

# Chapter 6

## Fungal Biotechnology: Role and Aspects



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**Abstract** Sustainability has become the prominent necessity in every human event in today's time, which can initiate from the household and leads to this planet earth. The Earth Summit organized by the United Nations has played the significant role in creating awareness about sustainable development to retort against the natural calamities, due to over-exploitation of natural resources and exponential growth of the human population for centuries. For the quest of sustainability, fungi have emerged as a suitable candidate. Fungi play a pivotal role in fundamental and modern processes of biotechnology. Nowadays many processes such as baking, brewing and the synthesis of alcohols, antibiotics, enzymes, organic acid as well as the additional pharmaceutical product are carried out using fungal bioproducts. Due to recent advances in genomics and rDNA technology, yeast and fungi have attained the forefront position because of their present industrial purposes. In general, the term "mycotechnology" is used, which states about the various roles of fungi with the addition to its impact on biotechnology as well as economy.

Fungi play the significant role in sustaining the health and terrestrial ecosystem. During disastrous event which leads to disruption of the earth ecosystem, fungi prepare themselves to prevail in the future. The aid of fungal population in sustaining the environment is showing promising result. About 90% of plant grows in symbiosis with fungi such as vesicular-arbuscular mycorrhizal (VAM) fungi, mycorrhizae, out of which *Glomus* is the most exploited genera (Van der Heijden et al. 1998). Fungi persisting on this earth have widespread complex relationship

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among the range of microbes, which can be arthropods, bacteria and nematodes. The dwelling zone of these fungi is named as the “rhizosphere”.

A fungus belongs to the group of Eukaryotes, which consists of microbes like moulds and yeast along with more familiar mushrooms. All of them are categorized under the kingdom *Fungi*. The fungi are omnipresent in every environment and play critical role in complex biological processes. Thus, it can work as decomposers, which aid in nutrient cycles, exclusively as symbiont as well as saprotrophs, in disintegrating the organic constituents into inorganic constituents, which gets retraced in the anabolic pathways of metabolic activities taking place in organism as well as plants.

**Keywords** Fungal biotechnology · Fungal enzymes · Bioprocessing · Fermentation · Secondary metabolites

## 6.1 Introduction and Definitions

Fungi belong to the lower class of eukaryotes according to contemporary biologist and sometimes are also regarded as the fifth kingdom on the basis of the mode of nutrition. Fungi project different types of enzymes into their surrounding and after the action of these enzymes engulf the pre-treated food. With assorted morphology, ecology and physiology, many eminent fungi still have an adverse effect on the well-being of humans as they are involved in different plant diseases (like blights, rusts, smuts and wilts) and biodeterioration (like mildews and rots) as well as pathogenic to animal (by producing mycoses and toxins). Range of fungi starts from the micro-sized moulds and yeasts to macro-sized truffles as well as mushroom. Large number of macro-sized species of fungi are believed to be delicate; thus they are grown and accumulated for human uses such as food or its supplements, whereas there are micro-sized fungi, comprising of genera like *Aspergillus*, *Penicillium* and *Saccharomyces*, which have positive influence in context to human activities as their regulated metabolisms are utilized for synthesizing enzymes as well as metabolites. These abilities have made fungi one of the foundation stones in modern biotechnology.

There are numerous ways by which the term biotechnology can be explained. The Spinks Commission, UK, was the first group to give a formal explanation: “Biotechnology is the application of biological organisms, systems, or processes to manufacturing and service industries”. On the other hand, the European Federation has quoted the similar definition but with a broad range of aspect: “the integrated use of biochemistry, microbiology and engineering sciences in order to achieve technological (industrial) application of the capabilities of micro-organisms, cultured tissue cells, and parts thereof”. Further, the National Institute of Health and Food and Drug Administration, USA, defined: “Biotechnology is the application of biological systems and organisms to technical and industrial processes. The technologies included in this area include genetic selection, in vitro modification of genetic material, e.g. recombinant DNA, gene splicing, cell fusion, hybridoma technology etc.” and other novel techniques for modifying genetic material of living organisms (Bennett et al. 1997).

For better understanding, the meaning of biotechnology can be comprehended and incorporated to the fermentation processes which are employed for producing wine and penicillin. Thus, the term “mycotechnology” needs to be incorporated with various biotechnological practices, both old and new era, that rely on fungal products and processes.

## 6.2 Premodern Fungal Technology

The term “modernism” separates the twentieth century by violating the tradition set in the nineteenth century. Like in art, abstraction superseded by representation; in architecture, functionalization gets superseded by ornamentation; in literature, new style forms got superseded by conventional narrative. Daily the new applications are discovered for basic science to revolutionize the living standards of the people. Adjectives such as “premodern”, “modern” and “postmodern” are employed as descriptive terms for assessing the massive number of procedures as well as products which involves fungal biotechnology.

For millennia, the bread, beer, wine, koji and various fermented food along beverages have been integral part of human regime, but they have lost their relic (Table 6.1). Historical documents have made it clear that the individual knows about microbes like moulds and yeasts on the basis of the function. Pasteur on visualizing the microbes under the microscope during sugar fermentation process, considered the fermentation as “organized ferment”, whereas amendment taking place in the solution without the traces of any microbes were regarded as “unorganized ferment”. Later on, it was found that unorganized ferments were the metabolites which were synthesized by organized ferment. Kuhne coined the word “enzyme”. Finally, “enzyme” was predominantly for unorganized ferments irrespective of the microbe producing which can be bacteria, fungi or yeast (Lutman 1929).

**Table 6.1** Premodern examples of mycotechnology (Gray 1970; Chang and Hayes 1978; Hesselstine 1983)

Process/substrate for Asian food fermentations	Microorganisms involved
<i>Ang-kak</i>	<i>Monascus purpureus</i>
<i>Miso</i>	<i>Aspergillus oryzae</i>
<i>Ontjam</i>	<i>Neurospora crassa</i>
<i>Soy sauce</i>	<i>Aspergillus oryzae</i> , <i>Aspergillus sojae</i>
<i>Tempeh</i>	<i>Rhizopus niveus</i>
<i>Brewing and baking</i>	<i>Saccharomyces cerevisiae</i> , <i>Saccharomyces carlsbergensis</i>
<i>Mould-ripened cheeses</i>	<i>Penicillium roqueforti</i> , <i>Penicillium camemberti</i>
<i>Mushroom cultivation</i>	<i>Agaricus bisporus</i> , <i>Auricularia</i> sp., <i>Flammulina velutipes</i> , <i>Lentinus edodes</i> , <i>Pleurotus</i> sp., <i>Volvariella volvacea</i>

### 6.3 Modern Fungal Technology

Alcohols, enzymes, organic acids and different pharmaceutical products synthesized by fungi are the key for the advancement of the modern technology. Some of the chief industrial products produced by fermentation process along with synthesizing species are illustrated in Table 6.2.

Traditional enzyme, diastase (also known as amylase), is one of the most exploited enzymes which were isolated from the barley, and it is now applied for manufacturing beer. In 1894, Jokichi Takamine realized about the industrial importance of enzymes produced by these moulds and fungi. Takamine used *Aspergillus oryzae*, a Japanese koji mould, for synthesizing the diastase, for which he inoculated the spores of the mould onto the wheat bran/steamed rice and allowed it to proliferate so as to form a thin layer over it. In 1894, successive patents were filed, in which he protected the processes involved but advised the new role of diastase (partially purified) as a malting enzyme. Additionally, he also suggested that diastase can aid digestive enzyme for treating dyspepsia (Takamine 1894; Underkofler 1954). During the early period of the twentieth century, analogous procedures were established for various other enzymes. In 1983, there were 30 classes of enzyme which were being commercially exploited, out of which half of them were of fungal origin. Due to exponential exploitation of these commercial enzymes, Godfrey and West compiled the summary of these enzymes in 1996.

**Table 6.2** List of examples of modern industrial products by mycotechnology

Industrial product	Fungi
<b>Antibiotics/pharmaceutical product</b>	
Penicillins	<i>Penicillium chrysogenum</i>
Cephalosporin	<i>Cephalosporium acremonium</i>
Cyclosporin	<i>Tolypocladium inflatum</i>
Ergot alkaloids	<i>Claviceps purpurea</i>
Griseofulvin	<i>Penicillium griseofulvin</i>
Mevalonin	<i>Aspergillus terreus</i>
<b>Enzymes</b>	
a-Amylases	<i>A. niger</i> , <i>A. oryzae</i>
Cellulase	<i>Humicola insolens</i> , <i>Penicillium funiculosum</i> , <i>Trichoderma viride</i>
Glucoamylases	<i>Aspergillus phoenicis</i> , <i>Rhizopus delemar</i> , <i>R. niveus</i>
Glucose oxidase	<i>A. niger</i>
Invertase	<i>A. niger</i> , <i>A. oryzae</i>
Laccase	<i>Coriolus versicolor</i>
Pectinase	<i>A. niger</i> , <i>A. oryzae</i> , <i>Humicola insolens</i>
Proteinases	<i>A. oryzae</i> , <i>Aspergillus melleus</i> , <i>R. delemar</i>
Rennin (microbial)	<i>Mucor miehei</i> , <i>M. pusillus</i>
<b>Organic acids</b>	
Citric acid	<i>A. niger</i>
Itaconic acid	<i>A. terreus</i>

Another product synthesized by these filamentous fungi which is the centre of attraction in modern biotechnology is the citric acid. Formerly, the citric acid was isolated from the citrus fruit, but at end of the nineteenth century, it was enlightened that these filamentous fungi were responsible for citric acid production. Pfizer, Brooklyn, New York, USA, developed the conventional process which gained worldwide recognition for its application in beverage and food industry. Further, these processes are utilized to produce antifoaming agents, cosmetics, detergents, tablets, textile treatment and preservatives for storing blood. Different approaches of modern fermentation technology were improved focussing on improving the yield of citric acid by amending the growing conduction and by exploiting the submerged process for enhancing the process of product recovery (Crueger and Crueger 1982).

The turning point took place in the industrial microbiology when penicillin was discovered, and further derivatives of penicillin were named as “wonder drug” (Wainwright 1990). Exploration of secondary metabolites with additional antimicrobial activity was elicited after the penicillin discovery. Beside that invigorating research on physiology of fungi, fermentation technology and development of industrial strain was taking place. During 1940–1950, varied number of antibiotics was discovered, and this time is also regarded as the “golden era of antibiotics”. Great success was achieved by Selman Waksman and his colleagues, who were working in Rutgers University and Merck Corporation, New Jersey, as they were the only one to screen out the antimicrobial metabolites. Most of the antibiotics found by Waksman group were synthesized by soil isolates, notably actinomycetes. As moulds and actinomycetes form the complex filamentous network, it emerged as a challenge for chemical engineers to remodel the industrial-level fermentation method perfectly for both batch and continuous procedure (Smith and Berry 1975; Demain 1981). During the revolution of rDNA technology, minor alteration in genetic material of microbes was done in order to synthesize the genetically engineered product of microbial origin.

During the golden era of antibiotics, where research was focusing mainly on finding novel drugs, the laboratory in Japan under the supervision of Umezawa was screening for the microbes with antidiarrhoeal, antihypertensive, antimutagenic, antitumour and immune-stimulant potentials (Umezawa 1982). Cyclosporin (immune-suppressant) and mevalonins (antihypertensive) are the pharmaceutical product which are acquired from the filamentous fungi (Von Wartburg and Trabor 1986; Monghan and Tkacz 1990).

## 6.4 Postmodern Fungal Technology

The advent of rDNA technology has transformed biology. Under the term “post-modern”, mycotechnology insinuates to recent improvements procreated by embracing the techniques like gene splicing along with the additional post-rDNA techniques other than the traditional industrial techniques. Few examples of this hybrid mix are depicted in Table 6.3. The regulation of heterologous protein

**Table 6.3** Postmodern example of mycotechnology

Development	Examples
Expression of heterologous genes	Fungal, plant and mammalian
Amplification of homologous genes	Antibiotic pathways and enzymes
Manipulation of secondary pathways	New semi-synthetic antibiotics and hybrid antibiotics
Large-scale genomics	<i>Aspergillus nidulans</i> , <i>Neurospora crassa</i> , <i>Magnaporthe grisea</i> and <i>Phytophthora infestans</i>
DNA chips	High-density DNA arrays for screening gene expression
“Mining” fungal biodiversity of new pharmaceutical	Sampling environmental DNA genomics-based screening

synthesized by the filamentous fungi has attained substantial appreciation. Presently, the chief host fungi are *Aspergillus nidulans*, *A. niger*, *A. oryzae* as well as *Trichoderma reesei* (Davies 1991), whereas *Neurospora crassa* is under evaluation (Rasmussen-Wilson et al. 1997). Formerly, researchers wanted to synthesize the mammalian protein that is also in high amount and were successful in expressing bovine prochymosin (also known as rennin) in different species (Davies 1991). Moreover, the expression of mammalian protein was less than the expected level. The molecular stages of secretion pathway of fungi, the PTM (post-translational modification) of metabolic proteins and the discharging of these proteins into environment via hyphae are only limited to textual level (Wosten et al. 1991). Thus, extensive research at the molecular level is required to get the insight about the fungal gene expression and mode of secretion.

Molecular analysis also acquiesced the secondary metabolic pathways. Certainly, penicillin family was the first family of antibiotic which gained the profit from innovative approaches. On cloning the gene encoding for isopenicillin N synthase, it was uncovered that various gene involved in the pathway were present in cluster; thus it accelerated the isolation process (Skatrud 1991). The strains showing high yield were determined to have multiple copies of the gene which code for the main enzyme involved in the penicillin pathway. In few instances, the researchers were able to engineer the fraction of the metabolic pathway with the help of atypical precursor or the host organism, hence exaggerating chemical diversity of the nature (Skatrud 1992).

Molecular analysis has also benefited the group of secondary metabolites named polyketides. Till date, extensive research is done in actinomycetes targeting either mixing or matching of the polyketide synthases (Kao et al. 1994). Moreover, these strategies have benefited fungi a lot. The variation spawned by employing the diverse initiating units, by amending the oxidation as well as stereochemistry of chemical during elongation and by inducing different post-polyketide amendment which resulted in the synthesis of different theoretic molecules. By exploiting the genetically altered polyketide synthases, one is even able to synthesize the artificial natural product.

The advances in the genetic transformation techniques have enabled to amend the fungal strain and provide them the potency as some of the species lacks sexual as well as parasexual cycles (Esser 1997). Enhancement in the conventional

fermentation by fungi is observed along with that genetic modification that has enabled to amend the fungi to perform specific function. Incorporation of both homologous and heterologous gene into fungal host has improved the yield and properties of the enzyme. Now, the enzyme could be synthesized on the varied substrate and at different optimum temperatures (Kinghorn and Lucena 1994).

Even traditional approach of cultivating the mushroom got the boost by the latest mycotechnology. Most of the appealing species are difficult to culture; thus they are needed to amass in their sporadic phase. Model species like *Coprinus cinereus* (Pukkila 1993) and *Schizophyllum commune* (Raper and Horton 1993) were chosen to evaluate the genetic foundation responsible for formation of fruiting body. It has been forecasted that to obtain the commercially available improved strain of mushroom, the same breeding techniques using molecular tools are needed. However, it is believed that isolating the gene responsible for the development of mushroom in in vitro models will provide the perception of fruiting in the exotic species.

The automation in DNA sequencing has made it possible to sequence the genome, and this genomic is transforming the whole biology. The bacterium *Haemophilus influenza* Rd was the microbes whose whole genome was sequenced (Fleischmann et al. 1995); although 7 other genomes have been sequenced beside it, 100 species are under evaluation. The first eukaryotic species, which got whole genome sequenced, is yeast, *Saccharomyces cerevisiae* (Dujon 1996). *S. cerevisiae* comprises 16 chromosomes containing 12,067,266 bp excluding the repeats. Various yeast genes show similarity with mammalian gene; thus their functionality is assessed to study about cancer as well as other human diseases (Botstein et al. 1997). In contrast, the open reading frames recognized via the sequencing result went into vain as they didn't display any phenotypic characteristics.

Genomic project started with the sequencing of the yeasts *Candida albicans* and *Schizosaccharomyces pombe* and some filamentous fungi, such as *Aspergillus nidulans* and *Neurospora crassa* (Bennett 1997). The significant amount of data like genetic as well as biochemical makes it feasible to understand the correlation between sequence and phenotypic traits.

Microbial genomics assures to reform not only the fundamental biology, but it gears up the drug discovery procedure by regulating the clinical experiments. New possibilities arise on finding the correlation among the few gene sets with the drug potential. By developing a DNA microchip which encompasses the fixed oligonucleotide genomic sequence of the yeast onto silica chip with the help of photolithography, probes are employed to assess the intensity of the gene expression on treating it with the suitable reagent. Diagnostic based on genome is more liable in association with spatial chemistry as well as miniature assay. DNA chips accelerate the identification process of drug suitable for the target. Genomic analysis enables us to explore novel bioactive compounds from the environmental samples and unexplored areas, and it also includes the marine and macro-fungi (Blanchard and Hood 1996). Mycotechnology works according to the scientific frontiers in the same framework of biotechnology. New development in scientific frontiers leads to development of new techniques in the field of agriculture, industry and medicine. Molecular biology has revolutionized and procreated the social as well as political



controversies. On the other hand, sometimes regulatory issues get masked by the scientific matters. However, economic element is not the new aspect in the industrial microbiology but is taken in consideration during setting up of new biotechnology start-up company. This altogether has generated the huge amount of literature concerning the financing, government regulations, intellectual property as well as safety (Moses and Cape 1991).

## **6.5 Role of Fungi in Agriculture and Soil Science**

### **6.5.1 *The Role of Arbuscular Mycorrhizal Fungi in Agro- and Natural Ecosystem***

Symbiosis is the key which determines the growth and development of the plant. Arbuscular mycorrhizal fungi (AMF) are the most common variety of the mycorrhizal fungi. Advantages of these AMF are they aid in translocating the nutrients from the soil, maintaining soil integrity, guarding the plant from stress due to drought and pathogen persisting in the soil as well as generating diversity in the plants. AMF belong to the order of class *Glomales*, which presently encompasses six genera. AMF comprises the special structure in their root which is named as arbuscules, which help them in transferring of the nutrients among the fungus and plant (Dodd 2000).

### **6.5.2 *Plant Nutrient Uptake by AMF***

Phosphorus, element which is immobile in soil and important nutrient for growth and proliferation of plant, requires mycorrhiza. On incorporating the AMF in the plant-growing mixture, the plant growth system gets boosted as it was deprived of indigenous inoculum as in sterilized soil. The result can be visualized by assessing the branched root system of *Chenopodiaceae* and *Brassicaceae* family. On growing these plants in container, symbiosis will be effective if nutrition balance and environmental conditions like light are properly regulated (Dodd 2000).

### **6.5.3 *Alleviation of Environmental Stress by AMF***

AMF aids in elevating the resistance against drought not only in the plants growing in the arid but also in the area where drought prevails for short duration. The reason proposed is that it helps in nutrient absorption. AMF also induce the tolerance against different heavy metals like Cd (cadmium). The AMF metal-resistant strain which was isolated from the heavy metal-contaminated site proves its adaptation against the heavy metals (Dodd 2000).



### **6.5.4 Sustainable Production Using AMF**

Under natural conditions, the young seeds germinate and get plug into the hyphae of AMF, which possess the ability to go under the soil and adhere to different plants. Due to this ability, AMF has gained a lot of attention. Moreover, AMF requires less carbon in comparison to plants for photosynthesis as it has the pre-existing mycelium. However, agriculture permits only those AMF which have the potential to synthesize new ERM for their survival (Dodd 2000).

Soil microbes form the colonies over the root of higher plants and develop the symbiotic relation. Nodulating bacteria play a significant role during nitrogen fixation (such as rhizobia over legumes); symbiotic relation between the root and fungi is called mycorrhizae, which is essential for phosphorus uptake. Mycorrhizae are omnipresent in nature and can be isolated from any kind of soil. Chenopodiaceae, Cruciferae, Cyperaceae, Juncaceae and Proteaceae are the few main families which form the vascular system with the fungi (Bolan 1991). The adaptation of plant with surrounding microbes is stated as rhizosphere, which is the narrow region of soil nearby the roots (Johansson et al. 2004). Elevated microbial activity is observed in rhizosphere as seepage of organic constituents from the root takes place. The concept of rhizosphere has been extended on incorporating fungal constituents responsible for symbiosis, and it is termed as “mycorrhizosphere”. Thus, this progression in nature has directed us towards the traditional concentrated management to low-cost, sustainable crop cultivation (Johansson et al. 2004).

### **6.5.5 Effect of AM Fungi on Mycorrhizosphere Bacteria**

AM fungi directly and indirectly affect the bacteria colonizing on the roots of plants. Direct interaction involves the pH change which is stimulated by fungi and creates the competition for nutrient consumption. However, indirect interaction influences the growth of plant, soil texture as well as exudation from the roots (Mishra et al. 2016; Johansson et al. 2004).

### **6.5.6 Relevance of Mycorrhizosphere Interactions to Sustainable Agriculture**

AMF is considered to amend the phosphorus nutrition, and along with that it will extemporize disease resistance in the target plant. Thus, the mycorrhizal fungi are essential for sustaining the agriculture land where the nutrient inputs are very low. AM mycelia in collaboration with bacteria/fungi aid in mobilizing the nutrients (Johansson et al. 2004). In nodulating legumes, the N-fixing bacteria and AMF

work synergistically. The N-fixation gene in *Burkholderia* species that is present in the hyphae of AMF has been determined and advised that it may enhance N supply by fixing the atmospheric N for mycorrhizal plants.

### **6.5.7 Degradation of Pesticides by White-Rot Fungi and Its Relationship with Ligninolytic Potential**

The white-rot fungi comprise those fungi which have potential to degrade the lignin (polyphenolic polymer) present in the lignocellulosic substrate. White-rot got its name from the appearance of the wood which is infected by fungi, and leaching out of substrate during lignin removal causes the white-rot appearance. Basidiomycetes are most exploited white-rot fungi [13].

### **6.5.8 The Ligninolytic System of White-Rot Fungi**

White-rot fungi possess the ability to secrete more than one extracellular enzyme, having the potential to degrade the lignin. Along with these enzymes fusion of different processes will result in mineralization of the lignin, thus also known as the lignin-modifying enzymes (LMEs) (Orth and Tien 1995). These three enzymes are heme-containing glycosylated peroxidases, lignin peroxidase (LiP, E.C. 1.11.1.14) and Mn-dependent peroxidase (MnP, E.C. 1.11.1.13) and a copper-containing phenoloxidase, laccase (Lac, E.C. 1.10.3.2) (Thurston 1994).

The peroxidase enzyme synthesized during the white-rot has the ability to degrade different aromatic xenobiotics, which involve polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls, pentachlorophenol and group of pesticides (Bending et al. 2002). The ligninolytic enzyme has the ability to disintegrate the C-C as well as C-O bonds leading to depolymerization of the lignin, which subsequently leads towards cleaving of aliphatic/aromatic components (Hammell 1997). White-rot has been associated with Poly R-478 degradation to 3–5 rings of PAHs. The ability of ligninolytic enzyme for degrading mono-aromatic xenobiotic is still unclear (Bending et al. 2002). The chief objective of this experimentation was to evaluate the potency of white-rot fungi to disintegrate the mono-aromatic compounds and to establish the characteristic value of ligninolytic enzyme acquired from fungi to disintegrate the pesticides. Nine different types of fungi namely, *Agrocybe semi-orbicularis*, *Auricularia auricula*, *Coriolus versicolor*, *Dichomitus squalens*, *Flammulina velutipes*, *Hypholoma fasciculare*, *Phanerochaete velutina*, *Pleurotus ostreatus* and *Stereum hirsutum* having the white-rot traits were selected for investigation. The assessment was done to find the correlation between the potency of fungi in degrading the pesticides. Even the mechanism responsible for ligninolytic ability is still unknown (Bending et al. 2002).

### 6.5.9 Degradation of Atrazine by Soil Fungi for a Sustainable Environment

Microbial disintegration is the main process which provides the insight about the pesticide behaviour inside the soil (Singh et al. 2017a, b; Singh et al. 2016; Kumar et al. 2015). Various studies were conducted to observe the potential of pure isolates to degrade the  $^{14}\text{C}$ -ethylamino- and  $^{14}\text{C}$ -ring-labelled atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine). The chief objective of the experimentation was to uncover the potential of fungi isolated from the soil to degrade the atrazine. *Aspergillus fumigatus* is reported to degrade simazine, which on dealkylation leads to the formation of 2-chloro-4-amino-6-ethylamino-s-triazine. Successive catabolization of the intermediate molecule by the processes like dealkylation and dehalogenation leads to the synthesis of ammelide (Kaufman and Blake 1970; Kearney et al. 1965). A bioassay of oat seedling was done to assess the ability of fungi isolated from the soil to degrade the atrazine, simazine and propazine into non-toxic or medium toxic constituents. In another research, the cultures were inoculated into sandy loam of Lakeland procured in the unperforated pot. The culture solution inoculated was having different growth times as follows: 0, 3, 6, 12, 36 or 72 days. The composition of culture solution was 5 mg/l of atrazine, simazine or propazine dissolved in the basal medium. The comparison between culture solution containing s-triazine and sterile s-triazine was done for each culturing time. Whole experiment was executed in four replicas. After 3 weeks of growing period, the seedlings of oats were harvested, and their fresh weight was assessed and illustrated in percent to that of the sterile control. Basal medium comprising of  $^{14}\text{C}$ -ring- and chain-labelled ( $^{14}\text{C}$  ethyl) atrazine with the concentration of 5 ppm was inoculated with microbes and incubated at 24 °C inside a closed flask. Results were depicted as percent for initial radioactivity of  $^{14}\text{C}$ . Cells were collected, filtered and washed with 0.85% NaCl at the end. *A. fumigatus* was readily purified by the simazine, whereas partial detoxification of atrazine and minute detoxification took place in the case of propazine. Different fungal strains also showed the analogous result. As a conclusion for this study, microbes were able to disintegrate the herbicide s-triazine. On associating the result of in vitro study, N-dealkylation method was found as the effective mechanism for s-triazine detoxification by the fungi isolated from the soil (Kaufman and Blake 1970).

## 6.6 Conclusion

The exploration of the green endophytic growth of fungi persisting in the soil and water in symbiosis with marine creatures, functioning as nutrient translocation unit or the factory of synthesizing the bioactive molecules, is a major perspective. The exploitation is being done in far-fetched niches like remote ocean and hypoxic region. The microbes have become the part of our life, operating as bio-protector,

bio-fertilizers, bio-remediators as well as drug producers. Fungi being the conventional fermentation workhorses, for the processes like brewing, baking and synthesizing the antibiotics, enzymes, organic acids and various other pharmaceutical products, are economically important. Thus, fungi have become the frontiers of molecular biotechnology. These micro-sized eukaryotes have become the vital model for understanding the basic science and synthesizing the commercial product. Genomes of yeast act as platform for functional genomics as well as the DNA microchip method. In coming time, the researchers will keep on exploiting these filamentous fungi and yeast to gain knowledge about their physiology, different biochemical pathways, mechanism of developing resistance and production of secondary metabolites in order to build a strong foundation for developmental research.

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