

The Future Products: Endophytic Fungal Metabolites

Kartikeya Tiwari*

Department of Microbiology, Management and Science University, Shah Alam, Selangor - 40100, Malaysia

*Corresponding author: Kartikeya Tiwari, PhD, MS, MSU Sr. Lecturer, Department of Microbiology, Management and Science University, Shah Alam, Selangor - 40100, Malaysia, Tel: (60) 14-9294304; E-mail: Kartikeya_tiwari@msu.edu.my, tiwarikartik1@gmail.com

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Abstract

As far as microbial world concerned on earth, there are large number of domains has shown the potential for various applications. Fungal domain is one of them. Since the discovery of penicillin, various fungi has been harnessed for a large number of applications. Still there is a requirement of novel products for human welfare and development. So what are the fungi of next door? Are the fungal endophytes? Yes? This is the area untouched for a longer period of time. These fungi represent one of the most understudied and diverse group of organisms, 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 products of these fungi remains poorly characterized. A large number of endophytic fungi such as *Taxomyces*, *Pestalotiopsis*, *Muscodor*, *Piriformospora*, *Alternaria*, *Colletotrichum*, *Cryptosporiopsis*, produce enormous valuable products for medicinal, agricultural and industrial importance. Some of the well-known products are taxol (Paclitaxel), serine hydrolases for polyurethane degradation, Annulene (Volatile organic compounds), Colletotric acid and Cryptocandin. So what next, are endophytic fungal metabolites are the future products? The present review explore the potential of these endophytic fungi with respect to the production of various novel secondary metabolites/products in comparison with the traditional fungi of importance.

Keywords: Endophytic fungi; Mycelia sterilia; Antibiotic; Taxol; Volatile organic compounds (VOCs); *Taxomyces andreanae*; *Muscodor albus*; *Pestalotiopsis microspore*

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].

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 [2,3]. 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 [4,5].

Petrini 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 [6]. These organisms reside in the living tissues of host plant and do so in a variety of relationships, ranging from symbiotic to slightly pathogenic [7,8]. 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 [9]. 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 in 1987 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 [10-14]. 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 [15-17]. 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 [18].

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:

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

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

Out of 1.5 million fungal species estimated to be present [19], 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 microorganisms. 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.

Plants from unique environmental settings, especially those with an unusual biology, and possessing novel strategies for survival are seriously considered for study.

Plants that have an ethno botanical 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).

Plants that are endemic, that have an unusual longevity, or that have occupied a certain ancient land mass.

Plants growing in areas of great biodiversity also have the prospect of housing endophytes with great biodiversity [8,20].

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 can begin. 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 [8,20,21].

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 [21,22].

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 [23-27]. 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 [21,22].

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 9 cm 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 1A and 1B). 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 [21,22,28].

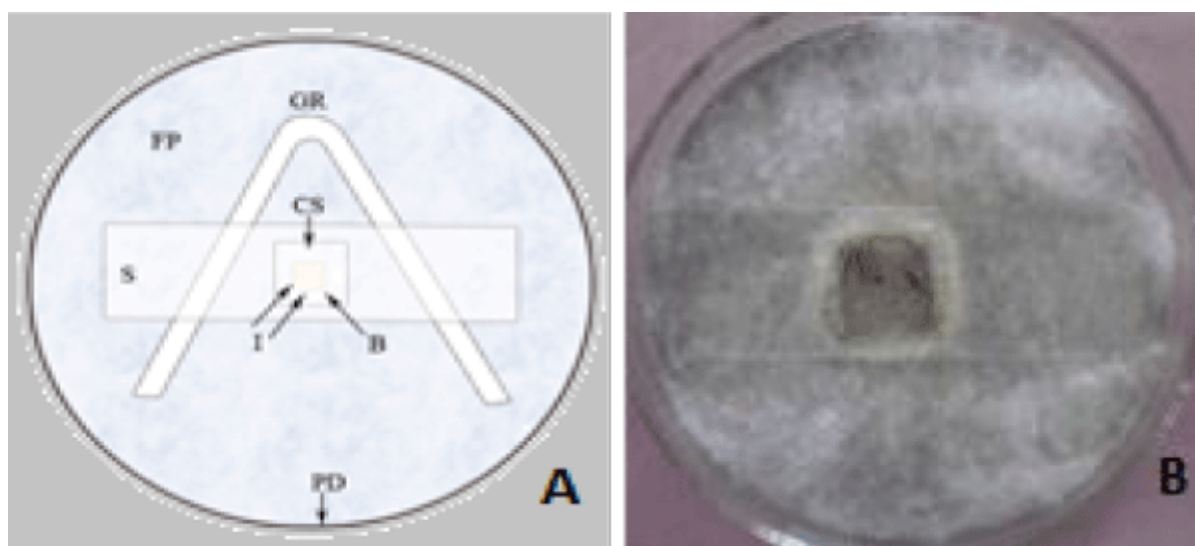


Figure 1: A- Showing the slide culture technique (PD- Petridish, FP- Filter paper, GR-Glassrod, S- Slide, B- Block, CS- Coverslip, I- Inoculated fungal culture). B- Sporulating endophytic fungi showing various conidial development stages by this technique.

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 wooly it may not be necessary to take the agar, but in the more common appressed 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 [1,29,30]. 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*.

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 Encephalosporan antibiotics and this is produced by *T. inflatum*. About 20% of the drugs produced by pharmaceutical industry today are derived from fungi [31].

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 [32]. It was originally isolated and characterized from the inner bark of Pacific yew, *Taxus brevifolia* [32]. An endophytic fungus, *Pestalotiopsis microspora*, obtained from the inner bark of *T. wallaciana*, 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. Biocin 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.

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 1: Major bulk Enzyme producing Companies [33].

Enzyme	Source
Acid, alkaline	<i>Aspergillus oryzae</i> ; <i>A. niger</i>
Neutral proteases	<i>A. flavus</i> ; <i>A. sojae</i>
Cellulase	<i>Trichoderma koningi</i>
Diastase	<i>Aspergillus oryzae</i>
Glucoamylase	<i>Aspergillus niger</i> ; <i>A. oryzae</i>
Invertase	<i>Saccharomyces cerevisiae</i>
Lactase	<i>S. lactis</i> ; <i>Rhizopus oryzae</i>
Ligninase	<i>Phanerochaete chrysosporium</i>
Lipase	<i>Rhizopus spp.</i>
Pectinase	<i>A. niger</i> ; <i>Sclerotinia libertine</i>
Serine hydrolases	<i>Pestalotiopsis microspora</i>

Table 2: The major enzyme producing fungi.

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.

Organic acid	Source
Colletotric acid	<i>Colletotrichum gleosporioides</i>
Citric acid	<i>Aspergillus niger</i>
Fumaric acid	<i>Rhizopus nigricans</i>
Gluconic acid	<i>Aspergillus niger</i>
Itaconic acid	<i>A. terreus</i>
Kojic acid	<i>A. oryzae</i>

Table 3: Organic acids produced from fungi.

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 [34]. Furthermore, in the near future engineered yeasts may provide new commercial processes to make lactic acid [35].

Fungi in bioremediation

Fungi are known to degrade, or cause to deteriorate, a wide variety of materials and compounds, processes known as mycodegradation and mycodegradation. The degradative activities of fungi have been recognized in various situations where they destroy different types of wood, stored paper, textiles, plastics, leather, and electroinsulating and various wrapping materials. Polyethylene, with a molecular weight of 4000 to 28,000, is degraded by the cultivation of *Penicillium simplicissimum* YK [36], 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 [37]. 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. [38]. *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 detoxification of ground waste tire rubber material prior to devulcanization [39].

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 [40].

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 [20].

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 [41].

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 andreanae* paved the way for next generation product discovery processes and new technology developments (Table 4).

Fungal endophyte	Novel compounds	Bioactivity
<i>Epichloe</i> (grass)	Methyl (Z)-3-methyldodec-2-enoate	Antimicrobial activity
<i>Penicillium</i> species	Penicidones A-C	Cytotoxicity
<i>Phoma</i> species	Phomodione	Antimicrobial activity
<i>Trichoderma harzianum</i>	Sesquiterpenes trichothee-9-en-4-ol,12,13-epoxy-, acetate, 4B – (8Cl, 9Cl)	Pesticide, Inhibitory effect against the plant pathogenic fungi
<i>Trichoderma koningii</i>	Koninginins A, E and F	Phospholipase A (2) inhibitors
<i>Xylaria</i> species	7-amino-4-methylcoumarin	Antimicrobial activity
<i>Taxomyces andreanae</i>	Paclitaxel	Anticancerous compounds
<i>Pestalotiopsis microspora</i>	Torreyanic acid, Pestatin and Isopestatin	Anticancerous and Antioxident activity
<i>Rhinocladiella</i>	Cytochalasins	Anticancerous compounds
<i>Nodulisporium</i> species	Nodulisporic acid	Antiinsecticidal activity
<i>Fusarium subglutinans</i>	Subglutinol A and B	Immunosuppressive activity
<i>Colletotrichum gleosporioides</i>	Colletotric acid	Antimicrobial activity
<i>Cryptococcus quercina</i>	Cryptocandin and Cryptocin	Antifungal activity
<i>Muscodor albus</i>	Annulene	Antimicrobial activity

Table 4: Metabolites/products harnessed from fungal endophytes [42].

- 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 andreanae* 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.
 - Unique peptide antimycotic termed cryptocandin will be purified, isolated from *C. quercina* useful for agriculture against plant pathogenic fungi especially against high yielding croppy plants [43-57].
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