ISJ 10: 141-150, 2013 ISSN 1824-307X

RESEARCH REPORT

The comparative study of immunity between two scallop species *Chlamys farreri* and *Argopecten irradians*

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Accepted November 12, 2013

Abstract

Zhikong scallop (*Chlamys farreri*) and Bay scallop (*Argopecten irradians*), two major aquaculture molluscan species in China, are different in some important traits such as life cycle, growth performance and temperature tolerance. In the present study, the malondialdehyde (MDA) content and four immune parameters including phagocytic activity, respiratory burst and activities of superoxide dismutase (SOD) and acid phosphatase (ACP) of scallops under heavy metal exposure and bacteria challenge were measured by flow-cytometric method and immunochemistry assays to compare the immunity of these two scallop species. In the non-treated scallops, the phagocytosis of hemocytes, MDA content, the activities of SOD and ACP in hepatopancreas of Bay scallops were all significantly higher than those of Zhikong scallops. After challenged with *Vibrio anguillarum*, the ROS level in hemocytes of Bay scallops was significantly lower than that of Zhikong scallops at 30 min, and the cumulative mortality of Bay scallop was also significantly lower than that of Zhikong scallop at 2^{nd}-5^{th} day. The exposure of Pb^{2+} with different concentration induced significantly higher phagocytic activity, ACP activities, SOD activities, MDA content in hepatopancreas and significantly stronger respiratory burst in hemocyte of Bay scallops compared with those of Zhikong scallops, while the hemocyte mortalities in Bay scallops were significantly lower than that in Zhikong scallops. The results collectively indicated that Bay scallops had a higher level of immune potential than Zhikong scallops, suggesting its greater capacity for stress response and immune resistance against pathogens as well.

Key Words: *Chlamys farreri*; *Argopecten irradians*; immune defense; phagocytosis; respiratory burst; enzyme activities; lead exposure

Introduction

The Bay scallop *Argopecten irradians* and Zhikong scallop *Chlamys farreri* are both dominant aquaculture species in China (Guo *et al.,* 1999; Zhang and Yang, 1999). The Bay scallop, a hermaphroditic bivalve distributed along the Atlantic coast of United States, was introduced to China as alternative species for aquaculture in 1982. The Zhikong scallop is a dioecious bivalve native to the coast of China, Korea, Russian and Japan. After having flourished for several years, massive summer mortality of both species has become a major constraint for the development of scallop aquaculture (Zhang and Yang, 1999; Xiao *et al.,* 2005). Although the cause of mortalities has not been accurately identified, it is believed that the mortalities are related to the combination of the deteriorating water quality, excessive stocking densities, pathogen infection and stock degeneration from inbreeding (linked to the original hatchery seed). Anthropogenic contaminants, such as heavy metals, may partly be responsible for the increase in disease incidence by adversely affecting immunity, thus enhancing susceptibility to infection (Pipe and Coles, 1995; Wootton *et al*., 2003). Numerous studies have demonstrated that heavy metals could affect the hemocyte functions in molluscs, such as cell viability, cytoskeletal organization and phagocytic activity (Fagotti *et al*., 1996; Olabbarietta *et al*., 2001; Sauvé *et al*., 2002; Duchemin *et al*., 2008). Among all of the heavy metals, lead is a toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. Pb^{2+} can be accumulated in bivalves and cause immunosuppression and even lead to mortality.

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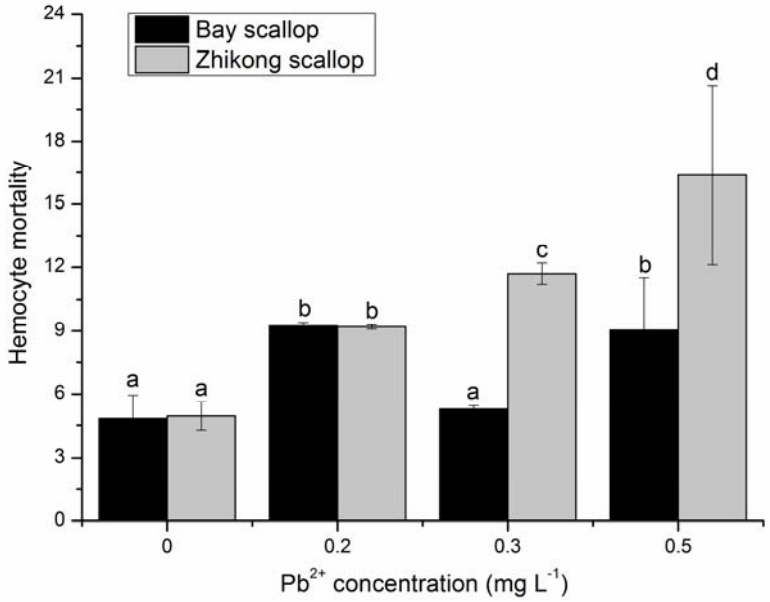

Fig. 1 Hemocyte mortality of Bay scallops and Zhikong scallops exposed to Pb^{2+} at different concentrations. Vertical bars represent the mean ± SD (n = 3), and bars with different letters are significantly different ($p < 0.05$).

Vibrio anguillarum has been reported one of the bacterial pathogens affecting scallops (Song *et al.,* 1997; Zhang *et al.,* 1999). Increasing evidence indicates that the mortalities of scallops sometimes appear to be species specific, and that Bay scallops not only endure higher temperatures in contrast with Zhikong scallops but also grow faster (Zhang *et al*., 2000; Xiao *et al.,* 2005). It can be inferred that there might be some immune mechanisms different from each other, which inspires our interests in the comparison of immune parameters in these two species after lead exposure and bacteria challenge.

Given the continuing prevalence of summer mortalities, there is a growing realization that knowledge of immunity and disease susceptibility in these aquaculture animals is still inadequate (Harvell *et al.,* 1999; Falco *et al.,* 2009; Li *et al.,* 2013). In the present study, the comparison of immune parameters (hemocyte mortality, phagocytic activity, respiratory burst, superoxide dismutase and acid phosphatase activities), malondialdehyde content, and the mortality between scallops *C. farreri* and *A. irradians* was conducted to better understand the immune potential of these two scallops, to improve health management practices as well as allow more efficient control in scallop farming.

Materials and methods

Source of specimens, lead treatment and mortality observation

Zhikong scallops (*Chlamys farreri*) and Bay scallops (*Argopecten irradians*) were collected from a commercial farm (Qingdao, China), and acclimated in a free-flowing aquarium system at salinity 30 ± 0.1 ‰, temperature 18 ± 1 °C, dissolved oxygen above 6.0 mg L^{-1} and pH from 7.7 to 8.2 for two weeks before assays. The scallops were fed on *Isochrysis galbana Parke* which was in logarithmic-growth phase at excess ration one time per day (at 10:00). The seawater was changed 100 % daily to ensure high water quality. Zhikong scallops were one year old, averaging 56 mm in shell length, and Bay scallops were 5 months old, averaging 51 mm in shell length. For the lead treatment experiment, 320 scallops (160 of Zhikong scallop; 160 of Bay scallop) were divided into eight groups. Two groups of 40 animals maintained in normal seawater were employed as control groups. Six groups of 40 scallops were exposed to $PbCl_2$ for a period of up to 10 days at final concentration of 0.2 mg L^{-1}, 0.3 mg L^{-1} and 0.5 mg L^{-1}, respectively, according to the previous study (Zhang *et al*., 2010). Simultaneously, another 90 scallops from each species were employed to evaluate cumulative mortality under bacteria challenge. *V. anguillarum* M3, kindly provided by Dr. Zhaolan Mo, was employed as pathogen in the challenge experiment. The bacteria were cultured in 2216E broth (Tryptone 5 g L^{-1}, yeast extract 1 g L^{-1}, $C_6H_5Fe{\cdot}5H_2O$ 0.1 g L^{-1}, pH 7.6) at 28 °C overnight, and centrifuged at 2000 g for 5 min. The pellet was suspended in PBS and adjusted to 1×10^8 CFU ml^{-1}. For each species, 90 scallops were randomly divided into three groups (30 for each group), and two groups received an injection of 200 µl *V. anguillarum* suspension (challenged group) and 200 µl of PBS (control group) into the adductor

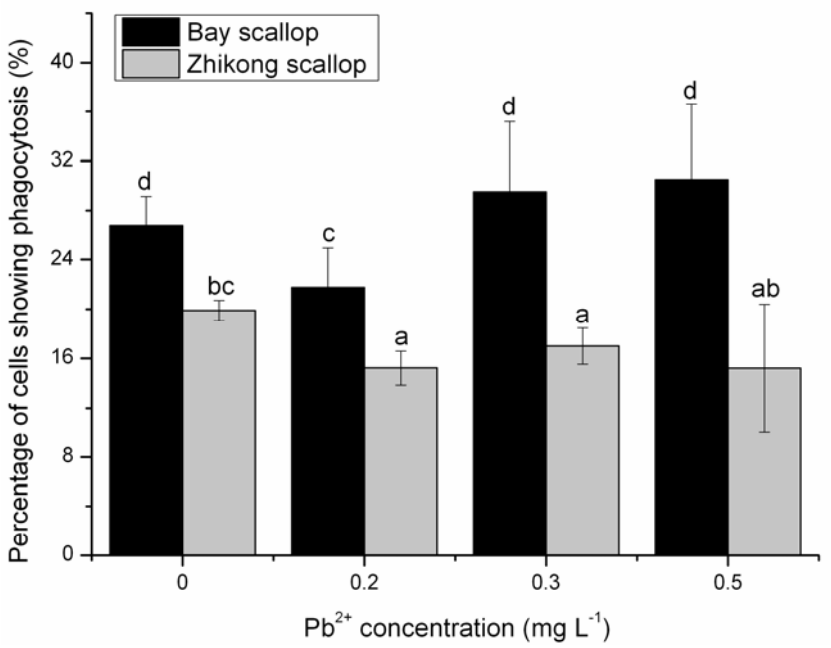

Fig. 2 Percentage of cells showing phagocytosis in Bay scallops and Zhikong scallops exposed to Pb^{2+} at different concentrations. Vertical bars represent the mean ± SD (n = 3), and bars with different letters are significantly different ($p < 0.05$).

muscles, respectively. The treated scallops were immediately returned to seawater tanks. Another 30 untreated scallops were used as the blank group. Dead scallops were removed immediately and cumulative mortality was calculated for both species.

Hemolymph and hepatpancreas collection

For the assays of phagocytosis, hemocyte mortality and respiratory burst assays, about 200 µl hemolymph was collected from the pericardium of each scallop by using a 23-gauge needle attached to a 2-ml syringe containing 1 ml TBS anticoagulant solution (0.05 mol L^{-1} Tris-HCl, pH 7.4; 2 % glucose; 2 % NaCl; 20 mmol L^{-1} EDTA). The hemolymph from ten scallops was pooled together immediately as one sample, and then divided into several aliquots for the following assays. Three samples collected from 30 individuals were analyzed for each species.The hepatopancreas were collected from 30 scallops of each species in the lead treatment experiment, and ten of them were pooled together as one sample. The samples were homogenized in glass homogenizers containing PBS buffer on ice (pH 6.41, 15 mmol L^{-1}). Each homogenate was centrifuged at 13,000 rpm at 4 °C for 1 h. The supernatants were collected and stored at -80 °C for SOD, ACP activities and MDA content analysis.

Quantitative analysis of phagocytosis

Two microliters of fluorescent beads (Fluoresbrite YG Microspheres, 2.00 µm; Polysciences, USA) were added to 1.5 ml of ultrafiltered (0.2 µm) seawater (FSW). Four hundred microlitre of each pooled hemolymph sample was immediately centrifuged at 3,000 rpm at 4 °C for 10 min. After removing the supernatant, the hemocyte pellet was resuspended in 400 µl of FSW. Then 200 µl of each suspension was mixed with the fluorescent bead suspension and incubated at 18 °C for 1 h in the dark and this reaction was terminated by adding 250 µl of Baker's formol solution (4 % formaldehyde, 2 % NaCl). The samples were analyzed by flow cytometer (FACS Vantage, BD, USA) and the flow cytometry data was analyzed by Flowjo software (Tree Star, Ashland, OR, USA). The FITC staining cells were considered as the candidates of phagocytic hemocytes, and the microscopic examination was used to ensure that beads were internalized instead for adhered to the cells. The hemocytes with FITC fluorescence were recorded as phagocytic cells, while the hemocytes without FITC fluorescence were considered as non-phagocytic cells. The percentage of phagocytic cells was calculated as the following. Percent of phagocytic cells = [number of hemocytes engulfing fluorescent beads (phagocytic hemocytes) / total number of hemocytes] × 100 %.

Observation of hemocyte mortality

Hemocyte mortality was recorded according to Delaporte's protocol (2003) with some modification. A total of 400 µl pooled hemolymph from control and Pb^{2+} treated groups was labeled with propidium iodide (PI, at the final concentration of 20 µg ml^{-1}) and incubated in dark for 10 min before flow cytometric analysis. PI fluorescence was measured at wavelengths above 630 nm. The hemocyte mortality was calculated as the percentage of hemocytes that had incorporated PI fluorescence relative to total hemocyte counts.

Table 1 SOD, ACP activities and MDA content in hepatopancreas of Zhikong scallops and Bay scallops under lead exposure

Pb^{2+} concentration (mg/L)	SOD (U/mg protein)		MDA (nmol/mg protein)		ACP (U/g protein)	
	Zhikong scallop	Bay scallop	Zhikong scallop	Bay scallop	Zhikong scallop	Bay scallop
Control	40.6 ± 6.9^{a}	114.0 ± 24.3^{bc}	29.0 ± 3.1^{b}	92.5 ± 13.8^{cd}	146.6 ± 13.3^{a}	432.4 ± 56.9^{bc}
0.2	58.9 ± 21.5^{ab}	218.7 ± 38.0^{d}	52.5 ± 9.1^{b}	155.6 ± 18.2^{ef}	176.1 ± 37.5^{a}	438.3 ± 111.6^{bcd}
0.3	91.0 ± 36.9^{cd}	212.1 ± 58.8^{bc}	81.5 ± 31.9^{abcde}	194.7 ± 34.5^{f}	295.4 ± 71.6^{ab}	544.3 ± 131.7^{d}
0.5	153.1 ± 43.1^{cd}	175.9 ± 27.4^{cd}	92.3 ± 14.5^{c}	135.5 ± 17.3^{def}	295.1 ± 65.1^{b}	536.9 ± 76.2^{cd}

The values were shown as means ± SD, n = 3. Significant difference between two scallop species, and the various concentration of Pb was indicated by different letters ($p < 0.05$).

Flow cytometric respiratory burst assay

The respiratory burst of both species was measured according to Bass's method using 2', 7'-dichlorofluorescin diacetate (DCFH-DA), a nonfluorescent fluorescein analogue, with some modifications (Bass *et al*., 1983; Hégaret *et al.,* 2003). DCF production, quantitatively related to the ROS production of hemocytes, was measured by evaluating the green fluorescence on the FL1 detector of the flow cytometer. For the detection of respiratory burst after Pb^{2+} exposure, a 400 µl of pooled hemolymph from control and Pb^{2+} treated scallops was mixed with 200 µl FSW. Then 6 µl of DCFH-DA (Sigma) was added at a final concentration of 0.01 mM, and the mixture was incubated in the dark at 18 °C for 60 min. DCF fluorescence was measured at 500 - 530 nm by flow cytometer. Respiratory burst of hemocytes was calculated as the geometric mean of the fluorescent peak on an FL1 histogram plot for each sample. For the detection of respiratory burst after bacteria challenge, a 400 µl aliquot from pooled hemolymph was mixed with 200 µl of *V. anguillarum* (OD_{600} = 0.4). Simultaneously, those hemolymph samples incubated with the FSW were used as control. Then 6 µl of DCFH-DA was added to each tube. The DCF fluorescence of each sample was measured with the flow cytometer at 30, 60, and 120 min after incubation with DCFH-DA, and respiratory burst of hemocytes was calculated as above description.

Measurement of SOD, ACP activities and MDA content in hepatopancreas

The activities of SOD and ACP and the content of MDA in hepatopancreas were measured by using the kits from Jiancheng Bioengineering Institute (Nanjing, China) according to manufacturer's protocols. The MDA content was expressed as nmol per mg of protein (nmol/mg protein). SOD activity was defined as the ability of 1 mg protein to cause 50 % inhibition in 1 mL reaction solution, and expressed as unit activity per mg of protein in the sample (U/mg protein). ACP activity was defined as the ability of 1 g protein to produce 1 mg phenol after incubation at 37 °C with the substrate for 30 min, and the activity was expressed as units per g of protein (U/g protein). The total protein content was measured with BCA assay (Beyotime biotechnology, China).

Statistical analysis

Data from the flow cytometer were processed by using the analysis software WinMDI 2.8. For the immune parameters and MDA content assays, the data were given in terms of means ± SD (n = 3). Survival data were graphed by the method of Kaplan-Meier and compared using log-rank analysis with GraphPad Prism 5.0 software (Lee and Wang 2003; Sohail *et al.,* 2008). All of the data were subjected to one-way analysis of variance (one-way ANOVA) followed by a multiple comparison using SPSS v15.0 (SPSS Inc., Chicago, Illinois). Differences were considered to be statistically significant at a p value of 0.05 or less.

Results

The hemocyte mortality

The flow cytometric analysis with PI fluorescence revealed that hemocytes mortality in the

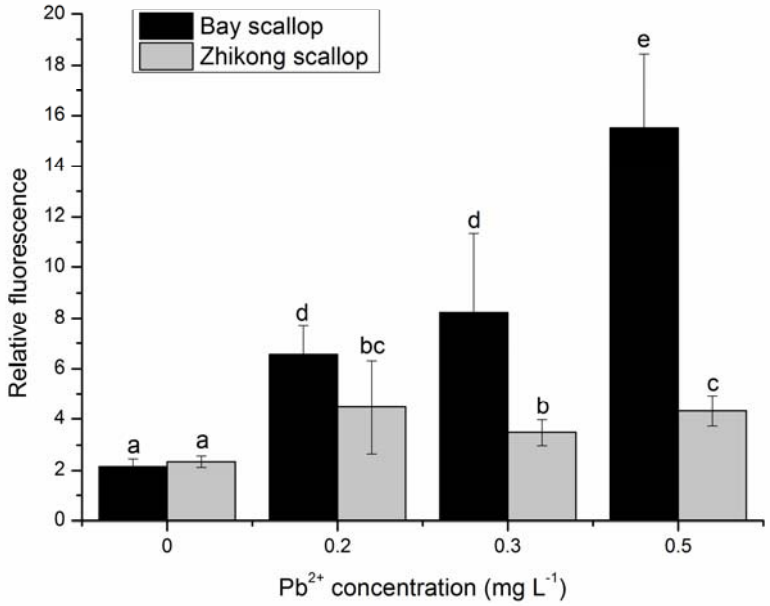

Fig. 3 ROS levels of Bay scallops and Zhikong scallops exposed to Pb^{2+} at different concentrations. Vertical bars represent the mean ± SD (n = 3), and bars with different letters are significantly different ($p < 0.05$).

control groups of Bay scallops and Zhikong scallops was 4.8 ± 1.1 % and 4.9 ± 0.7 % respectively. There was no significant difference between the controls of two species. After Pb^{2+} exposure, the hemocyte mortality of Zhikong scallops was significantly increased in a dose-dependent manner ($p < 0.05$). And the hemocyte mortalities of Bay scallops at 0.2 or 0.5 mg L^{-1} Pb^{2+} were significantly higher than those at 0.3 mg L^{-1} Pb^{2+}. Under the same condition, the hemocyte mortality in Bay scallops was lower than that of Zhikong scallops except at 0.2 mg L^{-1} Pb^{2+} (Fig. 1). A significant difference between the two scallop species was observed at 0.3 and 0.5 mg L^{-1} Pb^{2+} ($p< 0.05$).

Phagocytic activity of hemocytes

In control groups, the percentage of cells showing phagocytosis in Bay scallops (26.7 ± 2.4 %) was significantly higher than that of Zhikong scallops (19.9 ± 0.8 %) ($p < 0.01$) (Fig. 2). After Pb^{2+} exposure, the phagocytosis of Bay scallop hemocytes was significantly inhibited at the lowest Pb^{2+} concentration ($p < 0.05$), while those at other Pb^{2+} treatments were higher than that of the control group (Fig. 2). And the phagocytosis in the 0.2 and 0.3 mg L^{-1} Pb^{2+} treated Zhikong scallop groups was significantly lower ($p < 0.05$) than that in the control (Fig. 2). The percentage of cells showing phagocytosis in Bay scallops was significantly higher than that of Zhikong scallops ($p < 0.05$) in the 0.2, 0.3 and 0.5 mg L^{-1} Pb^{2+} treatments.

SOD, ACP activities and MDA content in hepatopancreas of control and lead treated scallops

SOD activity in hepatopancreas of Bay scallops in the control group was 114.0 U/mg protein, which was 2.8-fold of that in Zhikong scallops (40.6 U/mg protein), and there was significant difference between them ($p < 0.05$) (Table 1). After lead treatment, the significantly higher SOD activity was observed in 0.2 mg L^{-1} Pb^{2+} treated group of Bay scallops compared with that of Zhikong scallops ($p < 0.05$) (Table 1).

The ACP activity in hepatopancreas of the control Bay scallops (432.4 U/g protein) was significantly higher (2.9-fold) than that of Zhikong scallops (146.6 U/g protein) ($p < 0.05$) (Table 1). And Bay scallops had a significantly higher ACP activity in comparison with Zhikong scallops when they were treated with Pb^{2+} at corresponding concentration ($p < 0.05$), respectively. After Pb^{2+} treatment, there was no significant change in the ACP activity of Bay scallops in comparison with controls. The ACP activity in 0.5 mg L^{-1} Pb^{2+} treated Bay scallops were significantly higher than that of the untreated Zhikong scallops ($p < 0.05$) (Table 1).

The MDA content in hepatopancreas of Bay scallops in the control group was 92.5 nmol/mg protein, and it was significant higher than that in Zhikong scallops (29.0 nmol/mg protein) (3.19-fold, $p < 0.05$) (Table 1). After Pb^{2+} exposure, the MDA content in different Pb^{2+} treated Bay scallop groups was 1.68-fold ($p < 0.05$), 2.10-fold ($p < 0.05$) and 1.46-fold ($p > 0.05$) of the controls, while that in Zhikong scallop was 1.81-fold ($p > 0.05$), 2.81-fold ($p > 0.05$) and 3.18-fold ($p < 0.05$) of the controls, respectively. The MDA contents in the Pb^{2+} treated groups of Bay scallops were significantly higher than those in Pb^{2+} treated groups of Zhikong scallops at the Pb^{2+} concentration of 0.2 mg L^{-1}, 0.3 mg L^{-1} and 0.5 mg L^{-1} ($p < 0.05$).

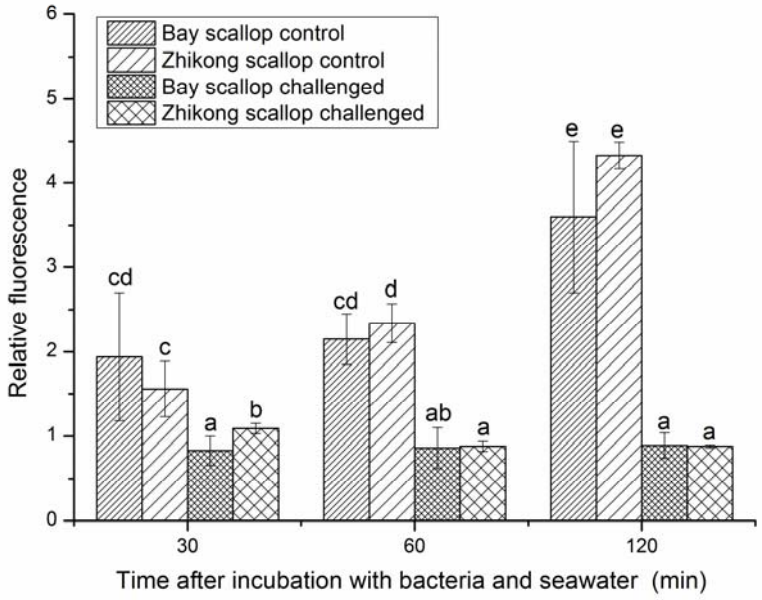

Fig. 4 ROS levels of Zhikong scallop and Bay scallop hemocytes challenged by *V. anguillarum*. Vertical bars represent the mean ± SD (n = 3), and bars with different letters are significantly different ($p < 0.05$).

Variation of respiratory burst after lead exposure and V. anguillarum challenge

There was no significant difference in the production of reactive oxygen species between Bay scallops and Zhikong scallops (1.56 Vs 1.94) under control conditions ($p > 0.05$) (Fig. 3). The respiratory burst in the two scallops both significantly increased ($p < 0.05$) after Pb^{2+} exposure. In Bay scallop, respiratory burst was about 3.05, 3.8 and 7.2 fold of the control after 0.2, 0.3 and 0.5 mg L^{-1} of Pb^{2+} exposure, respectively. The respiratory burst in Zhikong scallop was also significantly activated by 0.2, 0.3 and 0.5 mg L^{-1} of Pb^{2+} exposure, and it increased by 1.91, 1.48 and 1.84 fold of the control, respectively ($p < 0.05$) (Fig. 3). Bay scallop had a significantly higher respiratory burst than Zhikong scallop after the same level of lead exposure ($p < 0.05$) (Fig. 3).

The ROS level in hemocytes of scallops challenged by *V. anguillarum* was significantly lower than that in the control (incubation with seawater) ($p < 0.05$). In the control groups, there was no significant difference in the ROS production of two scallops ($p > 0.05$) (Fig. 4). After bacteria challenge, the ROS in the hemocytes of Bay scallops was significantly lower than that of Zhikong scallops at 30 min ($p < 0.05$), while it was comparable to that of Zhikong scallops post 60 min and 120 min challenge ($p > 0.05$). Compared to the control scallops, the respiratory burst in both scallops was significantly inhibited after *V. anguillarum* challenge ($p < 0.05$), which was different from the significant activation of respiratory burst after lead exposure. At 30 min after bacteria challenge, the respiratory burst was inhibited to be 0.43-fold of the control in Bay scallops ($p < 0.05$) and 0.70-fold in Zhikong scallops ($p < 0.05$), following 0.38 - 0.40 fold at 60 min ($p < 0.01$) and 0.20 - 0.25 fold at 120 min ($p < 0.01$) (Fig. 4). There was a significant difference in the ROS production of challenged Zhikong and Bay scallops at 30 min post challenge ($p < 0.05$).

Cumulative survival rate of scallops challenged by V. anguillarum

The similar cumulative survival rates were detected in the blank (0 h) and control groups of the two scallop species. No scallop died in the first 5 days in the untreated Zhikong and Bay scallops (blank groups). In the control groups of both Zhikong and Bay scallops, the died scallop was firstly observed at 4^{th} day, and mortality was of 3.3 % at the 4^{th} and 5^{th} day after injection (Fig. 5). After bacteria challenge, no Bay scallop died in the first two day, while the mortality of Zhikong scallops occurred since the first day and quickly increased to 41.2 % on the second day. On the third day, the mortality of the Bay scallops was 62.5 % and 94.1 % of the Zhikong scallops died at 3^{rd} day under the same condition. Afterwards, all the Zhikong scallops died in the 4^{th} day post challenge, while there was still 25 % of Bay scallops survival. The mortalities of Bay scallops at 2^{nd} - 5^{th} day post *V. anguillarum* challenge were significantly lower than those of Zhikong scallops ($p < 0.05$).

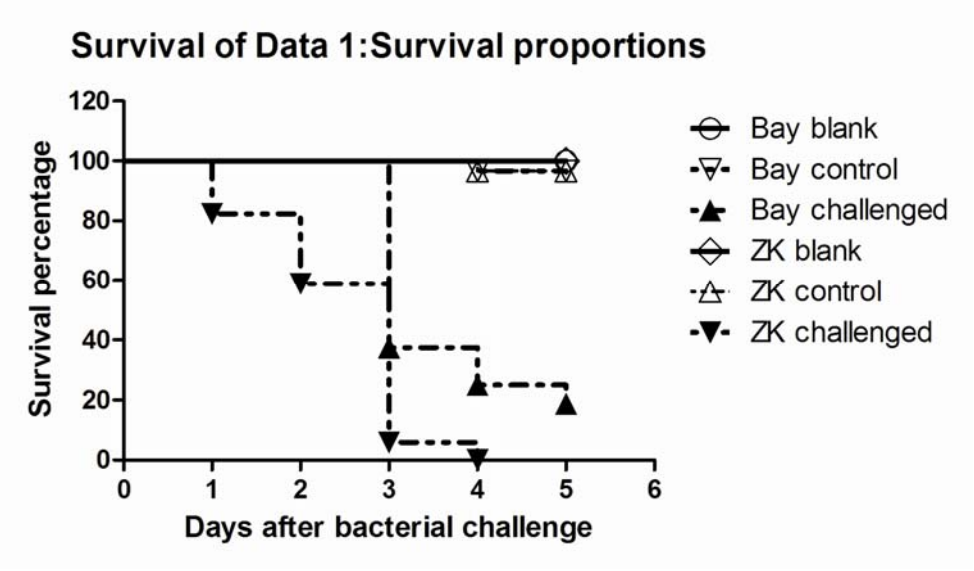

Fig. 5 Cumulative survival rate in Zhikong scallops and Bay scallops under *V. anguillarum* challenge. Bay: Bay scallop; ZK: Zhikong scallop. Cumulative survival rates of scallops (N = 30) treated by injecting 200 μl of *V. anguillarum* resuspended in PBS (OD 600 = 0.4) (challenged group) (▲ in Bay and ▼ in ZK) and 200 μl of PBS (control group) (▽ in Bay and △ in ZK), and without treatment (blank group) (○ in Bay and ◇ in ZK) were plotted against the duration after challenge.

Discussion

Bivalves respond to environmental stress depending largely on the viability and functional capability of hemocytes (Chu, 2000), and heavy metals as well as invading pathogens could affect the hemocyte functions in molluscs, such as cell viability, cytoskeletal organization and phagocytic activity (Fagotti *et al*., 1996; Song *et al.,* 1997; Zhang *et al.,* 1999; Olabbarietta *et al*., 2001; Sauvé *et al*., 2002; Duchemin *et al*., 2008). In the present study, the hemocyte mortalities were found to be less than 5 % in both healthy Bay scallop and Zhikong scallop under control condition, and this result was in agreement with previous reports from other bivalves. After Pb^{2+} exposure, the mortality of scallop hemocytes increased in a dose-dependent manner, but it differed between the two scallop species. It has been reported that the percentage of dead hemocytes is a good indicator of physiological status especially before or during mortality events (Paillard *et al*., 1996). In clams, a higher percentage of dead hemocytes was observed when they were experiencing massive mortalities (Soudant *et al.,* 2004). Bay scallop exhibited lower hemocyte mortality than Zhikong scallop under the same condition, indicating it bore stronger tolerance to environmental stress, and its physiological and immunological status were less influenced during heavy metal exposure compared with Zhikong scallop.

The phagocytosis assay has been developed as a common method to examine the ability of hemocytes to recognize and eliminate “non-self” material (Chu, 1988). In the present study, Bay scallops in the control and Pb^{2+} treated groups all displayed higher phagocytosis ability than that of Zhikong scallops. In eastern oyster *C. virginica*, the hemocytes with lower phagocytic ability exhibited a higher mortality (Hegaret *et al.,* 2004). Pacific oysters with higher phagocytosis ability were less susceptible to the protozoan parasite *Perkinsus marinus* infection (La Peyre *et al*., 1995). In shrimp *Litopenaeus vannamei*, the enhanced phagocytic activity could increase its resistance against *Vibrio alginolyticus* (Cheng *et al*., 2005). The percentage of cells showing phagocytosis in Bay scallops increased after Pb^{2+} exposure, while significantly decreased in Zhikong scallops. The higher phagocytic potential of Bay scallops in present study suggested that Bay scallops seemed to have a possible advantage of resisting to environmental exposure compared with Zhikong scallops.

Recent available evidence demonstrated that heavy metals enhanced the intracellular formation of ROS and induced cellular oxidative stress (Stohs and Bagchi, 1995; Gurer and Ercal, 2000; Pacheco *et al.,* 2007). In the present experiment, Pb^{2+} treatment significantly increased the ROS generation of scallops, and the increased ROS level lead to unspecific oxidation of proteins and membrane lipids, resulting in an increasing of malondialdehyde (MDA). A higher MDA content was observed in Bay scallops compared with Zhikong scallops before and after lead exposure. MDA content along with ROS results in present study possibly suggested the compromised immune function of scallops under metal exposure. The relatively gentle increase of MDA content in Bay scallops indicated they could offer a better buffer

capacity to alleviate metal induced damage than Zhikong scallops.

Endogenous enzymes excreted and released by hemocytes during exocytosis and degranulation have a close connection with the functions of hemocytes (Chu, 2000; Cima *et al*., 2000). Enzymatic activities in hemolymph have been studied as one of the immunity indicators in many bivalve species (Hine and Wesney, 1994; Carballal *et al*., 1997). The inhibitory effects of lead on various enzymes would probably result in impaired antioxidant defenses by cells and render cells more vulnerable to oxidative attacks (Gurer and Ercal, 2000). Significantly reduced superoxide dismutase (SOD) activities have been observed in lead-exposed scallops (Zhang *et al*., 2010). And higher SOD activity was detected in Japanese pearl oysters with low mortality from an infectious disease (Uchimura *et al*., 2003). The higher SOD activity after lead treatment indicated that Bay scallop should have a somehow stronger ability than Zhikong scallops to eliminate ROS and to prevent the injury of oxidative stress resulting from metal exposure.

Acid phosphatase is a lysosomal marker enzyme playing important roles in destructing pathogens in lysosome, and its activity appears to be altered by stress conditions (Cheng, 1989; Suresh and Mohandas, 1990). In the present study, ACP activities in both hemolymph and hepatopancreas of Bay scallop were higher than those of Zhikong scallop before and after Pb^{2+} treatment. The higher ACP activity in Bay scallop was consistent with its higher phagocytic ability (Cheng, 1978). Compared with Zhikong scallops, Bay scallops displayed higher ACP activities, especially in hepatopancreas, suggesting their predominance in resisting to the environmental stress and invading pathogens. Previous studies also revealed that some other immune factors in the hemolymph of *A. irradians* were significant higher than those in *C. farreri* (Wang and Sun, 2005).

In the present study, ROS in both scallop species was significantly lower than that in the control groups during the incubation of hemocytes with *V. anguillarum*, indicating the respiratory burst of both scallops was inhibited by bacteria challenge (Lambert *et al*., 2003; Labreuche *et al.,* 2006). The reduced respiratory burst was possibly associated with impaired ability to kill bacteria, and increased susceptibility to infectious diseases (Cai *et al*., 1994; Johnston, 2001). Furthermore, *V. anguillarum* was employed as the pathogen to challenge both scallops (Cavallo and Stabili, 2002), and the cumulative mortality of Zhikong scallop at 2^{nd} - 5^{th} day was significantly higher than that of Bay scallop ($p < 0.05$). Similarly, researches have previously reported that Bay scallops not only grow fast in contrast with Zhikong scallops but also endure higher temperatures (Zhang *et al*., 2000; Xiao *et al.,* 2005). These results collectively favored that Bay scallop had higher potential defense capabilities than Zhikong scallop against pathogens and environmental stress factors.

Acknowledgements

The authors would like to thank all the laboratory members for technical advice and helpful discussions. This research was supported by grants from National Science Foundation of China (No. 30925028 to L.S.), Shandong Provincial Natural Science Foundation (No. JQ201110 to L.W.), and the Knowledge Innovation Program of the Chinese Academy of Sciences (No. KZCX2-EW-QN201 to H.Z.).

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