. The conventional process for the removal of contaminants like heavy metals from the soil and waste water includes precipitation of certain chemicals, chemical oxidation or reduction, ion exchange, reverse osmosis, filtration, adsorption using activated charcoal, electrochemical treatment and evaporative recovery [7] These physicochemical types of techniques are very expensive. Their metal binding is non-specific. The microbial process of detoxification of contaminants is more significant than the conventional process [8]. The naturally occurring fungi have the potential to adapt to any ecological system and conditions, due to the presence of a large number of extracellular proteins, organic acids and other metabolites. The filamentous fungi like

Trichoderma sp. are reported in soil mostly in the agriculture systems [9]. Fungi are mostly found in soil, root, foliar environments and other environmental conditions. Some fungi have specific characters as they can grow faster, undergo sporulation and are also used as biocontrol agents. Fungi secrete cell wall degrading enzymes to undergo bioremediation and makes the myco-remediation process cost and eco-friendly in nature [10]. Fungi have high resistant capability against different types of contaminants like heavy metal toxins and xenobiotic compounds, such as antibiotics, fungicides, herbicides and pesticides, *etc* [11]. Filamentous fungi Trichoderma sp. have the potential and good antagonistic capabilities against some plant pathogenic fungi. Some heavy metals play a significant role in the metabolic activities of fungi in comparison to others [12]. *Monilinia fructicola* has the potential to decompose cellulose and hemicelluloses with the help of metal ions like copper and manganese. These metal ions directly participate in the process of lignin degradation in white-rot fungi [13]. The role of Mn in lignin degradation has been reported in many studies [14]. Some plants have the potential to detoxify soils contaminated with metal and persistent organic pollutants (POPs) which seek attention [15]. The rhizospheric detoxification or rhizospheric remediation is too cheap compared to other approaches and is eco-friendly [16]. The phytoremediation is the term widely used for the elimination of metal contaminants and polymer contaminants from the soil. This process leads to the accumulation of toxic metals by plants, as the plant tissues are the depository of the pollutants [17]. The plants which are used to detoxify persistent organic pollutants can be termed "rhizospheric remediation", as the persistent organic pollutants detoxification in most situation occur in the rhizospheric region rather than in the plant [18]. The fast bioremediation of polymers or mineralization in the rhizospheric region has been observed for a wide range of polyaromatic hydrocarbons (PAHs), hydrocarbons, surfactants, pesticides and chlorinated alkanes [19]. The plant has the highest microbiological activity in the rhizosphere region. Other factors like physical (pollutants drawn into the rhizosphere by the transpiration stream, alteration of soil structure) and biological (bacterial plasmid transfer) factors may also play a role. The microbes in the rhizospheric region do not utilize persistent organic pollutants as an energy source rather they metabolize them as a consequence of utilizing plant-derived cyclic compounds [20]. Enzymes that can be used to digest the xenobiotic compounds by free-living rhizospheric microbial biomass can also degrade persistent organic pollutants [21]. A single microorganism cannot process the detoxification of persistent organic contaminants by the complete secretion of a group of enzymes required to detoxify the persistent organic contaminants. The consortium of rhizospheric microorganisms or groups of rhizospheric microbes can detoxify the persistent organic contaminants and pesticide [22]. Plants that undergo detoxification of constant organic pollutants contaminated sites have multiple challenges. The

CHAPTER 22

Bioremediation of Cypermethrin by Fungi

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Abstract: All over the world, due to the extensive use of pesticides in the agriculture sector, the soil gets contaminated and polluted. In the agriculture field, the pyrethroid pesticide cypermethrin is extensively used to control cotton, vegetables, and fruit insects. Cypermethrin is harmful for the atmosphere, human beings, and animals, along with having exerted detrimental effects on the nervous and reproductive system. Cypermethrin is moderately toxic to the skin and also negatively affects health, such as neurotoxicity and reproductivity. The toxicity of cypermethrin may also lead to death. Due to the very slow degradation rate, low water solubility, and nonpolar nature, the residues of cypermethrin can persist in soil for a longer period with a significant threat. The metabolite of cypermethrin, 3-phenoxy benzoic acid, is also hazardous to humans and surroundings, in addition to causing serious widespread soil and groundwater pollution. Therefore, there is a need to identify and remove such hazardous chemicals. Bioremediation is the best alternative, which is the most effective, inexpensive, and eco-friendly method. This chapter shows an overview of the current status of biodegradation of cypermethrin in soil and simultaneously also highlights applications of the bioremediation process, which converts cypermethrin and 3-PBA into simple and harmless substances, which play a key role in protecting human lives and the environment. Fungi play a major role in the bioremediation of cypermethrin and 3-PBA contaminating soil. Fungi are more efficient than bacteria because fungi are critical to the biogeochemical cycle and responsible for the bulk of the degradation of xenobiotics like cypermethrin.

Keywords: 3-phenoxy benzoic acid (3-PBA), Biodegradation, Bioremediation, Neurotoxicity.

INTRODUCTION

The pesticides constitute the key control strategy for crop disease and recently, pest management has been making significant contributions to enhancing the crop

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yield per hectare in India. Pesticides include a wide range of herbicides, insecticides, fungicides, rodenticides, nematicides, *etc.* Huge quantities of pesticides are regularly used in agriculture throughout the world and most of them are toxic for animals and humans.

Nowadays, it has been observed that the demand for agro products increased regularly in the agriculture field. Pesticides are applied to enhance crop productivity however, in due course of time, they get accumulated in plant parts, water, soil, air and biota [1]. Extensive use of pesticides contaminates soil, water and air, reduces the crop yield and is responsible for showing very hazardous effects on the environment [2]. Cypermethrin is a synthetic pyrethroid insecticide that has high insecticidal activity and is commonly used in household applications for pest/insect management because of their target oriented mechanism of action. The half-life of the cypermethrin in the soil varies from 14.6 to 76.2 days, depending on the physicochemical properties of the soil. Microbial activity plays a crucial role in finding out the fate and performance of cypermethrin. Microorganisms are considered the most capable biodegradation and bioremediation sources. The main objective of this article is to study the possible applications of isolated fungi from soil, use of these fungi for the degradation of cypermethrin and 3-PBA and evaluate their applicability for soil bioremediation. Different fungal and bacterial strains like *Aspergillus* sp., *Candida* spp., *Trichoderma* spp., *Pseudomonas fluorescence*, *Raoultella ornithinoytica*, *Bacillus* spp., *etc.*, show the significant ability for the degradation of pyrethroid pesticide cypermethrin [3]. The pyrethroid pesticide like cypermethrin and its key metabolite/intermediate product 3-phenoxy benzoic acid both cause serious soil and groundwater pollution. The extreme use of these pesticides in the agricultural sector shows damaging effects to humans and biodiversity. Therefore, bioremediation is considered an effective remedy that is harmless, effective, cheaper, and eco-friendly [4 - 6]. Fungi are more effective than bacteria, and they are responsible for a vast amount of pesticide degradation [7]. Fungi are considered the most competent in biodegradation and bioremediation methods. The current approach is safest, as fungal cultures always help convert toxic material into simple and harmless substances and play a key role in protecting human lives and the environment. The pyrethroid pesticide cypermethrin and the major intermediate product of cypermethrin degradation 3-PBA; both cause serious soil contamination. Excessive use of this pesticide in agricultural areas has negative effects on humans and the entire biodiversity. Therefore, here we proposed a significant, economical, and eco-friendly method that is bioremediation. Fungi are considered the most effective in biodegradation and bioremediation methods.

PESTICIDE CLASSIFICATION

Pesticides are generally classified based on structure, and the structural classification of pesticides is divided into chemical pesticides [8] and biopesticides, which are mentioned below Fig. (**1**). Pesticides are also classified based on their use and include a variety of different types of chemicals like herbicides, insecticides, fungicides, and rodenticides.

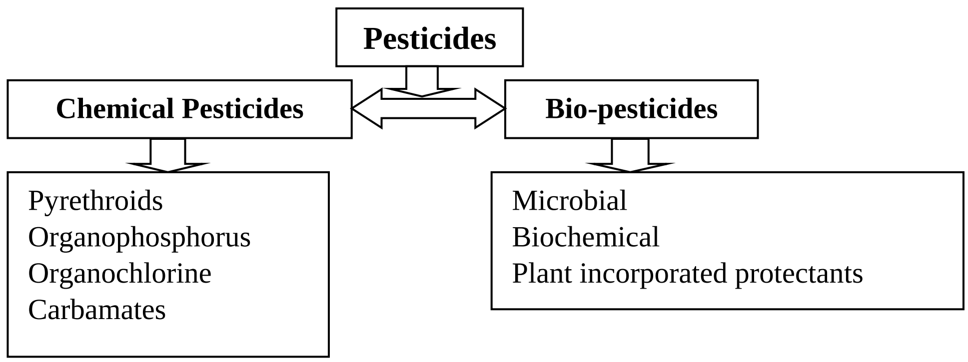

Fig. (1). Structural classification of pesticides.

Chemical Pesticides

Chemical pesticides are chemical materials that can kill pests and show detrimental and harmful effects on human beings, animals, and the environment. Chemical pesticides include several types of pesticides which are mentioned below.

Pyrethroid Pesticides

Pyrethroid pesticides are strong neurotoxins. As same as the organochlorine pesticides, pyrethroids are also endocrine disruptors. They are responsible for causing paralysis. These pyrethroid pesticides, including cypermethrin, cyfluthrin, permethrin, allethrin, *etc.*, play a decisive role in controlling household pests like bed bugs and are also responsible for showing a lethal effect on these bed bugs. These pesticides can cross the exoskeleton of the insect. Cypermethrin and deltamethrin are the best examples of pyrethroid pesticides. Due to the weak hydrophobicity, pyrethroid pesticides can easily migrate and accumulate in the soil [9].

Organophosphorus Pesticides

Organophosphorus pesticides are made up of chemical substances formed by the reaction between phosphoric acid and alcohols. Organophosphorus pesticides

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Dr. Shantipriya Ajmera Assistant Professor in the Department of Microbiology, Palamuru University. She has a bright academic record with research experience in Mycology working on the Coprophilous thermophilic fungi. Her research focuses on thermotolerant enzymes from thermophilic microorganisms and exploring fungal biomolecules in inhibiting the growth of multidrug-resistant pathogenic bacteria. Her research work has been published in National and International journals and presented in several National and International conferences. She is the recipient of the UGC Research fellowship Award-2013, Microbiologist Society India Young Scientist Award-2020 and honored as an Associate fellow of Telangana Academy of Sciences 2019, life member of many academic bodies like Indian women scientist's association (IWSA), Microbiologist's Society India (MSI), The Association of Microbiologist's India (AMI), Indian Academy of Neurology (IAN), Indian Science Congress Association (ISCA) and Coordinator of Microbiologist's Society India, Palamuru University, Telangana. She has been credited with two books, and published more than 20 research articles and 12 book chapters.

B. BHIMA

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