

# Heat Shock Proteins: An Alternative to Control Disease in Aquatic Organism

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The heat shock response was discovered by Ritossa and co-workers in 1962 when the exposure of *Drosophila* larvae to elevated temperature was observed to generate salivary gland polytene chromosome puffs, subsequently proven to reflect transcriptional induction of heat shock protein (Hsp) genes. Hsps, also referred to as stress proteins and molecular chaperones, normally account for 5-10% of total cellular protein, and they increase two or three times when cells are exposed to biotic and abiotic stressors [1]. Hsps occur in all living organisms and they are categorized into several families based on function, sequence and molecular mass. Major Hsp families include Hsp110, Hsp100, Hsp 90, Hsp70, Hsp 60, and the small Hsps (sHsps), with the latter having a molecular mass less than 40 kDa. Constitutive and inducible Hsps, some of which are organelle specific, perform vital functions generally by interacting with exposed hydrophobic surfaces of nascent and stress-induced non-native proteins [2]. Hsps help other proteins find their correct three-dimensional configurations by keeping them in folding-competent, folded, or unfolded states assist protein localization to specific organelles, and target non-native or aggregated proteins for degradation and removal from the cell. In addition to stress resistance, Hsps are involved in animal and plant development, aging, environmental adaptation, and the immune response, demonstrating the fundamental importance of these proteins to cell survival [3].

Hsp70 is the most widely studied stress protein in aquatic organisms and it is thought to function in induced thermo tolerance and cross tolerance [4,5]. It is now becoming clear that stress-induced Hsp70 enhances tolerance of aquatic organisms to disease, and work with several pathogenic *Vibrio* challenge models has raised many issues related to the role of Hsps in fish and shrimp pathology. In this context, a non-lethal heat shock (NLHS) of 37°C for 30 min followed by 6 h recovery maximally induced endogenous Hsp70 and optimally enhanced the resistance of *Artemia* larvae against *V. campbellii* and *V. proteolyticus* [6], *Vibrio* species known to infect brine shrimp. The two-fold increase in larval survival, in concert with stress protein synthesis, suggests a protective role for Hsp70. In a separate experiment, exposure of *Artemia* larvae to a combined hypo- and hyperthermic shock enhanced the amount of a 70 kDa polypeptide which reacted with antibody to Hsp70. Protection against infection by *V. campbellii* was significantly enhanced in these larvae, with the result again supporting a causal link between Hsp70 accumulation induced by heat stress and enhanced resistance to infection [7]. Similar observations were made in shrimp other than *Artemia* where Hsp70 build-up after a 24 h hyperthermic stress from 29 to 37°C correlates with attenuation of gill-associated virus (GAV) replication in the black tiger prawn [8]. The most frequently used protocol to stimulate Hsp expression in these experiments entails a short NLHS followed by incubation for several hours under non-stress conditions.

Other methods that enhance Hsp70 synthesis and prime aquatic organisms against disease include exposure to chemical inducers of Hsp70. Pro-Tex<sup>®</sup>, a soluble variant of Tex-OE<sup>®</sup>, a patented extract from the skin of the prickly pear cactus *Opuntia ficus-indica*, is a non-stressful inducer of high levels of endogenous or host-derived Hsps which has

become available for use in fish and shellfish. Stimulation of salmon and gilthead sea bream *Sparus aurata* L. with Pro-Tex<sup>®</sup> in the laboratory before exposure to *Vibrio anguillarum* infection reduces loss of fish to half of what occurs in fish not exposed to Pro-Tex<sup>®</sup>. When Pro-Tex<sup>®</sup> was used to stimulate fish before exposure to infection, circulating Hsp levels were detectable after incubation with little or no delay [9]. Treatment of *Artemia* with Tex-OE<sup>®</sup> (152 ppb) for 1 h promoted accumulation of Hsp70 and enhanced survival when subjected to *V. campbellii* challenge. Protection is perhaps due to enhanced prophenoloxidase (proPO) and nitric oxide (NO) production, important components of the innate immune system [10].

Supplying exogenous Hsps, either by feeding with Hsps encapsulated in bacteria or injecting recombinant Hsp70, represents another way to limit *Vibrio* infection in aquatic organisms. As one example, feeding with *E. coli* YS2 over-producing DnaK, the prokaryotic equivalent of Hsp70, enhances gnotobiotic *Artemia* larvae survival approximately two- to three-fold upon challenge with pathogenic *V. campbellii* [11]. Similar results were obtained when larvae were fed with heated bacterial strains LVS 2 (*Bacillus* sp), LVS 3 (*Aeromonas hydrophila*), LVS 8 (*Vibrio* sp), GR 8 (*Cytophaga* sp) and GR 10 (*Roseobacter* sp), all of which produce increased amounts of DnaK when compared to non-heated bacteria. Improvement in larval resistance to *V. campbellii* infection correlates with escalating amounts of DnaK, suggesting a protective role for this protein, either via chaperoning or by immune enhancement [12]. Support for an immunological effect is offered by the observation that feeding DnaK-enriched bacteria stimulates the Pro PO cascade system of *Artemia*, a mechanism important for pathogen melanisation by the innate immune system [10]. In a related study, feeding white leg shrimp *Litopenaeus vannamei* larvae with *E. coli* YS2 over-producing DnaK protects against pathogenic *V. harveyi*, boosting survival beyond 30% in a standardized challenge assay. As revealed by RT-q PCR, administration of DnaK enhances crustin mRNA transcript 7-fold more in whole larvae homogenates than those fed with YS2 cells that do not produce DnaK. Crustins are cationic cysteine-rich antimicrobial peptides and their up-regulation may protect shrimp larvae by suppressing *Vibrio* [13]. In fish, intra-coelomal injection with DnaK and GroEL, proteins equivalent to mammalian Hsp70 and Hsp60, combined with a non-lethal heat shock, safeguards *Xiphophorus*

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*maculatus* from death caused by *Yersinia ruckeri* [14]. These studies indicate that the resistance of aquatic organism to *Vibrio* infection is enhanced by endogenous DnaK/Hsp70.

To summarize, there are several mechanisms by which Hsp70 guards against bacterial infection. Hsp70 may stabilize cells against injury due to pathogen proliferation, assist the proper folding of cell proteins synthesized in response to bacterial pathogens and facilitate the storage and re-folding of partially denatured proteins. Hsps have the potential to improve tolerance to *Vibrio* sp. via immune stimulation. Hsps are thought to influence the production of cell surface peptides which are presented to the immune system, facilitating recognition of diseased cells [15,16] and they are involved with Toll-like receptors, a major element of the innate immune system. This possibility is currently under investigation, work that promises to yield findings of fundamental importance with applications in aquaculture, a major method of food production.

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