

- Brief Communication -

Trials for the control of scuticociliatosis in the cultured olive flounder (*Paralichthys olivaceus*) by bath treatment

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The scuticociliate, a histophagous ciliate, is known to cause high cumulative mortalities in juvenile olive flounder *Paralichthys olivaceus* rearing in land-based tank facilities. This study examined effects of bath treatment of 3 chemical agents including formalin, hydrogen peroxide and sodium chloride, and freshwater against scuticociliates infected olive flounder. Although 100 ppm formalin and freshwater did not completely eliminate the scuticociliates within the internal organ of fish, chemicals were effective to prevent scuticociliatosis from spreading. It confirms the efficacy of the chemical with treating the diseased fish for at least 4 consecutive days.

Key words: Scuticociliatosis, Olive flounder, Formalin, Freshwater

Introduction

Scuticociliate is a facultative parasite, opportunistically invading fish seriously stressed under unfavorable environment and the causal agent of 'scuticociliatosis' in cultured flounder (Yoshinaga and Nakazoe, 1993). One problem associated with the flounder farming in Korea is the scuticociliatosis (Jee et al., 2001). Scuticociliatosis can be a source of significant economic loss, which caused mass mortalities of fry and high cumulative mortalities of juvenile in cultured flounder (unpublished). Although *in vitro* efficacy of some chemicals against scuticociliates, *Anophryoides haemophila* and *Uronema nigricans*, have been demonstrated (Novotny et al., 1996; Crosbie & Munday, 1999), little is known on the efficacy for *in vivo* of these chemicals. Therefore, currently there are no known measures to effectively control scuticociliatosis. This study provides information on potential chemotherapeutants for the control of scuticocil-

iatosis of cultured olive flounder.

Materials and Methods

Scuticociliate-infected fish (mean body length: 12cm) were obtained from a local land-based flounder farm, located at Kijang, Pusan, Korea. The infection rate of the fish was confirmed by examination of 40 fish. The infection rate of total fish was 100%. Mildly infected fish showed white patches in the body surface; ciliate detected in the external organs including body surface was 70%. Heavily infected fish showed serious lesions in the infected area; ciliate detected in the internal organs including brain was 30%. A total of 480 fish were separated into two groups (240 mildly infected fish and 240 heavily infected fish) and acclimatized for 24 h prior to the experiment in both 500 l tank with aeration and continuous flow of filtered seawater (18 ± 1°C, salinity 33 ppt, dissolve oxygen 7.2 ppm).

Estimation of infection status was recorded on a

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4-point scale, after examination of infected area in wet mounts ($\times 100$), as follows: 'no' (-), ciliate not detected in any tissue or organ of the sample; 'minimal' (+), ciliate only detected in the body surface of the sample; 'low' (++) , ciliate detected in the external organ of the sample; 'high' (+++) , ciliate detected in the internal organ of the sample. The chemicals tested in the study are formalin, fresh water, hydrogen peroxide and sodium chloride.

Each chemical was evaluated in a 30 l plastic container with aeration at closed seawater ($18 \pm 1^\circ\text{C}$, pH 6.2, DO 7 ppm) containing 10 infected fish. The fish received either bath treatment for 0.5h or 1h per day on 2 or 4 consecutive days. Throughout the assay period the fish were monitored regularly to check for signs of toxicity. Twenty-four hours after the end of the assay, fish were killed for determination of infection status as mentioned above.

Table 1. Infection status of juvenile olive flounder (*Paralichthys olivaceus*) mildly infected with scuticociliates in the containers receiving the indicated substances (in all cases treated for 2 consecutive days), 24 h post treatment infection status is shown for each of the 10 fish included in the assay for that substance. Infection status: (-), no; (+), minimal; (++) , low; (+++) , high. Each value represents the mean of 2 replicated experiments

Chemical	Concentration	Treatment	Flounder number									
			1	2	3	4	5	6	7	8	9	10
Formalin	100 ppm	Bath, 1 h	-	-	-	+	+	+	+	+	-	+
Fresh water	100%	Bath, 1 h	-	-	-	+	+	+	+	+	+	-
Hydrogen peroxide (30%, Merck)	100 ppm	Bath, 1 h	+	+	+	+	-	-	+	+	++	+
Sodium chloride (99%, Sigma)	30%	Bath, 0.5 h	-	-	-	-	+	+	+	+	+	+
Control			+	++	++	++	+	++	+	+	++	++
Control ^a			+	+	++	+	+	++	+	+	+	+

^a The control was juvenile olive flounder infected with scuticociliates in the containers prior to treating with the chemicals.

Table 2. Infection status of juvenile olive flounder (*Paralichthys olivaceus*) mildly infected with scuticociliates in the containers receiving the indicated substances (in all cases treated for 4 consecutive days), 24 h post treatment infection status is shown for each of the 10 fish included in the assay for that substance. Infection status: (-), no; (+), minimal; (++) , low; (+++) , high; (D), fish died. Each value represents the mean of 2 replicated experiments

Chemical	Concentration	Treatment	Flounder number									
			1	2	3	4	5	6	7	8	9	10
Formalin	100 ppm	Bath, 1 h	-	-	-	-	-	-	-	-	-	-
Fresh water	100%	Bath, 1 h	-	-	-	-	-	-	-	-	-	-
Hydrogen peroxide (30%, Merck)	100 ppm	Bath, 1 h	+	-	-	-	D	-	-	-	+	+
Sodium chloride (99%, Sigma)	3%	Bath, 0.5 h	D	D	-	-	-	-	-	-	-	-
Control			+	+++	++	++	++	++	+++	++	+++	+
Control ^a			+	+	-	+	++	+	+	++	-	++

^a The control was juvenile olive flounder infected with scuticociliates in the containers prior to treating with the chemicals.

Table 3. Infection status of juvenile olive flounder (*Paralichthys olivaceus*) heavily infected with scuticociliates in the containers receiving the indicated substances (in all cases treated for 2 consecutive days), 24 h post treatment infection status is shown for each of the 10 fish included in the assay for that substance. Infection status: (-), no;(+), minimal;(++), low;(+++), high;(D), fish died. Each value represents the mean of 2 replicated experiments

Chemical	Concentration	Treatment	Flounder number										
			1	2	3	4	5	6	7	8	9	10	
Formalin	100 ppm	Bath, 1 h	D	D	D	D	++	+++	+++	+++	+++	+++	+++
Fresh water	100%	Bath, 1 h	D	+++	D	D	++	+++	+++	+++	+++	+++	+++
Hydrogen peroxide (30%, Merck)	100 ppm	Bath, 1 h	D	+++	D	D	D	++	+++	+++	+++	+++	+++
Sodium chloride (99%, Sigma)	3%	Bath, 0.5 h	D	D	D	D	D	+	+	+++	+++	+++	
Control			D	D	D	D	+++	+++	+++	+++	+++	+++	+++
Control ^a			+++	+++	++	++	+++	+++	++	+++	++	+++	+++

^aThe control was juvenile olive flounder infected with scuticociliates in the containers prior to treating with the chemicals.

Table 4. Infection status of juvenile olive flounder (*Paralichthys olivaceus*) heavily infected with scuticociliates in the containers receiving the indicated chemicals (in all cases treated for 4 consecutive days), 24 h post treatment infection status is shown for each of the 10 fish included in the assay for that chemical. Infection status: (-), no infection;(+), mild infection;(++), moderate infection;(+++), heavy infection;(D), fish died. Each value represents the mean of 2 replicated experiments

Chemical	Concentration	Treatment	Flounder number									
			1	2	3	4	5	6	7	8	9	10
Formalin	100 ppm	Bath, 1h	D	D	D	D	D	D	++	++	+++	+++
Fresh water	100%	Bath, 1h	D	+++	D	D	D	+++	+++	D	D	D
Hydrogen peroxide (30%, Merck)	100 ppm	Bath, 1h	D	D	D	D	++	+++	+++	D	D	D
Sodium chloride (99%, Sigma)	3%	Bath, 0.5h	D	D	D	D	D	++	D	D	D	+++
Control			D	D	D	D	D	D	D	+++	+++	D
Control ^a			+++	+++	+++	++	+++	+++	+++	+++	+++	+++

^aThe control was juvenile olive flounder infected with scuticociliates in the containers prior to treating with the chemicals.

Results

Bath treatment effect of the substance against mildly infected flounder is presented in Table 1 and 2. Although all groups treated for 2 days showed reduction in infection rate compared with control group did not show complete elimination of ciliates in the external organs of flounder (Table 1). However, 3 groups except for hydrogen peroxide treated group for 4 days showed a complete elimination of

ciliate infection (Table 2). The groups of fish treated with sodium chloride had a little toxic effect.

Bath treatment effect of the chemicals against heavily infected flounder is presented in Table 3 and 4. All groups were not effective (Table 3). The chemicals showed to be detrimental to fish infected on internal organs (Table 4). The results confirm the efficacy of the chemicals when treated for a mild infection or early infection stage, represented scuticociliates infected on external organs of the fish.

Discussion

The results indicate that mildly infected flounder, scuticociliates infecting the external organs of the fish, can be controlled effectively by bath treatment of 100 ppm formalin and freshwater.

All chemicals using in this study are commonly used in Korea, as parasiticide of ectoparasite. Formalin is currently approved for use in aquaculture as a fungicide and parasiticide, typically at 167 to 250 ppm for 1 h bath (Poupard, 1978; Scott, 1993). Hydrogen peroxide has been used to treat freshwater fish for ectoparasite, typically at 1.5 g/ℓ to 2g/ℓ (Bruno and Raynard, 1994). Sodium chloride and freshwater bath has been applied to eliminate of ectoparasite using osmoregulatory control of fishes (McVicar and Richards, 1981; Schnick, 1988; Sevendsen and Haug, 1991).

In our studies, 100 ppm hydrogen peroxide had no perceptible effect on mildly infected fish. Although 3% sodium chloride could completely eliminate the ciliate infecting external organs of flounder, the substance had a little toxic effect. Therefore, these chemicals may be of limited applicability in scuticociliates infecting flounder. Both 100 ppm formalin and freshwater had a reliable effect on mildly infected fish. Effectiveness of the chemicals was determined by treatment periods. Bath treatment for 4 day of the substances could completely eliminate the scuticociliate infected on external organs of flounder. Cheung *et al.* (1980) and Bassler (1983) recorded that scuticociliates infecting fish must be repeatedly treated by chemicals as similar to our treatment method. Although the pathological effects of formalin and the detrimental effects of fresh water bath on fish are documented (Bassler, 1983; Cruz and Pitogo, 1989), we could not observe any side effect such as histopathological change and tissue damage in the

fish treated the chemicals. Therefore, formalin and freshwater may be useful for control of mildly infected flounder.

The present study showed that all substances used in this study were not effective against heavily infected fish, on the contrary to be detrimental to heavily infected fish observed by Bassler (1983). It is probable that the substances do not act directly on scuticociliate within internal organs since these substances are not absorbed into the internal organs of fish. Once the scuticociliate has penetrated into host tissue (Dykova and Figueras, 1994), immediately settle down internal organs including brain of host fish (Munday *et al.*, 1997).

The elimination of scuticociliates within the internal organs would be worth future study.

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