

Injection vaccination of rainbow trout (*Oncorhynchus mykiss*) against *Flavobacterium psychrophilum*

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Abstract

Compared to European aquaculture, there have so far been very few reports on mortality associated with *Flavobacterium psychrophilum* in the Norwegian farming of salmonids. However, during 2008 *F. psychrophilum* was detected at 16 farm sites with rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar* L.) both at summer (15–16 °C) and winter (3 °C) temperatures. This was a drastic increase in the number of systemic infections compared to previous years, with only two outbreaks reported in 2004 and one in 2007, as recently summarized by Nilsen et al. [1]. Mortality in these cases were typically higher in fry than in smolts [2]. In later years, *F. psychrophilum* infections in rainbow trout have been a recurring challenge in the Osterøy area situated in the west of Norway (Hordaland County) in the period from May to August, linked to high levels of ice melting reducing sea water salinity.

Due to this development, field strains of *F. psychrophilum* have been collected from different outbreaks on post-smolts of rainbow trout in the period from 2008 to 2011. Using ALPHA JECT® 5-3 (containing *Aeromonas salmonicida*, *Vibrio anguillarum* O1 and O2, *Moritella viscosa* and *Vibrio salmonicida* antigens) as a basis, an autogenous vaccine has been produced for use in the affected areas. An efficient multi-component injection vaccine might be crucial to protect high value species such as salmonids against *Flavobacterium* throughout the production cycle. Here we present our ongoing work regarding establishment of a challenge model using a highly virulent field strain and documentation of vaccine efficacy, including antibody responses, ulcer development and post challenge survival on *F. psychrophilum*-vaccinated rainbow trout.

To establish a challenge model, prechallenge/virulence tests were performed using three different strains of *F. psychrophilum*; the vaccine isolate (V2008), a field strain (F2011) and P13-4/96, which is an isolate characterized as Th serotype isolated from rainbow trout in Finnish waters [3]. Rainbow trout (~36 g) were challenged with the F2011 strain by the intramuscular route (0.05 ml/injection) with bacterial concentrations ranging from 1×10^9 to 5×10^5 CFU/ml, resulting in mortality >80 % in all cases and 100 % in most cases. In addition, a virulence test using the three different isolates at equal doses (2×10^6 CFU/ml) demonstrated that the F2011 strain could be regarded as a highly virulent strain of *F. psychrophilum* as it resulted in 100 % accumulated mortality at day 18 post challenge (pc) (starting at day 9), while the V2008 gave 71 % mortality at day 18 pc (starting at day 12). The P13-4/96 did not yield any mortality pc.

For evaluation of vaccine efficacy, a group of rainbow trout was vaccinated with the multi-valent autogenous vaccine containing whole cells of *F. psychrophilum* (FLAVO AVM6), while another group was mock-vaccinated with an adjuvanted control (ALPHA JECT® 5-3

not containing *F. psychrophilum* antigens). A third group remained non-injected (negative control group). After 504 degree days, fish from each group were sampled for blood for antibody level determination. Challenge was then performed by intramuscular injection (0.05 ml) using two different concentrations of *F. psychrophilum* (F2011); 1×10^7 or 1×10^5 CFU/ml with 50 fish/challenge dose/group in two parallel tanks. As heavy ulceration is one of the most prominent clinical signs of *F. psychrophilum*, the presence of ulcerations was recorded on a yes or no basis during the challenge progression. At study termination, blood samples for antibody measurements were once again collected from each group.

The challenge experiment showed that, as opposed to the control treatments, the multivalent oil-based FLAVO AVM6 had capacity to induce significant protection in immunized rainbow trout resulting in RPS₆₀ levels up to 87 % (Fig. 1). Mortality progression and accumulated mortality was equal for the adjuvanted and non-injected control groups, indicating that any unspecific immune responses induced by the adjuvant were not capable of protecting the fish against challenge with virulent *F. psychrophilum*. The high level of protection induced in FLAVO AVM6 vaccinated groups correlated with the significantly lower ulceration rates found in this group compared to the controls (Fig. 1 columns inside graph). Furthermore, an ELISA analysis showed that rainbow trout vaccinated with the multivalent FLAVO AVM6 had significantly higher *F. psychrophilum*-specific antibody levels at the time of challenge compared to the non-injected group and the adjuvanted control group, the two latter showing equally low levels. At study termination, specific antibody levels were still highest in the FLAVO AVM6 vaccinated group, emphasizing the robustness of the response.

From our studies we conclude that the multivalent FLAVO AVM6 vaccine induces a high level of protection against *F. psychrophilum* in rainbow trout and has the capacity to reduce ulcer development during disease progression. We have furthermore demonstrated that vaccine induced protection against *F. psychrophilum* is dependent on initiation of specific immune mechanisms.

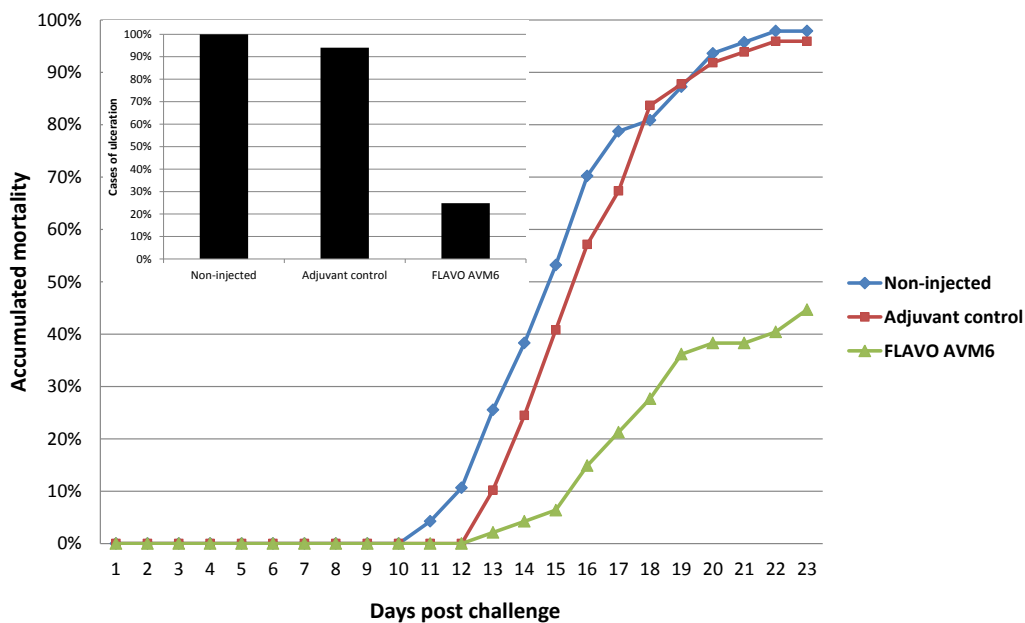


Figure 1. Accumulated per cent mortality after intramuscular challenge with a highly virulent field strain (F2011) of *F. psychrophilum* (0.05 ml/injection containing 10^5 CFU/ml). The columns inside the graph demonstrate the accumulated ulceration rates for the three different groups.

References

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