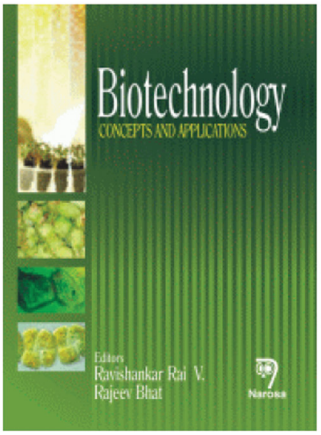

Biotechnology: *Concepts and Applications*

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About the book

Biotechnology has revolutionized the field of biological sciences worldwide. Escalating knowledge and understanding of the importance of technological advances, has created a renewed interest to further explore in depth about the subject and its applications. The present book covers a wide range of topics that involves recent developments in the field of animal, plant, clinical (pharmaceutical), food, environmental and microbial technology. The book has been comprehensively integrated and written by experts of International repute, in their respective fields. The information's presented can be easily assimilated by any person involved in the field of biotechnology, extending from students, researchers and academicians up to industrialists.

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Polysaccharides from Basidiomycetes: A Promising Source for Immunostimulating and Anticancerous activity

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SUMMARY

Owing to the fungal metabolic versatility, ecological diversity, complex life cycles and essential role in nature, fungi have attracted the attention of chemists, biochemists, biologists, ecologists and naturalists in myriad ways. The use of fungi for the production of commercially important products and drugs has a long tradition, but it has increased rapidly over the past half century. The antitumor compounds derived from mushroom fruit bodies, cultured mycelium and culture filtrate are grouped as polysaccharides. In this chapter, the polysaccharides derived from mushrooms and their role in immunostimulating and anticancer activity are briefly discussed.

***Key words*:** Medicinal mushrooms, polysaccharides, β-Glucan, anticancer activity, immunomodulation.

INTRODUCTION

In the developed countries, many causes of death or disability such as coronary heart diseases, strokes, diabetes, arthrosclerosis, obesity and certain forms of cancer can be attributed to diet (Barasi, 1997). Poor food selection and restricted dietary intake can affect the health status of an individual. Arising from the awareness, the US Academy of Science has defined functional foods as those that "encompass potentially healthful products". Since mushrooms are the source of both nutritive and medicinal properties, they are considered as

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functional foods. Nature has bestowed us with a large number of diverse types of mushrooms, which are grown in different parts of the world. In terms of taxonomy, they belong to the class Basidiomycetes or to the Ascomycetes (Mizuno, 1995). Commonly, mushrooms are described as macrofungi with distinctive fruiting bodies that can be grown on a wide variety of agricultural by-products and are large enough to be seen with human naked eyes and can be picked by hand. Based on this definition, more than 12,000 species have been considered as mushrooms. At least 2,000 of them are considered as edible (Chang, 1999), of which around 300 species belong to 70 genera have been reported from India (Arjunan and Dinakaran, 1999).

Since ancient days, mushrooms are valued as highly tasty and nutritional food by many societies throughout the world (Chang and Miles, 1989). To the ancient Romans, they were "the foods of the Gods" resulting from bolts of lightening thrown to the earth by Jupiter during thunder storms; the Egyptians considered them as "a gift from the God Osiris"; while the Chinese viewed them as "the elixir of life". Mushrooms are considered to be a good source of digestible proteins above most vegetables and somewhat less than meats and milk. Protein content varies from 10 to 40%, fiber by 3-35% (on dry weight basis) and carbohydrates by 3-21% (on fresh weight basis). Mushroom cultivation is a worldwide practice. The cultivation of edible mushrooms through solid state fermentation helps in recycling of agro wastes and also filling up the protein gap prevalent among large population of many countries (Bano et al. 1996). Of the 92% of total world production of leading 10 species, 87% of the total production (Chang, 1999) was the cultivation of the six species, viz: *Agaricus bisporus* (31.8%), *Lentinus edodes* (25.4%), *Pleurotus* spp. (14.2%), *Auricularia auricula* (7.9%), *Flammulina velutipes* (4.6%), and *Volvariella volvaceae* (7.9%).

The statistical records says that cancer is the second largest cause of death in people of various ages and has led to vast research efforts and clinical studies in the fight against the disease. The prevention of cancer by the ingestion of chemical agents, aimed at minimizing the risk of carcinogenesis has greatly reduced the morbidity and mortality rates. These agents include non-steroid anti-inflammatory drugs (NSAID) such as aspirin and indomethacin, which inhibit cyclooxygenase (COX) enzyme activity. Cyclooxygenase inhibition is necessary because COX catalyzes the conversion of arachidonic acid to pro-inflammatory substances such as prostaglandin, which can stimulate growth of tumor cells and suppress immune surveillance. It also activates carcinogens to take up forms that damage the genetic material (Jang et al. 1997; Wasser and Weis, 1999).

The immediate cause of cancer must be combination of behaviors and accidents that induce normal cells in a healthy human body to turn malignant (Gibbs, 2003). The elucidation of DNA as the genetic material ushered in the era of molecular genetics and cancer is now seen as a result of cumulative mutations that alter specific locations in a cell's DNA and thus change the particular protein encoded by cancer related genes at those locations. These mutations affect two kinds of cancer genes namely, tumor suppressors and oncogenes. Tumor suppressors normally restrain the cells ability to divide, mutations on this gene render it inactive. Oncogenes on the other hand stimulate cell growth and cell division. Mutations on the oncogenes make them permanently active thus promoting cell growth and progressive changes leading to the increase of the cancer genes (Gibbs, 2003). Consequently, cancer cells continue to divide even in situations in which normal cells will usually wait for a special chemical transduction signal. The tumor cells would ignore such stop signals that are

sent out by adjacent tissues. Cancer cells also have the character of immortality even *in vitro* whereas normal cells stop dividing after 50-70 generations and undergo a programmed cell death (Apoptosis). Cancer cells continue to grow invading nearby tissues and metastasizing to distant parts of the body. Metastasis is the most lethal aspect of carcinogenesis. The phenomenon is such that, before a tumor is discovered and removed by surgery, a few cells of the tumor would detach from the initial mass, float through the circulation system and start a new colony in a different organ from the one that gave birth to it.

The theory that cancer results from cumulative mutations that alter specific genes in the DNA has come under great challenge recently. Biologists now trace the cause of tumor to include other abnormalities at work inside the cell nucleus that though not yet malignant is prone to become so. Breakdown in DNA duplication or repair leads to many thousands of random mutations in cells, this damage to a few "master" genes mangles the chromosomes, which then becomes dangerous, plunging the cells into disorder. Finally, the abnormal number of chromosomes in a cell may be the milestone on the road to cancer (Gibbs, 2003). However, there is a great expectation that one day science will produce a definitive answer to the root cause of cancer. It may probably be a very complicated answer or a simple one, which may force us to shift our hope from curative drugs to chemopreventives. It has been difficult to develop specific remedies against cancer as with the development of vaccine against viral infections and antibiotics. Cancer cells originate from normal cells and a novel drug able to select and destroy only affected cells preventing carcinogenesis without injury to normal cell would be an ideal chemotherapy against cancer. Recent investigations have been channeled on the development of immunotherapy to target and remove cancer cells as well as on substances such as immunopotentiators, immunoinitiators and biological response modulators (BRM) that act to prevent carcinogenesis and induce carcinostasis (Wasser and Weis, 1999).

Mushrooms belonging to the family Polyporaceae have been effective against esophageal, stomach, prostate and lung cancers. Lucas et al. (1957) have demonstrated the antitumor effect of higher Basidiomyces (specifically extracts of fruiting bodies of *Boletus edulis*). Yohida et al. (1962) have isolated an active agent against Ehrlich carcinoma of the mouse from *Lampteromyces japonicus* (Kowamura) Sing. Gregory (1966) experimented on more than 7000 cultures of higher Basidiomycetes for antitumor activity against rodent tumor systems and positive inhibitory effects were obtained using fermentation media materials against sarcoma 180, mammary adenocarcinoma 755, and leukemia L-1210. Ikegawa et al. (1968) have reported that an essence obtained from the fruit body of edible mushrooms exhibited remarkable host-mediatory antitumor activity against grafted cancer in animals such as sarcoma 180. The antitumor essence was later discovered as β-D-glucan, a polysaccharide yielding D-glucose only by acid hydrolysis (Mizuno, 1999). Daba and Ezeronye (2003) found that *Pleurotus ostreatus* mushrooms cultivated on date fruits waste possess a potent antitumor activity against 'Ehrlich ascites carcinoma' and further biochemical studies on the effect of mushrooms on the tumors transplanted in mice resulted in the isolation of polysaccharides. β-D-glucan has a different mode of action from the conventional chemotherapeutic agents in that it is immunotherapeutic, inhibiting the growth of cancer cells by activating and reenforcing the host immune system. This chapter elucidates the antitumor polysaccharides from mushrooms, their chemical nature, antitumor and immunomodulatory properties and their mode of action.

MUSHROOMS IN FOLK MEDICINES

In addition to their nutritional value, many edible mushrooms have long been used for medicinal purposes. Many non-edible species have also gained important medicinal usage. There are at least 270 species of mushroom that are known to have various therapeutic properties (Ying et al. 1987). Since ancient times, the practice of using fungi, especially mushrooms, in Chinese herbal medicines has been recorded to possess medicinal effects that includes: *Ganoderma lucidum, Poria cocos, Tremella fuciformis* and others (Bensky and Gamble, 1993). Many species of mushrooms and fungi utilized as folk medicines for thousands of years are under intense study by ethnobotanists and medical researchers. Maitake, shiitake, and reishi are prominent among those being researched for their potential anti-cancer, anti-viral and immunity-enhancement properties. Psilocybin, originally an extract of certain psychedelic mushrooms, is being studied for its ability to help people suffering from mental disease, such as obsessive-compulsive disorder. Minute amounts of psilocybin have been reported to stop cluster and migraine headaches.

Medicinal mushrooms have become even more widely used as traditional medicinal ingredients for the treatment of various diseases and related health problems. As a result of large number of scientific studies on medicinal mushrooms in Japan, China and Korea over the past three decades, many of the traditional uses have been confirmed and new applications have been developed (Wasser and Weis, 1999). While much attention has been drawn to various immunological and anticancer properties of these mushrooms, they also offer other potentially important therapeutic properties including antioxidants, antihypertensive, cholesterol-lowering, liver protection, anti-fibrotic, anti-inflammatory, anti-diabetic, anti-viral and anti-microbial activities.

Hot water extracts of many mushrooms used in traditional Chinese medicine and other folk medicines have long been said to be efficacious in the treatment of various diseases including many forms of cancer. The use of medicinal mushroom extracts in the fight against cancer is well known and documented (Mizuno et al. 1995). They have been extensively screened for medical properties especially for anticancer application (Mizuno, 1999). Many species of mushrooms have been found to be highly potent immune system enhancers, potentiating animal and human immunity against cancer (Borchers et al. 1999; Wasser and Weis, 1999; Kidd, 2000; Ikikawa, 2000; Feng et al. 2001). While at least 30 mushroom species have yielded compounds with pronounced anticancer actions in xenographs, only a small number have taken the next step, viz. objective clinical assessment for anticancer potential in humans. Mushrooms, which belong to Basidiomycetes, contain biologically active polysaccharides. Many pharmaceutical companies are viewing medicinal mushrooms as a rich source of innovative biomedical molecules. Many polysaccharide-bound proteins produced by Basidiomyceteous fungi have been classified as antitumor chemicals by the US National Cancer Institute (Jong and Donovick, 1989).

POLYSACCHARIDES DERIVED FROM MUSHROOMS

Higher fungi are abundant source of a wide range of useful natural products. During the past decades, much interest has been generated in the polysaccharides produced by numerous fungi especially mushrooms because of their various biological and

pharmacological activities including antitumor, immuno-stimulating, and hypoglycemic activities etc. (Cohen, et al. 2002; Ooi and Liu, 2000). Polysaccharides are generally present in the cellular walls of fruits and vegetables and these can give a thickening, stabilizing or gelling effect to maintain fruit texture. These polysaccharides can be found in several different sources. They are found in several mushroom sources such as shiitake, maitake and reishi. Other sources include: yeast cell walls, seaweeds and certain bacteria. They are also found in the whole grains of oat and barley. Polysaccharides are structurally diverse group of biological macromolecules of widespread occurrence in nature. They are composed of repetitive structural features that are polymers of monosaccharide residues joined to each other by glycosidic linkages. In this way, they differ structurally from proteins and nucleic acids. Polysaccharides are present in maximum capacity for carrying biological information since they have the greatest potential for structural variability. The amino acids in proteins and the nucleotides in nucleic acids can interconnect in only one way, while the monosaccharide units in oligosaccharides and polysaccharides can interconnect at several points to form a wide variety of branched or linear structures (Sharon and Lis, 1993). As a consequence, this enormous potential variability in polysaccharide structure allows for the flexibility necessary for the precise regulatory mechanisms of various cell-cell interactions in higher organisms.

Many, if not all, Basidiomyceteous mushrooms have been shown to contain biologically active antitumor and immunostimulative polysaccharides. Reshetnikov et al. (2001) have listed 650 species and 7 intraspecific taxa from 182 genera of higher Hetero- and Homo-basidiomycetes that contain pharmacologically active polysaccharides that can be derived from fruit bodies, culture mycelium and culture broths which are either water-soluble β-D-glucans, β-D-glucans with heterosaccharide chains of xylose, mannose, galactose and uronic acid or β-D-glucan-protein complexes proteoglycans. As a general rule, the protein-linked glucans have a greater immunopotential activity than the corresponding glucans. Wasser and Weis (1999) have reported some of the antitumor polysaccharides isolated from mushrooms (Table 1).

In general, higher levels and a number of different polysaccharides have been extracted from fruit-bodies than from other cultural sources. Submerged culture is one of the excellent sources to produce reliable, consistent and safe products of mushroom polysaccharides. Enormous efforts have been made to obtain optimal submerged culture condition for bioactive polysaccharide production from several mushrooms. The production of exopolysaccharides (EPS) is sensitive to many factors such as temperature, pH, incubation period, carbon sources and nitrogen sources (Kim et al. 2002). The production of exopolysaccharides increases with biomass, suggesting that it may be produced constitutively. After a few days, its level in the culture medium declined due to the secretion of 1, 3 glucanases by *Phanerocheate chrysosporium* and *Pleurotus florida* (Bes et al. 1987; Burns, et al. 1994). In order to obtain polysaccharides from mushrooms, investigations (Kues and Liu, 2000) have exerted their efforts to cultivate edible and medicinal mushrooms on solid artificial media (for fruit body production) rather than submerged culture (for mycelial extract and/or EPS production).

Table 1 Antitumor active polysaccharide isolated from mushrooms.

Taxa	*Fruiting body*	*Submerged cultured mycelial biomass*	*Liquid cultured broth*
Homobasidiomycetes			
Aphyllophoromycetideae Polyporales			
Schizophyllaceae			
Schizophyllum commune Fr.: Fr	-	-	Sonifilan, SPG or Schizophyllan (β-glucan)
Polyporaceae			
Dendropolyporus umbelliatus (Pers.:Fr.) Jul.	GU-2, GU-3, GU-4, AP (β-glucan)	-	β-glucan
Grifola frondosa (Dick.:Fr.) S.F. Gray	Grifolan (β-glucan), Fa-1a-β (acidic β-glucan), FIII-2c (hetero-β-glucan), xyloglucan, mannoglucan, fucomannoglucan	Heteroglucan protein, manogalactofucan, heteroxylan, fucoxylan, galactomannoglucan	-
Fommes fomentarius (L.:Fr.)	β-glucan	β-glucan	-
Fomitopsis pinicola (Schw.:Fr.) P. Karst	F-1a-2-β (β-glucan) α-(1-6)- linked	α- and β-glucan	-
Albatrellus confluens (Alb. et Schw.:Fr.) Kotl. et Pouz.	(1-3)- β-D-glucan	(1-3)- β-D-glucan	-
Trametes versicolor (L.:Fr.) Lloyd	β-glucan	Coriolan, PSK, Krestin (β-glucan-protein)	-
Lenzites betulinus (L.:Fr.) Fr.	β-glucan	-	-
Wolfiporia cocos (Schw.) Ryv. et Gilbn.	Pachymaran (β-glucan)	-	-
Hericium erinaceus (Bull.:Fr.) Pers.	β-glucoxylan, glucoxylan protein, galactoxyloglucan protein	-	-
Ionotus obliquus (Pers.:Fr.) Bound.et Sing.	Polysaccharide fraction in the Allium-test	-	-
Ganodermatales			
Ganodermataceae			
Ganoderma lucidum (Curt.:Fr.) P. Karst.	Fl-1a (β-glucan), FIII-2b (hetero-β-glucan), acidic heteroglucan, chitin xyloglucan	-	β- glucan
G. applanatum (Pers.) Pat.	Fl-1-B-1 (β-glucan)	F-1a-1-b (β-glucan), heteroglucans, peptidoglucans	-
G. tsugae Murr.	Heteroglucan, heterogalactan, β-glucan, glucan	Heteroglucan, α-glucan	-
Gasteromycetideae, Phallaceae			

Table 1 Contd.

Table 1 Contd.

Dictyophora indusiata Fisch.	T-2 HN (O-acetylated-(1-3)- β-D-mannan), T-3-M1 (β-(1-3) linked D-mannan), T3-G, T-4-N, T-5-N (three kinds of β-D-glucans), T-3 Ad (Neutralheterogalactan)	-	-
Phallus impudicus L.:Pers.	PI-2 (glucomannan)	PI-2 (glucomannan)	-
Lentinus edodes (Berk.) Sing.	Lentinan (β-D-glucans)	KS-2-a-mannan-peptide, LEM, LAP (heteroglucan-protein), EP3	LEM, LAP (heteroglucanprotein), EP3
Pleurotus ostreatus (Jacq.:Fr.) Kumm.	Acidic polysaccharide fraction, HA (β-glucan)	-	β-glucan, heteroglucan
P. citrinopileatus Sing.	Heteroglucan, β-glucanprotein, glycoprotein (FI, FII, FIII)	-	-
P. pulmonarius (Fr.:Fr.) Quel. (=P.sajor-caju Fr.:Fr.)	Xyloglucan, xylanprotein	-	-
Tricholomataceae			
Panellus serotimus (Pers.:Fr.) Kuhn.	Heteroglucan, (1-6)- β- glucosyl branched (1(2-3)- β- D-glucans	-	-
Omphalina epichysium (Pers.:Fr.) Quel	EL-2 (β-glucan)	-	-
Flammulina velutipes (Curt.:Fr.) P.Karst.	EA6, EA6-PII (β-glucan-protein)	Proflamin (glycoprotein)	-
Leucopaxillus giganteus (Fr.) Sing.	Mannoxyloglucan, heteroglucan, glucan, xyloglucan, xylogalacetoglucan, galactoxyloglucan	-	-
Hypsizygus marmoreus (Peck) Bigel.	β-(1-3)-D-glucan	-	-
Agaricaceae			
Agaricus blazei Murr.	FI1-a-β (β-glucan), FIII2-β (β-glucan-protein), FA-1a-β(hetero-β-glucan), FA-2b-β(RNA), FV-1 (insoluble β-glucan)	ATOM (glucomannan-protein)	AB-FP (mannan-protein)
A. bisporus (J.Lge) Imbach	β-glucan	-	-

Table 1 Contd.

Table 1 Contd.

Pluteaceae			
Volvariella volvacea (Bull.:Fr.) Sing.	VVG (β-1-3)-D-glucans, β-manno-β-glucan	-	-
Strophariaceae			
Pholiota nameko (T.Ito) S.Ito et Imai	Galacto-β-glucan	-	-
Crepidotaceae			
Crepidotus mollis (Schaeff.:Fr.) Kumm.	CPS (β-glucan)	-	-
Agrocybe aegerita (Brit.) Sing.	β-(1-3)- β-glucans	-	-
Phragmobasidiomycetes			
Auriculariales			
Auriculariacea			
Auricularia auricula-judae (Bull.) Wettst.	(1-3)- β-glucan	-	-
Tremellales			
Tremellaceae			
Tremella fuciformis Berk.	Glucuronoxylomannan, T-7, T-19 (exopolysaccharides), mannose, xylose, glucuronic acid	Glucuronoxylomannan	Xylose, glucuronic acid, Mannose-
T. mesenterica Ritz.:Fr.	β-D-glucuronosyl (epitope)	-	-

POLYSACCHARIDE EXTRACTION AND PURIFICATION

Two decades of intensive research on medicinal mushrooms, by Mizuno in Japan resulted in reliable procedures for successful extraction, fractionation and purification of polysaccharides from fruiting bodies or culture mycelia (Mizuno, 1996, 1999). In general, this scheme involves elimination of low molecular weight substances from mushroom material using 80% ethanol, followed by three successive extractions with water (100°C, 3 h), 2% ammonium oxalate (100°C, 6 h) and 5% sodium hydroxide (80°C, 6 h). The first extraction results in water-soluble polysaccharides, the other two in water-insoluble polysaccharides. Polysaccharides extracted are further purified using a combination of techniques, such as ethanol concentration, fractional precipitation, acidic precipitation with acetic acid, ion-exchange chromatography, gel filtration, and affinity chromatography. Ion-exchange chromatography through DEAE-cellulose columns assures the separation of neutral polysaccharides from acidic ones. Neutral polysaccharides are then separated into β-glucans (adsorbed fraction) and β-glucans (non-absorbed fraction) with the help of gel filtration and affinity chromatography. The same procedure with acidic polysaccharides (after elution with 1 M NaCl) yields purified polysaccharides (Mizuno, 1999).

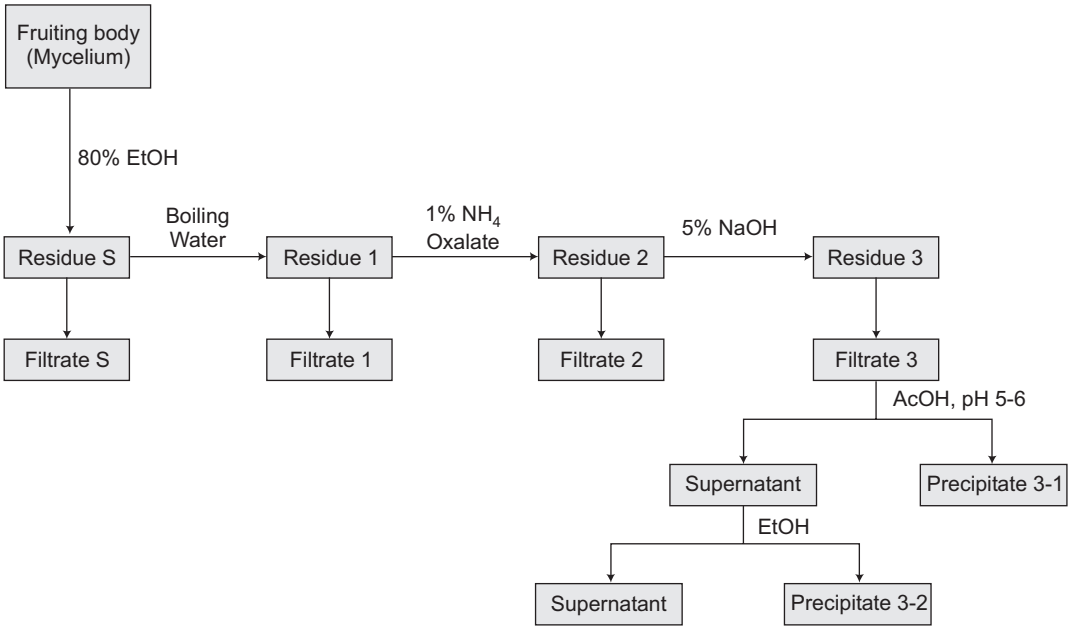

Fig. 1 General schemes for fractional preparations of polysaccharides from mushrooms (Mizuno, 1999).

A recent study by Yap and Ng (2001) has established a more efficient procedure for the extraction of β-D-glucans from *Lentinus edodes* (Fig. 2). β-D-glucan has been isolated through ethanol precipitation and freeze-drying in liquid nitrogen. Purity testing, using a carbohydrate analysis column, gave 87.5% purity. From a commercial aspect, this method is

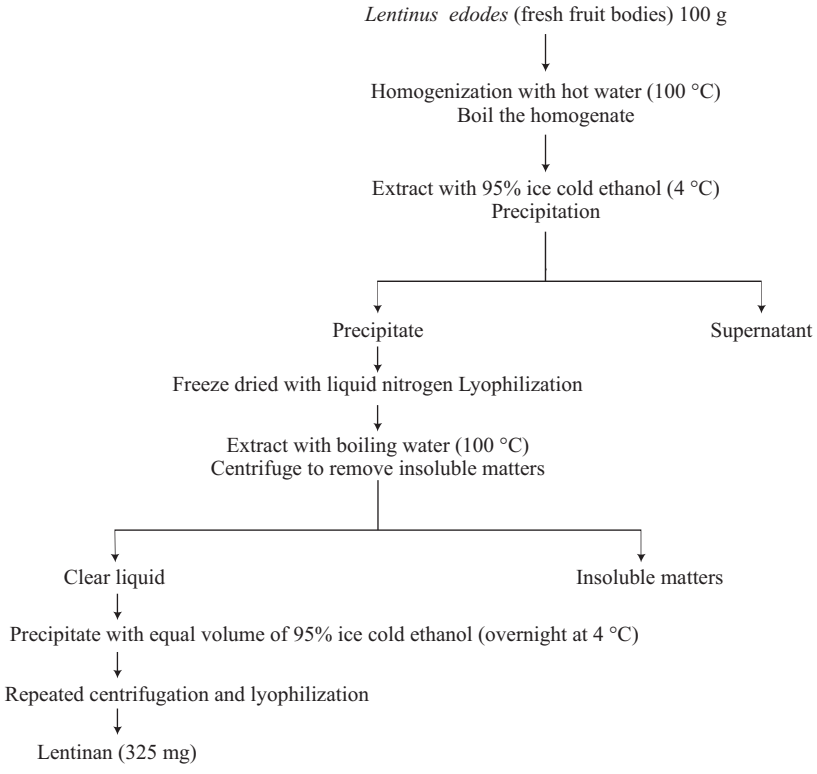

Fig. 2 Extraction method of Lentinan from *L. edodes* (Yap and Ng, 2001)

less time-consuming, more efficient and of relatively low cost when compared to the original methods proposed by Chihara et al. (1970) and Mizuno (1999).

β-D-GLUCAN

β-D-Glucan is a collective name of D-glucose units of high molecular weight joined together in linear chains by β-bonds. These can extend from carbon 1 of one saccharide ring to carbon 3 of the next (β-1-3), from carbon 1 to carbon 4 (β-1-4) or from carbon 1 to carbon 6 (β-1-6). Mostly, there is a main chain which is either β-1-3, β-1-4 or mixed β-1-3, β-1-4 with β-1-6 side chains. Levels of anticancer activity are related to their molecular weight, branching and solubility in water. The study of their steric structures by NMR analyses and X-ray diffractions clarified that active β-D-glucan shows a triple-stranded right-winding helix structure (Bluhm and Sarco, 1977). Not all β-D-glucans from fungi exhibit antitumor activity. The extent of occurrence of this activity seems to be influenced by solubility in water, size of molecules, and the β-(1-6)-bonding system in the β-(1-3) major chain. β-Glucans are water-soluble fiber that consists of chains of glucose molecules. Being fibrous in nature, β glucans

are not absorbed in the small intestine like most nutrients. They are partially digested in the large intestine and are broken down into oligosaccharides (long sugar and starch molecules), which can then be absorbed. Some of the water insoluble β-glucans are soluble in dilute alkali and then can show marked antitumor activity (Bohn and BeMillar, 1995).

BIOSYNTHESIS OF β-GLUCAN

The fungal cell is encapsulated by an extracellular matrix, the cell wall, which protects it from osmotic pressure and environmental stress, and determines cell shape. The cell wall has been described on one hand as a rigid layer of glycoproteins and polysaccharides, and on the other hand as a dynamic structure flexible enough to cope up with cell growth. The cell walls of most fungi consist of five major components: (1-3)-β-glucan, (1-4)-β-glucan, (1-6)-β-glucan, chitin, and glycoproteins. The cell-wall components are organized in a layered structure, in which (1-3)-β-glucan forms densely interwoven microfibrils which occupy the innermost layer, followed by (1-6)-β-glucan and mannoproteins (Osumi, 1998; Cabib et al. 1982). β-Glucan synthase is a plasma-membrane localized enzyme that catalyzes the intracellular synthesis of (1-3)-β-glucan and that is thought to facilitate extrusion of the newly synthesized (1-3)-β-glucan through the plasma membrane into the extracellular space via a putative membrane-spanning pore. It has been postulated that the synthesis of β-glucan begins in the **endoplasmic reticulum** with the formation of protein-bound primer structures and that these primer structures are extended in the **Golgi complex** by two putative glucosyl transferases that are functionally redundant, Kre6 and Skn1. The glucan synthesis inhibitors are, collectively, agents that are presumed to block fungal cell wall synthesis by inhibiting the enzyme 1,3-β glucan synthase. Inhibition of this enzyme results in depletion of glucan polymers in the fungal cell, resulting in an abnormally weak cell wall unable to withstand osmotic stress. As technical issues in the laboratory have made formal proof of the assertion that this enzyme is actual target of these compounds, it is most correct to speak of them at present as being glucan synthesis inhibitors rather than glucan synthase inhibitors. At present, there are three such agents, all three belonging to the chemical family known as the echinocandins or caspofungin, micafungin and anidulafungin. As would be predicted from the angle of their mechanism of action, these agents appear to be well-tolerated and have relatively less toxicity than polyene or azole-class antifungals. Indeed, maximum tolerated dosages were not reached, in human volunteers, for these agents in phase II clinical trials.

β-Glucans are considered to be immune system primers. They bind to specific receptors on macrophages, a special white blood cell that initiates the immune reaction towards bacteria and viruses. By binding to the macrophage, certain immune signaling molecules known as IL-1 and TNF are produced. These signaling molecules increase the production of specialized T cells and NK cells. It also effectively reduces cholesterol levels. Although, the exact mechanism is not fully known, it is assumed to increase the bile excretion; β-glucans are soluble fibers, they also known to increase the excretion of bile salts in the feces. Bile salts help to bind cholesterol and therefore by increasing their excretion, the absorption of cholesterol is decreased. β-Glucans have been used for cholesterol lowering effects, enhancing immune system, improving wound healing, anticancer activity, and mild blood sugar control.

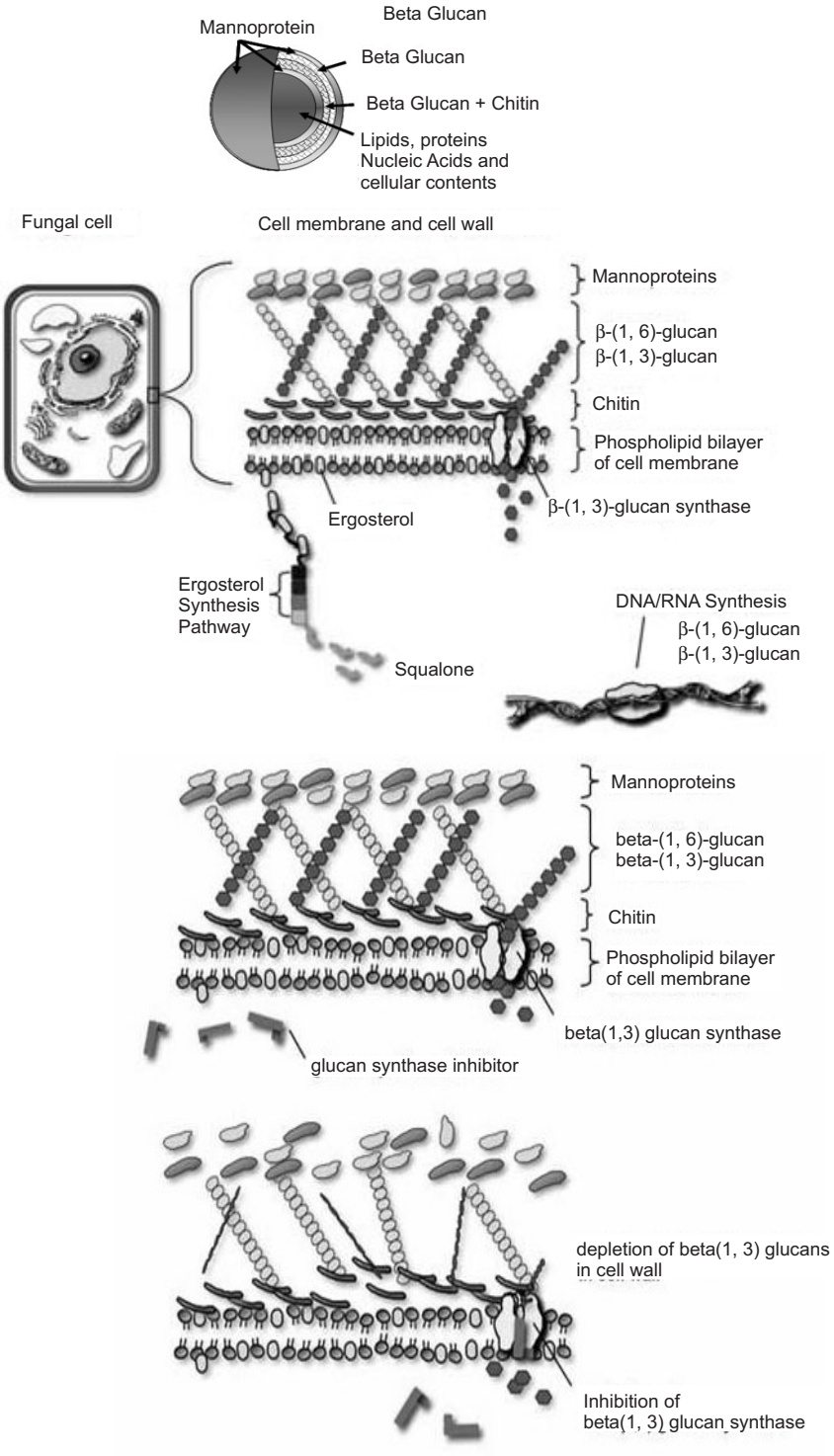

Fig. 3 Biosynthesis pathway of β-glucan

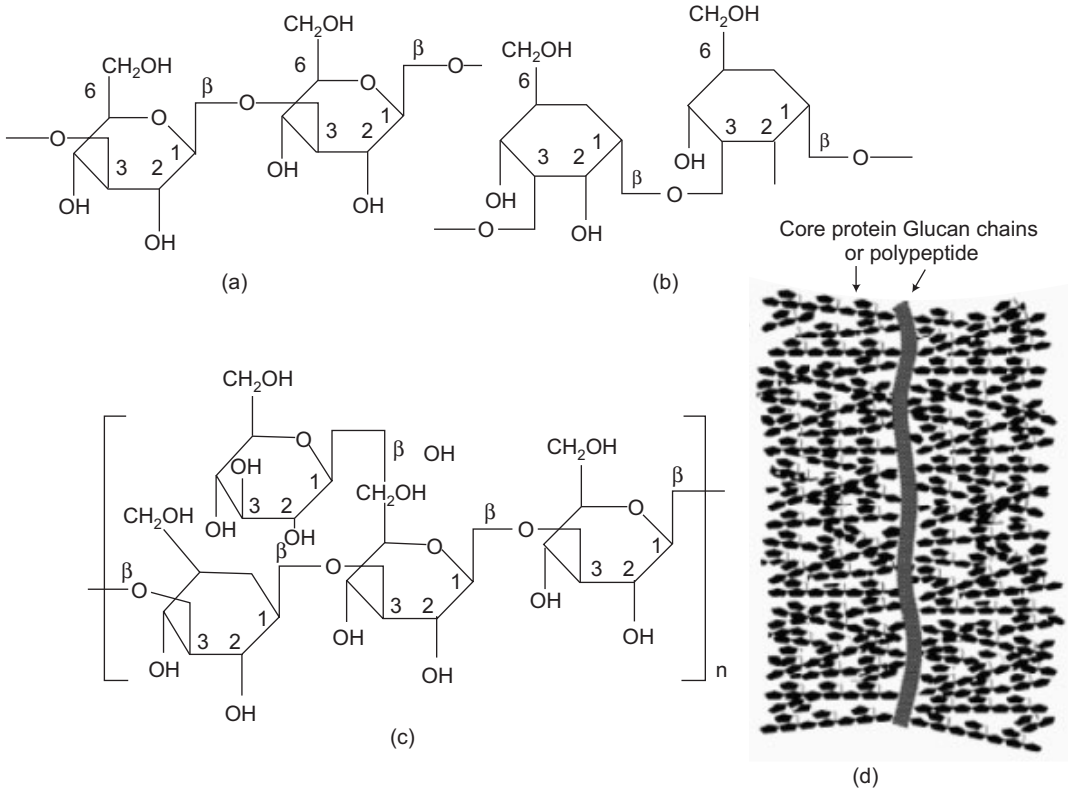

Fig. 4 Schematic representation of the molecular structure of β-(1-3)-D-glucan (a), β- (1-3)-D-glucan (b), 1,6-Monoglucosyl-branched 1,3-β-D-glucan (c), polysaccharide peptide/protein complex (d.) (Mohammad-Fata Moradali et al. 2007).

MOLECULAR CLONING OF β-GLUCAN SYNTHASE GENES

The fungal cell wall is mainly constituted of polysaccharides such as β-glucan and chitin, and among them β-1,3- glucan is the most prevalent. The biosynthetic pathways of β-glucans are possible targets for the design of novel chemotherapeutics because mammals are not known to possess β-glucan synthases. Although β-1,3-glucan synthesis has been intensively studied, the biosynthetic pathway is not fully understood. Molecular cloning of the *FKS1* gene might lead to the elucidation of the biosynthetic pathway of β-glucans. β-1,3-Glucans are synthesized from uridine 52 -diphosphate (UDP)-glucose by a membrane protein complex, β-1,3-glucan synthase. It seems that β-1,3-glucan biosynthesis occurs on the cytoplasmic side of the plasma membrane and β-1,3-glucan chains are extruded toward the periplasmic space. The β-1,3-glucan synthase complex has been shown to be composed of the catalytic subunit Fksp, a large molecular size polypeptide with transmembrane domains, and the regulatory subunit Rho1p, a small molecular size GTPase (Douglas et al. 1994; Mazur and Baginsky, 1996; Beauvais et al. 2001). The fungal *FKS1* gene encodes an integral membrane protein that

is the catalytic subunit of β-1,3-glucan synthase. The *Saccharomyces cerevisiae FKS1* (*ScFKS1*) gene encodes a 215-kDa integral membrane protein (ScFks1p), and *FKS2* (*ScFKS2*), a homologue of *FKS1* encoding a 217-kDa integral membrane protein (ScFks2p), has also been cloned (Mazur et al. 1995). Simultaneous disruption of *ScFKS1* and *ScFKS2* is lethal, suggesting that ScFks1p and ScFks2p are alternative subunits with overlapping function. β-1,3-Glucan biosynthesis has been studied at the molecular level almost exclusively in yeast and filamentous fungi, but poorly in mushrooms.

LENTINAN

The shitake mushroom (*Lentinus edodes*) is a common culinary mushroom used in traditional oriental cooking and herbal medicine. *Cortinellus edodes*, *Armillaria edodes* and *Cortinellus shiitake* are the synonyms of *Lentinus edodes* (Chihara, 1970). Extracts of shitake mushroom are now being incorporated into over-the-counter dietary supplements designed to improve the status of the immune system. Lentinan is a polysaccharide derived from the vegetative parts of shiitake mushroom. It is the cell wall constituent extracted from the fruiting bodies or mycelium of *L. edodes*. Lentinan is found in very low concentrations in fresh shiitake mushrooms. Lentinan is a water-soluble, β-1,3 glucan polysaccharide characterized by β-1,6 branched glucan linkages. At least 5 additional polysaccharides have been isolated from *L. edodes* (Chihara, 1970). Lentinan is a high molecular weight polysaccharide in a triple helix structure, containing only glucose molecules with mostly (1-3)-β-D-Glucan linkages in the regularly branched main chain with two β-(1,6)-D-glucopyranoside branchings for every five β-(1,3)-glucopyranoside linear linkages (Hobbs, 1995). It is a complex polysaccharide that possesses immuno-stimulating antitumor properties.

The antitumor activity of lentinan has been known for almost 30 years, eventhough a number of naturally occurring polysaccharides had previously been found to have antitumor activity, lentinan was considered for detailed evaluation. In addition to antitumor activity, lentinan also possesses immune-regulatory effects, antiviral activity, antimicrobial properties and cholesterol-lowering effects and activate the host immune system. Lentinan has been proved successful in prolonging the survival rates of cancer patients, especially those with gastric and colorectal carcinoma (Furue et al. 1981).

KRESTIN

The turkey tail fungus (*Tramates versicolor* or *Coriolus versicolor*) is commonly found throughout North America, Asia, and Europe. The polysaccharides of the fruiting bodies are commercially marketed as a tea that is commonly used in Asian and European traditional medicine (Kidd, 2000). The water-extracted protein-bound polysaccharide krestin (PSK) and polysaccharide peptide (PSP), polysaccharides of *C. versicolor* have immunomodulating and antitumor activity. They are chemically similar but distinguished by fucose in PSK and rhamnose and arabinose in PSP. PSP has a molecular weight of 100-kDa. The polypeptide component contains mostly glutamic and aspartic acids and the polysaccharides contain

primarily alpha-1,4 and β-1,3 glucosidic linkages (Tsukagoshi et al. 1984; Ng, 1998; Kobayashi, 1995). PSK consists of a β-(1-4) main chain and β-(1-3) side chain, with β (1-6) side chains that bond to a polypeptide moiety through O-N- linked glycosidic bonds (Sakagami and Aoki, 1991). Polysaccharide Krestin is an approved anti-cancer drug. Like many of the more interesting non-toxic anticancer agents, such as Lentinan, and Bovine Cartilage, PSK is a polysaccharide complex with immune stimulating effects. The polysaccharides are large molecules made up of chains of linked sugar molecules. PSK is actually a polysaccharide-protein complex.

Folklore remedies of turkey tail include the treatment of lung and liver infections. In China, turkey tail has been used as a preventive and curative agent for liver infections and liver cancer. In Japan, it is considered a panacea for a variety of cancers. Moreover, the mycelium and fruiting body of the mushroom are considered as immune stimulant and is believed to have anticarcinogenic activity (Tsukagoshi et al. 1984; Ng, 1998; Kidd, 2000). Clinical research with PSK began around 1970 and has focused on its immunotherapeutic efficacy in stomach, colorectal, esophageal, nasopharyngeal, lung, and breast cancers. The polysaccharide PSK reportedly increases gamma-interferon production, interleukin-2 production, and T-cell proliferation, therefore improving immune system functioning (Kidd, 2000). Other studies have focused on the antimicrobial, antiviral, and antioxidant properties of PSK. PSK extracted from a mycelial strain CM- 101 is with approximately 62% polysaccharide and 38% protein. This compound has been systematically tested against a wide range of human cancers with some considerable success (Ikuzawa et al. 1988; Kidd, 2000).

SCHIZOPHYLLAN

The polysaccharide derived from mushroom *Schizophyllum commune* is a β-(1,3)-D-glucan with β-(1,6)-D-glucan side-chains and is called Schizophyllan (or Sonifilan, Sizofiran, Sizofilan). In the case of Schizophyllan, the molecules are large and are normally administered in the clinical setting by the intramuscular or intraperitoneal route. Schizophyllan has been shown to be cytostatic in Sarcoma 180 tumor xenographs. The survival of Sarcoma 180 was not affected by pretreatment with Schizophyllan, while combined pre and post treatment and post treatment alone resulted in increased survival. Schizophyllan had no effect on the survival of Sarcoma 37, Ehrlich carcinoma or Yoshida sarcoma ascites tumors (Wasser and Weis, 1999).

GRIFOLIN

Grifola frondosa, one of the traditional and edible mushrooms in Southeast Asia, is commonly used in the treatment of various diseases, due to its considerable biological activities such as anticancer, antioxidant, antiviral activities (Inoue et al. 2002; Mayell, 2001; Lee and Nishikawa, 2003). However, the polysaccharides extracted with alkali from *G. frondosa* are mainly insoluble in water and less suitable for pharmaceutical study.

GANODERAN

The reishi mushroom (*Ganoderma lucidum*) is known to be high in polysaccharide content, including β-D-glucan (Ganoderan) and GL-1. Triterpene constituents also have been analyzed. Triterpene antioxidants, including ganoderic acids A, B, C, and D, lucidenic acid B, and ganodermanontriol have been found in reishi. Terpenoids 1, 2, and 3, and terpenes lucidenic acid O and lucidenic lactone also exist in the mushroom. Peptidoglycan from reishi containing approximately 7% protein and 76% carbohydrate has been reported. Certain enzymes from reishi also have been reported. The reishi mushroom contains elements including calcium, magnesium, and potassium. Germanium, lanostan, coumarins, ergosterol, and cerevisterol also are found in reishi. Some species of *Ganoderma* viz., *G. lucidum*, *G. applanatum* and *G. tsugae* produces polysaccharides in their different stages. The reishi mushroom produces several polysaccharides like β-glucan, hetero β-glucan, acidic heteroglucan, peptidoglucan, chitin xyloglucan and β-glucans. These polysaccharides used as anticancer drug and immunostimulant.

An antitumor active β-glucan-protein has been isolated from the fruit body of *Flammulina velutipes,* while a new antitumor glycoprotein has been isolated from cultured mycelium. This glycoprotein, "Proflamin" (mw = 16,000) is water soluble and contains 90% protein and 10% polysaccharide and has activity against tumor (Zhang et al. 1999).

While all of the mushroom polysaccharides have been successfully administered intravenously in animal and human cancer treatments, several of them can also be effective by oral administration. Mushroom polysaccharides (Lentinan and Schizophyllan), both large molecules, are only effective by intravenous or intraperitoneal administration. The antitumor β-1,6-glucan from *Agaricus blazei* showed that intravenous administration gave highly satisfactory results while no effect was seen with oral administration in mice. However, a simple acid treatment of the whole β-1,6-glucan produced molecular masses of 10 kDa which when administered orally to mice demonstrated activity (Fujimiya et al. 2000). This study has significant application with the other large β-glucans and improving their oral bioavailability and increased use as immuno-nutraceuticals.

MUSHROOM POLYSACCHARIDES AND IMMUNOMODULATING RESPONSE

Medicinal mushroom research has focused on discovering compounds that can modulate positively or negatively the biological response of immune cells. Those compounds which appear to stimulate the human immune response are being sought for the treatment of cancer, immunodeficiency diseases, or for generalized immunosuppression following drug treatment; for combinational therapy with antibiotics; and as adjuncts for vaccines. Those compounds that suppress immune reactions are potentially useful in the remedy of autoimmune or certain gastro-intestinal tract diseases. Several classes of compounds, such as proteins, peptides, lipopolysaccharides, glycoproteins, and lipid derivatives of mushrooms have all been classified as molecules that have potent effects on the immune system (Tzianabos, 2000). The immune function is very complex and employs different mechanisms

to destroy various infectious agents. In fact, it is clear that even within a species such as bacteria not all organisms are killed by the same mechanisms. β- Glucan's initial role is to prepare the innate immune function, the body's first line of defense, to respond to challenges from foreign invaders such as bacteria, virus, fungi, cancer, parasites, etc. The innate immune function then sends messages that the body is under attack to the components of the acquired immune function, the body's second line of defense.

The first definitive studies on these anticancer substances came in the late 1960s with the reports by Ikekawa et al. (1968, 1969) and Chihara et al. (1969, 1970). They demonstrated that extracts of several different mushroom species exhibited remarkable host-mediating antitumor activities against xenographs, e.g. Sarcoma 180. In both studies, the compounds were easily extracted with hot water and shown to be various types of polysaccharides. The polysaccharides are non-toxic and appear to affect tumors indirectly following administration, suggesting that the anticancer action is mainly host-mediated.

Whilst polysaccharides are generally considered to be classic T lymphocyte- dependent antigens that do not elicit cell-mediated immune responses (host defenses that are mediated by antigen-specific T lymphocyte cells and various non-specific cells of the immune system), certain polymers have been shown to act as potent immunomodulating agents. Compounds that are capable of interacting with the immune system to up regulate or down regulate specific aspects of the host response can be classified as immunomodulators. Whether immunomodulators enhance or suppress immune responses can depend on a number of factors such as dosage, route of administration, and timing and frequency of administration (Tzianabos, 2000).

Immunomodulating polysaccharides, derived from a variety of diverse microbial genera include *Streptococcus* spp. (hyaluronic acid), *Bacteriodes fragilis* (Polysaccharide-A), *Candida albicans* (Mannan) and *Saccharomyces cerivisiae*, have shown significant promise in the treatment of infectious diseases (Garner et al. 1990). Antitumor effects were another promising biopharmacological activity of polysaccharides from these sources. Many other polysaccharides, from bacteria such as *Escherichia coli*, *Streptococcus pyogenes* (OK-432), *Proteus vulgaris*, *Acetobacter xylinum* and *Salmonella typhimurium,* have also been reported to exhibit cytotoxicity against solid tumors (Whistler et al. 1976).

Polysaccharides act mainly as immune stimulants with little or no adverse drug reactions. Furthermore, several of these extracts have been shown to stimulate apoptosis in cancer cells. Antitumor polysaccharides isolated from mushrooms (fruit-body, submerged cultured mycelial biomass or liquid culture broth) are water-soluble β-D-glucans, β-D-glucans with heterosaccharide chains of xylose, mannose, galactose, and uronic acid or β-D-glucan-protein complexes- proteoglycans. As a general rule, the protein-linked glucans have a greater immunopotential activity than the corresponding glucans (Mizuno, 1999).

Researchers are now beginning to define the mechanism in which β-glucan primes and enhances the immune response. Orally consumed, β-glucan reaches the small intestine intact where it is taken up by the M-cells in specialized colonic lymph nodes called Peyer's patches. Within the Peyer's patch one type of white blood cells, known as macrophages, phagocytze (engulf) and degrade the β-glucan. Macrophages then circulate and release soluble fragments of β-glucan throughout the body to prime other immune cells or lymphoid tissue. The

macrophages process and secrete soluble fragments of β-glucan, which are then taken up by cell membrane receptors on other types of white blood cells, neutrophils and natural killer cells. The primed macrophages and neutrophils are now on alert and are ready to respond to foreign material. As a part of the normal antigen-presenting function, primed macrophages release various chemical messengers (cytokines) which prepare the state of readiness of other components of the innate immune function and activate subsets of the T- and B-cells, key cellular components of the acquired immune function.

Neutrophils are the most abundant and effective defender against hostile attacks. Neutrophils destroy foreign cells by phagocytosis and by releasing oxidative compounds. The macrophage-degraded β-glucan particles are taken up by a complement receptor (CR3) on a neutrophil's cell wall. This priming of neutrophils increases their killing capacity and enables neutrophils to migrate faster to the sight of the hostile challenges. In addition, the β-glucan fragments now enable the primed neutrophils to more efficiently differentiate between self and non-self cells resulting in heightened ability to identify, attack and destroy foreign cells with an attached antibody.

Several researches documented the immune-enhancing ability of β-glucan. Supplementation of two milligrams of β-glucan per kilogram of body weight has been found to prime and improve the innate immune response. The activity of the innate immune cells in turn enhances the immune response of acquired immunity improving the body's ability to better (maintain health) combat illness caused by germs. The likely mode of immunopotentiation by (1-3)-β-D-glucan involves activation of cytotoxic macrophages, helper T-cells and NK cells, and the promotion of T-cell differentiation (Bohn and BeMiller, 1995). Macrophages are one of the many critical components in the immune system, co-operation between which is necessary for tumor rejection. Bohn and BeMiller (1995) also reported that macrophages have a highly selective cytotoxicity towards cancer cells *in vitro*; and there is evidence that they may also destroy malignant cells *in vivo*. T-cell competence appears necessary for selection of macrophage resistance, which suggests that these two cell types interact in the intact host in response to a tumor challenge. Scientific research revealed that β-glucan supplementation

- Primes neutrophils to more effectively meet immune challenges.
- Enhances the migration of immune cells to sites of challenges.
- Enhances macrophages' and neutrophils' phagocytic activity (engulfing and destroying non-self cells).
- Improves intra-cellular communication in the presence of non-self intruders.
- Results in better presentation of foreign antigens to the T- and B-cells of the acquired immune function.

β-Glucan does not initiate an immune response but rather increases the state of preparedness of the innate immune system so that the body's immune function can respond at peak efficiency to a challenge from a foreign material. Through a progression of events, primed macrophages prepare additional components of both the innate and acquired immune functions to more effectively respond to foreign challenges. Fig. 5 shows the possible immune mechanism of polysaccharide against cancer cells.

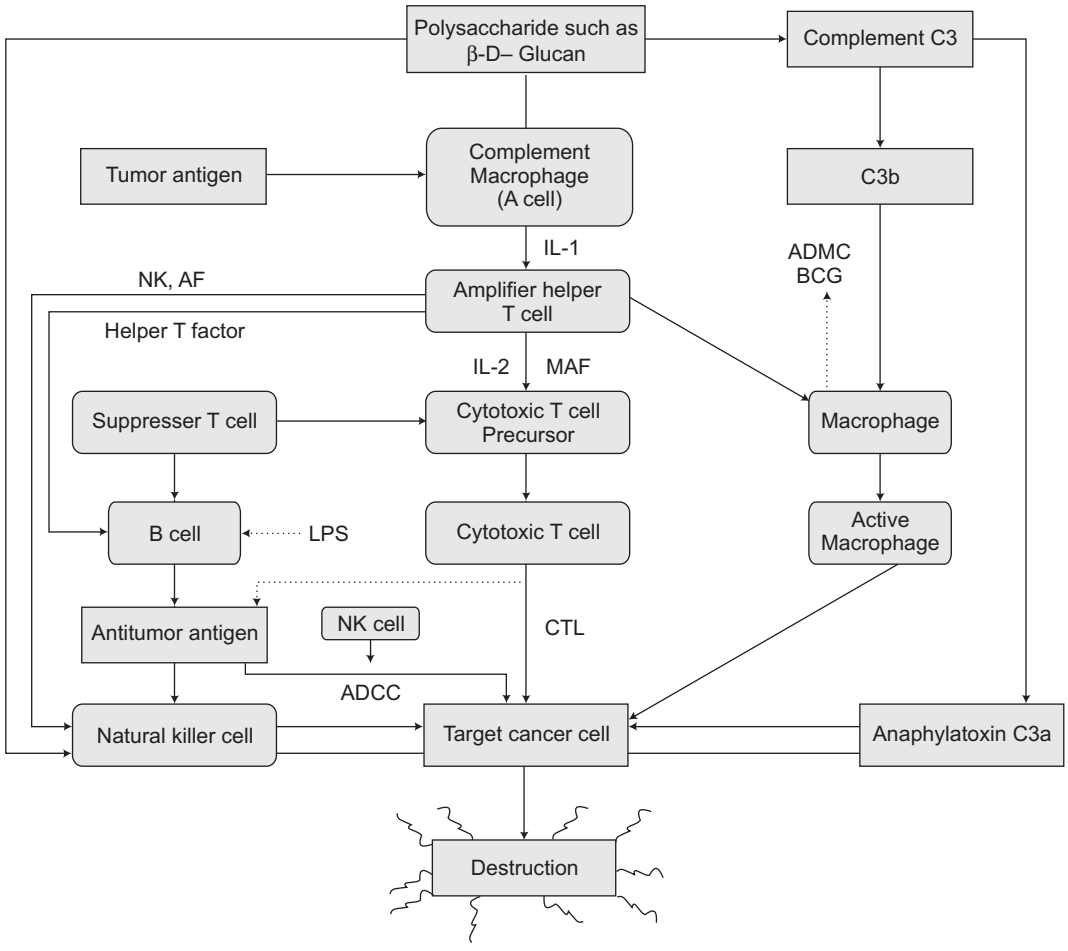

Fig. 5 Possible immune mechanism of β-D-glucan (Mizuno, 2002).

Lentinan is the most studied polysaccharide which was isolated from *Lentinus edodes.* Lentinan does not attack cancer cells directly, but produces its antitumor effect by activating different immune responses in the host. It has been shown that Lentinan is a true immunopotentiator, as administration of this bioactive polymer had a clearly augmenting effect on the proliferation of peripheral mononuclear cells (PMNCs) from healthy donors (Hobbs, 2000). Indeed, Lentinan appear to act as a host defense potentiator that is able to restore or augment the responsiveness of host cells to lymphocytokines, hormones, and other biologically active substances. Evidence suggests that this immune-potentiation occurs by stimulating the maturation, differentiation or proliferation of cells involved in host defense mechanisms. Thus, Lentinan has been shown to increase host resistance against various kinds of cancer and has the potential to restore the immune function of affected individuals (Chihara et al. 1989; Chihara, 1992).

Lentinan has displayed various kinds of immune activities in both animals and in humans. Until recently, the interactions of Lentinan with many kinds of immune cells are not known. An insight into receptor-binding in immune cells by β- glucans from fungi has been provided by Ross et al. (1999). These authors showed that β-glucans from yeast bind to iC3b-receptors (CR3, CD11b/CD18) of phagocytic cells and natural killer (NK) cells, stimulating phagocytosis and/or cytotoxic degranulation. Thus, research has shown previously that Lentinan stimulates various kinds of immune cells including macrophages, NK-cells and lymphocytes (T- and B-cells).

The antitumor activity has been shown to be abolished in neonatally thymectomised mice and it was decreased by the administration of antilymphocyte serum. Both practices reduce or eliminate T-lymphocyte production that is central to cell-mediated immunity. This supports the concept that Lentinan requires immunocompetent T-cell compartments (Maeda et al. 1971; Maeda and Chihara, 1973). Lentinan can activate NK-cells *in vitro* in the same concentrations that are achieved in the blood plasma of patients treated clinically with Lentinan. NK cell activity is involved in tumor suppression and while these cells do not stimulate certain T-killer cell activity, or do so only under certain conditions, they are strong T helper cell stimulants both *in vitro* and *in vivo*. Lentinan can inhibit prostaglandin synthesis, which can slow T-cell differentiation in animals and humans, as well as inhibiting suppressor T-cell activity *in vivo*, and in addition, increase the ratio of activated T-cells and cytotoxic T-cells in the spleen when administered to gastric cancer patients with chemotherapy (Hobbs, 2000).

The immune mediators like interleukin-1 (IL-1) (Fruehauf, et al. 1982), IL-3 (Izawa et al. 1984), IL-6 (Maeda, et al. 1992), colony stimulating factor(s) (Izawa et al. 1984) are the serum factors which are mainly produced by macrophages or T-lymphocytes and act on lymphocytes, hepatocytes, vacular endothelial cells, and other cells. Lentinan has been shown previously to increase the capacity of peripheral blood mononuclear cells of patients with gastric cancer, resulting in the production of IL-1α, IL-1β and TNF-a (Chihara, 1992). In its role as a host defence potentiator, Lentinan triggers the increased production of colony stimulating factors (CSFs) and IL-3, which correlates with the IL-1-producing activities of macrophages (Izawa, et al. 1984; Chihara, et al. 1989). Increased production of IL-1 results in augmented maturation capable of inducing IL-2, natural killer activating factor, and macrophage-activating factor (MAF). IL-1 also amplifies maturation of effector cells (type of lymphocyte that actively engaged in secreting antibodies) and augments responsiveness to lymphcytokines such as IL-2, MAF and others.

An overview of the host immune responses involved in Lentinan-mediated recognition and destruction of cancer cells is presented in Fig. 6. Interestingly, accumulating evidence suggests that Lentinan-stimulation of dendritic cells (antigen-presenting cells that are found in lymph nodes, spleen and thymus; follicular and interdigitating dendritic cells, skin: Langerhans cells, and other tissues; interstitial dendritic cells) has an important impact on immunomodulation and antitumor activity. Moreover, dendritic cell tumor-infiltration in association with killer cytotoxic T-cell stimulation and activation has been shown to have a governing role in tumor attack and elimination (Chihara, 1987).

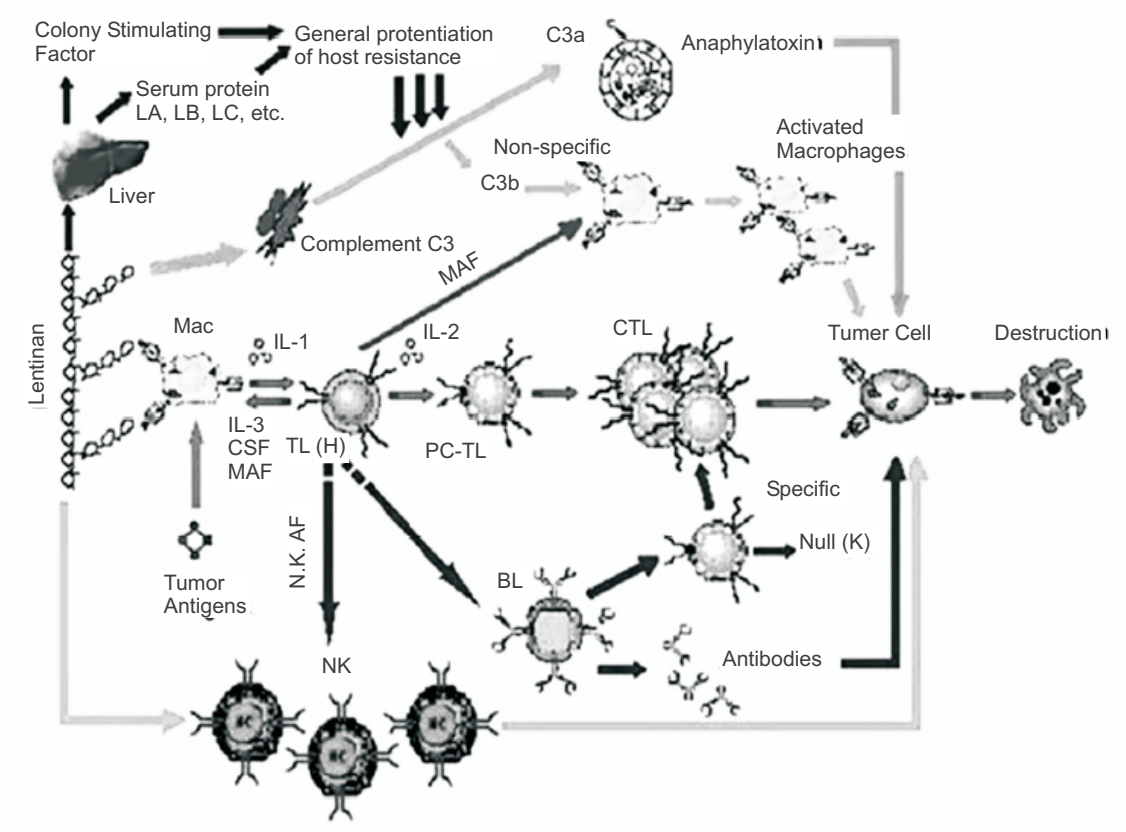

Fig. 6 Mode of action of Lentinan on cancer cells (Chihara et al. 1992; Mohammad-Fata Moradali et al. 2007).

Thus, Lentinan has been shown to restore or augment the ability of host cells to respond to lymphocytokines or other intrinsic bioactive factors and protect patients from infectious disease or cancer metastases (Medha et al. 1971). Lentinan can also improve the physiological constitution of host defense mechanisms by restoring homeostasis and enhancing intrinsic resistance to disease. Homeostasis is a term given to cellular processes, by which both negative and positive control are exerted over the values of a variable or set of variables, and without which control the system would fail to function. Lentinan may restore and augment immunological responsiveness of host cells, but it has no direct cytotoxicity against tumors.

Polysaccharide krestin has remarkable immune-enhancing activity and a broad antineoplastic scope. It has been shown to prolong the survival time of radiated mice, stimulate phagocytotic activity of macrophages, and improves the functions of the reticuloendothelial system (Zhu, 1987). With regard to its antitumor properties, it acts directly on tumor cells, as well as indirectly in the host to boost cellular immunity (Hobbs, 1995; Stamets, 2000). It has shown antitumor activity in animals with adenosarcoma, fibrosarcoma, mastocytoma, plasmacytoma, melanoma, sarcoma, carcinoma, and mammary, colon, and lung cancer (Sugimachi et al. 1997). An intriguing feature of this compound is that

injection of PSK at one tumor site has been shown to inhibit tumor growth at other sites, thus helping to prevent metastasis. PSK has been used both orally and intravenously in clinical medicine. It has been shown to be effective against many types of cancer (Hobbs, 1995; Stamets, 2000), but seldom with satisfactory results if administered alone.

Protein bound polysaccharides PSK (Krestin) and PSP are potent immunostimulators with specific activity for T-cells and for antigen-presenting cells such as monocytes and macrophages. The biologic activity is characterized by their ability to increase white blood cell counts, IFN-γ and IL-2 production and delayed type hypersensitivity reactions (Tzianabos, 2000). *In vitro* experiments with spleen T-lymphocytes cultured in solutions containing various concentrations of PSP showed that, when compared to the physiological saline control group, concentrations in excess of 100 μg/ml produced an increase by a factor of 1.5 to 4 times in T-lymphocytes. It was also determined that PSP can appreciably increase the secretion of IL-2 in mice (Yang, et al. 1993). Human white blood cells (WBCs) were cultivated in solutions containing different concentrations of PSP. Using vesicular stomatis virus as the challenge virus, the PSP induced interferon in human WBCs. γ-Interferon levels in the PSP group were twice those of the control group, while γ-interferon levels were two to four times higher. PSP can promote the expression of the IL-6 gene of peripheral blood lymphocytes (PBL) in humans and hence induce the production of interleukin 6 (IL-6) (Yang et al. 1993; Yu et al. 1996).

PSP stimulates lymphokine-activated-killer (LAK) cell proliferation, and reduces the concentration of IL-2 needed to produce a cytotoxic response. Qian et al. (1999) also showed that PSP (2 g/kg/day) possessed immunopotentiating activities, being effective in restoring cyclophosphamide (CPA) induced immunosuppression such as depressed lymphocyte proliferation, NK cell function, production of white blood cells and the growth of spleen and thymus in rats. In addition, PSP increased both IgG and IL-2 production where CPA had significant inhibitory effects. PSP effectively stimulated the generation of INF-γ reaching level of 800-1000 IU/ml when the concentration of PSP was 100 μg/ml, and improved yields of INF-γ were reported (Yang, et al. 1999). PSP in concentrations of 50-100 μg/ml promoted the proliferation of phytohaemagglutinin (PHA) - activated human peripheral blood lymphocytes (Liang et al. 1996). These researchers observed a greater increase in the $CD4^+$ cell group levels compared with $CD8^+$ cells, thereby raising $CD4^+/CD8^+$ ratio.

Polysaccharide krestin has been shown to have no substantial effect on immune responses of the host under normal conditions (Ehrke et al. 1983; Tsukagoshi et al. 1974; 1984). It can restore the immune potential to the normal level after the host was depressed by tumor burden or anticancer chemotherapeutic agents (Tsukagoshi et al. 1984; Dong et al. 1996; 1997). In ICR mice, antibody production against trinitrophenyl that had depressed the immunity in Sacroma 180-bearing mice can be restored by PSK administration. Oral administration of PSK can improve the impaired antitumor $CD4^+$ T-cell response in gut-associated lymphoid tissue of specific-pathogen free mice (Harada et al. 1997). PSK enhances the cytotoxic activity of peripheral blood lymphocytes (PBLs) *in vivo* and *in vitro*. On a related issue, it may accelerate the interaction of PBL with tumor cells such as T24 human urinary bladder tumors when effector cells and target cells are exposed to PSK simultaneously. Interestingly, Okazaki et al. (1995) have showed that PSK exerts tumorcidal activity by inducing T-cells that recognize PSK as an antigen and kill tumor cells in an antigen-specific manner.

More than 100 types of polysaccharides have been isolated from *Ganoderma lucidum*. (Mizuno et al. 1981, 1982). The major immunomodulating effects of these active substances derived from *G. lucidum* include mitogenicity and activation of immune effector cells such as macrophages, NK and T-cells (Gao and Zhou, 2002). Stimulation of these immune effector cells results in the production of cytokines such as interferon (INF), interleukins (IL) and tumor necrosis factor (TNF)-α. Extracts from *G. lucidum* containing poylsaccharides have shown mitogenic effects on human peripheral blood mononuclear cells (PBMC). Both *in vitro* and *in vivo* studies in mice have shown that water-soluble extracts from *G. lucidum* can stimulate the production of interleukin-2 (IL-2) by splenocytes in the presence of hydrocortisone. It is also the potent activator of human T-lymphocytes, where they induce the production of cytokines such as IL-1β, INF-γ, TNF-α, IL-2, IL-6 and IL-10 (Wang, et al. 1997; Mao, et al. 1999). A polysaccharide fraction from *G. lucidum* (GLB) was shown to promote the production of IL-2 in a dose-dependent manner. GLB augmented the toxicity of cytotoxic T-lymphocytes by as much as 100% when administered at a concentration of 200 μg/ml.

A water-extracted polysaccharide fraction from *G. lucidum* enhanced the cytotoxicity of splenic NK cells in tumor-bearing mice (Lee, et al. 1995). Murine and human macrophages are also activated by polysaccharides from *G. lucidum* (Lee et al. 1995). The macrophage responses (such as the release of cytokines, nitric oxide and other mediators) are associated with antitumor and anti-inflammatory effects. CR3 receptors on human macrophages bind β-D-glucans and become internalised. This initiates a cascade of events including the production of IL-1β, IL-6, INF-γ and TNF-α which cause anti-proliferation and the induction of apoptosis in HL-60 and U937 leukemic cells (Lee et al. 1995; Wang et al. 1997). Antibody neutralization studies have shown that INF-γ and TNF-α released from macrophages act synergistically to inhibit the growth of leukemic cells (Li et al. 2000). β-D-glucan (Ganoderan) and a protein-polysaccharide fraction from *G. lucidum* are potent stimulators of macrophages. Ganoderan has been shown to increase the expression of MHC class II molecules on these antigen-presenting macrophages (Oh et al. 1998). There is also evidence to suggest that extracts from *G. lucidum* can influence humoral or B cell immunity.

The polysaccharide schizophyllan shows antitumor activity against both the solid and ascite forms of Sarcoma 180, as well as against the solid form only of sarcoma 37, Erlich sarcoma, Yoshida sarcoma and Lewis lung carcinoma (Hobbs, 1995). Schizophyllan has also increased cellular immunity by restoring suppressed killer-cell activity to normal levels in mice with tumors (Borchers et al. 1999). Best results against radiation damage were found when schizophyllan was administered shortly after or at the same time as radiation, and schizophyllan restored mitosis of bone marrow cells previously suppressed by anticancer drugs (Zhu, 1987). Human clinical studies proved the beneficial activity of treatment with schizophyllan for patients with recurrent and inoperable gastric cancer, stage 2 cervical cancer, and advanced cervical carcinoma (Hobbs, 1995).

Schizophyllan is relatively similar to Lentinan in composition and biological activity, and its mechanism of immunomoduation and antitumor action appears to be quite similar (Jong, et al. 1991). The induction of gene expression of cytokines by schizophyllan has been studied *in vitro* and *in vivo* (Okazaki et al. 1995). After schizophyllan is administered intraperitoneally to ICR mice, the kinetics of gene expression of cytokines is different in peritoneal exudate cells, splenocytes, and hepatocytes (Ooi and Liu, 1999). It is generally accepted that protein

synthesis and gene expression of cytokines are regulated separately. Therefore, the antitumor activity of Schizophyllan is mainly due to host-mediated immune responses (Okazaki et al. 1995).

Neither Schizophyllan nor Lentinan demonstrated any anti-tumor activity against Sarcoma 180 under *in vivo* experiment with cyclosporin A as a T-cell suppressor, which suggests that an immunocompetent T-cell component is necessary for developing antitumor activity (Kraus and Franz, 1991, 1992). These results indicate that Schizophyllan and Lentinan are T-cell oriented immunopotentiators and therefore, require a functional T-cell component for its biological activity and that the action of (1-3)-β-D-glucans on the host's immune system might: (1) increase helper T-cell production, (2) increase macrophage production, (3) bring a non-immunological increase of the host defense mechanisms through stimulation of acute phase proteins and colony stimulating factors, which in turn effects proliferation of macrophages, PMNC, and lymphocytes and activation of the complement system (Bohn and BeMiller, 1995).

Another (1-3)-β-glucan, Grifolan, from *Grifola frondosa* is similar to schizophyllan in primary structure (Adachi, et al. 1994). Enhancement of mRNA levels of IL-6, IL-1 and TNF-α of macrophages by Grifolan treatment is detected *in vitro* by reverse transcription-polymerase chain reaction (RT-PCR), showing that grifolan is a novel macrophage activator that increases cytokine production (Ooi and Liu, 1999). A novel polysaccharide-bound protein (PSPC) (Mol. Wt. 15.5 KDa) has been isolated from cultured mycelium of *Tricholoma mongolicum* Imai (Wang, et al. 1996). PSPC activated both lymphocytes and macrophages from BALB/c mice and showed no direct cytotoxic activity against fibroblasts, hepatoma cells, and choriocarcinoma cells.

Similarly, immunomodulatory and antitumor PSPC with a molecular weight of about 154×103 has been purified and characterised from the culture filtrates of *Tricholoma lobayense* Heim (Liu, et al. 1995; 1996). It inhibited the growth of Sacroma 180 implanted in mice intraperitoneally or subcutaneously, with no sign of toxicity *in vivo* (Liu et al. 1995). PSPC has been able to restore the phagocytic function of peritoneal exudate cells and the mitogenic activity of T-cells of tumor-bearing mice.

Pharmacologically, these mushroom compounds are classified as biological response modifiers and have antitumor activity, a result of activation or augmentation of the host's immune system or immunocompetency rather than direct cytotoxicity. However, recent evidence suggests that some mushroom polysaccharides may also possess cytotoxic properties. In search for a more effective treatment for hormone-refractory prostate cancer, the potential antitumor effect of Grifron-D (a unique β-glucan from the Maitake mushroom *Grifola frondosa*) on androgen-independent prostatic cancer PC-3 cells was investigated (Fullerton et al. 2000).

β-GLUCAN BINDING RECEPTOR

β-Glucan from fungi binds to specific iC3b-receptors (CR3, CD11b/CD18) of phagocytic cells and NK cells, stimulating phagocytosis and/or cytotoxic degranulation (Ross, et al. 1999). The iC3b-receptor, CR3, also known as Mac-1 or αMβ2-integran, has two major functions. As Mac-1 adhesion molecule, it mediates the diapedesis of leukocytes through the endothelium and it stimulates phagocytosis and degranulation in response to microorganisms or immune

complexes opsonised (i.e., coated with) iC3b (Ross, et al. 1999). Most β-glucan that have immuno-modulatory properties are derived from yeast and fungi (mushrooms) and have a backbone structure of linear β-1,3-linked D-glucose molecules with β-1,6-linked side chains (Bohn and BeMiller, 1995). Although somewhat controversial, Czop and Kay (1991) and Zimmerman et al. (1998) suggested that CR3 serves as the major, if not only receptor for β-glucans with human (Thornton, et al. 1996) or mouse (Xia et al. 1999) leukocytes, and therefore, may be responsible for all reported functions of β-glucans *in vitro* and *in vivo*. These β-glucans polymers specifically target macrophages, neutrophils, and NK cells to tumors that are opsonised with antibody and C3 (complement 3).

The recently described leukocyte-glucan receptor, Dectin-1, was almost exclusively responsible for the glucan dependent, nonopsonic recognition of zymosan (a type of β-glucan) by primary macrophages (Brown et al. 2002). As Dectin-1 acts as a pattern recognition receptor for a variety of β-1,3 and/or β-1,6 glucan linked carbohydrates in transduced murine fibroblasts, Dectin-1 as a new target for examining the immunomodulatory properties of glucans for therapeutic drug design.

MUSHROOM POLYSACCHARIDES IN THE TREATMENT OF CANCER

The anticancer properties of β-glucan are due to direct stimulation of macrophages, neutrophils and NK cells and indirect stimulation-via activated macrophages of cytotoxic T-lymphocytes. The relative roles of each of these cell types in β-glucan tumoricidal activity are not fully understood, but studies indicate a primary role for macrophages. β-Glucan binds to macrophages and activates them to phagocytose tumor cells that have been "tagged" for destruction by antibodies and small serum proteins (complement proteins), which bind to the tumor cell. (This process is called opsonisation.) Macrophage attachment to tagged (opsonised) tumor cells is primed by β-glucan, which optimises binding to the complement proteins on the tumor cell, thus enhancing phagocytosis of tumor cells.

Activated macrophages also produce cytokines that prime Natural Killer (NK) cells and T-lymphocytes, both of which are cytotoxic to tumor cells, via different mechanisms. NK cells secrete chemical substances that destroy tumor cells, by bursting open cell membranes. Cytotoxic T-lymphocytes secrete cytokines that stimulate macrophages to produce other tumoricidal chemical substances.

Barley β-glucan has been shown to stimulate tumoricidal activity in both macrophages (Thornton et al. 1996; Roubroeks et al. 2000) and NK cells (Di Renzo et al. 1991; Vetvicka et al. 1996) *in vitro* and has shown exceptionally strong enhancement of monoclonal antibody (mAb) cancer therapy of a range of common human cancers (Cheung and Modak, 2002; Cheung, et al. 2002; Hong, et al. 2004). This impressive antitumor activity gives barley β-glucan huge potential as a cancer therapy agent in conjunction with mAb.

More than 50 mushrooms species have yielded potential immunoceuticals that exhibit anticancer activity *in vitro* or in animal models. Andrea et al. 2004 listed the antitumor activity of some mushroom and/or their constituents. Six of these polysaccharides that have been investigated in human cancers include Lentinan, Schizophyllan, active hexose correlated compounds (AHCC), Maitake D-fraction, polysaccharide-K and polysaccharide-P. Lentinan, produced from Shiitake mushroom, *Lentinus edodes,* is a β-(1-3), β-(1-6) glucan. There is an immense literature related to the anticancer effect of lentinan on animals and

Table 2 Antitumor activities of a variety of Mushrooms and/or their constituents.

Mushroom/constituent	*Dose and route of administration*	*Mouse strain*	*Tumor model*	*% Inhibitionor increase in life span*	*Reference*
Lentinus edodes polysaccharides LC-1, LC-33, EC-11 and Ec-14	Samples injected as I.P daily for 10 days 24h after tumor implantation. The effective doses of LC-1, LC-33, EC-11, and EC-14 are 100mg, 5mg, 50mg and 50mg /Kg	Swiss albino	Sarcoma 180	71.3, 97.5, no effect and 42.1% inhibition found	Chihara et al. 1969
Schizophyllum communae Schizophyllan Sp, Sme, and Sc	10mg/ kg intramuscular administration for 31 days	ICR/JCL	Sarcoma 180	30.53, 34.09 and 99.5%	Kojima et al 1986
Cryptoporus volvatus polysaccharide fractions H3B, SH3B, and SPG	Intramuscular injection of samples to hind paws at a dose of 5 or 10 mg per kg of mouse.	ICR/JCL	Sarcoma 180	97.6, 89.0 and 97.0% inhibition was found respectively.	Kitamura et al. 1994
Agaricus blazei/ATF = acid treated fraction Fraction containing mostly (1-4)-a-D-glucan and (1-6)-b-D-glucan	1 mg/ mouse intratumorally in to the right flank on Days 3,4, and 5 after MethA injection into the right and left flank	BALB/c	MethA, double-grafted	70% in both flanks	Fujimiya et al. 1998
Agaricus blazei Polysaccharide fractions	0.5 or 2 mg/mouse I.P., 5 doses on alternate days starting 7 days after tumor implantation	ICR	Sarcoma 180	No effect and 77-90%	Ohno et al. 2000
Hot water extract	2 mg p.o., 35 doses (no further detailsprovided)		Sarcoma 180	47%	
Sparassis crispa/several polysaccharide fractions	0.020, 0.1, or 0.5 mg/mouse I.P., three doses on Days 7, 9, and 11 after tumor implantation	ICR	Sarcoma 180	0.02 mg: 54 to 84; 0.1 mg: 95 to 100; 0.5 mg: 91 to 99%	Ohno et al. 2001
***Lyophyllum decastes* Sing.** Ethanol precipitate of hot water extract	10 mg/kg I.P. for 10 d starting 24 hrs after tumor implantation	ICR	Sarcoma 180	88%	Ukawa et al. 2000
Polysaccharide fractions IV-2 and IV-3	10 mg/kg I.P for 10 days starting 24 hrs after tumor implantation		Sarcoma 180	97% with complete regression in 9/10 mice	
***Lentinus edodes* (Shiitake)** Lentinan	3 mg/mouse/day p.o. starting 7 days before K36 inoculation	AKR	K36 murine lymphoma	94%	Ng and Yap, 2002
Crude extract	"Equivalent volume" to lentinan p.o.starting 7 days before K36 inoculation		K36 murine lymphoma	55%	

Lentinan	As above		Various colon carcinoma cell lines	90-93%	
***Phellinus rimosus* (Berk) Pilat.**					
Ethyl acetate Methanol Aqueous	50 mg/kg p.o., for 10 consecutive days starting 24 hrs after tumor implantation	Swiss albino	Ehrlich ascites carcinoma (EAC)	65% increase in life span 33% increase in life span No effect	Ajith and Janardhanan, 2003
Ethyl acetate Methanol Aqueous	50 mg/kg p.o., for 10 consecutive days starting 24 hrs after tumor implantation	Swiss albino	Dalton's lymphoma ascites	96% 84% 88%	
Ethyl acetate Methanol Aqueous	50 mg/kg p.o., for 10 daysstarting 13 days aftertumor implantation	Swiss albino	Dalton's lymphoma ascites	64% 49% 57%	
***Pleurotus pulmonarius* (Fr.) Que I.** methanol extract	250, 500, or 1000 mg/kg I.P, five doses on alternate days starting 24 hrs after I.P tumor cell injection	BALB/c	Ehrlich ascites carcinoma	No effect	Jose et al. 2002
	250, 500 or 1000 mg/kg I.P 10 doses on consecutive days staring 24 hrs after s.c. tumor cell injection		Ehrlich ascites carcinoma	52, 67, and 82% (volume); 50, 64, 81% (weight)	
***Lepista inversa* (Scop.: Fr.)** Pat./CE = methanol crude extract	75 mg/kg I.O., starting 3 days after tumortransplantation	DBA/2	L1210 (lymphocytic leukemia)	50% increase in lifespan	Bezivin et al. 2003
	75 mg/kg I.P., starting 5 days after tumortransplantation		3LL (Lewis lung carcinoma)	No significant effect	

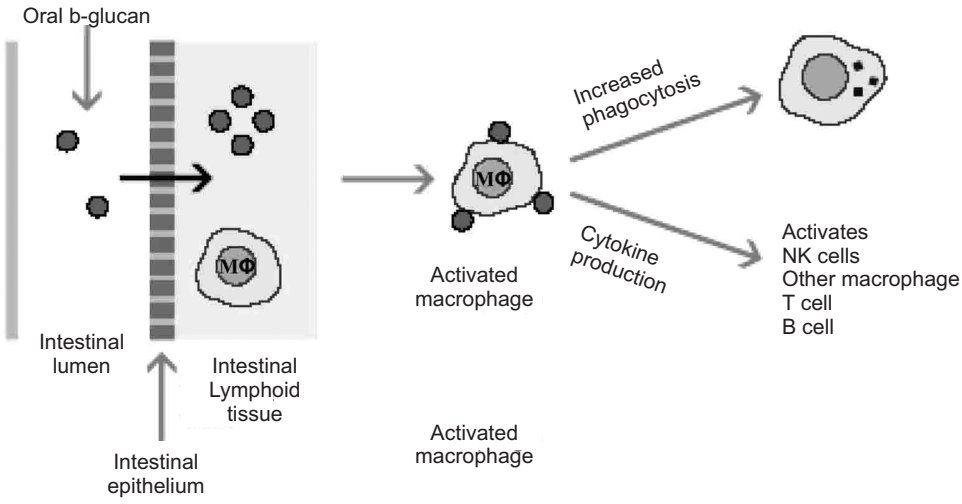

Fig. 7 β- Glucan activation of macrophage

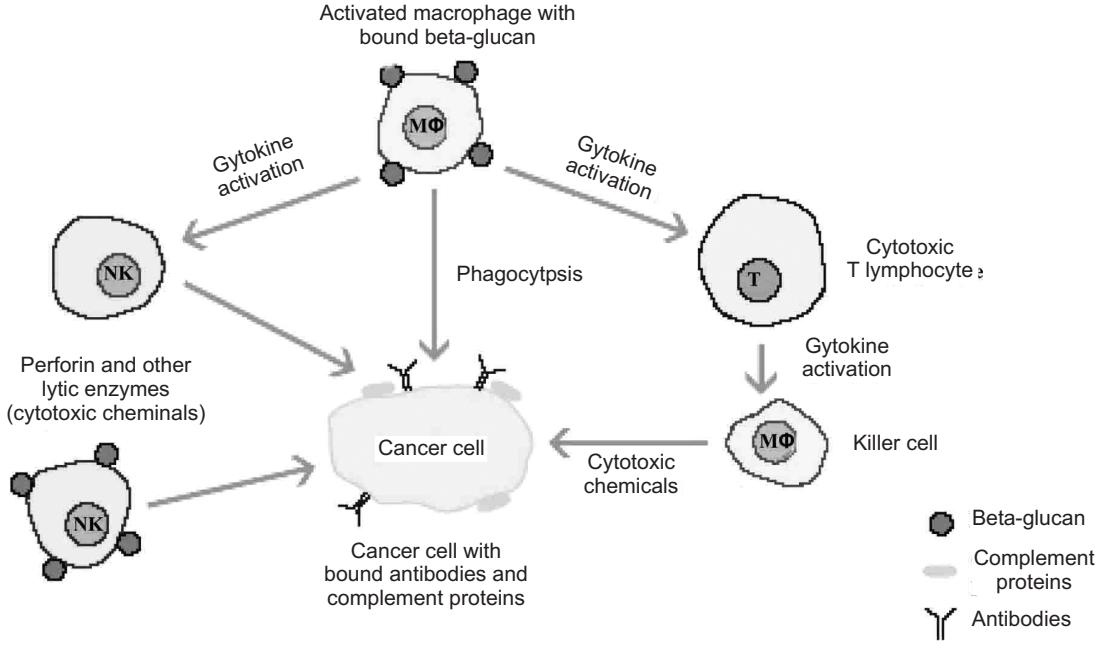

Fig. 8 β -Glucan stimulation of antitumor immune response

human carcinomas. It was first isolated and studied by Chihara et al. (1970) who demonstrated that its antitumor effects were greater than other mushroom polysaccharides. Maeda et al. (1974) however reported that lentinan was active for some but not all types of tumors. There have been numerous clinical trials of lentinan in Japan, and the drug is now manufactured and sold by several pharmaceutical companies.

Lentinan has proved successful in prolonging the overall survival of cancer patients, especially those with gastric and colorectal carcinomas (Furue and Kito, 1981 Taguchi, et al. 1985). In patients with recurrent gastric cancer, tumor responses and prolonged median survival were also noted. In a randomized controlled study of patients treated with tegafur or a combination of lentinan and tegafur overall survival was significantly prolonged in the lentinan plus tegafur group. More patients with the combined therapy appeared to survive longer: 19.5% survived more than one year, 10.4% more than two years and 6.5% more than three years. Using the criteria of the Japan society for cancer therapy for evaluation of clinical effects of cancer chemotherapy on solid tumors, patients treated with lentinan had a significantly higher response rate (14.9% than patients in the control arm).

A few adverse reactions to lentinan have been noted. In a detailed study of 469 patients, 32 (6.8%) experienced an adverse reaction, only 2 patients required discontinuation of treatment due to unacceptable tolerance. Perhaps the most intriguing aspects of lentinan use in conjunction with other chemotherapeutic agents is its apparent ability to greatly reduce the debilitating effects of the chemotherapy, e.g. nausea, pain, hair loss and lowered immune status.

The polysaccharide Schizophyllan derived from the mushroom *Schizophyllum commune* has been shown to be cytostatic in sarcoma 180 tumor xenographs. The survival of these xenographs was not affected by pretreatment with schizophyllan. Schizophyllan had no effect on the survival of sarcoma 37, Ehrlich carcinoma or Yoshida sarcoma ascites tumors (Wasser and Weis, 1999).

Various clinical trials have been carried out in Japan, although many are not blinded. Despite this, schizophyllan has been approved for clinical use in Japan. Early clinical studies with schizophyllan in combination with conventional chemotherapy (tegafur or mitomycin C and 5-fluorouracil) in a randomized controlled study of 367 patients with recurrent and inoperable gastric cancer resulted in a significant increase in median survival (Furne, 1985). However, a similar study was unable to confirm this apparent success with schizophyllan (Fugimoto and Furue, 1984). Schizophyllan has also been shown to increase overall survival of patients with head and neck cancers (Kimura et al. 1994). In a randomized controlled study of schizophyllan in combination with radiotherapy, Schizophyllan significantly prolonged the overall survival of stage II cervical cancer patients but not stage III (Okamura et al. 1986, 1989). In a prospective, randomized clinical trial involving 312 patients treated with surgery, radiotherapy, chemotherapy (fluorouracil) and schizophyllan in various combinations, patients treated with schizophyllan had a better overall survival than patients who had not received the polysaccharide (Miyazaki et al. 1995). Schizophyllan is currently produced commercially by several Japanese pharmaceutical companies.

Active hexose correlated compound (AHCC) is a proprietary extract prepared from co-cultured mycelia of several species of basidiomyceteous mushrooms, including Shiitake *(Lentinus edodes).* Animal research and preliminary human studies have indicated that AHCC has anticancer efficacy. Beginning in 1992, Kamiyama conducted a trial in Japan to evaluate the preventive effect of AHCC against recurrence of hepatocellular carcinoma following surgical resection. The investigators reported that after one year the AHCC group showed a significant higher survival rate than the control group, as well as significant lowering of certain tumor markers in the serum. The AHCC Research Association was formed in Japan in 1996 to foster the development of AHCC as an anticancer therapy. In their circulating

abstracts they report that of 300 cancer patients administered AHCC, 58 were effectively treated, with 46 showing complete or partial regression and 12 experiencing no change of tumor size.

Several studies have shown that β-D-glucan derived from *Grifola frondosa* (also known as Maitake) has strong antitumor activity in xenographs (Kurashiga et al. 1997) and there have also been limited number of clinical trials. A highly purified extract, β-glucan (β-1,6-glucan branched with a β-1-3-linkage) (Grifron-D, GD) has become available. GD has considerable immunomodulating and antitumor activities in animal models, and is orally bioavailable (Nishida et al. 1988). Maitake D-fraction and crude Maitake powder have demonstrated remarkable inhibition of metastasis in a mouse model, especially in the prevention of hepatic metastases, which in one series of experiments was reduced by 81% (Maitake powder) to 91% (D-fraction) (Nanba, 1995). GD has been shown to have a cytotoxic effect on human prostate cancer cells (PC9) *in vitro*, possibly acting through oxidative stress, and causing 95% cell death by an apoptosis (Fullerton et al. 2000). Vitamin C addition reduced the effective level of GD required.

An early pilot study from China involving 63 cancer patients showed a response rate (partial and complete) against solid tumor at 95% and for leukaemia 90% (Jones, 1998). Nanba (1997 a) observed tumor regression or significant symptomatic improvement in 11 out of 15 hepatocellular carcinomas with D-fraction plus Maitake. When D-fraction plus Maitake was combined with chemotherapy, the overall response rates were increased by 12-28% when results from cancer types were combined. The Food and Drug Administrarion (USA) has approved Grifon-D (GD) for trial under an Investigational New Drug Application (IND) for patients with advanced cancer and some US-based clinical trials are underway at various Institutions (Nanba, 1997 b).

Conclusions

The literature published over the past decade supports the concept that mushroom polysaccharides and mushroom extracts offer a lot of hope for cancer patients and sufferers of many devastating diseases. A fundamental principle in oriental medicine is to regulate homeostasis of the whole body and to bring the disease person to their normal state. Of the physiological constitution in favor of host defense results in the activation of many vitally important cells for the maintenance of homeostasis. A variety of polysaccharides from a number of mushrooms have been demonstrated to enhance the immune system. All of them have shown significant antitumor activity as a result of their ability to activate the host immune system rather than direct cytotoxicity. The researches conclude that mushrooms have more nutritional values especially enriched proteins. Apart from nutritional values, they are also a good source of bioactive compounds that have antimicrobial activity, cholesterol lowering and anticancer activity. Therefore, it is suggested that, the society can consume mushrooms and get immunity against diseases.

Future Outlook

Polysaccharide based carcinostatic (immunotherapeutic) agents have been developed from mushroom. These are used currently in the treatment of cancer of the digestive organs, lung and breast, as well as cancer of the stomach and cervical cancer. Several mushroom species

belonging to the Polyporaceae family are now regarded as the next drug producers. Mushroom polysaccharides are also expected to be developed into multipurpose medicines that are not only carcinostatic but also anti-inflammatory, antiviral (against AIDS), hypoglycaemic and antithrombotic. The mushroom polysaccharides appear to be well tolerated and compatible with chemotherapy and radiation therapy. However, studies that identify the molecular mechanisms that occur in specific immune modulation by mushroom polysaccharides such as receptors, the downstream events triggered by the binding of these polymers to their target cells, detailed study and cloning of the β-glucan synthesis genes are urgently needed.

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