

Abstract

Diseases are the main cause of economic losses in the aquaculture sector that's why researching and improving this immunomodulation technique is fundamental. In this research work, the immunomodulatory effect of the lipopolysaccharides (LPS) of *Vibrio alginolyticus* was evaluated in a species of commercial interest, the seabass, through the cytokines, one kind of humoral components. Three groups of fish were evaluated, one unstimulated control, another positive stimulated with Poly I: C and another stimulated with LPS. The results showed statistically significant differences between the stimulants, where it was observed that the expression of the inflammatory (IL-1 β , IL-6 and TNF α) and anti-inflammatory (IL-10) cytokines was directly related. The antiviral response (Mx) was different between the treatments and it was seen that with LPS the stimulating effect was more prolonged in time, although weaker.

keywords: Immunomodulation, cytokines, seabass, immunostimulants and lipopolysaccharides

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P-071.

Circadian rhythmic expression of TNF- α gene regulated by clock gene in the Japanese medaka (*Oryzias latipes*)

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Abstract

To date, little information is available on the effects of circadian oscillation on immune regulation in lower vertebrates, such as teleost fish. In the present study, regulation of circadian rhythmic expression of inflammatory cytokine: TNF- α gene by clock genes (Bmal1 and Clock1) was investigated using Japanese medaka (*Oryzias latipes*). Firstly, structural analysis of clock gene was performed, which revealed that medaka Bmal1 and Clock1 conserve functionally important domains, such as basic helix-loop-helix (bHLH) and period-aryl hydrocarbon receptor nuclear translocator-single-minded (PAS), seen in their counterparts in other vertebrates. Expression of medaka Bmal1, Clock1 and Per1 genes was confirmed in central and peripheral tissues. Moreover, the expression of these clock genes and TNF- α genes in medaka acclimated to a 12:12 light (L) - dark (D) cycle showed circadian rhythm. In addition, higher expression of TNF- α gene was detected in medaka embryo cells (OI-Hdr R-e3) overexpressing Bmal1 and Clock1 genes. It was suggested that this increase was mediated by transcriptional regulation by clock proteins, which target E-box sequence in the cis-element of TNF- α gene as was detected by luciferase reporter gene assay. Moreover, in vitro head kidney stimulation with LPS at different zeitgeber time (ZT) under LD12:12 condition affected the degree of TNF- α gene expression, which shows high and low responsiveness to LPS stimulation at ZT18 and ZT10, respectively. These results suggested that medaka TNF- α exhibited circadian rhythmic expression regulated by clock proteins and its responsiveness against immune-stimulation depends on time zone.

keywords: Medaka, TNF- α , Circadian rhythm, Clock gene, Transcriptional regulation

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P-072.

Molecular characterizations and ligand-induced responds of type I interferon receptor (IFNR1) in orange-spotted grouper (*Epinephelus coioides*)

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Abstract

Grouper fishes are known as high economical species in aquaculture industry. However, outbreak of diseases caused chronic death rate of grouper juveniles. To increase survival rate of grouper, mechanism of first line of defense against virus diseases in teleost fish is studied. Previous result indicated up-regulation of gene expression level of grouper IFN and downstream antiviral Mx protein gene in response to NNV virus infection. However, signalling cascade of IFN system is unclear. To investigate relationship between type I 2C-IFN to its receptor (IFNR), full length orange-spotted grouper IFNR1 was firstly cloned and identified. Phylogenetic analysis indicated osgIFNR1 shared high homology similarities with other teleosts. Relative expression of osgIFNR1 was determined using real-time qPCR in regards to interferon treatment using immune organ primary cell culture. Gene expression of osgIFNR1 and downstream osgMx1 which act as marker was seen up-regulated approximately 0.5 and 10-fold respectively in 4 hours post-stimulation indicating involvement of transcriptional level responses. We also observed transcript level of osgIFNR1 in response to immune-stimulant (LPS and Poly I:C) with increased fold of around 2 and 6 times respectively and nodavirus infected larvae of around 0.5-fold downregulation. We further used GF-1 cell line to observe sub-cellular localization and interaction between ligand and receptor. The present study revealed effect of IFNR1 through up-regulation of gene expression level during 4 hours of interferon (IFN) treatment. This study contributed different insights in analysis of IFNR role in type I interferon system in orange-spotted grouper.

keywords: Orange-spotted grouper, interferon receptor, nervous necrosis virus, immune system, molecular cloning

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P-073.

Effects of recombinant IL-4/13A on the phagocytic capacity of salmonid leukocytes

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Abstract

The professional phagocytes described on teleost fish are macrophages, neutrophils and dendritic cells, and B lymphocytes. In mammals, several cytokines have effects on the phagocytic capacity of these cells, for example, IL-4 induces increase of phagocytosis by macrophages, as well as increase of reactive oxygen species production. In fish, it has been reported that IL-4/13 increases phagocytic capacity in Japanese pufferfish and Grass carp, however the function of this cytokine in salmonids has not been studied. In this work we evaluated the effect of recombinant IL-4/13A on the phagocytic capacity and ROS production of SHK-1 and