Effects of Sodium Nifurstyrenate and Tetracycline on the Bacterial Flora of Rotifers (Brachionus plicatilis)

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The effects of sodium nifurstyrenate (NFS-Na) and tetracycline (TC) on the aerobic bacterial flora of rotifers (Brachionus plicatilis) were evaluated both in vivo and in vitro.

In vivo, the bacterial numbers on ZoBell's 2216e agar of rotifers exposed to 5 µg/ml of NFS-Na decreased slightly, but the numbers on BTB teepol agar rapidly declined from 10⁷ to 10⁵ CFU/g after 5 h exposure. At the same time, a decrease in the incidence of Vibrio accompanied by an increase of Moraxella or Acinetobacter was observed in the NFS-Na medicated rotifers as compared with the controls. The viable counts on the two media and microflora of rotifers exposed to 1 or 5 µg/ml of TC exhibited similar pattern to those of the controls throughout the exposure period.

In vitro, 60 strains of bacteria isolated from the rotifers were tested for their susceptibility to the two chemotherapeutics. All the Vibrio strains tested were sensitive to NFS-Na, the MIC values ranging from 0.1 to 1.6 µg/ml. The strains of Acinetobacter, Moraxella and Pseudomonas were less susceptible or resistant to the drug. With respect to TC, most of the strains of the four genera were resistant.

Mass seed production of various marine fishes has been successfully developed in Japan since rotifer (Brachionus plicatilis) was introduced as larval feed. Seed production with the aid of this food organism was first established in red sea bream (Pagrus major), and the methodology was further extended to other economically important species such as black sea bream (Acanthopagrus schlegeli), Japanese flounder (Paralichthys olivaceus), rockfish (Sebastes schlegeli), and tiger puffer (Takifugu rubripes) (NAGATA and HIRATA, 1986; FUKUHARA, 1987). However, high mortalities of fish larvae fed rotifers have occurred even after the nutritional value of this live diet was raised by incorporating essential fatty acids and vitamins (WATANABE et al., 1983a, 1983b). These mortalities have been often ascribed to infectious diseases. Some species of the genus Vibrio, for instance, have been reported to cause abnormalities in the alimentary tracts of the larvae of Japanese flounder, red and black sea breams (IWATA et al., 1978; KUSUDA et al., 1986; MURATA, 1987). Despite the fact that the pathogenesis of these intestinal infections are poorly understood, live diets are thought to be the source of the infections. Consequently, in actual seed production facilities, rotifers are often treated with some chemotherapeutic agents in order to diminish the bacterial contamination prior to feeding to the fish larvae. However, the effects of these drug treatments are not obviously known.

The present study aimed at ascertaining the effects of sodium nifurstyrenate, the most commonly used chemotherapeutant in hatcheries, and tetracycline hydrochloride on the microflora of rotifers.

Materials and Methods

Rotifer samples
Cultured batches of rotifers (B. plicatilis) were obtained from Hiroshima Prefectural Fish Farming Center on April 25 for experiment 1, and May 2, 1988 for experiment 2.

Drugs
Sodium nifurstyrenate (NFS-Na) (KASHIWAGI et al., 1977) or Erubaju (Ueno Fine Chemicals Industry Ltd.), a commercially available form containing 10% NFS-Na, and tetracycline hy-
drochloride (TC) (Sigma Chemical Co.) were used in this study.

**Treatment of rotifers with drugs**

Two experiments were conducted in three beakers each containing 1 l of sand-filtered sea water. In experiment 1, a control beaker had no chemotherapeutant and the remaining 2 beakers contained NFS-Na (Erubaju was used as NFS-Na) and TC at concentrations of 5 and 1 µg/ml, respectively. Then, 2 g (wet weight) of rotifers were placed into each of the test beakers, followed by incubation at 20°C with aeration. After 1, 3 and 5 h exposure, 300 ml of each suspension was taken and filtered through a plankton net. The collected rotifers were rinsed thoroughly in sea water for 2 min and then subjected to bacteriological examinations. Bacterial flora of rotifers just prior to the experiment (0 h) was also examined. Experiment 2 was performed in the same procedure, except enhancing TC concentration to 5 µg/ml.

**Bacteriological examination**

Each group of rotifers weighing 0.1 g was aseptically homogenized with 0.85% saline in a glass homogenizer. Serially 10-fold diluted samples at 0.1 ml were spread onto ZoBell's 2216e and BTB teepol agar (Eiken Chemical Co., Ltd.) media. After incubation at 25°C for 48 h, colony forming units on the two media were determined. For taxonomical analysis, 8 or 9 representative isolates...
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on ZoBell agar were selected at each exposure time. A total of 60 isolates was tested for 35 morphological and biochemical characteristics. The identification was based on the scheme described by Muroga et al. (1987) and Bergey's Manual of Systematic Bacteriology Vol. 1 (Krieg and Holt, 1984).

Drug susceptibility test

All the strains were tested in vitro for their susceptibility to NFS-Na and TC. The minimal inhibitory concentrations (MICs) were determined by broth dilution method (Mitsuhashi, 1979). Each drug was serially 2-fold diluted into three different liquid media to an appropriate concentration. The media employed were heart infusion (HI) broth (Eiken) containing 3% NaCl, HI dissolved in sea water and ZoBell's 2216e liquid medium with a pH of 7. Following inoculation with a calibrated suspension of the bacterial isolates, incubation was maintained at 25°C for 24 h. The lowest concentrations of the drugs inhibiting the growth of bacteria were recorded as MICs.

Results

Bacterial counts of rotifers

The effects of the two chemotherapeutics on the aerobic bacterial counts of rotifers are depicted in Fig. 1. NFS-Na at the given concentration (5 µg/ml) was found to have reduced the bacterial counts of rotifers. While the bacterial numbers on ZoBell agar decreased slightly, the numbers on BTB agar rapidly declined from $2.0 \times 10^7$ to $1.0 \times 10^5$ CFU/g in experiment 1 and from $3.9 \times 10^7$ to $3.3 \times 10^6$ CFU/g in experiment 2 after 5 h exposure to NFS-Na. On the contrary, the viable counts of rotifers exposed to 1 or 5 µg/ml of TC remained approximately constant as that of the controls on both media throughout the exposure period.

![Fig. 2. The effects of NFS-Na and TC on the bacterial flora of rotifers in experiment 1.](image-url)
Bacterial flora of rotifers

The bacterial flora associated with rotifer samples in the two experiments consisted of only Gram-negative bacteria. Three different genera were distributed throughout the samples examined in experiment 1 (Fig. 2). However, difference in the incidence of these bacterial groups was obvious between the rotifers exposed to 5 µg/ml NFS-Na and control. In the control group, Moraxella (45.0%) was the most predominant, followed by Pseudomonas (30.9%) and Vibrio (12.7%). The incidence of Moraxella decreased with the lapse of time, and instead of this, the proportion of Vibrio increased. In the NFS-Na treated group, the prevalence of Moraxella became greater and reached 78.2% and Vibrio remained as a minor component after 5 h exposure. The microflora of rotifers treated with 1 µg/ml TC exhibited similar pattern to those of the control throughout the experiment.

In experiment 2 (Fig. 3), in addition to the three bacterial types predominating in experiment 1, Acinetobacter was also detected from the rotifers. Again, the distribution of the bacterial groups in NFS-Na medicated rotifers differed clearly from that in control. While Vibrio (41.7%), Acinetobacter (27.8%) and Pseudomonas (22.2%) were preponderant in the control, Acinetobacter and Vibrio constituted 78.6 and 5.9% respectively of the bacterial flora of rotifers treated with NFS-Na for 5 h. On the other hand, no significant difference in the distribution of the bacterial groups between rotifers exposed to 5 µg/ml TC and the control was noted.

MICs of drugs

The 60 strains isolated from rotifers were grouped into Vibrio (15), Acinetobacter (5), Moraxella (12) and Pseudomonas (28). The MICs of NFS-Na against these strains are illustrated in Fig. 4. All the Vibrio strains tested were sensitive to NFS-Na in any media used, the MIC values determined in ZoBell medium ranging from 0.01 to 1.6 µg/ml. On the other hand,
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Fig. 4. The minimal inhibitory concentrations of NFS-Na against the bacterial flora of rotifers tested in three different media.

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Discussion

Rotifer has been thought to serve as the source of infection in seed production of ayu (Plecoglosus altivelis) (Hayashi et al., 1975; Tabata et al., 1982; Tatani et al., 1985) and marine fishes (Iwata et al., 1978). In order to find a proper method for reducing the bacterial contamination of rotifers, the effects of antibacterial agents and ultraviolet irradiation have been investigated (Hayashi et al., 1975, 1976; Hatai et al., 1981; Tabata et al., 1982; Yamanoi and Sugiyama, 1987; Miyakawa and Muroga, 1988). According to these studies, ultraviolet irradiation together with NFS-Na treatment seems to be the most efficient method to control the bacterial multiplication in rotifers. Among the drugs tested, NFS-Na is likely the best choice eventhough an exceptional result was reported (Hatai et al., 1981).

The effect of NFS-Na was demonstrated by the reduction of the bacterial counts measured on BTB teepol agar, a selective medium for vibrios (Yamanoi and Sugiyama, 1987; Miyakawa and
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Fig. 5. The minimal inhibitory concentrations of TC against the bacterial flora of rotifers tested in three different media.

Muroga, 1988). In the present study, it was confirmed that the total bacterial counts of the rotifers was reduced by NFS-Na and this was due to the decrease in the number of vibrios. Furthermore, it was demonstrated that Vibrio strains isolated from rotifers were all sensitive to NFS-Na and others were rather resistant to the drug. This indicates that the chemotherapeutant might be effective in preventing the bacterial diseases in seed production, because some Vibrio species are thought to be associated with various disease conditions in fish larvae as mentioned before. However, a practical dosage of the drug for controlling diseases cannot be concluded from the present study and further study in this aspect is necessary.

TC had no effect in diminishing the bacteria of rotifers and this is in agreement with the previous study (Hatai et al., 1981). As shown in Fig. 5, MIC values determined in sea water HI or ZoBell liquid medium were higher than 5 μg/ml and this coincides with the ineffectiveness in vivo experiment. An interesting observation is that the MICs of this drug determined in HI containing 3% NaCl were much lower than those tested in the other two sea water media. This suggests that some factors in sea water might inactivate TC. Thus, in order to evaluate the effect of a certain chemotherapeutant in sea water environments, media dissolved in sea water must be used for drug sensitivity test.

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References


