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Single cell protein potential of endophytic fungi associated with bamboo using rice bran as substrate

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ABSTRACT

The study was undertaken to identify the isolated endophytic fungi associated with bamboo and to determine their potential in single cell protein production using rice bran as substrate. Nine endophytic fungi were identified which include Cladosporium cladosporioides, Aspergillus ochraceus, Aspergillus niger, Aspergillus flavus, Penicillium citrinum, Monascus ruber, Fusarium semitectum, Fusarium sp1 and Fusarium sp2. After which, their potential as sources of single cell protein using rice bran as substrate was evaluated through their crude protein content(CPC) after 20 days of solid state fermentation. Results of the study revealed that inoculation of endophytic fungi had increased the crude protein content of rice bran. Aspergillus niger- treated rice bran had the highest CPC of 10.63% followed by A. flavus- treated rice bran with 10.46% and A. ochraceus and F. semitectum- treated rice bran both with 10.25% On the other hand, uninoculated rice bran registered the least CPC of 9.53% followed by C. cladosporioides- treated rice bran of 9.69%. For the percentage increase CPC, A. niger, A.flavus and A.ochraceus and F. semitectum- treated rice bran registered the highest percentage increase of 11.51%, 9.48% and 5.25%, respectively. Hence, indicating the potential of the endophytic fungi in enriching the CPC and possible sources of single cell protein.

Key words: crude protein content, endophytes, fungi, solid state fermentation

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INTRODUCTION

Endophytic fungi includes filamentous fungi that grow intra and intercelullarly in plant tissues without causing over symptoms on the plants in which they colonize and have proven to be rich sources of bioactive natural products [17, 34]. Suryanarayanan et al. [33] also discussed many fungal secondary metabolites with various chemical structures and their wide ranging biological activities which reflect the high synthetic capability of fungi. In addition, Mishra et al. [19] and Miller [21] have already proven the ability of endophytic fungi to produce new and interesting bioactive secondary metabolites, which are of pharmaceutical, industrial and agricultural importance. Thus, the utilization of the endophytic fungi associated with bamboo as possible sources of single cell protein.

Single cell protein (SCP) is used as protein rich source in human and animal foods [4]. These are the dried cell mass of algae, fungi, molds and bacteria grown in large scale to supplement the protein in animal and human foods [36]. Initially yeast was used for human foods but later the research was diverted in using it in animal feeds due to acute shortage of soy bean and fish meal across many countries [3, 31]. In addition, most of the agricultural wastes such as rice bran, sugarcane bagasse, corn and other vegetable wastes are used as substrate for SCP production.

Rice bran is a by – product of milling in rice processing countries. It is normally used for extracting oil and as animal feed and a food ingredient [10]. It contains 19.97% oil, 14.12% protein, 18.22% total dietary fiber, 22.04% starch, 8.81% ash, 8.71% moisture, and 8.13% other components [10]. Furthermore, it is a source of lipids, protein, soluble and insoluble dietary fibers, iron, B vitamins, and a number of small molecules like phytosterols, phenolic acids, and antioxidants that can aid in disease prevention, control, and treatment [14]. Previous research of Godber

& Juliano [6] shown that rice bran is beneficial for human health. It contains high protein significant amounts of antioxidants such as tocopherols, tocotrienol, gamma-oryzanol.

Hence the conduct of the study to identify the endophytic fungi associated with bamboo and to determine their potential as sources of single cell protein using dried rice bran as substrate.

MATERIALS AND METHODS

Methodology was based from the previous work of Valentino et al [35] with some modifications.

Sterilization of the lateral stem of bamboo explants
For sterilization, the bamboo explants were soaked in 10 % Sodium Hypochlorite (Chlorox) bleach for 40 minutes, then it was rinsed five times with sterile distilled water. The plant explants was then blot dried. Explants were individually inoculated to the sterilized ½ strength MS basal medium. The cultures were incubated at 25°C, 16hr light, 8hr dark. Cultures were incubated until bamboo shoot proliferation and mycelia growth were observed.

Isolation and Identification of Endophytic fungi
Endophytic fungi were isolated from the tissue culture bottles of micro propagated lateral stem of bamboo plants. Endophytic fungi were picked at the developing bamboo shoot of the cultured bamboo. Then the isolated fungi were grown into pure culture using Potato Dextrose Agar and were sent to the Laboratory Services Division of Philippine Center for Postharvest Development and Mechanization for identification.

Single Cell Protein Production
Preparation of Substrates
One hundred fifty (150) grams of rice bran was placed in a clean bottle and 176 ml of distilled water was added to the rice bran to obtain 60 – 65% moisture content. This was covered with plastic and was sterilized at 15 psi at 121° C for one hour.

Preparation and Inoculation of the Fungal Endophytes
Inoculum was prepared by growing the endophytic fungi in Potato Dextrose Agar for seven days. Then, 20 ml of sterile water was added to the cultures and it was adjusted to 5.0×10^6 cells per ml using a haemocytometer. Twenty (20) ml of the adjusted spore suspension of different endophytic fungi were aseptically transferred to the sterile rice bran. Cultures were covered with plastic and a sterile bamboo stick was inserted into the plastic cover to facilitate the mixing of cultures for every 48 hours. The inoculum was allowed to acclimatize in the substrate for 20 days at room temperature

Harvesting and Drying
After 20 days of solid state fermentation, the cultures were sterilized at 15 psi for one hour. It was spread in a clean paper individually and was air dried for seven days. Dried samples were pulverized using mortar and pestle. After which, samples were sent to Philippine Carabao Center, Science City of Muñoz, Nueva Ecija for the crude protein content analysis of the samples. Finally, the increase in crude protein content was computed.

Statistical Analysis
Data was analyzed using Analysis of Variance (ANOVA) and Comparison Among Means by Duncan's Multiple Range Test (DMRT). All tests of significance were done at 5% and 1% probability levels.

RESULTS AND DISCUSSION

Endophytic fungi were isolated during shoot proliferation of the micro propagated lateral stem of bamboo. Description and characterization were based from Pitt & Hockings [27] and Navi et al. [24] and identification was done by the LSD Division of PhilMech, Science City of Munoz Nueva Ecija, Philippines.

Nine species of endophytic fungi were described, characterized and identified. These include three species of *Aspergillus* (*Aspergillus niger, Aspergillus flavus* and *Aspergillus ochraceus*), three species of *Fusarium (Fusarium semitectum, Fusarium* sp1, *Fusarium* sp 2), *Monascus ruber, Penecillium citrinum* and *Cladosporium cladosporioides.* This corresponds to recent studies wherein species of *Aspergilli, Fusarium, Penecillium* and *Cladosporium* were endophytes of several crops [2, 19, 29, 30]. Similarly, Shen et al. [32] reported that the same genera of fungi were isolated from several species of bamboo.

***1. Aspergillus niger* van Teigh**
Colonies on PDA consist of a compact to fairly loose while to faintly yellow basal mycelium, which bears abundant erect and usually crowded conidial structures, typically carbon black but sometimes deep brown-black, covering the entire colony except for a narrow growing margin. Conidial heads are typically large and black, compact at first, spherical, or split into two or more loose to reasonably well-defined columns which are about 700-800 µm in diameter. Conidiophores borne from surface hyphae, 1.0–3.0 mm long, with heavy, hyaline, smoothwalls; vesicles spherical, usually 50–75 mm diameter, bearing closely packed metulae and phialides over the whole surface; metulae 10–15 mm long, or sometimes more; phialides 7–10 mm long; conidia spherical, 4–5 mm diameter, brown, with walls conspicuously roughened or sometimes striate, borne enlarged with radiate heads [24, 27].

***2. Aspergillus flavus* Link**
Colonies on PDA are very light yellow-green, deep yellow-green, olive brown, or brown. Conidiophores are swollen apically and bear numerous conidia-bearing cells (phialides) with conidia in long, dry chains. Conidia are typically spherical to subspherical, conspicuously spiny and variable, 3-6 µm in diameter, and sometimes oval or pear-shaped [24, 27].

***3. Aspergillus ochraceus* K. Wilh**.
Colonies on CYA are plane or sulcate, low and velutinous or lightly floccose. Its mycelium is white with conidial heads closely packed, light yellow to golden yellow. Sclerotia are sometimes produced, white when young and later pink to purple. Clear exudates are sometimes present, some exuded from stipe walls; reverse greyish orange to brown. Conidiophores are borne from surface hyphae, stipes of about 1.0–1.5 mm long with yellowish to pale brown walls which are finely to conspicuously roughened. Vesicles are spherical, 25–50 mm in diameter, bearing tightly packed metulae(15–20 mm long) and phialides (9–12 mm long) over the entire surface. It produces yellow brown (ochre) conidia, borne on long stipes; vesicles bear metulae and phialides over the entire surface [24, 27].

***4. Cladosporium cladosporioides* (Fresen.)G.A. de Vries**
Colonies on CYA are low and dense, lightly wrinkled or plane with its surface velutinous or lightly floccose. Conidia are abundant, olive in obverse and bluish grey in reverse. Conidiophores are in situ dendritic (tree-like) which are closely packed, with stipes bearing branching structures of acropetally produced cells. Conidia heavy walled, pale olive brown, larger ones non- or singly septated with 10–30 to 2–5 mm diameter, smooth to finely roughened walls [24, 27].

***5. Fusarium semitectum* Berk. & Rav. (W&R, GBJ)**
Colonies on PDA are pink turning white in color. Microconidia are borne on mycelium and are spindle-shaped, straight to slightly curved. The other type is sickle-shaped and is borne in sporodochia. These are slightly curved, with a foot-shaped basal cell. Conidiophores are unbranched and monophialides and polyphialides are branched [24, 27].

***6. Monascus ruber* Tiegh**
Colonies on PDA are sparse, surface texture is floccose to deeply floccose. Mycelia are white turning pale brown as cleistothecia and aleurioconidia develop and sometimes becoming dark brown. Cleistothecia are spherical, 30–60 mm diameter, borne as a hyphal knot from a well-defined stalk, with cellular walls, becoming brown during maturation. Ascospores are ellipsoidal, hyaline, 5–7 to 4.0–4.5 mm and smooth walled. A leurioconidia are sometimes borne on pedicels from the sides of hyphae, butmore commonly terminally, sometimes borne singly but more often in chains of up to 10 cells long, spherical to pyriform, often rounding at maturity, 10–14 mm in diameter or 10–18 to 8–14 mm in diameter, with thick, smooth, brown walls [24, 27].

***7. Penicillium citrinum* Thom**
Colonies on CYA 25–30 mm in diameter, radially sulcate with marginal areas velutinous, sometimes floccose. Its mycelium is white in peripheral areas, while greyish turquoise at the center. Conidia are often abundant, dull green in obverse while pale, dull brown to yellow brown in reverse. Conidiophores borne from subsurface or surface hyphae, stipes 100–300 mm long, smooth walled, characteristically terminating in well defined verticils of 3–5 divergent metulae, less commonly with a divergent ramus, or metulae produced subterminally or along the stipe. Metulae usually of uniform length, commonly apically enlarged or vesiculate, phialides ampulliform, 7–8mm long. Conidia are spherical to sub spheroidal, 2.2–3.0 mm with walls smooth or very finely roughened which are typically borne in long well defined columns arranged in a characteristic whorl on each conidiophores [24, 27].

Crude Protein Content Profile of the Single cell protein enriched rice bran
Solid state fermentation for 20 days was implemented to determine the potentiality of nine endophytic fungi as sources of single cell protein using rice bran as substrate. Iluyemi et al. [12] mentioned that fungal growth under

solid state fermentation has also been found to be more suitable for low technology applications and there is hardly any waste disposal at the end of the process because the whole product may be used directly in animal feeds. Additionally, rice bran is one of the most abundant and locally available agricultural wastes which contain variable ingredients such as carbohydrate that maybe used as a carbon and energy source for the growth of fungi in the production of single cell protein [15].

Results as presented in Table 1 showed that *Aspergillus niger* – treated rice bran had the highest CPC of 10.63%, followed by *A. flavus* – treated rice bran with 10.46% and *A. ochraceus* and *Fusarium semitectum* – treated rice bran both with 10.25% . On the other hand, uninoculated rice bran registered the least CPC of 9.53% followed by *Clasdosporium cladosporioides* – treated rice bran of 9.69%. Statistical analysis for the CPC of the fungal enriched rice bran revealed significant differences among the treatment means. Treatment means of the fungal enriched rice bran except for *C. cladosporioides* – treated rice bran were significantly higher than the CPC of the uninoculated rice bran. For the percentage increase CPC, *A. niger*, *A. flavus A.ochraceus* and *F. semitectum* – treated rice bran registered the highest percentage increase of 11.51%, 9.48% and 5.25%, respectively. Thus, results indicate the potential of the endophytic fungi in enriching the CPC of rice bran thus they could be a possible source of single cell protein.

Results of the study corresponds with the report of Iyayi [13] that the CPC of the wheat opal also increased significantly by *A. niger, A. flavus* and *Penicillium* sp. since they are known to have the ability to produce a variety of enzymes. *A. niger*, *A. flavus* and *Penicillium* spp. have been reported to be main sources of cellulase, amylase, hemicellulase, catalase, pectinase and xylanase [8]. Similarly, Oshoma et al. [25] observed increased biomass yield on rice bran medium in submerged fermentation by *Aspergillus niger*. Also, Ikenebomeh & Chikwendu [11] and Yigitoglu [37] reported that *A. niger* was superior to other species of *Aspergillus* and strains of fungi in biomass yield from agricultural waste due to its high specific amylolytic, cellulytic and hemicllulytic activity. Additionally, *A. niger* were use in the production of extra cellular enzymes including cellulase, amylase and xylanase which could be attributed to the increase in crude protein [23]. Moreover, Pandey [26] reported that most species of fungi including *Aspergillus* and *Rhizopus* produces carbohydrate-hydrolyzing enzymes (cellulases, xylanases, pectinases, α-amylase, glucoamylases, glucanases, lipases, hemicellulases and proteases) since these are used in nature by fungi for their growth and can be contributed to the production of crude protein.

Filamentous fungi (*Aspergillus*, *Neurospora*, *Rhizopus* and *Trichoderma*) biodegrade the substrate through exocrine enzymes which include pectinase, cellulase, hemicellulase, glucanase, xylanase, protease, lipase, tannase and phytase. Additionally, predigesting fiber compounds via secreting carbohydrases increases bioavailablity of these compounds for target microorganism and consequently causes to produce nutritive protein biomass [9].

Increase in crude protein content of rice as animal feeds is important since based on the study of Anugwa et al. [1] high protein diet could lead to a significantly higher absolute and relative liver weight and relative kidney weight, but significantly lower relative stomach and leaf fat weights. Furthermore, increasing the protein level also reduced the fat deposition in the carcass, indicating less net energy, providing extra energy. At the high level of protein, additional energy gave a marked increase in protein deposition Miller [21]. Similar study conducted by Forbes [5] stated that feeding of high protein diets has been shown to limit voluntary feed intake in growing pigs.

Table 1. Mean percentage crude protent content of the endophytic fungi enriched rice bran

TREATMENTS	Crude Protein Content (%)	% increase in Crude Protein
Uninoculated Rice Bran	9.53^{a}	
Aspergillus flavus-treated rice bran	10.46^{bc}	9.48
Aspergillus niger -treated rice bran	10.63^{d}	11.51
Aspergillus ochraceus -treated rice bran	10.25^{bcd}	7.59
Clasdosporium cladosporioides-treated rice bran	9.59^{a}	1.68
Monascus ruber -treated rice bran	10.25^{bcd}	7.55
Penicillium citrinum-treated rice bran	10.19^{bc}	6.89
Fusarium semitectum -treated rice bran	10.03^{b}	5.25
Fusarium sp.1 -treated rice bran	10.19^{bc}	6.89
Fusarium sp. 2 -treated rice bran	10.17^{bc}	6.68

**Treatment means with the same letter are not significantly different*

Thus, results of the study proved the ability of the eight species of endophytic fungi associated with bamboo namely *Aspergillus ochraceus, Aspergillus niger, Aspergillus flavus, Penicillium citrinum, Monascus ruber, Fusarium semitectum, Fusarium sp*1 and *Fusarium sp*2. as possible sources of single cell protein using rice bran as substrate.

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