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The effect of vaccinating turbot broodstocks on the maternal immunity transfer to offspring immunity

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ABSTRACT

The study was designed to explore the effect of vaccinating turbot broodstocks on the immunity of offsprings. The expression of IgM, C3, TGF-β1, IL-1β, transferrin, lysozyme and their concentrations were detected respectively with quantitative real-time PCR and Elisa kits in offsprings at different stages from both naive and vaccinated brood stocks. The survival rate of offsprings in pathogen challenge experiment and their pathogen inhibition rate were also detected. The results shown that the maternal transfer of genes and proteins both existed in turbot. In the expression level, from eggs to 5 dpf (days past fertilization), the immune related genes expressed far more in vaccinated broodstocks offsprings, after hatching, the phenomenon disappeared. In protein level, transferrrin, C3 and IL-1β kept a stable level in concentration from eggs to 25 dph (days past hatching), and increased sharply at 45 and 55 dph. While, IgM and lyzozyme had a much higher concentration at 5 dpf in the embryos from vaccinated broodstocks, and we concluded that vaccinating broodstocks enhanced the production or maternal transfer of IgM and lyzozyme. *Vibrio anguillarum* challenge experiment showed that the vaccinated offsprings of 4 dpf and 14 dph had a better anti-pathogen ability than the ones from naive brood stocks. Throughout the study, it was concluded that vaccinating broodstocks could strength the maternal transfer of immune factors in gene and protein level, and the gene expression level during hatching was also enhanced at the early stage of development.

1. Introduction

The aquaculture industry is recently facing a serious setback due to infectious diseases [1]. Due to the limited immune ability at the early stage [2] maximum mortalities in all the cultivated fish species quite often erupt at larval stages.

Vaccination and medical treatment are the main methods in treating with the disease in fishing industry. Vaccination will be not effective unless the immune system has developed [3]. The remaining drug in the body or environment will cause the second pollution under medical treatment. With no drug remaining and high efficiency, vaccinating broodstocks is becoming more and more important [4]. Vaccinating broodstocks means vaccinating the broodstocks in advance before spawning, and provides the offsprings with higher immune ability through enhancing the maternal immunity transfer to offspring which has been proved in birds and mammals [5,6], while the concrete mechanism is not clear.

Maternal immunity refers to the immune factors transferred from mother to offspring. The animals at early stage of ontogeny usually have poor or limited immune ability, this makes maternal immunity the main protection to resist the attack of pathogenic microorganism.

A series of immune factor have been found in maternal course of many fish [7] and *Dicentrarchus labrax* [8,9]. Several kinds of innate immune factors have also been found having maternal transferring phenomenon in fish, for example, C3 has also been studied in spotted wolfish [10], Carp [11], Atlantic salmon [12] and zebrafish [13,14]; lectins were also found in steelhead trout [15] and *Katsuwonus pelamis* [16]. Lysozymes were detected in zebrafish [17] and cathelicidin in Atlantic cod [18].

Ontogeny of the lymphoid organs in turbot has been studied by a light and electronmicroscope study [19,20]. Several immune-related genes including specific immune gene IgM, and non-specific ones like lysozyme, transferrin, interleukin-1β (IL-1β) were also detected in kidney, spleen and gill with RT-PCR [21]. However, the concrete protection phases during the development of offspring and the tangible protection mechanism of maternal immunity is still little known.

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This study was set to explore the effect of vaccinating broodstocks on the maternal immunity transfer to the offsprings. Four experiments were designed to explore this: (1) Real-time PCR was taken to detect the expression of IgM, C3, TGF-β1, IL-1β, Transferrin, lysozyme; (2) the concentration of the above 6 kinds of proteins were detected with Bradford method; (3) the whole anti-*Vibrio anguillarum* ability of embryos homogenate were detected through co incubating with *V. anguillarum* on 2216E medium; (4) the survival rate of embryos 4 days after fertilization were detected when challenged with *V. anguillarum*

2. Materials and methods

2.1. Broodstocks rearing and vaccinating

The turbots were supplied with Tong Yong Fishing Industry. Mature turbots weighing 1.35 ± 0.095 kg were allocated at a ratio of 1 male: 3 female in a pond as broodstocks. The broodstocks were divided into two groups: (1) naive brooders – all female fish which were not subjected to any treatment; (2) vaccinated brooders (test group) – all female fish were similarly injected twice with 0.1 ml containing 1×10^7 cfu/ml live *V. anguillarum* at the interval of two weeks by intraperitoneal injection. The last injection was performed 1 month before spawning. The fish were kept in well-aerated water at 11–12 °C, and fed with frozen fish.

2.2. Sampling of gametes of embryos

Gametes were carefully stripped from the gonad end to the cloacal aperture by hand by a professional stuff from the industry. Gametes were collected separately and subsequently mixed gently in a dish for artificial fertilization, several ml of sea water were added to the dish to activate the sperm. Then each dish was moved gently in circles for about 5 s. About 5 min later, the dishes were filled up and left undisturbed for at least 40 min for egg hardening. The mean water temperature during the fertilisation process was 10–11 °C. After egg hardening, the freshly fertilised eggs which suspended in the dish were transported to a net cage, and discarded the eggs which failed to fertilize. The net cage were located in the pool with the temperature was maintained at 10–11 °C. 24 h later, removed and discarded the fertilized eggs on the bottom of the net cage with siphon principle once a day.

2.3. Pathogens-challenge experiment

2.3.1. Preparation of the pathogen solution

The opportunistic pathogen *V. anguillarum* was cultivated on 2216E medium (containing 20 g agar, 5 g tryptone, 1 g yeast extract powder in 1 L sea water). After sterilization and cooling, each 20 ml of the medium was poured into the sterilized culture dish. Inoculated the *V. anguillarum* into the 2216E medium and cultivate at 28 °C for 24 h. Collected the *V. anguillarum* on the 2216E medium carefully with seawater into a sterilized beaker and mixed gently. Diluted the pathogen solution with seawater and determined its OD at 550 nm with a microplate reader. The pathogen solution was diluted to 1.0×10^8 cfu/ml with the formula $y = 8.712x - 0.596$ (x: OD; y: concentration, cfu/ml).

2.3.2. The survival rate of embryos assay

Transferred the embryos which had fertilized for 4 days from net cage to breakers. The breakers were set into 4 groups, (1) embryos from naive brooders with no pathogen solution injected; (2) embryos from naive brooders with 5 ml 1.0×10^8 cfu/ml pathogen solution injected, which had been prepared in 2.3.1; (3) embryos from vaccinated brooders with no pathogen solution injected; (4) embryos from vaccinated brooders with 5 ml 1.0×10^8 cfu/ml pathogen solution injected. Each group included 3 breakers and each breaker contained about 0.4–0.5 g embryos, and were filled with 2 L sea water. Ventilated the system with air bump and maintained the temperature ranging from 13 to 14 °C for 24 h, then weighed the embryos which failed to hatch and got the survival rate.

2.3.3. The survival rate of larvaes analysis

Twenty tails of larvaes aged 14 dph from the above two kinds of broodstocks were challenged with the pathogen solution which had been prepared in 2.3.2 for 24 h, and then recorded the number of survival larvaes to get the survival rate. The experimental system was same with 2.3.1.

2.4. Pathogen inhibition rate analysis

About 100 mg embryos of 1, 5 dpf(days after fertilization) and 5, 15, 25, 40, 55 dph (days after hatching)days after fertilization and eggs from both naive and vaccinated brooders were homogenized and centrifuged at 8000 rpm for 15 min, the supernatant was removed to sterile eppendorf tubes. *V. anguillarum* was cultured on 2216E medium for 24 h at 28 °C. Collected the *V. anguillarum* carefully with seawater into a sterilized beaker and mixed gentally. Detected the OD of *V. anguillarum* under 550 nm 100 μL supernatant of homogenate and 100 μL *V. anguillarum* solution were mixed gently and were separated uniformly on the 2216E medium under 28 °C for 24 h. The mediums were set to 5 groups. (1) 100 μL naive embryos homogenate supernatant +100 μL *V. anguillarum* solution. (2) 200 μL naive embryos homogenate supernatant. (3) 100 μL vaccinated embryos homogenate supernatant +100 μL *V. anguillarum* solution. (4) 200 μL vaccinated embryos homogenate supernatant. (5) 200 μL *V. anguillarum* solution. After 24 h, washed the medium with 4 ml sterile water and collected the mixture on the 2216E medium in to a sterile breaker, then mix gently and detected the OD at 550 nm.

2.5. RNA isolation and quantitative real-time PCR

Total RNA of whole fish aged 1, 2, 3, 4, 5 dpf (days after fertilization) and 5, 15, 25, 40, 55 dph (days after hatching) and eggs from the naive and vaccinated broodstocks were isolated in triplicate for each age group using TRIzole Reagent (Life Technologies) at a concentration of not more than 100 mg tissue/ml Trizol reagent as described in the manufacturer's protocol. The RNA concentrations were determined by OD260 measurements and 0.5 μg of total RNA was treated with Takara PrimeScript TM RT regent kit with gDNA Eraser (Takara Perfect RealTime Code: RR047A).

The real-time PCR was performed using Takara SYBR Premix Ex TaqTM^{II} (Takara RR820A) on Light Cycler System in a volume of 20 μl with final concentration of 1 × SYBR Premix Ex TaqTM^{II}, 0.4 μM of each primer and 2 μl of first strand cDNA mixture, and 6.4 μl dH_2O. The primers and conditions used for both the conventional PCR and quantitative real-time PCR are listed in Table 1. Two-step method was taken in this experiment: stage (1) initial denaturation 95 °C 30 s 1cycle. Stage (2) PCR 95 °C 5 s. 55–60 °C 50 s 40 cycles. The PCR products quantitative real-time PCR were sequenced by Life Technology and blased by the BLAST function of NCBI (National Center for Biotechnology) online. The results were all specific no amplification was observed in negative controls (water blanks and total RNA without reverse transcription). The formula of $2^{-\Delta\Delta t}$ was used to calculate the relative expression of immune relative genes.