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A novel heat-shock protein inducer triggers heat shock protein 70 production and protects *Artemia franciscana* nauplii against abiotic stressors

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Abstract

Induction of **heat shock protein 70** (Hsp 70) is important in the tolerance to subsequent deleterious environmental stresses. It would therefore be of great benefit to develop non-toxic Hsp70-inducing compounds that are able to induce **Hsp70** in advance, for animals which are subjected to various environmental stresses. This study aimed to investigate whether Pro-TEX[®], a soluble version of Tex-OE[®] a chaperone-stimulating factor isolated from the **prickly pear** cactus (*Opuntia ficus indica*), could manipulate Hsp70 expression in a gnotobiotically cultured **brine shrimp** *Artemia franciscana* and subsequently protect against abiotic stressors. Results showed that Tex-OE[®] enhanced Hsp70 expression in a dose- and time-dependent manner in *Artemia*. In addition, **pretreatment** of *Artemia* with Tex-OE[®] (152ppb) for 1h protected the **shrimp** against thermal challenge. Interestingly, the expression level of Hsp70 coincided well with the extent of protection against thermal challenge, suggesting that the protective effect of the compound is mediated by Hsp70 induction. Results also demonstrated that Tex-OE[®] can function in synergy with a non-lethal heat shock (37 °C for 30min followed by 6h recovery) conferring maximum protection to *Artemia* against thermal and hypersalinity stresses at either optimal (152ppb) or sub-optimal (76ppb) dose. From these results, it is suggested that Tex-OE[®] is a potential inducer of Hsp70 and in the presence or **absence** of a bona fide stress, it could be an ideal candidate for use as an anti-stressor during various aquaculture practices.

Highlights

- Here we tested cross protective effect of Hsp-inducing compound Tex-OE[®] on *Artemia*.
- We found Tex-OE[®] to enhance Hsp70 level and survival of *Artemia* after heat stress.
- Tex-OE[®] acts synergistically with non-lethal stress conferring maximum protection.
- The maximum cross protection was against either heat or salinity stress.
- In essence, Tex-OE[®] can be an ideal anti-stressor candidate for use in aquaculture.

Introduction

Shrimp production by aquaculture is a high value activity worldwide. Recently it was estimated to have reached about three million metric tons which are valued in excess of US\$ 12 billion (FAO, 2010). Farming of shrimp has been carried out in either extensive or intensive/semi-intensive culture system. However, under the latter culture systems, environmental conditions can degrade rapidly causing significant stress to the shrimp (Capy et al., 2000). The consequence of such stress includes decreased immune defense and increased susceptibility to pathogens (Horowitz and Horowitz, 2001, Le Moullac and Haffner, 2000). In shrimp production systems, many potential pathogens, such as bacteria, fungi and viruses, co-exist with the shrimp without causing a negative impact on production (de la Vega et al., 2004, de la Vega et al., 2006, Vidal et al., 2001). However, some quiescent bacterial or viral infections may develop into acute diseases if shrimp become stressed and this has repeatedly led to significant industry losses (Hall and de la Vega, 2004, Vidal et al., 2001). Therefore, management of such stress is of great relevance in aquaculture due to its negative impact on the welfare and economic production of shrimps and other aquaculture species.

Accumulating evidence over the past decades suggested that sudden exposure of cells, tissues and organisms to sub-lethal heat stress (temperature well above the ambient condition but still within the physiological range of the organism) activated the

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production of an array of endogenous proteins known as heat shock proteins (Hsps) (de la Vega et al., 2006, DuBeau et al., 1998, Rahman et al., 2004). Functionally, these Hsps, mainly the 70kDa Hsp (Hsp70), are involved in the cross protection or cross-tolerance in animals and plants, i.e. a general stress response and a transient increase in the resistance to a second heterologous physiological and environmental insult (Sabehat et al., 1998). The protective function of the Hsp70 is documented to be due to its chaperone activity maintaining protein homeostasis by protecting nascent polypeptides from misfolding, facilitating co- and post-translational folding, assisting in assembly and disassembly of macromolecular complexes, and regulating translocation (Bukau et al., 2006, Morimoto, 2008, Ron and Walter, 2007). In a variety of experimental models an early peak of Hsp70 has been shown to confer thermal resistance (Frankenberg et al., 2000, Lei et al., 2005, Periago et al., 2002, Sejerkilde et al., 2003), protect against osmotic stress (DuBeau et al., 1998, Neta et al., 2005, Todgham et al., 2005), prevent oxidative toxicity and damage (Arieli et al., 2003, Collins and Clegg, 2004, Todgham et al., 2005) and improve desiccation tolerance (Ma et al., 2005). These observations clearly illustrated that Hsp70 protects multiple organisms against a further and eventually, more severe environmental insults. Besides, by heat shock, Hsp70 expression is also up-regulated by various physiological perturbations such as oxidative stress, nutritional deficiencies, ultraviolet radiation, chemicals, viral infection and anoxia (Pockley, 2003, Rahman et al., 2004). In an aquaculture system, however, sub-lethal heat shock is possibly not the best way to enhance Hsp production because acute temperature shifts are often detrimental, adversely affecting physiological balance and causing significant mortality. It would therefore be useful and beneficial to find less traumatic approaches for up-regulation of Hsp expression in aquatic organisms

Recently, Pro-TeX®, which contains the active molecule Tex-OE®, a patented extract from the skin of the prickly pear fruit, *Opuntia ficus indica*, has been reported as a non-stressful effector that induces high levels of endogenous or host-derived Hsps in animal tissues (Roberts et al., 2010). In this study, we investigated whether Tex-OE® (hereinafter mentioned as Hspi, for "HSP inducer") could manipulate the expression of stress protein in a gnotobiotically cultured brine shrimp *Artemia franciscana* and subsequently protect against abiotic stressors. The brine shrimp was chosen as an experimental organism in this study because it represents an ideal animal model to study crustacean stress response studies due to its ability to tolerate environmental perturbations (Clegg et al., 2000). The possibility to culture this animal under axenic/gnotobiotic conditions also eliminates the possibility of microbial interference in mechanistic studies (Baruah et al., 2011).

Herein we present findings demonstrating that pretreatment of *Artemia* with Hspi confers successful protection against abiotic stressors and that protection by Hspi is associated with the induction of endogenous Hsp70.

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Axenic hatching of *Artemia*

Axenic *Artemia* were obtained following decapsulation and hatching (Baruah et al., 2011). Briefly, 1.5g of *Artemia* cysts originating from the Great Salt Lake, Utah, USA (EG® Type, batch 21452, INVE Aquaculture, Dendermonde, Belgium) was hydrated in 89mL of distilled water for 1 h. Sterile cysts and nauplii were obtained via decapsulation using 3.3mL NaOH (32%) and 50mL NaOCl (50%). During the reaction, 0.22µm filtered aeration was provided. All manipulations were carried out under a laminar flow ...

In vivo induction of Hsp70 by Hspi

In the initial study, in order to determine whether Hspi has an inductive effect on Hsps in vivo, we analyzed Hsp70 expression by SDS-PAGE and Western blot in *Artemia* after 1-h treatment with different doses of Hspi. As shown in Fig. 1A, there was a constitutive expression of Hsp70 in the control and the expression was almost the same to that in the negative control. However, Hspi treatment at concentration as low as 7.6ppb markedly increased Hsp70 expression compared with the controls. The ...

Discussion

The production of Hsps in fish and shellfish has been shown to have profound protective effects under detrimental conditions (Baruah et al., 2010, Baruah et al., 2011, Iwama et al., 1998, Roberts et al., 2010, Sung et al., 2007, Sung et al., 2008). These observations justify attempts to characterize novel Hsp inducers, which could be used

to increase environmental stress resistance of different aquaculture species.

In this in vivo study, we examined the effect of an Hsp stimulating factor ...

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