Kasetsart J. (Nat. Sci.) 43 : 348 - 357 (2009)

Polymorphism of Major Histocompatibility Complex Class I alpha cDNA and Resistance against Streptococcosis of Six Strains of Nile Tilapia (*Oreochromis niloticus* Linnaeus)

Sita Poonsawat[1],[2] Nontawith Areechon[3]*, Prapansak Srisapoome[3], Masashi Maita[3] and Makoto Endo[4]

ABSTRACT

The partial cDNA encoding major histocompatibility complex class I alpha (MHC class Iα) gene was obtained from a cDNA library of Nile tilapia (*Oreochromis niloticus*) spleen. 5′ RACE was then conducted to get the full-length of MHC class Iα cDNA. Sequence analysis revealed that this cDNA consisted of 2,131 bp including a 1,071 bp open reading frame with 5′ untranslated and 3′ untranslated regions (UTR) of 128 bp and 932 bp respectively. The highly polymorphic characteristic of the MHC class Iα gene in six different strains of Nile tilapia (ST1-ST6) was evident. At least 15 different alleles were observed when the specific primers were used to amplify nucleotide sequences of the α1 and α2 domains. Additionally, resistance against streptococcosis of these strains was compared by intraperitoneal injection with viable *Streptococcus agalactiae*. The results revealed that the ST1 strain showed a significantly higher survival rate than the other five strains (ST2-ST6) with 100±0.0% survival after seven days of injection ($P<0.05$), while the others had 86.67±5.77, 73.33±5.77, 70.00±0.00, 60.00±0.00 and 43.43±5.77% survival, respectively. Some hematological and immunological parameters were also compared. The findings provide useful information for further investigation to select disease-resistant strains of Nile tilapia using MHC class Iα as the molecular marker.

Key words: MHC class Iα, cDNA, RACE, *Streptococcus agalactiae*, *Oreochromis niloticus*

INTRODUCTION

Classical major histocompatibility complex (MHC) class I (or class Iα) molecules are involved in the presentation of peptide antigens at the cell surface which are recognized by cytotoxic T cell receptors (TCR) of the CD8 T lymphocyte (Townsend and Bodmer, 1989; Germain, 1994). MHC class I molecules have been found in all classes of gnathostomata (jawed vertebrates) but not in more primitive species (Hashimoto *et al*., 1999; Flajnik and Kasahara, 2001). MHC class I molecules consist of two noncovalently linked, polypeptide chains: an MHC-encoded α chain (or heavy chain) of 44 to 47 kD and a non-MHC-encoded, 12-kD subunit called α_2-microglobulin (Abbas, 2005). Each heavy chain is encoded by a polymorphic gene

1 Center for Agricultural Biotechnology, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.
2 Center for Agricultural Biotechnology (AG-BIO/PERDO-CHE)
3 Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok 10900, Thailand.
4 Graduate School of Fisheries Sciences and Technology, Tokyo University of Marine Science and Technology, Tokyo, Japan.
* Corresponding author, e-mail: ffisnwa@ku.ac.th

Received date : 03/11/08 Accepted date : 04/02/09

containing a leader peptide, three extracellular domains (α1, α2 and α3) and transmembrane and cytoplasmic regions (Cuesta *et al*., 2007).

One characteristic of the MHC class I molecule is extensive polymorphism. The polymorphism results in the presentation of different sets of antigenic peptides by each allomorph (protein expressed by an allele), and may result in differences in disease resistance (Xia *et al*., 2002). In aquaculture, there is extensive interest in the characterization of unique strains within different fish species (Chevassus and Dorson, 1990). Characterization of strains with respect to their histories and relationships will facilitate strain improvement. One advantage of the MHC class I molecule, as a genetic trait marker, is that allelic differences may be related to differences in disease resistance. Genes of MHC are obvious candidates, as they have an important role in both the innate and adaptive immune response. Moreover, specific MHC alleles have been well documented to correlate with disease resistance in chicken (Briles *et al*., 1983), mice (Medina and North, 1998), sheep (Paterson *et al*., 1998) and salmonid species (Gjedrem *et al*., 1991; Grimholt *et al*., 1994; Lohm *et al*., 2002; Wynne *et al*., 2007).

Nile tilapia (*Oreochromis niloticus*) is a tilapiine fish that is widely distributed across Africa and the Middle East (Trewavas, 1983). There has been a rapid increase in aquaculture production in recent years, much of which has come from increasing the intensity of existing systems. High stocking densities can make fish more susceptible to stress and disease, which in turn may affect or cause severe losses of stock (Iwana and Nakanishi, 1996). The use of antibiotics has partially solved the problem, but has raised concerns regarding antibiotic residues, environmental pollution and antibiotic resistance development. There is extensive interest in enhancing the resistance of the cultured fish to diseases. Therefore, it is necessary to assist selective breeding of a resistant strain via molecular techniques.

This study was conducted to determine the polymorphism of MHC class Iα molecules in the α1 and α2 domains, using six strains of Nile tilapia in association with resistance against pathogenic *Streptococcus agalactiae*. The results will be used as a guideline for the development of disease-resistant strains of Nile tilapia.

MATERIALS AND METHODS

Genotype of MHC class Iα cDNA of 6 strains of Nile tilapia

Six strains of Nile tilapia were selected from commercial and government sources in Thailand. Fish were held in a flow-through 2.0 m^3 concrete tank for 1 month prior to the experiment.

Rapid Amplification of cDNA Ends (RACE), Cloning and Sequencing of Full-length MHC class Iα cDNA

Total RNA was extracted from the spleen of each tilapia strain using TRIzol reagent (Molecular Research Center, Cincinnati, Ohio, USA) according to the manufacturer's instructions. The extracted RNA was further studied using both qualitative and quantitative techniques by formaldehyde-agarose gel electrophoresis and spectrophotometer. The RACE technique was utilized to clone full-length cDNAs in the 5′ end using a BD Smart™ RACE cDNA Amplification Kit (BD Biosciences ClonTech, Qume Drive, San Jose, USA) according to the manufacturer's instructions. Approximately 1 μg of mRNA was used for first-strand cDNA synthesis. For the 5′ -RACE-ready cDNA, 1 μg of mRNA, 1 μl of BD Smart II A oligo (5′-AAGCAGTGGTATCAA CGCAGAGTACGCGGG-3′) and 1 μl of 5′ -CDS primer (5′ -$(T)_{25}$VN-32 , N = A, C, G or T; V = A, G or C) were used to generate the nucleotide sequences at the 5′ end. After first-strand cDNA

synthesis, the 5′-RACE-ready cDNAs were used as templates to generate the nucleotide sequences at the 5′. For RACE PCR reactions, the 50-µl PCR reaction mixture consisted of : 2.5 µl of 5′-RACE-ready cDNA; 5 µl of 10x BD Advantage 2 PCR buffer; 1 µl of 10mM dNTP Mix (each at 2.5 mM); 1 µl of 50x BD Advantage 2 Polymerase Mix; 5 µl of 10x Universal Primer Mix (UPM: Long (0.4 µM), 5′ CTAATACGACTCACTATAGGGC AAGCAGTGGTATCAACGCAGAGT-3′ and Short (2 µM), 5′-CTAATACGACTCACTATA GGGC-3′ and 1 µl of 10 µM gene-specific primer (GSP). 5′-ACCATCTCGGCTCTGTTAGGATA G-3′ with design from part of the nucleotide of cDNA SP216 clone (GenBank accession number FF28081955139855). The 5′-RACE was carried out using reverse primer SP216RACE_N and Universal Primer Mix (UPM) under the following conditions for 25 cycles: 94°C for 3 min, 94°C for 30 s, 60°C for 30 s and 72°C for 3 min.

The RACE products were gel-purified using Wizard® SV Gel and a PCR Clean-Up System Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. The DNA fragment was ligated into vectors using pGEM®-T Easy Vector (Promega, USA). The positive clones were selected for extraction of the plasmid using an Aurum™ Plasmid Mini Kit (Bio-Rad, Hercules, USA) according to the manufacturer's instructions. Three µl of extracted plasmid DNA were sequenced using a Thermo Sequence Fluorescent Labeled Primer Cycle Sequencing Kit (Amersham Pharmacia Biotech, Uppsala, Sweden). The M13 forward and reverse primers were used for sequencing the 5′ and 3′ ends of the cDNA. The cDNA sequences were searched for homology using the BLAST programs (NCBI, http:// www.ncbi.nlm.nih.gov) (Altschul *et al*., 1997).

Sequence alignment and phylogenetic analysis

The 5′ and 3′ UTR sequences of the full-length of MHC class Iα cDNA were removed using Genetyx version 7.0. The Clustal W program (Thompson *et al*., 1994) was used for multiple sequence alignment of the amino acids from MHC class Iα gene of: gilthead seabream type1; *Sparus aurata* (DQ211540); aulonocara, *Aulonocara jacobfreibergi* (AF038550); bastard halibut type1, *Paralichthys olivaceus* (AB126917); Nile tilapia, *Oreochromis niloticus* (FJ457118); Atlantic salmon, *Salmo salar* (AF504019); rainbow trout, *Oncorhynchus mykiss* (AF287485); Atlantic cod, *Gadus morhua* (AJ132511); common carp, *Cyprinus carpio* (BAA33884); and humans, *Homo sapiens* (NM002116), which were used to construct the MHC class Iα phylogenetic tree by the neighbor-joining method (Saitou and Nei 1987) and bootstrapped for 1000 replicates using MEGA version 3.1 (Kumar *et al*., 2001).

Sequence analysis of each tilapia strain

Total RNA was extracted from the spleen of six different strains with five fish per strain and three clones per fish using TRIzol reagent (Molecular Research Center), according to the manufacturer's instructions. Approximately 1 µg of total RNA was used for first-strand cDNA synthesis using a RevertAid™ First Strand cDNA Synthesis Kit (Fermentas) according to the manufacturer's instructions.

PCR was performed using cDNA as a template to amplify the nucleotide sequence of the α1 and α2 domains with the primer designed from the full length of the MHC class Iα gene (GenBank accession number FJ457118). The PCR reaction mixture contained 1x *Taq* buffer, 0.75 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 µM of each forward (MHFP) 5′-CCAGAGTTTCTGACTGTGGGG AT-3′ and reverse primer (MHRP) 5′-CTCTGTTA GGATAGAAACCTGTAG-3′, 1 U *Taq* DNA polymerase (Fermentas) and 0.5 µl of first-strand cDNA in a total volume of 25 µl. The PCR was carried out for 25 cycles under the following conditions: 94°C for 30 sec, 58.8°C for 30 sec and 72°C for 1 min. Expression of β-actin was used as

an internal control. Ten µl of PCR products were subjected to electrophoresis through a 1.5% agarose gel in 1x TBE buffer at 100 volts with 100 bp DNA marker. Multiple sequence alignment of the amino acid from the six strains was carried out by the Clustal W program (Thompson *et al*., 1994).

Disease resistance of 6 strains of Nile tilapia

A total of 600 fish of six strains of Nile tilapia (100 fish per strain, mean weight of 10 g) were acclimated in a flow-through 2.0 m^3 concrete tank for 15 days prior to the experiment. Three replicates of 10 fish from each strain were used in a challenge test, which was conducted in a glass aquarium.

Pathogenic bacteria, *Streptococcus agalactiae* isolated from diseased tilapia were cultured at 35°C to mid-logarithmic growth on brain heart infusion agar (BHIA) and then resuspended after centrifugation to approximately 10^7 colony forming units (cfu) ml^{-1} in sterile 0.85% normal saline solution (NSS). Fish were injected intraperitoneally (i.p.) with 0.1 ml of the bacterial suspension. The same volume of sterile 0.85% NSS was injected into control fish. The fish were observed at 4 h intervals for behavioral changes and mortality. All dead fish were removed and bacterial isolation was confirmed for *S. agalactiae* using conventional methods and the API 20 STREP system (Biomerieux). Survival rates were statistically compared after seven days of challenge.

Hematological parameters and immune functions analysis

Hematological parameters

Blood samples (6 fish/strain) were collected from the caudal vein for the analysis of total erythrocyte count (cell ml^{-1}), haemoglobin content (g dl^{-1}) and haemotocrit (%) using a semi-automated microcell counter machine, model F-820.

Immune functions

Separation of leukocytes

Leukocytes were separated from the blood sample by density gradient centrifugation. One part of heparinized blood was diluted with three parts of RPMI-1640 medium and then 3.0 ml of diluted blood were carefully put into the 15 ml PE tubes containing 3.0 ml histopaque and centrifuged at 400 x g for exactly 30 min at 25°C in a swing rotor. After centrifugation, the opaque component was carefully obtained with a Pasteur pipette and transferred into a clean, conical, centrifuge tube, an equal amount of PBS added and then centrifuged (2-3 times) at 400 x g for 10 min. The number of leukocytes was counted by a haemacytometer.

Phagocytic assay (Modified from Puangkaew *et al*., 2004)

The adjusted leukocyte component (2×10^6 cells/ml) was dropped on a cover slip and allowed to attach by incubating for 2 hr at 25°C (a yeast suspension was prepared after 1 hr had passed), with the unattached cells being lightly washed three times with RPMI medium. Then 200 µl of yeast suspension (2×10^7 cells/ml) were added and incubated at 25°C for 1 hr. The samples were washed three times or more with RPMI medium to remove excess yeast and non-adherent cells, then stained with Diff-Quick staining dye and observed under a microscope. At least 300 cells were counted for the total number of both phagocytizing and non-phagocytizing cells. The percent phagocytosis (PP) was calculated from Equation 1:

$$PP = \frac{\text{Total number of phagocytizing cells}}{\text{Total number of counted cells}} \times 100$$

Statistical analysis

Survival rates after challenging by *S. agalactiae* and hematological and immunological parameters of the six strains of Nile tilapia were statistically compared by ANOVA and

Duncan's new multiple range test (*P=0.05*).

RESULTS

Genotype of MHC class Iα cDNA of each tilapia strain

Complete sequence of MHC class Iα cDNA

The complete cDNA sequence for MHC class Iα cDNA consisted of 2,131 bp which encoded 357 amino acid residues (Figure 1). To determine the regions of the three alpha domains (α1, α2, α3) and the transmembrane and cytoplasmic regions, the cDNA sequence of Nile tilapia was compared with that of gilthead seabream, *Sparus aurata*. The deduced amino acid sequence of Nile tilapia MHC class Iα (348 residue) included α1 domain (position 9-90), α2 domain (position 91-183), α3 domain (position 184-282), transmembrane (position 283-326) and cytoplasmic regions (position 327-357) (Figure 2).

Sequence alignment and phylogenetic analysis

The deduced amino acid of Nile tilapia MHC class I gene shared 55.24, 54.22, 53.21, 50.56, 49.86, 47.71, 47.51 and 30.59% identity with the MHC class I gene of bastard halibut type1, aulonocara, gilthead seabream type1, Atlantic salmon, Atlantic cod, rainbow trout, common carp and humans respectively (Figure 3).

Polymorphism of MHC class Ia

Nucleotide sequences of the α1 and α2 domains of each strain of *O. niloticus* showed a polymorphic domain consisting of 567-576 bp in length (Figure 4), which encoded for 189-192 amino acid residues. The polymorphism in the α1 and α2 domains of MHC class Iα cDNA was different in six strains of Nile tilapia and was evident in at least 15 different alleles (*Orni*a-Orni*o*) (Figure 5).

Disease resistance of six strains of Nile tilapia

The resistance of these strains against streptococcosis was investigated by intraperitoneal

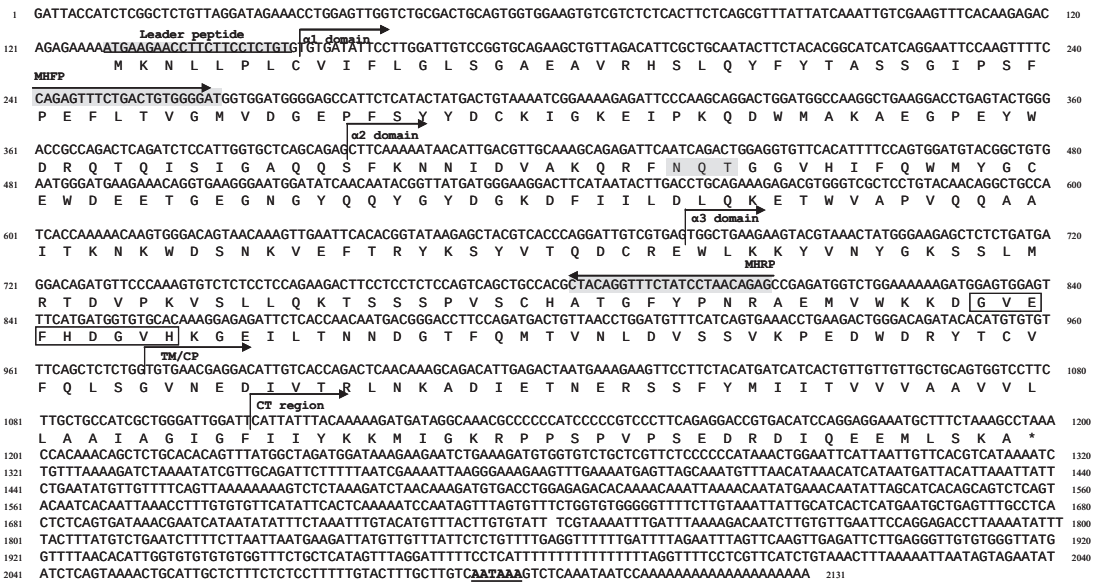

Figure 1 The complete cDNA and deduced amino acid sequences of the MHC Class Iα gene. The name and direction of the primer are indicated with arrows and potential N-linked glycosylation sites are highlighted. Boxes show the CD8 binding loop. Poly-adenylation and the leader peptide are underlined. (*) indicates a stop codon.

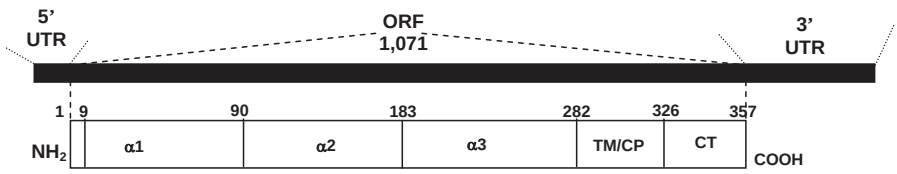

Figure 2 Schematic illustration of *O. niloticus* of MHC Class Iα cDNA (thick black line) and encoding sequence. Five major domains are represented with boxes and the figures indicate the number of amino acid residues. UTR = untranslated region; ORF = open reading frame; α1 = alpha-I; α2 = alpha-II; α3 = alpha-III; TM/CP= transmembrane/connecting peptide and CT = cytoplasmic domain.

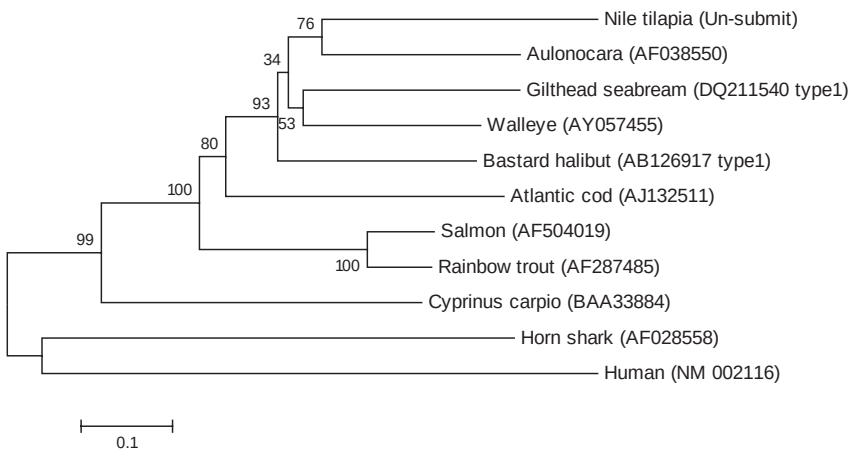

Figure 3 Phylogenetic tree based on the amino acid sequences of MHC Iα from fish and other organisms. The horn shark (AF028558) and humans (NM002116) were used as the out-group. The numbers indicate bootstrap values from 1000 replicates. The bottom scale refers to percentage divergence.

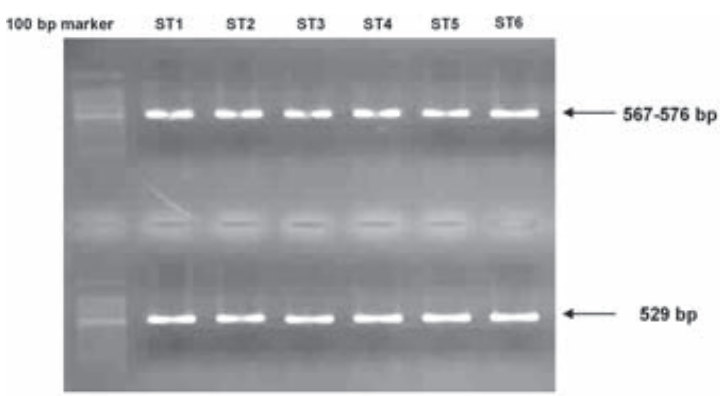

Figure 4 The upper band illustrates cDNA of MHC class Iα amplified nucleotide sequences of the α1 and α2 domains and the lower band illustrates β-actin (analysed on 1.5 % (w/v) agarose and stained with ethidium bromide).

```
                  *       20        *       40        *       60        *       80        *      100
Orni*Nilo : .........GEPFS...CKIG...........EG....D........A.....N..D.A.............W.......E....G.........K....L.. : 107
Orni*a    : ......................AE........NT.Q.........A.EQ.....V................F..Q.......E...D....M.........S.E. : 107
Orni*b    : ......................A.........NT.Q.......ANCW.........DTA.............V.................R.D........S... : 107
Orni*c    : ..........................ND.........N.D..RA...MS......A.G.....E......Q.........D..Y............S... : 107
Orni*d    : ..........................ND.........N.D..R......E.....A..............Q..S.............G.........G... : 107
Orni*e    : ....................M.M.......NG.........N.GNL.................A.........V...C...........K..D......K...AL.. : 107
Orni*f    : ..............G......A.M.........NT.Q...DS.......Q.H................A.....A..T..........K.K..DD.........KL.. : 107
Orni*g    : ..............E........L.S.....NG.......D.......TN......S..N..P..........L.V.......E.....T..R.............. : 107
Orni*h    : ......................K......F..NT.Q....EG...RK.Y.....V...T............TTMI..........D..T..RRD............ : 107
Orni*i    : .........E...VQ.....M.T.S.....N.N..E....T..GGF.SA..V..V....A............V....S.......N..................... : 107
Orni*j    : ....................G.D......F..NT.Q.....N...LR.R........DT..............N.......E........F..........S... : 107
Orni*k    : .........GEPFS....KIG............EG....D........A.....N..D.A.............W.......E....G.........R....L.. : 107
Orni*l    : ..............E......A.M.........NT.Q...DS.......Q.H.....................A..T..........K.K..DD.........KL.. : 107
Orni*m    : ....................M.M.......NG.........N.GNL.................A.........V...C...........K..D......K...AL.. : 107
Orni*n    : ...........Q..EH...I.G.AQV..E....Y.ADSDP.YSERNAQTYMGNQPSFKAN.EIAKGRFNQTGGVHI.QQMYGC.WDE.TGDVNG.RQF.Y.GAD.L. : 107
Orni*o    : .........GEPFS....KIG............EG....D........A.....N..D.A.............W.......E....G.........K....L.. : 107

                *       120         *       140         *       160         *       180         *
Orni*Nilo : .K..........A..........VEFTR..S....D.R.................D..K.........................--- : 189
Orni*a    : K.A....AK.......Q.L.R...LS..Q....I.E.........T.....................F..L..............--- : 189
Orni*b    : ........K.......T.......T.T.............G.....................................------ : 189
Orni*c    : K........H......Y...R..F.TR.A.....................................P..................--- : 189
Orni*d    : K..............Y......F.TR.A.....................................P..................--- : 189
Orni*e    : ..K.....TP......H...K..G....E.....................................P..................--- : 189
Orni*f    : ........TP..........N...L...K....F............K.R.................P..R..............--- : 189
Orni*g    : K.A.........FR..M.......VNTRDM..F..E.........K.........D..K..........S..............--- : 189
Orni*h    : K....T.TTP......H..EN......GR.........D.....................P..................--- : 189
Orni*i    : ........K.......H.L.......TSV...Y..........C............P............A..............--- : 189
Orni*j    : KD......K...E...R.......L.TND.........................R............SPVTCHATG.YPNR-- : 190
Orni*k    : .K..........A..........VEFTR..S....D.R...........N.....D..K.........................--- : 189
Orni*l    : ........TP..........N...L...K...F............K.R.....................P..R..............--- : 189
Orni*m    : ..K.....TP......H...K..G....E.........K.....................P..................--- : 189
Orni*n    : FDLKTETW.APVQQAVITK.KCNSNR.LITSDKNYFTQLCPEWLKK.VNYGRSSL.RTD.PK.SLLQK.SS.PVTCHATGFY... : 192
Orni*o    : .K....V.....A..........VEFTR..S....D.R.................D..K.........................--- : 189
```

Figure 5 The 15 alleles (*Orni*a-o*) from the six strains of Nile tilapia based on the α1 and α2 domains.

challenging with viable *Streptococcus agalactiae*. The results revealed that the ST1 strain showed a significantly higher survival rate than the other five strains with 100±0.0% survival after 7 days of injection ($P<0.05$), while the other strains had 86.67±5.77, 73.33±5.77, 70.00±0.00, 60.00±0.00 and 43.43±5.77% survival, respectively (Table 1).

Hematological parameters and immune functions of six strains of Nile tilapia

Some hematological parameters and immune functions were compared, with some showing a positive correlation with superior disease resistance of ST1 including total erythrocyte, haemoglobin content and percent phagocytosis (Table 1).

DISCUSSION

In a previous study, Grimholt *et al*. (2003) investigated Atlantic salmon (*Salmo salar*) infected with the anemia virus (ISAV) causing anemia in infected salmon and with *Aeromonas salmonicida* causing furunculosis. They found highly significant associations between resistance against both pathogens and MHC class I and class II polymorphism in Atlantic salmon. Evidence also suggested the possible use of the polymorphism of MHC alleles with disease resistance such as *Vibrio anguillarum* in Japanese flounder (*Paralichthys olivaceus*) (Zhang *et al*., 2006), *Aeromonas salmonicida* in brook charr (*Salvelinus fontinalis*) (Croisetiere *et al*., 2008) and infectious hematopoietic necrosis virus in Atlantic salmon (Miller *et al*., 2004).

Cnaani *et al*. (2004) found that two out of 42 markers utilized in genome-scan analysis for QTLs were polymorphic genes for MHC class I and transferrin, which were related to the immune system and were previously mapped in the tilapia genome (Cnaani *et al*., 2002, 2003). Some hypotheses were reported to explain the vast polymorphism of the MHC genes, such as heterozygous advantage, over-dominant selection and frequency dependent or balancing selection (Parham and Ohta, 1996). The pathogen-driven

Table 1 Hematological and immunological parameters and survival rate after challenging with *S. agalactiae* of six strains of Nile tilapia.

Parameter	ST1	ST2	ST3	ST4	ST5	ST6
Total erythrocyte count ($x10^9$ cell ml^{-1})	2.04 ± 0.05 ^{a}	1.75 ± 0.35 ^{bc}	1.54 ± 0.02 ^{c}	1.91 ± 0.11 ^{ab}	1.52 ± 0.07 ^{c}	1.28 ± 0.04 ^{d}
Total leukocyte count ($x10^7$ cell ml^{-1})	1.57 ± 0.31 ^{b}	2.70 ± 0.70 ^{a}	2.68 ± 0.15 ^{a}	2.10 ± 0.26 ^{ab}	1.53 ± 0.058 ^{b}	1.93 ± 0.42 ^{b}
Hemoglobin content (g dl^{-1})	11.08 ± 0.81 ^{a}	8.60 ± 0.38 ^{bc}	9.00 ± 0.70 ^{b}	10.65 ± 0.94 ^{a}	7.53 ± 0.81 ^{c}	9.23 ± 0.78 ^{b}
Hemotocrit (%)	15.4 ± 1.86 ^{ab}	8.65 ± 0.69 ^{c}	9.30 ± 0.74 ^{c}	18.53 ± 5.57 ^{a}	13.13 ± 4.36 ^{bc}	12.08 ± 2.66 ^{bc}
Percent phagocytosis (%)	51.67 ± 1.53 ^{a}	44.68 ± 2.52 ^{b}	45.60 ± 3.24 ^{b}	47.0 ± 2.65 ^{ab}	50.53 ± 1.46 ^{a}	36.17 ± 3.4 ^{c}
Survival rate after challenge with *S. agalactiae* (%)	100.00 ± 0.00 ^{a}	86.67 ± 5.77 ^{b}	73.33 ± 5.77 ^{c}	70.0 ± 0.00 ^{c}	60.0 ± 0.00 ^{d}	43.33 ± 5.77 ^{e}

selection was considered to favor genetic diversity of the MHC genes through both heterozygote advantage (over-dominance) and frequency-dependent selection (Grimholt *et al*., 2003).

The sequence analysis of six strains of Nile tilapia from this study revealed high polymorphism within the peptide-binding region (α1 and α2) of the MHC class Iα gene. Of the 15 different alleles, two alleles (*Orni*a and Orni*j*) were found in ST1 that showed the highest resistance to *S. agalactiae*. The number of alleles within a tilapia strain was diverse, ranging from one to five, which was similar to rainbow trout (*Oncorhynchus mykiss*) (Aoyagi *et al*., 2002) and tilapia (*Oreochromis niloticus*) (Sato *et al*., 2006). This study also showed a certain level of association between polymorphism of MHC class Iα and disease resistance and some immune functions in which the ST1 strain of Nile tilapia showed superior activity over other strains.

This study provides valid information for further investigation to select the *S. agalactiae* resistant strain of Nile tilapia using MHC class Iα (*Orni*a* and *Orni*j* alleles) as the molecular marker for the strain selection and breeding program.

CONCLUSIONS

Sequence analysis of cDNA encoding major histocompatibility complex class I alpha (MHC class Iα) of six strains of Nile tilapia (*Oreochromis niloticus*) indicated high polymorphism characteristics with at least 15 different alleles using a specific primer to amplify the nucleotide sequence of the α1 and α2 domains. A certain level of association between the polymorphism of MHC class Iα and disease resistance and some immune functions were also observed. The development of a molecular marker for the selection of certain strains and a future breeding program regarding disease resistance merit further investigation.

ACKNOWLEDGEMENT

This research is supported by a the Center for Agricultural Biotechnology, Postgraduate Education and Research Development Office, Commission on Higher Education, Ministry of Education.

LITERATURE CITED

Abbas, A.K. 2005. **Cellular and Molecular Immunology**. Philadelphia, Pa.: Elsevier Saunders. 564 p.

Altschul, S.F., T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller and D.J. Lipman. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. **Nucleic Acids Res.** 25: 3389–3402.

Aoyagi, K., J.M. Dijkstra, C. Xia, I. Denda, M. Ototake, K. Hashimoto and T. Nakanishi. 2002. Classical MHC class I genes composed of highly divergent sequence lineages share a single locus in rainbow trout (*Oncorhynchus mykiss*). **J. Immunol.** 168: 260–273.

Briles, W.E., R.W. Briles, R.E. Taffs and H.A. Stone. 1983. Resistance to a malignant lymphoma in chickens is mapped to subregion of major histocompatibility (B) complex. **Science** 219: 977-979.

Chevassus, B. and M. Dorson. 1990. Genetics of resistance to disease in fishes. **Aquaculture** 85: 83-107.

Cnaani, A., B.Y. Lee, M. Ron, G. Hulata, T.D. Kocher and E. Seroussi. 2003. Linkage mapping of major histocompatibility complex class I loci in tilapia (*Oreochromis* spp.). **Anim. Genet.** 34: 390-391.

Cnaani, A., M. Ron, B.Y. Lee, G. Hulata, T.D. Kocher and E. Seroussi. 2002. Mapping the transferrin gene in tilapia. **Anim. Genet.** 33: 78-80.

Cnaani, A., N. Zilberman, S. Tinman and G. Hulata. 2004. Genome-scan analysis for quantitative trait loci in an F2 tilapia hybrid. **Mol Gen Genomics** 272: 162-172.

Croisetiere, S., P.D. Tarte, L. Bernetchez and P. Belhumeur. 2008. Identification of MHC class IIâ resistance/susceptibility alleles to *Aeromonas salmonicida* in Brook charr (*Salvelinus fontinalis*). **Mol. Immunol.** 45: 3107–3116.

Cuesta, A., J. Meseguer and M.A. Esteban. 2007. Cloning and regulation of the major histocompatibility class I alpha gene in the teleost fish gilthead seabream. **Fish Shellfish Immunol.** 22: 718-726.

Flajnik, M.F. and M. Kasahara. 2001. Comparative genomics of the MHC glimpses into the evolution of the adaptive immune system. **Immunity** 15: 351–362.

Germain, R.N. 1994. MHC-dependent antigen processing and peptide presentation: providing ligands for T lymphocyte activation. **Cell** 76: 287-299.

Gjedrem, T., R. Salte and H.M Gjoen. 1991. Genetic variation in susceptibility of Atlantic salmon to furunculosis. **Aquaculture** 97: 1-6.

Grimholt, U., I. Olsaher, C.V. Lindstrom and O. Lie. 1994. A study of variability in the MHC class II α1 and class I α2 domain exons of Atlantic salmon (*Salmo salar*). **Anim. Genet.** 25: 147-153.

Grimholt, U., S. Larsen, R. Nordmo, P. Midtlyng, S. Kjoeglum, A. Storset, S. Saebo and R.J. Stet. 2003. MHC polymorphism and disease resistance in Atlantic salmon (*Salmo salar*); facing pathogens with single expressed major histocompatibility class I and class II loci. **Immunogenetics** 55: 210–219.

Hashimoto, K., K. Okamura, H. Yamaguchi, M. Ototake, T. Nakanishi andY. Kurosawa. 1999. Conservation and diversification of MHC class I and its related molecules in vertebrates. **Immunol. Rev.** 167: 81-100.

Iwana G.K. and T. Nakanishi. 1996. **The fish immune system, organism, pathogen and environment.** London: Academic Press. 380 p.

Kumar, S., K.Tamura, I.B. Jakobsen and M. Nei. 2001. MEGA2: molecular evolutionary genetics analysis software. **Bioinformatics** 17: 1244–1245.

Lohm, J., M. Grahn, A. Langefors, O. Andersen, A. Storset and T.V. Schantz. 2002. Experimental evidence for major histocompatibility complex-allele-specific resistance to a bacterial infection. **Proc. Biol. Sci.** 269: 2029-2033.

Medina, E. and R.J. North. 1998. Resistance ranking of some common inbred mouse strains to *Mycobacterium tuberculosis* and relationship to major histocompatibility complex haplotype and Nramp1 genotype. **Immunology** 93: 270-274.

Miller, K.M., J.R.Winton, A.D. Schulze, M.K. Purcell and T.J. Ming. 2004. Major histocompatibility complex loci are associated with susceptibility of Atlantic salmon to infectious hematopoietic necrosis virus. **Environ. Biol. Fishes** 69: 307-316.

Parham, P. and T. Ohta. 1996. Population biology of antigen presentation by MHC class I molecules. **Science** 272: 67-74.

Paterson, S., K. Wilson and J.M. Pemberton. 1998. Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population (*Ovis aries* L.). **Proc. Natl. Acad. Sci. USA** 95: 3714-3719.

Puangkaew, J., V. Kiron, T. Somamoto, N. Okamoto, S. Satoh, T. Takeuchi and T. Watanabe. 2004. Non specific immune response of rainbow trout (*Oncorhynchus mykiss* Walbaum) in relation to different status of vitamin E and highly unsaturated fatty acids**. Fish Shellfish Immunol.** 16: 25-39.

Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. **Mol. Biol. Evol.** 4: 406–425.

Sato, A., R. Dongak, L. Hao, N. Takezaki, S. Shintani, T. Aoki and J. Klein. 2006. MHC class I genes of the cichlid fish *Oreochromis niloticus* . **Immunogenetics** 58: 917–928.

Thompson, J.D., D.G. Higgins and T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. **Nucleic Acids Res.** 22: 4673–4680.

Townsend, A. and H. Bodmer. 1989. Antigen recognition by class I-restricted T lymphocytes. **Annu. Rev. Immunol.** 7: 601-624.

Trewavas, E. 1983. **Tilapiine Fishes of the Genera *Sarotherodon*, *Oreochromis* and *Danakilia***. British Mus. Nat. Hist., London, UK. 583 p.

Wynne, J.W., M.T. Cook, B.F. Nowak and N.G Elliott. 2007. Major histocompatibility polymorphism associated with resistance towards amoebic gill disease in Atlantic salmon (*Salmo salar* L.) **Fish Shellfish Immunol.** 22: 707-717.

Xia, C., I. Kiryu., J.M. Dijkstra, T. Azuma, T. Nakanishi and M. Ototake. 2002. Differences in MHC class I genes between strains of rainbow trout (*Oncorhynchus mykiss*). **Fish Shellfish Immunol.**12: 287-301.

Zhang, Y., S. Chen, Y. Liu, Z. Sha and Z. Liu. 2006. Major histocompatibility complex Class IIB allele polymorphism and its association with resistance/susceptibility to *Vibrio anguillarum* in Japanese flounder (*Paralichthys olivaceus*). **Mar. Biotechnol.** 8: 600-610.