important link in food chain. Photosynthetic bacteria present just above the anoxic zone could serve as food for zooplankters also[16]. The photosynthetic bacteria also flourish in the area and in the mudflats having fine organic detritus and rich in humic substances.

The strains isolated in the cultures of purple and green sulphur bacteria identified as belonging to *Chromatium* sp (*Family: Thiorhodaceae*), *Chloroflexus* sp (*Family: Chloroflexaceae*) respectively were found to utilize H_2S for their growth in the laboratory studies.

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NOTE ADDED IN PROOF

Since sending our communication, we have come across recently in *Current Science* (April 20, 1986) 55(8) pp. 426–427, a similar report by P. A. Lokabharathi and D. Chandramohan, from the Lakshadweep area. The findings are interesting. Our purple sulphur bacteria do not seem to belong to the species *Chromatium violascens*. Green sulphur bacteria have been tentatively identified as, *Chloroflexus* sp, because of their characteristic gliding nature, filamentous appearance and specific absorption peaks in contrast to *Prosthecochloris* sp of Lokabharathi and Chandramohan. The differences in species composition of bacteria could be due to the habitats from where the samples were collected in both the reports.

USE OF DIGESTIVE ENZYMES OF INDIGENOUS SNAIL *ARIOPHANTA LINGULATA* FOR YEAST PROTOPLAST PRODUCTION

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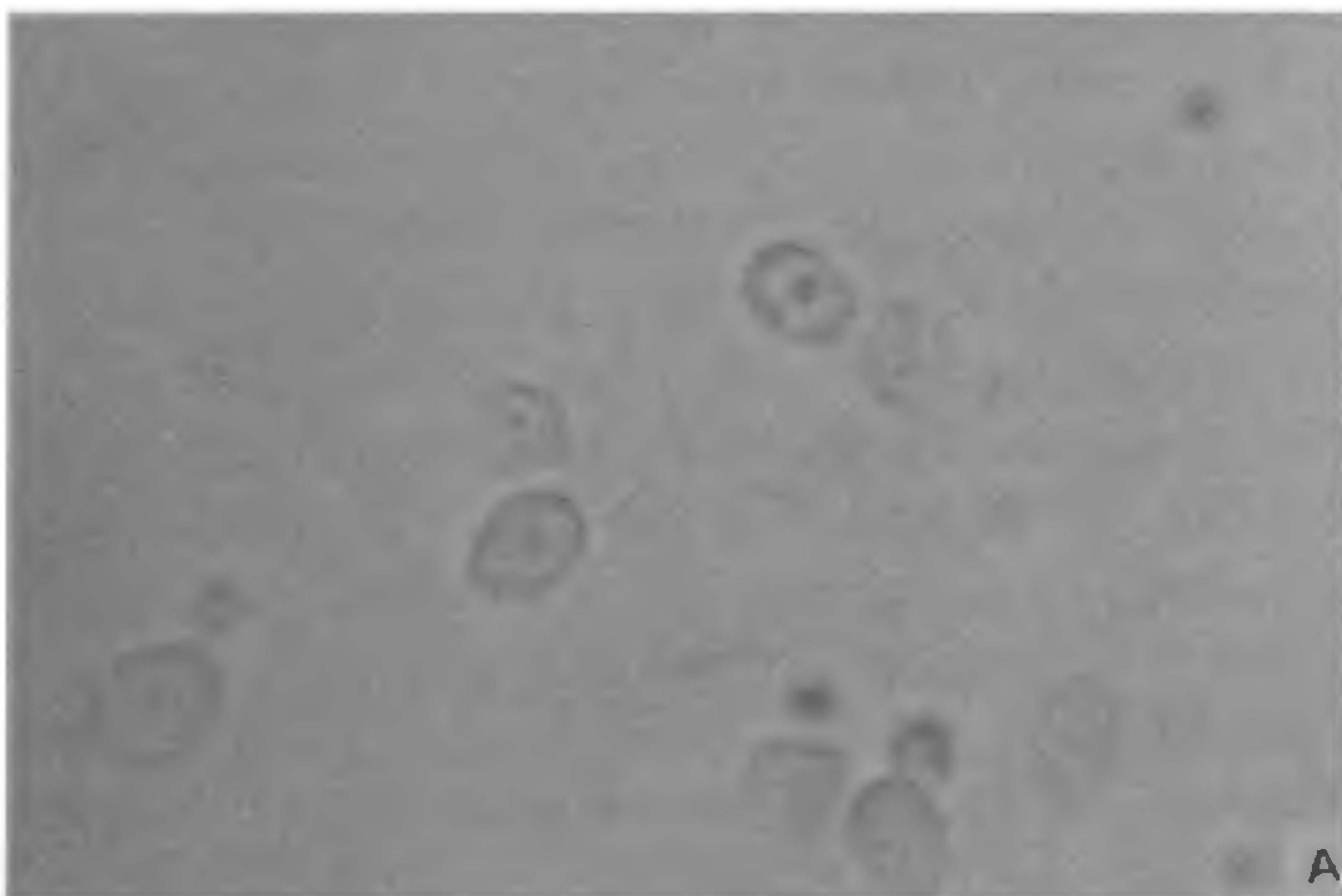
PROTOPLASTS have become a very important tool for genetic manipulation and in the breeding of yeasts. For protoplasting, commercial enzyme preparations like digestive enzymes of the snail *Helix pomatia* or enzymes from *Arthrobacter, Cytophaga* or *Streptomyces* are in common use[1-3]. Since snails

belonging to the genus *Helix* are not encountered on the Indian subcontinent, the enzyme is imported. In this context we present a simple procedure for preparing a crude enzyme extract of the digestive enzymes of the indigenous snail *Ariophanta lingulata* and a method for obtaining protoplasts from *Saccharomyces cerevisiae* using crude enzyme extract. Use of digestive enzymes of this snail also eliminated many of the conventional steps in the preparation of protoplasts.

A. lingulata is available in large numbers during rainy season. Well-developed snails were collected and maintained in the laboratory for a few days on a diet of common leafy vegetables. In order to facilitate the dissection of snails, they were immersed in lukewarm water containing a crystal of menthol for 8 hr. During this treatment it is necessary that air be completely excluded from the container. This treatment relaxes the muscles of the snail and reduces the mucus secretion. Snails were then carefully dissected to obtain intact digestive tracts. The digestive tracts were then suspended in previously chilled 50% glycerol and stored in the freezer compartment. After collecting sufficient number of digestive tracts (about 25), they were homogenized using a high speed *Remi* tissue homogenizer. This homogenate (crude enzyme preparation) was then stored in an airtight container in the refrigerator. The preparation retains activity even after 2 years of storage.



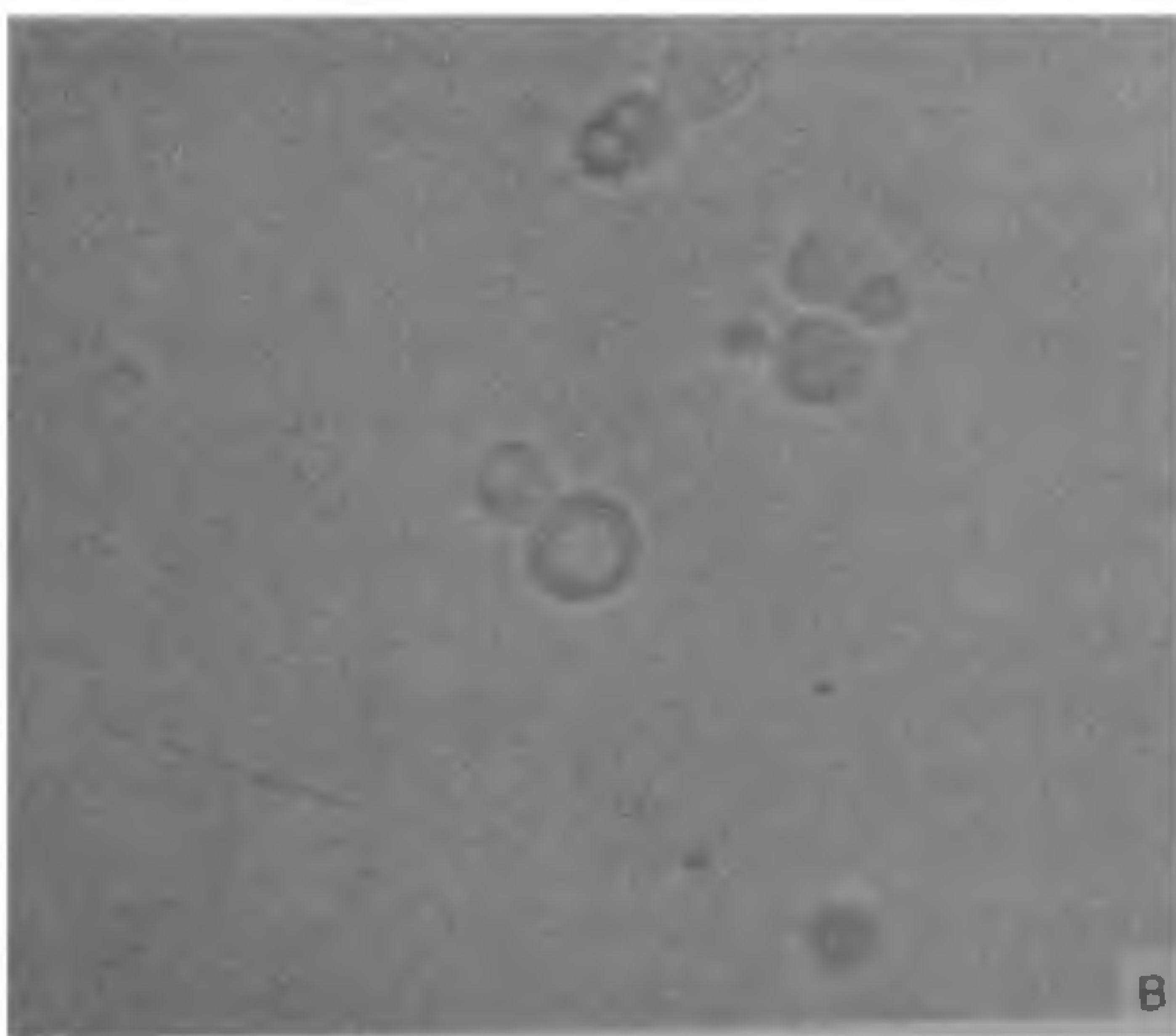


Figure 1 (2000×) *Saccharomyces cerevisiae* cells (A) before and (B) after enzyme treatment.

Two strains of *S.cerevisiae* were used in the study. Stock cultures were maintained on yeast extract peptone dextrose (YEPD) agar medium (yeast extract—1%; peptone—2%; dextrose—2%; agar—3%; pH—5.4). *S. cerevisiae* was grown in YEPD medium to obtain a late log phase culture (about 20 hr old). Cells were then washed twice with distilled water and resuspended in distilled water to get a final concentration of about 800 mg (wet weight) of cells per 100 ml of distilled water. The suspended cells were incubated for 20 hr at 30°C with rotary agitation at 250 rpm. Germ tubes were induced by suspending starved cells in prewarmed YEPD medium and incubated at 30°C. A 16 hr old culture was used for protoplast formation. The cells were harvested by centrifugation, washed twice with distilled water, resuspended in 5 mM Na EDTA at pH 7.5 and incubated for 1 hr at 30°C. Cells were then harvested by centrifugation, resuspended in 0.5 molar sodium thioglycolate in 0.1 molar Tris at pH 9.3 (pretreatment medium)[1] and incubated at 30°C for 90 minutes. Cells were harvested by centrifugation, washed twice with distilled water and then washed with 1 M $MgSO_4 \cdot 7H_2O$, adjusted to pH 4.1 with citric acid.

Cells were harvested by centrifugation and suspended in diluted crude enzyme preparation (10 mg of

Table 1 *Protoplast yield after 135 minutes of digestive juice treatment*

Sample number	Total number of cells per RBC chamber	Number of protoplasts per RBC chamber	Percentage yield
1	43	27	62.9
2	30	18	60.0
3	32	21	65.6
4	42	29	69.0
5	38	24	63.4
6	50	35	70.0
7	25	18	68.0

crude extract per ml) at a conc. of 150 mg (wet weight) per ml for 120–180 min. Samples of the digested suspension were diluted eight times with 1 molar mannitol and examined by light and phase contrast microscopy. Protoplasts can easily be differentiated on the basis of morphological characteristics (figures 1 A and B). The number of protoplasts as determined by differential cell count using a haemocytometer were expressed as a percentage of the total cell count. (table1)

Table 1 shows that the yield of protoplasts from *S. cerevisiae* after the treatment with the crude digestive juice for 135 minutes, lies between 60 and 70 %.

This yield of protoplasts could be increased by pretreatment with thiol reagents if desired[1]. It was also found that the increase/decrease in the concentration of crude enzyme also decreases protoplast yield.

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STUDIES ON THE TOXIC EFFECT OF *CALOTROPIS GIGENTEA* LATEX ON *ASPERGILLUS JAPONICUS*

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PLANT latices are known to show potent antimicrobial activity[1,2]. A hydrocarbon degrading strain of *A. japonicus* was isolated[3], which could transform the components of *Euphorbia nerifolia* and *Calotropis gigentea* latices[4,5]. Cell lysis took place within 72 hr, during transformation of *C. gigentea* latex. In the present investigation we demonstrate that one of the toxic effects of *Calotropis* latex is lysis of mitochondria of *A. japonicus*. Further, *in vitro* studies indicated that succinate dehydrogenase (SDH) activity was inhibited by two lipid components isolated from the *Calotropis* latex.

The cultivation of *A. japonicus* and the growth conditions are the same as described earlier[5]. The preparation of cell free extract (CFE) was essentially the same as reported earlier[6], except that Tris-HCl buffer (pH 7.2) was used for the preparation of CFE. In order to isolate mitochondria, the mycelia were frozen and subjected to mild grinding with glass powder. The extract was then suspended in TSE buffer pH 7.5 (0.44 M sucrose, 0.2 M EDTA, 10 mM Tris-HCl)[7]. The preparation was centrifuged at 1,000 g for 10 min to remove cell debris and the resulting supernatant was centrifuged at 34,000 g for 45 min. The mitochondrial pellet thus obtained, was washed twice with TSE buffer and recentrifuged at 34,000 g for 45 min. SDH (EC 1.3.99.1) activity in mitochondria was assayed from the supernatant after the mitochondrial pellet was disrupted by sonication (six 30 sec. bursts with 30 sec intervals). The method employed for assaying the enzyme activity was that of Arrigoni and Singer[8].

Intracellular lipids were extracted as reported by Gunasekeran *et al*[9]. Total lipids were estimated by the method of Bragdon[10]. Qualitative and preparative thin layer chromatographic (TLC) analysis was performed on 0.25 mm and 0.5 mm thick silica gel G (Ranbaxy) plates respectively, using a mobile phase of heptane:ether:acetic acid (80:20:2). The bands were visualized by exposing the plates to iodine vapours.

Upon incubation of mitochondrial pellets (obtained from glucose grown cells) with latex, SDH activity appeared in the supernatant (table 1). The ratio of SDH activity in the supernatant to that in the mitochondrial pellet increased with time suggesting that mitochondria were lysed in the presence of latex. The corresponding ratios in the control set did not show a significant increase, indicating that lysis of mitochondria in buffer was negligible.

Latex also inhibited SDH activity *in vitro*. A 56 % inhibition of SDH activity was observed when 0.4 % (v/v) latex was added to the assay system. The lipid content in *C. gigentea* latex is about 2.9 g %. Two lipid spots, having R_{fs} 0.64 (lipid 1) and 0.77 (lipid 2) were detectable by TLC in untransformed latex and in cells of *A. japonicus* grown in latex. They were, however absent in extracts of glucose grown cells. Therefore it was of interest to see the effect of these two lipids on SDH activity. Both the lipids were purified separately by preparative TLC. Lipid 1 (9.04 mg/ml) causes 22 % and lipid 2 (9.7 mg/ml) 19 % inhibition of SDH activity within 24 hr when incubated with CFE at 4°C