

# EARLY IMMUNE RESPONSE IN ATLANTIC SALMON VACCINATED WITH INACTIVATED WHOLE-CELL BACTERIN FROM *Piscirickettsia salmonis* OR INFECTED WITH PATHOGENIC ISOLATES

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## INTRODUCTION

Piscirickettsiosis (SRS) is the most challenging disease affecting the Chilean salmon industry. The control of SRS has focused mainly on chemotherapy and vaccination. Although the available vaccines based on bacterins, recombinant subunits and/or live-attenuated bacteria have not prevented SRS in Chile, they have delayed onset of the first outbreak.

## METHODOLOGY

To evaluate the expression of immune response genes we used head kidney samples obtained from Atlantic salmon i.p. infected with the LF-89 and EM-90 isolates of *P. salmonis* (Fig. 1). Other group was i.p. injected with 0.1 ml of inactivated whole-cell bacterin from *P. salmonis* ( $5.7 \times 10^5$  to  $2.5 \times 10^6$  *P. salmonis* and oil adjuvant). Five fish were sampled from each tank at 1, 3, 5, 7 and 14 days post inoculation. RNA was extracted from head kidney and relative quantification of immune-related genes was performed for the fish from each experimental group by normalized RT-qPCR (Fig. 2).

### Intraperitoneal model

Fish: *S. salar*, 118.4 g  
Health status: Negative  
Isolates: LF-89; EM-90 (0.1 ml / fish)  
DO<sub>625</sub>: 0.057, 0.056 (10<sup>5.6</sup> CFU / mL)  
Vaccine: Inactivated whole-cell bacterin  
No. of replicas: 2  
No. tanks: 6  
Water salinity: 15 ppt  
Water temperature: 12°C  
Duration: 4 weeks

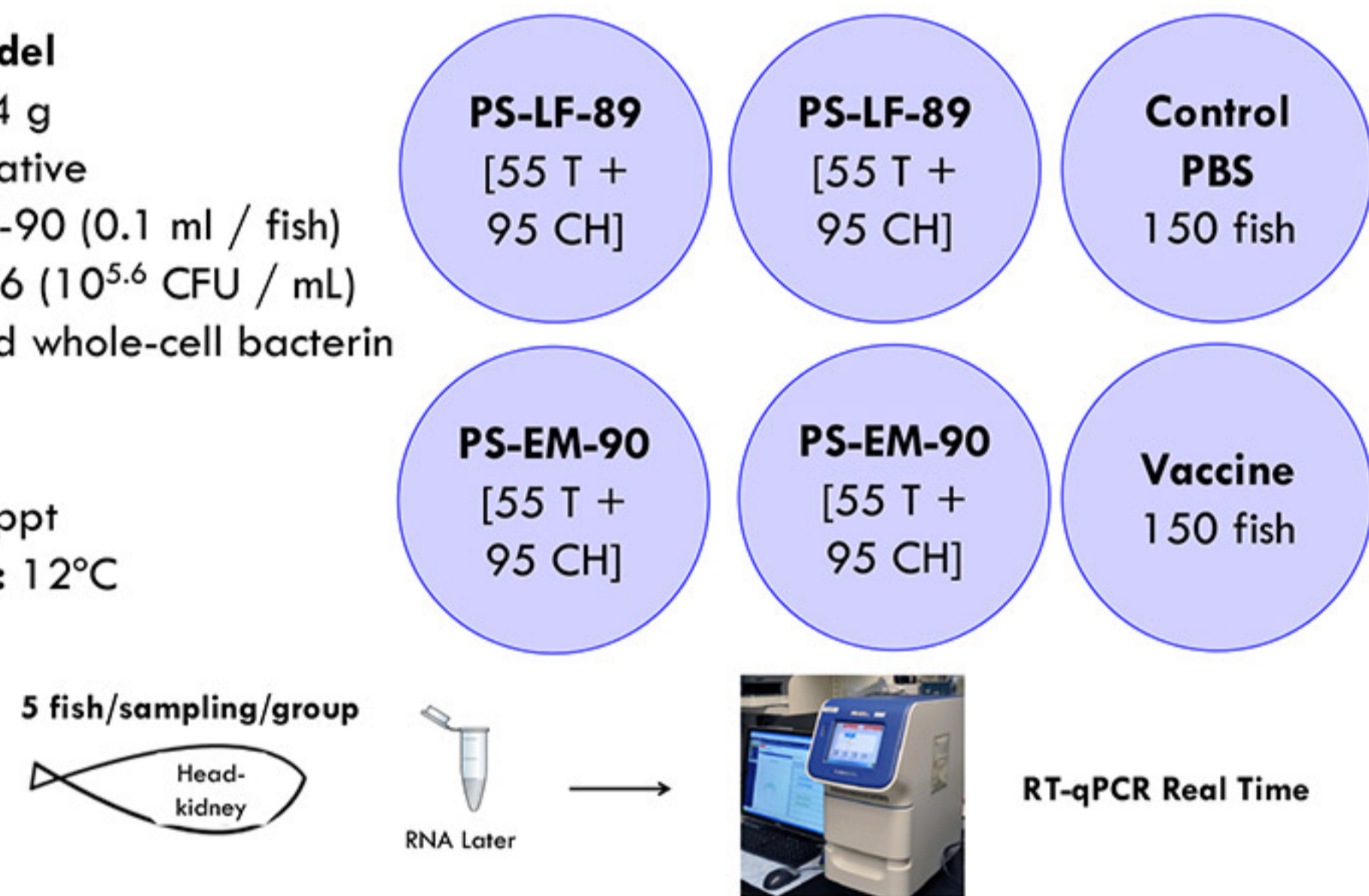


Figure 1. Schematic summary of the experimental design.

## RESULTS

1. Fish infected with PS-LF-89 showed an anti-inflammatory response, but this finding was not observed in PS-EM-90-infected fish or vaccinated fish.
2. Fish infected with both *P. salmonis* isolates showed *mhc1-mhc2*, *cd4-cd8b* and *igm* overexpression, suggesting that *P. salmonis* promotes CD4+ and CD8+ T cell responses and the humoral immune response.
3. Vaccinated fish exhibited *mhc1*, *mhc2* and *cd4* overexpression but a significant downregulation of *cd8b* and *igm*, suggesting that the vaccine supported the CD4+ T cell response but did not induce an immune response mediated by CD8+ T cells or a humoral response.

Gene	P. salmonis isolate	Relative expression, head-kidney (days post-inoculation, dpi)				
		1	3	5	7	14
<b>Mediators of innate immunity and proinflammatory cytokines</b>						
IFN $\gamma$	LF-89	7.44	2.70	2.49	3.05	2.47
	EM-90	7.69	1.60	4.90	1.30	2.82
	Vaccinated	8.38	2.59	3.23	1.69	1.68
TNF $\alpha$	LF-89	5.08	1.00	-1.58	-5.64	2.43
	EM-90	3.21	-2.11	14.25	-1.54	1.46
	Vaccinated	2.58	0.96	1.15	1.14	1.44
IL-1 $\beta$	LF-89	7.83	1.72	1.65	2.57	6.13
	EM-90	1.72	1.59	25.20	2.10	3.33
	Vaccinated	4.38	1.42	2.00	-1.13	2.68
IL-2	LF-89	37.80	7.93	1.90	2.19	4.40
	EM-90	2.95	-2.07	1.73	-1.20	2.08
	Vaccinated	31.75	8.32	9.50	2.31	-5.74
IL-6	LF-89	9.64	1.04	5.11	2.46	1.68
	EM-90	3.34	-2.30	3.84	-2.24	-3.39
	Vaccinated	5.27	-2.40	3.40	3.53	-2.30
IL-8	LF-89	3.17	-2.62	-1.90	-2.37	2.07
	EM-90	3.72	-3.77	2.52	-1.80	1.76
	Vaccinated	2.18	-1.85	-1.54	-2.99	1.53
IL-10	LF-89	28.32	2.57	1.79	2.57	2.73
	EM-90	45.18	4.78	6.43	1.47	4.10
	Vaccinated	3.78	1.84	3.44	8.84	1.91
IL-12 $\beta$	LF-89	5.22	-1.04	-1.86	-1.16	2.11
	EM-90	8.02	2.12	1.84	1.70	-1.45
	Vaccinated	5.70	-1.18	1.18	-1.56	-1.57
IL-15	LF-89	104.48	26.95	33.46	17.95	113.78
	EM-90	146.56	15.57	47.44	12.61	126.55
	Vaccinated	183.78	48.73	62.83	5.79	1.17
IL-18	LF-89	230.88	70.92	48.14	48.83	109.11
	EM-90	498.11	105.12	100.95	154.70	83.69
	Vaccinated	462.86	88.41	105.18	28.98	25.65
<b>Cell-mediated immunity &amp; Humoral immunity</b>						
MHC-I	LF-89	18.30	2.70	3.08	3.20	10.83
	EM-90	18.12	6.60	9.84	9.75	11.35
	Vaccinated	18.28	7.07	7.52	2.49	2.94
MHC-II	LF-89	4.52	1.90	2.17	1.08	2.23
	EM-90	3.65	2.11	1.59	1.24	2.31
	Vaccinated	2.16	1.81	2.85	1.49	1.96
CD4	LF-89	11.04	3.44	4.09	4.72	5.33
	EM-90	9.94	4.74	3.72	2.30	4.57
	Vaccinated	6.22	3.44	5.98	2.23	6.31
CD8 $\beta$	LF-89	1.98	1.36	2.81	-2.17	2.23
	EM-90	2.98	1.23	1.14	1.06	5.54
	Vaccinated	2.76	2.65	2.27	-3.98	-1.70
IgM	LF-89	7.35	3.38	3.22	1.16	4.41
	EM-90	15.84	4.14	3.87	3.31	6.22
	Vaccinated	2.99	3.85	5.78	-1.11	-1.36

Figure 2. Relative expression of genes related to the innate immune response and the adaptive humoral and cell-mediated immune responses, as determined by RT-qPCR. Each box represents the average expression level in five fish, which is presented as the fold change  $\pm$  SEM compared with the average expression level in five control fish.

## CONCLUSIONS

The expression patterns of genes related to the humoral and cell-mediated adaptive immune responses showed upregulation in fish infected with *P. salmonis* and downregulation in vaccinated fish. The results of this study contribute to our understanding of the immune response against *P. salmonis* and can be used in the optimization of SRS prevention and control measures.