Recent Developments in Global Shrimp Health Management

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AQUA2018, August 25-29 2018; Montpellier, France

FORUM: PRODUCTION OF PENAID SHRIMP IN EUROPE - RISKS AND OPPORTUNITIES
The expansion of farmed shrimps is not without diseases.
Early Mortality Syndrome in Shrimps – The Perfect Killer

IMPACT: > billion dollar loss

Countries affected: South America, China, Vietnam, Thailand
Control Measures for Bacterial Diseases in Aquaculture Animals

**DISEASE CONTROL STRATEGIES**

- Probiotics
- Immunostimulants
- Vaccines
- Acidifiers
- Good husbandary

**NOT BROADSPECTRUM**
**LACK OF CONSISTENCY**

**KEY FOR EFFICACY**
Controlling Diseases in Human – Practice Followed

ISOLATION & INTENSIVE CARE
Controlling Diseases in Terrestrial Animals – Practice Followed

ISOLATION & INTENSIVE CARE
Role of Environment in Host-Pathogen Interaction

HOST
(Human, cow)

ENVIRONMENT

SPECIES INTERACTION

PATHOGEN

REGULATION OF DISEASE OUTCOME
Multiple agents interact to cause diseases in aquaculture animals.

External
- Environmental factors (pond water)
- Microorganisms

Internal
- Defense system
- Gut flora

Diet
- Stress
Multiple Agents Cause Diseases in Aquaculture Animals

- Environmental factors (pond water)
- Microorganisms
- Diet
- Stress
- Gut flora
- Defense system

• Inconsistency of anti-infective strategy – Single disease target
A Paradigm Shift in Shrimp Health Research

Aquaculture Stress/Health Management
Designing of Anti-infective Strategy

ENVIRONMENT

PATHOGENS

HOST

Holistic Approach
Critical Factor in Host-Pathogen Interaction Studies

Model organism
Test System - Development of Disease Control Strategies

Under laboratory conditions: no risk for production, more control, yet labor intensive.

Under real production conditions: trial and error, uncontrolled, risk for production.

Inhibition of growth

Virulence factor activity

Intermediate step: GART, the Gnotobiotic Artemia screening platform
Shrimp Health and Microbial Management

- Genetics
- Glucans
- Polyphenols
- Organic acids
- Biofloc
- Heat Shock Protein
- Probiotics
- Epigenetics
- Quorum Sensing
Findings Snapshot

- Genetics
- Probiotics
- Heat Shock Protein
- Epigenetics
- Glucans
- Polyphenols
- Butyric acid
- Biofloc
- Quorum Sensing
- Shrimp Health/Microbial Management

Shrimp Health/Microbial Management
Quorum Sensing
Quorum Sensing

• **Before**: bacteria = Separate entities

• **Now**: bacteria sense and respond to environment and to each other
  - Extracellular signal molecules
  - ≈ hormones in higher organisms
• QS in vibrios: multi-channel systems:

• Documented in:
  - V. alginolyticus
  - V. anguillarum
  - V. campbellii / V. harveyi
  - V. ichthyoenteri
  - V. mimicus
  - V. parahaemolyticus
  - V. salmonicida
  - V. scophthalmi
  - V. vulnificus
QS-REGULATED PHENOTYPES

- **Virulence factors**
  - *In vitro* activity assays
  - *In vitro* gene expression (RT realtime PCR)

**Quorum sensing**

+ Metalloprotease
  - “Extracellular toxin”

- Type III secretion system
  - Siderophore
  - Phospholipase
  - Chitinase

Chitinase assay

INTERACTION: Vibrio – brine shrimp

- Brine shrimp (*Artemia*): model organism for shrimp

- Creating gnotobiotic cultures starting with axenic larvae (Instar II nauplii)
  → Only bacteria added to cultures are present!
Artemia Model in Bacterial QS Research

- *In vivo challenge tests* with gnotobiotic *Artemia* and weak (BB120) or more virulent (LMG21363) strain

20 mg/l furanone: protection against both vibrios

Furanone is toxic at 50 mg/l

Artemia survival (%)
Disruption of QS to Control Bacterial Infections

- **Use of QS inhibitors** (e.g. plant extracts/plant-derieved compound)
- **Effect of the furanone on bioluminescence of *V. harveyi***

The furanone blocks all three channels of the quorum sensing system
Disruption of QS to Control Bacterial Infections

- *In vitro* growth of luminescent vibrios without and with furanone (50 mg/l)

The furanone does not affect growth of the vibrios
Epigenetics
Teaching Shrimps Self-Defence to Fight Infections

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impose major yield-limiting effects on production, causing significant losses [5]. To avoid production losses, several veterinary drugs are commonly used. However, their prudent use has resulted in the development of antimicrobial resistance (AMR) in many shrimp pathogens (including bacteria, fungi, viruses, and parasites). Consequently, veterinary drugs are no longer effective in treating shrimp disease more predictable, reliable, cost-effective, and ultimately more sustainable disease-control strategy. The results of a trans-generational study using the shrimp model organism *Artemia* suggested that training induced by exposing the parental population of *Artemia* at their early life stages to challenge with *Vibrio campbellii* (an important shrimp bacterial pathogen) significantly increased the resistance of
Experimental Design

Parthenogenetic Artemia

(clonal population)

Daily non-lethal heat shocks (T)

Iso-thermic (C)

TF1

TF2

TF3

ISO

THERMIC

CF1

CF2

CF3
Common garden test - Verifying stress-resistant phenotypes

- 42°C for 15 min
- Or
- \(Vibrio\ campbellii\ 10^7\) cells/ml

20 instar II nauplii in 6 replicates
Thermo-tolerance test

TF1 > CF1

TF2 > CF2

TF3 > CF3
V. campbellii resistance

Environmental heat stress induces epigenetic transgenerational inheritance of robustness in parthenogenetic Artemia model
HSP-INDUCING COMPOUNDS
IN HEALTH & DISEASE
Function of Intracellular Hsp

- Conserved proteins acting 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

- Immunogenic proteins that modulate both innate and adaptive immune responses
Hsp-inducing Compound in Shrimp Health Management

Phloroglucinol

Pyrogallol

Carvacrol

CONTAINS Tex-OE™ PATENTED CACTUS FRUIT EXTRACT
Protective Effect of Phloroglucinol against *V. parahaemolyticus*

“Whole-animal Response”

Negative control: No pretreatment, no challenge
Positive control: No pretreatment, *Vibrio* challenge

Kumar et al. 2018. Frontiers in Immunology. 9:1091
Mode of Action of Phloroglucinol: Induction of Hsp70

Kumar et al. 2018. Frontiers in Immunology. 9:1091
**In vivo knockdown of *Artemia* Hsp70 using RNAi**

Figure: Knockdown of hsp70 mRNA in brine shrimp larvae. Equal amounts of RNA from brine shrimp larvae were amplified by RT-PCR and the products were resolved by electrophoresis in agarose gels.

- M - 100bp DNA ladder,
- Lane 1 - Wild type brine shrimp larvae,
- Lane 2 - Brine shrimp larvae injected with dsRNA gfp,
- Lane 3 - Brine shrimp larvae injected with dsRNA hsp70,
- Lane 4 - Wild type brine shrimp larvae pretreated with phloroglucinol,
- Lane 5 - Brine shrimp larvae injected with dsRNA gfp and pretreated with phloroglucinol,
- Lane 6 - Brine shrimp larvae injected with dsRNA hsp70 and pretreated with phloroglucinol

Kumar et al. 2018. Frontiers in Immunology. 9:1091

**Phloroglucinol induces anti-*Vibrio* effect by inducing Hsp70**
Mode of Action of Phloroglucinol

Phloroglucinol

Damage Control System (Hsp70)

+ 

Reduce virulence factor production in *Vibrio*

Kumar et al. 2018. Frontiers in Immunology. 9:1091
Anti-\textit{Vibrio} Effect of Phloroglucinol – Validation Study Ongoing
The Health Beneficial Activities of Tex-OE®

Findings Snapshot

- Temperature stress
- Osmotic shock
- *V. campbellii*
- *V. harveyi*

FUTURE PERSPECTIVES

Heat Shock Protein

Polyphenols

Quorum Sensing Disruption

Probiotics

OMNIBIOTIC - A HOLISTIC STRATEGY

BROADSPECTRUM

CONSISTENT
ACKNOWLEDGMENTS