

IgM) and IgM-IgD-IgT+ cells. The IgM+ and IgT+ cells were scattered throughout the parenchyma, and grouped around large vessels and surrounding melanomacrophage centres (MMC). Cell proliferation was estimated using a combination of anti-IgT, anti-IgM and anti-PCNA antibodies. Proliferation of both IgM+ and IgT+ B cells was observed in several areas of the spleen. In addition, vaccinated fish showed a mass of vaccine and cells (CVM) associated to the spleen. The CVM contained scattered PCNA+/IgM+ and PCNA+/IgT+ B cells, possibly indicating its importance during vaccination, above and beyond its role as a site for phagocytosis and material exchange.

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**keywords:** Turbot, Spleen, vaccination, B lymphocytes, Immunoglobulins

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#### P-043.

##### Protective immune responses of recombinant outer membrane proteins OmpF and OmpK of *Aeromonas hydrophila* in European eel (*Anguilla anguilla*)

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#### Abstract

Outer membrane proteins (Omps) of Gram-negative bacteria were proved to be efficient subunit vaccines against bacteriosis. In this study, OmpF and OmpK of *Aeromonas hydrophila* were expressed and evaluated their immune protective effects on European eel (*Anguilla anguilla*). The genomic DNA of *A. hydrophila* 322A was used as the template, and two kinds of prokaryotic expression plasmids pET-32a-OmpF and pET-32a-OmpK were constructed, respectively. Recombinant OmpF protein (r-OmpF) and r-OmpK were purified and proved to have antigenicity by Western-blot analysis. The r-OmpF and r-OmpK were used as immunogens to immunize European eel by the intraperitoneal injection. The mRNA expression of 6 immune-related genes (*IgM*, *IL-10*, *IRF3*, *IRF7*, *LysG4*, and *HexB*) in liver tissues of eels at 1 h, 3 h, 6 h, 12 h, 24 h, 72 h, and 10 d post-immunization was analyzed by real-time PCR. At 30 dpi, serum antibody response was measured by ELISA. Fish were attacked at 15 dpi by live 322A in order to assess the protective immunity of r-OmpF and r-OmpK. Both r-OmpF and r-OmpK could up-regulate the expression of all 6 genes in varying degrees. The serum antibody titer of r-OmpF- and r-OmpK-immunized groups was 1: 1600 and 1: 3200, respectively. In addition, r-OmpF could give 35.5% of relative immune protection rate to European eels, while r-OmpK gave 70.0%. By analyzing the protective immunity and the regulatory role in the immune-related gene expression of the two recombinant proteins provided, it could be found that r-OmpK was a potential vaccine candidate of *A. hydrophila*.

**keywords:** *Aeromonas hydrophila*; outer membrane protein; subunit vaccine; protective immunity; European eel (*Anguilla anguilla*)

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#### P-044.

##### Transcriptome analysis of immune-related gene expression in hybrid snakehead (*Channa maculata* ♀ X *Channa argus* ♂) after challenge with *Nocardia seriolae*

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#### Abstract

Hybrid snakehead fish (*Channa maculata* ♀ x *Channa argus* ♂), a new species used in freshwater aquaculture in China, is the common host of an epizootic bacterial infection by *Nocardia seriolae*. However, the information on the functions and mechanisms of hybrid snakehead immune pathways with the *N. seriolae* infection is limited. Thus, the peripheral blood lymphocytes from hybrid snakehead were used for transcriptome analysis to understand the host immune response after challenge with *N. seriolae*. A total of 49,839,332 and 50,059,283 raw reads were obtained from the *N. seriolae*-challenged group (Ns group) and phosphate-buffered saline control group (Ctr group), respectively. The 75.50% and 74.25% reads from the Ns and Ctr groups were matched to reference genomic sequence after cleaning the raw reads, respectively. Additionally, there were 2892 significant differentially expressed genes (DEGs) among the 17,196 expressed genes between the Ns and Ctr groups, including 1387 upregulated and 1505 downregulated genes. All the DEGs were classified into three gene ontology categories, and 2502 DEGs had significant matches, which were allocated to 246 Kyoto Encyclopedia of Genes and Genomes pathways. Immune-related genes were detected from immune system pathways among the top 20 enriched pathways. Moreover, the regulation of several observed effective genes was confirmed by real-time quantitative Polymerase chain reaction. Altogether, this study offers deep-sequence data of hybrid snakehead peripheral blood lymphocyte via transcriptome analysis and lays the foundation for further study on the immunogenetics of hybrid snakehead. Moreover, it provides insights into the pathogenic mechanism of *N. seriolae*, facilitating the prevention and treatment of fish nocardiosis.

**keywords:** *Nocardia seriolae*, Hybrid snakehead, Transcriptome analysis, Immune-related genes, Fish nocardiosis

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#### P-045.

##### A recombinant vaccine targeting the parasitic ciliate *Ichthyophthirius multifiliis*

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#### Abstract

New vaccine candidates were identified targeting the one celled parasite *I. multifiliis*, which negatively affects aquaculture freshwater fish productions all over the world. In silico selection with the use of artificial