Genome-wide Characterization of Heat Shock Proteins in Channel Catfish (*Ictalurus punctatus*) and Determination of Their Involvement in Diseases

by

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Abstract

Heat shock proteins (HSPs) consist of a large group of chaperones whose expression is induced by a number of stresses such as exposures to high temperature, hypoxia and infection.

In this study, I identified a total of 93 HSPs in channel catfish (*Ictalurus punctatus*), with 13 members of small Hsp family, 57 members of Hsp40 family, 16 members of Hsp70/110 family, one member of Hsp60 family, one member of Hsp10 family and 5 members of Hsp90 family, through *in silico* analysis using RNA-Seq and genome databases. Phylogenetic and syntenic analyses were conducted with all the families of HSPs, which provided strong evidence in supporting the orthologies of these HSPs. Besides, two tandem repeat members of Hsp70/110s, *hsp70.2* and *hsp70.3* were not found located within the MHC-complex, which is a group of molecules that are essential in the antigen presenting process, suggesting different process of presenting the antigens or epitopes.

Meta-analyses of bacterial challenged RNA-Seq datasets were conducted to analyze expression profile of catfish HSPs following bacterial infection of *Flavobacterium columnarae (F. columbare)* and *Edwarsiella ictaluri (E. ictaluri)*. HSPs expression was analyzed through the early phase of immune response. As a result, a majority of catfish HSPs were found significantly expressed in gill and intestine after bacterial infections during the early immune response phase. A total of thirty Hsp40 genes were regulated under disease situations involving two tissues after two bacterial infections. Five Hsp90 genes, one Hsp60 gene, one Hsp10 gene and nine sHsp genes were significantly up/down-regulated after two bacterial infections. Twelve Hsp70/110s genes

were significantly up/down-regulated after two bacterial infections. Different responses by the Hsp70/110s expression after the infection of *F. columnarae* and *E. ictaluri* revealed the different pathogenesis mechanism of the two bacteria. As an agonist ligand of TLR4, an import Pattern recognition receptor of innate immunity, Hsp70s showed a time pattern through the two bacterial infections in two tissues. Also a pathogen-specific response in host Hsp90 had been revealed. Hsp90s showed the most significant involvement at the 24 hours after challenging with *F. columnarae*. Both pathogen-specific and tissue-specific pattern were found in small Hsp family after both two bacterial infections. Additional meta-analysis were conducted on the comparisons of differences in gene expression profiles between resistant and susceptible fish at 0 h, and 1 h, 2 h, and 8 h after *F. columnare* challenge. A total of 18 catfish HSPs genes showed significant expression at basal level prior to *F. columnare* challenge, which is our great interest as these signatures could potentially serve as QTL or biomarkers for selection.

Keywords: Heat shock protein, catfish, genome, immune response, infection

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List of Abbreviations

HSPs	Heat Shock Proteins
ESC	Enteric Septicemia of Catfish
TLRs	Toll-like Receptors
DCs	Dendritic Cells
IL	Interleukin
IFN	Interferon
NKs	Natural Killer cells
MHC	Major Histocompatibility Complex
APCs	Antigen Presenting Cells
CD	Cluster of Differentiation
TCR	T cell receptor
Tc	cytotoxic T cell
Th	helper T cell
PAMPs	Pathogen-associated Molecular Patterns
PRRs	Pattern Recognition Receptors

Chapter 1. Introduction

1.1 Overview

Catfish is the primary aquaculture species in the U.S., accounting for over 60% of all U.S aquaculture production. It is also an important model for the study of the teleost immune system. In recent years, the catfish industry in the United States has encountered unprecedented challenges due to increased feed costs, international competition, and devastating diseases. Feed costs have increased more than 200% in the last decade, making the industry extremely difficult to maintain a profit margin that is sustainable. International competition has become more and more keen with exponential levels of increases in catfish imports from other parts of the world including Vietnam, China, among other countries. Disease problems are also increasing with intensifies aquaculture.

Of the serious disease problems, bacterial diseases are the major threats to the catfish industry. In addition to the bacterial diseases, several other diseases caused by virus and parasites are also important. For instance, channel catfish virus disease can cause significant problems (Plumb, 1986). Parasitic disease Ich caused by *Ichthyophthirius multifiliis* can also lead to heavy mortalities (Xu and Klesius, 2004). However, these diseases are relatively less frequent or less severe as compared to bacterial diseases. Of the bacterial diseases, several are very severe and cause major economic losses to the catfish industry. These include enteric septicemia of catfish (ESC) caused by *Edwardsiella ictaluri* (Hawke et al., 1981), columnaris disease caused by *Flavobacterium columnare* (Bullock et al., 1986), and Aeromonas bacterial disease caused by *Aeromonas hydrophila* (Pridgeon and Klesius, 2011).

Columnaris disease is the most frequently occurring disease in fish including catfish (Bullock, Hsu et al., 1986). It is responsible for significant economic losses in freshwater fish aquaculture worldwide. Columnaris disease costs the catfish industry about $40 million annually. Compared with columnaris disease, ESC disease is less frequent in occurrence, but is more severe. It too can cause very significant economic losses to the catfish industry. It accounts for approximately 30 percent of all disease cases submitted to fish diagnostic laboratories in the southeastern United States. Economic losses to the catfish industry are in the millions of dollars yearly and continue to increase steadily with the growth of the industry. Besides the diseases, the stresses from the low water quality and other factors such as low oxygen, high or low water temperature can also cause major mortalities. More importantly, bacterial disease is more frequent and more severe under stress conditions. As a matter of fact, stresses often are the direct trigger of diseases, especially with columnaris diseases.

Fish respond to diseases and stresses in various ways. Much work has been conducted in studies of immunological or physiological responses after infection of catfish with bacterial infections (Dunham et al., 2002; Austin and Austin, 2007), and therefore this aspect will not be the focus of this study.

1.1.1 Heat shock proteins and their homologues

Heat shock proteins (HSPs), which were originally identified after heat shock being highly regulated, are well conserved in all the organisms from bacteria to mammals. They are present in all cells in all organisms and in a variety of intracellular locations, including the cytosol of prokaryotes, and the cytosol, nucleus, endoplasmic reticulum (ER), mitochondria and chloroplasts of eukaryotes (Lindquist and Craig, 1988). HSPs constitute up to 5% of the total intracellular proteins; however, under stresses, such as exposure to high temperatures, toxins and low oxygen

conditions, their levels can rise to 15% or more (Srivastava, 2002). HSPs are classified based on their molecular weight to include Hsp10, Hsp40, Hsp60, Hsp70, Hsp90, Hsp110 and small HSPs (sHSPs). However, Kampinga et al., (2009) proposed a new guideline for the nomenclature of the human HSP families based on functional domains, thus heat shock proteins can be classified into five families: HSPA/HSPH (HSP70/110), HSPC (HSP90), , DNAJ (HSP40), and HSPB (small HSPs) as well as for the human chaperonin families HSPD/E (HSP60/HSP10) (Table1-1) (Kampinga et al., 2009). The amino acid homolog between HSP families are very little, however members of each family are highly related and share same domains. All HSP families are present in every organism, but individual HSPs can be vary in distribution both within and between organisms.

Table 1-1. Structures and functions of Heat shock protein families

HSPs families	Structure	Function
Hsp90	Dimeric protein; each monomer has an amino-terminal domain that hydrolyzes ATP, a substrate–binding middle domain and a carboxyl-domain that interacts with co-chaperones	Folds nascent and denaturing proteins; interacts with kinases and other regulatory molecules; protein degradation
Hsp70/110	Monomeric protein with a conserved ATP binding /hydrolysis site in the amino-terminus, a linker region and a variable carboxyl substrate-binding domain	Binds and folds nascent and denaturing proteins; protects against stresses such as bacterial infection
Hsp60/10	Two back-to-back rings of eight to nine subunits each with an apical capped domain that binds substrate, an equatorial region with an ATP hydrolysis site and a linking domain	Binds substrates via hydrophobic, polar and charged residues; required by actin and tubulin for folding
Hsp40	Monomeric protein with a conserved N-terminus J-domain, and variable C-terminus domain (CTD)	Regulate the ATPase activity of Hsp70 proteins.
Small Hsp	Multimeric complexes composed of monomers containing an amino-termial region, an α-crystallin domain and a carboxyl-extension; the domains cooperate in oligomerization and substrate binding	Prevent stress-induced irreversible protein denaturation; inhibit apoptosis; ATP independent

1.1.2 Chaperone and housekeeping functions of HSP

Basically, the HSPs are divided into two categories, inducible HSPs and constitutive HSPs (Kelly and Yenari, 2002). The inducible HSPs are induced by any form of stresses including heat, toxins or diseases, and they act as chaperone proteins by binding to misfolded protein. The constitutive HSPs function as housekeeping protein who are essential and have a constant expression in normal or non-stressed cells. Both of the functions are feasible due to their binding ability to target substrates.

Molecular chaperones include a large and diverse group of proteins that assist in folding or unfolding of other proteins or macromolecule structures in cells (Ellis, 1987). Among these, heat shock proteins (HSPs) constitute one of the largest groups of chaperones. The discovery of heat inducible chromosome puffs in salivary glands of Drosophila by Ritossa in 1962, started a wide expanded field of Heat shock response studies (Ritossa, 1962; Moran et al., 1978). Several studies found that a mild non-lethal dose of heat shock could protect the cells from death which were caused by the later severe heat or other stresses (Gerner et al., 1976; Sapareto et al., 1978; Henle et al, 1978; Petersen and Mitchell, 1981). Soon it was clear that the protection came from the induced and accumulated heat inducible proteins which were designated as Heat shock proteins (HSPs). Several heat shock proteins function as intra-cellular chaperones for other proteins. They play an important role in protein-protein interactions such as folding and assisting in the establishment of proper protein conformation (shape) and prevention of unwanted protein aggregation. By helping to stabilize partially unfolded proteins, HSPs aid in transporting proteins across membranes within the cell.

Besides this chaperone function, it also had been discovered that some HSPs were constitutively expressed in non-stressed cells as housekeeping proteins, such as Hspa8 in Hsp70

family. The house keeping HSPs are distributed all through the body and act like a "monitor" for the maintenance. The housekeeping functions of HSPs include transport of proteins between cellular compartments, degradation of unstable and misfolded proteins, prevention and dissolution of protein complexes, folding and refolding of proteins, uncoating of clathrincoated vesicles, and control of regulatory proteins. Unlike Hsp90, Hsp70 doesn't require a "client" protein. Instead, they bind to any exposed hydrophobic residue of the newly synthesized protein or misfolded proteins caused by stresses to prevent their aggregation. Hsp70s also can directly unfold misfolded proteins, in an adenosine triphosphate (ATP)-dependent way (16). In vivo, sHsps have been implicated in an astounding variety of processes, such as enhancing cellular stress resistance (Landry et al., 1989), regulating actin and intermediate filament dynamics (Wieske et al., 2001; Quinlan, 2002), inhibiting apoptosis (Arrigo et al., 2002), modulating membrane fluidity (Tsvetkova et al., 2002), and regulating vasorelaxation (Flynn et al., 2003). Mutants of human sHsps are responsible for various forms of hereditary cataract (Pras et al., 2000; Mackay et al., 2003), muscular diseases (Vicart et al., 1998; Selcen and Engel, 2003) and neuropathies (Evgrafov et al., 2004; Irobi et al., 2004).

1.1.3 Immunological functions of HSP

HSPs have been proved to involve in both innate and adaptive immune response. For innate immunity, Hsps are thought to mediate both humoral and cellular innate immune responses (Sung and MacRae, 2011). The presence of Hsps in the extracellular environment served as a danger signal to activate innate immune such as dendritic cells (DCs) and macrophages (Singh-Jasuja et al., 2000; Chen and Syldath, et al., 1999; Kol et al., 2000); Several cytokines can be induced by Hsps, including tumour-necrosis-α (TNF-α), interleukin-1β (IL-1β), interleukin-12 (IL-12), nitric

oxide and some chemokines (Basu et al., 2000; Lehner et al., 2000; Moré et al., 2001; Panjwani et al., 2002).

For adaptive immunity, or the intermediate phase between innate and adaptive immunity, HSPs can stimulate adaptive immune responses as potent antigen carriers. They even can show an immunogenicity by binding the peptides derived from specific antigens. The studies of immunological functions of HSPs began to emerge in the 1980s when HSPs were found an ability of eliciting immunity to cancer after they were isolated from cancer cells, whereas corresponding HSPs isolated from normal tissues did not (Srivastava, 1998). The earliest studies were carried out with the HSP gp96 (Ullrich et al., 1986), however in the later studies HSP70, HSP90, HSP110 and GRP170 showed the similar results (Udono and Srivastava, 1993; Tamura et al., 1997; Basu, 1999; Wang et al., 2001). The immunogenicity of cancer-derived HSPs preparation resulted from the binding of HSP molecules to peptides that were generated by the degradation of tumour-specific antigens, which was expressed by the tumor cell where the HSPs were purified. Besides binding to tumour-specific antigens (Ishii et al., 1999), the HSPs also bind to the peptides from normal proteins, whereas HSPs from normal tissue were associated solely with peptides derived from normal proteins. So, the HSPs chaperone the "peptide fingerprint", which includes the antigenic peptides of the cells from which they are isolated. However, this association of peptides with HSPs occurs only in vivo and does not result from purification of HSPs in vitro (Ménoret et al., 1999). Peptide-binding pockets of HSP70 (Zhu et al., 1996) and HSP90 (Linderoth et al., 2000) have been defined, providing a firm structural basis for the HSP–peptide association/binding. Similar in HSPs' ability to bind the antigenic fingerprint of cells, the major histocompatibility complex (MHC) molecules also can bind a wide range of intracellular antigens. However, the HSPs chaperone a wider range of peptide than MHC molecules. In fact, HSPs have the ability of "cross-priming" that

scan all the available peptides in cell and then transfer the peptides of antigen to MHC. The antigen peptide can be transfer to MHC either within the cell or between the neighbouring cells, when the HSPs released due to cell lysis and carry the chaperoned peptides to the MHC class II molecules of the neighbouring professional antigen-presenting cells, which can later present the epitopes to CD4+ helper T cell to release cytokine to active the other immune cells. Therefore, both of these two molecules have the roles that contribute to the T-cell response. HSP70 genes have been mapped within the MHC loci in the short arm of chromosome 6 in human (6p21), but the significance of this mapping is not clear (Sargent et al., 1989).

Hsp60, Hsp70, Hsp90 have been proposed to interact with immune cells as a ligand for a variety of cell-surface receptors such as Toll-like receptors and a number of clusters of differentiation (CDs) such as CD14 and CD91 (Basu *et al.*, 2001; Ohashi *et al.*, 2000; Vabulas *et al.*, 2001; Habich *et al.*, 2002). Extracellular and membrane bound heat-shock proteins, especially Hsp70 are involved in binding antigens and presenting them to the immune system. Members of the Hsp70 cytosolic group are either constitutively expressed (Hspa8) or can be induced by a broad range of stress factors. The inducible Hsp70 has been recently characterized as a potent maturation stimulus for Dendritic Cells (DCs), which is an important professional antigen presenting cell (APC) that express MCH (Major Histocompatibility Complex) class II molecule on its surface and process the antigen into epitopes while migrating to the thymus to present the epitopes to the CD4+ T cell. This process is considered as the bridge of innate immunity and adaptive immunity (Kuppner, et al., 2001, Vanbuskirk, et al., 1989). One rare type of CD4+ T cells, which is called γδ T cell, are believed to be triggered by alarm signals such as HSPs and it is only found abundant in gut mucosa. However, common with the immune cells in innate immunity, this 'unconventional' T cell have the ability of the antigen recognition by its T cell receptor (one of the Pattern

Recognition Receptors-PRR), whereas the other T cells can't recognize the free antigen and require the APC or MHC molecules to present the processed epitopes to them. Therefore, this γδ T cell is an immune cell that bear both characters of innate immune and adaptive immune. Heat shock proteins, participating as a danger signal when the host is injured or infected, play an important role in between of the two immune phases - innative and adaptive immunity. Moreover, studies have suggested that Hsp60 plays a key role in preventing apoptosis in the cytoplasm by forming a complex with proteins responsible for apoptosis and regulates the activity of these proteins (Itoh and Komatsuda, et al., 2002). The cytoplasmic Hsp60 is also involved in immune response (Ranford *et al.*, 2000) and cancer (Itoh and Futenma, et al., 2002; Urushibara et al., 2007; Lebret et al., 2003). Hsp10 aids Hsp60 in protein folding by acting as a dome-like cover on the ATP active form of Hsp60 (Ranford *et al.*, 2000).

1.1.4 HSPs in the lumen of the ER and Mitochondria

The members of HSPs show a character of localization-specific. While most of HSPs localized in cytosol, still some members are reside in the lumen of the ER (Hsp90b1, Hspa5, Hsp47 and Hyou1), since protein synthesis often occurs in this area. gp96 is one of the most abundant components in the lumen of the ER, where it associates with a large array of peptides; it is an ATP-binding protein and an ATPase (Li and Srivastava, 1993). I have recently shown that gp96 is also an aminopeptidase that can trim amino-terminal-extended 19-mer precursors of an octamer epitope that are derived from the vesicular stomatitis virus to the octamer. It is increasingly apparent that peptides generated in the cytosol due to proteasomal action are extended on the amino terminus with respect to the MHC class I-binding epitopes (Serwold et al., 2001), even though they are more precisely processed on the carboxy terminus. These peptides must be trimmed in the lumen of the ER. However, the preliminary evidence for participation in antigen presentation exists only for Hsp90.

Heat shock protein 60 (HSP60) is a mitochondrial chaperonin that is typically responsible for the transportation and refolding of proteins from the cytoplasm into the mitochondrial matrix. Through the extensive study of groEL, which is the HSP60's bacterial homolog, HSP60 has been deemed essential in the synthesis and transportation of essential mitochondrial proteins from the cell's cytoplasm into the mitochondrial matrix. HSPA9 of heat shock protein 70 family also resides in mitochondria and acts as a role of housekeeping protein.

1.1.5 Role of HSPs in immune response of aquatic organisms

Based on their function as chaperones for other proteins and their role in immune responses, Hsps has been thought as an approach to disease management in organisms. More and more studies have showed that aquatic organisms respond to pathogen infection by the production of Hsps (Chen and Cao, 2010; Dong et al., 2006; Forsyth et al., 1997; Ackerman and Iwama, 2001; Sung and MacRae, 2011). Increasing Hsps in aquatic organisms by heat shock, chemical application and feeding exogenous Hsps also enhanced resistance to infection (Wilhelm et al., 2005; Sung and MacRae, 2011) and the level of tolerance correlated with the amount of accumulated Hsps (Sung and MacRae, 2011). Endogenous Hsp70 increases significantly after bacterial and viral infection of fin-fish and shrimp (Roberts et al., 2010; Baruah et al, 2010; Wang et al., 2010). Platyfish are protected against *Yersinia rukeri* by injecting them with bacterial Hsps, an effect enhanced by non-lethal heat shock (Ryckaert et al., 2010). Because microbial Hsp60 (GroEL) and Hsp70 (DnaK) are frequently major pathogen-derived antigens that invoke high antibody response, they have the potential to function as highly specific potent vaccines against harmful biotic agents (Marshall et al., 2007; Dang et al., 2011). Control of disease caused by vibriosis in the crustacean *Artemia franciscana* is achieved by employing non-lethal heat shock to boost endogenous Hsp70 (Sung et al., 2007; Sung et al., 2008) and by feeding the exogenous DnaK, the prokaryotic equivalent of

Hsp70 (Sung and Ashame, et al., 2009; Sung and Dhaene, et al., 2009). RNA-seq studies of transcriptome level after catfish bacterial infections showed that the expression of Hsps in channel catfish significantly changed after fish infected by pathogen. Eight Hsp genes in intestine showed significant differential expression for at least one time-point following *Edwardsiellla ictaluri* infection (Li et al., 2012), 23 Hsp genes differentially expressed in gill post *Flavobacterium columnare* challenge in at least one timepoint (Sun et al., 2012), eight Hsp genes exhibited differential expression in resistant fish when being compared with the susceptible fish after infected by *E. ictaluri* (Wang et al., 2013).

However, still limited studies of full set teleost HSPs for their roles in the immune response are available so far. Small HSPs are almost entirely unexplored, and the extra-large (larger than 100 kDa) HSPs have just begun to be explored immunologically. Similarly, the overwhelming majority of studies have been carried out with the constitutively expressed HSPs. The immunological roles of inducible HSPs might not be similar to those of their constitutive siblings and could provide new insights into inflammation and fever. Thus, a systematically research into the catfish heat shock proteins and how they involve in immune response after bacterial infection is needed as it will not only fill the blank of the full members of heat shock protein's role in immune response but also possibly provide a guide to control the diseases in catfish and improve the catfish culture industry in future.

HSPs in catfish have not been well studied, and their expression in relation to stresses and disease infections are unknown before this work. The goal of my dissertation work is to characterize the HSPs from channel catfish through their identification, phylogenetic analysis, comparative genome analysis, and meta-analysis of their expression in relation to different types

of stresses, thereby providing insights into the roles of HSPs during stress responses, particularly after bacterial infection.

1.2 Long-term goals and specific objectives

The long-term goal of such studies is to determine the roles of HSPs under stress conditions or after disease infection, and determine the relationship of their expression with resistance against diseases or with tolerance against adverse environmental conditions such as low oxygen, high temperature, low water quality, among many other factors, and apply such knowledge in genetic programs for the development of genetically enhanced catfish brood stocks. The overall hypothesis is that heat shock proteins are involved in the disease and stress responses, and their expression may be correlated with disease resistance or stress tolerance. To reach these long-term goals, my dissertation project will accomplish the following specific objectives:

1) Identify members of heat shock proteins in the channel catfish genome;
2) Phylogenetic analysis and syntenic analysis of HSPs;
3) Meta-analysis of their expression after infections.

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Chapter 2. Genome-wide Identification of Hsp40 Genes in Channel Catfish and Their Regulated Expression after Bacterial Infection

2.1 Abstract

Heat shock proteins (HSPs) consist of a large group of chaperones whose expression is induced by a number of stresses such as exposures to high temperature, hypoxia and infection. Among all the HSPs, *Hsp40* is the largest HSP family, which bind to Hsp70 ATPase domain in assisting protein folding and stress release. In this study, I identified 57 *hsp40*s in channel catfish (*Ictalurus punctatus*) through *in silico* analysis using RNA-Seq and genome databases. All these genes can be classified into three different subfamilies, Type I, Type II and Type III, based on their structural similarities. Phylogenetic and syntenic analyses provided strong evidence in supporting the orthologies of these HSPs. Meta-analyses of RNA-Seq datasets were conducted to analyze expression profile of *Hsp40*s following bacterial infection. Nineteen *hsp40*s were found to be regulated in the intestine after infection with *E. ictaluri*; and nineteen *hsp40*s were found to be regulated in the gill following infection with *F. columnare*. Altogether, a total of 30 Hsp40 genes were regulated under disease situations involving two tissues and two bacterial infections. The regulated expression of Hsp40 genes after bacterial infection suggested their involvement in disease defenses in catfish. Additional RNA-seq dataset was used to profile gill Hsp40 expression differences between resistant and susceptible fish group post *F. columnare* challenge at both basally (before infection) and at three early timepoints (1 h, 2 h, and 8 h). Twenty genes showed significant differential expression between groups for at least one timepoint following infection.

Among them, thirteen gene were found significantly differentially expressed at the basal level between resistant and susceptible fish family, which could potentially serve as expression QTL and biomarkers for selection.

Keywords: Heat shock protein; Hsp40; catfish; genome; immunity; infection.

2.2 Introduction

Hsp40 proteins, also known as DnaJ proteins, constitute one of the largest families among heat shock proteins. They regulate the ATPase activity of Hsp70 proteins whose function is reversibly binding to partially denatured protein substrates to avoid the aggregation of themselves or with other molecules (Beckmann et al., 1990; Peter Walsh et al., 2004). In the Hsp70-Hsp40 co-chaperone system, the association between Hsp70 proteins and substrates requires an ATP binding to ATPase domain and then being hydrolyzed to change conformation of the binding domain. Thus, various Hsp70's substrates could specifically bind to its least conserved C-terminal at a higher affinity. However, because the ATPase activity of Hsp70s is extremely weak, The J-domain of Hsp40s is needed for activating the ATPase domain of Hsp70s (Kelley, 1998; Qiu et al., 2006).

Hsp40s share a 70-amino acids conserved J-domain, similar to the 73-amino acid-domain of prototypical DnaJ protein in *Escherichia coli* (Hennessy et al., 2005). The DnaJ of *E. coli* typically consists of four regions: N-terminus J-domain, glycine/phenylalanine-rich region, Cysteine repeats and variable C-terminus domain (CTD) (Hennessy et al., 2005). According to the homology of the DnaJ protein of *E.coli,* Hsp40 proteins were classified into three types: Type I DnaJ proteins (DnaJA) possess all four parts of DnaJ protein in *E. coli*; Type II DnaJ proteins (DnaJB) possess the N-terminus J-domain and the Gly/Phe-rich region; Type III DnaJ proteins (DnaJC) only have a J-domain, which is not necessarily located at N-terminus of the protein (Cheetham and Caplan, 1998; Kampinga et al., 2009). Recently, type IV DnaJ protein family was added, which differs from the other three types of DnaJ proteins in that it owns a 'J-like' domain (Botha et al., 2007; Morahan et al., 2011; Peter Walsh et al., 2004) containing various mutations in a highly conserved histidine, proline, and aspartic acid--HPD motif located between helices II

and III in DnaJ domain (Douglas, 1996; Hennessy et al., 2000; Mayer et al., 1999; Peter Walsh et al., 2004). However, Peter Walsh et al. (2004) proposed that the term J-proteins should be used more strictly to describe only J proteins with well-conserved J-domain in the HPD motif, while structurally less-conserved proteins should be referred to as J-like proteins.

Though heat shock proteins are induced by heat and other stresses, more and more studies indicate HSPs play an important role in immune responses (Srivastava et al., 1998; Roberts et al., 2010). For example, HSPs are considered to mediate humoral and cellular innate immune responses (Sung and MacRae, 2011); HSPs in extracellular environment serve as a danger signal to activate innate immune cells such as dendritic cells and macrophages (Chen et al., 1999; Kol et al., 2000; Singh-Jasuja et al., 2000). Several cytokines can be induced by HSPs, including TNF-α, IL-1β, IL-12, nitric oxide and some chemokines (Srivastava, 2002); HSPs can also stimulate adaptive immune responses as potent antigen carriers. Hsp60, Hsp70, Hsp90 have been reported to interact with immune cells as a ligand for a variety of cell-surface receptors such as Toll-like receptors (Ohashi et al., 2000; Vabulas et al., 2001) and a number of CDs such as CD14 and CD91 (Basu et al., 2001; Habich et al., 2002).

In teleost, hsp70 genes have been found to be involved in bacterial kidney disease in coho salmon (Forsyth et al., 1997) and vibriosis in rainbow trout (Ackerman and Iwama, 2001). In olive flounder, Hsp40 proteins were found to be up-regulated in flounder embryonic cells (FEC) after viral infection and a flounder hsp70 gene was also expressed in heat-shocked and virus treated FEC cells (Dong et al., 2006), indicating *hsp40* and *hsp70* functioned as co-chaperone in antiviral immune responses. In the kidney of olive flounder, *dnaja4, dnajb6* and *dnajb11* were found to be expressed after being infected by *Streptococcus parauberis* (Cha et al., 2013).

Channel catfish (*Ictalurus punctatus*) is the leading aquaculture species in the United States. Its genomic resources have been well developed in recent years, particularly ESTs (Li et al., 2007; Wang et al. 2010), transcriptome sequences generated by RNA-Seq (Liu et al., 2011; Liu et al., 2012) and draft whole genome sequence (unpublished data). These resources make it feasible to conduct systematic analysis of hsp40 genes in channel catfish genome. The objective of this study was to determine the involvement of hsp40 genes in disease responses after bacterial infection in catfish. Here I report the genome-wide identification of a full set of 57 hsp40 genes, their phylogenetic and syntenic analyses, and their involvement in disease responses after bacterial infection with ESC and columnaris using RNA-Seq datasets.

2.3 Materials and Methods

2.3.1 Database mining and sequence analyses

In order to identify the full set of hsp40 genes in channel catfish, I collected all Hsp40 proteins from teleost fishes (zebrafish, three-spined stickleback, medaka, tilapia and fugu) and other species (human, mouse, platypus, chicken, turtle and frog) (supplementary table 3-1). These sequences were retrieved from NCBI (http://www.ncbi.nlm.nih.gov) and Ensembl (http://www.ensembl.org) databases and used as queries to search against channel catfish RNA-Seq datasets. The e-value was set at intermediately stringent level of e^{-10} for collecting as many as potential *hsp40*-related sequences for further analysis. The retrieved sequences were then translated using ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Further, the predicted ORFs were verified by BLASTP against NCBI non-redundant (Nr) protein sequence database. The simple modular architecture research tool (SMART) (Letunic et al., 2012) was used to predict the conserved domains based on sequence homology and further confirmed by conserved domain prediction from BLAST. The predicted catfish Hsp40s proteins and all other query sequences were

utilized to search against catfish genome database using TBLASTN program. The retrieved genome scaffolds were then predicted by FGENESH in SoftBerry (http://linux1.softberry.com/berry.phtml?topic=fgenesh&group=programs&subgroup=gfind).

2.3.2 Phylogenetic and conserved syntenic analyses

All the amino acids from channel catfish and other species were used to construct phylogenetic tree. Multiple protein sequences alignments were conducted using the Clustal W2 program (Larkin et al., 2007) and MUSCLE 3.8 (Edgar, 2004). Three alignment methods: L-INS-i, E-INS-i and G-INS-i were applied from MAFFT 7.01 (Katoh and Standley, 2013) and the best alignment with highest score was evaluated by program MUMSA (Lassmann and Sonnhammer, 2006). JTT+I+G model (Jones-Taylor-Thornton (JTT) matrix incorporated a proportion of invariant sites (+I) and the gamma distribution for modeling rate heterogeneity (+G)) was selected as the best-fit model by ProtTest 3 program (Darriba et al., 2011) according to the Bayesian information criterion. Maximum likelihood phylogenetic tree was constructed using MEGA5.2.2 (Tamura et al., 2011) with bootstrap test of 1,000 replicates. Final phylogenetic tree was separated into three different phylogenetic trees according to classification of subfamilies due to the large size.

Conserved syntenic regions surrounding the relevant hsp40 genes were searched by examining the conserved co-localization of neighboring genes on scaffold of channel catfish and other species based on genome information from Ensembl (Release 74) and NCBI database. Neighbor genes of channel catfish Hsp40 genes were predicted by FGENESH (Salamov and Solovyev, 2000) and BLASTP.

2.3.3. Meta-analysis of expression of hsp40 genes and bacterial challenge

The Illumina-based RNA-Seq reads were retrieved from bacterial challenge experiments in catfish: intestine samples challenged with *Edwardsiella ictaluri* (SRA accession number SRP009069) (Li et al., 2012) and gill samples challenged with *Flavobacterium Columnare* (SRA number SRP012586) (Sun et al., 2012). Trimmed high-quality reads were mapped onto the catfish Hsp40 genes using CLC Genomics Workbench software (version 5.5.2; CLC bio, Aarhus, Denmark). Mapping parameters were set as ≥ 95% of the reads in perfect allignment and ≤ 2 mismatches. The total mapped reads number for each transcript was determined and normalized to analyze RPKM (Reads Per Kilobase of exon model per Million mapped reads). The proportions-based Kal's test was performed to identify the differently expressed genes comparing with control sample and fold changes were calculated. Transcrirps with absolute fold change *p*-value ≤ 0.05, value ≥ 1.5 and total reads number ≥ 5 were included in the analyses as significantly differently expressed genes.

Another set of RNA-seq dataset (SRA accession number SRP017689) was used to analyze the differently expressed catfish Hsp40s between columnaris resistant and columnaris susceptible catfish family (Peatman et al., 2013). The two families of channel catfish utilized were previously revealed to have differing susceptibilities to columnaris disease (Beck et al., 2012). Both before and at early timepoints (0h, 1h, 2h and 8h) following *F. columnare* challenge in gill tissue of the fish from resistant and susceptible families of channel catfish were examined. The Illumina-based RNA-Seq reads were retrieved from the NCBI Sequence Read Archive (SRA) under Accession SRP017689. Trimmed high-quality reads were mapped onto the catfish Hsp40 genes using CLC Genomics Workbench software (version 6.5.2; CLC bio, Aarhus, Denmark). Mapping parameters were set as ≥ 95% of the reads in perfect allignment and ≤ 2 mismatches. The total mapped reads

number for each transcript was determined and normalized to analyze RPKM (Reads Per Kilobase of exon model per Million mapped reads). The proportions-based Kal's test was performed to identify the differentially expressed genes comparing samples from columnaris resistant family with samples from columnaris susceptible family at each time point. After scaling normalization of the RPKM values, fold changes were calculated. Transcrirps with p-value ≤ 0.05, absolute fold change value ≥ 1.5, and total reads number ≥ 5 were included in the analyses as significantly differently expressed genes.

2.4 Results

2.4.1 Identification of Hsp40 genes in catfish

A total of 57 Hsp40 genes were identified in channel catfish. All the information of classification, domain structures and GenBank accession numbers are summarized in Table 2-1. According to the structures of Hsp40 (Type I, Type II, Type III), 57 sequences could be classified into 3 subfamilies, including 6 Type I Hsp40 genes, 16 Type II Hsp40 genes and 35 Type III Hsp40 genes (Table 2-1). Among all these genes, almost all sequences were identified in both transcriptome and genome databases with full-length or nearly full-length hsp40 except one—dnajb9 with partial sequences in both databases. These catfish hsp40 genes were named following Zebrafish Nomenclature Guidelines (https://wiki.zfin.org/display/general/ZFIN+Zebrafish+Nomenclature+Guidelines).

Six type I genes were identified in the catfish genome including *dnaJa1, dnaJa2, dnaJa2-like, dnaJa3a, dnaJa3b* and *dnaJa4*. These catfish type I proteins are homologous to DnaJ of *E.coli*, whose structure is conservatively comprised of N-terminal J-domain, glycine/phenylalanine-rich region, cysteine repeats motif and variable C-terminus domain (CTD) (Figure 2-1 and Table 2-1).

Sixteen type II genes were identified in channel catfish including *dnaJb1a, dnaJb1b, dnaJb2, dnaJb4, dnaJb5, dnaJb5-like, dnaJb6a, dnaJb6b, dnaJb9, dnaJb9-like1, dnaJb9-like2, dnaJb11, dnaJb12a, dnaJb12b, dnaJb13* and *dnaJb14*. This subfamily contained the most widely expressed and most heat-inducible human DNAJ member, DNAJB1 (Kampinga et al., 2009). All catfish type II proteins are type II Hsp40 proteins, which harbored one N-terminal J-domain and one glycine/phenylalanine-rich region (Figure 2-1 and Table 2-1).

A total of thirty five type III genes were identified from the catfish transcriptome and confirmed with the genome database including *dnaJc1, dnajc2, dnajc3, dnajc3 (prkri), dnajc4, dnajc5aa, dnajc5ab, dnajc5gb, dnajc6, dnajc7, dnajc8, dnajc9, dnajc10, dnajc11, dnajc12, dnajc13, dnajc14, dnajc15, dnajc16, dnajc16-like, dnajc17, dnajc18, dnajc19, dnajc20 (hscb), dnajc21, dnajc22, dnajc23 (sec63), dnajc24, dnajc25, dnajc26 (gak), dnajc27, dnajc28, dnajc29 (sacs), dnajc30a* and *dnajc30b*. Catfish type III Hsp40 proteins only have one J-domain in their structure, which is not necessarily located at N-terminus of the protein (Figure 2-1 and Table 2-1). Orthologies were established for all the type III *Hsp40s* among human, zebrafish and catfish. However, several type III *Hsp40s*, i.e., *Dnajc3 (Prkri), Dnajc20 (Hscb), Dnajc23 (Sec63), Dnajc26 (Gak),* and *Dnajc29 (Sacs)* have not been annotated as DnaJC members. They are currently named according to aliases of human DNAJC proteins respectively (Kampinga et al., 2009).

2.4.2 Phylogenetic analysis of channel catfish Hsp40s

A total of 57 channel catfish Hsp40 genes have been phylogenetically analyzed. Each subfamily of Hsp40 was subsequently analyzed separately. The type III hsp40 proteins are divided into three parts due to the enormous size of the phylogenetic tree (Figure 2-4). In a few cases where it was difficult to establish orthologies due to duplications (*dnajb9, dnajb12* and *dnajc30*), syntenic

analyses were also conducted (see below). I renamed some of the ambiguous names of hsp40 genes from other fish according to their relationship with the relevant zebrafish genes on the phylogenetic tree. The phylogenetic trees were then reconstructed after standardizing all the names. In the phylogenetic tree, all the members of catfish *Hsp40* were well distributed into distinct clades and grouped with corresponding genes of zebrafish and other fishes, which were supported by strong bootstrap value (Figure 2-2, 2-3, 2-4).

2.4.3 Syntenic analysis of channel catfish Hsp40s

Though phylogenetic relationships provide strong support for the identities of most Hsp40 genes, syntenic analyses were required to provide additional evidence for orthologies or otherwise the paralogies for several hsp40 genes including the duplicated hsp40 genes such as *dnajb9, dnajb12 and dnajc30*. Positions of these catfish hsp40s genes and their neighbor genes were identified from the draft genome scaffolds. And the genes were also identified from the zebrafish genome. As shown in Figure 2-5, three *dnajb9*-related genes were analyzed. The gene with the highest level of conservation in gene contents and gene orders as compared with the human DNAJB9 was named *dnajb9* in zebrafish (accession number from ensembl: ENSDARP00000094644). Two other genes similar to *dnajb9* in zebrafish with NCBI accession number: NP_001020355.1 and NP_001019564.1 were named *dnajb9L1* and *dnajb9L2* (L refers to "like") respectively. All those genes of catfish, as well as those related genes from other fish species, were therefore named as *dnajb9L1* and *dnajb9L2* accordingly.

As shown in Figure 2-6, of the two *dnajc3*-related genes, the neighboring genes surrounding the DNAJC3 gene is clearly well conserved among human, zebrafish and catfish (Figure 2-6A), suggesting their orthologies. However, the genes surrounding the other *dnajc3*-related gene were quite different between the fish genes and the human genes (compare Figure 2-

6A and Figure 2-6B), suggesting that they were not orthologous. However, the genes surrounding this gene are well conserved between zebrafish and catfish. I therefore annotated this genes as prkri as annotated in zebrafish.

Two *dnajc30*-related genes were found in catfish and zebrafish. As shown in Figure 2-7, these two genes were located on two different chromosomes of zebrafish, but genes surrounding the duplicated gene are well conserved among human, zebrafish and catfish, suggesting that they were derived from the whole genome duplication event. Therefore, they were annotated as *dnajc30a* and *dnajc30b*, following zebrafish gene nomenclature system.

2.4.4 Hsp40s copy number variation among species

Catfish have almost all the orthologues of Hsp40s in human and zebrafish (Table 2-2), with exception of several genes existing in humans, but absent from teleost fishes including *Dnajb3*, *Dnajb7,* and *Dnajb8.* It is interesting that quite a few of the Dnaja genes and Dnajb genes have duplicated copies in various teleost species, however, most of the Dnajc genes have only a single copy in the teleost genomes (Table 2-2). Specifically, *Dnaja2, Dnaja3, Dnajb1, Dnajb5, Dnajb6, Dnajb12, Dnajc3, Dnajc7, Dnajc11, Dnajc16* and *Dnajc30* were found to have two duplicates, and *Dnajb9* was found to have three copies in catfish and most of the teleost fish while only one copy was found in other species. Note that *Dnajc5* have been found to have five copies in zebrafish, three copies in catfish and three copies in human as well. This is the only gene that has more than one copy in the human genome. Compared to zebrafish, channel catfish has fewer copies for *Dnajb12, Dnajc5, Dnajc7 and Dnajc11* (Table 2-2).

2.4.5 Regulated expression of hsp40 genes in catfish after bacterial infection

Using two bacterial challenged RNA-Seq datasets (gill sample infected by *F. columnare* and intestine sample infected by *E. ictaluri*), the involvement of hsp40 genes after bacterial

infection was determined. As shown in table S2, 30 out of a total of 57 *hsp40s* were involved in disease defense responses. Specifically, 19 *hsp40s* were found regulated in the gill following *F. columnare* infection. Among them, 12 genes were up-regulated and 7 genes were down-regulated after columnaris infection (Figure 2-8). Among these regulated hsp40 genes, some of them are transiently up- or down-regulated while others are gradually induced or suppressed. For instance, *dnajb9L2, dnajb11, dnajb12b, dnajc3, dnajc20, dnajc21,* and *dnajc29* were up-regulated at only one time point; similarly, *dnajb13* was only down regulated at 24h after infection. In contrast, *dnaja4, dnajb1a, dnajc5aa, dnajc6,* and *dnajc16L* were up-regulated in at least two time points after infection, suggesting their up-regulated expression was more lasting. Similar patterns were observed for down-regulated genes including *dnajb1b, dnajb4, dnajc12, dnajc19, dnajc24,* and *dnajc30a* (Figure 2-8).

A total of 19 hsp40 genes were found to be regulated in the intestine after *E. ictaluri* infection. Among these, 17 were up-regulated while two were down-regulated. The upregulated hsp40 genes included *dnaja4, dnajb1a, dnajb1b, dnajb2, dnajb5, dnajb6b, prkri, dnajc5aa, dnajc6, dnajc12, dnajc13, dnajc16, dnajc18, dnajc22, dnajc27, dnajc29,* and *dnajc30b*. The two down-regulated hsp40 genes were *dnaja2L,* and *dnajc17*. Regulated expression of most of these genes were observed in more than one time points with exception of *dnajb6b, dnajc12, dnajc13, dnajc16,* and *dnajc22* where the regulated expression was observed at only one time point (Figure 2-9).

The level of regulated expression varied among the genes as well as tissues and time after infection. For most of the hsp40 genes, up- or down-regulation was less than five-fold as compared with the controls. Only three genes were regulated more than five times after bacterial infections.

Dnajc5aa was up-regulated 6-8 times after *F. columnare* infection (Figure 2-8). No hsp40 genes were up- or down-regulated more than 5-fold in the intestine after *E. ictaluri* infection.

2.4.6 Differentially expressed catfish Hsp40 gene between columnaris resistant and susceptible fish families

Additional meta-analysis were conducted on the comparisons of differences in hsp40s gene expression profiles between resistant and susceptible fish at 0 h, and 1 h, 2 h, and 8 h after *F. columnare* challenge. Designating the susceptible family as the control group, a comparison of catfish Hsp40s expression levels was made between resistant and susceptible families at each timepoint. A total of 24 genes showed significant differential expression between groups for at least one timepoint following infection. Among them, 13 genes was found significantly differently expressed at the basal level between resistant and susceptible fish family (Figure 2-10). The basal differential expression of hsp40s genes could potentially serve as expression QTL and biomarkers for selection.

2.5 Discussion

Hsp40 proteins play key roles in assisting protein folding by activating the ATPase domain of Hsp70. In spite of their importance, only a few Hsp40 genes were characterized from channel catfish (Chen et al., 2010). Systematic analysis of channel catfish Hsp40 genes has been lacking. In this study, I identified a full set of 57 catfish Hsp40 genes in the catfish genome. This was achieved by thorough analysis of rich genomic and transcriptomic resources including several hundred thousands of ESTs (Li et al., 2007; Wang et al., 2010), RNA-Seq transcriptome assemblies (Liu et al., 2011; 2012), and the draft genome sequences (unpublished).

Phylogenetic and syntenic analyses allowed annotation of Hsp40 genes. It is apparent that catfish harbored most of hsp40 genes. Compared with the human genome, catfish lacked only three

hsp40 genes, similar to the situation of other teleost fish species. However, a number of paralogues were also discovered in catfish due to duplication. The phylogenetic analysis strongly supported the nomenclature of catfish Hsp40 genes in all three subfamilies. Most Hsp40s are conserved through evolution, while there are still special preferences made by teleost and catfish. First, the teleost tended to have more duplications than mammals, likely as a consequence of whole genome duplication. Among various fish species, *DnaJa2, DnaJa3, DnaJb1, DnaJb5, DnaJb9, DnaJb12, DnaJc3, DnaJc7, DnaJc11, DnaJc16,* and *DnaJc30* were found to be duplicated while in mammals, birds and amphibians only one copy of these genes were found.

RNA-Seq-based expression analysis has become a robust method to assess transcriptional profile to different challenge experiments (Oshlack et al., 2010). In our recent RNA-Seq studies, I have successfully obtained comprehensive transcriptome assemblies from catfish intestine after *E. ictaluri* infection and from catfish gill after *F. Columnare* infection (Li et al., 2012; Sun et al., 2012). The expression patterns of differentially expressed genes from these two studies were validated by quantitative real-time RT-PCR with average correlation coefficient around 0.9 ($p < 0.001$). Meta-analysis of disease challenges revealed a gene fold change profile of catfish *hsp40s* involving in disease defense and stress protection. As mentioned in the introduction, although regulated expression of HSPs after infection have been reported in several fish species, systematic analysis of their involvement in diseases has not been conducted. This work, therefore, represents the first systematic analysis of Hsp40 involvement after bacterial infection among all species.

Surprisingly, a large number of hsp40 genes, 30 in total, were up- or down- regulated after bacterial infection, suggesting their extensive involvement in disease responses. Specifically, a total of 19 genes were found regulated in intestine after *E. ictaluri* infection, and 19 genes were found regulated in gill following *F. columnare* infection. Of these 30 regulated hsp40 genes: 7

genes (*dnajb1b, dnajc29, dnaja4, dnajb1a, dnajc5aa, dnajc6 and dnajc12*) were commonly regulated both after *F. columnare* infection in the gill and after *E. ictaluri* infection in the intestine, suggesting their importance as general disease response HSPs.

In spite of a set of commonly regulated HSPs, disease- and tissue-specific genes in each challenge experiment were observed. For instance, after *F. columnare* infection, eleven genes (*dnajb4, dnajb9L2, dnajb11, dnajb12b, dnajb13, dnajc3, dnajc16L, dnajc19, dnajc20, dnajc21,* and *dnajc30a)* were found to be specifically regulated in the gill. Similarly, twelve hsp40 genes (*dnaja2L, dnajb2, dnajb5, dnajb6b, dnajc3, dnajc13, dnajc16, dnajc17, dnajc18, dnajc22, dnajc27* and *dnajc30b*) were found to be specifically regulated in the intestine after *E. ictaluri* infection, suggesting their specific roles in a tissue- and time-dependent manner.

The vast majority of hsp40 genes were found to be regulated soon after infection at 3h or 24h after both infections, suggesting their involvement in the early phases of disease response. Only *dnajb6b and dnajc13* were not up-regulated until 3 days after infection. This findings could be explained by the co-chaperon system of Hsp40 and Hsp70 (Freeman et al., 1995; Houry, 2001). Hsp70 regulates the intracellular function and fate of proteins through the formation of direct protein-protein interactions that occur largely through an EEVD-binding domain in its C terminus (Brinker et al., 2002; Liu et al., 1999; Scheufler et al., 2000). In innate immune system, Hsp70 can serve as the endogenous ligand of TLR2 and TLR4 and aid to recognize the bacteria (Asea et al., 2002). Previous work demonstrated that TLRs were up-regulated at 1h post challenge (Peatman et al., 2013) by *F. columnare* and most up-regulated at 6h and 24h post challenge by *E. ictaluri* (Pridgeon et al., 2010) in catfish. It is believed that since Hsp70 serves as a TLR4 agonist, there is a positive correlation between HSP70 and TLR4. Therefore, the increased or decreased fold of Hsp40s after infection of bacteria can reflect the fold change of its co-chaperone Hsp70 and thus

the associated TLRs (Asea et al., 2002; Nair et al., 2013). Furthermore, it was also reported that TLRs are down-regulated after 36h. This could be the explanation to the large number of down-regulated in liver 3 days and 14 days post challenge from our meta-analysis. It is interesting to observe that seven genes were down-regulated after *F. columnare* infection whereas only two genes were down-regulated after *E. ictaluri* infection at early stages of disease development. This observation indicated that the immune response to the *E. ictaluri* was faster than that to *F. columare*. In general, hsp40 genes were up-regulated at early stages of diseases, and with time, they tended to be down regulated as the diseases progressed.

The level of regulated expression varied among the genes as well as tissues and time after infection. For most of the hsp40 genes, up- or down-regulation was less than five-fold as compared with the controls. Only three genes were regulated more than five times after bacterial infections. Dnajc5aa was up-regulated 6-8 times after columnaris infection (Figure 2-8). No hsp40 genes were up- or down-regulated more than 5-fold in the intestine after ESC infection (Figure 2-9).

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Table 2-1 Summary of 57 Hsp40 genes identified in the catfish genome

Name	Type	ORF	Domain Structure	Accession number
dnaja1	I	complete	DnaJ-CXXCXGXG-DnaJ_C	JT413966
dnaja2	I	complete	DnaJ-CXXCXGXG-DnaJ_C	JT408916
dnaja2l	I	complete	DnaJ-CXXCXGXG-DnaJ_C	JT412411
dnaja3a	I	complete	DnaJ-CXXCXGXG-DnaJ_C	JT425696
dnaja3b	I	complete	DnaJ-CXXCXGXG-DnaJ_C	JT410497
dnaja4	I	complete	DnaJ-CXXCXGXG-DnaJ_C	JT340875
dnajb1a	II	complete	DnaJ-DnaJ_C	JT410595
dnajb1b	II	complete	DnaJ-DnaJ_C	JT405623
dnajb2	II	complete	DnaJ-3*(UIM)	JT413231
dnajb4	II	complete	DnaJ-DnaJ_C	JT418024
dnajb5	II	complete	DnaJ-DnaJ_C	JT410284
dnajb5L	II	complete	DnaJ-DnaJ_C	JT407526
dnajb6a	II	complete	DnaJ	JT280415
dnajb6b	II	complete	DnaJ	JT477624
dnajb9L1	II	complete	DnaJ	JT406977
dnajb9L2	II	complete	DnaJ	JT244156
dnajb9	II	Partial	DnaJ	JT383860
dnajb11	II	complete	DnaJ-DnaJ_C	JT410155
dnajb12a	II	complete	DnaJ-DUF1977	JT411397
dnajb12b	II	complete	DnaJ-DUF1977	JT348699
dnajb13	II	complete	DnaJ-DnaJ_C	JT413284
dnajb14	II	complete	DnaJ-DUF1977	JT417082
dnajc1	III	complete	DnaJ-SANT	JT407463
dnajc2	III	complete	(DnaJ)-2*(SANT)	JT342108
dnajc3(prkri)	III	complete	7*(TPR)-DnaJ	JT407497
dnajc3	III	complete	7*(TPR)-DnaJ	JT410821
dnajc4	III	complete	DnaJ	JT400171
dnajc5ab	III	complete	DnaJ	JT412549
dnajc5aa	III	complete	DnaJ	JT483994
dnajc5gb	III	complete	DnaJ	JT406264
dnajc6	III	complete	(PTPc_DSPc)-DnaJ	JT410375
dnajc7	III	complete	7*(TPR)-DnaJ	JT278330
dnajc8	III	complete	DnaJ	JT278586
dnajc9	III	complete	DnaJ	JT223126
dnajc10	III	complete	DnaJ-4*(Thioredoxin)	JT424718
dnajc11	III	complete	DnaJ-DUF3395	JT406478
dnajc12	III	complete	DnaJ	JT411276
dnajc13	III	complete	DnaJ	JT411283
dnajc14	III	complete	DnaJ	JT340805
dnajc15	III	complete	DnaJ	JT417797
dnajc16	III	complete	DnaJ-Thioredoxin	JT405377
dnajc16L	III	complete	DnaJ-Thioredoxin	JT413761
dnajc17	III	complete	DnaJ-RRM_1	JT411855
dnajc18	III	complete	DnaJ-DUF1977	JT414201
dnajc19	III	complete	DnaJ	JT348880
dnajc20(hscb)	III	complete	DnaJ-HSCB_C	JT410908
dnajc21	III	complete	DnaJ-ZnF_U1- ZnF_C2H2	JT414736
dnajc22	III	complete	TM2-4*(Transmembrane)- DnaJ	JT405460
dnajc23(sec63)	III	complete	DnaJ-Sec63	JT409443
dnajc24	III	complete	DnaJ-CSL zinc finger	JT407343
dnajc25	III	complete	DnaJ	JT406198
dnajc26(gak)	III	complete	S_TKc-STYKc-PTPc_DSPc-PTEN_C2-	JT418500
dnajc27	III	complete	Small GTPase-DnaJ	JT464820
dnajc28	III	complete	DnaJ-DUF1992	JT413325
dnajc29(sacs)	III	complete	UBQ-2*(HATPase_c)-DnaJ-HEPN	JT399814/JT345237
dnajc30a	III	complete	DnaJ	JT399756
dnajc30b	III	complete	DnaJ	JT199100

Table 2-2

Comparison of copy numbers of HSP40s genes among selected vertebrate genomes. Yellow shaded boxes indicated absence of HSP40 gene from chicken, frog, and teleost fish; orange-shaded boxes indicated absence of HSP40 gene from frog and teleost fish species.

	Gene	human	chicken	frog	zebrafish	catfish	medaka	tilapia	fugu
Type I	***Dnaja1***	1	1	1	1	1	1	1	
	Dnaja2	1	1	1	2	2	2	2	2
	Dnaja3	1	1	1	2	2	2	2	2
	Dnaja4	1	1	2	1	1	1	1	1
	Subtotal	4	4	5	6	6	6	6	5
Type II	***Dnajb1***	1		1	2	2	2	2	2
	Dnajb2	1	1	1	1	1	1	1	1
	Dnajb3	1							
	Dnajb4	1	1	1	1	1	1	1	1
	Dnajb5	1	1	1	2	2	2	2	3
	Dnajb6	1	1	2	2	2	2	2	2
	Dnajb7	1							
	Dnajb8	1	1						
	Dnajb9	1	1	1	3	3	2	3	1
	Dnajb11	1	1	1	1	1	2	1	1
	Dnajb12	1	1	1	3	2	2	2	2
	Dnajb13	1	1	1	1	1	1	1	1
	Dnajb14	1	1	1	1	1	2	1	1
	Subtotal	13	10	11	17	16	17	16	15
Type III	***Dnajc1***	1	1	1	1	1	1	1	1
	Dnajc2	1	1	1	1	1	1	1	1
	Dnajc3	1	1	1	2	2	2	2	2
	Dnajc4	1		1	1	1	1	1	1
	Dnajc5	3	1	2	5	3	2	1	2
	Dnajc6	1	1	1	1	1	1	1	1
	Dnajc7	1	1	1	2	1	2	2	2
	Dnajc8	1	1	1	1	1	1	1	1
	Dnajc9	1	1	1	1	1	1	1	1
	Dnajc10	1	1	1	1	1	1	1	1
	Dnajc11	1	1	1	2	1	2	2	2
	Dnajc12	1	1	1	1	1	1	1	1
	Dnajc13	1	1	1	1	1	1	1	1
	Dnajc14	1	1	1	1	1	1	1	1
	Dnajc15	1	1	1	1	1	1	1	
	Dnajc16	1	1	1	2	2	2	2	2
	Dnajc17	1	1	1	1	1	1	1	1
	Dnajc18	1	1	1	1	1	1	1	1

	Dnajc19	1	1	1	1	1	1	1	1
	Hscb(Dnajc20)	1	1	1	1	1	1	1	1
	Dnajc21	1	1	1	1	1	1	1	1
	Dnajc22	1	1	1	1	1	1	1	1
	Dnajc23	1	1	1	1	1	1	1	1
	Dnajc24	1	1	1	1	1	1	1	1
	Dnajc25	1	1	1	1	1	1	1	1
	Dnajc26	1	1	1	1	1	1	1	1
	Dnajc27	1	1	1	1	1	1	1	1
	Dnajc28	1	1	1	1	1	1	1	1
	Dnajc29	1	1	1	1	1	1	1	1
	Dnajc30	1	1	1	2	2	1	1	2
	Subtotal	32	29	31	39	35	35	34	35
	Total	49	43	47	62	57	58	56	55

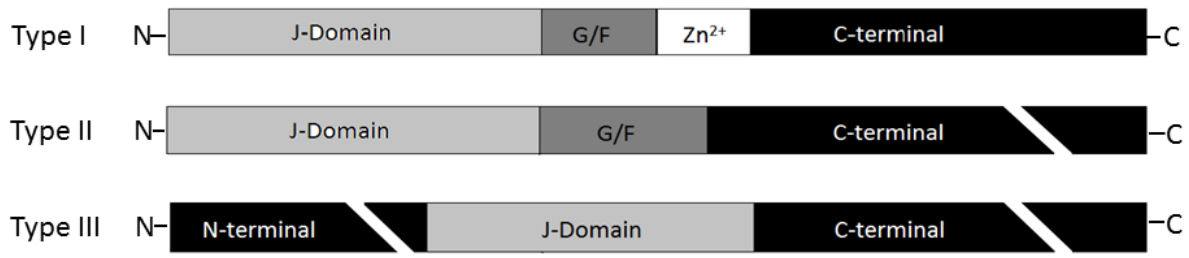


Figure 2- 1.

Schematic presentation of three sub-types of HSP40 family. Type I DnaJ proteins have full domain conservation with *Escherichia coli* DnaJ, type II DnaJ proteins have a J domain and G/F (Gly/Phe-rich region) motif at N-terminus, type III DnaJ proteins only have a J domain anywhere in the protein.

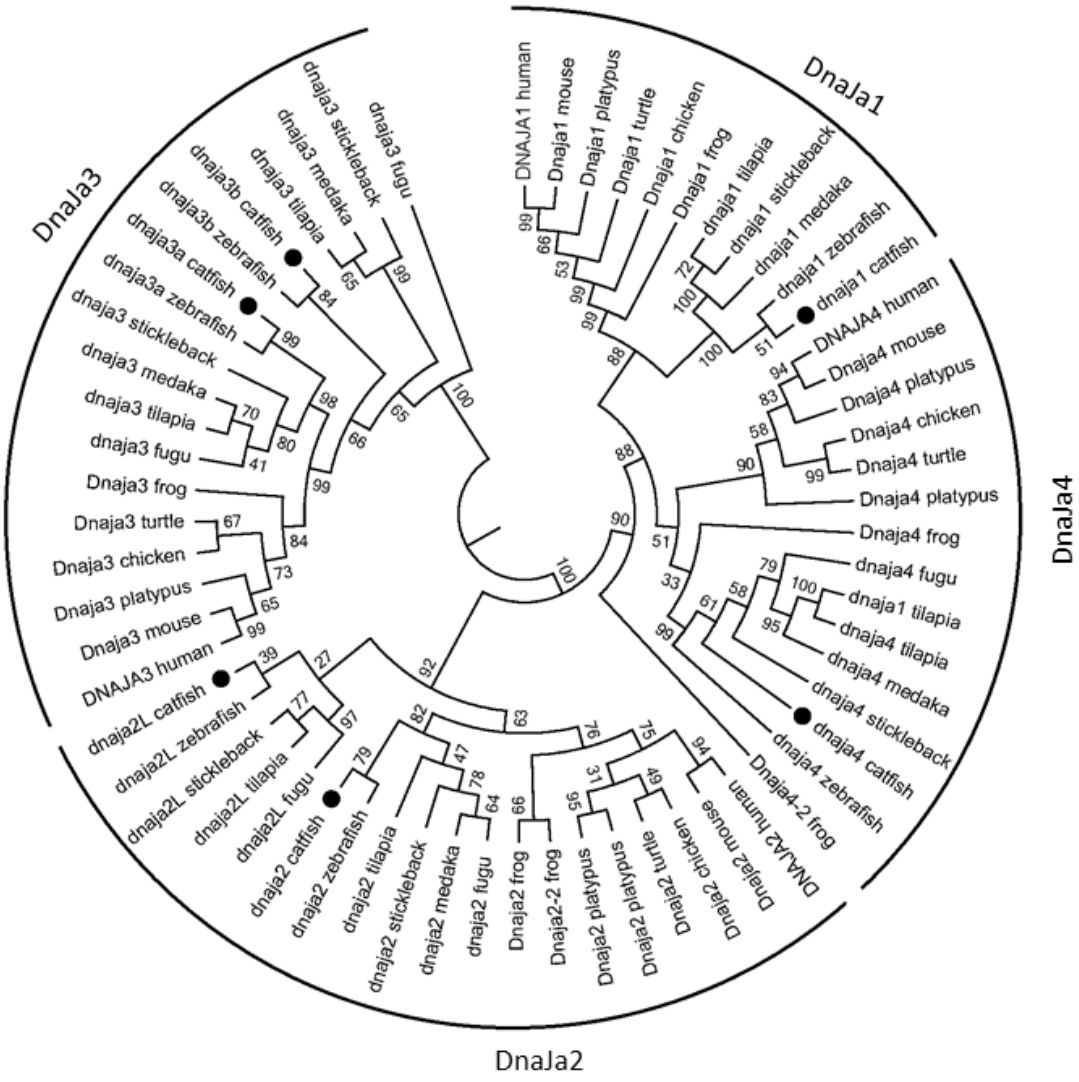


Figure 2- 2.

Phylogenetic tree of Hsp40s subfamily A. The phylogenetic tree was constructed by Mega5.2.2 using the Maximum Likelihood method based on the JTT matrix-based model of amino acid substitution as described in detail in Material and Method section. Numbers around the nodes correspond to bootstrap support values in percentages. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.1981)). The rate variation model allowed for some sites to be evolutionarily invariable ((+I), 2.8567% sites). All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps,

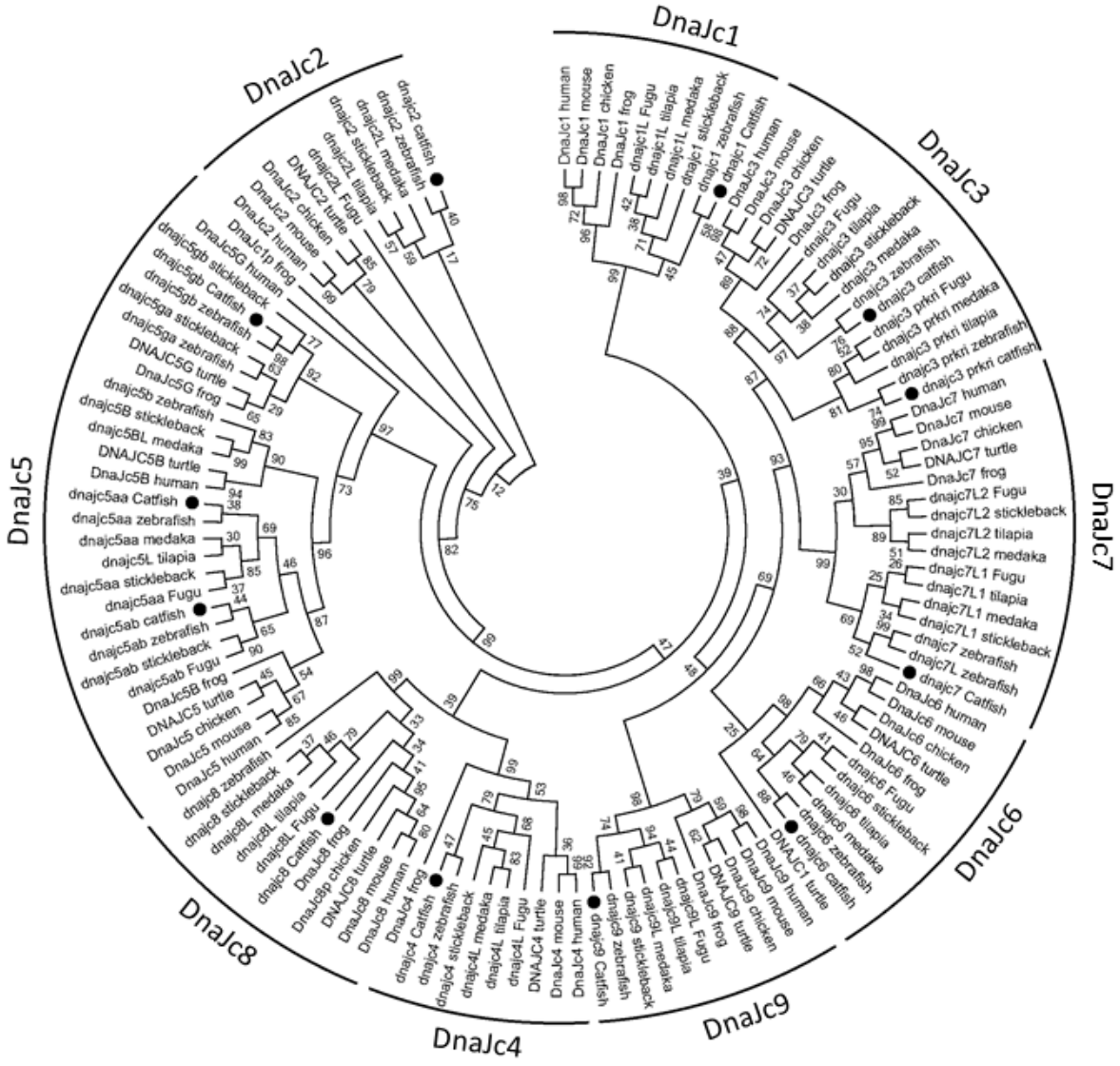
missing data, and ambiguous bases were allowed at any position. There were a total of 332 positions in the final dataset. Accession numbers for all protein sequences used in the analysis are provided in Table S1. The black dots indicate catfish dnaja genes. Suffix “L” indicated “-like”, for instance, dnaja2L means dnaja2-like.

Figure 2- 3

Phylogenetic tree of Hsp40s subfamily B. The phylogenetic tree was constructed by Mega5.2.2 using the Maximum Likelihood method based on the JTT matrix-based model of amino acid substitution as described in detail in Material and method section. The bootstrap consensus tree inferred from 1000 replicates is taken. Numbers around the nodes correspond to bootstrap support values in percentages. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 1.1669)). The rate variation model allowed

for some sites to be evolutionarily invariable ((+I), 0.2358% sites). All positions with less than 95% site coverage were eliminated. There were a total of 158 positions in the final dataset. Accession numbers for all sequences are provided in Table S1. Suffix “L” indicated “-like”, for instance, dnajb5L means Dnajb5-like.

Figure 2-4A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DnaJc3
DnaJc7
DnaJc6
DnaJc9
DnaJc4
DnaJc8
DnaJc5
DnaJc2
DnaJc1 human
DnaJc1 mouse
DnaJc1 chicken
DnaJc1 frog
dnajc1L Fugu
dnajc1L tilapia
dnajc1L medaka
dnajc1 stickleback
dnajc1 zebrafish
dnajc1 Catfish
DnaJc3 human
DnaJc3 mouse
DnaJc3 chicken
DNAJC3 turtle
DnaJc3 frog
dnajc3 Fugu
dnajc3 tilapia
dnajc3 stickleback
dnajc3 medaka
dnajc3 zebrafish
dnajc3 catfish
dnajc3 prkri Fugu
dnajc3 prkri medaka
dnajc3 prkri tilapia
dnajc3 prkri zebrafish
dnajc3 prkri catfish
DnaJc7 human
DnaJc7 mouse
DnaJc7 chicken
DNAJC7 turtle
DnaJc7 frog
dnajc7L2 Fugu
dnajc7L2 stickleback
dnajc7L2 tilapia
dnajc7L2 medaka
dnajc7L1 Fugu
dnajc7L1 tilapia
dnajc7L1 medaka
dnajc7L1 stickleback
dnajc7 zebrafish
dnajc7L zebrafish
dnajc7 Catfish
DnaJc6 human
DnaJc6 mouse
DnaJc6 chicken
DNAJC6 turtle
DnaJc6 frog
dnajc6 Fugu
dnajc6 stickleback
dnajc6 tilapia
dnajc6 medaka
dnajc6 zebrafish
dnajc6 catfish
DNAJC1 turtle
DnaJc9 human
DnaJc9 mouse
DnaJc9 chicken
DNAJC9 turtle
DnaJc9 frog
dnajc9L Fugu
dnajc9L tilapia
dnajc9L medaka
dnajc9 stickleback
dnajc9 zebrafish
dnajc9 Catfish
DnaJc4 human
DnaJc4 mouse
DNAJC4 turtle
dnajc4L Fugu
dnajc4L tilapia
dnajc4L medaka
dnajc4 stickleback
dnajc4 zebrafish
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DnaJc4 frog
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DnaJc8 human
DnaJc8p chicken
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dnajc8 zebrafish
DnaJc5 human
DnaJc5 mouse
DnaJc5 chicken
DNAJC5 turtle
DnaJc5B frog
dnajc5ab Fugu
dnajc5ab stickleback
dnajc5ab zebrafish
dnajc5ab catfish
dnajc5aa Fugu
dnajc5aa stickleback
dnajc5L tilapia
dnajc5aa medaka
dnajc5aa zebrafish
dnajc5aa Catfish
DnaJc5B human
DNAJC5B turtle
dnajc5BL medaka
dnajc5B stickleback
dnajc5b zebrafish
DnaJc5G frog
DNAJC5G turtle
dnajc5ga zebrafish
dnajc5ga stickleback
dnajc5gb Catfish
dnajc5gb stickleback
DnaJc5G human
DnaJc1p frog
DnaJc2 mouse
DnaJc2 human
DnaJc2 chicken
DNAJC2 turtle
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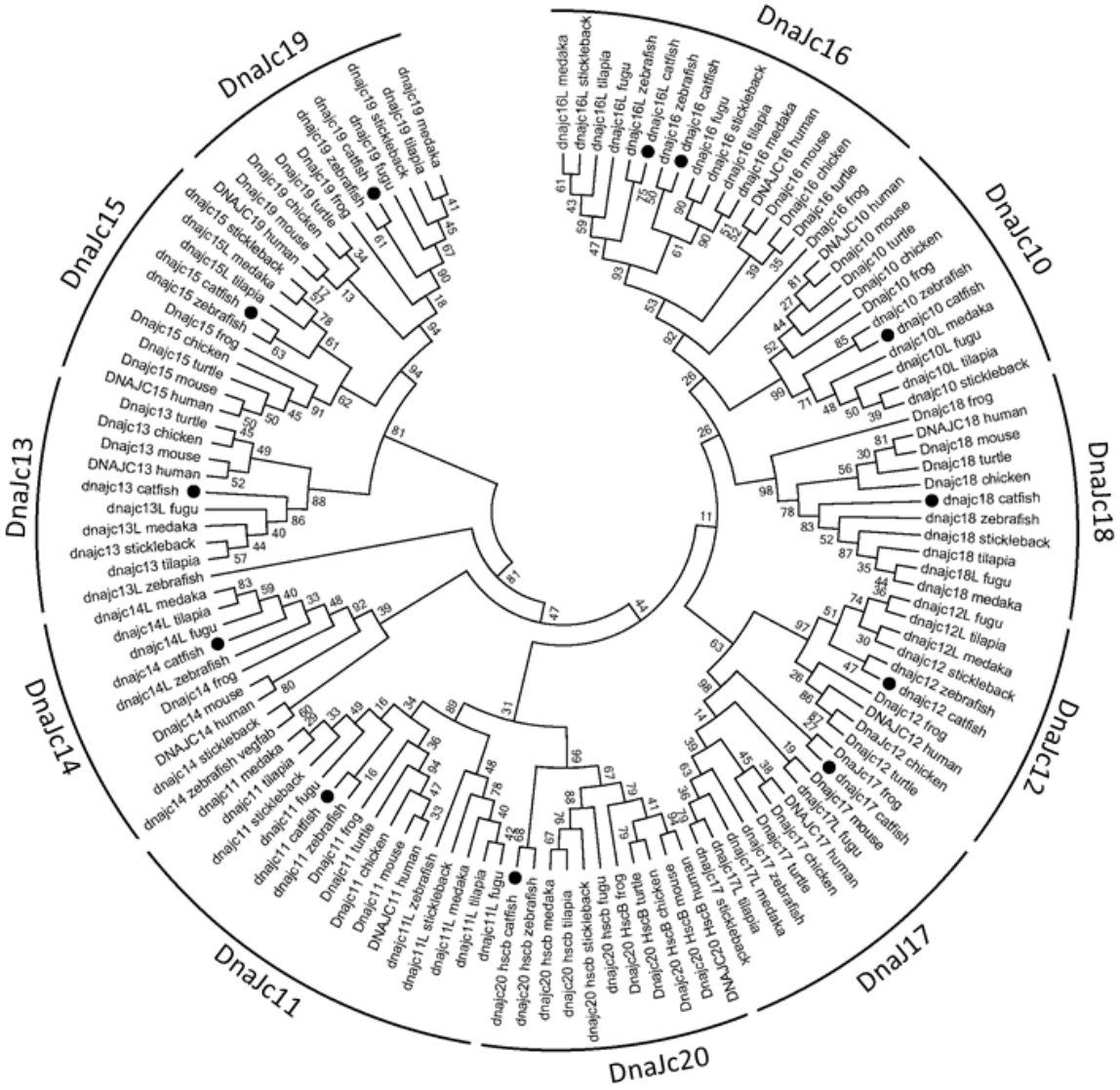
dnajc2L medaka
dnajc2 zebrafish
dnajc2 catfish

Figure 2-4B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DnaJc18
DnaJc12
DnaJ17
DnaJc20
DnaJc11
DnaJc14
DnaJc13
DnaJc15
DnaJc19
dnajc16L medaka
dnajc16L stickleback
dnajc16L tilapia
dnajc16L fugu
dnajc16L zebrafish
dnajc16L catfish
dnajc16 zebrafish
dnajc16 catfish
dnajc16 fugu
dnajc16 stickleback
dnajc16 tilapia
dnajc16 medaka
DNAJC16 human
Dnajc16 mouse
Dnajc16 chicken
Dnajc16 turtle
Dnajc16 frog
DNAJC10 human
Dnajc10 mouse
Dnajc10 turtle
Dnajc10 chicken
Dnajc10 frog
Dnajc10 zebrafish
dnajc10 catfish
dnajc10L medaka
dnajc10L fugu
dnajc10L tilapia
dnajc10 stickleback
Dnajc18 frog
DNAJC18 human
Dnajc18 mouse
Dnajc18 turtle
Dnajc18 chicken
dnajc18 catfish
dnajc18 zebrafish
dnajc18 stickleback
dnajc18 tilapia
dnajc18L fugu
dnajc18 medaka
dnajc12L fugu
dnajc12L tilapia
dnajc12L medaka
dnajc12 stickleback
dnajc12 zebrafish
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Dnajc12 chicken
Dnajc17 turtle
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dnajc17 catfish
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Dnajc17 mouse
DNAJC17 human
Dnajc17 chicken
Dnajc17 zebrafish
Dnajc17 turtle
dnajc17L medaka
dnajc17L tilapia
dnajc17 stickleback
DNAJC20 HscB human
Dnajc20 HscB mouse
Dnajc20 HscB chicken
Dnajc20 HscB turtle
dnajc20 HscB frog
dnajc20 hscb fugu
dnajc20 hscb stickleback
dnajc20 hscb tilapia
dnajc20 hscb medaka
dnajc20 hscb zebrafish
dnajc20 hscb catfish
dnajc11L fugu
dnajc11L tilapia
dnajc11L medaka
dnajc11L stickleback
dnajc11L zebrafish
DNAJC11 human
Dnajc11 mouse
Dnajc11 chicken
Dnajc11 turtle
Dnajc11 frog
dnajc11 zebrafish
dnajc11 catfish
dnajc11 fugu
dnajc11 stickleback
dnajc11 tilapia
dnajc11 medaka
dnajc14 zebrafish vegfab
dnajc14 stickleback
DNAJC14 human
Dnajc14 mouse
Dnajc14 frog
dnajc14L zebrafish
dnajc14 catfish
dnajc14L fugu
dnajc14L tilapia
dnajc14L medaka
dnajc13L zebrafish
dnajc13 tilapia
dnajc13 stickleback
dnajc13L medaka
dnajc13L fugu
dnajc13 catfish
DNAJC13 human
Dnajc13 mouse
Dnajc13 chicken
Dnajc13 turtle
DNAJC15 human
Dnajc15 mouse
Dnajc15 turtle
Dnajc15 chicken
Dnajc15 frog
Dnajc15 zebrafish
dnajc15 catfish
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dnajc15 stickleback
DNAJC19 human
Dnajc19 mouse
Dnajc19 chicken
Dnajc19 turtle
Dnajc19 frog
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dnajc19 medaka

Figure 2-4C

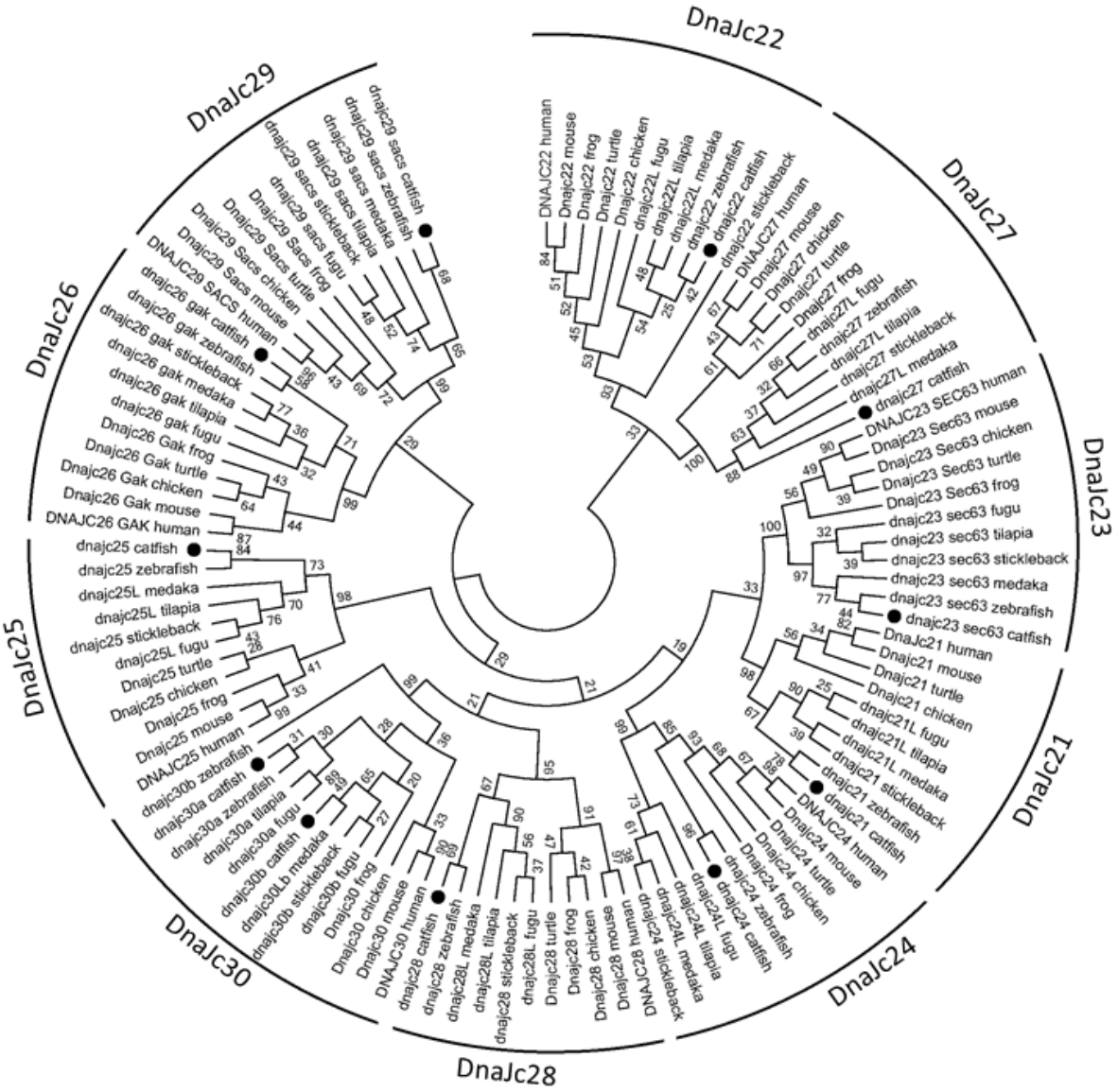

Figure 2- 4

Phylogenetic tree of Hsp40s subfamily C. Members of Dnajc1-Dnajc9 are shown in Figure 2-4A. Members of Dnajc10-Dnajc20 are shown in Figure 2-4B. Members of Dnajc21-Dnajc30 are shown in Figure 2-4C. The phylogenetic tree was constructed by Mega5.2.2 using maximum

likelihood algorithm under the JTT+I+G model of amino acid substitution as described in detail in Material and method section. Numbers around the nodes correspond to bootstrap support values in percentages. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 4.0831 for Fig 4A.; (+G, parameter = 12.3665) for Figure 2-4B.;) (+G, parameter = 6.1230) for Figure 2-4C.). The rate variation model allowed for some sites to be evolutionarily invariable ((+I), 0.0000% sites for all the Figures). All positions with less than 95% site coverage were eliminated. There were a total of 162 positions in the final dataset of Figure 2-4A, 94 positions in Figure 2-4B and 93 positions in Figure 2-4C. Accession numbers for all sequences are provided in Table S2-1. The black dots indicate catfish Dnajc genes. Suffix “L” indicated “-like”, for instance, Dnajc7L means Dnajc7-like.

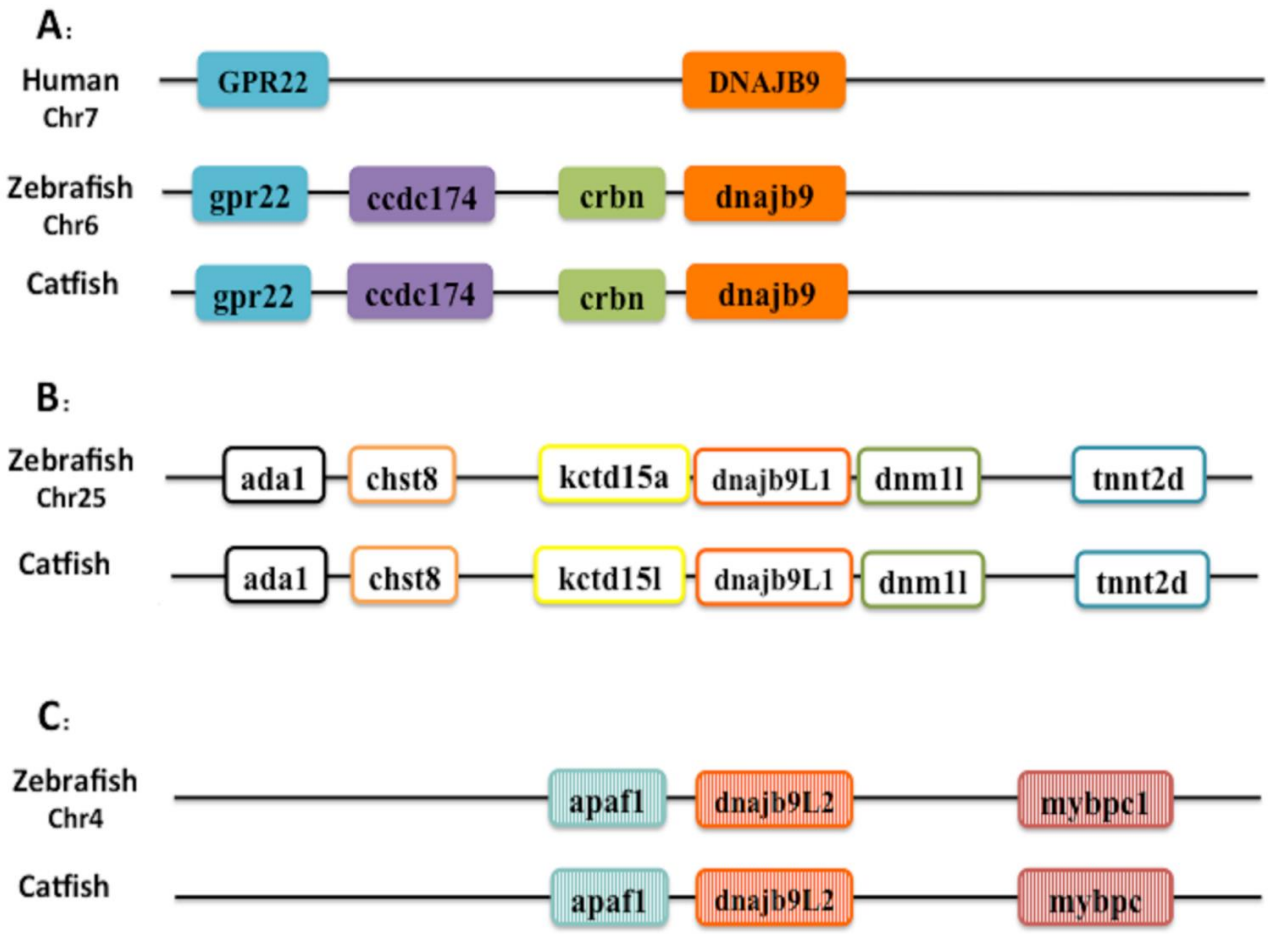

Figure 2- 5

Schematic presentation of the conserved synteny blocks neighboring Dnajb9 gene (A) Dnajb9-like1 gene (B) and Dnajb9-like2 gene (C). Note that in each case, the gene order and orientation was relatively conserved. Abbreviations: (A) GPR22, G Protein Receptor 22; Dnajb9, Dnaj (Hsp40) homolog, subfamily B, member 9; ccdc174, Coiled-Coil Domain Containing 174; crbn, cereblon. (B) ada1, Adenosine Deaminase; chst8, carbohydrate (N-acetylgalactosamine 4-0) sulfotransferase 8; kctd15a, potassium channel tetramerisation domain containing 15a; dnajb9L1 DnaJ (Hsp40) homolog, subfamily B, member 9 like 1; dnm1l, dynamin 1-like; tnnt2d, troponin T2d, cardiac. (C) apaf1, apoptotic protease activating factor 1; dnajb9l2 DnaJ (Hsp40) homolog, subfamily B, member 9 like 2; mybpc, myosin binding protein C.

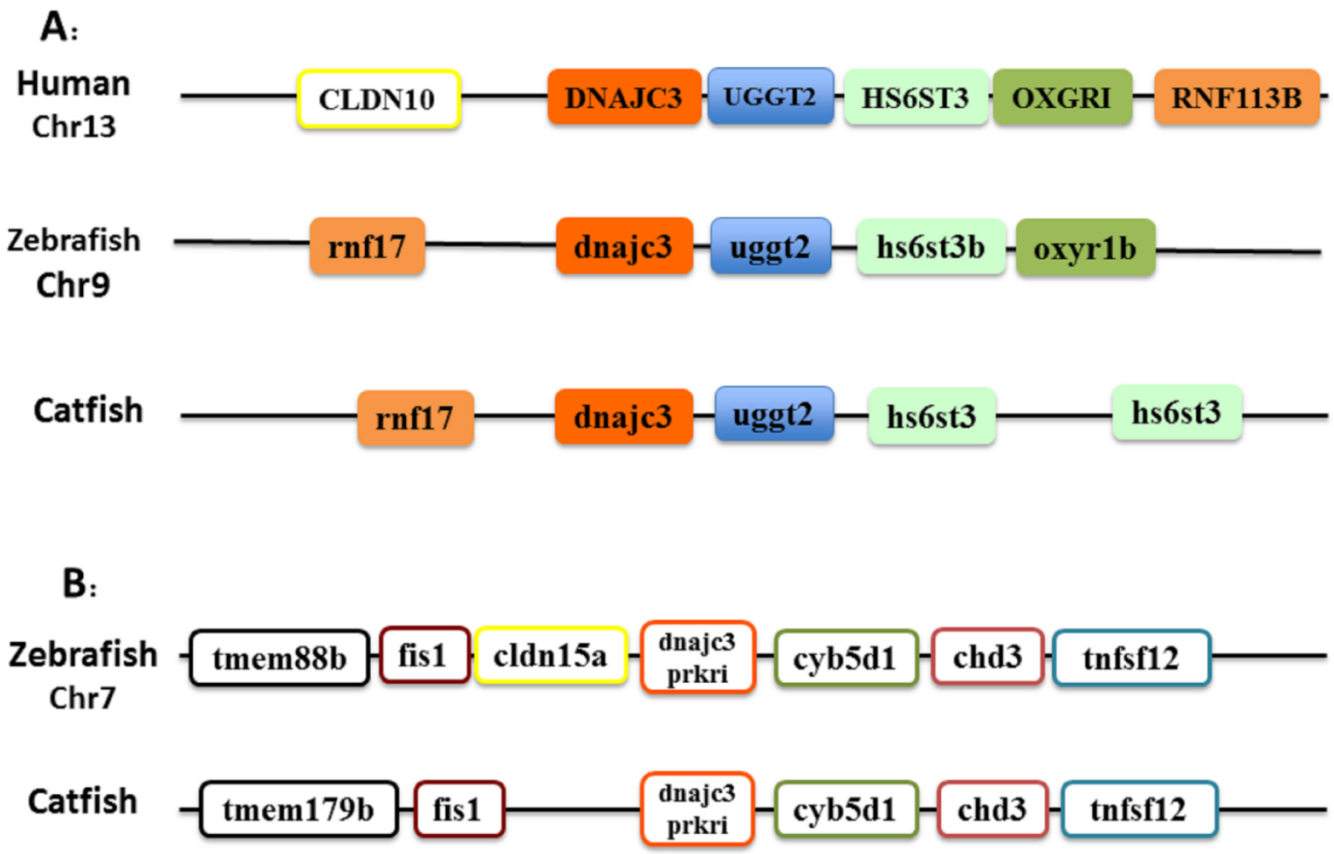

Figure 2- 6

Schematic presentation of the conserved synteny blocks neighboring DnaJc3 (A) and DnaJc3-prkri gene (B). Note that in each case, the gene order and orientation was relatively conserved. Abbreviations: (A) CLDN10, claudin 10; rnf17, ring finger protein 17; DnaJc3, DnaJ (Hsp40) homolog, subfamily C, member 3; uggt2, UDP-glucose glycoprotein glucosyltransferase 2; hs6st3b, heparan sulfate 6-O-sulfotransferase 3b; oxyr1b, oxoglutarate (alpha-ketoglutarate) receptor 1b; RNF113B, ring finger protein 113B. (B) tmem88b, transmembrane protein 88 b; fis1, fission 1 (mitochondrial outer membrane) homolog (S. cerevisiae); cldn15a, claudin 15a; dnajc3 prkri, protein-kinase, interferon-inducible double stranded RNA dependent inhibitor; cyb5d1, cytochrome b5 domain containing 1; chd3, chromodomain helicase DNA binding protein 3; tnfsf12, tumor necrosis factor (ligand) superfamily, member 12.

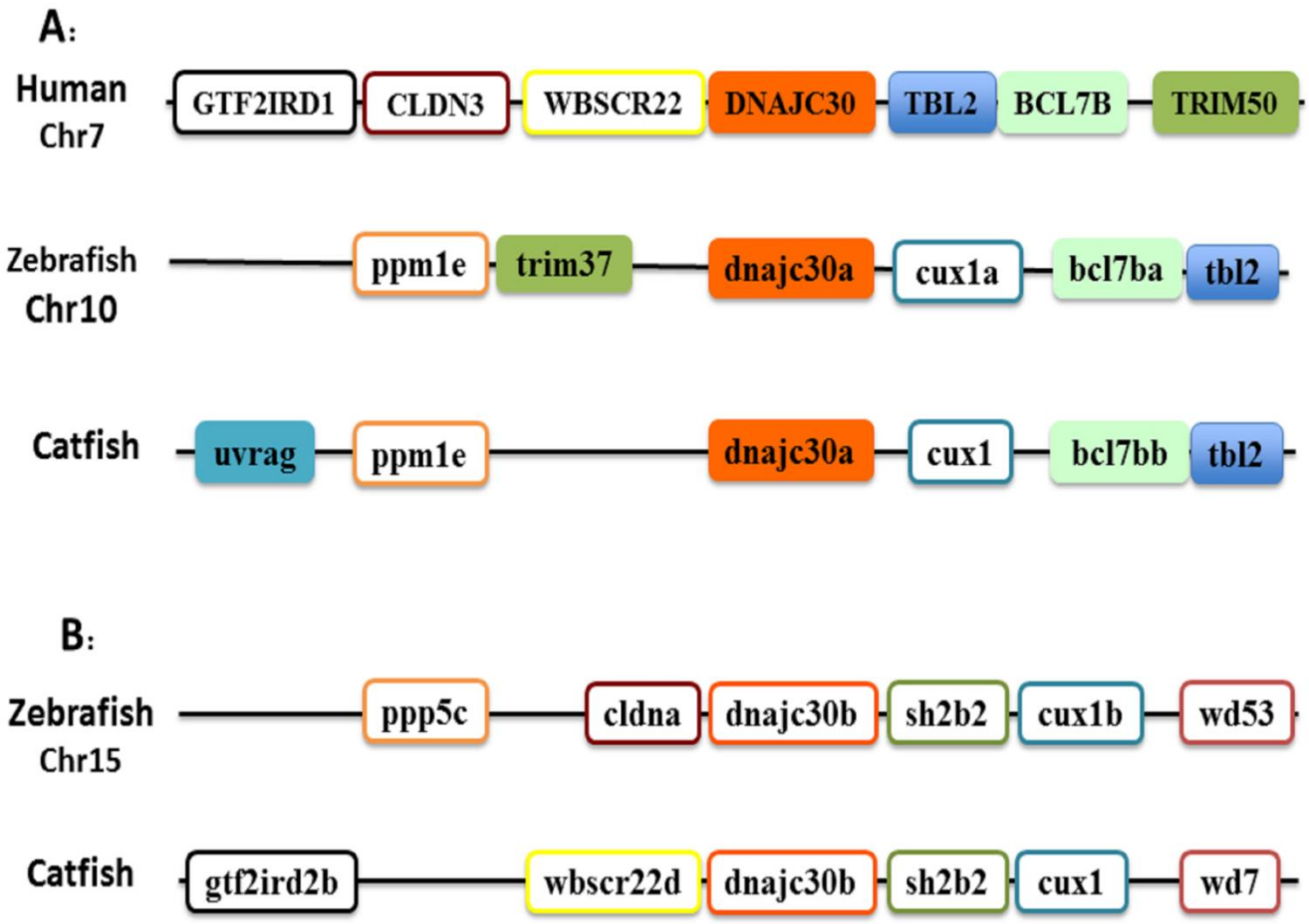

Figure 2- 7

Schematic presentation of the conserved synteny blocks neighboring DnaJc30a (A) and DnaJc30b gene (B). Note that in each case, the gene order and orientation was relatively conserved.Abbreviations: (A) GTF2IRD1, GTF2I repeat domain containing 1; CLDN3, claudin 3; WBSCR22, Williams Beuren syndrome chromosome region 22; DnaJc30, DnaJ (Hsp40) homolog, subfamily C, member 30; TBL2, transducin (beta)-like 2; BCL7B, B-cell CLL/lymphoma 7B; TRIM50, tripartite motif containing 50; ppm1e, protein phosphatase 1E (PP2C domain containing); trim37, tripartite motif containing 37; cux1a, cut-like homeobox 1a; uvrag, UV radiation resistance associated. (B) ppp5c, protein phosphatase 5, catalytic subunit; cldna, claudin a; dnajc30b, DnaJ (Hsp40) homolog, subfamily C, member 30;sh2b2, fission 1 (mitochondrial outer membrane) homolog (S. cerevisiae); wd53, WD repeat domain 53; wd7, WD repeat domain 7; gtf2ird2b, GTF2I repeat domain containing 2b.

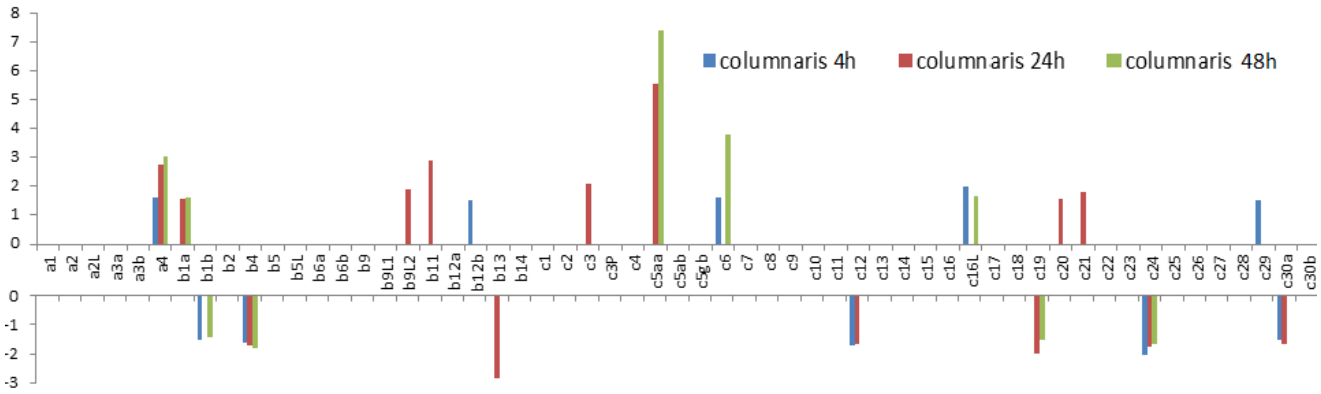

Figure 2- 8

Column bar chart showing the fold change of Hsp40s expression in *F. Columnare* challenge experiments. Vertical axis shows the value of fold change. Gene names are shown as clipped name without "dnaj". For instance, a1 means "*dnaja1*". "c3P" represents "*dnajc3_prkri*".

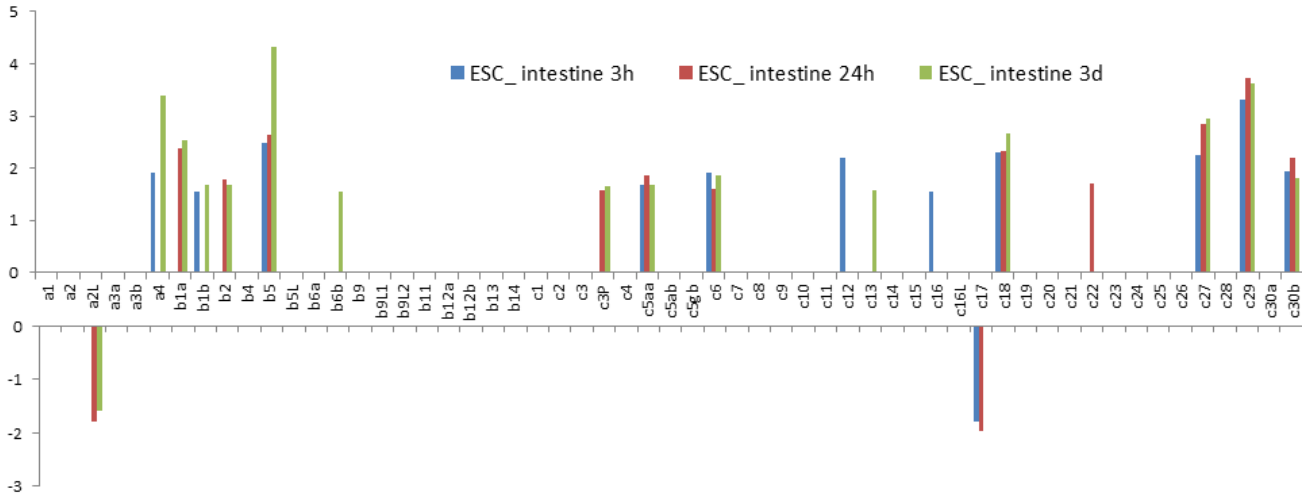

Figure 2-9

Column bar chart showing the fold change of Hsp40s expression in *E. ictaluri* challenge experiments. Vertical axis shows the value of fold change. Gene names are shown as clipped name without "dnaj". For instance, a1 means "*dnaja1*". "c3P" represents "*dnajc3_prkri*".

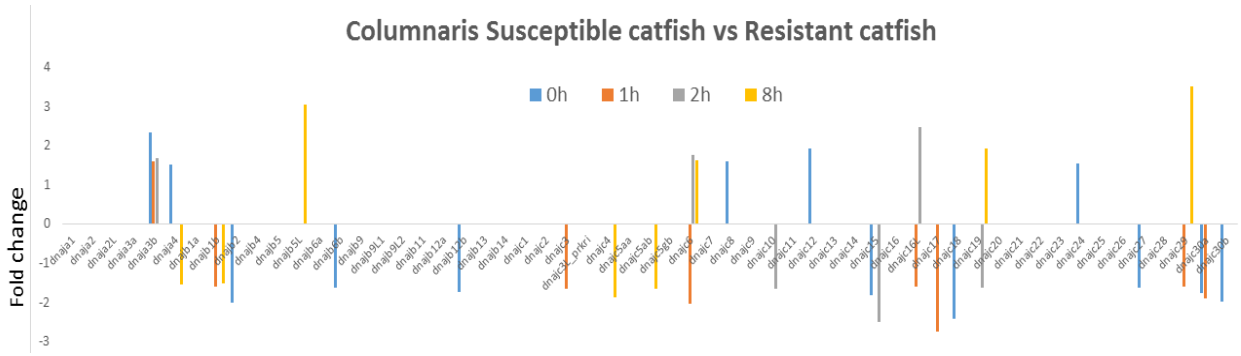

Figure 2-10. Differentially expressed hsp40 genes in the gill between catfish resistant and susceptible to *F. columnare*.

Chapter 3. Genome-wide Identification of Hsp70/110 Genes in Channel Catfish and Their Regulated Expression after Bacterial Infection

3.1. Abstract

In this study, I identified 16 *hsp70/110*s in channel catfish (*Ictalurus punctatus*) through *in silico* analysis using RNA-Seq and genome databases. Among them 12 members of hsp70 (hspa) family and 4 members of hsp110 (hsph) family were identified. Phylogenetic and syntenic analyses provided strong evidence in supporting the orthologies of these HSPs. Besides, two tandem repeat members of Hsp70/110s, *hsp70.2* and *hsp70.3* were not found located within the MHC-complex, which is a group of molecules that are essential in the antigen presenting process, suggesting a different process of presenting the antigens or epitopes. Meta-analyses of RNA-seq datasets were conducted to analyze expression profile of *Hsp70/110*s following bacterial infections. Hsp70/110s expression was analyzed through the early phase of immune response. Twelve out of sixteen genes were significantly up/down-regulated after bacterial challenges. Specifically, ten genes were found significant expressed in gill after *F. columnare* infection. Ten genes were found significant expressed in intestine after *E. ictaluri* infection. Pathogen-specific pattern and time pattern are found in the two infections. The significant regulated expressions of catfish Hsp70 genes after bacterial infection suggested their involvement in immune response in catfish. Additional RNA-seq dataset was used to profile gill Hsp70 expression differences between resistant and susceptible fish group post *F. columnare* challenge at both basally (before infection) and at three early timepoints (1 h, 2 h, and 8 h). Six genes showed significant differential expression between groups

for at least one timepoint following infection. Among them, one gene, *hsp70.3* was found significantly differentially expressed at the basal level between resistant and susceptible fish family, which could potentially serve as expression QTL and biomarkers for selection.

Keywords: Heat shock protein; Hsp70; catfish; genome; immunity; infection.

3.2. Introduction---Heat shock protein 70 and Heat shock protein 110 (HSPA and HSPH):

The discovery of heat inducible chromosome puffs in salivary glands of Drosophila by Ritossa in 1962, started a wide expanded field of Heat shock response studies (Ritossa, 1962; Moran et al., 1978). Several studies found that a mild non-lethal dose of heat shock could protect the cells from death, which were caused by a later and more severe heat or other stresses (Gerner et al., 1976; Henle et al., 1978; Petersen and Mitchell, 1981; Sapareto et al., 1978). Soon it was clear that the protection came from the induced and accumulated heat inducible proteins, which were designated as Heat shock proteins (HSPs). Among all the HSPs, 70 KDa protein (Hsp70) is one of the most heat inducible and protective proteins. In 1984 Hugh Pelham suggested that the ability of Hsp70 protect the proteins from heat stress was due to their abilities to help the damaged ribosomal proteins reassemble (Pelham, 1984). Shortly after the chaperone function was revealed, it also had been discovered that some HSPs were constitutively expressed in non-stressed cells as housekeeping proteins, such as Hspa8 in the Hsp70 family. The housekeeping functions of Hsp70 include transport of proteins between cellular compartments, degradation of unstable and misfolded proteins, prevention and dissolution of protein complexes, folding and refolding of proteins, uncoating of clathrin coated vesicles, and control of regulatory proteins. Unlike Hsp90, Hsp70 doesn't require a "client" protein. Instead, they bind to any exposed hydrophobic residue of the newly synthesized proteins or misfolded proteins caused by stresses to prevent their aggregation. Hsp70s also can directly unfold misfolded proteins, in an adenosine triphosphate (ATP)-dependent way (Bukau et al., 2006; Hartl, 1996; Jäättelä, 1999; Lindquist and Craig, 1988; Watowich and Morimoto, 1988).

HSPs are classified based on their molecular weight and their functional domains to include Hsp110 (HSPH), Hsp90 (HSPC), Hsp70 (HSPA), Hsp60 (HSPD), Hsp40 (DNAJ), Hsp10 (HSPE),

and small HSPs (HSPB) (Feder and Hofmann, 1999; Kampinga, 2009). Among them, Hsp70 is one of the most conserved proteins in evolution. It is found in all organisms from archaebacteria and plants to humans. A high amino acid identity of approximately 50% is shared between the prokaryotic Hsp70 protein DnaK and eukaryotic Hsp70 proteins (Takayama et al., 1999).

Another group of Hsps, Hsp110, share a high level of similarity in domain structure with Hsp70, and therefore, the Hsp110 (HSPH) were often studied and discussed with Hsp70 (HSPA) as Hsp70/110 family. Due to the gene duplication during evolution, the numbers of gene coding for the Hsp70/110 members varied in different organisms. For example, there are 13 HSPA members in human whereas 3 members in *Escherichia coli*. This feature satisfied the need for tissue specific or developmental expression and provided functional diversity during evolution.

Hsp70 proteins of all known species display highly conserved amino acid sequences and domain structures consisting of: (i) a conserved ATPase domain; (ii) a middle region with protease sensitive sites; (iii) a peptide binding domain; and (iv) a G/P-rich C-terminal region containing an EEVD-motif enabling the proteins to bind co-chaperones and other (Daugaard et al., 2007; Mayer and Bukau, 2005). Furthermore, the members localized to specific cellular compartments have a localization signal in their N-terminus. The conserved domain structure consolidates the chaperone function of the Hsp70 proteins and enables them to bind and release extended stretches of hydrophobic amino acids, exposed by incorrectly folded globular proteins in an ATP-dependent manner. The C-termini contain the least conserved sequences that may explain the non-redundant functions of Hsp70 family members (Munro and Pelham, 1987). The Hsp110 proteins have a high homology to Hsp70 members except for the existence of a longer linker domain between the N-terminal ATPase domain and the C-terminal peptide binding domain. In fact, two members (with Human nomenclature), HSPA4 (HSPH2) and HSPA4L (HSPH3), were also considered as HSPA

members in the Entrez Gene database. In addition to variations in their sequences, Hsp70 /110 family also exhibit compartment-specific expression patterns (Dragovic et al., 2006; Raviol et al., 2006). In humans, for example, the Hsp70/110 family comprises 17 unique gene products that differ from one another by amino acid sequences, expression levels and sub-cellular localization. Thirteen of them are belong to HSP70 family, whereas the remaining four are classified into HSP110 family. The localization of HSPA5 (also known as Bip or Grp78) is within the lumen of the ER and HSPA5 has a C-terminal retention signal sequence that inhibits its exit from the ER (Gidalevitz et al., 2013). HSPA9 is the mitochondrial housekeeping HSPA member (HSPA9 is also known as mortalin/mtHSP70/GRP75). HSPA13 (also known as stch) is found in microsomes and may yet be another compartment-specific HSPA member. HSPA13 may function in regulating cell proliferation and survival, and modulating the TRAIL-mediated cell death pathway. HSPA13 gene is a candidate stomach cancer susceptibility gene; a mutation in the NBD coding region of HSPA13 has been identified in stomach cancer cells (Bae et al., 2013). The remaining Hsp70 proteins reside mainly in the cytosol suggesting that they either display specificity for their client proteins (Tavaria et al., 1996). HSPA1A and HSPA1B are the most studied genes, the products of which only differ by two amino acids. Together with HSPA6, these three proteins are the most heat-inducible family members. HSPA1L and HSPA2 are two constitutive expressed cytosolic family members with high level in the testis. HSPA8 is the cognate HSPA and was designated previously as Hsc70 (or HSP73). It is an essential “house-keeping” HSPA member and is involved in co-translational folding and protein translocation across intracellular membranes (Goldfarb et al., 2006; Liu et al., 2012b; Ziemienowicz et al., 1995). HSPA12A is predominantly expressed in neuronal cells. It may also play a role in the atherosclerotic process (Dansky et al., 2002; Han et al., 2003). HSPA12B is predominantly expressed in endothelial cells, is required for angiogenesis,

and may interact with known angiogenesis mediators. HSPA12B may also be important for host defense in microglia-mediated immune response. Its expression is up-regulated in lipopolysaccharide (LPS)-induced inflammatory response in the spinal cord, and mostly located in active microglia; this induced expression may be regulated by activation of MAPK-p38, ERK1/2 and SAPK/JNK signaling pathways. Overexpression of HSPA12B also protects against LPS-induced cardiac dysfunction and involves the preserved activation of the PI3K/Akt signaling pathway (Steagall et al., 2006). HSPA14 is a potent T helper cell (Th1) polarizing adjuvant that contributes to antitumor immune responses.

In Hsp110 family has four members. Three of the four Hsp110 proteins, HSPH1, HSPA4, HSPA4L, are cytosolic members whereas one, HYOU1, is compartment-specific and present in the ER (Rossetti et al., 2011; Wan et al., 2004). Recent evidence indicated that Hsp110 members are nucleotide exchange factors for the HSPA family (Dragovic et al., 2006; Raviol et al., 2006).

Besides the chaperone and housekeeping functions, there is plenty of evidence suggesting the involvement of Hsp70 in immune response in vertebrates. Rammas et al. showed that HSP70 induced interleukin-12 (IL-12) and endothelial cell-leukocyte adhesion molecule-1 (ELAM-1) promoters in macrophages (Vabulas et al., 2002). Members of the Hsp70 cytosolic group are either constitutively expressed (Hspa8) or can be induced by a broad range of stress factors (refs). The inducible Hsp70 has been recently characterized as a potent maturation stimulus for Dendritic Cells (DCs), which is an important professional antigen presenting cell (APC) that express MCH (Major Histocompatibility Complex) class II molecule on its surface and process the antigen into epitopes while migrating to the thymus to present the epitopes to the CD4+ T cell. This process is considered as the bridge of innate immunity and adaptive immunity (Kuppner et al., 2001; Vanbuskirk et al., 1989).

Heat shock proteins, participating as a danger signal in the host immune response, play an important role in innate immune response or even in adaptive immunity. The acute immune response is organized and executed by innate immunity influenced by the neuroendocrine system. This response starts with sensing of danger signals by pattern-recognition receptors on the immune competent cells and endothelium. TLR4 is involved in signaling of both exogenous and endogenous danger signals. Hsp70s are one of these danger signals and act as the agonist of TLR4 (Castellheim et al., 2009). One rare type of CD4+ T cells, which is called γδ T cell, are believed to be triggered by alarm signals such as HSPs and it is only found abundant in gut mucosa. However, common with the immune cells in innate immunity, this 'unconventional' T cell have the ability of the antigen recognition by its T cell receptor (one of the Pattern Recognition Receptors-PRR), whereas the other T cells can't recognize the free antigen and require the APC or MHC molecules to present the processed epitopes to them. Therefore, this γδ T cell is an immune cell that bear both characters of innate immune and adaptive immune (Haregewoin et al., 1989).

In teleost, hsp70 genes have been found to be involved in bacterial kidney disease in coho salmon (Forsyth et al., 1997) and vibriosis in rainbow trout (Ackerman and Iwama, 2001). In olive flounder, Hsp40 proteins were found to be up-regulated in flounder embryonic cells (FEC) after viral infection and a flounder hsp70 gene was also expressed in heat-shocked and virus treated FEC cells (Dong et al., 2006), indicating *hsp40* and *hsp70* functioned as co-chaperone in antiviral immune responses. In channel catfish, Hsp70 was suggested involving in the early stages of the systemic response of channel catfish to bacterial infection. Upon exposure to *E. ictaluri*, expression of one member of Hsp70s significantly increased in the anterior kidney (48, 72, and 96

hpi) and spleen (48 and 72 hpi) but had little effect on liver expression (Elibol-Flemming et al., 2009).

Channel catfish (*Ictalurus punctatus*) is the leading aquaculture species in the United States. It is also an important model for the study of the teleost immune system (Bengten et al., 2006). However, in recent years, the catfish industry has encountered huge losses due to diseases outbreaks. Columnaris disease is the most frequently occurring disease in fish including catfish (Bullock et al., 1986). It is responsible for significant economic losses in freshwater fish aquaculture worldwide. Compared with columnaris disease, ESC disease is less frequent in occurrence, but is among the most severe diseases of catfish (Klesius, 1992). Economic losses to the catfish industry are in the tens of millions of dollars annually and the problem may become even more severe with the growth of the catfish industry. The catfish genomic resources have been well developed in recent years, particularly ESTs (Li et al., 2007; Wang et al., 2010), transcriptome sequences generated by RNA-Seq (Liu et al., 2012a; Liu et al., 2011) and draft whole genome sequence (unpublished data). These resources make it feasible to conduct systematic analysis of interested genes in channel catfish genome. The objective of this study was to determine the involvement of *hsp70*/110 genes in disease responses after bacterial infection in catfish. On the basis of my previous work on Hsp40 genes, the objective of this chapter is to systematically characterize Hsp70 genes: their identification, annotation, and analysis of their expression after bacterial infections. Here I report the genome-wide identification of a full set of 16 hsp70 genes, their phylogenetic and syntenic analyses, and their expression in disease responses after bacterial infection with ESC and columnaris through meta-analysis of RNA-Seq datasets. The nomenclature in this article is updated and based on the ZFIN (zebrafish Information Network) and HGNC (HUGO Gene Nomenclature Committee).

3.3. Materials and Methods

3.3.1 Database mining and sequence analyses

In order to identify the full set of hsp70/110 genes in channel catfish, I collected all Hsp70s and Hsp110s proteins from teleost fishes (zebrafish, three-spined stickleback, medaka, tilapia and fugu) and other species (human, mouse, platypus, chicken, turtle and frog) (supplementary table 1). These sequences were retrieved from NCBI (http://www.ncbi.nlm.nih.gov) and Ensembl (http://www.ensembl.org) databases and used as queries to search against channel catfish RNA-Seq datasets. The e-value was set at intermediately stringent level of e^{-10} for collecting as many as potential *hsp70/110*-related sequences for further analysis. The retrieved sequences were then translated using ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Further, the predicted ORFs were verified by BLASTP against NCBI non-redundant (Nr) protein sequence database. The simple modular architecture research tool (SMART) (Letunic et al., 2012) was used to predict the conserved domains based on sequence homology and further confirmed by conserved domain prediction from BLAST. The predicted catfish Hsp70/110 proteins and all other query sequences were utilized to search against catfish genome database using TBLASTN program. The retrieved genome scaffolds were then predicted by FGENESH in SoftBerry (http://linux1.softberry.com/berry.phtml?topic=fgenesh&group=programs&subgroup=gfind).

3.3.2 Phylogenetic and conserved syntenic analyses

All the amino acids of Hsp70/110s from channel catfish and other species were used to construct phylogenetic tree. Multiple protein sequences alignments were conducted using the Clustal W2 program (Larkin et al., 2007) and MUSCLE 3.8 (Edgar, 2004). Three alignment methods: L-INS-i, E-INS-I and G-INS-i were applied from MAFFT 7.01 (Katoh and Standley,

2013) and the best alignment with highest score was evaluated by program MUMSA (Lassmann and Sonnhammer, 2006). JTT+I+G model (Jones-Taylor-Thornton (JTT) matrix incorporated a proportion of invariant sites (+I) and the gamma distribution for modeling rate heterogeneity (+G)) was selected as the best-fit model by ProtTest 3 program (Darriba et al., 2011) according to the Bayesian information criterion. Maximum likelihood phylogenetic tree was constructed using MEGA5.2.2 (Tamura et al., 2011) with bootstrap test of 1,000 replicates. Final phylogenetic tree was separated into three different phylogenetic trees according to classification of subfamilies due to the large size.

Conserved syntenic regions surrounding the relevant hsp70/110 genes were searched by examining the conserved co-localization of neighboring genes on scaffold of channel catfish and other species based on genome information from Ensembl (Release 77) and NCBI database. Neighbor genes of channel catfish Hsp70 genes were predicted by FGENESH (Salamov and Solovyev, 2000) and BLASTP.

3.3.3. Meta-analysis of expression of Hsp70/110 genes and bacterial challenge

The Illumina-based RNA-Seq reads were retrieved from bacterial challenge experiments in catfish: intestine samples challenged with *Edwardsiella ictaluri* (SRA accession number SRP009069) (Li et al., 2012) and gill samples challenged with *Flavobacterium Columnare* (SRA number SRP012586) (Sun et al., 2012). Trimmed high-quality reads were mapped onto the catfish Hsp70 genes using CLC Genomics Workbench software (version 6.5.2; CLC bio, Aarhus, Denmark). Mapping parameters were set as ≥ 95% of the reads in perfect allignment and ≤ 2 mismatches. The total mapped reads number for each transcript was determined and normalized to analyze RPKM (Reads Per Kilobase of exon model per Million mapped reads). The proportions-based Kal's test was performed to identify the differently expressed genes comparing each

timepoint sample with control sample and fold changes were calculated. Transcrirps with *p*-value ≤ 0.05, absolute fold change value ≥ 1.5, and total reads number ≥ 5 were included in the analyses as significantly differently expressed genes.

Another set of RNA-seq dataset (SRA accession number SRP017689) was used to analyze the differently expressed catfish Hsp70s between columnaris resistant and columnaris susceptible catfish family (Peatman et al., 2013). The two families of channel catfish utilized were previously revealed to have differing susceptibilities to columnaris disease (Beck et al., 2012). Both before and at early timepoints (0h, 1h, 2h and 8h) following *F. columnare* challenge in gill tissue of the fish from resistant and susceptible families of channel catfish were examined. The Illumina-based RNA-Seq reads were retrieved from the NCBI Sequence Read Archive (SRA) under Accession SRP017689. Trimmed high-quality reads were mapped onto the catfish Hsp70 genes using CLC Genomics Workbench software (version 6.5.2; CLC bio, Aarhus, Denmark). Mapping parameters were set as ≥ 95% of the reads in perfect allignment and ≤ 2 mismatches. The total mapped reads number for each transcript was determined and normalized to analyze RPKM (Reads Per Kilobase of exon model per Million mapped reads). The proportions-based Kal's test was performed to identify the differentially expressed genes comparing samples from columnaris resistant family with samples from columnaris susceptible family at each time point. After scaling normalization of the RPKM values, fold changes were calculated. Transcrirps with *p*-value ≤ 0.05, absolute fold change value ≥ 1.5, and total reads number ≥ 5 were included in the analyses as significantly differently expressed genes.

3.4. Results

3.4.1 Identification of Hsp70/110 genes in catfish

A total of 16 Hsp70/110 genes were identified in channel catfish. All the information of domain structures and GenBank accession numbers are summarized in Table 1. Only one of these catfish hsp70 genes were identified before as 'heat shock cognate 70 protein' (NP_001187202.1) and now is named as *hspa8a.2* after our phylogenetic and syntenic analysis (Luft et al., 1996). Among all these genes, almost all sequences were identified in both transcriptome and genome databases with full-length or nearly full-length hsp70/110 in both databases. These catfish hsp70/110 genes were named following Zebrafish Nomenclature Guidelines (https://wiki.zfin.org/display/general/ZFIN+Zebrafish+Nomenclature+Guidelines).

Twelve heat shock protein 70 (*hspa*) genes were identified in the catfish genome including *hsp70.2, hsp70.3, hspa8a.1, hspa8a.2, hspa8b, hsc70 hspa5, hspa9, hspa12a, hspa12b, hspa13* and *hspa14*. Four heat shock protein 110 (*hsph*) genes were identified in channel catfish including *hspa4a, hspa4b, hspa4L* and *hyou1* (Table 3-1). However, the *hsph1* (hsp105) didn't identified from either transcriptome database or genome database of catfish. (Kampinga et al., 2009).

3.4.2 Phylogenetic analysis of channel catfish Hsp70/110s

A total of 16 channel catfish Hsp70/110 genes have been phylogenetically analyzed. In a few cases where it was difficult to establish orthologies due to duplications and ambiguous names (for example, *hspa8a.1, hspa8a.2* and *hspa8b*), syntenic analyses were conducted and their names were standardized followed by the zebrafish or human orthologues. The phylogenetic trees were then reconstructed after standardizing all the names. In the phylogenetic tree, all the members of catfish *Hsp70/110* were well distributed into distinct clades and grouped with corresponding genes of zebrafish or other fishes, which were supported by strong bootstrap value (Figure 3-1).

3.4.3 Syntenic analysis of channel catfish Hsp70/110s

Though phylogenetic relationships provide support for the identities of most Hsp70 genes based on the similarity of gene sequences, it can't provide a strong and clear classification of the sequence share very similar sequence or the tandem repeats which occur specifically in the catfish. Hspa1, Hspa2, Hspa6 and Hspa8 genes have a common domain called HSP1_2_6_8Nucleoid Binding Domain. In catfish, there were six genes in this subfamily were found through the genome. However, it was hard to assign the names to each of them according to the clade of the phylogenetic tree due to some tandem repeat and gene duplications. Sytenic analyses were required to provide additional evidence for orthologies or otherwise the paralogies for these hsp70 genes. Positions of these catfish *hsp70s* genes and their neighbor genes were identified from the draft genome scaffolds. And the genes and their neighborhoods were also identified from the zebrafish and human genome. As shown in Figure 3-2, the neighbor genes of Hsp70 members related to Hspa1 were analyzed in human, zebrafish and catfish chromosomes. The genes in same color showed a homology of each other, except one situation that the yellow color shows the homology to all human counterpart genes. Three tandem repeat genes, *HSPA1A*, *HSPA1B*, *HSPA1L*, were found on human chromosome 6 within the MHC-complex loci (6p21), which was mapped to short arm of chromosome 6 from 29.7M to 33.3M. Four genes from zebrafish were related to HSPA group. Two of them, *hsp70.2* and *hsp70.3* as tandem repeats located on chromosome 3, were found orthologous to human HSPA genes. The other two gene, *hspa-like* and *hsp70-like*, were located on the chromosome 8 and were less related to human HSPA. Two copies of catfish hsp70 genes were found highly orthologous to zebrafish *hsp70.2* and *hsp70.3*. Both of them were named after the zebrafish orthologues. One member of MHC-complex molecules was found on the same chromosome of catfish *hsp70.2* and *hsp70.3*. Even though the MHC complex loci or locus were

found on the same chromosome of all these fish hspa genes, however, the loci are relative far ahead of the hspa genes, which is different with the situation in human genome.

Three hspa8-related genes were analyzed (Figure 3-3). One copy of zebrafish hspa8 gene has the highest level of conservation in gene contents and gene orders as compared with the human *HSPA8*. In addition most of the neighboring genes are duplicated genes and named as "*gene a"*, whereas the other zebrafish hspa8 gene neighbored by several "*gene b*". Thus, I renamed the first hspa8 gene as *hspa8a* and the latter hspa8 gene as *hspa8b*. The counterpart catfish hspa8 genes were named according to zebrafish hspa8 genes. However, a tandem repeat was found in the catfish hspa8a gene, therefore, they were named as *hspa8a.1* and *hspa8a.2* respectively (Figure 3-3). Another copy of hsp70 gene was found on the same chromosome of all fish hspa8 genes though in a distance. In zebrafish, this copy of hsp70 was named as *hsc70*, which means an alias of hspa8 by name. However, I couldn't find a strong evidence from both phylogenetic and syntenic analysis to support that this gene is an orthologue of hspa8, instead, according to the information from the database online (NCBI and ensembl), I found this gene is more close to Hspa1a. However, I still named this gene as *hsc70* following the zebrafish *hsc70*.

3.4.4 Hsp70/110 copy number variation among species

The human Hsp70/110 family comprises 16 unique gene products that differ from each other by amino acid sequences, expression levels and sub-cellular localization. Thirteen of them are belong to HSPA (HSP70) family, whereas the remaining four are classified in to HSPH (HSP110) family. Catfish have almost all the orthologues of HSP70/110 in human and zebrafish, with exception of several genes existing in humans, but absent from teleost fishes including *Hspa2*, *Hspa6*, and *Hspa7*. *Hsph1* wasn't found in any teleost except zebrafish (Table 3-2).

It is interesting that a few of the hsp70/110 genes have more duplicated copies in teleost species than in mammal, bird and amphibian such as hspa8, hspa4. Compared to zebrafish, channel catfish has fewer copies for *hspa1* and *hapa12* (Table 3-2). Generally speaking, hsp70 is quite conserved from teleost to human.

3.4.5 Regulated expression of hsp70/110 genes in catfish after bacterial infection

Using two bacterial challenged RNA-Seq datasets (intestine sample infected by *E. ictaluri* and intestine sample infected by *F. columnare*), the involvement of Hsp70/110 genes after bacterial infection was determined. As shown in table S3-2, 12 out of a total of 16 *Hsp70/110s* were involved in disease defense responses. Specifically, 10 *Hsp70/110s* were found regulated in the gill following *F. columnare* infection. Among them, eight genes were up-regulated and two genes were down-regulated after *F. columnare* infection (Figure 3-4). Among these regulated Hsp70/110 genes, some of them are transiently up- or down-regulated while others are gradually induced or suppressed. For instance, *hspa4L, hspa12a* and *hspa12b* were up-regulated at only one time point of 48h; similarly, *hspa13* was only down regulated at 48h after infection. In contrast, *hspa4a, hspa4b, hyou1, hspa5* and *hspa9* were up-regulated in at least two time points after infection, suggesting their up-regulated expression was more lasting. Similar patterns were observed for down-regulated gene, *hsc70* (Figure 3-4).

A total of 10 hsp70 genes were found to be regulated in the intestine after *E. ictaluri* infection. Among these, six were up-regulated while four were down-regulated. The up-regulated hsp70 genes included *hsp70.3, hspa4a, hspa4L, hyou1, hspa5* and *hspa12b*. The four down-regulated hsp70 genes were *hsc70, hspa4b, hspa12a* and *hspa14*. Regulated expression of most of these genes were observed in more than one time points with exception of *hspa4b, hspa12a,*

hspa12b, and *hspa14* where the regulated expression was observed at only one time point (Figure 3-5).

As shown in Figure 3-4 and Figure 3-5, the expression of these 16 hsp70/110 genes after bacterial infections can be characterized in the following general patterns: (1) Different bacterial infection has different response pattern. Even though the total number of significantly expressed hsp70 genes is same after both bacterial challenges, different Hsp70s expression patterns still were found between two challenges. Most of the significant expressed genes (nine out of eleven) after *F. columnare* infection were up regulated whereas four out of ten significant expressed genes were down regulated after *E. ictaluri* infection. Though same trends were found in five genes in response of both of two bacterial challenges, including *hsc70, hspa4a, hspa4L, hyou1* and *hspa5*, there are seven genes shows different expression patterns, which are *hsp70.3, hsp4b, hspa9, hspa12, hspa13* and *hspa14*. The most inducible hspa1a homologue, catfish *hsp70.3* were only significantly up-regulated after the infection of *E. ictaluri* at both 4h and 3d timepoints. Hspa1a can bind to TLR4 as the stimulus and agonist (Klink et al., 2012) thus involves in the recognition of LPS, which is the virulent factor of *E. ictaluri* (Arias et al., 2003; Klesius, 1992). (2) Time patterns were presented after both two bacterial infections. Four genes were significantly up-reglated at the 4h timepoint while six genes were significantly expressed at 24h and eight genes were regulated at 48h after *F. columnare* infection, suggesting the involvement of Hsp70 in disease response increases with time during early stage of inflammation. Similar time pattern was found after *E. ictaluri* infection. Four genes were significantly up-reglated at the 4h timepoint while eight genes were significantly expressed at 24h and six genes were regulated at 48h after *E. ictaluri* infection, suggesting the involvement of Hsp70s in disease response increases with time during early stage of inflammation.

3.4.6 Differentially expressed catfish Hsp70/110 genes between columnaris resistant and susceptible fish families

Additional meta-analysis were conducted on the comparisons of differences in hsp70/110s gene expression profiles between resistant and susceptible fish at 0 h, and 1 h, 2 h, and 8 h after F. columnare challenge. Designating the susceptible family as the control group, a comparison of catfish Hsp70/110s expression levels was made between resistant and susceptible families at each timepoint. Six genes showed significant differential expression between groups for at least one timepoint following infection. Among them, one gene, *hsp70.3* was found significantly differently expressed at the basal level between resistant and susceptible fish family (Figure 3-6). The RPKM (Reads Per Kilobase of exon model per Million mapped reads) of *hsp70.3* was found twice higher in resistant fish family than that in susceptible fish family. However, after infection at the early time, the differences were reduced and modest induction of *hsp70.3* expression was observed within either group at 8 h post-infection (Figure 3-7).

3.5. Discussion

HSP70s has been proved to involve in both innate and adaptive immune response (Sung and MacRae, 2011). In the innate immunity, the Hsp70s could bind to the TLRs directly, which are one class of most important germline-encoded Pattern Recognition Receptors (PRRs) that interact with in the Pathogen-association molecular patterns (PAMPs) on the surface of an effector cell such as dendritic cells (DCs) and macrophages (Fang et al., 2011; Triantafilou, 2004). Thus, the presence of Hsp70s can served as a danger signal to activate innate immune response. Besides, the recombinant Hsp70s can induce the maturation of DCs and the production of cytokines from monocytes as well as enhance the proliferation of NK cells and their cytotoxicity (Kuppner et al., 2001). However, the time course of innate immunity, which acts immediately when pathogen

adheres and is the first barrier against the pathogen invasion, only last for few hours (0h-4h) and followed by an "early induced response" phase (4h-96h) which can be activated by infection but can't provide long lasting protection (Janeway et al., 2001). The adaptive immunity with generation of specific effector cells as T and B cell, occurs only when those early lines of defense are broken. Since the T cell can't recognize antigen by itself, the Antigen Presenting Cells (APC) recognize and phagocytize the antigens then immigrate to lymphoid tissue and present the antigens to the naïve T cell there. During the APC immigration, the antigens are digested into epitopes and are bounded to the MHC complex molecules, which can be recognized by the T cell receptor (TCR), on the membrane of the APC. Hsp70s involve in adaptive immunity by help MHC complex molecules select and bind to epitopes in this antigen-presenting step. Hsp70s chaperone a wider range of peptides than MHC molecules and have an ability of "cross-priming" which means they scan all the available peptides in cells first and then transfer the peptides (epitopes) of antigen to MHC complex, which can later present the epitopes to CD4+ helper T cell to release cytokine to active the other immune cells or to CD8+ cytotoxic T cell to release toxic granules and powerful enzymes to kill the infected host cells (Srivastava, 2002). Therefore, Hsp70 contribute to the adaptive immune response during the "bridge" period that connects the innate and adaptive immunity. Thus, I carried out the bacterial challenge experiments according to Hsp70s' function period from 0 hour to 3 days, which are just before the adaptive immunity are triggered. Three human HSP70 genes (*HSPA1A, HSPA1B* and *HSPA1L*) have been mapped within the MHC loci in the short arm of chromosome 6 (6p21), which could be an evidence to support the theory (Milner and Campbell, 1990; Sargent et al., 1989; Wu et al., 1985; Wurst et al., 1989). From the syntenic analysis of HSPA genes (Figure 2), I found two MHC complex loci, MHC complex I and MHC complex II, located on the same chromosome of zebrafish hsp70.2/hsp70.3 and hspa1-like on

chromosome 3 and 8 respectively. However, in catfish, I found one member of MHC complex, mhc1, on the same chromosomes of the catfish orthologs, hsp70.2/hsp70.3 of chromosome 3. And a large MHC complex loci was found on the catfish chromosome 26. The differences in the locations of MHC complex and Hsp70 on genome may lead to the different function of Hsp70 in the immunity among organisms. And thus could probably explain the *hsp70.2* and *hsp70.3* wasn't induced too much in our experiment after *E. ictaluri* infection.

At the beginning of infection, fish gills, mucous surfaces of skin and the epidermis act as the first barrier against infection (Ellis, 2001; Ingram, 1980; Shephard, 1994). The roles of the mucus in disease defense are trapping and sloughing of pathogens. Besides, fish mucus contains immune parameters like lectins, pentraxins, lysozyme, complement proteins, antibacterial peptides and IgM (Alexander and Ingram, 1992; Aranishi and Nakane, 1997; Rombout et al., 1993). For the columnaris, the gill is the primary site of invasion by *F. columnare*, whereas in the enteric septicemia (ESC), intestine is the primary invasion site by *E. ictaluri*. Therefore, the Hsp70s expression data I obtained from these two tissues after the bacterial infections could represent the early response of Hsp70s to the infections. A set of expression profile of hsp70/110 gene in catfish after bacterial infections has been generated. Generally, these genes showed patterns in different bacterial infections and time of infection.

Disease-specific pattern: More hsp70s genes were down-regulated after *E. ictaluri* infection, than *F. columnare* infection. However, the most inducible *hspa1a* homologue, catfish *hsp70.3* were only significantly up-regulated after the infection of *E. ictaluri* at both 4h and 3d timepoints while no *hspa1a* homologue genes were significantly expressed after *F. columnare* infection. This suggests a different pathogenesis mechanism of these two diseases. *E. ictaluri*, a Gram negative pathogen with flagella (Hawke et al., 1998), enters catfish through the gut, where

is the primary invasion site. One of the most important virulence factors of *E. ictaluri* is the lipopolysaccharide (LPS), which can be recognized by TLR4 of the host immune cell (Arias et al., 2003; Klesius, 1992). Furthermore, the TLR5 is a receptor for flagella and acts as one member of TLRs which then active the innate and even adaptive immune response. This pathogenesis could explain the up-regulated *hsp70.3* after infected by *E. ictaluri*, in that Hspa1a could bind TLR4 directly and function as TLR4 agonist to active the immune cells afterwards (M. Triantafilou and K. Triantafilou, 2004). However, the pathogenesis of *F. columnare* is different, even though it is a Gram negative bacterial too. The most important ability for *F. columnare* is adhesion on the gill of fish. The mucus from the skin and gills of channel catfish is a chemoattractant to *F. columnare*. This positive chemotactic response may be an important first step for *F. columnare* colonization of channel catfish skin or gills (Decostere et al, 1999; Klesius et al, 2008). And the major virulence factors of *F. columnare* are tissue degradation enzyme and chondroitin AC lysate (Kunttu et al., 2011; Srivastava, 2002; Suomalainen et al., 2006). The different pathogenesis of the two bacteria may be one of the reasons that explain the different hsp70 expression response after two bacterial infections. However, the expression level of catfish *hsp70.2* and *hsp70.3* were not expressed as high as expected after the *E. ictaluri* infection. The Hspa1, which is the homologue of them, is the most inducible gene response to disease infection and believed that it involves in antigen presenting process by interacting with MHC complex. From the sytenic analysis, hspa1 orthologues of human and zebrafish were found located within the MHC loci. However, situation is different in catfish as only one MHC gene (*mhc 1*) was found on the same chromosome of catfish hspa1 orthologues (*hsp70.2, hsp70.3*) and in the distance of them as well. Therefore the antigen presenting process may be different with other organisms.

Common expressions between diseases or within the two tissues of each disease: In this study I found some particular catfish hsp70/110 genes, such as *hsc70, hspa4a, hspa4L, hyou1* and *hspa5*, responded to both infections. The catfish *hspa8* paralogues, *hspa8a.1, hspa8a.2* and *hspa8b* were not found significantly expressed in either infection due to the Hspa8 is a housekeeping protein that constitutively expressed in the normal cell and stressed cells.

Differentially expressed gene between columnaris resistant and susceptible catfish family: Six genes in total were found significantly differentially expressed between columnaris resistant and susceptible catfish family for at least one timepoint post *F. columnare* infection. Only one gene, *hsp70.3* showed significant expression at basal level between two catfish families. RPKM (Reads Per Kilobase of exon model per Million mapped reads) of channel catfish *hsp70.3* in the columnaris resistant and susceptible catfish families in gill before and after *F. columnare* infection reflect the expression differences from basal level and early time after infection. Level of *hsp70.3* were higher in the gill of resistant fish at basal level when compared to susceptible fish. This suggests the *hsp70.3* may play as critical innate immune component and potentially reveal genetic differences linked with differential rates of infection. *Hsp70.3* is one of the most inducible chaperones during stress and involve in innate immunity by binding the misfolded peptides and interacting with TLRs. However, the expression level of *hsp70.3* decreased as the infection progresses in both catfish families. The *hsp70.3* from resistant fish group even showed a lower RPKM compared to susceptible family at 2h post infection. However, *hsp70.3* level increased in both of the two groups at 8h post infection, suggesting the process of infection of *F. columnare* suppressed the expression of *hsp70.3* during innate immune phase.

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Table 3-3

Summary of 16 Hsp70 genes identified in the catfish genome

Name	Accession number	Domain
hsp70.2		HSPA1-2_6-8-like_NBD
hsp70.3	JT412120.1	HSPA1-2_6-8-like_NBD
hspa8a.1	JT315838.1	HSPA1-2_6-8-like_NBD
hspa8a.2	NP_001187202.1	HSPA1-2_6-8-like_NBD
hspa8b	JT418846.1	HSPA1-2_6-8-like_NBD
hsc70	JT408276.1	HSPA1-2_6-8-like_NBD
hspa4a	JT406739.1	HSPA4_NBD
hspa4b	JT407108.1	HSPA4_NBD
hspa4L	JT407194.1	HSPA4L_NBD
hyou1	JT411259.1	HYOU1_like_NBD
hspa5	JT415554.1	HSPA5-like_NBD
hspa9	JT408761.1	HSPA9-like_NBD
hspa12a		HSPA12A-like_NBD
hspa12b	JT411717.1	HSPA12B-like_NBD
hspa13	JT413763.1	HSPA13-like_NBD
hspa14	JT406759.1	HSPA14-like_NBD

Table 3-4
Comparison of copy numbers of HSP70s genes among selected vertebrate genomes.

The shades indicate absence of genes of that species.

Gene	Human	Bird	Amphibian	Zebrafish	Catfish	Medaka	Tilapia	Fugu
hspa1	3		4	6	3	2	2	2
hspa2	1	1	1					
hspa6	1							
hspa7	1							
hspa8	1	1	2	2	3	3	3	2
hspa4	2	2	1	3	3	3	1	2
hsph1	1	1	2	1				
hyou1	1	1	1	1	1	1	1	1
hspa5	1	1	2	1	1	1	2	1
hspa9	1	1	2	1	1	1	1	1
hspa12	2	2	1	3	2	2	2	2
hspa13	1	1	1	1	1	1	1	1
hspa14	1	1	2	1	1	1	1	1
Total	17	12	19	20	16	15	14	14

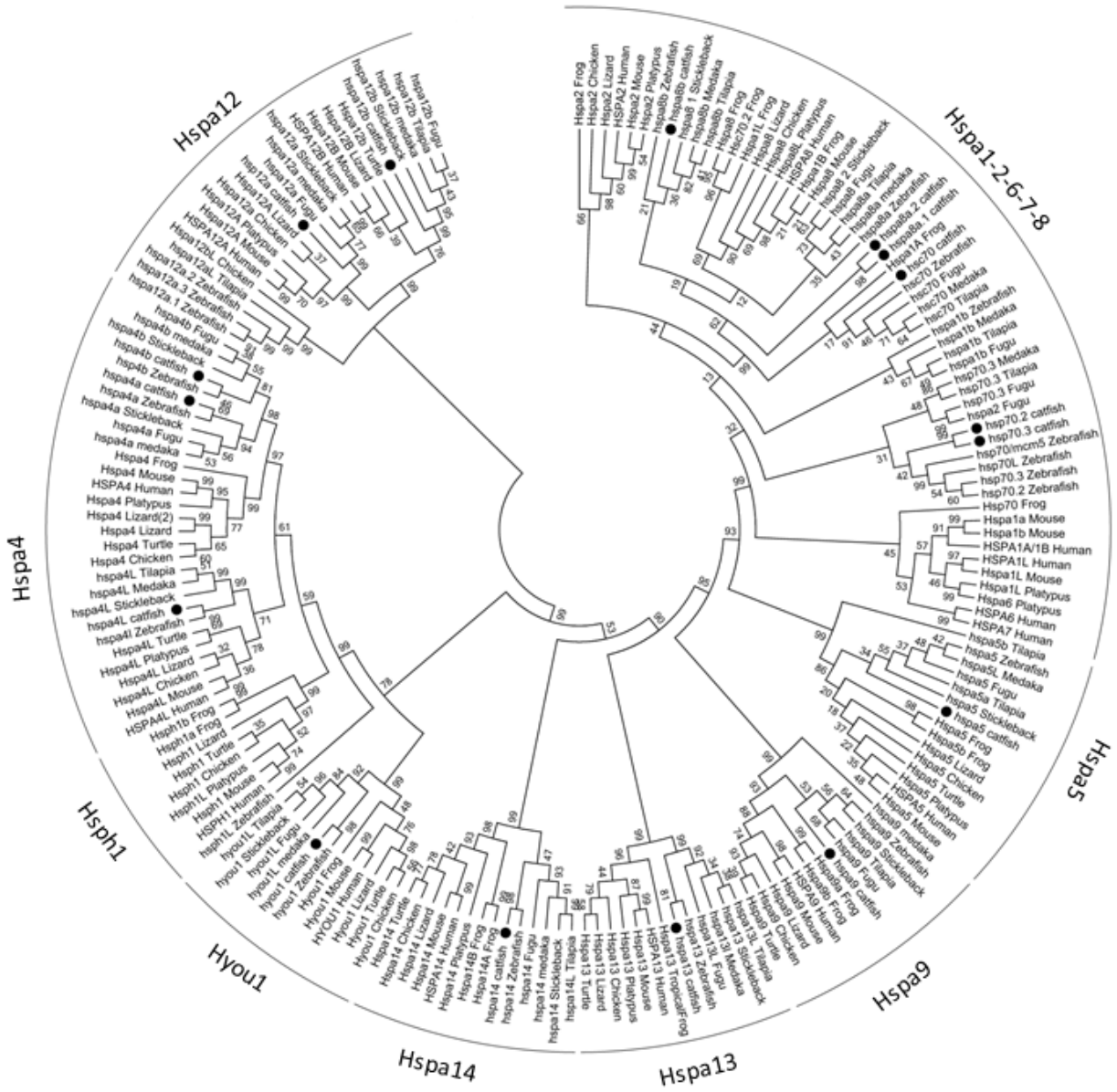

Figure 3-1.

Phylogenetic tree of Hsp70 family. The phylogenetic tree was constructed by Mega5.2.2 using the Maximum Likelihood method based on the JTT matrix-based model of amino acid substitution as described in detail in Material and method section. The bootstrap consensus tree inferred from 1000 replicates is taken. Suffix “L” indicated “-like”, for instance, Hspa4L means Hspa4-like.

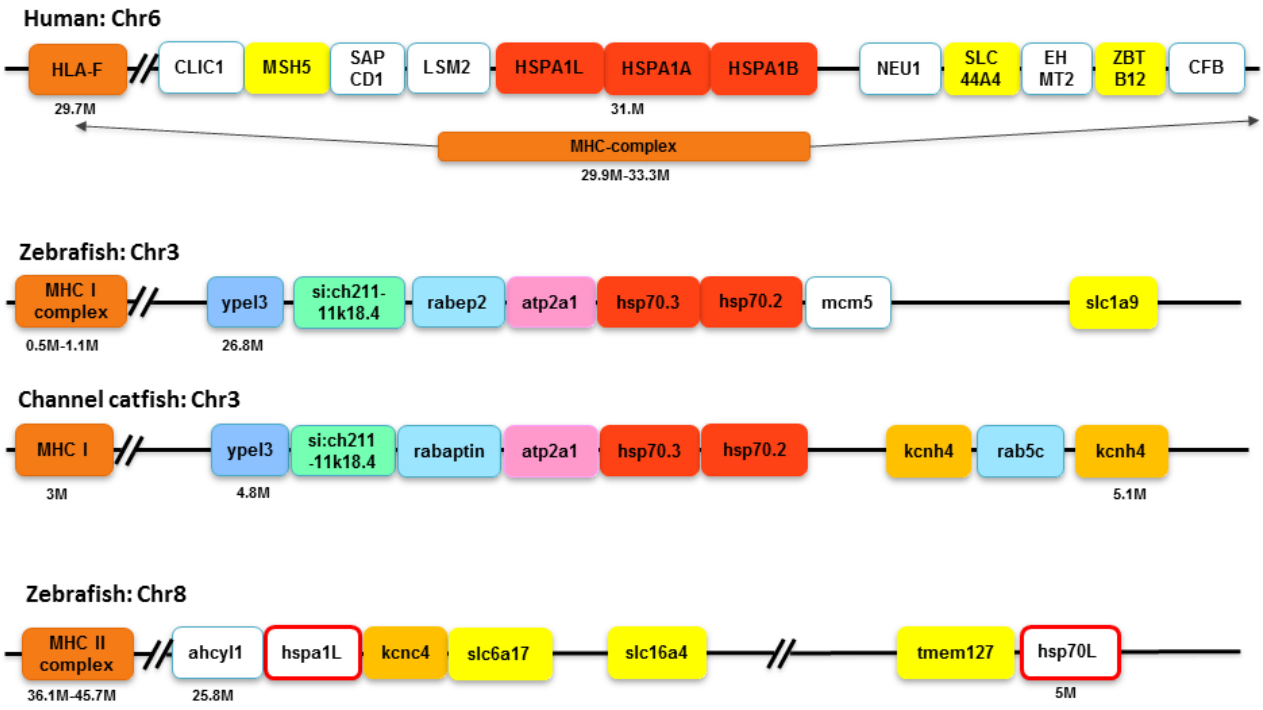

Figure 3-2.

Schematic presentation of the conserved synteny blocks neighboring homologues of human HSPA1A gene.

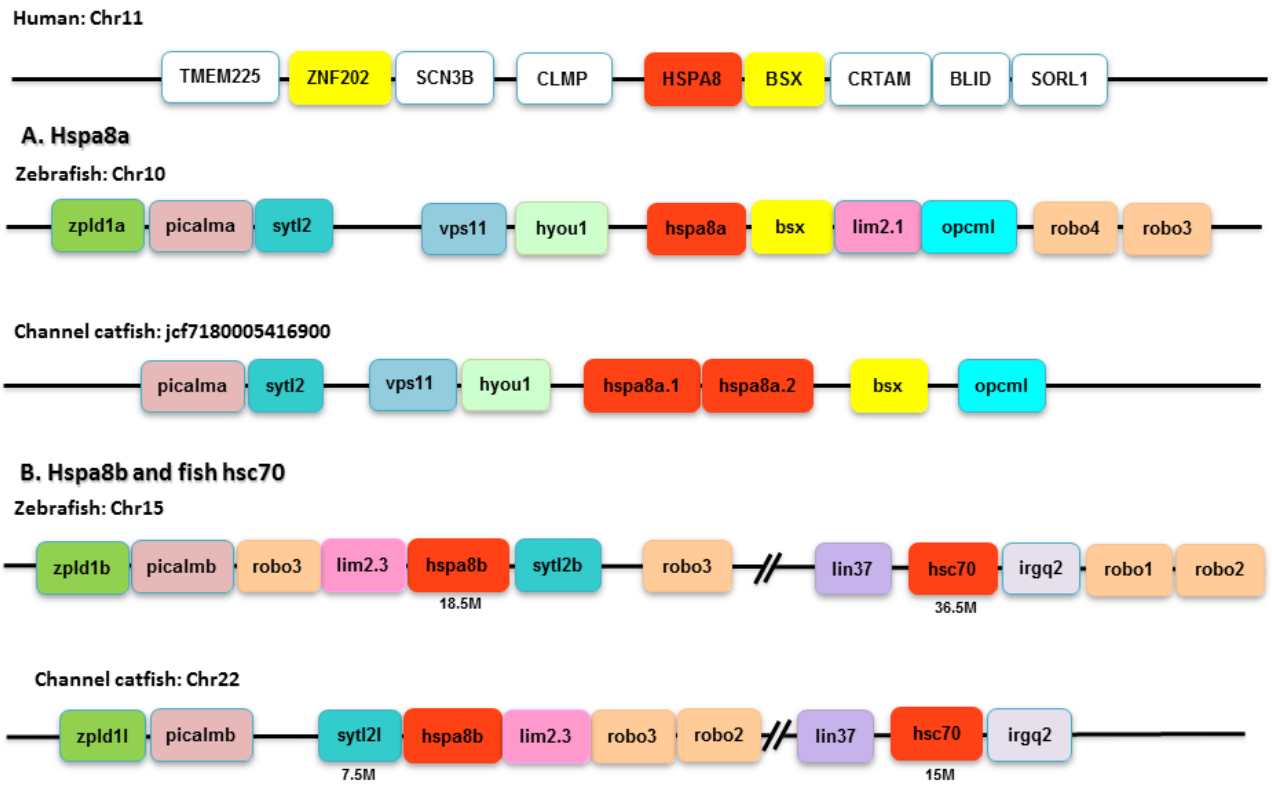

Figure 3-3.

Schematic presentation of the conserved synteny blocks neighboring homologues of human HSPA8 gene.

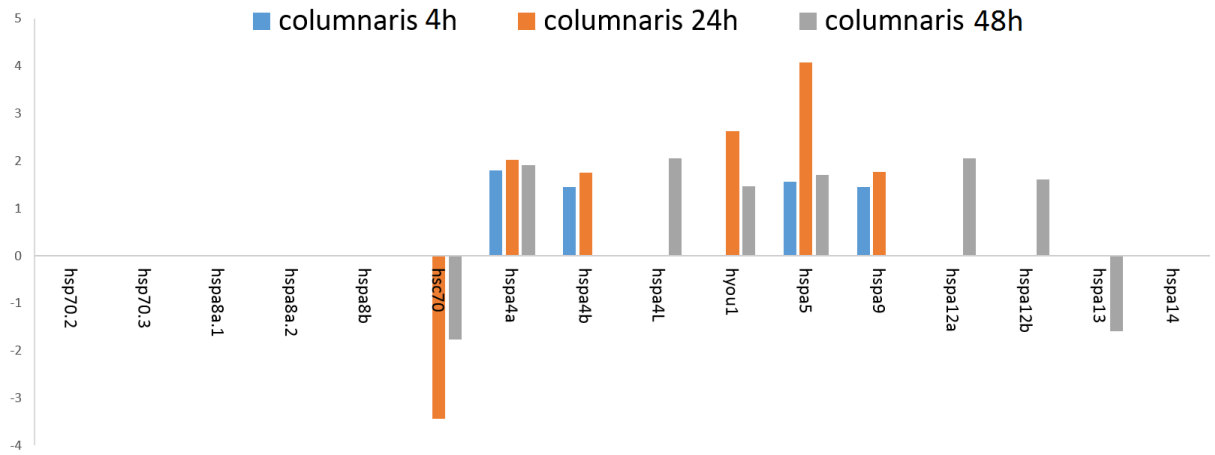

Figure 3-4.

Column bar chart showing the fold change of Hsp70/110s expression in gill after *F. Columnare* challenge. Vertical axis shows the value of fold change.

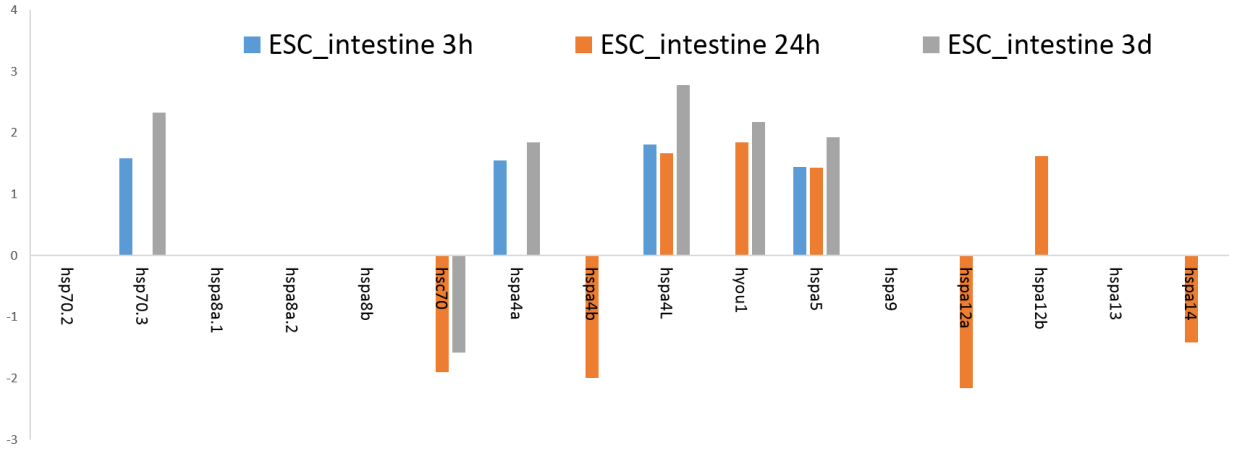

Figure 3-5.

Column bar chart showing the fold change of Hsp70/110s expression in intestine after *E. ictaluri* challenge. Vertical axis shows the value of fold change.

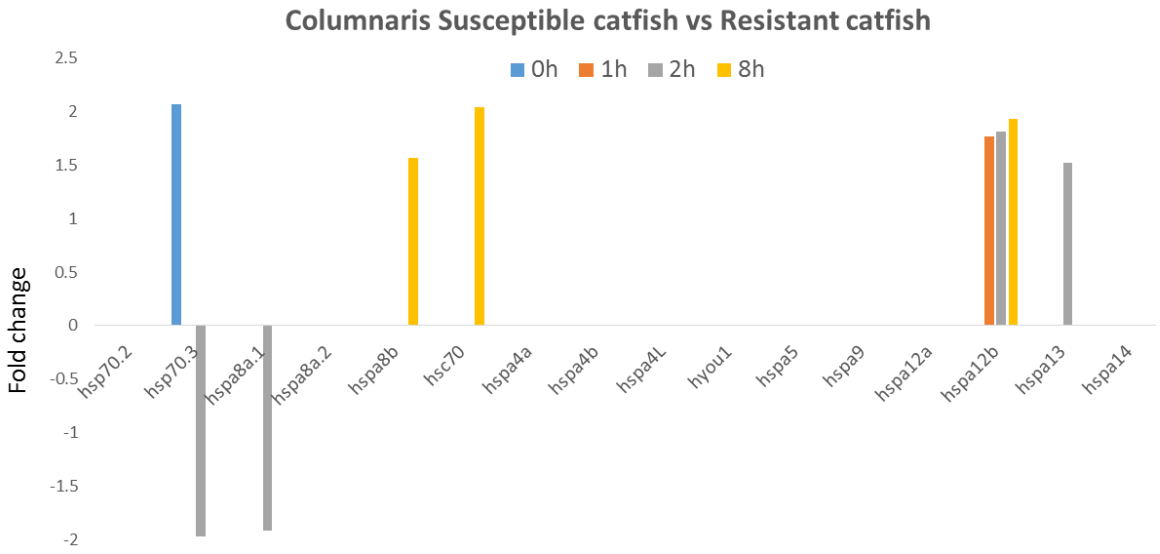

Figure 3-6. Differentially expressed hsp70/110 genes in the gill between catfish resistant and susceptible to *F. columnare.*

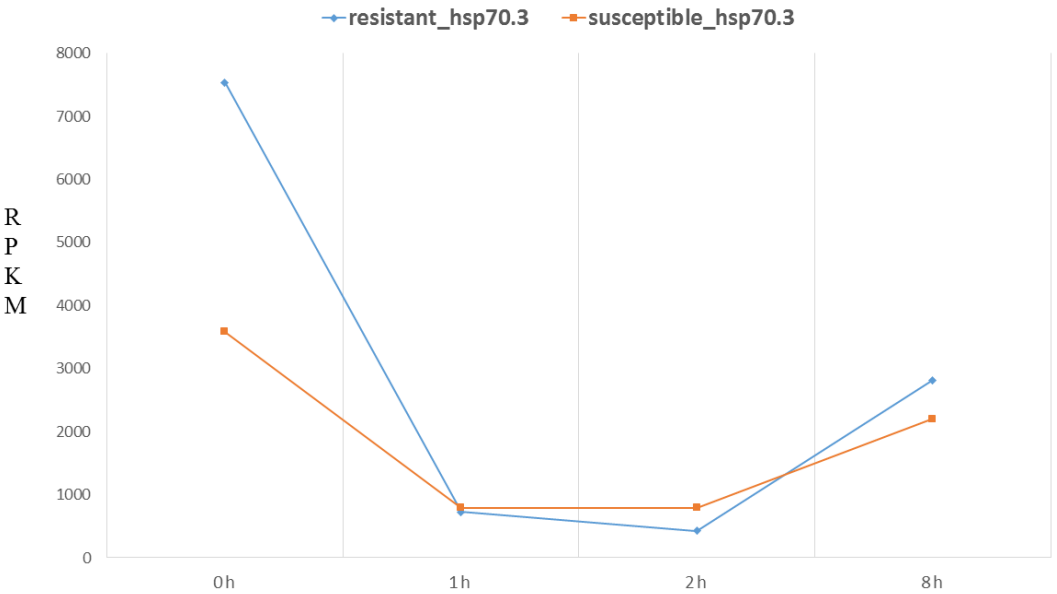

Figure 3-7. RPKM (Reads Per Kilobase of exon model per Million mapped reads) of channel catfish *hsp70.3* in the columnaris resistant and susceptible catfish families in gill before and after *F. columnare* infection.

Chapter 4. Genome-wide Characterization of Heat Shock Protein Genes in Channel Catfish (*Ictalurus punctatus*) and their Expression under Disease Stress

4.1. Abstract

Heat shock proteins (Hsps) are a suite of highly conserved proteins whose expression are generally induced after exposure to elevated temperature. However, many Hsps play important roles in both innate and adaptive immunity. On the basis of our previous work on Hsp40 gene family, the objective of this study was to characterized Hsp90s, Hsp60s, Hsp10s, and small Hsps genes. A total of 20 Hsp genes were identified and annotated including five hsp90 genes, one hsp60 gene, one hsp10 gene, and 13 shsp genes. Of the 20 Hsp genes characterized, six were differentially expressed after *Edwardsiella ictaluri* infection, and 13 were differentially expressed after *Flavobacterium columnare* bacterial infection. Although expression of these genes exhibited both temporal and spatial regulation, the induced Hsp genes tend to be differentially expressed soon after bacterial infection, while suppressed Hsp genes were differentially expressed at later time points, suggesting their roles in disease responses and disease defenses. A pathogen-specific expression pattern of Hsp90 was observed. After *F. columnare* infection, all hsp90 genes were found up-regulated except *hsp90ab1,* which was not significantly regulated. However, after *E. ictaluri* infection, only one hsp90 gene was found significantly down-regulated in intestine. Both pathogen-specific and tissue-specific pattern of expression were found with small Hsps after both ESC and columnaris bacterial infections. These results suggested that most of the Hsp genes may play important roles in disease response and/or disease defense in channel catfish. Additional

RNA-seq dataset was used to profile gill Hsp70 expression differences between resistant and susceptible fish group post *F. columnare* challenge at both basally (before infection) and at three early timepoints (1 h, 2 h, and 8 h). Five genes showed significant differential expression between groups for at least one timepoint following infection. Among them, four genes were found significantly differentially expressed at the basal level between resistant and susceptible fish family, which could potentially serve as expression QTL and biomarkers for selection.

Keywords: Heat shock protein; Hsp90; Hsp60/10; small Hsp; catfish; genome; immunity; infection.

4.2. Introduction

Heat shock proteins (Hsps) are a suite of highly conserved proteins whose expression is mostly increased when cells are exposed to elevated temperature or other stresses such as disease and hypoxia (Feder and Hofmann, 1999; Iwama et al., 1998; Srivastava, 2002). They are generally classified into five families based on their molecular weight as well as on domain structures and functions: Hsp90, Hsp70/Hsp110, Hsp60/Hsp10, Hsp40, and small heat shock protein (sHsp) families (Gething, 1997). I previously reported 57 Hsp40 genes and 16 Hsp70 genes from channel catfish (Song et al., 2015a; 2015b), the objective of this project was to characterize the remaining three families of Hsp genes: Hsp90, Hsp60/Hsp10, and small Hsps, and their expression after bacterial infections.

In addition to their roles as chaperones, Hsp90s has a number of other functions including involvement in intracellular transport (Jakob et al., 1995; Miyata and Yahara, 1992; Wiech et al., 1992), protein degradation (Correia et al., 2005; Imai et al., 2003; Kimura et al., 1994), and cell signaling (Grad and Picard, 2007; Pratt et al., 2006). Hsp90s also stabilize a number of proteins

required for tumor growth, which is why Hsp90 inhibitors are investigated as anti-cancer drugs (Csermely et al., 1998; Goetz et al., 2003; Subbarao Sreedhar et al., 2004). In most of the species previously studied, Hsp90 genes are abundantly expressed, accounting for 1–2% cellular proteins (Csermely et al., 1998). They can be found in the cytosol, nucleoplasm, endoplasmic reticulum (ER), mitochondria, and chloroplasts (Csermely et al., 1998; Felts et al., 2000; Krishna and Gloor, 2001). In the human genome, 17 Hsp90 genes were found, of which six, HSP90AA1, HSP90AA2, HSP90AB1, HSP90B1, TRAP1 and HSP90N, were recognized as functional genes while the remaining 11 were considered to be putative pseudogenes (Chen et al., 2005a). Functional Hsp90s are dimeric, each monomer consists of four structural domains: a highly conserved N-terminal domain (NTD) that has a high-affinity ATP-binding site, a "charged linker" region that connects the N-terminus with the middle domain, a protein-binding middle domain (MD) and a C-terminal domain (CTD) that interacts with co-chaperones (Didenko et al., 2012; Krukenberg et al., 2011; Pearl and Prodromou, 2002; Prodromou and Pearl, 2003; Street et al., 2011).

Hsp60/Hsp10 generally act as a chaperonin in mitochondria although they can be found in the cytoplasm as well. In addition, they play important roles in the transportation of mitochondrial proteins into the mitochondrial matrix from the cytoplasm (Koll et al., 1992). Moreover, studies have suggested that Hsp60 plays a key role in preventing apoptosis in the cytoplasm by forming a complex with proteins responsible for apoptosis and regulates the activity of these proteins (Itoh et al., 2002). The cytoplasmic Hsp60 is also involved in immune response (Ranford et al., 2000) and cancer (Itoh et al., 2002; Lebret et al., 2003; Urushibara et al., 2007). Hsp10 aids Hsp60 in protein folding by acting as a dome-like cover on the ATP active form of Hsp60 (Ranford et al., 2000).

Most sHsps display in vitro chaperone-like activity (Haslbeck and Buchner, 2002; Horwitz,

1992). In vivo, sHsps have been implicated in an astounding variety of processes, such as enhancing cellular stress resistance (Landry et al., 1989), regulating actin and intermediate filament dynamics (Quinlan, 2002; Wieske et al., 2001), inhibiting apoptosis (Arrigo and Müller, 2002), modulating membrane fluidity (Tsvetkova et al., 2002), and regulating vasorelaxation (Flynn et al., 2003). Mutants of human sHsps are responsible for various forms of hereditary cataract (Mackay et al., 2003; Pras et al., 2000), muscular diseases (Selcen and Engel, 2003; Vicart et al., 1998) and neuropathies (Evgrafov et al., 2004; Irobi et al., 2004). Small heat shock proteins (sHsps) are probably the most diverse in structure and function amongst the various families of stress proteins (Narberhaus, 2002; van Montfort et al., 2001). Functional sHsps are multimeric complexes made up of one or more kinds of sHsp's monomers (Kim et al., 1998; van Montfort et al., 2001). Each sHsp's monomer contains an amino-terminal region, an α-crystallin domain (ACD) and a carboxyl-extension (Hilario et al., 2011; Jehle et al., 2011; Kim et al., 1998; Laganowsky et al., 2010; Mchaourab et al., 2009; Sun and MacRae, 2005; van Montfort et al., 2001).

Hsps play important roles in both innate and adaptive immune responses (Roberts et al., 2010; Srivastava, 2002). For innate immunity, Hsps are thought to mediate both humoral and cellular innate immune responses (Sung and MacRae, 2011). The presence of Hsps in the extracellular environment served as a danger signal to activate innate immune such as dendritic cells (DCs) and macrophages (Chen et al., 1999; Kol et al., 2000; Singh-Jasuja et al., 2000); Several cytokines can be induced by Hsps, including tumour-necrosis-α (TNF-α), interleukin-1β (IL-1β), interleukin-12 (IL-12), nitric oxide and some chemokines (Basu et al., 2000; Lehner et al., 2000; Moré et al., 2001; Panjwani et al., 2002). For adaptive immunity, Hsps can stimulate adaptive immune responses as potent antigen carriers. For instance, Hsp60, Hsp70, Hsp90 have been proposed to interact with immune cells as a ligand for a variety of cell-surface receptors such as Toll-like

receptors and a number of clusters of differentiation (CDs) such as CD14 and CD91 (Basu et al., 2001; Habich et al., 2002; Ohashi et al., 2000; Vabulas et al., 2001). Elevated expression of Hsps after pathogen infection has been demonstrated in aquatic organisms (Ackerman and Iwama, 2001; Chen et al., 2010; Dong et al., 2006; Forsyth et al., 1997; Sung and MacRae, 2011). Increasing Hsps in aquatic organisms by heat shock, chemical application and feeding exogenous Hsps also enhanced resistance against infection (Sung and MacRae, 2011; Wilhelm et al., 2005), and the level of tolerance correlated with the amount of accumulated Hsps (Sung and MacRae, 2011).

Channel catfish is important aquaculture species in the United States and around the world. However, in recent years, catfish industry has encountered great challenges including devastating diseases which cause the largest economic losses. Of the serious disease problems, bacterial diseases are the major threats to the catfish industry. Enteric septicemia of catfish (ESC), caused by Gram-negative bacterium *E. ictaluri* (Hawke et al., 1981), is the most significant disease facing the catfish industry (Rogge and Thune, 2011; Williams et al., 2012; Bao et al., 2005; Baoprasertkul et al., 2004; Baoprasertkul et al., 2006; Chen et al., 2005b; Liu et al., 2011a; Liu et al., 2010; Peatman et al., 2006; Sha et al., 2009; Sha et al., 2008; Takano et al., 2008; Zhang et al., 2012). Columnaris, caused by Gram-negative bacterium *F. columnare*, (Decostere et al., 1999), is the most frequently occurring disease in fish including catfish, causing huge economic losses worldwide.

In this study, I identified Hsp90, Hsp60, Hsp10 and sHsp gene families of channel catfish, and analyzed their expression profile after bacterial infections, thereby providing insights into their roles against diseases and apply knowledge in genetic programs for the development of genetically enhanced catfish brood stocks.

4.3. Materials and Methods

4.3.1 Database mining and sequence analysis

To identify the Hsp genes, the channel catfish transcriptome database (Liu et al., 2012; Liu et al., 2011b; Lu et al., 2011) and the whole genome database of channel catfish (unpublished data) were searched using available teleosts (zebrafish (*Danio rerio*), stickleback (*Gasterosteus aculeatus*), medaka (*Oryzias latipes*), tilapia (*Oreochromis niloticus*), fugu (*Takifugu rubripes*)) and other vertebrate species from amphibian to mammals (xenopus (*Xenopus laevis* or *X. tropicalis*), turtle (*Pelodiscus sinensis*), lizard (*Anolis carolinensis*), bird (chicken (*Gallus gallus*) or turkey (*Meleagris gallopavo*)), platypus (*Ornithorhynchus anatinus*), mouse (*Mus musculus*), human (*Homo sapiens*)) 90 kDa heat shock proteins (HSP90AA1/Hsp90aa1.1, HSP90AA2/Hsp90aa1.2, Hsp90ab1, Hsp90b1, Trap1), 60 kDa heat shock proteins (Hspd1), 10 kDa heat shock proteins (Hspe1) and small heat shock proteins (Hspb1, Hspb2, Hspb3, Cryaa, Cryab/ (Cryaba, Cryabb), Hspb6, Hspb7, Hspb8, Hspb9, Hspb10, Hspb11, Hspb12, Hspb15) sequences as queries (Supplementary Table 1). The e-value was set at intermediately stringent level of e-10 for collecting as many as potential Hsps.

The retrieved sequences from transcriptome database were translated into amino acid sequences using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). The predicted ORFs were verified by BLASTP against NCBI non-redundant protein sequence database. The simple modular architecture research tool (SMART 7) (Letunic et al., 2012) was used to predict the conserved domains based on sequence homology and further confirmed by conserved domain prediction from BLASTP.

To remove the redundancy among the database, the catfish Hsps amino acid sequences were used as query to search the draft catfish genome sequences (unpublished data) to obtain the

genomic scaffold containing the catfish Hsps genes. The genome scaffold contigs were retrieved and predicted by Fgenesh of Molquest software (Softberry Int.) (Salamov and Solovyev, 2000). A manually confirmation was conduct to make sure there is no repeat gene translated from same site of the scaffold. This step also can find out and verify the multiple copies of one gene and have a chance to complete partial genes from the RNA-seq contigs.

The catfish Hsp genes were named following the ZFIN (Zebrafish Nomenclature Guidelines) and the Guidelines for the nomenclature of the human heat shock proteins (Kampinga et al., 2009). First, the genes are named after the zebrafish or mammalian orthologues whenever possible. When zebrafish or mammalian orthologues are known, the same name and abbreviation are used, except all letters are italicized and in lower case. When a gene is homologous to a zebrafish gene or human gene, but orthology is ambiguous, the gene should be named after the closest zebrafish homologue or mammalian homologue with the word 'like' appended to the name of the homologue and the letter 'L' appended to the gene symbol of the homologue. Finding the orthology was done by syntenic analysis. The protein symbol is the same as the gene symbol, but non-italic and the first letter is uppercase. Detailed phylogenetic analyses and in some cases syntenic analyses were conducted for each Hsp gene.

4.3.2 Phylogenetic tree construction and analysis

The amino acid sequences of Hsps from other vertebrate organisms were retrieved from Ensembl genome databases or NCBI database for phylogenetic analysis. Multiple protein sequences alignments were conducted using the Clustal W2 program (Larkin et al., 2007), Muscle v3.8 (Edgar, 2004) and the Auto (FFT-NS-1, FFT-NS-2, FFT-NS-i or L-INS-i; depends on data size), L-INS-i, E-INS-i and G-INS-i strategies from MAFFT v7.01 (Katoh and Standley, 2013)

with default parameters. The program MUMSA (Lassmann and Sonnhammer, 2006) was employed to select the best-scoring multiple alignment.

To find a best-fit model of Hsps' evolution, the ProtTest program was used (Darriba et al., 2011) according to the Bayesian information criterion. The best-fit model was the LG+G model for Hsp90 family, which uses a LG amino acid model (Le and Gascuel, 2008) and the gamma distribution for modeling rate heterogeneity (+G); while the JTT+G model for Hspd/Hspe family and sHsp family, which uses a Jones-Taylor-Thornton (JTT) matrix and the gamma distribution for modeling rate heterogeneity (+G). The phylogenetic and molecular evolutionary analyses were conducted by using MEGA 6 (Tamura et al., 2013). Using parameters of Bootstrap test of 1000 replicates and 95% partial deletion method, the Maximum Likelihood trees, Minimum Evolution trees and the Neighbor Joining Trees were generated. Separate phylogenetic analyses were constructed per family with other representative vertebrate species including zebrafish, medaka, fugu, stickleback, tilapia, Xenopus, turtle, lizard, chicken/turkey, platypus, mouse and human (Supplementary Table 4-1).

4.3.3 Syntenic and orthology analysis

In order to clarify the ambiguous from the phylogenetic tree and nomenclature problems, syntenic analysis was conducted by analyzing syntenic regions harboring Hsp genes from several vertebrates based on genome information from Ensembl (Release 77) and NCBI. Thus the additional evidence for gene identification and orthology were provided. Briefly, the catfish Hsps amino acid sequences were used as query to search the draft catfish genome sequences to obtain the genomic scaffold containing the catfish Hsps genes. After the assembly of the scaffold, the neighboring genes were identified by Fgenesh program (Salamov and Solovyev, 2000) and

BLASTP. The orders of these neighboring genes were compared with those from zebrafish and human using the software Genomicus (Louis et al., 2012).

4.3.4 Meta-analysis of bacterial challenge

The trimmed high-quality reads from stress related RNA-seq experiments in catfish, including intestine sample in response to *E. ictaluri* challenge (SRA accession number SRP009069) (Li et al., 2012) and gill sample in response to *F. columnare* (SRP012586) (Sun et al., 2012) were mapped onto channel catfish Hsp genes' transcript sequences using CLC Genomics Workbench software (V5.5.2). Mapping parameters were set as ≥ 95% of the reads in perfect allignment and ≤ 2 mismatches. The total mapped reads number for each transcript was determined and normalized to detect RPKM (Reads Per Kilobase of exon model per Million mapped reads). The proportions-based kal's test was performed to identify the differently expressed genes comparing with control sample and fold changes were calculated. Transcrirps with absolute fold change value ≥ 1.5, *p*-value ≤ 0.05 and total gene reads ≥ 5 were included in the analyses as significantly differently expressed genes.

Another set of RNA-seq dataset (SRA accession number SRP017689) was used to analyze the differently expressed catfish Hsps between columnaris resistant and columnaris susceptible catfish family (Peatman et al., 2013). The two families of channel catfish utilized were previously revealed to have differing susceptibilities to columnaris disease (Beck et al., 2012). Both before and at early timepoints (0h, 1h, 2h and 8h) following *F. columnare* challenge in gill tissue of the fish from resistant and susceptible families of channel catfish were examined. The Illumina-based RNA-Seq reads were retrieved from the NCBI Sequence Read Archive (SRA) under Accession SRP017689. Trimmed high-quality reads were mapped onto the catfish Hsps genes using CLC

Genomics Workbench software (version 6.5.2; CLC bio, Aarhus, Denmark). Mapping parameters were set as ≥ 95% of the reads in perfect allignment and ≤ 2 mismatches. The total mapped reads number for each transcript was determined and normalized to analyze RPKM (Reads Per Kilobase of exon model per Million mapped reads). The proportions-based Kal's test was performed to identify the differentially expressed genes comparing samples from columnaris resistant family with samples from columnaris susceptible family at each time point. After scaling normalization of the RPKM values, fold changes were calculated. Transcrirps with *p*-value ≤ 0.05, absolute fold change value ≥ 1.5, and total reads number ≥ 5 were included in the analyses as significantly differently expressed genes.

4.4. Results

4.4.1 Identification and phylogenetic analysis of Hsp90 gene in channel catfish

Five hsp90 genes were identified from the channel catfish transcriptome and its genome. They are annotated as *hsp90aa1.1, hsp90aa1.2, hsp90ab1, hsp90b1* and *trap1l*. Full-length coding sequences were obtained for all five channel catfish hsp90 genes (Table 4-1). Of the five Hsp90 genes, *hsp90aa1.1* and *hsp90aa1.2* were arranged in the genome as a head-to-tail tandem. All Hsp90 proteins comprised of HATPase_c and HSP90 domain in structure (Table 4-1). Phylogenetic analysis supported the annotation of catfish hsp90 genes. They were placed into distinct clades well, grouped with other fishes first and then grouped together with tetrapods (Figure 4-1).

4.4.2 Identification and phylogenetic analysis of Hsp60 and Hsp10 genes in channel catfish

One Hsp60 gene (*hspd1*) and one Hsp10 gene (*hspe1*) were identified from the channel

catfish genome. Full-length coding sequences of both genes were obtained (Table 4-1). *hspd1* and *hspe1* are present in the genome as a head-to-head gene pair on the same scaffold. The Hspd1 protein comprised of GroEL/cpn60 domain while the Hspe1 protein comprised of GroES/cpn10 domain (Table 4-1). Phylogenetic analysis supported the annotation of catfish *hspd1* and *hspe1* genes. Hspd1 and Hspe1 were separated to two distinct groups on phylogenetic tree. In each group, the channel catfish gene clustered with its counterpart of zebrafish (Figure 4-2).

4.4.3 Identification and phylogenetic analysis of sHsp genes in channel catfish

A total of 13 sHsp genes were identified from the channel catfish genome. Full-length coding sequences were obtained for all of them (Table 4-1). All channel catfish sHsps have Alpha crystallin domain (ACD) (Table 4-1). *Hspb2* and *cryabb* form a head-to-head gene pair on the same scaffold. The phylogenetic analysis supported the annotation of catfish sHsp genes, each catfish sHsp gene clustered with its respective counterpart of other species, and most of them clustered with their respective counterpart of zebrafish (Figure 4-3). Of the small Hsps, *hspb10* was not found in the channel catfish genome.

4.4.4 Syntenic analysis of Hsp genes in channel catfish

In most cases, phylogenetic analysis provided strong evidence for the identities of Hsp genes, with exception of *trap1*, *hsp90aa1.1*, *hspb9* and *hspb12*. To provide stronger evidence for the identities of these Hsp genes, syntenic analysis was conducted. As shown in Figure 4-7, conserved syntenies were found between channel catfish and other species for *hsp90aa1.1*, *hspb9* and *hspb12*. For *trap1*, the neighboring genes were not conserved between catfish and zebrafish (Figure 4-4). Due to the lack of evidence for conserved synteny, the catfish *trap1* gene was named as "*trap1-like* (*trap1L*)".

4.4.5 Meta-analysis of expression of Hsp genes after bacterial infection

Expression of the Hsp genes after bacterial infection was determined using RNA-Seq datasets (intestine sample of *E. ictaluri* infection (SRP009069) (Li et al., 2012) and gill sample of *F. columnare* infection (SRP012586) (Sun et al., 2012)). The significantly expressed genes after bacterial infection from each RNA-seq dataset were determined based on Proportions fold change using RPKM (reads per kilobase per million). A total of 20 Hsp genes were analyzed (Supplementary Table 4-2). Of these 20 Hsp genes, 16 Hsp genes were differentially expressed after bacterial infection, with 13 genes differentially expressed after columnaris infection (Figure 4-8), and six genes differentially expressed after ESC infection (Figure 4-9).

In the gill after *F. columnare* infection, 13 of 20 Hsp genes were found to be significantly differentially expressed. Eight Hsp genes (*hsp90aa1.1*, *hsp90aa1.2*, *hsp90b1*, *trap1l*, *hspd1*, *hspe1*, *hspb6* and *hspb11*) were up-regulated, while five (*hspb1*, *hspb2, hspb3, hspb9* and *cryabb*) were down-regulated (Figure 4-8). However, the patterns of up-regulated or down-regulated expression of these genes are quite different; in general, the up-regulated genes were induced quite rapidly after bacterial infection with significant up-regulation detectable 4 hours or 24 hours after infection, whereas significant down-regulation was not observed until at least 24 hours after infection (Figure 4-8). Among these significantly regulated genes, *hsp90b1* was most significantly up-regulated, approximately 6.3 times up at 24 h after challenge. Among down-regulated genes, *cryabb* was most down-regulated, approximately 3.3 times down at 24 h after challenge. The extent of induction or suppression for all other differentially expressed genes were modest, 1.5-4 fold up or down after the bacterial infection. It should be noted that of the 20 genes under analysis, seven did not exhibit differential regulation after the bacterial infection, of which three (*hsp90ab1*, *haspb7*, and *hspb8*) were not differentially expression before and after infection, while the

remaining four (*cryaa*, c*ryaba*, *hspb12*, *hspb15*) had low levels of expression that were excluded from analysis.

In the intestine after *E. ictaluri* infection, six of 20 Hsp genes (*hsp90ab1*, *hspd1*, *hspe1*, *cryabb*, *hspb7* and *hspb8*) were found to be significantly regulated. Three genes (*hsp90ab1*, *hspd1* and *hspe1*) were down-regulated, while three (*cryabb*, *hspb7* and *hspb8*) were up-regulated after *E. ictaluri* infection (Figure 4-9). The level of induction or suppression was modest for all six genes, within 2-3 folds. Similar to the situation of bacterial infection with columnaris, the up-regulation appeared to be more rapid than down regulation, with the significant up-regulation being detectable as early as 3 hours after challenge. However, down-regulation was much slower, with significant down-regulation being detectable three days after the bacterial challenge (Figure 4-9).

Additionally, expression of the Hsp genes of Susceptible and Resistant families after *F. columnare* infection was determined using RNA-Seq datasets (gill sample of *F. columnare* infection (SRP017689) (Peatman et al., 2013). Of these 20 Hsp genes, four Hsp genes (*hsp90aa1.2*, *hspe1*, *hspb1* and *hspb8*) were differentially expressed between Resistant family and Susceptible family before *F. columnare* infection, and three Hsp genes (*hspb1*, *hspb7* and *hspb8*) were differentially expressed between these two families at 1h, 2h or/and 8h after *F. columnare* infection (Figure 4-10). Gene *hspb1* of both families were significantly up-regulated after *F. columnare* infection, while *hspb7* and *hspb8* of susceptible family were significantly down-regulated, but *hspb8* of Resistant was up-regulated after *F. columnare* infection (Figure 4-10; 4-11).

4.5. Discussion

4.5.1 Hsp genes in channel catfish

In this work, systematic analysis of Hsp90, Hsp60, Hsp10 and sHsps genes was conducted.

A total of 20 Hsp genes were identified and annotated in the channel catfish genome, and their expressions after bacterial infections were examined to determine their involvement in defense responses. The 20 Hsp genes included five Hsp90 genes, one Hsp60 gene, one Hsp10 gene, and 13 sHsp genes. In most cases, their identities were readily determined by phylogenetic analysis, but with four Hsp genes, syntenic analysis was required to provide additional evidence to properly annotate these genes. Of the four genes, three were found to be within well conserved syntenic region in the genomes, thus their identities were determined. However, with one gene, Trap1, phylogenetic analysis alone did not provide concrete answer to its identity, and the genomic region was not conserved among analyzed teleost genomes. Therefore, it was annotated as Trap1-like.

4.5.2. Copy numbers of Hsp genes

The copy numbers of the analyzed Hsp genes are generally conserved among many organisms across a broad evolutionary spectrum (Table 4-2). However, several differences were noted. First, the teleost and amphibian have two copies of *hsp90aa1*, while mammal, bird and reptile possess only one copy, suggesting that one copy of this gene was lost during evolution between amphibian and reptile. Second, *Hspb10/Odf1* was not found from the teleost and amphibian genomes, but was found from the genomes of reptiles, birds and mammals, suggesting that the gene was gained in higher vertebrate during evolution (Elicker and Hutson, 2007; Franck et al., 2004). Third, catfish as well as zebrafish have two copies of *cryab* gene, *cryaba* and *cryabb*, while other species have only one *cryab* genes (Table 4-2).

4.5.3 Hsp genes of channel catfish involved in disease defense

Although reports were available for the involvement of the Hsp genes in disease defenses (Chen et al., 2010; Roberts et al., 2010; Sung and MacRae, 2011), there was no previous studies conducted systematically examining the expression of the Hsp genes after bacterial infection. In

this study, I took advantage of the existing RNA-Seq datasets obtained after ESC and columnaris challenges (Li et al., 2012; Sun et al., 2012). Among 20 Hsp genes being studied, five Hsp90 genes (*hsp90aa1.1*, *hsp90aa1.2*, *hsp90ab1*, *hsp90b1*, *trap1L*), one Hsp60 gene (*hspd1*), one Hsp10 gene (*hspe1*), and nine sHsp genes (*hspb1*, *hspb2*, *hspb3*, *cryabb*, *hspb6*, *hspb7*, *hspb8*, *hspb9*, *hspb11*) were found to be differentially expressed after bacterial challenges (Figure. 4-8; Figure. 4-9). Although their roles and the mechanisms of regulation are not known at present, the findings that they are significantly regulated after bacterial challenges suggest that they are involved in disease responses, and perhaps also in disease defenses against infectious bacteria.

Hsp90 proteins were known to be abundantly expressed in most cells under normal conditions. In terrestrial animal species, there are four Hsp90 gene paralogues: two (Hsp90aa and Hsp90ab) are cytosolic, Hsp90b1 is distributed in the endoplasmic reticulum (ER), and Trap1 is mitochondrial. Hsp90s are chaperone proteins playing a number of important roles including interacting with immune cells as a ligand for a variety of cell-surface receptors such as Toll-like receptors and CDs (Basu et al., 2001; Vabulas et al., 2002). In this study, *hsp90aa1.1* was up-regulated in gill at first (4h) and then returned to the normal level (24h) after *F. columnare* infection (Figure 4-8). However, it was not expressed in intestine after *E. ictaluri* infection (Figure 4-9). Both *hsp90aa1.2* and *hsp90b1* were significantly up-regulated in all three time points after *F. columnare* infection, but they did not show significant fold change in intestine after *E. ictaluri* infection. Due to the constitutive expression, *hsp90ab1* had the highest total RPKM values in both experiments, but it did not show significant fold change with the exception of being down-regulated at 3d in intestine after *E. ictaluri* infection (Figure 4-8; Figure 4-9). *trap1L* was significantly up-regulated in gill at 24h after *F. columnare* infection, but was not differentially expressed in intestine after *E. ictaluri* infection. These results demonstrated the different

expression patterns between Hsp90aa and Hsp90ab genes: the former is inducible but the latter is constitutively expressed (Chen et al., 2005a). Additionally, *hsp90aa1.1* and *hsp90aa1.2* had different expression profile though they were tandem repeated genes. Furthermore, four Hsp90 genes showed up-regulation in gill after *F. columnare* infection (Figure 4-8), but they were not significantly regulated in intestine after *E. ictaluri* infection challenge at all timepoints after infection (Figure 4-9), suggesting pathogen-specific gene regulation.

Hsp60 is an important chaperonin, and was also proposed to interact with immune cells as a ligand for a variety of cell-surface receptors such as Toll-like receptors and CDs (Habich et al., 2002; Ohashi et al., 2000; Vabulas et al., 2001). Hsp60 requires interacting with Hsp10 for proper function (Zeilstra-Ryalls et al., 1991). In this study, both *hspd1* and *hspe1* showed up-regulation in gill after *F. columnare* infection (Figure 4-8), however both were down-regulated in intestine at 3d after *E. ictaluri* infection (Figure 4-9), indicating that their expression was regulated temporally as well as by the functions of pathogenesis with different diseases.

sHsps are the most diverse family amongst the heat shock proteins. Amongst 13 sHsp genes identified in channel catfish, nine sHsp genes exhibited differential expression after *E. ictaluri* or *F. columnare* infections (Figure 4-8; Figure 4-9). *hspb1* was down-regulated in gill at 24h and 48h after *F. columnare* infection, but was not significantly regulated in intestine after *E. ictaluri* infection. *hspb2*, *hspb3* and *hspb9* were down-regulated expression in gill after *F. columnare* infection but their expression was low or not detected in intestine after *E. ictaluri* infection (Supplementary Table 4-2), suggesting tissue-specific expression. In this study, RNA-Seq datasets were from intestine (ESC) and gill (columnaris). These findings are consistent with previous reports that expression of *hspb2* and *hspb3* was restricted to heart and skeletal muscle cells in mammals (Sugiyama et al., 2000; Verschuure et al., 2003) because gill has skeletal muscle cells

while intestine has not.

cryabb was significantly down-regulated at 24h in gill after *F. columnare* infection but was significantly up-regulated at 3h and 24h in intestine after *E. ictaluri* infection (Figure 4-8; Figure 4-9). As the major structural protein of eye lens, Cryaa (αA-crystallin) is abundantly expressed together with Cryab (αB-crystallin) in the eye lens. In spite of their high degree of homology, Cryab is expressed in many non-lenticular tissues; it has the greater chaperone-like activity than Cryaa and its heat-induced conformational change and aggregation is more susceptible than that of Cryaa (Bhat and Nagineni, 1989; Horwitz et al., 1998; Iwaki et al., 1989; Liang et al., 2000; Sun et al., 1997). This could explain why *cryaa* was not expressed in gill or intestine while *cryabb* was expressed in both tissues.

One interesting observation was that all up-regulated Hsp genes tended to be differentially expressed at early times after infection, whereas the down-regulated Hsp genes exhibited differential expression at much later times after infection. The exact reason of this observation is unknown at present, but it is possible that higher levels of Hsp gene expression at early stages of pathogenesis could be beneficial to the host because chaperone activities are demanded to fight against the bacterial infection. However, as the pathogenesis progressed, additional high levels of Hsp expression could be detrimental to the host. Alternatively, the regulated Hsp gene expression could also be a consequences of pathogenesis rather than proactive or active defense mechanisms of the host because it is well known that Hsps can be induced by many stresses including infections (Ackerman and Iwama, 2001; Chen et al., 2010; Dong et al., 2006; Forsyth et al., 1997; Sung and MacRae, 2011).

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Table 4-1.

Gene list of Hsp90 family, Hsp60/10 family and small HSP family in channel catfish

Gene	Accession Number	Domain
hsp90aa1.1	JT341208.1	HATPase_c--HSP90
hsp90aa1.2	JT411683.1	HATPase_c--HSP90
hsp90ab1	chao_comp184115	HATPase_c--HSP90
hsp90b1	JT417742.1	HATPase_c--HSP90
trap1L	JT406894.1	HATPase_c--HSP90
hspd1	JT407863.1	GroEL/cpn60
hspe1	JT408710.1	GroES/cpn10
hspb1	JT419201.1	ACD_HspB1_like
hspb2	JT416075.1	Crystallin--ACD_HspB2_like
hspb3	JT413022.1	ACD_HspB3_Like
cryaa	JT408754.1	Crystallin--ACD_alphaA-crystallin_HspB4
cryaba	comp150340_c0_seq1	Crystallin--ACD_HspB4-5-6
cryabb	JT407340.1	Crystallin--ACD_HspB4-5-6
hspb6	JT410786.1	metazoan_ACD
hspb7	JT408248.1	ACD_HspB7_like
hspb8	JT417241.1	ACD_HspB8_like
hspb9	JT412125.1	metazoan_ACD
hspb11	JT473518.1	ACD_HspB9_like
hspb12	JT471744.1	ACD_HspB7_like
hspb15	JT426179.1	ACD_HspB1_like

Table 4-2.

Comparison of copy numbers of Hsp90, Hsp60/10 and sHsp gene families among selected vertebrate genomes

Gene Name	**Human**	**Bird**	**Amphibian**	**Tilapia**	**Fugu**	**Medaka**	**Zebrafish**	**Catfish**
Hsp90aa1	1	1	2	2	2	2	2	2
Hsp90ab1	1	1	1	1	1	1	1	1
Hsp90b1	1	1	1	1	1	1	1	1
Trap1	1	1	1	1	1	1	2	1
Total of Hsp90 family	**4**	**4**	**5**	**5**	**5**	**5**	**6**	**5**
Hspd1	1	1	1	1	1	1	1	1
Hspe1	1	1	1	1	1	1	1	1
Total of Hsp60/10 family	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Hspb1	1	1	1	1	1	1	1	1
Hspb2	1	1	1	0	0	0	1	1
Hspb3	1	1	1	1	1	1	1	1
Cryaa	1	1	1	1	1	1	1	1
Cryab	1	1	1	1	1	1	2	2
Hspb6	1	0	1	1	1	0	1	1
Hspb7	1	1	1	0	1	0	1	1
Hspb8	1	1	1	1	1	1	1	1
Hspb9	1	0	0	1	0	0	1	1
Hspb10/Odf1	1	1	0	0	0	0	0	0
Hspb11	1	0	1	1	1	1	1	1
Hspb12	0	0	1	1	1	0	1	1
Hspb15	0	0	0	1	1	1	1	1
Total of sHsp family	**11**	**8**	**10**	**10**	**10**	**7**	**13**	**13**
Total	**17**	**14**	**17**	**17**	**17**	**14**	**21**	**20**

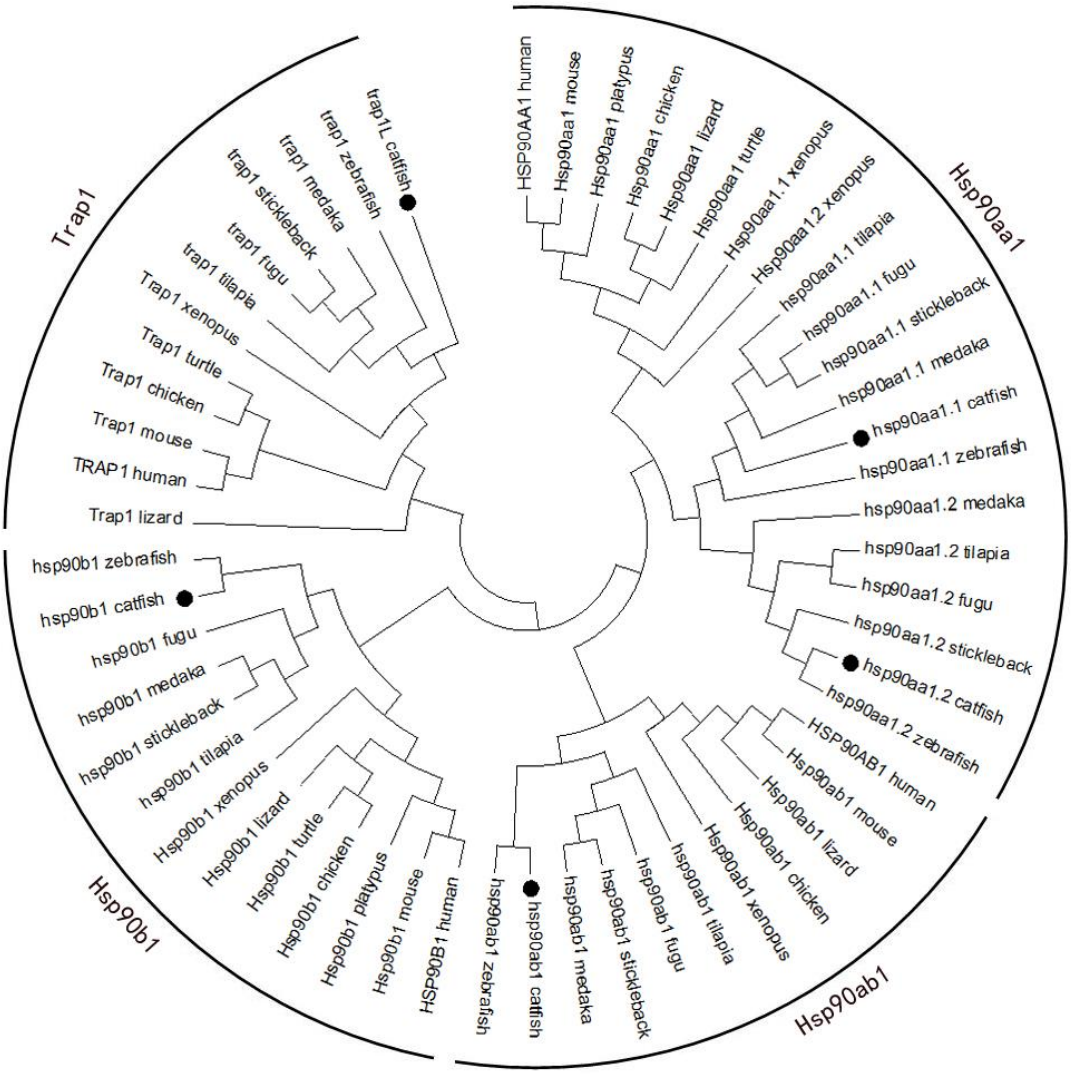

Figure 4-1.

Phylogenetic analysis of channel catfish Hsp90 genes with other species. Multiple amino acid sequences were aligned with E-INS-i strategies from MAFFT v7.01 with default parameters. The phylogenetic tree was constructed by using the maximum likelihood method with LG+G model and 95% partial deletion method in MEGA6. The statistical robustness of the tree was estimated by bootstrapping with 1000 replicates. Bootstrap values are indicated by numbers at the nodes.

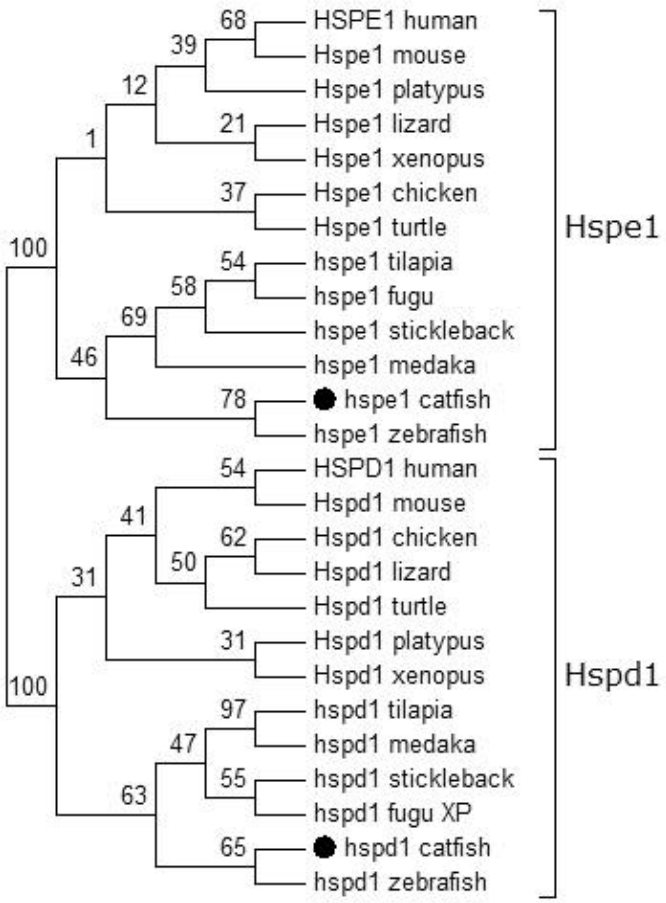

Figure 4-2.

Phylogenetic analysis of channel catfish *hspd1* and *hspe1* with other species. Multiple amino acid sequences were aligned with E-INS-i strategies from MAFFT v7.01 with default parameters. The phylogenetic tree was constructed by using the maximum likelihood method with JTT+G model and 95% partial deletion method in MEGA6. The statistical robustness of the tree was estimated by bootstrapping with 1000 replicates. Bootstrap values are indicated by numbers at the nodes.

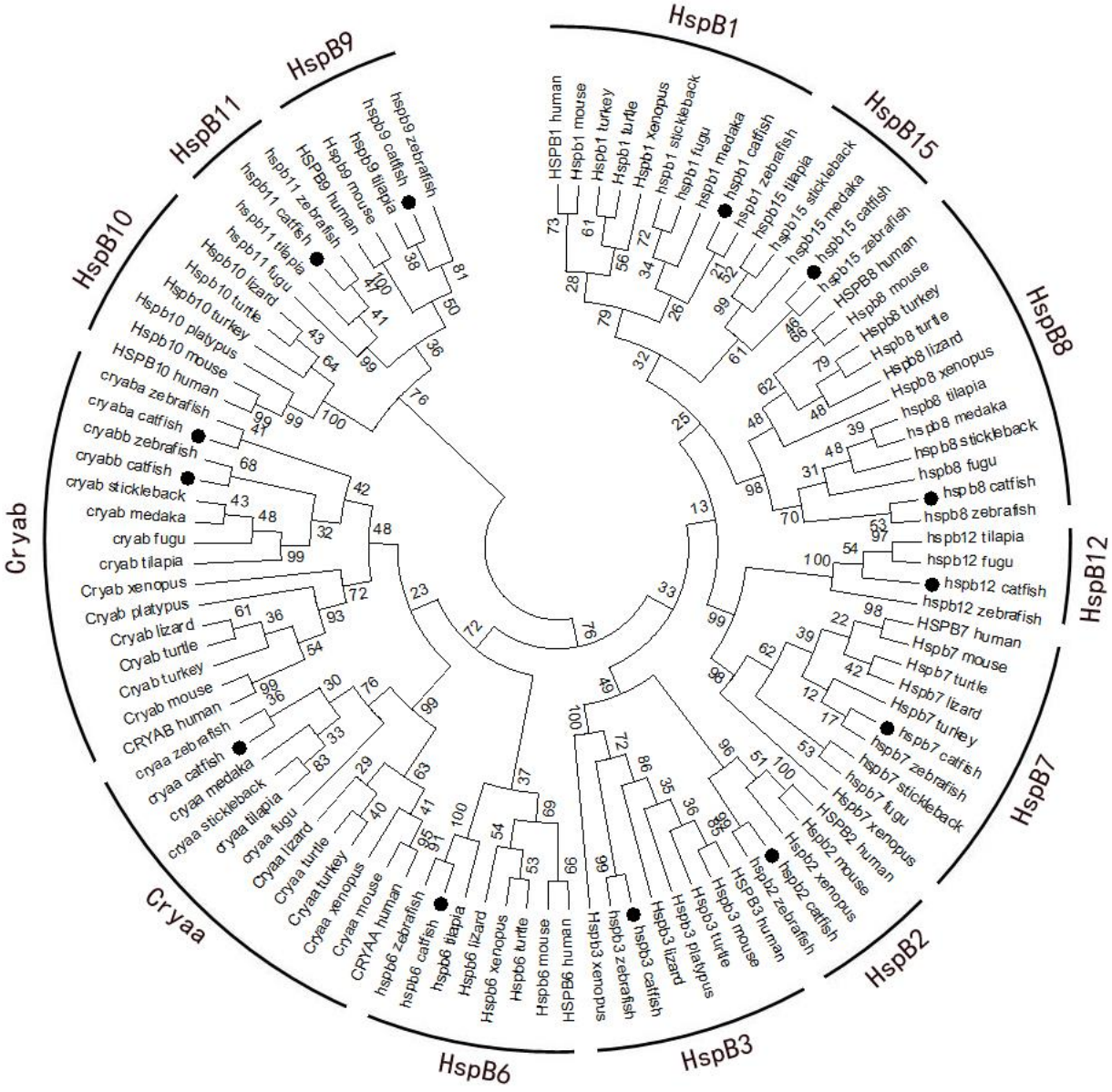

Figure 4-3.

Phylogenetic analysis of channel catfish sHsp genes with other species. Multiple amino acid sequences were aligned with L-INS-i strategies from MAFFT v7.01 with default parameters. The phylogenetic tree was constructed by using the Minimum Evolution method with JTT+G model and 95% partial deletion method in MEGA6. The statistical robustness of the tree was estimated by bootstrapping with 1000 replicates. Bootstrap values are indicated by numbers at the nodes.

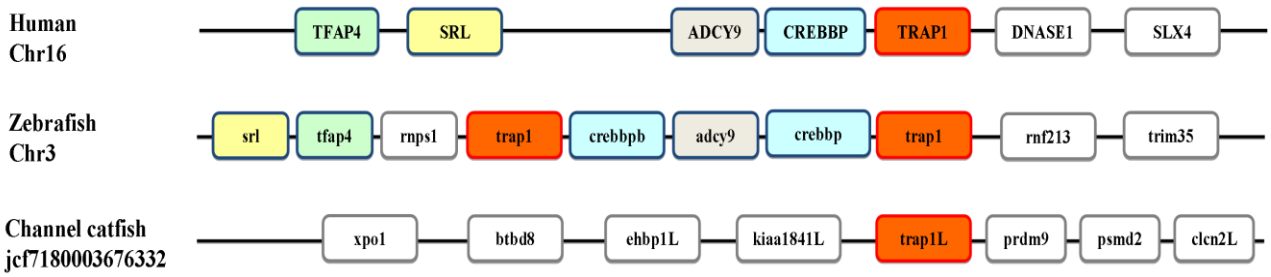

Figure 4-4.

Syntenic analysis of channel catfish *trap1L* gene with zebrafish and human. *trap1* in channel catfish has no common neighbored genes as that of zebrafish and human. Chr indicates chromosome.

adcy9: adenylate cyclase 9; btbd8: BTB (POZ) domain containing 8, isoform CRA_c; clcn2L: chloride channel protein 2-like; crebbp: CREB-binding protein; crebbpb: CREB binding protein b; DNASE1: deoxyribonuclease I; ehbp1L: EH domain-binding protein 1-like; kiaa1841L: uncharacterized protein KIAA1841-like; prdm9: histone-lysine N-methyltransferase PRDM9; psmd2: 26S proteasome non-ATPase regulatory subunit 2; rnf213: ring finger protein 213; rnps1: RNA binding protein S1, serine-rich domain; SLX4: SLX4 structure-specific endonuclease subunit; srl: sarcalumenin; tfap4: transcription factor AP-4; trap1: TNF receptor-associated protein 1; trap1L: TNF receptor-associated protein 1-like; trim35: tripartite motif containing 35; xpo1: exportin-1.

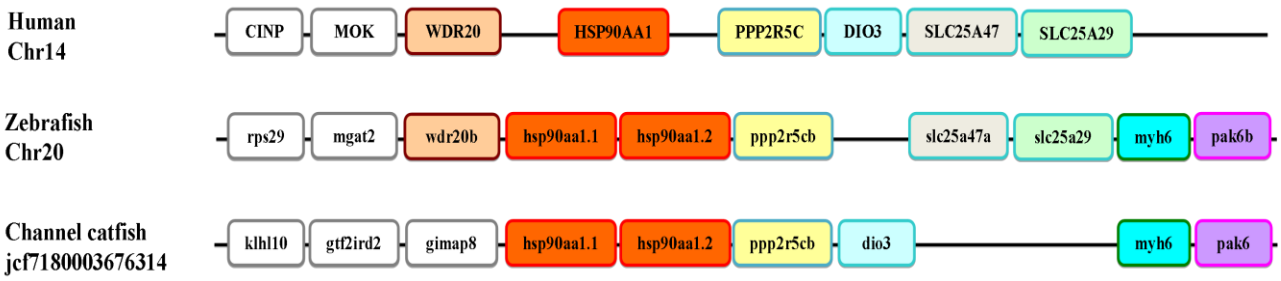

Figure 4-5.

Conserved syntenic analysis of channel catfish *hsp90aa1.1* gene with zebrafish and human. *hsp90aa1.1* in channel catfish has some common neighbored genes as that of zebrafish and human. Chr indicates chromosome.

CINP: cyclin-dependent kinase 2 interacting protein; dio3: iodothyronine deiodinase type 3; gimap8: GTPase IMAP family member 8; gtf2ird2: general transcription factor II-I repeat domain-containing protein 2; HSP90AA1: heat shock protein 90kDa alpha (cytosolic), class A member 1; hsp90aa1.1: heat shock protein 90, alpha (cytosolic), class A member 1, tandem duplicate 1; hsp90aa1.2: heat shock protein 90, alpha (cytosolic), class A member 1, tandem duplicate 2; klhl10: Kelch-like protein 10; mgat2: mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase; MOK: MOK protein kinase; myh6: myosin, heavy polypeptide 6, cardiac muscle, alpha; pak6: serine/threonine-protein kinase PAK 6; pak6b: p21 protein (Cdc42/Rac)-activated kinase 6b; PPP2R5C: protein phosphatase 2, regulatory subunit B', gamma; ppp2r5cb: protein phosphatase 2, regulatory subunit B', gamma b; rps29: ribosomal protein S29; slc25a29: solute carrier family 25, member 29; SLC25A47: solute carrier family 25, member 47; slc25a47a: solute carrier family 25, member 47a; WDR20: WD repeat domain 20; wdr20b: WD repeat domain 20b.

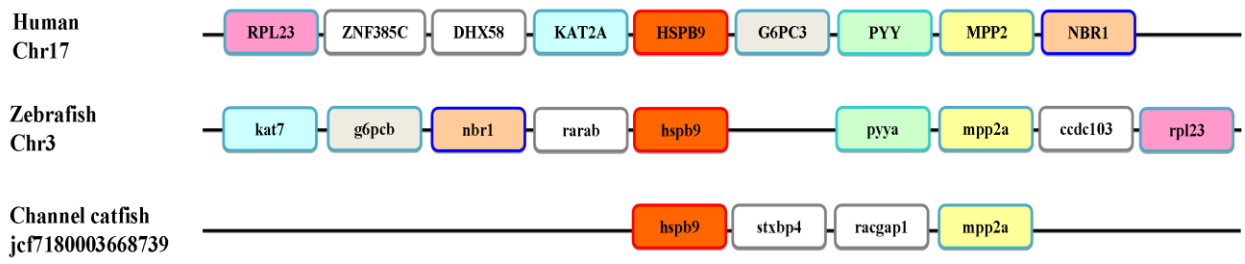

Figure 4-6.

Conserved syntenic analysis of channel catfish *hspb9* gene with zebrafish and human. *hspb9* in channel catfish has one common neighbored gene as that of zebrafish and human. There are only four identified genes in Scaffold embodied *hspb9*. Chr indicates chromosome.

ccdc103: coiled-coil domain containing 103; DHX58: DEXH (Asp-Glu-X-His) box polypeptide 58; G6PC3: glucose-6-phosphatase 3, catalytic; g6pcb: glucose-6-phosphatase b, catalytic; hspb9: heat shock protein, alpha-crystallin-related, 9; KAT2A: K(lysine) acetyltransferase 2A; kat7: K(lysine) acetyltransferase 7; MPP2: MAGUK p55 subfamily member 2; mpp2a: membrane protein, palmitoylated 2a; nbr1: neighbor of BRCA1 gene 1; PYY: peptide YY; pyya: peptide YYa; racgap1: Rac GTPase-activating protein 1; rarab: retinoic acid receptor, alpha b; rpl23: ribosomal protein L23; stxbp4: syntaxin-binding protein 4; ZNF385C: zinc finger protein 385C.

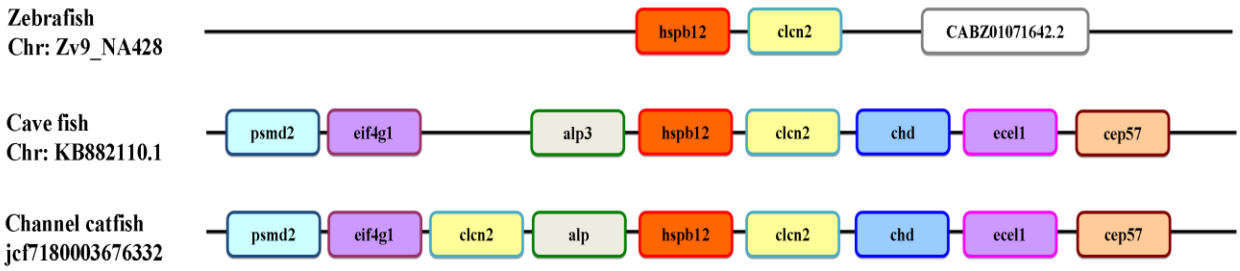

Figure 4-7.

Conserved syntenic analysis of channel catfish *hspb12* gene with zebrafish and cave fish. *Hspb12* in channel catfish has some common neighbored genes as that of cave fish and has one common neighbored gene as that of zebrafish which only two neighbor genes were known so far. Chr indicates chromosome.

alp: alkaline phosphatase, tissue-nonspecific isozyme precursor; alp3: alkaline phosphatase 3; CABZ01071642.2: Uncharacterized protein (ENSDARG00000087003); cep57: centrosomal protein 57kDa; chd: Chordin; clcn2: chloride channel protein 2; ecel1: endothelin-converting enzyme-like 1; eif4g1: eukaryotic translation initiation factor 4 gamma 1; hspb12: heat shock protein, alpha-crystallin-related, b12; psmd2: 26S proteasome non-ATPase regulatory subunit 2.

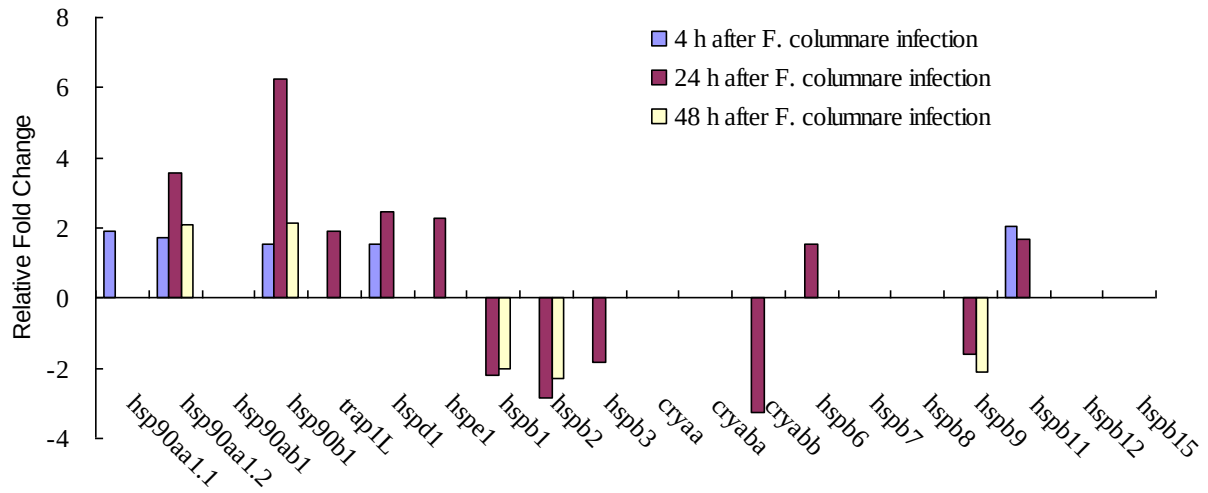

Figure 4-8.

Significant expression of channel catfish Hsp90, Hsp60/10 and sHsp genes in gill after *F. columnare* infection (Absolute fold change value≥1.5). Gene expressions were presented as fold-change relative to control samples.

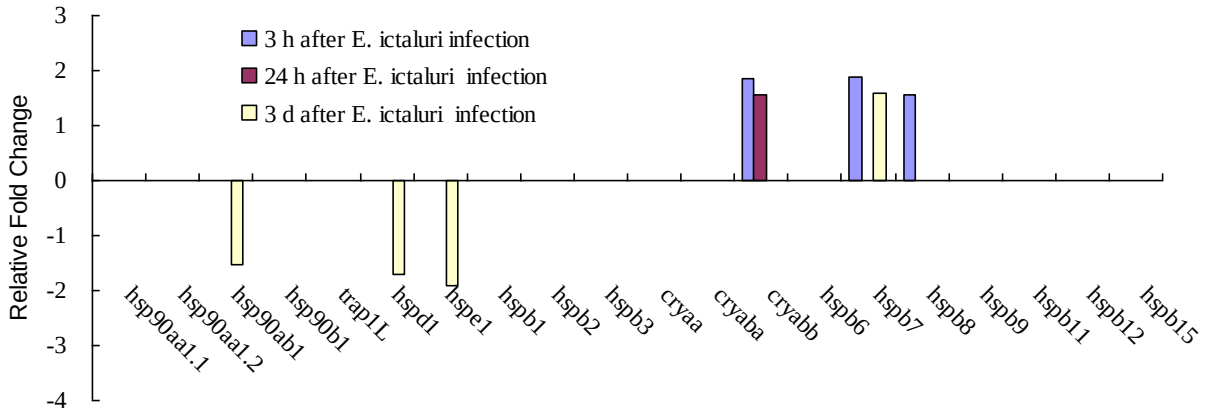

Figure 4-9.

Significant expression of channel catfish Hsp90, Hsp60/10 and sHsp genes in intestine after *E. ictaluri* infection (Absolute fold change value≥1.5). Gene expressions were presented as fold-change relative to control samples.

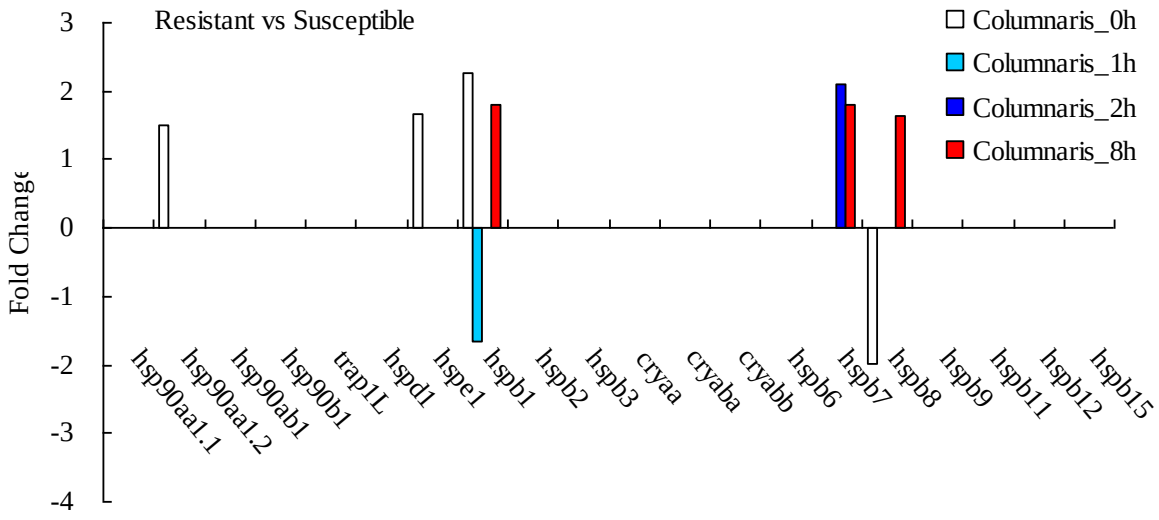

Figure 4-10.

Significant expression of Resistant and Susceptible channel catfish Hsp90, Hsp60/10 and sHsp genes in gill before and after *F. columnare* infection (Absolute fold change value≥1.5). Gene expressions were presented as fold-change of columnaris resistant channel catfish relative to columnaris susceptible channel catfish.

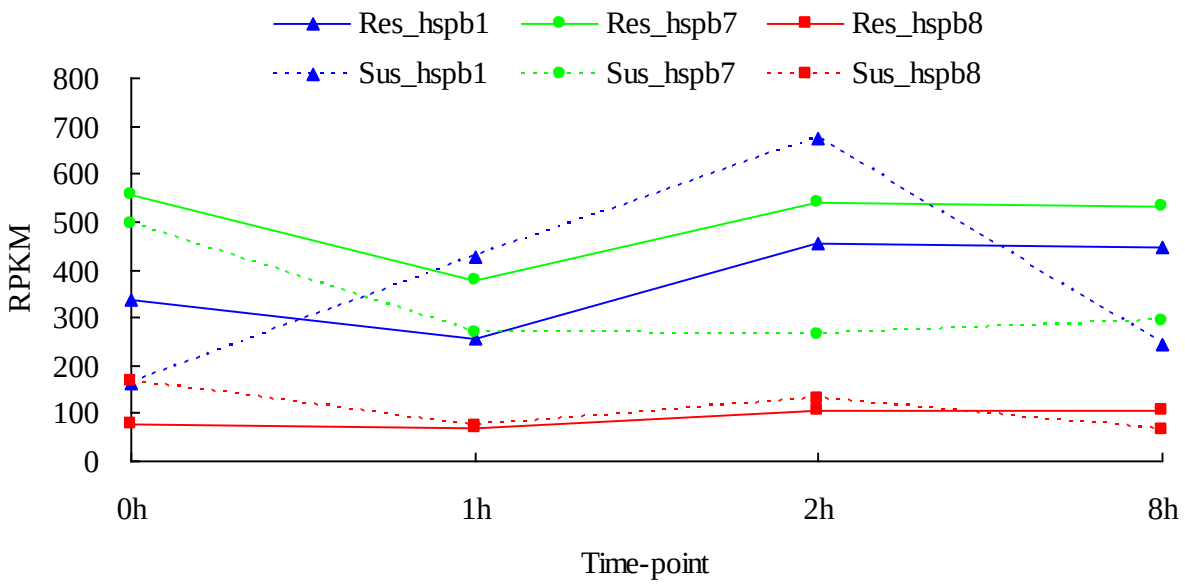

Figure 4-11.

RPKM (Reads Per Kilobase of exon model per Million mapped reads) of columnaris resistant channel catfish and columnaris susceptible channel catfish *hspb1*, *hspb7* and *hspb8* in gill before and after *F. columnare* infection. Res: Resistant family; Sus: Susceptible family.

Chapter 5 General conclusions

Heat shock proteins play key roles in assisting protein folding by their binding ability in not only stressed cells but also normal cells. In spite of their importance, there was no systematic analysis of heat shock proteins in teleost fish before my work reported in this dissertation. In channel catfish, only a few Hsp40 and hsp70 genes were characterized (Chen et al., 2010; Luft et al., 1996). Systematic analysis of channel catfish HSPs genes has been lacking, as the situation with all fish species. In this study, I identified a total of 93 HSPs in channel catfish (*Ictalurus punctatus*), with 13 members of small HSP family, 57 members of Hsp40 family, 16 members of Hsp70 family, one member of HSP60 family, one member of Hsp10 family and 5 members of HSP90 family. These were achieved by thorough analysis of genomic and transcriptomic resources including several hundred thousands of ESTs (Li et al., 2007; Wang et al., 2010), RNA-Seq transcriptome assemblies (Liu et al., 2011; 2012), and the draft genome sequences (unpublished). This work allowed the identification and characterization of the most complete set of HSPs among teleost fish species, which account for over 50% of all vertebrate animal species. This complete set of HSPs from channel catfish made up the void of HSPs sequences among teleost fish species. As HSPs are highly conserved proteins and they are frequently used for phylogenetic analysis, the complete set of Hsps identified here will allow more thorough analysis for phylogenetics.

Phylogenetic and syntenic analyses allowed annotation of all the families of HSPs and thus provided strong evidence in supporting the annotations of these HSPs. It is apparent that catfish harbored most of HSPs genes. Compared with the human genome, the catfish genome lacked only

three hsp40 genes, three hsp70 genes, one hsp110 gene and one small hsp gene, which is similar to the situation of other teleost fish species. However, a number of paralogues were discovered in channel catfish due to the whole genome duplication event of teleost that occurred during evolution of the phylum Chordata (Postlethwait, 2007). The phylogenetic analysis strongly supported the nomenclature of catfish HSP genes in all five families. Most HSPs are conserved through evolution, while there are still special preferences made by teleost and catfish. First, the teleost tended to have more duplications than mammals, likely as a consequence of whole genome duplication. However still some HSPs genes gained by terrestrial species due to the physiological demands of surviving in new environment. Besides, two tandem repeat members of Hsp70/110s, *hsp70.2* and *hsp70.3* were not found located within the MHC-complex, which is a group of molecules that are essential in the antigen presenting process, suggesting different process of presenting the antigens or epitopes comparing with other species.

RNA-Seq-based expression analysis has become a robust method to assess transcriptional profiles to different challenge experiments (Oshlack et al., 2010). In our recent RNA-Seq studies, I have successfully obtained comprehensive transcriptome assemblies from catfish intestine after *E. ictaluri* infection and from catfish gill after *F. Columnare* infection (Li et al., 2012; Sun et al., 2012). The expression patterns of differentially expressed genes from these two studies were validated by quantitative real-time RT-PCR with average correlation coefficient around 0.9 ($p < 0.001$). Meta-analysis of disease challenges revealed a gene fold change profile of catfish HSPs involving in disease defense. HSPs expression was analyzed through the early phase of immune response. As a result, a majority of catfish HSPs were found significantly expressed in gill and intestine after bacterial infections during the early immune response phase. A total of 30 Hsp40 genes were regulated under disease situations involving two tissues after two bacterial infections.

Five Hsp90 genes, one Hsp60 gene, one Hsp10 gene and nine sHsp genes were significantly up/down-regulated after two bacterial infections. Twelve Hsp70/110s genes were significantly up/down-regulated after two bacterial infections. As an agonist ligand of TLR4, an import Pattern recognition receptor of innate immunity, Hsp70s showed a time pattern through the two bacterial infections in two tissues. Also a pathogen-specific response in host Hsp90 had been revealed. A pathogen-specific expression pattern of Hsp90 was observed. Both pathogen-specific and tissue-specific pattern were found in small Hsp family after all three bacterial infections. These results suggested that most of the Hsp genes might play important roles in disease response and/or disease defense in channel catfish.

As mentioned in the introduction, although regulated expression of HSPs after infection have been reported in several fish species, systematic analysis of their involvement in diseases has not been conducted. This work, therefore, represents the first systematic analysis of HSPs involvement after bacterial infection among all species.

Besides, the systematically phylogenetic and syntenic analysis of catfish HSPs are provide important information for evolution due to they are very conserved in almost all the organisms. Teleost species count more than 50% of the vertebrate species. They display a more complex and disrupt genome due to the rapid expansion and contraction species caused by evolution of whole genome duplication (Postlethwait, 2007). Thus, a gap of phylogenetic and systematic information of evolution has been filled.

Although this work is most relevant to evolutionary studies or gene expression studies, it may have some implications for aquaculture. This study also provides information for aquaculture industry as a guide in disease control management. Control of disease caused by vibriosis in the crustacean *Artemia franciscana* is achieved by employing non-lethal heat shock to boost

endogenous Hsp70 and by feeding the organism with bacteria enriched in DnaK, the prokaryotic equivalent of Hsp70. Platyfish are protected against *Yersinia rukeri* by injecting them with bacterial Hsps, an effect enhanced by non-lethal heat shock. Application of Hsp stimulants such as Tex-OE®, a patented extract of the prickly pear cactus *Opuntia ficus indica* that non-traumatically enhances stress protein synthesis in fish and shrimp, is useful against several bacterial and viral diseases. Because microbial Hsp60 (GroEL) and Hsp70 (DnaK) are frequently major pathogen-derived antigens that invoke high antibody response, they have the potential to function as highly specific potent vaccines against harmful biotic agents. Besides, HSPs used as an adjuvant can enhance the protectiveness and efficacy of the vaccine. Thus the products of HSPs have a more promising future to apply in the aquaculture industry. However, I must say that the information is not directly applicable to aquaculture. Perhaps one applicable aspect is the possibility of using expression of Hsps as indicators of disease infection and stages of disease progression because the expression profiles of Hsps with each disease or during the course of pathogenesis is quite characteristic of the infection or time course after infection.

There are still more work need to be done in future.

a. Based on their expressions after infections, they are believed to involve in disease response. However certainly additional functional analysis with respect to certain disease is still required to obtain a solid conclusion due to different heat shock proteins have preferential responses to the diseases.

b. For applying in aquaculture, the novel product of vaccine with HSPs adjuvant can be developed according to the previous work. And the safety and efficacy need to be validated.

c. By applying to genomic improvement program as marker assistant selection, the specific SNPs at or near the loci of HSPs of disease resistant families should be analyzed for their applicability as markers for disease resistance in selective breeding programs.

Appendices and Supplementary Tables

Table S2-1 Gene names and accession numbers of reference Hsp40s used in this study.

Associated Gene Name	NCBI Protein ID	Species Name	Latin name
DNAJA1	NP_001530.1	human	*Homo sapiens*
DNAJA2	NP_005871.1	human	*Homo sapiens*
DNAJA3	NP_005138.3	human	*Homo sapiens*
DNAJA4	NP_061072.3	human	*Homo sapiens*
DNAJB1	NP_006136.1	human	*Homo sapiens*
DNAJB2	NP_001034639.1	human	*Homo sapiens*
DNAJB3	NP_001001394.1	human	*Homo sapiens*
DNAJB4	NP_008965.2	human	*Homo sapiens*
DNAJB5	NP_001128476.2	human	*Homo sapiens*
DNAJB6	NP_005485.1	human	*Homo sapiens*
DNAJB7	NP_660157.1	human	*Homo sapiens*
DNAJB8	NP_699161.1	human	*Homo sapiens*
DNAJB9	NP_036460.1	human	*Homo sapiens*
DNAJB11	NP_057390.1	human	*Homo sapiens*
DNAJB12	NP_001002762.2	human	*Homo sapiens*
DNAJB13	NP_705842.2	human	*Homo sapiens*
DNAJB14	NP_001026893.1	human	*Homo sapiens*
DNAJC1	NP_071760.2	human	*Homo sapiens*
DNAJC2	NP_001123359.1	human	*Homo sapiens*
DNAJC3	NP_006251.1	human	*Homo sapiens*
DNAJC4	NP_005519.2	human	*Homo sapiens*
DNAJC5	NP_079495.1	human	*Homo sapiens*
DNAJC5B	NP_149096.2	human	*Homo sapiens*
DNAJC5G	NP_775921.1	human	*Homo sapiens*
DNAJC6	NP_001243793.1	human	*Homo sapiens*
DNAJC7	NP_001138238.1	human	*Homo sapiens*
DNAJC8	NP_055095.2	human	*Homo sapiens*
DNAJC9	NP_056005.1	human	*Homo sapiens*
DNAJC10	NP_001258510.1	human	*Homo sapiens*
DNAJC11	NP_060668.2	human	*Homo sapiens*
DNAJC12	NP_068572.1	human	*Homo sapiens*
DNAJC13	NP_056083.3	human	*Homo sapiens*
DNAJC14	NP_115740.5	human	*Homo sapiens*

DNAJC15	NP_037370.2	human	*Homo sapiens*
DNAJC16	NP_056106.1	human	*Homo sapiens*
DNAJC17	NP_060633.1	human	*Homo sapiens*
DNAJC18	NP_689899.1	human	*Homo sapiens*
DNAJC19	NP_001177162.1	human	*Homo sapiens*
DNAJC20(HSCB)	NP_741999.3	human	*Homo sapiens*
DNAJC22	NP_079178.2	human	*Homo sapiens*
DNAJC24	NP_859057.4	human	*Homo sapiens*
DNAJC25	NP_001015882.2	human	*Homo sapiens*
DNAJC26	NP_005246.2	human	*Homo sapiens*
DNAJC27	NP_001185488.1	human	*Homo sapiens*
DNAJC28	NP_001035282.1	human	*Homo sapiens*
DNAJc29(SACS)	NP_001264984.1	human	*Homo sapiens*
DNAJC30	NP_115693.2	human	*Homo sapiens*

Associated Gene Name	**NCBI Protein ID**	**Species Name**	**Latin name**
Dnaja1	NP_001158143.1	Mouse	Mus musculus
Dnaja2	NP_062768.1	Mouse	Mus musculus
Dnaja3	NP_001128584.1	Mouse	Mus musculus
Dnaja4	NP_067397.1	Mouse	Mus musculus
Dnajb1	NP_061278.1	Mouse	Mus musculus
Dnajb2	NP_001153355.1	Mouse	Mus musculus
Dnajb3	NP_032325.2	Mouse	Mus musculus
Dnajb4	NP_080202.1	Mouse	Mus musculus
Dnajb5	NP_063927.1	Mouse	Mus musculus
Dnajb6	NP_001033029.1	Mouse	Mus musculus
Dnajb7	NP_067292.2	Mouse	Mus musculus
Dnajb8	NP_064348.1	Mouse	Mus musculus
Dnajb9	NP_038788.2	Mouse	Mus musculus
Dnajb11	NP_001177733.1	Mouse	Mus musculus
Dnajb12	NP_064349.2	Mouse	Mus musculus
Dnajb13	NP_705755.2	Mouse	Mus musculus
Dnajb14	NP_001028327.1	Mouse	Mus musculus
Dnajc1	NP_001177746.1	Mouse	Mus musculus
Dnajc2	NP_033610.1	Mouse	Mus musculus
Dnajc3	NP_032955.2	Mouse	Mus musculus
Dnajc4	NP_065591.1	Mouse	Mus musculus
Dnajc5	NP_001258513.1	Mouse	Mus musculus
Dnajc6	NP_001158055.1	Mouse	Mus musculus
Dnajc7	NP_062769.2	Mouse	Mus musculus
Dnajc8	NP_765988.2	Mouse	Mus musculus
Dnajc9	NP_598842.1	Mouse	Mus musculus
Dnajc10	NP_077143.2	Mouse	Mus musculus
Dnajc11	NP_766292.2	Mouse	Mus musculus

Dnajc12	NP_001240614.1	Mouse	Mus musculus
Dnajc13	NP_001156498.1	Mouse	Mus musculus
Dnajc14	NP_083149.3	Mouse	Mus musculus
Dnajc15	NP_079660.1	Mouse	Mus musculus
Dnajc16	NP_758841.1	Mouse	Mus musculus
Dnajc17	NP_631878.2	Mouse	Mus musculus
Dnajc18	NP_083945.1	Mouse	Mus musculus
Dnajc19	NP_001021382.1	Mouse	Mus musculus
DnaJc20(HscB)	NP_705799.2	Mouse	Mus musculus
Dnajc21	NP_084322.2	Mouse	Mus musculus
Dnajc22	NP_789805.1	Mouse	Mus musculus
DnaJc23(Sec63)	NP_694695.3	Mouse	Mus musculus
Dnajc24	NP_081268.1	Mouse	Mus musculus
Dnajc25	NP_001028337.2	Mouse	Mus musculus
DnaJc26(GAK)	NP_705797.1	Mouse	Mus musculus
Dnajc27	NP_694722.2	Mouse	Mus musculus
Dnajc28	NP_001093208.1	Mouse	Mus musculus
DNAJc29(SACS)	NP_766397.2	Mouse	Mus musculus
Dnajc30	NP_079638.2	Mouse	Mus musculus

Associated Gene Name	**NCBI Protein ID**	**Species Name**	**Latin name**
Dnaja1	XP_001515385.1	platypus	*Ornithorhynchus anatinus*
LOC100075633	XP_001507642.1	platypus	*Ornithorhynchus anatinus*
LOC100681728	XP_003428977.1	platypus	*Ornithorhynchus anatinus*
LOC100091767	XP_001520560.2	platypus	*Ornithorhynchus anatinus*
Dnaja4	XP_001511550.1	platypus	*Ornithorhynchus anatinus*
LOC100681988	XP_003429902.1	platypus	*Ornithorhynchus anatinus*
LOC100093557	XP_001515558.1	platypus	*Ornithorhynchus anatinus*
LOC100082006	XP_001512712.2	platypus	*Ornithorhynchus anatinus*
LOC100075106	XP_001506650.1	platypus	*Ornithorhynchus anatinus*
LOC100078804	XP_003428958.1	platypus	*Ornithorhynchus anatinus*
LOC100081129	XP_001511968.2	platypus	*Ornithorhynchus anatinus*
LOC100074770	XP_003430853.1	platypus	*Ornithorhynchus anatinus*
LOC100081705	XP_001512463.2	platypus	*Ornithorhynchus anatinus*
LOC100081687	XP_001512447.2	platypus	*Ornithorhynchus anatinus*
DnaJb12	ENSOANG00000031022	platypus	*Ornithorhynchus anatinus*
LOC100090819	XP_001519855.1	platypus	*Ornithorhynchus anatinus*
LOC100079285	XP_001510257.2	platypus	*Ornithorhynchus anatinus*
Dnajc3	XP_003430813.1	platypus	*Ornithorhynchus anatinus*
LOC100092598	XP_001521166.2	platypus	*Ornithorhynchus anatinus*
LOC100073724	XP_001509762.1	platypus	*Ornithorhynchus anatinus*
LOC100081140	XP_001511979.2	platypus	*Ornithorhynchus anatinus*
LOC100092623	XP_001521187.2	platypus	*Ornithorhynchus anatinus*

LOC100091537	XP_001520384.1	platypus	*Ornithorhynchus anatinus*
DnaJc9	ENSOANG00000001118	platypus	*Ornithorhynchus anatinus*
Dnajc10	XP_001515735.2	platypus	*Ornithorhynchus anatinus*
LOC100085712	XP_001515988.1	platypus	*Ornithorhynchus anatinus*
Dnajc11	XP_001516061.2	platypus	*Ornithorhynchus anatinus*
LOC100091451	XP_001520320.2	platypus	*Ornithorhynchus anatinus*
Dnajc13	XP_003430514.1	platypus	*Ornithorhynchus anatinus*
LOC100081012	XP_001511861.2	platypus	*Ornithorhynchus anatinus*
LOC100090053	XP_001519302.2	platypus	*Ornithorhynchus anatinus*
DnaJc19	ENSOANG00000011044	platypus	*Ornithorhynchus anatinus*
HscB(LOC100077444)	XP_001508664.2	platypus	*Ornithorhynchus anatinus*
DnaJc22	ENSOANG00000002644	platypus	*Ornithorhynchus anatinus*
DnaJc23(Sec63) (LOC100080660)	XP_001511532.2	platypus	*Ornithorhynchus anatinus*
LOC100074667	XP_001506256.2	platypus	*Ornithorhynchus anatinus*
Dnajc25	NP_001191967.1	platypus	*Ornithorhynchus anatinus*
DnaJc26(GAK)	XP_001512602.2	platypus	*Ornithorhynchus anatinus*
DnaJc27	ENSOANG00000021092	platypus	*Ornithorhynchus anatinus*
LOC100083377	XP_001513941.1	platypus	*Ornithorhynchus anatinus*
DNAJc29(SACS)	XP_001519222.2	platypus	*Ornithorhynchus anatinus*

Associated Gene Name	**NCBI Protein ID**	**Species Name**	**Latin name**
Dnaja1	NP_001012963.1	chicken	*Gallus gallus*
Dnaja2	NP_001005841.1	chicken	*Gallus gallus*
Dnaja3	XP_414967.2	chicken	*Gallus gallus*
Dnaja4	XP_413746.3	chicken	*Gallus gallus*
Dnajb2	XP_424624.4	chicken	*Gallus gallus*
Dnajb4	XP_422386.1	chicken	*Gallus gallus*
Dnajb5	XP_424983.2	chicken	*Gallus gallus*
Dnajb6	NP_001012574.1	chicken	*Gallus gallus*
Dnajb8	XP_001233013.1	chicken	*Gallus gallus*
Dnajb9	NP_001025906.1	chicken	*Gallus gallus*
Dnajb11	XP_422682.1	chicken	*Gallus gallus*
Dnajb12	NP_001026395.1	chicken	*Gallus gallus*
Dnajb13	XP_417251.3	chicken	*Gallus gallus*
Dnajb14	NP_001026546.1	chicken	*Gallus gallus*
Dnajc1	XP_418609.3	chicken	*Gallus gallus*
Dnajc2	NP_001186254.1	chicken	*Gallus gallus*
Dnajc3	NP_001008437.1	chicken	*Gallus gallus*
Dnajc5	XP_417428.1	chicken	*Gallus gallus*
Dnajc6	NP_001170886.1	chicken	*Gallus gallus*
Dnajc7	NP_001026673.2	chicken	*Gallus gallus*
Dnajc8	XP_417717.3	chicken	*Gallus gallus*
Dnajc9	NP_001186454.1	chicken	*Gallus gallus*
Dnajc10	XP_421968.2	chicken	*Gallus gallus*

Dnajc11	XP_425731.2	chicken	*Gallus gallus*
Dnajc12	NP_001186459.1	chicken	*Gallus gallus*
Dnajc13	XP_418787.2	chicken	*Gallus gallus*
Dnajc14	XP_001232019.2	chicken	*Gallus gallus*
Dnajc14	XP_004950802.1	chicken	*Gallus gallus*
Dnajc15	XP_417034.1	chicken	*Gallus gallus*
Dnajc16	NP_001034419.1	chicken	*Gallus gallus*
Dnajc17	NP_001254504.1	chicken	*Gallus gallus*
Dnajc18	NP_001020780.1	chicken	*Gallus gallus*
Dnajc19	XP_003641821.1	chicken	*Gallus gallus*
Dnajc19	XP_003641821.1	chicken	*Gallus gallus*
Dnajc20(Hscb)	XP_003642255.1	chicken	*Gallus gallus*
Dnajc21	XP_425006.2	chicken	*Gallus gallus*
Dnajc22	XP_428430.3	chicken	*Gallus gallus*
Dnajc23	XP_004940397.1	chicken	*Gallus gallus*
Dnajc24	NP_001177825.1	chicken	*Gallus gallus*
Dnajc25	XP_003643130.1	chicken	*Gallus gallus*
Dnajc26	XP_424873.4	chicken	*Gallus gallus*
Dnajc27	NP_998723.1	chicken	*Gallus gallus*
Dnajc28	XP_001233945.2	chicken	*Gallus gallus*
Dnajc29	XP_004938853.1	chicken	*Gallus gallus*
Dnajc30	XP_003642425.1	chicken	*Gallus gallus*

Associated Gene Name	**Esembl Protein ID**	**Species Name**	**Latin name**
dnaja1	ENSPSIP00000010811	Chinese soft shell turtle	*Pelodiscus sinensis*
dnaja2	ENSPSIP00000015086	Chinese soft shell turtle	*Pelodiscus sinensis*
dnaja3	ENSPSIP00000016890	Chinese soft shell turtle	*Pelodiscus sinensis*
dnaja4	ENSPSIP00000010789	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajb1	ENSPSIP00000007250	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajb2	ENSPSIP00000006570	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajb4	ENSPSIP00000015113	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajb5	ENSPSIP00000004751	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajb5	ENSPSIP00000004753	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajb5	ENSPSIP00000004756	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajb6	ENSPSIG00000018102	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajb8	ENSPSIP00000000350	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajb9	ENSPSIP00000008080	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajb11	ENSPSIP00000018615	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajb12	ENSPSIP00000019669	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajb13-201	ENSPSIP00000015043	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajb13-202	ENSPSIP00000015069	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajb14	ENSPSIP00000010049	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc1	ENSPSIP00000020439	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc2	ENSPSIP00000013855	Chinese soft shell turtle	*Pelodiscus sinensis*

dnajc3	ENSPSIP00000002176	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc4-201	ENSPSIP00000011692	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc4-202	ENSPSIP00000011707	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc5-201	ENSPSIP00000018525	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc5b-201	ENSPSIP00000008876	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc5g-201	ENSPSIP00000006211	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc6	ENSPSIP00000010174	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc7	ENSPSIP00000008701	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc8	ENSPSIP00000019990	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc9	ENSPSIP00000016511	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc10	ENSPSIP00000002118	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc11	ENSPSIP00000005117	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc12	ENSPSIP00000007307	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc13-201	ENSPSIP00000012606	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc13-202	ENSPSIP00000012618	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc15	ENSPSIP00000007756	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc16	ENSPSIP00000005416	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc17-201	ENSPSIP00000014828	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc17-202	ENSPSIP00000014837	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc18	ENSPSIP00000014430	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc19	ENSPSIP00000008121	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc20(hscb)	ENSPSIP00000017976	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc21	ENSPSIP00000012304	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc22	ENSPSIP00000003064	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc23(sec63)	ENSPSIP00000006317	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc24-201	ENSPSIP00000013921	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc24-202	ENSPSIP00000013931	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc25	ENSPSIG00000017981	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc26(gak)	ENSPSIP00000019681	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc27	ENSPSIP00000011021	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc28-201	ENSPSIP00000001980	Chinese soft shell turtle	*Pelodiscus sinensis*
dnajc29(sacs)	ENSPSIP00000008975	Chinese soft shell turtle	*Pelodiscus sinensis*

Associated Gene Name	**NCBI Protein ID**	**Species Name**	**Latin name**
dnaja1	NP_001080365.1	African clawed frog	*Xenopus laevis*
dnaja2	NP_001079686.1	African clawed frog	*Xenopus laevis*
dnaja2	NP_001080625.1	African clawed frog	*Xenopus laevis*
dnaja3	NP_001091364.1	African clawed frog	*Xenopus laevis*
dnaja4.1	NP_001079772.1	African clawed frog	*Xenopus laevis*
dnaja4.2	NP_001079642.1	African clawed frog	*Xenopus laevis*
dnajb1	NP_001008112.1	African clawed frog	*Xenopus laevis*
dnajb2	NP_001085074.1	African clawed frog	*Xenopus laevis*
dnajb4	NP_001087928.1	African clawed frog	*Xenopus laevis*
dnajb5	NP_001088287.1	African clawed frog	*Xenopus laevis*
dnajb6-a	NP_001089244.1	African clawed frog	*Xenopus laevis*

dnajb6-b	NP_001088302.1	African clawed frog	*Xenopus laevis*
dnajb9	NP_001080793.1	African clawed frog	*Xenopus laevis*
dnajb11	NP_001086343.1	African clawed frog	*Xenopus laevis*
dnajb12	NP_001085946.1	African clawed frog	*Xenopus laevis*
dnajb13	NP_001089893.1	African clawed frog	*Xenopus laevis*
dnajb14	NP_001080644.1	African clawed frog	*Xenopus laevis*
dnajc1	NP_001085833.1	African clawed frog	*Xenopus laevis*
dnajc2	AAH68855.1	African clawed frog	Xenopus laevis
dnajc3	NP_001080099.1	African clawed frog	Xenopus laevis
dnajc4	NP_001079836.1	African clawed frog	Xenopus laevis
dnajc5b	NP_001083797.1	African clawed frog	Xenopus laevis
dnajc5g	NP_001087399.1	African clawed frog	Xenopus laevis
dnajc6	NP_001120147.1	African clawed frog	Xenopus laevis
dnajc8	NP_001087268.1	African clawed frog	Xenopus laevis
dnajc9	NP_001089275.1	African clawed frog	Xenopus laevis
dnajc10	NP_001084933.1	African clawed frog	Xenopus laevis
dnajc11	NP_001086042.1	African clawed frog	Xenopus laevis
dnajc12	ENSXETG00000025659	African clawed frog	Xenopus laevis
dnajc13	AAH94194.1	African clawed frog	Xenopus laevis
dnajc14	XP_004911947.1	African clawed frog	Xenopus laevis
dnajc15	NP_001005145.1	African clawed frog	Xenopus laevis
dnajc16	ENSXETG00000006830	African clawed frog	Xenopus laevis
dnajc17	NP_001080020.1	African clawed frog	Xenopus laevis
dnajc18	NP_001096348.1	African clawed frog	Xenopus laevis
dnajc19	NP_001091424.1	African clawed frog	Xenopus laevis
dnajc21	AAH98972.1	African clawed frog	Xenopus laevis
dnajc22	NP_001107370.1	African clawed frog	Xenopus laevis
dnaJc23(Sec63)	NP_001088542.1	African clawed frog	Xenopus laevis
dnajc24	NP_001037943.2	African clawed frog	Xenopus laevis
dnajc25	NP_001089380.2	African clawed frog	Xenopus laevis
dnaJc26(GAK)	NP_001016350.2	African clawed frog	Xenopus laevis
dnajc27-a	NP_001080762.1	African clawed frog	Xenopus laevis
dnajc27-b	NP_001088891.1	African clawed frog	Xenopus laevis
dnajc28	NP_001090075.1	African clawed frog	Xenopus laevis
DNAJc29(SACS)	XP_004912121.1	African clawed frog	Xenopus laevis
dnajc30	XP_002933669.1	African clawed frog	Xenopus laevis
DnaJc20(HscB)	NP_001086103.2	African clawed frog	Xenopus laevis

Associated Gene Name	**NCBI Protein ID**	**Species Name**	**Latin name**
dnaja2L1	XP_003967154.1	torafugu	*Takifugu rubripes*
dnaja2L2	XP_003969457.1	torafugu	*Takifugu rubripes*
dnaja3	XP_003964717.1	torafugu	*Takifugu rubripes*
dnaja3L	XP_003976469.1	torafugu	*Takifugu rubripes*
dnaja4L	XP_003967491.1	torafugu	*Takifugu rubripes*

dnajb1L	XP_003964756.1	torafugu	*Takifugu rubripes*
dnajb1L	XP_003972089.1	torafugu	*Takifugu rubripes*
dnajb2	XP_003962049.1	torafugu	*Takifugu rubripes*
dnajb4	XP_003975507.1	torafugu	*Takifugu rubripes*
dnajb5L	XP_003978956.1	torafugu	*Takifugu rubripes*
dnajb5L	XP_003965279.1	torafugu	*Takifugu rubripes*
dnajb5L	XP_003975197.1	torafugu	*Takifugu rubripes*
dnajb5L	XP_003978855.1	torafugu	*Takifugu rubripes*
dnajb6L	XP_003968117.1	torafugu	*Takifugu rubripes*
dnajb6L	XP_003975561.1	torafugu	*Takifugu rubripes*
dnajb9	XP_003972872.1	torafugu	*Takifugu rubripes*
dnajb11	XP_003961678.1	torafugu	*Takifugu rubripes*
dnajb12	XP_003964091.1	torafugu	*Takifugu rubripes*
dnajb12	XP_003961211.1	torafugu	*Takifugu rubripes*
dnajb13	XP_003971267.1	torafugu	*Takifugu rubripes*
dnajb14	XP_003972448.1	torafugu	*Takifugu rubripes*
dnajc1	XP_003976767.1	torafugu	*Takifugu rubripes*
dnajc2	XP_003978560.1	torafugu	*Takifugu rubripes*
dnajc3	XP_003961871.1	torafugu	*Takifugu rubripes*
dnajc3	XP_003966940.1	torafugu	*Takifugu rubripes*
dnajc4	XP_003970268.1	torafugu	*Takifugu rubripes*
dnajc5aa	ENSTRUP00000011543	torafugu	*Takifugu rubripes*
dnajc5ga	ENSTRUP00000030930	torafugu	*Takifugu rubripes*
DNAJC6	ENSTRUP00000047508	torafugu	*Takifugu rubripes*
dnajc7	XP_003961244.1	torafugu	*Takifugu rubripes*
dnajc7	XP_003964932.1	torafugu	*Takifugu rubripes*
dnajc8	XP_003969328.1	torafugu	*Takifugu rubripes*
dnajc9	XP_003963778.1	torafugu	*Takifugu rubripes*
dnajc10	XP_003961647.1	torafugu	*Takifugu rubripes*
dnajc11	XP_003973667.1	torafugu	*Takifugu rubripes*
dnajc11	XP_003963503.1	torafugu	*Takifugu rubripes*
dnajc12	XP_003963739.1	torafugu	*Takifugu rubripes*
dnajc13	XP_003968164.1	torafugu	*Takifugu rubripes*
dnajc14	XP_003973569.1	torafugu	*Takifugu rubripes*
dnajc16	XP_003978530.1	torafugu	*Takifugu rubripes*
dnajc16	XP_003973687.1	torafugu	*Takifugu rubripes*
dnajc17	XP_003962640.1	torafugu	*Takifugu rubripes*
dnajc18	XP_003971210.1	torafugu	*Takifugu rubripes*
dnajc19	ENSTRUP00000010352	torafugu	*Takifugu rubripes*
dnajc21	XP_003965256.1	torafugu	*Takifugu rubripes*
dnajc22	XP_003963213.1	torafugu	*Takifugu rubripes*
DnaJc23(Sec63)	XP_003971615.1	torafugu	*Takifugu rubripes*
dnajc24	XP_003976045.1	torafugu	*Takifugu rubripes*
dnajc25	XP_003978958.1	torafugu	*Takifugu rubripes*
DnaJc26(GAK)	ENSTRUP00000043299	torafugu	*Takifugu rubripes*
dnajc27	XP_003966627.1	torafugu	*Takifugu rubripes*

dnajc28	XP_003977172.1	torafugu	*Takifugu rubripes*
DNAJc29(SACS)	ENSTRUP00000001580	torafugu	*Takifugu rubripes*
dnajc30	XP_003968455.1	torafugu	*Takifugu rubripes*
dnajc30	XP_003962744.1	torafugu	*Takifugu rubripes*
HscB	XP_003975248.1	torafugu	*Takifugu rubripes*

Associated Gene Name	**NCBI Protein ID**	**Species Name**	**Latin name**
dnaja1	XP_003454242.1	Nile tilapia	*Oreochromis niloticus*
dnaja1	XP_003440484.1	Nile tilapia	*Oreochromis niloticus*
dnaja2	XP_003440442.1	Nile tilapia	*Oreochromis niloticus*
dnaja2	XP_003437760.1	Nile tilapia	*Oreochromis niloticus*
dnaja3	XP_003450123.1	Nile tilapia	*Oreochromis niloticus*
dnaja3(mitochondrial-like)	XP_003456961.1	Nile tilapia	*Oreochromis niloticus*
dnaja4	ENSONIP00000019282	Nile tilapia	*Oreochromis niloticus*
dnajb1	XP_003450019.1	Nile tilapia	*Oreochromis niloticus*
dnajb1	XP_003442154.1	Nile tilapia	*Oreochromis niloticus*
dnajb2	XP_003445391.1	Nile tilapia	*Oreochromis niloticus*
dnajb4	XP_003448498.1	Nile tilapia	*Oreochromis niloticus*
dnajb5	XP_003451516.1	Nile tilapia	*Oreochromis niloticus*
dnajb5	XP_003451721.1	Nile tilapia	*Oreochromis niloticus*
dnajb6	XP_003456774.1	Nile tilapia	*Oreochromis niloticus*
dnajb6	XP_003439273.1	Nile tilapia	*Oreochromis niloticus*
dnajb9	XP_003443955.1	Nile tilapia	*Oreochromis niloticus*
dnajb9	XP_003450755.1	Nile tilapia	*Oreochromis niloticus*
dnajb9	XP_003441534.1	Nile tilapia	*Oreochromis niloticus*
dnajb11	XP_003456324.1	Nile tilapia	*Oreochromis niloticus*
dnajb12 isoform X1	XP_003451995.1	Nile tilapia	*Oreochromis niloticus*
dnajb12	XP_003441846.1	Nile tilapia	*Oreochromis niloticus*
dnajb13	XP_003456870.1	Nile tilapia	*Oreochromis niloticus*
dnajb14	XP_003452267.1	Nile tilapia	*Oreochromis niloticus*
dnajc1	XP_003459885.1	Nile tilapia	*Oreochromis niloticus*
dnajc2	XP_003458720.1	Nile tilapia	*Oreochromis niloticus*
dnajc3	XP_003447336.1	Nile tilapia	*Oreochromis niloticus*
dnajc3	XP_003449390.1	Nile tilapia	*Oreochromis niloticus*
dnajc3	XP_005454478.1	Nile tilapia	*Oreochromis niloticus*
dnajc4	XP_003444653.1	Nile tilapia	*Oreochromis niloticus*
dnajc5	XP_003438968.1	Nile tilapia	*Oreochromis niloticus*
dnajc6	ENSONIG00000008789	Nile tilapia	*Oreochromis niloticus*
dnajc7isoform1	XP_003441887.1	Nile tilapia	*Oreochromis niloticus*
dnajc7	XP_003442078.1	Nile tilapia	*Oreochromis niloticus*
dnajc8	XP_003452483.1	Nile tilapia	*Oreochromis niloticus*
dnajc9	XP_003438345.1	Nile tilapia	*Oreochromis niloticus*
dnajc10	XP_003449632.1	Nile tilapia	*Oreochromis niloticus*
dnajc11	XP_003438988.1	Nile tilapia	*Oreochromis niloticus*
dnajc11	XP_003441584.1	Nile tilapia	*Oreochromis niloticus*

dnajc12	XP_003441103.1	Nile tilapia	*Oreochromis niloticus*
dnajc13	ENSONIG00000006278	Nile tilapia	*Oreochromis niloticus*
dnajc13	ENSONIP00000007910	Nile tilapia	*Oreochromis niloticus*
dnajc14	XP_003441404.1	Nile tilapia	*Oreochromis niloticus*
dnajc15	XP_003451607.1	Nile tilapia	*Oreochromis niloticus*
dnajc16	XP_003455394.1	Nile tilapia	*Oreochromis niloticus*
dnajc16	XP_003441432.1	Nile tilapia	*Oreochromis niloticus*
dnajc17	XP_003447791.1	Nile tilapia	*Oreochromis niloticus*
dnajc18	XP_003446878.1	Nile tilapia	*Oreochromis niloticus*
dnajc18	ENSONIP00000006297	Nile tilapia	*Oreochromis niloticus*
dnajc19	ENSONIP00000004700	Nile tilapia	*Oreochromis niloticus*
DnaJc20 HscB	XP_003454205.2	Nile tilapia	*Oreochromis niloticus*
dnajc21	XP_003451544.1	Nile tilapia	*Oreochromis niloticus*
dnajc22	XP_003448295.1	Nile tilapia	*Oreochromis niloticus*
dnaJc23(Sec63)	XP_003440753.1	Nile tilapia	*Oreochromis niloticus*
dnajc24	XP_003449286.1	Nile tilapia	*Oreochromis niloticus*
dnajc25	XP_003440229.1	Nile tilapia	*Oreochromis niloticus*
dnaJc26(GAK)	XP_005459852.1	Nile tilapia	*Oreochromis niloticus*
dnajc27	XP_003457822.1	Nile tilapia	*Oreochromis niloticus*
dnajc28	XP_003451296.1	Nile tilapia	*Oreochromis niloticus*
dnajc29	ENSONIP00000006838	Nile tilapia	*Oreochromis niloticus*
dnajc30	ENSONIP00000004649	Nile tilapia	*Oreochromis niloticus*

Associated Gene Name	**NCBI Protein ID**	**Species Name**	**Latin name**
dnaja1	XP_004065928.1	medaka	*Oryzias latipes*
dnaja2	XP_004067524.1	medaka	*Oryzias latipes*
dnaja2	none	medaka	*Oryzias latipes*
dnaja3	XP_004066111.1	medaka	*Oryzias latipes*
dnaja3（mitochondrial -like）	XP_004071710.1	medaka	*Oryzias latipes*
dnaja4	XP_004069682.1	medaka	*Oryzias latipes*
dnajb1	XP_004066365.1	medaka	*Oryzias latipes*
dnajb1	XP_004071429.1	medaka	*Oryzias latipes*
dnajb2	XP_004081751.1	medaka	*Oryzias latipes*
dnajb4	XP_004078653.1	medaka	*Oryzias latipes*
dnajb5	XP_004072668.1	medaka	*Oryzias latipes*
dnajb5	XP_004074609.1	medaka	*Oryzias latipes*
dnajb6	XP_004078669.1	medaka	*Oryzias latipes*
dnajb6	XP_004081344.1	medaka	*Oryzias latipes*
dnajb9	XP_004082935.1	medaka	*Oryzias latipes*
dnajb9	XP_004069284.1	medaka	*Oryzias latipes*
dnajb11	XP_004085479.1	medaka	*Oryzias latipes*
dnajb11	XP_004084591.1	medaka	*Oryzias latipes*

dnajb12	XP_004077409.1	medaka	*Oryzias latipes*
dnajb12	XP_004080472.1	medaka	*Oryzias latipes*
dnajb13	XP_004076543.1	medaka	*Oryzias latipes*
dnajb14	XP_004076413.1	medaka	*Oryzias latipes*
dnajb14	XP_004085524.1	medaka	*Oryzias latipes*
dnajb14	XP_004084687.1	medaka	*Oryzias latipes*
dnajc1	XP_004078927.1	medaka	*Oryzias latipes*
dnajc2	XP_004084683.1	medaka	*Oryzias latipes*
dnajc3	XP_004079872.1	medaka	*Oryzias latipes*
dnajc3	XP_004081825.1	medaka	*Oryzias latipes*
dnajc4	XP_004073195.1	medaka	*Oryzias latipes*
dnajc5	XP_004070906.1	medaka	*Oryzias latipes*
dnajc5B	XP_004078813.1	medaka	*Oryzias latipes*
dnajc6	ENSORLP00000012508	medaka	*Oryzias latipes*
dnajc7	XP_004071335.1	medaka	*Oryzias latipes*
dnajc7 isoform 2	XP_004071336.1	medaka	*Oryzias latipes*
dnajc7	XP_004078296.1	medaka	*Oryzias latipes*
dnajc8	XP_004073836.1	medaka	*Oryzias latipes*
dnajc9	XP_004077109.1	medaka	*Oryzias latipes*
dnajc10	XP_004081681.1	medaka	*Oryzias latipes*
dnajc11	XP_004070887.1	medaka	*Oryzias latipes*
dnajc11	XP_004069222.1	medaka	*Oryzias latipes*
dnajc12	XP_004077551.1	medaka	*Oryzias latipes*
dnajc13	XP_004081204.1	medaka	*Oryzias latipes*
dnajc13 isoform 2	XP_004081205.1	medaka	*Oryzias latipes*
dnajc14	XP_004069306.1	medaka	*Oryzias latipes*
dnajc15	XP_004081485.1	medaka	*Oryzias latipes*
dnajc16	XP_004069327.1	medaka	*Oryzias latipes*
dnajc16	XP_004070366.1	medaka	*Oryzias latipes*
dnajc17	XP_004082400.1	medaka	*Oryzias latipes*
dnajc18	ENSORLG00000006189	medaka	*Oryzias latipes*
dnajc19	ENSORLP00000014656	medaka	*Oryzias latipes*
dnajc21	XP_004074771.1	medaka	*Oryzias latipes*
dnajc22	XP_004070498.1	medaka	*Oryzias latipes*
DnaJc23(Sec63)	XP_004083757.1	medaka	*Oryzias latipes*
dnajc24	XP_004069429.1	medaka	*Oryzias latipes*
dnajc25	XP_004075214.1	medaka	*Oryzias latipes*
DnaJc26(GAK)	ENSORLP00000001720	medaka	*Oryzias latipes*
dnajc27	XP_004066494.1	medaka	*Oryzias latipes*
dnajc28	XP_004085996.1	medaka	*Oryzias latipes*
DNAJc29(SACS)	ENSORLP00000016333	medaka	*Oryzias latipes*
dnajc30	XP_004075684.1	medaka	*Oryzias latipes*
HscB	XP_004072108.1	medaka	*Oryzias latipes*

Associated Gene Name	Protein ID	Species Name	Latin name
dnaja1-201	ENSGACP00000023632	stickleback	*Gasterosteus aculeatus*
dnaja1-202	ENSGACP00000023633	stickleback	*Gasterosteus aculeatus*
dnaja2-201	ENSGACP00000019663	stickleback	*Gasterosteus aculeatus*
dnaja2l-201	ENSGACP00000017001	stickleback	*Gasterosteus aculeatus*
dnaja3a-201	ENSGACP00000014932	stickleback	*Gasterosteus aculeatus*
dnaja3a-202	ENSGACP00000014936	stickleback	*Gasterosteus aculeatus*
dnaja3b-201	ENSGACP00000026251	stickleback	*Gasterosteus aculeatus*
dnaja4-201	ENSGACP00000013757	stickleback	*Gasterosteus aculeatus*
dnajb1a-201	ENSGACP00000013775	stickleback	*Gasterosteus aculeatus*
dnajb1-203	ENSGACP00000024866	stickleback	*Gasterosteus aculeatus*
dnajb1-201	ENSGACP00000024859	stickleback	*Gasterosteus aculeatus*
dnajb1-202	ENSGACP00000024864	stickleback	*Gasterosteus aculeatus*
dnajb2-201	ENSGACP00000008481	stickleback	*Gasterosteus aculeatus*
dnajb2-202	ENSGACP00000008484	stickleback	*Gasterosteus aculeatus*
dnajb4	ENSGACP00000021885	stickleback	*Gasterosteus aculeatus*
dnajb5 (1 of 2)	ENSGACP00000012840	stickleback	*Gasterosteus aculeatus*
dnajb5 (2 of 2)-201	ENSGACP00000021316	stickleback	*Gasterosteus aculeatus*
dnajb5 (2 of 2)-202	ENSGACP00000021318	stickleback	*Gasterosteus aculeatus*
dnajb6 (1 of 2)	ENSGACP00000005180	stickleback	*Gasterosteus aculeatus*
dnajb6 (2 of 2)-201	ENSGACP00000019589	stickleback	*Gasterosteus aculeatus*
dnajb6 (2 of 2)-202	ENSGACP00000019591	stickleback	*Gasterosteus aculeatus*
dnajb9 (1 of 2)	ENSGACP00000005437	stickleback	*Gasterosteus aculeatus*
dnajb9 (2 of 2)	ENSGACP00000024973	stickleback	*Gasterosteus aculeatus*
dnajb11	ENSGACP00000011965	stickleback	*Gasterosteus aculeatus*
dnajb12 (1 of 2)-201	ENSGACP00000011310	stickleback	*Gasterosteus aculeatus*
dnajb12 (1 of 2)-202	ENSGACP00000011344	stickleback	*Gasterosteus aculeatus*
dnajb12 (2 of 2)	ENSGACP00000003121	stickleback	*Gasterosteus aculeatus*
dnajb13	ENSGACP00000026904	stickleback	*Gasterosteus aculeatus*
dnajb14	ENSGACP00000023040	stickleback	*Gasterosteus aculeatus*
dnajc1-201	ENSGACP00000003308	stickleback	*Gasterosteus aculeatus*
dnajc1-202	ENSGACP00000003327	stickleback	*Gasterosteus aculeatus*
dnajc1-203	ENSGACP00000003337	stickleback	*Gasterosteus aculeatus*
dnajc2-201	ENSGACP00000025668	stickleback	*Gasterosteus aculeatus*
dnajc2-202	ENSGACP00000025671	stickleback	*Gasterosteus aculeatus*
dnajc3	ENSGACP00000005504	stickleback	*Gasterosteus aculeatus*
dnajc4	ENSGACP00000022455	stickleback	*Gasterosteus aculeatus*
dnajc5 (1 of 2)	ENSGACP00000013696	stickleback	*Gasterosteus aculeatus*
dnajc5 (2 of 2)	ENSGACP00000015856	stickleback	*Gasterosteus aculeatus*
dnajc5b	ENSGACP00000001990	stickleback	*Gasterosteus aculeatus*
dnajc5g (1 of 2)-201	ENSGACP00000008060	stickleback	*Gasterosteus aculeatus*
dnajc5g (1 of 2)-202	ENSGACP00000008065	stickleback	*Gasterosteus aculeatus*
dnajc5g (2 of 2)-201	ENSGACP00000015477	stickleback	*Gasterosteus aculeatus*
dnajc5g (2 of 2)-202	ENSGACP00000015482	stickleback	*Gasterosteus aculeatus*
dnajc6	ENSGACP00000009523	stickleback	*Gasterosteus aculeatus*

dnajc7 (1 of 2)	ENSGACP00000011616	stickleback	*Gasterosteus aculeatus*
dnajc7 (2 of 2)	ENSGACP00000008220	stickleback	*Gasterosteus aculeatus*
dnajc8	ENSGACP00000004222	stickleback	*Gasterosteus aculeatus*
dnajc9-201	ENSGACP00000013574	stickleback	*Gasterosteus aculeatus*
dnajc9-202	ENSGACP00000013582	stickleback	*Gasterosteus aculeatus*
dnajc10	ENSGACP00000010844	stickleback	*Gasterosteus aculeatus*
dnajc11 (1 of 2)	ENSGACP00000016420	stickleback	*Gasterosteus aculeatus*
dnajc11 (2 of 2)	ENSGACP00000010515	stickleback	*Gasterosteus aculeatus*
dnajc12	ENSGACP00000004461	stickleback	*Gasterosteus aculeatus*
dnajc13-201	ENSGACP00000006323	stickleback	*Gasterosteus aculeatus*
dnajc13-202	ENSGACP00000006335	stickleback	*Gasterosteus aculeatus*
dnajc14	ENSGACP00000000574	stickleback	*Gasterosteus aculeatus*
dnajc15	ENSGACP00000002739	stickleback	*Gasterosteus aculeatus*
dnajc16 (1 of 2)	ENSGACP00000007394	stickleback	*Gasterosteus aculeatus*
dnajc16 (2 of 2)	ENSGACP00000010244	stickleback	*Gasterosteus aculeatus*
dnajc17	ENSGACP00000008182	stickleback	*Gasterosteus aculeatus*
dnajc18	ENSGACP00000027323	stickleback	*Gasterosteus aculeatus*
dnajc19	ENSGACP00000008063	stickleback	*Gasterosteus aculeatus*
dnajc20（hscb）	ENSGACP00000018564	stickleback	*Gasterosteus aculeatus*
dnajc21	ENSGACP00000021760	stickleback	*Gasterosteus aculeatus*
dnajc22-201	ENSGACP00000012010	stickleback	*Gasterosteus aculeatus*
dnajc22-202	ENSGACP00000012013	stickleback	*Gasterosteus aculeatus*
dnajc23(sec63)	ENSGACP00000014702	stickleback	*Gasterosteus aculeatus*
dnajc24	ENSGACP00000003251	stickleback	*Gasterosteus aculeatus*
dnajc25	ENSGACP00000024165	stickleback	*Gasterosteus aculeatus*
dnajc26(gak)	ENSGACP00000018093	stickleback	*Gasterosteus aculeatus*
dnajc27-201	ENSGACP00000020389	stickleback	*Gasterosteus aculeatus*
dnajc27-202	ENSGACP00000020391	stickleback	*Gasterosteus aculeatus*
dnajc28	ENSGACP00000016328	stickleback	*Gasterosteus aculeatus*
dnajc29(sacs)	ENSGACP00000009097	stickleback	*Gasterosteus aculeatus*
dnajc30	ENSGACP00000013138	stickleback	*Gasterosteus aculeatus*

Associated Gene Name	**NCBI Protein ID**	**Species Name**	**Latin name**
dnaja	XP_002666797.3	zebrafish	*Danio rerio*
dnaja	XP_002666797.2	zebrafish	*Danio rerio*
dnaja1l	NP_955956.1	zebrafish	*Danio rerio*
dnaja2	NP_998658.1	zebrafish	*Danio rerio*
dnaja2l	NP_997830.1	zebrafish	*Danio rerio*
dnaja3a	NP_958470.1	zebrafish	*Danio rerio*
dnaja3b	NP_958499.1	zebrafish	*Danio rerio*
dnaja3b	NP_001116708.1	zebrafish	*Danio rerio*
dnajb1a	NP_001003571.1	zebrafish	*Danio rerio*
dnajb1b	NP_956067.1	zebrafish	*Danio rerio*
dnajb2	NP_001073462.1	zebrafish	*Danio rerio*

dnajb4	NP_001003455.1	zebrafish	*Danio rerio*
dnajb5	NP_001093510.1	zebrafish	*Danio rerio*
dnajb5-like	NP_001032663.2	zebrafish	*Danio rerio*
dnajb6a	NP_001002353	zebrafish	*Danio rerio*
dnajb6b	NP_956599.1	zebrafish	*Danio rerio*
dnajb9a	NP_001020355.1	zebrafish	*Danio rerio*
dnajb9b	NP_001019564.1	zebrafish	*Danio rerio*
zgc:152986-201	ENSDARP00000094644	zebrafish	*Danio rerio*
dnajb11	NP_942116.1	zebrafish	*Danio rerio*
dnajb12a	NP_997824.1	zebrafish	*Danio rerio*
dnajb12b	NP_001082977.1	zebrafish	*Danio rerio*
DnaJb12blike LOC100536510	XP_003199718.2	zebrafish	*Danio rerio*
dnajb13	NP_001017606.1	zebrafish	*Danio rerio*
dnajb14	NP_001071255.1	zebrafish	*Danio rerio*
dnajc1	NP_001071003.1	zebrafish	*Danio rerio*
dnajc2	NP_997976.1	zebrafish	*Danio rerio*
dnajc3	NP_955904.2	zebrafish	*Danio rerio*
c3_prkri	NP_571705.1	zebrafish	*Danio rerio*
dnajc4	NP_997842.1	zebrafish	*Danio rerio*
dnajc5aa	NP_001002464.1	zebrafish	*Danio rerio*
dnajc5ab	XP_001338363.1	zebrafish	*Danio rerio*
dnajc5b	XP_700383.1	zebrafish	*Danio rerio*
dnajc5ga	NP_955917.1	zebrafish	*Danio rerio*
dnajc5gb	NP_956694.1	zebrafish	*Danio rerio*
dnajc6	XP_001336673.2	zebrafish	*Danio rerio*
dnajc7	NP_998455.1	zebrafish	*Danio rerio*
dnajc7-like-LOC100535346	XP_003198135.1	zebrafish	*Danio rerio*
dnajc8	NP_001038771.1	zebrafish	*Danio rerio*
dnajc9	NP_001002433.1	zebrafish	*Danio rerio*
dnajc10	NP_001077016.1	zebrafish	*Danio rerio*
dnajc11	NP_997796.1	zebrafish	*Danio rerio*
dnajc11-like-LOC100329402	XP_002667092.2	zebrafish	*Danio rerio*
dnajc12	XP_001334518.1	zebrafish	*Danio rerio*
dnajc13-like-LOC100538099	XP_003201306.2	zebrafish	*Danio rerio*
dnajc14_vegfab	NP_001038320.2	zebrafish	*Danio rerio*
dnajc14-like-LOC555824	XP_683557.3	zebrafish	*Danio rerio*
dnajc15	NP_001003476.1	zebrafish	*Danio rerio*
dnajc16	XP_688223.3	zebrafish	*Danio rerio*
dnajc16-like-LOC556590	XP_002663742.2	zebrafish	*Danio rerio*
dnajc17	NP_956540.1	zebrafish	*Danio rerio*
dnajc18	NP_001107060.1	zebrafish	*Danio rerio*
dnajc19	NP_957055.1	zebrafish	*Danio rerio*

dnajc21	NP_956338.1	zebrafish	*Danio rerio*
dnajc22	XP_001335380.1	zebrafish	*Danio rerio*
dnaJc23(Sec63)	NP_001002588.1	zebrafish	*Danio rerio*
sec63-like protein-like-LOC100334249		zebrafish	*Danio rerio*
dnajc24	NP_956746.1	zebrafish	*Danio rerio*
dnajc25	NP_001191956.1	zebrafish	*Danio rerio*
dnaJc26(gak)	XP_003198642.2	zebrafish	*Danio rerio*
dnajc27	NP_001007163.1	zebrafish	*Danio rerio*
dnajc28	NP_001017648.1	zebrafish	*Danio rerio*
dnajc29(sacs)	XP_005157537.1	zebrafish	*Danio rerio*
dnajc30-like-LOC565734	XP_694098.1	zebrafish	*Danio rerio*
si:ch211-207k7.4 (hscB)	NP_001121870.1	zebrafish	*Danio rerio*

Table S2-2 Fold changes of catfish Hsp40s under each bacterial challenges.

Gene name	Columnaris_gill			ESC_ intestine		
	3h	24h	3d	3h	24h	3d
dnaja1						
dnaja2						
dnaja2l				-1.485	-1.789	-1.574
dnaja3a						
dnaja3b						
dnaja4	1.602	2.768	3.021	1.927	1.490	3.389
dnajb1a	-1.028	1.575	1.598	1.201	2.372	2.541
dnajb1b	-1.548	-1.346	-1.415	1.550	1.405	1.684
dnajb2				1.401	1.789	1.694
dnajb4	-1.601	-1.723	-1.797			
dnajb5				2.484	2.632	4.313
dnajb5l						
dnajb6a						
dnajb6b				1.415	1.081	1.564
dnajb9						
dnajb9l1						
dnajb9l2	1.006	1.899	-1.224			
dnajb11	1.401	2.890	1.296			
dnajb12a						
dnajb12b	1.542	1.236	1.036			
dnajb13	-1.262	-2.848	-1.446			
dnajb14						
dnajc1						
dnajc2						
dnajc3	1.052	2.080	1.449			
dnajc3_prkri				1.309	1.571	1.646
dnajc4						
dnajc5aa	1.110	5.558	7.398	1.681	1.874	1.694
dnajc5ab						
dnajc5gb						
dnajc6	1.602	1.084	3.819	1.926	1.597	1.853
dnajc7						
dnajc8						
dnajc9						
dnajc10						
dnajc11						
dnajc12	-1.712	-1.661	-1.466	2.197	1.394	1.040
dnajc13				1.330	1.456	1.582
dnajc14						
dnajc15						
dnajc16				1.560	1.238	1.132
dnajc16l	1.987	1.149	1.673			
dnajc17				-1.784	-1.957	-1.312
dnajc18				2.293	2.323	2.657
dnajc19	-1.456	-2.015	-1.513			
dnajc20(hscb)	-1.081	1.549	1.316			
dnajc21	1.225	1.814	1.426			
dnajc22				1.109	1.699	1.145
dnajc23(sec63)						
dnajc24	-2.062	-1.779	-1.646			
dnajc25						
dnajc26(gak)						
dnajc27				2.245	2.845	2.946
dnajc28						
dnajc29(sacs)	1.510	-1.056	1.390	3.314	3.734	3.617
dnajc30a	-1.538	-1.684	-1.300			
dnajc30b				1.952	2.190	1.815

Table S3-1 Gene names and accession numbers of reference Hsp90s used in this study

Associated Gene Name	NCBI Protein ID	Species Name	Latin name
HSPA1A	NP_005336.3	human	*Homo sapiens*
HSPA1B	NP_005337.2	human	*Homo sapiens*
HSPA1L	NP_005518.3	human	*Homo sapiens*
HSPA2	NP_068814.2	human	*Homo sapiens*
HSPA4	NP_002145.3	human	*Homo sapiens*
HSPA4L	NP_055093.2	human	*Homo sapiens*
HSPA5	NP_005338.1	human	*Homo sapiens*
HSPA6	NP_002146.2	human	*Homo sapiens*
HSPA7	UniProtKB/Swiss-Prot: P48741.2	human	*Homo sapiens*
HSPA8	NP_006588.1	human	*Homo sapiens*
HSPA9	NP_004125.3	human	*Homo sapiens*
HSPA12A	NP_079291.2	human	*Homo sapiens*
HSPA12B	NP_443202.3	human	*Homo sapiens*
HSPA13	NP_008879.3	human	*Homo sapiens*
HSPA14	NP_057383.2	human	*Homo sapiens*
HSPH1	NP_006635.2	human	*Homo sapiens*
HYOU1	NP_001124463.1	human	*Homo sapiens*

Associated Gene Name	NCBI Protein ID	Species Name	Latin name
Hspa1a	NP_034609.2	Mouse	*Mus musculus*
Hspa1b	NP_034608.2	Mouse	*Mus musculus*
Hspa1l	NP_038586.2	Mouse	*Mus musculus*
Hspa2	NP_001002012.1	Mouse	*Mus musculus*
Hspa4	NP_032326.3	Mouse	*Mus musculus*
Hspa4l	NP_035150.3	Mouse	*Mus musculus*
Hspa5	NP_001156906.1	Mouse	*Mus musculus*
hspa8	NP_112442.2	Mouse	*Mus musculus*
Hspa9	NP_034611.2	Mouse	*Mus musculus*
Hspa12a	NP_780408.1	Mouse	*Mus musculus*
Hspa12b	NP_082582.1	Mouse	*Mus musculus*
Hspa13	NP_084477.1	Mouse	*Mus musculus*
Hspa14	NP_056580.2	Mouse	*Mus musculus*
Hsph1	NP_038587.2	Mouse	*Mus musculus*
Hyou1	NP_067370.3	Mouse	*Mus musculus*

Associated Gene Name	NCBI/ Ensemble Protein ID	Species Name	Latin name
Loc100091897	XP_001510204.2	platypus	*Ornithorhynchus anatinus*
Loc100080044	XP_001510947.2	platypus	*Ornithorhynchus anatinus*
Hspa2-201	ENSOANP00000015027	platypus	*Ornithorhynchus anatinus*
Hspa4-201	ENSOANP00000011834	platypus	*Ornithorhynchus anatinus*

Hspa4l-201	ENSOANP00000032051	platypus	*Ornithorhynchus anatinus*
Hspa5-201	ENSOANP00000020985	platypus	*Ornithorhynchus anatinus*
Hspa6-201	ENSOANP00000008582	platypus	*Ornithorhynchus anatinus*
Hspa12a-201	ENSOANP00000022296	platypus	*Ornithorhynchus anatinus*
Hspa13-201	ENSOANP00000004718	platypus	*Ornithorhynchus anatinus*
Hspa14-201	ENSOANP00000025044	platypus	*Ornithorhynchus anatinus*
Loc100078184	XP_001509055.2	platypus	*Ornithorhynchus anatinus*

Associated Gene Name	**NCBI Protein ID**	**Species Name**	**Latin name**
Hspa2	NP_001006686.1	chicken	*Gallus gallus*
Hspa4	XP_003642142.1	chicken	*Gallus gallus*
Hspa4l	NP_001012594.1	chicken	*Gallus gallus*
Hspa5	NP_990822.1	chicken	*Gallus gallus*
Hspa8	NP_990334.1	chicken	*Gallus gallus*
Hspa9	NP_001006147.1	chicken	*Gallus gallus*
Hspa12a	XP_421779.3	chicken	*Gallus gallus*
Loc770082	XP_001233402.2	chicken	*Gallus gallus*
Hspa13	NP_001025964.2	chicken	*Gallus gallus*
Hspa14	XP_416996.3	chicken	*Gallus gallus*
Hsph1	NP_001153170.1	chicken	*Gallus gallus*
Hyou1	NP_001006588.1	chicken	*Gallus gallus*

Associated Gene Name	**Ensemble Protein ID**	**Species Name**	**Latin name**
Hspa2-201	ENSACAP00000015494	lizard	*Anolis carolinensis*
Hspa4-201	ENSACAP00000013089	lizard	*Anolis carolinensis*
Hspa4-202	ENSACAP00000023153	lizard	*Anolis carolinensis*
Hspa4l-201	ENSACAP00000011642	lizard	*Anolis carolinensis*
Hspa5-201	ENSACAP00000004078	lizard	*Anolis carolinensis*
Hspa8-201	ENSACAP00000004798	lizard	*Anolis carolinensis*
Hspa9-201	ENSACAP00000015698	lizard	*Anolis carolinensis*
Hspa12a-201	ENSACAP00000009931	lizard	*Anolis carolinensis*
Hspa12b-201	ENSACAP00000004138	lizard	*Anolis carolinensis*
Hspa13-201	ENSACAP00000000965	lizard	*Anolis carolinensis*
Hspa14-201	ENSACAP00000001088	lizard	*Anolis carolinensis*
Hyou1	ENSACAP00000013983	lizard	*Anolis carolinensis*
Hsph1	ENSACAP00000004913	lizard	*Anolis carolinensis*

Associated Gene Name	**NCBI/ Ensemble Protein ID**	**Species Name**	**Latin name**
hspa4-201	enspsip00000014785	chinese softshell turtle	*pelodiscus sinensis*
hspa4l-201	enspsip00000010121	chinese softshell turtle	*pelodiscus sinensis*
hspa5-201	enspsip00000005890	chinese softshell turtle	*pelodiscus sinensis*
hspa9-201	enspsip00000014345	chinese softshell turtle	*pelodiscus sinensis*
hspa12b-201	enspsip00000004856	chinese softshell turtle	*pelodiscus sinensis*
hspa13-201	enspsip00000018220	chinese softshell turtle	*pelodiscus sinensis*
hspa14-201	enspsip00000018312	chinese softshell turtle	*pelodiscus sinensis*

hyou1	xp_006123893.1	chinese softshell turtle	*pelodiscus sinensis*
hsph1	enspsip00000009634	chinese softshell turtle	*pelodiscus sinensis*

Associated Gene Name	**NCBI Protein ID**	**Species Name**	**Latin name**
hspa1a	NP_001167480.1	African clawed frog	*Xenopus laevis*
hspa1b	NP_001091238.1	African clawed frog	*Xenopus laevis*
hspa1l	NP_001080068.1	African clawed frog	*Xenopus laevis*
hsp70	NP_001121147.1	African clawed frog	*Xenopus laevis*
hspa2	NP_001086039.1	African clawed frog	*Xenopus laevis*
hspa4	NP_001083317.1	African clawed frog	*Xenopus laevis*
hspa5	NP_001081462.1	African clawed frog	*Xenopus laevis*
hspa5b	NP_001165648.1	African clawed frog	*Xenopus laevis*
hspa8	NP_001079632.1	African clawed frog	*Xenopus laevis*
hsc70.ii	NP_001165656.1	African clawed frog	*Xenopus laevis*
hspa9-a	NP_001079627.1	African clawed frog	*Xenopus laevis*
hspa9-b	NP_001080166.1	African clawed frog	*Xenopus laevis*
hspa13	NP_001017223.1	African clawed frog	*Xenopus laevis*
hspa14	NP_001092168.1	African clawed frog	*Xenopus laevis*
hspa14-b	NP_001091353.1	African clawed frog	*Xenopus laevis*
hsph1-a	NP_001085637.1	African clawed frog	*Xenopus laevis*
hyou1	UniProtKB/Swiss-Prot: Q566I3.2	African clawed frog	*Xenopus laevis*

Associated Gene Name	**NCBI/ Ensemble Protein ID**	**Species Name**	**Latin name**
hsp70.3	NP_571472.1	zebrafish	*Danio rerio*
hsp70.2	XP_003198158.1	zebrafish	*Danio rerio*
mcm5(hsp70)	ENSDARP00000109199	zebrafish	*Danio rerio*
LOC798846(hspa1b)	NP_001093532.1	zebrafish	*Danio rerio*
hsp70l	NP_001107061.1	zebrafish	*Danio rerio*
hspa4a	NP_999881.1	zebrafish	*Danio rerio*
hspa4b	NP_956151.1	zebrafish	*Danio rerio*
wu:fc07b10(hspa4l)	XP_690505.2	zebrafish	*Danio rerio*
hspa5	NP_998223.1	zebrafish	*Danio rerio*
hspa8a	NP_001103873.1	zebrafish	*Danio rerio*
hspa8b[hsc70.2(LOC562935)]	NP_001186941.1	zebrafish	*Danio rerio*
hsc70	NP_956908.1	zebrafish	*Danio rerio*
hspa9	NP_958483.2	zebrafish	*Danio rerio*
si:dkey-61p9.8 (hsp12a.1)	NP_001038342.1	zebrafish	*Danio rerio*
si:dkey-61p9.4(hsp12a.2)	XP_003198604.1	zebrafish	*Danio rerio*
si:dkey-61p9.6(hsp12a.3)	NP_001038346.2	zebrafish	*Danio rerio*
hspa13	NP_001082948.1	zebrafish	*Danio rerio*
hspa14	NP_001038541.1	zebrafish	*Danio rerio*
LOC557824	XP_001919957.1	zebrafish	*Danio rerio*
hyou1	NP_997868.1	zebrafish	*Danio rerio*

Associated Gene Name	NCBI/Ensemble Protein ID	Species Name	Latin name
hsp70.3(hspa)	XP_004071143.1	medaka	*Oryzias latipes*
hsp70-5(hspa1b)	NP_001098384.1	medaka	*Oryzias latipes*
hsc70	NP_001098385.1	medaka	*Oryzias latipes*
hsc70.2(hspa8b)	XP_004075396.1	medaka	*Oryzias latipes*
hspa8a	UniProtKB/Swiss-Prot: Q9W6Y1.1	medaka	*Oryzias latipes*
hspa4a-201	ENSORLP00000001795	medaka	*Oryzias latipes*
hspa4b-201	ENSORLP00000007499	medaka	*Oryzias latipes*
hspa4l	XP_004082341.1	medaka	*Oryzias latipes*
hspa5l	XP_004074796.1	medaka	*Oryzias latipes*
hspa9-201	ENSORLP00000013340	medaka	*Oryzias latipes*
hspa12a-201	ENSORLP00000001447	medaka	*Oryzias latipes*
hspa12b-201	ENSORLP00000007349	medaka	*Oryzias latipes*
hspa13l	XP_004075919.1	medaka	*Oryzias latipes*
hspa14-201	ENSORLP00000015785	medaka	*Oryzias latipes*
hyou1l	XP_004084567.1	medaka	*Oryzias latipes*

Associated Gene Name	NCBI Protein ID	Species Name	Latin name
hspa1lpartial(hsp70.3)	xp_003442504.1	nile tilapia	*oreochromis niloticus*
loc100704606 (hspa1b)	xp_003444871.1	nile tilapia	*oreochromis niloticus*
hspa8a	xp_003448938.1	nile tilapia	*oreochromis niloticus*
hsc70	xp_003454400.1	nile tilapia	*oreochromis niloticus*
hspa8b	xp_003455104.1	nile tilapia	*oreochromis niloticus*
hspa4l	xp_003453147.1	nile tilapia	*oreochromis niloticus*
hspa5a	xp_005470418.1	nile tilapia	*oreochromis niloticus*
hspa5b	xp_003459659.1	nile tilapia	*oreochromis niloticus*
hspa9	xp_003459471.1	nile tilapia	*oreochromis niloticus*
loc100699432	xp_003457416.1	nile tilapia	*oreochromis niloticus*
hspa12b	xp_003452414.1	nile tilapia	*oreochromis niloticus*
loc100708509	xp_003441638.1	nile tilapia	*oreochromis niloticus*
loc100697637	xp_003455685.1	nile tilapia	*oreochromis niloticus*
loc100691644	xp_003448981.1	nile tilapia	*oreochromis niloticus*

Associated Gene Name	NCBI/ Ensemble Protein ID	Species Name	Latin name
loc101067318(hsp70.3)	XP_003964983.1	torafugu	*Takifugu rubripes*
loc101067989(hspa1b)	XP_003963154.1	torafugu	*Takifugu rubripes*
hspa2-201	ENSTRUT00000005983	torafugu	*Takifugu rubripes*
loc101063324(hspa8)	XP_003977939.1	torafugu	*Takifugu rubripes*
loc101075813(hsc70)	XP_003966054.1	torafugu	*Takifugu rubripes*
loc101063130	XP_003965205.1	torafugu	*Takifugu rubripes*
loc101077300	XP_003977088.1	torafugu	*Takifugu rubripes*
loc101063656	XP_003968291.1	torafugu	*Takifugu rubripes*
hspa4a-201	ENSTRUT00000021036	torafugu	*Takifugu rubripes*

hspa4b-201	ENSTRUT00000016139	torafugu	*Takifugu rubripes*
hspa12a-201	ENSTRUT00000009556	torafugu	*Takifugu rubripes*
hspa12b-202	ENSTRUT00000006941	torafugu	*Takifugu rubripes*
hspa14-201	ENSTRUT00000031936	torafugu	*Takifugu rubripes*
loc101065326	XP_003977948.1	torafugu	*Takifugu rubripes*

Associated Gene Name	**Ensemble Protein ID**	**Species Name**	**Latin name**
hspa4a	ENSGACP00000027410	stickleback	*Gasterosteus aculeatus*
hspa4b	ENSGACP00000024055	stickleback	*Gasterosteus aculeatus*
hspa4l	ENSGACP00000010866	stickleback	*Gasterosteus aculeatus*
hspa5	ENSGACP00000021969	stickleback	*Gasterosteus aculeatus*
hSPA8a	ENSGACP00000013930	stickleback	*Gasterosteus aculeatus*
hspa8b	ENSGACP00000026579	stickleback	*Gasterosteus aculeatus*
hspa9	ENSGACP00000025843	stickleback	*Gasterosteus aculeatus*
hspa12a	ENSGACP00000019311	stickleback	*Gasterosteus aculeatus*
hspa12b	ENSGACP00000026246	stickleback	*Gasterosteus aculeatus*
hspa13	ENSGACP00000008388	stickleback	*Gasterosteus aculeatus*
hspa14	ENSGACP00000025513	stickleback	*Gasterosteus aculeatus*
hyou1	ENSGACP00000026575	stickleback	*Gasterosteus aculeatus*

Table S3-2 Fold changes of catfish Hsp70s under each bacterial challenges.

Gene name	**Columnaris-gill**			**ESC-intestine**		
	4h	**24h**	**48h**	**3h**	**24h**	**3d**
hsp70.2						
hsp70.3				1.585		2.333
hspa8a.1						
hspa8a.2						
hspa8b						
hsc70		-3.438	-1.769		-1.897	-1.580
hspa4a	1.795	2.017	1.914	1.543		1.844
hspa4b	1.454	1.745			-1.991	
hspa4L			2.046	1.813	1.666	2.778
hyou1		2.627	1.462		1.842	2.180
hspa5	1.555	4.082	1.696	1.447	1.429	1.922
hspa9	1.454	1.772				
hspa12a			2.047		-2.160	
hspa12b			1.612		1.620	
hspa13			-1.593			
hspa14					-1.420	

Table S4-1 Gene names and accession numbers of reference Hsp90s used in this study.

Associated Gene Name	NCBI/ Ensemble Protein ID	Species Name	Latin name
HSP90AA1	ENSP00000216281	human	*Homo sapiens*
HSP90AB1	ENSP00000360709	human	*Homo sapiens*
HSP90B1	ENSP00000299767	human	*Homo sapiens*
TRAP1	ENSP00000246957	human	*Homo sapiens*
Hsp90aa1	ENSMUSP00000091921	mouse	*Mus musculus*
Hsp90ab1	ENSMUSP00000024739	mouse	*Mus musculus*
Hsp90b1	ENSMUSP00000020238	mouse	*Mus musculus*
Trap1	ENSMUSP00000006137	mouse	*Mus musculus*
Hsp90aa1	ENSOANP00000009131	platypus	*Ornithorhynchus anatinus*
Hsp90b1	ENSOANP00000008830	platypus	*Ornithorhynchus anatinus*
Hsp90aa1	NP_001103255.1	chicken	*Gallus gallus*
Hsp90ab1	ENSGALP00000016523	chicken	*Gallus gallus*
Hsp90b1	ENSGALP00000020744	chicken	*Gallus gallus*
Trap1	ENSGALP00000012445	chicken	*Gallus gallus*
Hsp90aa1	XP_006120052.1	turtle	*Pelodiscus sinensis*
Hsp90b1	XP_006138079.1	turtle	*Pelodiscus sinensis*
Trap1	ENSPSIP00000006661	turtle	*Pelodiscus sinensis*
Hsp90aa1-like	ENSACAP00000000156	lizard	*Anolis carolinensis*
Hsp90ab1	ENSACAP00000014598	lizard	*Anolis carolinensis*
Hsp90b1	ENSACAP00000015990	lizard	*Anolis carolinensis*
Trap1	ENSACAP00000012640	lizard	*Anolis carolinensis*
Hsp90aa1.1	NP_001016282.1	xenopus	*Xenopus (Silurana) tropicalis*
Hsp90aa1.2	NP_001072765.1	xenopus	*Xenopus (Silurana) tropicalis*
Hsp90ab1	NP_001025655.1	xenopus	*Xenopus (Silurana) tropicalis*
Hsp90b1	NP_001084280.1	xenopus	*Xenopus laevis*
Trap1	ENSXETP00000058756	xenopus	*Xenopus laevis*
hsp90aa1.1	ENSTRUP00000031409	fugu	*Takifugu rubripes*
hsp90aa1.2	ENSTRUP00000031536	fugu	*Takifugu rubripes*
hsp90ab1	XP_003971791.1	fugu	*Takifugu rubripes*
hsp90b1	ENSTRUP00000043499	fugu	*Takifugu rubripes*
trap1	ENSTRUP00000010455	fugu	*Takifugu rubripes*
hsp90aa1.1	ENSONIP00000001042	tilapia	*Oreochromis niloticus*
hsp90aa1.2	ENSONIP00000001024	tilapia	*Oreochromis niloticus*
hsp90ab1	ENSONIP00000007472	tilapia	*Oreochromis niloticus*
hsp90b1	ENSONIP00000010722	tilapia	*Oreochromis niloticus*
trap1	ENSONIP00000006501	tilapia	*Oreochromis niloticus*
hsp90aa1.1	ENSGACP00000017020	stickleback	*Gasterosteus aculeatus*
hsp90aa1.2	ENSGACP00000017047	stickleback	*Gasterosteus aculeatus*
hsp90ab1	ENSGACP00000017886	stickleback	*Gasterosteus aculeatus*
hsp90b1	ENSGACP00000013300	stickleback	*Gasterosteus aculeatus*
trap1	ENSGACP00000015006	stickleback	*Gasterosteus aculeatus*

hsp90aa1.1	ENSORLP00000021928	medaka	*Oryzias latipes*
hsp90aa1.2	ENSORLP00000021939	medaka	*Oryzias latipes*
hsp90ab1	ENSORLP00000014900	medaka	*Oryzias latipes*
hsp90b1	ENSORLP00000005467	medaka	*Oryzias latipes*
trap1	ENSORLP00000010499	medaka	*Oryzias latipes*
hsp90aa1.1	ENSDARP00000022302	zebrafish	*Danio rerio*
hsp90aa1.2	ENSDARP00000026065	zebrafish	*Danio rerio*
hsp90ab1	ENSDARP00000014978	zebrafish	*Danio rerio*
hsp90b1	ENSDARP00000013441	zebrafish	*Danio rerio*
trap1	ENSDARP00000107323	zebrafish	*Danio rerio*

Table S4-2 Gene names and accession numbers of reference Hsp60/10s used in this study.

Associated Gene Name	NCBI/ Ensemble Protein ID	Species Name	Latin name
HSPD1	ENSP00000373620	human	*Homo sapiens*
HSPE1	ENSP00000233893	human	*Homo sapiens*
Hspd1	ENSMUSP00000027123	mouse	*Mus musculus*
Hspe1	ENSMUSP00000074724	mouse	*Mus musculus*
Hspd1	ENSOANP00000016514	platypus	*Ornithorhynchus anatinus*
Hspe1	ENSOANP00000016516	platypus	*Ornithorhynchus anatinus*
Hspd1	ENSGALP00000013122	chicken	*Gallus gallus*
Hspe1	ENSGALP00000014746	chicken	*Gallus gallus*
Hspd1	XP_006117980.1	turtle	*Pelodiscus sinensis*
Hspe1	ENSPSIP00000020294	turtle	*Pelodiscus sinensis*
Hspd1	ENSACAP00000009377	lizard	*Anolis carolinensis*
Hspe1	ENSACAP00000009286	lizard	*Anolis carolinensis*
Hspd1	NP_001083970.1	xenopus	*Xenopus laevis*
Hspe1	ENSXETP00000016357	xenopus	*Xenopus laevis*
hspd1	XP_003961681.1	fugu	*Takifugu rubripes*
hspe1	ENSTRUP00000039965	fugu	*Takifugu rubripes*
hspd1	ENSONIP00000017312	tilapia	*Oreochromis niloticus*
hspe1	ENSONIP00000017320	tilapia	*Oreochromis niloticus*
hspd1	ENSGACP00000011870	stickleback	*Gasterosteus aculeatus*
hspe1	ENSGACP00000011889	stickleback	*Gasterosteus aculeatus*
hspd1	ENSORLP00000025853	medaka	*Oryzias latipes*
hspe1	ENSORLP00000025855	medaka	*Oryzias latipes*
hspd1	ENSDARP00000073057	zebrafish	*Danio rerio*
hspe1	ENSDARP00000118521	zebrafish	*Danio rerio*

Table S4-3 Gene names and accession numbers of reference sHsps used in this study.

Associated Gene Name	NCBI/ Ensemble Protein ID	Species Name	Latin name
HSPB1	ENSP00000248553	human	*Homo sapiens*
HSPB2	ENSP00000302476	human	*Homo sapiens*
HSPB3	ENSP00000303394	human	*Homo sapiens*
CRYAA	ENSP00000291554	human	*Homo sapiens*
CRYAB	ENSP00000227251	human	*Homo sapiens*
HSPB6	ENSP00000004982	human	*Homo sapiens*
HSPB7	ENSP00000364870	human	*Homo sapiens*
HSPB8	ENSP00000281938	human	*Homo sapiens*
HSPB9	ENSP00000347178	human	*Homo sapiens*
HSPB10/ODF1	ENSP00000285402	human	*Homo sapiens*

Associated Gene Name	NCBI/ Ensemble Protein ID	Species Name	Latin name
Hspb1	ENSMUSP00000005077	mouse	*Mus musculus*

Hspb2	ENSMUSP00000042374	mouse	*Mus musculus*
Hspb3	ENSMUSP00000054193	mouse	*Mus musculus*
Cryaa	ENSMUSP00000019192	mouse	*Mus musculus*
Cryab	ENSMUSP00000034562	mouse	*Mus musculus*
Hspb6	ENSMUSP00000039172	mouse	*Mus musculus*
Hspb7	ENSMUSP00000099544	mouse	*Mus musculus*
Hspb8	ENSMUSP00000037007	mouse	*Mus musculus*
Hspb9	ENSMUSP00000130551	mouse	*Mus musculus*
Hspb10/Odf1	ENSMUSP00000080632	mouse	*Mus musculus*

Associated Gene Name	NCBI/ Ensemble Protein ID	Species Name	Latin name
Hspb3	ENSOANP00000003206	platypus	*Ornithorhynchus anatinus*
Cryab	NP_001229681.1	platypus	*Ornithorhynchus anatinus*
Hspb10/Odf1-like	XP_001516448.1	platypus	*Ornithorhynchus anatinus*

Associated Gene Name	NCBI/ Ensemble Protein ID	Species Name	Latin name
Hspb1	ENSMGAP00000003402	turkey	*Meleagris gallopavo*
Cryaa	ENSMGAP00000015452	turkey	*Meleagris gallopavo*
Cryab	ENSMGAP00000004537	turkey	*Meleagris gallopavo*
Hspb7	ENSMGAP00000005006	turkey	*Meleagris gallopavo*
Hspb8	ENSMGAP00000009350	turkey	*Meleagris gallopavo*
Hspb10/Odf1	ENSMGAP00000012965	turkey	*Meleagris gallopavo*

Associated Gene Name	NCBI/ Ensemble Protein ID	Species Name	Latin name
Hspb1	ENSPSIP00000010835	turtle	*Pelodiscus sinensis*
Hspb3	ENSPSIP00000001651	turtle	*Pelodiscus sinensis*
Cryaa	ENSPSIP00000007929	turtle	*Pelodiscus sinensis*
Cryab	ENSPSIP00000017841	turtle	*Pelodiscus sinensis*
Hspb6	ENSPSIP00000008988	turtle	*Pelodiscus sinensis*
Hspb7	ENSPSIP00000013058	turtle	*Pelodiscus sinensis*
Hspb8	ENSPSIP00000014963	turtle	*Pelodiscus sinensis*
Hspb10/Odf1	ENSPSIP00000000031	turtle	*Pelodiscus sinensis*

Associated Gene Name	NCBI/ Ensemble Protein ID	Species Name	Latin name
Hspb3	ENSACAP00000015243	lizard	*Anolis carolinensis*
Cryaa	ENSACAP00000017563	lizard	*Anolis carolinensis*
Cryab	ENSACAP00000006339	lizard	*Anolis carolinensis*
Hspb6	ENSACAP00000016379	lizard	*Anolis carolinensis*
Hspb7	ENSACAP00000019633	lizard	*Anolis carolinensis*
Hspb8	ENSACAP00000004004	lizard	*Anolis carolinensis*
Hspb10/Odf1	ENSACAP00000008965	lizard	*Anolis carolinensis*

Associated Gene Name	NCBI/ Ensemble Protein ID	Species Name	Latin name
Hspb1	ENSXETP00000019657	xenopus	*Xenopus laevis*
Hspb2	ENSXETP00000022445	xenopus	*Xenopus laevis*

Hspb3	ENSXETP00000050520	xenopus	*Xenopus laevis*
Cryaa	ENSXETP00000044922	xenopus	*Xenopus laevis*
Cryab	ENSXETP00000022441	xenopus	*Xenopus laevis*
Hspb6	ENSXETP00000038123	xenopus	*Xenopus laevis*
Hspb7	NP_001086558.1	xenopus	*Xenopus laevis*
Hspb8	ENSXETP00000054022	xenopus	*Xenopus laevis*

Associated Gene Name	**NCBI/ Ensemble Protein ID**	**Species Name**	**Latin name**
hspb1	ENSTRUP00000010646	fugu	*Takifugu rubripes*
cryaa	ENSTRUP00000006669	fugu	*Takifugu rubripes*
cryaba	ENSTRUP00000043693	fugu	*Takifugu rubripes*
hspb7	ENSTRUP00000007348	fugu	*Takifugu rubripes*
hspb8	ENSTRUP00000041438	fugu	*Takifugu rubripes*
hspb11	XP_003970936.1	fugu	*Takifugu rubripes*
hspb12	ENSTRUP00000031119	fugu	*Takifugu rubripes*

Associated Gene Name	**NCBI/ Ensemble Protein ID**	**Species Name**	**Latin name**
cryaa	ENSONIP00000004728	tilapia	*Oreochromis niloticus*
cryaba	ENSONIP00000006222	tilapia	*Oreochromis niloticus*
hspb6	ENSONIP00000003447	tilapia	*Oreochromis niloticus*
hspb8	ENSONIP00000013264	tilapia	*Oreochromis niloticus*
hspb9	ENSONIP00000007955	tilapia	*Oreochromis niloticus*
hspb11	ENSONIP00000026520	tilapia	*Oreochromis niloticus*
hspb12	ENSONIP00000013579	tilapia	*Oreochromis niloticus*
hspb15	ENSONIP00000017540	tilapia	*Oreochromis niloticus*

Associated Gene Name	**NCBI/ Ensemble Protein ID**	**Species Name**	**Latin name**
hspb1	ENSGACP00000026734	stickleback	*Gasterosteus aculeatus*
cryaa	ENSGACP00000020229	stickleback	*Gasterosteus aculeatus*
cryaba	ENSGACP00000027305	stickleback	*Gasterosteus aculeatus*
hspb7	ENSGACP00000007001	stickleback	*Gasterosteus aculeatus*
hspb8	ENSGACP00000018827	stickleback	*Gasterosteus aculeatus*
hspb15	ENSGACP00000022388	stickleback	*Gasterosteus aculeatus*

Associated Gene Name	**NCBI/ Ensemble Protein ID**	**Species Name**	**Latin name**
hspb1	ENSORLP00000025314	medaka	*Oryzias latipes*
cryaa	ENSORLP00000025613	medaka	*Oryzias latipes*
cryaba	ENSORLP00000007409	medaka	*Oryzias latipes*
hspb8	ENSORLP00000001457	medaka	*Oryzias latipes*
hspb15	ENSORLP00000010251	medaka	*Oryzias latipes*

Associated Gene Name	**NCBI/ Ensemble Protein ID**	**Species Name**	**Latin name**
hspb1	ENSDARP00000060161	zebrafish	*Danio rerio*
hspb2	ENSDARP00000068795	zebrafish	*Danio rerio*
hspb3	ENSDARP00000088297	zebrafish	*Danio rerio*

cryaa	ENSDARP00000070021	zebrafish	*Danio rerio*
cryaba	ENSDARP00000062522	zebrafish	*Danio rerio*
cryabb	ENSDARP00000124531	zebrafish	*Danio rerio*
hspb6	ENSDARP00000079957	zebrafish	*Danio rerio*
hspb7	ENSDARP00000006705	zebrafish	*Danio rerio*
hspb8	ENSDARP00000113545	zebrafish	*Danio rerio*
hspb9	ENSDARP00000103607	zebrafish	*Danio rerio*
hspb11	ENSDARP00000026207	zebrafish	*Danio rerio*
hspb12	ENSDARP00000111674	zebrafish	*Danio rerio*
hspb15	ENSDARP00000104334	zebrafish	*Danio rerio*

Table S4-4 Fold changes of catfish Hsp90s, Hsp60/10 and sHsp under each bacterial challenges.

Gene name	Columnaris-gill			ESC-intestine		
	4h	24h	48d	3h	24h	3d
hsp90aa1.1	1.86		-1.16			
hsp90aa1.2	1.67	3.68	2.58			2.14
hsp90ab1			-1.07			
hsp90b1	1.51	6.44	2.63			
trap1		1.95	1.34			
hspd1	1.52	2.51	1.54			
hspe1		2.34	1.35			
hspb1		-2.15	-1.63			
hspb2						
hspb3		-1.79	-1.07			
cryaa						
cryaba						
cryabb		-3.16	1.10	2.06	1.60	
hspb6		1.57	1.82			1.83
hspb7			1.17	1.70	1.54	2.08
hspb8			1.23	1.65	1.51	1.80
hspb9		-1.54	-1.71			
hspb11	2.00	1.74	1.38			1.75
hspb12						
hspb15						