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The role of hybridization in improving the immune response and thermal tolerance of abalone

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ABSTRACT

Recently, frequent death of cultured abalone drew our attention to the stress tolerance of abalone. Hybridization is an effective way of genetic improvement in aquaculture, which can introduce improved traits to the hybrids. In this study, we challenged the hybrids between *Haliotis discus hannai* and *Haliotis gigantea*, and their parents with bacteria (*vibrio harveyi, vibrio alginolyticus and vibrio parahemolyticus*), then held them at 20 °C and 28 °C, survival rates of the parental populations and hybrid populations were recorded. Then we tested the immune responses and thermal-induced responses of the four populations at different temperatures. Total hemocyte count (THC), respiratory burst, superoxide dismutase activity (SOD), acid phosphatase activity (ACP), alkaline phosphatase activity (AKP), myeloperoxidase activity (MPO), and HSP70 expression were determined on day 1 and day 7 of the temperature exposure. Results showed higher survival rates of the hybrids than their parents against bacteria challenge. For immune parameters, THCs were evaluated at 28 °C, while increased THC was also observed in *H. discus hannai* ♀ × *H. gigantea* ♂ (DG) and *H. discus hannai* ♀ × *H. discus hannai* ♂ (DD) at 12 °C (day 7); at 28 °C, respiratory burst was activated (day 1 and 7), while SOD activity first rose then fell over 7-days exposure; AKP activity was elevated at 12 °C and 28 °C (day 1), most notably in DG, and an increased level of ACP was observed in DG at 28 °C (day 7); MPO activity was suppressed at 12 °C and 28 °C on day 1, but recovered on day 7. For HSP70, increased HSP70 levels were observed in all populations at 28 °C (day 1), and DD got the lowest HSP70 level after 7-days exposure at 28 °C. Overall, the results suggest that temperature changes could significantly affect the physiological status of abalone, and hybrids may be more resistant to disease and thermal stresses than their parents.

1. Introduction

Abalone, which belongs to genus Haliotis and family Haliotidae, is cultured worldwide because of its high commercial value [1,2]. Rapid development of abalone aquaculture has happened in recent years, to balance the decreasing wild stocks and increasing demands of abalone supply [3,4], as a result, the world abalone fishery and aquaculture production reached 63245t in 2010 (FAO). Abalone aquaculture in China started in the late 1980s and developed vastly since then [1,5–7], the annual production of abalone in China was 56511t in 2010 (FAO). Recently, in northern and southern provinces of China, cultured abalone encountered frequent mass mortalities, which was supposed to be caused by high temperature or bacterial infection. This brought heavy losses to China abalone aquaculture industry [1,5–9].

Hybridization is an effective method of genetic improvement. It has been widely used in aquaculture for many fish and shellfish species, including abalone, oyster, scallop, carp, catfish, salmonid, sparid, sunfish and so on [1,2,10–12]. Hybridization can bring improved traits to the hybrids, such as enhanced growth, survival rate, thermal tolerance, disease resistance and so on. This could be explained by heterosis theory (the offspring produced through hybridization either gains better phenotypic traits than both parents, or gains intermediate phenotypic traits between parents) [2,10,11]. Lots of studies have proven hybrids' heterosis in stress tolerance. For example, "Pacific" scallop, offspring of the Canadian native weathervane scallop and Japanese scallop, showed advantage in growth rate and disease resistance ability over its parents [12]. Two strains of oysters, MSX/Dermo-resistant and JOD-resistant strains were obtained after several generations of selective breeding. MSX/Dermo-resistant strain was characterized by

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strong resistance to multinucleated sphere X (MSX) disease and Dermo disease, while the JOD-resistant strain showed rapid growth and strong resistance to juvenile oyster (JOD) disease. Hybrids of the two strains were both resistant to Dermo and rapid-growing [13]. Hybridization of the white bass *Morone chrysops* (Rafinesque) ♀ and the striped bass *M. saxatilis* (Walbaum) ♂ created the sunshine bass, which had several advantages over both parents, such as rapid growth, higher survival rate, and stronger temperature tolerance. Similar phenomena also exist in abalone. The hybrid, *Haliotis discus hannai* ♀ × *H. discus discus* ♂, was superior in weight increase and shell growth than its parents, and it showed higher adaptability to high temperature. Stronger tolerance of low temperature was found in the hybrid, *H. discus hannai* ♀ × *Haliotis kamtschatkana* ♂, which exhibited higher growth rate at both 8 °C and 18 °C than its parents [14]. Comparison between the HSP70 expression levels among four stocks of *H. discus hannai* (two inbred populations and two hybrid populations) indicated that the hybrid offspring had higher thermal tolerance than the inbred offspring [15]. Overall, hybridization is a useful tool in aquaculture and fisheries; it is meaningful to study the relationship between hybridization and stress resistance improvement.

Water temperature is an important environmental factor in aquaculture and fisheries, and it's always relevant to disease outbreaks [16–21]. Occurrence of diseases in marine organisms usually depends on two factors: (1) the efficiency of the host's immune system and (2) the ability of the pathogenic bacteria to invade the host [17]. Both of the two factors can be influenced by water temperature [16]. Many researches have been done to determine the effects of temperature on immune responses of marine life. For instance, green-lipped mussel (*Prena viridis*) exhibited lower levels of esterase, reactive oxygen species, lysosome content, and phagocytosis at high temperatures [22]. In hard clam (*Mercenaria mercenaria*), cellular and humoral immune system components (such as total hemocyte count, reactive oxygen species, phagocytosis and lysozyme) exhibited significant variations at different temperatures [17]. Monthly changes in the immune responses of European abalone (*Haliotis tuberculata*) were measured from early to late summer (with temperature increased). Reduction of phagocytosis and phenoloxidase production, and increase of basal reactive oxygen species production and agglutination titers were observed [19]. Similar changes also happened in *Haliotis rubra* and *Haliotis diversicolor*, in which the increase of temperature could elevate the levels of THC and superoxide anion, the susceptibility to vibrio infections, or the antiviral and antibacterial ability [16,23]. These studies greatly improved our knowledge of the interaction between temperature changes and marine life's immune systems, and may help us in generating strategies to deal with frequent disease outbreaks. However, to the best of our knowledge, few studies have focused on the promotion of hybridization on stress tolerance in marine organisms.

H. discus hannai is naturally growing in north China, and it was introduced into south China in late 1990s. Since then, it was widely cultured and became the dominant commercial abalone species in China [24]; its optimal growth temperature is 15–22 °C [25]. *Haliotis gigantea* is a Japanese warm-water species which was introduced into China in 2003 [25], it also became a market-favored species because of its crisp and tender meat, and its excellent disease resistance ability [25]. In recent years, disease outbreaks frequently happened in *H. discus hannai* during the summer and winter days, which may be due to its declined genetical characterization or declined thermal adaptability. To solve this problem, interspecies hybridization between *H. discus hannai* and *H. gigantea* was conducted in 2006. Their offspring, *H. discus hannai* ♀ × *H. gigantea* ♂ (DG) and *H. gigantea* ♀ × H. *discus hannai* ♂ (GD) were obtained [2,24]. The hybrids DG and GD had shown heterosis in growth and survival rate [26]. Therefore, we hypothesized that through hybridization, the hybrids' resistance to thermal stress and bacterial infection were elevated.

To test our hypothesis, two hybrid populations (DG and GD) and two parental populations (DD and GG) were challenged with bacteria, and then held at ambient (20 °C) or high temperature (28 °C); their survival rates were recorded and compared. Besides, we exposed the four populations to different temperatures for 7 days, in order to compare their immune system efficiency and thermal resistance ability, we examined two cellular immunity components (total hemocyte count and respiratory burst level), activity of four immune-related enzymes (superoxide dismutase, acid phosphatase, alkaline phosphatase, myeloperoxidase), and heat shock protein 70 mRNA expression during the exposure. Through these experiments, we expect to learn whether hybrids perform better than their parents when challenged with bacteria and stressed temperatures. We believe this knowledge would provide guidance for the practical application of heterosis theory in abalone aquaculture and fisheries.

2. Materials and methods

2.1. Acclimation of abalone

Two hybrid populations, *H. discus hannai* ♀ × *H. gigantea* ♂ (DG) and *H. gigantea* ♀ × *H. discus hannai* ♂ (GD), and two parental populations, *H. discus hannai* ♀ × *H. discus hannai* ♂ (DD) and *H. gigantea* ♀ × *H. gigantea* ♂ (GG) were reared in standard abalone cages (10 abalones per cage) placed in the culture ponds in Zhangpu Hongyun Abalone Company. For acclimation, 15-cages adult abalones of each population (healthy, 60 ± 10 mm in-shell length) were divided equally into five PVC tanks filled with aerated seawater, each PVC tank contained 120 abalones (30 abalones from each population), placement of abalones was shown in Fig. 1. The culture conditions were set at 20 ± 1 °C, 33‰ salinity, and pH of 7.8 for 14 days before experimentation. Abalones were fed red alga (*Gracilaria lemaneiformis*) and the seawater was changed every 24 h. Survival rates of each population were examined every half a day.

2.2. Temperature setting

After 2 weeks of acclimation, two tanks were kept at 20 °C (control group), another two tanks were elevated to 28 °C using 1000 W titanium heaters (Weinuo, China), and the last tank was cooled down to 12 °C using a cooling-water machine (Haili, China), temperature settings of the five tanks were shown in Fig. 1. The speed of elevating temperature or reducing temperature was 2 °C/day. The target temperatures were then kept for 7 days using an electronic thermostat (Jingchuang, China).

2.3. Bacterial challenge and thermal stress

Two PVC tanks of abalones (at 20 °C and 28 °C) were used for bacterial challenge experiment (E1, Fig. 1). For each tank, 20 abalones of each population were injected with a mixture of *Vibrio harveyi*, *Vibrio alginolyticus* and *Vibrio parahemolyticus* (in same proportion) at a dose of 5×10^5 cfu, and the other 10 abalones were injected with an equal volume of sterile saline solution (isosmotic with the seawater where we reared the abalones). Survival rates of each population at 20 °C and 28 °C were recorded at 12 h, 18 h, 24 h, 36 h and 48 h after injection. Three tanks of abalones (at 12 °C, 20 °C and 28 °C) were used to evaluate immune parameters in hemocytes and HSP70 expression in foot muscle after 1 and 7 days of exposure to the experimental conditions tested (E2, Fig. 1).