PROTECTION OF RAINBOW TROUT (*ONCHORYNCHUS MYKISS* L.) AGAINST *FLAVOBACTERIUM PSYCHROPHILUM* BY IMMERSION AND INJECTION VACCINATION USING A NOVEL POLYVALENT VACCINE

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Rainbow Trout Fry Syndrome (RTFS) is a disease caused by the Gram-negative bacterium *Flavobacterium psychrophilum*, responsible for economic losses in the trout industry worldwide. The diversity of *F. psychrophilum* strains, inherent difficulties in vaccinating juvenile fish and lack of a reproducible immersion challenge model has hampered the development of a vaccine for this disease. Disease episodes tend to occur between 10-14 ºC, with necrotic lesions seen on the skin surrounding the dorsal fin and tail; in small fry often no clinical signs are apparent and death occurs due to septicaemia. At present no commercial vaccines are available, leaving antibiotics as the only course of action to contain disease outbreaks. The development of a vaccine is required, due to the potential risk of resistance developing in the antibiotics presently licensed for use in aquaculture.

We report on the development and efficacy of a polyvalent, whole cell vaccine containing formalin-inactivated *F. psychrophilum*. Significant strain variability between the *F. psychrophilum* isolates (>300) in our collection was confirmed by PFGE, $(GTG)_5$-PCR, 16S rRNA allele PCR, plasmid profiling and serotyping and these data were used to select representative isolates to produce a potentially cross-protective vaccine. A bath challenge model previously reported using hydrogen peroxide as a pre-stressor was improved by increasing the challenge period resulting in specific mortalities of 50-60%. This model was subsequently applied to test the efficacy of our polyvalent vaccine following immersion vaccination of Rainbow trout fry (5 g). The vaccine was administered as a 30 s dip with a booster vaccination given 315 degree days post-primary vaccination. A vaccine formulation combined with an immersion adjuvant (IMS1312Vg, Seppic) was also tested using the same vaccination regime. Challenge was by immersion with a virulent heterologous isolate of *F. psychrophilum* (630 degree days post-primary vaccination). Significant protection was achieved with a Relative Percentage Survival of 84% achieved using the immersion vaccine without adjuvant; RPS of the immersion vaccine with adjuvant was 60%. Long term efficacy of the vaccine applied via the intraperitoneal route alone or in combination with two adjuvants, Montanide and a novel adjuvant squalene/aluminium hydroxide, was tested in 80 g Rainbow trout. The fish were challenged by intramuscular injection 1155 degree days post-vaccination with a virulent heterologous isolate of *F. psychrophilum*. The vaccine emulsified with Montanide provided 100% protection, whereas survival in the groups given vaccine alone and vaccine combined with squalene/alum adjuvant was not significantly different from unvaccinated groups.

Keywords: Rainbow Trout Fry Syndrome, *F. psychrophilum*, immersion vaccination, immersion challenge, adjuvants

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