DETERMINATION OF PRO-IMMFLAMATORY CYTOKINES EXPRESSION IN SALMONIDS MACROPHAGES INDUCED BY OUTER MEMBRANE VESICLES PURIFIED FROM *PISCIRICKETTSIA SALMONIS*.

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ABSTRACT

*Piscirickettsia salmonis* is a Gram-negative intracellular bacterium that causes Piscirickettsiosis in salmonids farms in Chile. It was recently reported that *P. salmonis* produces exotoxins that play a role in the pathogenesis. However a delivery system has not yet been identified. Outer membrane vesicles (OMVs) are 10-300 nm spherical-bilayer structures discharged from the surface of many Gram-negative bacteria, which are able to deliver toxins and virulence factors. Recently, we described the production of OMVs by *P. salmonis* in both, in normal growth in broth and inside the host cells after infection. However, pro-immunflamatory cytokines expression induced by *P. salmonis* OMVs has not yet been characterized. Thus, the aim of this study was to investigate if OMVs purified from *P. salmonis* are able to generate an immflamatory immune response in fish macrophages.

*P. salmonis* was grown in basal broth supplemented with Cysteine (3.18 mM) and ferric chloride (0.05 mM) at 18°C until early stationary phase. Bacteria were removed by centrifugation (5000 x g, 10 min at 4°C) and the supernatant were filtrated through a 0.22-μm-pore-size filter. Then, vesicles present in the bacterial free supernatant were isolated by ultracentrifugation (125,000 x g, 2h at 4°C) and analyzed by SDS-PAGE. For gene expression analysis, specific primers for IL-1ß, IL-8 and TNF-α genes were designed to evaluate changes in gene expression in primary culture of macrophages isolated from head kidney. Thus, 1x10⁶ cells were incubated with 5, 10 or 20 µg of purified OMVs and gene expression was evaluated by real time PCR. 18S and EF-1α were used as reference genes for normalization of the relative transcription levels.

*P. salmonis* is able to produce OMVs. The purified OMVs were able to generate a strong pro-immunflamatory immune response in fish macrophages. Thus, IL-1ß, IL-8 and TNF-α genes were differentially expressed in fish macrophages after incubation with *P. salmonis* OMVs, suggesting a potential role in fish immune cells. Additionally, *P. salmonis* OMVs could be a potential candidate for vaccine development against Piscirickettsiosis.

KEYWORDS

Piscirickettsia salmonis, SRS, OMVs, Proteome, fish immune response
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