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Characterization and immunomodulatory effects of glucans from *Pleurotus albidus*, a promising species of mushroom for farming and biomass production

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ABSTRACT

Polysaccharides from a number of mushroom species are recognized as functional food ingredients with potential health benefits, including immunomodulatory effects. In this study, polysaccharides extracted from the basidiome with cold water (BaCW), hot water (BaHW), and hot alkali (BaHA) solution, and exo- (MyEX) and endopolysaccharides (MyEN) from the submerged culture of *Pleurotus albidus*, a promising species for farming and biomass production, were analyzed for their chemical composition and structure and immunomodulatory effects on macrophages. Compositional (HPAEC-PAD and HPSEC-RID/MWD) and structural (FT-IR, 1D- and 2D-NMR) analyses identified BaCW and MyEX as β-(1,6)-branched β-(1,3)-glucans, BaHW and MyEN as α-(1,3)-(1,2)-branched α-(1,6)-glucans, and BaHA as a mixture of α-(1,6)- and β-(1,3)-glucans. BaCW and MyEX stimulated the production of tumor necrosis factor alpha (TNF-α) and nitric oxide (NO), but not interleukin-6 (IL-6), and decreased phagocytosis of zymosan particles. In contrast, BaHW and MyEN induced TNF-α, NO and IL-6 production, and increased zymosan phagocytosis, while BaHA displayed intermediary effects in comparison the other polysaccharides. In conclusion, the basidiome and the submerged culture of *P. albidus* are sources of easily extractable α- and β-glucans with potential immunomodulatory effects.

1. Introduction

Mushroom polysaccharides comprise a large group of biopolymers which serve structural functions, provide an energy source or work as a mechanism for fungal protection or attachment to other surfaces [1]. In humans, the consumption of these macromolecules may enhance cell-mediated immune responses largely through interacting with macrophages, which are phagocytic cells that play a pivotal role in immune responses [2]. Thus, the consumption of edible mushrooms and the use of their polysaccharides as functional food ingredients have been increasing due to their potential immunomodulatory effects [3]. However, because these effects are largely dependent on polysaccharide structure, which in turn is related to the source organism, the study of polysaccharides from different edible mushrooms is required [1–3].

In this context, *Pleurotus albidus* (Berk.) Pegler, a South American species belonging to a genus comprised of several edible mushrooms which are consumed worldwide, has been proposed for commercial production due to its high biological efficiency in culture conditions and the qualities of the edible basidiome [4]. Moreover, *P. albidus* may be used for bioconversion of residual substrates as it can be easily cultivated on agricultural waste, producing

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relatively large amount of biomass [5]. Cultivation of *P. albidus* may therefore contribute to a reduction in the environmental impact of the biofuel industry, as it has been shown to have a high efficiency in bioconversion of the vinasse, a by-product of sugarcane fermentation during alcohol production [6]. Thus, *P. albidus* is promising both for farming production and for the biomass conversion of agricultural by-products or waste; however, the potential health benefits of their polysaccharides remains unknown.

In the present study, polysaccharides obtained from the basidiome of cultivated *P. albidus* are analyzed for their chemical composition and structure and effects on the production of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and nitric oxide (NO) by macrophages, in order to estimate their immunomodulatory effect. Furthermore, as the abundant mycelial biomass produced by the bioconversion of residual substrates may be an alternative source of polysaccharides with potential to be used as functional food ingredients, polysaccharides from submerged culture of *P. albidus* are also extracted and analyzed for their chemical composition and structure and effects on macrophages function.

2. Materials and methods

2.1. Chemicals

Yeast extract, peptone and agar were from BD Biosciences (San Jose, CA, USA). Potato dextrose agar (PDA) and malt extract were from Kasvi (Curitiba, Brazil). Heat-inactivated fetal bovine serum (FBS) and Dulbeccoís modified Eagleís medium (DMEM) containing penicillin (100 UI/mL) and streptomycin (100 μg/mL) were from Cultilab (Campinas, Brazil). The water used was filtered using a Milli-Q purification system from EMD Millipore (Bedford, MA, USA). Unless stated otherwise, other reagents and chemicals used were from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Production of the basidiome and submerged culture

Basidiomes of *P. albidus* were collected from *Araucaria angustifólia* (Bertol.) Kuntze trunk in Serra da Bocaina (Visconde de Mauá, Rio de Janeiro, Brazil). The mycelium pure culture was obtained by tissue culture from the fresh basidiocarp, which is currently deposited at the collection from the Algae, Cyanobacteria and Fungi Culture Collection of the Botanic Institute of São Paulo (CCIBt) under the number CCIBt4244. After incubation in petri dishes containing PDA (7 d; 25 °C), mycelial discs (7 mm) from the culture were used for spawn production in wheat grain. The wheat grain was soaked overnight in tap water, drained and autoclaved (121 °C; 15 min). Grains were allowed to cool and inoculated with mycelial discs (15 d; 25 °C) in order to produce the spawn. Then, polyethylene bags containing 8 kg of *Brachiaria brizantha* (Hochst.) Stapf. hay were humidified, sterilized and inoculated with the spawn (2% w/w). The spawn run was performed in a mushroom house with controlled light (500 lux) and temperature (25 °C). Three days after primordia initiation, the basidiome was collected, freeze-dried and stored for later analysis.

The morphological data used for the identification were recovered from the dried basidiomes, which was deposited at Herbarium SP from Botanic Institute of São Paulo under the number SP466412. In order to perform microscopic analyses, the material was wetted with 70% ethanol, rehydrated in 5% KOH and examined by light microscopy. For morphological identification the complete description was compared to that in the study by Lechner et al. [7]. For molecular identification [8], DNA was obtained from fragments of the dried basidiomes used for morphological identification. PCR was conducted using the ITS1F and ITS4 primer sets [9,10] according to the reaction method previously described [11]. The Internal Transcribed Spacer (ITS) sequence generated was deposited in GenBank and a maximum likelihood analysis was run using RAxML servers [12] with identical parameters and dataset (in addition to the sequence herein generated) to those previously described [11].

The production of submerged culture [13] was performed using mycelial discs of *P. albidus* CCIBt2930 stored in distilled water obtained from CCIBt. Discs cultured in petri dishes containing malt extract agar (MEA; 30 g/L malt extract, 3 g/L peptone, 15 g/L agar) were incubated (7 d; 25 °C) and mycelial discs (7 mm) were used for spawn production as described for basidiome production. After incubation, the spawn was inoculated (1% w/v) in Erlenmeyer flasks containing 500 mL of culture broth (20 g/L glucose, 2 g/L yeast extract, 2 g/L peptone, 1 g/L NaH_2PO_4, 0.5 g/L $MgSO_4$) on an orbital shaker at 120 rpm for 12 d at 28 °C [13]. Aliquots (10 mL) of submerged culture were taken at day 0, 5, 7, 10 and 12 for analysis of the biomass and endo- and exopolysaccharides contents. The biomass was determined as the weight of dried solid residue obtained per volume of culture broth. Endo- and exopolysaccharides were obtained as described below.

2.3. Polysaccharide extraction

Polysaccharides from the basidiome were extracted as described by Palacios et al. [14] with slight modifications [15]. A freeze-dried basidiome was extracted using chloroform:methanol (1:1, v/v) for 2 h at 70 °C. After extraction, the material was centrifuged (8000 × *g*; 10 min) and the supernatant was discarded. Remaining solids were washed with ethanol, acetone, dried at ambient temperature and submitted to successive 8 h extractions with (1) water at 25 °C, (2) water at 100 °C, and (3) 1 M KOH/20 mM $NaBH_4$ at 100 °C. Supernatants containing polysaccharides were collected after centrifugation (8000 × *g*; 10 min) and the pH was adjusted to 7. Proteins were removed from extracts after precipitation with trichloroacetic acid (TCA; 20% w/v) and centrifugation (8000 × *g*; 10 min). Polysaccharides in the supernatants were precipitated with ethanol (70% v/v) and washed with acetone. Polysaccharides from the basidiome extracted with cold water, hot water, and hot alkaline solution were designated BaCW, BaHW and BaHA, respectively.

Polysaccharides from the submerged culture were extracted similar to the method described by Yang et al. [13]. The submerged culture was centrifuged (8000 × *g*; 10 min) to separate the mycelium and supernatant. Endopolysaccharides from mycelium were extracted with water at 100 °C for 2 h. After extraction, the material was centrifuged (8000 × *g*; 10 min) and the supernatant was collected. Polysaccharides from supernatants were precipitated with ethanol (70% v/v) and separated after centrifugation (8000 × *g*; 10 min). Polysaccharides were washed with acetone and solubilized in water. After protein removal with TCA (20% w/v), the supernatant was dialyzed (MWCO 3.5 kDa, Spectrum Labs, Los Angeles, CA, USA) against water for 72 h (4 °C) and freeze-dried to yield endopolysaccharides. Exopolysaccharides from the supernatant of submerged culture were precipitated with ethanol (70% v/v) and separated after centrifugation (8000 × *g*; 10 min). Then, polysaccharides from the supernatants of the submerged culture were purified as described above for endopolysaccharides. Endo- and exopolysaccharides obtained from the submerged culture were designated MyEN and MyEX, respectively.

2.4. Total sugars and proteins analyses

Total sugars were determined using the phenol-sulfuric method, using glucose as the standard. Proteins were determined using the Bradford method, using BSA as standard, and were confirmed using