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CRUDE PROTEIN CONTENT PROFILING OF ONION STALK (*Allium cepa* L.) AS INFLUENCED BY ENDOPHYTIC FUNGI

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ABSTRACT

The study ulitized onion stalk as substrate for the production of single cell protein of the fungal endophytes associated with bamboo using solid state fermentation. Crude protein content and the moisture content of the fungal enriched onion stalk were analyzed.Results showed that onion stalk enriched with *P.citrinum*- had the highest crude protein of 19.06% followed by *A. flavus* and *Fusarium*sp.1–enriched onion stalk both with 18.7%. Similarly, for the percentage increase in crude protein content, *P.citrinum*–enriched onion stalk had the highest percentage increase with 18.80% followed by *A.flavus– enriched* onion stalk with 16.59% while *A.niger* -enriched onion stalk had the lowest percentage increase with 5.21%. Meanwhile, reduction in moisture content of the substrates to about 56% was also recorded. Thus, an indication of fungal growth and production of enzymes which led to the production of single cell protein and increased in crude protein content of the fungal enriched onion stalk.

Keywords: Crude protein content– fungi- moisture- onion stalk-single cell protein

INTRODUCTION

Onion (*Allium cepa* L.) belongs to the family, Amaryllidaceae, of the genus, Allium. They are perennial herbs having bulbous and scented underground stems and are classified as vegetable which add to taste and flavor to food. Onion contains carbohydrate, total sugar, vitamins-, proteins, fats, volatile compounds and other trace elements [1, 2]. Additionally, onion has been reported to possess hypocholesterolemic, thrombolitic and antioxidant effects [3-8]. However, increase in onion production also produces a large amount of onion wastes, which produce foul odor during decomposition and could pose possible harm to the environment. Thus, utilization of this waste is yet to be explored.

Onion wastes are rich in dietary fiber, mainly in insoluble fraction, and in total phenolics and flavonoids, with high antioxidant activity, high concentration of quercetinaglycone and calcium [9]. These characteristics make it a good candidate as substrate for single cell production of the fungal endophytes.

Single cell proteins (SCP) also known as biomass, bioprotein, or microbial protein can be produced due to the ability of the microorganisms to proliferate in expensive substrates utilizing them as carbon sources converting it to protein[10]. Hence the conduct of the study to determine the crude protein enhancing capability of the nine fungal endophytes using onion stalk.

METHODOLOGY

Methodology was adapted from previous studies dealing with single cell production using the same fungal endophytes with different substrates [11-16].

Preparation of the Inocula

Fungal inoculants which include *Aspergillus niger, Aspergillus flavus, Aspergillus ochraceus, Fusarium* sp.1, *Fusarium* sp.2, *Fusarium semitectum, Monascus ruber, Penicillium citrinum, Cladosporium cladosporioides* were grown in potato dextrose agar for seven days. The fungal spore concentration was standardized to 5.0 x 10^6 cells per ml with sterile distilled water.

Preparation of Substrates

Onion stalks were collected from the fields of Bongabon, Nueva Ecija. It was sun dried and pulverized into powdered form. Dried onion stalks were analyzed for its Crude Protein Content (CPC) using Kjeldahl method. This CPC served as the initial CPC of the substrate. Then, 100 grams of dried onion stalk were placed in a clean mayonnaise bottle and 150 ml distilled water was added. These were covered with plastic and were sterilized at 15 psi at 121˚C for one hour.

Single Cell Protein Production

The sterile substrate was inoculated with 10ml of fungal inoculants. Solid state fermentation took place for 20 days. After which, the fermented fungal enriched substrate were sterilized at 15 psi at 121˚C for one hour, was air dried for seven days and finally it was pulverized manually using sterilize mortar and pestle. Dried and pulverized samples were analyzed for the crude protein content (CPC) using Kjeldahl method.

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 DISCUSSION

Crude protein profiling of the fungal enriched onion stalk was done to determine the potential of onion stalk as substrate for SCP of the fungal endophytes.

Crude Protein Content

The crude protein content (CPC) of the protein enriched onion stalk with their corresponding percentage increase in CPC is shown in Table 1. Results showed that onion stalk enriched with *P.citrinum* had the highest CPC of 19.06% followed by *A.flavus* and *Fusarium sp.1* –enriched onion stalk both with 18.7%. Moreover, the unfermented onion stalk had the least CPC of 16.04% followed by *A. niger*- enriched onion stalk with 16.87%. Similarly, for the percentage increase, *P.citrinum*–enriched onion stalk had the highest percentage increase with 18.80% followed by *A.flavus*– enriched onion stalk with 16.59% while *A.niger*-enriched onionstalk had the lowest percentage increase with 5.21%. Statistical analysis for the CPC of the fungal enriched onion stalk shows significant differences among the treatment means. Treatment means of fungal enriched onion stalk were significantly higher from the untreated dried onion stalk. This signifies that the nine endophytic fungi has the potential in increasing the CPC of onion stalk, thus could be a possible source of single cell protein.

Findings of the recent study coincides with the previous studies using the same fungal endophytes in different agricultural wastes such as pineapple peel [18], rice bran [11], sugar cane and rice straw [12]. These results give proof that these fungal endophytes are able to increase the crude protein content of different substrates using solid state fermentation and are potential candidate as sources of single cell protein. Additionally, onion stalk can be considered as good source of carbon for the growth of fungal inoculants.

Results can also be attributed to the microorganisms, substrate and method of fermentation used [18, 19]. According to Farinas et al [20], filamentous fungi are best suited to solid state fermentation since it mimics their natural habitat, wherein thereof they are capable of synthesizing amounts of enzymes and other metabolites. Whereas, agricultural wastes composed by cellulose, hemi-cellulose, lignin, starch, pectin and other fibers are potential substrate for microbial growth and synthesis several enzymes as products [19, 20]. Hence, the increment in protein contents could be due to the ability of the organisms to secrete extracellular protease during the fermentation process [21]. The multiplication of the fungi in the form of single cell protein could also contribute to the increase in the protein contents [22]. These waste products has been converted to biomass, protein concentrate or amino acids using proteases derived from certain microorganisms and are rich in some growth factors required by microorganisms [23]. Various hydrocarbon, nitrogenous compounds, polysaccharides and agricultural wastes such as hemicelluloses and cellulose waste from plants and fibrous proteins such as horn, feather, nail and hair from animals are also abundant waste products for the production of SCP [24].

Also, based on several studies, filamentous fungiare sources of enzyme amylase [24], proteases [25], pectinases [26], cellulase [27], xylanase [28], gluco amylase [29] and beta-glucodase [29]. Fungi have been reported to synthesize fungal proteins and subsequently add these to the protein content of the substrate [30-32].

Moisture Content

Moisture content of the fungal enriched onion stalk is presented in table 2. Moisture content was evaluated to determine the fungal growth as indicated by the decreases in the moisture content. Reduction in moisture content, can be attributed to enzymatic activities and microbial growth. As presented on table 2, results in moisture content showed that the untreated dried onion stalk had the highest moisture content which is 19.94% followed by *M.ruber*-enriched onion stalk with 15.26%. On the other hand, *A.flavus*- enriched onion stalk had the lowest moisture content which is 8.74% followed by *P. citrinum,* and *Fusarium sp.2* –enriched onion stalk with 10.95% and 11.58%, respectively. Statistical analysis showed that the moisture content of the fungal enriched dried onion stalk was significantly lower than the untreated dried onion stalk. Thus, prolific microbial growth and enzymatic activities which led to crude protein enrichment.

Table 1:Crude protein content (CPC) fungal enriched onion stalk

Treatments	Crude Protein Content (%)	Percent increase in CPC
Control (Uninoculated onion stalk)	16.04[d]	0.00
Aspergillusflavus- enriched onion stalk	18.70[a]	16.59[a]
Aspergillusniger- enriched onion stalk	16.87[cd]	5.21[c]
Aspergillusochraceus- enriched onion stalk	17.35[bc]	8.14[bc]
Cladosporiumcladosporoides- enriched onion stalk	18.36[a]	14.48[ab]
Fusariumsemitectum- - enriched onion stalk	18.35[a]	14.30[ab]
Fusarium sp.1 treated- - enriched onion stalk	18.70[a]	16.58[a]
Fusarium sp.2 treated- - enriched onion stalk	18.10[ab]	12.82[ab]
Monascusruber- - enriched onion stalk	17.41[bc]	8.52[bc]
Penicilliumcitrinum- - enriched onion stalk	19.06[a]	18.80[a]

* Treatment means with the same letter are not significantly different

Table 2: Moisture content of the fungal enriched dried onion stalk

Treatments	Moisture (%)	% reduction in moisture content
Control (Uninoculated onion stalk)	19.94[a]	
Aspergillusflavus- enriched onion stalk	8.74[e]	56.17
Aspergillusniger- enriched onion stalk	12.82[c]	35.71
Aspergillusochraceus- enriched onion stalk	14.78[b]	25.88
Cladosporiumcladosporoides- enriched onion stalk	12.63[c]	36.66
Fusariumsemitectum- - enriched onion stalk	12.79[c]	35.86
Fusarium sp.1 treated- - enriched onion stalk	12.59[c]	36.86
Fusarium sp.2 treated- - enriched onion stalk	11.58[c]	23.47
Monascusruber- - enriched onion stalk	15.26[b]	41.92
Penicilliumcitrinum- - enriched onion stalk	10.95[d]	45.08

* Treatment means with the same letter are not significantly different

CONCLUSION

Based from the results of study, onion stalk can be used as substrate of single cell production of the nine fungal endophytes resulting to the increment of crude protein content and reduction of the moisture content of the fungal enriched onion stalk.

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