PROPAGATION OF YEAST IN WHEY, A BY-PRODUCT FROM LEAF PROTEIN PRODUCTION PLANT

The exploitation of leaves as a source of protein in human nutrition has long been advocated. Protein calorie malnutrition is widely prevalent in many developing countries including India. Therefore a need for cheap, effective and acceptable form of protein, as a food supplement in malnutrition is essential. The results obtained from animal experiments were sufficiently encouraging to justify leaf protein as a valuable source of protein, which may be used as human and animal food.

Leaf protein can be extracted from fresh leaves very conveniently by IBP pulper and press. Heating the juice at 80°C results in the precipitation of total protein. The protein curd separates out by centrifugation and the uncoagulated supernatant or the de-proteinised juice from leaf protein production plant, which by analogy with cheese manufacture is often loosely called whey and has biological oxygen demand (B.O.D.) more or less equal to that of the sewage, as it chiefly contains carbohydrates, minerals, lipids, vitamins, colouring matter and soluble nitrogenous compounds. It should be used up to avoid local pollution. Attempts are being made to utilize the whey samples in place of costly microbiological growth media. Deproteinized juice from some plants (Brassica campestris, Raphanus sativus, B. napus, B. oleracea, Spinacia oleracea and Psophocarpus tetragonolobus) was collected after complete precipitation of the protein. The whey samples sterilized by autoclaving at 10 lbs. pressure for 10 minutes were stored for yeast propagation without any additional nutrients. The whey of each plant was analysed for soluble sugar (C-source) and total nitrogen content (N-source) by standard methods. The results are shown in Table I. Salts of Ca, Na, K, Fe and inorganic phosphate were also found to be present in all the whey samples.

Cultures of common yeast (Saccharomyces cerevisiae) were maintained in Sabouraud's agar medium Sucrose 20 g, peptone 10 g, agar 20 g, distilled water 1000 ml, pH—7.2 and this spore suspension (inoculum) was prepared from one day old culture in sterile water and was aseptically added to the flasks which were incubated on a rotary shaker (100—120 rpm) at 28°C for 48 hours. Growth of the yeast cell microorganism was measured by:

1. Turbidimetric method using a systronic colorimeter equipped with a red filter having transmittance near 600 m (Each whey sample which was inoculated and kept at —5°C (to check further growth) was used as blank.

2. Counting the number of viable cells/ml of the whey sample which was inoculated and incubated for 48 hours was done by dilution plate method. No. of viable cells per ml of the original inoculum (20 × 10^3 cells/ml) was taken as control.

The results are shown in Table I. All the results are the mean of triplicates.

### Table I

<p>| Chemical composition and propagation of Yeast in different whey samples** |
|---------------------------------|-----------------|------------------|----------------------|</p>
<table>
<thead>
<tr>
<th>Chemical composition of different whey samples</th>
<th>Dry weight g/100 ml</th>
<th>Anthron positive materials g/100 ml</th>
<th>Total nitrogen g/103 ml</th>
<th>Propagation of yeast in whey samples (after 48 hrs of growth) viable cell count/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Brassica campestris (Turnip)</td>
<td>3.6</td>
<td>0.80</td>
<td>0.250</td>
<td>80 × 10^6</td>
</tr>
<tr>
<td>2. Brassica napus (Mustard)</td>
<td>3.6</td>
<td>0.90</td>
<td>0.238</td>
<td>60 × 10^6</td>
</tr>
<tr>
<td>3. Raphanus sativus (Raddish)</td>
<td>2.3</td>
<td>0.80</td>
<td>0.220</td>
<td>75 × 10^6</td>
</tr>
<tr>
<td>4. Brassica oleracea var. botrytis (Cauliflower)</td>
<td>2.0</td>
<td>0.24</td>
<td>0.115</td>
<td>2 × 10^6</td>
</tr>
<tr>
<td>5. Spinacia oleracea (Spinach)</td>
<td>3.3</td>
<td>0.01</td>
<td>0.055</td>
<td>2 × 10^4</td>
</tr>
<tr>
<td>6. Psophocarpus tetragonolobus (Winged bean)</td>
<td>3.5</td>
<td>0.075</td>
<td>0.175</td>
<td>4 × 10^3</td>
</tr>
</tbody>
</table>

* Glucose equivalent  ** pH of whey samples—6
Table I shows that the yeast (Saccharomyces cerevisiae) has propagated more or less in all the samples. Out of 6 samples turnip whey was found to be the best basal medium for yeast propagation.

Bulk production of proteins is bound to create liquid waste disposal problems because for every 2 kg of leaves in a leaf protein production plant, a litre of the whey is produced. Utilization of the whey by the yeast would help to remove of carbonaceous and nitrogenous matter and at the same time production of yeast.

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3. — —, Street, Ibid., 1969, 11, 529.

OCCURRENCE OF "INTRACORTICAL AZOTOBACTER CHROOCOCCUM" IN SOME MONOCOTS

Bhide and Purandare reported, for the first time, the occurrence of Azotobacter chroococcum within the root cells of Cynodon dactylon (L.) Pers. and Cyperus rotundus L.2. Earlier, Dobereiner and Day showed the occurrence of the nitrogen fixed Spirillium lipferum (now Azospirillum lipoferm) in the root cells of Digitaria decumbens cv. transvaal, where the bacterium was found to be localized in the inner cortex and occasionally in the endodermis.

So far, the presence of the "Intracortical Azotobacter" (a term proposed by Purandare) has been reported in the case of Cynodon dactylon and Cyperus rotundus only, but the possibility of its occurrence in other monocot species cannot be ruled out. Therefore, to study the extent of such a type of Azotobacter grass association in the monocots, particularly in the grasses, a survey was undertaken. Commonly occurring monocot plants and grasses were collected from lawns, crop fields and barren lands around Pune and from deciduous forests of Mahabaleshwar, Dist. Satara. The roots of these plants were tested for the presence of bacteria by subjeing them to the tetrazolium technique described by Dobereiner and Day and used by Bhide and Purandare. Majority of the samples showed positive reaction to the tetrazolium test as evidenced by even reddening of sites harbouring tetrazolium reducing bacteria. Transverse sections through such areas showed presence of bacteria in the cortical cells under a high power microscope.

These bacteria could be isolated from the cortex of these plants on Jensen's N-free agar and were Azotobacter chroococcum as per Bergey's Manual 8th edition. Cultures of Intracortical Azotobacter isolated from inside the roots fixed from 3.0-6.0 mg/l of sucrose in Jensen's medium in 4 to 5 days at 30°C.

The following species showed the presence of "Intracortical Azotobacter chroococcum":


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