

RTS-11 cell lines and salmon spleen B cells. The phagocytic capacity was evaluated with fluorescent latex beads and ROS production with the probe 2,7-dichloro-4-hydroxyfluorescein diacetate by flow cytometry. The results showed that rIL-4/13A increases the percentage of phagocytosis in trout and salmon spleen B cells. Also this protein, increases the phagocytic capacity in RTS-11 and SHK-1 cell lines, and in addition the mean fluorescence intensity of all tested cells. Besides, rIL-4/13A increases ROS production in RTS-11 cells and trout and salmon spleen B cells. In conclusion, rIL-4/13A increases phagocytosis and the production of reactive oxygen species indicating that this cytokine plays a role stimulating phagocytes to effectively engulf and eliminate invading microorganisms in salmonids.

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P-074.

Atlantic salmon lymphocytes: Infection target cells for *Piscirickettsia salmonis*?

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Abstract

Piscirickettsia salmonis is a facultative intracellular gram-negative bacterium of variable size (0.5–1.5 µm of diameter) causing salmonid rickettsial septicaemia in salmonids. During 2017, *P. salmonis* was responsible of almost 70% of mortality of salmon due to infectious causes in Chilean marine aquaculture centers. The pathogen produces prominent lesions in the liver, kidney, spleen and intestine, and one of the main target cells are macrophages. To date, only few studies investigate the cell targets of infection and the strategies and kinetics of *P. salmonis* infection at the cellular level. Thus, this study aimed to characterize *P. salmonis* infection of Atlantic salmon leukocytes using confocal microscopy and flow cytometry. We hypothesized that not only macrophages, but also lymphocytes were able to internalize *P. salmonis*. Thus, leukocytes isolated from the spleen of Atlantic salmon were infected with *P. salmonis*-727 conjugated to FITC (MOI 10). First, a time-course study was performed to follow up salmon splenocytes infection with *P. salmonis* for 6 hours using confocal microscopy. Video recording showed that 5 min post-infection (p.i.), *P. salmonis* was attached to the cell membrane of salmon splenocytes, whilst the bacteria were on the cell surface and/or internalized 30 min p.i. Thirty minutes later almost all splenocytes had the bacteria internalized. During the 6 hours of infection, splenocytes of different size showed protrusions of cell membrane that seems to engulf the bacteria, which is a characteristic process during phagocytosis of a pathogen. Apoptotic bodies and dead cells were also observed during this period of time. We then examined if the lymphoid population internalize the bacterium. This was evaluated by flow cytometry, using salmon spleen lymphocytes, infected with *P. salmonis* 727-FITC (MOI 10) for 0.5, 4, 8 and 24 hours. The percentage of lymphocytes able to incorporate *P. salmonis*-FITC was evaluated. An increase in the number of live lymphocytes containing the bacteria was observed during the first 8 h, that was decreasing when approaching 24 hours after infection. Similarly, an increase in the percentage of dead lymphocytes with *P. salmonis* was observed, supporting results observed in confocal microscopy. In summary, we reported that spleen lymphocytes of Atlantic salmon can internalize *P. salmonis* that might be due to the phagocytic capabilities of fish lymphoid cells or because they are target cells for *P. salmonis* infection or both.

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keywords: Atlantic salmon, lymphocytes, *Piscirickettsia salmonis*, infectivity, phagocytosis.

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P-075.

Comparative modulation of lncRNAs in wild-type and RAG1 heterozygous mutant zebrafish exposed to an immune challenge with Spring Viremia of Carp Virus (SVCV)

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Abstract

Although the modulation of immune-related genes after viral infection have been largely described in vertebrates, the potential implication non-coding RNAs (ncRNAs), and especially the long non-coding RNAs (lncRNAs), in the immunity is still an incipient research field. The model species zebrafish could serve as a very useful organism to study the functionality of lncRNAs due to the numerous advantages of this teleost, including the existence of numerous mutant lines. In this work, we conducted whole-transcriptome analysis of kidney from wild-type (WT) and heterozygous *rag1* mutants (*rag1*+/-) after infection with the pathogen Spring Viremia of Carp virus (SVCV). The RAG1 of vertebrates is one of the endonucleases with a role in the assembly of immunoglobulins and T cell receptor (TCR) genes. The absence of functional RAG1 protein results in the impossibility to develop mature B and T lymphocytes. However, heterozygous *rag1* zebrafish, which are partially deficient in RAG1, could allow us to detect lncRNAs potentially involved in the adaptive immunity due to the compensatory processes induced after infection. We identified 12,165 putative lncRNA in zebrafish, most of them shared by both zebrafish lines. However, by comparing the lncRNA profile induced after SVCV infection in both WT and *rag1*+/-, we found that the majority of the lncRNAs significantly induced after viral challenge were exclusive of each line, reflecting a highly differential response to the virus. Analysis of the neighbor genes of lncRNAs exclusively modulated in WT revealed a high representation of metabolism-related terms, whereas those from *rag1*+/- showed enrichment in terms related to the adaptive immune response, among others. As was expected, commonly modulated lncRNAs were surrounded by genes involved in numerous antiviral processes. These results clearly indicate that, after SVCV infection, zebrafish are able to induce the expression of an array of lncRNAs with a function in different aspects of the immunity.

keywords: zebrafish, lncRNAs, RNA-Seq, SVCV, immunity, *rag1*

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P-076.

Two regulator of complement activation group 2 genes in rainbow trout: Gene duplication and divergent evolution

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Abstract

The complement system is a crucial part of the immune system of vertebrates, protecting hosts from invading pathogens. The optimal activation of the complement system is tightly regulated by regulators of complement activation (RCAs) for host cell protection. In vertebrates, the RCAs can be categorized into two groups, including group 1 and group 2. Despite increasing researches on complement components of rainbow trout, the RCA group 2 genes are still unknown. In this study, two RCA group 2 genes were cloned and identified in rainbow trout, named TRC1 and TRC2. The TRC1 is comprised of a signal peptide and ten SCR domains, while TRC2 only comprised of six SCR domains. Protein sequence alignment, gene structure comparison, phylogenetic and syntenic analysis revealed that TRC1 and TRC2 are two homologous genes, which may duplicate and evolved from an ancestral gene by the salmonid-specific whole-genome duplication (WGD) event. Further analysis revealed that the SCR domains of fish group 2 RCAs can be clustered into four types (A, B, C and D), and the SCR orders of TRC1 and TRC2 are ADBAABADCA and ADBADC, respectively. Expression analysis showed that TRC1 and TRC2 are constitutively expressed in various tissues and leukocyte subpopulations, with the expression level of TRC1 higher than that of TRC2, especially in IgT+ and IgM+ B cells. Previous studies have shown that vertebrate RCA group 2 genes are closely located in one chromosome, the first time we report two homologous, but significantly different RCA group 2 genes located in two different chromosomes in rainbow trout, providing new insights into the duplication and evolution of RCA genes in vertebrates.

keywords: Complement, regulator, duplication, evolution, rainbow trout

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P-077.

Influence of traditional Chinese medicine and *Bacillus* species (TCMBS) on growth, immune response and disease resistance in Nile tilapia, *Oreochromis niloticus*

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Abstract

Multispecies herbs and probiotic bacteria offered to fish might enhance their immune response and increase disease resistance, but the dose effects of herbal-probiotic application remain unclear. Therefore, the effect of herbal-probiotic mixtures of traditional Chinese medicine (TCM) of composition *Astragalus membranaceus*, *Angelica sinensis*, *Crataegus hupehensis* and probiotic *Bacillus* species (BS) of composition *Bacillus subtilis*

and *Bacillus lincheniformis* as natural immunostimulants in tilapia *Oreochromis niloticus* have been investigated. Fish were randomly divided into four groups: control diet (CT), TCMBS1 [TCM at 3 g/kg and BS at 7 g/kg], TCMBS2 [TCM at 5 g/kg and BS at 5 g/kg], TCMBS3 [TCM at 7 g/kg and BS at 3 g/kg]. Tilapia in the TCMBS3 group showed significant improvement in weight gain, specific growth rate, and lowered feed conversion ratio compared with other treated groups and the control. Concerning immune indexes, all treated groups significantly enhanced lysozyme, superoxide dismutase, catalase, protease and antiprotease activities, with highest values in catalase and antiprotease activities in TCMBS3 compared with control. TCMBS3 demonstrated higher expression of beta-defensin, lysozyme, heat shock protein 70, catalase and transforming growth factor-beta compared with other treated groups or the control group in both mid-intestines and head-kidney. After challenge with *Streptococcus agalactiae*, the best survival was found in TCMBS3 (97%), followed by TCMBS2 (73%), and TCMBS1 (69%) compared with the CT (35%). Collectively the present results suggest that TCMBS3 dose might potentiate a higher immune response and disease resistance in fish.

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P-078.

Serotransferrin and C-type lectin could mediated NCC activity by interacting with NCCRP-1 in Nile tilapia (*Oreochromis niloticus*)

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Abstract

Non-specific cytotoxic cells (NCCs) are precursor cells of mammalian natural killer cells and play an important role in innate immunity of fish. NCCRP-1, as an activated receptor of NCC, could combining many exogenous substances or endogenous proteins to mediate NCC activity. In this study, we obtained two proteins, On-C-type lectin and On-serotransferrin, that could interacting with NCCRP-1 through screening of Yeast Two-hybrid library. These two recombinant proteins can effectively induce some toxic effector molecules of NCC high expression. At the same time, On-C-type lectin and On-serotransferrin stimulated NCC could effectively activating FHM apoptotic signal, but blocking on-NCCRP-1 by anti-On-NCCRP-1 antibody could inhibit this apoptotic signal. These results indicate that on-C-type lectin and on-serotransferrin could mediate the killing activity of NCC through NCCRP-1. This study contributes in better understanding the mechanism of NCC activation in teleost.

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