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SINGLE CELL PROTEIN PRODUCTION FROM SUGAR BEET PULP

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Introduction

For the last few years efforts are being made to find out the possible application of Single Cell Protein (SCP) as solution to the world wide problem of protein deficiency[1-5]. The SCP may be used as food for direct human consumption or as animal feed stuff[6,7]. Various combinations of waste materials (bagasse and straws) and fungi have been tried to get SCP of acceptable nature[8,9].

Keeping in view the general programme designed to explore the potential of various cellulosic materials for their conversion into food and feed protein, the sugar beet pulp, a waste of sugar industry was used as a substrate for the production of SCP. Its feasibility as food and feed protein was studied by conducting feeding trials on albino rats.

Experimental

Four cellulolytic fungi: *Myrothecium verrucaria*, *Paecilomyces* sp., *Gliocladium* sp. and *Aspergillus terreus* were procured from the Department of Microbiology, Punjab Agricultural University, Ludhiana. These cultures were maintained by monthly transfers on glucose yeast extract agar medium and were stored at low temperature for further use.

The basal medium given by Chahal and Gray[13] with sugar beet pulp as cheap carbon source was employed in the present study. Solid material which remained after the extraction of juice was referred as beet pulp or shreads.

The flasks containing 50 ml of basal medium (pH 5·5) and one gram of sugar beet pulp were inoculated with different fungi and were incubated at 28± 0.5° for seven days. The cultures were harvested and cellulase activity in the culture filtrate was estimated by the method of Miller *et al*[11]. The biomass was dried at 60° C till constant weight was obtained. The crude fibre and crude fat were determined from the biomass by the standard methods of AOAC[12] and crude protein was estimated by the method of McKanzie and Wallace[13].

The amino acid analysis of the protein in biomass was done by a Beckman Model 116 amino acid analyser after hydrolysing the biomass with 6 N HCl at 110° C for 22 hours.

Feeding trial was conducted to study the effect of fungal protein and its supplementation to wheat flour on growth rate, Protein Efficiency Ratio and also its effect on different organs of albino rats. Diet

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TABLE I. SCP Production From Sugar Beet Pulp

Organism	Final Ph	CMCase activity (units)	Crude fibre (%)	Fat (%)	Wt. of biomass plus residual substance (mg/g of substrate)	Protein (%)	Total protein (mg/g of substrate)
Paecilomyces sp.	6·90	5·5	29·3	2·3	655	20·1	131·65
Myrothecium verrucaria	6·70	4·5	13·0	5·8	539	35·6	191·89
Aspergillus terreus	6·70	5·0	24·9	2·8	644	24·5	157·78
Gliocladium sp.	6·95	4·5	13·0	3·8	498	33·7	167·82

D_1, a control diet contained casein; diet D_5 had wheat flour only; diet D_2 and D_3 had 30 and 60 per cent SCP supplemented to wheat flour respectively, while D_4 had 100 per cent SCP only as a protein source. All the diets were maintained at 10 per cent protein level with adequate amounts of vitamins and minerals. The composition of various diets is shown in Table III.

Pure bred albino male rats 30 days old were weighed individually and randomly divided into five groups each containing five rats. The rats were housed individually in wire mesh bottom cages which were maintained in a well ventilated room.

Food and water were provided for *ad libitum* consumption in all experiments. Individual body weight (weekly) and food consumption were recorded (daily). At the end of the experiment, the rats were anaesthetised with ether and blood was collected from the aorta. Liver, kidneys and spleen were taken out from rat, and these organs were weighed after removing connective tissue. The liver nitrogen was determined by micro-kjeldahl method and plasma after centrifugation was also analysed for nitrogen. The haemoglobin content of blood of individual rats was estimated[14].

Results and Discussion

Four fungi viz. *Paecilomyces* sp., *Myrothecium verrucaria*, *Aspergillus terreus* and *Gliocladium* sp. were tried to investigate if these could utilize sugar beet pulp as a carbon source (Table-I). The biomass recovery was highest in case of *Paecilomyces* sp. (655 mg) followed by *Asp. terreus* (644 mg), the lowest by *Gliocladium* sp. (498 mg) per g of substrate.

TABLE II. Amino Acid Composition of Biomass (*M. verrucaria*)

Amino acids	g/100 g of Protein
Lysine	4·612
Arginine	3·814
Asparagine	8·180
Threonine	4·058
Serine	3·492
Glutamic acid	14·094
Proline	8·198
Glycine	3·938
Alanine	5·464
Valine	4·858
Methionine	1·124
Isoleucine	3·138
Leucine	6·060
Tyrosine	2·598
Phenylalanine	2·664

The values of essential amino acids are at par with FAO standards except that of methionine.

On the other hand protein percentage was highest in *Myrothecium verrucaria*

TABLE III. Composition of Diets

Ingredients	D_1	D_2	D_3	D_4	D_5
Vitamin mixture*	1·00	1·00	1·00	1.00	1·00
Salt mixture**	4·00	4·00	4·00	4·00	4·00
Dalda Ghee+	10·00	9·50	9·00	8·60	9·18
Cellulose	5·00	3·36	2·62	1·37	4·10
Casein	11·30	—	—	—	—
Whole wheat flour	—	50·00	25·00	—	76·20
Fungal biomass	—	8·43	16·86	28·10	—
Sucrose	5·00	5·00	5·00	5·00	5·00
Starch	63·70	18·73	36·52	51·17	0·52
	100·00	100·00	100·00	100·00	100·00

* Recommended by Chapman *et al*[17] 1959 for rats

** Recommended by Hawk *et al* 1954[18]

\+ Hindustan Lever Ltd., Bombay

D_1 — Casein; D_2 — SCP 30%; D_3 — SCP 60%; D_4 — SCP 100%; D_5 — Wheat flour

(35·6%) followed by *Gliocladium* sp.(33·7%) and the lowest by *Paecilomyces* sp. (20·1%)

M. verrucaria was grown on sugar beet pulp as a shake culture. SCP was harvested and dried in a hot air oven at 60°C and ground to a fine powder. Fungal biomass was analysed for amino acids. All the essential amino acids were present in sufficient quantities except that of methionine (Table II).

Then the nutritional evaluation of *M. verrucaria* was made using albino rats. The food intake, total weight gained and Protein Efficiency Ratio (PER) are shown in Table IV and Fig. The rats fed on diets of wheat flour supplemented with SCP (D_2 and D_3 diet groups) had sufficiently higher food intake, average weight gain and PER values, than the group D_5 rats fed on wheat flour only. However, the rats fed with SCP (100%) only had less PER value (1·82) as compared to D_2 (2·36) and D_3 (2·23) diet fed groups. This might be due to low digestibility, difference in palatability and presence of less amount of methionine in SCP as it was evident from Table II, however, the visual appearance of test animals as compared to control animals was normal. The amino acid profile of SCP from *M. verrucaria*, which was grown on sugar pulp, was

TABLE IV. Protein Efficiency Ratio of Values of Fungal Protein (10% protein level)

Diet	Feed intake (g)	Protein intake (g)	Gain in weight (g)	Mean PER
D_1 (Casein)	117·5	11·75	33·66	2·95
D_2 (SCP 30%)	105·2	10·52	24·83	2·36
D_3 (SCP 60%)	100·8	10·08	22·48	2·23
D_4 (SCP 100%)	101·4	10·14	18·45	1·82
D_5 (Wheat flour)	96·6	9·66	15·17	1·57

$$\text{PER} : \frac{\text{Gain in weight}}{\text{Protein intake}}$$

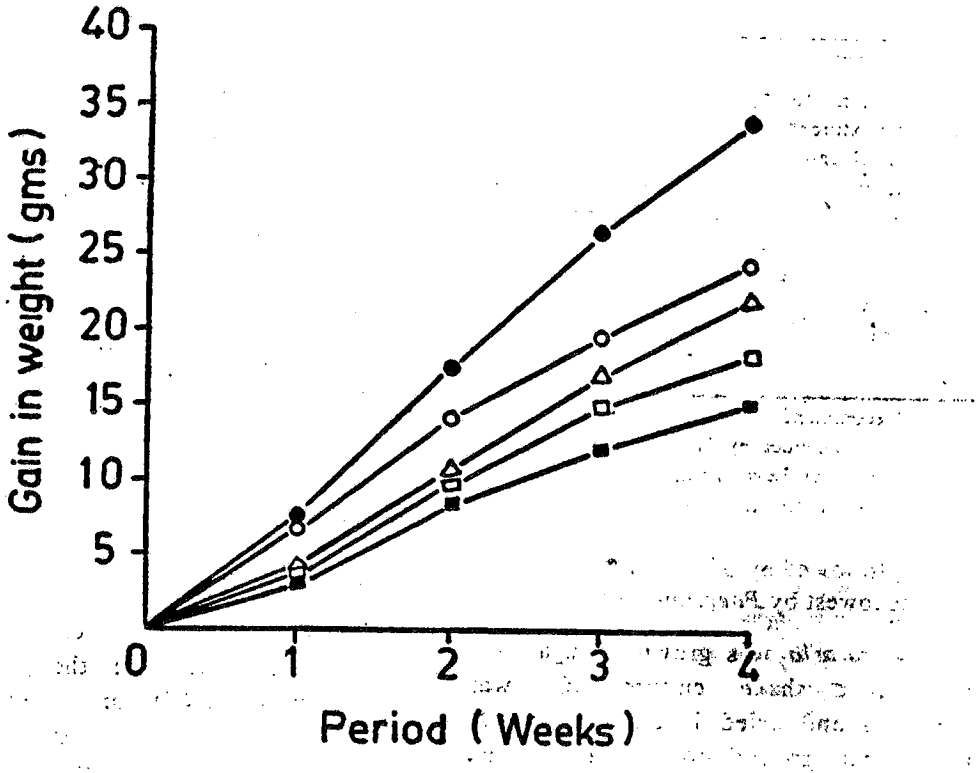

Fig Effect of SCP on growth rates of rats
●, Casein; ○, SCP 30%; △, SCP 60%;
□, SCP 100%; ■, Wheat flour.

TABLE V. Effect of Different Diets on Weight of Organs

Diets*	Liver wt. (g)		Spleen wt. (g)		Kidney wt. (g)	
	Total	Per 100 g body wt.	Total	Per 100 g body wt.	Total	Per 100 g body wt.
D_1 (Casein)	2·517	3·706	0·132	0·183	0·594	0·761
D_2 (SCP 30%)	1·988	3·202	0·093	0·155	0·473	0·789
D_3 (SCP 60%)	1·740	3·000	0·105	0·184	0·475	0·833
D_4 (SCP 100%)	1·710	3·160	0·080	0·149	0·455	0·860
D_5 (Wheat flour)	1·410	2·820	0·090	0·180	0·343	0·686

* As described in Table I.

determined. It was evident from the Table II, that methionine (1·124g/100g of protein) content was less in SCP as compared to FAO reference protein. The methionine content was also found less in the amino acid profile of *Cellulomonas* (1·8 g/100 g) and torula yeast (1·58 g/100 g)[15]. Yang[15] further reported that supplementation with methionine 0·3 per cent improves the growth rate. Presence of lesser amount of even a single essential amino acid lowers the biological value of protein. In an earlier communication from our laboratory[6] similar findings were reported when rats were fed with *Penicillium crustosum* SCP which had also lesser amount of sulphur containing amino acids[16].

TABLE VI. **Effect of Different Diets on Haemoglobin and Plasma Protein**

Diets*	Plasma protein	Haemoglobin
	g/100 ml. of plasma	g/100 ml. of blood
D_1 (Casein)	5·011±0·2	10·8±0·4
D_2 (SCP 30%)	4·314±0·1	10·4±0·3
D_3 (SCP 60%)	4·218±0·6	10·1±0·5
D_4 (SCP 100%)	4·286±0·3	9·5±0·2
D_5 (Wheat flour)	4·306±0·4	9·6±0·4

* As already described in Table I.

Effect of different diets on weight of organs (Table V) did not show significant difference among diets D_2, D_3 and D_4. However, significance in weight difference was quite evident as compared to control diet D_1. Liver nitrogen was the highest in rats fed with wheat flour diet and its value was the lowest in rats fed with flour D_5.

The effect of different diets on haemoglobin and plasma protein is given in Table VI. The values of plasma protein were slightly higher in rats of group D_1 while the values in rats of group D_2, D_3, D_4 and D_5 were almost same. Haemoglobin content of blood was 10·8±0·4 (g/100 ml of blood) for control group D_1. Among the remaining four groups of rats, the value of group D_2 rats was the highest (10·4±0·3).

Summary

Four cellulolytic fungi were grown on sugar beet pulp. *Myrothecium verrucaria* showed highest percentage of protein (35·6%) in biomass. The essential amino acid content of this protein (SCP) was at par with the standards laid down by FAO except that of methionine. Feeding experiments conducted on rats with protein from *Myrothecium verrucaria* revealed that its supplementation to wheat flour at a level of 30 per cent improved the growth rate and PER value as compared to wheat flour alone. The fungal protein thus can be supplemented at a level of 30 per cent to enhance nutritive value of wheat flour.

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