LITERATURE REVIEW

2.1 Medicinal Mushrooms

Since ancient times mushrooms has been consumed as food by man. Initially, it was probably due to the pleasing flavor and texture that was so attractive. Besides that in some societies their use was limited to royalty only and for well over two thousand years, some species of mushroom had also been used as medicinals and tonics. In modern times the cultivation of mushroom has steadily increased with the annual production where 4.27 million merits of mushrooms was consumed in an era in which people have become more concerned about human nutrition (Philip and Shu, 1997).

In China, there is a long history of the usage of many mushroom species as medicinal and tonics. Modern studies of Chinese medicines have succeeded in isolating and identifying compounds from many of these mushrooms and such compounds have been proven to be beneficial in the treatment of certain ailments. Furthermore, an active area of modern research involves the search in mushroom species for compounds that can be used in the treatment of various cancers, cardiovascular disease, viral disease, etc (Philip and Shu, 1997).

In Japan, about 1500 species of mushroom are now known, of which 300 are edible and 1200 are inedible, with 50 being toxic. A number of inedible mushroom have bitter and pungent tastes, especially those belonging to the *Polyporaceae* which have been used as medicinal drugs such as anticancer in China since ancient times. Little attention has been paid to the chemical constituents of inedible mushroom in Japan, except for the poisonous species (Makiko *et al*., 2002).

Mushrooms play an extremely important role in the world. The usefulness to man as food, tonics, medicinal and also in the bioconversion of organic materials to the forms that can enter the major nutrient cycles are all of great benefit to both man and nature (Philip and Shu, 1997).

2.2 Introduction to the Fungi Kingdom

Fungi are usually regarded as plants, yet they appear suddenly and mysteriously, often overnight, out of the soil or from tree trunks. Their sudden appearance has, in the past, provoked talk of "spontaneous generation". This old theory, common before the work of Pasteur, stated that microorganisms were capable of appearance of fungi, however, has nothing to do with magic. What we see as the fungus is only a fruiting body–one stage of a complex life history that takes place mostly underground (Ian, 1977).

The damp of autumn or the warmth of spring is usually adequate to trigger fungi into producing their fruiting bodies, giving a sudden "flush" of fungi at these times of the year. They appear in virtually every colour, from snow–white to flame–red and lemon–yellow to vivid orange; there is certainly no shortage of brown or ochreous, grey, black or violet shades. Blue and green mushrooms are rare and come in rather pale hues, usually grayish–blue where the former are concerned and tending to olive–green in the latter (Ian, 1977).

2.2.1 History and Discovery

Mycology, derived from Greek work, Mykes which means mushroom and logos means discourse. Etymologically, it is the study of mushroom. And indeed that was how mycology began in the dim past. With the invention of the microscope by Antonio Van Leeuwenhoek in the 17th century the systematic study of fungi began (Alexopoulos and Constantine, 1996). The man who deserves the honor of being called the founder of the science of Mycology is Pier' Antonio Michelli, the Italian botanist who in 1729 published Nova Plantarum Genera in which his researches on fungi were included (Alexopoulos and Constantine, 1996).

Nowadays, there are many scientists, botanist and biologist studying the fungi. Many new discoveries are repeated everyday. Many people have a very negative concept of fungi as they are disease-causing organisms. Although this impression is not entirely wrong, fungi are so much more than that. They are also beneficial organisms. A number of useful antibiotics are produced by them, including the wonder drug "penicillin". Without fungi we would not have leavened bread, cheeses, beer, wine and other alcoholic beverages (Idaya, 2001).

2.2.2 Importance of Fungi

Although the biggest use of fungi was traditionally for the gastronomic and nutritional appeal, there has always been interest in certain fungi for their tonic or medicinal attributes. This interest increased greatly in the 1970's and much research was done to determine the validity of claims of medicinal properties and the natural of the action of compound present in these fungi. In the past decade this research has accelerated and the products of importance have been marketed in larger amounts (Philip and Shu, 1997).

For example, in 1991, fungal products marketed for medicinal or tonic properties were estimated to be US$1.2 billion. This was based on the sale value by the products from *Coriolus, Ganoderma, Lentinula, Schizophyllum* and a few other mushrooms (Chang *et al*., 1993).

In the same year fungi produced as food had an estimated value of US$8.5 billion. Thus, one can see that the value of mushroom products was not at insignificant amount (Philip and Shu, 1997).

Actually, the study of medicinal mushrooms through the last three decades has proven its many beneficial outcomes and has been followed by the rapid development of manufacturing businesses dealing with commercial cultivation of mushrooms. In 1999, world production of mushrooms amounted to US$18 billion, roughly equal to the value of coffee sales (Solomon, 2002).

2.3 Fungal Systematics

Fungi represent a heterogenous group i.e. they are polyphyletic. While mycologist have learned a great deal about the fungi in last 30 – 35 years, there was still no agreement as to how the best to classify the fungi nor will there likely be any agreement at a later time (Idaya, 2001).

The fungi comprising the phylum Basidiomycota are commonly known as basidiomycetes. Basidiomycetes are a large and diverse lot and include forms commonly known as mushrooms, boletes, puffballs, earthstar, stinkhorns, bird's–nest fungi, jelly fungi, bracket or shelf fungi, rust and smut fungi (Tan, 2001).

Basidiomycetes are characterized primarily by the facts that they produced their sexual spores, termed basidiospores, on the outside of a specialized, microscopic, spore–producing structure called the basidium. The basidiospores of most species are balisopores and are discharged forcibly from their basidia by mean of an elaborate discharge mechanism (McLaughlin *et al*., 1985).

Basidiospores form after plasmogamy, karyogamy and meiosis. The last two processes occur in the basidium and four basidiospores typically are produced on each basidium. Although haploid, basidiospores may be uninucleate at maturity. Basidiospores exist in various sizes, shape, colors and may be either thin or thick walled and smooth or ornamented (Tan, 2001).

The morphology of the basidium is variable. Until recently the morphology of the basidium was believed to be a key to determine the relationship of the basidiomycetes. Basidial morphology is one of the basic characteristic for fungi classification. However, rRNA sequence analysis (Eric and John, 1993) of septal pore morphology and cell wall biochemistry have determined that far to much emphasis was placed on this characteristics and all members of the basidiomycetes that produce basidiocarps are now included in single class, the Basidiomycetes and the morphology of basidiocarps and basidium are characteristics that are now used to classify fungi into the various orders of this class (Idaya, 2001).

2.3.1 *Aphyllophorales order*

The species in this order are often coriaceous, leathery to woody, but may also be fleshy. The basidiocarps and hymenia are more variable than in the *Agaricales.* As in the case of the *Agaricales*, the basidiospores are forcibly ejected from basidium to be dispersed by wind (Idaya, 2001).

The order *Aphyllophorales* is an important group of Basidiomycota comprising 1200 species. Without microscopic examination it is sometimes difficult to recognize a thin appressed basidiocarp. On the other hand, many species produce conscious basidiocarps that could be visible even from a distance. Forms with more obvious basidiocarps are known as pore fungi, tooth fungi, bracket fungi, shelf fungi and beef steak fungus. These common names are based on various shaped. Usually large basidiocarps and hymenophores, the tissues are directly supporting the hymenia. However, many interesting species go unnoticed on the underside of dead wood.

A few species of Aphllophoralean fungi are edible, including the basidiocarps of the *Fistulina hepatica* (beef steak fungus, reported to taste as well as look like beef); *Sparassis crispa*; *Albatrellus* sp.; *Grifola frondosa*; *Hericium ernaceum* (bear’s head fungus) and *Laetiporus sulphureus* (sulfur fungus, which incidentally has been reported to sicken some individuals). “Tuckahoes”, the underground tuberlike sclerotia of *Wolfiporia cocos*, are eaten by Native Americans. Sclerotia of other species including *Polyporus tuberaster*, the Canadian tuckhoe, also have been used as food.

The order *Aphyllophorales* is very large and heterogenous. It is clear that it is not monophyletic and while progress is being made on the systematic of the group, a major revision is not yet possible. The Friesian system was used to describe “polypores”, “corticoids” or “hydnaceous” forms, but these former taxonomic names are now serve merely as descriptive terms for convergent basidiocarp and hymenophore morphology (Alexopoulos and Constantine, 1996).

2.3.2 *Polyporaceae* Family

The largest, most diverse group of poroid *Aphyllophorales* is *Polyporaceae*. There are over 700 species that vary widely in microscopic structure, decay characters and chemical staining reactions. The family is one of those that have been called a “chaotic mass”, one that is admittedly artificial and holds many forms but not yet removed and placed in monophyletic groups. Hyphal construction may be mono-, di-, or trimitic and both white-and brown-rotters were placed in the family. The basidiospores are hyaline and usually lack ornamentation.

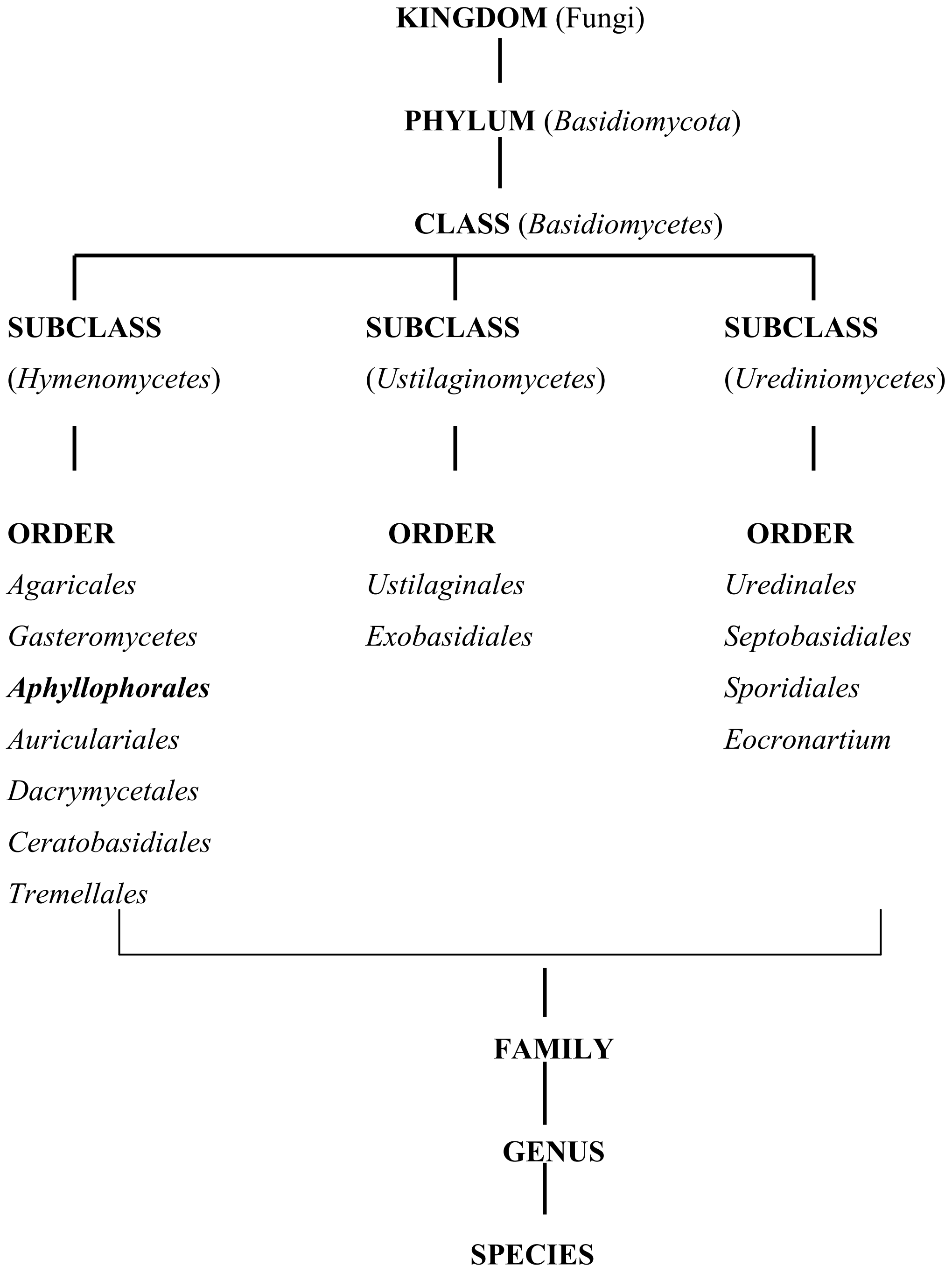
The fruiting bodies in *Polyporaceae* species may resemble crusts, shelves or even mushrooms. The shelf and mushroom-like forms are, of course, much more conspicuous than the resupinate or effused-reflexed ones. The basidiocarps of some may be soft and pliable when young, but at maturity most are tough, leathery, corky or woody. Although the hymenium is lamellate in a few species, more typically the basidia line the inner surface of tubes. The mouths of the tubes may be circular or angular or even elongated (Tan, 2001).

Although the family is artificial, there has been emphasis on the delimination of monophyletic genera. For example, the type genus *Polypores* has been reduced to include only those species with stipitate basidiocarps having a dimitic hyphal system with binding hyphae and producing white rot. Segregates have been placed in many genera including *Nigroporus, Fomes, Pycnoporus* and *Rigidoporus* within the family. *Poria* is another large genus that is currently undergoing dissection (Tan, 2001).

An enormous amount of morphological data on many genera of *Polypores* also had been provided by Corner. In the introduction to the order *Aphyllophorales,* it mentioned *Laetiporus, Fomes, Grifola* and *Wolfiporia*, all members of *Polyporaceae*. The most common species of *Polyporaceae* is probably *Trametes versicolor*, a white–rotter of dead hardwood. Although basidiocarp morphology may vary from collection to collection, most fresh specimens have a velvety pileus surface with distinctive concentric zones of brown, gray or reddish colors. Known commonly as turkey tails, the often imbricate (overlapping) fruiting bodies were sometimes used in floral arrangements (Pegler, 1997).

Polyporales have 23 families, which include Polyporaceae and Albatrellaceae. There are 64 genus of Polyporaceae and one genus of Albatrellaceae. Among the genus of Polyporaceae, there are 3318 species of *Polyporus*, 809 species of *Trametes*, 450 species of *Microporus* and 10 species of *Pycnoporus*. Among the genus of *Albatrellaceae*, there are 32 species of *Albatrellus* (Dictionary of Fungi, 2001).

2.4 Basidiomycetes Systematics



(Source: Dictionary of Fungi, 2001)

2.5 Importance of Basidiomycetes

Basidiomycetes produce a large number of secondary metabolites which have antibacterial, antifungal, antiviral, cytotoxic and hallucinogenic activity or which can be a source of plant growth regulators or flavours (Inmaculada *et al*., 2000). Beside these, it is well known that higher Basidiomycetes have a wide spectrum of therapeutic and prophylactic properties (Shamtsyan *et al*., 2004).

In fact, several compounds that inhibit the growth of a large spectrum of saprophytic and phytopathogenic fungi have been isolated from basidiomycetes. Furthermore, these fungi are able to inhibit the development of bacteria, actinomycetes and other fungi in their microhabitat, indicating that the antimicrobial substances produced by them have important ecological implications (Rosa *et al*., 2003). Basidiomycetes also are known to produce a series of biologically active compounds. Recently from the wood-inhibiting fungus *Aporpium caryae*, two indole metabolites, possessing antifungal activity, have been isolated (Giorgio *et al*., 2002).

Aqueous extracts of fruiting bodies and mycelium of various higher basidiomycetes have been studied for reliable biological effects (Shamtsyan *et al*., 2004). This is because mushrooms contain large amounts of well–balanced essential amino acids. Dietary fibers which are abundant in the tissue of all mushrooms absorb bile acids or hazardous materials in the intestine and thus decrease carcinogens and other poisons. The overall harmonizing effect of a diet balanced with mushroom, which is so highly praised by the ancient Chinese, is not a myth, but is being continually supported by modern scientific investigations (Solomon, 2002).

Several other healths–promoting effects of the mushrooms should not be overlooked. Not only polysaccharides and triterpenoids are known as biologically active compounds; wide range of substances from high basidiomycetes that belong to different classes of chemical compounds had been described and their medicinal properties were also evaluated. These substances represented glycolipids (schizonellin), compounds derived from the shikimic acid (strobilurins and oudemansins), aromatic phenols (drosophilin, armillasirin, omphalone), fatty acid derivatives (filiboletic acid, podoscyphic

A–H), nucleosides (clitocine, nebularine), different sesquiterpenes (protoilludanes, marasmanes, hirsutanes, caryophyllanes, etc.), diterpenes (cythin, striatal), sesterterpenes (aleurodscal) and many other substances of different origin (Solomon, 2002).

The bioactive substance from higher basidiomycetes posses antifungal, antibacterial and antiviral properties. In medicine they are used to immunomodulate both humoral and cellular immune factors in the body. For an example, polyfunctional acidic glucuronoxylomannan isolated from *Tremella* sp., for instance, stimulated vascular endothelial cells, possessed pronounced anti-radiating effects, stimulated hematogenesis, demonstrated antidiabetic, anti-inflammatory, hypocholesterolemic, anti-allergic activities and had hepatoprotective effects. It could be recommended to improve immunodeficiency, including that induced by AIDS, physical stress or aging and it prevented senile degeneration of micro vessels, maintaining better blood perfusion conditions in vital organs (Solomon, 2002).

By the way*, Polyporus* species are basidiomycetes belong to the Polyporales, which are wood-rotting fungi. They grow on living trees or dead wood and possess exoenzymes that will degrade cellulose and lignin. Many of them, fruiting bodies collected from host trees or grown in liquid cultures, have been chemically investigated and yielded alkaloids and quinines, linear compounds, alkaloids, pyrones and terpenoids (Gabriela *et al*., 2002).

In nature, there are notorious wood decaying basidiomycetes fungi including *T. versicolor*, *Heterobasidion annosum* (syn. *Fomes annosus*), *Ganoderma applanatum* and *Pycnoporus cinnabarinus* to name a few (Watanabe *et al*., 2003).

2.5.1 Distribution of Basidiomycetes in Malaysia

Most of the fungi species remaining to be described are probably to be found in the tropics because of the vastness of the tropical area, the number of unexplored habitats there and the existence of a latitudinal biodiversity gradient with the tropics richer in taxa. The under explored tropical rain forests represent the richest ecosystem on earth in term of the variety of micro–habitats, individual genomes present and morphological diversity.

Besides the great tropical richness in ecosystems through climatical and topological opportunities and since superstructures of species were no robust, different theories have been advanced to explain the reason for the taxonomic richness in the tropics (Moncalvo and Ryvarden, 1997).

We can find a variety species of fungi in Malaysia as we have tropical-rain forest. The monsoon, climate, temperature and rainfall ensure the good growth of the fungi. Many species of basidiomycetes can be found at Gunung Mulu, Sarawak, Gunung Kinabalu, Sabah and other mountains in Peninsular Malaysia as the cloudy and moisture environment enhance the fungi to grow well.

Wood decay fungi belong to the *Aphyllophorales* were recorded and collected from six sites in Peninsular Malaysia which included: forest reserves at Forest Research Institute of Malaysia (FRIM) Kepong, Pasoh and Jeram Lenang; and plantation forests at Kemasul, Mata Ayer and Ulu Sedili. Fungi were described and identified based on cultural characteristics, ecology and competition studies. Their ability to degrade three timbers was also studied. A total of 327 specimens of wood degrading fungi comprising 52 species were recorded from the forest sites (Salmiah and Thillainathan, 1998).

The most common species recorded, based on the abundance of basidiomes in nature were: *Earliella scabrosa, Trametes feei, Lentinus sajorcaju, Lenzites elegans, Microporus affinis, Microporus xanthopus, Nigroporus vinosus, Pynoporus sanguineus* and *Schizophyllum commune*. Similarity indices showed the highest species diversity at Pasoh Forest Reserve. Comparison between forest reserve and plantation forests showed that there were greater species diversity in natural forests and lowest in plantation forests. Factors considered to affect the abundance of fungi studied were the type of forests (especially the tree species and forest management) and environmental conditions (Salmiah and Thillainathan, 1998).

Beside, less than 20 species of macro fungi were encountered from the limestone slopes of Tasik Dayang Bunting or the sandy beach areas of Tanjung Rhu compared to over 50 species on the more humid, moist and forested slopes of Tanjung Kuala Teriang.

Daldinia concentrica, Calvulina gracilis, Fomes lignosus, M. xanthopus, P. sanguineas, S. commune, T. versicolor and *Auricularia polytricha* were found often abundantly, in all three areas (Idaya, 2001).

In Langkawi the fungus was evidently capable of tolerating salt and desiccation and it was found on many different types of fallen wood including coconut trunks and Casuarina branches that were exposed during low tides and submerged at least during high tides. The moist forest floor at Tanjung Kuala Teriang was also rich in clavaroid fungi particularly *Clavaria fossicola* and *C. gracilis*. In addition to the more transient soft – fleshed basidiomycetes, Tanjung Kuala Teriang was also rich in the woody members of *Polyporaceae* and *Hydnaceae*. The sporophores of particularly the Polyporaceae were several layered thick indicating their perennial nature. There was some species diversity among the agarics encountered (Kuthubutheen, 1981).

2.6 Alternative Medicine

The uses of plants in the indigenous cultures of developing countries are numerous and diverse. For many people they still form an important economic basis and are used in medicine (Kool *et al*., 2005).

Plant are not only important to the millions of people to whom traditional medicine serves as the only opportunity for health care and to those who use plants for various purposes in their daily lives, but also as a source of new pharmaceuticals (Hugo *et al*., 2005). Ostermayer (1993) concludes that 21% of the people that went to public health services in Dar es Salaam, the capital of Tanzania, consulted a traditional healer before doing so (Kool *et al*., 2005). Samuelsson (1999) also concluded that traditional medicine has provided western medicine with more than 40% of all pharmaceuticals.

Alternative medicine is frequently used to prevent or treat many human diseases including cancer. According to a review, alternative therapies were immensely popular and their prevalence in the general population is 40% in the US, 50% in Australia and 65% in Germany. In Asian, African and South American countries the prevalence of alternative methods, especially phytotherapy may be higher than in the above-mentioned countries because of the widespread use of the traditional medicine. Among the alternative therapies, the use of mushroom teas was one of the most popular and millenary practice, especially in Asian countries (Kaneno *et al*., 2004).

Fungi are an important source of materials in traditional Chinese medicine. Polysaccharide extracts from many species of fungi exhibit immunostimulating and/or anti-tumor activities. For instance, *Ganoderma lucidum* (Lingzhi) was a number of the fungus family (*Polyporaceae*) that naturally grows on fallen trees and logs of other broad leaf trees. The used of Lingzhi as a longevity- and vigor-promoting “magic herb” dates back more than 2000 years in China. Scientific investigations have repeatedly confirmed Lingzhi’s beneficial effect on health in general; it is now frequently promoted as an effective agent against cancers in the Pacific Rim areas, such as China, Japan, Korea and other Asian countries (Shao *et al*., 2004).

So, mushrooms were well known for their nutritional and medicinal value and also for the diversity of bioactive compounds they contained. The mushroom *T. versicolor* (Yun Zhi) was recorded in the Compendium of Materia Medica by Li Shi Zhen during the Ming Dynasty in China, as being beneficial to health and able to bring longevity if consumed regularly. Various products derived from this mushroom and claimed to have medicinal value are commercially available (Ng, 1998).

Recently, peptide–bound and protein–bound polysaccharides with immunomodulatory and antitumor activities have also been purified from other mushrooms, for example, *Tricholoma* species (Ng, 1998). Some study also demonstrated that polysaccharides extracted from the fruit bodies, mycelium and spores of *G. lucidum* can promote the function of macrophages, B cells, T cells as well as dendritic cells (Shao *et al*., 2004). Thus, *G. lucidum* is one of the medicinal mushrooms that have antidiabetic, antioxidant, immunomodulatory, antitumor and antimetastatic activities. However, very few studies on its protein components exist (Wang and Ng, 2005).

Directly, edible and medicinal mushroom (macrofungi), as the most popular alternative medicine, not only can convert the huge lignocellulosic biomass waste into human food, but most remarkably can produce notable mycopharmaceuticals, myconutriceuticals and mycocosmeceuticals (Solomon, 2002). The most significant aspect of mushroom cultivation, if managed properly, is to create zero emission of lignocellulosic waste materials. Mushroom biotechnological products have multi beneficial effects to human welfare (e.g. as food, health tonics, medicine, feed, fertilizers and to protect and regenerate the environment). Pharmaceutical substances with potent and unique health–enhancing properties have been isolated from medicinal mushrooms and distributed worldwide (Solomon, 2002).

Of course, many of them are pharmaceuticals products, while other are present with a novel class dietary of supplements or "nutraceuticals." Several antitumor polysaccharides, such as hetero-glucans and their proteins complexes (e.g. xyloglucans and acidic-glucan containing urinic acid) as well as dietary fiber, lectins and terpenoids, have been isolated from medicinal mushrooms. In Japan, China, Russia and Korea, several different polysaccharides antitumor drugs have been developed from fruiting bodies, mycelia and culture media of various medicinal mushrooms, such as *Lentinus edodes* (shiitake), *G. lucidum* (reishi), *T. versicolor* (turkey tail), *S. commune* (split gill), *Phellinus linteus* (mulberry yellow polypore) and *Inonotus obliquua* (chage or cinder conk) (Wang and Ng, 2005).

Obviously, the potential of medicinal mushrooms was enormous but mostly untapped. It could and should evolve into a successful biotechnological industry for the benefit of humankind (Solomon, 2002).

2.6.1 Antifungal Agents

Antifungal agents have a wide application in human medicine, agriculture and veterinary medicine. Five major classes of systemic antifungal compounds were currently in clinical use: the polyene antibiotics, the azole derivatives, the allylamines and thiocarbamates, the morpholines and the nucleoside analogs (Gupte *et al*., 2002).

Among the different types of drug prevailing in the market, antifungal antibiotics are a very small but significant group of drugs and have an important role in the control of mycotic diseases (Gupte *et al*., 2002). The first member of the group of polyene macrolide antifungal antibiotics, tentatively named fungicidin, was discovered by Hazen and Brown and was later renamed nystatin (Gupte *et al*., 2002).

Antifungal peptides and proteins have great economic implications because they protect crops from the devastating damage brought about by fungal infections (Ng, 2004). Antifungal agents now constitute 15 – 16% of the total activity in the infective area and the world market for the antifungal is reported to be expanding at a rate of 20% per annum (Gupte *et al*., 2002).

Antifungal proteins and peptides have been isolated from a vast number of animals, plants and fungi. Despite the common role of defense against fungal pathogens, antifungal proteins were in fact an assembly of structurally diverse proteins (Guo *et al*., 2005). Antifungal proteins have been intensively studied because they have the potential of protecting organisms from the deleterious consequences of fungal attack (Wang *et al*., 2004).

In crops the economic implications were substantial. Animals and plants produce a host of antifungal peptides and proteins with a spectacular diversity of structures, including thaumatin-like proteins, embryo-abundant proteins, allergen-like peptides, chitinases, glucanases, miraculin-like proteins, cyclophilin-like proteins, protease inhibitors, peroxidases, lectins, arginine- and glutamate-rich proteins, ribosome inactivating protein and peptides, ribonucleases and deoxyribonucleases (Wang *et al*., 2004).

In contrast to the large number of aforementioned antifungal proteins isolated from animal and plants, only a few mushroom antifungal proteins were known to date, including those from the shitake mushroom *L. edodes*, *Lyophyllum shimeiji*, *Pleurotus eryngii* and *Polyporus alveolaris* (Patrick *et al*., 2005). An antifungal protein was isolated from the mushroom *Tricholoma giganteum* var. golden blessings exhibited antifungal activity against *F. oxysporum* (Guo *et al*., 2005).

Nowadays, the antifungal drugs that are routinely used for treatment of deep mycoses include the polyenes, azoles and the newest class, the echinocandins. An additional drug that sees infrequent use, primarily for life threatening yeast infections, is flucytosine (5–fluorocytosine). For superficial mycoses, particularly infections cause by dermatophytes, azoles, allylamines and griseofulvin are most commonly used (Richard, 2005).

The unfortunate news, however, was that with the exception of two or three “me–too” azole and echinocandins drugs currently in late–stage development for use in humans, there were no new antifungal agents or classes of compounds described in the primary or patent literature that hold promise of advances in the treatment of mycoses. As such, the drugs that are available today are likely to be the only choices for the foreseeable future (Richard, 2005).

So, there was an acute need to develop new drugs to treat fungal infections. However, the urgency stems from the therapeutic limitations of the two current antifungal drug classes, polyenes (such as amphotericin B) and azoles (such as fluconazole and itraconazole), which display toxicity and a limited spectrum of efficacy, respectively (Jiang *et al*., 2002).

As example, amphotericin B was used in the treatment of serious disseminated dimorphic fungal and yeast infections, caused by *Blastomyces, Candida, Cryptococcus* and *Histoplasma* spp. However, it causes nephrotoxicity, reduction of renal blood flow, vomiting and anorexia. Nystatin, although too toxic for systemic use, was mainly applied topically in cases of mucous membrane candidiasis. Thus, a number of factors affect and limit the use of some of the existing antifungal antibiotics, which necessitates a search for newer antifungal antibiotics to control existing problems (Gupte *et al*., 2002).

The need for new, safe and more effective antifungal is a major challenge to the pharmaceutical industry today, especially with the increase in opportunistic infections in the immunocompromised host. The history of new drug discovery processes shows that novel skeletons have, in the majority of cases, come from natural sources. This involves the screening of microorganism and plant extracts, using a variety of model (Gupte *et al*., 2002).

From the fruiting bodies of the edible mushroom, *L. edodes*, a novel protein designated lentin with potent antifungal activity was isolated (Ngai and Ng, 2003). Lentin, which had a molecular mass of 27.5 kDa, inhibited mycelial growth in a variety of fungal species including *Physalospora piricola*, *Botrytis cinera* and *Mycosphaerella arachidicola* (Ngai and Ng, 2003). The isolation of a protein with inhibitory activity toward these fungi has important implications for human health (Wang and Ng, 2005).

2.6.2 Antitumor Agents

Only at the end of the 1069, Eastern and Western scientists started to investigate the mechanisms of the health effects of mushrooms. The first successful research discovered the antitumor effects of hot water extracts from several mushroom species. The main active components was proved to be polysaccharides, specifically D-glucans. Chihara (1969) isolated from the fruiting bodies of shiitake water–soluble antitumor polysaccharide, which was named “lentinan” after the generic name of this mushroom. It was a major discovery. Lentinan demonstrated powerful antitumor activity; preventing chemical and viral tumor development in mice and experimental models (Solomon, 2002).

It has been known for many years that most mushrooms with known effects against cancer belong to the family *Polyporaceae.* However, the components responsible for such action have not been clearly defined. In Japan, in 1968, a hot water extract from some edible mushrooms belonging to the *Polyporaceae*, showed a marked host-mediated antitumor activity. Since then, numerous researchers have isolated active polysaccharides and have identified them to be 1-3-beta-D-glucopyranans with a 1-6-beta-D-glucosyl branch containing protein. Several antitumor polysaccharides, some hetero-beta-glucans and their protein complexes, such as xyloglucans and acidic beta-glucan containing uronic acid, are insoluble in water. Several trials have been made to enhance activity by chemical modification, such as polyalcohols formed by a mild Smith degradation and the products formed by BH4-reduction after 104-oxidation. Mushroom polysaccharides are considered to be biological response modifiers (BRM) or immunopotentiators, because of their action mechanism (Mizuno, 1999).

Polysaccharides displaying remarkable antitumor activity *in vivo* (that is in screening studies against sarcoma 180 in mice using intraperitoneal or oral methods of administration) have been isolated from various species of mushrooms belonging to the order *Auriculariales, Tremellales, Polyporales* and *Gasteromycetales* (Solomon, 2002).

In the 1970's and 1980's, three polysaccharides with antitumor properties were isolated and develop from *L. edodes* and *Coriolus versicolor* (Ooi *et al*., 2000). An anticancer substance was prepared from cultured mycelia of *C. versicolor*. Mycelial extract was composed of 10.5% protein and 42.2% polysaccharide (containing of L–glucose, mannose and xylose). It showed that polysaccharides have anti–tumor activity (Lee *et al*., 1992). In addition, krestin from mycelium of *T. versicolor* or *C. versicolor* also have been developed from medicinal and edible mushroom in Japan (Mizuno, 2000).

On the other hand, hypoglycaemic and antitumor activities of some heteroglycans of *G. lucidum* fruit bodies were reported (Hikino and Mizuno, 1989). The application of modern analytical techniques have revealed the presence of numerous bioactive compounds including polysaccharides, anticancer and antitumor properties that appear to be based on an enhancement of the host immune system rather than a direct cytocidal effect (Chang *et al*., 1999).

There has been an increase in the level of commercial interest in *G. lucidum* products, with the market value of this fungus in 1995 in the region of US $1.6 billion. The increased demand has stimulated improvements in artificial cultivation methods (Chang *et al*., 1999). Recent advance in research of pharmacology and clinical application of *Ganoderma* spp. have been done. The pharmacological properties of *Ganoderma* sp. were included effects on brain ischemic/reperfusion injury, hyperlipaemia, antitumor–drug induced toxicity and immune function, antitumor, anticarcinogenic and antiaging properties. Clinical studies demonstrating effects on the immune system and reproductive system and against chronic viral hepatitis were briefly described (Liu, 1999).

2.6.3 Enzymes Activity

Lignin peroxidase has been reported to be produced by numerous species of microorganisms. However, ability to decompose lignin peroxidase is much less among bacteria then among fungi (Kalaiwaney, 2001).

Lignin peroxidase has been isolated and characterized in most of the white rot fungi such as *Phanerochaete chrysosporium, Phlebia radiata* and *Phlebia tremellosa. Bjerkandera* sp. strain BOS55, a newly isolated wild–type white rot fungus can produce lignin peroxidase (LiP) in nitrogen (N)–sufficient, glucose–peptone medium. *Pleurotus ostreatus* (Akhamedova, 1996) is a common fungus used in research of lignin peroxidase, which has been reported to form two kind of lignin peroxidase (I and II) detected by isoelectrofocusing (Kalaiwaney, 2001).

The enzymes have potential applications in wood–pulp processing, detoxification of chloroaromatic environment pollutants, assay for such contaminants and also potential uses in household products.

Lignin ranks second, after cellulose, in abundance in the biosphere as a renewable organic compound. The biodegradation of lignin was a rate-limiting step in the carbon cycle. Ligninolytic enzymes were highly non-specific on account of the complex structure of lignin and can be used in the degradation of structurally different environmental pollutant. Laccases form a class of ligninolytic enzymes that are phenol oxidase capable of catalyzing one-electron oxidation of aromatic substrates and the concominant reduction of oxygen to water. Laccases can also act on non-phenolic lignin subunits in the presence of readily oxidizable primary substrates, which are electron-transfer mediators (Ng, 2004).

The ligninolytic system of fungi that cause white-rot has been extensively studied in recent years, especially with respect to their enzymatic potential for the bioremediation of persistent pollutants. White-rot fungi and their ligninolytic enzymes have the potential to detoxify toxic xenobiotic compounds by partial degradation or complete mineralization (Rothschild *et al*., 2002). Among the many basidiomycetes, only few have been successfully utilized in bioremediation endeavors.

As a conclusion, fungal oxidases and peroxidases have been suggested to play a key role in lignin degradation. Manganese-dependent lignin peroxidase (MnP), versatile peroxidase (VP) and laccase (Lac) have been reported to be synthesized by many white-rot fungi. Lignin peroxidase (LiP) activity, on the other hand, has only been reported in few of them (Rothschild *et al*., 2002). Engineering of these proteins may lead to a higher potency of the desirable activities and minimization of the undesirable activities (Ng, 2004).

2.7 Culture Media for Fungi

The media for growing fungi contain a carbon source (usually glucose though a host of other substrates may be used), nitrogen source (usually ammonia or nitrate but often an amino–acid), phosphate, sulphate, magnesium, potassium and the trace elements: iron, manganese, zinc, molybdenum and copper. In addition to and sometimes in place of, these chemically defined constituents, complex natural materials were often added to the medium; these include corn–steep liquor, yeast extract, vegetable juices and protein hydrolysates (Connie and George, 1998).

2.7.1 Aqueous Culture System

In aqueous cultures, secondary metabolites accumulate both in the medium and in the mycelium. For related compounds, the distribution between medium and mycelium can often be correlated with water–solubility, though this apparent correlation may be a result of some other factor such as ease of transport across cell membranes (Connie and George, 1998).

2.7.2 Surface Culture System

When a nutrient medium was inoculated with fungal spores or mycelium, the mycelium grows over the surface of the liquid to form what was variously referred to as a felt, a pad or a mat. This form of cultivation was the simplest and cheapest but suffers from several disadvantages (http:// biology.about.com).

Growth was not homogeneous so that the felt contains mycelium at various stages of development and in a variety of environments that at the surface of the felt was under more aerobic conditions than that at, or below, the surface of the medium, while that in contact with the medium is better provided with nutrients. Moreover, since nutrient taken up by the mycelium is only slowly replaced by diffusion from the lower parts of the medium, a gradient is set up within the medium so that the process was inefficient in terms of nutrient uptake and therefore relatively slow compared with the submerged techniques.

The main use of surface culture was in screening where the non-homogenous mycelium becomes an advantage in that, if an organism has the ability to produce a given compound, some of the mycelium should be at the right stage and in the right environment for its production. The slowness of the process, too, becomes an advantage, increasing the chance of sampling at the right time.

2.7.3 Agitated Culture System

For physiological or biochemical experiments or for the large-scale production of fungal products, submerged conditions, either shaken or stirred, are always preferred, though they are often extremely difficult to obtain the production of a desired metabolite, known to be produced in surface culture and submerged cultures.

In this technique the medium is shaken after inoculation with spores or mycelium so that growth occurs in the body of the liquid. The advantages over surface cultures are that nutrient uptake is more efficient, giving more rapid growth that is more homogeneous. Even in shaken or in stirred aerated conditions it may be difficult to obtain sufficiently homogeneous growth for physiological experiments, especially in those organisms whose mycelium tends to form pellets rather than loose aggregates (conditions at the center of a pellet will be very different from those at the surface) (Solomon, 2002).

The additional advantage of submerged culturing is the fact that most medicinal mushrooms do not produce fruiting bodies under commercial cultivation. Reliable industrial cultivation techniques are known for only 37 mushroom species, but medicinal mushrooms included many mycorrhizal or parasitic species that needed several years for development of normal fruiting bodies on trees. Such species could not be grown commercially, but their mycelia could be grown easily and economically with the help of submerged culturing (Solomon, 2002).

Mushroom is an abundant source of a wide range of useful natural products with biological activities. Since the field cultivation of mushroom takes several months to yield the fruiting body with a low productivity of bioactive compounds, submerged cultivation of mushroom is viewed as a promising alternative for producing valuable substances. However, there have been few investigations on the bioprocess development of mushroom submerged fermentation (Mao *et al*., 2005).

As a conclusion, to obtain bioactive polysaccharides from mushrooms, most investigators have spent their efforts cultivating edible or medicinal mushroom on solid artificial media (for fruit body production) rather than in submerged cultures (for mycelial extract and/or exopolysaccharide production). Submerged cultures obviously have the potential for higher mycelial production in a compact space and in shorter time with fewer chances for contamination (Lee *et al*., 2004).

2.7.4 Solid Substrate Fermentation

Solid substrate fermentation (SSF) holds tremendous potential for the production of enzymes and bioactive agents. It can be of special interest in those processes where the crude fermented product may be used directly as the enzyme source. In addition to the conventional applications in food and fermentation industries, microbial enzymes have attained significant role in biotransformations involving organic solvent media, mainly for bioactive compounds.

Some of the substrates that have been used included sugar cane bagasse, wheat bran, rice bran, maize bran, gram bran, wheat straw, rice straw, rice husk, soyhull, sago hampas, grapevine trimmings dust, corncobs, coconut coir pith, banana waste, tea waste, cassava waste, palm oil mill waste, aspen pulp, sugar beet pulp, sweet sorghum pulp, apple pomace, peanut meal, rapeseed cake, coconut oil cake, mustard oil cake, cassava flour, wheat flour, corn flour, steamed rice, steam pre – treated willow, starch, etc. wheat bran however holds the key and has most commonly been used, in various processes (Biesebeke *et al*., 2002).