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ORIGINAL RESEARCH ARTICLE

The effect of temperature and nitrogen deprivation on cell morphology and physiology of *Symbiodinium*

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Summary Nutrients and temperature are the major elements in maintaining stable endosymbiotic relationships. The mechanisms and response of cultured *Symbiodinium* cells in the absence of nitrogen, and at various temperatures are still unclear. The present study investigated the influence of different temperatures and nitrogen-deprivation on free-living *Symbiodinium* cultures. The physiological responses of free-living *Symbiodinium* cells cultured at different temperatures during nitrogen deprivation under a 12:12 h light:dark were measured. *Symbiodinium* cell growth was significantly lower in response to lower temperatures. Transmission electron micrographs (TEMs) revealed the formation of lipid droplets induced by nitrogen deprivation under different temperatures. The results of this study will increase our understanding of adaptive responses occurring in *Symbiodinium* under environmental stress.

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1. Introduction

Symbiodinium sp. is a unicellular microalga that has mutualistic associations with invertebrates such as corals and anemones (Davy et al., 2012). The coral host provides nutrients and carbon dioxide to *Symbiodinium* in hospite (Muscatine and Porter, 1977; Rodrigues and Grottoli, 2007). In return, these microalgae transfer more than 90% of their photosynthetically fixed carbon to the host cytoplasm where they reside (Muscatine, 1980). This mutualistic relationship enables coral to survive and remain healthy.

In more than a decade, the phenomenon of global climate change has contributed to the decline of coral reef around the world (Wilkinson, 2008). In such case, this change might generate huge impacts to the ocean ecosystem at all levels, such as higher mean sea water level, warmer ocean, stratified ocean, increasing CO_2 level in global scale, ice cover and ocean chemistry changes (Doney et al., 2009; Duce et al., 2008; Jackson et al., 2001; Kroecker et al., 2013; Poloczanska et al., 2013). The coral bleaching is expected to happen more frequently and become more severe due to the climate change. Environmental factors, such as nutrient levels and temperatures, play an important role in maintaining the stability of mutualistic associations between cnidarians and unicellular dinoflagellates (Belda et al., 1993; Hastie et al., 1992; Hoegh-Guldberg and Smith, 1989; Steen and Muscatine, 1987). It was reported that coral bleaching appeared in the seawater of southern Taiwan due to the exposure of temperature at 30–31°C (Mayfield et al., 2013, 2011). Furthermore, when corals were exposed at 14°C under the full sunlight, the reduction in photosynthetic ability caused the coral bleaching (Saxby et al., 2003). In terms of elevated sea temperature, the stability of corals and *Symbiodinium* endosymbiotic relationship is yet to be determined by anthropogenic stress factor such as coastal water quality (Wooldridge, 2014). Previous studies show that increasing nutrient levels, like dissolved nitrogen (N), in coastal water could induce some physiological impact on coral-dinoflagellate, for example, coral bleaching and reduced symbiont density (Koop et al., 2001; Marubini and Davies, 1996; Stimson, 1991; Szmant, 2002; Wiedenmann et al., 2013). For instance, the organic carbon supply by *Symbiodinium* to hosts could be established depending on nutrients from various sources including exogenous sea water, host catabolism and host heterotrophy (Steen, 1986; Szmant-Froelich and Pilson, 1984). Nitrogen, a major nutrient, is excreted as ammonium by the host (Rahav et al., 1989). Several investigations have reported that endosymbionts could survive in nitrogen-limited environments (Jiang et al., 2014; Peng et al., 2012). Therefore, nitrogen deprivation could alter symbiont physiologies (Weng et al., 2014). It is likely that symbiotic cnidarians may maintain the endosymbiont density through regulation of nitrogenous waste (McAuley, 1987; Rees, 1989). Moreover, temperature elevation alone can damage *Symbiodinium* cells in hospite (Sammarco and Strychar, 2013). It has been reported that the nutrient uptake in symbionts differed under different temperatures due to stress susceptibility among corals hosting different symbionts (Baker et al., 2013). There have been numerous studies using *Symbiodinium* treated with either differing temperatures or nitrogen deprivation (Jiang et al., 2014; Nitschke et al., 2015; Weng et al., 2014).

Several studies have reported that nitrogen starvation or other environmental stressors can affect growth, morphology, and metabolism of microalgae (Hockin et al., 2012; Pasaribu et al., 2014). For example, reduced nitrogen concentration increases lipid production in microalgae, which is stored in lipid droplets (Li et al., 2010; Piorreck et al., 1984). Recent studies have shown that increasing the temperature variation induced lipid content accumulation in *Nannochloropsis oculata* (Converti et al., 2009). Macedo and Alegre (2001) observed that lipid content increased in *Spirulina* cultured with nitrogen and decreased temperatures. However, the synergistic effects between nitrogen source and different temperature treatment on cellular mechanisms of *Symbiodinium* are poorly known.

The present study describes the influence of different temperatures and the absence of nitrogen in the medium on cultured free-living *Symbiodinium*. The aim was to examine changes in cellular biology, including cell proliferation, lipid classes, and ultrastructure in free-living *Symbiodinium*. *Symbiodinium* cell proliferation was slower when samples were cultured at temperatures of 15°C, than at 25 and 30°C. Results showed increased formation of different lipid droplets in *Symbiodinium* when cultured in extreme temperatures (i.e. 15 and 30°C).

2. Material and methods

2.1. *Symbiodinium* culture and treatment

The free-living *Symbiodinium* sp. (clade B) used in this study was obtained from National Museum of Marine Biology and Aquarium. They were maintained in the f/2 medium in filtered seawater (FSW) at room temperature under a photosynthetically active radiation (PAR) of 40 μmol m^{-2} s^{-1} in a 12-h light/12-h dark (12L/12D) cycle. For treatment, three-batch cultures were grown in the nitrogen-deficient artificial seawater with temperatures at 15, 25 and 30°C, separately.

2.2. *Symbiodinium* clade identification

The genetic identity (18S rDNA) of the cultured *Symbiodinium* was examined by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) analysis, and it was determined as clade B. *Symbiodinium* DNA was extracted using a plant genomic DNA extraction miniprep system (VIOGENE, Taipei). Basically, *Symbiodinium* nuclear small subunit (n18S-rDNA) was amplified by PCR from 3 replicate extracts of each of the two cultures using the primers, ss5z (an equimolar mixture of the oligonucleotides 5′-GCAGTTATAATT TATTTGATGGTCACTGCTAC-3′ and 5′-GCAGTTATAGTTTATTT-GATGGTTGCTGCTAC-3′) and ss3z (5′-AGCACTGCGTCAGTCC-GAATAATTCACCGG-3′). The PCR products were digested by restriction enzymes, *Taq* I and *Sau*3A I (Promega, USA). The digestion products were separated by electrophoresis on 1.5% 0.5× TAE (Amresco, USA) agarose gels, to generate the RFLP pattern. The RFLP pattern was compared to the literature (Rowan and Powers, 1991) to identify the clade of each culture.