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Depth-dependant response to light of the reef building coral, *Pocillopora verrucosa*: Implication of oxidative stress

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Abstract

Several environmental factors have been described to trigger bleaching in cnidarian/dinoflagellate endosymbiosis. However, the molecular mechanisms underlying this process still need more investigations. Symbiosis breakdown is known to result from physiological damage to animal host cells and/or symbionts. Cellular oxidation appears to be an essential player in this damage. Indeed, oxidative stress is a direct consequence of increase in irradiance and temperature, the two main environmental factors involved in bleaching. In this study, we examined the role of irradiance in inducing dissociation and oxidative stress in cnidarians and dinoflagellates. We used the bleaching-sensitive scleractinian coral *Pocillopora verrucosa* in a field cross-transplantation experiment performed between 5 m and 20 m depth at Grande Glorieuse Island (Indian Ocean), a preserved area subject to minimal anthropogenic influence. Cellular damage and increase in antioxidant defense were correlated with bleaching in upward transplanted samples. Downward transplanted colonies presented no associated alterations similar to the controls. We therefore conclude that increasing light induced bleaching via a prooxidative period. Remarkably, the distribution of *Symbiodinium* over depth was invariant; all colonies were monomorph for clade C, suggesting that bleaching sensitivity of *P. verrucosa* might not be associated with clade specificity.

Keywords: Antioxidant defenses; Bleaching; Cnidarian; Mozambique channel; *Symbiodinium*; Symbiosis; Transplantation experiment; Western Indian Ocean; Channel

1. Introduction

Over the past three decades, massive bleaching events of endosymbiotic dinoflagellated cnidarians (mainly corals and sea anemones) have been widely reported in tropical oceans. Coral bleaching is defined as a response to environmental stress that leads to expulsion of the symbiotic dinoflagellate (commonly called zooxanthellae) from the host cnidarian tissues, causing a paling or whitening of the affected coral (Hoegh-Guldberg, 1999; Douglas 2003; Lesser, 2004). Bleaching of cnidarians, observed in all oceans, is the primary cause of coral death however adaptation and recovery phases have also been observed (Buddemeier and Fautin, 1993; Stobart et al., 2005). When mortality occurs, bleaching induces tragic consequences such as the disappearance of essential ecological niches that house thousands of diverse marine species (in addition to having a devastating economic impact). Among environmental factors that have been described to trigger coral bleaching, thermal stress is proposed as the principal cause. However, other factors, such as solar radiation, have been shown to act synergistically to lower the threshold temperature at which coral bleaching occurs (Hoegh-Guldberg, 1999).

Abbreviations: CuZnSOD, copper/zinc superoxide dismutase; FeSOD, iron superoxide dismutase; MnSOD, manganese superoxide dismutase; PAR, photosynthetically active radiation; RFLP, Restriction fragment length polymorphism; TOSC, Total oxiradicals scavenging capacity; UVR, Ultraviolet radiation.

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Recent work on *Porites* corals, demonstrated a bleaching occurrence under increasing irradiance at continuous elevated seawater temperature (Smith and Birkeland, 2007). Indeed, increased bleaching events have been reported during the periods of clear and calm waters (doldrums periods) that are more favorable to UVR penetration (Harriott, 1985; Drollet et al., 1994).

Modification of environmental factors, such as temperature and UV, are known to induce the reduction of oxygen into reactive oxygen species (ROS), leading to cellular damage such as protein oxidation, lipid peroxidation and DNA degradation (Halliwell and Gutteridge, 1999). Consequently, oxidative stress has been proposed as a unifying mechanism for the several environmental factors that cause bleaching (Lesser, 1996). In endosymbiotic cnidarians, oxidative stress is furthermore exacerbated in animal cells due to the photosynthetic process from the symbiont releasing oxygen in tissues of whole association (D'Aoust et al., 1976; Dykens and Shick, 1982; Harland and Davies, 1995; Richier et al., 2003). Although antioxidant defenses in the animal host have been shown to be proportional to photo-oxidative damage (Dykens and Shick, 1982; Dykens et al., 1992; Richier et al., 2003), additional environmental stress inducing cellular ROS production in the host (Dykens et al., 1992; Nii and Muscatine, 1997) or zooxanthellae (Lesser, 1996) can overwhelm the protective enzymatic defense and therefore result in toxic hydroxyl radical production (Asada and Takahashi, 1987). The pro-oxidant state consequently affects cellular integrity and can lead to zooxanthellae exocytosis from the coral host cells (Gates et al., 1992; Lesser, 1996, 1997; Smith et al., 2005) or apoptosis (Dunn et al., 2002, 2004; Franklin et al., 2004; Lesser and Farrell, 2004; Strychar et al., 2004).

Susceptibility to bleaching has been suggested to be linked to inter- and intraspecific symbiont diversity. Symbiotic zooxanthellae belong to the genus *Symbiodinium* in which seven distinct clades (A through H) have been identified (Baker et al., 2004; Pochon et al., 2004; Coffroth and Santos, 2005). Although many corals are relatively flexible in the type(s) of symbiont they contain, one type is usually dominant for a given species and environment (Rowan, 1998; Baker, 2003). As a result of bleaching induced by changes in environmental conditions, a new symbiotic combination could be favored, providing a potential mechanism by which corals would adapt to global warming (Coles and Brown, 2003; Rowan, 2004; Berkelmans and van Oppen, 2006).

We chose to investigate, in a field cross-transplantation experiment (from 5 to 20 m depth and *vice versa*), oxidative stress involvement in the bleaching reaction of the stony coral *Pocillopora verrucosa* in response to light-induced variation. This species has been chosen for its bleaching-sensitive property (Hoegh-Guldberg and Salvat, 1995) and its homogeneous bathymetric distribution from shallow to 20 m depth water.

Bleaching was visually detected and confirmed by chlorophyll measurements and biomarkers for damage and cellular defenses were monitored. Correlations were established between irradiance variation, oxidative stress and bleaching events. In order to test the implication of *Symbiodinium* clade diversity in bleaching sensitivity, RLFP analyses were carried out on corals distributed at different depths from 5 to 20 m depths.

2. Materials and methods

2.1. Location

Experiments were conducted from the 5th to the 15th of December 2003 at Grande Glorieuse Island (11°30 S', 47°20 E') in the northern Mozambique Channel (western Indian Ocean). In 1975, the French government has declared the island "protected area". All human impact has since been rigorously minimized but surrounding coral reefs are still subject to global warming, such as temperature and irradiation increase (Naim and Quod, 1999). The experimental site was located on the outer reef slope (11°35.4′ S 47°17.1′ E), ca. 500 m from the shore at the southwest side of the island. This site was chosen because of its gentle slope along which colonies of *Pocillopora verrucosa* are present, down to 20 m deep. Transplantations were done in two stations located ca. 200 m apart: a deep station at 20 m and a shallow one at 5 m deep (±2 m according to tide).

2.2. Experimental design

Three colonies of *P. verrucosa* (Ellis and Solander, 1786) were collected at 5 m and at 20 m deep and identified respectively as s1, s2 and s3 for shallow colonies and d1, d2 and d3 for deep colonies. From each colony, 14 small nubbins (40–50 mm long) were cut at their respective depth and mounted onto small plastic holders, without any glue or resin potentially toxic for the physiology of the coral. Coral nubbins were handled on sites and never emerged. Holders were then arranged on two plastic racks identified as shallow and deep plates, each containing 42 nubbins. After 5 days of acclimation period on the plate, half of the nubbins from the shallow plate (7 nubbins from each three shallow colonies) were transplanted from the 5 m to the 20 m plate; and reversely, half of the deep plate was cross-transplanted from 20 m to 5 m. Fig. 1 illustrates the organization of the shallow plate during acclimation and transplantation periods. In both plates, non-transplanted nubbins were used as reference. For each shallow (s1 to s3) and deep (d1 to d3) colony, independent non-transplanted ($N=3$) and transplanted ($N=3$–6) nubbins were then collected after 1, 2, 3 and 5 days of transplantation. Seawater temperature was monitored using two calibrated underwater temperature loggers (Hobo Water Temp Pro, accuracy: 0.2 °C, Onset Computer Corporation, Pocasset, USA).

2.3. Sampling and tissue extraction

After collection, samples were immediately dipped into nitrogen dry shipper and stored at −80 °C until analyzed. Frozen coral nubbins were weighed, powdered in a mortar and resuspended at 100 µl g^{-1} of coral powder in extraction buffer [50 mM phosphate buffer pH 7.8; 0.1% w/v ascorbic acid; 2 mM phenylmethyl sulfonyl fluoride (PMSF); 10 µg ml^{-1} protease