



**PROTEIN ENRICHMENT OF RICE STRAW AND SUGARCANE BAGASSE WITH
ENDOPHYTIC FUNGI ASSOCIATED WITH BAMBOO USING SOLID STATE
FERMENTATION**

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ABSTRACT

The present study performed the comparative study on the ability of the nine species endophytic fungi in enriching the crude protein content rice straw and sugarcane bagasse by solid state fermentation. Evaluation was based on the remarkable increase in crude protein of the aforementioned substrates and comparisons were based on the percentage increase in their crude protein content (CPC) after 20 days of solid state fermentation. The highest crude protein content of rice straw was recorded in *Aspergillus flavus* of 6.34% followed by *Aspergillus niger*, *Aspergillus ochraceus* and *Cladosporium cladosporoides* with 6.31%, 6.16% and 5.98% respectively. Meanwhile, the three species of *Fusarium* had the least CPC (*Fusarium* sp.2 with 5.78% and *Fusarium* sp.1, *Fusarium semitectum* both with 5.91%). For the CPC of the endophytic fungi-treated sugarcane bagasse, *Monascus ruber* obtained the highest CPC of 3.01%, followed by *Fusarium semitectum* with 2.57%. On the other hand, uninoculated sugarcane bagasse registered the least CPC of 1.57% followed by *Penicillium citrinum* of 2.05%. All of which were significantly higher than the untreated substrate indicating their potential in enriching the protein content of both rice straw and sugarcane bagasse. Apparently, sugar cane bagasse was found to be a better substrate as compared o rice straw in the production of single cell protein, thus resulting to higher percentage increase in CPC.

The ability of the endophytic fungi in the production of single cell protein and the potential of sugarcane and rice straw as substrate for the SCP protein were probed in the study.

Keywords: crude protein content, endophytic fungii, rice straw, single cell protein, sugarcane bagasse

INTRODUCTION

Sugarcane bagasse and rice straw are amongst the most common agricultural waste products in the Philippines as well as in other tropical countries. Sugarcane bagasse is the highly fibrous residue that remains after the juice has been removed consisting of the cellulose fiber of rind, vascular tissue and the pith of the cane stem. In addition, bagasse is composed of 43.8% cellulose, 28.6% hemicelluloses, 23.5% lignin, 1.3% ash and 2.8% other components (Luz *et al.* 2007; Sun *et al.* 2004 & Allen 1997). Whereas, rice straw is the vegetative part of the rice plant which is being cut and remain in the field after harvest (Kadam *et al.* 2000) Rice straw consists predominantly of cell walls with 19.64% lignin, 32.15, 29.8% cellulose, 28.0% hemicellulose, 11.33% ash, 3-5% crude protein and silica with 5 to15% (Shawky *et al.* 2011; Vadiveloo 1992; Agbagla-Dohnani *et al.* 2003, and Schiere *et al.* 1989). And due to the abundant availability of rice straw and sugarcane, with few known importance, their accumulation can lead to a serious pollution. Thus, further modes of utilization

of such agro industrial wastes are essential. They can possibly serve as an ideal substrate for microbial processes for the production of mycoprotein or single cell protein.

Single cell protein is dried microbial biomass grown in a carbon rich substrate and harvested primarily as protein source of human and animal feeds. These microorganisms has the ability of enhancing the protein, nucleic acids, carbohydrate cell wall material, lipids, minerals, vitamins and other nutritiobal attributes of the substrates (Reed & Nagodawithana 1995; Sivasanker 2002; Nigam 2000. As stated *et al.* by Bhalla (2007) and Kurbanoglu & Algur (2002), actinomycetes and filamentous fungi produce about 50-55% proteins when grown in from various substrates. Production of single cell protein by fungal organisms using waste materials as substrate provides an economically feasible protein source which can be use to solve the problem of worldwide protein shortage and reduces the pollution effects of these waste materials (Kurbanoglu *et al.* 2002;

Algur & Gokalp 1991; Anupama & Ravindra 2000; Paraskevopoulou *et al.* 2003).

Several studies have already established the ability of the fungal endophytes in the production of various enzymes such as xylanases, hemicellulase, non-specific peroxidases and laccases, chitinase and glucanase pectin and exhibiting lipolytic activity (Leuchtmann *et al.* 1992; Li *et al.* 2007; Promputtha *et al.* 2005; Suto *et al.* 2002; Tomita 2003; Seiber *et al.* 1991). And their presence within plant tissues could explain their capacity to produce substances which is useful in industrial, agricultural, and medicinal applications (Huang *et al.* 2008).

Therefore, the study was undertaken to evaluate the comparative ability of the endophytic fungi associated with bamboo which include, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Cladosporium cladosporioides*, *Fusarium semitectum*, *Fusarium sp1*, *Fusarium sp2*, *Monascus ruber* and *Penicillium citrinum* in enriching the crude protein content of rice bran and sugarcane bagasse through solid state fermentation.

MATERIALS AND METHODS

Methodology was based from the previous work of Valentino *et al.* 2015 with some modifications.

Collection of substrates:

Rice straw was collected from Bongabon, Nueva Ecija and sugarcane bagasse was obtained in Gerona, Tarlac, Nueva Ecija, Philippines. These were then sun dried and were pulverized into powder.

Preparation of substrates:

One hundred (100) grams of rice straw and sugarcane bagasse were placed in clean fermentation bottles separately. For rice straw 150 ml of distilled water while 200ml of distilled water was added to sugarcane bagasse to obtain the desired moisture content for the optimum growth of the fungal inocula. This was covered with plastic and was sterilized at 15 psi at 121° C for one hour.

Preparation and inoculation of the endophytic fungi:

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 the spores were counted using haemocytometer and it was adjusted to 5.0×10^6 cells per ml. Twenty (20) ml of the adjusted spore suspension of different endophytic fungi were aseptically transferred to the sterile rice bran. 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

According to Kuhad *et al.* (1997) and Oseni & Ekperigin (2007) microorganisms are very attractive feedstuffs, because they can be cultivated on agro-industrial waste, with production of large amounts of cells rich in proteins that commonly contain all the essential amino acids. In addition, Azzam (1992) used a defined mixed culture for biomass production on bagasse, and found that the growth of microorganisms was followed by the production of biomass protein.

The crude protein content (CPC) of the protein enriched rice straw and sugarcane bagasse is shown in Table 1. Results revealed that rice straw enriched by *Aspergillus flavus* had the highest CPC of 6.34% followed by *Aspergillus niger*, *Aspergillus ochraceus* and *Cladosporium cladosporoides* with 6.31%, 6.16% and 5.98% respectively. Meanwhile, three species of *Fusarium* had the least CPC (*Fusarium* sp.2 with 5.78% and *Fusarium* sp.1, *Fusarium semitectum* both with 5.91%). For the CPC of endophytic fungi-treated sugarcane bagasse, *Monascus ruber* obtained the highest CPC of 3.01%, followed by *Fusarium semitectum* with 2.57%. On the other hand, uninoculated sugarcane bagasse registered the least CPC of 1.57% followed by *Penicillium citrinum* of 2.05%. Apparently, the CPC of the fungal enriched rice straw indicates significant differences among the treatment means. This proves the potential of the endophytic fungi sources of single cell protein thus enhancing the crude protein content of the substrates.

These confirm the reports that microorganisms have high protein content and short growth times, leading to rapid biomass production which can be continuous and independent from the environmental conditions and they can be easily propagated using cheap raw

materials (Martin 1991). Results were also in line with several reports wherein the crude protein contents of products were increased upon solid state fermentation with filamentous fungi in rice bran cotton waste, rice straw, cocoa pod husk, cassava waste, wheat offal, maize offal, palm kernel meal, sugar cane baggase, orange peel, wheat straw (Iyayi & Aderolu 2004; Iyayi 2004; Ofuya & Nwajiuba 1990; Pothiraj *et al.* 2006; Oshoma & Ikenebomeh 2005; Yakoub Khan & Umar Dahot 2010).

Increase in crude protein content of the substrate can be due fungal growth and proliferation of the fungal biomass in the form of proteins (Raimbault 1998). Additionally, during colonization they synthesize and excrete hydrolytic enzymes and some extracellular enzymes (proteins) such as amylase, linamarase, zylanase, cellulose and cellulase, amylase, hemicellulase, catalase, pectinase and

xylanase which degrades the non starch polysaccharides (Raimbault 1998; Hamylyn, 1998, Oboh& Akindahunsi 2003).

For the rice straw, three species of genus *Aspergilli* recorded the highest crude protein content which can be attributed to its fast growth and ph tolerance therefore ability to produce more cellulolytic enzymes within a short period. *Aspergillus niger* has been reported to have high specific activity for cellulases and hemicellulases extra cellular enzymes including cellulase, amylase and xylanase (Oboh & Akindahunsi 2003; Nair *et al.* 2008; Howard *et al.* 2003).

Meanwhile, *Monascus ruber* superiorly increased the CPC of sugarcane bagasse. *Monascus* contains pigments which are frequently associated to proteins (Wong & Koehler 1983).

Table 1: Crude protein content of the endophytic fungi-treated rice straw and sugarcane bagasse

ENDOPHYTIC FUNGI	RICE STRAW	SUGARCANE BAGASSE
Control (Uninoculated substrate)	5.60 ^a	1.57 ^a
<i>Aspergillus niger</i>	6.31 ^c	2.12 ^b
<i>Aspergillus flavus</i>	6.34 ^c	2.21 ^b
<i>Aspergillus ochraceus</i>	6.16 ^{bc}	2.15 ^b
<i>Cladosporium cladosporioides</i>	5.98 ^{bc}	2.28 ^{bc}
<i>Fusarium sp.1</i>	5.91 ^{bc}	2.26 ^{bc}
<i>Fusarium sp.2</i>	5.78 ^{ab}	2.35 ^{bc}
<i>Fusarium semitectum</i>	5.91 ^{bc}	2.57 ^c
<i>Monascus ruber</i>	5.93 ^{bc}	3.01 ^d
<i>Penicillium citrinum</i>	5.94 ^{bc}	2.05 ^b

* Treatment means with the same letter are not significantly different

As stated by Khan *et al.* (2010), carbohydrate substrates are the most widely used for SCP production due to the fact that carbohydrates are natural microbial

substrates and also because carbohydrates constitute a renewable feedstock. Additionally, the degree of fungal biomass

growth depends on the type of substrate used (Ugalde & Castrillo 2005).

It can be depicted in Table 2 that among all the treatments used in the study, *Monascus ruber*- treated sugarcane bagasse had the highest % increase in CPC of 92.40, followed by 63.81% of *Fusarium semitectum*- treated sugarcane bagasse. While the least % increase in CPC was obtained in *Fusarium sp 2* and *Fusarium sp 1*- treated rice straw with 3.27 and 5.53%, respectively. Statistical analysis also revealed significant differences among the treatment means. *Monascus ruber*, *Aspergillus ochraceus*, *Cladosporium cladosporioides*, *Fusarium semitectum*, *Fusarium sp1*, *Fusarium sp2*, and

Penicillium citrinum-treated sugarcane bagasse were significantly higher than all the treatments. Whereas, all fungal-treated rice straw were significantly lower than those grown in sugarcane bagasse. These suggest that sugarcane bagasse is a better substrate for the growth and the production of single cell protein of the endophytic fungi. This can be attributed to the fact the sugarcane bagasse contains more sugar content which could be very useful as carbon source for the growth of fungal growth. Further colonization and proliferation of the endophytic fungi to the substrate would lead to rapid bioconversion of organic compounds to protein.

Table 2: Comparison on the treatment means of the % increase in CPC of the substrates

TREATMENTS	% increase in CPC
<i>Aspergillus flavus</i> –treated sugarcane bagasse	38.11 ^{bc}
<i>Aspergillus flavus</i> – treated rice straw	13.41 ^{ab}
<i>Aspergillus niger</i> –treated sugarcane bagasse	36.71 ^{bc}
<i>Aspergillus niger</i> – treated rice straw	12.62 ^{ab}
<i>Aspergillus ochraceus</i> –treated sugarcane bagasse	40.73 ^c
<i>Aspergillus ochraceus</i> – treated rice straw	9.94 ^a
<i>Cladosporium cladosporioides</i> –treated sugarcane bagasse	46.88 ^c
<i>Cladosporium cladosporioides</i> – treated rice straw	6.72 ^a
<i>Fusarium semitectum</i> –treated sugarcane bagasse	63.81 ^c
<i>Fusarium semitectum</i> – treated rice straw	5.53 ^a
<i>Fusarium sp2</i> –treated sugarcane bagasse	50.55 ^c
<i>Fusarium sp2</i> – treated rice straw	3.27 ^a
<i>Fusarium sp1</i> –treated sugarcane bagasse	44.01 ^c
<i>Fusarium sp1</i> – treated rice straw	5.53 ^a
<i>Penicillium citrinum</i> –treated sugarcane bagasse	45.77 ^c
<i>Penicillium citrinum</i> – treated rice straw	6.07 ^a
<i>Monascus ruber</i> –treated sugarcane bagasse	92.40 ^d
<i>Monascus ruber</i> – treated rice straw	5.83 ^a

* 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 enrich the crude protein content of both sugarcane bagasse and rice straw were disclosed in the study. Thus, their potentials as sources of single cell protein.

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To God be the Highest Glory! Thy Will Be Done

REFERENCES

- [1] Adenipekun, C.O., and Okunlade, O. A., 2012. Biodegradation of Rattan Wood and Maize Stovers by *Pleurotus ostreatus*. *Nature and Science*, 10 (5):49–57.
- [2] Agbagla-Dohnani, A., P. Noziere, B. Gaillard-Martinie, M. Puard, and M. Doreau, 2003. Effect of Si content on rice straw ruminal degradation. *Journal Agric. Sci.* 140:183–192.
- [3] Allen, M., T. Moore and M. Christensen, 1997. Phytohormone changes in *Boutellouagracillis* infected by vesicular-arbuscularmycorrhizae. *Canadian Journal of Botany*, 58:371-374.
- [4] Algur, O.F., and H.Y. Gokalp, 1991. Batch culture of *Rhizopus arrhizus* and *Actinomucor elegans* on vinasse medium for biomass production and BOD reduction. *Doga Trends in Cell Biology* 5: 198–205.
- [5] Anupama, P. and L. Ravindra, 2000. Value-added food: single cell protein. *Biotechnology Advances* 18: 459–479.
- [6] Azzam, A.M., 1992. Production of metabolites, industrial enzymes, amino acids. *J. Environ. Sci. Eng.*, 56: 67–99.
- [7] Bhalla, T., N. Sharma, and M. Sharma, 2007. Production of metabolites, industrial enzymes, amino acids, organic acids, antibiotics, vitamins and single cell proteins. National Science Digital Library, India.
- [8] Hamlyn, P.F., 1998. Fungal Biotechnology. *Brit Mycol Soc Newslett.*
- [9] Howard, R.L., E. Abotsi, E.L van Renseburgel and S. Howard, 2003. Lignocellulose biotechnology: issues of bioconversion and enzyme production. *Afr. J. Biotechnol.* 2(12):602-619.
- [10] Huang, Z., X. Cai, C. Shao, Z. She, X. Xia, Y. Chen, J. Yang, S. Zhou, and Y. Lin, 2008. Chemistry and weak antimicrobial activities of phomopsins produced by mangrove endophytic fungus *Phomopsis* sp. ZSU-H76. *Phytochemistry*, 69:1604–1608.
- [11] Iyayi, E.A. and A.Z. Aderolu, 2004. Enhancement of the feeding values of some agro- industrial by-products for laying hens after their solid state fermentation with *Trichoderma viride*. *Afr. J. Biotech.* 3, 182 – 185.
- [12] Iyayi, E. (2004). Changes in the cellulose, sugar and crude protein contents of agro-industrial by-products fermented with *Aspergillus niger*,

- Aspergillus flavus and Penicillium sp. 3 (3).
- [13] Kadam, K. L., L.H. Forrest, and W.A. Jacobson, 2000. Rice straw as a lignocellulosic resource: collection, processing, transportation, and environmental aspects. *Biomass and Bioenergy*, 18: 369-389
- [14] Khan, M., S.S. Khan, Z. Ahmed, and A. Tanveer, 2010. Production of Single Cell Protein from *Saccharomyces cerevisiae* by utilizing Fruit Wastes. *Nanobiotechnica Universale* Vol. 1(2), 127-132
- [15] Kuhad, R.C., A. Singh, K.K. Tripathi, R. K. Saxena and K.E.L. Erikson, 1997. Microorganisms as an alternative source of protein. *Nutrition Reviews* 55: 65–75.
- [16] Kurbanoglu, E.B., and O.F. Algur, 2002. Single-cell protein production from ram horn hydrolysate by bacteria. *Bioresource Technology* 85: 125–129.
- [17] Leuchtmann, A., O. Petrini, L.E. Petrini, and G.C. Carroll, 1992. Isozyme polymorphism in six endophytic *Phyllosticta* species. *Mycological Research* 96, 287–294.
- [18] Li, W., J. Zhou, Z. Lin, and Z. Hu, 2007. Study on fermentation condition for production of huperzine A from endophytic fungus 2F09P03B of *Huperzia serrata*. *Chinese Medicinal Biotechnology*. 2007; 2: 254–259.
- [19] Luz, S., A. Goncalves, P. Ferrao, M. Freitas, A. Leao, and Del Arco, 2007. Water absorption studies of vegetable fibers reinforced polypropylene composites, In: *Proceedings of 6th International Symposium on Natural Polymers and Composites*.
- [20] Martin, A., 1991. *Bioconversion of waste materials to industrial products*. Elsevier Applied Science, London, UK.
- [21] Nair, S.G. R. Sindhu, and S. Shashidhar, 2008. Fungal xylanase production under solid state and submerged fermentation conditions. *Afr. J. Microbiol. Research*. 2:082–086.
- [22] Nigam, N.M., 2000. Cultivation of *Candida langeronii* in sugarcane bagasse hemi cellulose hydrolysate for the production of single cell protein, *World Journal of Microbiology and Biotechnology*, 16, 367–372.
- [23] Oboh, G. and A. A. Akindahunsi, 2003. Biochemical changes in cassava products (flour and garri) subjected to *Saccharomyces cerevisiae* solid state fermentation. *Food Chemistry*. Vol.82 (4): 599–602.

- [24] Ofuya, C.O., and C.J. Nwajiuba, 1990. Microbial degradation and utilization of cassava peel. *World J. Microbiol. Biotechnol.* 6: 144 – 148.
- [25] Oseni, O.A., and M. Ekperigin, 2007. Studies on the biochemical changes in maize wastes fermented with *Aspergillus niger*. *Biokemistri*, 19: 75–79
- [26] Oshoma, C., and M. Ikenebomeh, 2005. Production of *Aspergillus niger* Biomass from Rice Bran. *Pakistan Journal of Nutrition* 4 (1): 32–36,
- [27] Paraskevopoulou, A., I. Athanasiadis, M. Kanellaki, A. Bekatorou, G. Blekas, and V. Kiosseoglou, 2003. Functional properties of single cell protein produced by kefir microflora. *Food research International* 36: 431–438.
- [28] Pothiraj, C., P. Balaji, and M. Eyini, 2006. Raw starch degrading amylase production by various fungal cultures grown on cassava waste. *Microbiology*, 34:128e30.
- [29] Promputtha, I., R. Jeewon, S. Lumyong, E. Mckenaie, and K. Hyde, 2005. A phylogenetic evaluation of whether endophytes become saprotrophs at host senescence. *Microbial Ecology* 53, 579–590.
- [30] Raimbault M.J., 1998. General and microbiological aspects of solid substrate fermentation. *Electronic J. Biotechnol.* 1(3):395-399.
- [31] Reed, G. and T. Nagodawithana, 1995. *Biotechnology enzymes, biomass, food and feed*, Bibliographic Citation.9, 168-215.
- [32] Schiere, J.B., M.N.H. Ibrahim, V. J. H. Sewalt, and G. Zimmelink, 1989. Response of growing bulls given rice straw to lickblocks containing urea and molasses. *Animal feed Science and Technology* (in press).
- [33] Seiber, T., F. Sieber-Canavesi, and C. Dorworth, 1991. Endophytic fungi on red alder (*Alnus rubra*) leaves and twigs in British Columbia. *Canadian Journal of Botany* 69, 407–411.
- [34] Shawky, B.T., M.G. Mahmoud, E.A. Ghazy, M.M.S. Asker, and G.S. Ibrahim, 2011. Enzymatic hydrolysis of rice straw and corn stalks for monosugars production. *Journal of Genetic Engineering and Biotechnology*, 9(1), 59–63
- [35] Sivasanker, B., 2002. *Food processing and reservation*. Prentice- Hall. New Delhi
- [36] Sun, X., R. Sun, and J. Sun, 2004. Acetylation of sugarcane bagasse using NBS as a catalyst under mild reaction conditions for the production of oil sorption- active

- materials, *Bioresource Technology*, 95(3), 343-350.
- [37] Suto, M., M. Takebayashi, K. Saito, M. Tanaka, A. Yokota, and F. Tomita, 2002. Endophytes as Producers of Xylanase, *Journal of BioScience and Bioengineering* 93, 88–90
- [38] Tomita, F., 2003. Endophytes in Southeast Asia and Japan: their taxonomic diversity and potential applications. *Fungal Diversity* 14, 187–204.
- [39] Vadiveloo, J., 1992. Varietal differences in the chemical composition and in vitro digestibility of rice straw. *Journal Agric. Sci.*, 119: 27-33.
- [40] Valentino, M.J.G, S.P. Kalaw, C.T. Galvez, and R.G. Reyes, 2015. Mycota of distillery yeast sludge as source of single cell protein. *Mycosphere* 6(3), 241–247.
- [41] Ugalde, O., and J. Castrillo, 2005. Single Cell Proteins from Fungi and Yeast, *Applied Mycology and Biotechnology. Agriculture and Food Production*, Vol.2, Elsevier, London, pp.123-150
- [41] Wong, H., and P., Koehler, 1983. Production of water-soluble *Monascus* pigments. *J Food Sci* 48:1200–1203.
- [42] Yakoub Khan, M., and M. Umar Dahot, 2010. Effect of Various Agriculture Wastes and Pure Sugars on the Production of Single Cell Protein by *Penicillium Expansum* *World Applied Sciences Journal* 8 (Special Issue of Biotechnology & Genetic Engineering): 80-84