Extraction of fungal mycelium beta-glucan: a source for immunomodulator

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Abstract

This research screened and identified the saprobic fungal mushrooms in order to analyze for amount of beta-glucan. Fresh mushroom samples were collected from Dongyai forest, Umnatchareon Province, Thailand. All samples were inoculated on agar media to get the fungal spore shooting. The isolated fungi were cultivated in enriched liquid media to obtain fungal mycelium, which then was subjected to enzymatic extraction of beta-glucan. Megazyme Beta-Glucan Assay Kit was used for quantitative analysis of extracted beta-glucan. THP-1 monocyte was used as a model for anti-inflammation property of beta-glucan. From 497 collected mushroom samples, 93 isolates were identified as the saprobic fungi and used for further study. All of them grew well on both agar and liquid media, however, showed different levels of beta-glucan. Among those, the mycelium of Auricularia polytricha (RSPG00622) in liquid media resulted in highest beta-glucan content. Beta-glucan from this strain was enzymatically extracted to 50-60% purity. It was also proved to possess the immune-modulating property on THP-1 monocytes against the inflammation by Escherichia coli LPS.

Keywords: beta-glucan, fungal mycelium and immune-modulating agent

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Introduction

Nowadays, food is not only to provide humans with necessary nutrients but also assist in maintaining the best physical and psychological condition and also help in disease avoiding. This idea has induced occurrence of functional food which can influence human health. Functional food includes inter alia fungi, those are abounding source of many bioactive compounds, e.g. beta-glucans.

Beta-glucan is polysaccharides, containing D-glucose connecting by 1,3-ß-glycosidic bonds. It is commonly found in cell walls of yeast, bacteria, fungi, algae and grains. Numerous researches reveal the beneficial impacts of beta-glucan on humans. The structures of beta-glucan, including tertiary structure, water solubility, molecular weight and chain length, are complex and have been shown to influence its bio-activity. Owing to its importance, the Food and Drug Adminstration (FDA) allowed its use in food products and made it obligation for labeling requirement to acquire health claim (Ahmed et al., 2012). Beta-glucan has been extensively applied in human supplements to help reduction levels of cholesterol and sugar in blood (Rop et al., 2009), to improve skin health (Du et al., 2014). Moreover, beta-glucan is also applied in animal feeds in order to help stimulating the growth and development (Zhang et al., 2012), to modulate the immune system (Pionnier et al., 2014), to balance the gastrointestinal microorganisms (Yuheng et al., 2013) or to reduce the odor and release of ammonia from fecae (O'Shea et al., 2011).

The saprobic fungi, including Ganoderma sp., Schizophyllum sp., Lentinus sp., Phellinus sp. and Pleuortus sp., were widely reported as the potential producers for beta-glucan. Furthermore, they also contain high fiber, amino acids, unsaturated fatty acids, vitamins and minerals. Thus, the application of fungi-derived beta-glucan in healthcare and cosmetic innovations is of interest. Extraction of beta-glucan requires special attention to obtain consistent raw material. The beta-glucan extraction from saprobic fungi can be performed from the fruiting body, mycelium and sclerotium, which represent all forms of the macrofungi life cycle. The fungal mycelium, however, is recommended as source of beta-glucan extraction in case that the interest fungal strain can not be cultured to fruiting body, or the shorter working period is concerned.

Methodology

Cultivation of fungal mycelium

Ninety-three fresh mushroom samples were collected from Dongyai forest, Umnatchareon Province, Thailand. All samples were inoculated on potato dextrose agar (PDA; 200 g potato, 20 g glucose, 20 g agar, 1,000 ml water) to get the fungal spore shooting. The isolated fungi were re-streaked on PDA and incubated at room temperature (28+2 °C) for 5-8 days or until the fungal mycelium covered almost agar. The cork borer was used to cut the growing edge of mycelium for further cultivation in enriched liquid media (potato dextrose broth (PDB); 200 g potato, 20 g glucose, 1,000 ml water) to obtain fungal cells, which then was subjected to further studies.

Extraction of beta-glucan from fungal mycelium (adapted from Borchani et al., 2016)

The fungal cells from above section were collected by centrifugation, water was added to obtain the ratio of cells: water = 1:3, and the mixture was incubated at -20 °C for 72 h. After that, the cells were separated by centrifugation. Water was added to the cells (cells: water = 1:1), boiled at 100 °C for 10 min, and centrifuged. The cells were resuspended in water (cells: water = 1:1), 0.3% protease enzyme was added, and the mixture was incubated at 55 °C for 5 h. The precipitate was separated by centrifugation, ethanol was added at the ratio of 1:2 (precipitate: ethanol) and left for 24 h. The resulting precipitate

(extracted beta-glucan) was collected by centrifugation and dried.

Characterization of extracted beta-glucan

The quantitative analysis of extracted beta-glucan was performed using the Megazyme Enzymatic Mushroom and Yeast Beta-Glucan Assay Kit (Megazyme International Ireland Ltd, Bray, Ireland). The contents of moisture, protein, lipid, ash, fiber and carbohydrate were analysed according to the Association of Official Agricultural Chemists (AOAC) 2000. The anti-oxidation activities of extracted beta-glucan were assayed using DPPH radical scavenging assay (Zhu et al., 2006) and ferric reducing antioxidant power (FRAP) assay (Kubola and Siriamornpun, 2008). Ascorbic acid, alpha-tocopherol and butylated hydroxytoluene (BHT) were used as references. Cell toxicity and anti-inflammation property of extracted beta-glucan were tested according to Chanput et al. (2012). THP-1 monocyte was used as a model.

From total 476 collected mushroom samples in phylum Basidiomycota, 93 isolates of saprobic fungi (classified based on their roles in habitats) were cultured in laboratory and screened for the ability to produce beta-glucan. The top 10 isolates showing highest beta-glucan contents were as follows: *Auricularia polytricha* (RSPG00622) 42.27% (Figure 1), *Resupinatus sp.* (RSPG00581) 40.28%, *Hohenbuehelia sp.* (RSPG00580) 39.16%, *Phellinus sp.* (RSPG00048) 28.86%, *Auricularia sp.* (RSPG00336) 38.21%, *Coriolopsis byrsina* (RSPG00722) 38.10%, *Lentinus sp.* (RSPG00278) 36.71%, *Perenniporia sp.* (RSPG00139) 35.78%, *Lentinus sajor-carju* (RSPG00224) 35.77%, *Lentinus fasciatus* (RSPG00590) 35.41% as shown in Figure 2.

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Figure 1. *Auricularia polytricha* (RSPG00622)

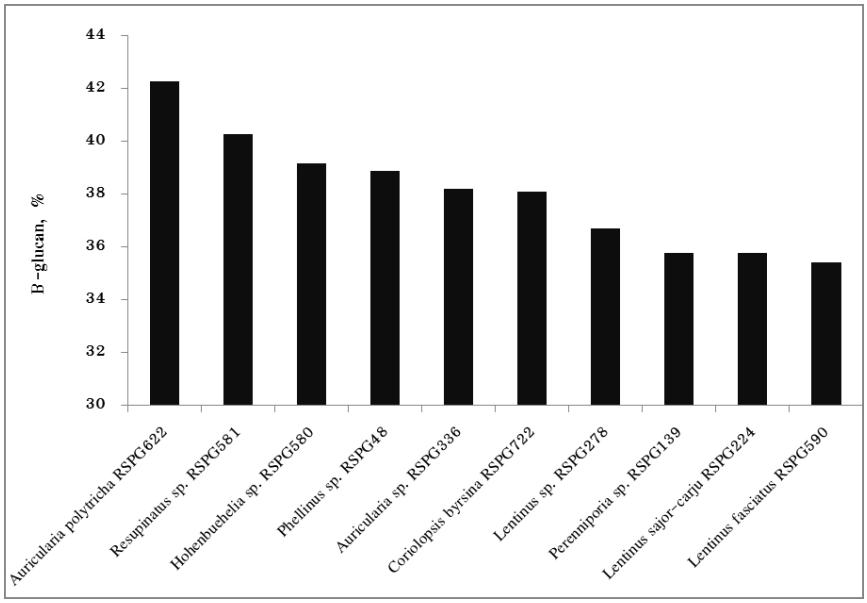


Figure 2. Beta-lucan contents in selected fungal mycelium

Auricularia polytricha are popular in China, Japan and Korea. These peculiar mushrooms with soft, jelly-like texture and ear-shaped fruiting bodies commonly grow on the wood of deciduous trees and shrubs (Sekara et al. 2015). It has been primarily recognized as functional dietary supplement, especially in Asian countries due to its economic importance, as well as a wide spectrum of its health properties, including anti-inflammatory, antioxidant and antimicrobial. Hot water extraction is a popular approach for obtaining water-soluble polysaccharides (Zhang et al., 2007). Up to now, many attempts have worked on extraction of beta-glucan from this fungal specie in the form of its fruiting body grown on solid culture, but none is reported from its mycelium. The extraction method basically is to break cell wall and removes impurities with mild to strong extraction conditions. In this research, beta-glucan was extracted from *A. polytricha* (RSPG00622) cells through several steps. The proximate composition of extracted beta-glucan was shown in Table 1. The 59.54% of the extract was carbohydrate (in which 47.25% was proved as beta-glucan by the Megazyme Enzymatic Mushroom and Yeast Beta-Glucan Assay Kit), whereas 32.83% was protein.

Table 1. Proximate composition of extracted beta-glucan from *A. polytricha* (RSPG00622)

Proximate composition	%based on dry weight
Moisture	1.29 ± 0.07
Lipid	3.70 ± 0.10
Protein	32.83 ± 0.27
Carbohydrate	59.54 ± 0.59
Ash	2.12 ± 0.04
Fiber	0.52 ± 0.04

All extracted beta-glucan were also analysed for their antioxidant activities as shown in Figure 3, and the top 10 isolates possessing highest antioxidant activities were as follows: *A. polytricha* (RSPG00622) IC50 5.79±0.29 mg/ml (DPPH), 3.53±0.07 µmol Fe (II)/g (FRAP), H*ohenbuehelia sp*. (RSPG00580) IC50 7.32±0.20 mg/ml (DPPH), 2.81±0.07 µmol Fe (II)/g (FRAP), *Resupinatus sp.* (RSPG00581) IC50 8.57±0.40 mg/ml (DPPH), 3.53±0.29 µmol Fe (II)/g (FRAP), *Phellinus sp.* (RSPG00048) IC50 11.71±0.22 mg/ml (DPPH), 1.26±0.07 µmol Fe (II)/g (FRAP), *Lentinus sp.* (RSPG00278) IC50 15.69±1.59 mg/ml (DPPH), 1.38±0.02 µmol Fe (II)/g (FRAP), *L. fasciatus* (RSPG00590) IC50 16.19±0.73 mg/ml (DPPH), 0.74±0.02 µmol Fe (II)/g (FRAP), *L. sajor-carju* (RSPG00224) IC50 16.24±1.07 mg/ml (DPPH), 1.20±0.01 µmol Fe (II)/g (FRAP), *Perenniporia sp.* (RSPG00139) IC50 17.07±0.45 mg/ml (DPPH), 1.03±0.07 µmol Fe (II)/g (FRAP), *Auricularia sp.* (RSPG00336) IC50 20.16±1.78 mg/ml (DPPH), 0.68±0.02 µmol Fe (II)/g (FRAP) and C. byrsina (RSPG00722) IC50 20.38±2.73 mg/ml (DPPH), 0.46±0.02 µmol Fe (II)/g (FRAP).

The extracted beta-glucan from *A. polytricha* (RSPG00622) was further tested for its anti-inflammatory activity, considering the stimulation of related genes expression (IL-8, IL-1β, TNF-α, IL-10). Figure 4 shows %viability of THP-1 monocytes after incubation with extracted beta-glucan from *A. polytricha*

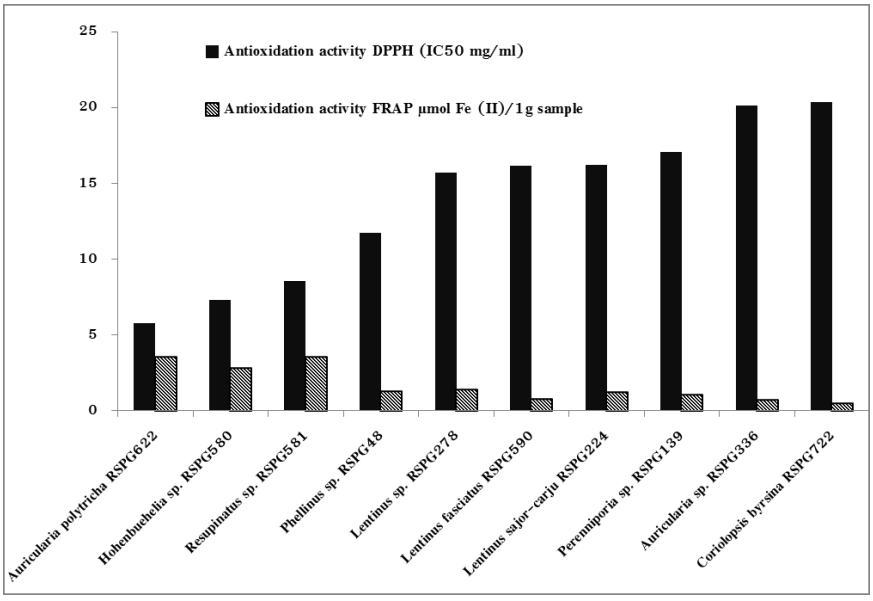


Figure 3 Anti-oxidant activity of extracted beta-glucan

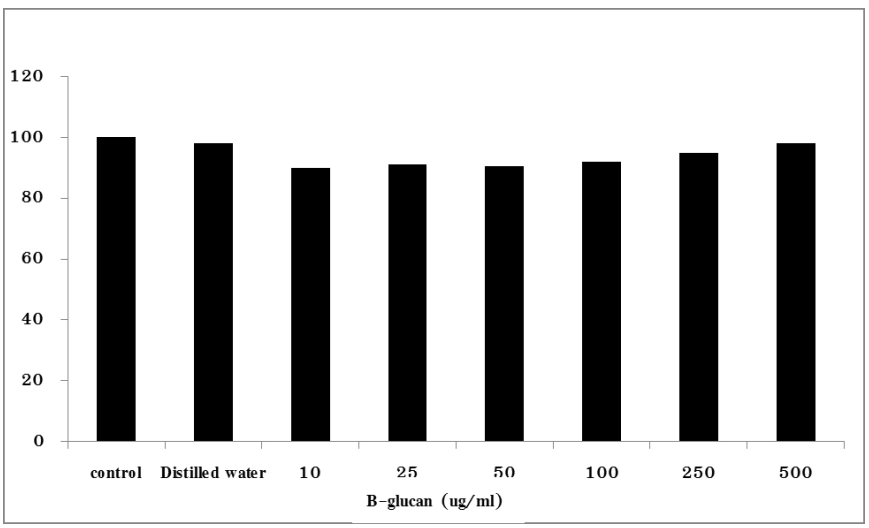
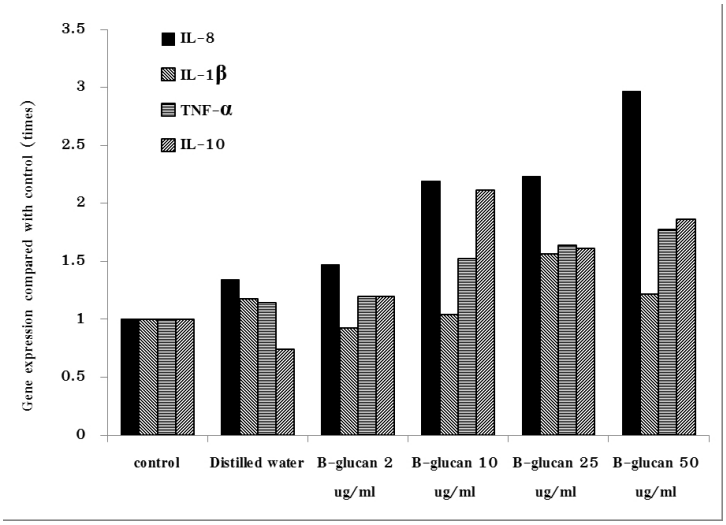
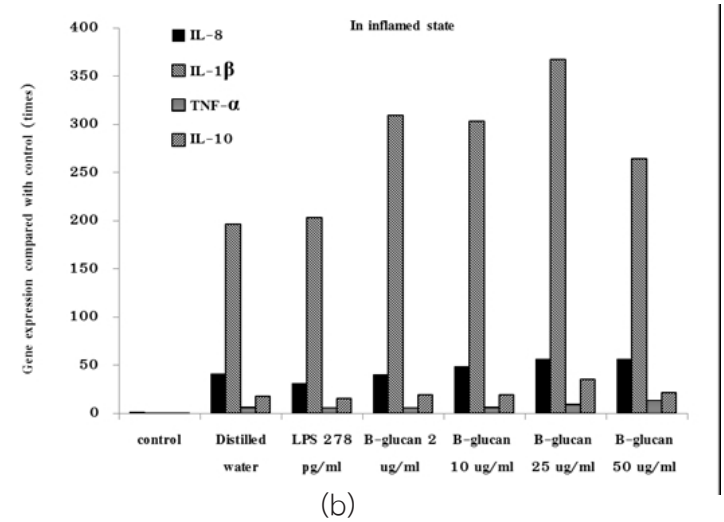


Figure 4 %viability of THP-1 monocytes after incubation with beta-glucan

(RSPG00622) for 3 h. More than 90% of THP-1 monocytes survived in every tested concentration of beta-glucan, demonstrating no cytotoxicity of beta-glucan on THP-1 monocytes. After that, the effect of beta-glucan on inflammatory related gene expression was performed in resting and LPS-inflamed states as shown in Figures 5(a) and 5(b), respectively. The results show that in the resting state (Figure 5(a)), concentration of beta-glucan obviously influenced the expression of inflammatory related genes: IL-8, IL-1β, TNF-α, IL-10, thus indicating its role in stimulating of inflammatory related genes. Extracted beta-glucan was further tested in the state inflamed with Escherichia coli lipopolysaccharides (LPS) for 3 h. Figure 5(b) clearly demonstrates that beta-glucan upregulated inflammatory related gene expression, and the intensity of expression depended on beta-glucan concentration, especially IL-1β (Interleukin-1β) which is a key mediator of the inflammatory response and crucial for host-defence responses to infection and injury. Qin et al. (2010) also similarly reported that mushroom derived glucans could act as immunomodulators by activating host immune cells (such as cytotoxic macrophages) or chemical messengers (cytokines such as interleukins). In 2013, Zhou et al. showed the antimutagenic activity of beta-glucan against the in vivo DNA-damaging effect of the indirectly acting alkylating agent, cyclophosphamide. It was assumed that beta-glucan probably modulated the response of the immune system.



(a)



(b)

Figure 5. Effect of beta-glucan on inflammatory gene expression in (a) resting state and (b) LPS-inflamed state

Conclusion

This research screened and identified the saprobic fungal Auricularia polytricha (RSPG00622) in Thailand, which contained high beta-glucan content. An easy and convenient cultivation technique and extraction of fungal mycelium to obtain high amount of beta-glucan extract is promising to be applied as main ingredient in healthcare product. The extracted beta-glucan was shown to contain antioxidation activity and anti-inflammatory activity. The extract, thus, could be developed as a potential supplement for various applications in functional food, cosmetic or nutraceutical industries. The effect of extraction process on the rheology, viscosity, gel formation, molecular weight profile of beta-glucan, however, needs to be further determined.

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