Preliminary studies on the bleaching mechanism using coral cell aggregates

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INTRODUCTION

To investigate bleaching mechanisms of coral-zooxanthella symbiotic systems, it is important to study response of corals to stress conditions at cellular or tissue level. We attempted to establish an experimental system to study response of coral cells to stress treatment and chemical reagents such as antioxidant. Dissociated coral cells aggregate to form spherical bodies, which start to rotate by ciliary movement. These spherical bodies (tissue balls) stop their rotation and become disintegrated when exposed to stress. The first objective of our study is to evaluate the use of tissue balls for bleaching studies. The second objective is to test the hypothesis that zooxanthellae produce harmful substances such as active oxygen when exposed to high temperature under light.

MATERIALS AND METHODS

We exposed tissue balls containing various numbers of zooxanthellae to high temperature and examined the relationship between the zooxanthella density and survival time of the tissue balls. Tissue balls were prepared from dissociated cells of *Fungia* sp. Dissociated cells were allowed to form cell aggregates (tissue balls) in a petridish for one night. Tissue balls of similar size were put in each well of a 96 multi-well plate containing 300 µl FSW and allowed to recover overnight. The volume of tissue balls was estimated from video print assuming that they are ellipsoid. Tissue balls were then exposed to thermal stress (31°C) or control condition (25°C). Tissue balls were observed at 2-6 h intervals during the daytime until all the tissue balls died. In some cases, antioxidants (125 uM ascorbic acid and 250 Uml-1 catalase) were added during high temperature treatment. Tissue balls were scored as healthy, stopped, or degraded at each observation to make survival curves under high and normal temperature conditions. After the tissue balls disintegrated, the number of zooxanthellae within each tissue ball was counted to calculate zooxanthella densities of the tissue balls.

RESULTS AND DISCUSSION

Survival curves of tissue balls were markedly different between 31 and 25°C. At 31°C tissue balls died more quickly than those kept at 25°C. Addition of antioxidants extended the survival time of tissue balls at 31°C. There was a negative correlation between the survival time and zooxanthella density of tissue balls at 31°C, while no significant correlation between survival time and zooxanthellae density was found at 25°C or at 31°C in the presence of the antioxidants. There was no significant correlation between the survival time and the size of tissue balls at both temperatures. The present results showed that the higher the zooxanthellae density was the more quickly the tissue balls died and supported our hypothesis that zooxanthellae produce harmful substances and cause damage to coral cells under stressful condition. The finding that the antioxidants extended the survival time of tissue balls at high temperature suggests that zooxanthellae produce active oxygen species under the stress condition. This study also showed that tissue balls provide us a good experimental system to study the effect of stress condition and various chemical reagents on corals cells.

Key words: coral, bleaching, active oxygen, zooxanthellae, symbiosis