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A STUDY ON SYNTHESIS OF FUNGAL PROTEIN BY *ASPERGILLUS ORYZAE* ON MOLASSES, A BY-PRODUCT OF SUGAR INDUSTRY

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Aspergillus oryzae, Paecilomyces elegans and *Trichoderma viridae* were tried for the selection of a medium which will support rapid growth and form a thick mycelial mat with high fungal protein values. Protein values were high on molasses containing 9 % sugar as a source of carbon. The effect of the addition of different concentrations of KH_2PO_4 and $(NH_4)_2SO_4$ was also studied on the formation of the thick mycelial mat and protein values. The time required for optimum production of mycelium and fungal protein was also determined.

It was observed that *A. oryzae* was the most suitable fungus for these studies. 0.1% KH_2PO_4 and 1.4 % $(NH_4)_2SO_4$ when added to the medium did exercise beneficial effect. An incubation period of 5 days was found to be optimum for best mycelial growth and the production of fungal protein.

Key words: Fungal protein; *Aspergillus oryzae* and Molasses.

INTRODUCTION

The world population is increasing at an accelerated rate and though in few areas there is food surplus but vast segments of population suffer from food shortage, particularly protein shortage. In order to increase protein supplies several fungi have been tried by investigators to convert sugar and starches into protein [1,2,3,4].

Molasses is a cheaper source of carbon and a by-product of sugar industry. It was, therefore, considered worthwhile using it as a base material for the production of fungal protein [5,6,7]. Different concentrations of molasses were used to get optimum results. The time required for best growth of fungi was also studied. Besides, the effect of the addition of certain chemicals to molasses was also determined.

MATERIALS AND METHODS

A. oryzae, P. elegans and T. viridae were used in the present studies. These fungi were maintained on modified Czapeck's Dox Agar slants ($NaNO_3$, 3g;KH_2PO_4,1g; $MgSO_4.7H_2O$,0.5g; KCl, 0.5g; $FeSO_4.7H_2O$, 0.01g; Agar 20g and glucose 50g). Distilled water was added to make up to 1 lit. The above mentioned fungi were inoculated on Czapeck's Dox Agar Slants and incubated at 24-26^{o} for 7 days. These 7-day old cultures were inoculated on different media for studying the effect of cultural conditions on the production of fungal protein [8]. The following media were used :

1.	Molasses	6% (Sugar)
	$(NH_4)_2SO_4$	1.2%
	KH_2PO_4	0.3%
	$CaCO_3$	1%
2.	Molasses	6% (Sugar)
	Peptone	0.5%
	*CSL	3%
	$NaNO_3$	0.2%
3.	Molasses	6% (Sugar)
	Peptone	0.5%
	*CSL	3%
	$(NH_4)_2SO_4$	1.2%
4.	Molasses	6% (Sugar)
	$NaNO_3$	0.3%
	$(NH_4)_2SO_4$	1%
5.	Molasses	6% (Sugar)
	$(NH_4)_2SO_4$	1.2%
	KH_2PO_4	0.3%
	*CSL	3%

*CSL = Corn Steep Liquor.

Distilled water was added to make up to 1 litre and all the above mentioned media were distrubuted in 1 litre conical flasks containing 200 ml. of each medium.

Preparation of molasses. Molasses was diluted with water to get 6% sugar value in 200 ml of solution. The

diluted molasses was treated with potassium ferrocynide by boiling to avoid undesirable materials present in it. Diluted and treated molasses was thus used in media preparation.

Selection of media and organism. A batch of 10, 1 litre conical flasks, each containing 200 ml. of the above mentioned media, was separately inoculated with seven days old cultures of *T. viridae, P. elegans* and *A. oryzae.* The flasks were incubated at 24-26° for 7 days. After seven days *A. oryzae* showed satisfactory growth while *T. viridae* and *P. elegans* produced little mycelium. *A oryzae,* was therefore, selected for further work. Flasks containing *A. oryzae* were harvested and mycelial mats were removed and dried at 60° for 48 hr. The dried mycelia were then ground and assessed for protein values by the Kjeldhal method [9]. Protein values were calculated by multiplying proteinaceous nitrogen with the factor 6.25. It may be mentioned here that medium No. 5 was selected for use in further studies as it was found to be the best suited for growth of *A. oryzae* mycelium and yielded better protein values than on other media.

After selection of the medium (No. 5), the ingredients of the medium were varied in order to get maximum yield of mycelium with high protein values. After several experiments a concentration of ingredients was evolved to yield the maximum mycelial mat and best protein values. To determine optimum duration for the best yield of protein, *A. oryzae* was inoculated on medium No. 5 in four sets of 5 flasks (200 ml in each 1-litre conical flask) and incubated for 3,5,7, and 9 days.

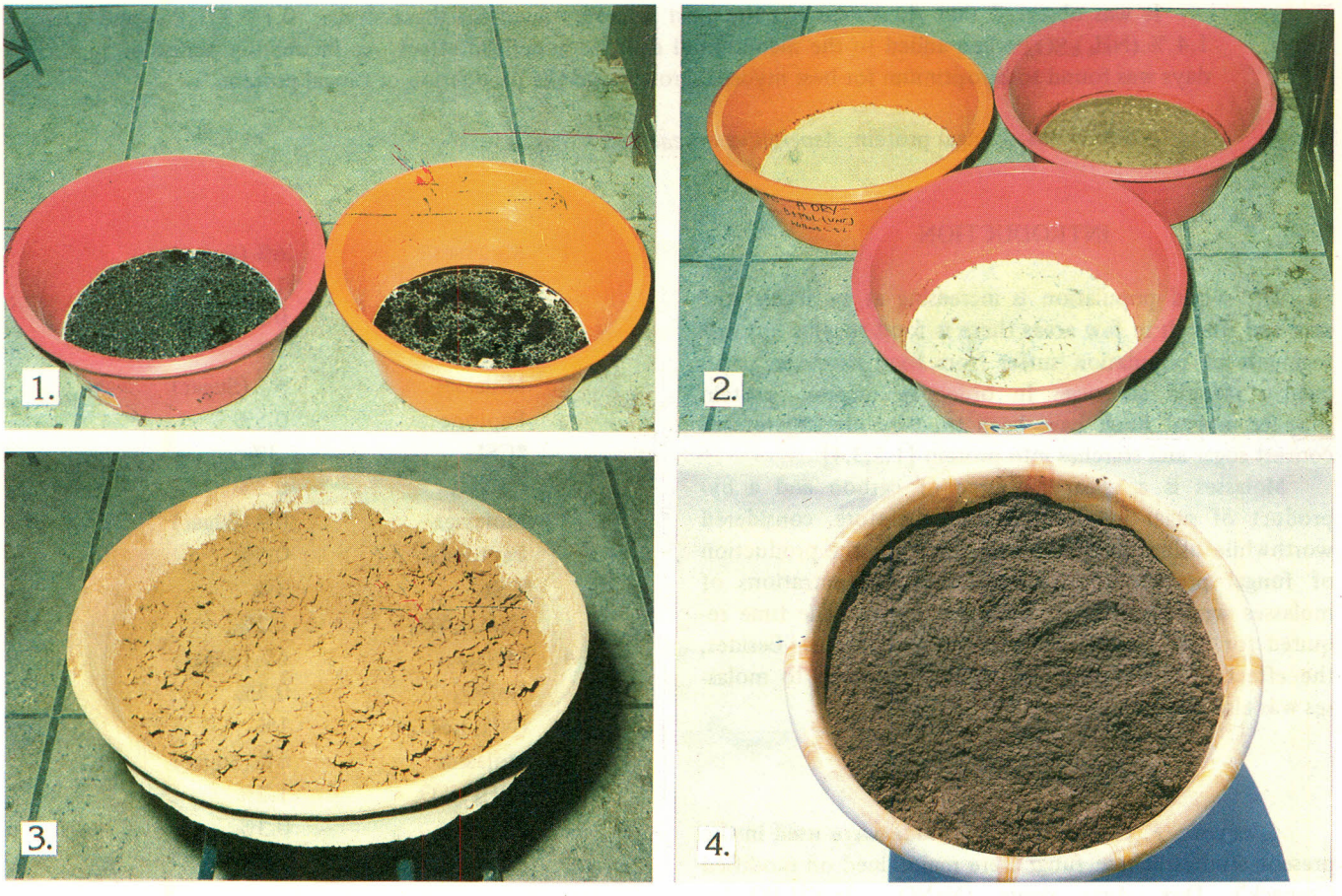

Fig. 1. 24 hr growth of *Aspergillus oryzae* on molasses.

Fig. 2. *Aspergillus oryzae* growing on molasses (bottom) 3 days after inoculation (top left); 5 days after inoculation (top right); 7 days after inoculation.

Fig. 3. Dried fungal mat (mycelium) of *Aspergillus oryzae.*

Fig. 4. Powdered mycelium of *Aspergillus oryzae.*

Table 1. Composition of different media alongwith dry weight of mycelium and protein values

S. No.	Composition of medium (1 lit.)		Dry weight mycelium (in gms.)	Protein values %
1.	Molasses containing	6.0% Sugar	24.5	37
	$(NH_4)_2SO_4$	1.2%		
	KH_2PO_4	0.3%		
	$CaCO_3$	0.1%		
2.	Molasses containing	6.0% Sugar	22	32.6
	Peptone	0.5%		
	CSL	3 %		
	$NaNO_3$	0.2%		
3.	Molasses containing	6.0% Sugar	23	34
	Peptone	0.5%		
	CSL	3.0%		
	$(NH_4)_2SO_4$	1.2%		
4.	Molasses containing	6.0% Sugar	20	36
	$NaNO_3$	0.2%		
	KH_2PO_4	0.3%		
	$(NH_4)_2SO_4$	1.0%		
5.	Molasses containing	6.0% Sugar	27.5	44.34
	KH_2PO_4	0.3%		
	CSL	3.0%		
	$(NH_4)_2SO_4$			

Table 2. Variation in medium No. 5 effect of varying concentration of molasses along with other ingredients on the production of mycelium and protein.

S. No.	Composition of medium (1 lit.)		Dry weight of mycelium (g)	Protein values (%)
1.	$(NH_4)_2SO_4$	12 g	32.3	43.2
	KH_2PO_4	0.5 g		
	CSL	50 g		
	Molasses	6 % (Sugar)		
2.	$(NH_4)_2SO_4$	12 g	30.5	40
	KH_2PO_4	0.5 g		
	CSL	60 g		
	Molasses	6 % (Sugar)		
3.	$(NH_4)_2SO_4$	14 g	31.0	43
	KH_2PO_4	1 g		
	CSL	40 g		
	Molasses	6 % (Sugar)		
4.	$(NH_4)_2SO_4$	14 g	38.3	44.23
	KH_2PO_4	1 g		
	Molasses	9 % (Sugar)		
5.	$(NH_4)_2SO_4$	14 g	32.6	34
	KH_2PO_4	1 g		
	CSL	70 g		
	Molasses	12 % (Sugar)		

RESULTS AND DISCUSSION

Three different moulds, *A. oryzae, T. viridae* and *P. elegans* were grown on different media for the production of fungal protein. The results show that *A. oryzae* was the best organism for the production of fungal protein, since it grew faster than the other two moulds.

A. oryzae was grown on five different media, all of them containing molasses as the carbon source. Among the five media used, medium No. 5 was the most suitable for best mycelial growth as well as fungal protein production. Other media did not support good growth of the fungus and did not yield appreciable protein values. The results are summarized in Table 1.

Table 3. Duration for the production of mycelium and protein values.

S. No.	Incubation period	Dry weight mycelium (g)	Protein values (%)
1.	3 days	23.7	46.5
2.	5 "	35.5	48.4
3.	7 "	37.8	44.14
4.	9 "	38.3	42.75

The effect on the production of fungal protein were also studied by varying the concentration of different ingredients of the selected medium [10]. From the results it was concluded that molasses containing 9% sugar in combination with 0.1% potassium dihydrogen phosphate and 1.4% ammonium sulfphate produced a thick mycelial mat which was also rich in protein values (45% approx. Table 2).

After varying the ingredients, duration for the production of high-value proteins, was also determined. It was observed that best protein values were obtained after five days of incubation (Table 3; Fig. 1 and 2 showing the growth of *A. oryzae* on molasses after 24 hr. and 3,5 and 7 days after inoculation). Figure 3 and 4 show the dried and powdered fungal mat of *A. oryzae* respectively.

REFERENCES

1. H.J. Bunker, Chem, Ind., London, 179 (1948).
2. D.W. Ribbons, Chem. Ind., London, **26**, 867 (1968).
3. William D. Gray (Southern IIIinois Univ. Carbon-dale), Advan. Chem. Ser. No. 57, 261-68 (1966 Eng).
4. A. Spicer, (Lord Rank Res. Cent., High Wycombe, Bucks., Engl.) Proc. Int. Symp. Convers. Manuf. Foodst. Microorganisms, 1971. (Pub. 1972), 221 - 3 (Eng).
5. L.A. Goncharova, (Technol. Inst. Food Ind., Leningard) Komovye Belki i Biastimulayatory dlya Zhivolnovodslva, Akad, Nauk S.S.S. R,Vases. Microbiol Obshchestvo, Sb Rabot. 1961,127-38.
6. J.S. Garcha, Charanjeet Kaur, Ajit Singh; R.K. Raheja, (Dept. Chem. Biochem, Punjab Agr. Univ. Ludhina, India), Indian J. Aim. Res., 7, 19 (Eng) (1973).
7. J.P. Shukla and S.M. Sarkara Dutta, 7, 102 (1966) (Eng).
8. C.W. Donald Graham, (Cornell Univ., Ithaca, N.Y) Diss. Abst. Int. B. **32**, 1723 (1971).
9. A.O.A.C, Official Methods of Analysis, (Washington, D.C. 1975), 12th ed., p. 309, 18.021.
10. N. Murtaza, S.A. Husain, I.H. Qureshi and M. Shameemullah, Pakistan J. Biochem., **12**, 18 (1979).