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Review

Research progress in submerged mycelial culture of *Grifola frondosa*, a culinary-medicinal mushroom

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Grifola frondosa* or maitake, a kind of edible and medical fungus, has become a hotspot. This paper advances how to increase the mycelial growth and bioactive polysaccharide production of *G. frondosa* by submerged fermentation at home and abroad. It focus on two ways: one is improvement and optimization of *G. frondosa* self-strain, culture medium and culturing methods, and the other is the effects of adding some stimulators into submerged culture of *G. frondosa.* The last ten years have seen an unprecedented study on characteristics of bioactive polysaccharide from *G. frondosa* by submerged fermentation. Finally, a simple summary of the extracts and product of *G. frondosa* and some rare reports are given. The current review illustrates the role of *G. frondosa*, especially its polysaccharide, with the primary aim of illustrating the latest developments in research on *G. frondosa.

Key words: *Grifola frondosa*, maitake, submerged fermentation, culture, mycelial growth, biomass, polysaccharide.

INTRODUCTION

Grifola frondosa also known as maitake in Japan, a Basidiomycete fungus, belongs to the order *Aphyllophorales* and family Polyporeceae. It is not only an edible mushroom, but a type of medical fungus that is widely used as a culinary material and dietary supplement in Asia.

G. frondosa fruit body tastes tender and delicious. It is reported that *G. frondosa* is rich in various nutrients such as protein, amino acids, sugars, vitamins, minerals, etc. (Tao et al., 2007). Among them, *G. frondosa*'s active substances, especially its polysaccharide has the most biological and pharmacological activities, such as anti-tumor (Suzuki et al.,1989; Nanba, 1995; Lee et al., 2004; Shi et al., 2007; Cui et al., 2007b), hypoglycemic activities (Lee et al., 2004; Lei et al., 2007), enhancing immunity (Nanba, 1993; Adachi et al., 1987; Kodama et al., 2003; Deng et al., 2009), anti-HIV infections (Nanba et al., 2000), antioxidant and superoxide anion scavenging (Lin, 2011; Yeh et al., 2011; Chen et al., 2012), promoting longevity as a tonic and improving the quality of life (Kuo et al., 1996; Yang et al., 2000; Cui et al., 2007a). Among them, a polysaccharide called D-fraction from *G. frondosa* (Figure 1) seems to be a hotspot. Its various bioactivities were analyzed mostly in detail and systematically (Nanba, 1993, 1995; Nanba et al., 2000; Kodama et al., 2002, 2003, 2004, 2005).

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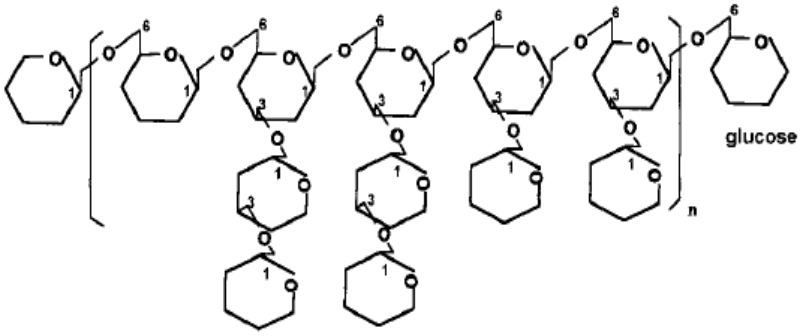

Figure 1. Chemical structure of D-fraction (Kodama et al., 2002).

Accordingly, based on these previous studies, the current review focuses on improving the production of biomass and bioactive polysaccharides of *G. frondosa*. Many investigators, including our groups, have spent time and effort in cultivating *G. frondosa* in submerged culture (Lee et al., 2004, 2008; Hsieh et al., 2006, 2008; Shih et al., 2008; Zhang et al., 2012; Xu et al., 2012; Wang et al., 2012, 2013; Wu et al., 2013).

Additionally, it is reported that as early as 2009, the total output of dry *G. frondosa* around the globe was up to about 10000 tons, of which Japanese, the country that first had development in maitake health food, has about 36000 tons of fresh mushroom every year (Xu et al., 2010). Accordingly, the industry of *G. frondosa* has formed an established downstream chain. Now there are maitake mushroom tea, capsule of maitake extract and its drops (D-fraction), compound capsule of maitake and gold maitake 404, etc. in the United States market. In China, *G. frondosa* health product is mainly capsule of maitake extract made by different producers. Consequently, *G. frondosa* health care products, as a new kind of anticancer health products have a broad prospect.

The purpose of this review was to stay up-to-date with the latest trends of *G. frondosa*, including its theoretical research and application. As great value is placed on our health; both theoretical and applied research on *G. frondosa* and its application will certainly be done in-depth and extensively.

RESEARCH ADVANCES ON MYCELIAL GROWTH AND BIOACTIVE POLYSACCHARIDE PRODUCTION OF GRIFOLA FRONDOSA BY SUBMERGED FERMENTATION

Because of medical functionality and health benefit from maitake polysaccharide, especially its extracellular polysaccharide (EPS), it has become important to know how to increase the mycelial growth and polysaccharide production of *G. frondosa* in maximum amount. We briefly summarize these reports at home and abroad, and two main aspects are included below:

1. Improvement and optimization of *G. frondosa* self-strain, culture medium and culturing methods (Table 1).
2. Effects of some stimulators added into submerged culture of *G. frondosa* (Table 2).

RESEARCH ADVANCES ON CHARACTERISTICS OF BIOACTIVE POLYSACCHARIDE FROM GRIFOLA FRONDOSA BY SUBMERGED FERMENTATION

It is reported that medical functionality and health benefits are mainly from maitake's polysaccharide; so now almost 70% of studies on *G. frondosa* are about characteristics of bioactive polysaccharide from *G. frondosa*. To illustrate the latest developments in research on *G. frondosa*, papers only in the last ten years are summed up in Table 3.

RESEARCH ADVANCES ON NON-POLYSACCHARIDE BASED ON EXTRACTS FROM GRIFOLA FRONDOSA IN SUBMERGED CULTURE

Compared with study on the activity of maitake polysaccharide extracts listed above, research on other extracts and product from *G. frondosa* in submerged culture is indeed scarce. However, we still sum up some studies on non-polysaccharide based extracts from *G. frondosa* in submerged culture (Table 4).

Additionally, Yang et al. (2013) analyzed fruiting bodies and mycelia of *G. frondosa*, respectively. The results showed that the fruiting bodies and mycelia contained 62 and 94 volatile compounds, respectively. Hereinto, 37 compounds in both fruiting bodies and mycelia accounted for 86.81 and 84.28% of total volatile substances and the content of isovaleraldehyde and lichen phenol reached 23.31 and 15.41%, respectively. Moreover, Chen et al. (2013) did a research on the speciation of iron in *G. frondosa* in details. After optimizing the extraction conditions, the soluble Fe was about 85% and the suspended Fe was about 15% in the water extracts. In

Table 1. Research progress in strategies to improve the *in vitro* growth of *G. frondosa.*

Procedure	Results	References
Comparison of mycelial growth and bioactive polysaccharide production in batch and fed-batch culture of *G. frondosa*	The fed-batch fermentation by glucose feeding greatly enhanced the accumulation of BIO and EPS; the BIO and EPS reached 8.23 g/L and 3.88 g/L at 13 d of cultivation after glucose feeding. In contrast, the BIO and EPS in the batch fermentation were 6.7 g/L and 3.3 g/L at 13 d of cultivation.	Shih et al., 2008
Two different series of experiments were carried out. In the first, the moisture in the solid substrate was maintained nearly constant while in the second the substrate was exposed to spontaneous drying by aeration.	Moistures in culture medium higher than 70% promote growth of *G. frondosa* mycelium and polysaccharide production. Four fractions of pure extracellular β-D-glucans with total mass 127.2 mg and four fractions of intracellular polysaccharides with total mass 47.2 mg were isolated. The extent of TNF-α induction was up to 322 pg mL^{-1} at a polysaccharide concentration of 200 µg mL^{-1} for the intracellular fraction.	Švagelj et al., 2008
Optimization of submerged culture conditions for mycelial biomass and exopolysaccharide (EPS) production by *G. frondosa* was studied.	Under optimal culture conditions, maximum biomass concentration and EPS production in a stirred-tank fermenter were 16.8 and 5.3 g/l, respectively.	Lee et al., 2004
A three-level Box-Behnken factorial design was employed combining with response surface methodology to optimize the medium composition.	A mathematical model estimated that a maximal yield of BIO (17.61 g/l) could be obtained when the concentrations of glucose, KH_2PO_4, peptone were set at 45.2 g/l, 2.97 g/l, 6.58 g/l, respectively; while a maximal exo-polymer yield (1.326 g/l) could be achieved when setting concentrations of glucose, KH_2PO_4, peptone at 58.6 g/l, 4.06 g/l and 3.79 g/l, respectively. Maximum BIO yield of 22.50 g/l was achieved in a 15-L fermenter using the optimized medium.	Cui et al., 2006
Different cereal grains for spawn production; and industrial by-products as substrates for mushroom production were evaluated.	1. The use of corn grains as substrate for spawn production results an important factor for reducing crop cycle time. A cold shock to 10°C was requisite for basidiome formation. 2. Coffee spent-ground was a good substrate for mycelial growth, but not for mushroom production. 3. When using oak sawdust plus corn bran as substrate, consistent yields with combined high biological efficiency (BE) (35.3%), best quality mushrooms, and a crop cycle of 12-14 weeks were obtained.	Barreto et al., 2008

the solution, the inorganic and organic Fe ratio was about 60 and 40%, respectively. Organic Fe was about 45 and 40% in protein combination and polysaccharide combination, respectively.

Interestingly, *G. frondosa* mycelium fermented liquid can be also used to produce its mycelium fermented wine. Zhu et al. (2012) developed a kind of maitake mycelium fermented wine in optimum fermentation conditions, and the produced wine was light yellow in color, clear and transparent without impurities. Furthermore, the wine tasted mellow and soft with its alcohol content as 11.75 % and its *G. frondosa* polysaccharide content as 1.56 g/L.

Finally, the fact that *G. frondosa* is a kind of medical and edible mushroom is not only proved by these evidences, but is also sustained by facts. In the year 2004, Tanaka et al. successfully treated a patient with occupational hypersensitivity pneumonitis (HP) caused by *G. frondosa* mushroom spore with an extra-fine aerosol corticosteroid; beclomethasone dipropionate (BDP) dissolved in hydrofluoroalkane-134a (HFA). A 49-year-old woman developed respiratory symptoms 3 months after beginning work on a mushroom farm. She was diagnosed as HP based on radiological and serological findings. Oral prednisolone therapy improved her HP and she returned to the same farm. Her HP relapsed after 5 months, and daily 400 µg of HFA-BDP was administered with gradual improvement. An extra-fine particle inhaled corticosteroid might reach appropriate alveoli to be effective therapy for mild HP (Tanaka et al., 2004).

Conclusion

Although, various strategies to improve the mycelial growth and bioactive polysaccharide production of *G. frondosa* have been proposed, practical application

Table 2. Research progress in effects of some stimulators' addition into submerged culture on *G. frondosa.*

Samples of stimulators	Results	References
Buckwheat	With addition of *Buckwheat* into the submerged culture of *G. frondosa*, and the EPS biosynthesis of *G. frondosa* increased by 0.5 g/L.	Zhao, 2008.
Rhizoma gastrodiae (*R. gastrodiae*)	1. With addition of *R. gastrodiae* into the submerged culture of *G. frondosa*, the EPS biosynthesis of maitake increased by 2.1 g/L (Zhao, 2008); 2. After screen and optimize maitake's medium with adding the ethanol extract of *R. gastrodiae* by Plackett-Burman, a further result showed that 3.91 g/L EPS could be obtained, which increased by 3.4% compared with the control (He and Wu, 2011); 3. With addition of 5%(v/v) ethanol extract of fresh *R. gastrodiae* into submerged culture of maitake, the BIO and EPS biosynthesis of maitake were both promoted from 0.564±0.09 to 1.324±0.25 g/L and from 71.69±0.53 to 107.08±0.85 mg/L, separately increased by 134.75 and 49.37%; However, intracellular polysaccharides (IPS) content declined from 60.38±0.87 to 45.71±0.66 mg/g, which decreased by 24.30% compared with the control group, respectively (Zhang et al., 2012); 4. From the perspective of fermentation kinetics and with addition of 7 %(v/v) alcohol extract of processed *R. gastrodiae* into the fermentation broth of *G. frondosa*, the biomass and EPS productions reached a maximum of 2.0630±0.0520 g/L and 89.3846±3.2422 mg/L, respectively after 10 and 8 of days' cultivation (Wang et al., 2012); 5. A maximum dry cell weight of 138.5 mg/L and the EPS at 0.606 g/L were obtained when the unprocessed *Gastrodia* tuber culture was added into submerged culture of *G. frondosa* (Xu et al., 2012); 6. *Rhizoma Gastrodiae* at 7 g/L significantly promoted the biosynthesis of EPS in *G. frondosa* when compared with blank control, increasing EPS yield from 3.72 g/L to 3.91 g/L (He et al., 2013).	Zhao, 2008; He and Wu, 2011; Zhang et al., 2012; Wang et al., 2012; Xu et al., 2012; He et al., 2013
Yam	With addition of *Yam* into the submerged culture of *G. frondosa*, and the EPS biosynthesis of *G. frondosa* increased by 1.2 g/L.	Zhao, 2008
Fructus arctii	(1) Some enzymes secreted from *G. frondosa*, such as β-glucosidase, would convert the glycosides (arctiin and caffeic acid derivatives) into aglycones (arctigenin and caffeic acid); (2) The fermented *Fructus arctii* extract with *G. frondosa* (G-FAE) had antioxidant and 5-lipoxygenase inhibitory activities.	Kim et al., 2010.
Olive oil	With 1% olive oil addition in 21% O_2 and 40% O_2, the production of BIO was enhanced and increased to 10.1 and 14.9 g/L, respectively, after 9 days' cultivation. And the EPS production increased from 0.7-0.9 g/L to 2.24 g/L and 3.00 g/L at day 13 with 21% O_2 and 40% O_2 aeration, respectively. In addition, the IPS increased rapidly and reached the maximum level of 28.2 mg/g at day 7 and this level remained till day 13 through the whole fermentation.	Hseih et al., 2006.
Plant oil and surfactant	(1) Olive, safflower seed, soy and sunflower oil were favorable plant oil sources to the mycelial growth of *G. frondosa*. The highest cell growth (~12.64 ± 0.47 g/L cell dry weight) could be obtained on day 13 of cultivation in the medium containing 1% all the plant oil sources. EPS production was slightly enhanced by olive oil but significantly inhibited by safflower seed oil and sunflower oil after 13 days of cultivation; (2) Amongst four plant oil sources examined, cell growth yielded relatively high BIO (11.22 ± 1.14 g/L) and that was achieved in 4% glucose medium with 0.5% soybean oil. The higher EPS production and slightly lower cell growth were found in 4% glucose media; the maximum EPS production was 2.248 ± 0.107 g/L found in 4% glucose media with olive oil addition; (3) Tween 80 and Span 80 addition had shown to increase cell growth and the maximal cell concentration of 9.10 ± 0.80 g/L was obtained with 1% Span 80 addition. Both EPS and IPS production were found to decrease with all the tested concentrations of Tween 80 and Span 80 addition. Span 80 added at the vegetative growth phase in 4% glucose media yielded the highest BIO of *G. frondosa* (8.95 ± 0.57 g/L); meanwhile Tween 80 added at the beginning cultivation had resulted in the highest EPS production (1.451 ± 0.098 g/L).	Hseih et al., 2008.
Olive oil press cakes	Olive oil press cakes reduced the mushroom yield, and the best biological efficiency was obtained on substrates supplemented with wheat bran and without olive oil press cakes. All extracts were capable of inducing splenocyte proliferation and were half as effective as the positive control (6.0 μg/mL phytohaemagglutinin). No correlation between substrate composition and bioactivity could be established. Extracts from wild-growing *G. frondosa* were superior to cultivated ones in respect to biological activity.	Gregori et al., 2009.

Table 3. Research progress in characteristics of bioactive polysaccharide from *G. frondosa* by submerged fermentation.

Samples of bioactive polysaccharide	Results	References
A polysaccharide of D-fraction	(1) The level of IL-10 as well as those of NO and IFN-γ were increased by D-fraction from *G. frondosa*. The result suggested that D-fraction induced a Th-2 dominant response through the activation of macrophages, resulting in the enhancement of humoral immunity rather than cell-mediated immunity. Furthermore, an increase in the percentage ratio of CD69 and CD89 expression on major histocompatibility complex II+ cells revealed activation of APCs 4 h after D-fraction administration. These results indicate that D-fraction enhances both the innate and adaptive arms of the immune response in normal mice. (2) D-fraction significantly enhanced the cytotoxicity against NK-sensitive YAC-1 cells and the expression of CD223 on NK cells. D-fraction also increased the expression of CD86 on macrophages. In addition, the levels of IL-12 in the culture supernatant of whole spleen cells and in serum increased, compared with the control corresponding to an increase in expression of IL-12 receptor βI on NK cells.	Kodama et al., 2004, 2005.
D-fraction from *G. frondosa* (GF-D)	D-fraction from *G. frondosa* (GF-D) or its combination with human interferon alpha-2b (IFN) alone could inhibit hepatitis B virus (HBV) DNA in HepG2 2.2.15 cells (2.2.15 cells) with the 50% inhibitory concentration (IC50) of 0.59 mg/ml and 1399 IU/mL, respectively. The combination of GF-D and IFN for anti-HBV activity synergistically inhibited HBV replication in 2.2.15 cells. In combination with 0.45 mg/ml GF-D, the apparent IC50 value for IFN was 154 IU/mL. This 9-fold increase in antiviral activity of IFN suggested that GF-D could synergize with IFN.	Gu et al., 2006.
Grifolan LE (GRN-LE)	The primary structure of Grifolan LE (GRN-LE), a purified β-D-glucan from liquid-cultured *G. frondosa*, comprised a 1, 3-β-D-glucan backbone with a single 1, 6-b-D-glucosyl side branching unit on every third residue.	Tada et al., 2009.
A chemically sulfated polysaccharide (S-GAP-P)	A chemically sulfated polysaccharide (S-GAP-P) from water-insoluble polysaccharide of *G. frondosa* mycelia significantly inhibited the tumor growth and enhanced the peritoneal macrophages phagocytosis in S180-bearing mice.	Nie et al., 2006.
Sulfated polysaccharide (S-GAP-P)	Chemically sulfated polysaccharide (S-GAP-P) from water-insoluble polysaccharide of maitake mycelia distinctly inhibited SGC-7901 cells growth in a dose-dependent manner and induced cell apoptosis evidenced by characteristic DNA ladder and sub-G_0/G_1 peak. Furthermore, the combination of S-GAP-P (10–50 μg/ml) with 1 μg/ml 5-FU resulted in a significant inhibition on SGC-7901 cells growth, meaning the beneficial interaction between the two drugs.	Shi et al., 2007.
Polysaccharide from *G. frondosa* (GFP-1, GFP-2 and GFP-3)	Three main fractions from the fruiting bodies of *G. frondosa* , GFP-1, GFP-2 and GFP-3, were obtained. Then the antioxidant activities of these three fractions showed that GFP-1, GFP-2 and GFP-3 possessed significant inhibitory effects on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, hydroxyl radical and superoxide radical; their reducing power, ferrous ions chelating effect and the inhibition ability of the rat liver lipid oxidation were also strong.	Chen et al., 2012.
Se-enriched *G. frondosa* polysaccharide (Se-GP)	A Se-enriched *G. frondosa* polysaccharide (Se-GP) was obtained from maitake enriched with Se by spraying a Na_2SeO_3 solution during fruit body growth. Under optimal conditions, polysaccharide yields and both the Se-GP and GP contents do not differ; however, the Se content of Se-GP (17.52μg/g) was 48.7 times that of GP. Three homogenous Se-GPs or GPs were obtained via DEAE-52 and Sephacryl S-400 purification. The antioxidant activity of Se-GP for the DPPH, ABTS and hydroxyl radicals was higher than that of GP and was highest for the hydroxylradical.	Mao et al., 2014.
Maitake liquid extract	No dose-limiting toxicity was encountered. Two patients withdrew prior to completion of the study due to grade I possibly related side effects: nausea and joint swelling in one patient; rash and pruritus in the second. There was a statistically significant association between maitake and immunologic function ($p < 0.0005$). Increasing doses of maitake increased some immunologic parameters and depressed others; the dose-response curves for many endpoints were non-monotonic with intermediate doses having either immune enhancing or immune suppressant effects compared with both high and low doses.	Deng et al., 2009.

among them is rare, and now the yield of its fruit body cannot meet the demand of people. From the above, all questions are put forward seemingly because of the same issue: *G. frondosa*'s bioactive polysaccharide.

Table 4. Research progress in non-polysaccharide based extracts from *G. frondosa* in submerged culture.

Non-polysaccharide based extracts	Results	References
A low-molecular-weight protein fraction (MLP-fraction)	A low-molecular-weight protein fraction (MLP-fraction) was obtained from the fruiting body of the maitake mushroom. The effect of the MLP-Fraction on the immune system resulted in a simultaneous increase in splenocyte proliferation and production of cytokines such as interleukin (IL)-1α, tumor necrosis factor-α, IL-10, IL-12, and interferon (IFN)-γ. The possibility was confirmed that the MLP-fraction acts as a BRM using colon-26 carcinoma-bearing mice. This fraction enhanced the production of IL-12 and IFN-γ by splenocytes in tumor-bearing mice and clearly showed an inhibitory effect on tumor cell growth.	Kodama et al., 2010.
A new protein (GFP)	It is the first to reveal the critical role of GFP, a new *G. frondosa* protein from maitake fruiting bodies, in modulating the immune response and to link the immune-enhancing effects of maitake to its antitumor activities. GFP is a nonglucan heterodimeric 83 kDa protein that consists of two 41 kDa subunits. GFP induced interferon-γ secretion by murine splenocytes and natural killer cells and activated the maturation of bone marrow-derived dendritic cells (BMDCs) via a TLR4-dependent mechanism. GFP-treated BMDCs promoted a Th1 response and exhibited significant antitumor activity when transferred into tumor-bearing mice.	Tsao et al., 2013.
Methanolic extracts	Methanolic extracts from maitake mycelium showed high antioxidant activities (85.4-94.7%) at 25 mg ml^{-1}. Reducing powers of the methanolic extracts were 0.97-1.02 at 25 mg ml^{-1}, and scavenging effects on 1, 1- diphenol-2-picrylhydrazyl radicals were 78.8-94.1% at 10 mg ml^{-1}. However, there was no scavenging effect on hydroxyl radicals. Chelating effects on ferrous ions were high (90.3-94.4%) at 10 mg ml^{-1}. Total phenols were the major naturally occurring antioxidant components found in methanolic extracts. EC50 value below 10 mg ml^{-1} indicated that the mycelium had a good antioxidant property except for the scavenging effect on hydroxyl radicals.	Mau et al., 2004.
Exo-biopolymer (EX-GF)	The exo-biopolymer (EX-GF) was fractionated into EX-GF-Fr. I, II, and III by Sephadex G-100 gel chromatography. Anti-complementary activity of EX-GF-Fr.III was highest (71.1%), and its activation system occurred through both classical and alternative pathways. Lysosomal enzyme activity and nitric oxide production ability of macrophage were also found to be mediated by EX-GF-Fr.III. The molecular weight of the three fractions was estimated to be about 163, 40, and 2.8 kDa, respectively. Total sugar and protein contents of the three fractions were 80.3, 61.9 and 89.3%, and 17.3, 35.2, and 10.7%, respectively.	Yang et al., 2007.
Anti-HSV-1 protein (GFAHP)	This antiviral protein from *G. frondosa* fruiting bodies (GFAHP), a molecular weight of 29.5 kDa, could inhibit herpes simplex virus type 1 (HSV-1) replication. Higher concentrations of GFAHP (125 and 500 µg/ml) also significantly reduced the severity of HSV-1 induced blepharitis, neovascularization, and stromal keratitis in a murine model. Topical administration of GFAHP to the mouse cornea resulted in a significant decrease in virus production.	Gu et al., 2007.
Proteolytic enzymes (ProGF)	1. Highly active proteolytic enzymes were found in the fruiting bodies of *G. frondosa* (ProGF). The optimal pH for ProGF activity was pH 3 or 7 using hemoglobin or Hammersten casein as a substrate, respectively. The optimal temperature were 55 °C; 2. These proteases were substrate-specific, mainly cleaving at Ala14-Leu15, Tyr16-Leu17, and Pro28-Lys29 bonds, with occasional cleavage of Phe24-Phe25 bonds in the oxidized insulin B-chain; 3. The ProGF also liberated hydrophobic amino acids, using the oxidized insulin B-chain as a substrate; 4. When soy protein was used as a substrate, valine, leucine, phenylalanine, and tyrosine were selectively released from the hydrolysate.	Nishiwaki et al., 2009.

Table 4. Contd.

Water extract (GFW)	GFW, a water extract of the fruiting body of *G. frondosa*, (1-100 µg/mL) dose-dependently inhibited vascular endothelial growth factor (VEGF)-induced angiogenesis. In addition, GFW inhibited VEGF-induced proliferation, chemotactic migration, and capillary-like tube formation of human umbilical vein endothelial cells (HUVECs). Upon stimulation by VEGF, HUVECs rapidly increased reactive oxygen species production, which was significantly blocked by the treatment with GFW. Moreover, phosphorylation of extracellular signal-regulated kinase 1/2, a downstream signaling molecule following VEGF receptor activation, was also inhibited by GFW.	Lee et al., 2008.
Ethanolic, cold-water and hot-water extracts	At 1 mg/mL, *G. frondosa* T1 and T2 cold-water extracts showed high reducing powers of 1.02 and 0.50, respectively. Chelating abilities on ferrous ions of *G. frondosa* T1 and T2 were higher for cold-water extracts than for ethanolic and hot-water extracts. For the scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radical, *G. frondosa* T1 and T2 extracts were effective in the following order: ethanolic > hot-water > cold-water. The *G. frondosa* hot-water extract showed high scavenging ability on superoxide anions. Total phenols, flavonoids, ascorbic acid and α-tocopherol were the major antioxidant components found in the various *G. frondosa* extracts.	Yeh et al., 2011.
A class I hydrophobin HGFI	A novel hydrophobin from *G. frondosa*, which was named HGFI and belongs to class I. The purified HGFI was found to have 83 amino acids. The protein sequence deduced from the cDNA sequence had 107 amino acids, from which a 24 aa signal sequence had been cleaved off in the mature protein. This signal sequence was 5 aa longer than had been predicted on the basis of signal peptide analysis of the cDNA.	Yu et al., 2008.
Lysophosphatidylethanolamine (LPE)	Lysophosphatidylethanolamine (LPE) from *G. frondosa* (GLPE) was confirmed to induce the activation of MAPK of cultured PC12 cells and was found to suppress cell condensation and DNA ladder generation evoked by serum deprivation, suggesting that the GLPE had antiapoptotic effects. Moreover, GLPE could induce the MAPK cascade [EGFR-MEK1/2-extracellular signal-regulated protein kinases (ERK1/2)] of PC12 cells, the activation of which induced neuronal differentiation and suppressed serum deprivation-induced apoptosis.	Nishina et al., 2006.
Lignocellulolytic enzyme	*G. frondosa* degraded both substrates (oak-sawdust plus corn bran, and oak/corn bran supplemented with coffee spent-ground) decreasing 67 and 50% of their lignin content, along with 44 and 37% of the polysaccharides (hemicellulose and cellulose) respectively. 35.3% biological efficiency was obtained when using oak sawdust plus corn bran as substrate. Coffee spent-ground addition inhibited mushroom production, decreased growth, xylanase and cellulase activities. Enzyme highest activities during colonization achieved were: endoglucanase 12.3, exoglucanase 16.2, β-glucosidase 2.3, endoxylanase 20.3, amylase 0.26, laccase 14.8 and Mn-peroxidase 7.4 U/g dry substrate.	Montoya et al., 2012.
Extracellular laccase	The optimal temperature and pH value for laccase activity were 65 ℃ and pH 2.2, respectively. Enzyme activity was also affected by buffer composition. *G. frondosa* laccase was relatively heat stable. Halide ions could strongly inhibit laccase activity.	Zhao et al., 2012.
Polysaccharide-peptide (GFPPS1b)	1. GFPPS1b had anti-tumor activity and could signigcantly inhibit the proliferation of SGC-7901 cells, whereas slightly influences the growth of human normal liver cell line L-02. When treated with GFPS1b, SGC-7901 cells showed typical apoptotic morphological features. The results showed that GFPS1b could reduce cell survival via arresting cell cycle and inducing apoptosis of tumor cells (2007a); 2. A 21-kDa heteropolysaccharide, coded as GFPS1b from the cultured mycelia of *G. frondosa* GF9801 by hot-water extraction, exhibited more potent anti-proliferative activity on MCF-7 cells than other polysaccharide fractions. GFPS1b was composed of D-glucose, D-galactose, and L-arabinose with a molar ratio of 4:2:1. A analysis revealed that GFPS1b had a backbone consisting of α-(1→4)-linked D-galacopyranosyl and α-(1→3)-linked D-glucopyranosyl residues substituted at O-6 with glycosyl residues composed of α-L-arabinose-(1→4)- α-D-glucose (1→ linked residues (2007b).	Cui et al., 2007a, 2007b.

However, it is the medical functionality and health benefit of bioactive polysaccharide from *G. frondosa* that have illustrated the important role of *G. frondosa*. Accordingly, further theoretical study on *G. frondosa*'s bioactive polysaccharide will play an important role in promoting maitake production.

Conflict of Interests

The authors have not declared any conflict of interests.

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