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Effect of immersion booster vaccination with *Yersinia ruckeri* extracellular products (ECP) on rainbow trout *Oncorhynchus mykiss*

Unal Ispir[1*], Mustafa Dorucu[2]

[1]*Departmant of Fisheries, Faculty of Agriculture, Bingol University, 12000, Bingol, Turkey*
[2]*Fisheries Faculty, Firat University, 23119, Elazig, Turkey*

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Abstract

Extracellular products (ECP) of bacteria is a factor responsible for a number of biological effects including immunostimulatory activities in different animals including fish. In this study, the effect of extracellular products (ECP) of *Yersinia ruckeri* on protective immunity in rainbow trout was investigated. To obtain an effective vaccine against the Yersiniosis, ECP preparation was obtained from the *Y. ruckeri* and the immersion twice into the rainbow trout was carried out.After 60 days vaccinated fish with first and second immunization by ECP were challenged via intraperitoneal injection with 9.8 x 10^6 cell/ml of *Y. ruckeri*. The protection against challenge was obtained by the vaccination (74.0–81.4% relative percentage survival (RPS)), but the fish from the control group had an 67.5% of mortality rate. The results indicated that the vaccinated fish showed higher non-specific immune activity and protective effect than did the unvaccinated fish. The results suggest that the administration of ECP at rainbow trout is safe and elicits protection upon pathogen challenge.

Keywords: Rainbow trout, *Yersinia ruckeri*, Extracellular products, ECP

Introduction

Bacterial fish diseases caused by a variety of pathogens are a significant problem in commercial aquaculture. Fish vaccination was successfully used to protect to the farmed fish from the common pathogens. Although the vaccinations are efficient, the vaccination mode remains a critical point to determine the efficiency of a vaccine. The vaccines have to be administrated by a way that can lead into a high level of immunization of the fish organism. The immunization has also to last as long as possible. On the other hand, the vaccination has to be quick, from statement with low labour and not to be stressful for the fish.At the same time, the minimum amount of the vaccine has to be used. Such a way is the immersion immunization.

In aquaculture,in spite of the need of vaccines against bacterial diseases, only few commercial vaccines are available.Particularly, typical components of commercial vaccines to protect fish against bacterial diseases are either

* Corresponding author. Email: uispir@yahoo.com. Tel: +90-426 213 2550.

killed bacterial whole cells or extracellular products (ECPs). The important role of the extracellular products in the pathogenesis of *Yersinia ruckeri* has been demonstrated (Romalde and Toranzo 1993) and thus it would be interesting to include inactivated ECP of this species as a potential vaccine.

The Gram negative bacterium *Yersinia ruckeri* is recognised as the ethological agent of enteric redmouth disease, a systemic infection of salmonid fish that causes important economic losses worldwide (Fernandez et al. 2002). *Y. ruckeri* was isolated approximately 50 years ago in the USA. However, over the last two decades, the disease has become established in salmon and trout raising areas across many countries (Tobback et al. 2007). In this study, the effectiveness of new developed vaccine was tested in *Yersinia ruckeri* in experimental infected fish. Additionally tests were performed to evaluate the possible effect of booster vaccination to extend the protection against the yersiniosis.

Materials and methods

For the challenge experimental study, 120 healthy fish, with the approximate weight of 6.3 g, were used. They were obtained from the Keban Fish Breeding Unit of IX. Region Directorate, the State Hydraulic Works in Turkey. On the day 0, 120 fish were divided in random into three groups (40 fish per group). The fish were transfered to an experimental tank containing 300 l of freshwater. The water was aerated by compressed air. The daily photoperiod was 11 h dark/13 h light and the water temperature was 15 ± 0.5 ^{o}C. Commercial dry pellets were used the feed the fish (2% body weight per day). Fish were allowed to acclimate for 15 days before the experiment.

A virulent isolate of *Yersinia ruckeri* was obtained from Fisheries Faculty, University of Firat, Elazig, Turkey. The bacteria was grown in Triptic Soy Agar (TSA). The ECP from *Yersinia ruckeri* were obtained by the cellophane plate technique (Liu et al. 1957). Briefly, sterilized cellophane sheets were placed on the surface of TSA plates and inoculated by spreading 0.3 mL of a 24 h-old broth culture with a sterile swap. After 24 h of incubation at 22 ^{o}C, cells were washed off the cellophane with PBS (pH 7.2). The cell suspensions were centrifuged at 10,000 × g for 30 min at 4 ^{o}C, and the resulting supernatants were filtered (0.45 µm Millipore membranes, Millipore Corporation, Bedford, USA) and stored at -20 ^{o}C until used. The protein concentration of ECP samples was determined by the method of Lowry (Lowry et al. 1951) with bovine serum albumin as the Standard. The inactivation of the ECP was performd by addition of formalin to achieve a final concentration of 1%. The sterility of the final vaccine was confirmed by the absence of bacterial growth after the inoculation of the bacterin in TSA and inoculation at 22 ^{o}C for 5 days.

The immersion vaccination on the rainbow trout took place in a ECP (16 µg/ml) for 2 min under aeration. Fish were re-immunized immersion with the same quantity of antigen 20 days after the first immunization to evaluate if the booster effect took place. The control group fish were unvaccinated.

Fish were subjected at day 60 after vaccination for the first and second immunization with ECP, challenged via intraperitoneal injection with 9.8×10^6 cell/ml of *Y. ruckeri* in 0.1 ml of PBS. The fish in the control tanks were treated as the others but were not infected.Observations were made for a period of 14 days and data on mortality were recorded. The cause of mortality was established by reisolation of *Y. ruckeri* from the kidney. The protective index (Amend 1981) was calculated as the relative percentage survival (RPS):

RPS= [1 – (percentage mortality in vaccinated fish / percentage mortality in controls)] × 100.

A one-way ANOVA test was used to determine statistical differences in the mortality among the different groups.

Results and discussion

The percentage mortality rates obtained for 6.3 g fish immunized by one and booster immersion with extracellular producs of *Y. ruckeri* are summarized in Table 1.In this study,RPS level was associated with efficacy of the *Y. ruckeri* ECP with primo-vaccinated and booster vaccinated. Protection against pathogen by ECP as indicated by percentage mortality rate was observed, compared to control fish (Table 1). The rainbow trout vaccinated once or boosted vaccinated by immersion with the ECP of *Y. ruckeri* were protected significantly ($P < 0.05$) during the challange that took place 60 days after the first and booster vaccination. These results clearly demonstrate that the

immersion mode of vaccination could be very effective at least for the rainbow trout and against the yersiniosis proveked by the *Yersinia ruckeri*.

Table 1. Mortality among the fish of the three groups post-challenge

		Mortality each day post-challenge														
Groups	n	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
Booster	40	-	-	-	-	2	-	-	-	-	-	-	1	2	-	5*
Vaccine once	40	-	-	-	3	1	-	-	-	1	-	2	-	-	-	7*
Unvaccinated	40	-	-	3	7	9	-	6	-	-	2	-	-	-	-	27

*Significant at $P < 0.05$.

A lower mortality was achieved in all vaccinated groups in comparison with the control group, when the rainbow trout was challenged with a dose containing 9.8×10^6 cells/ml of a highly virulent *Y. ruckeri*. The RPS values obtained for *Y. ruckeri* using the ECP-vaccine ranged between 74.0 and 81.4%. These values are similar to or lower than RPS values reported previously for the vaccine against yersiniosis on the rainbow trout (Cagirgan and Tanrikul 1998; Kubilay and Timur 2001; Raida and Buchmann 2008). One the other hand, ECP antigens were reported to has a stronger immune response to Photobacterium damselea spp. piscicida (Magarinos et al. 1994); Vibrio harveyi (Zorilla et al. 2003) and Flavobacterum psychrophilum (LaFrentz et al. 2004) was induced in fish vaccinated with ECP. The booster vaccination significantly enhanced the efficacy of ECP, achieving RPS values in excess of 81.4% for *Y. ruckeri*. Thus, this result confirms the need for a booster after the initial immersion.

Tebbit et al. (1981) reported a reduced mortlity of about 84% in yersiniosis affected populations and found that level of protection can be used as an acceptable indicator for a succesful fish vaccine. Surprisingly, the results of the booster vaccination trial which was employed 29 days after the first vaccination, was disappointing as it did not give the anticipated additional and prolonged protection. Tatner and Horne (1985) showed a relatively high priming dose of *Y. ruckeri* vaccine on the brown trout, and a similar booster dose to increase protection from yersiniosis. The results in this study are in agreement with the results in previous investigations. Johnson and Amend (1984) also reported increased protection from furuncluosis due to boosting with Aeromonas salmonicida. Factors such as time intervals between primary and secondary exposures and temperature of vaccination still remain to be optimized.

The results presented in this paper showed that rainbow trout can be successfully vaccinated against infection of *Y. ruckeri* by injection of live bacteria. In conclusion, the protection afforded by the ECP of *Y. ruckeri* vaccine in cultured rainbow trout strongly suggested that it could be a good prophylactic measure against mortalities caused by pathogen.

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