Progressions of phototrophic bacteria and sulphur chemistry in decomposition models

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Decomposing cultures of macro-organisms in sea water, with no added mineral media, produced faithful enrichments of phototrophic sulphur bacteria. The enrichments brought about spectacular colouration of the sea water solutions and specific bacterial progression, depending on the type of decomposing biomass. These patterns were remarkably consistent in enrichments from geographically isolated marine locations on the east coast of India. Chemical analysis of sea water in decomposition experiments with or without lighting indicated incomplete sulphide oxidation by the phototrophs, yielding primarily elemental sulphur. Contrary to the presumption that marine sponges are the only exceptional aerobic habitat for phototrophic sulphur bacteria, the present results strongly suggest that these anaerobic bacteria may generally subsist in association with macro-organisms and are prevalent in coastal waters.

PHOTOTROPHIC sulphur bacteria are a specialized group of microorganisms that carry out the oxidation of sulphide under anaerobic conditions[1,2]. The phototrophs are coloured by virtue of their pigment and carotenoid components. They occur regularly in sulphide-rich environments such as anoxic lakes and fjords where mass blooms bring about spectacular colouration of the waters[3–5].

Numerous studies around the world oceans, including the Indian seas, have shown that phototrophic sulphur bacteria could be readily enriched and cultured[6-13]. Some investigators, notably Trüper[14] and Imhoff[15], suggest that these bacteria are generally absent in oxygenated parts of the ocean, limited only to anaerobic habitats. They presume that oxygen will discourage anaerobic microbial activity. Paradoxically, other anaerobic bacteria, e.g. sulphate-reducing bacteria, are now known to occur in oxygenated environment (in a dormant state) and to become active during favourable conditions[16]. Indeed, aerobic respiration has now been demonstrated in sulphate reducers, that accounts for their remarkable ability to cope with oxygen[17]. It has not been clearly established whether the phototrophs would also be present in oxygenic parts of the sea.

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It is also generally presumed that the only exceptional aerobic marine habitat for phototrophic sulphur bacteria is the body portion of Porifera[14,15,18]. Eimhjellen[18] isolated *Chlorobium limicola*, *Thiocystis violacea* and *Thiocapsa roseopersicina* from a marine sponge. Following up, Imhoff and Trüper[19] employed four sponge species and confirmed the findings. Using molecular tools, Webster *et al*.[20] have also demonstrated the association of phototrophic sulphur bacteria with sponges. Marine organisms other than the sponges, however, have not been traditionally employed in creating enrichments of the phototrophs.

Two basic inquiries prompted this work. Firstly, a detailed study was considered necessary that dealt with potential enrichments of phototrophic bacteria from macro-organisms further than the sponges. Secondly, the changes in sulphur chemistry associated with the growth of phototrophs also required particular examination. The main objective of the present work was hence to gain insights into the ecology of marine phototrophic sulphur bacteria.

Decomposing cultures were set-up as previously addressed[21]. Samples of *Caulerpa peltata* (green alga), *Gracilaria corticata* (red alga), *Crassostrea cucullata* (oyster) and *Tedanus anhelans* (sponge) were sampled from Tuticorin harbour area (southeast coast of India; 8°47′N; 78°9′E). The algae and soft portions of the animal species were let to decompose in separate 1 l stoppered glass vessels filled with freshly sampled coastal sea water (in 5% w/v ratios). The samples were placed adjacent to the northern windows of the laboratory in such a manner as to provide diffuse sunlight during the daytime. The light intensity (Kyoritsu 5200 illuminometer) was 1000 to 3000 lux in the early morning and evening hours, and between 6000 and 8000 lux during the rest of the day. No lighting was given at night, such as to provide a very nearly 1 : 1 light/dark cycle.

During the decomposition sequence, solution pH and dissolved oxygen were periodically measured. In separate experiments, changes in sea water sulphur chemistry were investigated in decomposing cultures of *C. peltata* (5% w/v). Two cases of lighting were employed – a truly darkened condition (D) in which the light level was below the detection limit (20 lux) of the illuminometer, and a light/dark (L/D) cycle as previously mentioned. At time intervals, estimations were made of sulphate, thiosulphate, sulphide[22] and elemental sulphur[23].

As phototrophic bacteria occurred in very high numbers in the enrichments, agar shake dilution technique was directly employed[2]. Media preparation and incubation for Chromatiaceae, Chlorobiaceae and Ectothiorhodospiraceae were done according to Pfennig and Trüper[2], and Imhoff[15]. Enrichments from the biomass and the purified isolates were looked at under a Hertel and Ruess trinocular microscope for colour, motility, shape and size. *In vivo* absorption spectra were recorded on a Shimadzu 160 UV spectrophotometer in whole cells suspended in 30% bovine serum[24].

The above experiments were all done between the years 1992 and 1996. Since Tuticorin harbour is particularly known for a high number of sulphur cycle bacteria in the waters[25], supplementary locations were considered necessary in order to ascertain that the observed patterns were unexceptional. As a result, enrichments were additionally set-up with samples and sea water taken from Mandapam region (9°12′N; 79°17′E) in the Gulf of Mannar during June 1996 to January 1997. Enrichments were also set-up in Andamans (August 1999 to December 2000), with samples and sea water taken from Port Blair Bay (11°40′N; 92°45′E) and sheltered beaches in the neighbouring Jolly Boys Island. These tests were limited to an examination of the progression in bacterial types and colour in decomposing cultures of *Ulva*, *Caulerpa* and *Halimeda* species.

Figure 1 illustrates the three types of colouring sequences in Tuticorin enrichments. The control in the figure corresponds to sea water with no added macro-organism and is coloured grey for clarity. The initial colouring pattern was identical regardless of the type of decomposing species, and the sea water turned purple-pink in 3–4 days. In the *C. peltata* vessel (as also with *T. anhelans*, not shown in the figure), there was a gradual change in colour from pink to green by the third week of decomposition. In contrast, sea water in the *C. cucullata* vessel exhibited conversion from pink to red during the same time period. One further sequence, namely a pink to green to red transformation, occurred with *G. corticata*. It was verified from innumerable trials that these colouring patterns were consistent and biomass-dependent.

In the Mandapam and Andaman enrichments (with green and calcareous algae), the pattern was identical to case 1 in Figure 1, where the solution initially turned purple-pink and then green. The one noteworthy variance with Andaman samples was a considerably long incubation time (two to several weeks) before the sea water became noticeably coloured.

Figure 1 also shows variations of sea water solution pH. The pH of sea water with no macro-organism decreased from 8.1 to 6.95 in 30 days. The decrease was more marked in decomposing cultures, and the pH minima occurred during the first 2–3 days. There was a return of solution pH later, which was most rapid and largest with *G. corticata*. Dissolved oxygen decreased to insignificant values by 36 h, indicating the incipience of anaerobic conditions.

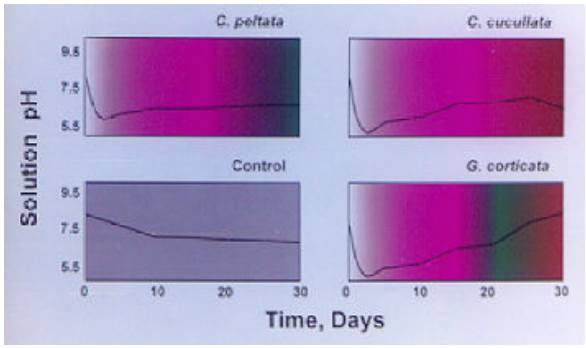

Figure 1. Patterns of pigmentation and solution pH variations in decomposing cultures of three marine macro-organisms in sea water. Control in the figure refers to sea water with no added macro-organism and is coloured grey for clarity.

Figure 2 shows sulphur chemistry variation in decomposing cultures of *C. peltata* with or without lighting. With either condition, there were small increments in sulphate concentration after 24 h, but the concentration decreased thereafter. The reduction was more progressive in the D vessel, while minor variations occurred in the L/D vessel. The thiosulphate increase in the initial periods was much higher than sulphate, followed by sharp decrease until 7 days. Thereafter, the trends were reversed for the two cases of lighting – thiosulphate concentration went up in dark, while it was further reduced in the L/D condition. Sulphide progressively increased in the first 10 to 12 days. Under darkened conditions this high level stayed fairly unchanged between 13 and 37 days whilst sulphide diminished rapidly in the L/D vessel. Levels of sulphur were low all through in the dark. Note that in the L/D case, there was a dramatic increase in sulphur concentration after the first week, and this was coincident with the sharp reduction in sulphide.

Table 1 summarizes the characteristics of all phototrophic bacteria that were enriched and/or purified from Tuticorin samples. The genus level assignment is based on microscopy and absorption spectra characteristics[2,26]. Only three of the listed organisms were obtained in purity, and the absorption spectra are shown in Figure 3. *Thiocystis* sp. shows a major peak at 831 nm and two minor peaks at 585 and 521 nm. The first two are typical of bacteriochlorophyll-*a*, while the peak at 521 is

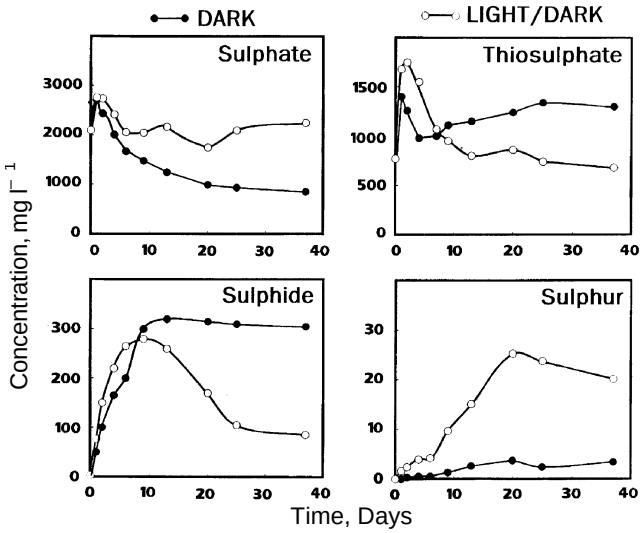

Figure 2. Variation of sulphate, thiosulphate, sulphide and elemental sulphur in decomposing cultures of *Caulerpa peltata* in sea water (5% w/v) with or without lighting.

Table 1. Characteristics of phototrophic bacteria detected in Tuticorin samples

Group/Organism	Size	Shape	Motility	Sulphur storage pattern
Purple sulphur bacteria				
Chromatiaceae				
Thiocystis sp.	2–3 μm dia	Ovoid to spherical	Motile	Along periphery of cytoplasm
Thiospirillum sp.	About 20 μm long	Rods	Strongly motile	Intracellular
Thiodictyon sp.	1–2 μm wide, 5 μm long	Rods	Non-motile	Along periphery of cytoplasm
Ectothiorhodospiraceae				
Ectothiorhodospira sp.	1 μm wide, 2–3 μm long	Bent rods to spiral	Motile	Outside the cells into medium
Green sulphur bacteria				
Chlorobium sp.	1 μm wide, 1–4 μm long	Rods	Non-motile	Outside the cells into medium
Oscillochloris-like	5 μm wide, indefinite length	Filamentous	Gliding motility	Unknown
Other bacteria				
Purple non-sulphur bacteria	1 μm wide, 2–4 μm long	Rods	Non-motile	None
Spirulina-like	3 μm wide, unknown length	Filamentous	Gliding motility	None

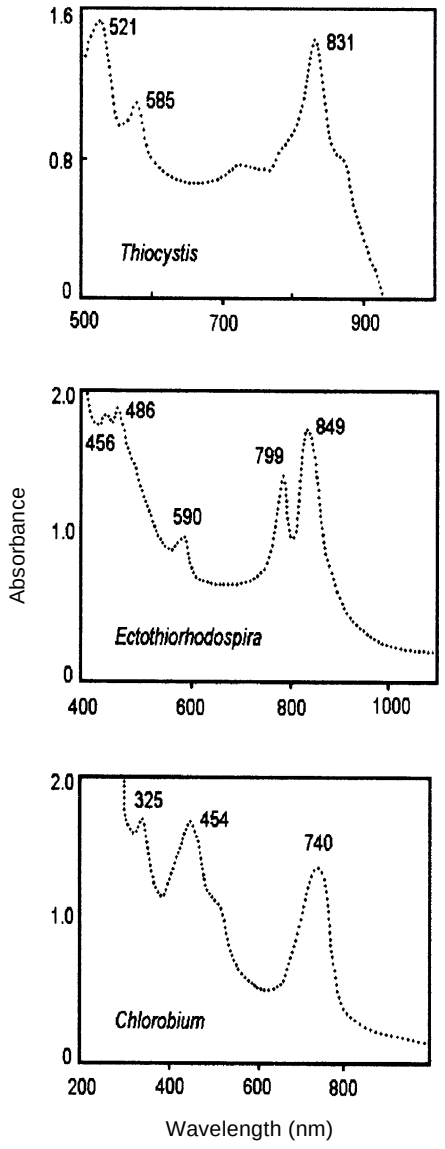

Figure 3. *In vivo* absorption spectra for *Thiocystis*, *Ectothiorhodospira* and *Chlorobium* species.

indicative of carotenoids of the okenone series. *Ectothiorhodospira* sp. presents bacteriochlorophyll peaks at 849 and 799 nm and minor peaks relative to spirilloxanthin. Long wavelength absorption maximum at 740 nm (bacteriochlorophyll-*c*) and minor peaks designating chlorobactene are characteristic of *Chlorobium* sp.

Enrichments from Mandapam produced essentially the same bacterial types as Tuticorin and, in addition, *Prosthecochloris*. In Andaman samples, *Chromatium*- and *Chlorobium*-like bacteria were most common besides several, as yet unidentified, cyanobacteria and purple non-sulphur bacteria. The *Chromatium*-like rods were 4–5 μm long, exceptionally motile, and showed intracellular sulphur along peripheral ends. Their absorption spectrum given in Figure 4 shows carotenoid peaks at 443 and 470 nm (probably relative to violaxanthin), bacteriochlorophyll peak at 840 nm and, rather curiously, the absorption maximum at 970 nm.

In the present work, the purple-pink bacteria occurred in the early periods of decomposition at lower pH and higher sulphide levels. The green sulphur bacteria came much later, when the pH and sulphide trends were reversed. At first sight, this appears somewhat anomalous because numerous records[3,4,27-29] indicate that green sulphur bacteria prefer to establish in areas of high sulphide and low pH, such as the bottom layers in stratified lakes. The discussion below will account for this anomaly.

To begin with, the green sulphur bacteria in this work appeared only after the purple bacteria 'coated' the walls of the vessels. Even during transition from purple to green colour of the sea water, the green sulphur bacteria were initially not directly visible from outside and were detected only by sampling. This could be perceived as the influence of an ideally low light condition inside the vessels, that was apparently necessary. The ability of *Chlorobium* to grow at low light intensities is of ecological significance[30], and these bacteria can endure light intensities as low as 5 lux (ref. 11). It is also possible that the spectral quality of light that passed through the

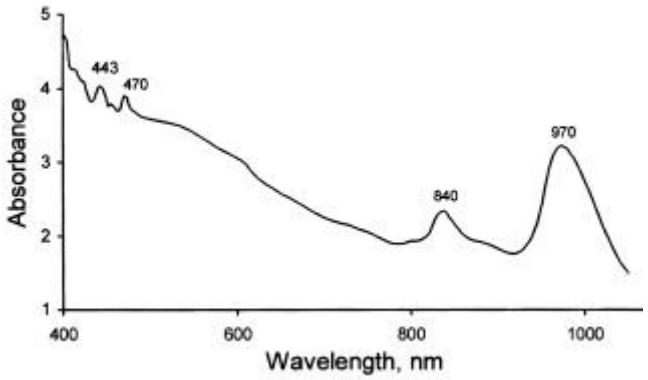

Figure 4. Absorption spectrum for *Chromatium*-like bacterium isolated from Andaman waters, showing unusual bacteriochlorophyll absorption maximum at 970 nm.

Chromatiaceae 'window' suited the green sulphur bacteria[4]. Next, the growth of these bacteria in the enrichments occurred over a wide pH range, 6.3 (*C. peltata*) to 8.0 (*G. corticata*).

Some results from coloured Wisconsin lakes[3,27] are also worth stating. In particular, the position of green bacterial layer in Kraack Lake was at 2–3 m below the surface, in contrast to most other lakes where the layer occurred around 10 m. The occurrence of green sulphur bacteria at an unusually high level was attributed to high amounts of dissolved organic matter, which apparently resulted in extreme light attenuation. The above issues together imply that light levels critically influenced bacterial progression in this work more than either pH or sulphide.

Earlier enrichments from the Indian seas have regularly produced *Chromatium*, *Rhodopseudomonas*, *Prosthecochloris* and *Chloroflexus*-like species[7–9,11]. In this work, *Chromatium* occurred only in Andaman samples and *Chlorobium*, generally sporadic in other reports, was dominantly present. The absorption maximum for the *Chromatium* at 970 nm (Figure 4) is rather curious. This appears somewhat related to the unusual absorption maximum at 986 nm that Pfennig *et al.*[31] recorded for *Rhodospira trueperi*, although the significance of this spectrum remains unclear.

In the process of organic matter decomposition, denitrification[32] occurs as the initial step followed by intense sulphate reduction. Hydrogen sulphide can be liberated from decaying protein[33] as well as through bacterial sulphate reduction[34]. Biomass in its proteins contains, on the average, 1% bound (organic) sulphur[33]. Liberation by putrefaction would thus yield approximately 10 g H_2S per 1000 g of biomass. If, however, the same amount of biomass is channelled into bacterial sulphate reduction, according to:

$$2(CH_2O) + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S,$$

(where CH_2O is a general average formula for biomass that allows one to estimate stoichiometries), it could in principle yield 570 g H_2S per 1000 g of biomass.

Many of the solution chemistry changes that followed H_2S production can be explained by reactions inherent to phototrophic sulphur bacteria. A rapid increase in pH is clearly an effect of the phototrophs. At pH close to 7 when they start to bloom, sulphide would occur as HS^- (50%) and H_2S (50%)[35]. Phototrophs, however, consume stoichiometrically, the undissociated species[36]. Withdrawal of CO_2 and H_2S will lead to OH^- ions and hence to an increase in pH. The overall reaction catalysed by phototrophic sulphur bacteria in this work can be hypothesized as:

$$HCO_3^- + H_2S + HS^- \rightarrow (CH_2O) + S^\circ + 2OH^-,$$

where the formula in the parenthesis again means cell material. Data for sulphide and sulphur (Figure 2) are in excellent agreement with the suggested reaction. The results are also consistent with the perception[23,37] that incomplete sulphide oxidation (to elemental sulphur) is a prevalent pathway in marine conditions.

Lastly, the consistency with which phototrophic sulphur bacteria could be enriched using algae and animal species and the dependence of bacterial progressions on the type of decomposing biomass together suggest that these bacteria may sustain ubiquitous occurrence in coastal waters, similar to their anaerobic counterparts, the sulphate reducers.

Note added in the proof: Lately, Kobler *et al.* (*Science*, 2001, **292**, 2492–2495) have reported that photosynthetic anaerobic bacteria are abundant in the upper open ocean and consist of up to 11% of the total marine microbial community. The above finding very much validates the ideas presented in this paper.

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Extraction of terrain parameters from IRS-1C PAN stereo data using photogrammetric techniques

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Digital Elevation Model (DEM) is used for extraction of terrain parameters such as elevation, slope, aspect, contour, drainage pattern, etc. These parameters are often required in preparation of development and conservation plan for natural resources, infrastructure development, town planning, etc. Indian Remote Sensing Satellites IRS-1C/1D carry on-board push broom Linear CCD PAN camera, providing high resolution (5.8 m) stereo imagery of the earth's surface. DEM has been derived from PAN stereo pair of IRS-1C/1D using suitable mathematical models. It has been used to generate contours and extract drainage patterns. DEM generated from stereo data has been validated using ground control points (GCPs) and also by the surface-to-surface comparison method. Evaluation of drainage pattern is carried out by morphometric analysis.

TERRAIN parameters such as elevation, slope, aspect, contour, drainage pattern, etc. are often required as input in a large number of applications such as landslide hazard zonation, cellular phone network planning, watershed management, selecting sites for sewage treatment plants, solid waste disposal in urban areas, alignment of rail/road network, catchment area treatment, sitting of rainwater harvesting structures, wetland conservation and management, etc. In addition, these are also needed in modelling of soil loss, run-off and site suitability analysis. Conventionally these parameters are obtained through field measurement/survey or from existing topographic maps depending on the purpose and level of information required. The above-mentioned terrain parameters can be extracted from DEM (Digital Elevation Model).

The DEM and ortho image can be generated from satellite stereo data using photogrammetric techniques. DEM extraction from SPOT stereo pair has been attempted by Giles and Franklin[1], Bolstad and Stowe[2], Rousan *et al.*[3], Richard *et al.*[4]. Srivastava *et al.*[5] discussed the use of IRS-1C panchromatic data for cartographic applications. They have given a theoretical assessment of cartographic potential of IRS-1C imagery and the early results from few stereo pairs.

Lawrence[6] used DEMs to automatically map the stream channel and divide networks of a watershed. Jenson and Domingue[7] developed algorithms to extract topographic structure and to delineate watersheds and overland flow paths from DEMs. Veregin[8] discussed the effects of vertical errors in DEM on the determination of flow-path direction.

The Indian Remote Sensing Satellite IRS-1C/1D PAN camera has a spatial resolution of 5.8 m and it consists of 3 CCD arrays, each having 4096 sensor elements. The

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