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Molecular cloning, genomic structure, polymorphism and expression analysis of major histocompatibility complex class II B gene of Nile tilapia (*Oreochromis niloticus*)

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ABSTRACT

Major histocompatibility complex (MHC) class II molecules play important roles in the immune system of vertebrates. In this study, full-length MHC II B cDNA was obtained from Nile tilapia (*Oreochromis niloticus*) by rapid amplification of cDNA ends polymerase chain reaction (RACE-PCR). In order to study the function of the MHC II B gene in Nile tilapia, the genomic structure, three-dimensional model, molecular polymorphism, tissue distribution, and immune response of the MHC II B gene to bacterial challenge were analyzed. Six exons and five introns were identified in the Nile tilapia MHC II B gene, which is different from the general five-exon–four-intron structure of other teleosts. Interestingly, the β-2 domain of Nile tilapia MHC II B was encoded by two exons. Sequence comparison showed that the deduced amino acid sequence had 26.7–69.6% identity with those of other species. Ten alleles were observed among five healthy individuals. However, a putative N-linked glycosylation site located at theβ-1 domain was observed in all the sequences except in Orni-DAB*0301-0501. Five different alleles observed in a single individual may imply the existence of at least three MHC II B loci. Moreover, d_N was significantly greater than d_S at protein-binding region (PBR) positions ($d_N/d_S = 3.073$, $P = 0.016$) in Nile tilapia MHC II B, which may provide evidence of strong positive selection at β-1 PBR positions among Nile tilapia sequences. Semi-quantitative reverse transcriptase PCR demonstrated that the Nile tilapia MHC II B mRNA was expressed in all tested tissues. Significant changes were observed in the liver, gill and kidney after bacteria challenge, while no obvious change was found in the intestine and spleen.

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1. Introduction

The major histocompatibility complex (MHC) is a large genomic region found in most vertebrates that encodes MHC molecules, which play important roles in the immune system and autoimmunity. MHC genes fall into two general classes, class I and class II, which differ in structure, peptide binding specificity, and the subset of T cells they activate (Rothbard and Gefter, 1991; Welsh, 1987). MHC class I is found on all nucleated cells, presents peptides to cytotoxic T cells and plays an important role in the primary immune defense system. The MHC class II molecules, consisting of one alpha and one beta chain, are found in certain immune cells such as macrophages, B cells, monocytes, and dendritic cells and have direct functional relevance to immune responses. As they are important to respond to infectious disease and vaccines, MHC genes have been intensively studied in all major vertebrates, including chondrichthyes (Bartl and Weissman, 1994), teleosts (Ono et al., 1992; Stet et al., 1998), amphibians (Liu et al., 2002), reptiles (Grossberger and Parham, 1992), birds (Burri et al., 2008), and mammals (Gao et al., 2009). Unlike mammals and other vertebrates, MHC class I and class II genes have been found to reside on different linkage groups in teleosts (Sato et al., 2000; Stet et al., 2003).

MHC class II genes have vital roles in the complex immunological dialogue that must occur between T cells and other cells of the body, and are good candidates for disease resistance study (Grimholt et al., 2003). Since the first identification in zebrafish (Ono et al., 1992), the MHC class II B gene has been isolated in numerous fish species, such as Atlantic salmon (Hordvik et al., 1993), rainbow trout (Glamann, 1995), common carp (Van Erp et al., 1996), channel catfish (Godwin et al., 1997), Japanese flounder (Srisapoome et al., 2004), red sea bream (Chen et al., 2006), turbot (Zhang and Chen, 2006), sea bass (Buonocore et al., 2007), half-smooth tongue sole (Xu et al., 2009), large yellow croaker (Yu et al., 2009), spotted halibut (Li et al., 2011), and Chinese longsnout catfish (Shen et al., 2011). There are many other reports on allelic polymorphism and expression of the MHC

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class II B gene in various tissues (Langefors et al., 2001; Rakus et al., 2009; Zhang et al., 2006). However, reports about the response of MHC genes in tissues infected with pathogenic bacteria in teleosts are relatively rare.

The Nile tilapia (*Oreochromis niloticus*), a representative species of the tilapiine branch of the Cichlidae, is considered to be one of the most important aquaculture species and widely cultured throughout the world. It was first introduced into China by the Yangtze River Fisheries Research Institute in 1978, and has become an economically important fish because of its rapid growth and good taste. However, diseases have occurred frequently and losses due to viral and bacterial diseases limit the profitability and the development of Nile tilapia. The application of antibiotics has solved the bacterial infection to some extent, but it also raised other problems, such as antibiotic residues in fish, environmental pollution, and antibiotic resistance development. Therefore, it is essential to improve the disease resistance through application of molecular techniques and selective breeding of resistant strains of Nile tilapia.

Breeding of cichlids with disease resistance currently is being conducted in China. However, molecular marker-assisted selective breeding is still lacking. In this study, molecular cloning, 3-D modeling and polymorphism analysis of the MHC II B gene from the Chinese population of Nile tilapia were performed, and then basal tissue expression and expression modulation upon stimulation with *Aeromonas hydrophila* were examined. These results will be helpful for advancing the understanding of vertebrate immunity and the cultivation of resistant varieties.

2. Materials and methods

2.1. Fish and sampling

One-year old Nile tilapia were provided by an experimental aquaculture farm (Taian, China) and then raised in aerated tanks. Twelve tissues (brain, fin, spleen, stomach, intestine, kidney, bladder, liver, gill, muscle, gonad, and heart) and blood samples were collected and kept at −80 °C until use.

2.2. Challenge of Nile tilapia

The fish were acclimated in the laboratory at 20 ± 2 °C for two weeks in aerated tanks before processing. The *A. hydrophila* B005 strain (isolated by the aquaculture laboratory, Shandong Agriculture University) was cultured at 28 °C to mid-logarithmic growth in LB medium. The culture was centrifuged and resuspended to approximately 1×10^8 CFU ml^{-1} in phosphate-buffered saline. In order to determine the median lethal concentration, a pre-challenge experiment was taken on fish of the same size as the test fish. Fish were anesthetized by immersion in MS222 and injected intraperitoneally with 0.5 ml bacterial suspension. As a control, fish injected with the same volume of PBS were maintained in separate tanks. Three individuals were randomly sampled at 5, 12, 24, 48 and 72 h post-infection, respectively, and five tissues (liver, spleen, kidney, intestine, and gill) were removed and kept at −80 °C until use.

2.3. DNA isolation and cDNA synthesis

Genomic DNA was extracted from blood samples of Nile tilapia with a DNA isolation kit (Tinagen, Beijing, China) following the manufacturer's instructions. Total RNA was extracted from various tissues using Trizol reagent (Invitrogen, Carlsbad, CA, USA), and treated by the RNase-free DNase I (Takara, Dalian, China) to remove genomic DNA contamination. First-strand cDNA was synthesized from total RNA with the First-Strand cDNA Synthesis Kit (Fermentas, Ontario, Canada) according to the manufacturer's protocol.

2.4. Primer design and PCR amplification

Two degenerate primers, MHCIIB-F and MHCIIB-R (Table 1), were designed according to the conserved sequences of the MHC II B gene from cichlids (AAA48547 and AAB27553), seabream (AAP20186), and turbot (AAY18805). To isolate full-length cDNA of Nile tilapia MHC II B gene, four specific primers (GSP5, NGSP5, GSP3 and NGSP3, Table 1) were designed according to the partial cDNA sequence obtained. The primers MHCIIB-ORF-F and MHCIIB-ORF-R were designed to determine the genomic organization and to analyze molecular polymorphism. The primer pairs MHCIIB-ex-F and MHCIIB-ex-R were used to analyze the expression of MHC II B gene.

PCR amplification was performed in a total volume of 25 μl containing 2.5 μl of 10× PCR buffer, 0.2 mmol of dNTP, 0.2 mmol of each primer, 1 unit of Pfu DNA polymerase (Fermentas) and 1 μl of cDNA/DNA template. The conditions of the PCR were as follow: predenaturation at 94 °C for 5 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing temperature for 30 s, extension at 72 °C for 1 min; and a final extension for 10 min at 72 °C.

2.5. Full-length cDNA and genomic sequences of Nile tilapia MHC II B

To isolate full-length cDNA of the Nile tilapia MHC II B gene, 5′-RACE and 3′-RACE were carried out. For 5′-RACE-PCR, cDNA was transcribed from total RNA using the oligo-dT primer and tailed with poly(C) at the 5′-end with terminal deoxynucleotidyl transferase (TdT, Fermentas). For 3′-RACE-PCR, cDNA was transcribed using an oligo-dT primer. Touchdown PCR was used to improve the specificity of PCR amplification. The conditions were as follow: 94 °C 5 min; (94 °C 50 s, 69 °C 50 s, and 72 °C 1 min) for 5 cycles; (94 °C 50 s, 67 °C 50 s, and 72 °C 1 min) for 5 cycles; (94 °C 50 s, 65 °C 50 s, and 72 °C 1 min) for 5 cycles; (94 °C 50 s, 63 °C 50 s, and 72 °C 1 min) for 20 cycles; and 72°C 10 min.

To identify the genomic organization of the Nile tilapia MHC II B gene, primers MHCIIB-ORF-F and MHCIIB-ORF-R were used to obtain the sequence of the corresponding genomic DNA. The PCR amplification was run as follows: initial incubation at 94 °C for 5 min, followed by 38 cycles of 94 °C for 45 s, 62 °C for 45 s, and 72 °C for 3 min, with a final extension of 10 min at 72 °C.

PCR products were resolved by electrophoresis on 1% agarose gels, and the fragments of interest were excised, and then purified using the OMEGA Gel Extraction Kit (OMEGA, Boston, USA). The purified fragments

Table 1
Primers used in this study.

Name	Primer sequences (5′–3′)	Amplification target
MHCIIBF	CGTCTWYGACTTCTWYCCCA	cDNA fragment
MHCIIBR	CTYTCWGACTCRGGCATGGA	of MHC II B
MHCIIB-GSP3	CTGGCTGAGAGATGGACAGGAAG	For 3′-RACE
MHCIIB-NGSP3	GAGGAGATGGCAGATGGTGATTGG	
Oligo-dT-AP	AAGCAGTGGTATCAACGCAGAGTAC$(T)_{30}$VN	
AP	AAGCAGTGGTATCAACGCAGAGT	
MHCIIB-GSP5	CAGAGGTGACTTCCTGTCCATC	For 5′-RACE
MHCIIB-NGSP5	CAGTCCTGAGGCTCCGATGGCG	
AAP	GGCCACGCGTCGACTAGTACG(G)14	
AUAP	GGCCACGCGTCGACTAGTACG	
MHCIIB-ORF-F	ATGGCTTCATCCTTCCTCTG	Complete ORF
MHCIIB-ORF-R	GTTAGTGGGAACCAGGATAC	of MHC II B
MHCIIB-ex-F	AGGAGATGGCAGATGGTGATTGGTA	Expression of
MHCIIB-ex-R	AGGACCAGGAACAGCCTCAGTTA	MHC II B
β-actin-F	CCATTGAGCACGGTATTG	Expression of
β-actin-R	CTGTGGTGGTGAAGGAGTAG	β-actin

Notes: degeneracy bases, Y = C/T, W = A/T, and R = A/G.

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