

Figure 2. Effect of chlorpromazine on the growth of *E. histolytica* NIH-200.

Table 1 Effect of EGTA on the growth of *E. histolytica* NIH-200 (initial inoculum 1×10^5 amoebae/ml)

Concentration (EGTA) (mM)	Time (hr)		
	24	48	72
Nil	++	++++	5.51
1	++	++++	5.02
2	++	++++	4.97
5	++	+++	2.45
10	++	++	1.50

modulin of *Tetrahymena* and *Trypanosomes* has been characterized¹⁵ and appears to be different from higher organisms. Partial cure of *Leishmania* infection by CPZ has been reported¹⁶. Further work is needed on the characterization of protozoal calmodulin and related reactions of calcium metabolism to understand the precise mode of antiprotozoal action of phenothiazine. Suitably modified phenothiazines and other modulators of calcium metabolism hold promise in antiprotozoal chemotherapy.

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1. WHO 1969, *Amoebiasis*, Tech. Rep. Series No.

421, World Health Organisation, Geneva.
2. Steck, E. A., *J. Protozool.*, 1981, **28**, 10.
3. Krishna Prasad, B. N., *Indian J. Exp. Biol.*, 1972, **10**, 43.
4. Anthony, P. and McCann, P. P., *Am. J. Physiol.*, 1982, **243**, C212-221.
5. Gillin, F. D., Reiner, D. and McCann, P. P., *J. Protozool.*, 1984, **31**, 161.
6. Bacchi, C. J., *J. Protozool.*, 1981, **28**, 20.
7. Kaul, S. M., Imam, S. A. and Shukla, O. P., *Curr. Sci.*, 1985, **54**, 800.
8. Klee, C. B., Crouch, T. H. and Richman, P. G., *Annu. Rev. Biochem.*, 1980, **49**, 489.
9. Gillies, R. J., *Trends Biochem. Sci.*, 1982, **7**, 233.
10. Kakiuchi, S. and Sobue, K., *Trends Biochem. Sci.*, 1983, **8**, 59.
11. Schuster, F. L. and Twomey, R. J., *J. Cell Sci.*, 1983, **63**, 311.
12. Europe-finner, G. N. and Newell, P. C., *FEBS Lett.*, 1984, **171**, 315.
13. McAlister, R. P. and Mishra, G. C., *J. Parasitol.*, 1983, **69**, 777.
14. Keegan, F. P. and Blum, J. J., *J. Protozool.*, 1983, **30**, 397.
15. Ruben, L., Egwuagu, C. and Patton, C. L., *Biochem. Biophys. Acta*, 1983, **758**, 104.
16. Person, R. D., Manian, A. A., Hall, D., Marcus, J. L. and Hewlett, E. L., *Antimicrob. Agents Chemother.*, 1984, **25**, 571.
17. Schuster, F. L. and Mandel, N., *Antimicrob. Agents and Chemother.*, 1984, **25**, 109.
18. Ockert, G. *Angew. Parasitol.*, 1984, **25**, 163.
19. Diamond, L. S., *J. Parasitol.*, 1968, **54**, 1047.

PHOTOSYNTHETIC BACTERIA FROM THE COASTAL BOARD ECOSYSTEMS

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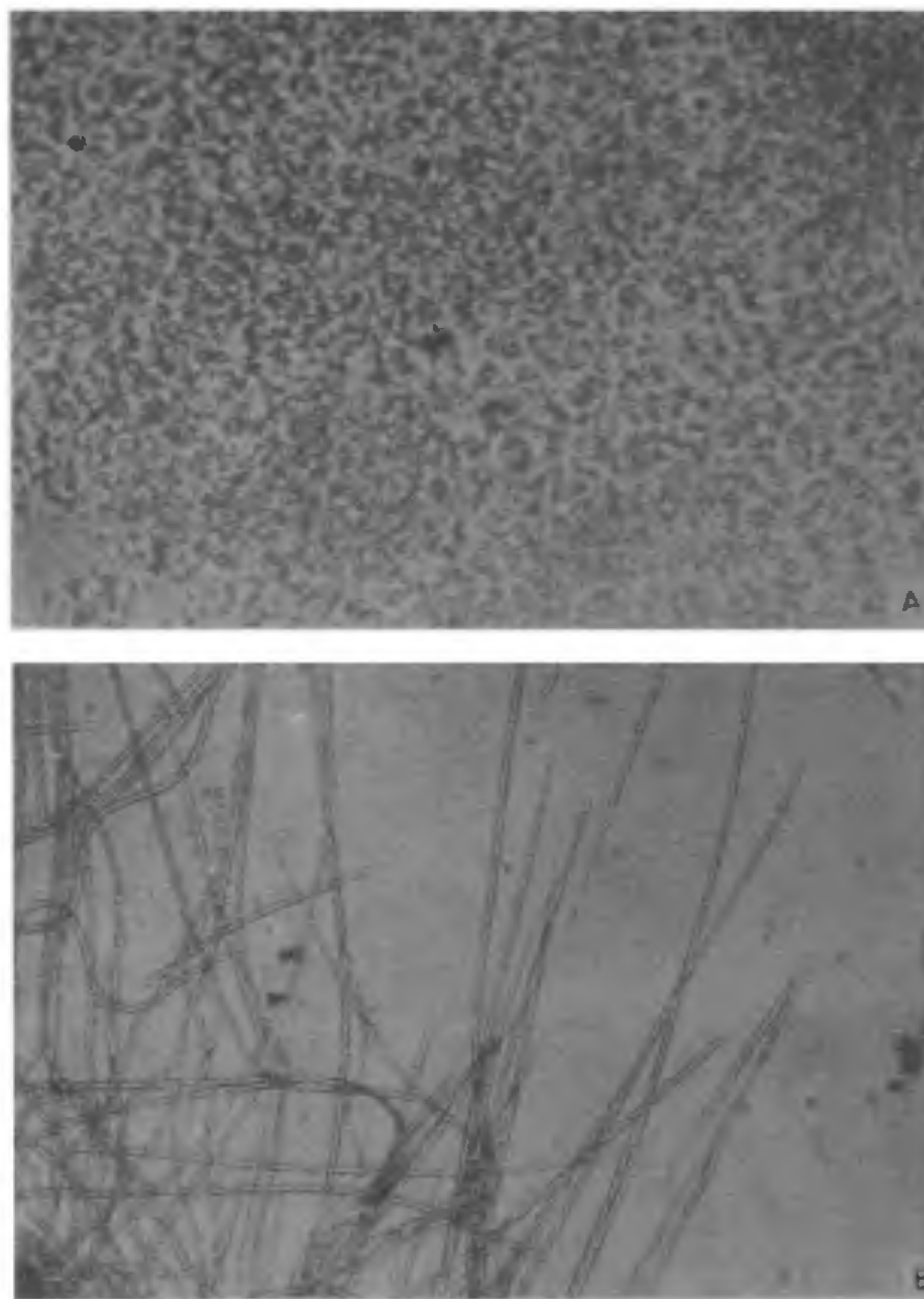
THE role of photosynthetic bacteria in the synthesis of organic matter in aquatic biotopes is well-recognized¹. Following the early work of Warming², who reported on the mass development of purple sulphur bacteria on the Danish Coast, many papers have appeared on the distribution of photosynthetic bacteria³⁻⁵. Generally,

the photosynthetic bacteria are more numerous in the estuarine environment than in the open ocean⁶⁻⁸. Recent reports clearly indicate that the productivity of photosynthetic bacteria is comparatively higher than that of phytoplankters^{9, 10}. Only limited information is available on the ecology of photosynthetic bacteria from the Indian waters¹⁰⁻¹². The present study deals with the enrichment and isolation of purple and green photosynthetic bacteria from the sediment of the Pichavaram mangroves near Porto Novo.

The sediment samples were collected below 40 cm water column from the mangrove vegetation-lined channels. Due to falling of leaves the sampling site had high organic matter deposition. The sampling site is influenced by semidiurnal tides. The samples were brought to the laboratory within 3 hr in a presterilized MacCartney bottle, to minimize any change in the microflora. Hydrographical parameters were also recorded during sampling.

For the culture of purple bacteria (*Chromatium* sp), a small amount of sediment was added to a flask containing sterile estuarine water, adding to it pieces of boiled egg to enable the production of H₂S to stimulate anoxic condition. The flask was sealed air tight and kept under constant fluorescent illumination¹³. After a week of incubation, the bacterial growth was discernible by the development of bright red colour in the medium. It was subcultured and pure cultures were made using the Agar-shake method¹⁴.

For the culture of the green bacteria (*Chlorobium* sp), the sample of mud was filled in a glass bottle (350 ml capacity) upto a third of its volume. The medium was prepared as recommended by Skermann¹⁴ and stoppered tightly and kept under fluorescent illumination. Subcultures were made using fresh medium¹⁴.



Figures 1A and B. Photomicrograph of bacterial colonies of A. *Chromatium* sp and B. *Chloroflexus* sp.

Estimations of bacteriochlorophylls were based on the methods of Strickland and Parsons¹⁵ and Takahashi and Ichimura¹⁶. Chlorophyll extracts from the bacterial cultures were made by using 90% acetone and visible light absorption spectra were recorded

Table 1 Bacterio-chlorophyll biomass (mg/m³) and the hydrographical data

Type of bacteria	Chl. a	Chl.650	Chl.660	B. Chl
1) Redsulphur bacteria	1.83	16.80	19.40	91.80
2) Green sulphur bacteria	0.60	49.95	87.95	20.19
<i>Hydrographical data</i>				
(i) Water salinity: 32 ‰				
(ii) Temperature				
a) Atmosphere = 34°C				
b) Water = 31.5°C				
(iii) Dissolved oxygen = 3.10 ml/l				
(iv) pH = 8.6				

(400–850 nm). The pure cultures were identified following the usual methods^{17, 18}.

The calculated values of bacteriochlorophylls and the hydrographical data observed at the time of sampling are given in table 1. Bacterial colonies of purple and green bacteria are shown in figure 1 (A & B). Light absorption spectra of purple and green bacteria are shown in figures 2 and 3.

The morphological features of the red sulphur bacterial isolates showed that they were microscopic, oval-shaped, highly motile, gram-negative cells with the deposit of sulphur globules inside their cells. Light absorption spectra showed two major peaks at the wavelengths of 480 and 772 nm and three minor peaks at 450, 470 and 680 nm. Of these observed peaks, the peak at 772 nm might be due to the contribution of Ba.Chl. *c, d* and carotenoids. Similar observations have been reported by Truper and Genovese³. The peak at 772 nm might indicate the presence of *Chromatium* sp observed earlier by Jimbo¹⁹.

Although *Chlorobium*-like organisms appeared initially in the green bacterial cultures they were subsequently outgrown by gliding filamentous green

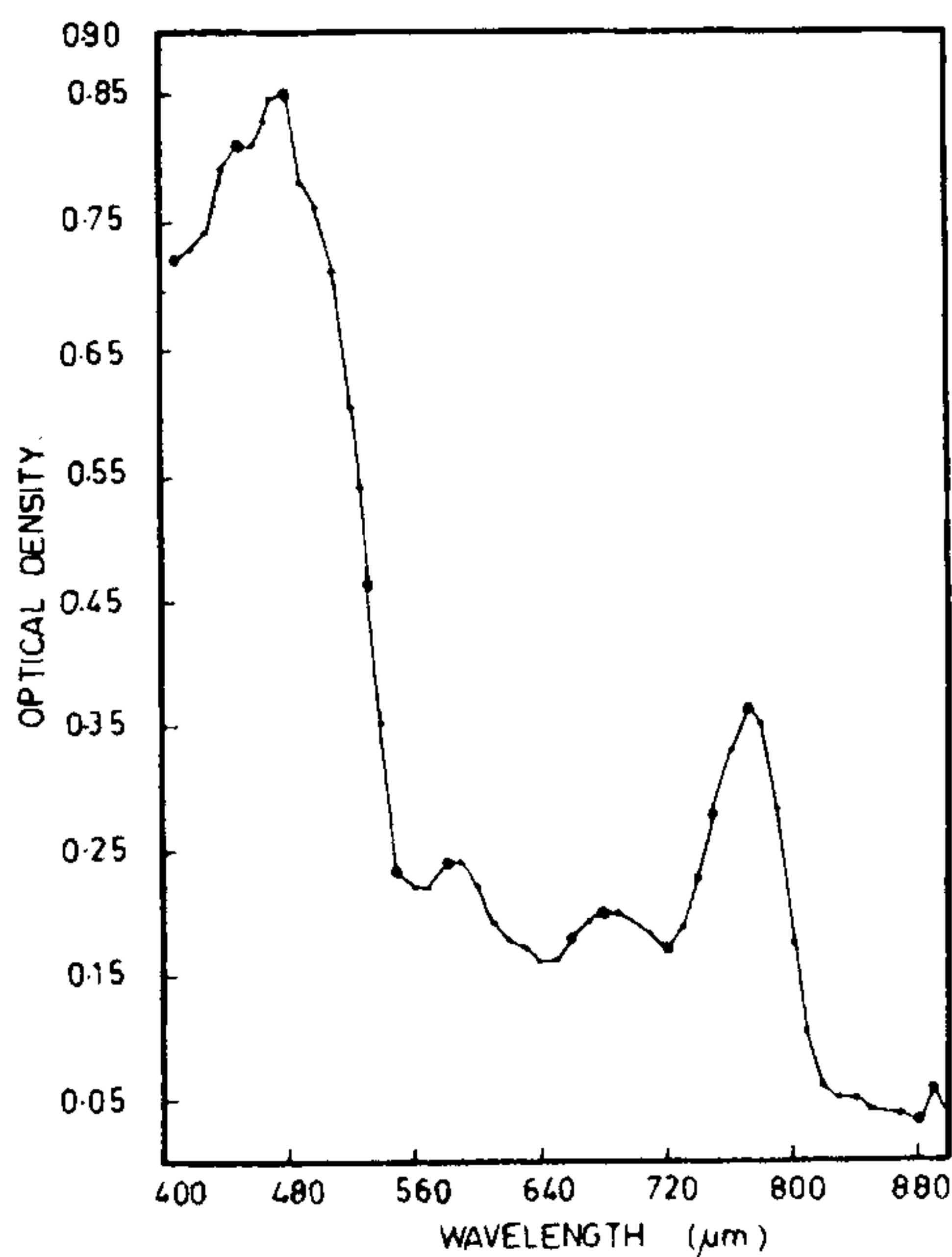


Figure 2. Light absorption spectra of purple-bacterial culture (*Chromatium* sp)

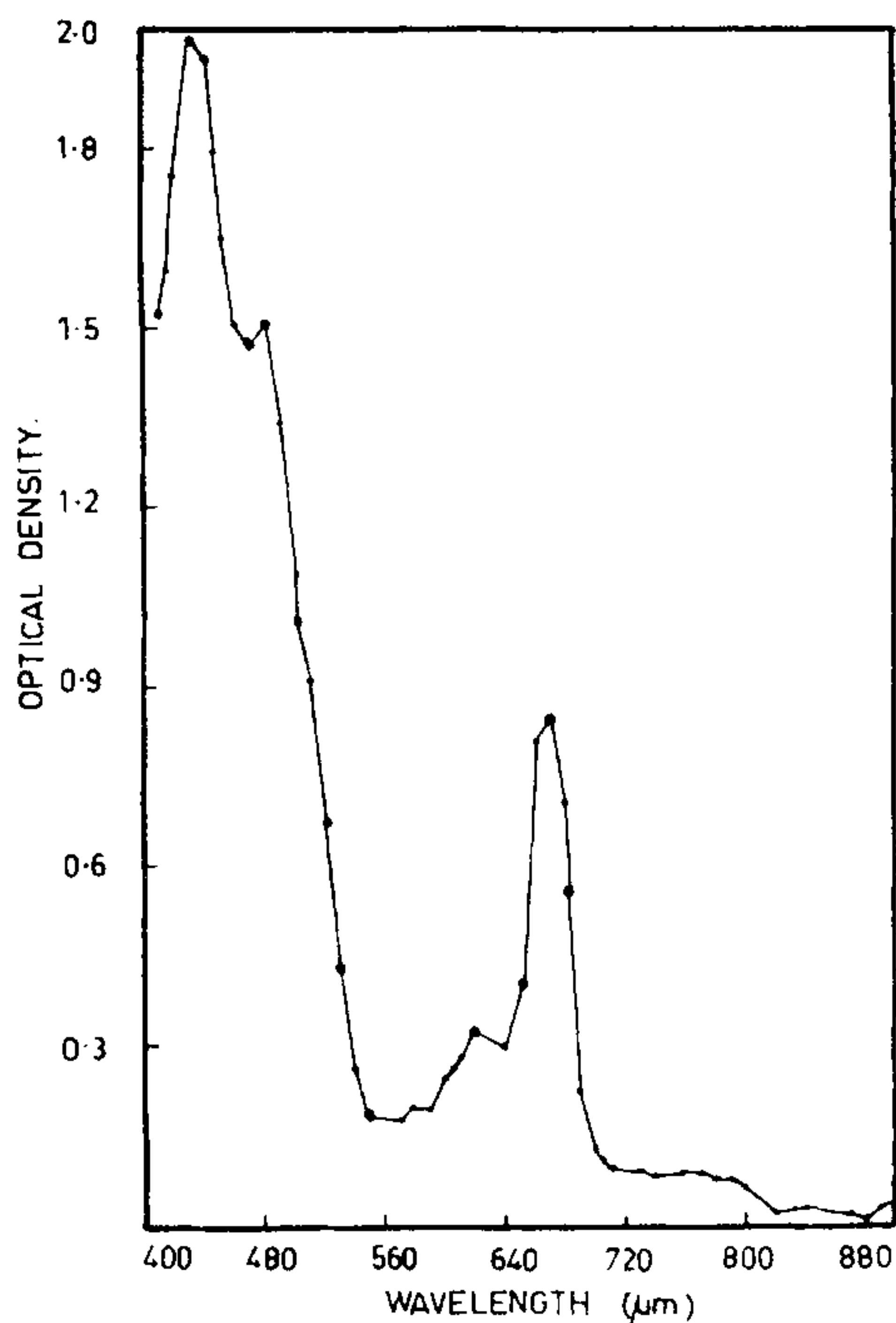


Figure 3. Light absorption spectra of gliding green bacterial culture (*Chloroflexus* sp).

organisms. Microscopic observation of the green mats showed the presence of sheathed green filaments, showing characteristic gliding movements with sulphur globules being deposited around these filaments. Spherical shaped yellow coloured organisms were also observed in small numbers. Light absorption spectra showed two major peaks at wavelengths of 430 and 670 nm and 3 minor peaks at 480, 620 and 760 nm. Of these, the peaks at 480 and 760 nm might be due to the contribution of Ba.Chl. *a* and carotenoids. The above characteristics recorded agree with the observations of Pierson and Castenholz¹⁸. Based on these characteristics the above mentioned bacterium is inferred to be a member of the species of the genus, *Chloroflexus*.

In the experimental studies the observed values of bacteriochlorophyll content showed higher value for purple bacteria (table 1). Santhanam and Krishnamurthy²⁰ found that abundance of sulphur bacteria coincided with the dominance of rotifers in the Pichavaram mangroves and that they formed an

important link in food chain. Photosynthetic bacteria present just above the anoxic zone could serve as food for zooplankters also¹⁶. 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 H₂S for their growth in the laboratory studies.

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1. Siefert, E., Iregens, R. E. and Pfennig, H., *Appl. Environ. Microbiol.*, 1978, **35**, 38.
2. Warming, E., *Vidensk. Meddr Dansk Naturb. Foren.*, 1875, 20/28, 3.
3. Truper, H. G. and Genovese, S., *Limnol. Oceanogr.*, 1968, **13**, 225.
4. Cohen, Y., Krumbein, W. E. and Shilo, M., *Limnol. Oceanogr.*, 1977, **22**, 609.
5. Truper Hans, G., Colloques Internationaux du C. N. R. S. No. 293-Biogeochimie de la matiere Organique a l'interface eau-sediment marin.
6. Eimhjellen, K. E., *Acta. Chem. Scand.*, 1967, **21**, 2280.
7. Imhoff, J. F. and Truper, H. G., *Microbiol. Ecol.*, 1976, **3**, 1.
8. Czezug, B., *Hydrobiology*, 1968, **31**, 317.
9. Panneerselvam, A., Kannan, L. and Krishnamurthy, K., *Indian J. Mar. Sci.*, 1979, **8**, 109.
10. Gore, P. S., *Curr. Sci.*, 1972, **41**, 737.
11. Aguiar, A. and D'souza, J., *Mahasagar*, 1978, **11**, 21.
12. Karanth, N. G. K., Shantha Nair and Loka Bharathi, P. A., *Indian J. Mar. Sci.*, 1977, **6**, 94.
13. Rodina, A. G., In: *Methods in aquatic microbiology*, (eds) R. R. Colwell and M. S. Zambruski, University Park Press, Baltimore and Butterworths, London, 1972, p. 329.
14. Skermann, V. B. D., *A guide to the identification of the genera of bacteria*, Waverly Press, Baltimore, 1967, p. 231.
15. Strickland, J. D. H. and Parsons, T. R., *Bull. Fish. Res. Bd. Canada*, 1968, **167**, 311.
16. Takahashi, M. and Ichimura, S., *Limnol. Oceanogr.*, 1968, **13**, 644.
17. Pfennig, H. and Truper, H. G., In: *Bergey's manual*

of determinative bacteriology, (eds) R. E. Buchanan and M. E. Gibbone, Baltimore, Williams and Welkins Co., 1974, 8th Edn., p. 1246.

18. Pierson, P. K. and Castenholz, R., *Arch. Microbiol.*, 1974, **100**, 5.
19. Jimbo, T., *Sci. Rept. Tohoku Univ.*, Fourth Ser., 1938, **13**, 259.
20. Santhanam, R., Krishnamurthy, K. and Subbaraju, R. C., *Bull. Dept. Mar. Sci. Univ. Cochin*, 1975, **7**, 899.

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 use¹⁻³. Since snails