

58. Biodegradation of Calcareous Shells of the Window-pane Oyster by Shell-boring Cyanobacteria

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Window-pane oyster shells collected from Siridaon Beach along the Zuari estuary in Goa were found to be heavily infested with shell-boring cyanobacteria identified as *Mastigocoleus testarum*, *Hyella caespitosa*, and *Plectonema terebrans*. Colonization of fresh healthy shells by these organisms was established in the laboratory by direct contact with infested shells. The infested shells were brittle, had low breaking strength, and heavy leaching of calcium and protein. A positive correlation between chlorophyll content and leaching of protein and calcium was observed, implicating the role of these cyanobacteria in the degradation of the shells. One of these shell-boring bluegreens, *H. caespitosa*, was successfully cultured.

INTRODUCTION

The disintegration of calcium-carbonate substrates by algae and fungi has been reported (Golubić 1970, Humm and Wicks 1980). The most successful penetrators of limestone are the cyanobacteria (blue-green algae).

The shells of the window-pane oyster, *Placuna placenta* L., which are plentiful in the intertidal zones of Siridaon Beach and Nauxim Bay of the Zuari estuary in Goa, are infested by cyanobacteria. This oyster is regularly exploited for food in Goa, except during the monsoon, when its population declines. The white shells are used to make window panes, lamp shades, screens, and many ornamental articles, and by the lime-processing industry. Infested shells are green or black in contrast to white, uninfested ones.

This paper reports on some ecological and physiological aspects of the shell-boring cyanobacteria and how they affect the shells that are commercially important to the local population.

MATERIALS AND METHODS

Window-pane oyster shells were collected during low tides from the inter-

tidal zones of Siridaon Beach along the Zuari estuary in Goa. The shells were always collected from rocky pools with muddy bottoms, and not from above the high-tide mark where the cyanobacteria become dessicated due to long exposures to sunlight. The shells were examined microscopically before and after decalcifying with 5% sodium EDTA solution (Aldermann and Jones 1967). The cyanobacterial mats recovered by this method were stained with 1% aniline blue for detection of mucilaginous sheaths.

Studies on infection were conducted in the laboratory using uninfested and infested shells, determined by microscopic examinations.

To estimate chlorophyll *a* of the cyanobacteria, the shell powder of green shells was extracted in acetone at 5°C, overnight, and absorbance of the extract read at 665, 645, and 630 nm. Richards with Thompson's (1952) method of calculation was used for estimation and calculation. Extraction of phycobilins was by the method described by Parry (1984).

Culturing of the blue-greens was in enriched seawater medium (ES liquid medium) (McLachlan 1973). D-glucose, glycerol, D-fructose, sucrose, and mannitol (10 mM) were tested as substrates for heterotrophic growth (Taylor 1983). The cultures were grown in 12:12 light:dark periods.

The infested and uninfested shells were washed with sterile seawater and incubated in sterile distilled water in Petri dishes. The amount of calcium leached out after three days was estimated by titration using the method described by Culkin and Cox (1966), and the amount of protein leached out was estimated using the method of Peterson (1977).

For estimation of the total calcium in the shells, 500 mg of healthy shell powder was digested with 5 ml of concentrated HCl, and the digest made up to 1 liter with distilled water. Ten ml of this sample was used for titration.

Maceration of infested and uninfested shells was compared by incubating the shells in 5% Na EDTA solution and testing the maceration with a pair of needles at hourly intervals for five hours.

The breaking strength of uninfested and infested shells was compared by applying weight loads to 1 cm strips of shells. The ends of the strips were glued to plastic frames using epoxy resin (Araldite) and hung by iron clips. Weight loads were added slowly to the weight pan, which was also hung from the iron clip. The load at which the strip broke in the middle was calculated as the breaking strength by using the following equation:

$$\text{Breaking strength in kg/mm}^2 = \frac{\text{Weight required for breaking}}{\text{Thickness of strip} \times \text{width of strip in mm}}$$

The broken strips were detached, weighed, powdered, and extracted in

acetone for chlorophyll estimation. Chlorophyll *a* content/g of shell versus breaking strength was used for comparing the amount of bluegreens and the relative breaking strength of shells.

Correlation coefficients of chlorophyll *a* versus calcium and protein leachates and versus the breaking points of the shells were calculated to estimate the significance of the values obtained.

RESULTS

The infested shells were green to black whereas the uninfested ones were white (figure 1a,b). On microscopic examination, three species of bluegreens were identified: *Mastigocoleus testarum* Lagerh., *Hyella caespitosa* Born. et Flah., and *Plectonema terebrans* Born. and Flah. Figure 2(a-d) shows the bluegreens photographed after decalcifying the shells. The presence of a mucilaginous sheath was detected by staining with 1% aniline blue. The presence of bluegreens in the shells was also confirmed by the extraction of phycobilins. Phycocyanin and phycoerythrin showed peaks at 625 and 560 nm, respectively.

When an infested piece of shell was placed in direct contact with an uninfested one at room temperature, fresh growth of cyanobacteria was established within ten days. Germinating and ungerminated heterocysts (figure 1 e, f) of *M. testarum* were observed on the healthy shell. No growth could be established when uninfested and infested shells were kept apart. *P. terebrans* grew readily from the infested shell onto a thin plastic film, which was floated on sterile seawater in a Petri plate. In this case, a piece of infested shell (0.5 cm in size) was placed on top of the floating film. Similar results were obtained in all the replicates.

Healthy shells, when incubated in wet mud taken from the intertidal region of Siridaon Beach, were infested with bluegreens within 20 days (figure 1 c, d).

The infested shells were brittle and could be broken easily whereas the healthy ones could not be broken that easily. The infested shells were macerated completely when treated with EDTA solution, whereas the healthy ones could never be completely macerated. This was quantified in terms of the breaking strength of the shell. There was an inverse correlation between chlorophyll content and breaking strength (figure 3). A shell having higher chlorophyll content, contributed by greater amounts of bluegreens, needed very little weight for breaking. These results were significant at the 0.1% level.

There was direct correlation between chlorophyll content and the calcium and protein leached out from the shells when these were placed in distilled water (figures 4, 5). The amounts of calcium and protein leached out, thus, increased with greater growth of bluegreens in the shells.

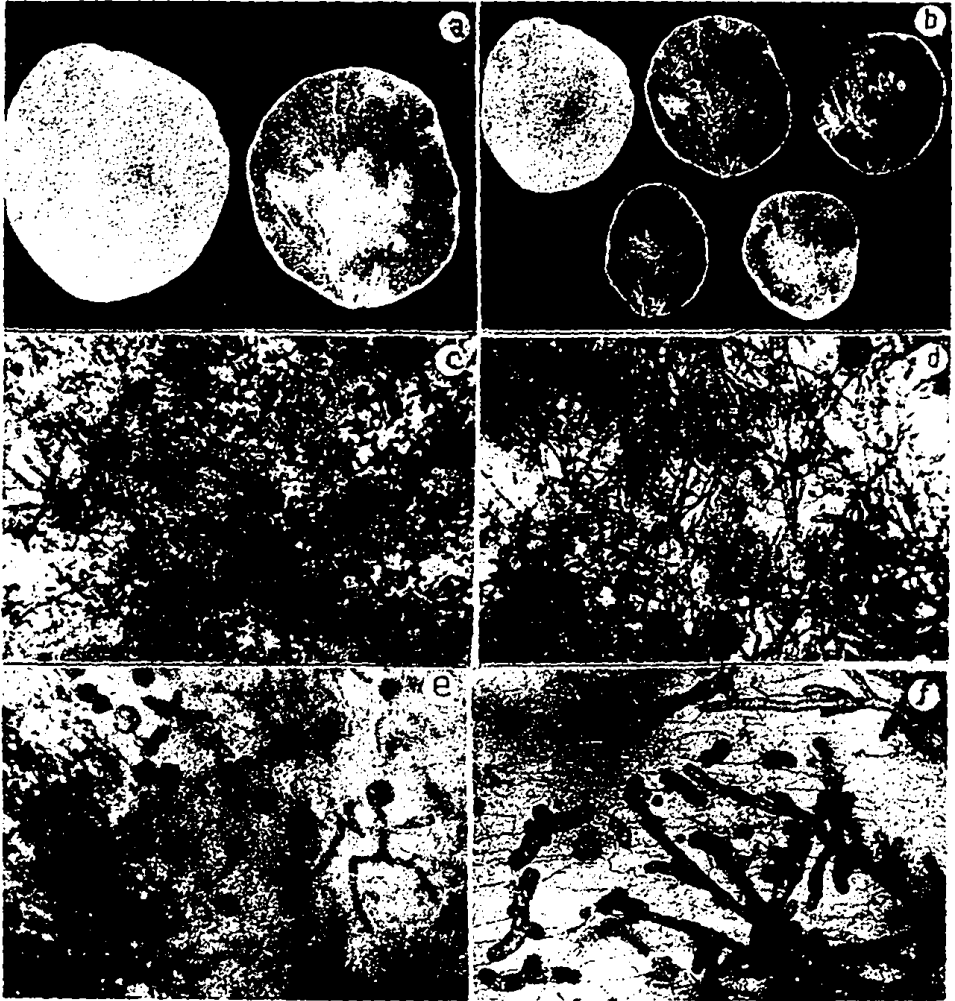


Figure 1 a. Healthy and infested shell, two thirds of original size. b. Healthy and infested shell with various degrees of infestation, half original size. c. Growth of *Mastigocoleus testarum* on the shell, 20 days after incubation in the mud from the collection site (x 100). d. Growth of *Plectonema terebrans* on the shell, 20 days after incubation in the mud from the collection site (x 100). e. Germinating heterocyst of *M. testarum* on a healthy shell, 10 days after direct contact with the infested shell (x 1600). f. Germinated and ungerminated heterocysts of *M. testarum* on the shell (x 600).

H. caespitosa grew in culture in the presence of mannitol on the bottom and along the wall of the culture flask. It did not show heterotrophic growth in the presence of other sugars tested, nor did it grow in the absence of mannitol.

DISCUSSION

The cyanobacteria reported here are common shell-boring organisms

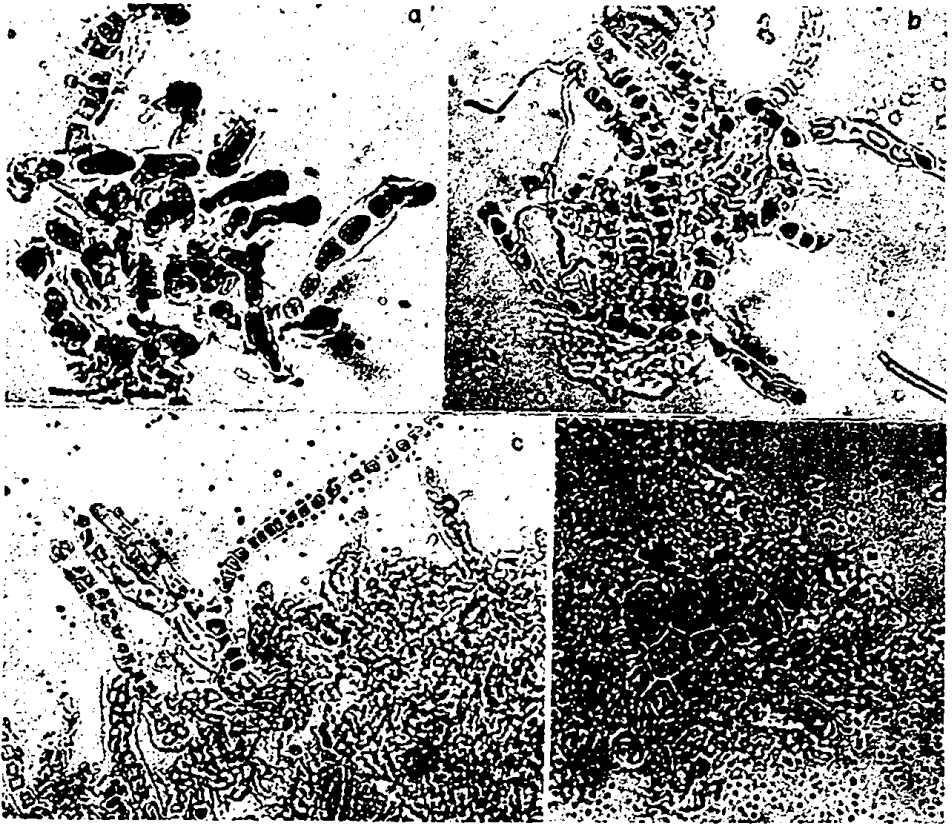


Figure 2 a-d. Bluegreens observed after partial decalcification of the shell with sodium EDTA solution, (x 450).

found on the beaches of India as reported by Desikachary (1959). They were detected in other shells and dead corals collected from the Lakshadweep Islands. In live oysters, they were restricted to the outer edges of their shells.

Boring of the shell by bluegreens is thought to be by dissolution processes (see Fogg et al. 1973, Golubić 1973) or by production of acids. During this process, they produce a tunnelling effect, making the shells brittle and resulting in a low breaking strength. With increased growth of bluegreens, this effect is more pronounced. Similarly, leaching of calcium ions and protein is four to five times greater than that of uninfested shells. Since white, uninfested shells provide the raw material for small scale industry in Goa, infestation resulting in green brittle shells is a problem. However, lately, completely black, infested shells are also being used to make decorative articles.

Healthy shells contain 300-400 mg of calcium per gram weight. If, on an average, 3-5 mg of calcium per day is leached out of an infested shell,

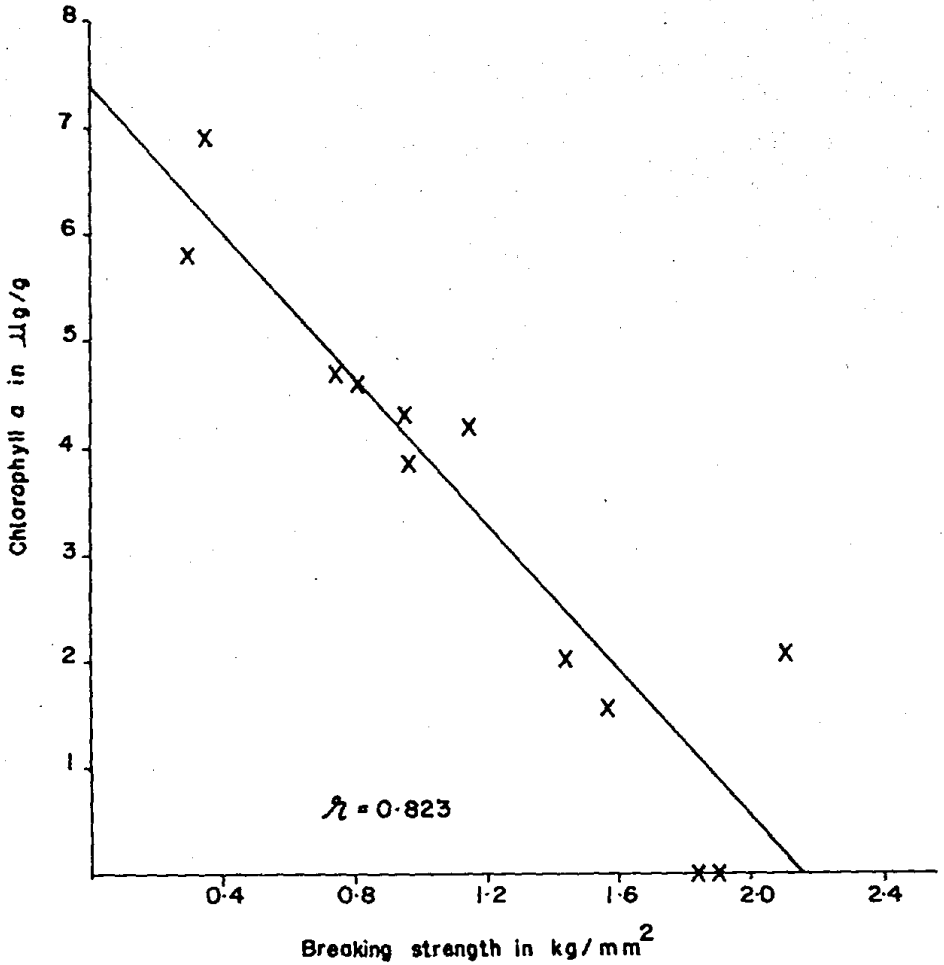


Figure 3. Relationship between the amount of cyanobacteria as determined by chlorophyll *a* content and breaking strength of shells.

(figure 4), the shell would be completely decalcified in about 90 days. In a healthy shell, 1-2 mg of calcium is leached out per day, which would take about 245 days for complete decalcification.

The cyanobacteria growing inside the shells would be highly adapted to alkaline conditions, high protein content, and low light. Under these circumstances, they might be adapting to a heterotrophic mode of growth. Until this study, none of the shell-boring cyanobacteria could be cultured in the laboratory. While other species of *Plectonema* were successfully cultured (Cannon et al. 1971), the shell-boring species *P. terebrans* was not. Therefore, the growth of *H. caespitosa* in the presence of mannitol is interesting. The coccolith covering of the unicellular alga, *Emiliania huxleyi*, contains mannose, xylose, and rhamnose in the calcium carbonate matrix (de Jung et al. 1976). The amount of this sugar alcohol,

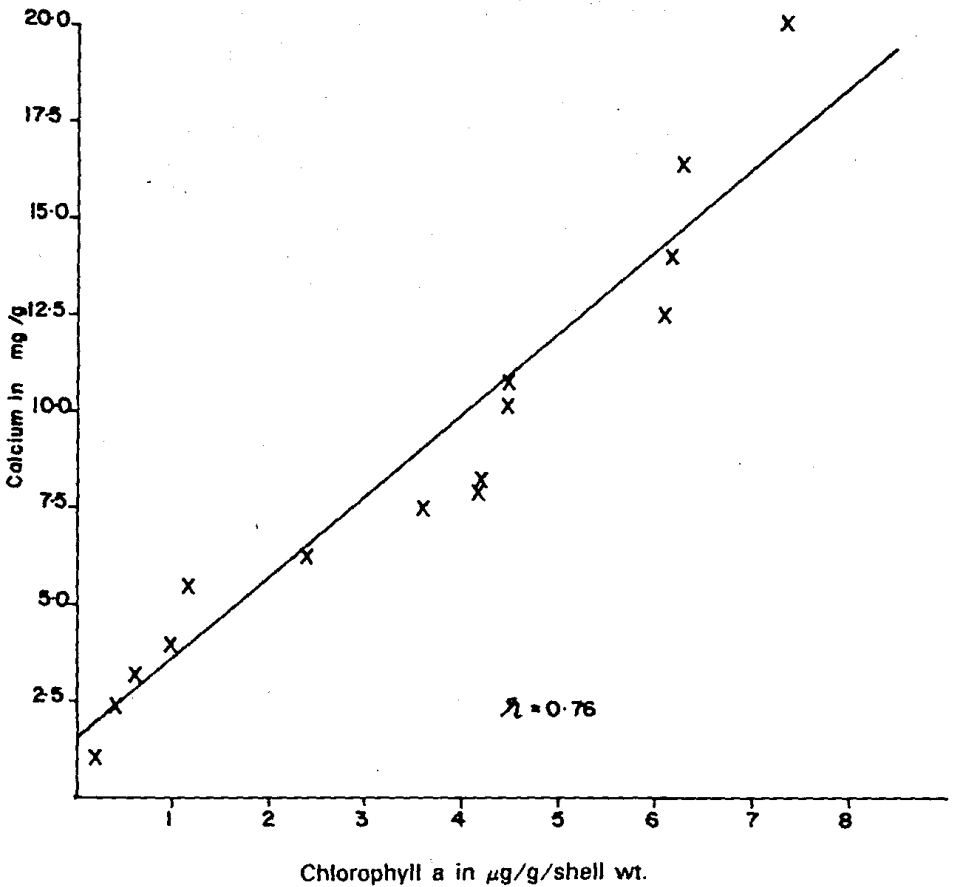


Figure 4. Relationship between the amount of cyanobacteria and leaching of calcium from shells.

mannitol, present in window-pane oyster shell is not known. Culturing of all species of shell-boring cyanobacteria might provide a clue to their nutritional requirements. Taylor (1983) reported the growth of *Phormidium corallyticum* in culture in the presence of organic compounds in the dark or with low light.

It would be interesting to determine if these shell-boring cyanobacteria, which were also found in dead corals in the Lakshadweep Islands, invade healthy ones and cause damage. Rützler et al. (1983) reported a "black band disease" of live corals in the Atlantic Reef caused by another cyanobacterium, *Phormidium corallyticum*. It attacks live, but damaged and stressed corals and, upon infestation, the causal organism forms a thick mat around the coral, resulting in the death of polyps in the infected region.

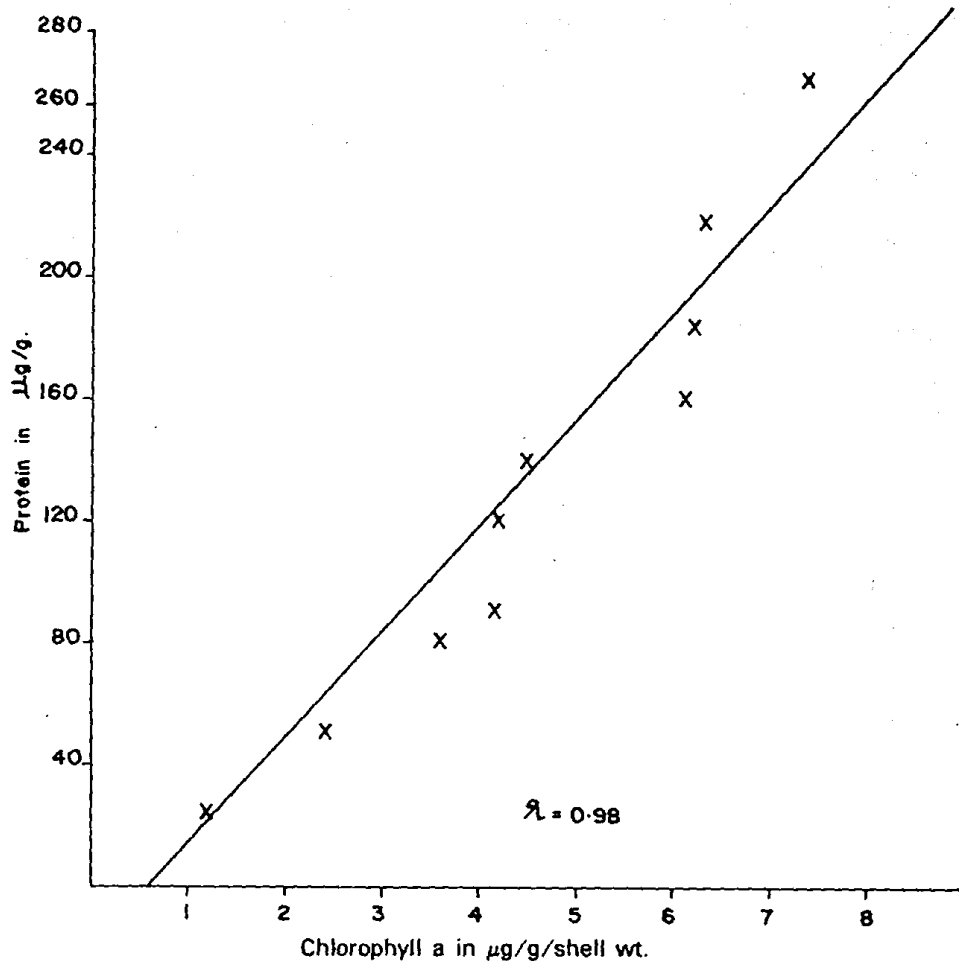


Figure 5. Relationship between the amount of cyanobacteria and leaching of protein.

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