P-024.

Hydrogen peroxide treatment modulates the immune and detoxification responses in the sea louse *Caligus rogercresseyi*

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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.

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P-025.

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

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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. baumanii*, *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 substractive 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

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P-026.

Recombinant immunotherapy against *Ichthyophthirius multifiliis* in *Oncorhynchus mykiss*

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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