

Most of the *Acacia* species in the tropical ecosystems are found to be self-incompatible and out-crossing. *Acacia sinulata* exhibits self-incompatibility, as indicated by Raju and Rao¹². Hand pollination experiments show that *C. religiosa* is a self-incompatible, obligate out-crosser. This breeding system is functional only when conspecifics bloom simultaneously and occur nearby, and requires vectors to mediate pollen among conspecific plants.

In *C. religiosa*, the natural fruit-set is low when compared to the high flower production. Different factors could affect fruit-set. First is the floral galls formation, which leads to loss of productivity. Secondly, the intensive pollen collecting behaviour of attending bees and their tendency to confine to the same plant that they first forage may result in more wasteful self-pollen transfer.

C. religiosa is of ornamental as well as medicinal importance and is an obligate out-crosser. For higher fruit-set, conspecific trees should be planted nearby and remedial measures should be adopted to combat infection of floral parts.

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Histological observations on the scleractinian coral *Porites lutea* affected by pink-line syndrome

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A pink-line syndrome (PLS) was reported in the reef building coral *Porites lutea* in Kavaratti island of the Lakshadweep archipelago. The affected corals had dead patches colonized by a cyanobacterium *Phormidium valderianum* and the bordering coral tissue was pink. We examined the histological changes associated with the PLS-affected tissue. Results showed that the zooxanthellae were released from the gastrodermal cells into the coelenteron. Gastrodermal cells undergo necrosis and detachment from the basal membrane. The basic staining of the cytoplasm in the gastrodermal cells bordering the calicoblastic layer suggests accumulation of calcium ions. The ectodermal epithelium and calicoblastic cells showed destruction through ‘apoptosis-like’ processes. Cell swelling and vacuolation were observed in the gastrodermal and ectodermal cells. In this communication we discuss how the presence of the cyanobacterium adjacent to the PLS-affected tissue could cause the observed damage, bring about imbalance and a shift in the coral-zooxanthellae symbiosis.

Keywords: Cellular changes, pink-line syndrome, *Porites lutea*, symbiosis, zooxanthellae.

EXISTENCE of corals is threatened by environmental changes, pollution and direct human interference in many coral reefs around the world. Diseases bring about destruction of corals to a greater extent than other factors. Investigations on diseases that help identify the factors which cause and manifest the disease will help in the management of diseases¹. We earlier reported partial mortality in the massive coral *Porites lutea* affected by pink-line syndrome (PLS)^{2,3}. Fungi and a cyanobacterium *Phormidium valderianum* were isolated from the PLS-affected specimens. The cyanobacterium was identified as the etiological agent in the PLS through Koch’s postulate experiments^{3,4}. This communication reports on the cellular changes associated with the PLS-affected corals and the interaction between the host tissue and associated organism.

Coral specimens of *P. lutea* affected by PLS and healthy ones were collected from a lagoon in Kavaratti island and were transported in sea water to the field laboratory. These specimens were fixed and preserved following the standardized method⁴. Healthy and PLS-affected specimens preserved in 70% ethanol were cut into small pieces of

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0.5–1 cm² size. These were placed in a glass vial and decalcified in a decalcification solution⁴ till the entire tissue was released from the skeleton within 3–4 h.

The decalcified specimens were washed several times with distilled water to remove salts and were dehydrated in 30, 50, 70, 90, 95 and 100% ethanol sequentially for 10 min each and cleared with 100% acetone. The decalcified tissues were embedded in paraffin wax blocks, following standard histological procedures. Thin sections of 6 µm size were cut using a rotary microtome (Weswox, India, Model 1090A) and double-stained with 70% heamatoxyline staining solution in distilled water (haematoxyline solution: haematoxyline 2 g; 100 ml of 96% alcohol; distilled water 100 ml; glycerol 100 ml; potassium alum 3 g; acetic acid 10 ml; sodium iodate 100 mg) and eosin (0.5% w/v in distilled water). The sections were observed under light microscope (Olympus BX60, Japan) and the cellular details were recorded by photomicrography.

Coral colonies comprising tubular polyps are interconnected through a narrow tissue called coenosarc. The walls

of these polyps have three dermal layers, namely the outer ectoderm, the middle mesogloea and the inner gastroderm (Figure 1). Some epithelial cells in the ectodermal layer are modified into calciblastic cells that are known to be involved in the calcification process⁵. Histological examinations of the PLS-affected tissues showed that they were degenerated and destroyed. The basic architecture of the three dermal layers was affected (Figures 2–4). Most of the calciblastic epithelium in the PLS-affected tissue

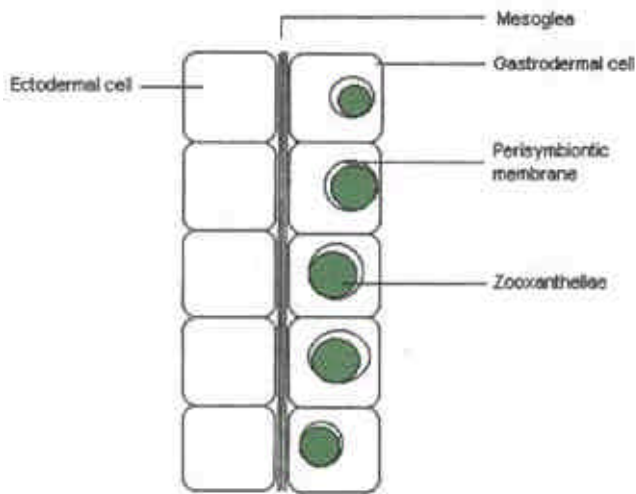


Figure 1. Schematic diagram of the dermal layers of a coral polyp.



Figure 2. Dermal layers as observed in sections of healthy polyp (bar = 25 µm). EC, Ectodermal cell; GS, Gastrodermal cell.

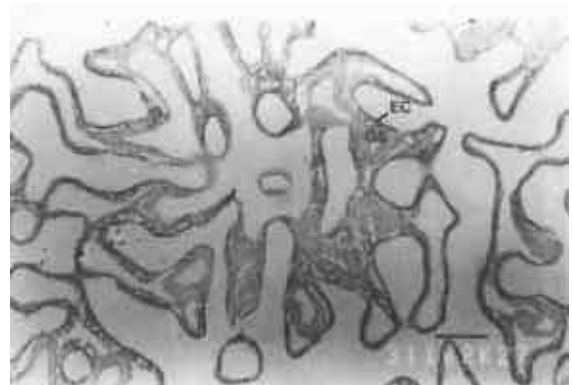


Figure 3. Destroyed tissue of PLS-affected polyp (bar = 100 µm).



Figure 4. Disturbed dermal layers of PLS-affected polyp (bar = 10 µm).

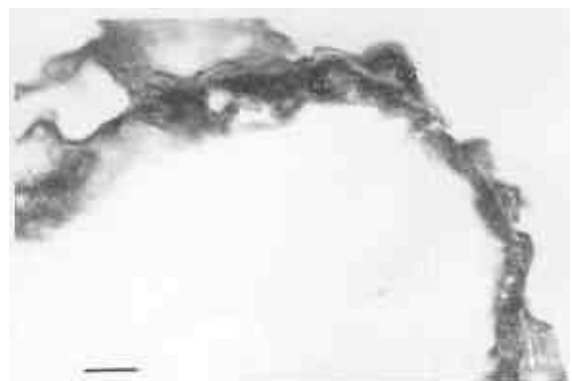


Figure 5. Disturbed calciblastic epithelium (CL) in PLS-affected polyp (bar = 10 µm).

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was degenerated (Figure 5). The gastrodermal layer bordering the calicoblastic layer showed extensive degeneration and stained dark with haematoxylin (Figures 4 and 5). The ectodermal and calicoblastic layers did not have the normal cellular organization and appeared granular (Figures 4 and 5). During the initial stages of destruction, the granules appeared as a layer and were eosin-positive (Figures 4 and 5). The granules were separated into small clusters of the size $7.6 \mu\text{m}$ (Figure 6). These granular packets got detached from the ectodermal layer and finally disappeared. The most prominent feature was the presence of large vacuoles in most of the gastrodermal cells having zooxanthel-

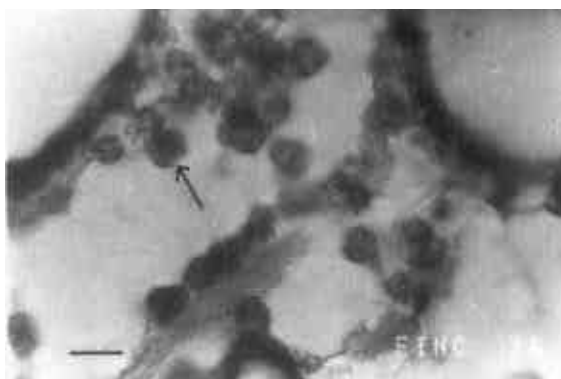


Figure 6. Granular pockets (arrow) of calicoblastic epithelium in PLS-affected polyp (arrow). Bar = $10 \mu\text{m}$.

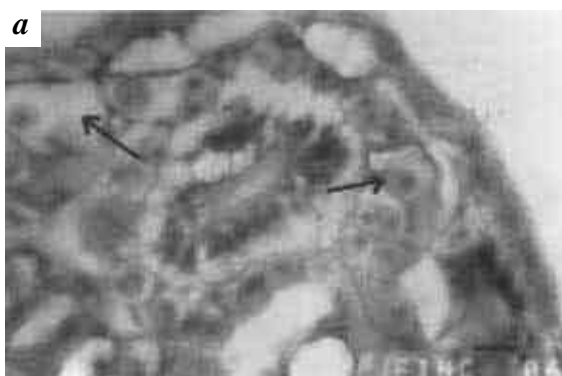


Figure 7 a, b. Large vacuoles (arrow) around the zooxanthellae (arrow). Bar = $10 \mu\text{m}$.

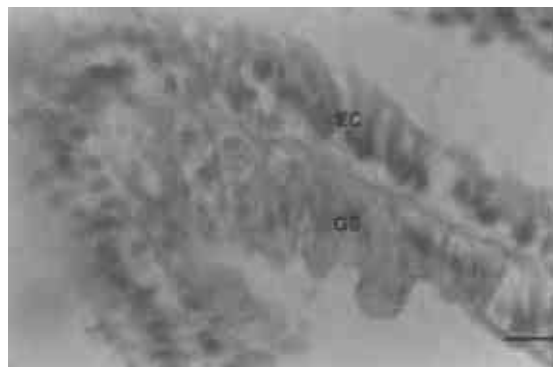


Figure 8. Gastrodermal cells of PLS-affected tissue showing swollen, non-staining brown patches. Bar = $10 \mu\text{m}$.

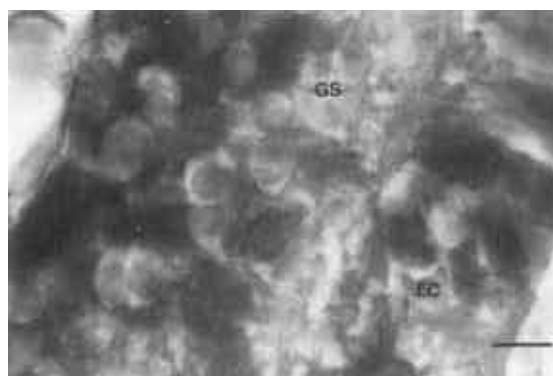


Figure 9. Cells in ectoderm and gastroderm showing necrosis and brown non-staining patches. Bar = $10 \mu\text{m}$.



Figure 10 a, b. Detachment of gastrodermal cells and expulsion of zooxanthellae in PLS-affected tissue. Bar = $10 \mu\text{m}$. Z, Zooxanthellae.

lae (Figure 7a arrow). Vacuoles were found initially around the zooxanthellae in most gastrodermal cells (Figure 7b arrow). It is not clear whether the vacuole was present in the cytoplasm or between the zooxanthellae and perisymbiotic membrane. In later stages, cells appear empty with only zooxanthellae in them because of the enlargement of the vacuole (Figure 7b). Another prominent feature of the gastrodermal cells was that they were swollen and their cytoplasm did not take the stain (Figure 8). Some of the swollen cells in the gastrodermal layer contained brown, non-staining patches (Figure 8). Both ectodermal and gastrodermal cells showed swelling and necrosis (Figure 9). These swollen cells were yellowish to brownish-green in colour and non-staining (Figure 9). During degeneration of the gastrodermal layers, zooxanthellae were released into the coelenteric cavity through various mechanisms. This included gastrodermal cell detachment (Figure 10a), and zooxanthellae expulsion from the lysed gastrodermal cells (Figure 10b), thereby releasing the zooxanthellae into the coelenteric cavity. The gastrodermal cells detached were mostly those that bordered the ectodermal epithelium. The ectodermal layers showed shrinkage of cytoplasm with darkly stained nuclear material. The layers also appeared empty due to shrinkage of the cytoplasm (Figure 11). Besides all these abnormalities,

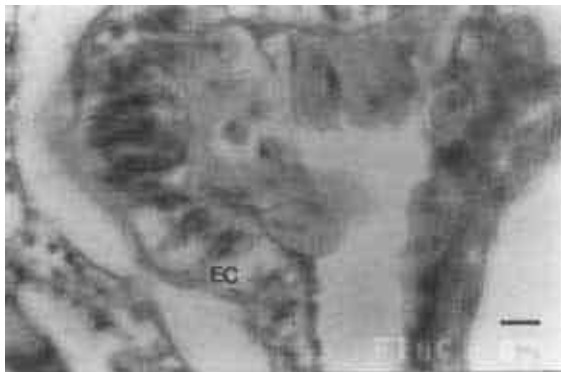


Figure 11. Shrinking of cytoplasm (arrow) and empty ectodermal cells. Bar = 10 μ m.

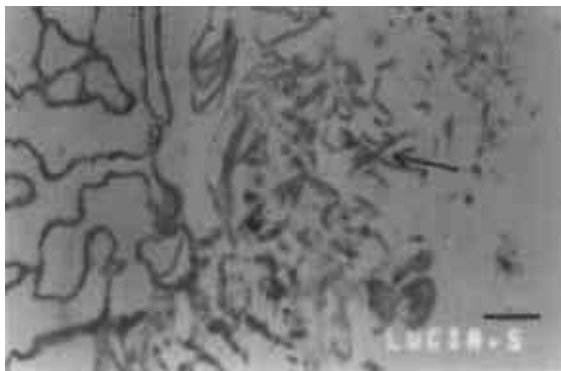


Figure 12. Cyanobacteria (arrow) in debris of PLS-affected polyp (bar = 100 μ m).

cyanobacterial filaments were also noticed in association with the debris of coral tissue in PLS-affected tissue (Figure 12).

In summary, the PLS-affected tissue of *P. lutea* showed the following histological abnormalities.

1. Calicoblast cells were destroyed and replaced by granular layer.
2. Some ectodermal cells were shrunken, while others swollen and showed necrosis.
3. The gastrodermal cells, in addition to swelling and necrosis, expelled their zooxanthellae.
4. Gastrodermal cells and ectodermal cells were highly vacuolated.
5. Invasion of cyanobacteria.

The fine balance between cell proliferation and cell death is regulated through a process termed programmed cell death or apoptosis⁶. A feature of apoptosis involves cell shrinkage, nuclear and cytoplasmic condensation, membrane blebbing, chromatin compaction and fragmentation of chromosomal DNA⁷. Appearance of the calicoblastic layer as granular pockets that stain acidic (eosin-positive), suggests that the pockets consist of cellular debris. These granules are cytoplasmic fragments bound by a membrane. This is characteristic of apoptic bodies and the calicoblastic layer itself may be undergoing apoptosis. The membrane-bound nature of the granules in the early stages, loss of the membrane boundary in the later stages and acidophilic staining of granules strongly suggest that these granules are apoptic bodies. The apparent cause for the apoptosis of calicoblastic cells seems to be acidification of the extracellular space by the presence of the cyanobacterium, *P. valderianum*.

Calcium (Ca^{2+}) efflux from calicoblast cells to external calcifying fluid is an energy-dependant process⁸. When the nutrient translocation from zooxanthellae to the corals is affected due to higher zooxanthellae proliferation, calcification is reduced resulting in the elevation of intracellular free calcium that is reported to cause cell damage⁹. Impairment of calcium efflux in PLS-affected tissues results in increased intracellular free calcium as observed in the gastrodermal cells, that is elucidated by staining with haematoxylin¹⁰. Increased pinocytic activity caused vacuolation in the brown shrimp, *Crangon crangon* (L) due to its exposure to elevated cadmium ions¹¹. Similarly, accumulation of calcium in the gastrodermal cell layer and its possible role in the formation of vacuoles in the cytoplasm need further investigation. It is hypothesized that dissolution of the skeleton may be proportional to the number of zooxanthellae in the corals, as observed in the boring sponge, *Anthosigmella varians*¹². Any breakdown of host control over the maintenance of zooxanthellae number may change the existing mutualism into parasitism.

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