Brief Review On Bioactive Metabolites Of Fungus Isolated From Soil

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1. Introduction

Fungi are very diverse group of organisms and an important components of ecosystem. It has been estimated that only 5% of the World's fungi have so far been discovered, and for most of these, little is known about their biology. They consist of microscopic single cell yeast to large macro-fungi like mushrooms and toad stools. They are ubiquitous organisms and adaptable in all habitats. They are mainly saprobes which help in decomposition and also play a key role in decomposition and mineralization.

Thermophilic fungi are present in the natural habitat, soil and where decomposition of plant, animal and birds takes place due to self heating masses. These fungi are those which can grow at temperature from 20-60°C. Fungi are the big decomposer of dead organic matter of forests, fruits etc. Some of the fungi form mycorrhizal association, the symbiotic association of plant and fungi. Several fungi produce bioactive compounds, secondary metabolite and chemical models having pharmaceutical importance. Fungi are the major components of bio-diversity, necessary for the survival of other living organism and are crucial in ecological processes.

Many fungi are very much important for humans, in industries and commercially preparing products. The oldest fungi used for fermentation is yeast, e.g: brewing, wine making and bread making. Yeasts and fungi plays important role in drugs production, food processing, biocontrol agent, enzymes production, enzymes biotechnology and research development. Fungi are also used in the production of cheese and different flavor in the industries due to methyl ketones. A large number of fungal extracts or extracellular products have been found to have antimicrobial activity, mainly from the filamentous fungi. It is believed that secondary metabolites are performing some useful secondary functions and serve as competitive weapons, used against predators, metal transporting agents, symbiotic agents, sexual hormones and differentiation effectors. Fungi plays an important role from the starting of world in many biological processes, hence fungal flora should be further investigated for their secondary metabolites to develop new antifungal and antibiotic drugs, fungicide and insecticides.

2. Soil Heterogenicity

Soil is a complex mixture of minerals, nutrients, organic matter and living organisms upon which all other terrestrial trophic systems are dependent (PerezdeMora et al., 2005). It is a vital resource for sustaining basic human needs such as food, fibre and shelter (Branzini et al., 2009). It is fundamental and irreplaceable part of life and it governs productivity of terrestrial ecosystems and maintains biogeochemical cycles utilizing microorganisms to degrade all organic compounds including persistent xenobiotics and naturally occurring polyphenolic compounds. The soil provides support to variety of life forms including macrofauna, mesofauna, microfauna, macroflora and microflora. Around 80–90% of the processes in soil reactions are mediated by microorganisms (Coleman & Crossley, 1996; Nannipieri & Badalucco, 2003). It is highly heterogeneous and complex microhabitat, which is reflected in the spatial distribution and enormous diversity of microorganisms and their metabolic versatility.

3. Soil Microbial Diversity

Soil represents a favorable habitat for microorganisms and is inhabited by a wide range of microorganisms. Soil supports richest number and kinds of microorganism interacting with each other and thus many modification or alteration in soil conditions greatly influence the microbial community and their activity in soil ecosystem. Soil physico-chemical and biological factors interact to provide rapid change in ecological niches and microbial components fluctuate in response to change in soil condition (Cook and Baker, 1983). Soil microbial communities are arguably the most diverse communities on earth. Microorganisms are found in prominent amount with great species diversity in the soil of the earth. Bacteria and fungi, the major contributors of soil microbial diversity are extremely flexible and can carry out almost all known biological reactions.

Microorganisms are found in large numbers in soil usually between one to ten million microorganisms are present per gram of soil with a dominant number of bacteria and fungi. Soil microorganisms are regarded as a sensitive indicator of the changes occurring in soil.
ecosystem. They contribute to important soil functions such as supporting the growth of plants both in natural plant communities and those grown for food, fibre, or energy and absorbing, neutralizing and transforming compounds that might otherwise become pollutants in the environment. Soil microorganisms are very important as almost every chemical transformation taking place in soil involves active contribution of these microbes. Soil organic matter is generally used to represent the organic constituents in the soil, excluding undecayed plants and animal tissues, their partial decomposition products and the soil biomass (Dick, 1992).

The soil organic matter provides a favorable habitat for the microorganisms to grow as compared to inorganic soil. This microbial diversity present in the soil is greatly influenced by organic matter. It has been consistently reported that soil organic matter favors the growth of microbes like bacteria, fungi, algae, protozoa and some nematodes which plays a vital role in performing essential biochemical transformations in soil to maintain soil productivity.

4. Soil Mycobiota: Role And Importance

Garrett (1950) surveyed upon ecological groups of soil fungi and substrate relationships. Accordingly, an ecological group of soil fungi was defined as an assemblage of species characterized by some peculiar advantage for pioneer colonization of a particular substrate. Five such ecological groups have been indicated as examples, viz. saprophytic fungi, root inhabiting fungi, lignin-decomposing fungi, coprophilous fungi, and predaceous fungi.

An acceptable number of fungi in 1g of fertile soil are around 400000 (Griffiths et al., 1999). The genera with the greater number of species in fungi were Aspergillus (8 species), Glomus (4 species), Penicillium (3 species), Cladosporium (3 species) in the serial dilution plate method. The most widely distributed and abundant colony forming taxa were Penicillium (16 colonies), Aspergillus (29 colonies), Rhizopus (10 colonies) Trichoderma (10 colonies), Fusarium (10 colonies), Glomus (8 colonies), Cladosporium (8 colonies) and Humicola (7 colonies) in both soil sample fields.

Coleman (2001) discussed on the soil biota, soil systems and major soil processes occurring in those ecosystems. The review describes how soils act as components of ecosystems, their role as organizing centers in ecosystems, various major soil processes and biodiversity in soils.

Cowan (2001) made a review on fungi as life support for ecosystems. Fungi are fundamental to the success and health of almost every ecosystem on the earth, both terrestrial and aquatic, and essential to the sustainability of biodiversity. They perform a crucial role in the transport, storage, release and recycling of nutrients. Despite their central role in ecosystems and their applications in biotechnology, knowledge about fungi remains at a low level.

Jenkins (2005) describes soil fungi as microscopic plant-like cells that grow in long threadlike structures or hyphae that make a mass called mycelium. The mycelium absorbs nutrients from the roots it has colonised, surface organic matter or the soil. It produces special hyphae that create the reproductive spores. Some fungi are single celled (e.g. yeast). Fungi have many different structures but they can act in similar ways and thus are not as plant specific in their needs as some soil bacteria such as Rhizobia. There are three functional groups of fungi: Pathogens, Mutualists and Decomposers that they perform important functions within the soil in relation to nutrient cycling, disease suppression and water dynamics, all of which help plants become healthier and more vigorous.

5. Fungi as Producers of Biologically Active Metabolites

It has been estimated that there may be 1.5 million fungal species, while only about 100,000 species are presently known (Hawksworth, 2004).

Berdy (2005) reported soil fungi have been the most studied typical soil genera such as Acremonium, Aspergillus, Fusarium and Penicillium have shown ability to synthesis a diverse range of bioactive compounds. More than 30% of isolated metabolites from fungi are from Aspergillus and Penicillium. Therefore the same fungal strains were re-isolated and this lead to the re-discovery of known compounds as the same taxa produce the same metabolites. Only a few taxa have tested for their biological applications including their ability for drug production and biological control. Thus it seems that the discovered percentage of economically valuable fungal metabolites is small. Microbial metabolites account for a majority of drugs available in the market. More than 20,000 bioactive metabolites are of microbial origin.

Fungi are among the most important groups of eukaryotic organisms that are well known for producing many novel metabolites which are directly used as drugs or function as lead structures for synthetic modifications (Kock et al. 2001, Bode et al. 2002, Donadio et al. 2002, Chin et al. 2006, Gunatilaka 2006, Mitchell et al. 2008, Studler & Keller 2008). The success of several medicinal drugs from microbial origin such as the antibiotic penicillin from Penicillium sp., the immunosuppressant cyclosporine from...
Tolypocladium inflatum and Cylindrocarpon luidum, the antifungal agent griseofulvin from Penicillium griseofulvum fungus, the cholesterol biosynthesis inhibitor lovastatin from Aspergillus terreus fungus, and β-lactam antibiotics from various fungal taxa, has shifted the focus of drug discovery from plants to microorganisms.

Suryanarayanan et al., (2009) discussed many fungal secondary metabolites with various chemical structures and their wide ranging biological activities and this reflects the high synthetic capability of fungi (Suryanarayanan & Hawksworth, 2005).

Pelae (2005) reported about 1500 fungal metabolites to show anti-tumor and antibiotic activity and some have been approved as drugs. These include micafungin, an anti-fungal metabolite from Coleophoma empetri (Frattarelli et al., 2004), mycophenolate, a product of Penicillium brevicipactum, which is used for preventing renal transplant rejection (Curran & Keating, 2005), rosuvastatin from *Penicillium citrinum* and *P.* brevicipactum, which used for treating dyslipidemias (Scott et al., 2004) and cefditoren pivoxil, a broad spectrum antibiotic derived from Cephalosporium sp. (Darkes & Plosker, 2002). Others include derivatives of fumagillin, an antibiotic produced by *Aspergillus fumigates* (Chu et al., 2005), and illudin-S, a sesquiterpenoid from *Omphalotus illudens* (McMorris et al., 1996) which exhibits anti-cancer activities. Also, fungal metabolites are important in agriculture applications (Anke & Thines, 2007).

### 6. Isolation of Fungi

Five steps were observed in the modern isolation methods. These included the choice of substrate, composition of the isolation medium, pre-treatment and incubation conditions, colony selection and purification (Cross, 1982; Nolan and Cross, 1988). Of the five factors, composition of the isolation medium, pre-treatment and incubation conditions are the most important since they determine which organism will develop on the isolation plates.

Collection of soil samples from both the organic and inorganic fields takes place, then 3 flasks (250 ml) were taken and 90 ml distilled water was transferred into each flask. Each flask was plugged properly, labeled 1-3 and autoclaved at 15lb/inch² for 30 minutes. 1 gm of soil sample was weighed and transferred into the flask 1 containing 90 ml. It gives the dilution 1:10 i.e. 10-1. Then, it was shaked for five minutes gently with a stirrer to get homogenous soil suspension. 1.0 ml soil suspension was transferred from 10-1 dilution into flask 2 containing 90 ml distilled water to get dilution 10-2 and then mixed it gently. Similarly 1ml of soil suspension was serially transferred from 10-2 dilution into flask 3 containing 90 ml water to get the final dilution of 10-3 and mixed it gently, this is known as serial dilution method. 1ml of soil suspension was aseptically poured from 10-3 dilution in different media plates. The plates were gently rotated so as to spread the suspension on medium. The plates were incubated at ± 250°C for 4-5 days. Different media like Czapek Dox Agar medium, Potato Agar medium, Martin’s Rose Bengal medium, etc. were prepared for isolation of fungi. For identification of fungi lacto phenol and cotton blue stain was used also called as mounting fluid. The slides were observed under microscope and fungi were identified by following the mycological literature (Dubey and Maheshwari, 2007).

The soil samples in an isolation process can be processed using the soil dilution plate method (Waksman, 1922) and soil washing methods (Gams et al., 1987) in the laboratory. Soil dilution plate method was to isolate the propagules of microfungi occurring inactively in the soil, whereas soil washing technique was to isolate active microfungus hypha. In the dilution plate method, there were remarkable variations in the numbers of organisms observed at different dilutions. A 10-4 dilution was found to be most suitable for isolating single colonies as it contained individual colonies of a large variety of organisms. Although particular attention was directed in this study to fungi, it was very important that each and every colony that developed on the isolation plates was treated as a potential antibiotic producer. The use of selective procedures minimized the laborious work involved in screening thousands of isolates.

Ottow and Glathe (1968) presented rose bengal-malt extract-agar, a simple medium for the simultaneous isolation and enumeration of fungi and actinomycetes from soil. Studies were done on fungi and actinomycetes in periodically water-logged soils (gleys) for which a simple rose bengal-malt extract-agar was developed, suitable for the enumeration of both fungi and actinomycetes: Commercial malt-extract (Bio-Malz), 20 g; K2HPO4, 0.5 g; FeC2+, Mn, Cu, Zn, Mo, B, Co, 1 ppm each (added as soluble salts, not as nitrate); rose bengal, 1 part in 15,000; agar, 20 g; tap water, 1 liter; pH 6.0 to 6.2. Bills et al., (2004) has elaborated the isolation techniques for filamentous fungi from soil along with the details of taxonomy, diversity, and distribution of soil saprobic fungi.

Saravanakumar and Kaviparasan (2010) examined the seasonal distribution of soil fungi and forty eight soil samples were collected from wet evergreen forest of Tamil Nadu, Southern India belonging to various groups viz., *Ascomycota, Zygomycota and Deuteromycota* were identified with the help of relevant literatures.

Swer et al., (2011) studied the population and
diversity of soil fungi in organically amended agricultural soils of Meghalaya, India. Fungal populations were much higher in organically fertilised plots as compared to the control. Altogether, 122 fungal species and two sterile mycelia were isolated from all the plots of which 25 fungal genera belonged to Deuteromycotina, seven to Ascomycotina, four to Zygomycotina and one to Mastigomycotina. The most common genera isolated from all the plots include Penicillium, Aspergillus, Acremonium, Fusarium, Mortierella, Mucor, Paecilomyces, Talaromyces, Trichoderma and Verticillium. The organic matter level in the organically managed soil systems can play a pivotal role in fungal growth, sporulation and diversity.

Gaddeyya et al., (2012) isolated a total of 15 species belonging to 6 genera of fungi from agricultural fields at Salur Mandal by using soil dilution technique and soil plate technique on Potato Dextrose Agar and Czapek Dox Agar medium supplemented by suitable antibiotics such as penicillin and streptomycin. Identification and characterization of the mycoflora were made with the help of authentic manuals of fungi. The most common among them viz., A. flavus, A. fumigatus, A. niger, A. nidulans, A. terreus, Penicillium chrysogenum, Penicillium frequentans, Penicillium funiculosum, Trichoderma viride, Trichoderma harzianum, Fusarium oxysporum, Fusarium solani, Curvularia clavata, Curvularia lunata, and Rhizopus stolonifer were isolated and characterized.

Khalil et al., (2013) analysed the mycobiota composition of the mangrove soil in Egypt by collecting 24 soil samples where almost all samples showed clay, sandy to sandy loam texture, pH of the soil samples ranged from 7.5 to 8.9 and water content ranged from 8% to 9%. Fifteen fungal species belonging to 9 genera were identified belongs to the Ascomycotina with fewer proportions belonging to the Deuteromycotina and Zygomycotina.

7. Antimicrobial Activity

According to Yamaç and Bilgili (2006), 52 species were reported as having antifungal activity; most of them are edible mushrooms. Most of the mushrooms that revealed antifungal activity are wild. Regarding Agaricus species, Ozturk et al., 2011 reported antifungal activity of A. bisporus, A. bitorquis and A. essettei methanolic extracts against Candida albicans and C. tropicalis, being Agaricus bitorquis the most active one for both species.

As in the case of Spilanthes calva when inoculated with Piriformospora indica, it produces a range of antifungal compounds, as plants inoculated with P. indica produced extracts that were inhibitory to soil-borne pathogens (F. oxysporum and Trichophyton mentagrophytes) suggesting induction of antifungal chemical production in the host (Rai et al., 2002).

Many endophytic fungi have been reported to produce novel antibacterial, antifungal, antiviral, anti-inflammatory, antitumor, and other compounds belonging to the alkaloids, steroid, flavenoid and terpenoid derivatives and other structure types (Guo et al., 2008, Yu et al., 2010).

Wang et al., (2007) investigated Cladosporium sp., displaying the most active antifungal activity and found to produce a secondary metabolite known as brefeldin A with antibiotic activity

The endophytic fungus Pestalotiopsis microspora was found to produce numbers of antifungal metabolites, like ambuic acid, pestaloside, and pestalotiopsins A and B Pestalachlorides A, which exhibit antifungal activity against a variety of plant pathogenic fungi (Li et al., 2008a). Pestalachlorides A was proven to display significant antifungal activity against three plant pathogenic fungi, Fusarium culorum, Gibberella zeae, and Verticillium albo-atrum (Li et al., 2001, Li & Strobel 2001, Li et al., 2008).

Investigation of endophytic Phomopsis cassia by Silva et al., (2005), Huang et al., (2008), ethyl 2,4-dihydroxy-5,6-dimethylbenzoate and phomopsilactone displayed strong antifungal activity against two phytopathogenic fungi, Cladosporium cladosporioides, and C. sphaerospermum.

Cryptocin and cryptocandin are antifungal metabolites obtained from the endophytic fungus Cryptosporiopsis cf. quercina. Cryptocandin demonstrated excellent antifungal activity against some important human fungal pathogens, including Candida albicans and Trichophyton spp and its related compounds are currently being considered for use against a number of fungi causing diseases of the skin and nails (Strobel & Daisy, 2003).

Anita et al., (2012) reported the antifungal activity of the compounds obtained from Trichoderma atroviride was able to inhibit the growth of pathogens and showed 100% inhibition at concentration of 500 ppm.

Idris et al., (2013) isolated endophytic fungi viz. Aspergillus flavus, Aspergillus sp., Curvularia lunata, Cladosporium sp. and three unknown species from the medicinal plant, Kigelia africana. The fungal species were screened for antibacterial test against E. coli, Bacillus subtilis, Staphylococcus aureus. The zones of inhibition ranged from 14-37 mm.

Sohail et al., (2014a) investigated the antimicrobial activity
activity of the fungal species Aspergillus flavus against different fungal A. niger, A. oryzae, C. albican, P. digitatum, F. oxysporum and bacterial pathogenic strain P. aeruginosa, E. coli, S. aureus, S. aureus (methicillin resistant), S. aureus (vancomycin resistant). The extract of Aspergillus flavus was found effective against all tested pathogenic strains.

Sohai et al., (2014b) reported the antimicrobial activity of the Rhizopus stolonifer extracts against different bacterial pathogenic strain P. aeruginosa, E. coli, S. aureus, S. aureus (methicillin resistant), S. aureus (vancomycin resistant) and fungal strains A. niger, A. oryzae, C. albican, P. digitatum, F. Oxysporum.

Tawfik et al., (2012) reported the antibacterial activity of fungal extracts of some fungi, against bacterial species, E.coli and S. aureus. The zone of inhibition by fungal extracts ranged between 22-28 mm diameters. MIC test showed that the extracts of D. australiensis exhibited a minimal inhibition values (12.5 and 6.25 ug/ml) against E. coli.

Castlebury et al., (1999) reported the antibacterial activity of the compound beauvericin isolated from fungal extract against Clostridium perfringens, Enterococcus faecium, Escherichia coli. Pseudomonas aeruginosa, Listeria monocytogenes, Salmonella enterica, Shigella dysenteriae, Yersinia enterocolitica and Mycobacterium tuberculosis.

8. Conclusion
In the respect of above review it can be stated that fungi are the most important components of ecosystem. Soil is an chief and significant habitat for fungi, bacteria, and actinomycetes etc. They have significant role in industries for the production of commercial products. Fungal Secondary metabolites are also responsible for medicinal activities and thus plays keen role in various pharmaceutical industries. Different phytochemical properties for curing various ailments and possesses potential leads to the isolation of new and novel compounds. A better basal understanding of the fungi will apparently result in much more straight forward or appropriate approaches than currently exist to screen for new valuable bioactive metabolites.

9. References


