

O-132.**White spot syndrome virus infection in a crustacean**

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Abstract

As a lethal pathogen for crustacean aquaculture, the mechanism of white spot syndrome virus (WSSV) infection remains largely unknown. By using a red claw crayfish *Cherax quadricarinatus* primary haematopoietic tissue (Hpt) stem cell culture suitable for WSSV propagation, we found that the fusion between WSSV and endosome was pH-dependent which was essential for WSSV infection. The internalized WSSV virion was detained into dysfunctional vacuoles in Hpt cells if pretreated by alkalizers, leading to the pH neutralization of endosome system or autophagosome. Importantly, a valosin-containing protein was found to positively regulate the viral delivery with endosome, whose dysfunction resulted in strong aggregation of the intracellular WSSV. When the valosin-containing protein activity was blocked by inhibitor, the co-localization of Cq-GABARAP puncta, an indicator of autophagy activity, on the aggregated virions was significantly increased, indicating the participation of autophagy in the elimination of WSSV. Furthermore, the autophagic sorting and ultimate degradation of the endocytic WSSV were all clearly enhanced in Hpt cells if pretreated with inhibitor, which demonstrated that the autophagy played a defensive role against WSSV infection. Taken together, our data shed new light on the pathogenesis of WSSV which will benefit the antiviral design and breeding selection against WSSV disease in crustacean aquaculture. This work was supported by NSFC (U1605214, 41676135).

Keywords: Endosome; γ -aminobutyric acid receptor-associated protein (GABARAP); Autophagy; White spot syndrome virus (WSSV); Crustacean

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O-133.**Prohibitin 2 is associated with WSSV infection by promoting stat translocation**

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Abstract

Prohibitins are mainly located at the inner membrane of mitochondrion and that are associated with aging, apoptosis, cancer formation and cell proliferation. In the previous studies, PHBs were found associated with virus infection and replication by interact with virus proteins. Jak/STAT signaling pathway play a key role in anti-virus immune response. The function of PHBs in the regulation of Jak/STAT remains largely unknown. In the present project, we identified that PcPHB2 was widely distributed in several tissues, and it was induced expression by white spot syndrome virus (WSSV). PcPHB2 significantly promote the amount of WSSV in crayfish, and the quantity of WSSV in PcPHB1 RNAi crayfish was decreased compared with those in the controls. We further confirmed the interaction of PcPHB2 with the STAT using pulldown and CoIP. Finally, we observed that the protein level of STAT in the nuclear was induced after PcPHB2 injected in the crayfish compared with control. Together, the location of STAT from cytoplasm to nuclear was inhibited by PcPHB2 knockdown. STAT was essential in the expression of early protein of WSSV. Therefore PcPHB2 is associated with the infection of WSSV by promoting the translocation of STAT.

Keywords: Prohibitin; White spot syndrome virus, Signal transducers and activators of transcription; RNA interference; *Procambarus clarkii*

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O-134.**Comparison of gene expression in post-smolt Atlantic salmon challenged by LF-89-like and EM-90-like *Piscirickettsia salmonis* isolates reveals differences in the immune response associated with pathogenicity**

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Abstract

Piscirickettsiosis is the main bacterial disease affecting the Chilean salmon farming industry and is responsible for high economic losses. The aim of this study was to describe and comparatively quantify the immune response of post-smolt Atlantic salmon infected by cohabitation with fish bearing LF-89-like and EM-90-like *Piscirickettsia salmonis*. The expression of 17 genes related to the immune response was studied in head kidney from cohabitant fish by RT-qPCR. Our results at the transcriptomic level suggest that *P. salmonis* is able to manipulate the kinetics of cytokine production in a way that might constitute a virulence mechanism that promotes intracellular bacterial replication in cells of Atlantic salmon. This strategy involves the creation of an ideal environment for the microorganism based on induction of the inflammatory and IFN-mediated response, modulation of Th1 polarization, reduced antigen processing and presentation, modulation of the evasion of the immune response mediated by CD8⁺ T cells and promotion of the CD4⁺ T-cell response during the late stage of infection as a mechanism to escape host defences. This response was significantly exacerbated in fish infected by PS-EM-90 compared with fish infected by PS-LF-89, a finding that is probably associated with the higher pathogenicity of PS-EM-90.

Keywords: Immune response, *Piscirickettsia salmonis*, RT-qPCR

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O-135.**The microbiome and transcriptome analysis of intestinal microbiota communities in larval and diseased grouper**

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Abstract

Grouper, the important economic aquaculture species in Taiwan; however, it is susceptible to infectious diseases such as nervous necrosis virus and *Vibrio alginolyticus*, and iridovirus during the fry and adult stages. We aimed this researches in aquatic disease control and bioinformatics to investigate the intestinal microbiota community and immunity in grouper by 16S metagenomics and transcriptomic analyses. The results will facilitate establishing database of health-related gut microbial metagenomics in grouper, which can assist in screening for healthy fish fry. Microbiota

analysis of *Epinephelus coioides* in metamorphosis stages revealed that dissimilarity in distribution of the individuals in 12dph, 18dph and 50dph. Analysis of immune gene (TLR-3, IL-1 β , IFN- α , IFN- γ , NF- κ B and Mx gene) showed the fish were in pathogen-free environment. The Proteobacteria in intestine microbiome is most abundant microbes at each development stages. In the other hand, NNV-, GIV-, and Vibrio- infected fish exhibit different phylum composition of microbes. The relationship of pathogen and microbiome in grouper will be investigated in the future, which might be the solution for the grouper disease control.

Keywords: Grouper, intestinal microbiota, development stages, disease, 16S metagenomics

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O-136.

Identification of novel biomarkers associated with infection and chronic stress in Russian sturgeon

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Abstract

Acipenser spp (sturgeon) are non-teleost fish species of ecological and economic value due to their exquisite meat and caviar. During 20th century, overfishing and pollution have led to a drastic decline of its natural reserves and nowadays many species are listed as critically endangered. Sturgeon aquaculture is thus an important tool for conservation and restoration of sturgeon populations, representing also an activity with valuable socio-economic impact. *A. gueldenstaedtii* (Russian sturgeon), one of the most cultured sturgeon species worldwide, is successfully farmed in Uruguay. However, for achieving a sustainable development, sturgeon aquaculture has to deal with the adaptation of sturgeon to a warmer climate, since increased mortality rates and economic losses are observed during Uruguayan summer. Our previous work demonstrated that warm temperatures ($\geq 24^\circ\text{C}$) alter fish innate defences by a chronic stress-dependent mechanism. Unfortunately, molecular tools for monitoring chronic stress and innate defences are not commercially available for sturgeons. Therefore, identification of biomarkers associated with inflammation and/or chronic heat stress in sturgeon is crucial for improving sturgeon aquaculture. With this aim, we follow two different experimental approaches: i) analysis of sturgeon acute phase proteins as putative biomarkers of inflammation and ii) analysis by RNA sequencing of differentially expressed genes in sturgeons subjected to chronic heat stress and challenged with pathogenic bacteria. Firstly, we identified several putative acute phase proteins in Russian sturgeon: hepcidin, haptoglobin, hemopexin, transferrin, serum amyloid A protein (SAA) and serum amyloid P protein (SAP). Hepatic expression analysis of these proteins in bacteria-challenged sturgeons showed that SAA could be a good inflammation biomarker candidate. We also obtained specific antibodies against SAA and SAP and analysed their serum protein levels by ELISA or Western blot. We found that SAA, but no SAP levels were significantly increased in serum of bacteria-challenged sturgeons, suggesting that SAA is a positive acute phase protein in Russian sturgeon. However, neither SAA nor SAP showed to be good biomarkers of chronic heat stress. Analysis of differentially

expressed genes in liver and spleen of bacteria-infected sturgeons subjected to chronic heat stress are currently being undertaken by Illumina sequencing in order to identify novel inflammation and chronic stress biomarkers. This identification will open new possibilities for monitoring sturgeon health in farms, contributing to less economic impact from disease outbreaks and favouring aquaculture development.

Keywords: *Acipenser gueldenstaedtii*, infection and chronic-stress biomarkers

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O-137.

A lectin with LDL receptor domain suppress the replication of WSSV

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Abstract

In invertebrates, C-type lectins (CTLs) play important roles in innate immunity against pathogens. Previous studies have focused mainly on the functions of CRD of CTLs in immune recognition, activation, and regulation. Conversely, limited research has been conducted on the relationship of CTLs and low-density lipoprotein (LDL). In this work, a lectin with LDL receptor domain was identified in *Procambarus clarkii* and designated as CTL-R. CTL-R was widely distributed in all tested tissues, and it was induced expression after white spot syndrome virus (WSSV) challenge. The recombinant CTL-R enhanced the anti-virus action of crayfish, whereas the anti-virus action was inhibited after CTL-R knockdown. The expression level of AMPs were suppressed after CTL-R knockdown in crayfish. Furthermore, CTL-R protein induce the activity of dorsal. NF- κ B is associated with anti-virus by regulating the expression of AMPs. Therefore CTL-R plays a key role in immune response of WSSV by positive regulation the activity of dorsal.

Keywords: Lectin; White spot syndrome virus; NF- κ B; *Procambarus clarkii*; Innate immunity

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O-138.

A comparative study on intestinal immune cells in two elasmobranchs species

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Abstract

The immune system of fish is of great relevance as it provides information on the evolution of immunity in vertebrates. All sharks, skates and rays are cartilaginous fish and together form a group called the elasmobranchs.