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AREA OF EXPERTISE: Microbiology, Zebrafish, Membrane vesicles

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DISSERTATION TITLE: Characterization of *Piscirickettsia salmonis* membrane vesicles and their use as a vaccine for aquaculture

Piscirickettsia salmonis er en intracellulær bakterie som forårsaker sykdom hos laks. Hensikten med oppgaven har vært å teste ut membraner vesikler isolert fra *P. salmonis* som en vaksinekandidat ved bruk av sebrafisk som modellsystem.

The Gram-negative intracellular bacterium *Piscirickettsia salmonis* is the etiologic agent of salmonid rickettsial septicemia (SRS), a chronic and often fatal disease in salmonid and a variety of marine fish species. *P. salmonis* was initially characterized from Coho salmon (*Oncorhyncus kisutch*) in 1989 after a devastating epizootic in the Chilean aquaculture. The bacterium has since then been recognized as an emerging problem as outbreaks of SRS have been reported across the world, including in Norway and Canada. As a result, continuous outbreaks of SRS have led to a recent decline in the Chilean salmon industry, due to a lack of efficient vaccines. As there are no efficient vaccines against SRS large amounts of antibiotics has been used in the Chilean aquaculture, leading to an emergence of antibiotic resistant strains. Thus, development of efficient vaccines against *P. salmonis* is important in order to provide a safe and sustainable aquaculture industry.

The results presented in this thesis describe the investigation of *P. salmonis* derived membrane vesicles (MVs) as a vaccine candidate against SRS. MVs are small spherical structures known to contain a variety of bacterial components, including proteins, LPS, DNA and RNA, which makes the vesicles similar to their mother cell in many aspects. MVs were isolated in large concentrations from broth-cultured *P. salmonis*. Comparative characterization of MVs from three different isolates of the bacterium revealed several strain specific differences, both *in vivo* and *in vitro*. When used for immunization in an adult zebrafish model the vesicles induced a protective response against *P. salmonis*. Incorporation of chitosan as an adjuvant, by MV encapsulation, did furthermore enhance the protective effect of the vesicles in adult zebrafish. Histological analysis indicated a reduced bacterial load upon challenge in the MV immunized group, and the RNA expression of several immune related genes altered, including *mpeg1.1, tnfα*, *il1b, il10* and *il6*. MVs induced the secretion of IgM upon immunization, suggesting an immunogenic effect of the vesicles. Taken together, the data demonstrate a vaccine potential of MVs against SRS.