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Constituents actually responsible for the antioxidant activities of crude polysaccharides isolated from mushrooms

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ABSTRACT

The major chemical constituents and antioxidant activities of water-soluble polysaccharide (PS) fractions isolated from three edible mushrooms were investigated. Crude PS (CPS) isolated from the mushroom hot water extract by ethanol precipitation contained 15–30% (w/w) total protein and 2–5% phenolics. The CPS were fractionated by ion exchange chromatography and the neutral, and slightly ionic PS fractions (eluted with 0 and 0.1 M NaCl) contained 70–80% carbohydrate with low protein and phenolic contents, while the ionic PS fractions (eluted with 0.3 M and 0.7 M NaCl) had higher contents of protein (20–70%) and phenolics (2–13%). The antioxidant activities of all PS fractions were significantly correlated with the total phenolic and protein contents but not with the carbohydrate content. Purified PS free of phenolics and proteins had no significant activities. Therefore, the phenolic and protein components instead of carbohydrates were mainly responsible for the antioxidant activities of mushroom PS.

1. Introduction

Edible fungi or mushrooms are regarded as healthy foods because of their favorable chemical composition which is abundant in carbohydrates, proteins, dietary fiber, important vitamins, and minerals but low in calorie and fats (Cheung, 2010). Many edible fungi also have notable medicinal properties such as antitumor, antiviral and antioxidant activities (Guo et al., 2012; Lindequist, Niedermayer, & Jülich, 2005). Mushrooms contain bioactive compounds including polypeptides/proteins and polysaccharides, and a variety of small organic molecules (secondary metabolites) such as phenolics, flavonoids and sterols (Lakhanpal & Rana, 2005). Polysaccharides (PS) including PS–protein complexes (PSPs) have been recognized as a class of major bioactive constituents of medicinal mushrooms (Ooi & Liu, 2000; Zhang, Cui, Cheung, & Wang, 2007). Anticancer and immunomodulation are the most notable and proven bioactivities of mushroom PS and some mushroom PS have been used as therapeutic agents, such as the β-glucans from *Lentinus edodes* (lentinans) and *Grifola frondosa* (Grifolan), and PSPs from *Trametes versicolor* (PSK or Krestin).

Abbreviations: CPS, crude polysaccharide; IEC, ion exchange chromatography; IEC0, IEC0.1, IEC0.3 and IEC0.7, PS fractions of CPS by IEC eluted with distilled water containing 0, 0.1, 0.3, and 0.7 M NaCl; IEC0.1–1 and IEC0.1–2, PS fractions of IEC0.1 by SEC; PS, polysaccharide; SEC, size exclusion chromatography

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Another widely reported activity of mushroom PS is antioxidative and radical scavenging (Cheung, Siu, Liu, & Wu, 2012; Fan et al., 2011; Kozarski et al., 2012; Lee et al., 2003).

Oxidative stress imposed by reactive oxygen species (ROS) can cause damage to biomolecules such as DNA, proteins, lipids and carbohydrates, which may be the contributing factors for many diseases such as cardiovascular diseases, cancer, arthritis and inflammation (Halliwell, 1996; Herman, 1998). Because of public concerns with the adverse effects of synthetic chemical antioxidants on human health, there has been increasing interest in the discovery and application of natural antioxidants. Polysaccharides (PS) extracted from many mushrooms have been reported to have significant antioxidant activities based on various *in vitro* and *in vivo* assays (Lindequist et al., 2005). However, many of the fungal PS tested for antioxidant activities in previous studies were crude or partially purified PS fractions with complex and undefined chemical composition, often mixed with proteins and low-MW pigments. Usually these PS were isolated from the hot-water extract simply by ethanol precipitation without further purification, such as the antioxidative PS from three well-known medicinal mushrooms, *L. edodes*, *G. frondosa* and *Ganoderma tsugae* (Fan et al., 2011; Kozarski et al., 2012; Lee et al., 2003). Purification of the crude PS attained by ethanol precipitation of aqueous extracts is usually accomplished by chromatographic methods such as size-exclusion (SEC) and ion-exchange chromatography (IEC) (Zhang et al., 2007). However, some studies have found that the purified PS had even lower antioxidant activities than the original crude PS, e.g. the PS from *Ganoderma atrum* (Chen, Xie, Nie, Li, & Wang, 2008) and tea leaves (Wang, Zhao, Andrae-Marobela, Okatch, & Xiao, 2013).

This study aimed to identify and clarify the chemical constituents actually responsible for the antioxidant activities of crude PS isolated from edible and medicinal mushrooms through chromatographic fractionation, chemical analysis and bioactivity assays, and examination of the correlation to three major components, carbohydrate, protein and phenolic contents. Three important edible/medicinal fungi (mushrooms) were selected for this study, *L. edodes*, *G. frondosa* and *T. versicolor*. Crude PS was isolated from each mushroom by hot water extraction followed by ethanol precipitation, and the crude PS was then separated by IEC and SEC into fractions with different chemical contents. Their antioxidant activities were determined with chemical and cell culture assays.

2. Materials and methods

2.1. Mushroom materials

Three important and well-known edible/medicinal mushrooms were chosen for this study, *L. edodes* (shiitake or Xianggu), *G. frondosa* (maitake or Hui-shu-hua) and *Trametes* (*Coriolus* or *Polyporus*) *versicolor* (turkey tail or Yunzhi). The mushrooms were supplied in dry fruit body form by Zhejiang Fangge Pharmaceutical Co., Ltd. (Qingyuan Country, Zhejiang, China). They were enclosed within plastic bags and stored at 25 °C before use.

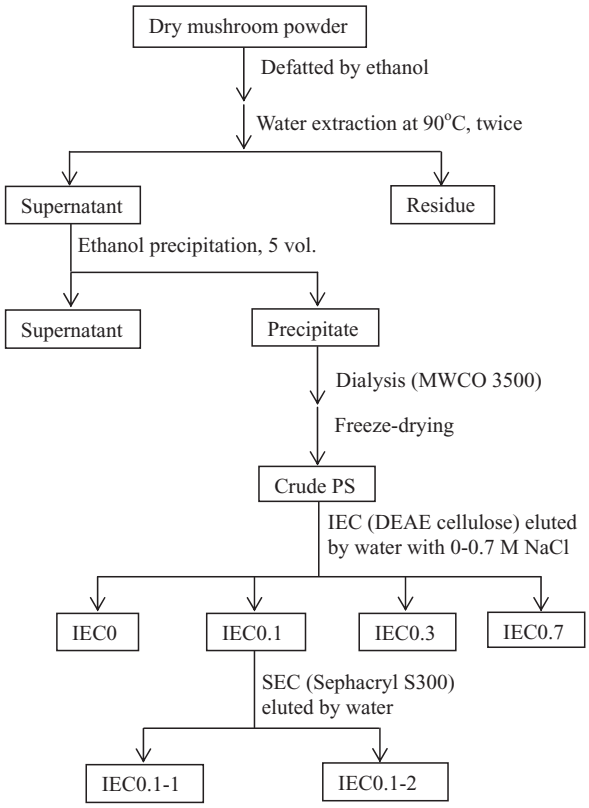

Fig. 1 – Experimental procedure for the extraction, isolation and fractionation of PS from mushrooms.

2.2. Extraction, isolation and fractionation of PS from mushrooms

Fig. 1 represents a flowchart showing the overall procedure for the extraction, isolation and fractionation of PS from the dry mushrooms. The mushrooms were dried at 40 °C in an oven till constant weight and were ground into a powder with an electric mill (maximum particle size about 1.0 mm). The dry mushroom powder (100 g) was first defatted with ethanol, followed by hot water extraction of the solid residue. Hot water extraction was performed twice, with the solid suspended in 2 l of distilled water and maintained at 90 °C in a water bath for 6 h. The liquid extract was separated from the solid residues by centrifugation at 14,980 g (Beckman Coulter, Avanti JE Centrifuge, Palo Alto, CA, USA), and concentrated by vacuum evaporation at about 50 °C. The concentrated liquid extract was mixed with 5 volumes of 95% ethanol at 4 °C overnight for PS precipitation. The PS precipitate was collected by centrifugation (14,980 g, 20 min), followed by dialysis using a regenerated cellulose dialysis membrane (MWCO 3500 Da, Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) against distilled water at room temperature for 3 days. The dialysate was lyophilized and collected as the crude PS (CPS) for further fractionation.

The CPS from each mushroom was fractionated by anion IEC using DEAE cellulose resin (Sigma, St. Louis, MO, USA). The freeze-dried CPS was redissolved in Millipore water by constant stirring overnight and then centrifuged at 3824 g (Mikro 22R, Hettich Zentrifugen, Tuttlingen, Germany) for 20 min. The

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