seeds in the diets showed significant influence on the non-specific and specific immune system of catla. Respiratory burst, nitric oxide synthase and serum lysozyme were significantly ($P<0.05$) higher in fish fed with diet D2 compared to other two groups. Highest bactericidal activity was recorded in D1 diet fed catla compared to D2 and D3 diets fed fish. The antibody response was significantly ($P<0.05$) higher in D1 diet fed fish compared to D3. There was no significant ($P > 0.05$) difference between D1 and D2 diets fed groups. In kidney, TNF-α gene expression was up-regulated in seed supplemented diets fed groups (23-36% higher) compared to the control one. It was significantly ($P<0.05$) higher in D2 diet fed fish. The expression of IL-10 was down-regulated (18%) in D2 diet fed fish compared to others. There was up-regulation of lysozyme c and g expression in seed supplanted diets fed catla compared to the control group. A direct relationship was found between the dose of seed in the diet and the expression of lysozyme c and g in kidney. In the gill, TNF-α expression was significantly ($P<0.05$) higher in D1 diet fed group compared to others. There was no expression of IL-10 in this tissue. Lysozyme c and g expression were significantly ($P<0.05$) higher in D2 diet fed group compared to other groups. There was no expression of TNF-α in the hepatopancreas of catla. IL-10 expression was up-regulated by the supplementation of seed in the diet. Lysozyme c and g expressions were significantly ($P<0.05$) higher in seed supplemented diet fed fish compared to the control one. This study showed tissue-specific gene expression pattern in catla. An inverse relationship was also found between the expression of TNF-α and IL-10 in various treatments.

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O-250.

Characterisation of a functional intracellular type I interferon system in rainbow trout *Oncorhynchus mykiss*

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Abstract
Type I interferons (IFNs) are encoded by intronless genes in amniotes and by intron containing genes in amphibians and fish. IFNs are known to activate a cellular antiviral response through cell surface receptors. In teleosts, multiple transcript variants have been reported and are shown to be transcribed from alternative splicing of introns. Whether these transcript variants are translated into functional proteins is unclear and if so, their physiological importance requires to be determined. In this report, two alternatively spliced IFN transcripts were identified in rainbow trout *Oncorhynchus mykiss* and predicted to encode proteins lacking a signal peptide. Expression studies have shown they are induced in cells after stimulation with polyI:C and in head kidney of fish infected with virus. The proteins have been confirmed to be made from the IFN transcripts and are located within the cytoplasm. Overexpression of intracellular IFN (iIFN) proteins in cells or stimulation of cells with bacteria-derived recombinant proteins significantly up-regulates expression of antiviral genes and enhances cell resistance to viral infection. Furthermore, two intracellular IFN receptor chains have been identified and found to be co-localised with the IFN ligands in the cytoplasm and are required to facilitate phosphorylation of STAT1 and STAT2. Taken together, our data demonstrate for the first time a functional intracellular IFN system in teleosts.

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O-432.

Activation of intracellular antiviral immune responses by antibacterial pattern recognition receptor NOD1

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Abstract
Intracellular pattern recognition receptors (PRRs) such as the nucleotide-binding oligomerization domain (NOD)-like receptors NOD1 and NOD2 are keys for innate immune recognition of microbial infection. In mammals, it has been shown that NOD2 but not NOD1 can also function as a cytoplasmic viral PRR by using the adaptor protein MAVS to activate IRF3. In this study, we evaluated the role of NOD1 transcript variant from teleost fish (*Danio rerio*), especially the role in the innate antiviral immune response. Expression studies showed that NOD1 was induced in ZF4 cells after the infection with *Edwardsiella tarda* and SVCV. PCR arrays data analysis revealed that overexpression of zebrafish NOD1 in embryos regulated expression of some genes involved in cytokine-cytokine receptor interaction, JAK-STAT, Toll-like receptor, RIG-I-like receptor signaling pathway, etc. In addition, overexpression of zebrafish NOD1 in ZF4 cells could inhibit intracellular bacterial growth and enhance cell resistance to SVCV infection. Furthermore, zebrafish NOD1 has a synergism effect with RIG-I/MDA5 in antiviral innate response. We also showed that NOD1 could form a direct interaction with RIG-I and MDA5, and the transfection of NOD1 significantly induced the production of RIG-I, MDA5 and Mx proteins with or without virus infection. Taken together, our data demonstrate for the first time that NOD1 likely acts as a positive regulator for RIG-I/MDA5 signaling.

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O-387.

Melanization cascade of shrimp and its importance for white spot syndrome virus infection

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Abstract
Melanization cascade, which is activated by the prophenoloxidase (proPO) system, is one of a potent effective defense mechanism that plays a key role in the production of cytotoxic intermediates as well as melanin products for microbial sequestration in invertebrates. In the present study, the effect of melanization cascade of the black tiger shrimp *Penaeus monodon* on viral infection was investigated. Analysis of the effect of melanization on white spot syndrome virus (WSSV) infection showed that melanization reaction of shrimp significantly reduced the viral replication, whilst the survival rate of shrimp injected with melanization reaction-treated WSSV was found to be significantly higher than that of the control shrimp injected with untreated-WSSV. The dsRNA-mediated gene silencing of shrimp *Pm*proPO genes followed by WSSV infection resulted in a significant increase in the cumulative mortality of the *Pm*proPO silenced shrimp. In addition, injection of WSSV significantly reduced the hemolymph phenoloxidase (PO) activity of the infected shrimp at day 2 and 3 post

infection compared to the uninfected shrimp and the reduction of PO activity was compromised after an exogenous trypsin was added to the reaction. This result suggests that WSSV probably affect the proteolytic activity of some proteinases in the proPO cascade of shrimp. Using yeast two-hybrid screening, a viral protein named WSSV453 was identified as a protein that capable to interact with a proPO-activating enzyme 2 (*Pm*PPAE2) of *P. monodon*. WSSV453 is an uncharacterized protein with no putative domains and its sequence consists of 306 bp encoding for a predicted 101 amino acid protein with a calculated molecular mass and an estimated p*I* of 11.92 kDa and 9.95, respectively. Recombinant protein of WSSV453 and *Pm*PPAE2 were then produced and co-immunoprecipitation assay indicated that WSSV453 interacts directly with the *Pm*PPAE2 protein. Additionally, *in vivo* gene silencing of WSSV453 showed a significant increase in the hemolymph PO activity of the WSSV-infected shrimp when compared to the control shrimp. These results suggest the important role of shrimp proPO system in the defence against WSSV infection and also demonstrate the mechanism on inhibition of shrimp proPO cascade by the proteinase inhibitory activity of the viral protein.

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O-188.

The peroxiredoxin genes and their functions in the antivirus immunity of Kuruma shrimp, *Marsupenaeus japonicus*

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Abstract
White spot syndrome virus (WSSV) is one of the major pathogenic viruses that cause extensive mortalities in aquaculture. WSSV infection can bring about oxidative stress through the release of reactive oxygen species (ROS) which are deleterious to the cells. Peroxiredoxin (Prx) is known to be thioredoxin-dependent peroxidase activity antioxidant protein that protects the organisms against various oxidative stresses and functions in the intracellular signal transduction. To investigate the composition and the function of peroxiredoxin in shrimp, one of the model animals for aquatic, we cloned five peroxiredoxin genes from Kuruma Shrimp, *Marsupenaeus japonicus* (*M. japonicus*). Phylogenetic analysis and multiple alignment showed the genes we cloned belong to the Prx I, Prx II, Prx IV, Prx V and Prx VI subfamily and named as MjPrx I, MjPrx II, MjPrx IV, MjPrx V and MjPrx VI separately. RT-PCR analysis of the tissue distribution of peroxiredoxin showed the transcript of MjPrx IV, MjPrx V and MjPrx VI can be detected in all the tissues tested (hemocytes, heart, stomach, gills, hepatopancreas, ovaries, spermary and intestine) while the MjPrx II was mainly detected in gonad and MjPrx I expressed mainly in stomach, intestine, testis and ovaries. The expression profiles of peroxiredoxin transcripts in gonad upon WSSV infection was investigated by Real-time PCR and showed different patterns. In spermary, the transcript of MjPrx II and MjPrx IV were upregulated after the 6 h WSSV challenge while MjPrx I and MjPrx VI were downregulated at the same time. In ovary, the transcripts of all MjPrxs were upregulated after the 6 h WSSV challenge. The MjPrx IV was selected for the further function study. The mature peptide of MjPrxIV was recombinant expressed in *Escherichia coli* system and showed peroxidase activity *in vitro*. Furthermore, suppression of MjPrxIV by dsRNA resulted in the increase replication of WSSV in shrimp while injection of rMjPRX IV into shrimp could decreased the replication of WSSV in vivo. To our knowledge, this is the first integrative research about the Prxs and their function in the antivirus immunity of shrimp.

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O-437.

Survival of *Edwarsiella tarda* in fish serum relates to baterial surface LPS

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Abstract

Edwardsiella tarda is the etiological agent of edwardsiellosis, a systematic disease that affects a wide variety of marine and freshwater fish worldwide. Survival in fish serum is an important feature for virulent strains of *E. tarda* to escape from host immune surveillance and plays essential roles for the bacterial establishment and infection in fish. In the study, we investigated the complement activity in turbot serum after incubating with virulent *E. tarda*. Our results indicated that over 80% of bacteria can survive in fish serum as the complement molecules in the serum could not be activated by the *E. tarda* bacteria. Further study suggested that the intact LPS layer plays important roles for virulent *E. tarda* against complement activation in fish serum. The detailed mechanisms were still under investigation.

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O-393.

Trout transcriptome induced by the non-virion (NV) protein of VHSV. Identification of NV targets

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Abstract
Viral haemorrhagic septicemia virus (VHSV) infects many species of freshwater and marine fish, leading to important economic losses mainly for aquacultured salmonids. VHSV belongs to Novirhabdovirus genus, which also includes Hirame rhabdovirus, infectious haematopoietic necrosis virus (IHNV), and snakehead virus. Novirhabdoviruses are characterized by the expression of the non-virion (NV) protein. While NV deletion (dNV) attenuated VHSV, the functional role of NV still remains uncertain in fish infections. We have obtained and compared the transcriptional profiles of internal organs from rainbow trout (*Oncorhynchus mykiss*) after 48 hours of intraperitoneal injection with recombinant NV, wild type (wt)-VHSV or dNV-VHSV. Trout transcriptional profiles were characterized by using probes from GenBank immune-related genes corresponding to interferons, VHSV-induced, macrophage-related, complement components, toll-like receptors, tumour necrosis factors, chemokines, interleukins, antimicrobial peptides, and cluster differentiation antigens. The results suggested that to favour VHSV infection, NV induces gene downregulation of interferons, myxovirus resistance genes (mx), apoptosis, some cytokines and other relevant genes involved in immune response. Thus, these data could depict specific-VHSV immune response pathways in trout that could be extrapolated to other affected species. Some of these newly discovered downregulated trout genes, after NV or VHSV exposition, might help to better understand the functional mechanism of NV protein as well as improving fish vaccines.

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