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Algal symbiont type affects gene expression in juveniles of the coral *Acropora tenuis* exposed to thermal stress

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ABSTRACT

Reef-building corals harbor symbiotic dinoflagellates, *Symbiodinium* spp., which are currently divided into several clades. The responses of corals associated with different *Symbiodinium* clades to thermal stress are not well understood, especially at a gene expression level. Juveniles of the coral *Acropora tenuis* inoculated with different algal types (clade A or D) were exposed to thermal stress and the expression levels of four putative stress-responsive genes, including genes coding green and red fluorescent proteins, an oxidative stress-responsive protein, and an ascorbic acid transporter, were analyzed by quantitative real-time PCR. The expression levels of the four genes decreased at high temperatures if juveniles were associated with clade A symbionts but increased if the symbionts were in clade D. The intensity of green fluorescence increased with temperature in clade D symbionts harboring juveniles, but not in juveniles associated with clade A symbionts. The present results suggest that genotypes of endosymbiotic algae affect the thermal stress responses of the coral juveniles.

1. Introduction

Coral reefs are experiencing increasing degrees of global and local environmental stresses (Hoegh-Guldberg, 1999; Hughes et al., 2003; Hoegh-Guldberg et al., 2007). Large-scale coral bleaching has been ascribed to increases in seawater temperature induced by climate change resulting from the greenhouse effect (Glynn, 1993; Crabbe, 2008). Coral bleaching is a breakdown of the symbiotic association between reef-building corals and their symbiotic algae, dinoflagellates of the genus *Symbiodinium*. *Symbiodinium* spp. consist of nine clades (A–I) (Baker, 2003; Coffroth and Santos, 2005; Pochon and Gates, 2010). Symbionts of clades A, B, C, and D are commonly associated with metazoan hosts (Baker, 2003), while clade C, E, F, G, H, and I are associated with large soritid foraminifera (Pochon and Pawlowski, 2006; Pochon and Gates, 2010). They are further divided into many subclades and strains (Coffroth and Santos, 2005).

Various clades of *Symbiodinium* show different levels of tolerance to stress and might be responsible for the differential tolerances of corals to bleaching stressors. The clade D *Symbiodinium* has been characterized as heat- or stress-tolerant based on its increased prevalence in the Caribbean and Indo-Pacific corals after bleaching events (Glynn et al., 2001; Toller et al., 2001; Baker et al., 2004; van Oppen et al., 2005; Jones et al., 2008). However, few studies have compared stress responses among corals hosting different *Symbiodinium* clades. In the high temperatures and turbid conditions of the north-eastern Indian Ocean, clade D *Symbiodinium* were more diverse and frequently observed than clade C in coral hosts (LaJeunesse et al., 2010). Colonies of *Acropora millepora* that had shuffled their dominant endosymbiont from C2 to D following bleaching exhibited higher photochemical efficiency and greater symbiont densities than C2-dominated colonies when exposed to heat stress (Berkelmans and van Oppen, 2006). On the other hand, *Acropora tenuis* juveniles associated with clade C1 symbionts showed much greater thermal tolerance than those associated with clade D symbionts based on photochemical efficiency measurements (Abrego et al., 2008). These results suggest that corals associated with different subclades of *Symbiodinium* might show different stress responses.

Changes in the expression patterns of stress-responsive genes are key indicators of the physiological response to stress and usually occur before metabolic or cellular damage becomes detectable. Recent studies have examined the gene expression patterns during thermal stress using cDNA microarrays as well as other gene expression analysis tools (Desalvo et al., 2008; Rodriguez-Lanetty et al., 2009; Voolstra et al., 2009). Heat shock

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proteins, superoxide dismutase, lectin, antioxidant proteins, and other genes have been identified as stress biomarkers in reef-building corals (Downs et al., 2000; Hashimoto et al., 2004; Csasezar et al., 2009; Vidal-Dupiol et al., 2009). Additionally, corals contain fluorescent proteins (FPs) (Dove et al., 2001), which have also been identified as stress response protein because the expression of a GFP-homolog was found to decrease under high temperatures (Smith-Keune and Dove, 2007). FPs also reduce the photoinhibitory effect of high levels of solar radiation, which in conjunction with thermal stress leads to bleaching (Salih et al., 2000). Finally, GFP might have the ability to quench reactive oxygen species (ROS) (Bou-Abdallah et al., 2006). FPs may therefore be possible indicators of the thermal stress within host coral cells.

While many researchers have reported molecular biomarkers of corals, few studies have examined stress responses of corals associated with different *Symbiodinium* clades at a molecular level (Desalvo et al., 2010). This may be in part due to the difficulty of maintaining corals associated with monoclonal *Symbiodinium* cells of different types for stress exposure experiments. We previously established a model system by infecting aposymbiotic *Acropora tenuis* primary polyps with a monoclonal population of cultured *Symbiodinium* cells (Yuyama et al., 2005). The objective of the present study was to compare the stress response of *A. tenuis* juveniles harboring different *Symbiodinium* clades. For this purpose, we infected aposymbiotic *A. tenuis* juveniles with a monoclonal population of cultured *Symbiodinium* cells. Two different *Symbiodinium* types (clades A and D) were used in this study, because we had difficulty to inoculate *A. tenuis* juvenile polyps with other cultures (clade B, F) or freshly isolated homologous *Symbiodinium* (clade C) (Yuyama et al., 2005, 2010). Changes in the gene expression patterns of FPs as well as FP content in juveniles were studied. Other stress-responsive proteins, including an oxidative stress-responsive protein and an ascorbic acid transporter, were also analyzed.

2. Materials and methods

2.1. Preparations of algae and coral juveniles

The monoclonal *Symbiodinium* strains PL-TS-1 (subclade A3) and CCMP 2556 (clade D) were obtained from the Bigelow Laboratory for Ocean Sciences (West Boothbay Harbor, ME, USA; https://ccmp.bigelow.org) and cultured in an IMK medium (Wako Chemicals, Osaka, Japan) at 25 °C under a 12-h light (100 μE m^{-2} s^{-1}):12-h dark cycle.

Colonies of *A. tenuis* were collected at the northern reef patch of Sesoko Island (26°39'48.3"N; 127°52'23.9"E), Okinawa, and kept in a running seawater tank at Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus (Okinawa, Japan) until spawning in June 2009. Two of five colonies spawned on 11 June. Eggs and sperms were mixed for fertilization and rinsed twice in filtered (0.22 μm) seawater (FSW) approximately 2 h after fertilization, after which they were kept in 2-L plastic containers (26 °C) (Harii et al., 2009). Seven days after fertilization, larvae were induced to metamorphose using Hym 248, a neuropeptide identified in Hydra (2 μM; Iwao et al., 2002). Approximately 50–100 larvae settled in two to three dishes. The FSW was replaced daily for both larvae and polyps.

2.2. Algal inoculation and stress treatment

Each *Symbiodinium* strain (2×10^3 cells·mL^{-1}) was introduced to primary polyps for 24 h. They were washed and maintained by changing the FSW daily at 26 °C until the number of symbionts in polyps increased. Two weeks after algal inoculation, the polyps were exposed to high temperature stress (32 °C) or control temperature (26 °C) under a 12-h dark: 12-h light (100 μE m^{-2} s^{-1}) cycle for 24 h. After 24 h treatment, algal density decreased in the polyps kept at high temperature (Fig. 1), indicating that the 24 h stress treatment was effective.

2.3. Count of Symbiodinium cells in polyps

Before and after stress treatment, three to five polyps from each treatment group were fixed and decalcified in 3% formaldehyde with 0.5M EDTA to estimate the algal density of the polyps. Following decalcification, polyps were homogenized, and the homogenate was diluted with a new fixation solution. The number of *Symbiodinium* cells in the dilute solution was counted using a hemocytometer under a florescence microscope (Optiphoto-2; Nikon, Tokyo, Japan).

2.4. Image analysis of fluorescence

Epifluorescence photomicrographs of juvenile polyps were taken under Multizoom AZ100 (Nikon) using a digital camera (Digital Sight DA-L1; Nikon). After the 12 h light period of the 24 h stress trestment, the polyps were moved to petri dishes (90 × 15 mm) containing filtered seawater and immediately observed. The intensity of green fluorescence within the polyp area was measured on digital micrograph images using the Photoshop (Adobe Systems, San Jose, CA, USA) and ImageJ software packages (Research Services Branch, National Institutes of Health, Bethesda, MD, USA). Green fluorescence was extracted using the green layer function of Photoshop and the average intensity of green fluorescence in the juveniles was calculated using the Mean Gray Value

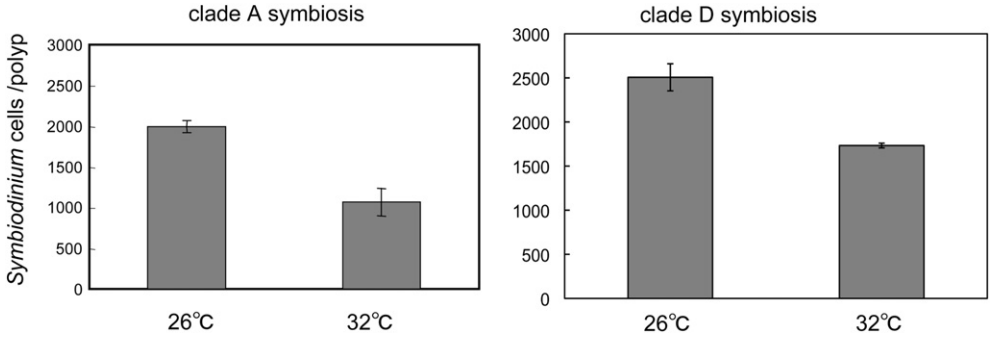

Fig. 1. Number of *Symbiodinium* cells per *Acropora tenuis* polyp of the control and high-temperature-treated groups. Polyps associated with clade A symbionts (left) or clade D symbionts (right) were kept at 26 °C or 32 °C for 24 h. Means ± SE ($n = 4$).