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Efficacy of a Formalin-Killed Vaccine against *Flavobacterium psychrophilum* in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

Vaccine development for rainbow trout fry syndrome (RTFS) has been based primarily on whole-cell bacterins. In this study, protective effects of candidate strain to develop a vaccine in future studies against to *Flavobacterium psychrophilum* were investigated. Alteration in the relative percentage of survival rate (RPS) of rainbow trout exposed to $1x10^8$ CFU/mL formalin-killed cells for 30, 60 and 90 min. Upon challenge with a strain the RPS of respectively, 21.74%, 34.78% and 43.48% was recorded. The results suggested that a weak protection had obtained with this strain, therefore; the strain was not suitable for use in vaccine development.

Keywords: *Oncorhynchus mykiss*, *Flavobacterium psychrophilum,* rainbow trout fry syndrome, relative survival rates, vaccine.

Gökkuşağı Alabalığı (*Oncorhynchus mykiss*)'nda *Flavobacterium psychrophilum'a* Karşı Formalinle Öldürülmüş Bir Aşının Etkisi

Özet

Gökkuşağı alabalığı yavru sendromu için aşı geliştirilmesi tam hücre bakterinlerine dayalı olarak yapılmaktadır. Bu çalışmada, *Flavobacterium psychrophilum'*a karşı sonraki çalışmalarda aşı geliştirilmesinde kullanılacak aday suşun koruyucu etkileri araştırıldı. 30, 60 ve 90 dakika için $1x10^8$ CFU/ml formalinle inaktive edilmiş hücrelere maruz kalan gökkuşağı alabalıklarının nisbi hayatta kalma oranlarındaki (RPS) değişimler belirlendi. RPS oranları sırasıyla %21.74, %34.78 ve %43.48 olarak kaydedildi. Sonuç olarak, bu suşun zayıf bir koruyucu etkiye sahip olduğu ve aşı yapımı için kullanımının uygun olmadığı kanaatine varıldı.

Anahtar kelimeler: *Oncorhynchus mykiss*, *Flavobacterium psychrophilum,* Gökkuşağı alabalığı yavru sendromu, nisbi hayatta kalma oranları, aşı.

Introduction

Flavobacterium psychrophilum, the causal agent of bacterial cold water disease (BCWD) or rainbow trout fry syndrome (RTFS), was first isolated from juvenile coho salmon, *Oncorhynchus kisutch*, in the USA in 1948 (Borg, 1960). The bacteria, a gram-negative, long rod have recently become one of the most crucial problems affecting salmonid culture worldwide (Wood and Yasutake, 1956; Pacha, 1968; Lorenzen and Olesen, 1997). The disease is characterized by skin erosions and ulcerations, darkening, fin and gill rot. In severe cases, the caudal fin is completely sloughed with the bare spine in the caudal peduncle area being completely exposed. In very young fish, the disease can also be associated with nervous manifestations such as erratic swimming behavior and spiral movements (Holt *et al.,* 1987; Kent *et al.,* 1989; Elsayed *et al.,* 2006). *F. psychrophilum* has been isolated mainly from diseased and covertly

infected salmonids (Holt *et al.,* 1993; Dalsgaard and Madsen, 2000) and other fish (Wakabayashi *et al.,* 1994; Lida and Mizokami, 1996).

RTFS causes high mortalities among reared rainbow trout fry in worldwide and at present antibiotics is the most effective treatment. The best results are obtained if the drug is administered immediately when the first fry show sign of disease, i.e. often from several weeks after start of feeding (Lorenzen *et al.,* 2010). Several studies on the effects of vaccination have been made although commercial vaccines are not yet available. Both bath vaccination and intraperitoneal injection have been evaluated with varying results (Holt, 1987; Obach and Laurencin, 1991; Lorenzen, 1994; Rahman *et al*., 2000; LaFrentz *et al*., 2002). However, vaccinating fish by injection is not practicable. The control of RTFS 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 formalin-killed inactivated whole-cell based immersion vaccine was tested for candidate vaccine strain against *F. psychrophilum* on rainbow trout fry.

Materials and Methods

Fish

Rainbow trout (approximately 1 g) were obtained from a commercial fish farm (Elazığ-Türkiye). All fish were maintained in dechlorinated city water in 150 L tanks. All fish were remained native to *F. psychrophilum* infection, which was confirmed as culture negative in fish stock.

The experimental fish were stocked at a rate of 50 fish per 50 L tank in 4 tanks. The fish were hold for 2 weeks prior to experimentation in order to acclimatize to tank conditions. Fish were fed as adlibitum with commercial dry fish food. The water temperature averaged 15^{o}C± 0.5^{o}C, dissolved oxygen was 6 mg/L. A photoperiod of 14h:10h (dark: light) was maintained and aeration was supplied by an air stone.

Bacteria and Vaccine Preparation

F. psychrophilum originally isolated (BU12-01) from a rainbow trout farm suffering from RTFS in Bingöl (Türkiye), was used for experiments. The strain were stored in Tryptone Yeast extract salts (TYES) Broth (0,4% Tryptone; 0,05% Yeast extract; 0.05% $MgSO_4.7H_2O$; 0,02% $CaCl_2.2H_2O$; pH: 7.2) supplemented with 15% sterile glycerol at -80^{o}C.

The vaccine was prepared according to Klesius *et al.* (1999). Bacteria was cultured in TYES Broth in a incubator at 15^{o}C for 3-5 days. Cultures were treated with formalin (final concentration 3%) at 15^{o}C for 24h. Bacteria were centrifuged at 10000 rpm for 20 min. Supernatant was 10 fold using a final concentrator. The final concentration was 1x10^{8} CFU ml^{-1}.

The Protocol of Vaccination

The rainbow trout were groups of 50 fish and immersed in aerated 3 L bath solutions for 30, 60 and 90 min. Control immersion baths was prepared by 3 L sterile culture medium.

Challenge

At 21 days post-vaccination, experimental and control groups were bath challenged with *F. psychrophilum* isolate at cell concentrations (1x10^{8} CFU/mL) for 2h. The monitored daily for clinical sings and mortality daily for 14 days. Dead fish were removed and bacterial samples were obtained aseptically from viscera organ of morbid and dead fish to confirm the presence of *F. psychrophilum*. The efficacy of the vaccine was calculated as Relative percent survival (RPS) (Amend *et al.,* 1981).

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

Table 1. Quantities of fish per day and total mortality in the groups

Days	Groups			
	Control	30 min	60 min	90 min
1	8	5	3	4
2	5	3	3	2
3	2	1	1	-
4	-	-	2	2
5	-	-	-	-
6	-	2	-	-
7	1	1	-	1
8	2	1	2	-
9	-	-	-	-
10	2	2	2	-
11	-	-	1	2
12	-	1	-	1
13	1	2	-	1
14	2	-	1	-
Total	23	18	15	13

Results and Discussion

The effect of vaccine treat on mortality performance of rainbow trout is shown in Table 1. Typical external symptoms of diseases were not obvious following experiments, and no internal lessons were noted in any of the fish.

Mortality, control and vaccinated fish began 24h after challenge, and almost all mortalities in groups occurred during the 14 days post challenge.

The control group experienced 46% mortalities while the vaccinated groups experienced 21.74%, 34.78% and 43.48% mortalities, respectively.

RPS and days of survival between control and vaccine groups at challenge period were different. The percent survival in control bath-immersion with sterile culture medium was 46% on 14 days, while the relative percent survival for the vaccinated was 21.74%, 34.78% and 43.48%, respectively (Figure 1).

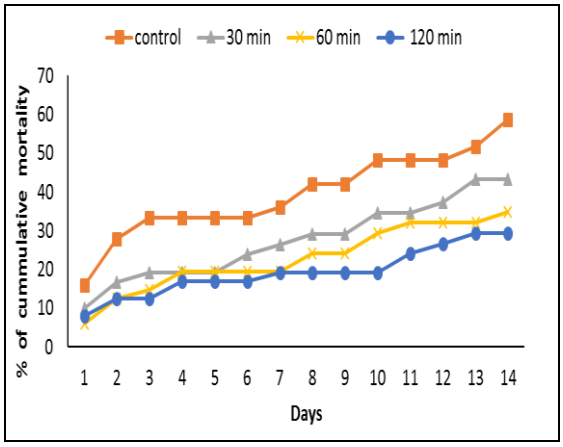

Figure 1. Relative Percentage Survival of fish in the group (RPS)

Rainbow trout, *O. mykiss* naturally infected show clinical signs such as trunk ulcers, lack of caudal fin edge, anemia, exophthalmia and hemorrhage of the gills, necrotic myositis, necrotic scleritis and cephalic osteochondritis (Holt *et al.,* 1993; Ostland *et al.,* 1997). But, in the present study, no clinical signs of RTFS were observed in the fish during this infection.

The present study showed that immersion exposure of fry in approximately 1 g for 90 min in formalin-killed *F. psychrophilum* cells was able to induce a lower level of immunity to immersion challenge 21 days later despite the control challenge group. Previous research had demonstrated that oral, immersion and intraperitoneal injection immunization of rainbow trout with formalin-killed *F. psychrophilum* (LaFrentz *et al.,* 2002; Kondo *et al.,* 2003; Madetoja *et al.,* 2006). In this study, protective effect against RTFS of a strain isolated from our region was investigated for the detection of a suitable strain for candidate vaccine. Palti *et al.* (2003) reported that variation of survival following *F. psychrophilum* infection. The weak protection obtained in this study.

Holt (1987) found that coho salmon vaccinated by direct immersion in formain-killed whole cell bacterins conferred protection, but that protection was not strong when compared to the survival of fish that had been vaccinated by injection of the whole cell bacterin. Obach and Laurencin (1991) similarly noted that heat inactivated vaccine administrated via immersion provided much reduced protection (RPS 14-47%) as compared to the 80% RPS reported when injection was used to vaccine. The RPS have been at least 50% for an effective vaccine (Amend, 1981), lower vaccine efficacy may have been related to the ability for the fish to respond to vaccination. Whether this difference in immune stimulation can be related to a higher number of bacteria entering the fish, e.g. caused by better adhesion to the fish mucus, or a higher multiplication rate inside the fish in case of low passage isolates of field strains, remains to be determined. Also, differences in the level of protection may be related to virulence differences of strain. Shoemaker *et al.* (1999) demonstrated that the marginal protection in the 10 min immersion vaccination, but protection was observed in the 2 min immersion vaccination of channel catfish.

In our study, the best protection against *F. psychrophilum* was seen in the group administered formalin-killed vaccine for 90 min.

In conclusion, these results suggest that low virulence of this study strain and no candidate vaccine strain for RTFS.

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