sequenced to assess taxonomical composition and structure of different epithelia 6 h and 72 h after a short bacterial bath infection (1 h). We also assessed the immune status of fish after bath infection through qPCRassays. Alpha diversity measurements (inverse Simpson and Shannon index) suggest that the different microbiotas are highly diverse but predominated by few taxa and that the bacterial infection does not affect these indices. On the other hand, beta diversity measurements showed a potential early infection through gills after 6 h,then affecting skin and caudal fin microbiotas. Negative binomial generalized linear model (nb-GLM) highlighted the increase of different opportunistic pathogens such as Aeromonas, Pseudomonas, etc 6 h after bath infection. These observations are consistent with immune assays (cytokines, humoral response, gene expressions) showing a response of the immune system after 6 h followed by a disorder in its functioning. This study suggests that furunculosis not only impair immune system in rainbow trout but also induce dysbiosis leading to the increase of opportunistic pathogens in the bacterial community.

Keywords: Aquaculture, Microbiota, pathogen infection, immune system, *Oncorhynchus mykiss*

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O-040.

TLR5M and TLR5S play opposite roles in NF-κB pathway in *Vibrio parahaemolyticus* flagellin stimulation in orange-spotted grouper, *Epinephelus coioides*

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Abstract

A family of Toll-like receptor (TLRs) acts as primary sensors, which detect various microbial components, activate the host innate immune response to eliminate invading pathogens. In mammals, nuclear factor (NF)-κB signaling pathway is critical to the inflammatory cytokines and effectors expression. However, in fish, the bidirectional regulation of TLRs on NF-κB pathway is poorly understood. Here, we investigated the potential molecular mechanism of orange-spotted grouper (*Epinephelus coioides*) TLR5M (*Ec*TLR5M) and TLR5S (*Ec*TLR5S) regulating the NF-κB pathway after *Vibrio parahaemolyticus* flagellin stimulation.

*Ec*TLR5M, a member of the conserved TLR protein family, is involved in specifically recognizing flagellin and activating the NF-κB pathway. After knockdown of *Ec*TLR5M in grouper spleen (GS) cell line, the phosphorylation of IκBα and the expression of downstream cytokines, such as interferon (IFN) -γ2, interleukin (IL) -6 and tumor necrosis factor (TNF) -α, were all significantly suppressed. The overexpression of *Ec*TLR5M induced not only the activation of NF-κB pathway, but also mRNA expression of *Ec*TLR5S.

*Ec*TLR5S, consisting of 17 extracellular leucine-rich repeat domains, is located in the cytoplasm and involved in flagellin recognition. Knockdown of *Ec*TLR5S enhanced the phosphorylation of IKKα/β and IκBα, promoted NF-κB p65 nuclear transport, and augmented the cytokines IFN-γ2, IL-6 and TNF-α mRNA expressions after flagellin stimulation. Consistently with these observations, over-expression of *Ec*TLR5S negatively regulated the NF-κB pathway activation. We verified that the N-terminal (aa 1-254) and C-terminal (aa 514-643) of *Ec*TLR5S are the major functional domains of negative regulation by deletion mutation.

Taken together, *Ec*TLR5M is identified as a positive regulator, activating the NF-κB signaling after flagellin recognition. And for the first time, *Ec*TLR5S is demonstrated as a negative regulator that suppresses flagellin-induced activation of NF-κB, suggesting an important role for *Ec*TLR5S in control of innate immunity homeostasis.

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Keywords: NF-κB; TLR5M; TLR5S; flagellin; cytokine

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O-041.

Bacterial outer membrane vesicles of *Aeromonas salmonicida* induce a proinflammatory immune response in vitro and in vivo

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Abstract

High mortality rates after bacterial infections cause huge annual losses for the aquaculture industry. As treatment with antibiotics is not an alternative, bacterial vaccines for intramuscular or intraperitoneal injection were developed resulting in protection but also in inflammatory granulomas and stress. Here we propose the design of a modular vaccine based on outer membrane vesicles (OMV's) of the bacterial fish pathogen *Aeromonas salmonicida* (*A. salmonicida*.). The simple preparation, the safety due to their non-replicative nature as well as the composition of natural surface exposed membrane antigens in their native confirmation are the advantages of such a vaccine design. In the present project, the innate immune response to OMV's in comparison to bacterial stimulation was characterized using a peritoneal model for rainbow trout (*Oncorhynchus mykiss*). The distribution, recruitment and kinetics of myeloid cell populations in peritoneum, blood, spleen and head kidney were compared using lineage marker specific monoclonal antibodies. Additionally, the monocyte/macrophage cell line RTS-11 was used to characterize the mRNA profile response of phagocytes to OMVs and *A. salmonicida* bacterial particles.
First results indicate similar patterns of cellular responses in vivo either by stimulation with OMVs or with bacteria in regards to cell kinetics as well as to the induction of pro-inflammatory genes.
Next steps will include engineering of recombinant *A. salmonicida*, which produce of OMVs, presenting the immunogenic G-proteins of Viral-hemorrhagic-septicemia-virus (VHSV), Infectious-hematopoietic-necrosis-virus (IHNV) and Spring-viraemia-of-carp-virus (SVCV).Those OMV's will be used to analyze the innate immune response against bacterial and viral pathogens in regard to induction of protective immune memory.

Keywords: *Aeromonas salmonicida*, outer membrane vesicles, *Oncorhynchus mykiss*, innate immune response, vaccine

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O-042.

Impact of sea lice (*Caligus rogercresseyi*) infection levels on skin transcriptome in Atlantic salmon (*Salmo salar*)

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Abstract

Skin is a complex mucosal tissue, which is exposed to the outer environment, interacts with a plethora of environmental insults and represents the first line of defence against many pathogens. Sea lice *Lepeophtheirus salmonis* and *Caligus rogercresseyi* constitute a major threat to farmed salmonids, in both Northern and Southern hemispheres, respectively. We examined the response of skin transcriptome to different levels of infection with *C. rogercresseyi* in Atlantic salmon (*Salmo salar*). The infections were carried out on post- smolts using copepods, with a non- infected, control group. On day 14 post- infection, fish were examined for the total number of lice and grouped into low- and high-infected individuals, with 25 ± 5.3 and 59 ± 9.2 lice per fish, respectively. Skin samples (~100 mg) were collected from the area anterior to the dorsal fin and midway to the lateral line. In total, 36 samples samples –with 12 fish allocated to control, low-infected and high- infected group, accordingly- were subjected to total RNA extraction, followed by library preparation and RNA- seq (Illumina NextSeq 500 platform, single reads 150bp long, sequencing depth 20M reads per sample). Raw reads were aligned to the Atlantic salmon reference genome (GCA_000233375.4 ICSASG_v2) and assessed for differential gene expression using EdgeR. Differentially expressed genes were identified following statistical analysis many of which could be defined as immune response genes. Of interest, genes from encoding mucins were detected as being highly enriched in the infected fish compared to control, suggesting an increase in transcription of this gene family. Other genes of interest included lectins and antimicrobial peptides such as hepcidin and cathelicidin. Many cytokines were also found modulated suggesting a complex immunological response. Full gene set enrichment is being performed to define more comprehensively the immune pathways responding to the lice infection.

Keywords: Atlantic salmon, transcriptomics, *Caligus rogercresseyi*, RNA-Seq, differential gene expression

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O-043.

Pesticide drugs used against sea lice cause transcriptome changes in the early stages of the non-target species *Choromytilus chorus*

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Abstract

Azamethiphos and deltamethrin are pesticide drugs commonly used in Chile as control treatments for sea lice outbreaks in salmon farms. However, their effect on many biological processes of non-target marine species is still unknown. Here we described transcriptome patterns of the mussel *Choromytilus chorus* associated to the effect of these drugs on the early stages of its lifecycle. Trocophore and D larvaes were submitted to continuous exposure to azamethiphos and deltamethrin drugs at 10 and 1000 ppb during 5 days. Samples were collected for RNA-sequencing and transcriptomic analyses were conducted to evaluate gene expression and annotation. Short-read sequencing were conducted in the Ilumina HiSeq platform and long-read sequencing on the Oxford's Nanopore MinION platform. Mortality was not observed in either trocophore or D larvaes exposed to both drugs. Also, fertilization was successful at 80% or more in all treatments. However, thousands of assembled contigs were differentially expressed ($FC > |4|$; $p\text{-value} < 0.01$) in RNA-seq analyses from samples exposed to drugs with respect to control. Time of exposure to drugs triggered larger expression changes with respect to control groups than different chemical concentrations. Annotation by Gene Ontology of differential expressed genes revealed that azamethiphos exposure were mostly associated to changes in genes related to general metabolic processes, while deltamethrin to binding proteins. This study contributes to the understanding of the abnormal effects at molecular level that pesticides over early stages of the mussel *C. chorus* as non-target species and can have potential impacts on the later development of this economically important species.

Keywords: Non-target species, mussel, sea lice, azamethiphos, deltamethrin.

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O-044.

Understanding virulence and pathogenesis in controlled conditions: The case of brown ring disease causing immunodepression in clams

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Abstract

The Brown Ring Disease (BRD) has caused high mortality rates since 1986 in the Manila clam *Venerupis philippinarum*, which was introduced in Western Europe in the 1970s. The causative agent of BRD is a Gram-Negative bacterium, *Vibrio tapetis,* which is also pathogenic to fish. Infection of clams inside the adductor muscle has been done and intra-cellular fate of *Vibrio tapetis* has been investigated inside hemocytes using Transmission Electronic Microscopy. Intracellular multiplication of *V. tapetis* inside hemocytes has been followed during three days, showing that *V. tapetis* could be able to interfere with the autophagy process. Comparative genomic analysis of 17 *Vibrio tapetis* strains allowed identifying a type IV secretion system (T4SS) only present in strains virulent to clams. To further characterize the mechanisms underlying pathogenicity in *V. tapetis* CECT4600, we developed a gene deletion approach to determine the role of different genes in infection. In particular, we created a Δ *virB4* null mutant by deleting the full *virB4* gene, which encodes a protein essential to the T4SS assembly and functioning. We performed *in vitro* virulence tests on the wild type and the deleted strains, and showed that the Δ *virB4* mutation cancelled the virulence of *V. tapetis* to clams. These results proved that our approach is appropriate to explore and understand the role of multiple genes in the *V. tapetis* pathogenicity. Our on-going experiments have the objective to further characterize the role of T4SS, as well as other genes, in the *V. tapetis* infection and the development of BRD in the Manila clam.

Keywords: Vibriosis, clams, autophagy, T4SS, mutagenesis.

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