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Maternal transfer of immunity in scallop *Chlamys farreri* and its trans-generational immune protection to offspring against bacterial challenge

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ABSTRACT

Maternal immunity plays a crucial role in protecting the offspring at early stages of life and contributes a trans-generational effect on the offspring's phenotype. In the present study, maternal transfer of immunity and its trans-generational effect on offspring in scallop *Chlamys farreri* were investigated. The proteins including CfLGBP, CfLBP/BPI, CfLYZ and CfCu/Zn–SOD existed in the scallop eggs with high level while CfLec-3 was not detected. In contrast, the mRNA levels of these proteins were extremely low except that of CfCu/Zn–SOD. The protein extracts of scallop eggs exhibited remarkable agglutination activity and bactericidal effect against gram-negative bacteria *Escherichia coli* and *Vibro anguillarum*, and fungi *Pichia pastoris*. When the maternal scallops were stimulated with heat-killed *V. anguillarum*, the mRNA levels of CfLBP/BPI and CfLYZ in their offspring were expressed significant higher in D-shaped larvae. Furthermore, the protein levels of CfLBP/BPI and CfCu/Zn–SOD in the offspring of maternal immune stimulation group were higher than that of control at almost all the developmental stages, while the level of CfLec-3 and CfLYZ was higher than that of control just in eggs or trochophore, respectively. A significant enhancement of Cu/Zn–SOD and antibacterial activities was also observed in eggs, 4-cell embryos and trochophore of offspring from immune stimulated mother scallops. Moreover, the mortality of offspring from the immune stimulated mother scallops was significantly lower than that of control after bacterial challenge, especially in trochophore. The results indicated that scallop eggs or embryos received maternal derived immune competence to defense against the invading pathogens, and the maternal scallops received an immune stimulation endowed their offspring with a trans-generational immune capability to protect them against infections effectively.

1. Introduction

Like other invertebrates, scallops exclusively rely on innate immunity to defense against the invasion of foreign pathogens. The innate immune response of scallop is mediated by various immunocytes and humoral immune factors. Over the past decade, lots of immune factors have been identified from scallop, such as pattern-recognition receptors (PRRs) including peptidoglycan-recognition protein (PGRP) (Su et al., 2007; Yang et al., 2010b), lipopolysaccharide-and β-1, 3-glucan binding protein (LGBP) (Su et al., 2004; Yang et al., 2010a) and c-type lectins (Yang et al., 2011; Zhang et al., 2009), and immune effectors including LPS-binding protein and bactericidal/permeability-increasing protein (LBP/BPI) (Gonzalez et al., 2007) and lysozyme (LYZ) (Zhao et al., 2007). Besides, as important antioxidants, superoxide dismutase (SOD) and catalase (CAT) have proved to play an essential role in immune defense response (Li et al., 2008; Ni et al., 2007). All those immune factors coordinating with immunocytes integrated a sophisticated immune system to protect the organism against pathogens. However, compared to adult scallop, the embryos and larvae of scallop do not possess the sophisticated immune system (Yue et al., 2013). It's meaningful to elucidate the mechanisms underlying the immune protection of the scallop at early developmental stages.

Maternal transfer of immunity defines as the immunity transferred from mother to offspring, which is considered to play an essential role in protecting the offspring against pathogens at early stages of life (Chucri et al., 2010; Shlichta and Smilanich 2012; Swain and Nayak 2009). Up to now, the transfer of maternal immunity has been found in many vertebrates (Hamal et al., 2006; Poorten and Kuhn 2009; Zhang et al., 2013) and some invertebrates

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(Kamiya et al., 1984; Little et al., 2003; Marchini et al., 1997) by the transmission of immune factors. Immunoglobulin, as the most important maternal immune factor in vertebrates, can be transferred to the offspring via the placenta (Malek et al., 2011) or colostrums (Weaver et al., 2008) in mammals, while mainly via the eggs in birds, reptiles and fishes (Hamal et al., 2006; Picchietti et al., 2006; Poorten and Kuhn 2009; Schumacher et al., 1999). Other innate immune factors such as the complement components (Huttenhuis et al., 2006; Løvoll et al., 2006), lectins (Bly et al., 1986; Hasan et al., 2009; Yousif et al., 1994), lysozyme (Magnadottir et al., 2005; Yousif et al., 1991), and antimicrobial peptides (Seppola et al., 2009) have also been found to be transferred maternally by protein or mRNA in several fish species. Those maternal-derived immune factors are proposed to involve in the immune defense of fish embryos and larvae (Tateno et al., 2002; Wang and Zhang 2010; Wang et al., 2009, 2008). Although several immune factors have been identified in eggs of insect and mollusc (Fernandez-Sousa et al., 2008; Hathaway et al., 2010; Marchini et al., 1997), and maternal antibacterial activity has also been observed in several invertebrates (Benkendorff et al., 2001; Esteves et al., 2009; Kamiya et al., 1984), the maternal immune components and its transmission mechanism are still far from well understood.

The physiological condition of parents has a profound effect on their offspring fitness by providing non-genetic factors, such as hormones and nutrients (Groothuis et al., 2005; Vijendravarma et al., 2010). Moreover, the maternal transferred immunity from mother with immune experience has positive impact on the offspring immunity or disease resistance, which is termed as trans-generational immune priming (TGIP) (Sadd et al., 2005). In vertebrate, trans-generational effect of immunity is well known as the maternal transfer of antibody that provides the progeny an early protection before the maturation of its own immune system (Grindstaff et al., 2003; Hanif, 2004). While in invertebrate, it has been first demonstrated in shrimp *Penaeus monodon* that offspring derived from glucan-injected mothers have significantly higher survival rates than the control group against WSSV infection (Huang and Song 1999). Recently, it has been found the eggs from immune-challenged bumblebee *Bombus terrestris* L. possessed increased internal antibacterial activity (Sadd et al., 2005; Sadd and Schmid-Hempel, 2007), while the parental beetle *Tribolium castaneum* priming with heat-killed bacteria significantly improved the survival of their offspring after challenge with live bacteria (Roth et al., 2009). Consequently, trans-generational immune priming has been considered as a beneficial survival strategy that infectious mother invests elevated immune competence to the offspring for improving their resistance to further pathogen encounter (Zanchi et al., 2012).

Chlamys farreri is one of important economic bivalve species cultured widely in the northern coast of China. However, the outbreak of diseases during early developmental stages of scallop has resulted in the high unpredictable mortality of larvae. As scallop eggs and embryos are released and developed independently in external seawater, they are facing with numerous pathogens capable of causing various diseases. There should be a sophisticated mechanism for the scallop eggs and embryos to protect themselves from pathogen infection. The aims of the present study were to examine if the scallop eggs possess the maternal derived immune competence, and whether the immune stimulation to mother scallop bestows their future offspring with increased immunity.

2. Materials and methods

2.1. Preparation of microbe

Escherichia coli TOP10F' and *Staphylococcus aureus* was cultured in Luria-Bertani (LB) broth at 37 °C, and *Vibrio anguillarum* and *Pichia pastoris* GS115 was cultured in 2216E or yeast extract-peptone-dextrose (YPD) broth at 28 °C, respectively. After growth to $OD_{600nm} = 0.4$, the microbe was harvested by centrifuging at 2000*g* for 5 min and then washed three times with sterile PBS. The *V. anguillarum* was re-suspended to 1×10^8 cells/ml and heat-killed used for the immune stimulation experiment of adult female scallop, while the *V. anguillarum* suspended in sterile seawater at 5×10^8 cells/L were used for the bacterial challenge experiment. Besides, the microbe was also suspended with sterile PBS used for the following agglutination and bactericidal activity assay. All of the microbe strains were acquired from the microbiology lab in Institute of Oceanology, Chinese Academy of Sciences.

2.2. Animal, immune stimulation and sample collection

Adult scallop *C. farreri* (120 females and 20 males) were collected from the Yixiang scallop hatchery in Shandong Province, China in April, 2012, and reared separately in filtered seawater. The temperature of seawater was raised from 10 °C to 18 °C within 30 days to induce the gonad maturation.

One hundred microliter of heat-killed *V. anguillarum* was injected intramuscularly into the sexually-mature female scallops as the maternal immune stimulation group, while scallops in the control group received an injection of 100 μl sterile PBS. After injection, the scallops were reared at 18 °C for another 7 days.

The spawning induction and larval rearing was carried out according to our previous report (Yue et al., 2013). Briefly, female scallops in the maternal immune stimulation and control groups were induced to spawn by thermal shock method, and then the eggs were fertilized with the same sperm. After fertilization, the developing embryos were cultured separately in filtered and aerated seawater at 20 °C. The embryo or larvae from the two groups at different stages were identified microscopically and the samples were collected as eggs, 4-cell embryos, blastula (11 h post-fertilization, hpf), gastrula (18 hpf), trochophore (22 hpf) and D-shaped larvae (2 days post-fertilization, dpf). For each stage of the two groups, some samples were stored in liquid nitrogen directly for protein extraction, and some were stored after adding 1 ml TRIzol reagent (Invitrogen, Carlsbad, CA) for RNA extraction.

2.3. Protein extraction

Eggs, embryos and larvae were thawed in ice and homogenized with ice-cold PBS, then centrifuged at 5000*g* at 4 °C for 30 min. The supernatants were collected, quantified according to Enhanced BCA Protein assay kit (Beyotime Institute of Biotechnology, Jiangsu, China), and adjusted to the same concentration for the following assays.

2.4. Agglutination assay

The agglutination assay was performed according to previous report (Yu et al., 1999). Briefly, the fluorescein isothiocyanate (FITC)-labeled Gram-negative bacteria *E. coli* TOP10F', *V. anguillarum*, Gram-positive bacteria *S. aureus* and fungi *P. pastoris* GS115 were suspended in PBS at 2.5×10^9 cells/ml. An aliquot of 20 μl microbe suspension was added to 100 μl protein extracts of scallop eggs from control group, or to 100 μl PBS as negative control. The mixtures were incubated at 18 °C for 2 h in the dark and the cells were then observed under fluorescence microscopy.

2.5. Bactericidal activity assay

Bactericidal effect of scallop egg protein extracts was examined using scanning electron microscopy according to previous report (Wang and Zhang 2010). Briefly, an aliquot of 20 μl microbe