WHAT HAPPENS WHEN CORALS BLEACH: A NEW PERSPECTIVE

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Bleached *Montastrea annularis*, Discovery Bay, Jamaica


Agaricia agaricites held at 32°C for six hours, the left half illuminated with 800 µmol/m²/s, the right half shaded.

INTRODUCTION:

Coral bleaching is the loss of the host's symbiotic algae (zooxanthellae). It is usually associated with high temperature but may also involve light as "solar" bleaching[1]. Other possible causes are cold shock[2], increased UV[3], vibrios[4], reduced salinity[5] and oxygen stress[6]. The current explanation is that there is a temperature driven detachment of host cells with their complement of zooxanthellae[7]. This study was undertaken to investigate the role of light in coral bleaching.

RESULTS AND DISCUSSION.

Observations.

A microscope built into a chamber in which both temperature and illumination could be controlled was used to observe and photograph the behaviour of coral gastrodermal tissue under a range of temperatures and light intensities. Tissue, scraped with a needle from between parallel septa from the edge of thin plates of *Agaricia agaricites*, rounded off (Fig. 1); zooxanthellae moved to the surface; protruded (Fig. 2); and after a delay began to detach, singly, or in groups of two or three, surrounded by a thin layer of host cytoplasm, inclusions and plasma membrane. Fragmentation, by the ballooning out and detachment of small spheres of cytoplasm (bleb formation), took place simultaneously (Fig. 2,3). Eventually all that was left was a layer of algae and fragments (Fig. 4).

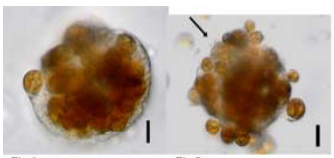
Fig 1

Fig 2

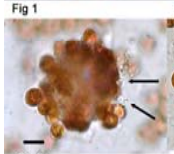
Fig 3

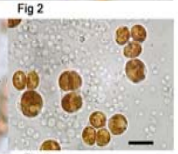
Fig 4

Observations were made of similarly sized clumps of gastrodermal tissue at 30°C, 32°C and 34°C and at four different levels of illumination, dark, 850, 1250 and 2000 µmol/m²/s. In light the length of delay before detachment started and the rate at which algae detached were inversely related to both temperature and illumination (Fig. 5). In the dark there was no detachment or fragmentation over the temperature range 30-34°C; however at 36°C fragmentation was complete within two hours (as in Fig. 4).

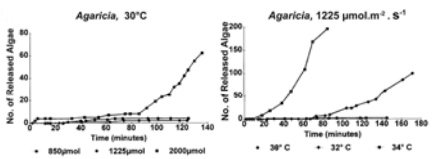

Fig 5

Coral sections.

Ground, polished and stained thin acrylic sections (Fig. 6) of corals held at 32°C in 600-800 µmol/m²/s light showed zooxanthellae in the coelenteron after three hours (Fig. 6b) and degraded gastrodermal layer and considerable fragmentation after six hours (Fig. 6c).

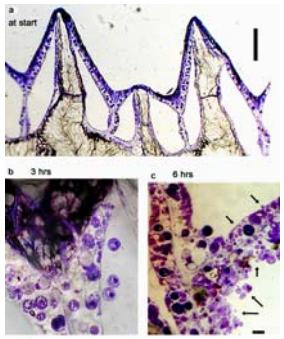

Fig 6

Nature of the fragments.

The fragmentation suggested that apoptosis might be involved. Exclusion of the vital stain, trypan blue, confirmed the integrity of the membranes, but with bisbenzimide staining the condensation of the chromatin characteristic of apoptosis in higher organisms was not seen. The thin layer of tissue surrounding the zooxanthellae contained inclusions, but not always nuclei (Figs.7,8). It seems likely that the detachment of algae involves the same processes as the blebbing of smaller fragments. It is possible that the fragments may be recognised, ingested and recycled.


Fig 7 Pairs of zoozanthellae with 0, 1 and 2 host nuclei

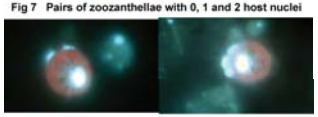
Fig 8 Detached zooxanthellae with 2 and 3 host nuclei

Role of Ca^{2+} in bleaching.

Calcium plays a major role in maintaining the structural integrity of the cell. A low level of Ca^{2+} is maintained by a calcium exclusion system (calcium pump)[8]. The exclusion system is temperature sensitive[9]. Disruption of the system can lead to apoptotic or necrotic death[8]. A consequence of increased Ca^{2+} in the cell is blebbing or ballooning out of the plasma membrane[8]. To test for temperature related changes of calcium ions in coral tissues small pieces of Agaricia were loaded with the cell permeant calcium indicator 'calcium orange'. These were placed in the sample vial of a fluorometer (Fig. 14), operated in a temperature controlled chamber. The coral was kept in the dark except for brief periods of excitation with green laser light (532nm). Between 35-36°C there was a rapid increase in emitted fluorescence (Fig.9) indicating a build-up of intracellular calcium. This would account for the observed blebbing and fragmentation.

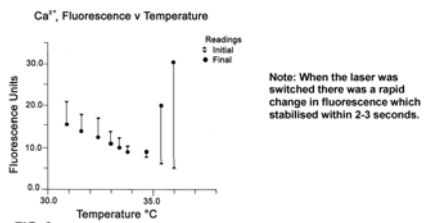

FIG. 9

Note: When the laser was switched there was a rapid change in fluorescence which stabilised within 2-3 seconds.

The role of hydrogen peroxide in "solar" bleaching.

Under photo-inhibitory conditions singlet oxygen and superoxide radicals are produced. H_2O_2 is formed by dismutation of the superoxide radical, and is able to pass easily through biological membranes[8]. It is known to induce apoptosis in higher organisms and to cause membrane damage via lipid peroxidation. It is also known to damage the Ca^{2+} exclusion system and the structural integrity of cells by oxidizing –SH groups of trans-membrane channels and cytoskeletal proteins[8].

Under illumination of 300 µmol/m²/s isolated zooxanthellae released up to 150ng H_2O_2 /hr/10^6 algae (Fig. 10), mean, 90ng, (n=12). Small pieces of *Agaricia* released up to 3.5 µg/hr/cm², mean 0.61 (n=7). The rate of release was constant for about two hours then dropped to a lower rate (Fig. 11). At this point some pieces of *Agaricia* showed visible symptoms of bleaching. The rates of H_2O_2 release increased with light and temperature and were much lower than rates recorded for red tide dinoflagellates (0.1-2.0 nmol/min/10^4 algae).

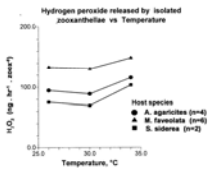

Fig 10

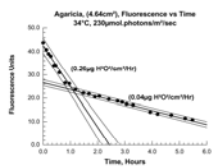

Fig 11

Small pieces of Agaricia loaded with a cell permeant analog of dichlorofluorescin diacetate and excited briefly with blue LED light (peak, 480nm) showed little change in fluorescence at 530nm over a 28-36°C temperature range. However, when the blue light was kept on permanently, the fluorescence and the rate of change of fluorescence increased exponentially as the temperature was raised slowly by steps (Fig. 12). This confirms that under illumination H_2O_2 accumulates in the tissues and the rate of accumulation increases with temperature.

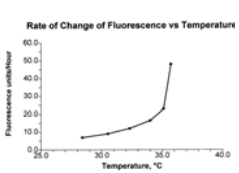

Fig 12

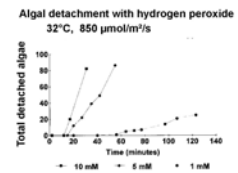

Fig. 13

When gastrodermal tissue was exposed to 1,5 and 10 mmolar H_2O_2 (Fig. 13) the tissue responded in the same way as it did to high light and temperature (Fig. 5): the higher the concentration, the shorter the delay period and the higher the rate of algal detachment.

Light enhanced lipid peroxidation took place in *Agaricia,* glutathione was present and probably acts in a protective/repair capacity[8].

These experiments indicate that H_2O_2 is produced by algae in light, accumulates and diffuses out of the coral. If the rate of production exceeds the rate of loss by diffusion H_2O_2 will accumulate and cause the detachment of zooxanthellae and fragmentation of gastrodermis at lower temperatures than those required for dark bleaching. The detachment of xoozanthellae in high light took place at 30°C (Fig. 5) which is below the temperature at which corals bleach in Jamaica. This suggests that on a very localized level this may be the mechanism that limits algal density. The levels of H_2O_2 produced are much lower than those recorded for other free-living dinoflagellates and this may be a requirement for the symbiotic mode of life.

CONCLUSIONS:

Bleaching involves the detachment of zooxanthellae surrounded by a thin layer of host cytoplasm and the simultaneous fragmentation of the gastrodermis by blebbing. In the dark these events are triggered by increases of internal Ca^{2+} resulting from a temperature related breakdown of the calcium exclusion system. In "solar" bleaching, under the synergistic action of light and temperature, H_2O_2 accumulates and leads to the same results, but at lower temperatures than for "dark" bleaching.

Below "bleaching" temperatures the same processes may be involved, on a localised level, in controlling algal density. The fragments and coated zooxanthellae may be recycled within the coral.

One action of H_2O_2 is to make cell membranes leaky to Ca^{2+}. The calcium pump would work continuously. This may underlie the higher calcification rates of corals in light.

Any condition that increases H_2O_2 (UV, heavy metals, oxygen stress), or pathogens that release H_2O_2 or other toxins that interfere with the Ca^{2+} exclusion system may thus contribute to bleaching. This mechanism probably applies to other relationships between animals and internal photosynthetic symbionts.

Fragmentation may be missed with some sectioning techniques.

TECHNIQUES

Sections: Corals were fixed in a modified Karnovsky solution (2.5% glutaraldehyde, 2% paraformaldehyde in 0.1 M Sorensen buffer). Small pieces from the edge of Agaricia colonies were dehydrated, embedded in LR White (hard) acrylic resin (Sigma) and cured at 60°C under nitrogen. Sections were cut with a diamond saw, mounted on glass slides with thermo-plastic cement, ground (< 0.05mm) with a series of abrasives, polished on both sides and stained with toluidine blue and mounted in immersion oil.

Light: Intensities were measured with a LI-COR Quantum Radiometer, Model LI-250. Illumination for experiments was provided by a 60-watt narrow beam halogen spotlight with a heat filter and 80b optical filter.

Fluorometer: A vial containing the 0.8 ml sample is at one focus of an ellipsoid reflector which collects fluorescence and brings it to a focus on the filters/photovoltaic sensor at the second conjugate focus (Fig.14). Three interchangeable lamps were used in the study: a UV LED at 350nm for H_2O_2 and glutathione measurement, a laser diode module (532 nm) for the calcium probe and a Luxeon LED (480 nm) and interference filters for the H_2O_2 probe.

H_2O_2 was measured with a technique based on the reduction of fluorescence in its reaction with scopoletin in the presence of peroxidase[11].

Glutathione was estimated with a technique based on its reaction with o-phthalaldehyde at pH8 to give a fluorescent compound (emission at 425 nm)[12].

Lipid peroxides were measured with the Fox2 technique[13].

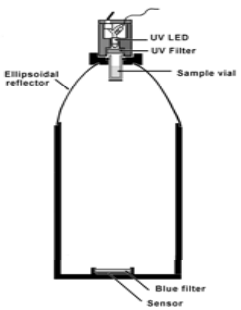

Fig. 14 Fluorometer

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