

Lysosomal Destabilization after Spawning in Pacific Oyster, *Crassostrea gigas*

WOO-GEON JEONG, SANG-MAN CHO, JONG-HWAN SEO

Division of Marine Bioscience, College of Marine Science, Gyeongsang National University, 445, Tongyeong, 650-160, Korea

INTRODUCTION

The defense systems of marine bivalves are varied with several factors such as alteration of environmental condition (Fisher & Newell, 1986; Genthner et al., 1999), exposure to xenobiotics (Auffret & Oubella, 1997) and toxicant from possible food organism (Jones et al., 1994) and infection with pathogen and parasites (Ford & Ashton-Alcox, 1998). Among the cellular organelles, lysosomes play an important role in defense system. Considering the properties of concentrating contaminants in the lysosome, the structural change in lysosome could be a threatening challenge for the cell causing damage in cellular immune system.

little information is available of the cost of spawning in the immune system, especially in oyster. Considering the periodical coincidence between summer mortality and spawning period, it has been suspected that the spawning is, to some extent, responsible for the summer mortality of farmed oysters.

The objective of this study was, therefore, to investigate the impact of the spawning activity on the lysosomal stability with neutral red retention assay, one of biomarker for health status of organisms, in Pacific Oyster, *Crassostrea gigas*.

MATERIALS AND METHODS

Spawning induction was carried out by introduction of gamete concentrate. Followed by weighing the wet and dry weight of the soft tissue, around 0.5ml hemolymph per each oyster were removed from the pericardial cavity with syringes. One drop of hemolymph was placed and dispersed onto each counting chambers of hemocytometer in a duplicate count. Neutral red retention (NRR) time assay was carried out by Lowe et al. (1995a, b). A duplicate examination was terminated when the dye loss of small granular hemocytes from lysosome to cytosol was numerically evident in 50% (Pipe, 1990). One-way analysis of variance (ANOVA) test was applied for the comparison of two means: weight, hemocyte and NRR time respectively.

RESULTS AND DISCUSSION

In our study, we observed a significant decrease of circulating hemocyte density after spawning (83.6×10^4 cells/ml to 40.8×10^4 cells/ml, $P < 0.05$). Considering the functions of hemocyte (Chen, 1984), a decrease in circulating hemocyte density could be possibly developed to a depression of defense system.

A significant decrease of lysosomal stability was also observed (74.8 min to 47.6 min, $P < 0.05$), which could be a possible disorder in the defense system. NRR assay is an integrated and rapid indicator of the health status of an organism that measures lysosomal damage, which has successfully been applied to marine organisms.

Among the studies on the summer mortality, growing interest has been focused on the gametogenesis and physiological changes after spawning. In this study, a significant decrease of NRR time was observed after the spawning. The energetic cost for spawning event may provide an internal threaten for the lysosomal stability, which can affect the cellular health state.

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