Review Article

Exploitation of Fungi: Redefined

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ABSTRACT

A long time back the discovery of penicillin opened the new door for fungal antibiotics. Gradually a large number of secondary metabolites harnessed by various scientists and used now a days as antibiotics. Although the new area to be yet explored is fungal endophytes. All plants in natural ecosystems appear to be symbiotic with fungal endophytes. This highly diverse group of fungi can have profound impacts on plant communities through increasing fitness by conferring abiotic and biotic stress tolerance, increasing biomass and decreasing water consumption, or decreasing fitness by altering resource allocation. Endophytic fungi are an important part, are ubiquitous and occur within almost all the plants, including a broad range of hosts in various ecosystems, and therefore play an important role in the natural environment. Despite extensive work in this field, the ecological significance of these fungi remains poorly characterized. More than 1 million species of endophytic fungi are estimated to exist based on a ratio of vascular plants to fungal species of 1:4 or 1:5. Nevertheless, our recognition of endophyte diversity is limited at present. In surveys of endophyte diversity, traditional techniques, such as cultivation-dependent methods, have been routinely used in previous studies collectively, more than 100 years of research suggests that most, if not all, plants in natural ecosystems are symbiotic with mycorrhizal fungi and/or fungal endophytes. The discovery of endophytic fungi in natural environments, however, has been limited by traditional methodology due to some non-sporulating and non-culturable fungi. Molecular techniques, such as DNA fingerprinting and sequencing methods, have been successfully employed in the detection and identification of endophytic fungi, and different endophyte diversity and community composition have been documented by cultivation-dependent and molecular techniques. So what next, are endophytes the future medicines? The present review explore the potential of fungi with respect to the production of various secondary metabolites/medicines.

KEYWORDS: Fungi, Endophytes, Metabolites, Medicines, Drug resistance, Microorganisms.

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INTRODUCTION

The need for new and useful compounds to provide assistance and relief in all aspects of the human condition is ever growing. Drug resistance in bacteria, severe infections caused by various pathogens, the increased human population and environmental degradation, loss of biodiversity and pollution of land and water added problems to society. Microorganisms have long served mankind by virtue of the myriad of natural products they produce. Preliminary examination confirms that we have by no means exhausted the world of the undiscovered microbes. Therefore, much more comprehensive

search of the Earth's various niches might yet reveal novel microbes which have direct usefulness to human societies. Furthermore, only a small number of microbes are used directly in industrial applications, in environmental cleanup operations and in the biological control of pests and pathogens. Even though actinomycetes became the more important source of antibiotics in the years to come, some new antibiotics are still being sourced from fungi. Following the antibiotics era, in the latter part of 20th century, scientists isolated from fungi many more products important in agriculture, industry and medicine. However, emphasis started shifting slowly towards other groups of microbes, such as bacteria and actinomycetes. In the 21st century, there are reasons to believe that fungi can again occupy the center stage in bioprospecting novel bioactive compounds useful in industry. In fact, with in this small group various molecular approaches were developed to harness maximum benefit from these fungal strains. Still, there is a general call for new antibiotics, chemotherapeutic agents and agrochemicals that are highly effective, possess low toxicity and have a minor environmental impact. The quest for this search leads to exploring the wide capacities and prospects of whole new domain of microbial society, "endophytes". The present article discusses the past, present scenario and novel process development from these microbes for the upcoming future.

History

The development of industrial microbiology can be divided into three distinct phases. A period of ignorance before 18th century, an era of discoveries (1800-1900) including the golden age of microbiology and a period of rapid industrial development (after 1900). Fungi have traditionally been the source of several useful chemical substances starting with the well known ethyl alcohol from yeast, which continues to influence human civilization all over the world. In 1928 Alexander Fleming discovered Penicillin opening up the era of antibiotics [1].

Classification & Description

Taxonomy and classification is based on morphological and molecular characterization. Vegetative characters and reproductive features help in the segregation of genera and species. However strain differentiation is difficult. Therefore, restricted fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), enzyme linked immune-sorbent assay (ELISA) and inter transcribed spacer amplification (ITS) have become important tools in the segregation of strains and biotypes.

The fungal world includes molds, yeasts, mushrooms, puffballs, jelly fungi, bracket fungi besides many others. These are classified in four divisions: the Chytridiomycota (chytrids), Zygomycota (bread molds), Ascomycota (yeasts and sac fungi), and the Basidiomycota (club fungi). Placement into a division is based on the way in which the fungus reproduces sexually. The shape and internal structure of the sporangia, which produce the spores, are the most useful characters for identifying these various major groups. Fungi are composed of filaments called hyphae; their cells are long and thread-like and connected end-to-end. The body of the organism is given the unique name mycelium, a term which is applied to the whole body of any fungus. In addition to being filamentous, fungal cells often have multiple nuclei. In the chytrids and zygomycetes, the cells are coenocytic, with no distinction between individual cells. Rather, the filaments are long and tubular, with a cytoplasm lining and large vacuole in the center. By contrast, the ascomycetes and basidiomycetes are septate; their filaments are partitioned by cellular cross-walls called septa. These spores, haploid hyphae grow and ramify, and may give rise to asexual sporangia, special hyphae which produce spores without meiosis. As part of their life cycle, fungi produce spores. The sexual phase is begun when haploid hyphae from two different fungal organisms meet and fuse. When this occurs, the cytoplasm from the two cells fuses, but the nuclei remain separate and distinct. The single hypha produced by fusion typically has two nuclei per "cell", and is known as a dikaryon, meaning "two nuclei". The dikaryon may live and grow for years, and some are thought to be many centuries old. Eventually, the dikaryon forms sexual sporangia in which the nuclei fuse into one, which then

undergoes meiosis to form haploid spores, and the cycle is repeated. When reproductive hyphae are produced, they form a large organized structure called a sporocarp, or mushroom. This is produced solely for the release of spores and is not the living, growing portion of the fungus.

Fungal Endophytes and diversity

Endophytes are defined as organisms that inhabit plant tissues at some stage in their life cycle without causing apparent harm to their host [2-4]. These endophytes are extremely common and highly diverse microorganisms that live within plant tissues, but usually remain asymptomatic. Microbial endophytes are one of the largest untapped resources and poorly investigated group of microorganisms that represent an abundant and dependable high value bioactive metabolites and chemically novel compounds with the potential for exploitation in the field of medicine, agriculture and industry. Plant breeders are using them for production of enzymes, medicines and biological control agents [5-6].

Petrini (1991) [7] reported that these microorganisms colonize in healthy tissues of plants, at least for a part of their life cycle, without causing apparent disease symptoms in their host. These organisms reside in the living tissues of host plant and do so in a variety of relationships, ranging from symbiotic to slightly pathogenic [8-9]. History shows that ancient civilizations had also discovered the utility and benefits of medicinal plants along with fungi grown on roasted green corn to treat intestinal ailments [10]. Since then, people have been engaged in the discovery and application of microbial metabolites with activity against both plant and human pathogens.

The most frequently isolated endophytes are the fungi. It turns out that the vast majority of plants have not been studied for their endophytes. Thus, enormous opportunities exist for the recovery of novel fungal forms, taxa, and biotypes. Hawksworth and Rossman (1987) [11] estimated that there may be as many as one million different fungal species, yet only about 100,000 have been discovered. As more evidences accumulate, estimates keep rising as to the actual number of fungal species.

Fungi are one of the major sources of natural bioactive compounds. The search for new metabolites has recently been expanded to the fungi living inside plants. Interest on endophytic fungi has recently surged, which has led to a considerable amount of research regarding the role of these fungi in host plants. Over 4000 bioactive metabolites of fungal origin have been described. It is well investigated that endophytes can increase ability of host plants to resist invasion of herbivores, insects, and pathogens [12-15]. They occupy a wide range of different habitats due to their vast nutritional diversity as heterotrophs and symbionts. Endophytic infection enhances the defense of the host plants; this has led to the hypothesis that plants might have accommodated endophytes to improve their fitness in a given environment under certain stress conditions [16-18]. Depending on the biotype and ecological niche, endophytic fungi may play an important role in the fitness of host plants by producing appropriate bioactive compounds. An intensive search for newer and more effective agents to deal with diseases is now under way as endophytes are a novel source of potentially useful medicinal compounds. Exploitation of endophytes for the production of several metabolites is now the upcoming area of research along with other group of organisms [19].

The term diversity refers to the variation in the form and function of living organisms in the natural environment. Fungal diversity can be recognized under the following aspects:

a) Morphological variations in the spore forms which has been primarily used as the basis for taxonomy and classification.

b) On a physiological basis, fungi can be grouped under thermophiles, mesophiles, halophiles etc.

Out of 1.5 million fungal species estimated to be present [20], only 97,861 fungal species has been described.

It seems obvious that endophytes are a rich and reliable source of genetic diversity and novel, undescribed species. Finally, in our experience,

novel microbes usually have associated with them novel natural products.

Of the myriad of ecosystems on earth, those having the greatest biodiversity seem to be the ones also having endophytes with the greatest number and the most biodiverse micro-organisms. Tropical and temperate rainforests are the most biologically diverse terrestrial ecosystems on earth. The most threatened of these spots cover only 1.44% of the land's surface, yet they harbour more than 60% of the world's terrestrial biodiversity. As such, one would expect that areas of high plant endemicity also possess specific endophytes that may have evolved with the endemic plant species. Tropical rainforests are a remarkable example of this type of environment. Competition is great, resources are limited, and selection pressure is at its peak. This gives rise to a high probability that rainforests are a source of novel molecular structures and biologically active compounds.

Isolation of fungal endophytes

Criteria for plant selection: It is important to understand the methods and rationale used to provide the best opportunities to isolate novel endophytic microorganisms as well as ones making novel bioactive products. Thus, since the number of plant species in the world is so great, creative and imaginative strategies must be used to quickly narrow the search for endophytes displaying bioactivity. A specific rationale for the collection of each plant for endophyte isolation and natural-product discovery is used. Several reasonable hypotheses govern this plant selection strategy and these are as follows. (i) Plants from unique environmental settings, especially those with an unusual biology, and possessing novel strategies for survival are seriously considered for study. (ii) Plants that have an ethnobotanical history (use by indigenous peoples) that are related to the specific uses or applications of interest are selected for study. These plants are chosen either by direct contact with local peoples or via local literature. Ultimately, it may be learned that the healing powers of the botanical source, in fact, may have nothing to do with the natural products of the plant, but of the endophyte (inhabiting the plant). (iii) Plants that are endemic, that have an unusual longevity, or that have occupied a certain ancient land mass. (iv) Plants growing in areas of great biodiversity also have the prospect of housing endophytes with great biodiversity [46, 48].

After a plant is selected for study, it is identified, and its location is plotted using a global positioning device. Small stem and leafy pieces (explants) are cut from the plant and placed in sealed plastic bags after excess moisture is removed. Every attempt is made to store the materials at 4°C until isolation procedures canbegin. In the laboratory, plant materials are thoroughly surface treated with 70% ethanol, sometimes they are flamed, and ultimately they are air dried under a laminar-flow hood. This is done in order to eliminate surface-contaminating microbes. Then, with a sterile knife blade, outer tissues are removed from the samples and the inner tissues are carefully excised and placed on water agar plates. After several days of incubation, hyphal tips of the fungi are removed and transferred to potato dextrose agar. The fungal endophytes are encouraged to sporulate on specific plant materials and are eventually identified via standard morphological and molecular biological techniques and methods [21-22].

Identification of fungal endophytes:

Morphological characteristics: During early stage of civilization, fungi were identified mostly on the basis of morphological characteristics. These include habitats and colonization on various substrate (terrestrial, lignicolous, coprophilous and fungicolous). Several fungi have been described and identified on the basis of morphological features like colony characterization, growth of fungi on different media, colour of colony (front and reserve), conidial development, size, shape, conidia, attachment of conidia, and shape of conidial head [23].

Slide culture technique: If a fungus is grown as a slide culture, sporulation characteristics and the spores of the fungus remain undisturbed and attached to the sporophores thus facilitating in identification. This technique was performed for various stages of conidia formation and proper identification of the sporulating fungi [24-27]. All experiments and observations were repeated at least thrice. Those cultures which

failed to sporulate were named as mycelia sterilia, and divided into different morpho species according to their cultural characteristics.

The bottom of a Petri dish (PD) is lined with filter paper (FP), then a bent glass rod (GR) is put, and a clean slide (S) is placed on top of it .The filter paper is moistened with 5% glycerin or simply by distilled water and sterilized. In 9cm Petri dish 15 ml of desired medium is poured, when solidified, is cut into 0.7-1 cm blocks (B) with a sharp scalpel. One or two agar blocks (2 cm apart) are placed on a sterile slide and inoculated (I) at the center of each block. A sterile square cover slip (CS) is placed on the inoculated agar block and incubated under favourable conditions for sporulation (Figure: A & B). When the growth of fungus is optimum, the cover slip is carefully lifted and mounted with a drop of appropriate mountant on a clean slide. The agar block is discarded and the fungal growth is similarly mounted on the culture slide [24-27].

Fig. A: Showing the slide culture technique (PD- Petridish, FP- Filter paper, GR-Glassrod, S- Slide, B- Block, CS- Coverslip, I- Inoculated fungal culture).

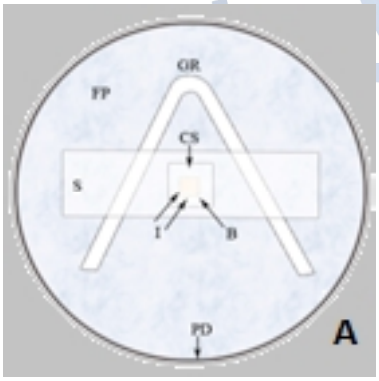
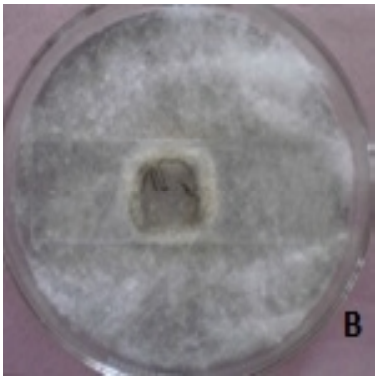


Fig. B: Sporulating endophytic fungi showing various conidial development stages by this technique.



Microscopic identification

Take a small drop of lactophenol-cotton blue stain (or water as per requirement) on microscope slide. Using sterilized dissecting needle, place a small portion of mould from the actively growing colony margin with a thin layer of agar surface. If colony are thick and woolly it may not be necessary to take the agar, but in the more common apprised type it is necessary. Spread the material properly and place the coverslip by lowering one edge on to the slide before the other so that air bubbles can escape. Observe the slide under the microscope at 10X and 40X magnification.

Eventually, when an endophyte is acquired in pure culture, it is tested for its ability to be grown in shake or still culture by the use of various media and growth conditions. It is also immediately placed in storage under various conditions, including 15% glycerol at -70°C. Ultimately, once appropriate growth conditions are found, the microbe is fermented and extracted and the bioactive compound(s) is isolated and characterized.

Distinguished roles of fungi

Fungi as an antibiotic producer: There is a general call for new antibiotics, bioactive compounds and agrochemicals that are highly effective possess low toxicity and have a minor environmental impact. Most of the synthetic drugs available in the market are less effective and possess various side effects in the body. Furthermore, the drug resistance in pathogens is a major problem for the treatment of various life threatening diseases. The arrival of new diseases such as AIDS, swine flu requires the discovery and development of new drugs to combat them. Therefore, for safety and environmental problems, many synthetic compounds/drugs have been and currently are being targeted for removal from the market, which creates a need to find alternative ways to control farm pests and pathogens. Novel natural products and the organisms that make them offer opportunities for innovation in drug and agrochemical discovery.

The first antibiotic to be produced was Penicillin and it was discovered through the sheer serendipity of Alexander Fleming in 1928.

This was derived from the ascomycetous fungus *Penicillium notatum*. The antibiotic was put into mass production and large scale therapeutic use because of the scale up work subsequently carried out by Howard Florey and Ernst Chain in the 1940s, and this work was spurred by the necessity to cure wounded soldiers of infections during the II world war [28-29]. With the discovery of Streptomycin from an actinomycete, by Selman Waksman in 1944, the era of antibiotics truly began, and during this period extending over two decades, more than 1000 antibiotics were discovered, many of them from actinomycetes, and some from fungi.

As on today, the important antibiotics derived from fungi, other than Penicillin, are: Cephalosporin from *Cephalosporium* spp., Griseofulvin from *Penicillium griseofulvum*, Lentinan from *Lentinus* sp., and Schizophyllan from *Schizophyllum commune*. Penicillin and Cephalosporin are antibacterial antibiotics acting against Gram-positive bacteria, whereas, Griseofulvin is an antifungal antibiotic useful in treating dermatophyte infections. Lentinan is active against *Mycobacterium tuberculosis*, *Listeria* sp., and Herpes Simplex Virus-1 (HSV-1). Schizophyllan is both antibacterial and antifungal in activity. It is useful in controlling *Candida albicans* and *Staphylococcus aureus*.

Mushrooms and polypores are rich source of natural antibiotics. The cell wall glucans are well known for their immunomodulatory properties, and the secondary metabolites are active against bacteria [30-31] and viruses [32-33]. Exudates from mushroom mycelia are active against protozoa such as the malarial parasite *Plasmodium falciparum* [34-35]. Since humans and fungi share common microbial antagonists such as *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, humans can benefit from the natural defense strategies of fungi to produce antimicrobials [36]. The general hypothesis increasingly substantiated is that polypores provide a protective immunological shield against a variety of infectious diseases [37-39].

Two other polypores are notable, *Fomes fometarius* and *Piptoporus betulinus*, both of which were found in the high Alpines near the border of Italy, buried along with the legendary 'ice man' 5300 years ago [40]. Scientists believe that the use of these fungi was for their antimicrobial properties.

In a recent *in vitro* study, more than 75% of polypore species surveyed showed antimicrobial property [41]. In particular, this study showed species of the genus *Ganoderma* such as *G. applanatum*, *G. lucidum*, *G. pfeifferi* and *G. resinaceum* to be effective against Gram-positive bacteria. In contrast, gilled mushrooms such as *Psylocibe semilanceata*, *Pleurotus eryngii*, and *Lactarius delicious* all strongly inhibited the growth of *Staphylococcus aureus* [42].

Fungi as an immunosuppressant drugs producer: There are non-antibiotic therapeutic agents obtained from fungi that have revolutionized medical practice. Cyclosporin is an important immunosuppressant drug that is used in organ transplantation surgery. Cyclosporin-A is derived from *Tolypocladium inflatum*, and *Aspergillus* sp. Isopenicillin-N is a common precursor of Penicillin and Ensuphulosporan antibiotics and this is produced by *T. inflatum.* About 20% of the drugs produced by pharmaceutical industry today are derived from fungi [43].

Lovastatin is a cholesterol biosynthesis inhibitor derived from *Aspergillus terreus*. It is one of the many drugs used as a cholesterol reducing agent. A similar cholesterol reducing drug is produced from *Penicillium citrinum*, and it is called Pravastatin.

Fungi as an anticancerous agents producer: Taxol is a highly functionalized diterpene that is found in extremely small quantities in all *Taxus* species [54]. It was originally isolated and characterized from the inner bark of Pacific yew, *Taxus brevifolia* [44]. An endophytic fungus, *Pestalotiopsis microspora,* obtained from the inner bark of *T. wallacbiana,* produces taxol in culture. This fungus typically possesses a brown to hyaline branched septate mycelium with 'Onidiomata that are and are brownish with a thin wall. The conidia are fusiform with four septa, with both the basal and terminal cells being hyaline and the median cells brown. The characteristic terminal cell appendages are not branched and may number three or four. The conidia are typically 24-26 pm x 5.5- 6.0 pm,

making them, on average, somewhat larger than many of the other representatives of this species. Furthermore, the taxol isolated from this source is biologically active against certain cancer cell lines, is spectroscopically identical to authentic taxol, and accumulates in cultures at the level of micrograms per litre. These results indicate that *P. microspora* is an excellent candidate for consideration in fermentation technology.

Fungi as an enzyme producer: Commercial microbial enzymes are increasingly replacing conventional chemical catalysts in many industrial processes (Table 2). Enzymes have several advantages over chemical catalysts, including the ability to function under relatively mild conditions of temperature, pH and pressure. Enzymes are specific, often stereo selective, catalysts which do not produce unwanted by products. The first fungal enzyme amylase was produced via SSF (Solid State Fermentation) from *Aspergillus oryzae* on moist rice or wheat bran. The process was initially developed by Jokichi Takamine and patented in the USA in 1884. However the large-scale production of microbial enzymes was carried out by submerged fermentation technology in 1940s. Nowadays there are several multinational companies having stake in manufacturing industrial enzymes from fungi. Biocon India Ltd. is a major bulk enzyme producer in India, but not a major at global level. The top 14 companies at the global level are listed in table 1.

Table-1. Major bulk Enzyme producing Companies (Ratlege and Kristiansen, 2001) [45].

· Amano Pharmaceutical Co., Japan
· Biocatalysis Ltd., Wales
· Enzyme Development Corp., USA
· Danisco Cultar, Finland
· DSM-GIST, Netherlands
· Meito Sankyo Co., Japan
· Nagase Biochemicals Ltd., Japan
· Novo Nordisk, Denmark
· Rhone-Poulenc, England
· Rohm gmbh, Germany
· Sankyo Co., Japan
· Shin-Nihon Chemical Co., Japan
· Solavy Enzymes gmbh, Germany
· Yakult Biochemical Co., Japan

Table-2. The major enzyme producing fungi.

ENZYME	SOURCE
· Acid, alkaline & neutral proteases	*Aspergillus oryzae; A. niger A. flavus; A. sojae*
· Cellulase	*Trichoderma koningi*
· Diastase	*Aspergillus oryzae*
· Glucoamylase	*Aspergillus niger; A. oryzae*
· Invertase	*Saccharomyces cerevisiae*
· Lactase	*S. lactis; Rhizopus oryzae*
· Ligninase	*Phanerochaete chrysosporium*
· Lipase	*Rhizopus spp.*
· Pectinase	*A. niger*; *Sclerotinia libertina*

Fungi as an organic acid producer: There are several organic acids produced on a commercial scale from fungi in which, four commercial organic acids produced by fungi are employed in high-volume, low-value applications (Table 3). For example, they are used in industrial metal cleaning or other metal treatments and in the food and feed industry as flavor enhancers, acidifiers, stabilizers, or preservatives. The commercial success of fungal bioprocesses is ultimately based on rapid and economic conversion of sugars to acid, but that alone does not explain the commercial situation for each of these acids. An understanding of the economic and business parameters that have contributed to the success of these four products may be useful in development and commercialization of new organic acid products from filamentous fungi.

Table-3. Organic acids produced from fungi.

Organic acid	Source
· Citric acid	*Aspergillus niger*
· Fumaric acid	*Rhizopus nigricans*
· Gluconic acid	*Aspergillus niger*
· Itaconic acid	*A. terreus*
· Kojic acid	*A. oryzae*

The (unicellular fungi) yeasts including *Saccharomyces cerevisiae*, species of *Rhodotorula, Pichia*, and *Hansenula* are important organisms in fungal biotechnology, they have not been significant for commercial organic acid production, with one exception. The yeast, *Yarrowia lipolytica*, and related yeast species, may be in use commercially to produce citric acid [33]. Furthermore, in the near future engineered yeasts may provide new commercial processes to make lactic acid [46].

Fungi in bioremediation: Fungi are known to degrade, or cause to deteriorate, a wide variety of materials and compounds, processes known as *mycodegradation* and *mycodeterioration*. The degradative activities of fungi have been recognized in various situations where they destroy different types of wood, stored paper, textiles, plastics, leather, and electro insulating and various wrapping materials. Polyethylene, with a molecular weight of 4000 to 28 000, is degraded by the cultivation of *Penicillium simplicissimum* YK [55] and bioremediation of polyethylene may be possible in the future. Enzymes of *Mucor rouxii* NRRL 1835 and *Aspergillus flavus* have produced changes in the mechanical properties and weight of disposable polyethylene bags [47]. The white-rot fungi are also efficient in polyethylene degradation. *Aspergillus flavus* colonized and degraded chitosan-graft polymethyl methacrylate film by 45% during 25 days of aerobic cultivation in a study by Harish Prashanth *et al.* (2005) [48]. *Phanerochaete chrysosporium* attached to fibers of polyamide-6 and reduced 50% of the polymer's molar mass after 3 months. Of 15 species of white- and brown-rot fungi, *Resinicium bicolor* was shown to be the most effective fungus for the detoxify cation of ground waste tire rubber material prior to devulcanization [49].

White-rot fungi for lignin degradation have been examined for more than half a century. After the discovery of the extracellular oxidative ligninolytic enzymes of the white-rot fungus *Phanerochaete chrysosporium*, [50] proposed the use of this fungus for bioremediation. Enzymes involved in the degradation of wood are also responsible for the degradation of a wide variety of persistent organic pollutants. The white-rot fungus *P. chrysosporium* has emerged as an archetypal model system for fungal bioremediation. *P. chrysosporium* has the ability to degrade toxic or insoluble compounds more efficiently than other fungi or microorganisms. The numerous oxidative and reductive mechanisms of degradation make its application attractive in different matrices. There has been a plethora of review articles on *P. chrysosporium* with respect to mechanisms of degradation of recalcitrant compounds and xenobiotics.

In addition to *P. chrysosporium*, several other white-rot fungi (e.g., *Pleurotus ostreatus*, *Trametes versicolor*, *Bjerkandera adusta*, *Lentinula edodes*, *Irpex lacteus*) are known to degrade these compounds. Based on the literature of the past two decades, it appears that the white-rot fungi account for at least 30% of the total research on fungi use in bioremediation. White-rot fungi have added a new dimension to the already complex system of fungal bioremediation.

Soil fungi comprise the majority of organisms screened for PUR degradation activity. Fungi of the genera *Alternaria*, *Aspergillus*, *Phoma*, *Penicillium*, *Plectosphaerella*, *Geomyces*, *Nectria*, and *Neonectria* were isolated with access to mixed nutrient sources from buried PUR samples. *Geomyces pannorum* was the most commonly isolated PUR-degrading organism with this method. Few organisms have been shown to degrade PUR as a sole carbon source. *Aspergillus niger* has some reported degradation activity; however, it was observed to be quite slow, with visible signs of degradation occurring only after 30 days.

The genus *Pestalotiopsis* is grouped in the *Xylariales* order and comprises several known plant pathogens. The fungus is not host specific and causes rot and disease in a wide variety of plant species, although these isolates were all endophytic and the plants showed no pathogenic symptoms. *Pestalotiopsis microspora* isolates have previously been shown to have a propensity for horizontal gene transfer. We found that two isolates of *Pestalotiopsis microspora* (E2712A and E3317B) were able to degrade PUR when grown anaerobically with Impranil DLN serving as the sole carbon source. For these two organisms, the level of activity was the same when grown under either aerobic or anaerobic conditions. This is in contrast to the control fungus *Aspergillus niger*, which showed substantially less activity when grown anaerobically. This observation may have practical significance in that fungal growth on and metabolism of PUR by *Pestalotiopsis microspora* could be used in anaerobic fermentation systems. The enzyme produced by *Pestalotiopsis microspora* that is responsible for PUR degradation appears to be a member of the

serine hydrolase family. Furthermore, activity extended throughout the medium at a distance well removed from the areas of fungal growth. This suggests that the enzyme responsible for degradation is extracellular, secreted, and diffusible. In comparison to an inactive cell-free filtrate from a fungal culture grown in rich medium, we found that the polyurethanase is inducible when *Pestalotiopsis microspora* E2712A is grown in minimal PUR-Lmin medium containing a suspension of Impranil DLN. By using activity-based probes, the active enzyme was identified as a serine hydrolase with an approximate molecular mass of 21 kDa. The protein was shown to be able to degrade PUR after subsequent purification, showing that activity is independent of other components of the culture filtrate [51].

Muscodor is a novel endophytic fungal genus that produces bioactive volatile organic compounds (VOCs). This fungus as well as its VOCs, has enormous potential for uses in agriculture, industry and medicine. *Muscodor albus* produces a mixture of VOCs that act synergistically to kill a wide variety of plant and human pathogenic fungi and bacteria. This mixture of gases consists primarily of various alcohols, acids, esters, ketones, and lipids. Artificial mixtures of the VOCs mimic the biological effects of the fungal VOCs when tested against a wide range of fungal and bacterial pathogens. Many practical applications for mycofumigation by *M. albus* have been investigated and the fungus is now in the market place [52].

Piriformospora indica (Hymenomycetes, Basidiomycota) is a newly described cultivable endophyte that colonizes roots. Inoculation with the fungus and application of fungal culture filtrate promotes plant growth and biomass production. Due to its ease of culture, this fungus provides a model organism for the study of beneficial plant-microbe interactions and a new tool for improving plant production systems [53].

Future Prospects

Endophytic fungal biology has emerged as a new field in harnessing various products. Fungal endophytes are the poorly investigated group of microorganisms that represent an abundant and dependable source of bioactive and chemically novel compounds with potential for exploitation in a wide variety of medical, agricultural, and industrial arenas. The mechanisms through which endophytes exist and respond to their surroundings must be better understood in order to be more predictive about which higher plants to seek, study, and spend time isolating microfloral components. The discovery of novel fungal endophytes such as *Muscodor albus*, *Pestalotiopsis microspora, Piriformospora indica, Taxomyces adrenae* paved the way for next generation product discovery processes and new technology developments.

In the upcoming future, the fungal endophyte *Piriformospora indica* formulation based biofertilizer/plant growth promoter directly can be used to increase the yield of various crops.

The fungal endophyte *Pestalotiopsis microspora* can be exploited upto molecular/gene level to obtain maximum amount of serine hydrolase enzyme. This enzyme will be used as a bio remedy for the degradation of polythene and polythene based compounds anaerobically.

Scientist will come out with the technology/ protocol by which the fungal endophyte *Taxomyces adrenae* will produce taxol at large scale and millions of cancerous patients can be benefitted from this magic anticancerous drug.

Similarly the *Muscodor albus*, a fungal endophyte can be harnessed to get volatile organic compounds (VOCs) with significant activity at large scale for various purposes.

REFERENCES

[1]. Jacobs, F. The True Story of Penicillin. Dodd Mead & Company, New York 1985.

[2]. Anthony, E. H. and Walkes, A. C. An improvement in slide culture technique. Can. J. Microbiol. 1962;8:929-930.

[3]. Azevadeo J. L, Maccheroni, W., Pereira, J. O. and de Araujo, W. L. Endophytic microorganisms: a review on insect control and recent advances on tropical plants. EJB Electronic Journal of Biotechnology 2000; 3:1-36.

[4]. Bacon, C.W. and White, J.F. Microbial Endophytes. Marcel Dekker Inc., New York, USA 2000.

[5]. Carroll, F. E., Muller, E. and Sutton, B. C. Preliminary studies on the incidence of needle endophytes in some European conifers. Sydowia 1977;29:87-103.

[6]. Schulz, B., Boyle, C., Draeger, S. and Römmert, A.K. Endophytic fungi: a source of novel biologically active secondary metabolites. Mycological Research 2002;106:996-1004.

[7]. Petrini, O. Fungal endophytes of tree leaves. In: Andrews, J.H., Hirano, S.S. (Eds.), Microbial Ecology of Leaves. Springer-Verlag, New York, USA 1991, pp. 179-197.

[8]. Schutz, B. Endophytic fungi: a source of novel biologically active secondary metabolites, p. 20. In British Mycological Society, international symposium proceedings 2001: bioactive fungal metabolites—impact and exploitation.University of Wales, Swansea, Wales.

[9]. Strobel, G.A. Useful products from rainforest microorganisms. Part2. Unique bioactive molecules from endophytes. Agro Food Industry Hi-Tech. 2002;13:12-17.

[10]. Buss, T. and Hayes, M.A. Mushrooms, microbes and medicines, p.75–85. In S. K. Wrigley, M. A. Hayes, R. Thomas, E. J. T. Chrystal, and N. Nicholson (ed.), Biodiversity: new leads for pharmaceutical and agrochemical industries. The Royal Society of Chemistry, Cambridge, United Kingdom 2000. p.75–85.

[11]. Hawksworth, D.L. and Rossman, A.Y. (1987): Where are the undescribed fungi? Phytopathology 1987;87:888-891.

[12]. Goggin, F.L. Plant-aphid interactions: molecular and ecological perspectives. Current Opinion in Plant Biology 2007;10:399-408.

[13]. Hyde, K.D. and Soytong, K. Understanding microfungal diversity-a critique. Cryptogamie Mycologie 2007;28:281-289.

[14]. Lambert, A.M. and Casagrande, R.A. No evidence of fungal endophytes in native and exotic Phragmites australis. Northeastern Naturalist 2006;13:561-568.

[15]. Valkama, E., Koricheva, J., Salminen, J.P., Helander, M., Saloniemi, I., Saikkonen, K. and Pihlaja, K. Leaf surface traits: overlooked determinants of birch resistance to herbivores and foliar micro-fungi? Trees-Structure and Function 2005;19:191-197.

[16]. Carroll, G. Fungal endophytes in stems and leaves-from latent pathogen to mutualistic symbionts. Ecology 1988;69:2-9.

[17]. Carroll, G.C. Forest endophytes: pattern and process. Canadian Journal of Botany 1995;73: 1316-1324.

[18]. Hyde, K.D. and Goh, T.K. Fungi on submerged wood in Lake Barrine, north Queensland, Australia. Mycological Research 1998;102:739-749.

[19]. Strobel, G.A. Endophytes as sources of bioactive products. Microbes and Infection 2003; 5: 535-544.

[20]. Hawksworth, D.L. The fungal dimension of biodiversity: magnitude, significance, and conservation. Mycological Research 1991;95:641-655.

[21]. Strobel, G.A. Useful products from rainforest microorganisms. Part2. Unique bioactive molecules from endophytes. Agro Food Industry Hi-Tech 2002;13:12-17.

[22]. Strobel G. Harnessing endophytes for industrial microbiology. Current opinion in microbiology 2006;9:240-244.

[23]. Nagamani, A., Kunwar, I.K., and Manoharachary, C. Hand Book of Soil Fungi I K international New Delhi 2006.

[24]. Cole, G. T., and Kendrick, W. B. A thin culture chamber for time-lapse photomicrography of fungi at high magnifications. Mycologia 1968;60:340-344.

[25]. Ellis, J.J. and Ajello. L. An unusual source for Apophysomyces elegans and a method for stimulating sporulation of Saksenaea vasiformis. Mycologia 1982;74:144-145.

[26]. Kohlmeyer, J. and Kohlmeyer, E. Permanent microscopic mounts. Mycologia 1972;64:666-669.

[27]. Zoberi, M. H. A simplified technique for preparing and preserving slide cultures. Trans. Br. Mycol. Soc. 1967;50:157-158.

[28]. Abraham, E.P. The beta-lactam antibiotics. Scientific American 1981;244:76-86.

[29]. Bottcher, H.M. Wonder Drugs: A History of Antibiotics. J.B. Lippincott, Philadelphia 1964.

[30]. Benedict, R.G. and Brady, L.R. Antimicrobial activity of mushrooms. Journal of Pharmaceutical Science 1972;61:1820-1822.

[31]. Kupra J., Anke, T., Oberwinkler, G., Schramn G. and Steglish, W. Antibiotics From basidiomycetes VII. Crinipellis stripitaria (Fr.) Pat., Journal of Antibiotics 1979;32:130-135.

[32]. Suzuki, H., Iiyama, K., Yoshida, O., Yamazaki, S. Yamamoto, N. and Toda, S. Structural characterization of the immunoactive and antiviral water-solubilized lignin in an extract of the culture medium of Lentinus edodes mycelia (LEM). Agric. Biol. Chem., 1990;54: 479-487.

[33]. Brandt, C.R., and Piriano, F. Mushroom antivirals. Recent Research Developments for Antimicrobial Agents and Chemotherapy 2000;4:11-26.

[34]. Lovy, A., Knowles, B., Labbe, R. and Nolan, L. Activity of edible mushrooms against the growth of human T4 leukemia cancer cells, and Plasmodium falciparum. Journal of Herbs, Spices & Medicinal Plants 1999;6:49-57.

[35]. Isaka, M., Tantichareon, M., Kongsaeree, P. and Theblaranonth, Y. (2001): Structures of cordypyridones A-D, antimalarial N-hydroxy- and N-methoxy-2-pyridones from the insect pathogenic fungus Cordyceps nipponica. Journal of Organic Chemistry 2001;66:4803-4808.

[36]. Hardman, A., Limbird, L., Gilman, A. (Eds.). The Pharmacological Basis of Therapeutics. Tenth edition, McGraw Hill, New York 2001.

[37]. Chihara, G. Immunopharmacology of lentinan, a polysaccharide isolated from Lentinus edodes: Its application as a host defense potentiator. Int. Journal Oriental Medicine 1992;17:55-77.

[38]. Hobbs, C. Medicinal Mushrooms. Interweave Press, Loveland Corporation 1986.

[39]. Mizuno, T., Saito, H, Nishitoba, T., and Kawagishi, H. Antitumor active substances from mushrooms. Food Reviews International 1995;111:23-61.
[40]. Peintner, U., Podder, R., and Plumpel, T. The Ice Man's Fungi. Mycological Research 1998;102:1153-1162.
[41]. Suay, I, Arenal, F., Asenio, F., Basilio, A., Cabello, M., and Diez, M.T. Screening of basidiomycetes for antimicrobial activities. Antonie von Leeuwenhoek 2000;78:129-139.
[42]. Smania, A., Monache, F.D., Logueric io-Lette, C., Smania, E.F.A., and Gerber, A.L. Antimicrobial activity of basidiomycetes. Int. Journal Medicinal Mushrooms 2001;3:87-88.
[43]. Churchill, A. Fungi: Uses as a Resource for Therapeutic Agents. American Medical Association Briefing on Food Biotechnology 2001; page 1-6.
[44]. Wani, M. C., H. L. Taylor, M. E. Wall, P. Goggon, and A. T. McPhail. (1971): Plant antitumor agents, VI. The isolation and structure of taxol, anovel antileukemic and antitumor agent from Taxus brevifolia. J. Am. Chem. Soc. 93:2325–2327.
[45]. Ratlege, C. and Kristiensen, B. (2001): Basic Biotechnology. Cambridge University Press, Cambridge.
[46]. Porro, D., Bianchi, M., Ranzi, B.M., Frontali, L., Vai, M.,Winkler, A.A., and Alberghina, L. (2002): Yeast strains for the production of lactic acid transformed with a gene coding for lactic acid dehydrogenase. US Patent 6,429,006.
[47]. El-Shafei, H A., El-Nasser, N H A., Kansoh, A L., Ali, A M. Biodegradation of disposable polyethylene by fungi and Streptomyces species. Polym. Degrad. Stab. 1998; 62:361-365.
[48]. Harish Prashanth, K. V. and Tharanathan, R. N. Depolymerized products of chitosan as potent inhibitors of tumor-induced angiogenesis. Biochim. Biopohys. Acta, 2005;1722, 22-29.
[49]. Bredberg, K., Andersson, B., Erik, L.E., Holst, O. Microbial detoxification of waste rubber material by wood-rotting fungi. Bioresource Technology 2002;83(3):221–224.
[50]. Bumpus J.A., Tier M., Wright D and Aust S D. Oxidation of persistent environmental pollutants by a white rot fungus. Science 1985;228:1434-1436.
[51]. Russell JR, Huang J, Anand P, Kucera K, Sandoval AG, Dantzler KW, Hickman DS, Jee J, Kimovec FM, Koppstein D, Marks DH, Mittermiller PA, Núñez SJ, Santiago M, Townes MA, Vishnevetsky M, Williams NE, Vargas MPN, Boulanger LA, Slack CB, Strobel SA. Biodegradation of polyester polyurethane by endophytic fungi. Appl Environ Microbiol 2011;77:6076–6084.
[52]. Strobel G. Harnessing endophytes for industrial microbiology. Current opinion in microbiology 2006;9:240-244.
[53]. Varma A., Verma S., Sudha., Sahay N., Butehorn B., and Franken P. Piriformospora indica a cultivable plant growth promoting endophyte. Applied and Environmental Microbiology 1999;65(6):2741-2744.

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