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Biodegradation potential and ligninolytic enzyme activity of two locally isolated *Panus tigrinus* strains on selected agro-industrial wastes

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ABSTRACT

The degradation potential and ligninolytic enzyme production of two isolated *Panus tigrinus* strains (M609RQY and M109RQY) were evaluated in this study. These strains were grown on three selected abundant agro-industrial wastes (rice straw; rice husk and cassava peel) under solid-state fermentation conditions. Degradation potential was determined by analyzing the chemical composition of the selected substrates before and after fermentation along with ligninolytic enzyme production. The strain M609RQY led to the highest lignin degradation of 40.81% on cassava peel, 11.25% on rice husk and 67.96% on rice straw. Both strains significantly increased the protein content of cassava peel. Rice husk stimulated maximum laccase (2556 U/L) and lignin peroxidase (24 U/L) production by the strains M109RQY and M609RQY, respectively. Furthermore, cassava peel stimulated maximum manganese-dependent peroxidase (141 U/L) production by the strain M109RQY. The de-lignified rice straw and the nutritionally-improved cassava peel could serve as potential animal feed supplements.

1. Introduction

Large amounts of lignocellulosic waste produced through forestry and agricultural practices, paper-pulp industries, timber industries and many agro-industries are posing serious environmental pollution problems (Howard et al., 2003). Unfortunately, most of these lignocellulosic wastes are disposed off by burning (Levine, 1996) and thereby causing a serious environmental threat. This has attracted the interest of many researchers in the valorization of lignocellulosic wastes.

Rice straw and rice husk are some of the wastes obtained during harvesting and processing of rice and they remained underutilized in all rice-producing countries. In Malaysia, the annual grain production is about 2.2 million tons, of which 0.44 million tons is rice husk (Vadiveloo et al., 2009). Similarly, cassava peel, a common waste in most cassava-producing countries, could make up to 10% of the wet weight of the root (Pandey et al., 2000). These wastes are considered as low-grade feed for livestock because of their low digestibility, high silica content (Van Soest, 2006) and poor nutritive value.

Agro-industrial wastes are lignocellulosic in nature consisting mainly of cellulose, hemicellulose and lignin. However, the highly-bounded nature of cellulose and hemicellulose by lignin in most lignocellulosic materials make these components difficult to digest by the animal enzymatic system (Arora and Sharma, 2009). Thus, the presence of high fiber in the diet of both ruminant and non-ruminant animals tends to decrease digestibility, thereby hindering its use as an ideal animal feed. Furthermore, the availability of lignocellulose material is higher than required by animals, as such any attempt to improve its utilization and at the same time exploiting its abundance is required.

Various methods have been used in the upgrading of agro-industrial wastes into animal feed, viz: physical, chemical and biological methods. However, the biological method has proven to be economical and environmentally friendly (Okano et al., 2005). This method is potentially useful in addressing the environmental problem caused by these wastes and in enhancing animal feed and human food supplies (Shojaosadati et al., 1999).

Several microorganisms have been used in the bioconversion of lignocellulosic materials into value-added products. However, white-rot fungi have shown to be efficient degraders of the cell wall components especially lignin (Shi et al., 2008). This is due to their capacity of secreting the three major extracellular lignin-modifying enzymes: lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and laccase (Lac) (Sarnthima et al., 2009; Mtui and Nakamura, 2007).

In the bioconversion of agro-industrial wastes intended for ruminant feeding, selective lignin degraders are better choice of

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fungi as such cellulose is left practically intact, and this aids in retaining the energy value of the wastes as far as possible. Whereas, a major priority for non-ruminant feed is the selection of efficient cellulose degrader as non-ruminant animals do not possess the enzyme to breakdown cellulose.

Solid-state fermentation (SSF) is a method of cultivating microorganisms on solid materials to serve as a source of nutrient or as physical support or both in the absence or near absence of free flowing water (Pandey, 1992). In view of this, solid-state cultivation provides similar conditions under which fungi grow in nature; thus, making it a better choice for the bioconversion of agro-industrial wastes into animal feed.

There are few studies on degradation of these selected agro-industrial wastes by white-rot fungi whereby their ligninolytic enzymes activities were studied simultaneously (Arora et al., 2002; Bisaria et al., 1997). Based on these, the present study aims at evaluating the biodegradation potential and the ligninolytic enzyme production of two locally isolated *Panus tigrinus* strains on rice straw, rice husk and cassava peel during solid-state fermentation of these wastes into animal feed.

2. Materials and methods

2.1. Fungi

Two locally isolated *Panus/Lentinus tigrinus* strains (M109RQY) and M609RQY (IMI 398363, CABI Europe -UK) of the class: Basidiomycetes, order: Polyporales and family: Polyporaceae (Tijani et al., 2011) were used in this study. The strains were cultured on Petri plates containing malt extract agar (MEA, Merck, Germany) at 30 °C for seven days, maintained at 4 °C and sub-cultured fortnightly.

2.2. Substrates

Rice straw and rice husk (*Oryza sativa*) were collected from MARDI (Malaysian Agricultural Research Development Institute). Rice straws were cut into small size (approx. 2 cm), washed with water to remove dust and dried in an air forced oven at 60 °C for 48 h. The chemical composition of rice straw as analyzed was 38% cellulose, 5% lignin and 158.9 mg/g protein using the method described by Goering and Van Soest (1970) and Lowry et al. (1951). Rice husk was also washed to remove dust and stones and dried at 60 °C in an air forced oven for 48 h. The chemical composition of rice husk as analyzed was 39% cellulose, 17% lignin and 84.73 mg/g protein. Cassava (*Manihot esculenta*) peels were collected from a small scale Kerepek (local snack) industry in Kuala Langat (Selangor, Malaysia). The peels were washed properly to remove sand and dried immediately at 60 °C in an air forced oven for 48 h to avoid deterioration and growth of unwanted microbes. The chemical composition of cassava peel as analyzed was 18% cellulose, 15% lignin and 26.27 mg/g protein. The substrates were milled to pass through 2 mm sieve.

2.3. Inoculum preparation

For inoculum preparation, each *P. tigrinus* strain was grown on four malt extract agar (Merck, Germany) plates for 7 days at 30 °C. On the 7th day, each plate was washed with 15 ml of sterilized distilled water into a sterilized Erlenmeyer flask (250 ml). To determine the concentration of the inoculum, the procedure for inoculum preparation described by Tijani et al. (2011) was used, except that the mycelia in the inoculum suspension was collected on a pre-weighed filter paper and dried for 24 h. The concentration of the mycelia suspension was calculated in gram per liter. Thus, the concentration of the mycelia suspension used in this study was 0.865 g/l.

2.4. Solid-state fermentation

The total fermentation media was 20 g, comprising of 5.5 g of substrate (rice straw, rice husk or cassava peel, according to the experiment), 0.5 g of co-substrate (wheat flour) and 14 ml of moisture content. The moisture content consisted of 11.8 ml of distilled water, 1.2 ml of inoculum and 1 ml of mineral solution. The composition of the mineral solution was as follows (g/l): $(NH_4)_2SO_4$, 2; malt extract, 5; $MgSO_4 \cdot H_2O$, 0.5; KH_2PO_4, 0.6; K_2HPO_4, 0.4 and $CaCl_2 \cdot H_2O$, 0.2. Initial pH of the media was adjusted to 5.5 using 1 M HCl or 1 M NaOH before autoclaving at 121 °C for 20 min. The flasks were allowed to cool down to room temperature before aseptically inoculating them. Uninoculated flasks were used as controls.

The bioconversion was carried out in cotton-plugged Erlenmeyer flasks (250 ml) incubated (Memmert) at 30 °C for 15 days in static position. At the end of each harvesting period (5, 10 and 15 days), three flasks for each substrate were withdrawn, making a total of nine flasks for each substrate and for each strain. The experiments were carried out in triplicate. Before enzyme extraction, the pH of the control and the fermented substrate was determined by dissolving 1 g of substrate in 10 ml of distilled water under orbital shaking at 150 rpm for 30 min.

2.5. Enzyme extraction

Enzyme extraction was carried out according to the method suggested by Arora et al. (2002) with some modifications. Sixty ml of sodium acetate buffer (10 mM, pH 5) was added into each flask and agitated on a rotary shaker at 150 rpm for 30 min. The content of each flask were filtered using Whatman No.1filter paper weighted beforehand, and dried at 60 °C for two days. The filtrate obtained was centrifuged at 10,000 rpm at 4 °C for 15 min and the supernatant was used for enzyme assay. The residue obtained was dried in an air forced oven at 60 °C for two days. The loss in weight on a dry matter basis was calculated and the residual ash was estimated by taking 1 g of sample in a pre-weighted crucible and kept at 550 °C in a muffle furnace for 24 h and then weighted to calculate the residual ash content.

2.6. Enzyme assays

2.6.1. Lignin peroxidase activity (LiP)

Lignin peroxidase assay method was based on the oxidation of the dye Azure B (Arora and Gill, 2000). One unit of enzyme activity was defined as the amount of the enzyme that oxidized 1 μmol of Azure B per min. The activities were reported in U/L.

2.6.2. Laccase activity

Laccase activity was determined by the method described by Bourbonnais et al. (1995) and modified by Mtui and Nakamura (2007), which is based on the oxidation of 2, 2′-azino-bis (3-ethylbenzothiazoline)-6-sulphonic acid (ABTS). The increase in absorbance was measured at 420 nm for 3 min. One unit of laccase activity was defined as the amount of enzyme that oxidized 1 μmol of ABTS per min. The activities were reported as U/L.

2.6.3. Manganese-dependent peroxidase activity (MnP)

Manganese-dependent peroxidase activity was determined using Phenol Red as the substrate according to Kuwahara et al. (1984). One unit of enzyme activity was defined as the amount that oxidized 1 μmol of Phenol Red per min and the activities were expressed in U/L.