VACCINATION WITH OUTER MEMBRANE VESICLES AGAINST INTRACELLULAR PATHOGENS FOR AQUACULTURE

THE CASE OF FRANCISELLOSIS IN A ZEBRAFISH MODEL

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ABSTRACT

Vaccines development against many extracellular bacteria has been a success for the sustainability of the aquaculture industry. In contrast, infection of fish with intracellular pathogens remains largely an unresolved problem. Francisellosis is a bacterial disease in fish that presents nonspecific clinical signs and can cause high mortalities. *Francisella noatunensis (Fn)*, the causative agents of francisellosis, is a non-motile, Gram-negative, facultative intracellular bacterium. *Fn* consists of two subspecies; *F. noatunensis* subsp. *orientalis* (*Fno*) that cause disease in "warm-water" fish, like tilapia, while *F. noatunensis* subsp. *noatunensis* (*Fnn*) cause disease in fish living in colder waters, like cod.

Production of membrane vesicles by cells is a conserved mechanisms occurring throughout all domains of life. Outer membranes vesicles (OMVs) secreted from Gram-negative bacteria are spherical, 10-300 nm in diameter, and consist of a phospholipid bilayer with outer membrane proteins, endotoxin and a lumen with periplasmic proteins. Their secretion is associated with a variety of traits including the discharge of virulence factors during infections. Isolated OMVs are promising vaccine candidates against diseases caused by intracellular bacteria and have been used successfully as a vaccine against meningitis in humans. In the present work, we show that intact OMVs can be isolated from broth-cultured *Fno.* Proteomic analyses reveal that the vesicles includes large sets of proteins involved in the host immune response, such as GroEL, OmpA and ClpB, which play an important role in the initial infection stages and the bacteria's overall virulence and survival within a host.

Furthermore, *Fno* OMV was tested as a vaccine in an *Fno*-zebrafish infection model, confirming its capacity to significantly reduce the development of francisellosis, inducing the secretion of cytokines as TNFα, IL-1β and IFNγ. Moreover, we report the transcriptional profile of important immune markers such as *mhcii* (major histocompatibility complex class 2) and *mpeg* (macrophage expressed gene), demonstrating that *Fno* mainly infect macrophages as their replication site in zebrafish.

Our research unfolds new possibilities to study the pathogenesis and develop treatments against intracellular pathogen by using zebrafish as an infection model.

KEYWORDS
OMVs, vaccine, francisellosis, zebrafish, immune response

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