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Short communication

Yield, polysaccharides content and antioxidant properties of *Pleurotus abalonus* and *Pleurotus geesteranus* produced on asparagus straw as substrate

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ABSTRACT

The aim of this work is to evaluate the potential of asparagus (*Asparagus officinalis* L.) straw as a raw material for cultivating *Pleurotus abalonus* and *Pleurotus geesteranus*. The effects of asparagus straw substrate on yield, polysaccharides content and antioxidant properties of these two mushrooms were studied. On non-supplemented asparagus straw substrate, the yield of *P. geesteranus* (199 g/bag) was significantly higher than that of *P. abalones* (140 g/bag) while the polysaccharides content of *P. abalones* was significantly higher than that of *P. geesteranus*. Addition of appropriate amounts of glucose, $MgSO_4$ and K_2HPO_4 to the substrate increased the mushroom yield of *P. geesteranus* significantly. Addition of saccharides and inorganic salts to the substrate had no remarkable effect on the polysaccharides content of *P. geesteranus* fruit bodies, whereas caused the significant decrease of the polysaccharides content of *P. abalones* fruit bodies compared with the control. According to the scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radicals and reducing power of ethanolic extracts from two mushrooms, it was shown that the antioxidant properties of *P. abalones* was superior to that of *P. geesteranus* on non-supplemented asparagus straw substrate, and the supplementation of sucrose + $MgSO_4$ to the substrate was favorable to the enhancement of antioxidant activity of two mushrooms.

1. Introduction

In some parts of the world, mushrooms constitute a very important and highly appreciated source of food, and are also widely consumed for their medicinal properties. In this regard, *Pleurotus* spp. (oyster mushrooms) comprises the groups of edible fungi with important medicinal properties and biotechnological and environmental applications, being the second most important in terms of mushroom production worldwide after *Agaricus bisporus* (Kües and Liu, 2000). The genus *Pleurotus* comprises about 40 species (Jose and Janardhanan, 2000). Among *Pleurotus* species, *P. abalones and P. geesteranus* are getting popularity because of their very pleasant flavor, richness of dietary fiber and high quality of proteins (Yang et al., 2009; Zhang et al., 2011). A number of reports on the polysaccharides and antioxidant activity of *P. abalones and P. geesteranus* are available (Li et al., 2007; Wang et al., 2011; Zhang et al., 2011). However, the effect of substrates for cultivation of these mushrooms, on their polysaccharides content and antioxidant activity still remains largely unknown.

P. abalones and P. geesteranus have been produced at large scale in China since 2000s, using a procedure based on the method of *P. ostreatus* (Yang, 1986). The main substrates for cultivation of *P. abalones and P. geesteranus* are paddy straw, wheat straw, corn cobs and hardwood sawdust. However, due to their limited availability and seasonality, in some parts of China, these raw materials may not be available or are available at relatively high prices. To reduce the cost and improve the productivity, there is a need to select local raw material as substrate for the production of *P. abalones and P. geesteranus* for axenic cultivation.

Asparagus officinalis L. is a well known healthy vegetable, native to most of Europe, northern Africa and western Asia, and now widely cultivated as an important economic crop all over the temperate world (ZHBCEB, 1999). In Shanxi Province of China, approximately 100,000 tons per year of asparagus is produced. Large amounts of asparagus straw (i.e. dried old stalks) are produced every year, which are generally considered as useless residue and discarded, causing not only environmental pollution but also a wastage of this resource. The asparagus straw could be a basic component of the substratum formulation used to grow various species of mushrooms in this region. Previously we showed the potential of asparagus straw in the cultivation of *Agaricus blazei* Murrill (Wang et al., 2010). In the present study, the potential of asparagus straw was assessed for *P. abalones and P. geesteranus* production. Further, the effects of asparagus straw substrate on yield, polysaccharides content and antioxidant properties of these two mushrooms were studied.

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2. Materials and methods

2.1. Microorganisms

P. abalonus and *P. geesteranus* were obtained from the Institute of Edible Fungi, Shanghai Academy of Agricultural Science, China, and routinely maintained on potato dextrose agar slants.

2.2. Substrates

Asparagus straw (*A. officinalis* L.) was collected in Yongji City, Shanxi Province, China. Wheat bran, corn meal and gypsum were obtained from a grower in the suburbs of Taiyuan.

2.3. Cultivation of P. abalonus and P. geesteranus

The cultivation of *P. abalonus* and *P. geesteranus* was processed using the method of Yang (1986). Asparagus straw was cut into small pieces (*ca.* 1 cm length). The asparagus straw substrate consisted of asparagus straw, wheat bran, corn meal, gypsum supplemented with saccharides and inorganic salts (Table 1). Dry ingredients were mixed and then warm tap water was added to reach 65% moisture content. Moistened substrate (650 g) was packed into polypropylene bags (16 cm × 33 cm), sealed with foam plugs held in place with plastic collars, and then autoclaved at 121 °C for 90 min. Cooled substrate was inoculated with 10 g (±0.1 g) of grain spawn. Bags were incubated for 25 days at 25 °C. After the complete colonization of substrate with mycelia, the bags were transferred to a cropping room set at 90% relative humidity, 25 °C and 8 h light/16 h dark cycle (using cool-white fluorescent bulbs). Foam plugs were removed once primordia emerged. Mushrooms were manually harvested (first flush only) when matured (pileus and margins flat) and lasted 30 days. At the end of the 30-day harvest period, yield (fresh mushrooms) and biological efficiency (BE) were calculated. Yield was expressed as fresh mushroom weight (g) per bag. BE was determined as the ratio of fresh mushroom weight/dry weight of the substrate, and expressed as a percentage.

2.4. Experimental design

The experiment included eight treatments. The eight treatments corresponded to eight different formulations for the preparation of substrate had been shown in Table 1. Ten bags each weighing 650 g of wet substrate (equal to 350 g of dry substrate) were used for each treatment. Each treatment was performed in triplicates. Eight different treatments were assigned at random in the cropping room. Data are presented as the mean values ± SE. Duncan's multiple range test (Du, 1985) was used to determine the significant differences among mean values at 5% level of confidence.

2.5. Extraction and determination of polysaccharides from fruit bodies

Dried fruit bodies of *P. abalonus* and *P. geesteranus* were mixed with distilled water at a ratio of 1:10 (w/v) and extracted three times at 100 °C for 3 h. The suspension was filtered and the filtrate was treated with cooled ethanol (8 °C) at a volume ratio of 4:1 (ethanol:sample). After 48 h under refrigeration (4 °C) the sample was centrifuged at 5000 × *g* for 10 min at 4 °C (Lee et al., 2003). Finally, the polysaccharides content was determined by the phenol-sulfuric acid method (Hsieh et al., 2005).

2.6. Ethanolic extracts from two mushrooms

The dried fruit bodies were milled until obtaining a fine powder. For ethanolic extraction, a sample (2 g) were extracted by stirring with 100 ml of ethanol, at room temperature at 20 × *g* for 24 h, and filtered through Whatman no. 1 paper. The residue was then extracted with two additional 100 ml portions of ethanol as described above. The combined ethanolic extracts were then rotary evaporated at 40 °C to dryness. The resultant extracts were stored in freezer until use.

2.7. Antioxidant properties

Two complementary assays, radical scavenging capacity and reducing power were used to screen the antioxidant properties of ethanolic extracts of two mushrooms.

2.7.1. Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

Four milliliter of each mushroom extract (0.5–20 mg/ml) was mixed with 1 ml of ethanolic solution containing DPPH radical, resulting in a final concentration of 0.2 mM DPPH. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm (Shimada et al., 1992). EC_{50} value (mg/ml) is the effective concentration at which DPPH radicals were scavenged by 50% and was obtained by interpolation from linear regression analysis. BHT (2,6-ditert-butyl 4-methyl phenol), a synthetic antioxidant used generally for food, cosmetics and pharmaceuticals was used as standard.

2.7.2. Reducing power

The reducing power was assayed as described in Kuda et al. (2005) with some modifications. Different concentrations of ethanolic extracts of mushrooms (1.0 ml) were mixed with 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 °C for 20 min. After the addition of 2.5 ml of trichloroacetic acid (10%), the mixture was centrifuged at 200 × *g* for 10 min. Finally, 1.25 ml supernatant was mixed with 1.25 ml of distilled water and 0.25 ml $FeCl_3$ solution (0.1%, w/v). The absorbance was measured at 700 nm. Increased absorbance values indicate a higher reducing power. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against the extract concentration. BHT was used as standard.

2.8. Chemicals

DPPH and BHT were obtained from Sigma Chemical Co. Other chemicals were of analytical grade.

3. Results

3.1. Effect of asparagus straw substrate on yield and BE

The mushroom yield and BE of the crop cycle of 30 days were shown in Table 2. There were apparent differences in the yield of harvest mushrooms among treatments. On non-supplemented asparagus straw substrate (Treatment 8), the yield of *P. geesteranus* (199 g/bag) was significantly higher than that of *P. abalonus* (140 g/bag). We examined the effect of selected saccharides (i.e. glucose, maltose, lactose and sucrose) on mushroom yield (Table 2). The result showed that supplementation of 4 types of saccharides to the substrate could not result in the significant increase of mushroom yield of *P. abalonus*. However, the mushroom yield of *P. geesteranus* increased compared with control (Treatment 8).