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Differences in structure and changes in gene regulation of murrel molecular chaperone HSP family during epizootic ulcerative syndrome (EUS) infection

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ABSTRACT

Heat shock proteins (HSPs) are immunogenic, ubiquitous class of molecular chaperones, which are induced in response to various environmental and microbial stressful conditions. It plays a vital role in maintaining cellular protein homeostasis in eukaryotic cells. In this study, we described a comprehensive comparative data by bioinformatics approach on three different full length cDNA sequences of HSP family at molecular level. The cDNA sequences of three HSPs were identified from constructed cDNA library of *Channa striatus* and named as *Cs*CPN60, *Cs*HSP60 and *Cs*HSP70. We have conducted various physicochemical study, which showed that *Cs*HSP70 (666 amino acid) possessed a larger polypeptides followed by *Cs*CPN60 (575) and *Cs*CPN60 (542). Three dimensional structural analysis of these HSPs showed maximum residues in α-helices and least in β-sheets; also *Cs*HSP60 lacks β-sheet and formed helix-turn-helix structure. Further analysis indicated that each HSP carried distinct domains and gene specific signature motif, which showed that each HSP are structurally diverse. Homology and phylogenetic study showed that the sequences taken for analysis shared maximum identity with fish HSP family. Tissue specific mRNA expression analysis revealed that all the HSPs showed maximum expression in one of the major immune organ such as *Cs*CPN60 in kidney, *Cs*HSP60 in spleen and *Cs*HSP70 in head kidney. To understand the function of HSPs in murrel immune system, the elevation in mRNA expression level was analyzed against microbial oxidative stressors such as fungal (*Aphanomyces invadans*) and bacterial (*Aeromonas hydrophila*). It is interesting to note that all the HSP showed a different expression pattern and reached maximum up-regulation at 48 h post-infection (p.i) during fungal stress, whereas in bacterial stress only *Cs*CPN60 showed maximum up-regulation at 48 h p.i, but *Cs*HSP60 and *Cs*HSP70 showed maximum up-regulation at 24 h p.i. The differential expression pattern showed that each HSP is diverse in function. Overall, the elevation in expression levels showed that HSPs might have potential involvement in murrel immune protection thus, protecting the organism against various external stimuli including environmental and microbial stress.

1. Introduction

Molecular chaperones are key components of cellular machinery, which play a crucial role in productive folding and assembly of cellular proteins into highly ordered oligomeric structures. It is evolutionary conserved, ubiquitous class of essential proteins present in all organisms from bacteria to mammals [1,2]. Chaperonins are a sub-class of molecular chaperones, which belongs to the family of heat shock proteins (HSPs). Chaperonins may be defined as a protein which ensures the correct folding of

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misfolded proteins to attain their native state [3]. In spite of their diverse origin and sequence homology, chaperonins are divided into two subfamilies i.e., group I and group II. The group I chaperonin contains the GroEL-GroES system with a high degree of *N*-terminal homology while group II possesses the thermosome, which shows low sequence homology and is found in eukaryotic cytosol [4]. The most well characterized chaperonins include the GroEL like chaperonin 60 in *Escherichia coli*, which functions in an ATP/Mg dependent manner [5]. Chaperonin 60 is tetradecameric protein, which consists of two stacked rings with seven subunits in each ring [6]. Moreover, these are constitutive proteins whose expression can be enhanced under various stress conditions [7]. A number of earlier studies have suggested that both in prokaryotes and eukaryotes, few of the chaperonins are known to be HSPs, whereas all the HSPs could possibly be chaperonins [8–10].

HSPs are a set of highly conserved well known group of molecular chaperones, whose expression can be significantly increased when cells are exposed to elevated temperature or other various biotic and abiotic stresses including heat shock, thermal stress and infections [11,12]. HSPs play prominent role in maintaining cellular protein homeostasis which prevent aggregation or unwanted conformation and promote protein folding in the cell [1,13]. The pioneer work that led to the discovery of HSPs was made by Ritossa [14] who reported the expression of HSPs under thermal stress condition in *Drosophila melanogaster,* hence HSPs are referred as stress proteins [15]. Moreover, HSPs can also be induced in cell in an un-stressed condition and their secretion may decrease with aging process [16]. In recent years, HSPs have received much attention because it possesses unique features that permit their use in innate immune reaction and stress response [17].

The HSPs mostly existed in the molecular weight ranged between 27 and 110 kDa. It is mainly classified into seven major families based on the sequence homology, domain, motif as well as their molecular weight. They are HSP110, HSP100, HSP90, HSP70, HSP60, HSP40 and low molecular weight or small heat shock protein (sHSP) [18,19]. Among different groups of HSPs family, HSP60 and HSP70 are the most studied and well characterized. The HSP60 and 70 are multigenic and stress inducible; present in all organisms studied so far [20]. However, Parsell and Lindquist [18] reported that HSP60 and 70 play important roles in many biological processes such as cell survival, cell signaling and in cellular stresses. HSP60 is a well characterized multi-functional immunogenic chaperone (or chaperonin) molecule, with a distinct ring shaped or a toroid quaternary structure [21]. It is predominantly localized in the mitochondria of eukaryotic cells and assists protein folding mechanism of both nascent and denatured proteins [22,23]. Also, some researchers have reported about the existence of HSP60 in cytoplasm. The cytoplasmic HSP60 is involved in a variety of autoimmune and inflammatory processes [24,25]. Moreover, it can be expressed in response to various external stressors such as oxidative stress, toxic metals and pathogens [26].

The 70 kDa mitochondrial HSP70 is the largest heat shock protein among all the studied HSPs, which primarily binds to the target protein and mediates their folding, stability and intracellular transport [27,28]. Like HSP60, HSP70 also interacts with immune molecules and plays a crucial role in the immune system. Moreover, HSP70 is highly induced during several stress conditions, which can enhance the cellular resistance against various environmental stresses and infectious diseases in aquatic animals. In aquatic environment, fishes are more frequently encounter a variety of physical, chemical and biological stressors and their innate immune system plays an important role to protect them against those external agents [29]. It is to be noted that in aquatic animals the HSPs do not always obey the same expression pattern, rather it varies with species to species, tissues, sex and age. Wang et al. [26] and Dowling et al. [30] also showed that the gene expression pattern of HSPs in various fish species was affected by a wide variety of environmental and microbial challenges.

Channa striatus commonly called as snakehead or stripped murrel is one of the most important farmed aquaculture species of freshwater air breathing teleost fish in China and Southeast Asian countries including India, due to its tasty fleshy meat and high commercial and medicinal value [31,32]. In recent years, this species has been greatly threatened by a diverse group of environmental stresses such as water temperature, hypoxia, heavy metals as well as infectious diseases, which are causing high mortality in fish and reducing their production [29]. Dhanaraj et al. [33] reported that epizootic ulcerative syndrome (EUS) is a major devastating disease in stripped murrel which is primarily caused by fungus (*Aphanomyces invadans*) followed by bacteria (*Aeromonas hydrophila*), resulting in the largest economic losses to murrel industry worldwide. HSPs may enhance the cellular tolerance and disease resistance in fishes against severe environmental and microbial stresses [34]. So far, different group of HSP families have been identified and reported in some fish species such as rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), cyprinidae fish (*Tanichthys albonubes*) [35–39] etc. Moreover, to the best of our knowledge, there is no report on the HSP gene families in *C. striatus* till now. Thus, it is necessary to characterize the murrel HSPs and understand its functions, especially in the immune system to combat such serious problem like EUS. This study could be imperative to significantly improve the defense mechanism and in enhancement of disease resistance and stress tolerance in stripped murrel.

Hence, in this study, we have reported the first comprehensive comparative statement based on the bioinformatics characterization including evolutionary and structural analysis of three families of large HSPs including chaperonin 60 (CPN60), HSP60 and HSP70 from *C. striatus* (*Cs*) at the molecular level using various computational and biological tools. Moreover, to investigate the role of HSP in immune response and defense mechanism in stripped murrel, we determined their comparative gene expression profile at various time intervals after exposure to fungal and bacterial infections.

2. Materials and methods

2.1. Collection of freshwater C. striatus fish

Healthy *C. striatus* (average body weight of 60 ± 10 g) were collected from Pour Lake, Chennai, India. The fishes were transported to our research lab in plastic pots. The fishes were maintained in 10 rectangular flat bottomed plastic tank (100 L) with aerated and filtered dechlorinated freshwater (water quality: dissolved oxygen, 5.8 ± 0.2 mg/L; water temperature, 28 ± 1 °C and pH, 7.2 ± 0.2). A maximum of 10 fish per tank was maintained during experimentation. The individuals left a week for acclimatization; during this time, they were fed up to the satiation level twice a day. Then, they were being challenged with various immune stimulants or oxidative stressors including fungus or bacteria.

2.2. Immune challenged using microbial stressors

C. striatus was challenged with microbial infection including fungus and bacteria as prescribed in our earlier studies [40–42]. Briefly, *A. invadans* were isolated from EUS infected *C. striatus* muscle tissue and injected (100 μl per fish) intraperitoneally at a concentration of 10^2 spores suspended in 1X phosphate buffer saline (PBS). Similarly, *A. hydrophila* isolation suspended in 1X PBS