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Production of Single Cell Protein (SCP) with *Aspergillus terreus* Using Solid State Fermentation

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Abstract: The deficiency of protein in human food and animal feed is well recognized due to the rapid growth of population. It is therefore, important to increase protein production by utilizing all the available ways and means. In the light of this, an attempt has been made in the present study by selecting a fungus, *Aspergillus terreus*. Its protein content was studied in various culture conditions and its nutritive value was assessed. Because of versatile nature of the fungus, lack of demand for any specific growth factors, rapidity of growth to turn out biomass in considerably large quantities and non pathogenicity of *A. terreus* strains, it is considered as useful species to examine it as a candidate species for SCP. The results have shown that the *A.terreus* possesses a high protein value and can be used as a better choice for SCP production using cheap energy sources like Eichornia and Banana peel.

Key words: Single Cell Protein · Eichornia, Biomass · Banana Peel · Solid State Fermentation

INTRODUCTION

The problem of providing protein rich food to the ever increasing population is more acute in the developing countries like India where the per capita availability of pulses have come down and the animal feed compounding industry is not yet well established. Under these conditions, use of microorganisms for protein could be a complementary solution.

The growing shortage of proteins and protein rich foods has stimulated the searching for new alternatives. Single cell protein (SCP) is used as protein rich source in human and animal foods [1]. Single cell protein is the dried cell mass of algae, fungi, moulds and bacteria grown in large scale to supplement the protein in animal and human foods [2]. Initially yeast was used for human foods but later the research was diverted in using it in animal feeds due to acute shortage of soy been and fish meal across many countries [3-5]. SCP was produced in Germany during First World War with *Sacharomyces cerviceae* by using molasses as carbon source and ammonium salts as nitrogen sources [6]. Single cell protein produced by culturing different microorganisms [7, 8] on different substrates such as whey [9], starch [10], cellulose [11, 12], hydrocarbon [13] and alcohol [14].

It is convenient to use fungi and bacteria for production of SCP when grown on inexpensive waste material. The rapid growth and high protein content have made them the prime candidates for use as sources of SCP. Many fungi like *A. niger AS101*with corn cobs [15], *Chrysonilia sitophilia* with lignin [16], Marine yeast with prawn shell waste [17], *Paecilomyces variolii* with sulfite liquor [18], White rot fungi with sugar cane baggase [19], *Candida utilis* with rice polishing [20], Kefir yeast with milk whey [21] and *A. niger* with rice bran [22] were opted for the production of SCP.

Currently, SSF is being used for the production of protein-enriched feed [23, 24]. Industrial scale application of SSF for production of SCP would help in increased yields and improved conversion efficiency, which would reduce the overall cost of the final product. In the present study an attempt has been made to produce SCP with *A. terreus* through SSF by using Eichornia and banana peel as cheap energy sources.

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MATERIALS AND METHODS

All the chemicals used in the present study were procured from Qualigens, Mumbai and ingredients for microbial media were procured from Hi-Media, Mumbai.

Isolation of Fungus: The fungus was isolated from soil of Harrison Institute Of Biotechnology, Nellore surroundings of Andhra Pradesh, India, subjected for serial dilution and inoculated into PDA plates and the fungi obtained was identified by using microbial atlas [25].

Preparation of Inoculums: Spores were harvested from a week old slant culture. The spores were gently scraped off with the help of a sterile inoculation needle. This was added to 100 ml conical flask containing 50 ml distilled water and was used as inoculum in all experiments.

Fermentation Media for Solid State Fermentation (SSF): One of the objectives of the study was to check the suitability of Eichornia and banana peels as the substrates for the production of SCP by SSF. In addition, the solid state fermentation was also carried out with different agricultural wastes individually and in combinations to compare the protein content of the fungi with different agricultural wastes with that of Eichornia and banana peels. The substrates used for SSF were, rice bran with and without pre-treatment, Eichornia with and without pre-treatment, wheat bran, banana peels, Eichornia+banana peels, Eichornia+wheat bran and banana peels+wheat bran. In the combination studies both the substrates were taken in equal proportions (1:1). Medium composition for SSF (g/100 ml); substrate 5.0, KNO_3 0.3, $MgSO_4$ 0.1, $ZnSO_3$ 0.1 and $FeCl_3$ 0.001.

Optimization Studies For SSF: Production of SCP by the fungal isolates using Eichornia and banana peels as the substrates in solid state fermentation (SSF) was optimized by controlling different physico-chemical parameters like concentration of substrate, nitrogen source and other components in the medium i.e. $MgSO_4$, $ZnSO_4$, $FeCl_3$, pH range and incubation temperature for the maximum yield of biomass with high protein content were studied. The optimization experiments were conducted uniformly by varying one compound at a time and keeping the other conditions constant. All the optimization studies were carried out at the room temperature for 5 days unless otherwise indicated.

Optimization Of The Substrate: In the present study, Eichornia and banana peels were used as the substrates. In a series of conical flasks, equal proportions of Eichornia and banana peels were taken in the concentrations of 1, 2, 3, 4 and 5%. The media were autoclaved and inoculated with the fungal isolates. The flasks were kept for 5 days at room temperature and on the 5th day, the biomass was collected and the total protein content was estimated by Lowry method [26].

Optimization of the KNO_3: To determine the optimum concentration of KNO_3 varied concentrations of KNO_3 were taken from 0.1to 0.5%. After the incubation for 5days, the protein content was estimated.

Optimization of $MgSO_4$: For the optimization of MgSo4, 0.1to 0.5% concentrations of MgSo4 were taken and fermentations were carried out. After incubation, protein content was estimated.

Optimization of $ZnSO_3$: To determine the optimum concentration of $ZnSO_3$, different concentrations of $ZnSO_3$ between 0.001and 0.005% were taken and the fermentations were carried out.

Optimization of $FeCl_3$: For the optimization of $FeCl_3$, the concentration of $FeCl_3$ varied between 0.001to 0.005%. The biomass was collected on the 5th day of incubation and estimated for the protein content by Fc- Lowry method [26].

Nutritional Value of SCP: Nutritional value of the product was estimated on the basis of total protein content, amino acid profile, total carbohydrates, ash content and nucleic acid content of the mycelium. For all the tests carried out for the nutritional value of SCP, the fungi were inoculated into Czapekdox medium and the mycelia mat after 120 h of growth was separated by centrifuging the broth at 3000 rpm for 15 minute. The biomass was homogenized mechanically in a mortar and the homogenized solution was centrifuged at 6000 rpm for 20 minutes. The supernatant was taken to carry out the tests for nutritional value of SCP.

The total amino acid content was estimated by Ninhydrin method [27] and total carbohydrates by Anthrone method [28]. The amount of RNA and DNA in the mycelium was estimated by Orcinol [29] and DPA [30] methods respectively. For estimating ash content, 2 g

of the sample was weighed accurately into a crucible. The crucible was placed on a clay pipe triangulars and heated over a low flame till all materials were charred completely followed by heating in a muffle furnace for about 3-5 hours at about 600°C. It was then cooled in a dessicator and weighed to ensure completion of ashing. Crucible was again heated in muffle furnace for 1 h, cooled and weighed. This was repeated till two successive weights remain constant and ash was almost white or grayish white in colour.

RESULTS AND DISCUSSION

Isolation of Fungus: From the serially diluted soil solution, *A. terreus* was isolated. The fungus was maintained on PDA slants and was sub cultured every 15 days and stored in the refrigerator at 4°C.

Characterization And Description Of The Isolated Fungus: *Aspergillus terreus* colonies on potato dextrose agar at 25°C were beige to buff to cinnamon. Hyphae were septate and hyaline. Conidial heads were biseriate and columnar. Conidia were formed in long columns from the upper portion of the vesicle. Conidiophores were smooth-walled and hyaline, 70 to 300 µm long, terminating in mostly globose vesicles. Conidia were small (2 to 2.5 µm), globose and smooth.

Solid State Fermentation Using Different Substrates: The results for SSF were shown in Figure1. The results were expressed as mg of protein yield per gram of moldy substrate. Akram *et al*. [31] also studied *A. terreus* for the production of SCP using wheat bran as the substrate. In the present investigation, we found that wheat bran gave the highest mycelial protein in comparison with other substrates. The protein value is also higher than the value reported by Akram *et al*. [31]. Among different combinations of substrates, *Eichornia* and banana peel combination was more suited for the SCP production as the protein yields were nearly comparable if not equal to wheat bran and also these are 0-value substrates to produce value added SCP product. This will make very big difference when the economics of commercial production is considered.

Ravinder *et al*. [32] studied the SCP production by *A. oryzae* mutants using deoiled rice bran as substrate, which yielded high amount of protein. But as the strain was a mutant it is difficult to maintain and moreover the strain can revert back in course of time. But the strain used in the present study was wild and also easy to maintain. The protein yield in the present study is equally comparable with the results of Ravinder *et al*. [32]. Anupama and Ravindra [33] studied the effectiveness of rice bran as the substrate for SCP production by *A. niger* and the results of their investigation are nearly comparable with the results of the present study.

Optimization Studies: All the fermentations for optimization were carried out for 5 days of incubation at room temperature.

Optimization Of The Substrate: *Aspergillus terreus* showed maximum protein yield at 5% concentration of the substrate. The results were depicted in Figure 2.

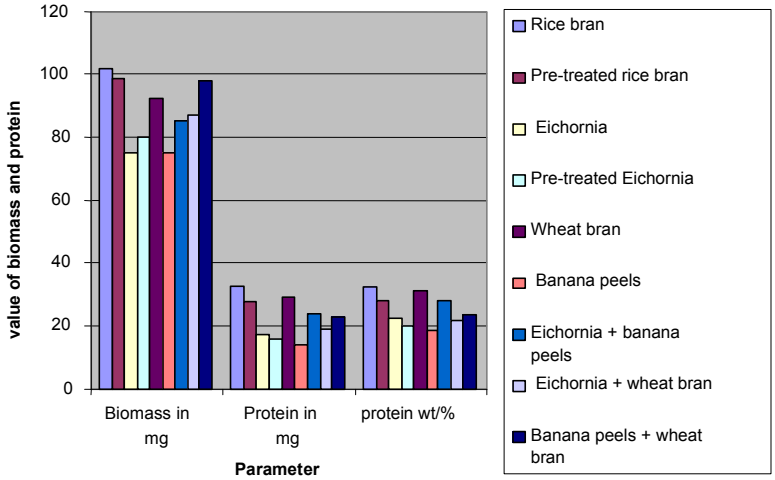

Fig. 1: Production of SCP with *A.terreus* using different cheap energy sources

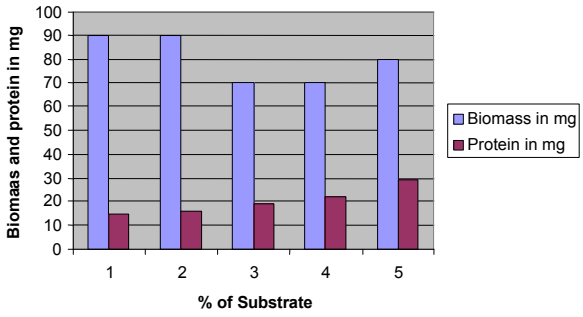

Fig. 2: Substrate optimization for the production of SCP with *A. terreus*

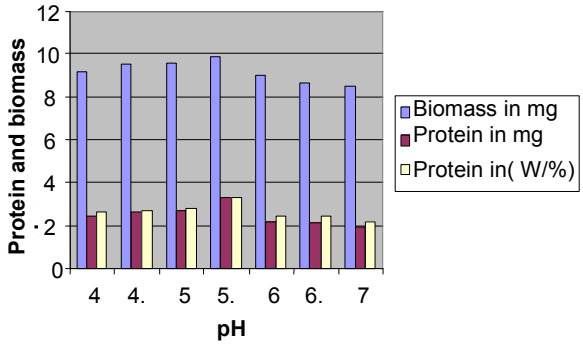

Fig. 3: Comparison of biomass and protein levels under different pH

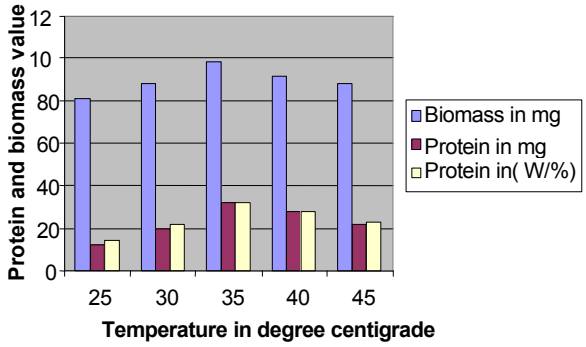

Fig. 4: Biomass production and protein levels under different incubation temperatures

Total protein content was expressed in mg/gm of moldy substrate. These results were deviated for *Candida utilis* when molasses is used as cheap energy source where the maximum production of protein was obtained at 2% concentration [34].

Optimization of KNO_3: The varied concentrations of KNO_3 were 0.1to 0.5%. *Aspergillus terreus* showed maximum protein yield at 0.3% concentration of the KNO_3. The results are almost similar with *Candida utilis* when ammonium nitrate was used as nitrogen source [35].

Optimization of $MgSO_4$: Similarly, the concentration of $MgSO_4$ varied from 0.1 to 0.5% and the fungus showed high protein yield at 0.1% of $MgSO_4$ concentration as the best concentration required for the high protein yield.

Optimization of $ZnSO_4$: For optimization of $ZnSO_4$, it was used in different concentrations ranging between 0.001 to 0.005%. The selected fungi showed high protein yield at 0.001%. As the concentration of the $ZnSO_4$ increased, the protein content was decreased.

Optimization of $FeCl_3$: The varied concentrations for $FeCl_3$ optimization were 0.001 to 0.005% and bets yield of protein with the selected fungi was obtained at 0.001%. As in the case of $ZnSO_4$, increasing concentrations of $FeCl_3$ decreased the biomass yield and the protein content.

Optimization of pH: The pH range for optimization was selected (4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0). *A. terreus* yielded high amount of protein at the pH of 5.5. The protein content of *A. terreus* increased gradually from the pH range 4.0 to 5.5 and then decreased rapidly (Figure 3). In a study with *Candida utilis* a pH of 6.5 was found to be best suitable for maximum protein production [35]. The same was also reported by Li *et al*. [36].

Optimization of Temperature: To determine the optimum temperature, the fermentations were carried out at different temperature i.e. 25, 30, 35, 40 and 45°C. The protein yield and the biomass turnover increased at 25 to 35°C and decreased gradually to 45°C (Figure 4). Ravinder *et al*. [32] reported that the effect of temperature on protein yield in *A. oryzae* mutants is increased from 20 to 35°C and decreased rapidly beyond that limit up to 45°C. Lemmal *et al*. [37] also showed that 32°C is best suited for *Candida utilis* [36]. The protein yield was maximum at 35°C i.e., 35 mg/g of deoiled rice bran. In the present investigation also the fungi showed luxuriant growth and high protein yield at 35°C. The results were deviated with *Chetomnium sps* and *A. niger* which showed maximum protein production at 32°C [38].

Nutritional Value of SCP: The nutritional value of the mycelium was assessed on the basis of guidelines given by FAO. Total amino acids content in the *A. terreus* was 37.5 % and total carbohydrate content was found to be 2.3%. While studying the nucleic acid content it was found that the DNA content is approximately 18.7mg/gm dry weight followed by the RNA content of 39.3mg.gm

Table 1: Nutritional value of *A. terreus*

Parameter	*Value*
Carbohydrates	2.3
Amino acids	37.5
DNA	18.7
RNA	39.3
Ash	8.9

dry weight of fungal mass (Table 1). In a study by Ravinder *et al*. [31], the nucleic acid content of *Asprgillus oryzae* mutants when deoiled rice bran was used as substrate for the production of SCP was higher than in the present study. The fungi in the present study showed much lesser amount of nucleic acid content than nucleic acid content reported in *Candida utilis* in RHH medium and *Candida krusei* [39]. The nucleic acid content is a bit higher than *Kluyveromyces fragilis* when the biomass was produced on deproteinized whey supplemented with 0.8% diammonium hydrogen phosphate and 10 ppm indole-3-acetic acid [40]. Ash content of *A. terreus* estimated with the help of muffle furnace. Which was 8.9%. The ash content of *Candida utilis* was higher than in the present observation when SCP was produced using Ram Horn Hydrolysate (RHH) [40] and when rice polishing was used as substrate [20]. The ash content of *Aspergillus niger* was higher when RHH was used as substrate [40]. A much greater ash content than in the present study was reported in *K. fragilis* when the biomass was produced on deproteinized whey supplemented with 0.8% diammonium hydrogen phosphate and 10 ppm indole-3-acetic acid [41].

The complete acceptability of fungi as animal nutritional supplement needs to be assessed before making any positive declaration. However, the present study in view of limited time frame, was restricted to the assessment or (and) improvement of fungal protein content and nutritional profile so that further work can be taken up in due course.

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