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# Potential Strain to Produce Bioprotein from Cheaper Carbon Source: Hope for Millions

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**Abstract:** Bioprotein production is one of the most promising breakthroughs of biotechnological innovations. Due to its increasing demand, the efficient strains, substrate and method must be used for high yield product. In this study, screening of five different *cerivisiea, Mucor hiemalis* and *Thricoderma harzianum*, was done for bioprotein production by liquid state bioconversion of wheat flour as a cheaper carbon source. Bioconversion was done with fixed wheat flour concentration of 2% (w/v) at a temperature 27°C, agitation of 150 rpm with 2% inoculum ($10^6$ spores $mL^{-1}$). Biomass production was recorded continuously for six days and the protein content was also determined every day. From the observed results, *Mucor hiemalis* was found to be the most potential strain with biomass of about 11.48 g $L^{-1}$ on the fourth day of treatment. With this promising result, the amount of bioprotein was further increased to 21.89 g $L^{-1}$ by optimizing few process factors. Further optimization developments are in progress. This study may provide a better alternative in agricultural products by converting cheaper carbon source to valuable and quality product bioprotein, which can be used as supplement and additive in the animal feed and food as well as in chemical and pharmaceutical industries.

**Key words:** *Mucor hiemalis*, screening, biomass, liquid state bioconversion, optimization

## INTRODUCTION

The significant increase in demand for livestock products in recent years in developing countries has required an increase in animal and human food supply. The importance of protein as food nutrient cannot be ignored because its deficiency can cause various malnutrition problems. This demands a search for new protein sources, with high nutritional value, economically feasible and locally available. Use of microbes as a food source is one of the biotechnological innovations that will certainly increase the availability of affordable protein in the world to solve the global food and feed problems. The production of bioprotein (protein derived from micro-organisms) by fermentation of wheat flour-a cheaper carbon source, is one of the most promising breakthrough of biotechnological innovations. This will certainly increase the availability of affordable good quality protein in the world. In addition to high quality, quantity will also be plentiful. It will reduce dependence on animal protein. This could be possible due to rapid growth rate of microorganisms and high production of bioproteins. Use of microbes as a food source may appear to be unacceptable for some people, but the idea of consumption of microbes as food for man is certainly innovative to successfully solve the global food problem[1]. Algae, fungi and bacteria are generally used as producers of bioproteins and can be utilized as a protein supplement because they are rich in protein, carbohydrates, fatty acids, vitamins and minerals. The protein extracted from cultivated microbial biomass, can be used for protein supplementation of a staple diet by replacing costly conventional sources like soymeal and fishmeal to alleviate the problem of protein scarcity[2]. The importance of protein in food nutrient cannot be neglected. Various malnutrition problems may occur due to protein shortage. This situation has created a demand for the formulation of innovative and alternative protein-rich food sources[2]. In addition to this, the food route represents highest immediate cash return because demand for food is huge and will remain stable and the technologies involved are too cost effective.

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Due to the increasing demand for bioprotein, the efficient strains, substrate and method must be used for higher level products. Various cheap carbohydrate sources are capable of supplying adequate calories to livestock such as wheat flour and cassava flour[3,4]. Wheat flour had long been are recognized as substrate that is full with nutrients and carbohydrate. Flour primarily consists of five nutrients: fat, minerals, moisture, starches and protein. Wheat flour could be a high quality substrate for bioprotein production due to its high carbohydrate (i.e. starch) value[5]. It is less expensive and easily available in Malaysia and other parts of the world. Therefore, in this research project, we introduced wheat flour as a cheaper carbon source for fermentation by a suitable microorganism to produce bioprotein. Selection of potential microorganism is necessary to produce maximum quantity bioprotein by liquid state bioconversion of substrate eg, wheat flour. Five different microorganisms-*Aspergillus niger (A.niger), Phanerochaete chrysosporium (P.chrysosporium), Saccharomyces cerivisiea (S. cerivisiea), Mucor hiemalis(M.Hiemalis)* and *Thricoderma harzianum (T. harzianum)* were selected from lab stock for screening. The experiment was conducted with fixed process conditions and the potential strain was selected on the basis of maximum biomass production and its protein content.

## MATERIALS AND METHODS

**Sample collection:** Wheat flour, bought from the local market, is used as raw material in this study.

**Microorganisms:** Five different microorganisms-*Aspergillus niger (A.niger), Phanerochaete chrysosporium (P.chrysosporium), Saccharomyces cerivisiea (S. cerivisiea), Mucor hiemalis(M.Hiemalis)* and *Thricoderma harzianum (T. harzianum)* were collected from lab stock at International Islamic University Malaysia, University Putra Malaysia and some other places. All strains were cultured, maintained on (PDA) slants and stored at 4ºC. Subculture was done once a month.

**Inoculum preparation:** Inoculum preparation (spore suspension) was done according to the popular and amicable method suggested[6]. Cultures grown on PDA medium in petri dishes at 32°C for 7 days were transferred into Erlenmeyer flask (250 mL) containing 100 mL of sterile distilled water. It was then shaken in a rotary shaker at 150 rpm for 24 hours. The suspended fungal cultures were filtered by Whatman #1 filter paper and the filtrate was used as inoculum after measuring its concentration (spores $mL^{-1}$) by Haemocytometer. Sterilization was done prior to addition of inoculum.

**Screening:** Screening was done in order to determine the potential strain for the maximum production of bioprotein. All selected strains were screened under controlled process conditions in 500 mL of Erlenmeyer flask having 2 % (w/v) of wheat flour. All experiments were conducted in a rotary shaker for six days by incubating at a temperature of 30°C, agitation of 150 rpm with 2% inoculum ($10^6$ spores $mL^{-1}$).Biomass was recorded (three replicates) on 2nd, $4^{th}$ and $6^{th}$ days.

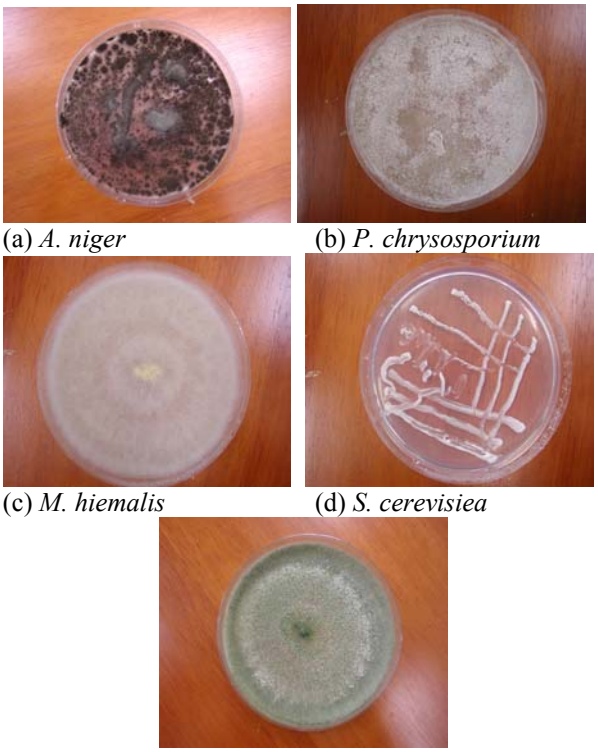
**Total protein determination:** Protein determination was done according to Lowry *et al.* (1951) method (Folin-Phenol Reagent)[7]. All reagents were prepared according to the suggested concentration and added to the sample solution as instructed in the method. Spectrophotometer reading was recorded at 660 nm after 20 minutes.

**Biomass analysis:** The biomass was filtered by vacuum filtration and washed three times with 20 mL of distilled water. Before taking the weight of the biomass, it was transferred into an aluminum disk and dried in an oven at 103ºC-105ºC for one hour followed by cooling in desicator to balance the temperature and weight[8].

## RESULTS AND DISCUSSION

**Evaluation of potential microorganism:** The images of each strain (*A. niger, M. hiemalis, P. chrysosporium, S. cerivisiea* and *T. harzianum*) cultured on PDA plate are shown in Fig. 1. Screening was done to determine the best microorganism that can produce highest protein and maximum amount of biomass by utilizing wheat flour.

**Biomass production:** Biomass concentration was one of the parameters used to evaluate the potentiality of microorganisms. The fermentation was conducted continuously for six days and each experiment was replicated three times. Biomass was determined on second, fourth and sixth days of the fermentation process. The concentration of biomass on different days of treatment period is shown in Fig. 2. All strains did not give similar trend for dried biomass concentration. *T. harzianum* and *M. hiemalis* obtained the optimum biomass of 10.7 g $L^{-1}$ and11.4 g $L^{-1}$ respectively on $4^{th}$



(a) *A. niger* (b) *P. chrysosporium*

(c) *M. hiemalis* (d) *S. cerevisiea*

(e) *T. harzianum*

Fig. 1: Culture of microorganisms on PDA plate

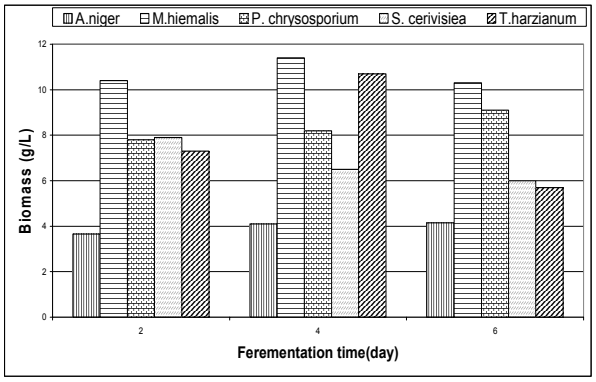



Fig. 2: Biomass concentration varies with fermentation time

day with an increase from day 2 to day 4 and then decreased on 6th day. This result indicated that these strains were already in exponential phase on day 4. After that they entered the death phase. *A. niger* showed a continuous growth until the last day of fermentation but the amount of biomass was lowest as compared to others. On the other hand, dried biomass of *S. cerevisiea* decreased gradually along the screening period from 7.9 to 6.5 g $L^{-1}$ and 6.0 g $L^{-1}$ on day 2, 4 and 6 respectively.

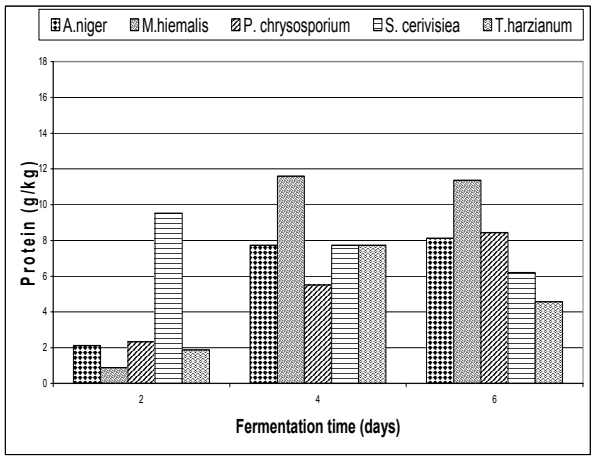



Fig. 3: Protein concentration varies with fermentation time

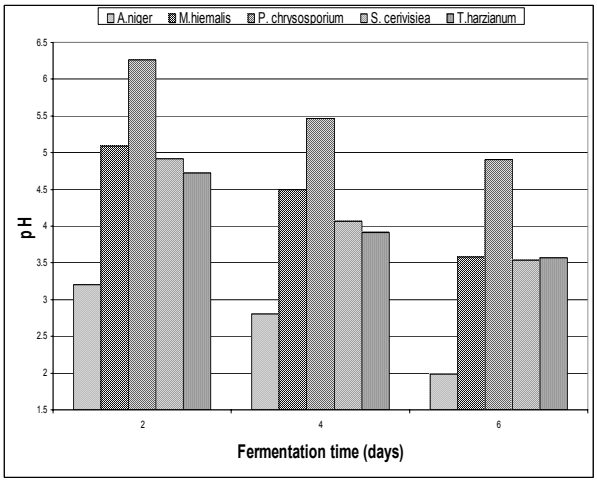



Fig. 4: pH varies with fermentation time

*P. chrysosporium* showed good growth of 9.1 g $L^{-1}$ on the sixth day of fermentation. This biomass concentration could not be considered as highest biomass concentration because this strain did not reach maximum growth yet and were still in growth phase. Considering the growth curve in batch fermentation, generally, the biomass should increase exponentially as the cell is growing and when the cells enter the decline phase or death phase, biomass will decrease[9].

**Protein concentration:** The concentration of protein in biomass during sixth days olong fermentation period is shown in Fig. 3. The protein productions by each strain slightly differ from each other. The fermentation time for maximum production of bioprotein was different for every microorganism. *A .niger* and *P. chrysosporium* showed highest concentration on sixth day, *M. hiemalis*

Table 1: Values of bioprotein with change in media composition

| Run | Wheat Flour ($g\ L^{-1}$) $X_1$ | $NH_4NO_3$ ($g\ L^{-1}$) $X_2$ | $KH_2PO_4$ ($g\ L^{-1}$) $X_3$ | Bioprotein concentration ($g\ L^{-1}$) |
|---|---|---|---|---|
| 1 | 1.5 | 0.2 | 0.05 | 7.308 |
| 2 | 1.5 | 0.2 | 0.15 | 9.026 |
| 3 | 1.5 | 0.4 | 0.05 | 8.99 |
| 4 | 1.5 | 0.4 | 0.15 | 10.146 |
| 5 | 3.5 | 0.2 | 0.05 | 19.18 |
| 6 | 3.5 | 0.2 | 0.15 | 20.29 |
| 7 | 3.5 | 0.4 | 0.05 | 21.7 |
| 8 | 3.5 | 0.4 | 0.15 | 21.13 |
| 9 | 0.5 | 0.3 | 0.1 | 3.249 |
| 10 | 4 | 0.5 | 0.1 | 21.89 |
| 11 | 2.5 | 0.1 | 0.1 | 14.69 |
| 12 | 2.5 | 0.5 | 0.1 | 20.53 |
| 13 | 2.5 | 0.3 | 0 | 10.35 |
| 14 | 2.5 | 0.3 | 0.2 | 16.322 |
| 15 | 2.5 | 0.3 | 0.1 | 18.44 |
| 16 | 2.5 | 0.3 | 0.1 | 18.368 |

and *T. harzianum* on fourth day while *S. cerevisiea* on second day of fermentation. The strain *M. hiemalis* showed the highest protein concentration of 11.6 g $kg^{-1}$ and 11.4 g $kg^{-1}$ on second and fourth day of fermentation respectively as compared to other strains. Although two strains, *A .niger* and *P. chrysosporium* had an increasing trend until the last day (sixth day), but the protein concentration was not as high as *M. hiemalis* on fourth day. Even though *S. cerevisiae* had highest biomass concentration of 9.5 g $Kg^{-1}$ on the second day itself as compared to other strains, we could not select it as a potential strain due to a decrease in concentration after that. One of the objectives of this project is to obtain maximum concentration of bioprotein. Therefore, *M. hiemalis* was more preferable in this case because this strain produced highest biomass as well as high concentration of protein.

**pH variation:** The values of pH for all the samples with different microorganisms can be seen in Fig. 4. All five microorganisms showed a decreasing trend in the pH values. The variation in pH is different for every strain. The lowest pH was observed for *Aspergillus niger*. On the day 6, the measured pH of *A. niger* was 1.98. It was expected so because *A. niger* had been widely used in citric acid production[10]. This pH is strongly acidic and not suitable for bioprotein or food production. For *P. chrysosporium,* it was less acidic on the last day as compared to other microorganisms. *T. harzianum*, *M. hiemalis* and *S. cerevisiea*, the value of pH was almost same on the day 6. On the fourth day, the pH of *M. hiemalis* was 4.5 and it seems to be suitable for maximum biomass production and protein concentration.

**Media optimization for bioprotein production:** After selecting *M. hiemalis* as the potential strain for the bioprotein production using wheat flour as a substrate, we evaluated its performance by improving media compositions. Three factors were selected for optimization of media; wheat flour concentration ($X_1$), nitrogen concentration ($X_2$) and nutrient supplement concentration ($X_3$). Bioprotein concentration was recorded on the 4th day of fermentation. The experimental results showed an increase in the bioprotein concentration up to 21.89 g $L^{-1}$ (Table 1).

## CONCLUSION

Screening was done to select the best strains for bioprotein production by evaluating the strains in terms of total concentration of biomass and bioprotein produced. From the observed results, *Mucor hiemalis* was identified as the most potential strain for the maximum bioprotein production having a concentration of about 11.598 g $Kg^{-1}$ on the fourth day of treatment. The maximum biomass of 11.4 g $L^{-1}$ was also obtained on fourth day of fermentation of wheat flour. The performance of the potential strain was satisfactory during media optimization. The study for the optimization of the process conditions is under progress and hopefully the amount of bioproteins can be further increased. This study may provide a better alternative by converting cheaper carbon source into useful and valuable bioprotein, which can largely be used as supplement and stabilizer in food and feed as well as an additive in the chemical and pharmaceutical industries.

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