Studies on Cultural Conditions of Marine *Chromatium buderi* Truper and Jannasch 1968

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Photosynthetic characteristics of a purple sulfur bacterium, *Chromatium buderi,* cultured under different ranges of pH, temperature, light intensities and ammonium chloride concentrations were examined. Maximum bacteriochlorophyll *a* synthesis was observed at pH 6.5 whereas the optimum growth was at pH 8.0. In general, higher temperature tended to inhibit the chlorophyll *a* synthesis and growth. 30°C is the optimum temperature both for chlorophyll *a* synthesis and growth. At 25μE $m^{-2}S^{-1}$ the bacteriochlorophyll *a* content and growth attained maximum level. The response to this low light intensity is an adaptation that ensure a high photosynthetic rate for the purple sulfur bacterium that usually occurs in dimly lit environment. Besides these, ammonium chloride at low concentration enhances both chlorophyll *a* synthesis and growth. Above 0.5% of it cause the nitrogen-chlorosis and also retard the growth of the bacterium. Possible chemical and structural mechanisms involved are discussed.

The ability of photosynthetic bacteria to grow in anaerobic environment and their dependance on the light, pH, temperature, hydrogen donor and nutrients were well documented by Van Niel (1944). They have an important role in the production of organic matter as well as the purification of polluted sewage waters in the aquatic environment (Takahashi & Ichimura, 1968). The photosynthetic response of the purple sulfur bacteria to the environmental conditions both in quantity and quality must be considered for the ecology of photosynthetic bacteria (Parson *et al.,* 1969). Bergstein *et al.* (1979) reported the temperature range in Lake Kinneret, Israel, in which they observed the bloom forming, *Chlorobium phacobacteriodes* and noted that the temperature had a specific implication over the formation of bloom in the stratified lake. Besides, the role of nitrogen as one of the limiting factors for productivity has relatively been neglected. Thus, because of increasing process of eutrophication, it is becoming important to study the physiological properties of the photosynthetic bacteria under varying levels of nitrogen sources. Recently Shilo and Rhimon (1982) observed that ammonia was the toxic inhibitory metabolite in the intensive fish breeding ponds and they utilized the bloom-forming cyanobacteria to breakdown the inhibitors leading to the build up of organic matter. Subsequently Dhevendaran (1984) studied the utilization of sulfur, as well as other organic compounds over the growth of a selected *Chromatium* sp. strain in Porto-Novo waters. Differential utilization of organic compounds under anerobic conditions resulted in the change of pH of the growth medium. It is conceivable that the fermentative pathways are functional in cells growing anerobically (Uffen&Wolfe, 1970). The occurrence and the functional significance of photosynthetic bacteria in Menai Bridge waters have not been studied previously. Truper & Pfennig (1981) reported the charactetistic features of the anoxygenic photosynthetic bacteria. Following their techniques in the present study an attempt has been made to optimize the different cultural conditions of *Chromatium buderi* in the laboratory.

Materials and Methods

Sediment samples were collected using Peterson grab and transferred aseptically

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to sterile polythene bags. One gram of wet sediment was aseptically removed and transferred into 100 ml of the sterile enrichment medium. The medium contained per litre of aged seawater; 1.0 g $MgSO_4$, 0.6 g K_2HPO_4, 3.0 g NH_4Cl, 1.0 g yeast extract, 2.58 g sodium malate, vitamin B_2 solution as described by Pfennig and Lippert (1966) and 0.5 g Na_2S. The pH of the medium was adjusted to 6.8.

The medium with sample was taken in glass stoppered bottle and incubated with continuous illumination at 20 $\mu Em^{-2} S^{-1}$ at room temperature (25 $\pm$ 2°C) for 10 days. After the incubation period, 0.1 ml of aliquot was taken and poured into the sterile

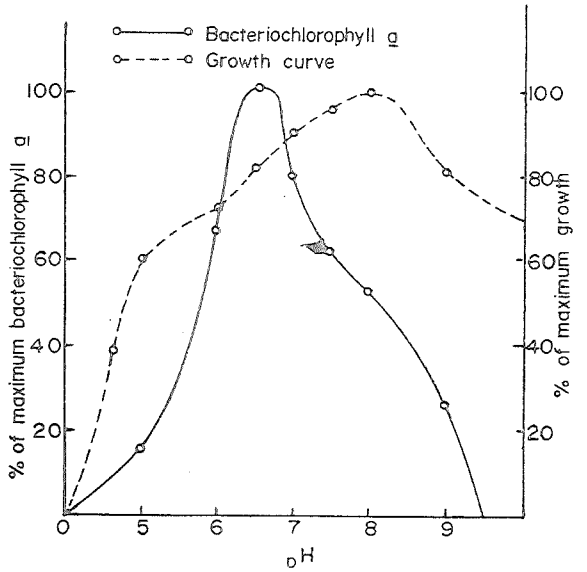

Fig. 1. Effect of pH on bacteriochlorophyll *a* synthesis and growth of *C. buderi*

petridishes following the method of Siefet *et al.* (1978) and mixed the above enrichment medium with 2% agar and incubated again under continuous illumination as before. Single pure colonies appeared were identified according to the method of Pfennig & Truper (1974) and Truper & Pfennig (1981). Protein content of the bacterial culture was estimated by the method of Lowry *et al.* (1951).

Bacteriochlorophyll *a* was extracted from pure culture of purple sulfur bacteria with acetone: methanol (7:2). 10 ml of cell supension was centrifuged, the pellet was resuspended in 1 ml of distilled water and 9 ml of solvent mixture was added. After 30 min at 4°C, the mixture was centrifuged at 7,000 rpm. The absorbance of the supernatant was measured at 775 nm. The bacteriochlorophyll *a* concentration was calculated using the extinction coefficient E 775 = 75 litres/n mol cm as given by Clayton (1963).

Results and Discussion

Fig. 1 shows the effect of pH on the synthesis of bacteriochlorophyll *a* and also on the growth of *C. buderi*. Maximum bacteriochlorophyll *a* synthesis was noticed at pH 6.5. At pH 8.0, only 50% of the maximum bacteriochlorophyll *a* synthesis was observed. Good growth of *C. buderi* occurred at pH range 6.5 to 9.0 with a maximum at pH 8.0. close to that of sea water. From this investigation it may be inferred that the bacteriochlorophyll *a* synthesis and growth of *C. buderi* are independent and the pH is one of the determining factors for the survival and productivity in the anaerobic condition in the marine environment, thereby determining the ecological niche of the isolate studied. Similar pattern of observation regarding the growth and longivity of bioluminescence of the bioluminescent bacteria was made by Lakshmanaperumalsamy *et al.* (1982) in tropical seawater. The isolatc seems to be more sensitive towards the acidic rather than alkaline conditions. Recently Dhevendaran *et al.* (1986) studied the cultural conditions on arylsulfatase activity in *Escherichia coli* and observed maximum

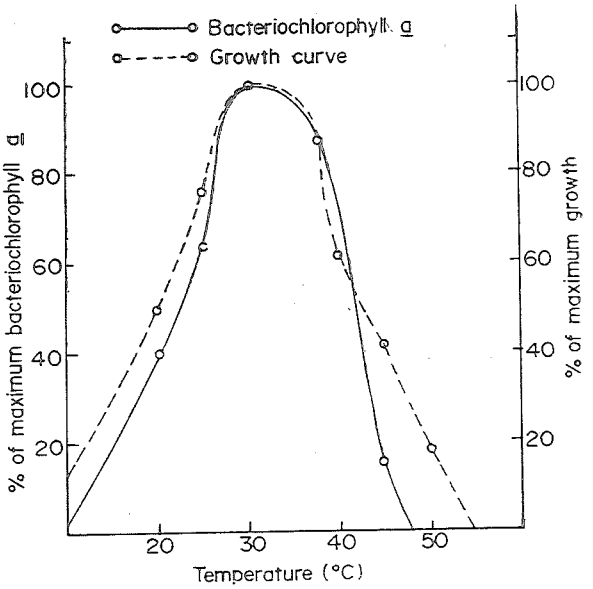

Fig. 2. Effect of temperature on bacteriochlorophyll *a* synthesis and growth of *C. buderi*

enzyme activity at pH 6.6 whereas the optimum growth of the strain occurred at pH 5.6 thereby confirming our results that the growth is independent of the physiological activity of the culture.

C. buderi has been tested further for its reaction towards temperature. Fig. 2 shows that both enhanced bacteriochlorophyll *a* synthesis and optimum growth noticed at 30°C. However, they were not affected to any considerable extent when it was incubated both at 25°C and 37°C. Milazzo & Fitzgerald (1967) recorded similar observation in *Proteus rettgeri* where maximum growth and highest arylsulfatase activity occurred at 28°C. Subsequently Dhevendaran *et al.* (1986) confirmed it with the same pattern of activity and growth of *Escherichia coli* at 29°C. When the temperature was raised above 40°C, there was a declining trend both in growth as well as in chlorophyll *a* synthesis (Bergstein *et al.*, 1979).

Photosynthesis and growth were determined in growing cells at different light intensities, ranging 10–50 $\mu E\ m^{-2}S^{-1}$ at 30°C (Fig. 3). The results indicated that light intensity exerted its most pronounced effects

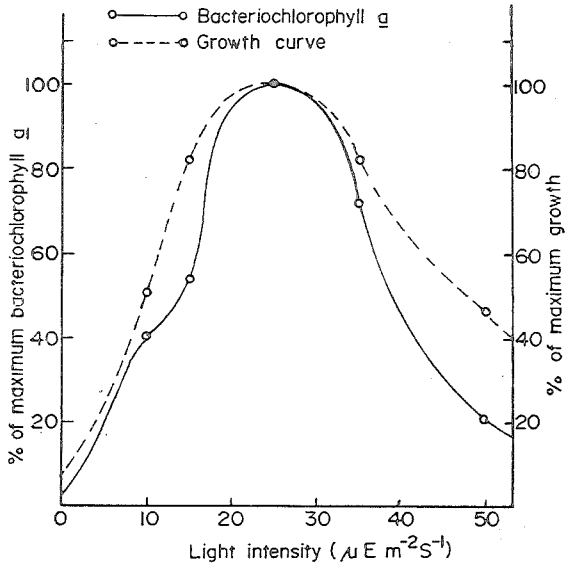

Fig. 3. Effect of light intensity on bacteriochlorophyll *a* synthesis and growth of *C. buderi*

on the bacteriochlorophyll *a* synthesis and also on growth of *C. buderi*. As the light intensity increased from 10 to 15 $\mu E\ m^{-2}S^{-1}$, there was an increase of 15% chlorophyll *a* synthesis. However, with further increase in the light intensity upto 25 $\mu E\ m^{-2}S^{-1}$, there was a sudden rise (about 3 fold) in the bacteriochlorophyll *a* content reaching the maximum concentration. Photosynthetic rate was related to the amount of bacteriochlorophyll *a* and growth of *C. buderi* was also attained the maximum level. From the present study, it became clear that the purple sulfur bacterium, *C. buderi* attained the maximum chlorophyll *a* synthesis per mg protein at a light intensity of 25 $\mu Em^{-2}S^{-1}$ and showed a higher photosynthetic rate similar to the report of Takahashi *et al.* (1972). The relationship between chlorophyll content and light intensity have already been reported by Sistrom (1962), Holt & Marr (1965), Takahashi *et al.* (1972) and Dhevendaran (1984) for purple bacteria, and Nielsen and Jorgensen (1968) for green algae. Their results showed continuous increase of chlorophyll per mg protein as zero light intensity was approached. In the present data, however, samples grown in very low light (10 and 15$\mu Em^{-2}S^{-1}$)showed lower chlorophyll levels than those at 25 $\mu E\ m^{-2}S^{-1}$.

Further, Fuller *et al.* (1963) and Cohen-Bazire (1963) have reported that the plastids of photosynthetic bacteria change morphologically depending on light intensities: samples grown in 'high' light had low numbers of chromatophores and sometimes fewer lamellae than corresponding samples grown in 'low' light. However, cells grown under anerobic dark condition (Uffen & Wolfe, 1970) have less membrane system of characteristic chromatophore than in anaerobic light condition. Further, *Rhodospirillum rubrum* which was grown anerobically, in the dark, was exposed to an actinic light beam at 874 nm, the cytochromes become oxidized as characterized by a decrease in absorption, and the absorption capacity was regained by cytochrome and became reduced when the actinic light was shutt off. These data indicate that cells of *R. rubrum* growing under anerobic dark condition continued to synthesize a complete light responsive pyotosynthetic apparatus. These changes in plastids and chromatophores might also influence the photosynthetic rates.

The uptake of ammonia by the photosynthetic bacteria plays an important role

in the reduction of toxicity of ammonia, which is one of the major inhibitory metabolites of the fish and is considered to be a powerful fish poison. It is also produced by the ammonification process of the organic nitrogenous matter anerobically in the sediments (Ram *et al.*, 1980). Besides this, the sediment serves both as a source from which ammonia may leak out and diffuse into the water column, as well as a sink and trap for the nitrogenous matter from the water-column. In order to establish the physiological role of *C. buderi,* an attempt has been made to know the uptake of ammonia for growth and utilization for synthesis of organic compound using ammonium chloride. The uptake of ammonium chloride is summarized in Fig. 4 and it shows that it is more rapid at lower concentration rather than in higher concentration. At 0.1% level the rate of bacteriochlorophyll *a* synthesis was

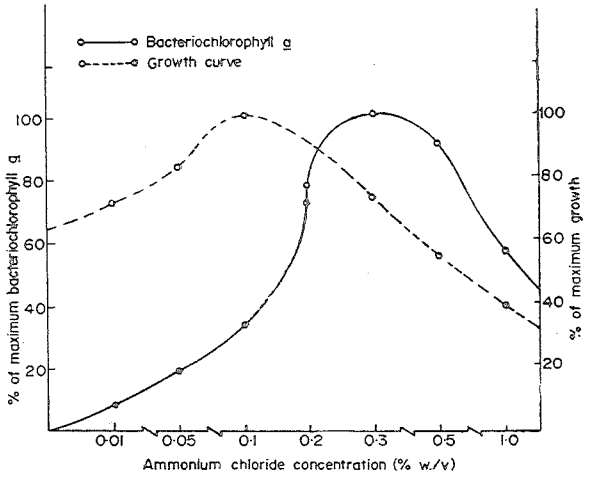

Fig. 4. Effect of ammonium chloride on bacteriochlorophyll *a* synthesis and growth of *C. buderi*

30% of maximum synthesis whereas between 0.1 and 0.3% level the rate of synthesis was maximum. It is understood that the utilization at this stage might be due to nitrogen assimilation with the carbon skeleton stored in the cell during photosynthetic activity. This is confirmed by the observation made by Shilo & Rimon (1982) with *Microcystis marginata.* With further increase in concentration it exerted a declining trend and it may inhibit the enzymatic pathways as suggested by Losada & Guerrero (1979). Subsequently, Flores *et al.* (1980) observed that ammonium at low concentration caused a rapid and effective inhibition of nitrate utilization in cyanobacterium, *Anacystis nidulas.* The actual factor determining the ammonium effect could thus be either the entry of ammonium into the cells or its incorporation into amino acids. Transport of ammonium into the cells might interfere with that of nitrate by competing for some common transport element or energy source that is present in limiting amounts. Evidence has been shown by Laane *et al.* (1980) and Jones & Monty (1979) indicating that short-term depressing effect of ammonia on nitrogen fixation in *Azotobacter vinclandil* and *Rhodopseudomonas sphacroides.* It is noted that excess ammonium accumulation resulted in the bleaching of the cells thereby causing the 'nitrogen chlorosis' as observed by Liere *et al.* (1977) in the blue-green algae, *Oscillatoria agardhil.*

It seems that there is a close relationship existing between the ammonium utilization and bacteriochlorophyll *a* synthesis to the specific concentration of ammonium chloride. The medium without the ammonium chloride showed only a feable concentration of bacteriochlorophyll *a* . It is understood that the actual factor for determining the ammonium effect for the normal metabolism of the bacterial cells is thus imperative. Since the role of marine photosynthetic bacteria in the nitrogen cycle of the sea is yet to be fully established, it could be more appropriate to study the metabolic pathways with intermediate enzymes.

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