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# Comparative analysis of sequence feature and expression of two heat shock cognate 70 genes in mandarin fish *Siniperca chuatsi*

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ABSTRACT

Heat shock cognate protein 70 (HSC70) is a molecular chaperone that plays essential roles in maintaining the cellular protein homeostasis. In this study, two *HSC70* isoforms were identified and characterized from mandarin fish *Siniperca chuatsi*. They have similar sequence structures, containing seven introns in their coding regions and sharing 94% similarity of their deduced amino acid sequences with 38 substitutions. Transcripts of both isoforms were detected throughout the embryogenesis, at low levels during the early developmental stages and up-regulated at blastula for *ScHSC70-1* and appearance of myomere stage for *ScHSC70-2*. They were ubiquitously expressed in tissues under normal conditions, whereas with tissue-specific variation. Following acute heat shock at 34 °C, the expression of *ScHSC70-1* showed no significant changes in the liver, and just a modest increase in the heart and head kidney, while the *ScHSC70-2* mRNA levels were markedly up-regulated in these tissues. Compared with their expression under gradual heat shock, the *ScHSC70-2* mRNA was rising at a higher rate under fast heat shock, whereas the *ScHSC70-1* mRNA increasing rate was lower under fast heat shock. Under hypoxia, transcripts of *ScHSC70-1* were not significantly changed, while the expression of *ScHSC70-2* was suppressed. *Aeromonas hydrophila* infection significantly increased the *ScHSC70-1* mRNA levels in the head kidney and spleen on early infective stages, while failed to have any significant impact on the expression of *ScHSC70-2* in both immune tissues. These results suggest that *ScHSC70-1* and *ScHSC70-2* are differently involved in the embryogenesis and the stress responses of high temperature, hypoxia and bacterial infection. This study will contribute to further study on enhancing stress tolerance and disease resistance of mandarin fish.

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## 1. Introduction

Heat shock proteins (HSPs), an evolutionary conserved multigene family of proteins, are ubiquitously essential molecular chaperones that maintain cellular protein homeostasis under normal and stress conditions (Basu et al., 2002; Roberts et al., 2010; Yamashita et al., 2010). HSPs are generally classified into HSP100, HSP90, HSP70, HSP60 and the small HSP families based on sequence homologies and molecular weights (Roberts et al., 2010). In the 70 kDa heat shock protein family, HSC70 is the constitutively expressed form, which is actively expressed under non-stressed cells and remains unchanged or only mildly induced upon stressful stimuli, while HSP70 is highly induced during stress (Erbse et al., 2004). HSC70 shares some of the structural and functional similarity with HSP70 and as molecular chaperones, they play important roles in protein folding/unfolding, assembly/disassembly, degradation and translocation, and are also involved in cellular protection when suffered with various stresses (Erbse et al., 2004; Liu et al., 2012; Yan et al., 2010). However, HSC70 also has its own functions in regulating apoptosis, embryo development and innate immune reactions (Dastoor and Dreyer, 2000; de la Rosa et al., 1998; Yan et al., 2010).

In the HSP70 family, HSP70 has been well studied on its regulation effects on the cellular resistance to stress (Ming et al., 2010), while compared with HSP70, much about HSC70 in this field remains to be known. Studies have shown that although the expression of HSC70 does not change or is only slightly up-regulated during stress, it does play pivotal roles in allowing cells to cope with stresses, including heat shock (Boone and Vijayan, 2002; Chu et al., 2001). In aquaculture, fish often encounter various environmental stresses, including changes in water temperature, pathogenic infection, hypoxia and heavy metals, sometimes even resulting in serious losses (Ming et al., 2010). Therefore, study on HSC70 in fish is of great significance to improving the animal's tolerance to environmental stresses. *HSC70* genes from a number of fish species have been cloned and characterized, and their functions during different stresses were studied preliminarily. For instance, acute heat shock significantly induced the expression of *HSC70* in silver sea bream (*Sparus*

*Abbreviations:* bp, base pare; BW, body weight; DO, dissolved oxygen; dph, day post-hatching; HRE, hypoxia response element; HSC, heat shock cognate protein; HSE, heat shock element; HSP, heat shock protein; MBT, midblastula transition period; ORF, open reading frame; PBS, phosphate buffered saline; RACE, rapid amplification of cDNA end; UTR, untranslated region.

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sarba) and *HSC70-2* in yellowtail (*Seriola quinqueradiata*) tailfin cells, suggesting that HSC70 might be responsible for cellular survival and adaptation under heat shock conditions (Deane and Woo, 2005; Yabu et al., 2011). The expression of *HSC70* in Wuchang bream (*Megalobrama amblycephala*), walking catfish (*Clarias macrocephalus*) and humphead snapper (*Lutjanus sanguineus*) were significantly increased when challenged with pathogenic bacteria, indicating that HSC70 might be involved in the immune response and played vital roles in resisting pathogenic infections (Poompoung et al., 2014; Zhang et al., 2011).

It is noted that there is more than one isoform of *HSC70* in a same organism. Distinct *HSC70* isoforms have been identified in several fish species, such as zebrafish (*Danio rerio*) (Graser et al., 1996; Santacruz et al., 1997), rainbow trout (*Oncorhynchus mykiss*) (Ojima et al., 2005; Zafarullah et al., 1992), carp (*Cyprinus carpio*) (Ali et al., 2003), yellowtail (Yabu et al., 2011), walking catfish (Poompoung et al., 2014) and tilapia (*Oreochromis niloticus*) (Zhang et al., 2014). Interestingly, distinct *HSC70* isoforms exerted different expression profiles under both stressed and non-stressed conditions. For instance, *HSC70-1* and *HSC70-2* in carp were expressed as one predominantly in a "complementary" manner in some organs under normal conditions (Ali et al., 2003). After treated by Cd (cadmium acetate), the expression of *HSC70-1* in liver of carp was markedly elevated, while the induction of *HSC70-2* was relatively modest (Ali et al., 2003). Bacterial infection did not affect the expression of walking catfish *HSC70-1* in most tissues whereas up-regulated the transcripts of *HSC70-2* in a tissue-specific manner (Poompoung et al., 2014). Although different expression profiles between *HSC70* isoforms have been found in given species, we don't know whether they exhibited similar differences in other fish species under the same or different stresses and how about their expression during the embryonic development.

The mandarin fish is an important cultured fish in China. Various environmental stressors, including the high temperature, hypoxia and pathogenic infection, caused severe economic losses to the aquaculture industry. Crucial roles of heat shock proteins in resistance to stress in aquaculture have been increasingly concerned. However, little information regarding heat shock proteins is available in mandarin fish. To provide molecular basis for further study the mechanism of anti-adversity and improving the ability of stress tolerance and disease resistance of mandarin fish, we identified and characterized two *ScHSC70* genes in the present study and comparatively studied their mRNA expression profiles on exposure to three heat shock regimes and acute hypoxia as well as challenged with *Aeromonas hydrophila*, the major bacterium caused bacterial hemorrhagic septicemia.

## 2. Materials and methods

### 2.1. Animals and sampling

The mandarin fish were obtained from BaiRong Aquatic Breeding Co., Ltd. (Guangdong, China). All fish were reared for at least three weeks in a circulating water system containing a series of 2000 L water tanks in Sun Yat-sen University. The water temperature was maintained at 25 °C and the fish were fed with juvenile *Cirrhinus molitorella* at a ratio of approximately 5% of the total biomass before any experiment. The fish were anesthetized with tricaine methanesulfonate (MS-222) before tissue collection. Tissues were sampled quickly and snap-frozen in liquid nitrogen, and then stored at −80 °C until RNA extraction.

### 2.2. Total RNA and genomic DNA isolation

Total RNA from tissues or embryos was isolated using E.Z.N.A. total RNA kit II (Omega Bio-Tec, USA) according to the manufacturer's instructions. Genomic DNA was extracted from the muscle using a TIANamp Genomic DNA Kit (Tiangen Biotech, China). The quality and quantity of RNA and DNA were assessed by the OD260/OD280 method and electrophoresis in 1% agarose gel.

### 2.3. Full-length cDNA cloning and genomic DNA amplification

Nested PCR was used to obtain the intermediate fragments of *Siniperca chuatsi HSC70* cDNAs. The first-strand cDNA was synthesized from total RNA with the First-Strand cDNA Synthesis Using M-MLV for RT-PCR kit (Invitrogen, USA). A mixture of cDNAs from multiple tissues (heart, liver, head kidney, gill and muscle) was used as the template. Two pairs of degenerate primers were designed and listed in Table 1. Amplification conditions of the nested PCR were: an initial preheating at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C (for the 1st round PCR) or 55 °C (for the 2nd round PCR) for 30 s, and elongation at 72 °C for 2 min, with a final extension at 72 °C for 10 min. Amplified PCR products were purified using a TIANgel Midi Purification Kit (Tiangen Biotech, China), and cloned into a pEASY-T1 vector using the TA cloning kit (TransGen Biotech, China). Recombinants were identified by blue/white screening and confirmed by PCR, and nine positive ones were selected and sequenced.

For 3′-RACE PCR, cDNA template was obtained from total RNA transcribed with the primer Oligo $(dT)_{20}$. Based on intermediate fragments of the two distinct *HSC70* cDNAs, forward gene-specific primers (Table 1) were designed for the two rounds of 3′-RACE PCR. An anchor primer (AP) and an abridged universal amplification primer (AUAP) were used as the reverse primers in the 1st and 2nd round, respectively. For 5′-RACE PCR, total RNAs were transcribed with primers of Oligo $(dT)_{20}$ plus the random primer. The first-strand cDNA was purified using a Universal DNA purification Kit (Tiangen Biotech, China), and a poly(C) end was added to the 5′terminal with a terminal deoxynucleotidyl transferase (TaKaRa, Japan). Based on the intermediate fragments and the 3′-cDNA end sequences, reverse gene-specific primers (Table 1) for 5′-RACE PCR were designed. An abridged anchor primer (AAP) and AUAP were used as the forward primers in the 1st and 2nd round, respectively. Cycling parameters for the 1st round of 5′- and 3′-RACE PCR were one cycle of 94 °C for 3 min, followed by 35 cycles (94 °C for 30 s, 55 °C for 30 s, 72 °C for 3 min) and a final extension step at 72 °C for 10 min; In the 2nd round, the annealing temperature was up-regulated to 58 °C and the extension time was reduced to 2 min.

Using the template of total genomic DNA and the primers that near the terminus of the full-length cDNAs (Table 1), the genomic DNAs of *HSC70s* were amplified. The PCRs were performed as follows: 94 °C for

**Table 1**
Primers and their applications in this study.

| Primer name | Primer sequences (5′–3′) | Objective |
|---|---|---|
| HSC-F1 | GGNACYACCTACTCCTGYGTNGG[a] | 1st round intermediate |
| HSC-R1 | TTAGTCNAYCTCYTCRATGGTNGG[a] | fragment amplification |
| HSC-F2 | ATCATHGCCAAYGACCAGGGNAA | 2nd round intermediate |
| HSC-R2 | TTRCAYACYTTCTCCARCTCCTT | fragment amplification |
| HSC70-1F1 | CGTAACACCACTATTCCTACCAAGCAG | 1st round 3′-RACE |
| HSC70-1F2 | CGTCCAACGTGACAAGGTGTCG | 2nd round 3′-RACE |
| HSC70-1R1 | CAAGGCAGCGACATCTCAGTAT | 1st round 5′-RACE |
| HSC70-1R2 | TGAACCTTTGGGCGAGTGTT | 2nd round 5′-RACE |
| HSC70-2F1 | GGAGGAGTCATGACTGTTCTCATTAAGAGG | 1st round 3′-RACE |
| HSC70-2F2 | AAGGCTGAGGATGATGTGCAGAGAG | 2nd round 3′-RACE |
| HSC70-2R1 | CTCCTGAGAAGACCGCCTACCA | 1st round 5′-RACE |
| HSC70-2R2 | GCAGGCACAGTCACTACAGCATT | 2nd round 5′-RACE |
| g-HSC70-1F | ATTCCAGACCCAACACCTAGTCAT | Genomic DNA cloning |
| g-HSC70-1R | CAAGGCAGCGACATCTCAGTAT | for HSC70-1 |
| g-HSC70-2F | ACACACAGACAGCGCGATATCAG | Genomic DNA cloning |
| g-HSC70-2R | CAGAAAAGCCACAGCGGAAG | for HSC70-2 |
| RT-HSC70-1F | AGTGAGAGGCTGATCGGAGATG | Real-time quantitative |
| RT-HSC70-1R | TGAACCTTTGGGCGAGTGTT | PCR |
| RT-HSC70-2F | TCTTCTCAGGAGACTATATTGCGTTC | Real-time quantitative |
| RT-HSC70-2R | CGACCATTTCCACCACATAACC | PCR |
| 18S-F | CTGAGAAACGGCTACCACATCC | Real-time quantitative |
| 18S-R | GCACCAGACTTGCCCTCCA | PCR |

[a] N, A/C/T/G; Y, C/T; R, A/G.

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