ANALYSE OF IMMUNE GENE EXPRESSION IN SHRIMPS: A TOOL FOR HEALTH MONITORING OR GENETIC SELECTION

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Introduction

The aquaculture of penaeid shrimps contribute to the economic development in many countries, but pathogenic virus and bacteria which cause massive mortalities in shrimp farms, represent the main limitation of this industry. Knowledge of immune gene expression in response to pathogens is of prime importance to understand the immune capability of shrimps and for further work on genetic selection but also on the establishment of health monitoring. The goal of the study is to establish quantitative assays for analyses of immune effector encoding genes, adapted to the evaluation of shrimp immune competence.

Materials and methods

Genomic approaches have been used to characterize immune genes in different shrimp species. Expression Sequence Tag programs have been developed from different tissues of shrimps. A subtracted hemocyte library (SSH) was used to isolate genes differentially expressed in *Litopenaeus stylirostris* that have survived to *Vibrio* infection. Database searches were carried out using BlastX program.
Expression profiles of selected genes were analysed by macro-array and real time PCR using the relative expression to a constitutive gene (Elongation factor-1alpha).

Results

EST and SSH sequences have been compared to nonredundant SwissProt and GenBank databases using both Blastn and Blastx (http//www.ncbi.nlm.nih.gov), and the sequences were categorized into functional groups. Among them, sequences for different classes of antimicrobial peptides (AMPs) have been identified in the shrimp species, such as *Penaeus monodon*, *Litopenaeus stylirostris, Fenneropenaeus chinensis*. *In silico* analyses have been made for design of primers specific for the different AMPs and of primers ubiquitous for the different shrimp species. The specificity of the PCR amplification has been controlled in the shrimp species for the different AMPs. Then, AMP gene profiling has been studied both by Real time PCR (Figure 1) and macro-arrays to investigate any differences in their expression levels between different shrimp populations and in response to infection.
From the SSH hemocyte library, beside AMPs, several genes have been isolated that appear potentially differentially expressed in hemocytes from surviving shrimps. Among

the known genes, the most two represented categories are immune genes and genes involved in cell proliferation.

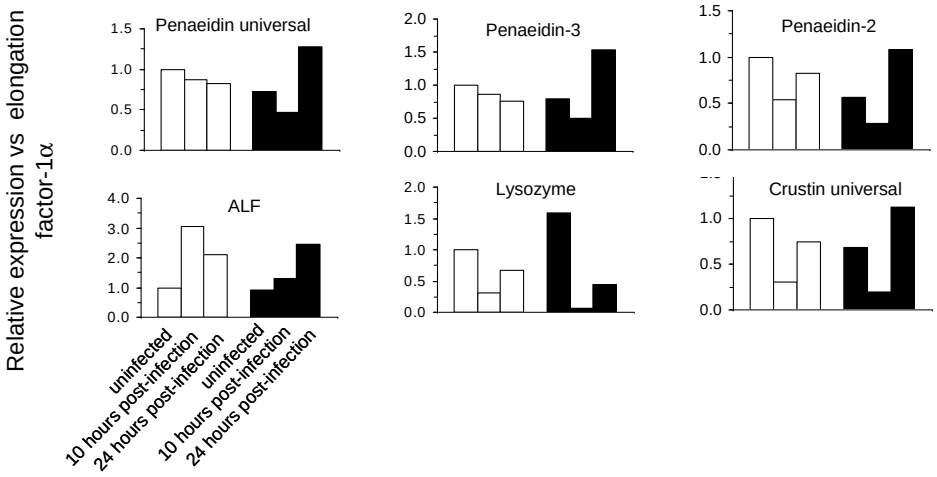

Figure 1
Relative expressions of six different genes studied by real time PCR. The expression profiles are determinate between three experimental conditions, they are uninfected shrimps, 10 hours post infection and 24 hours post infection and in two different groups of shrimp (black and white). The graphics showed relative expression of each gene normalized with the elongation factor 1-alpha (EF-1α).

Conclusions

From these results, expression profiles of selected genes, including genes coding for antimicrobial peptides, can be now considered at shrimp population levels using quantitative methods to evidence individual variability and any linkage of gene expression levels and better survival of animals to infections. Such heritable differences in gene expression should be further applied for identification of standard for Marker-Assisted Selection.

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http://www.ifremer.fr/Immunaqua