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O-102.

An improved genome assembly for *Larimichthys crocea* reveals hepcidin gene expansion with diversified regulation and function

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Abstract

Larimichthys crocea (large yellow croaker) is a type of perciform fish well known for its peculiar physiological properties and economic value. Here, we constructed an improved version of the *L. crocea* genome assembly, which contained 26,100 protein-coding genes. Twenty-four pseudochromosomes of *L. crocea* were also reconstructed, comprising 90% of the genome assembly. This improved assembly revealed several expansions in gene families associated with olfactory detection, detoxification, and innate immunity. Specifically, six hepcidin genes (LcHamps) were identified in *L. crocea*, possibly resulting from lineage-specific gene duplication. All LcHamps possessed similar genomic structures and functional domains, but varied substantially with respect to expression pattern, transcriptional regulation, and biological function. LcHamp1 was associated specifically with iron metabolism, while LcHamp2s were functionally diverse, involving in antibacterial activity, antiviral activity, and regulation of intracellular iron metabolism. This functional diversity among gene copies may have allowed *L. crocea* to adapt to diverse environmental conditions.

Keywords: *Larimichthys crocea*, Genome, Hepcidin

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O-103.

Role of sea lice secretome in host-parasite interaction: Immune modulation of SHK-1 cells exposed to *Caligus rogercresseyi* secretome

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Abstract

Caligus rogercresseyi is an ectoparasite that feeds on mucus, blood, and skin of its host. For a successful infestation, sea lice secrete proteins that allow avoiding host response. Among them, it has been described that trypsin and chymotrypsin have strong proteolytic activity in the peritrophic matrix of the intestinal parasite. In relation to the copepod *Lepeophtheirus salmonis*, it has been suggested that one of the strategies to successfully parasitize its host is given by the secretion of molecules such as proteases, prostaglandin synthetase E2 (PGE2) and cathepsin, causing immunodepression in fish. Moreover, from transcriptomic studies of *C. rogercresseyi*

developmental stage has been identified secretome-related proteins as cathepsin, trypsin, and serpin highly regulated during the infective stage, copepodid. The aim of this study was to evaluate the effects of *C. rogercresseyi* secretome over salmon immune and stress response by an in vitro approach using SHK1 cell line. Proteins identification and characterization were performed using the transcriptome database of *C. rogercresseyi*. Characterized sequences were cloned into an expression vector, pET30a and expressed in *Escherichia coli* system. Recombinant proteins were purified by His-tag affinity chromatography. SHK1 cell line was stimulated with 25 ng/mL, 50 ng/mL and 100 ng/mL of recombinant proteins for 24 hours. After cells stimulation, cells were collected for RNA extraction for immune-related genes expression analysis by RT-qPCR. A total of two isoforms of cathepsin, serpin, and trypsin were characterized. With a molecular weight of 36.4, 36.3, 43.5, 49, 26.4 and 27 kDa, respectively. After 24 h of stimulation cell damage was observed in all groups exposed to secretome proteins. Furthermore, differences in immune-related genes expression levels were observed among cells exposed to secretome proteins and control group. This study provides novel information associated with host-parasite interactions associated with *C. rogercresseyi* secretome effects on salmon.

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Keywords: Interaction parasite-host, *Caligus rogercresseyi*; secretome, SHK1 cell line, RT-qPCR

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O-104.

TLR-mediated type-I interferon production and the regulatory mechanisms in carp thrombocytes

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Abstract

In early stage of viral infection, type-I interferons (IFNs) are produced by signaling from innate immune receptors such as Toll-like receptors (TLRs), which recognize virus-specific molecular patterns including nucleic acids. The type-I IFN transcriptions induced by TLRs are regulated via nuclear factor κ B (NF- κ B) complex and interferon regulatory factors (IRFs), but these details are still unclear in fish. In the present study, we show that thrombocytes in common carp (*Cyprinus carpio*) have a potent ability to produce large amount of IFNs in response to TLR signaling. Magnetic-sorted HB-8 mAb+ carp thrombocytes and negatively sorted other peripheral blood leukocytes (PBLs) were incubated with resiquimod (also called R848, a potent agonist of TLR7/8), followed by qPCR analysis. The expression levels of the common carp type-I IFNs (ccIFN1 and ccIFN2) in thrombocytes were considerably higher compared with that of in other PBLs. Whereas the ccIFN1 expression was relatively lower than the ccIFN2, the R848 stimulant highly upregulated the ccIFN1 expression than ccIFN2. Although typical inflammatory cytokines including interleukin-6 were also upregulated in thrombocytes, the expression levels were still lower than those in other PBLs. These results indicate that activation of carp thrombocytes by R848 inclines immune system toward antiviral response, rather than inflammation. Expression levels of IRF3 and IRF7 were also upregulated by R848, implying that the IFN transcriptions were activated by these IRFs. The expression of the IFNs and inflammatory cytokines were decreased by several NF- κ B signaling inhibitors such as BAY11-7082 or phenethyl caffeate, however, sensitivities to each inhibitor were different between the IFNs and other cytokines. In the presence of those inhibitors, the ccIFN2 expression was correlated with the level of IRF3. In contrast, ccIFN1 expressions seem to be linked to IRF7, suggesting that these two IRFs regulates different IFN genes separately. Our finding suggests that fish thrombocytes are important components for antiviral immunity and can