Photosynthetic Bacteria in the Marine Environment at Porto-Novo

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Sediment and water samples were collected from mangrove and estuarine biotopes at fortnightly intervals. The physico-chemical characters of the overlying water were studied. In the mangrove biotope maximum temperature (31.5°C) and in the estuarine biotope maximum salinity (35.6‰) were recorded during the summer season, whereas in post-monsoon period the sulfate content was increased to 516 p.p.m. and the pH was reduced to 7.4. Invariably both in the enriched sediment and water samples four major peaks (at wavelengths 460, 705, 772 and 850 nm) and two minor peaks (at wavelengths 580 and 663 nm) of absorption spectra were noticed. A pure culture of *Chromatium* sp., isolated from mangroves sediment, showed three peaks of absorption spectra at wavelengths, 500, 580 and 850 nm. The effect of sodium chloride on the growth of *Chromatium* sp., was also studied and it was observed that maximum growth occurred in the range 1%–3% sodium chloride concentration. This isolate was also capable of utilizing various sulfur and carbon compounds. Glycerol and glucose did not show any specific effect whereas pyruvate, malate and acetate increased the growth.

Photosynthetic bacteria are widely distributed in all aquatic environments. Their ability to grow in anaerobic environment depends on the radiant energy and it was well documented in a review by Van Niel (1944). They have the leading role in the production of organic matter and in the purification of sewage in aquatic environment. These bacteria can be easily recognised by the formation of water-bloom and the bloom forming photosynthetic bacteria have been reported earlier and also they have been utilized as a feed by the copepods (Sorokin, 1966). Besides these, Cavari, *et al.* (1973) revealed a new phenomenon that the secondary peak of photosynthesis in the thermocline layer in the Lake Kinneret, (Israel) and attributed it mainly to photosynthetic sulfer bacteria *Chlorobium phaeobacteriodes*. As primary producers these photosynthetic bacteria are likely to contribute to the organic load and their contribution may reach 9 to 25% of the total primary production (Czeczuga, 1965, Takahashi & Ichimura, 1968). It is understood that the purple bacteria are particularly depending upon the degree of pollution of water, and in Japan and Germany the photosynthetic bacteria were utilized for the treatment of organic wastes (Kobayashi, 1975; Siefert *et al.*, 1978).

The occurrence and the functional significance of photosynthetic bacteria along the coastal waters of India have not been previously studied. Truper & Pfennig (1981) studied the characters and identified some of the anoxygenic photosynthetic bacteria. Following their techniques in the present study an attempt has been made to enrich and isolate the photosynthetic bacteria from Indian coastal waters and to characterize their distribution on the marine environment.

Materials and Methods

Fortnightly collections were made from February to July 1978 at two stations in two biotopes such as estuarine and mangrove. Description of the stations have been given by Dhevendaran (1978). Sediment samples were collected using Peterson grab and were transferred aseptically to sterile polythene bags and the overlying water was collected with a Knudsen water sampler for estimation of physico-chemical parameters following the method adopted by

Dhevendaran (1978). One g of wet sediment was aseptically removed from respective samples and transferred into 100 ml of the enrichment medium. The medium contained per litre of 50% aged seawater, 1.0 g of $MgCo_3$, 0.6 g of K_2HPO_4, 3.0 g of NH_4cl, 1.0 g of yeast-extract, 2.58 g of sodium malate, 10 ml of trace elements and the medium was prepared as described by Pfenning & Lippert (1966). 1 ml of water from each sample was pipetted out into the enrichment medium with sterile 1 ml pipette. The medium with samples was taken in glass-stoppered bottles and incubated with continuous illumination at 20 μ E m^{-2} S^{-1} at room temperature (28 ± 2° C) for 10 days unless otherwise stated, to study the absorption spectra of crude extracts in 90% acetone following the methods of Takahashi & Ichimura (1968) and of Truper & Pfenning (1981). For the isolation of purple photosynthetic bacteria, sediment sample was inoculated into the above mentioned enrichment medium and incubated as mentioned above. After 10 days of incubation at room temperature with continuous illumination (20 E m^{-2} S^{-1}) 0.1 ml of aliquot was taken and poured into the sterile petri dishes following the method of Siefert *et al.* (1978) and mixed the above enrichment medium with 2% agar and incubated again under continous illumination for 10 days. Single pure colonies appeared and the colonies were identified according to the method of Pfenning & Truper (1974), Truper & Pfenning (1981). After the incubation period, the crude extracts of the sediment and water were filtered separately through membrane filter paper under vacuum. The cell residues were suspended separately in about 10 ml of 90% acetone and incubated in dark at 4°C, for 30 min following the method of Siefert, *et al.* (1978) and the mixture was centrifuged at 10000 x g. Then the absorbance of the supernatant was measured in UNICAM Spectrophotometer at wavelength from 400–900 nm. The absorption spectra of the pure culture was also recorded. Beside this, the growth of the selected culture in different concentration of sodium chloride and also its growth in various sulfur and carbon compounds were measured by taking the optical density at 650 nm (Truper & Genovese, 1968).

Results and Discussion

Results of the physico-chemical parameters are summarized in Table 1. It is understood that the shallow mangrove biotope recorded maximum temperature and the deep estuarine biotope (Fig. 1) highest salinity during summer and they were 31.5°C and 35.6‰ respectively, but the salinity was lowered during monsoon season and this has been attributed to the high influx of freshwater. The pH ranged from 7.4 to 8.3 and the lowering of pH in mangrove biotope could be due to the humic acid and phenolic acid content of the sediment (Karanath *et al.*, 1975). The sulfate concentration in the mangrove biotope was higher (516 p.p.m.) and this could be due to the presence of thick vegetation as well as the direct influence of seawater and freshwater inflow from the adjacent paddy fields and this in turn facilitate the proliferation of sulphate reducing bacteria, photosynthetic bacteria and plankters during summer and pre-monsoon seasons. These seasons are noted for higher productivity (Krishnamoorthy &

Table 1. *Physico-chemical parameters in the mangrove and the estuarine biotopes*

Overlying water	Mangrove						Estuary					
	Feb.	Mar.	Apr.	May	June	July	Feb.	Mar.	Apr.	May	June	July
Temp., °C	28.0	29.5	31.5	31.5	30.0	27.5	29.0	29.0	31.0	31.0	30.0	29.0
Salinity, ‰	31.8	33.0	33.6	35.4	34.6	33.9	32.9	32.8	33.4	35.6	35.0	34.2
pH	7.4	7.9	8.2	8.2	8.1	7.8	7.9	8.3	8.2	8.3	8.3	8.0
Sulphate, p.p.m.	516.0	500.0	482.0	468.0	462.0	446.0	496.8	462.4	438.6	428.8	414.6	408.2

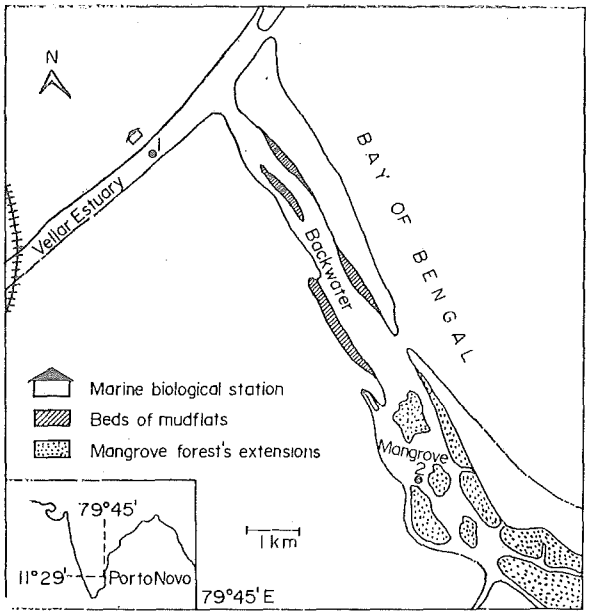

Fig. 1. The sampling stations

Sundararaj, 1973). The absorption spectra of selective enrichment of sediments as well as the overlying water were taken separately in order to study the nature of photosynthetic bacterial contribution of various pigments towards the sediments and the water (Fig. 2). Four major peaks (at wavelengths 460, 705, 772 and 850 nm) and two minor peaks (at wavelengths 580 and 663 nm) were noticed both in sediment and water. There is no marked variation in the absorption spectra but for the quantity. The absorption maximum at 460 nm is due to the contribution by typical carotenoids and the peaks at 705 and 850 nm are mainly by bacteriochlorophylls *d* and *a* respectively, thus indicating the predominance of Thiorhodaceae in sediments and water and the peculiar peak at 772 nm is also by Thiorhodaceae as suggested by Jimbo, (1938) and Truper & Genovese (1968). The presence of H_2S in the reductive zone of sediment attracted the photosynthetic sulfur bacteria. Besides this, they are present at the boundary between the oxidative and reductive zone thus confirming the report of Kuznetsov (1958). It is understood that the features of absorption spectra reveal that the photosynthetic bacteria are vertically distributed and they play an important role in the sulfur cycle. The suspension was pink, microscopically it showed *Chromatium* like bacteria of high motility in addition to a few non-motile *Chlorobium*-like forms (Truper & Genovese, 1968).

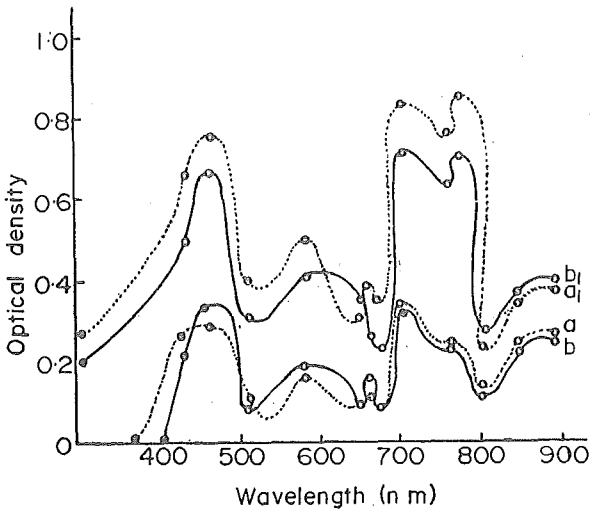

Fig. 2. Absorption spectra of enrichment cultures inoculated with sediment and water samples collected from mangrove and estuarine biotopes

Curve a Mangrove water
Curve a_1 Mangrove sediment
Curve b Estuarine water
Curve b_1 Estuarine sediment

One pure culture was isolated which is short, rod like, motile with sulfur globules inside the cell. The colour of the culture is pink and is *Chromatium*-like (Truper & Genovese, 1968). The absorption spectrum of the pure culture (Fig. 3) showed three peaks at 510, 580 and 850 nm. The *Chromatium* like bacterium contains typical carotenoids (maximum absorption at 510 and

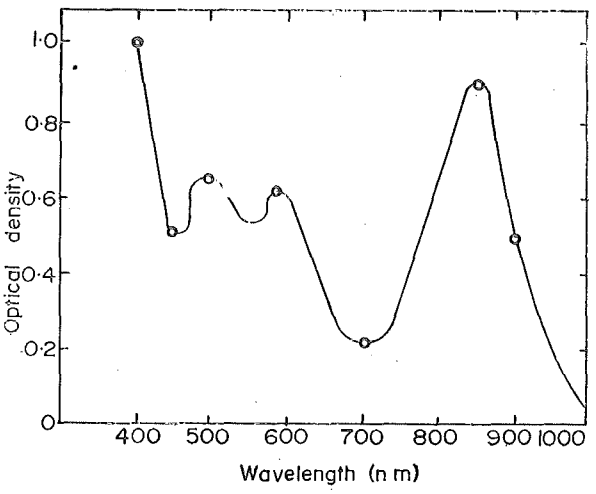

Fig. 3. Absorption spectra of pure culture of *Chromatium* sp.

580 nm) and bacteriochlorophyll *a* (850 nm) as shown in the absorption spectra of *Chromatium warmingii* and *Cromatium* sp. strain 3311 (Schlegel & Pfenning, 1961; Truper & Genovese, 1968).

The result of the sodium chloride concentration on the growth of the pure culture (*Chromatium* sp.) is given in Table 2.

Table 2. *Growth of the isolated strain at different sodium chloride concentrations*

	Nacl free	Nacl 1%	Nacl 2%	Nacl 3%	Nacl 5%
Chromatium sp.	+ +	+ + +	+ + +	+ + +	+

+ = Slight growth; + + = moderate growth; + + + = profuse growth

Sodium chloride was added to the medium at the concentrations of 1, 2, 3 and 5 g/100 ml of the medium. An inoculated control without sodium chloride for its growth was maintained to ascertain the importance of sodium chloride for its growth. The most distinct characteristic of the marine isolates, compared to the terrestrial counter parts, is a requirement of seawater. It is obvious that marine *Chromatium* gradually reduce their 100% demand of salinities but they need at least 10% seawater, whereas terrestrial and freshwater *Chromatium* would not tolerate seawater (Genovese *et al.*, 1963). In the present study, it was shown, that the maximum growth occurred from 1 to 3% sodium chloride concentration. However, in the absence of sodium chloride, growth still occurred to a acertain extent in this culture. Similar reports have already been made by Genovese *et al.* (1963) and Truper & Genovese (1968). Addition of sodium chloride to optimum levels substantially increased the growth rate and it may, therefore, be presumed that there is a demand for sodium chloride for its normal metabolic process. This indicates that this culture can be grouped under marine *Chromatium*. Further increase in the concentration of sodium chloride (5%) reduced the growth but it could tolerate that concentration. Similar halotolerant and halophilic strains of *Chloribium* and *Chromatium* have been reported (Genovese *et al.*, 1963; Truper & Pfenning, 1981).

Thiele (1966), Truper & Genovese (1968) and Truper & Pfenning (1981) studied the ability of Thiorhodacease to use a variety of different carbon and sulfur compounds. Following their technique we have done parallel experiments with our culture. Table 3

Table 3. *Utilization of sulfur and carbon compounds added to the enrichment medium*

Additions	Percentage	*Chromatium* sp. 3311 (Reference)	*Chromatium* sp.
Sulfide	0.05	Control	Control
Thiosulphate	0.1	+	+
Elemental sulfur	0.1	+	+
Glycerol	0.1	+	+
Glucose	0.1	+	+
Fructose	0.1	+	+
Casamino acids	0.1	+	+
Pyruvate	0.1	+	+
Acetate	0.1	+	+
Lactate	0.05	+	+
Malate	0.1	+	+
Succinate	0.1	+	+
Citrate	0.1	+	+
Nitrate	0.1	+	+

– = no growth; – = slight growth; + + = profuse growth

gives the results compared with those of Truper & Genovese (1968) for *Chromatium* sp. Their strain *Chromatium* 3311 has the strongest heterotrophic tendency. The similarity in the use of carbon and sulfur compounds between our strain and their strain 3311 is noticed. When *Chromatium* strain was supplied with fructose, pyruvate, acetate, malate and succinate, growth was markedly stimulated, while glucose, glycerol, citrate and nitrate showed no variation in growth, thereby indicating that there is not much difference in the metabolic activity. Thus the metabolism of *Chromatium* seems to be similar to that of *Chromatium* 3311 (Truper & Genovese, 1968) and that of *Chromatium* sp. (Truper & Pfenning, 1981).

The occurrence of *Chromatium* sp. in the marine environment shows that these bacteria are widely distributed. It was isolated from the anoxic, H_2S containing layer. But its role in the biogeochemistry and the primary production of the seawater is yet to be fully understood. Sulfate is the second most abundant anion in the sea forming a large reserve of bound oxygen and sulfur (Redfield, 1958). Beside, these photosynthetic sulfur bacteria, various other groups of sulfur oxidizing and sulfate reducing bacteria are known to take part in the sulfur cycle of marine environment. However, photosynthetic bacteria can also serve as food for zooplankton such as copepods as suggested by Sorokin (1966) and Takahashi & Ichimura (1968) and the work on this line is in progress.

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