Zooxanthellae ultrastructure affected in bleached corals

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Most reef-building corals are hermatypic: they harbour endosymbiotic algae of the dinoflagellates group (generally called zooxanthellae, genus *Symbiodinium*), within vacuoles in the gastroderm cells. Bleaching, loss of colour due to loss of these symbiotic algae and/or their photosynthetic pigments, on a large scale, appears to increase by intensity and geographic extent, certainly related to increasing sea surface temperatures. Most of oceans temperature rises occurred in the last half century (with greenhouse gas forcing). This warming may result in more uniform bleaching as more and more areas reach the temperature limits and light levels for bleaching. These ecosystems that harbour the highest marine biodiversity are in danger. A lot of tools are still needed to study the mechanisms of this phenomenon, not yet well understood.

To observe what are morphological changes appearing in zooxanthellae during bleaching, we sampled three coral species from environment and one coral heat-shocked in experimental aquarium. For each species, healthy and bleached fragments were cut, fixed in 2.5% glutaraldehyde and then rinsed and kept in 20 mM NaN_3. Coral fragments were kept for several weeks in 0.2 M EDTA until the complete dissolution of the calcified skeleton. They were then further fixed in 2.5% glutaraldehyde solution and post-fixed in 1% OsO_4 in distilled water, before embedding in epoxy resin according to a routine procedure (ethanol/epoxypropane dehydratation). Then, ultra-thin sections (~70 nm thick) were performed with a diamond knife on a Reichert-Jung ultra-microtome (Ultracut E), contrasted with uranyl acetate and lead citrate, and observed on a Jeol JEM 100-SX transmission electron microscope (TEM) at 80 kV of accelerating voltage.

Zooxanthellae of healthy corals present well-structured and visible organelles: a pyrenoid (chloroplast extension surrounded by a starch sheath), a peripheric chloroplast with parallel thylakoids, a nucleus with condensed chromosomes, and small reserve material globules (glucids). The algal cell wall and the symbiosome membranes stick to each other and to the vacuole membrane of the gastrodermal host cell.

In naturally bleached corals, zooxanthellae show signs of vacuolization. The spaces between membranes increase, as well as the spaces between organelles. The zooxanthellae of these corals show more reserve globules, that can be interpreted as an accumulation due to a metabolic dysfunction or after a signal preparing them to be expelled and to survive in the outer environment. Rupture of organelle membranes or cell membrane occur and are often the signs of cell necrosis. Angular holes are also found in the algae, and are probably places left by mineral crystals lost during cutting.

In heat-shocked corals, we have observed the same alterations as in naturally bleached ones. However, some characteristics are more marked: the number of zooxanthellae in division is higher, zooxanthellae are more vacuolized, with higher spaces between organelles, the rupture of some organelle membranes is visible and the number of holes left by mineral crystals is higher (with or without host cell lysis). Alterations are more important in these corals because a real shock (acute thermal stress) is applied in these case whereas a gradual stress appear in the natural environment. The division of algae (higher mitotic index) can be induced by the host to compensate the loss of algae due to bleaching (try to recovery) or by the zooxanthellae to induce their expulsion by increasing their density and therefore to leave an environment no more adapted.

These observations show that zooxanthellae are morphologically affected by environmental stress that causes bleaching and that natural and experimental bleaching have different effects on symbionts. Indeed, heat-shocked corals seem more heavily damaged than in nature. Ultrastructure of bleaching effects is an interesting complementary tool for the study of the mechanisms of this phenomenon that affect more and more coral reefs and threats global marine biodiversity.