

### Short Communications

## Marine photosynthetic bacteria from southeast coast of India

P T Kalaichelvan & B Rajkumar

Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Madras 600 025, India

Received 17 October 1988; revised 1 January 1990

Eighty isolates of marine photosynthetic bacteria belonging to Rhodospirillaceae, Chromatiaceae and Chlorobiaceae were isolated from seawater, marine sediments, decaying macroalgae and sea grass samples, collected from 8 stations along the southeast coast of India and the ecological significance of these forms was discussed.

Although photosynthetic bacteria (PSB) and their activities are known, there are only few reports<sup>1-3</sup> on these forms from the marine environment along the Indian coast. In the present study, some more marine PSB have been isolated from the southeast coast of India and are presented in this paper.

Samples of seawater, marine sediments, decaying macroalgae and sea grasses were collected from 8 stations along the southeast coast of India (Table 1). Seawater salinity and pH were recorded using CAT 10419 T/C refractometer (American Optical) and pH papers respectively at the collection stations. Dissolved oxygen (DO) content of water samples was also estimated. Each sample was divided into 2 portions. The first was inoculated into the marine photosynthetic non sulphur bacterial medium and the second into the marine photosynthetic sulphur bacterial medium obtained by the modification of Pfenning's medium<sup>4,5</sup>. Aged seawater was used instead of NaCl after adjusting the salinity according to that of collection station. Transparent glass reagent bottles (125 ml) completely filled with the liquid medium up to the

brim and tightly stoppered were used as culture vessels for the primary enrichment cultures. Cultures were incubated at room temperature ( $30^{\circ} \pm 3^{\circ}\text{C}$ ) in dark overnight and then incubated under illumination of 500-1000 lux intensity of incandescent light (100 w light bulb) at room temperature. Light intensity was measured by EEL portable photo electric meter. After 3-10 d of incubation, coloured spots appeared on the inner wall of culture bottles. These were aseptically removed and resuspended in 3% NaCl solution and subcultured in serial dilutions in 30 and 10 ml screwcapped tubes, and incubated as mentioned above. Pour plate and agar-shake culture techniques were employed for the isolation of pure cultures<sup>4</sup>. Pure cultures were examined under light and phase-contrast microscope for purity of cultures and to study the morphology of the bacteria. Light absorption spectra of cell suspensions were recorded in a Beckman DU-40 spectrophotometer, by dissolving 5 g of sucrose in 3.5 ml of cell suspension and using 5 g of sucrose dissolved in 3.5 ml of sterile culture medium as the blank. Characterization and identification of

Table 1—Characteristics of collection sites

Stations	Nature of stations	No. of samples	Salinity ( $\times 10^{-3}$ )	pH	DO ( $\text{ml.l}^{-1}$ )
Ennore	Estuary	8	29.5	7.0-7.5	1.5
Madras					
(Royapuram)	Splash water pool,	2	38.5	7.5	1.0
Fishing harbour)	Intertidal flat	2	32.0	7.0-7.5	1.5
Muttukadu	Backwaters	12	33.0	7.5	1.4
Rameswaram	Intertidal flat	4	30.5	7.5-8.0	1.25
Mandapam	—do—	12	31.0	7.5-8.0	1.0
Tuticorin	—do—	4	31.0	7.0-7.5	2.0
Tiruchendur	—do—	4	31.5	7.0-7.5	1.8
Kanyakumari	Splash water pool	4	33.5	7.5-8.0	1.0

Table 2—Characteristics and identification of isolated strains of photosynthetic bacteria

Colour of cell suspension	Shape and size ( $\mu\text{m}$ )	Gas vacuoles	Absorption maxima (nm)	Genus	No. of isolates
Rhodospirillaceae					
Purple-red	Rod 1.0/2.0	—	482,524,593,809,866	<i>Rhodopseudomonas</i>	8
Red-brown	Rod 0.8/1.5	—	478,526,580,808,854	<i>Rhodopseudomonas</i>	3
Brown	Spiral 0.8/4	—	525,552,582,803,885	<i>Rhodospirillum</i>	4
Red	Spiral 0.8/5.5	—	515,550,582,805,874	<i>Rhodospirillum</i>	5
Chromatiaceae					
Purple-violet	Rod 1.0/4.0	—	497,521,529,801,844	<i>Chromatium</i>	3
Brown-Red	Rod 2/3	—	497,529,591,810,855	<i>Chromatium</i>	8
Purple-red	Rod 1.5/6.5	—	452,518,805,851	<i>Chromatium</i>	12
Purple	Spheres 3	+	462,485,521,803,857	<i>Lamprocystis</i>	6
Pink-red	Spheres 2	—	486,515,552,801,853	<i>Thiocapsa</i>	5
Purple-violet	Rod 2/3.5	+	463,497,529,805,852	<i>Thiodictyon</i>	3
Chlorobiaceae					
Green	Vibrio 0.4/1	—	457,756	<i>Chlorobium</i>	3
Green	Rod 0.5/1.5	—	452,754	<i>Chlorobium</i>	6
Green-brown	Rod 0.8/2	—	402,458,517,723	<i>Chlorobium</i>	7
Brown	Vibrio 0.5/1.5	—	405,457,515,721	<i>Chlorobium</i>	1
Green	Rod 1/2	+	458,746	<i>Pelodictyon</i>	3
Green-brown	Rod 1/1.5	+	456,748	<i>Pelodictyon</i>	3

the isolated strains were made according to Truper and Pfenning's<sup>6</sup> procedure.

Eighty new isolates of marine photosynthetic bacteria belonging to the families Rhodospirillaceae, Chromatiaceae and Chlorobiaceae were isolated (Table 2). From all the sampling stations, only Rhodospirillaceae members could be isolated from decaying macroalgae and sea grasses. Marine sediment samples from Ennore and Madras (Royapuram) yielded Chromatiaceae and Chlorobiaceae members only.

Salinity and pH of the collection stations were in the optimum range required by the isolates as found in growth studies. All isolates were able to grow well in the salinity range  $25-35 \times 10^{-3}$ . Some of the strains were able to grow even at  $47 \times 10^{-3}$  salinity. Almost all the isolates grew well in the neutral pH. Though the DO content of ambient waters was not very low PSB were able to survive showing that, they can tolerate moderate aerobic conditions in the presence of other heterotrophs<sup>7</sup>. The optimum temperature range for the isolates was  $27^{\circ}-33^{\circ}\text{C}$ . Absorption spectrum of cell suspensions showed characteristic peaks at certain wavelengths and these were considered as important criteria for the characterization of the isolates apart from the morphology and the utilisation of the substrates (Table 2). All Rhodospirillaceae isolates were able to use ethanol and malate as electron don-

ors. Lactate was utilized by *Rhodospirillum* spp. Malate was utilized by all isolates of chromatiaceae as carbon source. Sodium sulphide and sodium thiosulphate served as electron donors to all isolates of Chromatiaceae and Chlorobiaceae.

Motile forms could not be subcultured in agar-shake cultures. At higher concentrations of sulphide and light intensity, motile chromatiaceae members got stuck to the walls.

The authors thank Prof. A. Mahadevan, Director, CAS in Botany, University of Madras and Prof. V. N. Raja Rao for facilities and valuable suggestions.

## References

- Lokabharathi P A & Chandramohan D, *Curr Sci*, 55 (1986) 426.
- Krishnamurthy K, Perumal P & Palaniappa R, *Curr Sci*, 55 (1986) 617.
- Karant N G K, Shantha Nair & Lokabharathi P A, *Indian J Mar Sci*, 6 (1977) 94.
- Van Niel C B, in *Methods in enzymology*, Vol. 21, edited by A. San Pietro (Academic Press, New York, London) 1971, 3.
- Raj Kumar B, *Molecular hydrogen photoproduction by marine photosynthetic bacteria: An alternative resources* M.Sc. thesis, University of Madras, 1988.
- Truper H G & Pfenning N, in *The prokaryotes*, Vol. 1, edited by M P Starr, H Stolp, H G Turper, A Balows and H G Schlegel (Springer-Verlag, Berlin, Heidelberg, New York) 1981, 299.
- Kobayashi M, Suzuki M, Yoshida T, Katayama H & Imai K, *Soil biology and conservation of the biosphere (Abstract)* 1985.