Short Paper

Uptake of Bacteria by Rotifer

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(Accepted December 22, 1986)

As seed production program is growing prosperous in various fishes in Japan, disease problems in larval and juvenile stages have emerged as a new research object. Especially in larval stage of red seabream *Pagrus major*, black seabream *Acanthopagrus schlegelii* and flounder *Paralichthys olivaceus*, disease conditions characterized by abnormalities in the alimentary tracts are known to result in mass mortalities, and some *Vibrio* species have been reported to be associated with these diseases.¹⁻³⁾

In order to reproduce the disease conditions, candidate pathogens must be contacted with fish larvae by water-born method or oral administration. Since the abnormalities are apparent in the alimentary tracts, the latter method seems to be of importance for artificial infection. At present, larvae of various marine fishes in seed production operations are usually fed with live diets represented by rotifer *Brachionus plicatilis* and brine shrimp *Artemia salina*. Therefore, a test bacterium must be incorporated into these planktonic diets. Kusuda *et al.* (1986)²⁾ were successful in making black seabream larvae infected with *V. alginolyticus* and other bacteria by feeding them with infected rotifer, but the data on the uptake of bacteria by rotifer and thus the inoculation doses for oral infection were not presented.

The present authors made an experiment using *V. anguillarum* as an example of fish pathogen to evaluate the uptake rate of a given bacterium by rotifer. Rotifers (so-called S form) harvested from a rearing tank, where they had been cultured at about 25°C receiving phytoplankton *Chlorella* sp. and yeast, were suspended in filtered sea water at about 3,000 individuals/ml. The rotifers used for this experiment contained bacteria at about 2×10^6 CFU/g (wet weight) but proved free from *V. anguillarum*. A plate culture of *V. anguillarum* strain PT-80149 (serotype J-O-1) was added to the rotifer suspensions at 2.5×10^5 or 2.5×10^6 CFU/ml and kept at 23–24°C without agitation. After 30 min, 1, 3 and 5 h, a swarm of rotifers was collected from each suspension and washed with filtered sea water to remove surface bacteria. The number of viable cells of *V. anguillarum* in these rotifer samples was deter-

Fig. 1. Uptake of *Vibrio anguillarum* by rotifer.

V. anguillarum was added into rotifer suspensions (3,000 individuals/ml) at 2.5×10^5 CFU/ml (●—●) or 2.5×10^6 CFU/ml (○—○).

mined by plate count method using nutrient agar (NaCl 0.5%).

The result is given in Fig. 1. The concentration of *V. anguillarum* rapidly reached 10^8 and 10^6 CFU/g in the rotifers exposed to 10^6 CFU/ml and 10^5 CFU/ml bacterial suspensions, respectively. It was also demonstrated in an additional experiment that the once attained bacterial concentration (10^8 CFU/g) in rotifers was retained for at least 2 h even after the rotifers were transferred to filtered sea water. These results suggest that the artificial infection with scheduled inoculum through oral route is practicable by using rotifers incorporated with a test bacterium.

The authors are grateful to Mr. K. Masumura, Hiroshima Prefectural Fisheries Experimental Station, for his kind help.

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