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PROTEOLYTIC ENZYME LIKE ACTIVITY OF FUNGAL ENDOPHYTES AND THEIR EFFECTS IN THE PROXIMATE COMPOSITION OF DRIED BAMBOO LEAVES

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ABSTRACT

The study aimed to assess the ability of the endophytic fungi associated with bamboo in the production of enzyme protease which act on the degradation of protein. Crude protein content and proximate composition of the bamboo leaves were evaluated after 20 days of solid state fermentation. Results revealed the capability of all the tested endophytic fungi (*Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Cladosporium cladosporioides, Fusarium semitectum, Fusarium sp1, Fusarium sp2, Monascus ruber* and *Penicillium citrinum*) in reducing the crude protein content of the bamboo leaves, thus the potential protease production . Similarly, reduction in the moisture and ash content; increase in crude fiber content of the substrate after solid state fermentation were noted in the proximate composition relative to the degradation of protein.

Keywords: bamboo, crude protein content, endophytic fungi, protease

INTRODUCTION

Proteolytic enzymes or proteases are group of hydrolases which causes the hydrolysis of proteins into smaller peptides and amino acids by catalyzing the breakdown of peptide bonds (Mukhtar & Ul-Haq, 2009; Salahuddin & Khan, 2008). It can be found in all forms of organisms, which include the plant, animal and microbial proteases (Oyeleke *et al.* 2010). Meanwhile, microbial proteases account for a two-third share of commercial protease production in the enzyme market across the

world and are considered as the most common sources of commercial enzymes (Rao *et al.*, 1998; Kumar & Takagi, 1999). Their physiological and biochemical properties, trite culture conditions and ease of cell manipulation could be of potential reasons (Godfrey and West, 1996; Adinarayana & Ellaiah, 2002; Dias *et al*., 2010). As mentioned by Jisha (2013), proteins are degraded by microorganisms as initiated by endopeptidases followed by hydrolysis of exopeptidases secreted by the microorganisms which largely depends on the strains or species of microorganism.

Additionally, endophytic fungi from various plants secretes hydrolysing enzymes such as cellulases, pectinases, and group of proteases as protection against invasion of pathogenic organisms and to obtain their nutrition from the host plants including (Caldwellet al. 2000; Petrini,et al 1992; Wang et al 2006). Furthermore, fungi are normally generally regarded as safe strains which are easier to be recovered from fermentation. Protease production can be employed both with solid state fermentation or submerged fermentation techniques (Pandey *et al.*, 2001; Tremacoldi *et al.*, 2004; Mukhtar & Haq, 2008). Whereas, fungal enzymes can be conveniently produced in a solid-state fermentation process and possess almost all the characteristics desired for their biotechnological applications. Proteases play a large variety of applications detergents, pharmaceutical, leather processing, food industry, feeds, chemical industries, waste treatments and agricultural industries (Nout & Rombouts, 1990; Deng *et al.*, 2010, Jisha *et al.*, 2013).

Agricultural by-products such as sunflower meal, wheat bran, soybean meal, lupin cake and cotton seed meal were found to be inducers of enzyme protease (Ul-Haq *et al*. 2003). Therefore the utilization of other plant materials in the likes of bamboo leaves. Bamboo leaves are very rich in nitrogenous material, fiber and vitamin A. They are a valuable fooder for livestocks and contribute to the pharmaceutical industry (Farrelly, 1984; Ruyle, 1993; Mirai & Yokohama, 2009).

The present study was carried out to determine the proteolytic enzyme like activity of nine endophytic fungi associated with bamboo and their influence in the proximate composition of bamboo leaves these could be of importance in agro industrial industries if found positive for protease production.

MATERIALS AND METHODS

Methodology was adapted from the works of Valentino *et al*. (2015), Valentino *et al*. (2016), Paynor *et al*. (2016) and Ganado *et al*. (2016) with some modifications. Bamboo leaves were

collected from Alalay Grande Lupao, Nueva Ecija, Philippines. Then it was sun dried and pulverized into powdered form. Dried bamboo leaves were analyzed for its Crude Protein Content (CPC) which served as the initial CPC of the substrate.

Preparation of the Fungal Inocula

Endophytic fungi were obtained from the collection of Valentino *et al.* 2016. Seven day old fungal endophytes were added with 20 ml sterile distilled water and spores were counted using hemacytometer and was adjusted to 5.0 x 10^6 cells per ml with sterile distilled water.

Preparation of Substrates

One hundred (100) grams of dried bamboo leaves was added with distilled water to obtain 60-65% moisture content. These were then sterilized at 15 psi at 121° C for one hour.

Inoculation of Fungal endophytes in Bamboo Leaves

Ten (10) ml of the adjusted spore suspension of different endophytes was aseptically transferred to the substrate. The inoculum was then 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 spreaded in a clean paper individually and was air dried for seven days. Dried samples were pulverized using mortar and pestle. Dried and pulverized samples were analyzed for the crude protein content (CPC) using Kjeldahl method.

Proximate Composition Analysis

Ground bamboo leaves (100 g) was sent to Lipa Quality Control Center– Bocaue, Bulacan, Philippines for proximate analysis of the nutritional content such as crude protein, crude fat, crude fiber, moisture and ash content value which were based on the guidelines of the Association of Official Analytical Chemist (2002).

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 DISCUSSIONS

The study was conducted to determine the ability of nine endophytic fungi associated with bamboo namely*; Cladosporium cladosporiodes, Aspergillus ochraceus, Aspergillus niger Aspergillus flavus, Penicillium citrinum, Monascus ruber, Fusarium semitectum, Fusarium sp.1* and *Fusarium sp. 2* in the production of protease through the evaluation on the decrease in crude protein content of bamboo leaves.

Presented in Table 1 are the CPC of the endophytic fungi treated bamboo and their corresponding % decrease in CPC. Remarkable decrease in crude protein content of the fermented bamboo leaves was recorded in all fungal treated bamboo leaves indicating the potential proteolytic activity of nine fungal endophytes (*Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Cladosporium cladosporioides, Fusarium semitectum, Fusarium sp1, Fusarium sp2, Monascus ruber* and *Panicillium citrinum*). Among the nine endophytic fungi used, *Aspergillus niger*- treated bamboo leaves had the least of CPC of 12.85 and the highest % decrease in CPC of 11.32%. It was seconded by *Fusarium sp* 1 and *Fusarium semitectum* of 12.86% and 12.90% CPC respectively with 11.25% and 10.97% decrease in CPC.

This coincides with the study of Chaven (2011) and Kakde & Chavan (2011), wherein decrease in crude protein content was observed in sunflower, sesame, soybean, safflower and groundnut when treated with several filamentous fungi (*Aspergillus niger, Aspergillus terreus, Aspergillus ustus, Aspergillus versicolor, Aspergillus parasiticus and Aspergillus fumigatus*). According to Kalpana Devi *et al*. (2008), molds of the genera *Aspergillus, Mucor, Fusarium, Penicillium* and *Rhizopus* are potent strains for proteases. Additionally, Paranthaman *et al.* (2009), revealed the ability of *Aspergilus niger* in the protease production. *Aspergillus niger* is one of the known fungi that have the potential in producing extracellular protease enzymes due to its cosmopolitan and ubiquitous nature (Benazir *et al.*, 2011). Peptidase production using wheat bran as substrate by *Penicillium* species was also demonstrated by Shimakage *et al.*(2004) and Raja *et al.* (2011). Consequently, Devi *et al.* (2008) revealed that molds of the genera *Aspergillus, Penicillium* and *Rhizopus* are especially useful for producing proteases, as several species of these genera are generally regarded as safe.

These results showed that the protease enzyme production can be attributed to the organism used and the effectiveness/suitability of the substrate as carbon sources that influence the fungal growth (Ferrero *et al.,* 1996; Nascimento & Martins, 1996; Johnvesly & Nailk, 2001; Pagare *et al.,* 2009). According to Adinarayana *et al.* (2003), the carbon sources are the utmost nutritional factors producing the necessary inducing compounds for the organism that triggers the protease production.

Table 1: Mean percentage of crude protein composition of the endophytic fungi enriched bamboo leaves

TREATMENTS	Crude Protein*	% decrease in Crude Protein
Uninoculated Bamboo Leaves	14.49[d]	
Aspergillus flavus-treated bamboo leaves	13.29[abc]	8.28[a]
Aspergillus niger -treated bamboo leaves	12.85[a]	11.32[a]
Aspergillus ochraceus -treated bamboo leaves	12.95[ab]	10.63[a]
Clasdosporium cladosporioides-treated bamboo leaves	13.19[abc]	8.97
Monascus ruber -treated bamboo leaves	13.45[c]	7.18[a]
Penicillium citrinum-treated bamboo leaves	13.34[a]	7.94
Fusarium semitectum -treated bamboo leaves	12.90[ab]	10.97[a]
Fusarium sp.1 -treated bamboo leaves	12.86[a]	11.25[a]
Fusarium sp. 2 -treated bamboo leaves	13.48[c]	6.97[a]

***Treatments with the same letters are not significantly different**

Analysis of the proximate composition of fungal treated dried bamboo leaves showed reduction in moisture, ash, crude fat while an increase in crude fiber of the fermented substrate (Table 2). *Fusarium semitectum*-treated bamboo leaves recorded the highest crude fiber with 29.55% followed by *Fusarium sp. 1* with 29.55% and *Aspergillus ochareus*- treated bamboo leaves with 28.58%, respectively. Increase in crude fiber could be due to the formation of resistant starch together with condensed tannin-protein complex (Perez hidalgo *et al.* 1997). High crude fiber content of bamboo leaves could therefore impart superior physical tone stamina to horses since fiber speeds up the process of digestion by improving peristalsis (Okaraonye & Ikewuchi, 2009).

Meanwhile, crude fat content of all the evaluated treated and untreated bamboo leaves were comparable with one another except by *Fusarium sp* 1 which registered the highest with 3.53%. Recorded crude fat ranged from 2.90 to 1.70%. Similarly, reduction of the moisture content from the initial of 7.79% to the least of 6.63% by the *Cladosporium cladosporioides* treated bamboo leaves was observed. The low moisture availability may favour the production of specific compounds that may not be produced (Mussatto, 2012). Finally, for the crude ash, all fungi treated bamboo leaves obtained values lower than the untreated bamboo leaves of 20.52%, followed by *Monascus ruber* with 19.51% and *Fusarium sp.2*- treated with bamboo leaves with 18.29%. The decrease in ash content, which is as indicator of total mineral content of powder was on similar trends as observed by Aziz *et al.*, (2000) who reported that *Aspergillus* depleted the zinc, copper and iron from the corn. Thus, there is direct correlation between metal uptake and the fungal growth (Tripathi & Mishra, 2008). Finally, reduction in its level during microbial fermentation could be as a result of the minerals being used up by the fermenting organisms as a mineral source during their metabolism (Aderiye & Ogunjobi, 1998).

Table 2: Mean percentage (%) of proximate composition of fungal enriched bamboo leaves

TREATMENTS	%Moisture	%Ash	%Crude Fat	%Crude Fiber
Uninoculated Bamboo Leaves	7.97^{d}	20.52^{d}	2.53^{abc}	21.16^{a}
A. flavus-treated bamboo leaves	6.63^{a}	19.51^{c}	2.43^{abc}	23.39^{bc}
A. niger -treated bamboo leaves	6.67^{ab}	17.81^{a}	2.33^{abc}	28.15^{e}
A. ochraceus -treated bamboo leaves	7.49^{c}	18.74^{b}	1.70^{ab}	28.58^{e}
C. cladosporioides-treated bamboo leaves	6.73^{ab}	19.76^{c}	2.90^{cd}	24.22^{c}
M. ruber -treated bamboo leaves	6.74^{ab}	20.14^{cd}	1.64^{a}	26.93^{d}
P. citrinum-treated bamboo leaves	6.70^{ab}	18.35^{ab}	2.43^{abc}	22.47^{b}
F. semitectum -treated bamboo leaves	6.56^{a}	18.58^{b}	1.90^{ab}	29.55^{e}
Fusarium sp.1 -treated bamboo leaves	6.85^{b}	18.58^{b}	3.53^{d}	29.29^{f}
Fusarium sp. 2 -treated bamboo leaves	7.57^{d}	18.29^{ab}	2.58^{bc}	26.70^{d}

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

CONCLUSION*:*

The ability of *Aspergillus niger, Aspergillus flavus, Aspergillus ochraceus, Cladosporium cladosporioides, Fusarium semitectum, Fusarium sp 1, Fusarium sp 2, Monascus ruber and Penicillium citrinum* to reduce the crude protein content of the dried bamboo leaves through solid state fermentation was revealed in the study. This also led to the depletion in crude ash content, moisture content, increase in crude fiber while no changes was recorded in crude fat of the dried bamboo leaves. This could further lead to the utilization of the endophytes as possible sources of proteases after further intensive studies.

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To God be the Highest Glory! Thy Will Be Done

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