Inducers of heat shock protein 70: A new disease preventive option in aquaculture production systems

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Inducer of Heat Shock Protein 70: A New Disease Preventive Option in Aquaculture Production Systems

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Bacterial diseases – Still a Threat to Larviculture

Abiotic Stressors

Diseases

Underdeveloped Immune System

Biotic Stressors

Diseases

Larval Mortality
Emphasis on **PREVENTION** which is likely to be more cost effective than the **CURE**
Alternative Solutions for Larval Diseases

- Probiotics
- Immuno-stimulants
- Vaccines
- Acidifiers
- Green water technique

Strategies successfully applied in aquaculture

More alternatives required as no anti-infective technique seems to be able to solve every problem alone.
Multiple agents cause disease outbreak.

- Biotic Stressors
  - Larval Mortality
  - Disease Outbreak

- Abiotic Stressors
  - Shell(fish) health

Larval Mortality
What are Heat Shock Proteins (Hsps)?

- Hsps are ubiquitous and highly conserved protein molecules, available in all prokaryotic and eukaryotic cells.

- Different types – sHsps, Hsp60, Hsp70, Hsp90, Hsp100.

- Hsp70s form one of the major Hsp families, most extensively studied one.

- Synthesized constitutively in the cells (heat shock cognate 70).

- Induced after exposure to stressors (cold, O₂ deprivation, etc.).
Function of Intracellular Hsp

- Inside the cell they act as molecular chaperones - bind to unfolded proteins (nascent polypeptides or denatured ones) - facilitate their refolding to the native state.

- Involved in protein translocation and degradation.
Due to necrosis, the Hsp70 gets released from the cell.

Extracellular Hsp70 serves as danger signal and modulates both innate and adaptive immune responses.
Hsp70 Induction in *Artemia*: Non-Lethal Heat Shock (NLHS)

Decapsulation & Axenic Hatching (28°C) → Instar stage 2 larvae

**Gnotobiotic Artemia**

NLHS at 37°C for 30 min followed by 6 h recovery period

Challenged with vibrios at 10⁷ cells/ml

Survival scored after 48 h

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Results

**Survival after *Vibrio* challenge** vs **Endogenous Hsp70 accumulation**

- Correlation exists between enhanced protection and Hsp70 accumulation
- Heat shock is not an ideal way to enhance Hsps in aquaculture animals.

- Is there any less traumatic approach to manipulate Hsps expression within (shell)fish?
Compounds extracted from plants

Can they induce Hsp70 production in aquaculture animals???

Can they induce protection against abiotic stressors???

Can they induce protection against pathogenic biotic stressors???
Compounds extracted from plants

- Compound A
- Compound B

- Unpublished work in progress
Pretreatment of axenic *Artemia* with compound

- Pretreatment of *Artemia* instar II with compound for 2 h (28°C)
- Washing of *Artemia* with sterile sea water (28°C)
- Given a recovery period of 2 h (28°C)
Challenge with abiotic or pathogenic biotic stress

**Thermal stress**
- Heat shock at 41°C for 20 min
- Survival scored after 6 h

**Vibrio challenge**
- Challenged at $10^7$ cells/mL
- Survival scored after 48 h
RESULTS

Compound A
Protective effect of compound A against vibrios

Positive control: No pretreatment, no challenge.

Negative control: No pretreatment, challenged with *Vibrio*

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Compound A conferred protection against vibrios and thermal stress coincides with Hsp70 production

Control: No pretreatment
RESULTS

Compound B
Protective effect of compound B against lethal heat shock

Control: No pretreatment

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Protective effect of compound B against *V. harveyi*

**Survival after Vibrio challenge** vs **Induction of Hsp70**

- **Survival (%)**
  - No Vibrio harveyi: 100%
  - Vibrio harveyi: 10%
  - 10 ppm: 75%
  - 25 ppm: 50%
  - 50 ppm: 75%
  - 75 ppm: 46%
  - 150 ppm: 20%

- **Relative production of Hsp70 in Artemia**
  - Hela cells: 1.5
  - Control: 0.5
  - Comp B: 1.0

Control: No pretreatment, challenged with *Vibrio*

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Collection of samples for Hsp70 and prophenoloxidase (proPO) genes

- Pretreatment for 2 h
- Rinsing
- Recovery for 2 h
- Challenged with V. harveyi for 24 h

Samples collected
Effect of Compound B on Hsp70 and proPO genes

Control: No pretreatment
0 h - Pretreatment without *Vibrio* challenge
6, 12 & 24 h: Pretreatment followed by 6, 12 & 24 h of *Vibrio* challenge

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CONCLUSIONS

- These plant-extracted compounds are potential inducers of Hsp70.
- They induced tolerance against subsequent abiotic (thermal) stress.
- They generated protective immunity in Artemia against pathogenic biotic (vibrios) stressor by priming the proPO system.
- These compounds represent potential preventive modality for vibriosis in Artemia and possibly in other commercially important aquaculture species.
Thank you
CLARIFICATIONS
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Prophenoloxidase Activation system

LPS + LBP

ß-glucan + ßGBP

peptidoglycan + PGBP

Serine proteinase cascade

Prophenoloxidase → Phenoloxidase

Phenols

Melanin ↔ Quinones
Experimental Model System

Host

Environment

Different micro organisms

Artemia

Host

Target micro organisms
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