"Anti-viral innate immunity in cultured aquatic species"

Contract number: ***QLK2-CT-2002-O1691***
Project acronym: ***AVINSI***

QUALITY OF LIFE AND MANAGEMENT OF LIVING RESOURCES PROGRAMME (1998 - 2002)
QoL action line: ***Key Action 2 - Control of infectious diseases***

PROJECT IDENTIFICATION

Contract number: *QLK2-CT-2002-O1691*
Title of the project: *"Anti-viral innate immunity in cultured aquatic species"*
Acronym of the project: *AVINSI*
Type of contract: *RTD Project*
QoL action line: *Key Action 2 - Control of infectious diseases*
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Project co-ordinator: Dr T. Renault - IREMER - Laboratiore de Génétique et Pathologie - 17390 La Tremblade - France - Telephone: +33 5 46 76 26 49 - Telefax: +33 5 46 76 26 11 - E-mail: trenault@ifremer.fr
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List of participants: ***Coordinator:*** Dr Tristan Renault (Participant no. 1) - IFREMER - DRV/RA Laboratoire de Génétique et Pathologie - 17390 La Tremblade - France - Phone: +33 5 46 36 98 36 - Fax: +33 5 46 36 37 51 - Email: trenault@ifremer.fr ***Participant no. 2:*** Dr.Abdenour Benmansour (Contractor) - Institut National de la Recherche Agronomique (INRA) - Unité de Virologie et Immunologie Moléculaires - F 78352 Jouy en Josas - France - Phone: +33 1 34 65 25 85 - Fax: +33 1 34 65 25 91 - Email: abdenour@jouy.inra.fr ***Participant no. 3:*** Pr Nathalie Bourgougnon (Contractor) - Université de Bretagne Sud (UBS) - Laboratoire de Biologie et Chimie Moléculaires - Tohannic - B. P. 573 Vannes - France - Phone: +33 2 97 68 26 51 - Fax: +33 2 97 68 16 39 - Email: Email: nathalie.Bourgougnon@univ-ubs.fr ***Participant no. 4/5/6:*** Dr Jean-René Bonami (Contractor) - CNRS/Université Montpellier 2 - 2 place Eugène Bataillon - F 34095 Montpellier cedex 5 - France - Phone: +33 4 67 14 47 10 - Fax: +33 4 67 14 46 22 - Email: bonami@crit.univ-montp2.fr ***Participant no. 7:*** Dr Andrew J. Davison (Contractor) - MRC Virology Unit - Institute of Virology - Church Street - Glasgow G11 5JR - United Kingdom - Phone: +44 141 330 6263 - Fax: +44 141 337 2236 - Email: a.davison@vir.gla.ac.uk ***Participant no. 8:*** Dr Beatriz Novoa (Contractor) - Instituto de Investigaciones Marinas (CSIC) - Eduardo Cabello, 6 - 36208 Vigo - Spain - Phone: +34 986 231930 - Fax: +34 986 292762 - Email: virus@iim.csic.es ***Participant no. 9:*** Dr Peter Dixon (Contactor) - CEFAS Weymouth Laboratory Virology Group Weymouth - Dorset, DT4 8UB - United Kingdom - Phone: +44 1305 206642 - Fax: +44 1305 206638 - Email : P.F.DIXON@cefas.co.uk

SCHEMATIC DESCRIPTION OF THE PROJECT

OBJECTIVES

The main objective of the project is to provide **knowledge of anti-viral innate immunity in cultured aquatic species** in order to **develop new approaches for the control of viral infections.**

These infections are among the most destructive diseases that affect vertebrate and invertebrate species in aquaculture. Despite the impact that viral diseases have on aquatic organisms, we know relatively little about what farmers can do to prevent and treat viral infections and how fish and shellfish fight viral diseases. Difficulties for control of viral infections in aquaculture come mainly from the absence of commercial vaccines and from the absence of specific therapeutic agents. In the long term, alternative treatments using anti-viral drugs may be developed and the most effective way for sustainable aquaculture production will certainly rely on the production of selected animals for disease resistance.

In this context, **anti-viral non-specific defence mechanisms (innate immunity)** are important because they constitute the first line of defence in vertebrates, and the only one in invertebrates. Therefore, innate immunity will be investigated in fish, molluscs and crustaceans. Through this project, we hope to identify conserved mechanisms and pathways of the innate immunity. In turn, this will be of benefit to the design of more potent vaccines in fish and anti-viral therapeutic agents, and to the identification of new targets for preventive actions in different cultured aquatic species.

The project has the following specific objectives:

- isolate and characterise at biochemical level anti-viral substances from bivalves, crustaceans and fish ;
- identify and characterise new genes induced by viral infections in fish, crustaceans and bivalves ;
- identify and characterise inhibitor of apoptosis proteins (IAPs) and study their expression during viral infections in bivalves, crustaceans and fish ;
- investigate the *in vitro* properties and anti-viral activities of selected molecules
- investigate the *in vivo* anti-viral activities of selected molecules and their expression during viral infections.

The expected achievements of this research project are:

(i) to characterise new genes and molecules involved in anti-viral innate immunity in bivalves, crustaceans and fish ;

(ii) to compile the functional data obtained in this project with genetic information already available through ongoing genomic projects in vertebrate and/or invertebrate species ;

(iii) to assess the efficiency of candidate genes and molecules in the control of viral diseases of aquatic species.

EXPERIMENTAL APPROACH AND WORKING METHOD

The project propose to use new approaches to increase knowledge of the molecular basis of innate immunity in cultured aquatic species in order to develop new tools for the control of viral diseases in aquaculture. The innate immune responses of fish, molluscs and crustaceans remains a vast domain to be explored and is very likely to present potential applications: (i) by the use of defined molecules as therapeutic agents, (ii) by the use of encoding genes as selection markers for improving resistance to infections and (iii) by the development of new vaccines in fish. The originality of the project is that characterisation of innate immune responses is carried out using different viral disease models including fish, bivalve and crustacean diseases (oyster herpesvirus 1/Pacific oyster (*Crassostrea gigas)*, White Spot Syndrome virus (WSSV)/crabs (*Liocarcinus puber* and *Carcinus mediterraneus*), nodavirus/sea bream (*Sparus aurata*), koi herpesvirus (KHV)/common and ghost carp (*Cyprinus carpio*) and Viral Haemorrhagic Septiceamia virus (VHSV)/rainbow trout (*Oncorhynchus mykiss*). This allows interchange of knowledge and methodological progress in the field of non-specific immunity. Given the diversity of viral infections in aquatic species and the lack of specific immune responses in invertebrates, three approaches to define anti-viral innate immunity are developed in the project: biochemical characterisation of anti-viral effectors, molecular characterisation of cellular genes induced by viral infections and searching for IAP genes in the genomes of aquatic species. Five animal models are studied in parallel. The selected models involving specific pathogens are relatively well documented. Pathogen characterisation and pathogenesis have already been studied. The background on the selected models may help in the analysis of anti-viral innate immunity in cultured aquatic species. Five viral infection models are used including diseases due to enveloped or non-enveloped viruses and to RNA or DNA viruses. **Biochemical characterisation of anti-viral molecules using tissue extracts from aquatic species** has previously been reported. However, these reports concern mainly plants, algae and unicellular organisms. We propose herein to apply already developed methodology to bivalve, crustacean and fish extracts in order to identify and characterise anti-viral compounds. For this purpose, experimentally infected and control animals are used. **New virus-induced genes in non-classical models such as fish and bivalves** are of interest, in studying the virus-host relationship in lower vertebrates and in invertebrates and in obtaining insights into the mechanisms involved in immune responses to viral stimuli. Nothing is known about this type of gene in invertebrates. It is important to note that function characterisation of conserved virus-induced genes may also add to discoveries in mammals. This is consistent with the current comparative approach to functional genomics and postgenomics, from bacteria to human. **Identifying inhibitor of apoptosis proteins (IAPs) in bivalves, crustaceans and fish** is innovative. The main goal of this work is to demonstrate the conservation of IAPs in invertebrates and lower vertebrates.

ACHIEVEMENTS AND RESULTS

Detection and biochemical characterisation of anti-viral molecules. Despite a cytotoxicity detected, a putative antiviral substance has been detected in Pacific oyster haemolymph. No antiviral activity was detected in crustacean (crab and shrimp) heamolymph as in sea bream and carp sera and tissue extracts. High cytotoxicity was observed in these samples.

Identification of virus-induced genes. RNAs have been extracted from sea bream macrophages, carp leucocytes from head kidney and oyster hemocytes after virus contact. The expected achievement of this work package is to characterize new genes involved in anti-viral innate immunity in different aquatic species**.** The Suppression Subtractive Hybridization technique (SSH) is used to carry out this step and promissing candidate genes have been obtained in the different models.

Molecular characterisation of cellular IAPs. Attemps to detect survin RNAs were unsuccessful in two tested fish species (sea bream and common carp). Research targeting genes related to mammalian IAP-1 and IAP-2 have been successful: a single IAP gene and two dffferent genes were identified in sea bream and common carp, respectively.