LIVER GENE EXPRESSION IN *SOLEA SENEGALENSIS* DURING *PHOTOBACTERIUM DAMSELAE* SUBSP. *PISCICIDA* INFECTION

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Introduction

Photobacterium damselae subsp. *piscicida (Phdp)* is responsible for important outbreaks affecting several fish species including flatfish *Solea senegalensis*. Information on the responses of *S. senegalensis* to *Phdp* infection is scarce. Thus, it is necessary to determine the scenario in which infection takes place in order to understand the progression of the disease. In the present work, expression of several genes with biological importance has been studied in *S. senegalensis* after infection with *P. damselae* subsp. *piscicida*.

Materials and methods

Senegalensis sole specimens (mean weight ± s.d. 130 ± 15g, n=60) were kept in two tanks at 20-22ºC and fed once a day. *Phdp* Lg41/01 strain was grown at 22ºC in tryptic soya broth supplemented with 1.5% NaCl, suspended in sterile saline (PBS) and intraperitoneally injected (dose 10^4 cfu per fish). Control fish were injected with sterile PBS. Liver samples were obtained after 24, 48 and 72 hours and stored at -80ºC. RT qPCR was carried out using primers for the following Senegalensis sole genes: heat shock protein gp96 (GP96), heat shock protein 90AA (HSP90AA), heat shock protein 90AB (HSP90AB), complement component 3 (C3), complement component 7 (C7), interleukin 1β (IL-1β), interleukin 6 (IL6), heat shock protein 70 (HSP70), glutathione peroxidase (GPx), g lysozyme (gLYS), natural resistance-associated macrophage protein 1 (NRAMP1), TNF receptor-associated factor 3 (TRAF-3), caspase 6 (C6), ubiquitin (UBQ), tumor necrosis factor α (TNF-α), transforming growth factor β 1 (TGF-β1), sequestosome 1 (SQSTM1), non-specific cytotoxic cell receptor protein 1 (NCCRP1), hepcidin (HAMP1), transferrin (TF), ferritin M (FERR_M) and haptoglobin (HP-1).

Results

Mortality in the infected fish group reached 35% and *Phdp* was reisolated from all dead fish in pure culture. Gene expression values were calculated by the $2^{(-\Delta\Delta Ct)}$ method relative to β-Actin 2 (ACTB2) and ribosomal protein S4 (RPS4) *S. senegalensis* internal reference genes. IL-1β, IL6, NRAMP-1, C6, TGF-β1 and SQSTM1 gene expression was not detected in liver tissues. In addition, TF, UBQ, FERR_M, HSP90AA, HSP90AB and HSP70 did not change transcription levels compared to non infected soles. On the contrary, LYS-G, HP-1, TNF-α, TRAF-3, NCCRP1, C7 and stress proteins GPx and GP96 were up-regulated 24h post-infection. Increased transcription of C3 gene was observed only 48h after infection (figure 1), whereas HAMP-1, HP and C3 were down-regulated 48 or 72h post-infection.

Discussion and conclusion

Understanding the immune response against *Phdp* infection in sole is a prerequisite to the development of effective treatments. NCCRP1 was highly up-regulated first days post-infection, playing an important role in the innate immune response to bacteria, with crucial functions in target cell recognition and cytotoxicity activation. Lysozyme activity is involved in innate immune responses after bacterial infections. Up-regulation of gLYS gene has been reported in several fish species after infection by *V. anguillarum* and *E. tarda* (Wang et al., 2013). In the present study, gLYS gene up-regulation has been observed in *Phdp* infected *S. senegalensis* liver tissues 24, 48 and 72h post-infection.

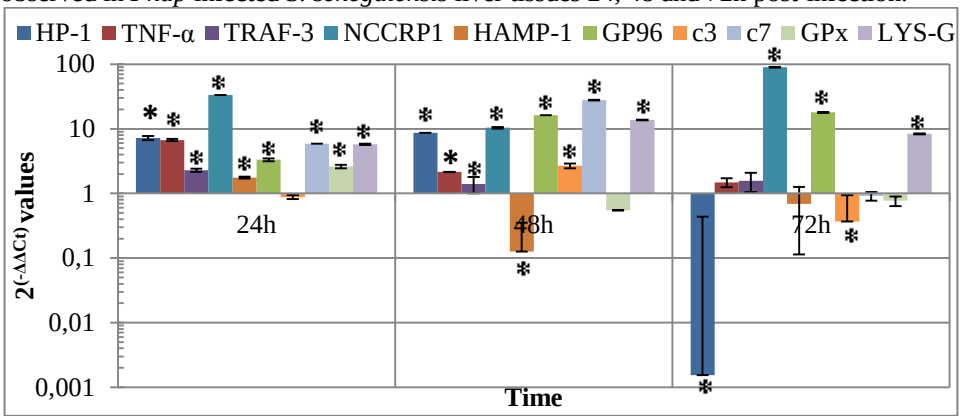

Fig. 1. qPCR analysis of upregulated or downregulated gene expression associated with immune response, apoptotic and iron metabolism. Statistical significance is denoted as: *$P < 0.05$ (ANOVA).

Activation of the complement system through any of the three pathways results in the activation of C3 (Wang et al., 2013). Up-regulation of C3 gene transcription started after 48h infection and maintained after 72h post-infection. On the contrary, C7 transcription is increased only 24 and 48h post-infection. C7 is a component involved in bacterial lysis. Although other components also participate in bacterial inactivation, lack of one of them may increase susceptibility to the pathogen. Higher transcription levels found in TNF-α gene at 24 and 48h may be responsible for cytokine accumulation in an inflammatory response. GPx and GP96 increases after 24h post-infection indicate fish response to stress due to *Phdp* infection as suggested by (Cha et al, 2013). Regarding iron metabolism, HP-1 transcription was increased 24h and 48h after infection. Decreased HP-1 protein levels resulting from gene down-regulation may lead to lower iron levels in plasma as host defense mechanism to deny bacteria access to the metal (Dunham el al., 2009).

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