P-016.

Filifolinone, a natural compound with adjuvant effect for optimization of a commercial vaccine for IPNV

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Abstract

Filifolinone is a natural compound isolated from resin of *Heliotropium huascoense*, that increases the expression level of pro-inflammatory and anti-inflammatory cytokines in kidney cells of salmon. Given that cytokines are generated in response to the presence of pathogens, we have studied their potential adjuvant effect for the optimization of commercial vaccines against IPNV. In Chile, infectious pancreatic necrosis (IPN) is an endemic, prevalent, highly contagious and economically important disease, since it is widely distributed in Chilean salmon farms, causing heavy economic losses, in freshwater crops such as sea crops. The negative economic impact is directly related to the increase in losses due to mortalities, elimination of carrier breeders, progeny from carrier breeders, decrease in growth rates and increase in control measures. Among the control measures implemented, is the application of biosecurity measures, egg disinfection, individual incubation systems and application of vaccines. Currently, there is a wide variety of vaccines, however none of them has been efficient enough to control or eradicate the disease. Therefore, we evaluated the adjuvant effect of Filifolinone searching new strategies for control. In addition to mortality, its effectiveness was determined in terms of the presence of erratic swimming, anorexia and exophthalmia. Besides, we evaluated the presence of total IgM antibodies and the viral load in the anterior kidney in terms of VP2 gene expression.

keywords: Adjuvant activity, Filifolinone, IPNV,vaccine, antiviral activity.

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P-017.

Cell surface markers for the identification of subpopulations of splenocytes from salmonids

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Abstract

The phylogenetic development of the immune system shows that the adaptive response appears in fish. However the structure of this response and the antigen presentation process that coordinates the transit between the innate and adaptive immunity has been poorly characterized in teleost, due to the low number of phenotypic tools available for aquatic organisms. The spleen is a secondary lymphoid organ in fish and the present study aimed to identify subpopulations of splenocytes in salmonids that express molecules associated with the antigen presentation (CD83, CD86 and MHC II). For this, splenocytes were co-cultivated, activated or not by interferon gamma, with lymphocytes. The results indicated the existence of cells expressing surface molecules capable of presenting antigens to T lymphocytes. These cells, derived from splenocytes, increase the expression of surface molecules induced by interferon gamma and decrease their phagocytic capacity over time. Activation of these cell types *in vitro* shows that there is a tendency to activate populations of T cells (previously isolated with anti-CD4.1 and anti-IgM antibodies) to T regulatory lymphocytes. Further research is necessary to validate if the events here described also occur *in vivo*, to understand if the link between innate and adaptive immunity in fish has an inhibitory phase component, which could explain the absence of memory or the low protection capacity of vaccines used in aquaculture. This work was support by the Program for Sanitary Management in Aquaculture of the Ministry of Economy, Development and Tourism of Chile (FIE-2015-V014 201708070149) and by the Norwegian Research Council (281052). BML is a fellow of Advanced Human Capital Formation of CONICYT, Chile (21151176).

keywords: *Salmo salar*, *Oncorhynchus mykiss*, primary culture, flow cytometry, qPCR

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P-018.

Betanodavirus infection modulates European sea bass immune system at short-term

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Abstract

Viral diseases are responsible for high mortality rates of several marine species and the associated economic losses on actual aquaculture. Special attention has been paid to *Nervous Necrosis Virus* (NNV), which affects many fish species and causes viral encephalopathy and retinopathy disease. Particularly, the European sea bass (*Dicentrarchus labrax*) is a very susceptible fish species to NNV and suffer mortalities up to 100% at larvae and juveniles stages. In this work, we aimed to evaluate the transcription of genes related to the cell-mediated cytotoxic activity (CMC), which is the main cellular immune response triggered by this virus, in different tissues of European sea bass. For this purpose, the virus was intra-muscularly injected to each individual and the effects upon NNV infection were evaluated in head-kidney and brain after 1, 7 and 15 days post-infection. Several immune-related genes were studied by real time PCR including those that code for interleukin (IL)−27 subunit beta-like (EBI3), the lymphocyte antigen 6-like secreted (SLURP1L), IL-12 beta subunit (IL12BA), the cytotoxic and regulatory T cell molecule (CRTAM) and the co-stimulatory receptor for the activation of naive T cells (CD28). In general, the expression of these genes were up-regulated after infection with NNV. Results of this study evidence that NNV modulate fish immune system at short-term (1 day post-infection). These findings suggest that CMC has an important role on immune response against virus-infected cells.

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keywords: Nervous necrosis virus (NNV), immune system, *Dicentrarchus labrax*, PCR

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P-019.

CpG oligodeoxynucleotides modulate innate and adaptive functions of IGM+ B cells in rainbow trout

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Abstract
Oligodeoxynucleotides (ODN) containing unmethylated CpG motifs have been widely postulated as vaccine adjuvants both in mammals and teleost fish. However, to date, the effects that CpGs provoke on cells of the adaptive immune system remain mostly unexplored in fish. Given that rainbow trout (Oncorhynchus mykiss) IgM+ B cells from spleen and blood transcribe high levels of toll like receptor 9 (TLR9), the receptor responsible for CpG detection, in the current work, we have investigated the effects of CpGs on both spleen and blood IgM+ B cells from this species. CpGs were shown to exert strong proliferative effects on both spleen and blood IgM+ B cells, also increasing their survival. The fact that CpGs increase the size of IgM+ B cells, reduce the expression of surface IgM and IgD and upregulate the number of IgM-secreting cells strongly suggest that IgM+ B cells differentiate to plasmablasts / plasma cells in response to CpG stimulation. Additionally, CpGs were
shown to modulate the antigen presenting capacities of trout IgM+ B cells through an
increased surface MHC II expression and transcriptional up-regulation of co-stimulatory molecules, although in this case, significant differences were observed between the effects exerted on spleen and blood cells. Similarly, differences were observed between spleen and blood IgM+ B cells when CpG stimulation was combined with B cell receptor (BCR) crosslinking. Finally, CpGs were also shown to affect innate functions retained by teleost IgM+ B cells such as their phagocytic capacity. These results demonstrate that CpGs regulate many adaptive and innate functions of teleost B cells, supporting their inclusion as adjuvants in novel vaccine formulations.
keywords: Rainbow trout, CpG, IgM, B cells, BCR.

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P-020.

Insights into the functions of piscidins in the European sea bass (*Dicentrarchus labrax*)

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ABTRACT
Antimicrobial peptides (AMPs) are one of the host's first line of defenses against a wide range of infectious agents. Apart from the antimicrobial activity, AMPs are known to influence other biological processes, such as immunomodulation and iron metabolism. Fish present a specific group of AMPs, the piscidins. These peptides have been characterized in several fish species, acting on multiple pathogens, being also altered when fish are subjected to an infection. Furthermore, several studies have shown the potential of using synthetic peptides to promote fish survival upon infection. However, in the European sea bass (*Dicentrarchus labrax*), a commercially important fish produced in aquaculture, only hepcidin has been extensively characterized. Thus, a comprehensive study on the functions of other AMPs, particularly piscidins, is missing. Here, we identified and characterized the different piscidins of sea bass. We evaluated the antimicrobial activity of piscidins against several bacteria known to cause massive mortalities in cultured marine fish. Furthermore, the expression of the different genes belonging to the piscidin family was assessed under distinct experimental conditions, particularly infection and iron modulation, at pre-determined time points. Our results show a diverse piscidin antimicrobial activity *in vitro* against the different bacteria, indicating that these AMPs have a direct role against these pathogens, depending on the pathogen and piscidin peptide. Our data also shows a piscidin response after infection, suggesting that piscidins are involved in the response against infection. Furthermore, preliminary data shows that piscidins also respond to iron modulation, indicating that these AMPs may have other yet undisclosed functions asides antimicrobial activity, such as a role in iron metabolism. Our findings imply that piscidins might be a complementary or alternative way that fish possess to deal with this essential element, apart from the major iron regulator hepcidin. It is known that iron is also essential for bacterial progression during infection. Thus, iron is in a continuous regulation to be available for body processes, being also modulated to ensure that is inaccessible to pathogenic microorganisms. Further work is necessary to fully understand the role and mechanisms of action of piscidins under the context of immune response and iron metabolism regulation, and to possibly uncover a novel function for these particular peptides in fish.
keywords: Antimicrobial peptides; Piscidins; Sea bass (*Dicentrarchus labrax*); Infection; Iron metabolism

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P-021.

Effects of 17α-ethynylestradiol (EE2) on the immune system of Juvenile European sea bass with a special focus on B and T cells

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Abstract
Synthetic compounds are known for their persistence and bioaccumulation in the environment. 17α-ethynylestradiol (EE2), a synthetic derivative of the natural hormone oestradiol, is present in human contraceptive pills, but also in livestock and aquaculture activity. Therefore, municipal wastewaters are one of the most important sources of this compound in the aquatic environment. Because EE2 induces oestrogenic effects even at trace level concentration, it has potent endocrine disrupting