

Abstract

Live food has been used for feeding and delivery of the compounds to larval stages of aquatic animals. *Artemia* can be used as a possible vector for the delivery of different substances such as nutrients, probiotics and immune-stimulants. Sulfated galactans (SG) from *Gracilaria fisheri* has been demonstrated to stimulate immune activity and against pathogenic infection in shrimp. In vitro study revealed that SG bound with shrimp haemocyte and activated the expression of immune related genes in haemocyte culture. The present study was carried out to investigate the bioencapsulation of *Artemia* with SG for delivery to tissues and activating the expression of immune genes in shrimp. SG conjugated with fluorescein isothiocyanate (FITC-SG) showed no significant toxic in *Artemia*. Bioencapsulation of *Artemia* with FITC-SG (0–100 µg/ml) revealed that FITC-SG was located in *Artemia* gut lumen with a time and dose dependent manners. Tracking of SG in shrimp, shrimp fed with *Artemia* bioencapsulated with FITC-SG demonstrated that FITC-SG presented in shrimp gut until 2 h after feeding. Twenty-four hours after feeding, shrimp tissues were collected and the distribution of FITC-SG was observed in gill, hepatopancreas and haemolymph. FITC-SG was also found to bind with shrimp haemocyte. In addition, shrimp continuously fed with *Artemia* bioencapsulated with SG for 3 days showed activated the expression of immune genes including IMD, IKKβ, dicer and proPO-I, and prolonged high expression levels for a week. These results indicated that *Artemia* bioencapsulation could deliver the SG passed through gut to tissues and bound with haemocyte, and subsequently activated expression of immune genes in shrimp.

Keywords: *Artemia* bioencapsulation; Sulfated galactans; FITC-SG; Shrimp haemocyte; Immunity

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O-005.**Vaccination strategies and IgM responses against PKD in rainbow trout**

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Abstract

PKD is one of the most serious diseases affecting trout aquaculture in the UK. Caused by the myxozoan parasite, *Tetracapsuloides bryosalmonae*, PKD is elicited by the temperature-dependent development of parasite spore sacs in colonial bryozoans. Since recovered fish are known to exhibit protective immunity to re-infection, a successful treatment, based on the reduction of kidney pathology and parasite burden, could markedly reduce fish mortalities leading to improved productivity and fish welfare. Our investigations have focused on the selection of putative *T. bryosalmonae* virulence factors to unravel host immune evasion mechanisms exploited by the parasite, whilst shortlisting candidates for vaccine studies.

Here we report the results of three DNA-vaccination field trials using selected antigens administered individually or in combination. Some vaccine groups were found to have a partial protective effect in reducing PKD-associated kidney pathology whilst decreasing parasite load. Pathology reduction was improved in successive trials by improving the vaccination strategy.

We have functionally characterized the most promising antigen from our vaccine studies, which represents a novel micro-exon gene (*Tb*-MEG1). MEGs, until now, were thought to be unique to helminth parasites. In schistosomes, they exhibit extensive antigenic variability that is thought to enable greater plasticity in host protein targeting as a mechanism of host immune subversion over the course of infection. Using a validated anti *Tb*-MEG1 MoAb, we have demonstrated the protein to be expressed in and on the surface of parasites and a subset of immune cells within the kidneys of

infected fish. We have also demonstrated potent *Tb*-MEG1-specific IgM responses in sera from parasite-infected (farmed) rainbow trout and have successfully induced a specific IgM response after protein vaccination.

DNA vaccines encoding molecules homologous to proteins involved in nutrition acquisition, cell-cell interactions or of unknown function, have shown promise towards the development of a future PKD vaccine, that may also be applicable to the generation of vaccines against other fish parasites. The discovery and characterization of *T. bryosalmonae* antigens has provided valuable insights into host immune evasion by myxozoan parasites, with the present *Tb*-MEG1 studies also having major implications towards understanding the evolution of antigenic variability in metazoan parasites.

Keywords: Vaccination, myxozoan, parasite, antigen, igm response

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O-006.**Demonstration of herd immunity effects in dna vaccinated rainbow trout**

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Abstract

While DNA vaccination by intramuscular injection is known to provide highly protective immunity against viral haemorrhagic septicaemia (VHS) in rainbow trout at an individual level, we here aimed at examination of herd immunity effects. Induction of clinical disease following experimental challenge by waterborne exposure of the fish to the pathogens represents a hurdle in vaccine potency testing for many pathogens, but in the case of VHS virus, challenge can be done efficiently by immersion. However, progression and level of mortality depends on the challenge dose. This has some implications for the experimental design and interpretation of the results. Initial experiments indicated that while mortality following a high challenge dose was independent on whether the fish were kept together in one aquarium or in individual aquaria, indirect infection by virus released from primary infected fish seemed to play an important role at low challenge doses. In experimental vaccination trials, it may be assumed that challenge of cohabitant naïve and vaccinated fish represents a harder test of vaccine potency compared to challenging the vaccinated fish separately due to secretion of high amounts of virus from the naïve fish. In challenge trials with high inoculum of virus, this seemed to be true for VHSV challenge of DNA vaccinated rainbow trout as illustrated by increased mortality among vaccinated fish cohabitated with naïve fish. However, at lower inoculum, the effect tended to be opposite in the sense that mortality among the naïve fish was reduced. Implications of the results for experimental vaccine testing will be discussed.

Keywords: Fish rhabdovirus, DNA vaccine, challenge setup, vaccine testing, herd immunity

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O-007.**Exposure to antibiotics affects saponin immersion induced immune stimulation and shift in microbial composition in zebrafish larvae**

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