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THE INFLUENCE OF A SELECTED BACTERIAL STRAIN *VIBRIO ANGUILLARUM* TR27 ON THE GROWTH RATE OF THE ROTIFER, *BRACHIONUS PLICATILIS* IN TWO CULTURE CONDITIONS

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Introduction

One of the most important factors in marine fish larval rearing is the production of live food. The rotifer *Brachionus plicatilis* is commonly used in the larval culturing of fish and crustaceans (Lubzens, 1987). Although rotifer culturing appears simple, sometimes a sudden and unexpected mortality or suppressed growth is observed (Hino, 1993). Because of the possible importance of the microflora in the development of a stable rotifer culture, experimental infection can be used as a tool to evaluate rotifer quality. The main purpose of this study was to investigate whether the dietary condition of the rotifer, *Brachionus plicatilis* influences its physiological performance and its reaction on a selected bacterial strain of *Vibrio anguillarum* TR27 isolated from a rotifer culture.

Materials and methods

The rotifer *Brachionus plicatilis* used for this study was kept in a stock culture on *Chlorella* for 2 weeks under standard conditions (25 °C, 25ppt salinity and 3000 lux light intensity). From this stock culture, the rotifers were transferred to a batch culture with increasing rotifer density and a constant volume (600ml). The culture was kept in seawater (25ppt) which was disinfected with sodium hypochlorite (5ppm). The initial density of the rotifers was maintained between 100 and 250 rotifers.ml^{-1}. The cultures were continuously aerated and were pre-adapted to the experimental diet (Culture Selco® (CS) INVE Aquaculture, Belgium) during two culture cycles. One cycle consisted of a 3 days culture period, after which the water was renewed before starting the next cycle . For the optimal rotifer culture, the daily ratio of CS was adjusted according to the density of the rotifers (Lavens et al., 1993). In order to

develop a sub-optimal culture with a lower growth rate and probably a poor physiological condition a 55% reduction of the optimal feeding regime was chosen based on preliminary experiments. At day 0 of the third cycle of each experiment, 1ml of a suspension of the *Vibrio anguillarum* strain TR27 (10^8 -10^9 CFU.ml^{-1}) was added in order to achieve a final concentration of (10^6-10^7 CFU.ml^{-1}). This strain had been isolated from a rotifer culture in a commercial hatchery and stored in liquid nitrogen. The strain was grown on marine agar (MA) (Difo) at 25°C for 24h. The bacterial culture was collected with a sterile swab and placed in 10ml of saline solution (0.85 %). To assess bacterial numbers 1ml of this suspension was diluted and plated on marine agar. In the last experiment samples were taken to trace back the *Vibrio anguillarum* strain 24h after bacterial inoculation. The samples were plated out on VAM (*Vibrio anguillarum* medium) and TCBS (Thiosulphate citrate bile sulphate agar) (Oxoid). After experiments were run, the *V. anguillarum* strain TR27 appeared to be serotype 06. In this way, it was impossible to trace back this strain from the media by means of a serological test (Mono-Va, Bionor Aqua, Norway) since serotype 06 cannot be detected by this test. The experiments were performed in three replicates for each treatment. For both dietary regimes, a control treatment was set up without addition of bacteria. The rotifer densities were counted daily .

At the end of a culture cycle (3 days) the rotifer growth rate was calculated using the following formula (Olsen et al., 1993):

$$\mu=(\ln N_t - \ln N_0)/t$$

μ = the specific max. growth rate, N_0 = initial rotifer density, N_t = rotifer density at day t , t = culture period in days.

Results and discussion

During the first and second cycle the growth rate showed a significant difference between the optimal culture (100% CS) and the sub-optimal culture (45% CS) in three consecutive experiments (Table I).

Table I. Growth rate of the rotifers in the first and the second cycle (before inoculation of bacteria) in three consecutive experiments (6 replicates for each dietary regime). Mean values ± SD

	Growth rate			
Experiment	Cycle 1		Cycle 2	
	100% CS	45% CS	100% CS	45% CS
1	0.20±0.02	0.09±0.01	0.21±0.03	0.12±0.02
2	0.22±0.02	0.10±0.01	0.33±0.02	0.13±0.02
3	0.23±0.03	0.10±0.02	0.32±0.03	0.13±0.02

Yu et al. (1990) reported about a bacterial strain, *Vibrio alginolyticus* Y5 ($2.5x10^4$ $CFU.ml^{-1}$), which caused a reduction in the growth rate of the rotifers. In this study the *Vibrio anguillarum* strain TR27 (10^6-10^7 $CFU.ml^{-1}$) caused a negative effect on the rotifer growth in the sub-optimal cultures in three consecutive experiments (Fig.1). On the contrary rotifers receiving an optimal diet were not affected by the bacterial strain. It seems that the overall condition of the rotifers has a profound influence on the capability of the animal to interact with its environment.

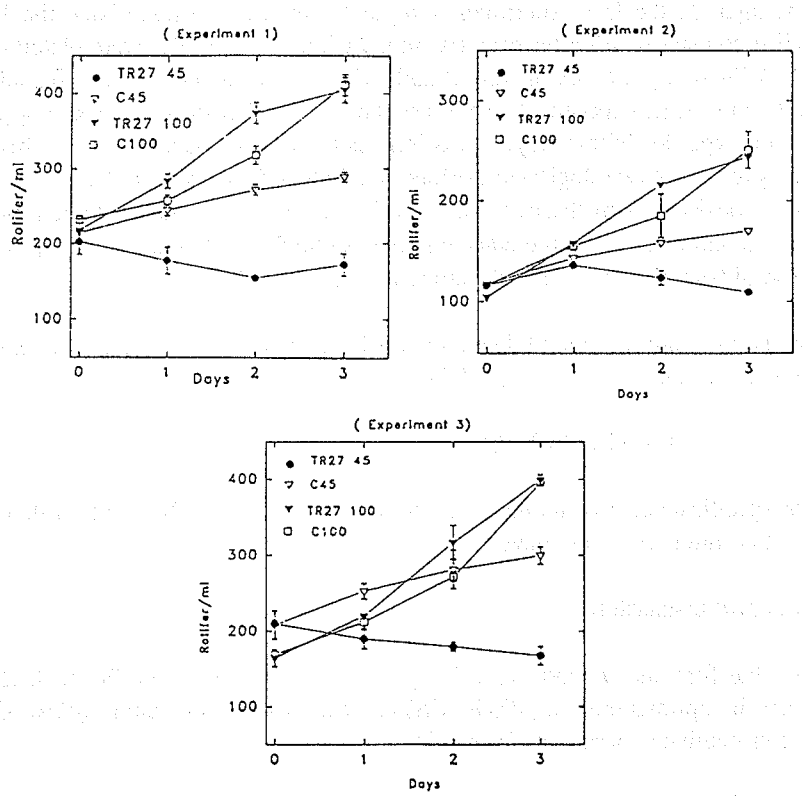

Fig. 1. Mean values and standard deviation of the rotifer density after infection with *Vibrio anguillarum* TR27 (Experiment 1, 2, 3). C100: control fed 100% CS; C45: control fed 45% CS; TR27 100:100% CS infected with TR27; TR27 45: 45% CS infected with TR27.

Nicolas et al. (1989) observed that in the presence of a poor quality micro-algae (*Pavlova lutheri*) the rotifers grazed more on bacteria in order to obtain the nutrients lacking in the algal food. He concluded that a diet better adapted to the rotifer needs, could decrease waste and grazing. Skjermo and Vadstein (1993) argued that the

grazing of bacteria by rotifers is strongly influenced by the physiological condition of the animals and that at low food concentration the rotifer, *Brachionus plicatilis* grazes more on bacteria. In the same way the lack of food in the sub-optimal rotifer culture can have caused a higher grazing rate on the *Vibrio anguillarum* strain TR27 in particular. This can be the explanation of the fact that the sub-optimal culture was more affected by the bacteria, compared with the optimal rotifer culture.

Conclusions

Based on the observations made, it can be concluded, that the culture conditions of the rotifer, *Brachionus plicatilis* influence its behaviour in the presence of selected bacterial strains, such as *Vibrio anguillarum* TR27. It also seems that experimental infection can be used as a tool to evaluate rotifer quality.

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