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Effects of Oxytetracycline Treatments on the Infection Potential of Scuticociliates in Cultured Olive Flounder, *Paralichthys olivaceus*

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The modulatory effects of oxytetracycline treatments at high concentrations on the infection potential of scuticociliates in cultured juvenile olive flounder (*Paralichthys olivaceus*) and density of the ciliates in culturing water were investigated. The groups bathed with 400 and 500 ppm of oxytetracycline showed significantly lower intensities of scuticociliates on the fish and considerably lower number of the ciliates in culturing water when compared with the control group. However, the intensity of scuticociliates on the fish in the group bathed with 300 ppm of oxytetracycline was not significantly different with that of the control group in spite of considerably lower number of scuticociliates in culturing water than in that of the control group. Although the intensities of scuticociliates on the fish intubated orally with 400 and 500 mg/kg of oxytetracycline were lower than that of the control group, there were no statistical significances. In contrast, the fish fed 300 mg/kg of oxytetracycline showed significantly lower intensity of scuticociliates when compared with other groups. The results of this study suggest that oxytetracycline treatments can modulate occurrence of scuticociliatosis in fish farms probably through change of bacterial density, damaging to scuticociliates, and immuno-suppression of fish.

Key words: Scuticociliates, Olive flounder, Oxytetracycline, Infection potential

Introduction

Several scuticociliate species belonging to the genera *Uronema, Miamiensis* and *Philasterides* are facultative histophagous parasites in marine fish (Thompson and Moewus, 1964; Cheung et al., 1980; Yoshinaga and Nakazoe, 1993; Dykov and Figueras, 1994; Dragesco et al., 1995; Gill and Callinan, 1997; Munday et al., 1997; Sterud et al., 2000; Iglesias et al., 2001). These ciliates are characterized by their high potential for systemic infection and fish tissue destruction, leading to high mortalities in cultured fish. In Korea, scuticociliatosis is a serious problem in culturing olive flounder, *Paralichthys olivaceus*.

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No effective, approved chemotherapeutant exist for the control or treatment of scuticocliatosis, and little information is available on the epizootiological factors which determine when ciliate infections progress to the disease in fish. The weakened physiological state of the host as a result of environmental or handling stress may create an opportunity for the facultative scuticociliates to gain entry into the host. Furthermore, the possibility of other pathogens such as bacteria serving as the primary invaders cannot be ruled out.

Frequently the olive flounder farms suffer from scuticociliatosis outbreaks immediately after handling or transportation of fish, antibiotic treatments, or an inflow of sediments around culturing farm because of typhoons. Although oxytetracycline is the most commonly used antibacterial drug in Korean

fish farming and routinely used to prevent bacterial diseases after applying handling stresses such as selection, the effects of this drug on the occurrence of scuticociliatosis have not been studied. The objective of this study was to evaluate the modulatory effects of oxytetracycline treatments at high concentrations on the infection potential of scuticociliates and density of the ciliates in culturing water.

Materials and Methods

Fish

Juvenile olive flounder (*Paralichthys olivaceus*) with an average body weight of 5 g were obtained from a local fish hatchery and acclimated in laboratory aquaria for 2 weeks prior to the experiment. The water temperature was kept at 20 ± 1℃ during the experimental period.

Isolation and culture of scutiociliates

Scuticociliates isolated asceptically from the brain of infected olive flounders were inoculated into minimum essential medium (MEM, Sigma Chemical Co., St Louis, MO, USA) and were incubated at 20 ℃. The ciliates were subcultured every 3 days for 2 months.

Experimental regime

A total of 80 fish were randomly distributed into 8 square tanks (10 fish in each tank) containing 3 L sea water with aeration. In each aquarium, 1 mL of bacterial suspension containing 0.2 g of bacteria, which were isolated from olive flounder culturing sea water and cultured in TSB broth medium, was inoculated. After 48 hours of bacterial inoculation, fish were treated with oxytetracycline by bath or oral administration. In bath treatments, oxytetracycline was dissolved at the final concentration of 0 (control), 300, 400, and 500 ppm in the culturing water. In oral treatments, fish were anaesthetized with MS222 (Sigma), and oxytetracycline was orally delivered at a dose of 300, 400, and 500 mg/kg B.W. using an intubation tube. The control fish were given saline without drug. Two hours post-treatments, all fish in each group were challenged with 2×10^6 scuticociliates. Two days after the treatments, whole dorsal skin and whole gill lamellae of each fish were sampled for evaluation of the intensity of scuticociliates. The culturing water of each aquarium, also, sampled for enumeration of the scuticociliates density.

Statistical analysis

A Mann-Whitney's U-test was used to test for differences in the number of scuticociliates on fish among experimental groups, and the difference was considered significant when $P<0.05$.

Results

Bath treatments

The groups bathed with 400 and 500 ppm of oxytetracycline showed significantly lower intensities of scuticociliates on the fish and considerably lower number of the ciliates in culturing water when compared with the control group (Fig. 1). However, the intensity of scuticociliates on the fish in the group bathed with 300 ppm of oxytetracycline was not significantly different with that of the control group in spite of considerably lower number of scuticociliates in culturing water than in that of the control group.

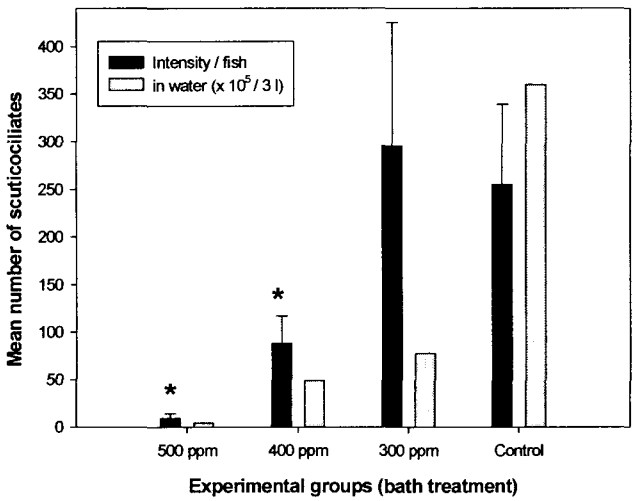

Fig. 1. The effects of bath treatments with oxytetracyclin on the intensity of scuticociliates in juvenile olive flounder, *Paralichthys olivaceus*, and number of the ciliates in culturing water (*, significantly different from control group at $P<0.05$).

Oral Treatments

Although the intensities of scuticociliates on the fish intubated orally with 400 and 500 mg/kg of oxytetracycline were lower than that of the control group, there were no statistical significances (Fg. 2). In contrast, the fish fed 300 mg/kg of oxytetracycline showed significantly lower intensity of scuticociliates when compared with other groups.

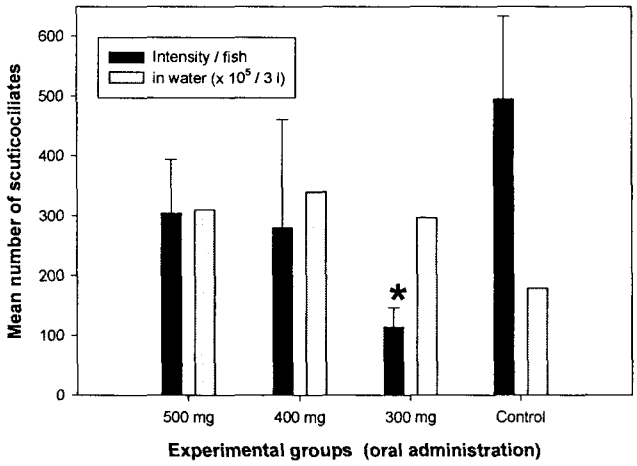

Fig. 2. The effects of oral treatments with oxytetracyclin (mg/kg) on the intensity of scuticociliates in juvenile olive flounder, *Paralichthys olivaceus,* and number of the ciliates in culturing water (*, significantly different from control group at $P<0.05$).

Discussion

The present results suggest that infection potential of scticociliates can be modulated by the treatments of olive flounder with oxytetracycline. Despite the strong histophagous tendencies demonstrated by several scuticociliate species, they are considered to be facultative parasites with infections being accidental or opportunistic in nature. Under free living conditions, scuticocilliates are bacteriovorous protozoans (Lom and Dykova, 1992). Therefore, antibiotic treatments would influence on the growth and survival of scuticociliates through reducing bacterial density in water and/or cytotoxic damages to the ciliates. Crosbie and Munday (1999) reported that growth of *Uronema nigricans* was sensitive to fluctuations in bacterial densities. The cytotoxic effect of oxytetracycline on scuticociliates was confirmed by *in vitro* inhibition of the ciliates growth (Novotny et al., 1996; Cribb et al., 1999).

Significantly reduced intensity of scuticociliates on the fish and a few number of scuticociliates in culturing water by bath with 500 ppm oxytetracycline indicate that not only bacteria but also scuticociliates are fatally damaged by the toxicity of high concentration of oxytetracycline. In contrast, higher intensity of scuticociliates on the fish and lower density of the ciliates in culturing water by bath with 300 ppm oxytetracycline when compared with those of control group suggests that scuticociliates change their feeding habit from bacteriovorous to histophagous because of shortage of bacterial food in water, or the ciliates shelter themselves from the toxic oxytetracycline in water by penetration into the fish skin. The typical ciliate response to starvation is rapid cell division with a subsequent decrease in cell volume, rapid motility and swarming behaviour (Fenchel, 1990). Therefore, olive flounder may be preferentially colonised in the absence of sufficient bacteria.

Stress-mediated immunosuppression has been proposed as a main cause of scutiociliatosis (Munday et al., 1997). In the present study, anesthetization and handling stressors during oral intubation should suppress the immunity of olive flounder, and this suppression might lead to the considerably higher density of scuticociliates on the fish in the control group of oral administration than that in the control group of bath treatments, which did not undergo handling stresses.

The significantly lower intensity of scuticociliates on the fish in 300 mg/kg of oxytetracycline administerd group than that in control group suggests that the ciliates evade fish tissues which contain oxytetracycline and relatively low density of bacteria. Although the intensities of scuticociliates on the fish intubated with 400 and 500 mg/kg oxytetra-cycline were lower than that of control, there were no statistical differences. This indicates that oral administration of oxytetracycline at extremely high concentrations may negatively affect on fish immunity. The suppression of fish immune responses by oxytetracycline treatments have been demonstrated by many authors (Rijkers et al., 1980; Lunden et al., 1998).

In conclusion, oxytetracycline treatments can modulate occurrence of scuticociliatosis in fish farms

probably through change of bacterial density, damaging to scuticociliates, and immuno-suppression of fish.

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References

Cheung, P.J., R.F. Nigrelli and G.D. Ruggieri. 1980. Studies on the morphology of *Uronema marinum* Dujardin (Ciliatea: Uronematidae) with a description of the histopatology of the infection in marine fishes. J. Fish Dis., 3: 295~303.

Cribb, A.E., B. Despres R.J. and Cawthorn. 1999. Tetrazolium -based cytotoxicityassay to determine anti-protozoal activity against the scuticociiate *Anohryoides haemophila*. Dis. Aquat. Org., 35: 213~219.

Crosbie, P.B.B. and B.L. Munday. 1999. Environmental factors and chemical agents affecting the growth of the pathogenic marine ciliate *Uronema nigricans*. Dis. Aquat Org., 36: 213~219.

Dragesco, A., J. Dragesco, F. Coste, C. Gasc, B. Romestand, J. Raymond and G. Bouix. 1995. *Philasterides dicentrarchi*, n. sp. (Ciliophora, Scuticociliatida), a histophagous opportunistic parasite of *Dicentrachus labrax* (Linnaeus, 1758), a reared marine fish. Eur. J. Protistol., 31: 327~340.

Dyková, I. and A. Figueras. 1994. Histopathological changes in turbot *Scophthalmus maximus* due to a histophagous ciliate. Dis. Aquat. Org., 18: 5~9.

Fenchel, T. 1990. Adaptive significance of polymorphic life cycles in protozoa: responses to starvation and refeeding in two species of marine ciliates. J. Exp. Mar. Biol. Ecol., 136: 159~177.

Gill, P.A. and R.B. Calinan. 1997. Ulcerative dermatitis associated with *Uronema* sp. Infection of farmed sand whiting *Sillago ciliata*. Aust. Vet. J., 75: 357.

Iglesias, R., A. Paramá, M.F. Alvarez, J. Leiro, J. Fernández, and M.L. Sanmartín. 2001. *Philasterides dicentrarchi* (Ciliophora, Scuticociliatida) as the causative agent of scuticociliatosis in farmed turbot *Scophthalmus maximus* in Galicia (NW Spain). Dis. Aquat. Org., 46: 47~55.

Lom, J. and I. Dykova. 1992. Development in aquaculture and fisheriesscience, Vol. 26. Protozoan parasites of fishes. Elsevier, Amsterdam, pp. 237~288.

Lunden, T., S. Miettinen, L.G. Lonnstrom, E.M. Lilius and G. Bylund. 1998. Influence of oxytetracycline and oxolinic acid on the immune response of rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol., 8: 217~230.

Munday, B.L., P.J. ODonoghue, M. Watts, K. Rough and T. Hawkesford. 1997. Fatal encephalitis due to the scuticociliate *Uronema nigricans* in sea-caged, southern bluefin tuna *Thunnus maccoyii*. Dis. Aquat. Org., 30: 17~25.

Novotny, M.J., R.J. Cawthorn and B. Despres. 1996. *In vitro* effects of chemotherapeutants on the lobster parasite *Anophryoides haemophila*. Dis. Aquat. Org., 24: 233~237.

Rijkers, G.T., A.G. Teunissen, R. Oosterom van and W.B. Muiswinkel van. 1980. The immune system of cyprinid fish. The immunosupprssive efct of the antibiotic oxytetracycline in carp (*Cyprinus carpio* L.). Aquaculture, 19: 177~189.

Sterud, E., M.K. Hansen and T.A. Mo. 2000. Systemic infecion with *Uronema*-like ciliates in farmed turbot, *Scophtalmus maximus* (L.). J. Fish Dis., 23: 33~37.

Thompson, C.L. Jr. and L. Moewus. 1964. *Miamiensis avidus* n.g. n.s., a marine facultative parasite in the ciliate order Hymenostomatida. J. Protozool., 11: 378~381.

Yoshinaga, T. and J. Nakazoe. 1993. Isolation and in vitro cultivation of an unidentified ciliate causing scuticociliatosis in Japanese flounder (*Paralichthys olivaceus*). Gyobyo Kenkyu, 28: 131~134.