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TRANSCRIPTOMIC PROFILES OF POST-SMOLT ATLANTIC SALMON CHALLENGED WITH *Piscirickettsia salmonis* REVEAL A STRATEGY TO EVADE THE ADAPTIVE IMMUNE RESPONSE AND MODIFY CELL-AUTONOMOUS IMMUNITY

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INTRODUCTION

Piscirickettsiosis (SRS) is the main bacterial disease affecting the Chilean salmon farming industry and is responsible for high economic losses. The development of effective strategies to control SRS has been limited in part by insufficient knowledge of the host response. The aim of this study was to use RNA sequencing to describe the transcriptional profiles of the responses of Atlantic salmon infected with LF-89 or EM-90 *Piscirickettsia salmonis*.

METHODOLOGY

The cohabitation challenge was conducted at the Clinical Salmon Trials station in Valdivia, Chile. Head-kidney tissues were collected from 5 Trojan fish and 5 control fish per tank at 5 days post-inoculation (dpi) and from 5 cohabitant fish and 5 control fish at 35 dpi of the Trojan fish per tank (Fig. 1).

Libraries for the fish from each tank were prepared using the TruSeq RNA Library Preparation Kit v2 Set A. For sequencing, 12.5 pM RNA from the pool and 1% PhiX were loaded into the Illumina MiSeq sequencer and sequenced using the MiSeq Reagents v3 600 cycles sequencing kit (Illumina Inc., San Diego, CA, USA) (Fig. 1).

To validate the reliability of the RNA-seq data, quantitative real-time PCR was conducted on 12 randomly selected representatives and differentially expressed genes.

Cohabitation model
Fish: S. salar, 118.4 g
Health status: Negative
Infection pressure: 30%
Isolates: LF-89; EM-90 (0.1 ml / fish)
DO625: 0.057, 0.056 (105.6 CFU / mL)
Marking: cut dorsal fin (T)
No. of replicas: 2
No. tanks: 5
Total fish number: 750
Water salinity: 15 ppt
Water temperature: 12°C
Duration: 9 weeks

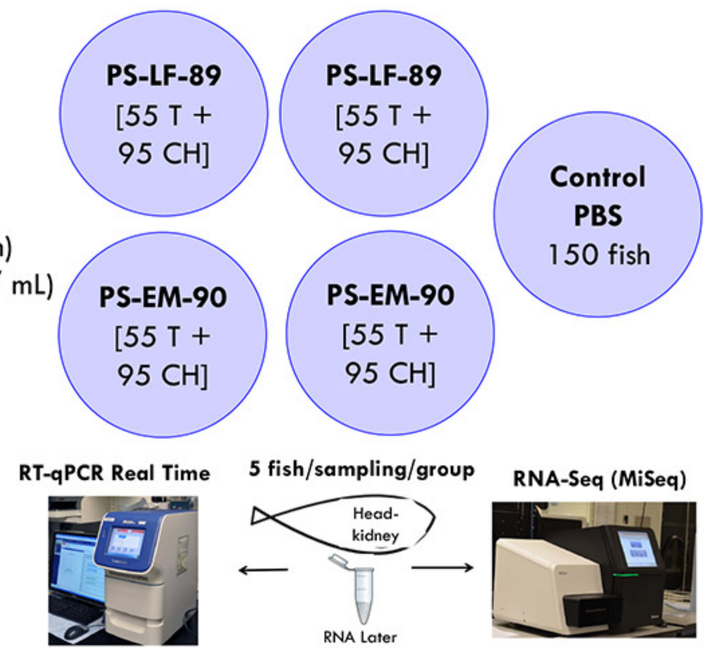

Figure 1. Schematic summary of the experimental design.

RESULTS

Six functional categories of genes were differentially expressed: 1) pathogen/antigen recognition; 2) cytoskeleton, cell interaction and signal transduction; 3) oxidative stress response; 4) adaptive immunity; 5) lysosome, phagosome, endosome and autophagy; and 6) cell cycle progression, proliferation and apoptosis.

Enrichment and pathway analyses of the differentially expressed genes revealed several central signatures following infection, including positive regulation of DC-SIGN and TLR5 signalling, which converged at the NF-kB level to modulate the pro- inflammatory cytokine response, particularly in the PS-EM-90-infected fish. *P. salmonis* induced an IFN-inducible response (e.g., IRF-1 and GBP-1) but inhibited the humoral and cell-mediated immune responses.

P. salmonis induced significant cytoskeletal reorganization but decreased lysosomal protease activity and caused the degradation of proteins associated with cellular stress. Infection with these isolates also delayed protein transport, antigen processing, vesicle trafficking and autophagy. Both *P. salmonis* isolates promoted cell survival and proliferation and inhibited apoptosis.

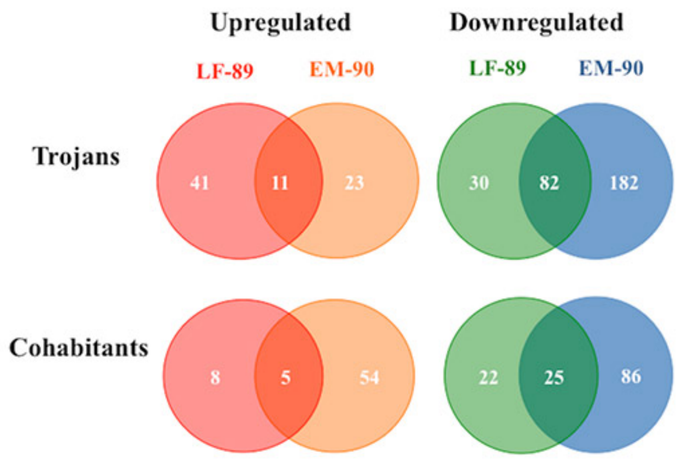

Figure 2. Venn diagram showing the number of transcriptionally up/downregulated genes determined by RNA-seq in the head kidneys of the Trojan and cohabitant fish infected with PS-LF-89 and PS-EM-90. The expression of all depicted genes was significantly altered (p .1) with > 1-fold or < 1-fold expression changes.

CONCLUSIONS

Our study showed the most important biological mechanisms used by *P. salmonis*, regardless of the isolate, to evade the immune response, maintain the viability of host cells and increase intracellular replication and persistence at the infection site. These results improve the understanding of the mechanisms by which *P. salmonis* interacts with its host and may serve as a basis for the development of effective strategies for the control of SRS.

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