National Journal of Biological Sciences, (2020), Volume 1, Issue I

Significance of Soil Fungi as an Inexaustible Source of Antibiotics

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Received: 27th January, 2020 / Revised: 25th February, 2020 / Accepted: 2nd March, 2020 / Published: 24th June, 2020

Abstract

Fungi are a diverse group of microorganisms and soil provides perfect habitat for them to produce new antibiotics. Fungi form different interactions with soil microbiota in which they produce secondary metabolites that show multiple biological activities. Yield of antimicrobial substances can be enhanced by optimizing extrinsic and intrinsic parameters. Thus, Fusarium, Penicillium, Aspergillus and Rhizopus spp. produce many antimicrobial substances. Different genes including cryptic or silent genes are involved for soil borne fungal antibiotics. These fungal antibiotics have many applications in different industries such as food, agriculture and pharmaceutical industries. Different methods are also used for the analysis of antibiotics production. Some pathogenic microorganisms adopt certain changes and show resistance to the currently available antibiotics and make them ineffective. Due to the occurrence of antibiotic resistance, the discovery of newly emerging antibiotics and semi-synthetic compounds is in high demand. The purpose of this study was to collect information about soil fungi and to highlight their importance to produce novel antibiotics.

Keywords: soil fungi, antibiotics, antibiotic resistance, semi-synthetic compounds, microbial interactions

Introduction

Antibiotics are low molecular weight substances which are produced by different microorganisms and act against other microbes. Antibiotics either kill microbes or stop their growth by interfering with their gene expression **(Dezkfully and Heidari, 2016)**. Psychrotolerant fungi are mostly preferred as valuable antibiotic producers because they can tolerate variation in temperature, pH and nutrients etc. **(Yogabaani *et al.*, 2017)**. Antibiotics production in fungi helps fungi to adjust in the multitude/variety of environments and make different associations with other microbial populations **(Gao *et al.*, 2017)**. Some antibiotics producing soil fungal species are *Cephalosporium*, *Penicillium*, *Tolypocladium*, and *Aspergillus* etc. In this way fungi act as a source of novel antibiotic production and are considered the future antibiotic factories.

In 1928, Alexander Fleming accidentally discovered penicillin. This achievement of Fleming straightened the way to produce other antibiotics. Later on, so many antibiotics were discovered such as chloramphenicol, cyclosporin,

chloromycetin, streptomycin, gliovirin and mycophenolic acid etc **(Goyal *et al.*, 2017)**. An Italian scientist Giuseppe Brotzu also contributed to the field of antibiotics by discovering cephalosporins in 1950s **(Silber *et al.*, 2016)**. Although antibiotics have been used for decades to treat various life-threatening microbial infections, but actual research on antibiotics started in the 21st century. In 1969, another cyclosporine was discovered in Norway by Sandoz scientists. It was the first drug used as an immunosuppressive agent, which initiated immunopharmacology era **(Aly *et al.*, 2011)**. The time period during 1940-1960 is considered the golden age of antibiotics which was initiated by the work of Waksman. He cultured many microbial species and discovered numerous antibiotics such as neomycin, streptomycin etc. During that era novel antibiotics were produced by the modification of cephalosporins, penicillin etc. However, in 1987 the golden era has diminished due to the problem of antibiotic resistance **(Goyal *et al.*, 2017)**.

Fungi due to interactions with other soil microorganisms, produce secondary metabolites that show multiple biological activities as well as stop the growth of other microbes **(Goyal *et al.*, 2016)**. Further study is needed to search environments that are rich of those fungal species which have the ability to produce a lot of new antibiotics **(Vander Heijden and Horton, 2009)**. A wide range of antibiotics are produced by soil fungi which play important roles in controlling diseases and numerous life-threatening infections. But due to over-use of antibiotics and mutations in infectious agents the microbes have become resistant to the current drugs by changing either the target sites or prohibiting antibiotics entry into the cell thereby making these drugs ineffective. Therefore further studies should be conducted to investigate and explore more effective antibiotics that can withstand antibiotic crisis **(Lihan *et al.*, 2014)**. This review provides a thorough insight about fungal antibiotics. Since antibiotics resistance-crisis is getting severe day by day therefore it is necessary to search for those soil fungal species with an ability to produce novel antibiotics that can withstand these problems and protect human lives.

History of Antibiotics

The term antibiotic is derived from Greek word ―antibiosis‖ which means against life **(Makut and Owolewa, 2011; Dezkfully and Heidari, 2016)**. In 1889, antibiotic word was coined by Louis Pasteur's pupil Paul Vuillemin **(Dezkfully and Heidari 2016)**. The term antibiosis was used in French Microbiological Literature in 1928 **(Rafiq *et al.*, 2018)** which was introduced by Selman Waksman in 1942 **(Goyal *et al.*, 2017; Rafiq *et al.*, 2018)**.

In 1893, an Italian physician Bartolomeo Gosio (1863-1944) isolated *Penicillium glaucum* fungi from spoiled corn later on this fungal strain was named as *Penicillium brevicompactum* which was producing mycophenolic acid. In 1896, Gosio isolated crystals of mycophenolic acid and observed that the compound could stop the growth of bacterium *Bacillus anthracis*. This work was forgotten but later on the same compound was rediscovered by Alsberg and Black which they named as mycophenolic acid. This antibiotic was one of the first antimicrobial metabolite which was showing broad spectrum activity **(Demain and Martens, 2016)**.

In 1928, Alexander Fleming discovered penicillin accidentally while he was studying *Staphylococcus aureus* bacteria. On that plate fungus *Penicillium notatum*

produced an active agent which he named penicillin **(Dezkfully and Heidari, 2016; Demain and Martens, 2016; Goyal *et al.*, 2017)**. The development of penicillin was a great advancement in the medicine field **(Gulshan and Moye-Rowley, 2007)**. The discovery of penicillin led to the beginning of microbial drug era. Natural antibiotics are produced by fermentation which is an old method that was initially used for wine and beer production **(Demain and Martens, 2016)**.

Although antibiotics have been used for medical purpose since 20th century but a new era of antibiotic research started from 21st century **(Berdy, 2005; Alanis, 2005; Dezkfully and Heidari, 2016)**. Antibiotics are low molecular weight (m.w. 3000) bioactive compounds which are produced by different microbes. They slow down/halt the metabolic activity of microbial cells **(Korzybski *et al.*, 1967; Walsh, 2003; Lorian, 2005; Dezkfully and Heidari, 2016)**. They can inhibit the growth of microbes even at very low amount **(Hugo and Russell, 1998; Makut and Owolewa, 2011)**. Antibiotics can either be germicidal or germistatic, they show antiviral, antitumor and antimicrobial activity. Some of them are narrow spectrum while other are broad spectrum **(Dezkfully and Heidari, 2016)**. In 1936, antifungal substance was isolated from a fungal strain of *Trichoderma lignorum* **(Ghisalberti and Sivasithamparam., 1991)**. In 1970, cyclosporin A was discovered which was produced by fungus *Tolypocladium nivenum* in an aerobic fermentation **(Demain and Martens, 2017)**. In 1982, another antibiotic gliovirin was discovered which was produced by fungus *Trichoderma virens*. Later this antibiotic was named as gliocladium. It showed antifungal as well as antibacterial activities **(Sherkhane *et al.*, 2017)**.

In 20th century, a new concept came into existence that microbes, when live together, affect each other by secreting some toxic metabolites which we call antibiotics **(Kaur *et al.*, 2014)**. More than 12,000 antibiotics have been discovered in the past 55 years **(Singh *et al.*, 2012)**. Fungi produce 30% of the total antibiotics that have been produced by microbes **(Dezkfully and Heidari, 2016)**.

There are many antibiotics that were discovered from soil fungi. But due to over-use of antibiotics and mutations in infectious agents, current antimicrobial agents have lost their effectiveness against pathogens. Therefore, further research is required to investigate and explore more effective antibiotics that can withstand antibiotic crisis.

Life in Soil and Importance of Soil Fungi

Soil provides a perfect habitat for fungi, bacteria and actinomycetes etc, as it contains nutrients, minerals and organic matter **(Bhardwaj *et al.*, 2017; Singh *et al.*, 2017)**. There are 10 billion microbes per gram of soil **(Geetanjali and Jain, 2016)**. Fungi can easily be found in the upper 10 cm of soil **(Ahmed *et al.*, 2019)**. Soil is a dynamic and versatile microhabitat which supports diverse groups of organisms **(Goyal *et al.*, 2017; Singh *et al.*, 2017)**. Soil consists of a diverse group of fungi which play role in nutrient cycles **(Ritz and Young, 2004; Tedersoo *et al.*, 2014; Geetanjali and Jain, 2016; Goyal *et al.*, 2017)**. Fungi are cosmopolitan in nature **(Singh *et al.*, 2017; Goyal *et al.*, 2017)**. Fungi can survive in almost all terrestrial habitats **(Tedersoo *et al.*, 2014)**. Most of them are saprophytic which feed upon dead organic matter. Some of them are

thermophilic which can tolerate 20-60°C temperature **(Singh *et al.*, 2017)**. They mostly prefer acidic and aerated soil environment **(Ahmed *et al.*, 2019)**. They can form different interactions either with other microbes or with plant roots i.e. mycorrhizal association **(Geetanjali and Jain, 2016; Singh *et al.*, 2017)**. In mycorrhizal associations, plants provide shelter to fungi and in return gain nutrients from them. Through the network of these fungal hyphae, plants obtain their phosphorus and nitrogen from soil. Soil fungi also provide protection to plants against pathogenic microbes and stressful conditions. These fungi help to increase the diversity of plants **(Vander Heijden and Horton, 2009)**.

Soil fungi are the main source of antibiotics **(Kaur *et al.*, 2014)**. Up till now 20% of the isolated antibiotics have been discovered from soil fungi **(Rafiq *et al.*, 2018)**. Fungi help in soil genesis as well as in decomposition of dead organic matter thereby they are integral part of the biotic interactions **(Ritz and Young, 2004)**. In order to survive, fungi compete with other organisms for nutrients. They live in a symbiotic relationship that could be antagonistic in which they produce secondary metabolites for their protection as well as for communication with other microbes **(Goyal *et al.*, 2017)**. Therefore, fungi (e.g. endophytic fungal species such as *Alternaria alternate* and *Grammothele lineata*) act as a source for novel bioactive compounds **(Kawaguchi *et al.*, 2013)**. Fungi produce pigments, enzymes, food products, antibiotics and pharmaceutical drugs. The potential of fungi to produce these substances can be increased by manipulating their growth parameters **(Goyal *et al.*, 2017)**. Fungal isolates of marine soil are the main source of important industrial products such as beverages, sugar, nutritious food and alcohol **(Ahmed *et al.*, 2019)**. Fungi perform important biological activities due to the production of valuable metabolites. Soil borne fungi provide many beneficial products such as alkaloids, aroma and flavor metabolites etc. They can be used as a source of potassium, selenium, niacin, riboflavin, proteins and vitamin D. Soil fungi can also be used for the treatment of different diseases such as hypertension, Parkinson, cancer and Alzheimer **(Goyal *et al.*, 2017)**.

A large group of antibiotics producing fungi are present in soil where they compete with other microbes by making an antagonistic interaction. In such interaction fungi produce secondary metabolites that show multiple biological activities as well as stop the growth of other microbes. Research is needed to discover and isolate more potent antimicrobial sources from soil.

Natural Products from Soil Fungi and Importance of Antibiotic Producing Fungal Strains

Microbes produce biologically active, organic compounds of low molecular weight that are known as secondary metabolic products **(Geetanjali and Jain, 2016)**. Microbes contribute 10% of the total 20-25% biologically active compounds, while fungi produce 40% of the total 22,500 microbial natural products **(Demain and Martens, 2016)**. Almost 338 fungal species are capable of antibiotic production **(Rafiq *et al.*, 2018)**. Current number of fungal metabolites is 466,000 **(Goyal *et al.*, 2017)**. Microbial metabolic natural compounds are widely used for therapeutic and prophylactic purposes **(Demain and Martens, 2016; Geetanjali and Jain, 2016; Bhardwaj *et al.*, 2017)**. Besides these applications these natural microbial

products are also used in agriculture, veterinary and pharmacy fields **(Geetanjali and Jain, 2016)**.

Fungi also produce a wide range of natural compounds which are beneficial for human nutrition and health **(Bhardwaj *et al.*, 2017)**. Most of these compounds have a role in defense system of organisms **(Geetanjali and Jain, 2016)**. These fungal metabolites are important for cellular functions as they are widely used in medicine field as immunosuppressant and antimicrobial agents **(Brakhage, 2013)**.

Fungi are the major source of antibiotics in industries **(Kaur *et al.*, 2014)**. Trichoviridin and dermadin were the first natural antimicrobials produced by TK-I fungal strain of *Trichoderma* **(Ghisalberti and Sivasithamparam, 1991)**. Fungal strains of *Cephalosporium* and *Penicillium* produce cephalosporin and penicillin, respectively **(Geetanjali and Jain, 2016)**. *Tolypocladium nivenum* fungus produces an antifungal metabolite i.e. cyclosporin. Fungal strains of *Monascus rubra* and *Aspergillus terreus* produce lovastatin **(Demain and Martens, 2016)**. *Fusarium* fungal strain KTY-07 produces antibacterial agent that inhibits *Staphylococcus aureus* growth **(Bose *et al.*, 2016)**. Antimicrobials produced by *Aspergillus fumigatus* and *Rhizopus stolonifera* fungi are active against *Escherichia coli* bacteria **(Rafiq *et al.*, 2018)**. Thus *Fusarium*, *Penicillium*, *Aspergillus* and *Rhizopus* fungi produce many antimicrobial substances **(Anees *et al.*, 2018)**.

Antibiotics produced by *Aspergillus*, *Trichoderma* and *Penicillium* fungal species showed antifungal and antibacterial activities **(Kawaguchi *et al.*, 2013)**. *Trichoderma virens* has two strains i.e. Q and P. Q strain produces gliotoxin while P strain produces gliovirin which show anticancer activity **(Sherkhane *et al.*, 2017)**. *Trichoderma* strains such as TMK-22, TMK-19 and TMK-20 produce antifungal metabolites against *Fusarium oxysporum* **(Anees *et al.*, 2018)**. *Acremonium caerelens* fungus produces cerulenin which shows antifungal activity by inhibiting the synthesis of fatty acids **(Demain and Martens, 2016)**. Glomalin is a glycoprotein produced by mycorrhizal fungal strains **(Ritz and Young, 2004)**. *Penicillium brevicompactum* produces mycophenolic acid which acts as antitumor, antiviral, antifungal as well as immunosuppressant drug that is used to treat many viral diseases such as dengue fever, yellow fever and Japanese encephalitis. Previously, mycophenolic acid has never been commercialized due to its high toxic nature but now its derivative i.e. mycophenolate mofetil is produced, which has revolutionized medicine field **(Demain and Martens, 2016)**. Soil fungi of Antarctic region, for instance *Aspergillus*, are also able to produce valuable antibiotics **(Abneuf *et al.*, 2016)**. Fungi that are found in marine soil produce metabolites with antimicrobial activity that can inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria **(Ahmed *et al.*, 2019)**.

Soil borne fungi produce important secondary metabolites that show biological activities. They play an irreplaceable role in many fields especially in pharmaceutical industry. Due to the toxicity of some of these natural products as well as their ineffectiveness against many infectious agents, more work is required to produce semi-synthetic antibiotics.

Physiochemical Conditions

Soil fungi can produce valuable bioactive, pharmaceutical products with

powerful antimicrobial activity, at optimum parameters **(Ramesh *et al.*, 2019)**. External and internal environmental factors such as pH, substrate availability, water, light and temperature greatly affect the production of metabolites **(Al-Shaibani *et al.*, 2013; Kaur *et al.*, 2014)**. Fungal population is also influenced due to slight variation in these parameters **(Geetanjali and Jain, 2016)**. Change in pH can affect fungal growth and their metabolites production. Fungi show proper growth at acidic pH. Their optimum growth temperature is almost 28°C **(Bose *et al.*, 2016)**.

Maximum production of antibiotics was evaluated on different physiochemical conditions such as carbon and nitrogen sources, availability of oxygen, pH, temperature etc **(Singh *et al.*, 2012)**. For maximum growth and metabolite production, fungi require optimum physiochemical conditions i.e. 24-192 hours of incubation time, 2-11 pH, and nutrients such as peptone, yeast extract, malt, casein, ground nuts, wheat bran, glucose, glycerol, sucrose, maltose, lactose, fructose and starch **(Jawaid *et al.*, 2019)**. Besides these nutrients, fungi require Sabouraud dextrose agar, ethyl acetate, gelatin, sodium hydroxide, hydrochloric acid, and some enzymes such as proteases and lipases for their proper growth **(Jawaid *et al.*, 2019)**.

Studies showed that optimum temperature for the growth of mesophilic fungal strains is 25°C, while that of psychrophilic fungi is 4°C, but psychrotolerant fungal strains can grow at 4°C as well as at 25°C **(Abneuf *et al.*, 2016)**. Psychrotolerant fungi are the major source of valuable secondary metabolites, as they can withstand harsh environmental conditions **(Des, 1995; Madronich *et al.*, 1998; Montiel, 2000; Yogabaani *et al.*, 2017)**.

Salt concentrations affect the growth and sporulation of fungi, it can also affect their biodiversity. These fungi produce extremozymes and extremolytes in order to compete during osmotic shock **(Raddadi *et al.*, 2018; Orwa *et al.*, 2020)**. Although overall physiochemical conditions are similar for most of the fungi but they deviate slightly, for instance, *A. terreus* fungi showed best growth and antibiotic production after 120 hours of incubation , at 5 pH and 60-80°C temperature when gelatin and carbon were used as nitrogen and carbon sources, respectively **(Jawaid *et al.*, 2019)**.

Fungi can grow in a wide range of intrinsic and extrinsic parameters. Yield of antimicrobial substances can be enhanced by optimizing these parameters such as pH, temperature and nutrients. Fungal strains which can tolerate extreme conditions, produce valuable antibiotics. Further studies should be carried out to know about other factors that affect the production of antibiotics.

Genes Responsible for Antibiotics Production Including Cryptic or Silent Genes in Soil Fungi

Many microorganisms acquire cryptic biosynthetic gene clusters (BGCs) that can synthesize new specialized products, but some of them produce lesser number of secondary metabolites (SMs) in lab than their genome indicates because of the inadequate expression of their BGCs **(Rutledge and Challis, 2015)**. For instance, *Aspergillus* species genome are analyzed through genome sequencing **(Brakhagea and Schroeckha, 2011)** which consists of long 30-40 Mb genome but only few genes can produce SMs while rest of them remain

inactive **(Kawaguchi *et al.*, 2013)**. Fungi with sequenced genomes, have shown greater number of genes and their clusters associated with SMs production compared to the actual number of known SMs **(Brakhage, 2013)**. For activating cryptic BGCs for SMs in fungi, three methods were predicted on the basis of genome mining; i) bioinformatic forecasting of polyketide synthase (PKS), or non-ribosomal peptide synthetase (NRPS), their substrates or their final product, ii) molecular and epigenetics based methods and, iii) to forecast natural condition for activation. But due to two main problems accession to new metabolites production is difficult i) incapability of cultivating potential producers of natural compounds in lab, ii) BGCs for SMs production become silent in lab **(Hertweck, 2009)**.

The cryptic genes of fungi are those gene clusters that encode for SMs production **(Brakhagea and Schroeckha, 2013)** from large scale sequencing data; thus, it is cleared that clustering provides the guideline for biosynthetic pathways of SMs **(Inglis *et al.*, 2013)**. *Aspergillus nidulans* contains two distant clusters of genes that have different loci and are essential for the biosynthesis of dehydro-austinol and meroterpenoids and clusters of *Fusarium fujikuroi* have specific pathway such as geranylgeranyl diphosphate synthase gene (*ggs2*) provides geranylgeranyl diphosphate for biosynthesis of gibberellin **(Brakhage, 2013)**.

BGCs are genes, comprising of more than 10,000 bases, that are physically linked in the chromosome and encode biosynthetic enzymes for SM production **(Pfannenstiel and Keller, 2019)**, such as genome of *Aspergillus* species contains 40 cryptic BGCs for secondary products **(Brakhagea, and Schroeckha, 2019)**. BGCs consist of a gene that encodes ―backbone‖ enzymes, PKS, NRPS, a polyketide synthase/non-ribosomal peptide synthetase hybrid (PKS-NRPS), a prenyltransferase known as dimethylallyl tryptophan synthase and/or a diterpene synthase (Inglis *et al.*, 2013), which helps to forecast number of BGCs in any sequenced genome **(Khaldi *et al.*, 2010; Medema *et al.*, 2011)**. For instance, studying genome of 19 *Aspergillus* species exhibit 21–66 BGCs in all species **(de Vries *et al.*, 2017*;* Pfannenstiel and Keller, 2019)**, that are more in number than the BGCs present in the genome **(Kawaguchi *et al.*, 2013)**.

Different fungal genes are involved in the synthesis of different antibiotics and SMs. Gene *mdp*G in *A. nidulans* is responsible for synthesis of arugosin, emodin, monodictyphenone, orsinellic acid, shamixanthones and sterigmatocystin and penicillin **(Inglis *et al.*, 2013)** which is transcriptionally initiated by disrupting *hda* gene **(Brakhagea and Schroeckha, 2019)**. In *Aspergillus terreus*, *Lov*D was necessary for synthesizing lovastatin and after that it is illustrated to be involved in the biosynthesis of simvastatin **(Kennedy, 1999; Jiménez-Osés *et al.*, 2014; Gao *et al.*, 2017)**. In the plant parasitic organism, *Gibberella fujikuroi*, Gibberellin (GA) production and activation of its biosynthetic genes is regulated by *FfAre*A **(Wagner *et al.*, 2010)** which acts as transcriptional co-activator and also helps in chromatin remodelling at GA cluster **(Gacek and Strauss, 2012)**. Some fungi are able to produce complex lactam-containing metabolites including high class lactam compounds through biosynthetic enzymes and pathway-specific transcriptional regulators of lactam synthesis **(Brakhage *et al.*, 2009; Khaldi *et al.*, 2010; Osbourn, 2010; Brakhage and Schroeckh, 2011)**. For example, genome of

Fusarium verticillioides and *Fusarium pseudograminearum* encodes hydrolytic action of lactamases (metallo-β-lactamase, MBL) **(Kettle *et al.*, 2015; Glenn *et al.*, 2016)**. In some fungi, proteins (LaeA, VeA and VelB) are involved in controlling specialized metabolism and sexual maturation. In *A. nidulans* and in other *Aspergillus* species, LaeA was recognized in 2004 as a universal specialized metabolic regulator i.e. removal of LaeA stops expression of SM BGCs while overexpression of LaeA influence expression of SM BGCs **(Rutledge and Challis, 2015).** Some fungal genomes encode biosynthetic enzymes for SMs production **(Brakhage, 2013)**, i.e. *Aspergillus fumigatus* genome have certain specific enzymes that catalyse synthesis of gliotoxin **(Sherkhane *et al.*, 2017)**. Some *Penicillium* species are also capable to produce SMs that have antibacterial and antifungal activities against *E. coli*, *Alcaligenes faecalis* and *Acinetobacter baumannii* **(Ramesh *et al.*, 2019)**. Sometimes horizontal gene transfer (HGT) controls antibiotics gene clusters in some fungi, thus HGT **(Brakhage and Schroeckha,2011)** is accountable for sterigmatocystin gene cluster in *Podospora anserine* **(Slotand Rokas, 2011)**.

Some metabolic pathways such as production of penicillin, sterigmatocystin and aflatoxin in *Aspergillus* species or deoxynivalenol and zearaleonon in *Fusarium* species, acts as an ideal system to better understand the genetics of SMs **(Diez *et al.*, 1990; MacCabe *et al.*, 1990; Hohn *et al.*, 1995; Yu *et al.*, 1995; Brown *et al.*, 1996; Gacek and Strauss, 2012)**.

Different cryptic BGCs in different fungal species are involved in the production of different antibiotics but due to the inactivation of these genes in lab, we should provide natural environmental conditions *in vitro* so that fungi could produce novel antibiotics.

Microbial Interactions Involving Antibiotic Production in Soil Fungi

Production of antibiotics due to microbial interaction is a protective or offensive approach for microorganisms to adjust in the environment and helps in microbial population in the rhizosphere **(Lynch et al., 2004; Gao *et al.*, 2017)**. Close association of microorganisms in soil has strong effect on them which constitutes a driving potential for SMs production and produces plenty of metabolic products **(Daniel, 2004)** in soil. Some SMs have biological benefit for the producer in a specific habitat **(Firnand, 2000; Brakhage and Schroeckha, 2011)**, for example when two *Aspergillus* strains are grown together so the extracts of one *Aspergillus* (producer) strain have higher antifungal activity over another **(Brakhagea and Schroeckha, 2011)**. Another important advantage of Fungal Bacterial Interactions (FBIs) **(Schroeckh *et al.* 2009)** is the activation of SMs BGCs that are silent in artificial (laboratory) growth conditions **(Rutledge and Challis, 2015)** and has emerged as a new strategy for antibiotic production **(Tarkka and Deveau, 2016)**. Different interactions can be found between different fungi and bacteria in different habitats such as upper soil, in rhizosphere and with plants etc **(Kobayashi and Crouch, 2009; Brakhagea and Schroeckha, 2011)**. Such as mycorrhizal fungi that are present in the endo-rhizosphere, rhizosphere and bulk soil and associate with other soil microbiota **(Rutledge and Challis, 2015)**.

The association of FBIs may be beneficial or harmful **(Rutledge and**

Challis, 2015) including antagonistic tissue population, synthesis of antimicrobial agents, organic acid, and lytic enzymes, neutralization of toxins and virulence factor degeneration **(Tarkka and Deveau, 2016)**, parasitic interactions, the mutual interaction of bacteria with mycorrhiza, **(Bonfanta and Anca, 2009)** bacterial endosymbionts in fungi **(Partida-Martinez and Hertweck, 2005)**, or mutualistic association in lichen holobionts **(Grube and Berg, 2009)**. Endobacterium *Burkholderia* species commensally interact with fungal host *Rhizopus microspores* **(Partida-Martinez *et al*., 2007)** and helps fungi to produce SM, rhizoxin **(Partida-Martinez and Hertweck, 2005; Brakhagea, and Schroeckha, 2011)**. Co-cultivation of *Streptomyces endus* S-522 with *Trihcoderma pulmonis* TP-B0596 produce new antibiotic alchivemycin A **(Rutledge and Challis, 2015)**. *A. fumigatus*, when grown with *Streptomyces peucetius* **(Zuck *et al*., 2011)** produce formyl xanthocillin congener, fumiformamide and N,N′-((1Z,3Z)-1,4-bis(4methoxyphenyl)buta-1,3-diene-2,3-diyl)diformamide. When *A. fumigatus* is co-cultivated with *Streptomyces bulli*, it generates ergosterol and seven new metabolites **(Rateb *et al*., 2013)**. When *A. fumigatus* is co-cultivated with *Sphingomonas* strain, it produces novel diketopiperazine, glionitrin A **(Park *et al*., 2009)** that shows antimicrobial activity against methicillin resistant *Staphylococcus aureus* (MRSA) and high cytotoxic activity against four human cancer cell lines. Co-culturing of endophytic fungus, *Alternaria tenuissima* with other microorganisms produce antifungal polyketides, stemphyperylenol for plant parasitic endophytic fungus, *Nigrospora sphaerica*. Commensalism between *Streptomyces halstedii* with *A. nidulans* produces antibiotic concanamycin A, which changes proteomics outline of *A. nidulans* and has an effective role in defense associated channels of fungi **(Melin *et al*., 2002; Netzker *et al*., 2015)**.

Fusarium species are in competitive interaction with soil microbes. The antagonistic association of *Fusarium* with soil biota involved nutrients and chemicals competition **(Gao *et al*., 2017)**. Ectomycorrhizal fungi in mycorrhizal association with soil-borne plant parasitic bacterium, *Pythium ultimum*, produces antibacterial agent *in vitro* due to acidified growth medium and inhibits growth of this bacterium **(Rasanayagam and Jeffries, 1992)**. Some fungi in association with algae also produce SM, i.e. Lecanoric acid which helps to defend fungi in response to other microorganisms **(Netzker *et al*., 2015)**.

In some FBIs, bacteria help to activate silent SM BGCs of fungi. Therefore, silent genes of plant parasitic fungi **(Hertweck 2005)** are activated due to mutual FBI and produces virulence factors which improve fungal growth and produce diverse fungal-bacterial biofilms **(Tarkka And Deveau 2016)**. When *A. nidulans* physically interacts with soil dumping bacterium *Streptomyces rapamycinicus* it activates large silent fungal BGCs of Lecanoric acid, orsellinic acid and cathepsin K inhibitors **(Netzker *et al*., 2015)** that decompose elastin, collagen, gelatine, bone and cartilage **(Rutledge and Challis, 2015)**.

When *A. fumigatus* is co-cultured with *S. rapamycinicus*, the silent polyketide BGCs of *A. fumigatus* becomes active and produces unique polyphenols, fumicyclines which help in *A. fumigatus* protection by inhibiting growth of *S. rapamycinicus* **(Rutledge and Challis, 2015)**. However, gene modulating mechanism that take part in SM production during microbial

association are not described in detail yet **(Netzker *et al.*, 2015)**.

Some fungi, when interacting with plants produce plants beneficial SMs, i.e. *Trichoderma* helps in development and enhancement of plants growth by producing SM, which also has a main role in the association with other organisms **(Cornejo *et al.*, 2020).** While some fungi when interacting to the exterior of plants produce SMs and protect them from antagonists that reach to their nutritive source **(Goyal *et al.*, 2017)**.

Soil fungi make different interactions with different soil microorganisms and produce useful SMs and antibiotics. Thus, the fungal interactions with other microbiota helps in the attainment of new antibiotics.

Antibiotics Produced By Soil Fungi

Soil fungi are the producers of antibiotics and bioactive compounds such as enzyme inhibitors, insecticides, anti-helmintics, antioxidants, and vitamins having pharmaceutical and industrial importance **(Ahmad *et al.*, 2016)** anticarcinogens (asperlin), antiviral drugs (beauvericin), immune-suppressive (mycophenolic acid) and immunomodulatory drugs. **(Ramesh *et al.*, 2019)**. Two basic β-lactam antibiotics penicillin and cephalosporin are primarily produced by fungal species, *Penicillium chrysogenum* and *Acremonium chrysogenum*, respectively, and are used to treat bacterial infections **(Brakhage *et al.*, 2009; Rutledge and Challis, 2015)**.

Antimicrobial agents of fungi are divided on the basis of their capability in two types. i) broad spectrum antibiotics which are active against broad range of gram positive and gram negative bacteria while ii) narrow spectrum antibiotics are active against only few groups of bacteria **(Kaur *et al.*, 2014)**. Different classes of fungal antibiotics on the basis of mode of action towards different target sites of bacteria are;

- Inhibitors of cell wall synthesis e.g. Penicillin
- Inhibitors of protein synthesis e.g. Aspirochlorine Class and Gentamicin **(Al-Dammy *et al.*, 2018)**.

Tolipocladium inflatum, an ascomycete fungus, produce the antibiotic cyclosporine and acts as immunosuppressive drug **(Azizan *et al,* 2016)**. *A. terreus* produce the antibiotics, lovastatin which is a cholesterol lowering drug **(Ahmad *et al.*, 2016)** and Terrequinone A which is a cytotoxic agent produced by *A. flavus* and used against tumour cells *in vitro* **(Rutledge and Challis, 2015)**.

SMs of *Trichoderma* species include different bioactive metabolites with antibiotic activities that could be used against many bacterial and fungal parasites **(Cornejo *et al.*, 2020)**. Gliotoxin is an antibiotic produced by *Trichoderma virens* that protects plants from root rot caused by *Rhizoctonia solani* **(Cornejo et al, 2020)**. Gliovirin, viridins and viridiol are antifungal antibiotics produced by *Gliocladum virens.* Gliovirin is effective against fungal parasite, *P. ultimum* and viridins have little antagonistic action against many fungi and bacteria **(Howell and Stipanovic, 1984)**.

Some ectomycorrhizal fungi are also capable to produce antibiotics, i.e. mycorrhizin A and chloromycorrhizin A are produced by fungi found on the roots of Monotropa hypopitys **(Trofast and Wickberg, 1977)**. Other antifungal antibiotics are p-hydroxy-benzoyl-formic acid and (R)-(-)-p-hydroxy-mandelic acid

that are produced by *Pisolithus arhizus* which were later given the names of pisolithin A and pisolithin B **(Kope *et al.*, 1990)**.

FKI-1079 strain of *Metarhizium* species produce two novel insecticidal antibiotics, hydroxyfungerins A and B in addition to fungerin. Fungerin is an antifungal antibiotic produced by *Fusarium* specie **(Uchida *et al.*, 2005)**. Ascofuranone (AF) and Ascochlorin (AC) are produced by *Ascochyta viciae*. AF is used against African trypanosomiasis and cryptosporidiosis while AC has significant action on mammalian respiratory chain **(Hijikawa *et al.*, 2016)**. Pyrrocidine A and B are antifungal antibiotics produced by *Saccharomyces zeae* are used against *A. flavus* and *F. verticillioides* **(Wicklow et al., 2005)**. Another novel antibiotic produced by fungi is monocyclic N-thiolated β-lactams and has antifungal effect on seven *Candida* specie **(O'Driscoll et al., 2008; Gao *et al.*, 2017)**.

Many antibiotics are produced from soil fungi and classified in different classes on the basis of their target sites. Fungal antibiotics are useful pharmaceutical drugs that are used for the treatment of infectious diseases caused by bacteria as well as fungi.

Methods to Analyze Antibiotics Production in Fungi

Number of methods could be used to check the antimicrobial activity of fungi. These methods are pour plate method, agar disk diffusion method, agar well diffusion method, microdilution method and dual culture technique.

In microdilution method, fungal liquid extracts are inoculated in microplates containing bacteria to find out values of minimum bactericidal concentration (MBC), or a concentration that stops 50% growth of bacteria **(Estrada *et al.*, 1998)**. However, advanced methods could also be used for the analysis of fungal antibiotics. One of them is isolation chip (iChip) method. iChip is a method that cultures the microorganisms in the natural environmental conditions and also analyses their antimicrobial activities. iChip have semipermeable membranes which acquires a single microbial cell. These perforated membranes adhere on each side of the plate and are placed on opposite side in the soil to isolate the microbes in their original condition, i.e. form soil **(Rutledge and Challis, 2015)**.

Antibiotic analysis and extraction from soil fungi are done by using different methods such as agar disk diffusion, microdilution, dual culture technique, iChip methods and dilution platting technique. We still need some advanced methods for easy isolation and purification of antibiotics from soil fungi.

Antibiotic Resistance in Fungi

Antibiotics are used to protect human health throughout the world, but with the passage of time microorganisms showed resistance against these drugs so the antibiotics either became less active or they are completely losing their effectiveness against infectious diseases. The occurrence of antibiotic-resistance against present drugs is raising enormously that demands the discovery of new anti-microbial agents **(Lihan *et al.*, 2014)**. Resistance of infectious agents towards antibiotics is one of the greatest challenges in control of diseases. This resistance is due to mutations in fungal genes that in turn result in alteration and/or inactivation of drug targets in fungal species (Table: 1). Emergence of

antibiotic resistant infectious agents has increased the rate of pandemics and epidemics **(Sheikh, 2010; Svahn *et al.*, 2012)**. Antibiotic resistance has been favored by many human activities such as traveling, delay in infection diagnosis, poor hygiene and high doses of antibiotics **(Bhardwaj *et al.*, 2017)**.

Amphotericin B and fluconazole are ineffective against fungi *Aspergillus fumigatus* and *Candida glabrata*, respectively. Molecular studies showed that the resistance of *C. glabrata* was due to the increase in number of 14a-lancosterol demethylase producing CYP51 (ERG11) genes **(Helmerhorst *et al.*, 1999)**. Resistance of fungi *Candida Lusitaniae*, *Candida guilliermondii*, *Candida lipolytica*, *Aspergillus flavus* and *Aspergillus terreus*, towards amphotericin B is due to the reduction of ergosterol amount in the plasma membrane of these fungal spp. Mutation in genes that encode cytosine deaminase and uracil phosphoribosyl transferase enzymes can also cause the resistance of *Aspergillus* and *Candida* species towards 5-FC (antibiotic).

Ergosterol reduction is also responsible for the azole resistance in *Candida albicans*. Point mutation in the gene cluster that encodes beta (1,3)-glucan synthase enzyme is responsible for echinocandin resistance in *C. albicans* **(Niimi *et al.*, 2010)**. In *C. albicans* resistance to azole is due to the amplification of genes. This mechanism involves aneuploidy, in this process the target genes for azole ERG11 are duplicated. Pleiotropic Drug Resistance (PDR) is responsible for resistance of the yeast *Saccharomyces cerevisiae* towards many antibiotics. PDR and some other genes express multiple proteins which protect the microbial species from antibiotics by stopping their activity **(Gulshan and Moye-Rowley, 2007)**.

Echinocandin resistance of *C. glabrata* is due to the mutation in FKS2 gene, which codes for 1,3- β-D-glucan synthases, this enzyme is involved in the formation of cell wall component. Mutation in CDC6 gene, which initiates DNA replication is also responsible for echinocandin resistance.

Table 1: Antibiotic Resistance and its Mechanism in Fungi

Antibiotics	**Fungal Species**	**Mechanism of Resistance**
Amphotericin B and fluconazole	*Aspergillus fumigatus* and *Candida glabrata*	14a-lancosterol demethylase producing CYP51 (ERG11) genes
Amphotericin B	*Candida lipolytica*	reduction of ergosterol in the plasma membrane
Echinocandin	*C. glabrata*	Mutation in CDC6 gene and FKS2 gene
Phenamacril	*Fusarium graminearum*	Mutation in gene that codes for myosin-5
Calcoflour	*Aspergillus nidulans*	Mutation in C1198T

Mutation in these two genes led to the demand of new antibiotics that can overcome the results of these mutations. In *A. nidulans* mutation in C1198T gene leads to the reduction of chitin amount in the cell wall of this fungal strain. Due to this mutation, *A. nidulans* is resistant to antifungal calcofluor. An antifungal agent phenamacril is inactive against fungal species of *Fusarium graminearum* due to mutation in gene that codes for myosin-5. Myosin-5 plays roles in transcription, signal transduction, cell polarization, intracellular transport and cytokinesis **(Sekyere and Asante, 2018)**.

Many scientists keep on searching for those microorganisms which have the ability to produce new antibiotics because the number of drugs resistant pathogenic strains is rapidly increasing compared to the discovery of new antibiotics **(Lihan *et al.*, 2014)**. Thus, the discovery of novel antibiotics is the need of this era as more antibiotic resistant infectious agents have emerged which have inactivated the efficiency of current drugs **(Valgas *et al.*, 2007)**. Furthermore, this scenario is favored by overuse and inappropriate use of antibiotics **(Jawaid *et al.*, 2019)**.

Antibiotics are used to treat infectious diseases and save life throughout the world. But with the passage of time the microbes have become resistant to the current drugs by changing either the target sites or prohibiting antibiotics entry into the cell thereby making these drugs ineffective, therefore the discovery of new antibiotics is very important.

Significance of Semi-Synthetic Compounds in Fungi

Semi-synthetic antibiotics are those compounds that are synthesized by making certain chemical changes and structure modifications in the original drugs that improve their effectiveness against infections **(Demain and Martens, 2016)**. **Suryanarayanan *et al.* (2009)** carried out a study on fungal metabolites and their biological activities which showed that fungi are important source of semi-synthetic antibiotics **(Singh *et al.*, 2014)**. Increase in antibiotic resistance has enhanced the need for novel antibiotic discovery. Due to which pharmaceutical industries have started struggling for the production of semi-synthetic compounds of secondary metabolites **(Goyal *et al.*, 2017).**

Microbial metabolites are extremely versatile. Due to their unique capacity of isomerization, condensation, extensive branching, series of alternative reactions, oxidation, polymerization, alkylation etc. these metabolites can be modified into multiple derivatives **(Berdy, 2005)**. Fungal metabolites are either directly used as a medicine or they are modified to produce their derivatives which have many applications in medical field (Figure:1) **(Kock *et al.*, 2001; Bode *et al.*, 2002; Donadio *et al.*, 2002; *Chin et al.*, 2006; Gunatilaka, 2006; Mitchell *et al.*, 2008; Stadler and Keller, 2008)**. Zocor is a lipid-lowering agent obtained synthetically from a fermentation product of *A. terreus*. Zocor is the semi-synthetic compound of lovastatin, which is used to treat hypertension and viral infections. Another semi-synthetic compound is simvastatin; it is derivative of Zocor which shows activity against hepatitis C virus and RNA viruses **(Demain and Martens, 2016)**. Due to the importance of fungal drugs such as penicillin, lovastatin, griseofulvin etc fungi are now considered as a major source for the discovery of novel antibiotics **(Singh *et al*, 2017)**.

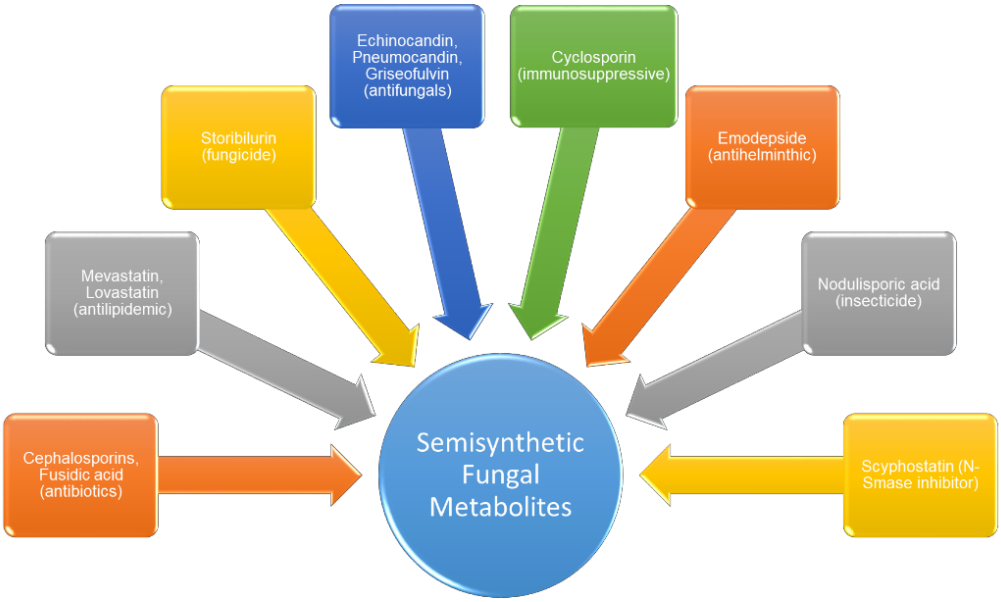

Figure 1: Uses of Semisynthetic Fungal Metabolites

Biosynthetic techniques have revolutionized the field of medicine by designing semi-synthetic compounds of natural antibiotics. But still the number of these compounds is very low, therefore scientists should continue their efforts in order to explore further methods for the designing of more potent synthetic compounds.

Applications of Antibiotics Produced by Fungi

Fungi have the capability to produce many secondary metabolites **(Devi *et al.*, 2020)**. The most significant fungal genus is *Trichoderma* which is identified for their capability to yield variety of antibiotics. This fungus exists in soil and it is active against fungal parasites **(Sivasithamparam and Ghisalberti, 1998)**. Since microbes are the main source of biologically active metabolites, therefore industries are struggling to screen these metabolites and to explore their role in different diseases **(Makut and Owolewa, 2011)**.

Fungal metabolites are used as immunosuppressants, insecticides, antiparasitic, herbicides, enzyme inhibitors, ruminant growth stimulants, hypocholesterolemic drugs and antitumor agents etc, due to these characteristics, fungi are widely used in pharmaceutical industry. Mold species *Monascus purpurea* are used in medicine. Some fungi produce metabolites such as camptothecin and taxol, which have roles in medicine. Taxol is a diterpene alkaloid. It is used as an anticancer agent because it inhibits uncontrolled mitosis by enhancing polymerization of tubulin. It is used against advanced stages of Kaposi' sarcoma **(Bhattacharyya and Jha, 2011; Demain and Martens, 2016)**.

Some fungal metabolites such as cephalosporin, fusidic acid and penicillin are used as antibacterial and antifungal agents **(Rafiq *et al.*, 2018)**. Out of 60% of known fungal metabolites, 14000 have

antimicrobial activity, 5000 have antitumor and 15000 of them show antiviral activity **(Berdy, 2012)**. Some of these antibiotics are used as chemotherapeutic drugs **(Makut and Owolewa, 2011)**. These drugs are used for the treatment of several bacterial infections by destroying bacterial cells **(Lihan *et al.*, 2014)**. Approximately 1.5 million of fungal species exhibit ecological activities. The most important fungal antibiotic is penicillin which is used to treat *Staphylococcus* and syphilis infections. They are used to control bacterial infections especially that of Gram-positive bacteria. It can even kill intestinal flora **(Fatima *et al.*, 2019)**.

Some fungal compounds are used as sweetening agents in food additives. Fungus *Penicillium roqueforti* and *Aspergillus niger* produce thaumatin which is used in food industry as a sweetening agent **(Demain, 2009)**. Another sweetener is xylitol which is produced by yeast *Saccharomyces cerevisiae* and *Pichia stipitis.* Mold specie *Monascus purpurea* is used in Chinese food and Koji food. Mold specie *Monascus rubramin* is used in the preparation of cheese, fish, meat, wine, red rice and soybean etc. **(Demain and Martens, 2016; Devi *et al.*, 2020)**. *Penicillium chrysogenum* have the ability to spoil food due to which this specie has drawn the attention of industries to be used for penicillin production **(Fatima *et al.*, 2019)**.

Fungi produce pigments such as carotenoids, quinones, flavins and violace which are used in cosmetics, medicine and food industry. **Sanchez *et al.* (2013)** reported that more than 600 carotenoids have been discovered that are produced by microbes. Fungal pigments and their derivatives are used as immune modulators, antioxidants and nutritional supplements. The yeasts *Xanthophyllomyces dendrorhous, Dunalliela salina* and *Blakslea trispora* are widely used for large scale production of carotenoids. *Monascus* specie produce 54 pigments that are used as anticancer, antimicrobial, antidiabetic, antimutagenesis, anti-inflammatory, antiobesity, hypotensive, immunosuppressive and hypocholesterolemic drugs etc. **(Stobel and Daisy, 2003; Rao *et al.*, 2017)**.

Some toxic metabolites of fungi such as ergot alkaloids, cyclosporin A, zearelanone and gibberellins have also been used in agriculture and medicine fields. The statins are secondary metabolic products of soil fungi that show hypolipidemic activity by lowering cholesterol level in plasma thereby preventing stroke and other circulatory diseases **(Demain and Martens, 2016)**.

Antibiotics are able to kill or stop the growth of harmful bacteria and protect the human health. One of the most important sources for the discovery of novel antibiotic compounds is fungi. These metabolites are used in medicine as chemotherapeutic agents as well as in agriculture.

Conclusions

There is a great need for research to enhance the standardization of physiochemical conditions in order to produce good quality antibiotics from fungi. It was shown that the issues of antibiotic resistance can be resolved by producing semi-synthetic antibiotics. Soil fungi form an antagonistic interaction with other microorganisms that can be studied to discover more potent antibiotics. This study provides information about newly emerging antibiotics to treat serious infections. However, we need to find effective fungal antibiotics that can withstand antibiotic crisis. There is also a need to search some advanced methods for best isolation of

antibiotics producing fungi from soil. We need to examine novel antibiotics producing fungal species that can kill the antibiotics resistant microorganisms and safeguard human health. Suitable environmental conditions play an important role in antibiotics production by fungi, so the determination of optimized physiochemical conditions is also needed.

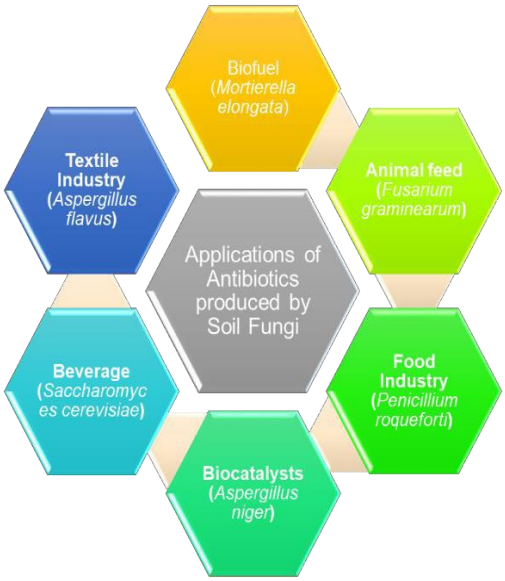

Figure 2: Applications of Antibiotics Produced by Soil Fungi

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