

(*Solea senegalensis*). A NNV reassortant (Ss160.03), combining genomic segments from red-spotted grouper nervous necrosis virus (RGNNV) and striped jack nervous necrosis virus (SJNNV) genotypes, has been previously isolated from Senegalese sole, being highly virulent to this fish species. The RNA-Seq technology has been used in a previous study to comparatively analyse Senegalese sole transcriptomes in two organs (head kidney and eye/brain) after infection with two NNV virus with different levels of virulence to that fish species, a highly virulent reassortant isolate (wSs160.03) and a less virulent mutant reassortant obtained by reverse genetics (rSs160.03247+270). To validate previous RNA-Seq results, a 112-essay OpenArray® platform (ThermoFisher) has been designed. This platform included 89 genes chosen according to transcriptomic changes observed by RNA-Seq (covering PRRs, type I IFN response, signal transduction, inflammation, virus responsive genes, and apoptosis), 17 genes selected based on their previously described relation with the immune response against fish viral infections, and 6 control genes (including 3 endogenous genes and 3 viral genes). A total of 63.25% differentially expressed genes (DEGs) detected by RNA-Seq were validated by the OpenArray designed, showing similar expression levels and a 100% expression tendency accuracy. Furthermore, this tool brings new information about the infection process that was not shown by the RNA-Seq analysis, such as the expression profiles of *mda5*, *ifng*, *c9*, *c3*, *mx*, *ifit-1*, *myd88*, *tbkbp1*, and *ube1* genes in different samples at 48 h post-infection (pi). Moreover, a consistent decrease in the number of DEGs was observed at 72 hpi, confirming that 48 h is an adequate time point to study innate immune response of sole against NNV infection. In conclusion, this molecular platform has been confirmed as a good tool for further studies on the sole immune response against NNV mutant infections, which will contribute to the knowledge of the mechanisms of the pathogen-host interaction.

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Keywords: *Solea senegalensis*, Reassortant Nervous Necrosis Virus, OpenArray®, differentially expressed genes (DEGs), Immune response.

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P-009.

***Salmo Salar* glucocorticoid receptors analysis of alternative splicing variants under stress conditions**

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Abstract

Cortisol is the main glucocorticoid in teleost, where exerts multiple functions mediated through the glucocorticoid receptors (GR). Currently, it is known that many fish species have two GR genes, gr-1 and gr-2. Additionally, some teleost has also two different splice variants for GR1; gr-1a and gr-1b. In this study, we report for first time the identification of 2 gene copies for GR1 and GR2, located on chromosomes 4q-13q (gr1) and 5p-9q (gr2) of *Salmo salar* genome. Furthermore, our results describe gr1 splice variants in each chromosome, sharing typical teleost GR elements, such as the 9 amino acids insertion in DNA binding domain (DBD) and variations in length of the ligand binding domain (LBD). For GR2 gene copy on chromosome 5, three splice variants were predicted and differentiated by 5 amino acids insertion and length in the DBD. Also, we identified an uncommon truncated gr-2 gene copy on chromosome 9, lacking the DBD and LBD domains and expressing its mRNA in salmon. Finally, through of

specific primers design for each predicted splice variants, we validate and determine the expression of its transcripts in *S. salar* subjected to stress by stoking density. The results showed differences in the expression of all identified mRNAs, revealing that gr1 and gr2 splice variants were up-regulated in head kidney and gills of post-stressed fish. In conclusion, our findings suggest that from specific salmonid genomic duplication (125 MYA), two gene copies of each GR receptor were generated in *S. salar* and the splice variants identified, could contribute to the variability of the complex modulation of the receptors expression during stressful events, leading to different physiological responses in fish. Fondap 15110027.

Keywords: Glucocorticoids Receptor, splicing variant, *Salmo salar*, cortisol, fish stress

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P-010.

MyD88 dependence on the activation of induced innate effector mechanisms by TLR5M and TLR5S in salmonids

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Abstract

The innate immune response (IIR) in teleosts is essential in the defense against pathogens because of adaptive immune response limitations. Also, the innate immune effector mechanisms are activated by the recognition of conserved structures among pathogens, through Pattern Recognition Receptors, such as Toll-like Receptors (TLRs). Specifically, the membrane-anchored and soluble Toll-like Receptor 5 (TLR5M and TLR5S, respectively) from teleosts recognize bacterial flagellin as do orthologs in mammals. However, it has not been demonstrated whether the induced signaling pathway by these receptors depends on the Myeloid Differentiation Protein 88 (MyD88) to generate a pro-inflammatory response, in addition to activating of IIR effector mechanisms in salmonids. Therefore, in this work we study the MyD88 dependence on the induction of TLR5M/TLR5S signaling pathway mediated by flagellin as ligand, at both predictive and experimental level. CellDesigner program was used for the construction and mathematical characterization of the TLRs model, and simulations were carried out to predict the its dynamics. On the other hand, at the experimental level, we studied the key components response of the TLR5M/TLR5S signaling pathway against flagellin stimulation, as well as the functional participation of MyD88. Additionally, the activation of some IIR effector mechanisms was evaluated against the induction of the signaling pathway under study and related dependence of MyD88. For these experimental assays, treatment kinetics were performed by immuno-stimulants and pre-treatments with a MyD88 inhibitor in *S. salar* Head Kidney Leukocytes (HKLs) primary culture, as cell model; and the key components expression of the signaling pathway was analyzed by RT-PCR. Moreover, the stimulation of some IIR effector mechanisms was evaluated (like Reactive Oxygen Species -ROS- production) in RT-S11 cells stimulated with flagellin and pre-treated with a MyD88 inhibitor.

Our results for the simulations predicted that the MyD88 inhibition produced a delayed response downstream of the signaling pathway against

stimulation with flagellin. Hence, the stimulation assay in HKLs corroborated the previous predictions, and also showed a relative expression kinetic of genes concordant with a positive feedback mechanism between TLR5M/MyD88/TLR5S. Finally, the assays of RII effector mechanisms demonstrated that flagellin stimulates ROS production (extracellular and intracellular) and that these processes also depend on MyD88. In conclusion, we demonstrate that the activation of flagellin-mediated TLR5 (M and S) signaling, as well as the activation of final effector functions, depend on MyD88.

keywords: Toll-like Receptor, salmonids, MyD88, flagellin, system biology.

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P-011.

Differential immune responses of European sea bass (*Dicentrarchus labrax*) upon nodavirus infection by bath or intramuscular injection

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Abstract

Viral nervous necrosis virus (VNNV) produces the viral encephalopathy and retinopathy (VER) disease causing great mortalities in aquaculture fish. As is known, the innate system of teleost fish plays an important role in the defence against viral infection. Therefore, the aim of this study was to evaluate several immune-related enzymes (i.e. lysozyme, peroxidase and proteases) in serum, as well as the expression of immune-related genes (i.e. lysozyme, IgM, MHC I, TCR β , CD8 α , IL-1 β , IFN α , IL-10, IL-8, MAVS and Mx) in European sea bass (*Dicentrarchus labrax*) infected with VNNV by two methods with demonstrating effectiveness. In order to do this, European sea bass juveniles were infected with VNNV by either bath (1 hour, 10⁶ TCID50 mL⁻¹) or intramuscular injection (10⁶ TCID50 mL⁻¹). After 7, 15 and 30 days post-infection (dpi), fish serum was collected to measure innate immune parameters whilst the brain (tissue target of VNNV), spleen and intestine (as lethal samples), caudal fin, gills and skin (as low-invasive samples) were collected into RNA-later for the analysis of relative gene expression using Real-Time PCR (qPCR). The results demonstrated that serum peroxidase activity decreased in both infected groups at 15 dpi respect to the values found in control groups whilst this activity remained unchanged. In the case of protease activity, the results showed an increase in the serum of fish infected by bath method at the end of the trial (30 dpi) compare to unchallenged fish. The rest of immune-related enzymes measured not showed significant variations. Regarding to gene expression, the results revealed a modulation dependent on the type of infection and the organ studied (invasive or minimally invasive sample), which could be of great interest for the detection and treatment of this viral disease in aquaculture industry.

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keywords: Viral Encephalopathy and Retinopathy (VER), nodavirus (VNNV), innate immune system, RT-qPCR, European sea bass (*Dicentrarchus labrax*).

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P-012.

Identification of peritrophins as potential vaccine candidates against sea lice: A reverse vaccinology approach

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Abstract

Peritrophins are structural proteins of the peritrophic membrane (PM). This protein has been described in arthropods and have been described in some species of crustaceans. Peritrophins contribute to the maintenance of structural characteristics, including strength, elasticity and, permeability of the PM, and are capable to affect homeostatic regulation at intestinal level. In some copepod species of commercial interest such as *Caligus rogercresseyi* and *Lepeophtheirus salmonis* have not been characterized. These species are ectoparasite pathogens known as sea lice. The development of vaccine-based control strategies for sea lice control in salmonid aquaculture has been desired. The aim of this study was to use a reverse vaccinology approach to identify the *C. rogercresseyi* and *L. salmonis* peritrophins isoforms and identified by *in silico* analysis linear epitopes. We analyzed databases obtained by Illumina sequencing of the different stages of sea lice development. The contigs were compared against crustacean peritrophic membrane proteins database using BLAST and UniProt. Expression levels of different isoforms were evaluated by RNA-Seq and validated by RT-qPCR. *In silico* prediction tests of linear T and B epitopes were performed by the online software BepiPred and TEPITOPE, respectively. As results, in both sea lice species was identified different peritrophin isoforms, with differences in the number of chitin-binding domains. Furthermore, these proteins were differently expressed among sea lice developmental stages. In addition, linear epitopes of B lymphocytes were identified. These results demonstrate the antigenic potential of the peritrophins of both species. Further subsequent research will demonstrate the immunogenic action of peritrophins through *in vivo* tests against sea lice in farmed salmonids.

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keywords: Peritrophins; sea lice; reverse vaccinology; *Caligus rogercresseyi*; *Lepeophtheirus salmonis*.

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P-013.

Are related GABA and acetylcholine with the synthesis of IL-1 β , TNF- α and TGF- β in the immune system organs of the Nile tilapia?

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