Potential of *Vibrio parahaemolyticus* as a vector for fish vaccination

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Bacterial Coldwater Disease (BCWD) is the leading bacterial disease challenge for salmonid aquaculture and resource enhancement hatcheries in the Pacific Northwest. The etiologic agent is *Flavobacterium psychrophilum*, which is a Gram negative, rod-shaped bacterium. Presently there is no commercial vaccine available. Immunization studies with putative vaccine candidates rely upon injection immunization and injection challenge; immersion models are not reliable for *F. psychrophilum*. While injection immunization is practical in some cases (e.g., vaccinating brood stock), it is an impractical method for large immunization efforts. Consequently, an alternative heterologous expression system is needed for immersion vaccines against agents such as *F. psychrophilum*. *F. psychrophilum* offers ohter challenges because it is difficult to genetically manipulate and it grows slowly. Recombinant expression in conventional *E. coli* systems is expected to be poor due to a significant codon bias between these organisms.

This project was a preliminary investigation of an alternative heterologous expression system based on *Vibrio parahaemolyticus*. This fast growing halophile has a similar codon bias to *F. psychrophilum*, and it is relatively simple to genetically manipulate. Furthermore, our ability to manipulate two separate type three secretion systems (island I and island II) in *V. parahaemolyticus* offers some novel opportunities for both recombinant protein export and antigen delivery. This project addressed three questions: 1.) How rapidly will *V. parahaemolyticus* die in river water? 2.) Is *V. parahaemolyticus* more efficient for expressing *F. psychrophilum* proteins? 3.) Can we attenuate infection potential by manipulating the type three secretion systems? To test these questions, 10^6 organisms were inoculated into river water at 17°C and quantified using the drop plate method over timed intervals. Colony forming units of *V. parahaemolyticus* declined rapidly so that no recoverable bacteria were observed within 12 hrs. This is important because an immersion vaccine vector should not be a potential environmental contaminant. A putative *F. psychrophilum* hemolysin gene was cloned and expressed in *V. parahaemolyticus* and in two strains of *E. coli*. Western blots showed expression in all three hosts, but total yields were 2-10 fold lower in *E. coli* and included a number of truncated protein products; a single band of predicted molecular weight was produced by *V. parahaemolyticus*. Type three secretion system knockouts were prepared, and we demonstrated that wildtype cells are able to lyse fish cells (rainbow trout gonad) except when either the island I or both secretion islands were disrupted. Disrupting secretion island II had limited effect on host cell lysis.

Organisms such as *V. parahaemolyticus* may prove to be ideal vectors for vaccine production and delivery. *V. parahaemolyticus* is unable to survive in fresh water and it is clearly capable of producing recombinant proteins from *F. psychrophilum*. Finally, while we do not have an *in vivo* test of attenuation, the fact that an island I knockout attenuates the ability of *V. parahaemolyticus* to lysis host cells suggests that attenuation is feasible. These secretion islands might also be routes through which normally insoluble proteins can be secreted for simplified purification from culture supernatant.