



# Analyse of immune gene expression in shrimps: a tool for health monitoring or genetic selection

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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. Genomic approaches have been used to characterize immune genes in different shrimp species. From Expression Sequence Tag programs, in particular, antimicrobial peptides (AMPs) were shown to be ubiquitous in shrimp species. Suppression subtractive hybridization (SSH) led to the isolation of genes differentially expressed in shrimps that have survived to *Vibrio* infection. Expression profiles of selected genes, including genes coding for antimicrobial peptides, are considered at shrimp population and individual 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 applied for identification of standards for Marker-Assisted Selection.

## 1- EST programs developed in shrimps.

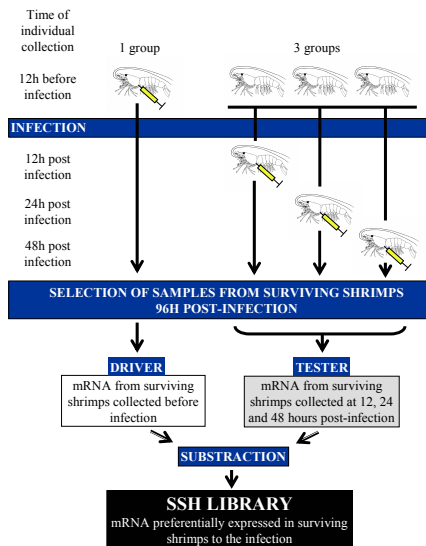
Species	Tissues	shrimp status	Reference
<i>Litopenaeus setiferus</i>	hemocyte	Not infected	Gross, 2001
	cephalothorax	Not infected	Gross, 2001
<i>Litopenaeus vannamei</i>	hemocyte	Not infected	Gross, 2001
	cephalothorax	Not infected	Gross, 2001
<i>Penaeus monodon</i>	hemocyte	Not infected	Supungul, 2002
	cephalothorax	Not infected	Lehnert, 1999
	ceystalk	Not infected	Lehnert, 1999
	pleopod	Not infected	Lehnert, 1999
<i>Penaeus japonicus</i>	hemocyte	Not infected	Rojtinnakorn, 2002
	hemocyte	Virus infection	Rojtinnakorn, 2002
<i>Fenneropenaeus chinensis</i>	hemocyte	Not infected	Xiang (pers. com.)
	cephalothorax	Not infected	Xiang (pers. com.)
<i>Litopenaeus stylirostris</i>	hemocyte	Vibrio infection	de Lorgeril, submitted

## 2- Experimental protocol for the SSH library construction to obtain mRNA preferentially expressed in surviving shrimps (*L. stylirostris*) to *Vibrio* infection.

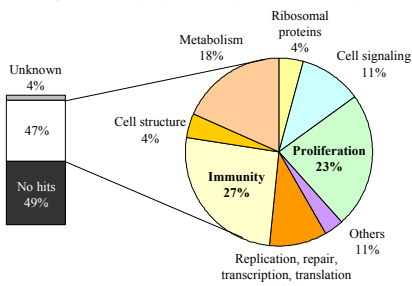
Shrimps were individually identified by silicon tagging and hemolymph was individually collected.

Infection with *Vibrio penaeicida* by balneation at the DL50 condition.

The hemolymph volume collected was limited to maintain shrimps alive.



## 3-The 260 cDNA from the SSH were analysed and clustered into ten functional categories according to the major function of their deduced proteins.



Higher proportion of genes involved in immunity and cell proliferation.

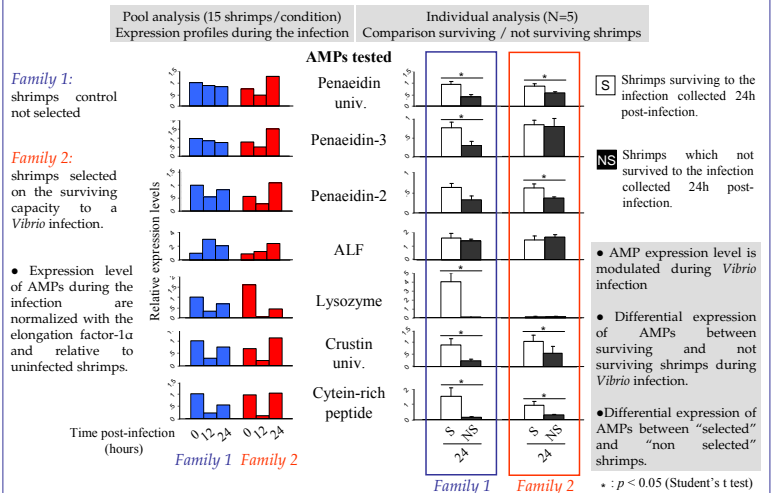
Weak proportion of genes encoding ribosomal protein compared to other EST programs in shrimps.

## 4- Design of universal AMPs gene primers (+ gene control) and test on hemocyte cDNA from different shrimp species.

Gene name	Sense/antisens primers	<i>L. stylirostris</i>	<i>F. chinensis</i>	<i>L. vannamei</i>	<i>P. monodon</i>	<i>P. japonicus</i>
Penaeidins	LsunivpenF1 / LsunivpenR1	Yes	Yes	Yes	Yes	N.D.
Crustins	CrusunivSTYF / CrusunivSTYR	Yes	Yes	No	Yes	No
Anti LPS factor	ALF34STYF / ALF34STYR	Yes	Yes	Yes	No	No
Lysozyme	Lyz-F2 / Lys-R	Yes	Yes	Yes	Yes	No
Transglutaminase	TGF / TGR	Yes	Yes	Yes	Yes	No
Cystein-rich peptide	CystF / CystR	Yes	N.D.	N.D.	N.D.	N.D.
Elongation factor-1 $\alpha$	EF1aFw / EF1aR	Yes	Yes	Yes	Yes	Yes

Yes: PCR product; No: no PCR product; N.D.: not determined.

## 5-Quantitative analyses by Real Time PCR of AMPs during a *Vibrio penaeicida* infection from hemocytes of two families of *L. stylirostris*.



Genomic programs in shrimps have permitted to isolated immune genes and functional categories of genes which could be implicated in the surviving capacity of shrimps.

AMPs have been characterized in the different shrimps species and quantitative method is developed to monitor their expression level.

AMP expression profiles differ between two families of shrimps and between surviving and not surviving shrimps.

AMP expression analyses could be further used to monitor shrimp health status and in selection programs.

J. de Lorgeril was supported by a doctoral funding from the New Caledonian Territories and the IFREMER.



IMMUNAQUA European Project (Contract n° ICA4-CT-2001-10023)

Antimicrobial Immune Effectors in Marine Invertebrates : Characterisation and Application for Disease in Aquaculture.

The goal of Immunaqua is to provide new tools and environment friendly technology for a sustainable development of aquaculture.

Project coordinator : Evelyne BACHÈRE

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