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Single Cell Protein Production by *Trichoderma harzianum* Using Waste Banana Peel

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Abstract: The aim of the present study was to apply the submerged fermentation (SmF) for the production of single cell protein (SCP) using banana peel waste as substrate. A local isolate of *Trichoderma harzianum*, produced significant level of biomass yield in the presence of different carbon and nitrogen sources. Sodium nitrate was found to be an effective nitrogen supplementing source, as it gave the higher sporulation and biomass yield. The maximum sporulation and biomass was observed when medium supplemented with sucrose as carbon source. Nutrient found in banana peel extract were 6.0±0.5% crude protein, 5.1±0.05% crude fibre, 3.7±0.03% fat and 57.1±0.02% carbohydrate.

Key words: Single cell protein · *Trichoderma harzianum* · Banana peel extract · Carbon sources · Nitrogen sources

INTRODUCTION

Most of the developing countries of the world have been facing malnutrition problem. The deficiency of protein in human food and animal feed is well recognized due to the rapid growth of population. Use of microbes as a food source may appear to be unacceptable to some people but the idea of consumption of microbes as food for man and animals is certainly innovative to solve the global food problem. Algae, Fungi and Bacteria are the chief of microbial protein that can be utilized as a protein supplement [1, 2]. Many fungal species are used as protein-rich food. They provide the B-complex group of vitamins and they also show a low level of nucleic acid content [3]. The amino acid composition of *Aspergillus niger* according to FAO standards is well balanced [4]. Single cell protein from mixed cultures of *Trichoderma reesei* and *Kluyveromyces marxianus* are reported to contain essential amino acids which compares favorably with FAO guidelines and soybean oil meal [5].

Banana (*Musa paradisiaca*) fruit peel is an organic waste that highly rich in carbohydrate content and other basic nutrients that could support microbial growth. In tropical climates, such as in India, the banana trees continue bearing fruit throughout the year. Sugar represents that part of the fruits which is used by microorganisms for single cell protein for food and feed applications [6-8]. The use of such a cheap and readily available substrate is desirable to lower the cost of production, reduce waste disposal and management problems, conserve natural resources and provide feed for livestock purposes. Therefore, the use of banana peel extract as a fermentation medium was taken into consideration as an alternative raw material for the production of *Trichoderma harzianum* biomass in this investigation. The effect of various carbon and nitrogen sources on biomass production was also studied.

MATERIALS AND METHODS

Microorganism: A local isolate of *Trichoderma harzianum*, from decaying wood in the soil, was used for the experimental studies. Identification of isolate was carried out based on cultural characteristics and microscopic morphology with reference to the manual of Rifai [9]. The stock culture was maintained on potato dextrose agar slants at 27±1°C. The subculturing was done once in fortnight.

Inoculum: A spore suspension was prepared by adding sterile distilled water to stock culture to get $80x10^6$ spores/ml.

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Preparation of Banana Peel Extract: A 500g ripe banana fruits were obtained from the fruit market, Hyderabad city. The fruits were washed with several changes of sterile water and peeled off. The peels were cleaned initially with 2% solution of H_2SO_4, cut into small pieces, rinsed in sterile water and pulverized into slurry using a sterile blender. The peel extract was obtained from slurry filtered with the help of cheese cloth. The extract was placed into a sterile container and the crude protein, fibre and total carbohydrate were determined.

Effect of Carbon and Nitrogen: From the extract, 100ml was measured into sterilized 250 ml conical flasks. To each different carbon sources were added i.e., sucrose, glucose, fructose, lactose, ribose, starch, mannose and maltose at 3g/100 ml only, sodium nitrate at 0.2g/100 ml was used as nitrogen source. Similarly for studying nitrogen sources i.e., sodium nitrate, potassium nitrate, ammonium nitrate, sodium nitrite, ammonium sulphate and ammonium oxalate at 0.2g/100 ml only, sucrose at 3g/100ml was used as carbon source supplement. The pH of the medium was adjusted to 6.5 with 0.1M NaOH. The conical flasks were plugged with sterile cotton wool and aluminium foil and autoclaved at 121°C for 15 min. On cooling, the medium in the flasks was inoculated with 1ml of inoculum ($80x10^6$ spores/ml). These flasks were incubated in an orbital cooled shaking incubator (Gallenkamp) at 200 rpm for 5-7 days at 25±1°C. The culture broth was separated from mycelium after incubation period by filtration through Whatman No 1 filter paper.

Determination of Mycelial Biomass: At the end of incubation, the mycelial dry weight was recorded after filtering the mycelial mats on Whatman No1 filter paper and dried at 60°C for 24 h in a hot air oven. The extent of sporulation was determined by Haemocytometer counts [10].

Analytical Methods: The method for the determinations of crude fat, crude fibre and total carbohydrate content were those recommended by AOAC as described earlier [11, 12]. Moisture content was determined by the method as described earlier [13], based on the principle of drying to constant weight. The biomass was expressed in terms of total protein content. The protein estimation was determined according to the method described by Lowry *et al.* [14] using bovine serum albumin as a standard. Estimation of inorganic phosphate and magnesium content was determined according to the method described by Cooper and Simmons [15]. Ash content was obtained by igniting the samples in a muffle furnace at 55°C as previously described by Pearson [16].

RESULTS AND DISCUSSION

The isolated fungal culture obtained from decaying wood in the soil was related mainly to the generic nomenclature *Trichoderma* known as *Trichoderma harzianum*.

The results of the chemical analysis of the banana peel extract are presented in Table 1. Banana peels which contain variable ingredients and these may be used as a carbon energy source for the growth of fungi in the production of single cell protein. The effect of different carbon sources on the mycelial dry biomass and protein of *T. harzianum* was recorded in Table 2. From the results it was revealed clearly that the *T. harzianum* showed marked selectivity in utilizing different carbon sources. Among all these carbon sources, the supplementation of banana peel extract with sucrose gave the highest protein yield. Sporulation and biomass yield was also maximum in sucrose followed by glucose and maltose. Poor growth and biomass yield was obtained when supplemented with ribose. In case of nitrogen sources, the supplementation of banana peel extract with sodium nitrate gave the highest protein yield of 0.78±0.01g/l followed by 0.69±0.03g/l when supplemented with ammonium nitrate (Table 3). Banana peel is one of the most abundant and locally available agricultural waste which contains variable ingredients such as carbohydrate that may be used as a carbon and energy source for the growth of fungi in the production of single cell protein [7, 8]. The addition of nutrient supplements provided available nitrogen source for the organism thereby enhancing its growth [17].

Table 1: Percentage chemical composition of banana peel extract

Ingredient	Percent (%)
Crude protein	6.0±0.50
Crude fat	3.7±0.03
Crude fibre	5.1±0.05
Carbohydrate	57.1±0.02
Moisture content	72.5±0.03
Inorganic phosphate	0.2±0.05
Total ash	11.0±0.02
Magnesium	-

*Data is expressed as mean ±SD of three replicate samples

Table 2: Effect of various carbon supplements on growth and protein yield of *T. harzianum*

Carbon sources	Mycelial dry wt. (mg)	10^6 spores/ml	Total protein (g/l)
Fructose	527.51±0.01	56.24±0.08	0.54±0.03
Glucose	840.35±0.03	62.57±0.01	0.67±0.01
Maltose	821.62±0.01	61.05±0.02	0.62±0.24
Mannose	456.00±0.15	52.61±0.01	0.51±0.02
Sucrose	961.57±0.24	69.56±0.01	0.73±0.05
Lactose	418.35±0.01	50.35±0.03	0.43±0.15
Ribose	256.00±0.10	42.36±0.24	0.37±0.01
Starch	523.51±0.01	53.57±0.1	0.53±0.03

*The values were the mean of five fermentations

Table 3: Effect of various nitrogen supplements on growth and protein yield of *T. harzianum*

Nitrogen sources	Mycelial dry wt. (mg)	10^6 spores/ml	Total protein (g/l)
Ammonium nitrate	967.57±0.01	69.57±0.01	0.69±0.03
Ammonium sulphate	952.36±0.03	67.00±0.03	0.67±0.00
Ammonium oxalate	493.97±0.02	46.00±0.01	0.43±0.02
Sodium nitrate	970.53±0.03	72.51±0.01	0.78±0.01
Sodium nitrite	859.51±0.01	65.35±0.02	0.54±0.05
Potassium nitrate	913.36±0.01	67.51±0.01	0.59±0.15

*The values were the mean of five fermentations

There was a higher growth in banana peels substrate due to the presence of proteins, minerals, vitamins and other soluble carbohydrates which served as source of nutrients [18]. The carbohydrate and protein content of banana peels is an indication that the waste could serve as a possible alternative substrate for cultivation of fungi [19].

In conclusion, a higher yield of biomass production from *T. harzianum* was possible by banana peels. The supplementation of banana peel extract with various carbon and nitrogen nutrition improved the *T. harzianum* growth. The biomass yield was best with banana peel extract medium with sodium nitrate as the nitrogen source and sucrose as the carbon source for single cell protein production. Banana peels offer a good option, if researches on the possibilities of augmenting its nutritional status are carried out, otherwise the potential of the moulds to utilize the substrate could be harnessed for effective waste management. It is recommended that the study on single cell protein production by *T. harzianum* using banana peels should be conducted on large scale. Extensive toxicological and acceptability tests should be performed before the product is approved for large scale consumption.

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