TRT10- DEVELOPMENT OF VACCINATION METHODS FOR THE CONTROL OF RAINBOW TROUT FRY SYNDROME (RTFS)

by R. E. Rangdale[1] and R. E Campbell[2]

Introduction

Over the last 15 years rainbow trout fry syndrome (RTFS) has caused persistent, high mortality in juvenile rainbow trout (*Oncorhynchus mykiss*). The Gram negative, yellow pigmented, rod-shaped bacterium *Flavobacterium psychrophilum* has been implicated in both RTFS and bacterial coldwater disease (BCWD). In the UK RTFS is considered to be among the most significant diseases in fry and fingerling production, costing the UK industry losses in excess of 5 % per annum. Previous research has demonstrated that existing vaccine preparations do not elicit adequate levels of protection or immune memory. This may be because they contain inappropriate antigens, protective antigens lack immunogenicity, or the disease itself causes immune suppression. Thus, currently, no commercial vaccine is available, and the development of such remains a long-term goal of the industry, particularly those involved in on-growing fish for the table market.

Scientific Objectives

The aim of the present LINK aquaculture research programme is to develop an immersion vaccine for fry and fingerlings for the control of RTFS.

Approaches

- Identification of antigens. Does the bacterial cell or part of the cell elicit a humoral immune response in fry and fingerlings?
- Determination of the nature of the antibody response and immune memory. Will an immunised fish be protected from disease and, for how long will this protection last?
- Determination of additional virulence factors. Are there molecules such as extracellular proteases, lipopolysaccharides or glycoproteins involved in pathogenicity that may be added to vaccines to increase their effectiveness?
- Determination of the various serotypes of *F. psychrophilum*. Is there a multitude of sub-types within the species *F. psychrophilum* that have different surface antigens? For a vaccine to provide adequate protection these will need to be included.
- Examination of factors known to effect the uptake of antigens. Does the concentration of the vaccine, the duration of immersion, the size of fish, the use of adjuvants or the physical state of the antigen (particulate or soluble) influence it's efficacy?
- Establishment of mass production techniques. Can the requisite antigen(s) be consistently produced, on a large enough scale to make a commercial product viable?
- Clinical field trials. Can it be demonstrated that a successful laboratory tested product is effective in the field?

Summary of progress

Preliminary Vaccination Trials.

- Immersion vaccination with heat and formalin inactivated whole cell preparations of virulent and non-virulent *F. psychrophilum* have produced no significant protection challenge trials.
- No detection of a circulating antibody response following vaccination has been possible.

Antibody Studies.

- Specific high to medium titres of anti *F. psychrophilum* antibody have been measured following intraperitoneal immunisation of rainbow trout with adjuvanted whole cell and crude soluble protein preparations (Figure 1).

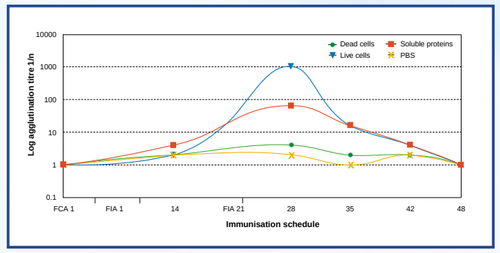

Figure 1: Antibody Response to live and heat inactivated cells

- However, the duration of the antibody response is relatively short, and is undetectable 48 days after immunisation (Figure1).
- Similar antibody responses have been measured in overtly infected fry/ fingerlings from commercial rainbow trout hatcheries (Figure 2).
- No detectable antibody response has been measured in covertly infected or non-diseased fry (Figure 2).

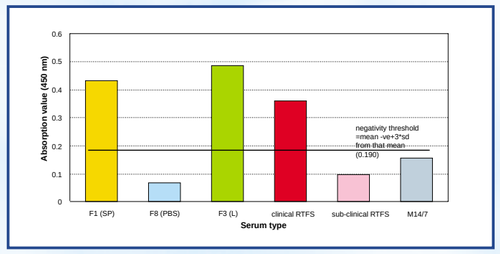

Figure 2: Detection of Serum Antibody in Naturally and Artificially Infected Fry

- There is negligible circulating antibody response to heat or formalin inactivated cells.
- Antibody raised against *F. psychrophilum* is highly bacteriostatic *in vitro* (Figure 3).

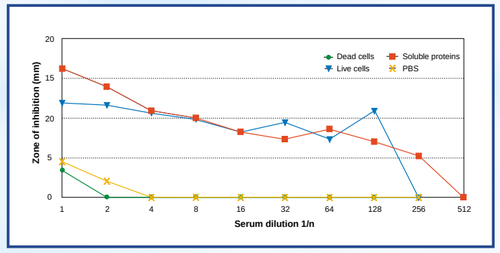

Figure 3: Inhibitory Ability of Artificially Immunised Rainbow Trout Sera

Identification of immunogens.

- Protein profiling of bacteria grown in standard and iron restricted media show homologous protein banding.
- Immuno-dot and Western blotting studies have indicated the presence of soluble protein antigens.
- Immune sera shows strong avidity for particular cellular sub-units of *F. psychrophilum* (Figure 4).

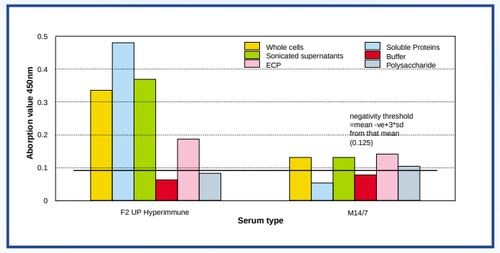

Figure 4: Avidity of antisera from RTFS infected fry for F. psychrophilum *cellular sub-units*

- Periplasmic proteins, extracellular proteases and lipopolysaccaride appear less antigenic in preliminary studies (Figure 5).

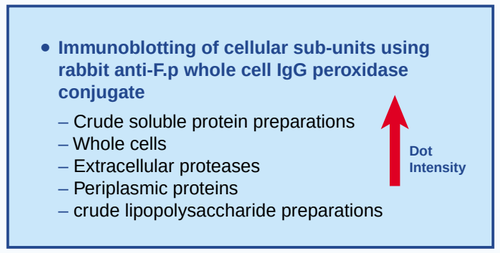

Figure 5: TRT10- Identification of Immunogens

Auto-agglutination/pathogenicity studies.

- Freshly isolated *F. psychrophilum*, virulent in rainbow trout fry, auto-agglutinate in liquid media.
- The ability to auto-agglutinate is lost after serial sub-culture in liquid media, concommittally virulence in susceptible fish is diminished.
- Light microscopy revealed progressive elongation and filamentation of cells during logarithmic growth followed by shortening and clumping at stationary phase.
- Protein content and profiles are identical in auto-agglutinating and non-agglutinating isolates.
- Polysaccharide component is significantly higher in auto-agglutinating (virulent) isolates. Therefore measurement of polysaccharide can be used as a determinant of prospective virulence in challenge trials.
- It is likely that polysaccharide is involved in attachment to host tissues and consequently initial infection.
- The polysaccharide component is not recognised by either trout or mammalian antibodies.

[1]The Centre for Enviroment, Fisheries and Aquaculture Science (CEFAS), Weymouth Laboratory, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB, UK http://www.cefas.co.uk

[2]Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA