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### Studies on Photosynthetic Bacteria Isolated from an Estuarine Beach of Goa

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A bacterial strain capable of fixing CO<sub>2</sub> was isolated from an estuarine beach of Goa. The strain grew in sea water containing a piece of boiled egg under anaerobic condition and in the presence of subdued sunlight or fluorescent illumination. The strain was gram negative and varied from spiral to Y shape. It had no sulphur deposit either inside or outside the cells and produced purple pigment. Based on these studies, it was inferred that the isolate belongs to the family *Athiorhodaceae* (non-sulphur purple bacteria). It had a characteristic absorption maximum at 850 nm which is a general feature of purple bacteria. The optimum pH for this strain was 7. Effect of different organic substances, nitrogenous compounds, and pollutants on CO<sub>2</sub> fixation by this isolate was also studied.

**R**OLE of photosynthetic bacteria in primary production and nitrogen fixation in the coastal ecosystem especially in nutrient deficient regions has been reported<sup>1</sup>. Distribution of these bacteria in anaerobic aquatic habitats such as salt marshes, estuarine muds and tidal pools has also been reported<sup>2</sup>. Although these groups of bacteria have been of considerable interest very little is known about their characteristic features in the Indian region except for a brief report on the green photosynthetic bacteria by Gore<sup>3</sup>. Therefore, an attempt has been made to study the purple non-sulphur photosynthetic bacteria isolated from an estuarine beach of Goa.

**Isolation** — Small amount of the sediment, collected from an estuarine beach near NIO, was added to a flask containing sterile sea water and a piece of boiled egg. The flask was sealed air tight and incubated in natural day light at room temperature<sup>4</sup>. After a week, bacterial growth was indicated by the development of purple colour. It was subsequently

sub-cultured once in 15 days under similar conditions. On examination, all the cells in the sub-cultures were found to be alike, thus indicating that the cultures were pure.

A loop of growing culture from the 5th sub-culture was smeared on a slide and observed under a microscope after gram staining. Also hanging drop preparation was made to examine the culture directly.

**Effect of light and air on CO<sub>2</sub> fixation** — To study the optimal condition for CO<sub>2</sub> fixation, the culture was grown using the same medium in test tubes in 10 ml quantities. Several test tubes, each containing 8 ml sea water were sterilized at 121°C for 20 min. After cooling, 1 ml of H<sup>14</sup>CO<sub>3</sub> was added (0.8 μCi obtained from BARC, Bombay) and inoculated with 1 ml of actively growing culture. These tubes were immediately sealed using cotton-wool, liquid paraffin and a rubber bung. The other set of tubes were kept for aeration and half the number of tubes were covered with blue cloth. All the tubes were kept in a container, maintained on a magnetic stirrer, and illuminated by 100 w lamp. The culture was allowed to grow at room temperature (28°C ± 2) for 48 hr. Duplicate samples were used for each treatment.

**Pigment measurement** — At the end of 48 hr growth, the culture was mixed well for 2 min using a vortex mixer. From this, 5 ml was pipetted out and centrifuged for 10 min at 10000 × g using a Serwall RC 2B centrifuge and the remaining part was used for taking the counts. To the pellet, after washing twice with cold sea water, 2 ml acetone (90%) was added and kept at 5°C for 12 hr. The supernatant was removed and the cells were extracted with 2 ml acetone and the supernatant was pooled. The cells were white at this stage and were discarded. The supernatant was made to 5 ml and its colour was read in Bausch and Lomb colorimeter at 490 nm against acetone blank. The optical density was corrected to 10 ml.

**Measurement of growth** — <sup>14</sup>C uptake into the cells was used as an index of growth of the culture. Culture (5 ml) was centrifuged and washed twice with cold sea water. To the pellet, 5 ml of 75% ethanol was added and kept in boiling water bath for 10 min, after which the cells were centrifuged at 10000 × g for 10 min. The supernatant was transferred to a test tube. The cells were again extracted twice with ethanol as above. The alcohol extract was pooled, made to a known volume (henceforth known as soluble fraction), from which 0.5 ml aliquot was pipetted out carefully on to a planchet, dried at 90°C in a hot air oven and counted using a Gas Flow Proportional Counter having 5% efficiency. From the counts, per cent incorporation was calculated.

**Effect of organic substrates on CO<sub>2</sub> fixation** — The culture technique was same as earlier, except that the egg was replaced by organic substrate (1%) and growth was measured following tracer technique. In addition to alcohol-soluble fraction, the incorporation of CO<sub>2</sub> was measured in alcohol insoluble fraction also.

**Activity in alcohol insoluble fraction** — After alcohol extraction, the pellet was digested overnight in

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0.5 ml NaOH (0.5 N) and its volume was made to 2 ml. From this, 0.1 ml aliquot was pipetted out into a planchet, dried and counted.

Similar experiments were carried out to study the effect of nitrogenous chemicals as well as some pollutants on CO<sub>2</sub> fixation.

*Carotenoid pigments*—The method followed for the extraction of carotenoid pigment was the same as given by Jungalwala and Cama<sup>5</sup>. The absorption spectra of the pigment was recorded using Cary-14 recording spectrophotometer. The near IR spectrum of 48 hr old culture was also recorded in the Cary-14 recording spectrophotometer.

*Morphological features* of the isolate showed that there is no deposition of sulphur either inside or outside the cell wall. The cells were gram negative and their shape varied from spiral to Y. The carotenoid pigment exhibited absorption maxima at 456, 487 and 510 nm and the growing culture showed peaks at 800 and 850 nm. This is a characteristic feature of purple bacteria.

Effect of 4 different combinations of air and light conditions on CO<sub>2</sub> fixation and pigment production in media containing egg or peptone is given in Table 1. The suitable condition for growth is anaerobic and the presence of light. <sup>14</sup>CO<sub>2</sub> incorporation and pigment formation were high in medium containing egg compared to peptone medium. That the present isolate is one of the photosynthetic bacteria was confirmed from the fact that good growth was attained only in the presence of light and under anaerobic condition. It has been reported by Gibson<sup>6</sup> that the presence of oxygen prevents

the synthesis of bacteria-chlorophyll in *Rhodospseudomonas* sp. This could be the reason attributed to the minimum growth under aerobic condition. Spiral to Y shaped cells, absence of sulphur in or around the cell, 3 banded nature of carotenoid pigment, absorption maximum at near infra-red region (850 nm) and the above mentioned growth conditions suggest that the bacterium is a member of the family Athiorhodaceae<sup>7</sup>.

Growth was maximum in the medium having pH 7 and subsequent experiments were carried out at pH 7.

Different organic substances were used to study their effect on CO<sub>2</sub> fixation. From Table 2, it is quite evident that medium containing egg is best suited for CO<sub>2</sub> fixation followed by that containing peptone. In all the other media there was a reduction in CO<sub>2</sub> fixation ranging from 40.3 to 94.3%. Like all the other organic substrates used, sodium formate and mannitol had also reduced the growth (76.7 and 78.1% respectively) which is in agreement with the studies of Stoker and Hoare<sup>8</sup>.

In the presence of most of the organic substrates there was a reduction in growth. However, maximum reduction was obtained in acetate (94.3%) which might have been due to the repressive effect (75%) of acetate on ribulose-1,5-diphosphate carboxylase as suggested by Anderson and Fuller<sup>9</sup>. Though we have not studied the inhibitory effect of citrate on ribulose 1,5-diphosphate carboxylase, the ultimate result was an inhibition of 87.7% which may be due to the inhibitory effect of citrate on ribulose 1,5-diphosphate carboxylase. In all the

TABLE 1 — EFFECT OF AIR AND LIGHT ON CO<sub>2</sub> FIXATION AND PIGMENT PRODUCTION

Conditions of Growth	Sea water with egg (1%) counts*, %		Pigment†		Sea water with peptone (1%) counts*, %		Pigment†	
	Incorporation	Reduction	OD at 490 nm	Reduction %	Incorporation	Reduction	OD at 490 nm	Reduction %
Air and light	3.2	80.83	0.022	88.24	3.4	77.93	0.002	98.83
Air and no light	3.5	78.94	0.018	90.38	4.0	74.03	0.015	91.18
No air and light	16.7	—	0.187	—	15.4	—	0.17	—
No air and no light	2.6	84.43	0.002	98.95	3.5	77.27	0.007	95.90

\*Counts incorporated into alcohol soluble fraction at the end of 48 hr growth

†Pigment at the end of 48 hr growth

TABLE 2 — EFFECT OF ORGANIC SUBSTRATES ON CO<sub>2</sub> FIXATION IN THE BACTERIAL ISOLATE AT THE END OF 48 HR GROWTH

Substrate (1%)	A Alcohol soluble fraction (%)		B Alcohol insoluble fraction (%)		Total %	
	Incorporation	Reduction	Incorporation	Reduction	Incorporation (A + B)	Reduction
Egg	17.7	—	47.5	—	65.2	—
Peptone	15.0	13.3	46.6	1.9	61.6	5.5
Sodium succinate	9.5	46.3	29.4	38.1	38.9	40.3
Potassium oxalate	0.5	97.2	20.7	56.4	21.2	67.5
Sodium formate	0.6	96.6	14.6	69.3	15.2	76.7
Mannitol	3.1	82.5	11.2	76.4	14.3	78.1
Sodium citrate	3.9	78.0	4.1	91.4	8.0	87.7
Yeast extract	3.1	82.5	4.0	91.6	7.1	89.1
Glucose	1.9	89.3	4.0	91.6	5.9	91.0
Sodium acetate	2.9	83.6	0.8	98.3	3.7	94.3

TABLE 3 — EFFECT OF NITROGENOUS CHEMICALS AND SOME POLLUTANTS ON CO<sub>2</sub> FIXATION BY THE BACTERIAL ISOLATE AT THE END OF 48 HR GROWTH

[Carbon source, egg 1%; chemicals, 1%; oil, 0.5%; and pesticide, 2 µmoles]

	A Alcohol soluble fraction (%)		B Alcohol insoluble fraction (%)		Total (%)	
	incorporation	Inhibition	Incorporation	Inhibition	Incorporation (A + B)	Inhibition
Control	17.7	—	47.5	—	65.2	—
Ammonium Sulphate	17.2	2.8	45.9	3.4	63.1	3.2
Sodium Nitrite	0.2	98.9	0.5	99.0	0.7	99.0
Sodium nitrate	15.2	14.1	12.5	73.7	27.7	57.5
Sodium Azide	3.7	82.5	12.4	73.9	16.1	75.3
Dicyston	26.1	47.5	30.5	35.8	56.6	13.2
Thiophamine	12.6	28.8	30.4	36.0	43.0	34.1
Dasanit	4.7	73.4	13.2	72.2	17.9	72.6
Fenitrothione	1.8	89.8	3.3	93.1	5.1	92.2
Crude oil	3.6	80.0	9.6	79.8	13.2	79.8
Kerosene	10.2	42.4	8.7	81.7	18.9	71.0
Petroleum	4.6	74.0	0.8	98.3	5.4	91.7

other cases, except in sodium acetate, CO<sub>2</sub> incorporation in the alcohol soluble fraction was less than that of alcohol insoluble fraction.

In the presence of nitrogenous chemicals, CO<sub>2</sub> fixation was inhibited to varying degrees (Table 3). Of the 4 nitrogenous chemicals used in the present study, ammonium sulphate was found to inhibit less than the others (3.2%) and sodium nitrite had the maximum inhibitory effect (99%). Nitrites and azides, known for their toxicity, have inhibited the CO<sub>2</sub> fixation by 99 and 75.3% respectively. Ammonium sulphate does not affect the bacterial enzyme<sup>10</sup> which explains its minimum inhibition (3.2%) in the present isolate.

To study the effect of pesticides and oil (pollutants) on CO<sub>2</sub> fixation, different pesticides and hydrocarbons were used (Table 3). Inhibition was maximum in fenitrothione (92.2%) among pesticides and in petroleum (91.7%) among the hydrocarbons. Similar observations were made by Walker and Colwell<sup>11</sup>, who reported that petrohydrocarbons limit the growth of microbial flora of both estuarine and marine environment. This holds good in the case of Athiorhodaceae also where all the hydrocarbons have inhibitory effect on their growth and CO<sub>2</sub> fixation to varying degree.

These studies show that the pollutants, as in present study, are always present in sea water and their inhibitory effect on photosynthesis affects the productivity and will in turn lead to disturbed food chain.

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### Diel Variation in Phytoplankton Pigments & Primary Production off Bombay

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Variations in biological parameters over 24 hr at 4 stations off Bombay indicated that chlorophyll *a* concentration, organic carbon production and particulate organic carbon (POC) were generally maximum during the day. Concentrations of pigments at any depth ranged from 0.09 to 1.74 (mg m<sup>-3</sup>). Similarly the production and POC varied from 1.15 to 86.12 (mgC m<sup>-3</sup> d<sup>-1</sup>) and 107.6 to 585.4 (mgC m<sup>-3</sup>). 'Smaller phytoplankton' contributed 50 to 100% to the pigment of the total phytoplankton. The maximum in assimilation number was found when NH<sub>4</sub>-N and NO<sub>3</sub>-N were maximum.