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DISINFECTANT EFFECTS OF ULTRAVIOLET IRRADIATION ON FISH PATHOGENS IN HATCHERY WATER SUPPLY

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Abstract

Disinfectant effects of U.V. irradiation were examined on cell suspensions of 5 species of fish pathogenic bacteria, and a punched agar medium disk covered with 10 strains of aquatic fungi and 7 strains of cell free fish pathogenic viruses. 99.99 % or more of the viable bacterial cells were effected by U.V. treatment of more than 2.2×10^3 μW·sec/cm² U.V. dosage. The hyphae of aquatic fungi showed relatively lower susceptibility to U.V. irradiation, that which inhibited the growth of hyphae was $1.5 - 2.5 \times 10^5$ μW·sec/cm². Fish viruses, IHNV, HRV, OMV, CCV and H. salmonis were found to be sensitive to U.V. irradiation, and a 99 % or more infectivity decrease (ID_{99}) was $1.0 - 3.0 \times 10^3$ μW·sec/cm². Susceptibility of IPNV and CSV were found to be low, ID_{99} measured $1.0 - 1.5 \times 10^5$ μW·sec/cm². The infectivity of IHNV, in virus contaminated river water and IHNV contaminated pond water, measured by the molecular filtration method was 0.56 and 5.6 $TCID_{50}$/l, respectively. U.V. treatment of river water with 10^3 μW·sec/cm² dosage could prevent an IHN outbreak. Furthermore, U.V. treatment of the hatchery water supply also decreases the viable bacterial counts and fungi infection rates of salmonid eggs.

Introduction

Disinfection of the hatchery water supply with ultraviolet (U.V.) irradiation has been studied (Bedell 1971; Kimura et al. 1976; Bullock and Stuckey 1977) and interest was generated in the use of U.V. for the prevention of diseases associated with aquaculture (Aboul-Ela 1958; Brown and Russo 1979; Fisher et al. 1976; Hoffman 1974; Sako and Sorimachi 1985; Waugh 1958). Recently rainbow trout farms in mainland Japan have been putting the U.V. irradiation to practical use, which could prevent the infectious hematopoietic necrosis (IHN) in U.V. irradiated river water which was contaminated with IHNV.

Here we review the results of our studies on the U.V. susceptibility of the fish pathogenic bacteria, fungi and viruses (Kimura et al. 1976, 1980a,b; Yoshimizu 1981; Yoshimizu et al. 1986) and consider the usefulness of a U.V. water treatment unit for the disinfection of hatchery water supplies by comparing the disinfection rates of bacteria and viruses, hatching rate of salmonid eggs, and survival rate of rainbow trout fry which were cultured with the U.V. irradiated water supply.

Materials and Methods

Fish pathogenic bacteria, fungi and viruses used:

Five species of fish pathogenic bacteria and Escherichia coli, 9 species of 10 strains of aquatic fungi and 6 species of 7 strains of fish viruses were used in this study (Table 1).

Preparation of pathogens for test:

Bacteria and fungi were cultured on fresh water agar (FWA; Kimura et al. 1976) and hemp seed broth or sabouraud agar medium, respectively, at 25 °C for 2 days. Fish viruses were cultured on rainbow trout gonad cells (RTG-2), chinook salmon embryo cells (CHSE-214), and channel catfish ovary cells (CCO) using Eagle's minimum essential medium (MEM) supplemented with 10 % fetal bovine serum and 100 IU/ml penicillin and 100 μg/ml streptomysin incubated at 15 °C for 7 days. Culture fluid was filtered and stored at -80 °C until used.

Measurement of U.V. susceptibility:

The U.V. susceptibility of the bacterial strains was measured using the FWA plates; 0.1 ml of bacterial suspension (10^4 CFU/ml) was spread on the agar plates (⌀ 90 mm) and irradiated with U.V. of different dosages. A dose of 99.9 % CFU reduction was determined. For the fungi, a punched agar medium disk covered with fungus hyphae was employed for the test. For fish viruses, 0.2 ml of cell free culture medium was spread on the dish (60 mm) and irradiated with U.V. in the same manner as the bacteria. A reduction of 99 % or more in their infectivity (ID_{99}) was measured. U.V. dosage was measured by a U.V. photometer (Topcon UVR-254).

Effect of the U.V. irradiation on the infectivity of viruses suspended in the dechlorinated city water:

Infectious hematopoietic necrosis virus (IHNV) and Oncorhynchus masou virus (OMV) were suspended in dechlorinated city water and the infectivity was adjusted to 100 $TCID_{50}$/ml. Viruses were treated by a U.V. water treatment unit (Nippo SF-INSM) with different flow rates and the infectivity of the viruses was measured.

Effect of the U.V. irradiation of the

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water supply on hatching rate of masu and chum salmon:

Eight thousand and five hundred masu salmon (O. masou) eggs, 6 days after fertilization, and 4,000 chum salmon (O. keta) eggs, just after fertilization, were employed for this study. Eggs were cultured in the Mori branch of Hokkaido Fish Hatchery and separated into 2 groups. U.V. irradiated water was supplied for one group of each species, the U.V. dosage was adjusted to 2.2 - 6.3 x 10^4 μW·sec/cm². Eggs were cultured until hatching and the eyeing rate and hatching rate were measured.

Effect of the U.V. irradiation of the water supply on the survival rate of rainbow trout:

Two groups of 1,000 rainbow trout (O. mikiss), average body weight 0.11 g, were cultured in 45 l aquariums, using river water and U.V. irradiated river water. The flow rate was 15 - 20 ml/sec and the U.V. dosage was 1.2 - 2.0 x 10^3 μW·sec/cm². Survival rates of these 2 groups of fish were observed for 25 days and virus infectivity in the water supplies was measured by the molecular filtration method (Watanabe et al. 1988) with tangential flow filtration (Pellicon Cassette System, Millipore Corp.). Before filtration, beef extract was added to the water (0.01 %) and the concentrated water was sterilized with a 0.45 μm filter (Millipore HA) pre-treated with 1% FBS. Pond water with an outbreak of IHN was also employed and the IHNV infectivity measured.

Table 1. Minimal bactericidal, fungicidal and virus inactivating U.V. dosage for fish pathogenic bacteria, fungi and viruses

Species				U.V. dosage*
Aeromonas hydrophila		IAM	1018	5.0 x 10^3
Aeromonas punctata		IAM	1646	4.0 x 10^3
Aeromonas salmonicida		ATCC	14174	4.0 x 10^3
Vibrio anguillarum		NCMB	6	4.0 x 10^3
Pseudomonas fluorescens		EFDL		5.0 x 10^3
Escherichia coli		O-26		4.0 x 10^3
Saprolegnia parasitica		IFO	897	2.3 x 10^5
Saprolegnia parasitica		ATCC	22284	2.0 x 10^5
Saprolegnia ferax		ATCC	10936	2.3 x 10^5
Saprolegnia diclina		CBS	3263	2.3 x 10^5
Saprolegnia anisospora		CBS	1784	1.5 x 10^5
Saprolegnia	sp.	Gifu		2.2 x 10^5
Saprolegnia	sp.	Tokyo		2.5 x 10^5
Saprolegnia	sp.	Shizuoka		2.3 x 10^5
Aphanomyces laevis		CBS	10752	2.1 x 10^5
Achlya flagellata		ATCC	14566	2.2 x 10^5
IPNV (Buhl)**				1.5 x 10^5
Chum salmon virus (CSV)				1.0 x 10^5
IHNV (ChAb)***				2.0 x 10^3
IHNV (RtTo)				3.0 x 10^3
Oncorhynchus masou virus (OMV)				2.0 x 10^3
Channel catfish virus (CCV)				2.0 x 10^3
Herpesvirus salmonis				2.0 x 10^3

* : To destroy at least 99.9 % of the viable bacteria, to inhibit the growth of hyphae or to decrease the virus infectivity more than 99%; μW sec/cm².
** : Infectious pancreatic necrosis virus.
*** : Infectious hematopoietic necrosis virus.

Table 2. Effect of U.V. disinfection of water supply on salmon egg hatching

	Experiment I		Experiment II	
	Masu salmon		Chum salmon	
	Test*	Control	Test**	Control
Number of eggs employed	4334	4333	1867	1974
Number of eyed eggs yield	3190	2393	1779	1512
Eyeing rate(%)	73.5	55.3	95.3	76.6
Number of fungal infected eggs	918	1940	155	573
Fungi infection rate (%)	26.4	44.2	8.3	29.1
Number of surviving alevin	2982	2007	1758	1493
Hatching rate (%)	68.8	43.3	94.2	75.6

* : Flow rate; 5.0 - 8.5 l/min.
U.V. dosage; 22-38 x 10^3 μW·sec/cm².
**: Flow rate; 3.0 - 5.0 l/min.
U.V. dosage; 38-63 x 10^3 μW·sec/cm².

Results

U.V. susceptibility of bacteria, fungi and viruses:

U.V. susceptibility of the 6 strains of bacteria, 9 species of 10 strains of aquatic fungi and 6 species of 7 strains of fish viruses used is listed in Table 1. U.V. dosage of 99.9 % CFU reduction measured 4 - 5 x 10^3 μW·sec/cm². The hyphae of 10 strains of aquatic fungi showed lower sensitivity than bacteria and the minimal fungicidal dosage of U.V. irradiation was 1.5 - 2.5 x 10^5 μW·sec/cm². Fish pathogenic viruses, i.e. IHNV, OMV, CCV and H. salmonis were found to be sensitive to U.V. and a 99 % or more reduction in their infectivity (ID_{99}) measured 2.0 - 3.0 x 10^3 μW·sec/cm². Susceptibility of IPNV and CSV was found to be low and the ID_{99} measured 1.0 - 1.5 x 10^5 μW·sec/cm².

Effect of the U.V. irradiation on the infectivity of viruses suspended in the dechlorinated city water:

U.V. treatment at the flow rate of less than 16.7 l/min (flow rate that was equivalent to more than 7.6 x 10^4 μW·sec/cm² U.V. dosage) was an effective means for disinfecting 100 $TCID_{50}$/ml of IHNV and OMV which were suspended in dechlorinated city water. The infectivity of both viruses decreased to a less than

detectable limit.

Effect of the U.V. irradiation of the water supply on hatching rate of masu and chum salmon:

Eyeing rates and hatching rates of masu and chum salmon using the U.V. irradiated and nonirradiated water supplies are shown in Table 2. In the case of the U.V. irradiated test groups, eyeing rates of masu and chum salmon were 73.5 and 95.3 %, respectively, but in the nonirradiated control groups, eyeing rates were 55.3 and 76.6 %, respectively. Hatching rates of test and control groups were 68.8 and 43.3 % in masu salmon and 94.2 and 75.6 % in chum salmon, respectively.

Effect of the U.V. irradiation of water supply on the survival rate of rainbow trout:

Virus infectivity of IHNV in non irradiated river water was 0.56 $TCID_{50}$/l and U.V. in irradiated water was less than 0.32 $TCID_{50}$/l. Infectivity of the IHNV in the pond water where IHN had broken out measured 5.6 $TCID_{50}$/l.

The cumulative mortality of the U.V. treated group and the nontreated group is shown in Fig. 1. In spite of the fact that 99.7 % of rainbow trout in nonirradiated river water died, cumulative mortality of U.V. irradiated group was 3.9 %.

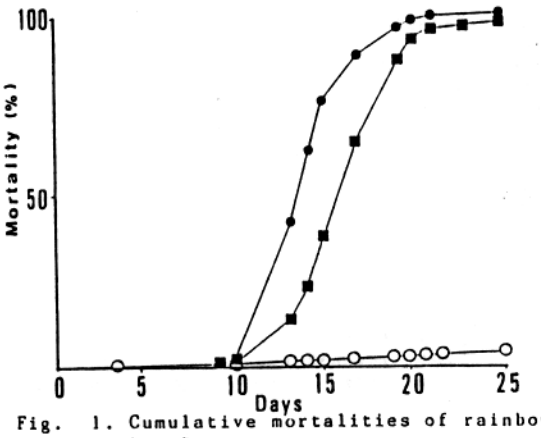

Fig. 1. Cumulative mortalities of rainbow trout.
●: Untreated water
■: After sedimentation
○: After U.V. irradiation

Discussion

We studied the U.V. susceptibility of the fish pathogenic bacteria, fungi and viruses. U.V. susceptibility of the 6 strains of Gram-negative bacteria and the 4 strains of fish pathogenic viruses, i.e. IHNV, OMV, CCV and H. salmonis employed was relatively high and with a U.V. dosage of 99.9 % CFU reduction and a 99 % or more reduction of their infectivity (ID) measured 4 - 5 and 1 - 1.5 x 10^3 μW sec/cm^2, respectively. This dosage of U.V. irradiation was almost the same as the data from Bullock and Stuckey (1977). The susceptibility of 10 aquatic fungi and viruses IPNV and CSV to U.V. was lower as compared with that of the bacteria and enveloped viruses, and with the minimal fungicidal dosage of U.V. irradiation, inhibition of growth of hyphae, and ID_{99} was 1.5 -2.5 x 10^5 $\mu W \cdot$ sec/cm^2, respectively. This susceptibility was a little higher than that of fish parasites (Vlasenko 1969; Hoffman 1974).

Regarding the usefulness of U.V. water treatment unit for disinfection of hatchery water supplies, U.V. treatment of less than 16.7 l/min flow rate, that was equivalent to more than 7.6 x 10^4 $\mu W \cdot$ sec/cm^2 U.V. dosage, is an effective means of disinfecting 100 $TCID_{50}$/ml of IHNV and OMV. We used a U.V. treatment unit for salmonid hatching water; eyeing rates and hatching rates of masu and chum salmon increased about 20 % as compared with control groups which were cultured with nontreated water.

Recently, rainbow trout farms in mainland Japan are using the U.V. treatment unit and this could prevent IHN from surviving in the river water which was contaminated with IHNV. Virus infectivity of IHNV in river water could not be determined by using the ordinary method, but infectivity of IHNV measured 0.56 $TCID_{50}$/l from nonirradiated river water and 5.6 $TCID_{50}$/l from pond water with an outbreak of IHN, when we used the molecular filtration method. IHNV was not isolated from U.V. irradiated water and the cumulative mortality of rainbow trout cultured in U.V. treated water was 3.9 %, but the cumulative mortality of the non treated control group reached 99.7 %, and demonstrated the effectiveness of U.V. treatment.

Generally, U.V. irradiation of water at wave length 2537 Angstrom (Å) units has proven effective and practical for the destruction of microorganisms and is nontoxic to fish. For this study we used this type of U.V. lamp. Wave lengths below 2000 Å produce ozone which was effective in destroying fish pathogenic bacteria, fungi, and viruses (Austin 1983; Conrad et al. 1975; Sako and Sorimachi 1985; Wedemeyer et al. 1977) but toxic to fish (Giese 1967; Wedemeyer 1978, 1979). The effectiveness of U.V.-ozone water sterilizer should be studied.

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