INTERACTION, INTRACELLULAR REPLICATION AND CYTOPATHIC EFFECT OF A HIGHLY PATHOGENIC CHILEAN ISOLATE OF *PISCIRICKETTSIA SALMONIS* IN SHK-1 CELLS.

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ABSTRACT

Piscirickettsia salmonis is a Gram-negative, facultative intracellular bacterium, which is the etiologic agent of Piscirickettsiosis, a systemic infection of multiple organs and tissues among as kidney, liver, spleen, brain, intestine, ovaries, and gills in several salmonids species such as Rainbow trout (*Oncorhynchus mykiss*), Atlantic salmon (*Salmo salar*) and Coho salmon (*Oncorhynchus kisutch*), causing high mortality and serious economic losses for fish farming in the south of Chile. *P. salmonis* is able to infect, evade the immune response and replicate inside of several fish cell lines such as RTS-11, SHK-1 and CHSE-214. However, there are no reports concerning the infection and lifestyle of *P. salmonis* inside SHK-1 cell line. Thus, the aim of this study was to characterize, in a time-course, the infection and intracellular lifestyle of *P. salmonis* in SHK-1 fish cell line.

SHK-1 cells were cultured at 20ºC in 75 cm^2 flask, in Leibovitz's L-15 medium supplemented with 10% FBS. *P. salmonis* AUSTRAL-005 isolate and LF-89 type strain were grown in *AUSTRAL-SRS* broth at 18°C with moderate agitation (50 rpm) until logarithmic phase. SHK-1 cells ($2x10^4$ cell/well) or ($3x10^5$ cells/well) were seeded onto 8-well chamber slides (Lab-Tek) or in 24-well culture plates with L-15 medium supplemented with 2% FBS for confocal microscopy or transmission electron microscopy (TEM), respectively. Then, monolayers containing adherent cells were infected with *P. salmonis* at multiplicity of infection (MOI) 20 bacteria per cell. For confocal microscopy, infected and control cells were fixed in 4% (wt/vol) freshly paraformaldehyde for 15 min at room temperature (RT) at the following post-infection (pi) times: 5, 15, and 30 min, 1, 2, 3, 4, and 6 h. *P. salmonis* was stained using an oligoclonal antibody anti-*P. salmonis* (BIOS Chile S. A). Additionally, BrdU was used to evaluate the intracellular replication. Samples were analyzed with an Olympus FluoView™ FV1000 confocal laser scanning microscope. For TEM, infected and control SHK-1 cells were fixed for 2 h at RT in 2.5% (vol/vol) freshly glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2. Finally, samples were observed in a JEOL JEM-1230 transmission electron microscope at 80kV.

Results showed that *P. salmonis* was found attached to the plasma membrane as early as 15 min pi, interacting tightly with the plasma membrane. Finally at 3 h pi and longer, *P. salmonis* was detected

inside discrete cytoplasmic vacuoles. After 4 days pi and longer, SHK-1 cells shown a high number of actively replicating bacteria labelled with BrdU inside the vacuole. Interestingly, outer membrane vesicles were detected near to *P. salmonis* inside the vacuole.

KEYWORDS
Piscirickettsia salmonis, SHK-1, BrdU, infectivity, intracellular replication

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