

higher number of GRP78/BiP transcripts in cells inoculated with the pathogenic VHSV suggests a role of the unfolded protein response in the VHSV immune evasion.

**Keywords:** Rainbow trout, VHSV, transcriptome, RNA-Seq, immune evasion, host-pathogen interaction

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### O-035.

#### Studies into B-glucan recognition in fish suggests a key role for the C-type lectin pathway

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#### Abstract

Immune-modulatory effects of  $\beta$ -glucans are generally considered beneficial to fish health. Despite the frequent application of  $\beta$ -glucans in aquaculture practice, the exact receptors and downstream signalling remains to be described for fish. In mammals, Dectin-1 is a member of the C-type lectin receptor (CLR) family and the best-described receptor for  $\beta$ -glucans. In fish genomes, no clear homologue of Dectin-1 could be identified so far. Yet, in previous studies we could activate carp macrophages with curdlan, considered a Dectin-1-specific  $\beta$ -(1,3)-glucan ligand in mammals. It was therefore proposed that immune-modulatory effects of  $\beta$ -glucan in carp macrophages could be triggered by a member of the CLR family activating the classical CLR signalling pathway, different from Dectin-1. In the current study, we used primary macrophages of common carp to examine immune modulation by  $\beta$ -glucans using transcriptome analysis of RNA isolated 6 h after stimulation with two different  $\beta$ -glucan preparations. Pathway analysis of differentially expressed genes (DEGs) showed that both  $\beta$ -glucans regulate a comparable signalling pathway typical of CLR activation. Carp genome analysis identified 239 genes encoding for proteins with at least one C-type Lectin Domains (CTLD). Narrowing the search for candidate  $\beta$ -glucan receptors, based on the presence of a conserved glucan-binding motif, identified 13 genes encoding a WxH sugar-binding motif in their CTLD. These genes, however, were not expressed in macrophages. Instead, among the  $\beta$ -glucan-stimulated DEGs, a total of six CTLD-encoding genes were significantly regulated, all of which were down-regulated in carp macrophages. Several candidates had a protein architecture similar to Dectin-1, therefore potential conservation of synteny of the mammalian Dectin-1 region was investigated by mining the zebrafish genome. Partial conservation of synteny with a region on the zebrafish chromosome 16 highlighted two genes as candidate  $\beta$ -glucan receptor. Altogether, the regulation of a gene expression profile typical of a signalling pathway associated with CLR activation and, the identification of several candidate  $\beta$ -glucan receptors, suggest that immune-modulatory effects of  $\beta$ -glucan in carp macrophages.

**Keywords:**  $\beta$ -glucan, primary macrophage, transcriptome analysis, C-type lectin-like domain, cyprinidae

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### O-036.

#### The immune proteome of the zebra mussel deciphered by deep proteogenomics

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#### Abstract

Bivalve immune system modulation appears to be a relevant strategy in environmental risk assessment. Indeed, immune system is known to be sensitive to different environmental and anthropogenic stresses. To date, the immune system of marine bivalves is well documented in comparison to continental bivalves. Among them, the freshwater mussel *Dreissena polymorpha*, a non-model organism, represents the counterpart of the marine mussel in ecotoxicological studies. While cellular responses of hemocytes are well characterized for *D. polymorpha*, the molecular immune mechanisms remain relatively scarce. In order to get insights into the immune proteome of the zebra mussel, proteogenomics was conducted on both hemocytes and plasma compartment of this non-model species. This strategy, combining transcriptomic sequences with mass spectrometry data acquired on proteins was relevant since 3,227 proteins were identified, which represent the largest protein inventory for this sentinel organism. Functional annotation and gene ontology (GO) analysis performed on the identified proteins described the main molecular players of hemocytes and plasma in the immune response of *D. polymorpha*. The GO analysis carried out on immune proteins showed that these two hemolymphatic compartments perform closely related and complementary immune functions: in signal transduction, adhesion and cellular mobility but also related to the recognition and elimination of microorganisms. Functional annotation revealed new mechanisms into the immune defence of the zebra mussel. Proteins rarely observed in the hemolymph of bivalves were pinpointed such as natterin-like proteins and thaumatin-like proteins. Furthermore, the high abundance of complement-related proteins observed in plasma suggested a strong implication of the complement system in the immune defence of *D. polymorpha*. This study contributes to a better understanding of the molecular mechanisms involved in immunity of bivalves and paves the way for their use as biomarkers in aquatic ecotoxicology.

**Keywords:** Hemolymph, bivalve, immunity, non-model organism, proteogenomics

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### O-037.

#### Salmonid IGH genes: From genomics to repertoire sequencing

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## Abstract

Rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) represent key species in aquaculture and are important models for the development of fish immunology. As in mammals, the basis of teleost humoral adaptive immune response is the clonal expression by B cells of somatically diversified immunoglobulins (IG), either as membrane bound or secreted in response to infections or immunizations. The IG repertoire sequencing has started to develop both in rainbow trout and in Atlantic salmon, reflecting a growing interest for an accurate and comprehensive description of the response against common pathogens and vaccines. In this context, a unified and standardized nomenclature and classification of IG genes is needed. In addition, these species are of particular interest because their IG loci are complex due to two additional whole genome duplication (WGD), compared to tetrapods: a WGD event that occurred during early teleost evolution, and a recent WGD that is specific to salmonids. This is reflected in the identification of IGH isoloci on two chromosomes. A good quality genome assembly is now available for rainbow trout and Atlantic salmon allowing a fully annotation that provide novel information. Here, we present how an IMGT-based nomenclature, numbering and structural description can be established in the frame of the Inferred Allele Review Committee (AIRC) working group, and how it helps comparing the diversity, the structure and the dynamics of antibody repertoires between fish and mammals.

**Keywords:** Immunoglobulin, locus, repertoire, nomenclature, Salmonids

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## O-038.

### Metagenome analysis of intestinal flora in the IL-17A/F1-knockout medaka

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## Abstract

In mammals, interleukin (IL)-17A and IL-17F are hallmark inflammatory cytokines, which are expressed by Th17 cells and play key roles in protection against infection and intestinal mucosal immunity. However, although fish IL-17A and IL-17F homologs named as IL-17A/F have been identified, their functional aspects, especially in intestinal mucosal immunity are still poorly understood. In this study, IL17A/F1-knock-out (IL17AF1-KO-) medaka (*Oryzias latipes*) was established using the genome-editing technique, CRISPR/Cas9 system, and a 7-bp deletion (-7bp) and a 11-bp addition (+11bp) were confirmed in the IL-17A/F1-KO-

medaka. After establishing F3 homo KO-medaka (+11bp), we conducted bacterial infection test with *Edwardsiella tarda* (E381 strain) to compare the defense capability in intestine of IL-17A/F1-KO-medaka to those of wild type (WT) medaka. After 24 hours immersion in freshwater containing 2.1Å~108 CFU/ml *E. tarda*, the number of bacteria was higher in posterior intestine than in anterior intestine in both WT and IL-17A/F1-KO-medaka. However, after 48 hours, bacterial number in posterior intestine decreased to the same extent as in anterior intestine at the same time. Furthermore, in comparison between WT and IL-17A/F1-KO-medaka, bacterial number of *E. tarda* in posterior intestine of IL-17A/F1-KO-medaka increased in 24 hours compared to those of WT. In addition, the results of gene expression in intestine by real-time PCR (qPCR) showed that antimicrobial peptide genes such as G-type lysozyme and transferrin a after infection were significantly down-regulated in IL-17A/F1-KO-medaka compared to those of WT. Furthermore, we performed 16S rRNA-based metagenome analysis to compare changes in composition of intestinal bacterial flora during naïve and infection between IL-17A/F1-KO and WT medaka. As a result of  $\alpha$  diversity analysis, under naïve condition, the diversity of bacterial flora was less in the WT medaka than in the KO medaka. After infection, although, the diversity of bacterial flora increased in both KO and WT medaka, bacterial species of WT medaka increased over twice in 24 and 48 hours after infection in comparison to those of naïve group, while there was a 1.5 times increase of bacterial species in IL-17A/F1-KO groups in 24 and 48 hours after infection. Furthermore, in weight-UniFrac analysis, it was revealed that WT and IL-17A/F1-KO group under naïve condition form different clusters. These results suggested that IL-17A/F1 induces a change in the composition of the intestinal bacterial flora in medaka.

**Keywords:** Interleukin 17, Japanese medaka, Antimicrobial peptide, 16S rRNA-based metagenome, Genome editing

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## O-039.

### Differential microbiota and immune modification in rainbow trout when facing bacterial infection

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## Abstract

Abstract must have a maximum of 2700 characters including spaces. In metazoans, the epidermal surface is important to maintain homeostasis of individuals. These epidermis are colonized by bacteria that have co-evolved with the host and that form communities with a complex network of interactions, called the microbiota. Communication between microbiota and the host was made possible by developing a suitable immune system. The microbiota is involved in many crucial functions for the host such as the maturation and stimulation of innate and adaptive immunity and the defense against pathogens by avoiding their colonization. Therefore, it is essential for the host to maintain homeostasis within the microbiota and between its mucosal immune system and the microbiota to keep functionality. However, this communication between those two compounds can be disrupted by various kinds of stressors present in the organism's environment. Such disturbance of this homeostasis is called a dysbiosis and can lead to detrimental, even mortal consequences for the host. Among these stressors, we can find some diseases caused by bacterial infection such as *Aeromonas salmonicida*. This pathogen is the causative agent of furunculosis and lead to important mortality in aquaculture. In this study, we have described the microbiota from different epithelial locations (skin, gills, caudal fin) exposed to a bacterial stressor (*Aeromonas salmonicida achromogenes*) using Next Generation Sequencing (Illumina HiSeq 2500). The hypervariable region V1-V3 16S rRNA gene was