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**SINGLE CELL PROTEIN PRODUCTION OF FUNGAL ENDOPHYTES IN
PINEAPPLE PEEL (*Ananas comosus* (L.) Merr.)**

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ABSTRACT

The study aimed to evaluate the potential of the previously isolated fungal endophytes associated with bamboo (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Fusarium* sp.1, *Fusarium* sp.2, *Fusarium semitectum*, *Monascus ruber*, *Penicillium citrinum*, *Cladosporium cladosporioides*) as enhancers of crude protein content of pineapple peel after 20 days of solid state fermentation.

Pronounced potential of the nine endophytic fungi in single cell protein production using pineapple peel as substrate was revealed in the study which led to the increased crude protein content of the pineapple peel fermented with fungal endophytes. Among which, *C. cladosporioides*- treated pineapple peel had the highest CPC of 9.24% while *P. citrinum* had the least of 3.61%. Similarly, *C. cladosporioides*- treated pineapple peel had the highest computed percentage increase in crude protein with 178%. Hence, the ability of the fungal endophytes as sources of single cell protein using pineapple peel as substrate.

Keywords: Crude protein – Endophytes – Pineapple peel – Single Cell Protein

INTRODUCTION

Pineapple which is scientifically known as *Ananas comosus* (L.) Merr. belongs to family Bromeliaceae and has originated in Brazil and Paraguay. It ranked as the second most important tropical fruits in the world and Philippines is next to Thailand in terms of its production. Pineapple is also called as queen of the fruits due to its flavor, taste and nutritional attributes. They are being processed into various nutritional products such as juice and canned products. It is also known as the queen of fruits due to its flavor, taste and nutritional attributes. [1, 2, 3, 4].

Meanwhile, only 22.5% of the fruit is edible which leads to accumulation of vast amount of under-utilized pineapple waste such as pineapple peel and it adds up to post agricultural wastes [5,6]. According to Hemalatha and Anbuselvi [7] pineapple waste contain non reducing sugars, carbohydrates, protein, crude fiber, protein, ascorbic acid and moisture content. This property makes it a good source of carbon and substrate for single cell protein production.

An array of microbes are involved in the conversion of various industrial wastes and substrates into biomass producing single cell protein or microbial protein which could

provide economically important protein source for animal feed or for human consumption [8].

Several studies on the single cell protein production of fungal endophytes associated with bamboo using different agricultural wastes such as rice bran [9], rice straw and sugarcane bagasse [10], corn cob [11], water hyacinth [12] and bamboo leaves [13], has been recently conducted which proved the capacity of these fungal endophytes in enriching the nutritional contents of the substrates, thus are possible sources of single cell protein. This study was conducted to explore further the potentials of the fungal endophytes in single cell protein production using other agricultural wastes such as pineapple peel.

MATERIALS AND METHODS

Methodology was adapted from previous works of Valentino *et al*[14], Gabres *et al* [13], Paynoret *al* [11], Zamora *et al* [12] and Valentino *et al*[9,10]with some modifications.

Preparation of the Inocula

Fungal inoculants were grown in potato dextrose agar for seven days. Then fungal spore concentration were counted using

hemocytometer and was adjusted to 5.0×10^6 cells per ml with sterile distilled water.

Preparation of Substrates

Pineapple peels were collected from the public market of San Jose City, Nueva Ecija, Philippines. It was sun dried and pulverized into powdered form. One hundred (100) grams of dried pineapple peels were placed in a clean wide mouth bottle and 200 ml distilled water was added to the substrate. It was sterilized at 15 psi at 121°C for one hour.

Inoculation of Fungal endophytes in

Pineapple peel

Ten ml of the adjusted spore suspension of different fungal isolates was aseptically transferred to the substrate. The inoculant 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 was pulverized using sterilize mortar and pestle. Dried and pulverized samples were analyzed for crude protein content.

Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA). Means were compared by Duncan's Multiple Random Test (DMRT) at 5% level of probability.

RESULTS AND DISCUSSIONS

Protein enrichment of pineapple peel with fungal endophytes (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ochraceus*, *Cladosporium cladosporioides*, *Fusarium semitectum*, *Fusarium* sp.1, *Fusarium* sp.2, *Monascus ruber* and *Penicillium citrinum*) was evaluated after 20 days of solid state fermentation.

Crude Protein Content (CPC) of Fungal Endophytes Treated Pineapple Peel

Single Cell Protein also termed as microbial protein are produced by culturing microorganism in different substrates which include agricultural wastes that can be utilized as carbon source for microbial growth. As revealed in the results of the study (Table 1), solid state fermentation of pineapple peel with nine fungal endophytes associated with bamboo had resulted to further enrichment of the crude protein content of the pineapple peel. Results showed that *C. cladosporioides*-treated pineapple peel had the highest CPC of 9.24% followed

by *Fusarium* sp. 2, *A. flavus* and *F. semitectum* - treated pineapple peel with 7.65%, 4.26% and 4.17%, respectively. Meanwhile, the inoculated pineapple peel had the least CPC of 3.31 % followed by *P.citrinum*-treated pineapple peel had CPC of 3.61% and *A. niger*- treated pineapple with 3.73%.

Statistical analysis for the CPC of the fungal enriched pineapple peel indicate significant differences among the treatment means. Treatments means of the fungal enriched pineapple peel were significantly higher than the CPC of the uninoculated pineapple peel. This suggests the potential of the endophytic fungi in enhancing the CPC of pineapple peel thus they could be sources of single cell protein.

Percentage Increase in Crude Protein Content of the Fungal Enriched Pineapple Peel

For the percentage increase in crude protein content of the fungal enriched pineapple peel, *C. cladosporioides*- treated pineapple peel obtained the highest computed percentage increase of 178%, followed by *Fusarium* sp 2- treated pineapple peel of 131% and *A. niger*- treated pineapple peel with 28.7%. *P.citrinum*- treated pineapple peel had the least percentage CPC with

8.95%. Statistically, significant differences among treatments were observed. Differences in the computed percentage increase in CPC can be attributed to varying physiological and nutritional requirements which influence the growth of the aforementioned fungal endophytes and the potential production of various microbial enzymes.

Results confirmed the previous reports obtained using the same fungal endophytes in different post agricultural waste such as rice bran [9], sugarcane bagasse and rice straw [10]. The mycoprotein production of genus *Fusarium* was proven by the studies of Weibe [15] and Hosseini and Khosravi-Darani [16], Hosseini et al [17] using *F. venenatum* and Ahangi et al [18] using *Fusarium oxysporium* wherein single cell protein was optimized using various carbon sources. Likewise, the psychrophilic lignocellulosic enzymes system of *C.cladosporioides* including ligninase and polysaccharide hydrolases has also been investigated [19]. Meanwhile, species of genus *Aspergillus* is one of the first filamentous fungi with SCP potential due its ability to produce wide range of carbohydrate –hydrolyzing enzyme which were considered as GRAS (Genetically Regarded as Safe) [20].

Apparently, the percentage increase in crude protein content vary depending on the growth of fungal inoculant. It was found that the mycelial yield of fungal biomass vary depending upon the substrates and the organisms used [21]. As cited by Misra et al. [22] various fungi can be used in fermenting crop residues under solid state fermentation which could lead to protein enriched feeds. They have the same potential as yeast which can assimilate sugars. Additionally, they have the ability to produce enzymes which can decompose and degrade various agricultural wastes [23].

Based from these findings, pineapple peel is suitable for the production of single cell protein by the nine fungal endophytes which would lead to further utilization of pineapple wastes. Correspondingly, about

40-50% of pineapple fruits are considered wastes which include the pineapple peel and core. These contain sucrose, glucose, fructose and other useful nutrients [24, 25]. Additionally, the conversion of these nutrients into useful products of higher value added products like ethanol or it can be utilize as raw material for other industries. Pineapple peel also contains total sugar of 9.75%, non-reducing sugar of 8.8% and proteins which are suitable as a nutrient medium and carbon source for microbial growth and fermentation to produce ethanol and single cell protein. Thus, these could be new sources of bioprotein production, with high nutritonal value which can be used mainly for animal feed [26, 27, 28, 29, 30].

Table 1: Crude protein content (CPC) of endophytic fungi-treated pineapple peel

Treatments	Crude Protein Content (%)
Control (Uninoculated pine apple peel)	3.31 ^f
<i>Aspergillusflavus</i> - treated pineapple peel	4.26 ^c
<i>Aspergillusniger</i> - treated pineapple peel	3.73 ^e
<i>Aspergillusochraceus</i> - treated pineapple peel	3.87 ^{ed}
<i>Cladosporiumcladosporioides</i> - treated pineapple peel	9.24 ^a
<i>Fusariumsemitectum</i> - treated pineapple peel	4.17 ^{dc}
<i>Fusarium sp.1</i> - treated pineapple peel	3.70 ^e
<i>Fusarium sp.2</i> - treated pineapple peel	7.65 ^b
<i>Monascusruber</i> - treated pineapple peel	3.89 ^{ed}
<i>Penicilliumcitrinum</i> - treated pineapple peel	3.61 ^{fc}

*Treatment means with the same letter are not significantly different at 5% level of significance

Table 2: Percentage increase in crude protein content of endophytic fungi-treated pineapple peel

Treatments	Percent increase in CPC
Control (Uninoculated pine apple peel)	0.00
<i>Aspergillusflavus</i> - treated pineapple peel	28.7 ^c
<i>Aspergillusniger</i> - treated pineapple peel	12.8 ^{ed}
<i>Aspergillusochraceus</i> - treated pineapple peel	16.8 ^{ed}
<i>Cladosporiumcladosporoides</i> - treated pineapple peel	178 ^a
<i>Fusariumsemitectum</i> - treated pineapple peel	25.9 ^{cd}
<i>Fusarium sp.1</i> treated- treated pineapple peel	11.7 ^c
<i>Fusarium sp.2</i> treated- treated pineapple peel	131 ^b
<i>Monascusrubertreated</i> - treated pineapple peel	17.4 ^{ed}
<i>Penicilliumcitrinum</i> treated- treated pineapple peel	8.95 ^c

CONCLUSION

The nine fungal endophytes have the ability to enrich the crude protein content of pineapple peel, thus they are possible sources of single cell protein. Interestingly, pineapple peel is a good source of carbon for fungal growth. Meanwhile, in depth investigation must be carried out to determine the toxicity of the fungal enriched pineapple peel before its utilization as substitute to various protein sources.

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