measuring different cytokine expression and biochemical parameters, as well as phagocytic activity.

keywords: *Dicentrarchus labrax*, fish cytokines, head-kidney macrophages, cameline oil, sea bass serum

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P-057.

Characterization and expressional analysis of *Salmo salar* heat shock proteins in response to *Piscirickettsia salmonis* infection under a cohabitation challenge

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Abstract

Heat shock proteins (HSPs) comprise several families of highly conserved proteins which function mainly as chaperonines that refold proteins in response to stressful stimuli (changes in pH, salinity, temperature, radiation, among others) and are players in the host immune system activation during infection. The different HSP families are roughly grouped according to their molecular weights (such as Hsp110, Hsp100, Hsp90, Hsp70, Hsp60 and small HSPs). HSPs have been widely characterized as being modulated by the aforementioned stimuli, and many have been linked to processes like signaling, apoptosis and regulation of homeostasis, in addition to their chaperonine functions. In previous studies exploring the modulation of gene expression for HSPs, results have been varying, mostly depending on the kind of stressing agent and host organisms. Infection by virus and bacteria show mostly an up-regulation of small HSPs and down-regulation of some constitutively expressed HSPs (cognates). The present study aims to characterize several HSPs (4 in the Hsp30 group, Hsp60, 2 Hsp70 proteins and 6 Hsp90-like proteins, for a total of 13 HSPs) in *Salmo salar* in terms of phylogenetic relationships, conserved synteny, basal gene expression in several tissues and modulation of gene expression at the transcript level in response to infection (via cohabitation challenge for 7 weeks) using two field strains of the bacterium *Piscirickettsia salmonis* (which is the most relevant pathogen in Chilean aquaculture and the etiological agent of Piscirickettsiosis) and under two different salinities (5 and 20%). The field strains used (AUS005 and AUS111) belong to two different genogroups (LF-like and EM-like) and were isolated from differing marine environments in terms of salinity (freshwater and estuary). Results show distinct patterns of gene expression for hsps in each tissue, with most of them expressed predominantly in liver and kidney. During infection, differential expression patterns were observed for most of the HSPs studied, generally showing a sharp up-regulation of *hsp30* genes in the initial phases of the challenge, up-regulation of *hsp90* genes and, interestingly, a slight down-regulation of *hsp60* and *hsp70* genes. These data demonstrate that *Salmo salar* HSPs possibly play a role in the immune response of fish against a bacterial infection and encourage further research in order to elucidate their concrete roles in those processes.

keywords: Heat shock proteins, *Salmo salar*, *Piscirickettsia salmonis*, cohabitation challenge, stress.

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P-058.

De novo assembly, characterization of tissue-specific transcriptomes and identification of immune related genes from the scallop *Argopecten purpuratus*

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Abstract

The scallop *Argopecten purpuratus* is one of the most economically important cultured mollusks on the coasts from Chile and Peru but its production has declined due, in part, to the emergence of mass mortality events of unknown origin. Driven by this scenario, increasing progress has been made on recent years in the comprehension of the immune response mechanisms in this species. However, it is still not entirely understood how different mucosal interfaces participate and cooperate with the immune competent cells, the hemocytes, in the immune defense. Thus, in this work we aimed to characterize the transcriptome of three tissues with immune relevance from *A. purpuratus* by next generation sequencing and de novo transcriptome assembly. For this, 18 cDNA libraries were constructed from hemocytes, gills and digestive gland tissues of scallops from different immune conditions, and sequenced by the Illumina HiSeq4000 platform. A total of 967.964.884 raw reads were obtained and 967.432.652 clean reads were generated. The clean reads were *de novo* assembled into 46.601 high quality contigs and 32.299 (69.31%) contigs were subsequently annotated. In addition, three de novo specific assemblies were performed from clean reads obtained from each tissue cDNA libraries for their comparison. Gene ontology (GO) and KEGG analyses revealed that annotated sequences from hemocytes, gill and digestive gland could be classified into both general and specific subcategory terms and known biological pathways, respectively, according to the tissue nature. Finally, several immune related candidate genes were identified, and the differential expression of a mannose receptor, C-type lectin, C1q, MPEG-1, IL-17 and a scavenger receptor CD163 genes was established as tissue-specific, suggesting they could display specific roles in the host defense. The data presented in this study provides the first insight into the tissue specific transcriptome profiles of *A. purpuratus*, which should be considered for further research on the interplay between the hemocytes and mucosal immune responses.

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keywords: RNA-seq; tissue-specific immune genes; scallop; mucosal interfaces; antimicrobial effectors

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P-059.

Cellular and humoral immune responses of meagre (*Argyrosomus regius*) juveniles to bacterial infection with *Photobacterium damselae piscicida*

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Abstract

One of the challenges of the fish farming industry is the occurrence of disease outbreaks that can lead to important monetary losses. In this context, the study of suitable biomarkers to assess the fish health status, such as haematological and immune responses during the first steps of infection could assist in the creation of measures of recognition and prevention of disease. The present study was conceived to evaluate meagre (*Argyrosomus regius*) innate immune response after infection with *Photobacterium damselae piscicida* (*Phdp*).

A time-course study was performed at CETEMARES (Instituto Politécnico de Leiria, Peniche, Portugal) facilities with 36 animals being sampled (79.3 ± 15.1 g). Among them, 12 fish were randomly selected and sampled before infection (time 0 h). Thereafter, the remaining animals were randomly selected and intraperitoneally injected (i.p.) with 100 µl PBS (control group) or 100 µl of bacteria (105 CFU/mL; infected group) and distributed as a randomized complete design in 6 recirculating systems (i.e. triplicates per experimental condition). Two animals per tank ($n=6$) were randomly selected and sampled at 6 and 24 h after i.p. injection. At each sampling point, fish were anaesthetized with 2-phenoxyethanol and blood samples were collected for haematological procedures such as total and differential counting of peripheral leukocytes and total circulating erythrocytes counts. The remaining blood was centrifuged and plasma was collected for innate humoral parameters determination (i.e. bactericidal, antiproteases and peroxidase activities). Results showed similarities among cellular and humoral parameters in challenged fish. Infected meagre presented an increased peripheral white blood cells concentration compared to control individuals. Peripheral lymphocyte numbers increased in infected meagre from 0 h to 24 h while circulating neutrophils decreased in challenged fish regardless time, most likely due to migration of these cells to the peritoneal cavity (inflammatory focus). Plasma bactericidal activity increased in infected specimens after 24 h. Samples of head-kidney tissue will be suited the assessment of mRNA immune-related gene expression in order to understand how *Phdp* infection influences meagre immune machinery.

keywords: Infection, leukocytes, immune response, bactericidal activity, neutrophils.

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P-060.

Impact of high temperature on the innate immune response in the intestine of orange-spotted grouper

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Abstract

Temperature is a well-known important factor to the immune response in poikilothermic animals, such as fish. Studies in teleost fishes have shown that production of many innate immunity and adaptive immunity factors are temperature-dependent. We report an investigation on the impact of increased water temperature on the innate immune response in different regions of the intestine (anterior gut, midgut, & posterior gut) of orange-spotted grouper (*Epinephelus coioides*). In the study, fish were subjected to two modes of temperature shifting: shock treatment (temperature increased from 28°C to 32°C sharply) and adaptive treatment (temperature increased from 28°C to 32°C at the rate of 1°C per day). Our study showed that the shock and adaptive treatments exerted differential impacts on the expression of immune-relevant genes in the three regions of intestine. In fish subjected to immune stimulations, including poly I:C, lipopolysaccharide (LPS) and CpG oligodeoxynucleotide (ODN), shock and adaptive treatments also exerted differential impacts on the induction of immune genes in the three regions of intestine. Interestingly, the negative impact by increased temperature on the induction of immune genes was most noted in the mid-section of the gut. The differential effects of increased temperature in different regions of intestine might reflect the diversity in cellular composition and even microbiota in the regions.

keywords: Temperature, immune stimulants, innate immunity, intestine, grouper

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P-061.

The turbot (*Scophthalmus maximus*) myeloperoxidase: Characterization and functional studies

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Abstract

Myeloperoxidase (MPO) is a major enzyme that is mainly present in fish neutrophils. This enzyme is well characterised in mammals but little is known about its structure and function in fish. In this study, we sequenced the turbot MPO and studied some of its functions in turbot. The 5690 bp turbot myeloperoxidase gene contains an ORF with 14 exons. In addition to the 13 introns of the ORF, there is one intron of 134 nucleotides located in the 5UTR region. The untranslated 5' and 3′regions have 111 bp and 970 bp respectively. The coding sequence contains 2301 nucleotides that encode a polypeptide of 767 aa with a predicted molecular mass of 86.21 kDa. BLASTp analysis revealed that turbot MPO displays high similarity to the MPO of other fish species (identity varied between 60 and 82%) and lower than those of mammals (identity 50%) and reptiles (identity 47%). Turbot MPO was found to have several conserved domains such as the signal peptide, propeptide (118 aa) and light (113 aa) and heavy chains (591 aa). Other important sites for regulation of MPO activity are also present in the turbot molecule, including distal haem cavities I and II and proximal haem cavities I and II. Several catalytic, haem linkage and cysteine residues, a Ca+2-binding motif and also eight potential N-linked glycosylation sites were identified. Western blot analysis and use of an anti-turbot MPO polyclonal antibody revealed that turbot MPO exists in its mature form as a homodimer of about 150 kDa in the anterior kidney, spleen, peritoneal fluid and serum, indicating that the protein loses the propeptide during maturation. The MPO transcripts were most strongly expressed in the anterior kidney, gill, white blood cells and spleen, and they were most weakly expressed in liver, muscle, heart and brain. Immunofluorescence was used to identify cells compatible with neutrophils containing MPO + granules in the anterior kidney, spleen, blood, gill and intestine. In an in vitro stimulation test, anterior kidney leukocytes (HKL) were isolated on a Percoll gradient and stimulated with purified MPO obtained by affinity chromatography. Incubation of HKL with turbot MPO generates positive regulation of the proinflammatory cytokines TNF-α and IL12-β, the cytokine IFNγ, the chemokine IL8 and the subunits of the CD11b / CD18 integrins, suggesting that, in addition to its microbicidal activity, the MPO may act as a mediator of the immune response in fish.

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