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Immunomodulatory roles of *Edwardsiella piscicida* and *Streptococcus parauberis* derived extracellular vesicles in Zebrafish

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Extracellular vesicles (EVs) are lipid bilayer vesicles (size 20-1000 nm) released by all three domains of life namely eukaryotes, bacteria, and archaea. Bacterial extracellular vesicles (BEVs) are involved in evolutionarily conserved mechanisms for intercellular communication and activation of immune signaling pathways. In this study, we performed a comparative analysis of BEVs isolated from two fish pathogenic bacteria namely *Edwardsiella piscicida* (*Ep*-EVs) and *Streptococcus parauberis* (*Sp*-EVs), and investigated their immunomodulatory activities. *Ep*-EVs and *Sp*-EVs were isolated using respective bacterial culture supernatants by ultracentrifugation process. Analysis of BEVs size and particle concentration was conducted using the Nano-Sight NS300. After confirming the cellular internalization, qRT-PCR and immunoblot were conducted to determine immune-related genes (kidney) and protein expression (spleen) in *Ep*-EVs and *Sp*-EVs-treated zebrafish. Transmission electron microscopy (TEM) results confirmed the spherical shape of *Ep*-EVs and *Sp*-EVs. The average particle size of *Ep*-EVs and *Sp*-EVs were 85.3 ± 1.8 nm, and 168.3 ± 6.5 nm, respectively. SDS-PAGE analysis confirmed that both *Ep*-EVs and *Sp*-EVs contain differently expressed proteins with varying molecular weights. Fluorescent-labeled *Ep*-EVs and *Sp*-EVs showed cellular internalization in fathead minnow (FHM) cells. qRT-PCR and western blot analysis results revealed *Ep*-EVs and *Sp*-EVs injected zebrafish modulate the transcription of immune functional genes (toll-like receptors, interleukins, chemokines, and heat-shock proteins) and proteins suggesting their potential in therapeutic applications.

Keywords: Extracellular vesicles, Edwardsiella piscicida, immunomodulation, Streptococcus parauberis, Zebrafish

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