Production of Fungal Protein from Cellulosic Plant Materials

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The ability of some fungal cultures, 5 strains of **Aspergillus niger**, *one strain each of* **Penicillium chrysogenum, Pestalotia** *sp. and a* **Basidiomycete** *to produce microbial protein on three different pretreated cellulosic substrates rice straw, bagassse and groundnut shells was studied.* **Penicillium chrysogenum** *strain St-F- 3B was found to be the best organism for the purpose and it produced maximum protein when grown on alkalitreated rice straw (ATS) as substrate. The growth pattern of this strain on ATS-mineral media was studied. Effect of some physical conditions like pH and substrate concentration on protein production was also studied. Maximum protein of 85 mg. per gram substrate was produced after 62 hrs. incubation on a rotary shaker, when the initial pH was kept in the range of 3.5 to 60 at 1% (W/V) substrate level.*

Cellulose is one of the most widely occurring renewable organic compounds. A. technology developed to utilize cellulose for the production of edible protein could alleviate to some extent the menace of protein malnutration in developing countries. Cultivation of fungal mycelium on cellulosic substrates protein product appears feasible. The use of fungi as food is not new. Pringsheim and Lichtenstein (1920) reported feeding animals with **A. fumigatus** grown on straw (2). Fungi have been shown to be an acceptable source of edible protein (1, 3 & 4). In the present study we have screened a few fungal isolates for their ability to produce biomass. A suitable culture and a suitable substrate have been selected and some of the conditions for cultivation optimized.

Materials and Methods

Five strains of *Aspergillus niger*, Rlm-1 Rlm-5, Rlm-6, Rlm-11 and F-2 were isolated in our laboratory by enrichment technique. Three cultures, *Penicillium chrysogenum* St-F-3B, *Pestalotia* sp. and a *Basidiomycete* F-88 were obtained from National Collection of Industrial Micro-organisms, National Chemical Laboratory, Poona. All the cultures were maintained on a modified potato dextrose agar medium which contained 0.01% yeast extract and 0.5% dextrose (instead of 2%) by monthly transfers at 27°C and stored at 4°C.

Penicillium chrysogenum St-F-3B was used for detailed studies Three cellulosic substrates viz. rice straw, powdered bagasse and powdered groundnut shells were subjected to a similar pre-treatment with 4% NaOH (1 : 50 W/V) and steaming for 60 min. for delignifying and to make them more susceptible for fungal growth. The substrates were used at 5% wet weight level (W/V) which corresponded to 1.65% rice straw, 1.2% of bagasse and 1.9% of groundnut shells on dry weight basis for screening studies. In optimization studies alkali-treated straw (ATS) was used at 1% (W/V) dry weight level after drying and grinding the treated material. In substrate concen-

tration studies 0.5 to 2% ATS were used, the mineral concentration being altered accordingly.

The mineral medium used in these studies was similar to that used by Srinivasan and Han (5). Initial pH was adjusted to 5.5 in all cases unless otherwise mentioned.

The inocula for these studies were obtained from 48 hr. fungal cultures grown on the corresponding media and were added at 5% (V/V) level.

Unless otherwise stated, 100 ml portions of the mineral medium were placed in 500 ml shaker flasks which contained cellulosic substrates as stated above and autoclaved at 121°C for 20 min. After cooling and inoculating the medium with selected fungi, the flasks were incubated on a rotary shaker (230 r. p. m.) at room temperature (23–28°C). For screening growth was examined under microscope in each flasks every 25 hrs. and were harvested after about a week's growth. For the following studies *P. chrysogenum* St.-F-3B was used. In kinetic studies the contents of the flasks were harvested at 0, 6, 12, 18, 24, 48, 72, 96 and 120 hrs. of incubation. For optimization of other physical conditions like pH and substrate concentration, the culture was cultivated for 72 hrs. For optimizing the pH the initial pH of the medium was varied from 2.0 to 8.0, pH being adjusted with HCl or NaOH solutions.

The contents of the flaks in all the cases were harvested, by filtering and washing through previously dried and weighed filter paper (Whatman No. 1). The product with the filter paper was dried at 55°C. and weighed. The products were powdered after scraping and then were taken for various analyses.

Estimation of crude protein was done by first analysing for total nitrogen by micro-Kjeldahl method and then convertig to protein by multiplying by a factor of 6.25 *Cellulose* was estimated by Updegraff's procedure (6).

Table 1 – Relative Growth of Some Fungal Isolates on Different Cellulosic Substrates

Organism	Substrate used		
	Alkali-treated rice straw	Alkali-treated bagasse	Alkali-treated groundnut shells
	Protein produced* (mg/gm substrate on dry weight basis)		
1. *Penicillium chrysogenum* St-F-3B	72.56	72.91	41.05
2. *Aspergillus niger* Rlm-11	60.45	—	27.64
3. *Aspergillus niger* F-2	65.45	31.88	23.74
4. *Aspergillus niger* Rlm-5	57.58	—	29.47
5. *Pestalotia* sp.	47.87	31.88	24.68
6. *Aspergillus niger* Rlm-1	23.33	18.84	29.47
7. *Aspergillus niger* Rlm-6	22.27	16.77	23.74

*mg protein calculated for gram substrate after substracting the initial protein value of the substrate,

Results and Discussion

Of the three cellulosic substrates tried, treated rice straw appeared most suitable for fungal growth. (Table 1). Out of the eight fungal cultures screened, *Penuicillium chrysogenum* St-F-3B utilized and produced more protein on all the three substrates. Maximum protein of 85 mg/gm of substrate was prooduced 72 hrs. incubation and remained constant although cellulose utilization continued upto 96 hrs. During growth the pH dropped from the initial 5.2 to 2.2 at 72 hrs. and remained constant thereafter.

The organism was capable of growing and producing maximum protein in the pH range of 3.5–6.6. pH values below 3.5 and above 6.5 appeared to have a deleterious effect on its growth and a pH of above 7.0 did not support growth at all.

An inverse relationship between the substrate concentration and the protein production per gram of substrate was observed (Table 2).

Table 2–The Effect of Substrate Concentration (Alkali-treated rice straw) on Protein Production by *P. chrysogenum* St-F-3B

Substrate concentration (gm/100m/on dry wt. basis)	Protein produced* (mg/gm substrate, dry weight)
0.50	100.0
0.75	82.0
1.00	74.2
1.25	74.7
1.50	63.8
2.00	58.5

* mg protein expressed after substrating the initial protein value of the substrate.

These observations suggested the possibility of increasing the efficiency of conversion of cellulose to protein by optimizing the composition of medium which was eventually done in our further studies.

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