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Abstract
Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional neuropeptide that is widely distributed in mammals and is capable of performing roles as a neurotransmitter, neuromodulator and vasodilator. This polypeptide belongs to the glucagon/secretin superfamily, of which some members have been shown to act as antimicrobial peptides in both mammalian and aquatic organisms. In teleosts, PACAP has been demonstrated to have direct antimicrobial activity against several aquatic pathogens, yet this phenomenon has never been studied throughout a live bacterial infection. The present study focuses on the influence of PACAP on the rainbow trout monocyte/macrophage-like cell line, RTS11, when exposed to the coldwater bacterial pathogen *Flavobacterium psychrophilum*. PACAP was shown to have direct antimicrobial activity on *F. psychrophilum* when grown in both cytophaga broth and cell culture media (L-15). Further, the ability of teleostean PACAP to permeabilize the membrane of an aquatic pathogen, *F. psychrophilum*, was revealed for the first time. The viability of RTS11 when exposed to PACAP was also observed using a trypan blue exclusion assay to determine optimal experimental doses of the antimicrobial peptide. Interestingly, when RTS11 was pre-treated with PACAP for 24 hours before experiencing exposure to live *F. psychrophilum*, growth of the pathogen was severely inhibited in a dose-dependent manner when compared to control cells receiving no PACAP pre-treatment. Relative expression of pro-inflammatory cytokines and PACAP receptors was also observed in RTS11 following PACAP exposure alone and in conjunction with live *F. psychrophilum* challenge. These qRT-PCR findings revealed that PACAP may have a synergistic effect on RTS11 immune function. The results of this study provide evidence that PACAP has immunostimulatory activity on rainbow trout immune cells as well as direct antimicrobial activity against aquatic bacterial pathogens such as *F. psychrophilum*. As there are numerous pathogens that impact the aquaculture industry, PACAP may stimulate the teleost immune system while also providing an efficacious alternative to antibiotic use.
Keywords: Rainbow trout, antimicrobial peptide, PACAP, RTS11, *Flavobacterium psychrophilum*

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O-108.

Efficient and long-lasting protection against the pacific oyster mortality syndrome through antiviral immune priming

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Abstract
The major economic and environmental consequences of recurring mortalities affecting the Pacific oyster *Crassostrea gigas* have initiated many research projects aiming at understanding these phenomena. The solutions anticipated to deal with these mortalities are mainly based on mass selection breeding programs but preventive treatments are still lacking. However, over the last decade, studies have been accumulating revealing the adaptive capabilities of innate immunity, the only component of defense mechanisms in invertebrates. Numerous findings have shown that a wide range of invertebrates can develop innate immune memory (also called immune priming) leading to improved survival during a second encounter with a pathogen. In this context, we undertook to study the possibilities of acting against mortalities by stimulating immune capacities of oysters.
In the present study, we show that the exposure of oyster juveniles to an immunostimulant (a viral mimic called poly (I: C)) can lead to enhanced survival capacities (up to 100%) following OsHV-1 infection or during a mortality episode in the field. That protection is specific to viral protection as poly(I:C) fails to protect oyster against a pathogenic bacteria. We also show that this priming phenomenon is durable as it can last more than 4 months suggesting for the first time the existence of mechanisms of immune memory in this invertebrate species. Finally, analysis of the molecular pathways underlying that protection using dual RNAseq, revealed that priming was based on the triggering of a strong and sustained antiviral response limiting replication of the virus, thus allowing the protection of oysters on the long term. Altogether these results bring new insights into the oyster capacities to build an innate immune memory, its adaptive capacities and provide a platform to further explore novel strategies to help mitigate disease threats upon marine bivalves.
Keywords: Immunity, antiviral, OsHV-1, priming, oyster

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O-109.

The immune recognition mechanisms in mollucs

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Abstract
Immune recognition is the first step of immune response, which plays key role in immune protection in all organisms. Since the proposal of pattern recognition theory, numerous pattern recognition receptors (PRRs) have been reported, especially in molluscs where expansive PRRs have been identified by genomic prediction. Taken the model bivalve oyster as example, oysters are constantly threatened by the invasion of pathogens and they have developed a sophisticated repertoire of PRRs to recognize diversiform microorganisms. So far, seven PPRs families, such as peptidoglycan recognition proteins (PGRPs), lectins (including C-type lectins, galectins and sialic acid-binding Ig-like lectins), toll-like receptors (TLRs), C1q domain containing proteins (C1qDCs), Gram-negative binding proteins (GNBPs), scavenger receptors (SRs) and fibrinogen-related proteins (FREPs) have been identified in oysters. What's more, some novel PRRs, such as DM9 domain containing proteins (DM9CPs), Caspases, interleukin 17 (IL-17) and arginine kinase have also been characterized from oysters, which expands our knowledge of PRRs in invertebrates. These various PRRs have been partially validated to be different in recognition specificity, down-stream signal pathway and immune effects. Most of the PRRs serve as multi-functional proteins, not only in immune recognition, but also in the elimination of invading microbes. In addition, these PRRs differentially expressed in mucosal immune tissues and systemic circulatory system where immune recognition taking place. And different effects could be induced by mucosal and systemic immune recognition, such as immune memory or immune tolerance. These results uncovered in molluscs have expanded our knowledge about the classical pattern recognition theory, and also provided theoretical basis for vaccine development in mollusc breeding-species in the future.

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

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O-110.

Hematopoiesis and regulatory signaling in molluscs

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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

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O-111.

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

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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

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O-112.

Immune responses of American oysters to bacterial and parasitic challenge

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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