Program International Cooperation with Developing Countries, INCO-DC (European Commission, DG XII) IMMUNAQUA

Immunaqua - Antimicrobial immune effectors in marine invertebrates: characterization and application for disease control in aquaculture

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As world aquaculture is facing economical losses due to infectious diseases, collaborative work is currently developed in the frame of the EC INCO-DEV program, Immunaqua (ICA4-CT-2001-10023) **http://www.ifremer.fr/immunaqua**

- ***Isolate and characterize antimicrobial molecules, effectors of innate immunity.***
- ***Investigate their* in vitro *activities against wide range of microorganisms and pathogens,***
- ***Monitor their effect on larvae and live food for their potential beneficial and/or protective effect against infections.***

➡ ***To develop methodologies alternative to the use of antibiotics and chemicals for larvae treatments.***

Objectives

The objectives are to provide new antimicrobial agents and new approaches for the control of infectious diseases in mollusc and shrimp aquaculture.

- ***develop quantitative methods for analyses of immune effectors gene expression at population level applicable for health monitoring and/or selection***

➡ ***To develop Marker-Assisted Selection programmes using immune genes as potential markers for increased non-specific response to diseases or for health improvement.***

1 *Characterise antimicrobial peptides/proteins in molluscs and shrimps at a proteomic level.*

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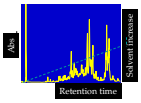
Antimicrobial peptides (AMPs) are purified according to chromatographic techniques from tissues and hemocytes. Then, amino-acid sequence and primary structure of the purified molecules are determined.

Reversed-phase HPLC



3 *Mass production of the antimicrobial effectors*

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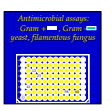
High amounts of pure molecules are necessary to determine their biological properties, range of *in vitro* antimicrobial activity and toxicity and *in vivo* assays on larvae and live food. Chemical synthesis and recombinant expression in yeast are used.



4 *Establish the in vitro activity and properties of AMPs*

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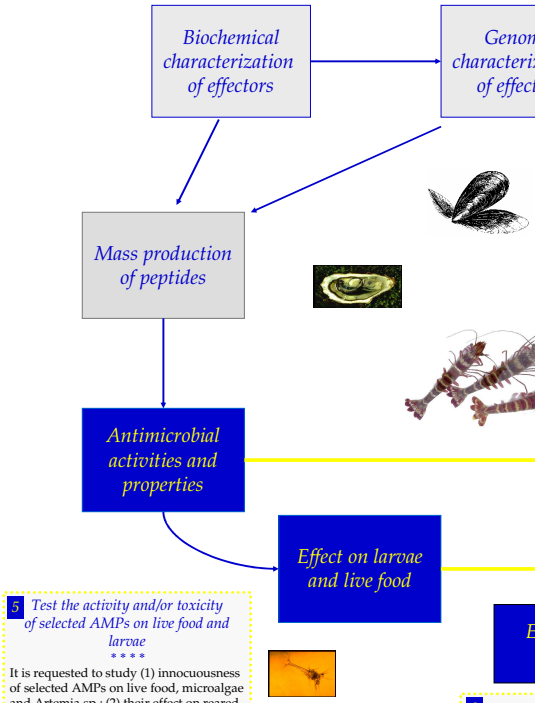
In vitro activity and properties of AMPs are studied against wide range of microorganisms and parasites to determine their minimum inhibitory concentration values.
These data are a prerequisite evaluation of molecules for potential use as therapeutic agents in aquaculture.



2 *Characterise anti-infectious effectors at the genomic level.*

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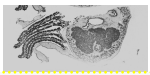
Genomic approaches, EST (Expressed Sequence Tag), mRNA Differential Display and SSH (Suppression Subtractive Hybridization) permit to identify immune and antimicrobial effectors orthologous to molecules or genes hitherto characterised in other species.



6 *Study spatio-temporal localisation and expression of AMP encoding genes*

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Analyses of AMP and immune gene expression are considered at animal population level under « normal » conditions then in response to infections. Quantitative methods are developed for further use in genetic selection or establishment of health monitoring.

7 *Investigate a genetic improvement approach in shrimps considering expression of AMP encoding genes.*

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The approach will consist first in checking the temporal stability of the level of effector gene expression at population level, and in the analyse of the genetic diversity of different populations. The response to selection in terms of gene expression and resistance to infection are assessed to establish an improved programme scheme or a Marker-Assisted Selection method in shrimps.

5 *Test the activity and/or toxicity of selected AMPs on live food and larvae*

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It is requested to study (1) innocuousness of selected AMPs on live food, microalgae and Artemia sp.; (2) their effect on reared larvae of fish used as running model and of shrimps and molluscs; (3) their protective efficiency against larva infection

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It is expected to transfer results to potentiel end-users and producers.
The project should contribute to generate environment friendly technologies for sustainable aquaculture activities.