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CHAPTER 8

Polysaccharides from Medicinal Mushrooms for Potential Use as Nutraceuticals

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Contents

8.1 Introduction

Mushrooms have been used for centuries in Asian and other traditional cuisine and traditional medicines due to their culinary and medicinal properties, but their properties remained unknown to the wide scientific community for a long time. In the last few decades, a large amount of research has focused on the types, sources, biosynthesis, and medicinal properties and applications of many mushrooms, mainly members of the Basidiomycetes family. The most common active ingredients in these higher fungi are their extracellular,

intracellular, or cell wall polysaccharides, which exhibit immunostimulating, antitumor, antimicrobial, anti-inflammatory, antioxidant, prebiotic, hypoglycemic, and hypocholesterolemic effects. In the last few years, some of these mushrooms or their biopolymers have been commercialized in pharmaceutical applications, but their application in food and nutraceuticals is still at an early stage. However, the fact that many of these mushrooms are edible (and thus nontoxic) as well as tasty makes them, or their polysaccharides, potentially ideal ingredients for the formulation of novel functional foods and nutraceuticals. However, their biological properties might be affected after addition to food, due to food processing and/or interaction with food ingredients. This chapter describes the most important and studied types and sources of bioactive mushroom polysaccharides, the biosynthesis and bioprocess conditions used for the production/cultivation in solid or liquid media, the relation between molecular/structural characteristics and bioactivity, their medicinal properties, and their existing or potential applications in human nutrition.

Microbial polysaccharides excreted or contained in the cell wall of bacteria, yeast, and fungi are long known to possess biological properties that promote human health, largely because they serve as dietary fiber, but few of them have been utilized to formulate novel drugs or nutraceuticals (Giavasis 2013, Giavasis and Biliaderis 2006). For centuries, a large number of medicinal mushrooms have been consumed in the Far East (China, Japan, Korea, and other countries where they are native), as part of a healthy diet and as a traditional cure for several diseases, since they can stimulate a general, nonspecific positive immune response, which is at the core of the holistic curative approach of traditional Asian medicine (Wasser 2002). In the last few decades, scientific research has focused on exploring the properties of several higher fungi, which produce a variety of bioactive polysaccharides in order to bridge the gap between traditional practice and the development of novel standardized, commercial functional foods, and nutraceuticals (Chang and Wasser 2012). This research has revealed that many of the members of the Basidiomycetes family (as well as some of the Ascomycetes family) are able to produce immunomodulating, hepatoprotective, antimicrobial, prebiotic, antioxidant, hypoglycemic, and hypolipidemic substances, which are usually β-glucans, or heteropolysaccharides, or proteoglucans (although other bioactive ingredients such as terpenes, sterols, phenols, and flavonoids also exist in many higher fungi) (Chang and Wasser 2012, Giavasis 2013, Mizuno and Nishitani 2013, Wasser 2011). These macromolecules are often described with the general term "biological response modifiers" (BRMs) due to their multiple biological effects and their indirect or nonspecific modes of action, which trigger several immune responses and potentiate the curative properties of the human body (Giavasis and Biliaderis 2006, Sullivan et al. 2006, Wasser 2002).

The bioactive polysaccharides from edible mushrooms in particular (in comparison to nonedible fungi) are very interesting and useful ingredients

for the formulation of functional foods and nutraceuticals, since they are already consumed as part of the traditional diet in Asian or South American countries, without any toxicological concerns. For instance, Lingzhi, Maitake, Shiitake, oyster, or other mushrooms deliver medicinal biopolymers that can be and have been adopted as the basis of novel nutraceuticals, in order to boost the immune system, act as anticancer or antiageing ingredients, reduce the side effects of chemotherapy/radiotherapy, protect against viral infections, reduce blood sugar and cholesterol, and stimulate the growth of probiotic bacteria (Giavasis 2013, Stachowiak and Reguła 2012, Wasser 2002, Xu 2001). However, despite the discovery of many medicinal mushrooms and the identification of their bioactive polysaccharides, only a handful of functional food products exist where these polysaccharides are utilized. This is probably because there are several concerns regarding the application of these important molecules in final food products, such as the molecular and structural diversity of the biopolymers that can be produced from the same fungus (which may affect the expression of pharmaceutical properties), the appropriate concentration of biopolymer that needs to be applied in nutraceuticals in order to express a health effect without inferring any toxic effects, the unstable quantity, quality, and availability of these mushroom polysaccharides, the impact of the purification process and food processing on the bioactivity of the biopolymers, and their production costs (Cho et al. 1999, Falch et al. 2000, Giavasis 2013, Maji et al. 2013, Wasser 2011, Zhang et al. 2011). Therefore, some of the issues that need attention and further investigation before the broad adoption of these biopolymers by the industry are the structure–function relationships of these polysaccharides (Falch et al. 2000, Giavasis and Biliaderis 2006, Maji et al. 2013), the standardization of the production/cultivation process and the purification process, the development of cost-effective strategies for controlled and stable productivity (e.g., by the cultivation of the fungal mycelium in bioreactors, which could shorten production time significantly and allow optimization and control of environmental process conditions) (Kim et al. 2002, Lee et al. 2004, Tang and Zhong 2003) and the conduction of more toxicological and clinical studies in humans, which will support the health claims that can be linked to the consumption of bioactive mushrooms polysaccharides as nutraceuticals (Aleem 2013, Jeurink et al. 2008, Zhou et al. 2005). Below, the issues already mentioned will be addressed and the most recent developments will be summarized regarding the types, properties, production process, and the utilization of medicinal mushroom polysaccharides as sources for novel functional foods.

8.2 Types and Sources of Bioactive Mushroom Polysaccharides

Lentinus (or *Lentinula*) *edodes*, also known as the Shiitake mushroom, is one of the most common, popular, and well-studied medicinal and edible

mushrooms in China and Japan, which produces a β-glucan known as lentinan. Lentinan contains a backbone of β-(1,3)-D-glucose residues to which β-(1,6)-D-glucose side groups are linked via glycosidic linkages (there is one branch to every third main chain unit) and has an average molecular weight of around 500 kDa, but may range from 400 to 800 kDa (Ganeshpurkar et al. 2010, Giavasis 2013, Ooi and Liu 2000, Thakur and Singh 2013). It is one of the most well-studied immunomodulating polysaccharides and has been used in commercial pharmaceutical applications (Giavasis 2013, Lakhanpal and Rana 2005). Glycoproteins have also been extracted from cultured mycelia of *L. edodes*. Among them, KS-2 is an α-mannan peptide known to have antitumor properties (Bisen et al. 2010, Lakhanpal and Rana 2005).

Schizophyllan (also called sizofiran) is similar to lentinan β-(1,3)-D-glucan with β-(1,6) branches, which is excreted by cultured mycelia of the edible fungus *Schizophyllum commune*. It has a lower molecular weight as compared to lentinan (in the range of 100–200 kDa) and acquires a triple helical conformation in solutions. Schizophyllan is also well-studied for its anticancer, antiviral, and other health effects, which have led to its utilization in industrial pharmaceutical applications, as an adjunct cancer therapy, together with conventional drugs (Ganeshpurkar et al. 2010, Giavasis 2013, Giavasis and Biliaderis 2006, Thakur and Singh 2013, Wasser 2002). The chemical structure (basic repeating unit) of lentinan and schizophyllan and the fruiting bodies of the producer mushrooms are depicted in Figure 8.1.

Hot water extracts or dry powders of *Ganoderma lucidum* (known as the Lingzhi mushroom in China, or Reishi mushroom in Japan, which means "supernatural mushroom") have also been used in traditional Asian medicine for over 2000 years, due to their immunostimulating, antiageing, and antioxidant properties (Bao et al. 2002, Eo et al. 2000, Wasser 2002). *G. lucidum* is an important medicinal mushroom of the Basiodiomycetes family, which produces a number of bioactive polysaccharides. The most typical is called ganoderan. It is a β-(1,3)-glucan with β-(1,6)-glucose branches at C-6, with a variable molecular weight and degree of branching, especially when isolated from the water extracts of the fruiting body. The equivalent glucan isolated from filtrates/centrifugates of liquid-cultured mycelia has a molecular weight of $1.2–4.4 \times 10^6$ Da (Bao et al. 2002, Ganeshpurkar et al. 2010, Giavasis 2013, Wasser 2002). In addition to these, *G. lucidum* also produces several other heteroglucans and proteoglucans with immunostimulating activity, most of which are isolated from water extracts of the fruiting bodies. This variety of isolated biopolymers is quite common among bioactive polysaccharides from mushroom fruiting bodies, which can be a problem with regard to the production of commercial purified and standardized ingredients for drugs and nutraceuticals (Giavasis and Biliaderis 2006, Wasser 2002, Wasser 2011). Ye et al. (2010) studied the structure and

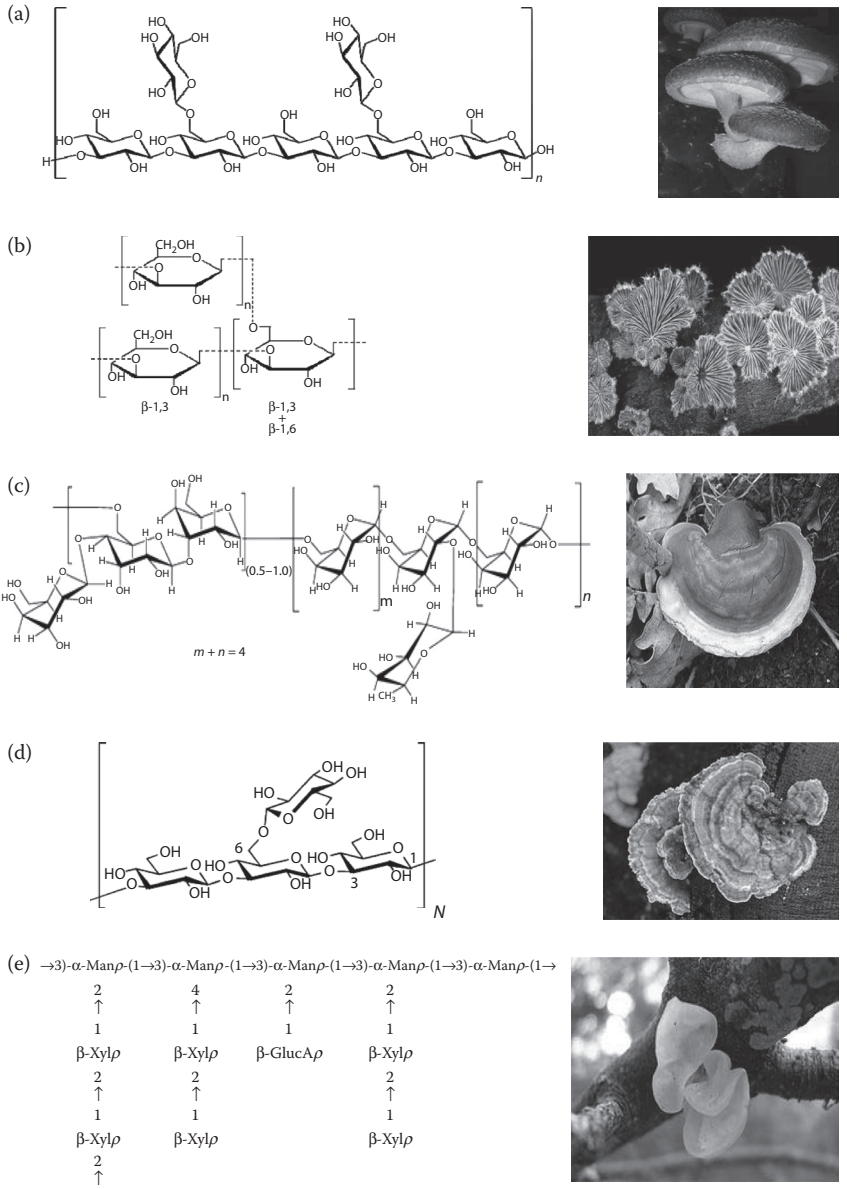

Figure 8.1 Chemical structures of polysaccharide repeating units (up) and the corresponding fruiting bodies (down) of (a) lentinan from *Lentinus edodes*, (b) schizophyllan from *Schizophyllum commune*, (c) the polysaccharide moiety of a *Ganoderma lucidum* proteoglycan, (d) the exopolysaccharide from *Coriolus (Trametes) versicolor*, and (e) the acidic heteroglycan from *Tremella mesenterica.*

composition of a complex polysaccharide moiety of a proteoglucan from *G. lucidum* fruiting bodies. This had a main chain of 1,6-α-galactopyranoside, 1,2,6-trisubstituted-α-galactopyranoside, 1,3-disubstituted-β-glucopyranoside, and 1,4,6-trisubstituted-β-glucopyranoside groups, with branches of 1-β-glucopyranoside and 1-α-fucopyranoside residues, and a relative molecular weight of 1.12 × 104 Da (Figure 8.1).

A close relative of *G. lucidum*, *Ganoderma tsugae* produces water-soluble and alkali-soluble antioxidant polysaccharides (Tseng et al. 2008), as well as water-soluble antitumor protein–heteropolysaccharide complexes with a mean molecular weight of ~62–82 kDa (Peng et al. 2005). It also yields (1,3)-β-D-glucans and (1,4)-α-D-glucans with antitumor properties, after submerged cultivation of the mycelium (Peng et al. 2005).

Another popular and tasty edible mushroom with bioactive polysaccharides is *Pleurotus ostreatus* (oyster mushroom), which forms pleuran, an insoluble β-(1,3/1,6)-D-glucan that exhibits immunostimulating and anticancer properties, especially after solubilization of the carboxymethylated derivatives (Bergendiova et al. 2011, Synytsya et al. 2009). Both *P. ostreatus* and *P. eryngii* produce a water-soluble branched 1,3/1,6-β-D-glucan, a linear alkali-soluble 1,3-α-D-glucan, and an insoluble glucan originating from the cell wall, which may serve as a prebiotic dietary fiber and source of nutraceuticals (Paulik et al. 1996, Synytsya et al. 2009). Similar glucans with pharmaceutical potential are found in other *Pleurotus* species, such as *P. florida*, *P. tuber-regium*, and *P. pulmonarius* (Bergendiova et al. 2011, Synytsya et al. 2009, Zhang et al. 2001).

Agaricus blazei, or *Agaricus brasiliensis*, as it was recently reclassified, is another well-studied edible medicinal mushroom that originates from Brazil, where it is called Piedade mushroom, medicinal mushroom (cogumelo medicinal), sun mushroom (cogumelo do sol), or God's mushroom (cogumelo de Deus) (Largeteau et al. 2011). It yields several antitumor polysaccharides contained in its fruiting body (Giavasis and Biliaderis 2006, Wasser 2002) such as a β-(1,6)/β-(1,3) glucan, an acidic β-(1,6)/α-(1,3) glucan, and an acidic β-(1,6)/α-(1,4) glucan. Unlike most mushroom glucans that are characterized by a β-(1,3)-linked backbone, *A. blazei* glucans have a main chain of β-(1,6) glycopyranose (Giavasis 2013, Mizuno 2002, Wasser 2002). Interestingly, the structure of these glucans can be influenced by the maturity stage of the fruiting bodies as a higher proportion of β-(1,3) and α-(1,4) glycosidic bonds are found in glucans from the most mature mushrooms (compared to immature ones) (Camelini et al. 2005). The fruiting body also contains an antitumor water-soluble proteoglucan with a molecular weight of 380 kDa, which has an α-(1,4)-glucopyranoside main chain and β-(1,6) glucopyranoside branches at a ratio of 4:1 (Fujimiya et al. 1998, Mizuno 2002, Wasser 2002). In addition, two immunostimulating heteroglucans have been isolated from *A. blazei* fruiting bodies, which are composed of glucose, galactose and mannose, one of which contains glucose and

ribose, and a xyloglucan (Giavasis and Biliaderis 2006). When the mycelium is cultivated in liquid cultures, *A. blazei* excretes an extracellular antitumor proteoglucan of very high molecular weight (1000–10,000 kDa), which is composed of mannose, glucose, galactose, and ribose groups (Mizuno 2002, Tsuchida et al. 2001).

Grifolan is also an important immunostimulating gel-forming β-(1,3)-D glucan with β-(1,6) glucosidic linkages at every third glucose residue, which is contained in the fruiting bodies of the edible fungus *Grifola frondosa* (Maitake mushroom) (Boh and Berovic 2007). In submerged cultures, *G. frondosa* produces extracellular antioxidant proteoglucans of high molecular weight (770–1650 kDa) and a protein content of 6–27%, depending on the composition of the fermentation medium as well as proteoglucans of lower molecular weight (around 500 kDa) in the cultured mycelia (Lee et al. 2003). Cui et al. (2007) have also reported the isolation of a low-molecular-weight (21 kDa) antitumor heteropolysaccharide extracted from the mycelia of submerged cultures of *G. frondosa*.

Krestin, or polysaccharide-K (PSK), is one of the most successful therapeutic mushroom polysaccharides (or polysaccharopeptide as it is often referred to) that have been marketed as anticancer drugs (in combination with chemotherapy) and nutraceuticals. It derives from the edible mushroom *Coriolous versicolor* (also known as *Trametes versicolor*) (Hobbs 2004). Ooi and Liu (2000) reported that it is a low-molecular-weight (94,000 Da) proteoglucan containing 25–38% protein residues. PSK has a main chain of β-(1,4)-glucopyranoside, with β-(1,6)-glucopyranosidic lateral chains at every fourth glucose unit. The major monosaccharide is glucose, but other sugar residues are also present, such as mannose, fucose, xylose, and galactose. The proteinaceous residue consists predominantly of acidic amino acids (glutamic and aspartic) and neutral amino acids (valine, leucine) (Ooi and Liu, 2000). However, other studies revealed that three fractions of different molecular weights can be extracted from the mushroom fruiting bodies, namely, a high-molecular-weight fraction (1200 kDa) with only 4.1% protein content, a medium-molecular-weight fraction (150 kDa), and a low-molecular-weight fraction (15 kDa), the latter having the highest proportion of protein. From these three fractions, the highest immunostimulatory activity was attained with the high-molecular-weight fraction, which contained glucose and arabinose moieties at a ratio of 4.3:1.0 (Jeong et al. 2004).

In addition, two similar polysaccharide–protein complexes (PSPC), but of lower molecular weight, one intracellular (28 kDa) and one extracellular (15 kDa), have been isolated from mycelia and liquid culture filtrates, respectively, of *Coriolus* (or *Trametes*) *versicolor* (Ooi and Liu 2000). In another study with submerged cultures of *C. versicolor*, two extracellular proteoglucans were identified, one of very high molecular weight (4100 kDa) and one of very low molecular weight (2.6 kDa) with a protein content of 2–3.6% (Rau et al. 2009). The main polysaccharide chain in the biopolymers is very similar

to schizophyllan as it is composed of β-(1,3)/β-(1,6)-linked D-glucose molecules, except that it does not adopt a triple helix conformation like schizophyllan (Figure 8.1) (Rau et al. 2009).

Another source of therapeutic (immunomodulatory and antidiabetic) polysaccharides are the edible medicinal *Tremmella* mushrooms (*T. mesenterica, T. fuciformis, T. auriantica, T. auriantialba,* and *T. cinnabarina*), which form a group of unusual jelly mushrooms of very high polysaccharide content (60–90% of the fruiting body, in contrast to 10–30% in other mushrooms) (Lo et al. 2006, Reshetnikov et al. 2000). *Tremella* polysaccharides are acidic with a pH of 5.1–5.6 in aqueous solutions as they are composed of a linear backbone of α-(1,3)-D-rhamnose, with side groups of xylose and glucuronic acid (Figure 8.1) (De Baets and Vandamme 2001, Khondkar 2009, Lo et al. 2006, Reshetnikov et al. 2000). Extracellular acidic heteroglycans from filtrates of *Tremella* species have also been studied; these are characterized by an α-(1 → 3)-mannan backbone with β-linked side chains and contain xylose, arabinose, mannose, galactose, glucose, and glucuronic acid residues (Khondkar 2009).

Cordyceps mushrooms belonging to the *Ascomycetes* group of fungi also have a long history of applications in traditional Chinese medicine. *Cordyceps sinensis* is a fungal parasite on the larvae of Lepidoptera and grows slowly to form a worm-like fruiting body. It produces several medicinal biopolymers that differ depending on whether they are isolated from fruiting bodies or cultured mycelia, the cultivation/fermentation conditions, the substrate used, and the purification method that is followed (Zhong et al. 2009). Polysaccharides extracted from the fruiting bodies or mycelia of *C. sinensis, C. militaris*, and other *Cordyceps* species exhibit antitumor, antiviral, and antioxidant activity (Khan et al. 2010). Wu et al. (2011) studied the antioxidant properties of a polysaccharide isolated from the fruiting bodies of *C. militaris*, which was composed mainly of mannose, glucose, and galactose in a molar ratio of 1.35:8.34:1.00, and linked by α-glycosidic linkages. Several highly branched galactomannans have been purified from fruiting bodies of *C. sinensis*. Their main chain contains predominantly (1,2)-α-D-mannopyranose groups, with branches of (1,3)-, (1,5)-, and (1,6)-linked D-galactofuranose and (1,4)-D-galactopyranose residues (Nie et al. 2013a). Also, a water-soluble protein–galactomannan complex from *C. sinensis* was reported, which has an estimated molecular weight of 23 kDa (Nie et al. 2013a). In addition, from extracts of cultured mycelia of *C. sinensis*, a 210 kDa polysaccharide with antioxidant and hypoglycemic properties was obtained that contained glucose, mannose, and galactose in a ratio of 1:0.6:0.75 (Li et al. 2003, 2006).

Chen et al. (2008) have extracted and characterized a hypolipidemic polysaccharide from fruiting bodies of the edible mushroom *Auricularia auricula* (AAP), which was found to contain 42.5% total carbohydrate, 19.6% uronic acids, 15.8% sulfate groups, 1.7% total nitrogen, and 20.3% ash. The

neutral sugar components were mainly rhamnose, xylose, glucose, and smaller amounts of mannose, galactose, and arabinose. In another study, an antithrombotic polysaccharide was obtained from alkali extracts of *A. auricula* fruiting bodies, which had a molecular weight of 160 kDa and was composed mainly of mannose, glucose, glucuronic acid, and xylose, without having any sulfate esters (Yoon et al. 2003).

Other less studied but still interesting polysaccharides from medicinal mushrooms with biological activities that could be exploited as the basis for novel nutraceuticals include the immunostimulatory and hypoglycemic heteroglycans from the edible *Hericium erinaceus* (Lakhanpal and Rana 2005, Mizuno 1998), the antitumor glucans from the bitter mushroom *Phellinus linteus* (Kim et al. 2004), the antitumor and hypoglycemic heteroglycans from the culinary *Morchella esculenta* (Duncan et al. 2002, Lakhanpal and Rana 2005), the immunostimulatory heteroglycans from *Flammulina velutipes* mycelium (the edible Enokitake mushroom) (Yin et al. 2010), and several others (Ooi and Liu 2000, Zhang et al. 2007).

8.3 Production of Bioactive Mushroom Polysaccharides

In the past, the consumption of mushrooms was limited in most countries (with the exception of China), and was based on the individual collection of wild mushrooms. In the last few decades, the industrial cultivation of mushrooms has increased rapidly, with China having a leading role in the worldwide production, followed by Italy, the United States, and several European countries (the European Union is the second worldwide producer) (Table 8.1) (F.A.O. 2013). This increase has been parallel to the increasing acknowledgment by scientists and consumers of the multiple dietary and health benefits of mushroom consumption (Stachowiak and Reguła 2012, Wasser 2002).

At the same time, there seems to be a shortage of commercial, industrialized polysaccharides from medicinal mushrooms, despite the extensive literature on their therapeutic properties, which is partly due to the fact that many of these fungi only grow as wild mushrooms in forests and are hard to cultivate in farms. But even for medicinal mushrooms that have been successfully cultivated as fruiting bodies in large scale, there are several concerns that limit their industrial exploitation for bioactive polysaccharide production, such as the long period of growing into mature fruiting bodies, the high purification costs, the erratic quality, and the limited availability. Furthermore, some scientists have raised the issue of the potential toxicity of mushrooms found in urban and industrial areas that may contain elevated levels of heavy metals or radioactive elements that the mushrooms can accumulate from the soil (Stachowiak and Reguła 2012, Vetter 2004, Vinichuk et al. 2010). These disadvantages could be overcome by the cultivation of the mycelia in fermentors (bioreactors) under controlled and sterile

Table 8.1 Worldwide Production of Mushrooms in the Years 1991, 2001, and 2011

	Production (Tons)		
Country	**1991**	**2001**	**2011**
Australia	24,394	39,394	49,696
Belgium	20,592[a]	40,500	41,556
Canada	53,020	86,357	78,930
China	775,000	2,660,000	5,000,000
France	198,500	196,254	115,669
Germany	56,000	63,000	62,000
India	4000	30,000	40,600
Indonesia	8000	25,500	45,851
Iran	6387	19,000	37,664
Ireland	39,000	68,000	67,063
Italy	79,536	72,900	761,858
Japan	78,000	66,100	60,180
The Netherlands	165,000	275,000	304,000
Poland	101,500	110,000	198,235
Republic of Korea	13,181	21,251	30,574
Spain	29,693	109,605	148,000
UK	123,300	92,600	69,300
USA	338,760	376,980	390,902
Vietnam	9000	16,000	21,957

Source: Data from FAO—Food and Agriculture Organization of the United Nations, 2011.

[a] Belgium-Luxemburg.

conditions, which shortens the production time from months to only a few days or a couple of weeks at the most, allows a close quality and process control, eliminates potential problems of toxicity and contamination, and offers practically unlimited quantity and constant, all-year-round availability, and usually entails lower purifications costs. Another potential advantage of some fungal fermentation processes is that the exopolysaccharides that are secreted to the process medium are more uniform in type, composition, and structure and easier to standardize as a commercial product, in comparison to the high diversity of polysaccharides that can be isolated from extracts of fruiting bodies (Donot et al. 2012, Giavasis and Biliaderis 2006, Lee et al. 2004, Wasser 2002). For all these reasons, the study of fungal physiology and the optimization of the fermentation conditions and the downstream processing for achieving a maximal bioactive polysaccharide production become critical factors for their commercial utilization (Giavasis 2013, Giavasis and Biliaderis 2006, McNeil and Harvey 2006). However, for several higher fungi, the submerged cultivation of mycelia is not feasible, or results in very low yields of biomass and biopolymer, thus the cultivation of

mushroom fruiting bodies is the only option for their commercial production (Chang 1993).

With regard to the large-scale cultivation of edible and/or medicinal mushrooms, nonectomycorrhizal mushrooms (without roots) can be grown on wood, straw, sawdust, or other cellulosic material (e.g., *Lentinus*, *Pleurotus, Auricularia, Tremella, Hericium*), animal manure (e.g., *Agaricus*), or soil (e.g., *Morchella*). On the contrary, ectomycorrhizal mushrooms (with roots under the ground) are very hard to cultivate artificially (Chang 1993). The artificial cultivation of mushrooms takes place on the appropriate substrate either in open farms, where mushroom productivity is limited by uncontrolled environmental conditions, such as temperature, moisture, and aeration, or in beds or sterile plastic bags of compost substrate, which are placed in indoor chambers where environmental conditions (pH, temperature, humidity, aeration) can be controlled, at the expense of energy. This process is of course very slow as it takes from several weeks up to 8–10 months until the fungal mycelia become mature fruiting bodies (Chang 1993). It is also susceptible to contamination by other fungi or bacteria that cause mushroom rot, or to pathogenic parasites and mites (Largeteau et al. 2011).

The isolation process of bioactive mushroom polysaccharides from mature fruiting bodies involves hot water or the alkali extraction of dried mushrooms, filtration, ethanol precipitation, and the removal of impurities (e.g., proteins, starch, and oligosaccharides) from the filtrate enzymatically or by a phenolic or other reagent, dialysis, and lyophilization (Synytsya et al. 2009, Zhang et al. 2007). If a higher purity is desired (e.g., for strictly pharmaceutical applications), gel permeation, ion-exchange, or affinity chromatography can be implemented, depending on the nature of the biopolymer (Zhang et al. 2007). A proposed process for the isolation and purification of different fractions of mushroom polysaccharides is depicted in Figure 8.2. Other processing methods such as ultrasonication have been proposed in order to enhance extraction yields of mushroom polysaccharides, by facilitating the disruption of the mushroom cell wall, the reduction of particle size, and the solubility of the biopolymers (Yang et al. 2011).

As concerns the fermentative or submerged culture of mycelia for the production of bioactive polysaccharides, several process parameters have to be taken into account in order to achieve high polysaccharide yields, such as the optimal pH, temperature, aeration, agitation, and process medium composition (Elisashvili 2012, Giavasis and Biliaderis 2006, Kim et al. 2002).

For instance, several mushroom polysaccharides are optimally synthesized at low pH, such as grifolan at pH 5.5 (Lee et al. 2004) and ganoderan at pH 4–4.5 (Yang and Liau 1998), or via a gradual pH drop from 5 to 4 in the absence of pH control (Kim et al. 2002). In contrast, Shu et al. (2004) observed that there was an increase in polysaccharide formation by *A. blazei* as the process pH increased from 4 to 7; however, at a low pH, a higher molecular weight (and biological activity) of the glucans was attained. According to

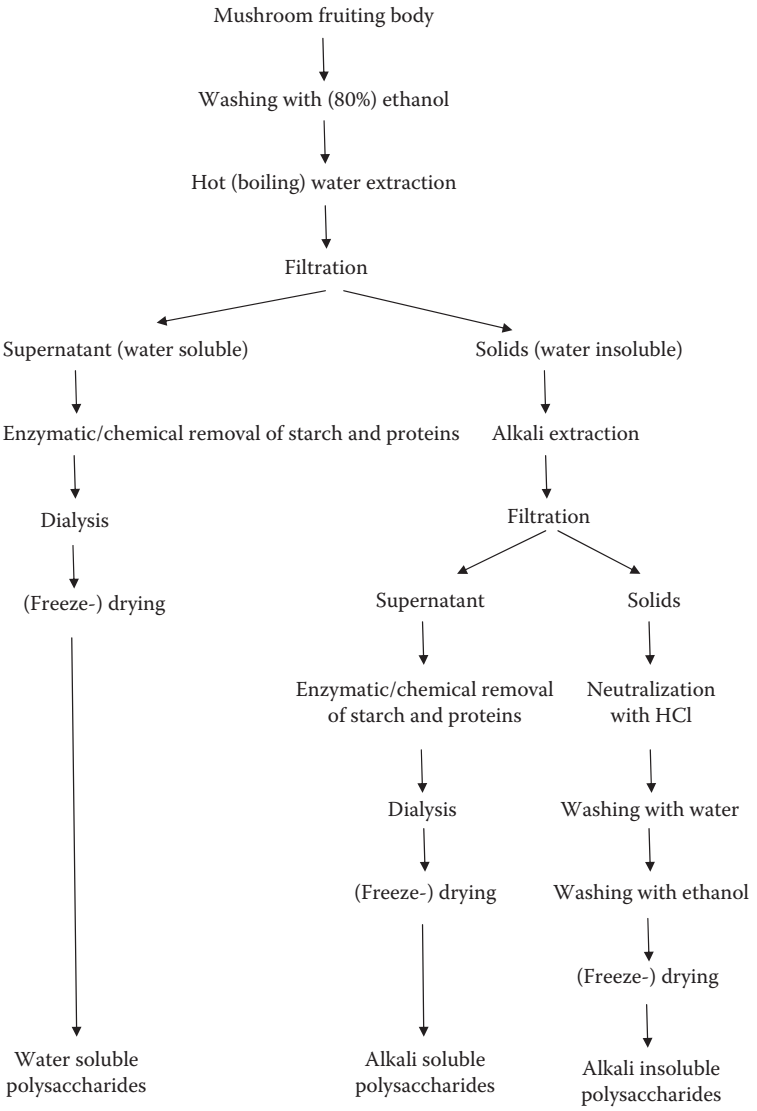

Figure 8.2 Proposed extraction and purification process for the isolation of water-soluble, alkali-soluble, and insoluble fractions of mushroom polysaccharides. (Adapted from Synytsya, A. et al. 2009. *Carbohydr Polym* 76:548–556.)

Fan et al. (2007), the optimum pH for exopolysaccharide production from *A. brasiliensis* was 6.1.

The optimum temperature for the growth of mycelia and polysaccharide production by medicinal fungi is usually around 30°C as in the case of *Ganoderma lucimum* (Yang and Liau, 1998) and *A. brasiliensis* (Fan et al.

2007), although for some fungal biopolymers such as grifolan from *G. frondosa*, a process temperature of 25°C has been proposed (Lee et al. 2004).

An agitation rate of 150 rpm was found to be the most preferable for the production of *G. lucidum* polysaccharide in shake flasks. Higher agitation may improve mixing, but high shear stress has a detrimental effect on the size and growth of mycelia and the synthesis of polysaccharides (Yang and Liau 1998). Fungal morphology is in fact a very important factor in submerged fungal cultures and polysaccharide production. Generally, as the culture grows, the morphology changes from a loose mycelium to unicellular pellets, the latter being associated with the maximum production of polysaccharides. In the case of *G. lucidum* polysaccharides, the pellet size was becoming more compact and small during a 13-day fermentation, at the end of which a maximum exopolysaccharide concentration of 5.7 g/L was obtained (Wagner et al. 2004).

Small–medium pellet size was also associated with an increased production of schizophyllan. Specifically, Shu et al. (2005) applied a pellet size control device in a bubble column fermentor where the pellet size of *S. commune* was reduced from 20.5 to 12.3 mm, causing an increase in schizophyllan yield and maximum concentration. However, at lower pellet size, schizophyllan production dropped, despite the increased specific growth rate of the smaller mycelia pellets (Shu et al. 2005).

Although all fungi are obligate aerobic organisms and an adequate aeration is expected to be necessary for submerged fungal cultures, it has been reported that low dissolved oxygen (DO) in the bioreactor, or even DO limitation, can stimulate the biosynthesis of schizophyllan or other fungal glucans, despite limiting cell growth (Rau et al. 1992). This can be attributed to the presence of oxygen-sensitive biosynthetic enzymes, or to a metabolic shift from catabolism and cell growth to glucan biosynthesis under growth-limiting conditions. Shu et al. (2005) tested the effect of different aeration rates upon schizophyllan production (0.05, 0.1, 0.2, 0.5 vvm) and observed that the maximum yield and polysaccharide concentration was achieved at the lowest aeration rate (0.05 vvm). This is a very low aeration rate and differs greatly from the high aeration rates usually applied for the production of bacterial polysaccharides (Borges et al. 2008, Giavasis et al. 2006). Similarly, low DO levels seem to favor exopolysaccharide production by *G. lucidum*, at the expense of cell growth (Tang and Zhong 2003). Lee et al. (2004) observed that the aeration rate had a great impact on the fungal morphology of *G. frondosa*, with low aeration favoring compact cell pellets and a high aeration rate leading to more freely suspended mycelia. This can probably explain the detrimental effect of high aeration for many mushroom polysaccharides, as described above. However, in the case of *G. frondosa*, the optimal morphology for exopolysaccharide production was that of loose mycelia hairy clumps (Lee et al. 2004) and an optimal aeration rate of 1.16 vvm was proposed.

The optimal medium composition for the production of bioactive polysaccharides via submerged cultures differs depending on each fungi and each polysaccharide. Generally, a glucose-based medium is used, although in many cases, a medium based on other sugars or potato extract has led to higher biopolymer yields (Kim et al. 2002). A phosphorous source such as phosphate salts, and a nitrogen source such as peptone, yeast extract, or ammonium salts are used to facilitate biomass growth, but high C/N ratios are usually required, so that nitrogen limitation can halt cell growth at some point during the fermentation and stimulate polysaccharide synthesis (Giavasis 2013). Kumari et al. (2008) noticed that sucrose was the most preferable sugar for schizophyllan production, while organic nitrogen sources such as beef or yeast extract gave rise to high biopolymer synthesis in comparison to inorganic nitrogen sources. For the production of exopolysaccharides of *A. brasiliensis*, glucose (at a relatively low concentration of 10 g/L) and yeast extract were the most suitable carbon and nitrogen sources, respectively (Fan et al. 2007), while the synthesis of exopolysaccharides by *G. lucidum* was optimal when using a medium containing complex carbon and nitrogen sources, namely, potato dextrose broth, peptone, and malt extract (Kim et al. 2002).

The presence of vegetable oils has been reported to enhance the production of fungal biomass and polysaccharide in liquid cultures (Hsieh et al. 2008, Huang et al. 2009), possibly because the assembly of polysaccharides generally takes place via the accumulation of sugar monomers to lipid carriers of the cytoplasmic membrane, where they are subsequently polymerized (Giavasis 2013, Sutherland 1990, Whitfield and Valvano 1993); thus the presence of lipids in the fermentation medium may facilitate this metabolic process. More specifically, Huang et al. (2009) reported a doubling of the mycelia biomass concentration and a ~50% increase in exopolysaccharide production by *G. lucidum* after addition of 2% corn oil in the process medium. Even more interesting was the fact that oil addition also changed fungal morphology, since the fungal pellets changed from spherical to oval, became darker inside, with apparent oil micelles adsorbed on the cell membrane, while the decay of pellets at the late stages of the fermentation was prevented after the addition of corn oil (Huang et al. 2009). Hsieh et al. (2008) reported an increase in biomass concentration in submerged cultures of *G. frondosa*, after the addition of several plant oils (olive, safflower, sunflower, and soybean oil) or surfactants such as Tween 80 or Span 80. However, the production of exopolysaccharide was only stimulated by olive oil, especially at 1% concentration. Taking into account the fact that some bioactive polysaccharide may be isolated from the mycelia (cell wall polysaccharides) while others from the fermentation medium (exopolysaccharides), these results show that one can design the appropriate medium composition according to the type of polysaccharides that need to be isolated.

In contrast to bacterial polysaccharides where genetic or biochemical engineering has been implemented to improve strains and productivity, such

approaches are very scarce among mushroom polysaccharides, probably due to the unexplored complex genome of such higher fungi, while information on basic biosynthetic routes is limited to a few industrial fungal biopolymers such as pullulan and scleroglucan, which do not derive from mushrooms (Cheng et al. 2011, Elisashvili 2012, Giavasis 2013, Schmid et al. 2011).

Another issue that must be taken into account with regard to the submerged cultures of fungal mycelia is the potential biosynthesis of fungal glucanases or other lyases that can degrade the biopolymer. Such fungal lyases have been described for pullulan production (Manitchotpisit et al. 2011); however, little is known about the biosynthesis of lyases during the submerged cultivation for the production of bioactive mushroom polysaccharides. Interestingly, Minato et al. (1999) found that lentinan glucanases are produced during the storage of *L. edodes* mushroom, so it is possible that such enzymes are also synthesized in liquid cultures, resulting in a decrease in biopolymer concentration. During the storage of *L. edodes* mushrooms for 7 days at 20°C, lentinan content dropped from 12.8 mg/kg of dry weight to 3.7 mg/kg, while at 5°C storage, the degradation was slower, resulting in a decrease of lentinan content to 9.3 mg/kg (Minato et al. 1999).

The isolation process of medicinal fungal polysaccharides from liquid cultures differs among cell wall polysaccharides, exopolysaccharides, and endopolysaccharides. For the isolation of exopolysaccharides, the downstream processing usually begins with the inactivation of cells and undesirable enzymes by heating. This also enables a better dissociation and separation of exopolysaccharides from the cell membrane. Hot alkali treatment or sonication can be applied to facilitate the separation of biopolymers from the cells (Morin 1998, Wang et al. 2010). Exopolysaccharides are separated from the biomass by filtration or centrifugation, precipitated by the addition of alcohol and further purified (if necessary) by ultrafiltration, gel permeation/ion-exchange chromatography, or dialfiltration. The final product is obtained after vacuum during spray-drying or freeze-drying (Donot et al. 2012, Fan et al. 2012, Giavasis and Biliaderis 2006, Wang et al. 2010). In the case of biopolymers from the mycelial cell wall, a hot water or alkali extraction process is followed, which is similar to the one applied to mushroom fruiting bodies (as described above). Intracellular bioactive polysaccharides, for example, those from *G. lucidum* cells, are obtained after enzymatic, chemical, thermal, or ultrasonic cell lysis in order for the biopolymer to be released from the cytoplasm, and the rest of the separation process is analogous to the one described above for extracellular biopolymers (Habijanic et al. 2001, Liu et al. 2012).

8.4 Medicinal Properties of Mushroom Polysaccharides

Medicinal mushroom polysaccharides act as BRMs, that is, they can alter and enhance immune responses against cancer cells, bacteria and viruses,

inflammation, and oxidative stress via an indirect, nonspecific mechanism that potentiates and alerts the human immune system. At the same time, they may act as dietary fibers with hypolipidemic, hypoglycemic, antiatherogenic, or prebiotic properties (Giavasis 2013, Kim et al. 2006, Mizuno and Nishitani 2013, Stachowiak and Reguła 2012, Wasser 2002).

Apart from dose dependence, these properties are closely related to the physicochemical and structural characteristics of each biopolymer, that is, composition of sugar monomers, molecular weight, type of side chains and the degree of branching, presence of a triple or double helix or random coil, presence of peptide–protein complexes and sulfate groups, and solubility. Generally, it has been observed that water solubility (often associated with short side chains and a low degree of branching) and a medium or large molecular weight usually leads to high immunomodulating activity, although low-molecular-weight bioactive polysaccharides have also been described (Bohn and BeMiller 1995, Giavasis 2013, Giavasis and Biliaderis 2006, Kim et al. 2006, Kulicke et al. 1997, Mizuno and Nishitani 2013, Sletmoen and Stokke 2008, Wasser 2002). Furthermore, the downstream and purification process followed for these biopolymers (filtration or water/alkali extraction alcohol/acetone precipitation of the polysaccharide, gel filtration, etc.), as well the drying method used for the formulation of the final product may affect their structural and functional characteristics (Giavasis 2013, Giavasis and Biliaderis 2006, Fan et al. 2012).

Below, the most recent findings on the medicinal properties of mushroom polysaccharides will be summarized, with reference to structure–function relationships and the mode of action.

8.4.1 Immunostimulating and Antitumor Properties

There is now a large volume of *in vitro* studies with human cell lines, as well as a fair amount of clinical studies that explore and verify the immunomodulatory effects of mushroom polysaccharides, especially in relation to their antitumor properties. In these studies, the active substances are mostly used in pure form (polysaccharide solution given intravenously, intraperitoneally, or orally) or sometimes in crude form (i.e., digested as dried mycelia/fruiting bodies), but since these mushrooms contain several medicinal ingredients, only studies on the application of pure polysaccharides can lead to safe conclusions on the bioactivity of specific molecules and their mode of action. At the same time, the incorporation of such biopolymers in functional foods and nutraceuticals may alter their functionality, and clinical studies based on the consumption of therapeutic biopolymers via food and nutraceuticals are needed to establish their medicinal properties in functional foods.

Lentinan and schizophyllan are two of the most well-studied mushroom glucans and many researchers underline their immunostimulating properties (stimulation of secretion of tumor necrosis factor-α (TNF-α) by human monocytes and activation of macrophages, or platelet hemopoietic

activity), and specifically their effects against gastric, breast, lung, cervical, or colorectal cancer, especially when combined with conventional (synthetic) antitumor drugs (chemotherapy) or radiotherapy, although they usually do not exhibit a direct tumor-killing capacity. Moreover, they can prevent metastasis and minimize the side effects of chemotherapy and radiotherapy upon the healthy tissue (Chang and Wasser 2012, Giavasis 2013, Ikekawa 2001, Lo et al. 2011, Stachowiak and Reguła 2012). Clinical studies on the therapeutic effects of lentinan showed that it improves the survival of patients with advanced gastric cancer significantly, when it is used in combination with standard chemotherapy (compared to chemotherapy alone) (Oba et al. 2009). These β-glucans exhibit no toxicity to humans even at high dosages, and are more effective when administered in the early stages of cancer treatment. Their bioactivity has been connected to the high molecular weight of these β-glucans and the presence of a triple helix conformation (Falch et al. 2000, Giavasis and Biliaderis 2006, Lo et al. 2011, Sletmoen and Stokke 2008). Both these mushroom glucans are poorly absorbed in the intestine after oral intake, so in most clinical or animal trials, they are injected intrapleurally, intraperitoneally, or intravenously (Chan et al., 2009, Giavasis 2013).

The glucans from *G. frondosa* and *T. versicolor* have also been used in clinical trials where they increased the survival rate and survival time on patients suffering from different types of cancer, especially when supplied in combination with chemotherapy or when administered after surgery, in order to prevent metastasis (Lindequist et al. 2005, Stachowiak and Reguła 2012).

In a phase I/II trial of a polysaccharide extract from *G. frondosa* administered orally to breast cancer patients, it was observed that the intake of the polysaccharide preparation increased cytokine production (IL-2, TNF-α, and IL-10) by immune cells by over 50% compared to a control group (Deng et al. 2009).

Krestin (also known as PSK), the medicinal biopolymer extracted from *T. versicolor*, is used in Asia as a complementary cancer treatment due to its immunostimulating, antimetastatic, and direct antitumor effects (Kobayashi et al. 1995). Krestin was also shown to have TLR2-agonist activities and to stimulate dendritic cells (DC) and T cells in murine models of breast cancer, where it significantly inhibited cancer growth (Lu et al. 2011). Similarly, in *in vitro* studies of mice with prostate cancer, PSK enhanced mRNA expression of interferon-γ (IFN-γ) compared to the control, and when used in combination with docetaxel drug it increased the survival rate of white blood cells and induced splenic natural killer (NK) cell cytolytic activity (Wenner et al. 2012). In clinical studies with crude preparation of *T. versicolor* dry mushroom powder given to women with breast cancer after standard chemotherapy and radiotherapy, up to 9 g/day appeared to be safe and tolerable in the postprimary treatment and could improve the immune status in the immunocompromised patients (Torkelson et al. 2012). However, clinical studies

with purified polysaccharides of *Trametes (Coriolus) versicolor* were not conducted in this study.

Ganoderan is another fungal biopolymer that has been used as adjuvant cancer therapy, as it increases the cytotoxic effect of chemotherapy and enhances the immune responses in patients with prostate cancer (Mahajna et al. 2009, Vannucci et al. 2013, Yuen and Gohel 2005). *G. lucidum* polysaccharides and proteoglucans are also reported to prolong the survival of mice with Lewis carcinoma, to increase the excretion of TNF-α and IFN-γ in blood serum in a dose-dependent manner, to stimulate the expression of macrophages and T-cell immunity, to facilitate the recovery of splenic and lymphokine NK cells in immunosuppressed mice, and to exhibit prophylactic activity toward chemically injured macrophages (Ganeshpurkar et al. 2010, Nie et al. 2013b, Stachowiak and Reguła 2012, You and Lin 2002, Yuen and Gohel 2005).

The glucans and proteoglucans from *A. blazei* have been very effective against different types of sarcomas and carcinomas in mice, in stimulating the excretion of IFN-γ, and also capable of suppressing allergic reactions (antiallergic immunomodulation) (Firenzuoli et al. 2008, Mizuno and Nishitani 2013). The *A. blazei* proteoglucans activate a number of immune system cells (macrophages, interferons, T cells, and NK cells) in order to halt the multiplication, metastasis, and recurrence of cancer cells (Fujimiya et al. 1998, Lakhanpal 2005).

Water or methanolic extracts of *P. ostreatus* β-glucans can induce apoptosis in human prostate cancer and suppress the proliferation of human breast and colon cancer cells (Asaduzzaman and Mousumi 2012). Similarly, the β-glucans from the fruiting body extracts of *P. tuber-regium* exhibit antiproliferative activity against several human cancer cell lines (Zhang et al. 2001), while the proteoglucan extracts from the sclerotia of the same mushroom were found to activate innate immune cells such as macrophages, and T-helper cells *in vivo* (in mice) in order to destroy tumor cells (Wong et al. 2011).

The antitumor and immunomodulatory polysaccharides from other medicinal mushroom exhibit a similar mode of action (Ooi and Liu, 2000, Zhang et al. 2007). This action is a combination of mechanisms that include a mitogenic activity of soluble glucans, stimulation of NK cells, T cells, DCs, neutrophils, and monocytes, enhanced phagocytosis, and excretion of cytokines (Chan et al. 2009, Giavasis 2013, Mizuno and Nishitani 2013, Wasser 2002). A simplified illustration of the immunostimulatory mechanisms of fungal glucans and proteoglucans is shown in Figure 8.3.

8.4.2 Antioxidant Properties

Polysaccharides extracted from *G. lucidum, T. versicolor, L. edodes, P. linteus,* and *Agaricus* mushrooms have reducing power abilities and chelating properties and can inhibit lipid oxidation or reduce oxidative stress. This effect

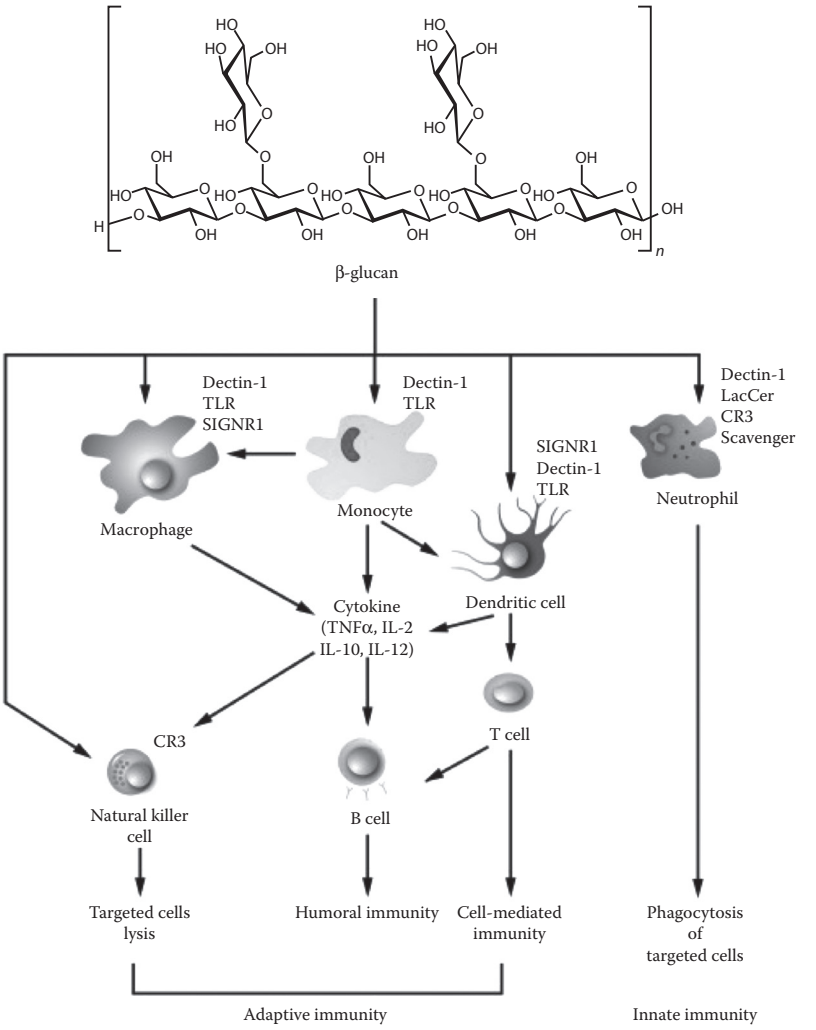

Figure 8.3 Postulated mechanisms of immunostimulatory fungal β-glucans: β-glucans are attached to membrane receptors of the immune cells (such as dectin, TLR) and subsequently activate macrophages, monocytes DCs, NK cells, B cells, T cells, and neutrophils, while they can also stimulate the excretion of cytokines, such as tumor necrosis factor (TNF)-α and interleukins (ILs). This immunostimulation may be either an innate or adaptive immune response. (Adapted from Chan, G. C. F., W. K. Chan, and D. M. U. Sze. 2009. *J Hematol Oncol* 2:25–36.)

was correlated with the presence of a β-glucan and a phenolic (mainly tyrosine and ferrulic acid) moiety bound to the β-glucan main chain by covalent bonds (Giavasis 2013, Kozarski et al. 2011, Yu et al. 2009). Although the phenolic compounds present in *G. lucidum* fruiting bodies and mycelia are the compounds with the highest antioxidant activity, *G. lucidum* polysaccharides

also exhibit free radical-scavenging properties, reducing power and inhibition of lipid peroxidation (Heleno et al. 2012, Tseng et al. 2008). Saltarelli et al. (2009) stated that among different polysaccharides of *G. lucidum* extracted from mycelia, the low-molecular-weight polysaccharides had the highest antioxidative activity, based on the chelating activity on Fe^{2+}, lipoxygenase assay, and 1,1-diphenyl-dipicrylhydrazyl (DPPH) free radical scavenging, while intracellular polysaccharides were ineffective as antioxidants. Also, *in vitro* studies showed that the *G. lucidum* peptidoglucan could protect the mitochondria, endoplasmic reticulum, and microvilli of macrophages from chemically induced damage and malfunction (You and Lin 2002). In addition, methanolic extracts of *G. lucidum* and *G. tsugae* glucans and proteoglucans act as antioxidants by scavenging reactive oxygen species (ROS), which are linked both to oncogenesis and to lipid oxidation (Lakhanpal and Rana 2005). Another mechanism of antioxidant activity is the ability of some mushroom polysaccharides (e.g., those from *G. lucidum*) to limit the production of oxygen-free radicals and the activity of peripheral mononuclear cells in murine peritoneal macrophages, which are related to the respiratory burst and the ageing process (Xie et al. 2012, You and Lin 2002), or to enhance the activity of antioxidant enzymes in blood serum (XiaoPing et al. 2009). Methanolic extracts from *G. frondosa, M. esculenta,* and *Termitomyces albuminosus* mycelia have also exhibited antioxidant properties, but these were mainly associated with the presence of phenolic compounds in the extract (Mau et al. 2004). In submerged cultures of *H. erinaceum* with the addition of selenium sources (sodium selenite and Selol) in the fermentation medium, a selenium-containing exopolysaccharide was produced, which showed excellent antioxidant capacity, based on reducing power, inhibition of lipid peroxidation, and DPPH radical scavenging (Malinowska et al. 2009). Interestingly, Selol also induced a 2.5-fold increase in the formation of the exopolysaccharide. Other antioxidant mushroom polysaccharides include the polysaccharides from water-soluble extracts of *A. auricula* and *C. militaris* fruiting bodies, which showed free radical-scavenging, chelating, and reducing power properties, and an increase in the total antioxidant capacity (Chen et al. 2008, Luo et al. 2009, Wu et al. 2011).

8.4.3 Antimicrobial Properties

Mushroom polysaccharides are active against bacterial and viral infections *in vitro* or *in vivo,* as they can stimulate the phagocytosis of microbes by neutrophils and macrophages. Lentinan has shown antimicrobial activity against *Listeria monocytogenes, Staphylococcus aureus, Salmonella enteritis, Escherichia coli,* as well as tuberculosis (Giavasis 2013, Mattila et al. 2000, van Nevel et al. 2003). In addition, crude extracts from different *Agaricus* fruiting bodies restricted the growth of *S. aureus, Bacillus subtilis,* and *B. cereus*, although the antimicrobial effect might also be due to nonpolysaccharide components of these extracts (Barros et al. 2008). Several mushroom

glucans are reported to have antiviral activity, which is believed to occur via the increased release of IFN-γ and enhanced proliferation of peripheral blood mononuclear cells (PBMC) (Lindequist et al. 2005, Markova et al. 2002, Sasidhara and Thirunalasundari 2012). Ganoderan has exhibited antiviral activity against the herpes virus, schizophyllan can enhance immune responses of hepatitis B patients, while lentinan has expressed significant antiviral activity against influenza virus and polio virus (Eo et al. 2000, Kakumu et al. 1991, Sasidhara and Thirunalasundari 2012). Notably, lentinan and an acidic *G. lucidum* proteoglucan have been applied successfully as adjunct therapy of HIV in combination with conventional anti-HIV drugs, as they could enhance host resistance to the HIV virus, and limit the toxicity of synthetic anti-HIV drugs. Similar anti-HIV activities were also reported for glucans from *G. frondosa* and *T. versicolor* (Giavasis and Biliaderis 2006, Lindequist et al. 2005, Markova et al. 2002, Sasidhara and Thirunalasundari 2012). Selegean et al. (2009) studied the antiviral activity of expolysaccharides of *P. ostreatus* against the infectious bursal disease virus in young broilers and noticed an enhanced antiviral activity, stimulation of maternal antibodies, and increased survival rate when the biopolymer was used (as part of the daily water intake) alone or in combination with conventional antiviral vaccines. As the antimicrobial activity of mushroom glucans seems to be indirect and related to immunomodulation (Giavasis and Biliaderi 2006), and to affect the intestinal microflora after oral administration (van Nevel et al. 2003), it seems feasible to formulate glucan-based nutraceuticals with prophylactic antimicrobial properties.

8.4.4 Hypolipidemic, Hypoglycemic, and Prebiotic Properties

Most of the bioactive mushroom polysaccharides can serve as dietary fiber, as they cannot be digested in the human intestine (Giavasis 2013). Fungal glucans from *L. edodes, G. lucidum, S. commune, P. ostreatus, G. frondosa, A. blazei, C. sinensis,* and *Cordiceps miltaris* are able to reduce blood sugar and serum cholesterol according to several animal and human studies (Chen et al. 2013, Lakhanpal and Rana 2005, Lindequist et al. 2005).

Hypoglycemic effects occur via the attachment of the indigestible polysaccharides to the intestinal epithelium, which decelerates glucose absorption, while hypolipidemic effects are due to the interruption of the enterohepatic circulation of bile acids, which favors their excretion in the feces (Giavasis 2013, Lakhanpal and Rana 2005).

Also, bioactive mushroom polysaccharides exert a hypoglycemic effect via the modulation of carbohydrate metabolism and insulin synthesis. For instance, glucans from *G. frondosa* exhibit antidiabetic and antiobesity properties (Lakhanpal and Rana 2005). An acidic xylomannan of *T. mesenterica* has been shown to regulate glycemic responses in normal and diabetic rats after ingestion, by decreasing serum concentration of fructosamines (Lo et al. 2006). The effectiveness of orally administered polysaccharides is important

as they can be utilized as active components in functional foods for diabetic people.

According to Zhou et al. (2009), polysaccharide extracts of *C. sinensis* exhibit hypoglycemic, hypolipidemic, and antihypertensive effects, while alcohol extracts of this mushroom protect against myocardial injury (induced by adriamycin in rats), due to the mannitol, amino acids, and polysaccharides present in the extracts, which have a nourishing effect upon the myocardium and induce its anti-injury capacity.

Lentinan has been used as a hypocholesterolemic agent in humans, as it reduces the blood levels of lipoproteins [both high density lipoproteins (HDL) and low density lipoproteins (LDL)] (Lakhanpal and Rana 2005). In addition, glucan-rich water extracts from *P. ostreatus* are reported to decrease the formation of atherogenic plaques in animal studies (Bobek and Galbavy 1999). Notably, in the case of *P. ostreatus* extracts, other nonpolysaccharide molecules the mushroom contains may also contribute to the cholesterol-lowering effect, such as plovatin, lovastatin, or menivolin, which inhibit 3-hydroxy-3-methylglutaryl CoA reductase, the major rate-limiting enzyme in the cholesterol biosynthetic pathway (Gunde-Cimerman 1993, Gunde-Cimerman and Cimerman 1995, Lakhanpal and Rana 2005). In recognition of the dietary fiber effects of *Pleurotus* mushroom, the National Mushroom Development and Extension Centre in Bangladesh recommends a daily dosage in human diet, which ranges from 5 to 10 g of dried mushroom for healthy individuals to 20 g for patients with diabetes, hypertension, cardiovascular complications, or cancer (Asaduzzaman and Mousumi 2012).

Chen et al. (2008) studied the hypolipidemic effects of polysaccharides extracted from *A. auricula* in hypercholesterolemic mice after a daily oral administration of 120 mg/kg body weight. Their results pointed out that the polysaccharides significantly lowered the concentrations of serum total cholesterol and LDL-cholesterol, improved lipoprotein lipase activity, and reduced lipid peroxidation and atherosclerotic index, showing a prophylactic activity against hypercholesterolemia. Similar hypolipidemic effects were also obtained by Luo et al. (2009) after an oral administration of 300 mg/kg/day of *A. auricula* polysaccharide in hyperlipidemic mice. The antithrombotic activity of purified acidic glucans from extracts of *A. auricula* was found to be equivalent to that of aspirin and depended largely upon the glucuronic acid content of the biopolymer, which activated antithrombin (a thrombin inhibitor), causing an inhibition of platelet aggregation in rats orally fed with the polysaccharide (Yoon et al. 2003).

In other studies, the hypocholesterolemic effect of *T. fuciformis* on hyperlipidemic mice was described, which reduced total blood cholesterol and LDL-cholesterol, without affecting the levels of the HDL fraction. Furthermore, the crude preparations of *T. fuciformis* reduced triacylglycerol levels in the serum and total cholesterol levels in the liver (Cheng et al. 2002, Guillamón et al. 2010).

The prebiotic effects of edible mushroom polysaccharides have also been described by many researchers. As the human digestive enzyme is unable to hydrolyze β-glucosidic bonds, several mushroom polysaccharides are capable of acting as a source of prebiotics (Aida et al. 2009). Synytsya et al. (2009) tested the prebiotic effect of glucans isolated from fruiting bodies of *P. ostreatus* and *P. eryngii* on several *Lactobacillus*, *Bifidibacterium*, and *Enterococcus* probiotic strains. Both water-soluble branched β-1,3/β-1,6-glucans and alkali-soluble linear α-1,3-glucans of these mushrooms were found to stimulate the growth of probiotic bacteria, especially those from *P. eryngii*. In another study, β-glucans from the sclerotia of *P. tuber-regium* were evaluated for their bifidogenic effect on *Bifidobacterium infantis, Bifidobacterium longum*, and *Bifidobacterium adolescentis* in liquid cultures, using inulin as a control prebiotic (Zhao and Cheung 2011). It was shown that after a 24-h fermentation, the populations of bifidobacteria supplemented with mushroom glucan had a 3–4 log increase in their population, which was similar to the increase achieved by the addition of inulin (Zhao and Cheung 2011).

Recently, Chou et al. (2013) reported that polysaccharides from mushroom waste, namely, *L. edodes* stipe, *P. eryngii* base, and *F. velutipes* base, can increase the survival rate of *Lactobacillus acidophilus*, *Lactobacillus casei*, and *B. longum* in refrigerated yogurt. The authors observed synergistic effects of these polysaccharides with the peptides and amino acids from a yogurt culture, which maintained probiotics above 10^7 cfu/mL during cold storage. They also noticed that the mushroom polysaccharides had a significant protective effect (increased survival rate) on the probiotic bacteria in simulated gastric and bile juice.

8.5 Applications of Mushroom Bioactive Polysaccharides in Functional Foods and Nutraceuticals

Many of the above-mentioned bioactive polysaccharides have been used as purified molecules in pharmaceutical studies *in vitro* or *in vivo*, and some of them have formed the basis of novel drugs (especially anticancer drugs) but few of them have been commercialized worldwide as novel nutraceuticals. In Asia, however, such dietary and therapeutic food products have entered the market in the last few years, and their regular intake is believed (or claimed) to boost human health (Lakhanpal and Rana 2005). It is thought that some issues of production economics, quality standardization, and stable availability need to be resolved and that clinical studies on the therapeutic effects and the effective dosages of such functional foods need to be performed in order to allow a more global commercialization (Giavasis 2013, Wasser 2011).

In order to consolidate a health claim for nutraceuticals based on mushroom polysaccharides, apart from the pharmacological studies with pure polysaccharide solutions, one needs to consider the potential impact of food processing (heating, high-pressure, irradiation, acidification, etc.) and

food physicochemical properties and composition (pH, moisture, presence of other biopolymers, enzymes, organic acids, and salts) on the bioactivity of the medicinal biopolymers. For example, ganoderan can be degraded by pectinases and dextranases, which may limit its bioactivity in a food matrix containing these enzymes (Xie et al. 2012). Moreover, when lentinan interacted with carrageenans, its antitumor capacity was reduced (Maeda and Chihara 1999). With regard to the effect of the heating and drying methods on the bioactivity of mushroom glucans, Fan et al. (2012) showed that freeze drying, in comparison to vacuum drying and heat drying, was the best method of drying *G. lucidum* polysaccharides in order to retain their antioxidant properties, measured as reducing powder and DPPH, hydroxyl radicals, and superoxide radical-scavenging ability. Heat drying was by far the least favorable method for retaining antioxidant activity (Fan et al. 2012). Apart from reducing antioxidant capacity, heating may also alter the composition of mushrooms. Indeed, after boiling several types of mushrooms (*Agaricus bisporus, L. edodes, G. frondosa*, and *F. velutipes*) for 10 min, it was observed that total dietary fiber content was increased while chitin and crude protein content was reduced. However, β-glucan concentration was largely unaffected by boiling (Dikeman et al. 2005).

There are several examples of novel nutraceuticals and functional foods that have recently been developed based on bioactive mushroom polysaccharides. Glucans from *L. edodes* have been successfully applied as a partial replacement of wheat flour in fiber-rich, low-calorie functional baked foods (with up to 2% glucan concentration), where they improved pasting parameters, batter viscosity, and elasticity, or in noodles where they conferred antioxidant and hypocholesterolemic effects and improved quality characteristics (Kim et al. 2009, 2011). Fan et al. (2006) produced a functional bread where wheat flour was partly substituted with *A. auricula* polysaccharides. In this study, an up to 9% substitution of flour with bioactive polysaccharides markedly increased the antioxidant capacity of bread (tested as DPPH free radical-scavenging ability), without altering the palatability and acceptance of the blended bread. The same mushroom was the main functional ingredient (along with Hawthorn fruit) in another experimental nutraceutical containing ethanolic extracts of *A. auricula* and Hawthorn, which exhibited antioxidant and hypolipidemic properties *in vitro* and in animal studies (Luo et al. 2009).

Interestingly, Okamura-Matsui et al. (2001) produced a functional cheese-like food containing live cultures of *S. commune*. In their novel approach, the fungus was added as a proteolytic, milk-clotting starter culture, which was also able to ferment lactose and produce up to 0.58% β-glucan in this novel "cheese," which had significant antithrombotic effects.

In other studies, the use of mushroom polysaccharides or mushroom powder has been proposed for the formulation of functional snack food. For instance, oyster mushroom powder has been added in an Indian papad snack

to improve its fiber content while *Agaricus* extracts were utilized in snacks offering high antioxidant and free radical-scavenging properties (Parab et al. 2012, Singla et al. 2009). Similarly, snack foods where starch was partially replaced (by up to 15%) by powdered extracts of *Agrocybe aegerita* (chestnut mushroom) had a low glycemic response after consumption, due to the dietary fiber content (Brennan et al. 2012). *A. brasiliensis* polysaccharides have also been suggested as a functional ingredient in antiobesity and antidiabetic functional foods (Yamanaka et al. 2013).

With regard to sensory characteristics and consumer acceptance, a potential additional advantage of using mushroom polysaccharides in functional foods may be the fact that some mushroom crude extracts also contain monosodium glutamate-like components and an intense umami taste, which might improve the flavor of the final product (Tsai et al. 2006, Tseng et al. 2005).

In China, there are nowadays several commercial nutraceutical products that are based on medicinal mushroom extracts and mushrooms biopolymers. For instance, a tonic liquor made of extracts of *L. edodes, Poria cocos,* and *G. lucidum*, which is claimed to have anticarcinogenic, antiviral, and hypolipidemic effects, and a similar potable extract of *C. sinensis, G. lucidum*, and some medicinal herbs, which is marketed as an antiageing dietary supplement that improves cardiovascular function and reduces blood lipids (Xu 2001). Also, in a traditional Chinese medicine recipe, which could form the basis of a novel nutraceutical, a soup of *A. auricula* and *Tremella* mushrooms is recommended for the treatment of hypertension (Xu 2001). Other marketed mushroom-based nutraceuticals (found mostly in Asia) include "Reishi Plus" (50% *G. lucidum* and 50% *L. edodes* extract), which is a daily nutritional supplement for good health vitality, "Trimyco-Gene" (33% *C. sinensis*, 33% *G. lucidum*, 33% *L. edodes*), which is a good immunomodulant that is supposed to promote good health and longevity, "Mycoplex-7" (14% *C. sinensis*, 14% *T. fuciformis*, 14% *A. blazei*, 14% *C. versicolor*, 14% *P. cocos*, 14% *S. commune*, 14% *Hericium erinaceous*), which is believed to promote general health vitality, and the "Garden of Life" (RM-120), which contains 10 medicinal mushrooms, along with *Aloe vera* and *Uncaria tomentosa*, and is said to stimulate the immune system, offer antitumor, antiviral, and antibacterial protection, regulate blood cholesterol, and facilitate the treatment of cardiovascular diseases (Lakhanpal and Rana 2005).

8.6 Conclusions

Medicinal mushrooms are a very interesting and versatile source of bioactive polysaccharides (and other medicinal compounds), which exhibit numerous therapeutic properties and are or can be at the core of the development of novel functional foods and nutraceuticals. As most of the studied mushroom biopolymers derive from edible mushrooms and are nontoxic, and some of

them are already used as pharmaceuticals in purified form, the idea of utilizing them as a general-purpose dietary and health-fortifying food, or as a specialized therapeutic edible supplement for groups of patients (e.g., suffering from cancer or cardiovascular diseases) becomes very attractive.

However, one has to bear in mind that the production of nutraceuticals and functional foods based on such biopolymers requires a thorough understanding of structure–function relationships, an economically viable and controllable production process and a stable polysaccharide quality. In addition, such products will require clinical studies to prove their therapeutic efficacy and the relative health claims, in order to be adopted in a global market. Although at a relatively early stage yet, the development of functional foods and nutraceuticals based on mushroom polysaccharides seems to have great potential and a bright future.

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