infected with both *P. salmonis* isolates showed mhc1-mhc2, cd4-cd8b and igm overexpression, suggesting that P. salmonis promotes a CD4+ T- and CD8+ T cell response and a humoral immune response. The vaccinated-fish exhibited mhc1, mhc2 and cd4 overexpression but a significant downregulation of cd8b and igm, suggesting that the vaccine supported the CD4+ T-cell response but did not induce an immune response mediated by CD8+ T cells or a humoral response.

keywords: *Piscirickettsia salmonis*, immune response, vaccination.

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P-049.

Macrophage-like cells from primary cell culture of Atlantic salmon are capable to phagocyte *Piscirickettsia salmonis*: Apoptosis of phagocytes also occurs during infection

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Abstract
Innate immune mechanisms of response in fish are essential as a first defense mechanism to fight against pathogens. The phagocytosis is one key process able to eliminate the pathogens and stop the infection. Once the pathogen is internalized, the phagocyte should destroy the pathogen by fusion of the phagosome with lysosomes. *Piscirickettsia salmonis* is a Gram-negative intracellular facultative bacterium, pleomorphic (coccoid predominant grouped in pairs) and size ranging among 0.5 to 1.5 um. This pathogen is the etiological agent of Piscirickettsiosis, the main infectious disease causing around 70% of mortalities in Atlantic salmon in Chilean farming, according the last sanitary report from Sernapesca (2017). Until now, there are just a few articles that poorly described the infection induced by this bacterium. In this study, we proposed that macrophage-like cells from Head Kidney of Atlantic salmon are capable to phagocytose P. salmonis as a mechanism of defense. To test this, we labelled *P. salmonis* using FITC and after testing viability of the bacteria, we used them to inoculate primary cell cultures of Head Kidney obtained from Atlantic salmon. The cells were infected with bacteria at MOI 10. Using confocal microscopy, we observed that the adherent cells internalized the pathogen and later underwent apoptosis. A video of six hours infection was made, and the results showed that the bacteria and cells attachment occur as quickly as five minutes post inoculation. The internalization can be observed for thirty minutes to four hours post infection while apoptotic cells were observed for thirty minutes post infection until the end of the experiment. In addition, other cells could be seen moving and looking for infected cells to destroy them and eliminate the threat. This was observed from the beginning of the experiment until the end of infection. Thus, macrophage-like cells from Head Kidney of Atlantic salmon are capable to phagocyte *P. salmonis*. In addition, the infected macrophage-like cells experienced apoptosis probably to diminish pathogen viability and avoid the spread of the microorganism. How apoptosis is induced is not very clear yet, but further analysis must be performed to solve this important question.

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keywords: Atlantic salmon, macrophages, Piscirickettsia salmonis, apoptosis, phagocytosis.

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P-050.

Transcriptional profile and serological studies of the European sea bass immune response against betanodavirus infections

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Abstract
European sea bass (*Dicentrarchus labrax*) culture is highly affected by outbreaks of viral nervous necrosis disease, provoked by the nervous necrosis virus (NNV). This virus displays a single-stranded, positive-sense RNA genome, which is composed of two segments, RNA1, encoding the viral polymerase; and RNA2, encoding the capsid protein. Only two genotypes of NNV have been detected in sea bass to date, although showing very different levels of virulence. Specifically, RGNNV is highly virulent to sea bass, causing high mortality, whereas SJNNV replicates in sea bass brain without causing clinical signs. In the present work, the comparative analysis of the European sea bass immune response against isolates belonging to both viral genotypes has been performed. The immune response has been evaluated in brain and head kidney of experimentally infected sea bass by relative real-time PCR of genes involved in the type I interferon (IFN I) system (ifn-I, mxA, isg15, isg12), and genes related to inflammatory (il-8, tnf-α, il-10, tgf-β) and adaptive responses (tr-γ, mhc-β). Ribosomal 18S RNA was used as reference endogenous gene. In addition, a serological study, consisting of the ELISA quantification of IgM in sera, was also performed. The transcription analyses of the innate defence-related genes point out the importance of this mechanism to control betanodavirus infections. The results obtained showed a strong induction of ifn-I, mxA, isg15 and isg12 in both organs analysed, especially in response to the virus highly virulent to sea bass (RGNNV). However, the response was quicker in head kidney of SJNNVinoculated sea bass, suggesting that this genotype induces a more rapid systemic response. Regarding the inflammatory response, RGNNV triggered a strong transcription of proinflammatory genes in brain, which provides evidences about the importance of the inflammatory process in betanodavirus infection. Thus, the massive inflammatory process may be responsible for the eventual damage in nervous tissues, which would lead to fish dead. Finally, the high values of tr-γ and mhc-β mRNA recorded in brain and the high IgM titer in sera, which was higher in SJNNV-inoculated fish, suggest that the adaptive response constitutes another important factor in the European sea bass immune response against betanodaviruses, both at systemic and at local level.

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P-051.

The nucleotide polymorphism of histone H2A and their functions of H2A variants in pathogen infection

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Abstract
Histones are well-known components of the nucleosome. Among core histones H2A, H2B, H3 and H4, the H2A family exhibits the greatest diversity including the largest number of various variants such as H2A.X, H2A.Z, MacroH2A and H2A.Bbd. In general, H2A variants differ mostly in their C-terminus, regarding both length and amino acid sequences. The importance of H2A variants in nucleosome stability and chromatin structure has been well established. Although a lot of progress was made regarding antibacterial peptides derived from the N-terminus of histone H2A in the past decade, the nucleotide polymorphism of H2A and their functions of H2A variants in pathogen infection are largely unknown. In the present study, we found that piscine H2A exhibited abundant nucleotide polymorphism. 15 H2A variants were cloned from zebrafish. Although only 1 to 2 amino acids differ, the similarities among these H2A variants are 90.1~99.5%. Strikingly, nucleotide polymorphism of H2A influenced the antibacterial and antiviral activities. Thus, our results provide insights into the functional differences of H2A variants in pathogen infection.
keywords: Histone H2a; nucleotide polymorphism; viral infection; bacterial infection

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P-052.

The effects of IFNΓ, IL-1B, and TNF-A on IFNΓ-IFNΓ R1/R2 pathway in macrophages from rainbow trout (*Oncorhynchus mykiss*)

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Abstract
In vertebrates, the cytokines: Interleukin-1β (IL-1β), Tumor Necrosis Factor-α (TNF-α) and Interferon-γ (IFNγ) are proinflammatory cytokines crucial for the inflammatory response. The cytokine IL-1β is important for inducing neutrophilia and imparting a signal required for optimal T and B cell function. The cytokine TNF-α is key for T and macrophage cell activation and has an important role in the induction of inflammatory mediators, such as nitric oxide and prostaglandins. While the IFNγ plays a central role controlling the host response to viral or bacterial infection, through the activation of the JAK/STAT pathway and the induction of iNOS in M1 macrophages. Studies described that IFNγ is capable of inhibits Il-1β and induce TNF-α in murine macrophages. Also, nitric oxide regulates IL-1β production in murine macrophages. In rainbow trout, the existence of IFNγ receptors (IFNγR1/R2) has been demonstrated, and Il-1β, TNF-α, IFNγ shows similar functions to their vertebrate's counterparts. However, there are no studies about the combinatory effect that different cytokines have on the IFNγ pathway and cytokine expression in rainbow trout. The aim of this work was to characterize at transcriptional level the IFNγ-IFNγ R1/R2 pathway, IL-1β and Interleukin-6 (IL-6) expression in trout macrophages. For this, a cell line of monocytes/macrophages from rainbow trout were stimulated with recombinant IFNγ/Il-1β and IFNγ/TNF-α during 4-6 and 12 hours. The results showed that IFNγR1 and TRIM8 were downregulated. While IFNγR2 has an oscillatory response and was not detected at 12 hours, also STAT1 was upregulated mainly at 12 hours for IFNγ/IL-1β treatment. The IFNγ/TNFα treatment showed an upregulation of STAT1 for all the hours, mainly at 4 hours, contrasting with the expression observed for the IFNγ/IL-1β. iNOS expression was downregulated in IFNγ/IL-1β at 12 hours. The evaluation of the cytokines showed downregulation of IL-1β at 6-12 hours and IL-6 at 4-6-12 hours for IFNγ/IL-1β experiment. However, in IFNγ/TNF-α just a downregulation of IL-6 was observed. An experiment with just IL-1β showed that IFNγR1 was upregulated at 12 hours. The results obtained suggest a different mechanism of regulation for IFNγ-IFNγ R1/R2 pathway and a difference in cytokine regulation, mainly with IL-6, a cytokine that was upregulated in mammals when IFNγ is used with IL-1β/TNF-α.
keywords: Cytokines - IFNγ - IL-1β - IFNγ-IFNγ R1/R2 pathway - Rainbow trout

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P-053.

Rainbow trout shape-shifted red blood cells

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Abstract
Rainbow trout Ficoll-purified red blood cells (RBCs) cultured *in vitro* undergo morphological changes, especially when exposed to stress, and derive into a cell stage that we have coined shape-shifted RBCs (shRBCs). We have characterized these shRBCs using transmission electron microscopy (TEM) micrographs, Wright-Giemsa staining, cell markers immunostaining, and transcriptomic and proteomic evaluation. shRBCs displayed reduced density of the cytoplasm, hemoglobin loss, decondensed chromatin in the nucleus. Also, shRBCs displayed striking expression of the B lymphocyte molecular marker IgM. shRBCs were transiently observed in heat-stressed rainbow trout bloodstream for three days. Functional network analysis of combined transcriptomic and proteomic studies resulted in the identification of proteins involved in pathways related to the regulation of cell morphogenesis involved in differentiation, cellular response to stress, and immune system process. In this regard, it has long been suggested that primitive nucleated erythroid cells in the bloodstream of mammals are more similar to nucleated red cells of fish, amphibians, and birds than the red cells of fetal and adult mammals. In addition, shRBCs increased interleukin 8 (IL8), interleukin 1 β; (IL1β), interferon ɣ (IFNɣ), and natural killer enhancing factor (NKEF) protein production in response to viral hemorrhagic septicemia virus (VHSV). Also, shRBCs conditioned medium triggered cytokine signaling in trout pronephros stroma (TPS-2) cell line. In conclusion, shRBCs may represent a novel cell stage that participates in roles related to immune response mediation, homeostasis, and the differentiation and development of blood cells.
keywords: Rainbow trout; shape-shifted red blood cells; VHSV; transcriptome; proteome

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