Studies on the mechanism of bleaching using coral cell aggregates

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Badrun Nesa and Michio Hidaka

Department of Chemistry, Biology and Marine Science, University of the Ryukyus, Nishihara, Okinawa 903-0213

INTRODUCTION

To investigate bleaching mechanisms of coral-zooxanthella symbiotic system, it is important to study response of corals to stressful 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. We exposed tissue balls containing various numbers of zooxanthellae to high temperature stress and examined the relationship between zooxanthella density and survival time of the tissue balls.

MATERIALS AND METHODS

Tissue balls were prepared from dissociated cells of *Fungia* sp and *Pavona divaricata*. Cells were removed from the skeleton and dissociated using Waterpik. 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 multiwell plate containing 300 µl FSW and allowed to recover at room temperature for 3-6 hours. Tissue balls were exposed to three treatments: elevated temperature at 31 °C, 25 °C as control and 31 °C in the presence of exogenous antioxidants, ascorbic acid (125 µm) and catalase (250 U ml^{-1}), or mannitol (10mM). Tissue balls were observed every 2 h for the first day, every 4 h for second day and then every 6 h. The volume of tissue balls was estimated from video prints assuming that they are ellipsoid. 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 the zooxanthella density of the tissue balls.

RESULTS AND DISCUSSION

Survival curves of tissue balls were markedly different between 31 and 25°C. At 31°C most of the tissue balls died within 24 h while at 25°C tissue balls survived for more than 24 h. There was a negative correlation between survival time and zooxanthella density of tissue balls at 31°C, while no significant correlation was found at 25°C or at 31°C in the presence of antioxidants. The present results showed that the higher the zooxanthellae density was the more quickly the tissue balls died under high temperature stress. This supports our hypothesis that zooxanthellae produce harmful substances and cause damage to coral cells under a stressful condition. Antioxidants increased the survival time of tissue balls indicating that the harmful substances might be produced active oxygen species. This study also showed that tissue balls provide us a good experimental system to study the effect of stress conditions and various chemical reagents on corals cells.