Immersion vaccination of rainbow trout (Oncorhynchus mykiss) against Flavobacterium psychrophilum

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Flavobacterium psychrophilum is a fish pathogenic bacterium responsible for bacterial cold water disease (BCWD) which causes serious disease outbreaks particularly in salmonid fish farms [1]. Since no commercial vaccines are available, the control of BCWD largely depends on the use of environmentally hazardous antimicrobial agents whereby an efficient immersion vaccine is urgently needed. In this study, the potential of an inactivated whole-cell based immersion vaccine (Flavovaccine, PHARMAQ) containing an immunostimulant was tested on rainbow trout fry. In the first experiment rainbow trout fry with a mean initial weight of 1.2 g were dip vaccinated for one minute and one group was booster vaccinated 3 weeks after the initial immunization using the same procedure. An intraperitoneal (i.p) challenge with virulent F. psychrophilum was performed both 3 (~2.0 g) and 6 weeks (~3.0 g) post-immunization after which the relative percent survival (RPS) was calculated by relating the number of diseased fish in the vaccinated groups with the corresponding number in the control group.

Blood samples (n=20) from each group were concurrently collected, pooled (4 samples/pool) and analyzed for specific IgM antibodies by an indirect enzyme-linked immunosorbent assay (ELISA) [2]. The results from the challenge performed 3 weeks after the initial immunization (n=32) showed no differences in survival between the vaccinated group and the control group (RPS=7.1). However, in the challenge performed six weeks after the initial immunization a clear difference in the mortality between the vaccinated groups and the mock-vaccinated group was observed (Fig. 1). Regardless of a relatively low level (mean titer<5000) of specific IgM antibodies in the blood plasma (Fig. 2), the mortality was significantly lower in the immunized groups, both with (n=36, RPS=75.8) and without a booster vaccination (n=50, RPS=65.2). In this experiment 3 weeks was not enough for immunity to build up. The results also implied that plasma IgM antibody levels are not necessarily the most important criteria of immunity in immersion vaccination. In addition to the specific immune response, other non-specific immune parameters in rainbow trout fry could be triggered during immersion vaccination and be equally important in combating F. psychrophilum infections.
Since the results from the first immersion vaccination trial were promising, a second experiment with the same vaccine was carried out. Flavovaccine (PHARMAQ) and its components were tested separately in order to assess whether the inactivated whole-cells alone, the immunostimulant or the combination of the two were responsible for the high level of protection. In the beginning of the experiment, rainbow trout fry with a mean initial weight of 2.5 g were dip vaccinated for one minute. Each group (n=80) were booster vaccinated 3 weeks after the initial immunization. The blood sampling (n=20) for antibody determination and the i.p challenge (n=60) were subsequently performed 6 weeks (~5.5 g) after the initial immunization. The results from the challenge trial showed that Flavovaccine (PHARMAQ) provided better protection (RPS=82.3) than the inactivated whole-cells (RPS=66.7) or the immunostimulant (RPS=44.4) alone (Fig. 3). Based on the results from the ELISA, the specific plasma IgM antibody levels induced by Flavovaccine (PHARMAQ) and the inactivated whole-cells alone were considered low (mean titer<2000) even though they were significantly higher compared to the level in the control group (Fig. 4).

Although vaccination with the immunostimulant alone did not increase the level of specific circulating IgM antibodies significantly, it provided protection in the i.p challenge trial, which implies that other non-specific immune responses were triggered by immersion vaccination which could be essential in neutralizing F. psychrophilum infections in rainbow trout fry.
Both experiments showed that Flavovaccine (PHARMAQ) gave good protection in the i.p challenge trials with *F. psychrophilum*. During both experiments before the challenge, however, a natural *F. psychrophilum* infection killed a number of fish in each group. Therefore, different sized groups had to be used in the challenge trial during the first experiment. It is difficult to assess the effects of the natural bacterial infection on the vaccination efficacy and on the outcome of the challenge trials. At the same time it is likely that the conditions during the experiments were reflecting those found in aquaculture environments. The lack of a reproducible bath challenge model with *F. psychrophilum* has made it difficult to evaluate the efficacy of a vaccine since injection-based challenge models completely bypass the protective function of the skin mucus layer which could serve as an important infection barrier to disease in small fry with an immature systemic immune system. Still, the results from our experiments are promising in the sense of immersion vaccination becoming an effective way of preventing BCWD outbreaks in the near future.

References
