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Molecular characterization and gene evolution of the heat shock protein 70 gene in snakehead fish with different tolerances to temperature

Ihlam Ibrahim Eid^{a, b}, Subha Bhassu^{a, b, *}, Zee Hong Goh^{a, b}, Li Teng Khoo^{a, b},
Geok Yuan Annie Tan^{a, b}

^a Aquatic Molecular Biology and Biotechnology Group Terra Aqua Lab, Centre for Research in Biotechnology for Agriculture (CEBAR), University of Malaya, 50603, Kuala Lumpur, Malaysia

^b Animal Genetics and Evolutionary Lab, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603, Kuala Lumpur, Malaysia

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ABSTRACT

Temperature tolerances (including lethal limits) and associated rates of thermal acclimation of fish are critical information in predicting fish responses to global climate changes. In this study, a partial sequence of the heat shock protein 70 gene (HSP70) from the fish species *Channa striatus* was isolated and characterized. Evolutionary process that led to the diversity of HSP70 specific to vertebrates was also analysed. Results revealed that HSP70 is highly homologous in other fish families. The conservation of the HSP 70 gene among fish families could be driven by forces of natural selection due to climatic change. We exposed *C. striatus* to heat shock (32 °C) and cold shock (16 °C) respectively, in order to examine the differences of temperatures in influencing the expression patterns of HSP70. We revealed that expression of HSP70 was higher at 32 °C than at 16 °C in most of the organs. Specifically, occurrence of chaperone activity of HSP70 was found at low temperature. Therefore, this fish was postulated that to seems to be able to survive at lower temperature compared to higher temperature indicating there is force of natural selection acting towards this HSP 70 gene. This will demonstrate the effect of global warming towards the fish survivability.

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1. Introduction

More than 99% of species on Earth (including fish) are ectothermic - that is, they rely primarily on external sources for their body heat. Temperature variations ultimately affects organisms' metabolism (Berg et al., 2010; Atkinson and Sibly, 1997). For example, fishes are frequently exposed to temperature variations. In combination of hydrological regime, temperature changes are also observed (Ashoka and Macusi, 2012). These conditions challenged their survival and reproduction (Jesus et al., 2013). Moreover, protein expressions of fishes toward global climate changes in tolerating the temperature (including lethal limits) as well as their associated rates for thermal acclimation provided important information for aquaculture. Different species of fishes showed varied levels of ability in adjusting their upper temperature tolerance limits with

* Corresponding author.

E-mail address: subhabhassu@um.edu.my (S. Bhassu).

increased acclimation temperatures (Roessig et al., 2004). Importantly, small increases (1 ± 2 °C) in temperature are sufficient to cause sub-lethal effects on tropical fish physiology, particularly reproduction. Effects of temperature changes are also observed when they are combined with the possible effects of an altered hydrologic regime (Ashoka and Macusi, 2012). Therefore, it is therefore important to know how organisms, particularly ectothermic, respond to high temperature.

Heat shock protein (HSP) or also known as stress protein, is a protein that will response to external stressful conditions. This protein protected cells from extreme physiological, pathological and environmental conditions. They played roles in including protein misfolding correction and preserving immature polypeptides from aggregation under stresses. Elevation of elevated HSPs repaired protein damage and facilitated normal cell growth until conditions improved (Multhoff, 2007; Wang et al., 2003). HSP is first reported in *Drosophila*, fruit flies. This protein a subset of cellular proteins that will induced upon heat shock. Several researches on HSP were also carried out on various organisms and its functions in stress tolerance (Feder and Hofmann, 1999). Through the researches, this HSP is found universally in living beings, from bacteria to humans, and is highly conserved genes among organisms (Molina et al., 2000). Furthermore, exponential increase in the level of interests and research activities in concerning the description, classification and functional significance of this HSP protein are exponential increased. Nevertheless, study of heat shock protein in fish is still in the early stages compared to bacteria, yeast and mammals (Alak et al., 2010).

Snakehead fish which is from Channidae family. It is widely recognized as an economically important species with great potential for aquaculture. This fish can be captured throughout Southern and Southeast Asia. In addition, some species are highly priced and popular in the ornamental fish trade. Consumption of snakeheads did has therapeutic effect for wound healing after trauma and surgical procedures such as caesarean sections. Therefore, it is important to understand the effect of thermal change correspond to the survivability of *Channa striatus*. In this study, HSP70 gene in Haruan fish, *Channa striatus* (designated as ChHSP70), was characterized. Phylogenetic analysis to determine the species'evolutionary relationship with different vertebrates based on mRNA was also carried out. The study further sought to experimentally investigate the effects of thermal stress on HSP70 gene expression in *Channa striatus*. Specifically, we examined HSP70 gene transcription patterns for *Channa striatus* exposed to high and low temperatures, and compared the gene expression patterns in various organs of the fish.

2. Materials and methods

2.1. Experimental fish

Snakehead fish (*Channa striatus*) weighing between 70 and 130 g were obtained from the Perak River, Malaysia, and transferred to laboratory ~30 L aquaria. The aquaria were equipped with dechlorinated freshwater recirculation and maintained at 28 ± 1 °C (mean temperature observed during sampling). All the fish were acclimatized for 1 week before being exposed to heat stress. A maximum of 10 fish per aquarium were maintained during the experiment.

2.2. Sequence identification and evolution

A partial length of ChHSP70 gene was identified from the DNA sequencing of the muscle of *Channa striatus* using Illumina Solexa sequencing technology. This gene was identified as heat shock protein 70 (ChHSP70) through a BLAST homology search against the NCBI database. The sequence was compared with other fish sequences which are available on the NCBI database. The similarities of these genes were analysed. The open reading frame (ORF) and amino acid sequence of ChHSP70 were obtained by an ORF finder program available online (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>). Furthermore the mRNA sequences of ChHSP70 from 54 different vertebrates were downloaded from the NCBI GenBank database. The sequences were aligned using online available ClustalO program (<http://www.ebi.ac.uk/Tools/msa/clustalo/>). A neighbour-joining phylogenetic tree was then constructed for the sequences, based on pairwise differences, by Mega v.6 software (Tamura et al., 2013) and using the maximum composite likelihood calculated with a bootstrap of 1000 replicates. Arlequin v.3.5 (Excoffier and Lischer, 2010) was applied to test the partitioning of genetic variations within and between the clustered groups generated by the phylogenetic tree, using an analysis of molecular variance (AMOVA) by computing conventional F-statistics from sequences with 16,000 permutations. The fixation index (F_{ST}) between different sequence classes was also calculated.

2.3. Temperature stress

The experimental fish were exposed to a natural photoperiod (11 h light: 13 h darkness) and fed once daily with squid. They were acclimated to these conditions for at least 2 weeks prior experiments. For temperature stress challenge test, the fish were divided into two groups, six individuals each, one day before experiment. One group was exposed to cold conditions, 16 °C; and the other was exposed to hot conditions, 32 °C. The fish were maintained at the respective chosen temperatures for 1 h. At the end experiments, tissue samples from the liver, kidney, stomach, gills, fins and muscle were removed, immediately frozen with liquid nitrogen and stored at -80 °C until use.

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