

P-040.**Effect of aloe vera natural additive on enteritis caused by diets that include soybean meal in Atlantic salmon**K. Fehrmann^{1,2,3,*}, M. Vega^{1,*}, M. Allende^{3,*}, C. Feijóo^{2,*}, A. Romero^{1,4,#}.¹ Instituto de Patología Animal, Facultad de Ciencias Veterinarias, Universidad Austral, Valdivia, Chile² Departamento de Ciencias Biológicas, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile³ Departamento de Biología, Facultad de Ciencias, Centro FONDAF de Regulación del Genoma, Universidad de Chile, Santiago, Chile⁴ Centro Interdisciplinario para la Investigación Acuícola (INCAR), Centro FONDAF, Concepción, Chile

Abstract

Soybean meal is one of the most widely used alternatives to replace fishmeal. However, its ingestion triggers an intestinal inflammatory process that compromises fish health. Finding strategies that reduce its deleterious effects will be relevant. In this work we analyzed the effects of aloe vera (*Aloe barbadensis miller*, AV) as additives in a soybean meal-based diet on intestinal inflammation in Atlantic salmon.

To determine the immunomodulatory effect of AV, we supplemented fishmeal (FM) and soybean meal (SBM) based diets with AV. 4 groups in duplicate of 40 Atlantic salmon each, with an average weight of 75 gr at the beginning of the study were fed for 28 days with the FM, SBM, FM + AV and SBM + AV diets. At the end of the feeding period, the length of fish fed with SBM was significantly lower than that of fish fed with the FM, FM + AV or SBM + AV diets. Weight gain was similar between fish fed with SBM, FM and FM + AV diets, whereas fish fed SBM + AV gained 11% more weight than the SBM group. Samples of the distal intestine of 12 fish per treatment were taken for histological analysis. A semi-quantitative scoring system was used to assess the degree of morphological changes induced by different diets. A higher score was evidenced for the SBM group compared with the FM group, suggesting SBM triggered an inflammatory process. On the other hand, the SBM + AV group had a significantly lower score compared to the SBM group, evidencing the intestinal protection granted by AV. To complement our result, we characterized the expression of cytokine markers *il-1 β* and *il-10* by qPCR, obtaining higher expression of inflammatory related genes in the SBM group when compared with SBM+AV or FM group. The present study suggests that aloe vera could be used as additive in farmed fish diets to facilitate the replacement of fishmeal by soybean meal without affecting intestinal health.

keywords: Soybean meal, enteritis, natural additive, distal intestine, histology

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P-041.**Identification and bioactivity of a granulocyte colony-stimulating factor b homologue from large yellow croaker (*Larimichthys crocea*)**Qihua Li¹, Jingjie Chen¹, Jingqun Ao³, Xinhua Chen^{1,2,#}.¹ Institute of Oceanology, College of Animal Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China² Laboratory for Marine Biology and Biotechnology, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266071, China³ Key Laboratory of Marine Biogenetic Resources, Third Institute of Oceanography, State Oceanic Administration, Xiamen, 361005, China

Abstract

Granulocyte colony-stimulating factor (GCSF) is a pleiotropic cytokine that plays a key role in regulation of hematopoiesis, innate and adaptive im-

mune responses in mammals. However, bioactivity of GCSF in teleost fish remains largely unknown. In this study, a GCSFb homologue from large yellow croaker (*Larimichthys crocea*) (*LcGCSFb*) was cloned by RACE-PCR techniques. The open reading frame (ORF) of *LcGCSFb* is 603 bp long and encoded a protein precursor of 200 amino acids (aa), with a 19-aa signal peptide and a 181-aa mature peptide. In healthy fish, the *LcGCSFb* was constitutively expressed in all examined tissues, with the highest levels in mucous tissues, such as gills, intestine, and stomach. Its transcripts in head kidney, spleen and gills were significantly induced by *Vibrio alginolyticus* challenge. *LcGCSFb* transcripts were also detected in primary head kidney leukocytes (PKL), primary head kidney macrophages (PKM), primary head kidney granulocytes (PKG) and head kidney cell line (LYCK), and markedly up-regulated by inactivated *Vibrio alginolyticus*. These data suggested that *LcGCSFb* may play a role in immune response against bacterial infection. *In vivo* administration of recombinant *LcGCSFb* protein (*rLcGCSFb*) significantly up-regulated the expression levels of the inflammatory cytokines (IL-6 and TNF α), and transcription factor C/EBP β , which is required for proliferation of neutrophils. Furthermore, *rLcGCSFb* showed an ability to strengthen the phagocytosis of PKL *in vitro*. Taken together, *LcGCSFb* may be involved in antibacterial immunity via promoting the inflammatory response and the phagocytic activity of leukocytes. To our knowledge, this is the first report on immunoregulatory roles of GCSF in teleost.

keywords: Granulocyte colony-stimulating factor (GCSF); Large yellow croaker (*Larimichthys crocea*); inflammatory response; bacterial infection; phagocytosis

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P-042.**Role of the spleen in the immune response of turbot (*Scophthalmus maximus*) to vaccination with the ciliate parasite *Philasterides dicentrarchi***F. Fontenla-Iglesias¹, I. Estensoro^{2,3}, A. Valle¹, M. Noia¹, C. Tafalla³, A. Sitjà-Bobadilla², J.M. Leiro⁴, J. Lamas^{1,#}.¹ Biología Celular, Dpto. de Biología Funcional, Facultad de Biología e Instituto de Acuicultura, Universidad de Santiago de Compostela, Santiago de Compostela, Spain² Fish Pathology Group, Institute of Aquaculture Torre de la Sal (IATS), Consejo Superior de Investigaciones Científicas (CSIC), Castellón, Spain³ Animal Health Research Centre, National Institute for Agricultural and Food Research, Valdeolmos 28130, Madrid, Spain⁴ Dpto. de Microbiología y Parasitología, I. de Investigación y Análisis Alimentarios, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain

Abstract

Fish spleen is rich in lymphocytes, particularly in B lymphocytes, and plays an important role in the adaptive immune response after vaccination. After vaccination of fish by intraperitoneal injection, there is a strong migration of antigen-containing cells to the spleen, where antigen presentation occurs. In the present study, we analysed the B cell populations (IgM, IgT or IgD positive cells) and gene expression (IgM, IgT, IgD, MHCII, and several immune-related genes) in the spleen of turbot immunized with a vaccine containing an oleous adjuvant and a particulate *Philasterides dicentrarchi* antigen. Fish were immunized on days 0 and 30, and samples were obtained on days 3, 7, 33, 37 and 60. The vaccine provoked a significant increase in specific and total serum IgM at 37 dpi and at 60 dpi, but the specific IgT levels did not vary significantly in vaccinated fish. No significant regulation of sIgT, mIgT, sIgM, mIgM and IgD occurred before 37 dpi, whereas at 37 and 60 dpi, overexpression of sIgT and mIgT was detected in fish injected with adjuvant alone or with the vaccine. Immunofluorescence analysis enabled classification of the B lymphocytes into four groups: IgM+IgD-IgT- (the majority of IgM+ cells), IgM+IgD+IgT-, IgM-IgD+IgT- (very few cells, probably corresponding to lymphocytes with low levels of

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.

ACKNOWLEDGEMENTS: This work was funded by EU H2020 program through ParaFishControl Project (634429), by the Ministerio de Economía y Competitividad (Spain) and FEDER (European Union) (AGL2017-83577-R) and by grant ED431C2017/31 from the Xunta de Galicia. I.E. was contracted under APOSTD/2016/037 grant by the “Generalitat Valenciana”, and F.F. was contracted by the Xunta de Galicia.

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