한국어병학회지 제 21 권 제 3 호 (2008)
J. Fish Pathol., 21(3) : 241 ~ 246 (2008)

Haemocytes responses of the pearl oyster, *Pincdata fucata*, at different temperatures

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The effects of temperatures on pearl oyster, *Pincdata fucata* were studied by evaluating some functional immune responses of the haemocytes. Water temperature is one of the most important factors in bivalve immune defense. Haemocytes comprise a primary line by inflammation, encapsulation and phagocytosis. These phagocytic abilities of haemocytes were observed in different temperatures. The number of the circulating haemocytes by migratory assay, phagocytic activities by MTT assay and reactive oxygen species production of haemocytes by CL assay were measured at different temperatures.

Results showed that pearl oyster maintained at 20℃ and 25℃ displayed significantly higher values for all the measured immune parameters in comparison to maintained at 10, 15, and 30℃.

Key words: Pearl oyster, Haemocyte, Phagocytosis, Temperature

Environmental changes and stresses are known to influence immune parameters in bivalves (Kaspar and Tamplin, 1993; Jones *et al*., 1995; Hauton *et al*., 2000; Paillard *et al*., 2004; Gagnaire *et al*., 2006). Temperature which is the most important environmental condition for bivalves, can affect defense-related activities of blood cells in determining the virulence of any infection (Parry and Pipe, 2004). Previous studies demonstrate that temperature strongly affects immune response in clam (Paillard *et al*., 2004), oyster (Parry and Pipe, 2004), and mussel (Carballal *et al*., 1997).

Haemocytes of bivalve mollusks are known to be responsible for many immunological functions (Bachere *et al*., 1990). Immune parameters such as phagocytosis, the number of circulating haemocytes and reactive oxygen species (ROS) production by haemocytes are often used as tools to investigate and diagnosis on health condition of bivalves. Phagocytosis is the most investigated functional aspect of haemocyte activity (Tripp, 1992; Carballal *et al*., 1997). Assessment of reactive oxygen species production (Anderson, 1994) and haemocyte migration (Lacoste *et al*., 2002), and aggregation (Auffret and Oubella, 1997) were also concerned for functional studies of bivalve haemocytes.

Farming of pearl oyster, *Pincdata fucata*, is a very important industry in Tongyeong and several pathological conditions can be considered as the most troublesome (Tomaru *et al*., 2001). However, in spite of the economic importance of this industry, there are not many studies on defense mechanisms of the pearl oyster.

The aim of the present paper was to study the

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ability of the haemocyte to evaluate the influence of temperature on immune parameters of the pearl oyster.

Materials and Methods

Two-year-old healthy oysters were obtained from a hanging culture bed in Tongyoung, southern Korea and acclimatized in the laboratory for 5 days before exposure to experimental temperatures. Exposure to experimental temperatures and preparation of haemocytes suspension were performed by following the method described previously (Monari *et al*., 2007).

As the minimum temperature needed of pearl oyster survival is reported to be 8℃, all experiments were tested at different temperatures, 10℃, 15℃, 20℃, 25℃ and 30℃. Each temperature was maintained throughout the experiment (i.e., 24 h incubation and counting) and each experiment was performed at least triplets for reproducible results. A statistical comparison (Tukey) test was conducted to compare the significance of differences among the treatments using the SAS computer software (SAS Institute Inc., USA). Values of $P < 0.05$ were considered significant.

Cellular responses were evaluated the reduction of a tetrazolium dye (MTT reduction), by haemocytes of individual oysters according to the published method (Volety *et al*., 1999).

The effect of opsonization of zymosan with serum (FBS) on the respiratory burst of haemocytes isolated from the pearl oyster was investigated. The reactive oxygen species (ROS) produced during the respiratory burst of haemocytes in response to the opsonized or unopsonized zymosan were measured using chemiluminescence (CL). Luminol (5-amino-2, 3-dihydro-1, 4-phthalazinedione) was prepared according to the published method (Cheng *et al*., 2004).

Migration assay was performed according to the published method (Oliver & Fisher, 1999). The migration of oyster hemocytes was quantified using chemotaxis chambers in which transwell inserts (6.5 mm diameter, 5.0-μm pore size polycarbonate filter, tissue culture treated; Costar, Cambridge, MA) and 24-well tissue culture plates (Cell Wells, Corning, Newark, CA) formed the upper and lower wells, respectively. 100 μL aliquots of hemocyte suspensions (2 x 10^6 cells/mL) in fresh sea water were added to the upper wells of the chemotaxis chambers and 600 μL FSW was placed in the lower wells. Volumes were allowed to equilibrate for 15 min at 15℃ to prevent net flow of solutions across the transwell membranes during chemotaxis assays; 15-30 μL of the putative chemoattractant (FBS) were then added to the lower wells. Cells were allowed to migrate through Transwell membranes for 2-6 h at 15℃. Following incubation, trypsin (Sigma Chemicals; 0.05% w/v final concentration) was added to the lower wells and the plates were incubated at 20℃ for 5 min. Data are presented as migration rate.

Results and Discussions

MTT reduction tended to be increased in pearl oysters exposed at 15℃, 20℃, and 25℃ and decreased at 30℃. Significant difference showed between 25℃ and the other temperatures (Fig. 1). These results are consistent with previous data showing temperature effects on oyster immune parameters (Parry and Pipe, 2004).

The CL response of haemocytes stimulated by zymosan particles was tested at different temperatures, 10℃, 15℃, 20℃, 25℃ and 30° C, in order to determine the effect of temperature. The CL generated at 20℃ and 25℃ in response to zymosan was

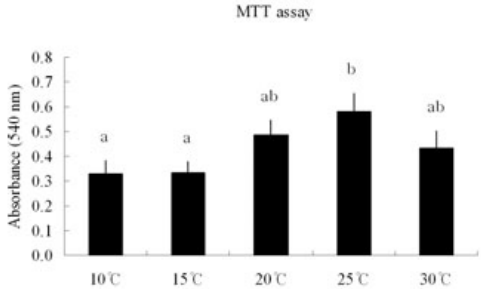

Fig. 1. Cytotoxicity of haemocytes isolated from pearl oyster, *Pincdata fucata* assessed by MTT assay at 10, 15, 20, 25, and 30℃. Each experiment was made in triplicate and comparisons were carried out using the SAS computer software (P<0.05).

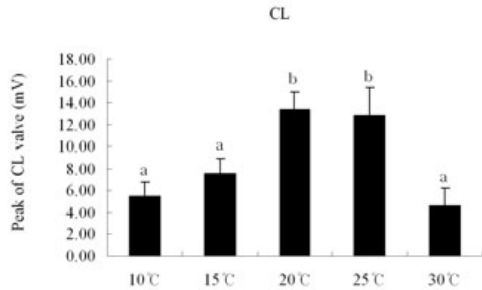

Fig. 2. Chemiluminescence response of haemocytes isolated from pearl oyster, *Pincdata fucata* at 10, 15, 20, 25, and 30℃. Presented as mean and standard errors of triplicate experiments. Statistical Comparisons were carried out using the SAS computer software (P<0.05).

significantly greater than at 10℃, 15℃ and 30℃ (Fig. 2). And the CL activity of pearl oyster haemocytes increased dramatically by 177% when temperatures were increased from 15 to 20° C and decreased dramatically by 36% when temperatures were increased from 25 to 30° C. For this study, temperatures during CL activity recording were stabilized at 20 and 25° C for pearl oyster haemocytes. Haemocyte respiratory burst activity has been investigated in oyster, mussels and clams using luminol-enhanced chemiluminescence (CL) (Bachere *et al*., 1990; Pipe, 1992; Anderson, 1994). The present results clearly show that temperature can provoke significant immune changes in the pearl oyster.

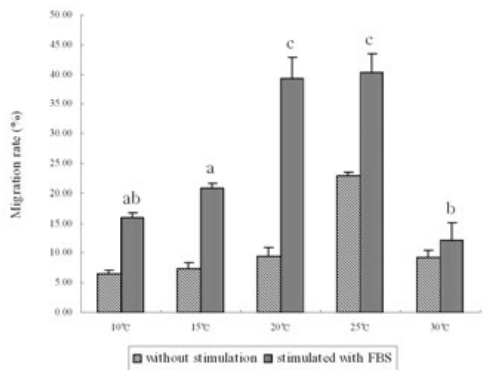

Fig. 3. Migration of haemocytes isolated from pearl oyster, *Pincdata fucata* at 10, 15, 20, 25, and 30℃. Statistical Comparisons were carried out using the SAS computer software (P<0.05), and presented as mean and standard errors of triplicate experiments.

Rate of migration with stimulated FBS were 16.05%, 20.86%, 39.37%, 40.30%, and 12.24%, for the assays performed at 10℃, 15℃, 20℃, 25℃ and 30℃, respectively. The percentage of migration cells was lower at low temperature than high temperature. Haemocyte migratory by FBS was significantly increased at 20℃ and 25℃ in comparison with 10℃, 15℃ and 30℃ (Fig. 3). Haemocyte migratory without FBS was also the highest level at 25℃. These results indicated that haemocyte migration is affected by temperature.

There are also some reports that the phagocytic ability of hemocyte was down regulated by low temperature in oyster (Parry and Pipe, 2004) and mussel (Tripp, 1992) and this was thought to be due to decreased haemocyte locomotion.

In this study, temperature also affects haemocyte reaction such as migratory, reactive oxygen species production, and MTT reduction in pearl oyster. In this study, increased temperature from 10℃ to 25℃

was enhanced haemocyte locomotion, ROS and phagocytosis of the pearl oyster. Enhanced phagocytic ability of haemocytes by environmental factors in bivalves has been reported previously and higher temperature induced the significant increase in phagocytic activity (Carballal *et al*., 1997; Gagnaire *et al*., 2006).

One of the causes of the mortality in pearl oyster was infectious disease with a marine birnavirus (Suzuki *et al*., 1998). This virus is stable in wide range of temperature (Mortensen *et al*., 1992). The detection rates of the virus on pearl oyster were higher in autumn and winter than in spring and summer (Mortensen *et al*., 1998; Kitamura *et al*., 2000; Kitamura *et al*., 2002), even though detected in July and August in blue mussels, *Mytilus galloprovincialis* at high rates (Kitamura *et al*., 2007). We suspect that higher temperature have an effect to induce phagocytosis of pearl oyster to inactivate or eliminate the viruses by haemocyte defense reaction (Bachere *et al*., 1990; Mortensen *et al*., 1992). But, some researchers found that grater phagocytosis capacity in American oyster, *C. virginica*, at high temperatures did not protect from *Perkinsus marinus* infection (Chu and Peyre, 1993). High temperatures can result in haemocyte stress so that they are less responsive. But temperature of 20-25℃ are not stressors for pearl oyster, higher temperature can effect immune response to the MABV. By contrast, mortality rate of hard clam, *Meretrix lusoria*, by MABV infection was increased at higher temperature (Chou *et al*., 1994) compare to which increased when temperature was decreased in pearl oyster. So further studies will be need to know about relationship among immune response, infection and temperature in pearl oyster, *Pincdata fucata*.

In summary, the present study examined the relationship between temperature and internal defense mechanisms of pearl oyster, *Pincdata fucata*. It provides further evidence that temperature is a potential factor activating immune system in aquatic organisms

Acknowledgement

This research was supported by a grant (04-RIS-024) from Regional Innovation System funded by the Ministry of Commerce, Industry and Energy, Republic of Korea.

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Manuscript Received : May 6, 2008
Revision Accepted : August 24, 2008
Responsible Editorial Member : Oh, Myung-Joo
(Chonnam National University)