Keywords: Mollusc; Immune recognition; Pattern recognition theory; Novel patter receptors; Mucosal recognition

Corresponding author.
E-mail address: lshsong@dlou.edu.cn (L. Song).

O-110.

Hematopoiesis and regulatory signaling in molluscs

Xiaorui Song [1,3], Weilin Wang [1,3], Linsheng Song [1,2,3,#].

[1] Liaoning Key Laboratory of Marine Animal Immunology, Dalian Ocean University, Dalian 116023, China
[2] Functional Laboratory of Marine Fisheries Science and Food Production Process, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266200, China
[3] Liaoning Key Laboratory of Marine Animal Immunology & Disease Control, Dalian Ocean University, Dalian 116023, China

Abstract
Hematopoiesis is a complex process by which different blood cells are formed and released from hematopoietic tissues. Due to lack of oxygen-carrying erythrocytes and blood cells of the lymphoid lineage, which are participating in adaptive immune defense, hematopoiesis in invertebrates offers a simple model system to study regulation of the blood cells of the innate immune system. Several transcription factors have been characterized as hemocyte-specific markers in molluscs, such as Tal-1/SCL, GATA2/3, Runx, CBFβ, ETS, and c-Myb, and are conserved across taxonomic groups from molluscs to chordates. They were highly distributed in the hemocytes as well as potential hematopoietic tissue gill, and the RNAi of Tal-1/SCL, GATA2/3 and Runx significantly reduced the hemocyte renewal rates in the hemocytes and gill tissue. The temporal and spatial expression pattern revealed the potential developmental events of hematopoiesis during ontogenesis of oyster, which initially occurred early in blastula stage and definitively resided in the dorsal region in trochophore larvae. A cytokine-like factor astakine was identified from Pacific oyster *Crassostrea gigas*, which could induce the regeneration of oyster hemocytes either receiving an injection of r*Cg*Astakine *in vivo*, or incubation with r*Cg*Astakine *in vitro*. Furthermore, critical components in signaling pathways, such as Notch signaling pathway, Wnt signaling pathway, were restricted to the potential hematopoiesis sites in the adult oyster, which hints at a possible role for them during the hematopoiesis. In oyster, three types of hemocytes were morphologically identified and separated as agranulocytes, semi-granulocytes and granulocytes by flow cytometry and Percoll® density gradient centrifugation. The granulocytes were proved to be the main immunocompetent hemocytes, and there was potential differentiation relationship among these three sub-population hemocytes. Several hemocyte-specific molecules, such as *Cg*AATase, *Cg*SPSB3, *Cg*CD-9 were identified, which could be employed as a potential marker for the isolation of each subtype hemocytes. Above researches of molluscan hematopoiesis may shed light on the study regulation of the blood cells of the innate immune system in invertebrates.
Keywords: Hematopoiesis; Mollusc; Transcription factors; Cytokines; Hemocyte-specific molecules

Corresponding author.
E-mail address: lshsong@dlou.edu.cn (L. Song).

O-111.

Deep transcriptome profiling sheds light on key players in nucleus implantation induced immune response in the pearl oyster *Pinctada martensii*

W. Wang [*], Y.Y. Wu [*], Q.N. Lei, H.Y. Liang[#], Y.W. Deng.

Fisheries College, Guangdong Ocean University, Zhanjiang, Guangdong, PR China

Abstract
Immunological rejection of the pearl oysters following nucleus implantation is a major issue limiting the successful rate of cultured pearls. To date, the molecular mechanism of immune tolerance during pearl formation in the pearl oysters is still largely unknown. Through the RNA sequencing platform and comparative transcriptomic analysis, we investigated the chronic gene expression changes at seven time points (0, 5, 10, 15, 20, 30, 60 days post implantation or dpi) over a period of 60 days following nucleus implantation in the pearl oyster Pinctada martensii. A total of 81,390 unique transcripts (or unigenes) with a combined length of 96.8 million bp and a N50 value of 2,227 bp were obtained. When compared with sequences in the nr, nt, Swiss-Prot, KEGG, COG and GO databases, 36,380 unigenes can find homologous genes. Pairwise comparison of gene expression among all the samples showed that the largest number (or 6,846) of differentially expressed genes was observed at 10 dpi. The number then decreased to below 5,000 at 15, 20 and 30 dpi and increased again to 6,679 at 60 dpi. PCA analysis further showed that the seven time points can be roughly divided into four groups. Comparative transcriptomic analysis between the four groups identified a variety of genes showing differential expression at different time points, including many immune-related genes such as those encoding for toll-like receptor, lectin, scavenger receptor, and peroxidase. In addition, GO and KEGG enrichment analysis revealed that these differentially expressed genes were mainly associated with metabolism, ribosome function, immune response, signaling transduction, and cytoskeleton organization. Notably, two KEGG pathways, namely “cell adhesion molecules” and “primary immunodeficiency” were significantly enriched during the whole process. This finding indicates that genes in these pathways are likely to play critical roles in the immune tolerance of the pearl oysters. To conclude, the data obtained contribute to a better understanding of the molecular mechanisms of allograft induced immune response in the Pearl oysters, and will facilitate the development of effective measures to improve the performance of pearl culture.
Keywords: Pearl oyster, *Pinctada martensii*, Nucleus implantation, Allograft, Transcriptome

Corresponding author.
E-mail address: zjlianghy@126.com (H.Y. Liang).
* These authors have contributed equally to this work.

O-112.

Immune responses of American oysters to bacterial and parasitic challenge

Marta Gomez-Chiarri [1,2,3,4,#], Tejashree Modak [1], Erin Roberts [1], Rebecca Stevick [2], David Nelson [3], David Rowley [4].

[1] Department of Fisheries, Animal and Veterinary Science, University of Rhode Island, Kingston, RI, USA
[2] Graduate School of Oceanography, University of Rhode Island, Kingston, RI, USA
[3] Department of Cell and Molecular Biology, University of Rhode Island, Kingston, RI, USA
[4] Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI, USA

Abstract
The American oyster Crassostrea virginica is an ecologically and economically important species in the Northwest Atlantic and the Gulf of Mexico. Wild and cultured populations of this organism are impacted by a variety of bacterial and parasitic pathogens. Taking advantage of the recently assembled sequence of the American oyster, we have performed a transcriptomic characterization of the immune responses of oysters to bacterial and parasitic challenge. Evaluation of the response of *C. virginica* larvae to probiotics *Bacillus pumilus* RI0695 and *Phaeobacter inhibens* S4 showed

immunostimulation, as evidenced by high levels of expression of the genes involved in pathogen recognition, immune signaling pathways, apoptosis and effectors such as protease inhibitors, mucins and perforin-2. In contrast, larvae exposed to the bacterial pathogen *Vibrio coralliilyticus* RE22 showed evidence of immunosuppression. Transcriptome analysis of lines of oysters with varying levels of resistance to the bacterial pathogen Aliiroseovarius crassostreae and the protozoan parasite *Perkinsus marinus* yielded information that confirmed the importance of serine protease inhibitors and other immune molecules in disease resistance. Finally, viral, bacterial, and parasite genomes deposited in genetic databases were indexed and used as a reference for the assembly of reads derived from oyster DNA resequencing and transcriptome (RNA) analysis projects. The coverage and expression of these microbial genomes was calculated and compared between samples. Analysis of resequencing data from 96 oysters collected at sites from Texas to Maine for the presence of known (and unknown) parasites of oyster show that genomic data could be used to mine for information on pathogen abundance and distribution. These experiments illustrate the potential of −omic tools to inform host-pathogen interactions in bivalves.

Keywords: bivalves, probiotic, protozoa, transcriptome, vibrio

Corresponding author.
E-mail address: gomezchi@uri.edu (M. Gomez-Chiarri).

O-113.

Changes in the host microbiota structure and diversity during the immune response of the scallop *Argopecten purpuratus*

K. Muñoz [1,], P. Flores-Herrera [1,*], A.T. Gonçalves [2], C. Rojas [3], C. Yañez [3], K. Brokordt [4], P. Schmitt [1,#].*

[1] Grupo de Marcadores Inmunológicos, Laboratorio de Genética e Inmunología Molecular, Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica de, Valparaíso
[2] Laboratorio de Biotecnología y Genomica Acuícola − Centro Interdisciplinario para la Investigación Acuícola (INCAR), Universidad de Concepción, Concepción, Chile
[3] Laboratorio de Microbiología, Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile
[4] Laboratory of Marine Physiology and Genetics (FIGEMA), Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Universidad Católica del Norte

Abstract
Traditionally it has been considered that invertebrates depend mainly on innate immunity mechanisms to defend against pathogens. However, new evidence suggests that the immune capacity of organisms is related with the symbiotic microorganisms. Indeed, all organisms live in close association with a variety of microorganisms, called microbiota, and several studies support a fundamental role of the composition of this microbiota in the health and homeostasis of vertebrates and invertebrates. In this context, the aim of this work was to determine the microbiota composition of the scallop *Argopecten purpuratus,* one of the most important reared bivalve species in northern Chile and Peru, and to assess changes in the scallop bacterial microbiota during the host immune response. For this, adult scallops were immune stimulated by an injection of heat-killed *Vibrio splendidus* and collected 24 h and 48 h after for analysis. The activation of the host immune response was established by the transcript overexpression of several scallop immune response genes in hemocytes and gills, and confirmed by the protein detection of the antimicrobial peptide big defensin in gills of *Vibrio*-injected scallops at 24 h post challenge. Next, the microbiota structure and diversity from pools of scallops were characterized using 16S rDNA deep amplicon sequencing. Results revealed that the injection of *A. purpuratus* with either filtered sea water or *Vibrio* resulted in a noticeable reduction of the order Bacteroidales and an increase in Mycoplasmatales, Clostridiales, Chlamydiales, among others when compared to non-injected scallops. Notably, particular shifts of some bacterial groups were observed between filtered sea water or *Vibrio* injected scallops, both at 24 and 48 hours. Thus, an overall modulation of the microbiota abundance and diversity according the scallop immune status was detected, allowing the prediction of some changes in the functionality of the microbial community. Finally, DGGE analysis was performed to identify any changes in the scallop microbiota at the individual level. Dendrograms constructed from the single scallop DGGE profiles demonstrate that the changes in the predominant bacterial groups are consistently detected according the immune status of scallops and evident changes occurs 48 hours after the immune challenge. Overall, the present study showed that changes in the structure and diversity of the bacterial communities associated to the scallop *Argopecten purpuratus* are detected after the activation of the host immune response. Now, the relevance of the disruption of the microbial balance in the immune capacity of the host remain to be elucidated. WORK FUNDED BY FONDECYT 11150009.

Keywords: Host-microbiota interactions; scallop; innate immune response; antimicrobial effectors; metagenome

Corresponding author.
E-mail address: paulina.schmitt@pucv.cl (P. Schmitt).
* These authors have contributed equally to this work.

O-114.

Proteomic profile and proteases characterisation of greater amberjack skin mucus after *Neobenedenia girellae* infection

A. Fernández-Montero [1,#,], S. Torrecillas [1,*], F. Acosta [1,*], M.J. Prieto-Álamo [2,*], J. Jurado [2,*], D. Montero [1,*].*

[1] Grupo de Investigación en Acuicultura. Ecoaqua institute. University of Las Palmas de Gran Canaria, Gran Canaria, Spain
[2] Department of Biochemistry and Molecular Biology, Agrifood Campus of International Excellence (ceiA3), University of Córdoba, Severo Ochoa Building, Rabanales Campus, 14071 Córdoba, Spain

Abstract
Skin mucus is known for being the first physical and immunological barrier in fish. Skin mucus is composed by a wide range of proteins, like glycoproteins, structural proteins, metabolic proteins and immune related protein-components. Likewise, proteome changes in Atlantic salmon (*Salmo salar*) have been observed due to sea lice infections, demonstrating the importance of protein-immune defenses against ectoparasite infections. Nowadays, greater amberjack (*Seriola dumerili*) pass-through a biosanitary bottleneck on its on-growing period related with monogenean ectoparasites, which could cause a 90% of mortality (Ogawa et al.,1998). For that reason, this study aimed to compare skin mucus proteome of non-infected and experimentally infected greater amberjack juveniles with *Neobenedenia girellae*, as well as to characterize proteases of this skin mucus. Thirty greater amberjack juveniles of 150 ±12 g were randomly distributed in 3 cylindroconical tanks of 500 liters. After 10 days of acclimation, skin mucus of non-infected fish was obtained, pooled and immediately freeze in liquid nitrogen. Cohabitation with *N.girellae* was conducted with 3 previously infected fish stored in cages for 15 days, when all the experimental fish were infected, skin mucus was sampled. The integrative proteomic approach was conducted using a label-free procedure as LC-MS/MS with a 2-DE-PMF-MS/MS. Protease activity was conducted using azocasein hydrolysis assay, while protease characterization was determined combining azocasein hydrolysis with inhibitors of metalloproteases and serine proteases. Results obtained with LC-MS/MS showed the first microbiota analyses in greater amberjack skin mucus, were the most abundant species belonged to gamma-proteobacteria group, and infected and non-infected fish bacterial presence only differed in 6 genus of bacteria.2-DE-PMF-MS/MS analyses showed differences in proteome profile at a qualitative level. Proteins of p/ 5 and molecular weight ranging between 36-66 KD, typically identified as structure proteins, were clearly affected by degradation for *N.girellae* infected fish. Protease activity analysis showed no difference among infected and non-