Shrimp Aquaculture - Case Studies

Using Standard BioTools Microfluidic Technology

Standard BioTools™
March 2023

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Gene Expression and Death

Any questions?

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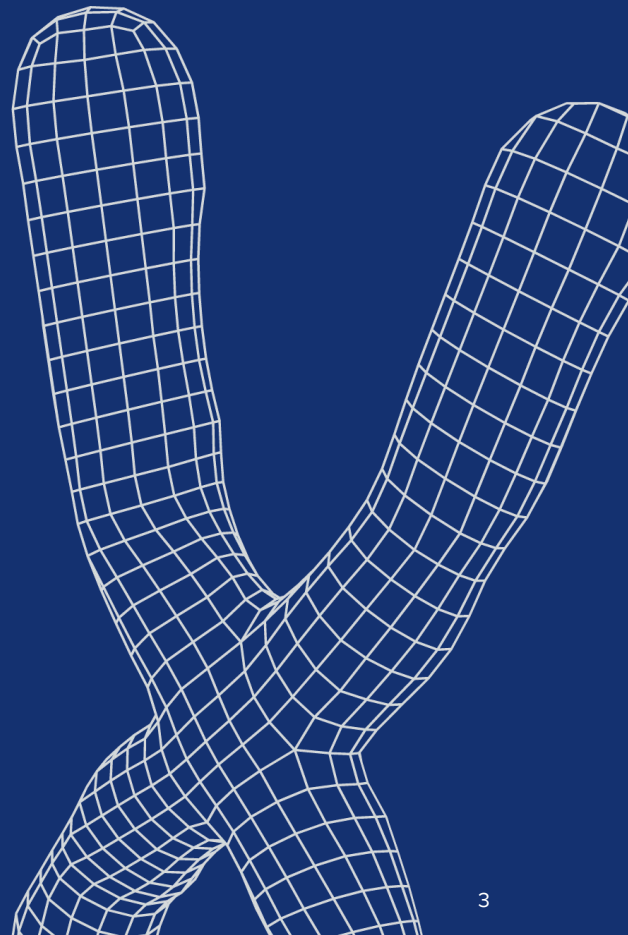

Gene Expression and Death

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Objective: The objective of this study was to measure gene expression levels of Hemocyte immune response genes when exposed to White Spot Syndrome Virus (WSSV) and Fusarium solani (fungal) in order to understand the molecular mechanisms driving shrimp-pathogen interactions to develop strategies to control disease outbreaks in shrimp production systems with *L. vannamei*.

Utilization of Standard BioTools products:

- Biomark System
- NanoFlex 4-IFC Controller
- 96.96 dynamic arrays
- Real-Time PCR Analysis Software

Conclusions:

- Genes that were monitored showed up regulation and down regulation over the time course study.
- Results reported can serve as immunomarkers for mortality events and could help reduce the loss in shrimp production systems

Goncalves, et al. "Molecular signatures at imminent death: Hemocyte gene expression profiling of shrimp succumbing to viral and fungal infections." Developmental and Comparative Immunology (2014)

Gene Expression and Death

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Background

- Infectious diseases represent the most serious threat to shrimp farming and the economic impact from mass mortalities in shrimp farming has intensified to develop strategies to overcome and control disease outbreaks.
- Researchers developed a panel to measure hemocyte-expressed genes involved in the immune defense of shrimp.
- Standard Biotools RT-qPCR technology that provided a high throughput platform to simultaneously measure expression levels for several genes during the time course study

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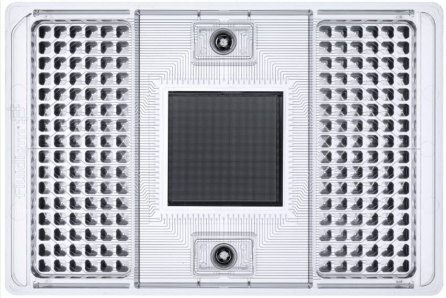

Methods:

- Data collected using the 96.96 GE IFC on the Biomark with Evagreen (DNA intercalating dye) chemistry
- Biomark Real-Time PCR analysis software was used to obtain Cq values and setting a cutoff of 2-fold change in expression level for analysis
- Pathogen-free shrimp were injected with lethal doses of WSSV and F. solani, separately, and gene expression levels were measured and compared to a control group over a time course of 24-48 hours
- A total of 68 hemocyte-expressed genes of broad relevance to immune system were chosen

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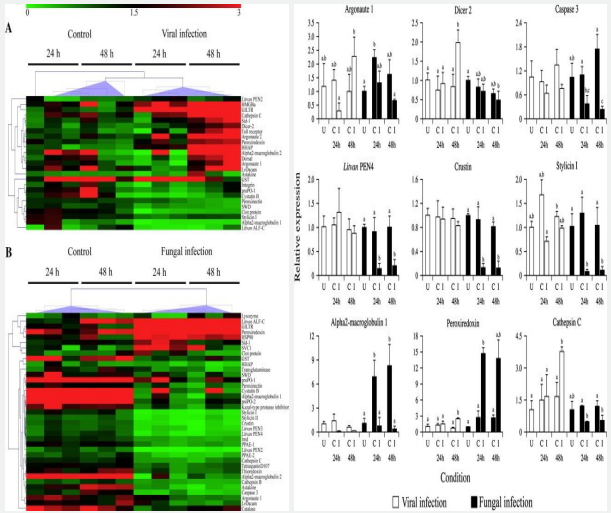

Results and conclusions

Results:

- WSSV infection showed an up-regulation of 11 genes and down-regulation of 11, while 3 showed time-dependent changes in gene expression.
- F. solani infection showed down-regulation of most modulated genes.
- Some genes analyzed were selective in their changes in expression, dependent upon the pathogen.

Conclusions:

- Molecular signatures identified uncovered promising RNAi pathways
- The transcriptional signatures identified here can serve as immunomarkers of mortality events
- At the time of publication, this was the first report of global gene expression profile of immune response of infected shrimp at the brink of death.

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Pathogen Detection Of AMR Genes

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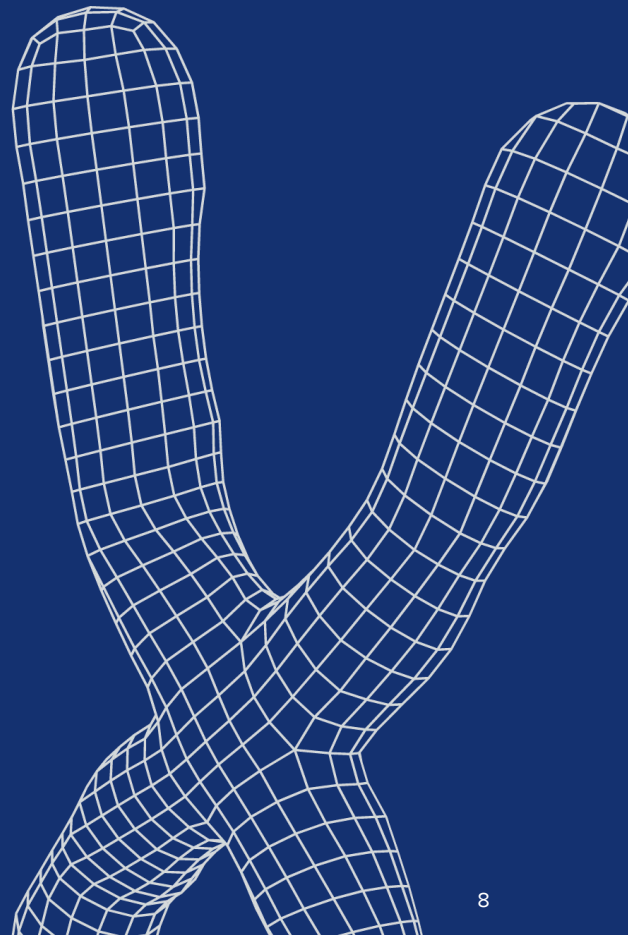

Pathogen Detection Of AMR Genes

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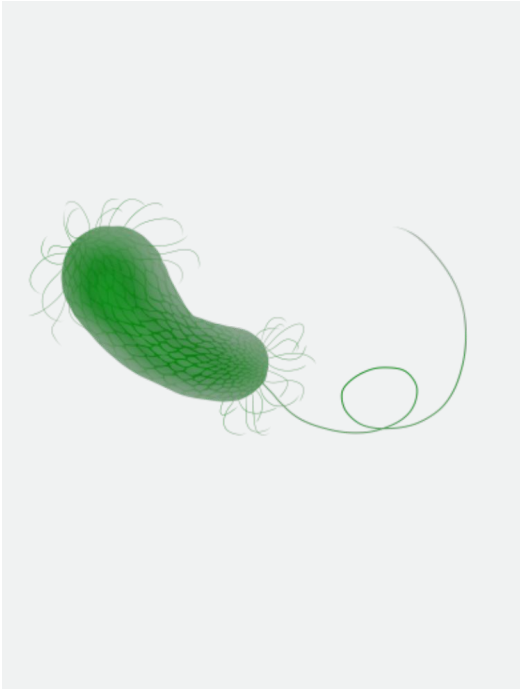

Objective: To investigate ARGs and MGEs in seafood bacteria in or to estimate AMR burden conveyed by the marine sector.

Utilization of Standard BioTools products:

- Biomark HD System
- 96.96 dynamic arrays
- Standard Biotools Real-time PCR Analysis software

Conclusions:

- Only 4.5% of isolates displayed ARG-MGE associated patterns which does not support the hypothesis of high-level human exposure through either direct exposure or through seafood consumption

Delannoy, et al. "High Throughput Screening of Antimicrobial Resistance Genes in Gram-Negative Seafood Bacteria." Microorganisms (2022)

Pathogen Detection Of AMR Genes

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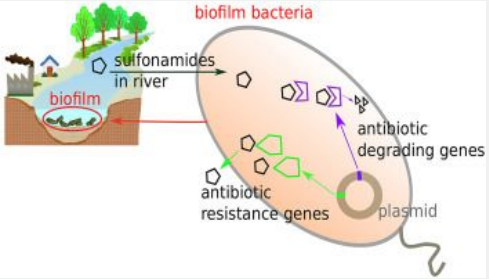

Background:

- AMR is a global public health safety issue because resistance can reduce treatment options in bacterial infections.
- These researchers wanted to analyze the prevalence of AMR and ARB in several different aquatic species.
- Researchers isolated gram-negative bacteria from five different aquatic species and analyzed them for the presence of antibiotic resistance genes (ARGs).

Delannoy, et al. "High Throughput Screening of Antimicrobial Resistance Genes in Gram-Negative Seafood Bacteria." Microorganisms (2022)

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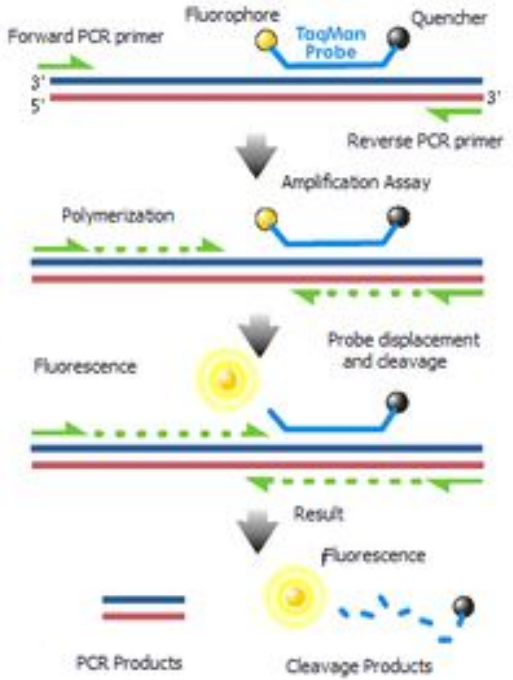
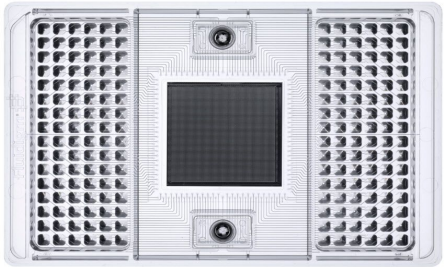


Methods:

- Bacterial strains isolated from cod, flat fishes, horse mackerel, shellfish and shrimp from their skin and gills
- 74 ARGs and MGEs (mobile genetic elements) identified as best for this study
- TaqMan chemistry used on 96.96 IFC leveraging FAM and HEX
- Positive controls used to validate the qPCR results consisted of already positively identified strains of isolated DNA by WGS or artificially made plasmid constructs



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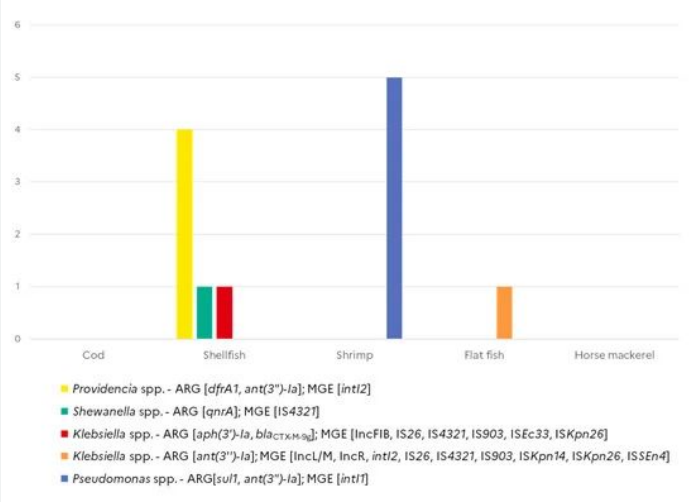

Results and conclusions

Results:

- 8 ARG markers detected positive, including sulfonamide resistance (sul1) in 5 of 50 Pseudomonas isolates, all in shrimp
- 6 MEG and 9 IS targets detected positive
- 76 of 268 strains (28.4%) gave positive signal for either ARG or MGE
- 12 of 268 (4.5%) gave positive signal for both ARG and MGE

Conclusions:

- ARB and ARGs decrease as the distance from sources of contamination increases
- Standard Biotools high throughput qPCR is a fast and convenient method for simultaneous investigation of large numbers of genes and hundreds of samples
- The technology is easy to implement, easily adaptable and new relevant markers can be added to accommodate an emergence of new AGRs
- Powerful tool for ARG and MGE investigation in complex DNA samples

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Modern aquaculture is powered by X9

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