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Short communication

Decomposition of asparagus old stalks by *Pleurotus* spp. under mushroom-growing conditions

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ABSTRACT

The aim of this work is to evaluate the decomposition of asparagus old stalks by five species of *Pleurotus* under mushroom-growing conditions. *P. ostreatus* gave the highest yield (270 g/bag) of fruit bodies at the first flush and the highest lignin degradation rate (68.4%) and cellulose degradation rate (56.2%) of asparagus old stalks substrate, followed by *P. geesteranus*, *P. abalonus*, *P. eryngii* and *P. nebrodensi*; *P. geesteranus* gave the highest hemicellulose degradation rate (35.8%). By contrast, the five species of *Pleurotus* had stronger decomposition ability to lignin and cellulose and weaker decomposition ability to hemicellulose in asparagus old stalks substrate. All the species reached their maximal carboxymethyl cellulose, xylanase, laccase, manganese dependent peroxidase and filter paper activities at the primordial stage. Moreover, the activity peaks of these lignocellulolytic enzymes of *P. ostreatus* were higher than that of other four species.

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) comprise the groups of edible fungi with important medicinal properties and biotechnological and environmental applications, being the second most important in terms of worldwide mushroom production after *Agaricus bisporus* (Kües and Liu, 2000). The genus *Pleurotus* comprises about 40 species (Jose and Janardhanan, 2000). *P. ostreatus*, *P. eryngii*, *P. nebrodensi*, *P. geesteranus* and *P. abalonus* are the mushrooms those are getting the popularity lately because of their very pleasant flavor, richness of dietary fiber and high quality of protein.

Pleurotus spp. have been produced at large scale in China, using a procedure based on the method of *P. ostreatus* (Yang, 1986). The main substrates for cultivation of *Pleurotus* spp. are cottonseed hull, 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 *Pleurotus* spp. 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 of asparagus is produced per year. Large amounts of asparagus old stalks (AOS) are produced every year, and generally, these AOS are considered a useless residue and discarded, which not only causes environmental pollution but is also a waste of this resource. The AOS could be a basic component of the substratum formulation used to grow various species of mushrooms in this region. Our previous experiments had showed that AOS had the potential for the cultivation of *Agaricus blazei* Murrill (Wang et al., 2010). However, decomposition of AOS by *Pleurotus* spp. under mushroom-growing condition still remains largely unknown. This report is about how to assess the potential of AOS as a major material for *P. ostreatus*, *P. eryngii and P. nebrodensi*, *P. geesteranus* and *P. abalonus* production and to evaluate the decomposition of AOS by the selected five species of *Pleurotus* under mushroom-growing condition.

2. Materials and methods

2.1. Microorganisms

P. ostreatus 33, *P. eryngii* 02, *P. nebrodensi* HB, *P. geesteranus* C1 and *P. abalonus* H01 were obtained from the Edible Fungi Research Institute, Shanghai Academy of Agricultural Science, China, and routinely maintained on potato dextrose agar slants.

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2.2. Substrates

Asparagus old stalks (AOS) (*A. officinalis* L.) were 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 pleurotus spp.

The cultivation of *Pleurotus* spp. was processed using the method of Yang (1986). AOS was cut into small pieces (*ca.*1 cm length). The AOS substrate was consisted of AOS (78%), wheat bran (10%), corn meal (10%), gypsum (1%) and sucrose (1%). Dry ingredients were mixed and then warm tap water was added to reach 65% moisture content. Moistened substrate (1000 g) was packed into polypropylene bags (16 × 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 at 25 °C. After the complete colonization of substrate with mycelia (including the fostering mycelia of *P. nebrodensi*), the bags were transferred to different cropping rooms set at 90% relative humidity, 15–25 °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 mature (pileus and margins flat). 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. Statistical analysis

A total of 250 bags of the substrate were prepared. Fifty bags each weighing 1000 g of wet substrate (was equal to about 350 g of dry substrate) were used for each fungus. Three bags were selected randomly and removed at different growing stages. The content of each bag was mixed and used for the chemical and enzymes analyses. Data are presented as the mean value ± 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. Determination of cellulose, hemicellulose and lignin contents

At the end of first flush mushrooms, the spent mushroom substrate (SMS) was removed from the bags and dried to constant weight at 60 °C. The cellulose, hemicellulose and lignin contents in SMS were determined by the method of Baker et al. (2015).

2.6. Enzymes extraction and assays

At different growing stages of five mushrooms (i.e. mycelia-growing stage, primordia-forming stage and fruit bodies-harvesting stage), 10 g content of each bag was suspended in 50 ml of sodium citrate buffer (50 mM, pH 5.0) for 30 min within an ice bath. Solids were separated by filtering through a gauze cloth, and filtrate was then centrifuged (4 °C, 10 000 rpm, 30 min). Supernatant was used for measurements of enzyme activity.

Manganese dependent peroxidase (MnP) activity was measured by oxidation of DMP (2, 6-dimethoxy phenol) in the presence of H_2O_2 and $MnSO_4$. Increase in absorbance was measured with spectrophotometer (λ = 469 nm) and one unit of enzyme activity was defined as the amount of enzyme catalyzing the oxidation of 1 μM of DMP/min (Martinez et al., 1996). Xylanase activity was estimated by DNS method (Miller et al., 1960) using a 0.5% solution of Birchwood xylan as substrate, previously dissolved in a sodium citrate buffer (50 mM, pH 5.3) according to Loera and Córdova (2003); laccase activity was determined by registering the oxidation of 2,2-azo-bis-(ethylbenzothiazoline-6-sulfonic acid) (ABTS) in an acetate buffer (0.5 mM, pH 5) every 20 s during 2 min (λ = 420 nm) (Membrillo et al., 2008);

carboxymethyl cellulase (CMCase) activity was estimated by DNS method (λ = 575 nm) using a solution of 1% carboxymethyl cellulose as a substrate, dissolved in a sodium citrate buffer (50 mM, pH4.8) according to Miller et al. (1960); filter paper activity (FPA) in the extracted filtrates was measured also at pH 4.8 employing a piece of filter paper (1 cm × 6 cm, No. 41) according to the procedures of Decker et al. (2003). Values were expressed in international units (IU), where one unit (IU) of enzyme activity is defined as the amount of enzyme required to release 1 μmol of product per minute under the given assay conditions. Activities were referred to initial substrate dry weight (IU/g dry weight).

Table 1
The spawn running time, yield and biological efficiency (BE) of five species of *Pleurotus*.

Species	Spawn running time (day)	Yield (g/bag)	BE (%)
P. eryngii	41 ± 1.5c	108 ± 9b	30.9 ± 2.0b
P. nebrodensi	57 ± 1.5d	89 ± 7a	25.4 ± 1.5a
P. abalonus	30 ± 1.2b	150 ± 11c	42.8 ± 3.1c
P. geesteranus	25 ± 1.2a	262 ± 14d	74.8 ± 5.2d
P. ostreatus	25 ± 1.2a	270 ± 14d	77.1 ± 5.1d

(1) Mean values in the same column followed by different letters are significantly different at the $P < 0.05$ level according to Duncan's multiple-range test.

3. Results and discussion

3.1. Yield and BE of five species of pleurotus on AOS substrate

The mushroom yield and BE at the first flush were shown in Table 1. The results showed that *P. ostreatus* gave the highest yield (270 g/bag) of fruit bodies at the first flush, which was significantly higher than that of *P. abalones* (150 g/bag), *P. eryngii* (108 g/bag) and *P. nebrodensi* (89 g/bag) ($P < 0.05$). There was no significant difference between the yields of *P. ostreatus* and *P. geesteranus* ($P < 0.05$). The BE of *P. ostreatus*, *P. geesteranus*, *P. abalones*, *P. eryngii* and *P. nebrodensi* on AOS substrate were respectively 77.1%, 74.8%, 42.8%, 30.9% and 25.4%.

Growers that produce *Pleurotus* spp. in bottles or bags typically harvest only one break before cleanout. BE, obtained from a single break of *P. eryngii* grown on substrate in bottles or bags, was average 50–80% (Rodriguez Estrada and Royse, 2008). It had been reported that the BE of *P. eryngii* in substrate consisted of broadleaf tree sawdust, sugarcane bagasse ground corncobs and wheat bran was about 76% (Zhang et al., 2014). In our experiment, the highest BE was 77.1% of *P. ostreatus*, and the lowest BE was 25.4% of *P. nebrodensi*. The results suggest that AOS has the potential for the cultivation of the five species of *Pleurotus*.

3.2. Decomposition of AOS substrate by five species of pleurotus

The cellulose, hemicellulose and lignin contents in SMS inoculated with five species of *Pleurotus* were shown in Table 2. *P. ostreatus* gave the highest lignin degradation rate (68.4%) and cellulose degradation rate (56.2%), followed by *P. geesteranus*, *P. abalonus*, *P. eryngii* and *P. nebrodensi*; *P. geesteranu* gave the highest hemicellulose degradation rate (35.8%). By contrast, the five species of *Pleurotus* had stronger decomposition ability to lignin and cellulose and weaker decomposition ability to hemicellulose in AOS substrate.

Plotting the cellulose degradation rates of these mushrooms versus their mushroom yields revealed a positive linear relationship ($R = 0.98$, $P < 0.01$): the higher the cellulose degradation rate, the higher the mushroom yield. Similarly, there existed positive linear relationship between the lignin degradation rates and the corresponding mushroom yields ($R = 0.94$, $P < 0.01$). This result indicated that stronger decomposition ability to lignin and cellulose in AOS substrate was favorable to the enhancement of mushroom yields of *Pleurotus* spp.