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PROPAGATION OF YEAST ON LEAF PROTEIN CONCENTRATE BY-PRODUCTS *

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Abstract. Nine strains of yeast were propagated on the filterates left after precipitation of proteins from leaf juices of *Trifolium alexandrinum*, *Phaseolus mungo* and *Cyamopsis psoralioides*. The growth of all the strains was supported by the filterates. Supplementation of the filtrates with glucose increased the cell yield. The combined effect of glucose and ammonium sulphate was maximum in case of *Candida utilis* No. 395.

Leaf juice extracted by the method of Morrison and Pirie[1] contains proteins and their breakdown products, lipids, carbohydrates, minerals, chlorophyll and other plant colouring matter. The proteins in this juice coagulate when subjected to steam at $70\pm2°C$ and can be separated by filtration through drill stockings. The fluid left after coagulation of proteins contains amino acids, amides, sugars and minerals, and is thus a rich medium for the growth of microorganisms.[2]

This paper deals with growth studies of nine strains of yeast on the fluid left after the precipitation of protein from leaf juices of *Trifolium alexandrinum*, *Phaseolus mungo* and *Cyamopsis psoralioides* with and without fortification with sources of carbon and nitrogen.

Materials and Methods

The leaf juice was extracted from lush green leaves of *Trifolium alexandrinum*, *Phaseolus mungo* and *Cyamopsis psoralioides*, using a Crypto electric mincing machine and by hand pressing. The proteins present in the juice were coagulated at $70\pm2°C$ by injecting steam and filtered through long cloth stockings. The filtrate, after sterilization, was stored for yeast propagation.

The total proteins present in the filtrate was estimated by a microkjeldhal method using $CuSO_4$–K_2SO_4–SeO_2 (1:9:0.02) mixture. Amide nitrogen was estimated after hydrolysis of 2 ml filtrate with 2 ml 4N H_2SO_4 at 100°C. The NH_3 present in the hydrolysate was estimated by titration against N/70 HCl after distillation. Sodium[3] and potassium[4] were determined flame photometrically, calcium by Versenate method[5] and iron by 2,4,6-tripyridyl-5-triazine method,[6] using Hellige direct reading colorimeter. Dry matter of the filtrate was estimated by keeping at 105°C till it attained a constant weight.

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Experimental

Strain Used. Nine strains of yeast were employed during these investigations, these were (1) *Candida utilis* NCYC No. 193; (2) *Candida utilis* No. 359; (3) *Torula utilis* NRC No. 862; (4) *Saccharomyces cerevisiae* IMI No. 39916; (5) *Rhodotorula* sp. U.A. M.H. 495 (6) *Torulopris magnoliac* IFO No. 0705; (7) *Candida guilliermondii* ATCC No. 9058; (8) *Debaryomyces subglobosus* NCYC No. 459, and (9) *Candida robusta* IFO No. 0735.

Preparation of the Inocula. The strains were maintained on a medium containing peptone 1.0%; yeast 0.5%; dextrose 0.3%; malt extract 0.3% and agar 2.00%. The 24-hr old cells were transferred into the broth of the same composition. The cells were allowed to grow for 24 hr, and a cell suspension of a definite optical density was prepared at 6000 Å, using a Beckman spectrophotometer.

Media Used. The filtrate, left after the coagulation of proteins from the leaf juice, was used as the basal media for yeast propagation. It was also fortified with glucose to bring reducing sugars level to 2%, and $(NH_4)_2SO_4$ content to 0.3%. The pH of the medium was adjusted to 5.0.

Propagation. Filtrate (49 ml) was sterilized at 15 lb/in² for 15 min in 250-ml Erlenmayer flasks. 1 ml of the inoculum was asceptically added to it. The flasks were kept at rotary shaker (100–120 rev/min) at $30\pm5°C$. The pH of the broth was maintained at 5.0 with dil NaOH after every 12 hr.

Harvesting. The flasks were removed from the shaker after 48 hr and the yeast cells were harvested by centrifugation. The cells were washed twice with distilled water and then dried at 105°C overnight.

Results and Discussion

Table 1 shows the chemical composition of the three leaf juice filtrates, i.e. *Trifolium alexandrinum*, *Phaseolus mungo* and *Cyamopsis psoralioides*. All the filtrates contained fair amount of sugars, nitrogen and minerals and can be used for the propagation of

TABLE 1. CHEMICAL COMPOSITION OF LEAF JUICE FILTRATE.

	Dry wt (g/100 g)	Reducing sugar (g/100 ml)	Total nitrogen (g/100 ml)	Amide nitrogen (g/100 ml leaf filtrate)
Trifolium alexandrinum	3·85	1·1	0·176	0·037
Phseolus mungo	4·50	1·0	0·259	0·017
Cyamopsis psoralioides	1·11	0·7	0·107	0·019

TABLE 2. MINERAL COMPOSITION OF LEAF JUICE FILTRATE (mg/litre).

	Ca	Fe	Na	K
Trifolium alexandrinum	200	0·7	3241·5	3616·7
Phaseolus mungo	165	2·5	689·7	3323·3
Cyamopsis psoralioides	44·8	0·6	1379·4	2150·5

TABLE 3. GROWTH OF YEAST ON LEAF JUICE FILTRATE (g/100 ml).

	Trifolium alexandrinum	*Phaseolus mungo*	*Cyamopsis psoralioides*
Candida utilis NCYC No. 193	0·72	0·58	0·50
Candida utilis No. NCYC 359	0·62	0·51	0·40
Torula utilis NRC No. 862	0·72	0·50	0·57
Saccharomyces cerevisiae IMI No. 39916	0·62	0·52	0·39
Rhodotorula sp. U.A.M. H495	0·59	0·29	0·25
Torulopsis mongolea IFO No. 705	0·52	0·55	0·42
Candida guiliermondii ATCC No. 9058	0·58	0·60	0·31
Debromyces subglobosus NCYC No. 459	0·51	0·36	0·35
Candida robusta IFO No. 735	0·33	0·34	0·29

yeast. *Trifolium alexandrinum* leaf juice filtrate, however, contained more reducing sugars and amide nitrogen. The concentration of Na, K, Ca, and Fe was also high in *Trifolium alexandrinum*, juice filtrate (Table 2).

Nine strains of yeast were separately tried on leaf juice filtrates of *Trifolium alexandrinum*, *Phaseolus mungo* and *Cyamopsis psoralioides*. Table 3 shows that all the strains propagated well on the filtrates. The cell yield of all the strains was comparatively more when *Trifolium alexandrinum* juice filtrate was used. This seems to be due to the fact that the filtrate contained higher amount of reducing sugars, nitrogen and minerals.

Four strains, i.e. *Candida utilis* NRC No. 193, *Candida utilis* No. 359, *Torula utilis* NRC No. 862, and *Saccharomyces cerevisiae* IMI No. 39916, which gave better cell yields were further examined. Table 4 shows the effect of supplementation of the filtrates with sources of nitrogen and sugars. It is evident that supplementation with a source of nitrogen resulted in a small increase in the cell yield. However, supplementation with glucose increased the cell yield by 22, 21.7 and 98.0% when *Trifolium alexandrinum*, *Phaseolus mungo* and *Cyamopsis psoralioides* filtrates were used respectively. Comparatively more increase, in the cell yield, after supplementation with glucose, in case of *Cyamopsis psoralioides* seems to be due to the fact that greater amount of glucose was added to it to bring its reducing sugar content to 2.0%.

Supplementation of the media with ammonium sulphate in addition to glucose resulted in increased growth of all the strains. However, *Candida utilis* No. 359 showed maximum growth in the medium, supplemented with both glucose and ammonium sulphate.

TABLE 4. EFFECT OF SUPPLEMENTATION ON THE GROWTH OF YEASTS (g/100 ml).

	Blank	Supplementation		
		$(NH_4)_2SO_4$	Glucose	$(NH_4)_2SO_4$ + glucose
Trifolium alexandrinum				
Candida utilis NCYC No. 193	0.720	0.736	0.764	0.886
Candida utilis No. 359	0.616	0.634	0.752	0.904
Torula utilis NRC No. 862	0.720	0.740	0.790	0.880
Saccharomyces cerevisiae IMI No. 39916	0.620	0.652	0.720	0.844
Phaseolus mungo				
Candida utilis NCYC No. 193	0.580	0.610	0.706	0.820
Candida utilis No. 359	0.680	0.702	0.768	0.840
Torula utilis NRC No. 862	0.728	0.744	0.794	0.886
Saccharomyces cerevisiae IMI No. 39916	0.520	0.538	0.614	0.752
Cyamopsis psoralioides				
Candida utilis NCYC No. 193	0.516	0.528	0.700	0.828
Candida utilis No. 359	0.406	0.526	0.804	0.900
Torula utilis NRC No. 862	0.620	0.654	0.826	0.886
Saccharomyces cerevisiae IMI No. 39916	0.390	0.420	0.484	0.852

References

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6. *Estimation of Iron by* 2,2′,2″*-Tripyridyl Method* (Standard methods for the examination of water and waste water APHA, AWWA, WPCF, 1960), p. 144.