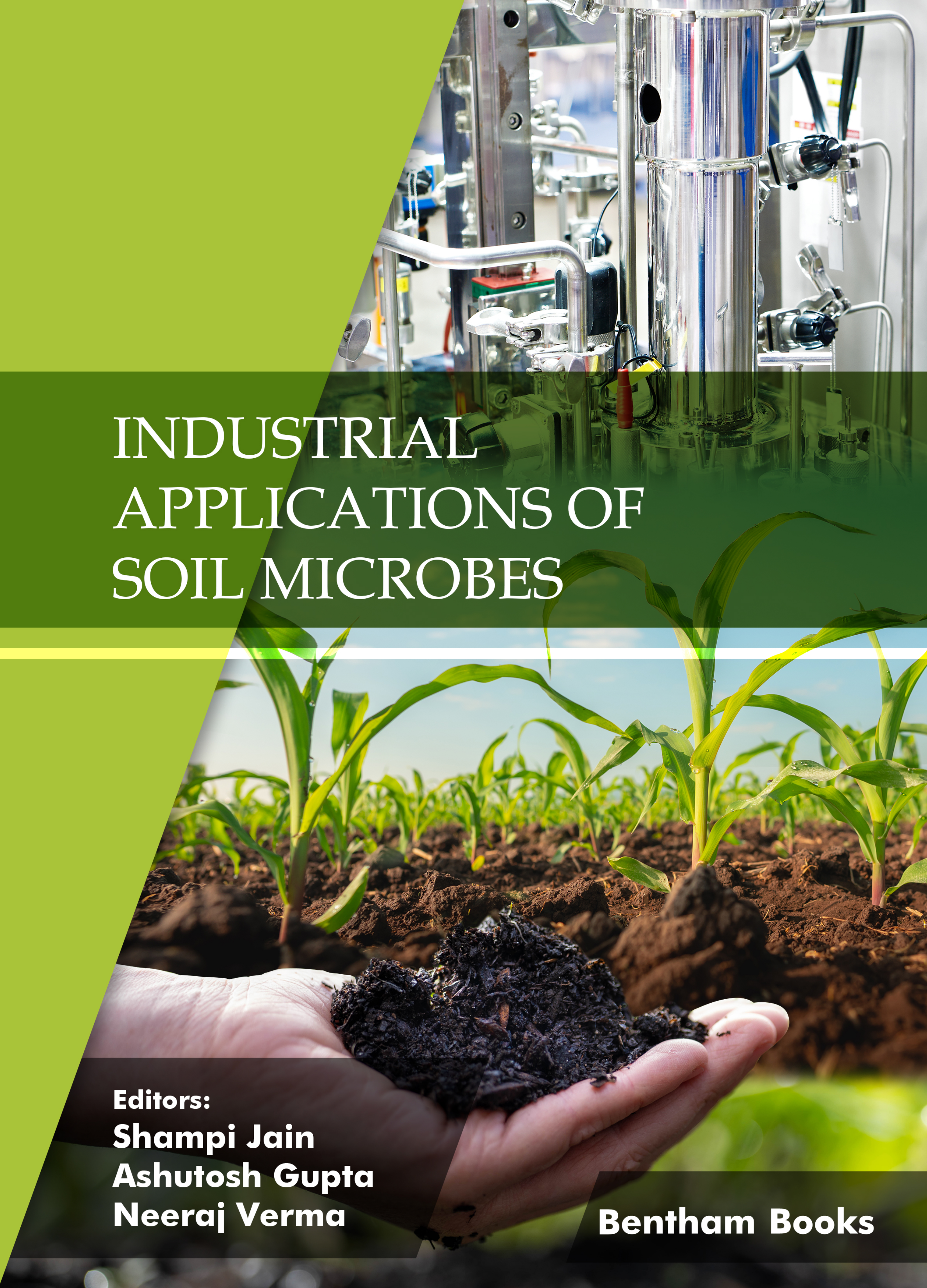

INDUSTRIAL APPLICATIONS OF SOIL MICROBES

Editors:

Shampi Jain
Ashutosh Gupta
Neeraj Verma

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Industrial Applications of Soil Microbes

(Volume 2)

Edited by

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FOREWORD

Microbes, or microscopic organisms, are widely used in large-scale industrial processes. They are crucial for the production of a variety of metabolites, such as ethanol, butanol, lactic acid, and riboflavin, as well as the transformation of chemicals that help in reducing the environmental pollution. For instance, microbes can be used to create biofertilizers or to reduce metal pollutants. Certain non-microbial products can also be used to produce certain microbial products, such as the diabetes medication insulin.

The immense use of chemical fertilizers, pesticides, fungicides, and weedicides has diminished the soil texture, quality, and ecosystem of the soil. It also leads to an unfavourable environment for favourable soil microbes. It may also lead to the development of resistant pathogenic strains, which may have devastating effects on the plants. Therefore, it seems that it is the best time to reduce the use of chemicals in agriculture and farmers should opt for biologically derived chemicals in their fields. The beneficial soil microbes, upon interaction with the plants, enhance productivity. They directly or indirectly take part in various enzymatic activities, leading to various byproducts beneficial to plants and ultimately animals, including human beings. Therefore, the use of natural bio-molecules will not only increase soil productivity but will also be environmentally eco-friendly.

This book, which deals with "INDUSTRIAL APPLICATION OF SOIL MICROBES" Vol. 2, is a timely, appreciable, and praise-worthy contribution to the field of Industrial Microbiology. The different chapters of this book, written by various experts in their fields, have made this book more valuable and highly scientific. A team of a galaxy of experts has explained the dynamics of soil microbes and the knowledge about their direct or indirect use in agriculture and industry. This book will help to launch a programme to make every family farm a bio-fortified farm in the days to come. I, therefore, hope that this book will be widely read by students, teachers, and researchers and will serve the purpose of being an important reference book.

I congratulate all the contributors and members of the editorial board for preparing this script of microbiological wisdom.

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PREFACE

Modern agricultural practices largely rely on high inputs of mineral fertilizers to high yields and involve the application of chemical pesticides to protect crops from diseases and pests. These practices are now being reevaluated and coming under scrutiny as our awareness of potential health and environmental consequences of excessive mineral fertilizers and chemical pesticide usage improves. It is widely recognized that the application of mineral fertilizers (especially nitrogen) can result in soil sickness and groundwater contamination by nitrates leaching through the soil profiles. To preserve our most precious natural resources like soil and water, this is high time to go for organic agriculture.

Thus, the present condition of soil and groundwater again recalls the scientists and researchers to maintain its productivity as well as quality. As the world population is increasing enormously, there is a need to sustain agriculture and increase the yield of agricultural products by using biochemicals instead of synthetic chemicals to reduce the negative impact on the soil and water environment. Using these biochemicals not only the productivity of soil will increase due to microorganism activity, but also the ecosystem could be saved.

For the use of soil microorganisms as biochemicals or the bioproducts obtained from them, one should know about the soil microorganisms effectively and their utility, which has been established over the years of research. Various chemicals like enzymes, vitamins, minerals, organic acids, biopigments, antibiotics, *etc.* have been successfully isolated from these microorganisms and are being exploited in various industrial fields like pharmaceuticals, Agriculture (biofertilizers, biopesticides, etc), food industries, beverages, and dairy industries.

Several studies have been conducted to exploit these microorganisms to produce biochemical helpful for not only humans but also for animals and the environment. However, these research findings are in scattered form. Although, the subject of microbes and their use in agriculture is very important there is a dearth of research-based books. Hence in this text, we have tried to collect the research findings of several eminent scientists and compile them in the form of a book. Therefore, this book will provide exhaustive knowledge about microbes and their uses for the welfare of mankind. The industrialists, professors, extension workers, scientists, and students will get most of the information about these soil microbes and their uses in one place.

The editors are highly thankful to the publisher of this text. Since this is the first edition, it may contain some errors that crept in inadvertently in spite of all editorial care. So, we are always interested to hear comments and suggestions from the learned readers.

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CHAPTER 1

Microorganisms and their Industrial Uses

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Abstract: For human beings, the diversity of microorganisms is still an undiscovered aspect. For the well-being of society, the huge microbial population performs many vital activities. Microorganisms play an important role in sustainable agriculture, environmental protection, and human and animal health. Microorganisms have a major contribution to agricultural issues like crop productivity, plant health protection, soil health maintenance, and environmental issues like bioremediation of soil and water from many pollutants. In addition to these activities, microorganisms also produce many products, either directly or through industrial processes, which are essential for human survival. In this chapter, we deal with various industrial products that are produced by microorganisms through different reactions, like antibiotics, enzymes, natural food preservatives, vitamins, fermentation products, amino acids, and agricultural products.

Keywords: Antibiotics, Biopesticides, Enzymes, Fermentation, Microbial Biotransformation.

INTRODUCTION

Microorganisms are mainly used to provide a large number of products and services. Because of the ease of their mass cultivation, speed of growth, use of cheap substrates (which are mainly wastes), and the diversity of potential products, they are considered very beneficial to human beings. As for the ease of their genetic manipulation, they have limitless possibilities for new products and services from the fermentation industry. The branch of biotechnology and microbiology that mainly deals with the study of various microorganisms and their applications in industrial processes is referred to as industrial microbiology. Even before the existence of microorganisms was known, they were used in industrial processes. Microorganisms play multiple roles in the industry. There are

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many ways to manipulate microorganisms so that product yields will be increased. The microorganisms are manipulated to produce many products like antibiotics, vitamins, enzymes, amino acids, solvents, alcohol, and dairy products. The discovery of microorganisms with their multiplicity of highly specific biochemical activities has stimulated a steady growth of industrial fermentation processes.

Microbes are widely used to synthesize several products valuable to human beings in industrial processes. Numerous industrial products have been derived from microbes such as:

- Antibiotics
- Enzymes
- Natural food preservatives
- Vitamins
- Fermentation products
- Amino acids
- Agricultural products

ANTIBIOTICS

For medical applications, the production of new drugs synthesized by a specific organism for medical purposes is the main focus of industrial microbiology. Antibiotic treatment is essential for many bacterial infections. Many naturally occurring antibiotics and precursors are produced through the fermentation process. As the microorganisms grow in a liquid medium, the population size can be controlled to maximize the amount of the product. During the production of antibiotics, the nutrient environment, pH, temperature, and oxygen should be controlled to produce a maximum number of cells without any mortality.

Certain microbes produce antibiotics, which function either by killing or retarding the growth of harmful microbes without affecting the host cells. Since 1928, when Alexander Fleming discovered the first antibiotic penicillin from the fungus *Penicillium notatum*, several microorganisms (fungal and bacterial) have been reported to produce many other antibiotics. Antibiotics such as streptomycin, tetracycline, chloramphenicol, erythromycin, vancomycin, and neomycin have been isolated from various *Streptomyces* spp. (to treat many bacterial infections), while antifungals such as Amphotericin B and Nystatin have also been isolated from *Streptomyces* spp. Bacitracin is an antibiotic that has been isolated from *Bacillus subtilis* [1].

Most of the antibiotics are produced by fungi or actinomycetes, whereas bacteria produce subtilin and bacitracin. But the latter is used very limitedly because of its toxic effect.

Steroids can be produced by microbial biotransformation. Steroids can be consumed either orally or by injection. Arthritis is mainly controlled by the use of steroids. Cortisone is an anti-inflammatory drug that fights against arthritis as well as several skin diseases. Testosterone is also a steroid, which is produced from dehydroepiandrosterone by using *Corynebacterium* sp.

ENZYMES

The biological catalysts that are mainly used to control certain biochemical reactions in the living system are enzymes. Enzymes have a wide range of applications both in the medical and non-medical fields. The enzymes, which are obtained from certain microbes, are referred to as microbial enzymes. Industrial enzymes are mainly produced by microorganisms through safe gene transfer methods. In the year 1896, from fungal amylase, the first industrially produced microbial enzymes were obtained and were used for indigestion and other digestive disorders. Some enzymes synthesized by microorganisms with their uses are described as follows [1]:

Proteases

These are obtained mostly as neutral proteases or zinc metalloprotease from *Aspergillus* spp. and *Bacillus* spp., along with some alkaline serine proteases.

Uses

a. Biological detergents: The alkaline proteases are used in this, which are produced by *Bacillus licheniformis* and *B. subtilis.*
b. Baking: dough modification/gluten reduction and flavour enhancement
c. Beer brewing: used to chill proof beer to remove protein haze.
d. Tendering and baiting with leather
e. Cheese processing: coagulation of milk protein, accelerated ripening, and flavoring.
f. Tenderization of meat and removal of meat from bones
g. Flavoring control and food product production
h. Waste management: Silver recovery from used photographic films

Lipases

These are mainly produced by various species of *Bacillus*, *Aspergillus*, *Rhizopus*, and *Rhodotorula.*

CHAPTER 2

Soil Inhabitant Bacteria: Morphology, Life Cycle and Importance in Agriculture and Other Industries

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Abstract: There are many bacteria in the soil, but they have less biomass because of their small size. Soil-inhabitant bacteria are an essential source of nutrients for plants. Some studies highlighted their industrial importance, like in the pharmaceutical industry, perfume manufacturing, and agriculture product scale-up production, including biofertilizers. Most of the studies have been carried out on Actinobacteria and *Nitrobacter* because of their potential to produce biofertilizers and chemical constituents on a large scale. This chapter discussed their taxonomic and morphological characteristics and gathered details about their practical applications from limited studies carried out in this field.

Keywords: Actinobacteria, *Azotobacter*, *Azospirillum*, Cyanobacteria, *Rhizobium*.

INTRODUCTION

Soil harbours a variety of bacterial communities. One gram of soil contains 10^3 to 10^6 unique species of bacteria. Most of these bacteria have not been fully characterized. However, through recent molecular advancements, we have gained in-depth knowledge of these bacteria from cellular to molecular level [1]. This data provided crucial information about the ecology of these bacteria and their importance to the soil ecosystem. Several taxa of these bacteria have been well studied in terms of their ecology, morphology, life cycle and importance in the industry. These are as follows:

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Contribution: Both authors contributed equally.

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1. **Actinobacteria** act as an essential nutrient (calcium, phosphorus and sodium) regulator in the soil [2].
2. ***Streptomyces*** act as a catabolic organism and increase soil fertility by converting xylan, lignin, cellulose and lignocellulose into organic matter [3].
3. ***Azospirillum*** acts as an influencer of growth due to its ability to fix nitrogen and stimulate auxin production [4].
4. ***Azotobacter*** acts as a phytohormone accelerators (*e.g.*, indole 3 acetic acid), metabolizes heavy metals, and pesticides and degrades oil globules [5].
5. ***Rhizobium*** acts as a nitrogen fixer [6].
6. ***Nitrobacter*** acts as a nitrite oxidizer by oxidizing nitrite into nitrate, which is a primary source of inorganic nitrogen [7].

This chapter discusses the physiological characteristics of soil-inhabiting bacteria and their importance in various industries.

ACTINOBACTERIA

Actinobacteria are freely distributed in both aquatic and terrestrial environments. These are Gram-positive filamentous bacteria. The feeding nature of these bacteria is saprophytic, which means they feed on organic matter. These bacteria are found abundantly in alkaline soil containing high organic matter content, whereas they are less abundant in water or air. These bacteria inhibit the upper surface and deep ground up to 1.5 m of soil [8].

Morphological Characteristics and Life Cycle

The characteristics of Actinobacteria mainly relate to cell wall composition, phospholipids, type of menaquinone, and sugar content of cells. Fragmented mycelium is regarded as a unique form of vegetative reproduction. However, these bacteria usually reproduce by asexual spores. Moreover, various morphologies have been observed in these bacteria, mainly the absence or presence of aerial and substrate mycelium. Besides, mycelial colouring and diffusible melanoid pigment production are also considered as one the criteria to distinguish between actinobacteria species [9]. It reproduces from both types of mycelia depending on the conditions, *i.e.*, it reproduces from aerial hyphae to form spores on a solid surface. On the other hand, substrate mycelia develop from germinating spores (Fig. **1**).

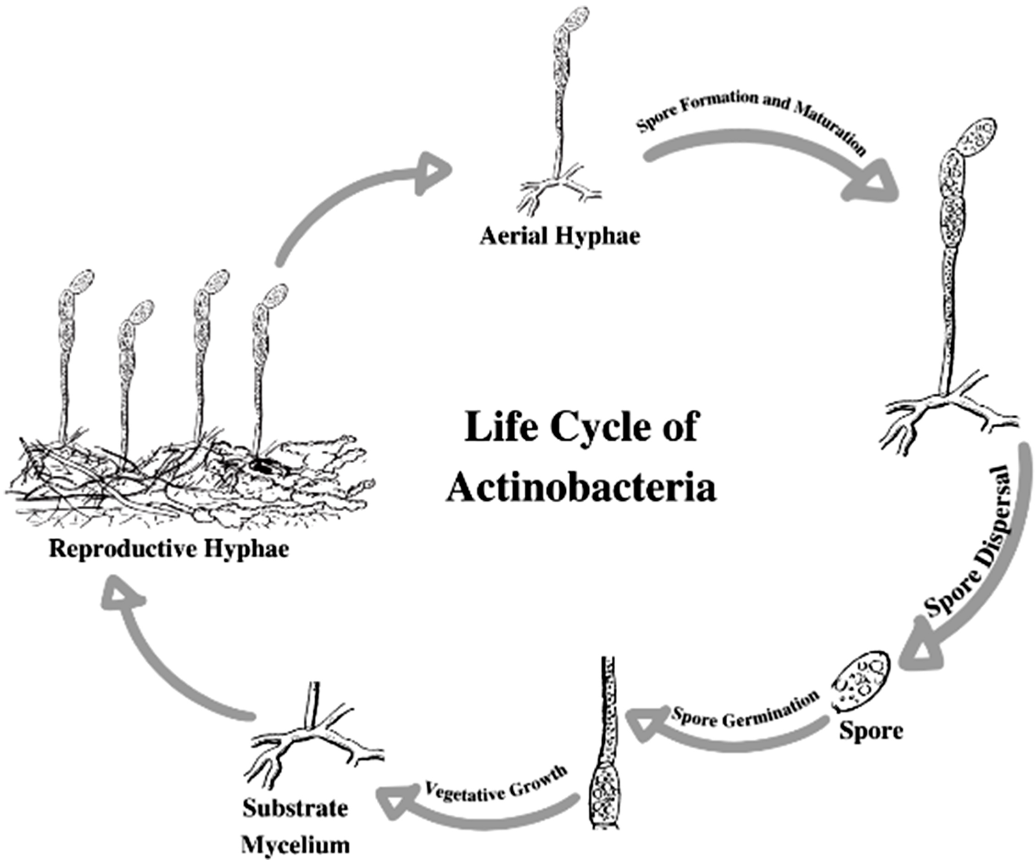

Fig. (1). Schematic illustration of the life cycle of typical actinobacteria.

Industrial Importance and Applications of Actinobacteria

Several genera of actinobacteria have been described for their industrial importance. The main industrial applications of these bacteria deal with biomedical science and biotechnology. Through recent advancements in bioinformatics tools and DNA sequencing, we can understand metaproteomics (proteomics for large-scale production of microbial enzymes) [10]. Actinobacteria have been deeply explored for their potential to produce amylases, proteases, cellulases, pectinases, chitinases, and xylanases. With their applications in paper, pulp, waste management, detergents, and agriculture, some of these industrially essential enzymes are listed in Table **1**.

CHAPTER 3

Role of Soil Microbes in the Sustainable Development: Agriculture, Recovery of Metals and Biofuel Production

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Abstract: Indiscriminate use of agrochemicals to ramp up production capabilities has caused a considerable decline in soil health status. The growing awareness of their ill effects on the environment and human health has called for a reversion to old organic agricultural practices blended with modern-day science and technology. Soil microorganisms with an identified ability to support plant growth are now being deployed in the form of biofertilizers and microbial biocontrol agents. Other than augmenting nutrition supply, these bio-inoculums can synthesize phytohormones and can also enhance the micronutrient and organic content of the soil. They can further induce resistance in plants against phytopathogens and compete against them by secreting secondary metabolites to keep the pathogenic population in check. Soil microorganisms, due to their omnipresence and survivability on varied substrates and in different environmental conditions, also find their use in other applications such as in the mining and energy industries. Unlike conventional metallurgical practices that deplete high-grade mineral ore reserves and cause wide-scale destruction of habitats, bioleaching provides a safe and cheap prospect for the recovery of metals. Other than the extraction of precious metals from low-grade ores, they also find their use in metal recovery from e-waste and can even remove heavy metals from soil. Moreover, the rapidly developing mining and the agrochemical industry count upon fossil fuels to meet their energy needs. In the final section of this chapter, we discuss a yet fascinating aspect of how non-conventional sources of energy are produced by the action of soil microorganisms to minimize strains on fossil fuel reserves. These biofuels, produced by the transformation of organic biomass, have an edge over fossil fuels as they emit low levels of particulate matter, sulphur dioxide, and carbon monoxide.

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Keywords: Bioleaching, Biocontrol, Biopesticide, Bioethanol, Biogas, Biofertilizer, Nitrogen.

INTRODUCTION

While the world was witnessing a serious food shortage owing to an exploding population and limited arable land, the increased yield losses due to the pest attack added to the growing concern. It was around the 1950s when the green revolution started, which employed hybrid seeds that could diminish the shortage of food grains. These 'high yield' variety seeds were indeed instrumental in substantially increasing crop production. However, they demanded extensive use of expensive chemical fertilizers, pesticides, and better irrigation, which had an immediate financial impact [1]. To achieve self-sufficiency and food security, this new agricultural practice took place around the world, ignoring the long-term environmental and health issues that we are facing today.

Soil is described as "the most complicated biomaterial on the planet" [2]. This complexity is associated with the chemical and biological heterogeneity of the soils around the globe. Overuse of inorganic fertilizers has induced a microclimatic change in the soil and reduced its organic carbon content, affecting the efficient uptake of nutrients and depleting micronutrient content. Moreover, it causes soil erosion and adds to environmental pollution. These chemicals deplete soil health by disturbing indigenous microbial diversity, soil fertility, pH, salinity, and other physico-chemical properties [3]. Thus, there is a mandate for an alternative that is sustainable in the long term, is cost-effective, and still increases the crop yield. The use of bio-inoculants comprising plant growth-promoting bacteria, fungi, and cyanobacteria effectively makes nitrogen, phosphorus, and other nutrients available to plants. Moreover, microorganisms are capable of secreting various compounds with antimicrobial properties or releasing certain factors that induce resistance in plants, suggesting their role as an alternate biocontrol agent. Further, large amounts of agrochemicals end up in the soil unutilized. This accumulation pollutes surface and groundwater and poisons tens of thousands of people worldwide [1, 3]. The use of bioinoculum could also prove significant in nutrient management and could save up to 30% of conventional chemical fertilizers [4, 5].

Other than their use in agriculture, soil microbes are also used to resolve environmental woes. For instance, unsustainable mining practices have not only depleted high-grade mineral ore reserves but have also caused wide-scale destruction of habitats. Traditional metallurgical practices, such as pyrometallurgy (smelting), are costly and may degrade the quality of the environment further. Furthermore, the mining industry has extensively contributed to the emission of greenhouse gases, which is associated with severe degradation of the environment. Nevertheless, it is the inadequacy of the legislature and the biohydrometallurgical procedures that have amplified the carbon footprints. These processes employ a variety of autotrophic bacteria and archaea, which are dexterous enough to fix carbon dioxide. Moreover, biomining, which works under two heads of bioleaching and bio-oxidation at the commercial scale, not only reduces the build-up of toxic compounds but also operates at lower temperatures and pressures, and hence reduces the rate of energy consumption.

The rapidly developing mining and agrochemical industries depend upon fossil fuels for their energy requirements. In their Annual Energy Outlook 2020 report, the Energy Information Administration (EIA) projected a nearly 50% increase in global energy consumption between 2018 and 2050, led by developing African and South Asian countries [6]. The industrial revolution has also heightened energy requirements, which ultimately increased the pressure on these conventional sources of energy. These non-renewable resources are extracted from the depths of the earth in an unsustainable manner and at an extravagant cost. Eventually, the cost of extraction will further escalate due to the depletion of these reserves. Therefore, the need for large-scale production of renewable energy is imperative for addressing the global energy crisis and negating the ill effects of conventional fossil fuels [7]. In the final couple of sections, we shall discuss how the over-exploitation of resources has led to a decline in the quality of the environment and the biotechnological methods for establishing a sustainable bioeconomy.

SOIL MICROBES AS BIOFERTILIZERS

Plant roots release exudates that directly influence the microorganisms in a narrow range of soil around roots called the rhizosphere. The rhizosphere microbiome is also influenced by many different factors, including environmental conditions, interaction with other microbes, and agricultural practices [8]. These microorganisms have a positive effect on the survival and growth of plants and can be free-living, or can form symbiotic relationships with plants, or can even live inside them as endophytes. They can improve nutrient availability and have a direct impact on the acquisition process, produce various phytohormones, decompose organic matter, and compete with pathogens [5, 9]. Biofertilizers contain a consortium of such microorganisms that can colonize the plant's rhizosphere and help in its growth and development by various natural processes to mobilize nutrients, including nitrogen fixation, phosphate and potassium solubilization, among others [10]. These microbial inoculants produce several other growth promoters like enzymes, vitamins, and bioactive compounds, and induce stress and disease resistance in plants [8].

CHAPTER 4

Industrial Applications of Soil Microbes: Production of Enzymes, Organic Acids and Biopigments

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Abstract: Commodity chemicals are the intermediates that are generally involved in the synthesis of other high-end products. The increasing demand for various industrial products has upscaled the requirement for commodity chemicals. Originally, the industrial sector was dependent upon conventional and toxic chemicals to sustain its processes. However, the advent of biotechnology led to the development of numerous microbial processes producing enzymes, extremozymes, organic acids, organic solvents, *etc.*, Moreover, the soil environment has diverse forms of microbial communities performing assorted functions. As a result, a thorough understanding of the soil microbiota involved in providing regulatory ecosystem services can aid in the development of exceptional microbial strains capable of meeting the high demand for these commodity chemicals. In addition, the exploitation of these excellent manipulative microbial systems can improve and customize the synthesis of commodity chemicals and thereby reduce the reliance on synthetic and petroleum-based products. This chapter will inform the readers about the applications of soil microbes in industry and their involvement in enzymes, extremozymes, organic acids, and biopigments production.

Keywords: Amylase, Microbial enzymes, Microbial pigments, Phytase, Thermophilic enzymes.

INTRODUCTION

Soil is the primary physical covering of the earth's crust, which is a distinctive product of geological parent material, geomorphological history, natural disturbances, and the presence of natural biota. Soil provides the habitat for a vast

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assortment of bacteria, fungi, algae, and many higher organisms. It is estimated that one gram of undisturbed soil may contain 10 billion microorganisms, representing an array of genomes. Despite the large numbers, these soil microbes comprise less than 1% of the total soil mass, but they play an indispensable role in supporting life on earth and influence countless ecosystems and commercial activities [1].

These microbial commodity chemicals find applications in diverse industries like medical, food and beverages, and agriculture and related fields. The enzymes like lipases, proteases, cellulases, amylases, *etc.*, obtained from these soil-dwelling microbial creatures represent the largest share in industrial applications. To list a few, (a) The textile industry utilizes a variety of proteases to eliminate undesirable fibres from products; (b) Enzymes from *Aspergillus* spp. are typically used in the processing of textiles, and (c) Lignin-degrading fungi like *Phanerochaete chrysosporium* have decolorizing properties which are also utilized by the textile industry for the treatment of wastewater [2]. Furthermore, microbial cultures play an important role in the production of a variety of food and dairy products. A wide range of organic acids are being procured by various fermentation processes. These acids are being utilized as acidulants, preservatives, emulsifiers, flavoring, sequestrants, and buffering agents.

Furthermore, metabolites obtained from the soil microbiota are being extensively manipulated for the development of various products of medical importance. Enzymes like asparaginase, which have a potential application against tumour cells, are produced by *Escherichia coli* and *Erwinia chrysanthemi* at a considerably lower cost [3]. Biofilms tend to confer antibiotic resistance and hence cause severe bacterial infections. Various biofilm inhibitory agents like cahuitamycins, obtained from soil microorganisms, find wide applications. Genetically engineered bioreporters, which have the potential to detect toxic compounds and generate quantifiable signals, are an emerging technology that has the potential to be utilized in various industries [4].

Techniques like microbiologically induced calcium carbonate precipitation are being employed in the cement and brick manufacturing industries. These alternative biobricks and biocement can possibly reduce environmental pollution by limiting the emission of carbon produced during the conventional manufacturing processes. Thus, microorganisms not only foster the products of commercial importance but also help in the sustainable development of economies [5]. Furthermore, growing public awareness has shifted research into microbiological alternatives to various products, such as various biological pigment molecules being used in the fabrication of bio-cosmetics [6].

The soil microbiota can be further explored for their potential to produce various powerful enzymes and drugs of medicinal value. Nevertheless, microengineering and synthetic biology tools can be employed to improve these soil microorganisms for the synthesis of the desired products. This chapter aims at providing an overview of various applications of soil microorganisms in industry and why there is a constant requirement for exploring soil microflora for novel products.

SOIL MICROORGANISMS IN ENZYME PRODUCTION

Modern enzyme technology began in 1874 when the Danish chemist Christian Hansen extracted rennet from calves' stomachs with the help of saline. This significant event was followed by a lengthy evolution, which brought about the parallel development of a multitude of industrial processes (Fig. **1**), which ultimately led to the need for further expansion of the enzyme industry.

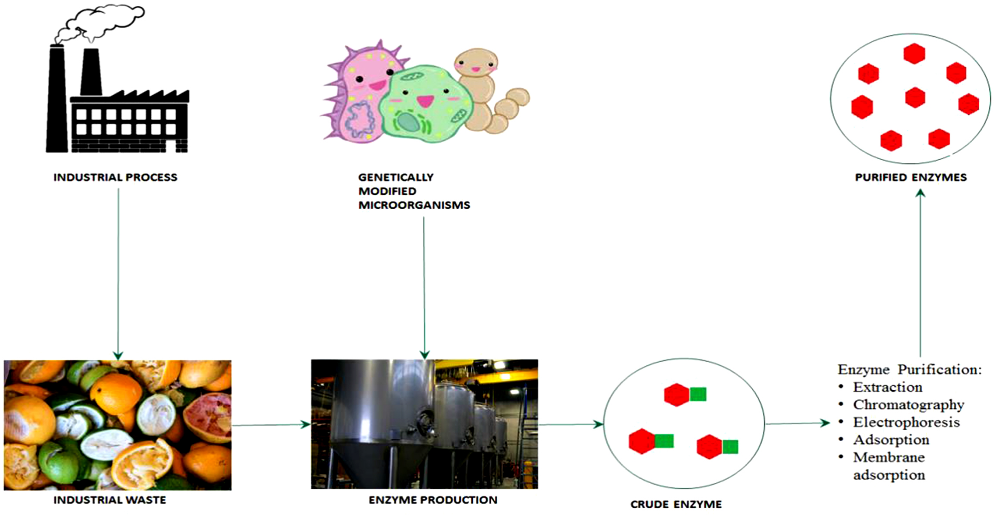

Fig. (1). Steps involved in the production of microbial enzymes from industrial waste.

Enzymes can be defined as organic compounds that catalyze various metabolic reactions occurring in a living organism. Proteins usually form the infrastructural backbone of enzymes. However, several enzymes contain non-protein components as well, which may be present in the form of a covalently attached carbohydrate group or a metal ion. Microbial enzymes have numerous industrial applications and hence have been applied to make several products. They are frequently used in the production of detergents, dyes, chemicals, wine and beer, laundry, food and beverages, paper and textiles, clothing, *etc.*

CHAPTER 5

Applications of Microbial Biopesticides

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Abstract: Microbial biopesticides involve various microorganisms such as bacteria, fungi, viruses, nematode-associated bacteria, protozoans, and endophytes working against invertebrate pathogens in agro-ecosystems. Such novel biopesticidal products, after extensive research work, have been explored in the global market to combat synthetic pesticide application adverse problems. Recent academic and industrial efforts are involved in the discovery of toxins and virulence factors from microbial species for the synthesis of commercial formulations. The current review is the expansion of the application of various bacteria, fungi, viruses, nematodes, protozoans, and endophytes for biopesticide formulations and their role in pest management.

Keywords: Entomopathogenic bacteria, Entomopathogenic nematodes, Endophytes, Fungi, Integrated pest management (IPM), Virus.

INTRODUCTION

Biopesticides are natural biochemicals, produced by plants, animals, fungi, bacteria, and some minerals such as baking soda and canola oil, that exert pesticide properties *via* nontoxic pathways. Approximately 195 registered biopesticidal active ingredients and 780 products were recorded at the end of 2001 [1]. Biopesticides play a crucial role in plant protection, either individually or commonly in combination with chemical pesticides, also known as integrated pest management (IPM). Biopesticides are expected to grow 10-15% faster than conventional chemical pesticides over the next decade [2], while Dar and colleagues [3] reported a 2.5 percent estimated annual growth rate for biopesticides in India. These biopesticides are eco-friendly, target pest-effective, and minimally threatening to human or environmental health [4 - 7]. Gupta and

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Dixit [8] elaborated on the potential applications of biopesticides for public and human health. Some plants like *Annona squamosa* (anonaine in the stem, leaf and unripe fruit), *Anacardium occidentale* or cashew (phenolic compounds in shell oil), and *Albizia lebbeck* (caffeic acid and quercetin in bark, stem and leaf) have been reported with insecticidal properties. The floral extract of *Butea monosperma* has chalcones and aurones (termiticidal) and the seed oil of *Madhuca longifolia* var. *latifolia* contains saponins, which might also serve as an excellent insecticidal and repellent [9].

Biopesticides involved in disease management are important since they are (a) Eco-friendly- causing minimum harm to the ecosystem, (b) Have environmental utility- small doses are enough to control a pest with easy decomposition, (c) Pest-specific- highly specific interaction with the pest, (d) High suitability- biopesticides contribute maximum during integrated pest management. The most common biopesticides are living genera like bioinsecticides (*Bacillus thuringiensis*, Bt), biofungicides (*Trichoderma* spp.) and bioherbicides (*Phytophthora* spp.). Such biopesticides involve plant-incorporated protectants (plants inserted with genetic material), biochemical pesticides, microbial pesticides, *etc.* Biopesticides are being used in agriculture against insects, weeds, and nematodes to improve the productivity of plants [10]. Recently, microbial biopesticides have been popularized amongst farmers worldwide [11]. Biopesticides include the following types:

Plant-Incorporated Protectants (PIPs)

The pesticidal compounds, or plant-incorporated protectants (PIPs), are produced by the plants upon insertion of genetic material, like the incorporation of Bt pesticidal protein into a plant genome. Consequently, such genetically modified plants kill the pest directly rather than the Bt bacterium.

Biochemical Pesticides

Biochemical pesticides (BP) involve naturally existing substances like fatty acids and plant extracts, which regulate pests *via* different nontoxic reactions, while synthetic pesticides commonly kill or inactivate the pest. Such BPs contain compounds like pheromones, which either attract or repel pests from mating, and also contain plant growth regulators (PGRs) that stimulate plant growth itself.

Microbial Pesticides

Microbial pesticides (MPs) are derived from naturally existing or genetically modified fungi, bacteria, viruses, algae, and protozoans. Microbial pesticides were found to be effective and safe substitutes for chemical insecticide formulations.

Microbial toxins are biological poisons derived from microorganisms, and their effects are significantly pest-specific. The uptake of such microbial toxins by entomopathogens occurs *via* (i) Invasion of the gut or integument of insects and (ii) With the multiplication of the microbes, leading to the death of insects due to the accumulation of insecticidal toxins. Such MPs were found to have efficiency, safety for non-target organisms like humans and animals, residual incorporation in food produced from crops, eco-friendly and potential targeted pest-specificity, and thus facilitate the survival of useful insects in treated crops. Therefore, such microbial insecticides have been exploited as biological control agents for three decades. These microbes contain active components that can control a wide range of pathogens either alone or in groups. For example, the *Bacillus sphaericus* strain has been shown to be effective against various types of mosquitoes such as *Culex quinquefasciatus*, whereas *B. thuringiensis* is more effective on *Aedes aegypti* alone [12].

Besides *B. thuringiensis*, novel bacterial species such as *Xenorhabdus* spp., *Photorhabdus* spp. (entomopathogenic nematode), *Yersinia entomophaga*, *Serratia* spp., *Pseudomonas entomophila*, *Burkholderia* spp., *Chromobacterium* spp., *Saccharopolyspora* spp., and *Streptomyces* spp. have received attention from an industrial point of view due to the presence of a diversity of metabolites or toxins exerting insecticidal properties [13, 14]. Gouli and coworkers [7] reported extensive utilization of microbial pesticides in pest management. Similar to synthetic pesticides, microbial pesticides have been mass cultivated or formulated from bacteria, fungi, and protozoans (single-celled organisms), and such biopesticidal formulations are regulated by the environmental protection agency and their application is controlled by the federal insecticide, fungicide, and rodenticide acts [3, 10, 11, 15].

BACTERIAL BIOPESTICIDES

Bacterial biopesticides are relatively cheaper and more common in microbial pesticides. Bacterial insecticides are usually specific to individual species of beetles, flies, butterflies, mosquitoes, and moths. An effective bacterial insecticide must be ingested by the gut of the target pest, followed by contact. The *Bacillus* genus is a rod-shaped, soilborne, and spore-forming bacterial insecticide that has been used against insects. This bacterium contains important traits that make it a potential microbial biopesticide [16]. It has crystalline inclusion bodies or endotoxins or Cry proteins and cytolytic (Cyt) toxins or δ-endotoxins or insecticidal crystal proteins. *Bacillus subtilis* has been recognized as a safe, non-pathogenic bacteria with the approval of the USFDA (US Food and Drug Administration). It is applied as a biopesticide and its spores are highly resistant to heat, dormancy, nutritional scarcity, and unfavourable pH [17]. Bacterial

CHAPTER 6

Soil Microflora - A Potential Source of Antibiotics

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Abstract: Soil is one of the principal ingredients of the universe, which supports all forms of life directly or indirectly. Soil consists of a mixture of various organic and inorganic matter, fluids, gases, and micro and macro-living systems and acts as a major living medium for a wide group of living organisms. One of the important soil inhabitants are microorganisms. Soil microorganisms can be categorized as bacteria, fungi, actinomycetes, protozoa, algae, and viruses. These microbes have varied features and functions. Most importantly, these microorganisms do not exist in isolation but interact with each other and contribute significantly to overall soil fertility. Many of these organisms have the capacity to produce antimicrobial substances as a defense mechanism to compete with other organisms for their survival and existence. Most of these antimicrobial substances, which are released as metabolites produced during trophophase as well as in idiophase, are medically significant in the treatment of many life-threatening infections in plants and animals. This chapter describes various soil microflora and their roles in the production of different kinds of antimicrobials. The primary goal is to familiarise readers with the various microflora found in soil and their ability to produce anti-microbial components.

Keywords: Actinomycetes, Antibiotics, Bacteria, Fungi, Isolation, Soil micro-flora.

INTRODUCTION

Certain groups of secondary metabolites released by specific groups of organisms possess antimicrobial properties against various microorganisms and are called antibiotics. These antimicrobial agents serve as a major compound in fighting against various infections caused by other microorganisms by either killing or inhibiting their growth and multiplication.

A variety of soil microorganisms are known to produce different types of antibiotics that are screened, isolated, purified and used against many life-threatening infections, mostly in human beings, animals and agriculture.

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The major groups of microbes capable of producing antibiotics include bacteria, fungi, and actinomycetes. These antibiotics serve as a primary defense mechanism against other groups of organisms in their vicinity for better survival [1].

Soil acts as a major habitat for a huge and diverse population of microorganisms because of its heterogeneous nature. It has been estimated that one gram of soil contains one to one hundred million fungi, fifty thousand to a million actinomycetes, one to ten million algae, ten thousand to fifty thousand protozoans, and many millions of bacteria [2]. The number and type of organisms vary depending on the type of the soil, the depth of the soil, climatic conditions, and various human, animal, and agricultural practices. The rhizosphere represents the thin layer of soil surrounding plant roots and the soil occupied by the roots, and it supports the growth of any number and variety of microorganisms [3]. The plant roots release a variety of biomolecules, including amino acids and sugars, that serve as food sources for microorganisms, greatly increasing microbial populations and activity in and around the rhizosphere [4]. Variation in the biotic and abiotic nature of soil makes its microbial inhabitants adapt and develop strategies for survival. The production of antimicrobial substances is one of the most powerful strategies employed in adaptation. The rhizosphere consists of three different zones based on their relative proximity to the root. The endorhizosphere consists of the endodermal along with the plant cortical region, in which the microbes interact significantly. It is followed by a region of rhizoplane, which is adjacent to the root and includes the root epidermis and the mucilage. The outer ectorhizosphere extends from the rhizoplane into the core soil with a comparatively lower number of microorganisms [5].

As discussed earlier, soil microorganisms are a major source of antibiotics and over a thousand different antibiotics have been isolated from various microflora, predominantly from bacteria, actinomycetes, and fungi. Many of the medically important antibiotics are produced by the genus *Bacillus*, filamentous fungi, actinomycetes, and other bacteria [6] (Table **1**).

Table 1. Some clinically important antibiotics produced from soil microbes.

Antibiotic	Source of Organism	Spectrum of Activity
Penicillin	*Penicillium chrysogenum*	Gram-positive bacteria
Cephalosporin	*Cephalosporium acremonium*	Broad spectrum
Bacitracin	*Bacillus subtilis, B. licheniformis*	Gram-positive bacteria
Polymyxin	*B. polymyxa*	Gram-positive and Gram-negative bacteria
Streptomycin	*Streptomyces griseus*	Gram-negative bacteria

(Table 1) cont.....

Antibiotic	Source of Organism	Spectrum of Activity
Kanamycin	*Streptomyces kanamyceticus*	Gram-positive bacteria, Gram-negative bacteria, and mycobacteria
Tetracycline	*Streptomyces rimosus*	Broad spectrum
Vancomycin	*Streptomyces orientalis*	Gram-positive bacteria
Neomycin	*Streptomyces fradiae*	Broad spectrum
Erythromycin	*Streptomyces erythreus*	Gram-positive bacteria
Amphotericin B	*Streptomyces nodosus*	Fungi
Trichomycin	*Streptomyces hachijoensis*	Fungi
Fusidic acid	*Acremonium fusidioides*	*Staphylococci* and Gram-negative bacteria
Cochliodinol	*Chaetomium cochlioides*	Fungi and bacteria
Polymyxin	*Bacillus polymyxa*	Gram-negative bacteria
Gramicidin	*Bacillus brevis*	Gram-positive bacteria
Zwittermicin	*Bacillus cereus*	Gram-positive, Gram-negative, and prokaryotic microorganism

ACTINOMYCETES - A POTENTIAL SOURCE OF ANTIBIOTICS

Actinomycetes are best known for their ability to produce antibiotics. Although most species in this group produce bioactive compounds, few selective forms are reported to synthesize the maximum types of antimicrobials. The best-known among them is *Streptomyces*, followed by other related actinomycetes, which include *Micromonospora*, *Actinomadura*, *Actinoplanes*, *Nocardia*, *Streptosporangium*, *Streptoverticillium*, *Thermoactinomyces*, *etc*.

Streptomyces are a group of Gram-positive bacteria that grow in different soil environments, and their shape resembles filamentous fungi. The typical characteristic feature *of Streptomyces* includes the formation of a layer of hyphae that differentiates into a chain of spores. They are predominantly found in soil and dead organic vegetation. Most *Streptomyces* spp. produce a characteristic earthy fragrance due to the production of a volatile metabolite called geosmin (Fig. **1**).

Apart from the ability to synthesize antibiotics, *Streptomyces* also has the capacity to produce other bioactive secondary metabolites such as antifungals, antivirals, antitumorals, anti-inflammatories, immunosuppressives, *etc*. The antibiotic production of these organisms is species-specific, and these metabolites are essential for Streptomyces sp. to compete with another group of organisms, even within the same genera [6] (Table **2**).

CHAPTER 7

Role of Nonpathogenic Strains in Rhizosphere

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Abstract: As the world's population is increasing rapidly, there is an urgent need to increase crop production. To achieve this goal, an eco-friendly alternative to chemical fertilizers and pesticides is required. Several types of microbes have been identified inhabiting the plant rhizosphere, such as nitrogen-fixing bacteria, plant growth-promoting rhizobacteria, fungi, proteobacteria, mycoparasitic and mycorrhizal fungi. These microorganisms not only influence the growth and development of plants but also suppress pathogenic microbes near plant roots through several different mechanisms. Non-symbiotic microbes play a crucial role in the biogeochemical cycling of organic and inorganic phosphorus (P) near the root zone *via* solubilization and mineralization of P from total soil phosphorus. Additionally, some non-pathogenic microbes have also been reported to induce systemic resistance in plants, which is phenotypically similar to pathogen-induced systemic acquired resistance (SAR). The present review summarizes the latest knowledge on the role of non-pathogenic strains of microbiomes residing in the rhizosphere and their commercial applications.

Keywords: Arbuscular mycorrhizal fungi, Non pathogenic, Rhizhobacteria.

INTRODUCTION

When we talk about the effects of the rhizosphere on plants and other organisms, the possibilities are nearly endless. For the sake of study, we are concerned here with only the beneficial aspects, otherwise known as non-pathogenic effects. It has been known for more than 300 years that endophytic microbes reside in plant roots. However, only recently has the value of these microbes been fully understood (Fig. **1**). Endophytes in plant roots improve crop yield and environmental buffering. Symbiotic interaction among plants and microbes was first reported by Malpighi in 1697. He described the formation of galls or nodules composed of both bacteria (Rhizobiaceae) and plant cells on the roots of plants as the mechanism to fix atmospheric nitrogen (N_2) and make ammonia (NH_3) availa-

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ble for leguminous plants [1]. In 1988, after almost two decades, plant roots were reported to be colonized by fungi called arbuscular mycorrhizal fungi (AMF). These fungi increase the productivity of plants by symbiotic means [2]. Soil-borne fungi *Trichoderma* were shown to have the ability to control pathogenic fungi of agricultural crops in the 1920s and 1930s [3]. Various other microbes are associated with roots. However, some true plant symbionts (Rhizobiaceae, *Trichoderma* strains, and AMF) have demonstrated the ability to serve as components of enhanced plant holobiomes [4].

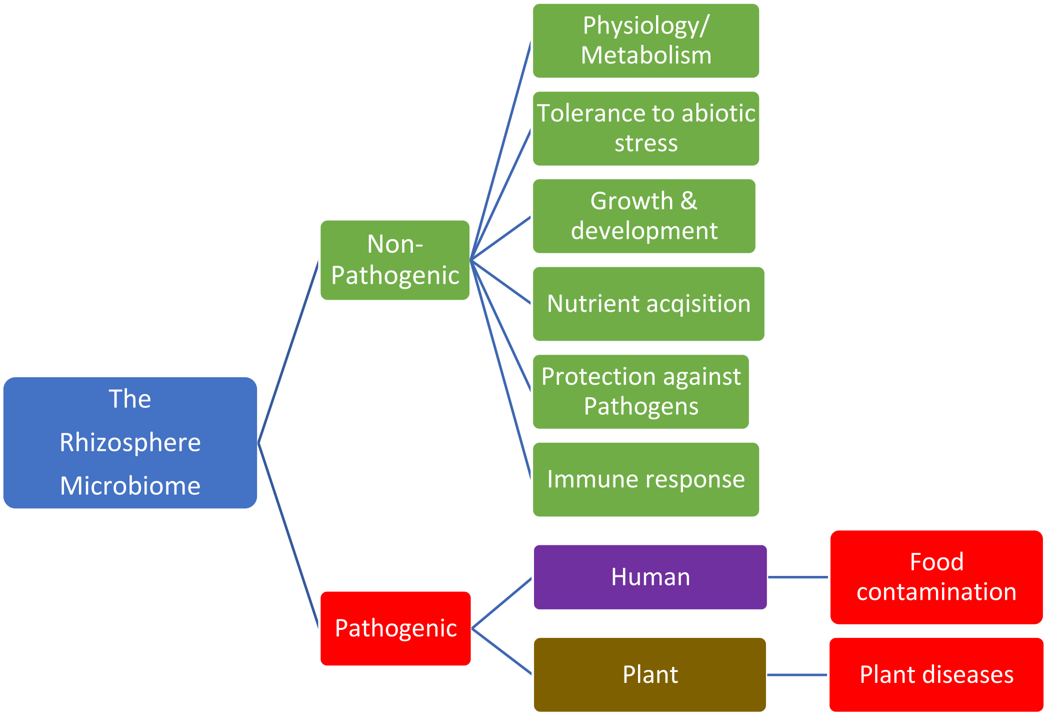

Fig. (1). Various effects of rhizosphere microbiome pathogenic (disease causing) as well as non-pathogenic (non-disease causing) strains.

IMPORTANCE OF NON-PATHOGENIC STRAINS OF RHIZOSPHERE

Rhizobiaceae

Bacteria in the Rhizobiaceae family are well known as nitrogen-fixers. However, two other groups of bacteria, comprising cyanobacteria and *Frankia*, can also fix atmospheric nitrogen in symbiosis with plants. Cyanobacteria can fix atmospheric nitrogen in a wide range of plants [5]. Bacteria in the Frankiaceace family fix di-nitrogen from the atmosphere like other symbiotic fixers of nitrogen. *Frankia* is capable of nodulating about 24 genera related to 8 varied angiosperm families [6].

Leguminous plants produce complex galls or nodules around the nitrogen-fixing cells referred to as bacteroids. These nodules are occupied with a protein containing iron called leghemoglobin that removes oxygen, consequently facilitating the hypoxic environment essential for the bacteroids to fix atmospheric nitrogen, reducing N_2 to NH_3 [7]. The symbiotic interactions of bacteria and plants that result in gall formation are highly specific, and only certain bacterial species or strains can colonize a specific host plant [8].

Trichoderma

The genus *Trichoderma* contains several species and strains isolated from forests and agricultural soil that can be easily cultured *in vitro*. *Trichoderma* sporulation is usually green and some strains possess coconut odour due to a biologically active compound called 6-pentyl-α-pyrone, which is volatile in nature [9]. Recently, *Trichoderma* isolates have been recognized as being capable of acting as endophytic plant symbionts. The strains become endophytic in roots. However, major variations in gene expression appear in shoots. These variations modify plant physiology and may result in the enhancement of photosynthetic efficiency, nitrogen uptake, and biotic and abiotic stress resistance. Generally, the overall result of these effects is excellent for plant growth and yield [10].

Arbuscular Mycorrhizal Fungi (AMF)

This association of host plant and fungus roots evolved around 400 million years ago. AMF are obligate fungi and require host plants for their growth and survival. Fungi form a complex with the roots of most terrestrial plants and involve chemical signalling pathways [11]. After the AM fungi enter the host root cortical cells, a prepenetration apparatus leads the fungi to an appropriate cell determined by the host. Once inside the host cell, the fungus forms lobed structures called arbuscules (sometimes vesicles) that are present between the host cell wall and cell membrane [12]. AM fungi are reported to induce the expression of ammonium and phosphorus transporter proteins, especially in nutrient-deficient soil conditions [13]. AMF also interacts with bacteria in the soil, which leads to significant effects in agriculture. In this interaction, soil bacteria bind with the fungi and degrade the fungal cell wall by releasing volatile compounds. This process affects the AMF's gene expression, which results in improved performance and yield [14].

BENEFICIAL ROLE OF NON-PATHOGENIC STRAINS IN RHIZOSPHERE

Studies in the past few years have shown the adverse effects of agrochemicals used to ensure the high yield of agricultural crops. Agrochemicals, generally

CHAPTER 8

Bioremediation Industry: A Microbial Perspective

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Abstract: Bioremediation of environmental pollutants and contaminants in soil is an emerging technology, which will gain relevance and importance in the near future. Microbiological bioremediation is not only cost-effective but also environmentally sustainable, as it does not cause undesirable effects like toxic byproducts or residues, requires heavy infrastructure, has on-site application, and is the least hazardous to human health. With new biotechnological tools, the microbes can be designed to have desirable effects for the bioremediation of more toxic wastes. However, the free release of genetically modified microbes for this purpose is still under risk assessment. This is an effective method to use indigenous microflora and harness their biodegradation properties to remove unwanted contaminants from soil, water bodies, underground water aquifers, ocean spills, *etc.* Currently, they are mostly used for cleaning oil spills and removing petroleum products and heavy metals from soil. Both *in situ* and *ex situ* methods are employed, where microbes can be used in varied ways. Much work is going on to explore and enhance the properties of microbes, especially bacteria, to be used as agents for contaminant removal from our environment. Global bioremediation is an emerging market that is slowly growing and will become a multibillion-dollar market worldwide in days to come. The current review tries to view the subject with microbes in perspective; their role in bioremediation; mode of action; technologies used; and their use for sustainable cleanup of the environment.

Keywords: Bioremediation, Biotransformation, Microflora, Pollutants, Pollutants.

INTRODUCTION

With the advent of the modern era with a rampant rate of development, anthropogenic activities have released several harmful chemicals and exposed the earth, air, soil, and water to some of the most destructive compounds. Pollution of our environment and its consequences are not new to us, and now several steps are being taken by humanity to negate and remove contaminants from the environment. Microbes are employed for the removal of pollutants by biological means. Bioremediation is one such process that is gaining importance.

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BIOREMEDIATION

It is the process of chemical removal by degradation/immobilization of a contaminant/pollutant from the environment by specific microbes. These microbes act singly, in sequence or in a consortium to achieve and complete the degradation process. Bioremediation is a safe option with the possibility of removing or rendering various harmless contaminants using biological entities. With the current advancement in microbial technology, bioremediation is an emerging industry where an integrated approach is sought to achieve the solution to the problem, *i.e.*, the removal of contaminants. The bioremediation process involves several microbial processes, some of which are: (a) Biodegradation is the natural modification of compounds by microbial metabolic pathways, such as mineralization of organic substrates; and (b) Biotransformation is the modification of a pollutant's chemical configuration or constituent to make it less toxic through the use of microbes.

The concept of using microbes to remove harmful chemicals is not new; they have always been part of domestic, agricultural, and industrial processes. About 50 years ago, Raymond and coworkers reported bioremediation in the work titled “Beneficial stimulation of bacterial activity in groundwater containing petroleum products” [1]. Bioremediation was invented by George Robinson in the 1960s for oil spill cleanup in California. Since then, it has been widely used for the cleanup of petrochemical spills, sewage, and leach fields, as well as for odour and pest control. It is widely used in the American continent and some of the most famous examples of bioremediation (microbial) are the Exxon Valdez oil spill in Alaska (1989), the crude oil spill in Minnesota (1979), sewage effluent cleanup, Cape Cod in Massachusetts and removal of chlorinated solvents in New Jersey [2].

POTENTIAL POLLUTANTS FOR BIOREMEDIATION

Organic Pollutants

A large range of chemicals have now been recognized as being biodegradable with the help of microbes. Chlorinated solvents, like trichloroethylene and perchloroethylene, are industrial-grade solvents used in textile, chemical, and allied industries. Some of the examples are polychlorinated biphenyls (4-chlorobiphenyl and 4,4-dichlorobiphenyl) produced in powerhouses and in the electrical manufacturing industry, chlorinated phenols (pentachlorophenol) from the timber industry, landfills, benzene and allied compounds (toluene, ethylbenzene, and xylene) obtained from the petroleum industry, paints, released from ports and airports, chemical manufacturing byproducts, and polyaromatic hydrocarbons (PAH) (naphthalene, anthracene, fluorine, pyrene, and benzopyrene) that are petroleum byproducts found in refineries, coke plants, landfills, tar production, power plants, gas plants, *etc.* Pesticides originate from agricultural runoff and contaminate soil, ground, and surface water. Some pesticides, where the bioremediation process is studied are atrazine, carbaryl, carbofuran, diazinon, glyphosate, parathion, propham, and 2-4 D [3].

The very first superbug (*Pseudomonas putida*) for bioremediation of oil spills was created in the 1970s when Anand Mohan Chakrabarty and co-workers [4] reported the development of a new strain of bacterium by the transfer of plasmids and named it superbug, which could biodegrade a number of toxic organic chemicals like octane, hexane, xylene, toluene, camphor, and naphthalene. The United States granted this patent, making it the first genetically engineered microorganism to be patented. This superbug was then used to clean an oil spill in Texas in 1990. Many bacteria are now reported and are used commercially to harness their ability to biodegrade organic compounds, *viz*., *Dechloromonas aromatica* (benzoate, chlorobenzoate, and toluene), *Nitrosomonas* sp. (nitrogenous pollutants), *Deinococcus radiodurans* (solvents, heavy metals, and radioactive), *Methylibium petroleiphilum* (methyl tert-butyl ether), and *Alcanivorax borkumensis* (various hydrocarbons) (Table **1**).

Table 1. Bioremediation of contaminants by bacteria.

Pollutant	Bacteria Bioremediating Agent	Reference
Polyaromatic hydrocarbons (PAH)	*Achromobacter* sp., *Arthrobacter* sp., *Bacillus* sp., *Mycobacterium* sp., *Burkholderia* sp., *Pseudomonas* sp., *Rhodococcus* sp., *Stenotrophomonas maltophilia*, *Sphingomonas* sp., *Xanthomonas* sp., *etc.*	[5, 6]
Benzene and allied compounds	*Pseudomonas putida*, *Acinetobacter johnsonii*, and *R. erythropolis*	[7]
Polychlorinated biphenyls	*Arthrobacter* sp., *Pseudomonas* sp., *Azotobacter* sp., *etc.*	[8, 9]
Pesticides	*Flavobacterium* sp., *Arthrobacter* sp., *Azotobacter* sp., *Burkholderia* sp., and *Pseudomonas* sp.	[10]

Inorganic Pollutants

These include several heavy metals, which come from industrial and other human activities like mining, tanneries, paints, battery wastes, pesticides, fertilizers, air emissions, fuel burning, and natural processes like weathering of minerals, erosion, volcanic activity, and forest fires. Heavy metals, which are prime candidates for the bioremediation process, are arsenic, cadmium, chromium, copper, mercury, nickel, and lead. Heavy metals are absorbed by microbes at cellular binding sites on the cell wall. Their extracellular polymers form complexes with heavy metals and immobilize them by various mechanisms, like

CHAPTER 9

Alleviation of Salinity Stress by Microbes

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Abstract: Agricultural production is majorly hampered by the negative impact of both biotic and abiotic stress in most developing countries. Among abiotic stresses, soil salinity is a major problem, affecting crop production and responsible for limiting the growth and productivity of plants in different areas of the world due to increasing use of poor quality of water, flooding, over-irrigation, seepage, silting, and a rising water table. In agriculture, salt-tolerant rhizospheric/endophytic microorganisms play an important role in helping alleviate abiotic stresses in plants. Under plant-microbe interactions, plant root-associated microbes, including endophytes, closely interact and cooperate with plants, and mediate important physiological and metabolic processes, thereby enhancing the plant's tolerance to salinity stress. Several mechanisms have been developed for microbial alleviation of salinity stress in plants, including the production of phytohormones, improving plant nutrient status, production of ACC deaminase, salt exclusion, and enhancing resistance to drought in plant cells. A wide range of micro-organisms are available that have diverse mechanisms for salt stress alleviation in plants. Future research needs to be directed towards field evaluation for the validation of the potential microbes.

Keywords: Mechanism of stress alleviation, Plant-microbe interaction, Soil salinity, Salt stress, Salt stress alleviation.

INTRODUCTION

Soil salinity is not a new phenomenon. This has been described for centuries, where salinity and humanity have lived one aside from the other. A better example is the publication '*Salt and silt in ancient Mesopotamian agriculture'* that reported the history of salinization in Mesopotamia where three episodes, *i.e.*, the earliest and most serious one, affected Southern Iraq from 2400 BC until almost

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1700 BC; a milder episode in central Iraq happened between 1200 and 900 BC; and in the east of Baghdad (which became salinized after 1200 AD) have been described [1]. The salinization of soil is a serious problem and is steadily increasing in many parts of the world, in particular in arid and semi-arid areas [2]. The main causes of soil salinization that have been reported are flooding, over-irrigation, seepage, silting, and a rising water table.

According to the FAO Land and Nutrition Management Service [3], saline soils occupy 7% of the Earth's land surface [4] and increased salinization of arable land has resulted in 50% land loss by the middle of the 21st century [5] (Table **1** and **2**). In 2008, about 77 million hectares out of 1-5 billion hectares of cultivated land around the world was affected by excess salt content [6]. Approximately 12 million hectares of land suffers from various kinds of salt-related afflictions. Over 2.1 million hectares of salt-affected land is located in the country's key breadbasket in the north [7]. Recent precise reports on the global extent of salt-affected soils are not recorded. Many countries have assessed their soils and soil salinization at the national level, such as the United Arab Emirates, Kuwait, the Middle East, Australia, *etc.* In consideration of the current extent of salt-affected soils, the damage to salt-induced land in 2013 was $441 per hectare, and annual economic losses were about $27 billion [1]. Salt stress presents an increasing threat to plant agriculture [8]. Among the various sources of soil salinity, irrigation combined with poor drainage is the most serious, because it represents losses of once productive agricultural land.

Salt Stress Damage to Plants

Combating soil pollution to feed the world’s growing population is very important and needs attention. Several environmental factors adversely affect plant growth and the final crop yield. Drought, salinity, nutrient imbalances (toxicities and deficiencies) and temperature extremes are some of the major environmental barriers to crop production. Adverse climatic conditions are among the principal limiting abiotic factors for the decline in agricultural production [9]. Of the world’s 5.2 billion ha of dryland agriculture, 3.6 billion ha is affected by the problems of erosion, degradation, and salinity [10].

Globally, soil salinity has become a major issue for plant growth and has reduced agricultural yield [11]. It is one of the major abiotic stresses faced by crops and has been reported to affect about 950×10^6 ha of land worldwide [12]. By salt accumulation, the amount of world agricultural land destroyed each year is estimated to be 10 million ha [13]. Due to soil salinity, the annual cost of land degradation in irrigated lands could be US $27.3 billion worldwide due to a loss in crop production [14]. The land area under ever-increasing salinization has reached almost 34 million irrigated hectares [15].

In plants, salt stress conditions cause various physiological and metabolic changes such as photosynthesis, nutritional imbalance, inhibition of water uptake, a decrease in growth and seed germination. In the natural environment, microorganisms colonize plants. Under plant-microbe interaction, plant root-associated microbes, including endophytes, closely interact, cooperate with plants, and mediate important physiological and metabolic processes, thereby enhancing the plant's tolerance to salinity stress [11].

Many plants can tolerate salinity to an optimal level, and yield decreases as salinity increases [16]. It has been recognized that a crop's sensitivity to salinity varies depending on the growth stage of the plant [17]. Most annual plants are tolerant at germination but are sensitive during emergence and early vegetative development [18]. A mature plant is more tolerant to salts, particularly during later stages of development. Most of the plants are tolerant during germination. Though salinity stress delays the germination process, there may be no difference in the percentage of germinated seeds [18].

However, higher salt concentrations have been shown to reduce seed germination in sorghum [18], cotton [19], tomato [20], and globe artichoke [21]. An increase in salinity levels significantly reduced the rate and percentage of seed germination of alfalfa [22].

Table1. Variation in salinity levels in the world, in million hectares (Mha) [3].

Regions	Total Area (Mha)	Saline Soils	Percentage	Sodic Soils	Percentage
Africa	1899.1	38.7	2.0	33.5	1.8
Asia, the Pacific & Australia	3107.2	195.1	6.3	248.6	8.0
Europe	2010.8	6.7	0.3	72.7	3.6
Latin America	2038.6	60.5	3.0	50.9	2.5
Near East	1801.9	91.5	5.1	14.1	0.8
North America	1923.7	4.6	0.2	14.5	0.8
Total	**12781.3**	**397.1**	**3.1**	**434.3**	**3.4**

Table 2. In drylands, salt-affected soils by continents [23, 24].

Continents	Total Salt Affected Area (mha)	Salt Affected Area (mha)	Salt Affected Area (mha)
		Saline soils	Sodic soils
Africa	209.6	122.9	86.7

CHAPTER 10

Lignocellulose Degrading Bacteria in Soil

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Abstract: The degradation of wood is a highly complex process involving the activities of several different microbes. It has been explored through research that microorganisms have developed various strategies (enzymatic and nonenzymatic) to utilize wood. In the present article, we are presenting the enzymes that originated from fungi and bacteria and their reactions to decomposing wood. Analysis of enzymes involved in wood degradation will not only be helpful in the study of the wood degradation process but also provide information about various ecological niches of the microorganisms. Genomic and secretome data have revealed the importance of the enzymes secreted by microorganisms such as fungi and bacteria in wood degradation in ecological niches.

Keywords: Lignocellulose, Biodegradation, Enzymes, Lignin-modifying enzymes.

INTRODUCTION

Organic matter is destroyed by microorganisms, such as bacteria and fungi, through biodegradation. There are three stages in the biodegradation process: biodeterioration, biofragmentation, and assimilation [1]. The process of biodeterioration can sometimes be described as a surface-level degradation of materials resulting in changes in mechanical, physical, and chemical properties [2]. Abiotic factors in the outside environment deteriorate the material's structure, allowing for further degradation. Abiotic factors such as mechanical pressure, temperature, light, chemicals, *etc*., can influence these initial changes. Biodeterioration usually occurs at the beginning of biodegradation, and sometimes it can occur in parallel to biofragmentation. Hueck defined biodeterioration as the unnecessary alteration to the structure of an object caused by the growth of living organisms, involving the breakdown of stone facade,

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corrosion of metals by microorganisms, or simply the aesthetic changes caused by the growth of living organisms on a structure [3].

Biofragmentation of a polymer is a lytic process in which bonds within a polymer are cleaved, resulting in oligomers and monomers. The steps taken to defragment these materials also differ based on the presence of oxygen in the system. Aerobic digestion refers to the breakdown of materials by microorganisms in the presence of oxygen. Anaerobic digestion is the breakdown of materials in the absence of oxygen. The anaerobic reactions produce methane, whereas the aerobic reactions do not (both reactions produce CO_2, H_2O, residual matter, and new biomass). Furthermore, aerobic digestion typically occurs faster than anaerobic digestion, whereas anaerobic digestion reduces the volume and mass of the material. Due to this, anaerobic digestion produces natural gas, while anaerobic digestion is widely used for waste management systems and as a source of local, renewable energy [4].

Biofragmentation products are later integrated into microbial cells, which is the assimilation stage. Some products of fragmentation are easily transported within the cell by membrane carriers. Others, on the other hand, must undergo biotransformation reactions in order to produce products that can then be transported inside the cell [5]. Once inside the cell, the products enter catabolic pathways that either produce adenosine triphosphate (ATP) or elements that contribute to the cell's structure.

FACTORS AFFECTING THE BIODEGRADATION PROCESS

Practically every chemical compound and material is subjected to biodegradation. But the significance lies in the relative rate at which these processes occur, such as days, weeks, years, or centuries. Several factors, such as light, water, oxygen, and temperature, determine the rate of degradation of organic compounds. Several methods are available to measure the rate of biodegradation. Respirometry tests are one of the methods that are used for aerobic microbes [6] in which the resulting amount of CO_2 serves as an indicator of degradation. Biodegradability can also be measured by anaerobic microbes and the amount of methane or alloy that they can produce [7].

It is important to note that factors that affect biodegradation rates during product testing ensure that the results produced are accurate and reliable. Several materials are tested as being biodegradable under optimal conditions in a lab for approval, but these results may not reflect real-world outcomes, where factors are more variable. For example, a material that may have been tested as biodegrading at a high rate in the lab may not degrade at a high rate in a landfill because landfills often lack light, water, and microbial activity that are necessary for degradation to occur. As a result, there must be standards for biodegradable plastic products, which have a significant environmental impact. In order to ensure that all plastics produced and commercialized will biodegrade naturally, it is important to develop and use accurate standard testing methods. The DINV54900 test has been developed for this purpose [8].

Due to the reaction with oxygen in the air, very large polymer molecules, which contain only carbon and hydrogen, enable plastic products to decompose in a week to one to two years. This reaction happens at a very slow pace without prodegradants. Therefore, conventional plastics persist in the environment for a long time. Formulations containing oxy-biodegradable ingredients catalyze and accelerate the biodegradation process. However, it takes an extensive amount of experience and skill to balance the ingredients to give the formulations a useful life, followed by degradation and biodegradation. Polyester is one of the known examples of a biodegradable product. In the bio-medical field, biodegradable technology is especially useful.

Biodegradable polymers can be classified into three groups, *viz*., (i) Medical, (ii) Ecological, and (iii) Dual application. In terms of origin, they can be divided into two groups, *viz*., (i) Natural and (ii) Synthetic. The Clean Technology Group (CTG) is exploiting the use of supercritical carbon dioxide, which under high pressure at room temperature is a solvent that can be used to make biodegradable plastics into polymer drug coatings. The polymer is used to encapsulate a drug before injecting it into the body and is based on lactic acid, a compound normally produced in the body, and is thus able to be excreted naturally. As a result, the coating is designed to release controlled levels of medicine over time, reducing the need for injections and maximizing the therapeutic benefit. Professor Howdle suggested that biodegradable polymers are particularly suitable for drug delivery since, once inside the body, they do not require retrieval or further manipulation and degrade into soluble, non-toxic byproducts. The degradation of different polymers may differ in the body, and therefore, the choice of polymer can be made in the right way to achieve the desired release levels [9].

BIODEGRADATION VS. COMPOSTING

There are several definitions for biodegradation and composting, leading to confusion between the terms. These terms are often combined, though they do not have the same meaning. Biodegradation occurs when materials are naturally broken down by microorganisms like bacteria and fungi or by other biological processes. A composting process involves human intervention and a particular set of circumstances leading to biodegradation [10].

CHAPTER 11

An Overview of Diverse Yeast Species Performing the Biocontrol Function in Agriculture

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Abstract: The agricultural economy has been suffering from various pathogenic diseases of crops, fruits, grains, and vegetables for a long time. Controlling these diseases is pivotal to the growth of agricultural production and the availability of harvests. Compared with relatively harmful chemical agents, biocontrol agents are now being used as safer and less toxic alternatives to control crop losses. Yeasts survive in all environmental conditions and have been described as potent antagonists to various plant pathogens. Due to their antagonistic activity towards pathogens, relatively simple cultivation requirements, and very limited biosafety concerns, many of these unicellular fungi are being considered for biocontrol applications. In this chapter, we have discussed the pros and cons of yeasts as biocontrol agents, various yeast species, and their modes of antagonistic action. To survive in the environment, yeasts need to tolerate various biotic and abiotic stresses. Those stresses have been discussed here, and how different yeast strains overcome these harsh conditions and carry out antagonistic activities have also been highlighted. Yeast biocontrol activities to date represent a largely unexplored field of research and plenty of opportunities remain for the development of commercial, yeast-based applications for plant protection.

Keywords: Anti-fungal, Biocontrol, Biofilm, Plant protection, Yeast.

INTRODUCTION

Various plant diseases caused by plant pathogens account for major crop losses throughout the year. It has huge socio-economic and food safety implications. Only plant diseases cause a worldwide estimated loss of US $40 billion each year. Reports confirmed that, since the year 2000, the total varieties of new fungal plant pathogens have increased seven-fold. Among crop diseases, the share of fungal-generated diseases constitutes 64–67% and a 20% loss of production. The application of chemical fungicides is one method that is used to prevent plant diseases and protect crops from pests and pathogens. But the redundant use of

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chemical fungicides confers the development of fungicide resistance in the fungal pathogens and, in the absence of other control measures, leads to the resurgence of the disease. Therefore, although the use of pesticides has brought clear improvements in crop production since their induction, their potency to treat some of the most harmful plant diseases is in decline. The other downside of chemical fungicides is their heavy impact on the microflora of agrarian soil and their indiscriminate destruction of beneficial microbes, *e.g.*, endophytic bacteria and fungi, as well as soil-inhabitant helminths, arthropods, and other animals.

One reliable approach to controlling fungal diseases of plants might be the use of biopesticides or biological control agents. In phytopathology, this term is used to define the use of introduced or resident living organisms to eliminate or suppress populations of pathogens. There are a few of these agents on the market, mostly used in the control of pests on a small scale. They comprise dry biomass of bacterial or fungal strains isolated from the endosphere or the rhizosphere of plants. These strains are cultured and live reproductive microorganisms are used as biocontrol agents. These include a diverse group of endophytic fungi that are characterized by their ability to colonize the plant host tissues without causing any external disease symptoms. These endophytes can prevent pathogen infection and propagation directly by competition, mycoparasitism, or antibiosis, or indirectly by inducing resistance responses in the host. Despite this, the biocontrol agents that could be applied in phytopathology are not restricted to these two groups of organisms. Endophytic microorganisms also include Ascomycota and Basidiomycota yeasts, found in many species of trees from very diverse climates, but also in agricultural species. The present review tabulates different kinds of yeast that are beneficial and can be used as biocontrol agents in agricultural ecosystems.

BENEFITS AND DRAWBACKS OF USING YEASTS AS BIOCONTROL AGENTS

The most desired characteristic of the organism to be used as an active biocontrol agent must be its effectiveness against a target disease. Other properties such as biosafety and registration issues, easy production and handling conditions, and the required application equipment are just as important as their effectiveness. Although in a snapshot, the lack of invasive, filamentous growth of most yeasts may seem a disadvantage, a closer analysis proves that the yeast-like morphology makes their culturability in fermenters easy and increases strategic options to prepare formulations, hence ample application options prevail [1]. Like bacteria, unicellular yeasts also favour attachment and biofilm formation, which influences their environmental persistence and survival, thereby improving biocontrol activity. Another important benefit of yeasts is their easy culturing conditions.

Most of the yeasts can be modified or improved using various biotechnological and molecular biology methods in the laboratory. Hence, direct intervention with recombinant DNA (rDNA) technology can improve their biocontrol potential. Budding yeast like *Saccharomyces cerevisiae* strains contains plasmids, whereas most non-conventional yeasts lack plasmids. But these yeasts can be engineered to maintain extra-chromosomal DNA by designing a plasmid vector from their sister species, which contain autonomously replicating and centromere sequences. Yeasts, thus, share growth characteristics and biocontrol activities like bacteria but are safer compared to antibiotic resistance, horizontal gene transfer, and toxin synthesis properties of bacteria.

Yeasts have been used for food and beverage production for generations. They are consumed directly as food supplements and are widely employed in the food processing and bakery industries. In some cases, yeasts used for consumption and biocontrol belong to the same genus or even species (*e.g.*, *Saccharomyces cerevisiae*, *Candida sake*, and *Metschnikowia pulcherrima*) [1 - 3]. Because yeasts are considered safe, their use in crops and food products causes less concern than introducing bacteria or filamentous fungi. Although some yeasts, such as certain *Candida* or *Cryptococcus* species, are also human pathogens. Among the drawbacks of yeast is dimorphism, *i.e.*, the switch to an invasive growth form needs to be kept in mind before considering yeasts for biocontrol. On the other hand, yeasts that show resistance to fungicides or common antifungals also need to be studied and carefully considered before making their application ready.

DIFFERENT YEASTS FOR BIOCONTROL AND THEIR MODES OF ACTION

Different microorganisms compete with each other and host species for nutrients, minerals, and space, and the winner generally predominates [4]. This competition is difficult to predict but is of immense importance in natural environments where resources are limited and highly competitive. In a natural environment, the limitation of space plays a minor role in species diversity and selection. Yeasts generally grow well on agar plates, but large differences in their antifungal activities are reported when compared between laboratory conditions and field trials. Most of the antifungal effects of yeasts are not species-specific. A particular yeast is either strongly or weakly antagonistic against most fungi in laboratory conditions. However, growing under field conditions activates diverse survival mechanisms, and the competition for the physical niche might gain importance in such circumstances.

CHAPTER 12

Macrophomina Phaseolina: An Agriculturally Destructive Soil Microbe

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Abstract: *Macrophomina phaseolina* (Tassi) Goid. is a destructive fungal soil microbe, a cause of charcoal rot disease and causes heavy losses in agricultural production. It is non-specific and appears in moderate to severe form every year worldwide. Due to the seriousness and economic importance of the pathogen as well as disease, it requires multiple approaches like epidemiological study, induction of systemic resistance through non-conventional chemicals, host-pathogen resistance and chemical as well as phytoextract application for its management. Epidemiological studies reveal that the onset of charcoal rot varied in different varieties during different dates of sowing. Timely sowing of crops is an important tool for reducing disease incidence. The intensity of disease in a timely sown crop is less, with higher production and productivity. The non-conventional chemicals like salicylic acid, acetylsalicylic acid, indole acetic acid, indole butyric acid, riboflavin, and thiamine induce systemic acquired resistance (SAR) and effectively inhibit mycelial growth of the pathogen. These non-conventional chemicals showed a reduction of charcoal rot disease under field conditions. It also enhances the yield-attributing traits and yield. It induces total phenol content, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, and catalase activity by the treatment of these chemicals. These activities showed a differential reaction after inoculation of the pathogen on different varieties. However, resistant varieties showed higher induction of biochemical activities than susceptible ones. Different phytoextracts showed inhibition of mycelial growth and a reduction of disease incidence in different crops. Seed treatment with fungicides is an effective method for controlling the pathogen and ultimately enhances the production of the crop. Genotype evaluation for host resistance is an effective, economical, and continuous way of managing the pathogen and disease.

Keywords: *Macrophomina phaseolina*, Soil microbes, Charcoal rot, Induction, Disease management.

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INTRODUCTION

Macrophomina phaseolina (Tassi) Goidanich is a destructive, omnipresent, and non-specific fungal pathogen with a vast host range. Tassi [1] first identified the pycnidial stage of the fungus as *Macrophomina phaseolina*. It is best known as the cause of a disease, which is aptly called “charcoal rot”. Taubenhaus [2] discovered a sclerotial stage, *i.e.*, *Sclerotium bataticola* Taub, and identified the fungus as a causal agent for charcoal rot of sweet potatoes in the USA. Petrak [3] established the genus *Macrophomina*, which was parasitic on sesame. Because of the sclerotial-bearing mycelial stage, the fungus known as *Macrophomina phaseolina* causes characteristic root rot disease. In India, Butler [4] identified a similar sclerotial-bearing fungus and compared it with isolates of Taubenhaus and named it *Rhizoctonia bataticola* (Taub.) Butl, and subsequently, it was transferred to *Macrophomina.* The pathogen attacks several crops in many parts of the world [5]. Generally, the pathogen appears to be non-specific with a wide host range and causes charcoal rot in maize, sesame, sorghum, soybean and other economically important crops and is responsible for huge losses every year in India [6]. *Macrophomina phaseolina* (Tassi) Goidanich belongs to the division Ascomycota, class Dothideomycetes, order Botryosphaeriales, family Botryosphaeriaceae, genus *Macrophomina* and species *phaseolina*.

The hyphae of the pathogen are branched at the right angle with constriction and a septum just after the constriction. Chaudhary and coworkers [7] reported that the pathogen continuously changes its nature, and rapidly resistant cultivars become susceptible. The absence of a known teleomorph stage has stalled its taxonomy for many years [8]. The severity of the disease is directly related to the presence of viable sclerotia in the soil. The hyphae are septate and filiform. Initially, hyphae are hyaline, afterward becoming grey to black and producing jet black oval to round microsclerotia of size 80-90 µm in diameter. Akhtar and coworkers [9] proved the necrotrophic behaviour of pathogens in sesame and found that the seed infection efficiency of *M. phaseolina* was 100% with a significant reduction in the seed index. Infection stages of the charcoal rot fungus *M. phaseolina* in sesame revealed a transition phase from biotrophy *via* BNS (biotrophy-to-necrotrophy switch) to necrotrophy [10]. The pathogen switched its strategy of infection; the host tailored its defence strategy to meet the changing situation. Less reactive oxygen species accumulation, up-regulation of its signalling genes and higher antioxidant enzyme activities post-BNS resulted in resistance. There was a greater accumulation of secondary metabolites and upregulation of secondary metabolite-related genes after BNS. A total of twenty genes functioning in different aspects of plant defence that were monitored over a period of time during the changing infection phases showed a coordinated response.

SURVIVAL OF THE PATHOGEN

M. phaseolina is a diverse, soil-borne pathogen causing stem and root rot on a number of economically important crops like pulses, oilseeds, vegetables, and fruit crops. It causes a monocyclic disease cycle, has a vast host range, and also survives in the seed. The occurrence of sclerotia in plant trash allows the fungus to grow in soil in the absence of the host for two or more years, depending on soil conditions [11]. Meyer and coworkers [12] did not consider the presence of mycelium in the soil as a primary source of inoculum. Reuveni and coworkers [13] observed that the pathogen survives in soil mainly as microsclerotia, which are the primary source of inoculum. Soil, seed, and plant remains are the primary sources of inoculums, and disease severity is directly related to the number of sclerotia present in the soil. These microsclerotia can survive from three months to three years under stressful field conditions [14]. Microsclerotia are formed in vascular tissues, giving a greyish-black appearance in sub-epidermal tissues of the stem. These are vegetative propagules highly resistant to unfavourable climatic conditions, developed on host tissues and then dispersed to the soil after the host plant dies [15]. The fungus survives in soil by multicellular jet black microsclerotia produced enormously during the saprophytic and/or parasitic phases [16]. According to Baggio and coworkers [17], the fungus surviving in the soil through the production of microsclerotia is reduced by pre-plant fumigation of the soil.

THE DISEASE

Charcoal rot is one of the most common, worldwide-dispersed, destructive root and stem rot diseases. In India, charcoal rot of sesame incited by *M. phaseolina* (Tassi) Goidinch was first reported from Uttar Pradesh [18]. Jain and Kulkarni [19] reported this disease from the Jabalpur and Gwalior divisions of Madhya Pradesh, India. It is now widely available throughout the sesame-growing states of Uttar Pradesh, Madhya Pradesh, Gujarat, Maharashtra, Haryana, Punjab, Bihar, West Bengal, Orissa, Tamil Nadu, Karnataka, and Kerala. Generally, the pathogen of charcoal rot appears to be non-specific in nature and causes disease in sesame, maize, sorghum, soybean, sunflower, and other economically important crops, resulting in huge losses every year in India [6]. Generally, about 25% yield losses by charcoal rot in the United States, Uruguay, Spain, and the Soviet Union have been observed, but under favourable weather conditions for the growth and development of pathogens, total crop failure in specific areas has also been recorded [20]. Murugesan and coworkers [21] reported that 1.8 kg/ha of sesame yield was lost for every one percent increase in charcoal rot disease intensity. The disease was reported from North and South America, Asia, Africa and Europe but was more prevalent in subtropical and tropical countries with a semi-arid climate.

CHAPTER 13

Beauveria Bassiana: An Ecofriendly Entomopathogenic Fungi for Agriculture and Environmental Sustainability

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Abstract: In the present day perspective, with the increasing cost of chemical pesticides along with increasing incidences of pesticide toxicity, the application of microbial pesticides holds good promise for crop protection around the world. *Beauveria bassiana* is a common soil fungus, having a broad host range and therefore is used for biological control of soil-dwelling insect pests. As this fungus is epizootic, it is being used worldwide as a biopesticide to control several pests, such as termites, whiteflies, and malaria-transmitting mosquitoes. The use of this fungus in different crop protection systems significantly controls the Colorado potato beetle, codling moth, and several genera of termites and bollworms. As insecticides, the spores are sprayed on affected crops as an emulsified suspension or wettable powder. Generally, *B. bassiana* is considered a nonselective pesticide because it parasitizes a very high range of arthropod hosts. This entomopathogenic fungus is also applied against the European and Indian corn borer, pine caterpillar, and green leafhoppers. The ability of *B. bassiana* to antagonize, parasitize, and kill insects endorses it as an efficient biocontrol agent. Although *B. bassiana* has a good share in the total biopesticide market, there is still ample scope for further development of this superior strain through advertisement among the farming community.

Keywords: *Beauveria bassiana*, Biopesticide, Soil fungus, Soil inhabiting organisms.

INTRODUCTION

Beauveria bassiana is an important and effective entomopathogenic fungus that causes a disease called white muscardine that occurs in a range of insects, including whiteflies, aphids, thrips, grasshoppers, and some types of beetles. It does not need to be ingested by the host; it only requires the spores to come into contact with one host. After infection, the fungus rapidly grows inside the insect. *B. bassiana* feeds on the nutrients present in the insect's body and produces toxins

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in the process. This causes the insect to die, and as the host dies, *B. bassiana* covers the carcass in a layer of white mould and produces more infective spores. *B. bassiana* is found naturally in the soil of many regions around the world and is primarily used to target foliar pests. Many soil-dwelling insects have developed a natural resistance to *B. bassiana* because it is native to many such areas. Therefore, the pest (s) should be closely examined for correct identification before being applied to the soil.

B. bassiana can be used for hard crops at the time of sowing and is not harmful to crop residues, although it is always good to wash the harvested material before consumption. All safety precautions set out on the label must be followed at all times when using *B. bassiana* products. *B. bassiana* is generally considered a low-risk pesticide, yet it is recommended that long-sleeved shirts and pants be worn with a mask and/or goggles when applying products containing the fungus so that the chances of getting into any kind of accident are very small [1, 2].

B. bassiana is a fungus that occurs naturally in soils around the world and grows in and acts as a parasite on various arthropod species, causing white muscardine disease. It is thus believed to be related to entomopathogenic fungi. It has been used for a long time as an organic pesticide to control many pests, such as termites, thrips, whiteflies, aphids, and various beetles. It is also used to control bedbugs and mosquitoes that spread malaria. *B. bassiana* was formerly also known as *Tritirachium shiotae*. The species is named after the Italian entomologist Agostino Bassi, who discovered it in 1815 as the cause of muscardine disease, also known as white muscardine disease. When microscopic spores of the fungus come into contact with the body of the insect host, they germinate on the insect itself, penetrate the cuticle, grow inside the insect, and take nutrients from the insect, weakening the insect and slowly dying. This spreads and colonizes the entire insect and thus deprives the insect of nutrients. The infected insects eventually die within a few days. Later, a white mould emerges from the carcass and produces new spores. A specific isolate of *B. bassiana* can also attack a wide range of insects. The fungus rarely infects humans or other animals, so it is generally considered safe as an insecticide.

Taxonomy

The systematic position of *B. bassiana* is given below [3]:

- Kingdom-Fungi
- Phylum-Ascomycota
- Class- Sordariomycetes
- Order-Hypocreales

- Family-Clavicipitaceae or Cordicipitaceae or ophiocordicipitaceae
- Genus-*Beauveria*
- Species- *bassiana*

MORPHOLOGY

B. bassiana (Bals.) Vuill. is saprophytic, ubiquitous and pathogenic for many insects belonging to different orders, such as Lepidoptera, Hemiptera, Coleoptera, Hymenoptera, Homoptera, Hemiptera, and Orthoptera. The hyphal diameter in these different types of insects varies from 2.5-25 µm. Conidiophores of long scaly transparent and septal filaments bear white to yellowish conidia (asexual spores).

Different types of conidia can be produced by different strains of *B. bassiana*, depending on environmental conditions. The fungus continues to produce spherical (1–4 µm in diameter) or oval (1–3 µm in diameter x 1–3 µm) conidia under aerobic conditions, but anaerobically it produces oval-shaped infective blastospores (2–2 in diameter x 1–3 µm) and conidia (3 µm), whose length can be up to 7 µm [4].

MODE OF ACTION AGAINST INSECTS

Conidia are wind-borne, although rain splashes or arthropod vectors can also help them establish infection in susceptible hosts. In the bodies of invertebrates, the *B. bassiana* infection cycle has been studied in detail by Douro and colleagues [5]. Host infection occurs mainly in four stages: (a) Adhesion, (b) Germination and differentiation, (c) Penetration, and (d) Proliferation.

Adhesion

This represents the first step in the recognition and compatibility mechanism of conidia to the host cuticle [6], which is attached to the insect's cuticle by electrostatic and chemical forces. The induced epicuticular modification led to the germination of conidia, possibly through the production of mucilage [3].

Germination and Differentiation

Environmental conditions and host physiology, such as the biochemical composition of the host cuticle, continue to influence the process of germination. Depending on these factors, the germination of spores can often be stimulated or inhibited. Under suitable conditions, conidia (blastospores) germinate to form a germ tube. This differentiation is characterized by the formation and establishment of appressorium (penetration peg), softening of the cuticle, and promoting penetration. Appresorium degrades host integument to allow hyphae

CHAPTER 14

Ergot, Ergotism and its Pharmaceutical Use

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Abstract: Many fungi are directly or indirectly toxic to humans and animals. Ergot, a fruiting body of the *Claviceps purpurea* fungus, contaminates grain after harvest and is toxic to humans and animals who consume contaminated grains. The lysergic acid diethylamide (LSD) that was widely used as a hallucinogen is best known as the ergot alkaloids. The main symptoms of the disease caused by consuming ergot-contaminated grain flour in humans and animals are blistering and reddening of the skin with a burning sensation. Ergot alkaloids such as agroclavine, ergovaline, ergotamine, ergonovine, lysergic acid, dopamine, *etc*., are the natural alkaloids produced by *Claviceps* spp. in many cereal crops (mainly wheat, barley, rye, bajra, jowar, and dallisgrass), but rye (triticale) is the most common host of this fungus. Contaminated grain may cause very harmful diseases to internal organs, the circulatory and nervous systems of animals and humans, and even they may die. Ergot alkaloids are very important in the pharmaceutical industry. Therefore, this soil-borne fungus, which can be used in the manufacturing of different types of medicines for human and animal welfare, is very important.

Keywords: *Claviceps purpurea*, Ergot, Ergotism, Ergot alkaloids, Holy fire, LSD.

INTRODUCTION

The French word "argot," meaning "a cock's spiny," is the fruit-like structure developed by *Claviceps purpurea* fungus instead of the seed of the plant [1] and contaminates the grain after harvest. Ergot is also the name of the disease of cereals and grasses caused by this and related fungi. Ergot, a plant disease, can reduce grain yields significantly, as each ergot completely replaces the kernel that it infects. Most of the damage to the crop, however, is because it makes the rest of the crop unfit for human or animal consumption unless the ergots are removed. The fungus, *Claviceps* spp., produces a large number of ergot alkaloids and infects a wide variety of grass species during the growing season.

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Structurally, ergot alkaloids are classified into three groups, namely, clavines, lysergic acid amides, and peptides (ergopeptines) [2]. The most commonly produced ergot alkaloids from *Claviceps* species are ergometrine, ergotamine, ergocristine, ergokryptine, ergosine, and ergocornine and their epimeric forms ergotaminine, ergometrinine, ergocristinine, ergokryptinine, ergocroninine, and ergosinine [3 - 6]. Ergotamine and ergovaline are two common alkaloids examined in ergot. These alkaloids have a therapeutic effect on some forms of migraine, post-partum hemorrhages, mastopathy, and a sedative effect on the central nervous system [7]. The most well-known of the ergot alkaloids, lysergic acid diethylamide (infamous LSD), was widely used as a hallucinogen by the hippie culture of the 1960s.

Several powerful alkaloids such as ergotamine, ergometrine, and ergonovine are used medicinally to induce labour and prevent post-partum haemorrhage during childbirth. Due to the importance of these drugs, rye and wheat fields are artificially inoculated in Europe and other countries to increase sclerotia production, which is a valuable source of a farmer's income. Pharmaceutical companies are much more interested in a method of growing the fungus in liquid culture in vats.

Depending on the climatic factors and plant species (rye, wheat, pearl millet, barley, sorghum, *etc*.) and *Claviceps* species, the quantity of ergot in the field and the severity of the symptoms of ergotism may vary. Rye is the most common host of the ergot, while wheat is the least common host of the ergot. These two grains were the source of feed for animals as well as food for poor people and food for rich men, respectively. The alkaloids produced by ergot can compress blood vessels and cause dead tissue by infection or lack of blood flow in humans and animals who consume contaminated food with ergot. Doctors and midwives used the ground fruiting body to stop severe bleeding during severe accidents and childbirth.

History

The disease (ergotism) seems to have existed since ancient times. A religious order was issued in 1093 against a severe outbreak of the disease in Southern France to help the people suffering from it. Because the order was issued by Saint Anthony, the disease was known as “St. Anthony’s Fire.” The disease varied in severity and occurrence from year to year and appeared to affect poor people more often [8]. During the Middle Ages, a Roman historian named Lucretius gave the name 'Ignissacer', meaning “Holy Fire,” to ergotism in 98–55 BC. In China, it was described in 1100 B.C., in Assyria in 600 B.C., and was reported to be very severe for the troops of Julius Caesar in one of his campaigns in France. Several epidemics of "holy fire" appeared in France, including ones of 857, of 994, (which killed between 20,000 and 50,000 people) and of 1093. About 20,000 soldiers in Peter the Great of Russia's army died of consuming bread made with severely contaminated wheat flour in 1722. Outbreaks of the "holy fire" have also occurred during the 20th century, *e.g.*, in 1926-1927 in Russia, about 10,000 people were affected by the disease; in England in 1927, more than 200 cases were reported; and about 200 people were affected in 1951 in the village of Poni-St. Esprit, France, by eating bread made from ergot-contaminated wheat flour [8]. Because of improper methods of cleaning the grains before grinding them for flour, human beings suffered from this horrible disease.

Some recent outbreaks occurred in India in 1975, where the effects were more of the nervous type symptoms of giddiness, drowsiness, nausea, and vomiting, and in Ethiopia in 1978, where gangrene and loss of limbs occurred.

PRODUCTION OF ERGOT ALKALOIDS

Ergot is a disease of grasses (Poaceae family) caused by several *Claviceps* sp. It is an important disease of crops like rye (*Secale cereale*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) caused by *C. purpurea* (Fr.) TuL., bajra (*Pennisetum typhoides*) caused by *C. fusiformis*, jowar (*Sorghum bicolor*) caused by *C. africana* and dallisgrass (*Paspalum dilatatum*) caused by *C. paspali*. Therye (triticale) is the most common host of this fungus, while oats (*Avena sativa*) are rarely affected. The fungus (*Claviceps*) belongs to the class of filamentous Ascomycetes (Pyrenomycetes) of the phylum Ascomycota. In India, the ergot disease of bajra was first observed in the early 1940s in the southern region of India as of minor importance. The first outbreak of the disease-causing considerable yield loss was reported in the Satara district of Maharashtra in 1956 [9]. Since then, the disease continued to appear in sporadic form until 1968, when it appeared in an epiphytotic form in the northern part of India. In the pearl millet growing regions of India, *C. purpurea* is a common cause of this disease, which was first recognized as a fungus in 1711 and whose life cycle was described in 1853 by Tulasne. On the host, the pathogen produces three distinct stages in its life cycle, *i.e.*, the sclerotial (ergot) stage, the perithecial stage, and the honeydew or sphacelia (conidial) stage (Fig. **1**). The sclerotia serve as resting structures in the offseason. In the next season, during blossoming, sclerotia produce 1–60 flesh-coloured stalks. An orange/pink coloured spherical head (stroma proper) is produced on the tip of each stalk. Several perithecia are developed at the periphery of the head. Each perithecium contains many cylindrical or clavate asci. Each ascus contains a bundle of eight long, slender, filiform, hyaline, multicellular ascospores, which are the source of primary infection, carried by insects or wind to newly opened flowers, where they cause infection in the ovaries

CHAPTER 15

Identification of Fungi on Rhizoplane and in Rhizosphere of Leguminous Crop by Adopting Different Techniques

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Abstract: Microbial inhabitants of soil are in an active state and change in response to modifications in environmental conditions, amendments, management, *etc.* Although various methods have been developed to study the biological properties of a disturbed soil sample or with their precincts, adoption of a method should be done keeping in mind the objective, limitations and assumptions of the method. Reproducibility of results is important to have broad applicability and comparisons, which in turn will depend upon effectiveness. Soil is a diverse medium having varying physicochemical properties, and hence, there should be modifications while standardizing the method. It will help in getting reproductive results. Sampling, processing, and storage of samples are equally important to have a true picture of the soil and need appropriate care. The numbers and kinds of fungi on the root surface, *i.e.*, rhizoplane of the leguminous crop plant and in the rhizosphere (near the roots), have been compared with the number and kinds in root-free soil. The crops showed a typical rhizosphere effect, and there were more microorganisms in the rhizosphere than in root-free soil. A total of 34 different species of fungi were identified. The majority belong to the *Aspergillus* genus. Roots and the rhizosphere of moth bean (*Vigna aconitifolia*) yielded a higher proportion of fungi than did root-free soil.

Keywords: Hyphae, Inhibition, Rhizosphere, Soil Factor, Soil Dilution.

INTRODUCTION

The rhizosphere is the narrow region of soil that is directly influenced by root exudates and associated soil microorganisms known as the root microbiome, and the rhizoplane is the region where the root surface is in contact with soil and corresponds to the inner limit of the rhizosphere. The microflora on the root surface may be more characteristic of a plant species than that of the rhizosphere. In the soil, a specific root effect may be diluted out or possibly obscured by the

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soil microflora. Organisms intimately associated with roots may also reflect a plant's growth and metabolism much more precisely than organisms in the rhizosphere soil [1 - 4].

Soil factors are known to affect both root disease development and rhizosphere microflora. These factors no doubt change with the growth of the plant. There is always more microbial activity in the soil in the vicinity of roots than in the soil away from them. Between these two zones is an area of transition (Fig. **1**) in which the root influence diminishes gradually with an increase in distance.

To study soil microorganisms, knowledge of suitable techniques and their methods is of utmost importance. There are some techniques for trapping specific microorganisms, while some are relatively common for others as well. Thus, a large number of methods have been devised to study microorganisms qualitatively and quantitatively as well as to isolate pure cultures of microorganisms from the soil.

Fig. (1). A photomicrograph showing transition zone antagonistic reaction between microorganisms isolated from rhizosphere soil.

SOIL SAMPLING

The soil factors vary from place to place, so methods for collecting soil samples are not standardized. The microflora is rarely free in soil but occurs largely as colonies attached to clay, humus, and organic matter particles (*e.g.*, small rootlets). The microbial population of soil should be considered as a composite of these microorganisms' environments. It is generally presumed that similar types of soil samples from a locality at the same depth may contain a nearly equal number of microorganisms. It is generally suggested that 3 to 5 composite samples should be taken from each soil for each determination, after removing about 1 cm of the soil from the surface.

Collected samples are taken in clean and sterilized plastic bags or other suitable containers. It is always preferred to shorten the gap between sampling and the results of their studies. Although some workers believe that the soil samples can be stored at a low temperature, the multiplication of microbial cells is slowed down. Furthermore, it is recommended that prior to the use of samples, they should be partially dried (preferably air dried). Sieving and mixing of soil are also necessary. However, it has been reported by some workers that there is no necessity for taking unusual precautions, including aseptic conditions during sampling [5].

ISOLATION OF FUNGAL SPECIES FROM RHIZOSPHERE AND RHIZOPLANE

Methods

Various methods were applied to study the microflora of the rhizosphere and rhizoplane of moth bean (*Vigna aconitifolia*). The details of them are given below:

Direct Inoculation

It is for studying fungi that produce viable mycelia in soil. The period for incubation is kept short so that the fungal spores do not germinate and produce a mass of mycelium about 1 cm in diameter. Soil is placed in the centre of a petri dish containing solidified agar medium and incubated for 24 hours at 20–22°C. The mycelium, which radiates out from the soil piece, is cut and transferred to agar slants [6].

The Soil Plate Method

The method was designed to study the distribution of various species of fungi. The spore masses remain more intact in the soil plate than in the dilution plate. A small amount of soil (0.05 to 0.015g) will be examined in a petri dish with the help of a micro spatula. It is preferable to mix the soil particles with a drop of water before the melted and cooled agar medium is added and soil particles are dispersed throughout the agar [7].

CHAPTER 16

Trichoderma: A Potential Arsenal for Industries

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Abstract: The genus *Trichoderma* (fungi) is a very large group of microorganisms that play a significant role in the environment. This is omnipresent in the climate, particularly in soils. *Trichoderma* species could be easily isolated from the soil by all traditional methods available because of their rapid growth and abundant conidiation. These are used both as biofungicides for biological plant protection as well as for bioremediation. In addition, the genus *Trichoderma* includes edible and medicinal mushrooms but also human pathogens. Members of the *Trichoderma* genus are often used primarily in the processing of enzymes, antibiotics, and other metabolites, but also for biofuel in various branches of industry. Several researchers have confirmed, based on phylogenetic analysis, that *Trichoderma* and *Hypocrea* form a single holomorphic genus. In which two can be differentiated by large clades. Several *Trichoderma* spp. positively affect plants by stimulating plant growth and protecting plants from fungal and bacterial pathogens. *Trichoderma* has entered the genomic period at present, and sections of the genome sequences are open to the public. For this purpose, *Trichoderma* can be used to an even greater degree than before for human needs. *Trichoderma* species possess diverse biotechnological applications, such as acting as biofungicides to control various plant diseases and as biofertilizers to promote plant production. *Trichoderma* secretes various volatile compounds, including alcohols, aldehydes, ketones, ethylene, hydrogen cyanide, and monoterpenes, as well as non-volatile compounds known to exhibit antibiotic activity, including peptaibols, and diketopiperazine-like gliotoxins and gliovirins. Nonetheless, further studies are required to make the application of these fungi more effective and safe.

Keywords: *Trichoderma*, Bioactive Compounds, Antibiotics, Hydrolytic Enzymes, Brewing Industry, Bioremediation.

INTRODUCTION

Trichoderma-type fungi are commonly found in all climatic zones. The most common habitats include soil and rotting wood. They can use various substrates and are considered immune to specific toxic chemicals. These fungi can be found

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in the soil environment on sclerotia and other propagating types of fungi. They are considered to dominate in all climatic areas, including agricultural, woodland, salt marsh, and desert soils. These were also separated from such rare sources as shellfish, sea bivalves, and termites. In addition, *Trichoderma* species are known to occur in rotting wood and soil due to their productive heterotrophic interactions, such as parasitism, decomposition, and even opportunistic endophytes. Such species produce different pigments, ranging from a greenish-yellow one up to a reddish tinge, but there are still some colourless specimens. The conidia can also have a variety of colours, from colourless to different shades of green, or even grey or brown tinges. This genus currently consists of more than 260 species, and approximately 35 known species are economically important either because of their ability to produce enzymes and antibiotics or because they are used as biocontrol agents. On the other hand, some members of the genus have been identified as emerging opportunistic human pathogens and as causative agents of green mould, a disease that causes significant losses in the production of agricultural edible mushrooms. This chapter covers the wild range of the biodiversity of a ubiquitous *Trichoderma* from different sources and its applications in industry as a producer of bioactive compounds and extracellular hydrolytic enzymes, and in agriculture as a prompter for plant growth and biocontrol.

BIOTECHNOLOGICAL APPLICATIONS

Production of Secondary Metabolites

Secondary metabolites are small organic compounds that do not participate directly in normal growth and reproduction but play an important role in the formation, signaling, and interaction with other species. The lack of secondary metabolites does not lead to the immediate death of the individual but results in a long-term impairment of the life, fecundity, or aesthetics of the organism or even no noticeable improvement at all. Often, there are some environmental conditions where secondary metabolites are essential for survival, such as siderophores that are necessary for growth where iron concentrations are poor. For plants, secondary metabolites are a significant defense against herbicides and other interspecies protection, whereas secondary metabolites are used by humans as medications, flavouring products, and therapeutic medicinal products [1]. Demain and Fang [2] reported that secondary metabolites are used as a strategic tool against other bacteria, fungi, amoebas, plants, insects, and animals for the transport of metals as well as agents for plant-to-organism symbiosis. Essential secondary metabolites are alkaloids, terpenoids, and phenolics.

Trichoderma spp. are economically important as they serve as biocontrol or biopesticide agents that inhibit the growth of phytopathogenic fungi. *Trichoderma* functions as a biocontrol agent due to the existence of a variety of extracellular lytic enzymes and secondary metabolites [3]. It produces numerous secondary metabolites as well as extracellular enzymes, including β-glucanase, chitinase, and proteinase [1]. They reveal a range of mechanisms of action for functioning as biocontrol agents in their antagonistic encounters with fungal pathogens, such as antibiotic activity, mycoparasitism, nutrient rivalry, cell wall lytic enzyme activity, and the development of systemic tolerance to plant pathogens [4, 5]. Antibiotic development by *Trichoderma* sp. is considered to play an important role in the case of biocontrol. A variety of antibiotics as well as antifungal toxins, such as trichodermin, gliovirin, and harzianic acid, have been known to be developed by *Trichoderma* species that have a direct effect on other organisms [6].

Table 1. Secondary metabolites /strains, bioactive compounds and their effects formed by *Trichoderma*.

Species	**Bioactive Compounds**	**Effects**	**References**
T. koningii and *T. viride*	Dermadin (U-21, 963)	Antimicrobial activity *S. aureus* and *Escherichia coli*	[7]
T. reesei	Cellulases	Degrade cellulase during root colonization to penetrate the plant tissue	[8]
T. longibrachiatum and *T. pseudokoningii*	Compactin	Act as a cholesterol-lowering agent	[9]
T. koningii	Koninginin A	Act as a regulator of plant growth	[10]
T. longibrachiatum	5-Hydroxyvertinolide	Antagonistic against the fungus *Mycena citricolor*	[11]
T. virens	Gliovirin	Antimicrobial against oomycetes and *Staphylococcus aureus*	[12]
T. longibrachiatum	Bisvertinolone	Antifungal properties	[13]
T. harzianum	Fleephilone	Inhibitory activity against the binding of REV-proteins to REV responsive element RNA	[14]
T. harzianum	Harziphilone	Cytotoxicity against the murine tumor cell line M-109	[14]
T. longibrachiatum	Trichodimerol	Inhibit tumor necrosis factor in human monocytes	[15]
T. harzianum	6-(1-pentenyl)-2H-pyran-2-one	Antifungal activity	[16]
T. virens	Trichocaranes A, B, D	Inhibit the growth of etiolated wheat coleoptiles	[17]

SUBJECT INDEX

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