keywords: Flagellin, cytokines proinflammatory, immune response, adjuvant

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Corresponding author.
E-mail address: roxana.gonzalez@uach.cl (R. González-Stegmaier).

P-065.

Development of a modular oral vaccine based in outer membrane vesicles for rainbow trout and characterization of the systemic and mucosal B and T cell response assembled

Ruth Montero [1,#], Lara Munkler [1,2], Sven Ostermann [1], Bernd Köllner [1].

[1] Friedrich-Loeffler-Institute, Institute of Immunology, Greifswald-Insel Riems, Germany
[2] Hochschule Esslingen, Department of Applied Natural Sciences, Esslingen am Neckar, Germany

Abstract

Infectious diseases cause serious economic losses due to the high-density fish stocks. Diversevaccines were developed to prevent this; however, they are not effective enough and theinjection route is linked to side effects and stress. It is known Gram-negative bacteria produce Outer Membrane Vesicles (OMVs) and are used for human research purposes; therefore, fish bacteria OMVs could be also used as vaccines platforms. The present work is focused on the development of an oral vaccine based on recombinant *Aeromonas salmonicida* OMVs, expressing the G-protein of VHSV, and the evaluation of the B and T cell response at mucosal and systemic level in Rainbow trout. In order to validate the oral stimulation formula, first fish were stimulated with inactivated bacteria intra-peritoneally or orally using vaccine pellets. Distribution and proliferation of B and T-cell populations were analyzed in gut, peritoneum, spleen and head kidney using monoclonal antibodies by flow cytometry. Additionally, cell populations were sorted for characterization of membrane and secreted markers, expressed cytokines and transcription factors. The immune response is characterized by an early proliferation of intraperitoneal B and T-cells (24-48h post stimulation). Comparing the kinetics of the cell populations observed in the peritoneum and in the gut as well as the recruitment of cells from spleen or head kidney will be further analyze. The next upcoming trial will be done with the OMVs from *A. salmonicida* to further studies.

keywords: Rainbow trout, oral vaccine, OMVs, *Aeromonas salmonicida*, adaptive immunity.

Corresponding author.
E-mail address: ruth.montero.m@gmail.com (R. Montero).

P-066.

Immune and pathogen interactions during experimental co-infection with *Piscirickettsia salmonis* and Piscine Orthoreovirus in *Salmo salar*

I. Aguirre [1,#], A. Mancilla [1], M. Navarrete [1], E. Paredes [1], R. González-Stegmaier [1,2], R. Enríquez [1,#].

[1] Laboratorio de Biotecnología y Patología Acuática. Instituto de Patología Animal. Facultad de Ciencias Veterinarias. Universidad Austral de Chile. Valdivia, Chile
[2] Centro FONDAP: Interdisciplinary Center for Aquaculture Research (INCAR), Chile

Abstract

Piscine Orthoreovirus (PRV) infections are widespreadly distributed in Chilean salmon cultivation. And it is estimated that over 80% of freshwater Atlantic salmon is infected predominantly with PRV-1. In this scenario, mixed infections with other viruses or bacteria are likely to occur, and typical clinical signs could be misdiagnosed due to different responses triggered during a simultaneous infection with two or more pathogens. On the other side, *Piscirickettsia salmonis (P. salmonis)* is the most important bacterial pathogen for Chilean salmon cultivation. The objective of our pilot study was investigating viral and bacterial presence, some aspects of innate immune responses and histopathological features during an experimental challenge with P. salmonis in a population of Atlantic salmon smolt infected with PRV-1.

From a population of 240, PRV-1 positive smolt (100g), 84 shedder fish were intraperitoneally infected with *P. salmonis* and then allocated with 156 co-habitant smolts. Sampling was carried out at 14, 21, and 30 days post-challenge (dpc). Co-habitant fish were euthanized and denervated. Blood, head kidney and spleen samples were directed to molecular analysis and head kidney, spleen, liver, heart, and gills were obtained for histological examination.

Our results showed that viral loads diminished significantly from 14dpc to 21dpc and to 30dpc, but they did not disappear. Meanwhile, the percentage of *P.salmonis* positive fish increased from 21 to 30dpc. In accordance with the decreasing viral load, a significant drop of IFN-I transcripts was detected from 21-fold change at 14dpc to 11,7 at 21 and to 1,4-fold change at 30dpc. On the other hand, Mx transcripts did not show any considerable change during the experiment. Cytokine transcripts related to inflammatory bacterial infections such as IL-8 transcripts were upregulated 12,4-fold change at 14 and 7,9-fold change at 21dpc and decreased 2,7-fold change at 30dpc. However, IL-12 and IL-1β _transcripts showed no variation at any time point evaluated. Most of the fish showed no lesion, and just a few evidenced only mild to moderate lesions concordant with HSMI or SRS at different time points. These results suggest that PRV-1 infection could exert an apparent protective effect on the host against the bacterial infection diminishing the severity of SRS clinical and histopathological signs. Grants: FIE-Sernapesca 2015-V014, VIDCA UACH.

keywords: Co-infection, Piscine Orthoreovirus, Piscirickettsia salmonis, antiviral immune response, antibacterial immune response

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Corresponding author.
E-mail addresses: isabel.aguirre@uach.cl (I. Aguirre), renrique@uach.cl (R. Enríquez).

P-067.

Stress regulation and tolerance in shrimp: The transcriptomic and physiological response to chronic ammonia exposure in the black tiger shrimp, *Penaeus monodon*

S.E. Berry [1,2,3,#], R. Huerlimann [1,2], S. McWilliam [3], A.R. Foote [1,2,3], D.R. Jerry [1,2], N.M. Wade [1,3].

[1] Australian Research Council Industrial Transformation Research Hub for Advanced Prawn Breeding, James Cook University, Townsville, QLD, 4811, Australia
[2] James Cook University, College of Science and Engineering, James Cook Drive, Townsville, QLD, 4811, Australia
[3] CSIRO Agriculture and Food, Aquaculture Program, 306 Carmody Road, St Lucia, QLD, 4067, Australia

Abstract

Elevated ammonia (NH3) is a significant challenge in penaeid shrimp aquaculture worldwide, and can compromise shrimp osmoregulation, growth, immune-competency, leading to substantial crop loss. Despite the impact of elevated NH3 levels to the well-being of farmed penaeids, little is known about physiological and transcriptomic responses to chronic NH3 exposure. NH3 is a toxic byproduct of the break-down of uneaten feed, faeces and metabolic processes, and high NH3 levels are often difficult to mitigate quickly in ponds. This study investigated the physiological and transcriptomic response of sub-adult black tiger shrimp, *Penaeus monodon*, after chronic exposure to moderate and high levels of NH3. An individual assessment was implemented wherein shrimp hemolymph metabolite profiles were assessed pre- and post-exposure to identify individual responses to 0 ppm (control), 10 ppm and 20 ppm NH3 across a 72 h period. Key metabolites that have previously been linked to condition and stress responses in penaeid shrimp were quantified, including total protein, glucose, triglycerides, and hemocyanin. Additionally, shrimp were held in individual respirometers throughout the trial and the oxygen consumption of shrimp was measured with fiber optic probes as a proxy of activity, or metabolism. Oxygen consumption in response to NH3 exposure showed wide variance in controls, while shrimp exposed to 10 ppm and 20 ppm NH3 decreased oxygen consumption in the 24 h period post-exposure. After 48h shrimp exposed to 10 ppm showed evidence of recovery through increased oxygen consumption, while 20 ppm remained low. Hemolymph triglycerides at 96 h post-exposure are significantly decreased in shrimp exposed to 20 ppm NH3 compared to 10 ppm and control shrimp, indicating an increase in energy expenditure. Other biochemical parameters measured including total protein, hemocyanin and glucose decreased pre-to-post exposure due to feed restriction, and were not significantly different between treatments. To compliment physiological data, the transcriptomic profile of gill tissue from shrimp post-exposure were obtained by Illumina RNASeq. Differentially expressed genes were identified in response to NH3 exposure, as well as in shrimp that showed physiological evidence of recovery. The pathways enriched and genes co-expressed were identified and demonstrate how shrimp respond to ammonia stress, and potentially how some animals are able to tolerate NH3.

keywords: Transcriptomics, physiology, stress, aquaculture, shrimp

Corresponding author.
E-mail address: sarah.berry3@my.jcu.edu.au (S.E. Berry).

P-068.

CQSIRT1 from red claw crayfish *Cherax quadricarinatus* promotes white spot syndrome virus infection VIA positively regulating PI3K-AKT-mTOR pathway

Shu-cheng Zheng, Wei-dong Li, Hai-peng Liu[#].

State Key Laboratory of Marine Environmental Science, Xiamen University, Fujian Collaborative Innovation Center for Exploitation and Utilization of Marine Biological Resources, Fujian Engineering Laboratory of Marine Bioproducts and Technology, Xiamen, 361102, Fujian, PR China

Abstract

Sirtuins (SIRT) are a family of evolutionarily conserved nicotinamide adenine dinucleotide (NAD+)-dependent deacylases that participate in antiviral immunity. In this report, the *SIRT1* gene (named as *CqSIRT1*) was identified with an open reading frame of 2256 bp that encoding 751 amino acids from red claw crayfish *Cherax quadricarinatus.* Tissue distribution analysis showed that *CqSIRT1* was ubiquitously expressed in all tissue tested with high expression in haematopoietic tissue (Hpt), haemocyte and gill, while low expression in hepatopancreas, musle and eyestalk. Unexpectedly, dysfunction of *CqSIRT1* by gene knockdown in red claw crayfish Hpt cell cultures resulted in markedly decrease expression of an early gene *ie1* and an envelope protein gene *vp28* of white spot syndrome virus (WSSV) at late stages post WSSV infection, indicating that CqSIRT1 was hijacked by WSSV to promote its replication. Importantly, the expression of *PI3K*, *AKT* and *mTOR,* which were involved in energy metabolism and autophagy, was significantly reduced after gene silencing of *CqSIRT1* during WSSV infection. Overall, these data suggest that CqSIRT1 could promote WSSV replication through positively modulating PI3K-AKT-mTOR signaling pathway in red claw crayfish *C. quadricarinatus*, which benefits further understanding of the molecular mechanism underlying the pathogenesis of WSSV in crustacean.

keywords: Sirtuins 1(SIRT1); PI3K-AKT-mTOR; White spot syndrome virus (WSSV); Haematopoietic tissue (Hpt); *Cherax quadricarinatus*.

Corresponding author.
E-mail address: Haipengliu@xmu.edu.cn (H.-peng Liu).

P-069.

The role of CD8α in the immune response of sea bass against nodavirus

Y. Valero[1,#], *J. Cortés*[2], *F. Ramírez-Cepeda*[2], *L. Mercado*[2], *E. Chaves-Pozo*[3], *M. Ángeles Esteban*[1], *A. Cuesta*[1].

[1] Fish Innate Immune System Group, Department of Cell Biology and Histology, Faculty of Biology, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, 30100 Murcia, Spain
[2] Grupo de Marcadores Inmunológicos, Laboratorio de Genética e Inmunología Molecular, Instituto de Biología, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
[3] Oceanographic Center of Murcia, Spanish Institute of Oceanography (IEO), Carretera de la Azohía s/n. 30860, Puerto de Mazarrón, Murcia, Spain

Abstract

The adaptive immune response is mediated by different mechanisms including humoral factors, cellular elements (T and B lymphocytes) and other specific proteins as major histocompatibility complex which act in coordination with diversified antigen receptors. Regarding cytotoxic T cells (CTLs) of vertebrates, they are direct effector lymphocytes in the fight against virus-infected cells by killing their cellular targets. The cluster of differentiation 8α (CD8α) is the distinctive marker of the lymphocyte subset of cytotoxic CTLs. However, teleost CTLs function is largely unknown mainly because of the lack of population-specific antibodies. European sea bass (*Dicentrarchus labrax*) is a very susceptible species to nodavirus (NNV) in which causes devastating mortality rates and, up to date, no solutions are available to prevent them. Previous studies pointed to the relevance of cell-mediated cytotoxicity in the response of European sea bass juveniles against NNV at transcriptional level. Thus, we have investigated the distribution, production and potential role on European sea bass CD8α+ cells by flow cytometry using a polyclonal antibody against European sea bass CD8α in naïve and NNV-infected cells. This work represents the first attempt to characterize CD8α+ cells in European sea bass.

Work partly funded by projects from MINECO and FEDER (AGL2013-43588-P and AGL2016-74866-C3-1-R), *Instituto Español de Oceanografía* (NODAMED), *Fundación Séneca* (*Grupo de Excelencia de la Región de Murcia* 19883/GERM/15) and National Commission for Scientific & Technological Research Chile (FONDECYT Nº 1140797).

keywords: *Dicentrarchus labrax*, nodavirus, CD8α, polyclonal antibodies.

Corresponding author.
E-mail address: yuvalero@um.es (Y. Valero).

P-070.

Study of the immunomodulatory and antiviral activity of bacterial lipopolysaccharides in seabass (*Dicentrarchus labrax*, Linnaeus 1758)

T.K. Segura-Bernal, J. Bravo[*], *B. Vega, F. Acosta*[#].

Grupo de Investigación en Acuicultura, Instituto Ecoaqua.Universidad de Las Palmas de Gran Canaria, Gran Canaria, Spain