Evaluation of Burma Reed as Substrate for Production of *Pleurotus eryngii*

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Abstract Burma reed (*Neyraudia reynaudiana*), a giant C₄ grass, was included in substrate at the rates of 0, 20, 40 and 66 % to partially or wholly substitute sawdust and cottonseed hulls to evaluate its suitability for *Pleurotus eryngii* cultivation. Inclusion of 20 and 40 % Burma reed did not significantly affect linear mycelial growth, dry matter loss, spawn run period and fructification, and achieved high fruiting body yields and biological efficiency of 336.67 g/bag, 67.33 % and 342.15 g/bag, 68.43 %, respectively, which were not significantly different from 350.08 g/bag to 70.02 % obtained from the control substrate. Enzyme assay revealed that on the mixed substrates laccase and manganese peroxidase activity were significantly enhanced, but cellulase was significantly reduced in the middle stage of incubation as compared with the control substrate. Even on Burma reed substrate without sawdust and cottonseed hulls, fruiting body yield (313.56 g/bag) and biological efficiency (62.71 %) were satisfactory, although significantly lower than that on the control substrate. Therefore, Burma reed was a promising potential substrate for *P. eryngii* production to largely substitute sawdust and cottonseed hulls.

Keywords *Pleurotus eryngii* · Burma reed · Cellulase · Laccase · Manganese peroxide · Biological efficiency

Introduction

Pleurotus eryngii, also known as the king oyster mushroom for its special flavor and texture, is one of the most valued and delicious mushrooms cultivated and consumed worldwide. Sawdust, cottonseed hulls, corn stover, wheat straw, sugarcane bagasse, combined with various amounts of soybean meal, peanut meal, wheat bran and wheat bran etc., have been the proven excellent substrates for commercial production of *P. eryngii* and other mushrooms [1, 2]. However, as the prices of sawdust and cottonseed hulls increased or fluctuated substantially with the rapid expansion of commercial production of mushrooms, substrate cost rise has become a major concern for growers. Therefore, cheaper and locally available alternative substrates including various grasses have been trialed for production of *P. eryngii* or other mushrooms [3–5].

In China, many studies have been made and commonly concluded that grasses were superior to crop straws for cultivation of mushrooms, achieving higher fruiting body yields and similar or better quality [6], but most of them were reported in Chinese and often without experimental details, and only a few mentioned using Burma reed or *Miscanthus floridulus* for *P. eryngii* cultivation [7].

Burma reed (*Neyraudia reynaudiana*), also called false reed in Chinese, is a reed-like perennial grass to 3 m tall with a hard stalk that resembles a bamboo, it is widely distributed as a dominant grass in woodless hills, roadsides in South Asian countries like China, India, Burma, Japan, Thailand etc. [8]. This paper was intended to evaluate the suitability of Burma reed for *Pleurotus eryngii* cultivation by partially or wholly substituting sawdust and cottonseed hulls with Burma reed as the main carbon source.

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Materials and Methods

Pleurotus eryngii Strain

Experiments of this study consist of two parts: mycelial culture of *P. eryngii* in petri plates were conducted in laboratory of School of Life Sciences, Jiaying University, and fruiting body cultivation was completed in Lantian Farming Co., Ltd, Fengshun County, Guangdong province, China. The strain AX-12 of *P. eryngii* var. *eryngii* was isolated by Lantian Farming Co., Ltd and maintained on glucose-peptone agar medium.

Substrate Preparation and Experimental Design

Burma reed (*N. reynaudiana*) used in this study was cut from an open land on the campus of Jiaying University, Meizhou city, Guangdong province, China. The stalks and leaves were cut into ca. 5 cm long pieces with pruning shears and ground with a grinding machine to pass an inner 1 mm-hole screen. The ground Burma reed had a fluffy physical property resembling sawdust.

Proximate Carbon Analysis of Burma Reed

Total carbon content of Burma reed was determined by potassium bichromate dilution-heat method (carried out in boiling water bath to increase oxidation rate of organic carbon); total nitrogen content was determined by the standard Kjeldahl procedure. Total soluble sugar of Burma reed was determined by sulfuric acid-anthrone colorimetric method. Determination of cellulose, hemicellulose and lignin content of Burma reed followed the procedures of Goering and Van Soest [9].

The ingredient composition of the substrates used in this study were provided in Table 1, in which SC stands for sawdust and cottonseed hulls as main carbon source, supplemented with other ingredients, representing the existing farm-used substrate; similarly, SCB₂₀ and SCB₄₀ stand for incorporating 20 or 40 % of Burma reed to correspondingly replace sawdust and cottonseed hulls; B₆₆ stands for replacing sawdust and cottonseed hulls wholly with Burma

reed (hereinafter referred to as whole-Burma reed substrate); the C/N ratio was set at about 40:1 for all substrates by referring to previous research conclusions [10] and based on our cultivation experience.

Sawdust was subject to weathering and composting in open air for half a year before being incorporated into substrates. All other ingredients including Burma reed were mixed into substrate fresh without any pretreatment.

All prepared substrates were moistened to contain about 65 % water for overnight before being filled in petri plates or polypropylene bags. In the mycelial culture test, five replicates (petri plates) were made for each formula. In each plate 25 g of wet substrate (10 g dry w.t.) was placed and autoclaved at 121 °C for 2 h. When cooled the plates were inoculated with a plug of 1 cm² agar culture for each plate buried in the center and incubated at 25 °C in dark with 60 % of air relative humidity. In the farm cultivation experiment, thirty 17 × 30 cm polypropylene bags for each formula were filled manually with 1.25 kg wet substrate. Sterilized substrate (121 °C for 2 h) was inoculated with about 40 ml liquid spawn for each bag.

Determination of Mycelial Growth Rate, Mycelial Density and Dry Matter Loss

Mycelial radical growth rate in petri plate experiment was calculated by recording colony diameter on day 5, 10 after inoculation. Relative mycelial biomass on day 30 was estimated by two means: mycelial density (whiteness) which was scored for each plate based on observation and the relative averaged values for different substrates were displayed by a number of “+’s”; the other parameter was substrate dry matter loss; the mycelial substrate in each plate was dried at 60 °C for 48 h and dry matter loss was calculated by deducting the dry substrate from the initial average dry weight of substrate (10 g).

Cellulase, Laccase and MnP Activity Assay

Crude enzyme was extracted by placing 2.0 g fresh mycelial culture into a 250 ml flask added with 20 ml 0.1 M citrate buffer (pH 5.0) which was shaken in a rotary shaker at 28 °C, 200 rpm for 3 h. The extracted solution together with

Table 1 Ingredient composition of substrates with different inclusion rates of Burma reed for *P. eryngii* cultivation (% dry w.t.)

Substrate	Ingredient composition (%)							
	Sawdust	Cottonseed hulls	Burma reed	Corn flour	Soy meal	Wheat bran	CaCO ₃	CaSO ₄
SC (CK)	36	30		5	3	23	2	1
SCB ₂₀	25	21	20	5	3	23	2	1
SCB ₄₀	14	12	40	5	3	23	2	1
B ₆₆			66	5	3	23	2	1

Fig. 1 Mycelial colonies of *Pleurotus eryngii* on 5 days (left) and 10 days (right) of incubation on substrates with different inclusion rates of Burma reed. Rows from top to bottom: SC, SCB₂₀, SCB₄₀ and B₆₆

substrate was centrifuged at 4,500 rpm for 10 min and the supernatant was used as crude enzyme for activity assay.

Carboxymethyl cellulase (CMCase) activity assay followed Ghose [11]. 1 unit CMCase activity was defined as the enzyme amount required to transfer substrate into 1 μ mol glucose and expressed in U/g fresh mycelial culture. Laccase activity and manganese peroxidase activity were measured by following the method described by Heinzkill et al. [12], with modification of doubling both the sample volume and reagent volume to suit a 1-cm cuvette. The enzyme activities were expressed as U/g substrate (fresh weight), where 1 U was defined as 1 μ mol of substrate oxidized per min.

Farm Spawn Run and Cropping

Spawn running was carried out at 22–24 °C and 65–70 % relative humidity under dark condition, air CO₂ concentration was controlled at less than 0.1 %. After full colonization, 10–12 days of incubation was extended before applying cold shock and light cycles for fruit body induction and development, in which the temperature was kept at 13–15 °C, relative humidity 80–85 %, light intensity at about 200–500 lux for 10 h/day, CO₂ concentration was controlled at 0.1–0.3 % during fruiting body development.

Spawn Run Period, Fruiting Body Yield and Biological Efficiency

The spawn run period (the number of days from inoculation to complete colonization of the substrate by the mycelium) was recorded. Only one flush of fruiting bodies was harvested on day 17 and 18 after cold shock and illumination was applied when the length of stipe of fruiting bodies reached over 10 cm and the edge of cap turned slightly flat from round shape. The fresh weights of fruiting body were recorded and biological efficiency (BE, %) was calculated by dividing fresh weight of fruiting body with dry substrate per bag.

Table 2 Colony diameter, mycelial density of *P. eryngii* and substrate dry matter loss

Substrate	Colony diameter 5 days (cm)	Colony diameter 10 days (cm)	Mycelium density 30 days	Dry matter loss (%) 30 days
SC (CK)	4.12 $\pm$ 0.08 ^a	7.06 $\pm$ 1.27 ^a	++++	11.44 $\pm$ 1.40 ^a
SCB ₂₀	3.98 $\pm$ 0.16 ^a	6.68 $\pm$ 0.41 ^a	+++	12.60 $\pm$ 1.05 ^a
SCB ₄₀	3.90 $\pm$ 0.20 ^a	7.60 $\pm$ 1.18 ^a	++++	11.74 $\pm$ 1.52 ^a
B	3.56 $\pm$ 0.76 ^b	6.52 $\pm$ 0.84 ^a	++	8.83 $\pm$ 0.84 ^b

Data are presented in means $\pm$ S.D. ($n = 5$). Means within each column bearing same superscripts are not significantly different ($P < 0.05$). Mycelial density are expressed semi-quantitatively with “+” signs

Data Statistical Analysis

Original data were processed using EXCEL (Microsoft, WA, USA) and Duncan’s multiple range tests were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Proximate Carbon and Nitrogen Composition of Burma Reed (% Dry w.t)

Results indicated that Burma reed had a total carbon content of 44.73 ± 2.68 % and total nitrogen content of 0.50 ± 0.02 %, therefore with a high C/N ratio of 89. It had high lignocellulose content, with 40.22 ± 1.08 % cellulose, 16.63 ± 0.30 % hemicellulose and 10.86 ± 1.73 % lignin, which renders it an ideal carbon source for mushroom production in terms of chemical composition. Its cellulose content was higher than sawdust and cottonseed hulls, whereas its lignin content was lower than the latter two, but higher than common softer grasses. Total soluble sugar content in Burma reed was low (1.03 ± 0.04 %), and ash content was 4.13 ± 2.31 %.

Fig. 2 CMCase activity of *P. eryngii* mycelia on different substrates in different stages of incubation (left to right for each period: SC, SCB₂₀, SCB₄₀, B₆₆). Multiple mean comparisons were carried out independently for each period and bars marked with different lower case letters were significantly different ($P < 0.05$)

Mycelial Growth Rate of *P. eryngii*

Mycelial colony diameter of *P. eryngii* on petri plates was measured on days 5, 10 of inoculation. As shown in Fig. 1 and Table 2, mycelial growth rate of *P. eryngii* on substrates SCB₂₀ and SCB₄₀ was not significantly different from that of on the control substrate (SC) through out the culture period. On substrate B₆₆ the growth rate on the first 5 days was significantly slower than that on the control substrate and the other two Burma reed substrates, but caught up the other substrates on day 10 when afterward the colony diameters on all substrate existed no statistically significant difference. Judged by mycelial density on day 30, mycelial growth of *P. eryngii* on SCB₂₀ and SCB₄₀ was comparable to on SC, and was slightly inferior on B₆₆.

Dry matter loss of SC, SCB₂₀ and SCB₄₀ over 30 days of mycelial culture exceeded 11 % and with no significant differences, but that of the whole-Burma reed substrate B₆₆ was only 8.83 %, which was significantly lower than those of the other three substrates, indicating inclusion of up to 40 % Burma reed did not affect substrate degradation, but using Burma reed alone without sawdust and cottonseed hulls would result a significantly lower rate of substrate degradation.

Lignolytic Enzyme Activities of *P. eryngii* During Mycelial Culture

Pleurotus eryngii and other white-rot fungi possess strong capability to degrade various of lignocellulosic residues because their mycelium can produce significant quantities of ligninolytic enzymes, e.g. cellulase, laccases, Mn-oxidizing peroxidases, and aryl-alcohol oxidase etc. [13–16]. In this study, cellulase (CMCase), laccase (Lac) and manganese peroxidase (MnP) activities were assayed on day 10, 18 and 25 of incubation.

A common trend was observed for the three enzymes that their activity in the late stage of mycelial culture (on day 25) were the lowest as compared with those in the early and middle stages, which was contrary to previous reports

Fig. 3 Laccase activity of *P. eryngii* mycelia on different substrates in different stages of incubation (left to right for each period: SC, SCB₂₀, SCB₄₀, B₆₆). Multiple mean comparisons were carried out independently for each period and bars marked with different lower case letters were significantly different ($P < 0.05$)

Fig. 4 Manganese peroxidase activity of *P. eryngii* mycelia on different substrates in different stages of incubation (left to right for each period: SC, SCB₂₀, SCB₄₀, B₆₆). Multiple mean comparisons were carried out independently for each period and bars marked with different lower case letters were significantly different ($P < 0.05$)

that the lignocellulosic enzyme activities increased during mycelial incubation and the highest values occurred before or during primordial initiation [17, 18]. The low values in this stage in the present study may be associated with the shallow substrate in the plates that caused the nutrients limitation after 25 days of culture, which might be no longer a problem in bag or bottle cultivation.

As shown in Fig. 2, in early stage of culture (10 days) CMCase activity of *P. eryngii* on SCB₂₀ (0.76 U/g) and

Table 3 Spawn running period, fruiting body yield, biological efficiency of *P. eryngii* on different substrates

Substrate	Spawn running period (day)	Number of fruiting body	Fruiting body yield (g/bag)	Biological efficiency (%)
SC (CK)	25.0 ± 0.93 ^a	5.25 ± 1.17 ^a	350.08 ± 23.97 ^a	70.02 ± 4.79 ^a
SCB ₂₀	25.0 ± 1.07 ^a	4.25 ± 1.16 ^a	336.67 ± 37.82 ^a	67.33 ± 7.18 ^a
SCB ₄₀	25.0 ± 1.31 ^a	4.09 ± 1.28 ^a	342.15 ± 32.70 ^a	68.43 ± 5.92 ^a
B ₆₆	25.6 ± 1.51 ^a	4.80 ± 1.78 ^a	313.56 ± 37.22 ^b	62.71 ± 7.04 ^b

Date are presented in means ± S.D. ($n = 5$). Means within each column bearing same superscripts are not significantly different ($P < 0.05$). Mycelial density are expressed semi-quantitatively with “+” signs

SCB₄₀ (0.71 U/g) were not significantly different from on SC (0.72 U/g), but surprisingly the activity on B₆₆ (0.97 U/g) was significantly higher, which maybe due to lower soluble sugar content combined with higher cellulose content in Burma reed. However, in the middle stage of incubation (18 days) *P. eryngii* on substrate SC had significantly higher CMCase activity (1.11 U/g) than the Burma reed substrates, among which no significant difference existed. On day 25 *P. eryngii* on SCB₄₀ had significantly higher CMCase activity (0.55 U/g) than on SC (0.30 U/g), and no significant differences existed among the rest substrates. Apparently, on Burma reed substrates as well as on any other types of substrates, cellulase activity requires to be raised in future research by large magnitude as it was hundreds folds lower than laccase and MnP activities.

Laccase and manganese peroxidase are two major enzymes involved in lignin degradation in *P. eryngii* [19–21]. As shown in Figs. 3 and 4, laccase activity and manganese peroxidase activity displayed very similar patterns.

Compared to the control substrate, although inclusion of 20–40 % Burma reed significantly reduced activities of laccase and manganese peroxidase in the early stage of mycelial culture (on day 10), but enhanced activities of these two enzymes in the middle stage of culture (18 days), with comparable peak values. Considering the larger total volume of mycelia in the middle stage, higher laccase and MnP activities in this stage is of more significance than in early stage for substrate utilization. The effect of Burma reed on stimulation of the enzyme activities might be due to the addition of more versatile substances (e.g. products of lignin breakage) that induced the excretion of enzymes or participated in redox cycling as mediators [22–24].

Spawn Running Period and Fruiting Body Yield of *P. eryngii* on Different Substrates

It can be seen from Table 3 that spawn running period for all substrates were at around 25 days with no significant differences, only variation among individual bags increased with the increase of Burma reed amounts in the substrate, which was consistent with results obtained in

plate mycelial culture. Fructification of *P. eryngii* on all four substrates were very well with number of primordia more than 30 and artificial bud thinning was applied once to all bags to leave 3–6 fruiting bodies in each bag. The fruiting bodies were harvested in two batches on 54, 55 days of inoculation (17, 18 days after cold and light application), and yield, BE were presented in Table 3.

The fruiting body yield and biological efficiency on the Burma reed-incorporated substrates (SCB₂₀ and SCB₄₀) were 336.67 g/bag, 67.33 % and 342.15 g/bag, 68.43 %, respectively, without significant differences from that on sawdust and cottonseed hulls substrate, which were 350.08 g/bag and 70.02 %. Fruiting body yield (313.56 g/bag) and BE (62.71 %) on the whole Burma reed substrate was significantly lower than that on the control substrate, indicating that Burma reed should be mixed with sawdust and/or cottonseed hulls to achieve optimal mushroom productivity. However, such yield and BE were still satisfactory obtained on a grass substrate without inclusion of sawdust and cottonseed hulls [3, 5, 25].

The significance of testing Burma reed (*N. reynaudiana*) as raw material for cultivation of *P. eryngii* and other mushrooms lies in the fact that Burma reed is able to grow vigorously on infertile waste or hilly lands. According to investigation made by Zeng et al. [26], the above ground dry biomass of *N. reynaudiana* in the wild reached as high as 29.0 t/hm² in low mountainous lands. As a fast growing and wide distributed giant C₄ grass, Burma reed could be cultivated on waste dry lands in subtropical and tropical regions with minimum invest and management and maximum harvest of biomass, therefore it represents a promising potential substrate for mushroom production to largely substitute sawdust and cottonseed hulls to cope with their increasingly less availability and rising prices.

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