

P-024.**Hydrogen peroxide treatment modulates the immune and detoxification responses in the sea louse *Caligus rogercresseyi***

C. Sáez-Vera^{*}, G. Núñez-Acuña^{*}, V. Valenzuela-Muñoz,
C. Gallardo-Escárate[#].

Laboratory of Biotechnology and Aquatic Genomics, Interdisciplinary Center for Aquaculture Research, Universidad de Concepción, Concepción, Chile

Abstract

The sea louse *Caligus rogercresseyi* is the main ectoparasite affecting Chilean salmon industry. Hydrogen peroxide (H₂O₂) has been applied as a chemical treatment to control sea lice infestations. The mode of action is based on forming bubbles in the copepod hemolymph inducing a mechanical paralysis detaching the parasite from the host. However, there are critical mechanisms underlying the defense responses of the ectoparasite to this chemical, but are poorly understood. This study is aimed to describe the molecular responses of *C. rogercresseyi* to H₂O₂ by gene expression analyses of selected candidate genes on parasites exposed to the chemical. Bioassays were conducted using 6 concentrations of H₂O₂ (180, 360, 540, 744, 900, 1080 ppm) plus one control group. Median-effective concentration (EC₅₀) and median-effective time (ET₅₀) values were obtained. This evaluation consisted in prolonged exposure of sea lice to H₂O₂ and counting affected animals during different intervals of time. Affected parasites at each examination time were collected for gene expression analyses. RT-qPCR was conducted to evaluate the expression of several immune-related genes, and others associated with the antioxidant system. Increased expression levels of genes related to defense response were obtained, such as genes of toll-like receptors and immune deficiency pathways. Genes of the antioxidant system associated as catalase and superoxide dismutase were also modulated. Here, novel immune and detoxification responses during exposure to hydrogen peroxide are evidenced. This study contributes to a better understanding of the innate immune response in sea louse and also provide new insights into the mechanism of action of hydrogen peroxide as a chemical treatment.

keywords: Hydrogen peroxide, *Caligus rogercresseyi*, immune response, gene expression, bioassays.

Corresponding author.

E-mail address: crisgallardo@udec.cl (C. Gallardo-Escárate).

* These authors have contributed equally to this work.

P-025.**Selection of protein candidates for vaccine development against *Piscirickettsia salmonis* using a reverse vaccinology approach**

P. Sánchez^{1,2}, A. Romero^{2,3,4}, C. Oliver^{3,4,#}.

¹ Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Isla Teja, Valdivia, Chile

² Interdisciplinary Center for Aquaculture Research (INCAR), Concepción, Chile

³ Instituto de Patología Animal, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Isla Teja, Valdivia, Chile

⁴ Immunology and Stress of Aquatic Organisms Laboratory, Facultad de Ciencias Veterinarias, Universidad Austral, Campus Isla Teja, Valdivia, Chile

Abstract

Piscirickettsia salmonis is an intracellular γ -proteobacteria, belonging to the order Thiotricales and the etiological agent of Piscirickettsiosis, which causes massive economic losses in the Chilean salmon industry and

generates an extremely high consumption of antibiotics during the production cycle. Despite experimental evidence for effective subunit vaccine formulations, currently available commercial vaccines for *P. salmonis* are mostly whole cell vaccines and vaccine combinations, which have been proven to be ineffective in generating long-term protection, thus the importance of developing new therapeutic tools.

Reverse vaccinology is the name given to a novel approach to vaccine/adjuvant design, which takes full advantage of the genome and protein information available for pathogens. The main goal in this method is to identify antigenic regions or epitopes in proteins that can stimulate different components of the immune system, using several *in silico* tools and immunological databases. This approach has been applied to several bacteria, such as *A. baumannii*, *Campylobacter*, *M. tuberculosis*, *S. pneumoniae*, *V. anguillarum*, among others. To our knowledge, no study has used this methodology to determine potential candidates for *P. salmonis* vaccine formulations. In this study, two datasets were used, the complete set of non-clustered protein sequences of the LF-89^T strain and a clustered *P. salmonis* 'pangenome' set of protein sequences, and their outputs were combined. As a result of our subtractive workflow, 12 potential proteins were identified, and annotated as hypothetical porins (mainly from the LbtU-like family), outer membrane proteins (OmpW), proteins belonging to secretion systems (TolC), LPS-related proteins and other lipoproteins. In addition, several T-cell and B-cell epitopes were determined for these proteins. Future work should focus on the validation of the epitopes obtained using structural *in vitro* tools for them to be applied in a hypothetical vaccine design for *P. salmonis*.

keywords: Reverse vaccinology, *Piscirickettsia salmonis*, Structural vaccinology, B-cell epitopes, T-cell epitopes

Corresponding author.

E-mail address: cristianoliver7@gmail.com (C. Oliver).

P-026.**Recombinant immunotherapy against *Ichthyophthirius multifiliis* in *Oncorhynchus mykiss***

Dagoberto Sepúlveda^{1,#}, Theodore G. Clark², Niels Lorenzen¹.

¹ National Institute of Aquatic Resources, Technical University of Denmark, Denmark

² Department of Microbiology and Immunology, Cornell University, Ithaca, New York, USA

Abstract

Ichthyophthirius multifiliis (Ich) is a fish protozoan parasite and the causative agent of white spot disease. During the Ich life cycle, fish epidermis and gill epithelia are disrupted, which could increase the susceptibility to a secondary infection, and even could cause mortalities when the fish are under a severe infection. Therefore, it is important to develop an effective measure to control this parasitic infection in farmed fish. Currently, the Ich control relies on treating the water containing infective Ich with chemical compounds that have a negative impact on both human health and the environment. Vaccines have raised as an alternative strategy to control Ich infection in farmed fish. Early observations have shown that rainbow trout were able to acquire protection after either a non-lethal infection or an intraperitoneal injection of live parasites. Antibodies seemed to play an essential role in the defense mechanism since fish were protected after being passively immunized with immobilizing monoclonal antibodies against Ich. Although several vaccines against this parasite have been evaluated, currently there is no commercial vaccine available. This work aims to develop an immunotherapy based on a recombinant Ich-immobilizing single chain variable fragment (Ich-scFv), which is a fusion of the variable regions of the heavy and light chains of an Ich immobilizing monoclonal antibody, connected by a short linker peptide. The steps to

reach this objective include: selecting a hybridoma clone that produces immobilizing monoclonal antibody against Ich, sequencing the variable regions of the IgG gene of the hybridoma clone, designing *in silico* an Ich-svFv, and evaluating *in vivo* the effectiveness of different delivery systems of the Ich-svFv. In this conference, preliminary results will be presented. This work was supported by the EU H2020 research and innovation programme ParaFishControl (634429).

keywords: *Ichthyophthirius multifiliis*, Ich, rainbow trout, single chain antibody, scFv.

Corresponding author.

E-mail address: dsep@aqu.dtu.dk (D. Sepúlveda).

P-027.

Effect of salinity on immunological responses in striped catfish (*Pangasianodon hypophthalmus*) in larvae and juvenile stages

Dang Quang Hieu^{1,2,#}, Bui Thi Bich Hang², Do Thi Thanh Huong², Nguyen Thanh Phuong², Frédéric Farnir³, Patrick Kestemont¹.

¹ Research Unit in Environmental and Evolutionary Biology, Institute of Life, Earth & Environment (LEE), University of Namur, Namur, Belgium

² College of Aquaculture and Fisheries, Can Tho University, Can Tho city, Vietnam

³ Department of Animal Production, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium

Abstract

Effect of salinity in aquatic animals has been investigated in many species with special attention to osmoregulation capacities but how it affects the immunomodulatory responses in fish remains largely unknown. In this study, striped catfish larvae (10-day post-hatching) and juveniles (20–25 g) were chronically exposed to different salinities (namely 0, 5, 10, 15 and 20 ppt during 10 and 20 days respectively). Then the larvae were heat shocked at 39°C while the juveniles were submitted to a challenge test with a virulent bacteria *Edwardsiella ictaluri*. The results showed that lysozyme activity in larval fish increased proportionally to the increase of salinity, with significant differences in groups submitted to 15 and 20 ppt (28.3 and 30.6 U/mg protein respectively). Additionally, lethal time LT50 after heat shock were 5 times higher in fish exposed to 5 and 10 ppt treatments in comparison with 0 and 15 ppt treatments ($p < 0.05$). In juveniles, hyperosmotic exposure led to a significant decrease of hematocrit during both salinity exposure period and bacterial challenge, with the highest hematocrit values found in 0 ppt treatment and the lowest in 20 ppt treatment ($p < 0.05$). On the other hand, blood osmolality and ion concentrations significantly increased with salinity exposure, confirming the low capacity of striped catfish to osmoregulate. The lysozyme activity reached the highest values in fish reared at 10 ppt after bacterial challenge ($p < 0.05$), without significant differences with fish at 15 ppt. Histopathological observations indicated some alterations of gills (e.g. reduction of interlamellar cell mass, increase of epithelial cell thickness) and head kidney (e.g. edema between melanomacrophages, reduction of hematopoietic tissue) after salinity exposure. Complement activity did not show any significant difference between salinity treatment, neither in larvae nor in juveniles. These results support the hypothesis that hyperosmotic stress may affect the striped catfish larvae and juveniles, with a higher sensitivity observed at the larval stage.

keywords: Hyperosmotic exposure, striped catfish, immunity, histopathology, salinity.

Corresponding author.

E-mail address: quanghiedang.87@gmail.com (D.Q. Hieu).

P-028.

Recombinant VP1 and VP2 of infectious pancreatic necrosis virus trigger lymphoid cell changes and induced cytokine transcriptional expression in rainbow trout head kidney

Wong Valentina¹, Trujillo Agustin¹, Barraza Felipe¹, Ruiz-Higgs Daniela¹, Vergara Claudio¹, M. Sandino Ana², Wang Tiehui³, Maisey Kevin⁴, J. Secombes Christopher³, Imarai Monica^{1,#}.

¹ Laboratorio de Inmunología, Centro de Biotecnología Acuicola, Departamento de Biología, Química y Biología, Universidad de Santiago de Chile, Santiago

² Laboratorio de Virología, Centro de Biotecnología Acuicola, Departamento de Biología, Química y Biología, Universidad de Santiago de Chile, Santiago, CL

³ Scottish Fish Immunology Research Centre, University of Aberdeen, Aberdeen, GB

⁴ Laboratorio de Inmunología Comparada, Centro de Biotecnología Acuicola, Departamento de Biología, Química y Biología, Universidad de Santiago de Chile, Santiago, CL

Abstract

Infectious pancreatic necrosis (IPN) is a disease that affects salmonid fish producing mortality and economic losses in the salmon industry. The agent responsible for this disease is the IPN virus (IPNV), which has a double-stranded RNA genome of two segments. The segment A encodes proteins VP2, VP3, VP4 and VP5 and the segment B encodes the VP1 protein, which is an RNA-dependent RNA polymerase.

In this work, we assessed the effects of VP1 and VP2 recombinant proteins on the lymphoid cell populations and cytokine expression in the head kidney of the rainbow trout (*Oncorhynchus mykiss*). Fish were i.p immunized with VP1 or VP2 and the IgM+, CD3+ and CD4+ lymphoid cells of the anterior kidney were analyzed by flow cytometry. The results showed that the percentage of IgM+ B cells did not change in the kidney of immunized fish whilst significant variations of CD3+ and CD4+ lymphoid cells were observed after VP1 and VP2 immunization, respectively. In addition, transcripts of cytokines related to the lymphoid immune response, i.e., IFN γ , IL-4/13A, IL-4/13B1, IL-4/13B2, IL-2, IL-22, IL-10 and TGF β were quantified by qRT-PCR. The results showed significant increase of the cytokine transcripts and distinct expression profile in VP1 and VP2 immunized fish. In summary, recombinant VP1 and VP2 trigger distinct immune responses in the head kidney of rainbow trout, which can be essential to develop fish immunity against IPNV.

keywords: Rainbow trout, Immune response, IPNV, lymphoid cells, cytokines.

Corresponding author.

E-mail address: monica.imarai@usach.cl (I. Monica).

P-029.

Immune responses of European seabass (*Dicentrarchus labrax*) juveniles to chronic inflammation

D. Peixoto^{1,2,#}, M. Machado^{1,2,3,4}, B. Costas^{1,2}.

¹ CIIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental, Matosinhos, Portugal

² ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

³ i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

⁴ IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal