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Characterization and antioxidant activities of polysaccharides from thirteen boletus mushrooms

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ABSTRACT

Water-soluble polysaccharides were extracted from the caps and stipes of thirteen boletus mushrooms representing five different species collected in Southwest China. Investigations of their structures and antioxidant activities allowed an evaluation of structure-function relationships. The polysaccharides were composed mainly of the monosaccharides arabinose, xylose, mannose, glucose and galactose. Most samples displayed a broad molecular weight range, with significant differences observed between the molecular weight ranges of the polysaccharides from the caps and the stipes. FT-IR spectral analysis of the polysaccharides revealed that most of polysaccharides from boletus mushrooms (except *Boletus edulis*) contained a pyranose ring. The antioxidant activities of the polysaccharides in stipes showed a significant correlation with their monosaccharide composition, and were also related to their molecular weight and anomeric configuration. *Suillellus luridus* collected in Pingwu, Mianyang, Sichuan, China had remarkably superior antioxidant activity and might be developed as a natural antioxidant.

1. Introduction

Humans have consumed mushrooms for centuries because of their high nutrient content and low lipid content, as well as their excellent flavor and texture [1]. Edible mushrooms possess notable medicinal properties and bioactivities, including antitumor, antiviral, antidiabetic, and antioxidant activities [2]. Polysaccharides, the most abundant biopolymers, are major active constituents in edible mushrooms [3]. Many studies have demonstrated that polysaccharides isolated from various kinds of mushrooms have wide-ranging bioactivities, especially strong antioxidant activities [4]. Therefore, there is increasing interest in characterizing the polysaccharides from various mushroom species and exploring their antioxidant activities for use as natural antioxidants.

The bioactivities of polysaccharides can be related to structural characteristics such as monosaccharide composition, molecular weight distributions and degree of branching, as well as degree of sulfation [5,6]. Sun, Wang and Zhou [7] demonstrated that the molecular weight (Mw) of polysaccharides had a notable effect on their biological activities, with low-Mw polysaccharides having better immunomodulatory effects than those of higher Mw. The antioxidant properties of polysaccharides may depend on the ratios of the different monosaccharide components; for example, rhamnose extracted from *Lentinula edodes* was the most significant determinant [8]. Recently, Ren et al. [9] also showed that polysaccharides with a low Mw or a beta configuration in the pyranose form had higher antioxidant activity. To date, although there are some studies on the structure and function of polysaccharides, the specificity and pertinence of these studies are strong, and there are lacking a variety of systematic comparisons and comprehensive studies.

Mushrooms of the genus *Boletus*, among the most delicious and widely consumed mushrooms, are distributed in many regions of China, but primarily in Sichuan and Yunnan [10]. Our preliminary study showed that five species of boletus mushrooms, among the most popular species in local markets, had high total carbohydrate contents and low crude fat contents. It also demonstrated that most of the mushrooms had antioxidant activities, and that samples of *Suillellus luridus* were remarkably superior among those tested [11]. However, more comprehensive studies of the polysaccharides of the boletus mushrooms from Southwest China are needed. Most of the available studies have investigated the crude or purified polysaccharides extracted from fruiting bodies and the mycelium [12,34]. Studies that characterize and investigate the antioxidant activities of polysaccharides isolated from caps and stipes of boletus mushrooms would be particularly useful.

In this study, water-soluble polysaccharides were extracted from the caps and stipes of 13 boletus mushrooms collected in Southwest China. These mushroom samples represent five different species: *Boletus*

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aereus, *Suillus bovinus*, *Suillellus luridus* [formerly *Boletus luridus*], *Boletus edulis* and *Boletus violaceo-fuscus Chiu*. The structures of the polysaccharides were characterized using gas chromatography (GC), high performance liquid chromatography (HPLC), and Fourier-transform infrared spectroscopy (FT-IR). Their antioxidant properties were investigated using in-vitro assays of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, reducing power, and metal chelating activity. These data were used to investigate the relationship between polysaccharide structure and antioxidant activity.

2. Materials and methods

2.1. Sample preparation and chemicals

The fruiting bodies of boletus mushrooms from the species *B. aereus*, *S. bovinus*, *B. luridus*, *B. edulis*, and *B. violaceo-fuscus Chiu*, were collected in Yunnan and Sichuan Provinces of Southwest China (Table 1) and identified by Prof. Douxi Zhu, a taxonomist from Mianyang Edible Fungi Research Institute in Sichuan, as previously described [13]. All samples were separated into caps and stipes, freeze-dried, ground into fine powders (40 meshes), and stored at −80 °C pending further analysis.

DPPH, 2, 6-ditert-butyl-4-methylphenol (BHT), ethylenediaminetetraacetic acid (EDTA) and standard monosaccharide samples were obtained from Sigma-Aldrich (Germany). All other chemicals used in this study were analytical reagent grade and purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Preparation of water-soluble polysaccharides

The water-soluble polysaccharides were prepared as previously described [14], with minor modifications. The powders (8 g) were soaked with 95% (v/v) ethanol for 12 h to eliminate low-Mw components. The residues were extracted with hot water (1:20, w/v) at 85 °C for 3 h. The supernatants were evaporated using a rotary evaporator at 45 °C under reduced pressure, proteins were removed using the Sevag reagent (chloroform: *n*-butanol, 4:1 (v/v)), and the resulting liquid was dialyzed (Mw cutoff 3000 Da) against tap water for 24 h and distilled water for 12 h. Finally, the solution was concentrated and precipitated with 4 volumes of 95% (v/v) ethanol for 24 h at 4 °C. The precipitates obtained by centrifugation (2654 ×*g*, 10 min, 4 °C) were lyophilized to obtain the crude polysaccharides.

Table 1
Sample species and collection site.

Abbreviation	Species	Collection site
Bolae.1-C Bolae.1-S	*Boletus aereus*	Qingchuan, Guangyuan, Sichuan, China
Bolae.2-C Bolae.2-S	*Boletus aereus*	Fumin, Kunming, Yunnan, China
Suibo.1-C Suibo.1-S	*Suillus bovinus*	Qingchuan, Guangyuan, Sichuan, China
Suibo.2-C Suibo.2-S	*Suillus bovinus*	Midu, Dali, Yunnan, China
Suilu.1-C Suilu.1-S	*Suillellus luridus*	Qingchuan, Guangyuan, Sichuan, China
Suilu.2-C Suilu.2-S	*Suillellus luridus*	Pingwu, Mianyang, Sichuan, China
Suilu.3-C Suilu.3-S	*Suillellus luridus*	Simao, Puer, Yunnan, China
Suilu.4-C Suilu.4-S	*Suillellus luridus*	Fumin, Kunming, Yunnan, China
Boled.1-C Boled.1-S	*Boletus edulis*	Qingchuan, Guangyuan, Sichuan, China
Boled.2-C Boled.2-S	*Boletus edulis*	Nanhua, Chuxiong, Yunnan, China
Boled.3-C Boled.3-S	*Boletus edulis*	Pingwu, Mianyang, Sichuan, China
Boled.4-C Boled.4-S	*Boletus edulis*	Luquan, Kunming, Yunnan, China
BolviCh-C BolviCh-S	*Boletus violaceo-fuscus Chiu*	Luquan, Kunming, Yunnan, China

C: mushroom caps; S: mushroom stipes.

2.3. Monosaccharide composition

The monosaccharide compositions of the polysaccharide samples were determined using GC according to an established method [15]. Briefly, the dried samples (10 mg) were hydrolyzed with 2 mL of 2 mol/L trifluoroacetic acid (TFA) at 110 °C for 2 h, and the solution was freeze-dried after removing the excess acid. Then, 10 mg hydroxylamine hydrochloride and 0.5 mL pyridine were added to the hydrolysate and this mixture was incubated for 30 min at 90 °C. After cooling to room temperature, 0.5 mL of acetic anhydride was added and reacted at 90 °C for 30 min in a water bath. Standard monosaccharide samples (glucose, galactose, arabinose, mannose, rhamnose, xylose) were derivatized under the same conditions. Monosaccharide compositions were identified by comparison with the retention times of the monosaccharide standards.

2.4. Molecular weight determination

The Mw distributions were measured using high performance gel permeation chromatography (HPGPC) with an Agilent 1100 HPLC system equipped with a Waters 2410 refractive index detector and a TSK-GEL G5000 PW x l column (7.8 × 300 mm, Tosoh Corp, Japan) [16]. The mobile phase was ultrapure water which flowed at a rate of 0.8 mL/min and a temperature of 30 °C. A 20 μL sample of polysaccharide solution (2.0 mg/mL) was injected in each run. Dextran standards ranging from 3.0 to 670 kDa (Sigma) were used to create the standard curve.

2.5. FT-IR and ultraviolet analysis

FT-IR spectra of polysaccharides were obtained using a Fourier-transform infrared spectrometer (Nexus 5DXC FT-IR, Nicolet). The polysaccharides (1 mg) were ground with 100 mg KBr powder, pressed into pellets, and then scanned for FT-IR measurement in the frequency range of 400–4000 cm^{-1}. Ultraviolet-visible (UV) absorption spectra were obtained using a UV–visible spectrophotometer (UV-2450, Shimadzu, Japan).

2.6. In-vitro antioxidant activities

2.6.1. DPPH radical scavenging activity

The DPPH radical scavenging activities of the polysaccharides were measured as reported by Blois [17], with some modifications. Polysaccharide solution samples (0.1 mL) of different concentrations were mixed with 2.9 mL of a methanolic solution of DPPH radical (6×10^{-5} mol/L). The resulting mixtures were shaken for 45 min at 25 °C in the dark, and then the absorbance at 517 nm was measured with a spectrophotometer (UV-2450, Shimadzu, Japan). Lower absorbance indicates higher free radical scavenging activity. In this study, the positive and negative controls were BHT and the reaction solution without polysaccharides, respectively. The DPPH radical scavenging ability was calculated according to the following equation:

$$\text{DPPH radical scavenging activity } (\%) = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

Where A_0 is the absorbance of the DPPH solution without a sample and A_1 is the absorbance of the solution containing a sample. The EC_{50} value, which was determined by plotting DPPH radical scavenging ability versus polysaccharide concentration, represents the concentration of extract producing 50% inhibition.