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SHORT COMMUNICATION

The influence of a selected bacterial strain *Vibrio anguillarum* TR 27 on the growth rate of rotifers in different culture conditions

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The rotifer *Brachionus plicatilis* is commonly used in the larval rearing of fish and crustaceans (Lubzens, 1987). Today almost all marine fish raised in hatcheries are first fed on rotifers followed by *Artemia* nauplii. *B. plicatilis* has been described by Walker (1981) as a non-selective filter-feeding plankton, whose diet may include various types of algae as well as bacteria and yeast. Food quantity (Walz, 1983) and quality (Yúfera and Pascual, 1984) appear to affect the embryonic development rate. According to Korstad *et al.* (1989) the feeding kinetics of *B. plicatilis* are influenced by the physiological status of the animal and the feeding rate shows a positive correlation with the food concentration and the growth rate.

B. plicatilis, are osmoconformers (Epp and Winston, 1977), tolerating salinities ranging from 1 to 97 g^{-1} (Walker, 1981). Optimal reproduction can only occur at salinities below 35 g l^{-1} (Lubzens, 1987). Both salinity and temperature will directly influence the reproduction rate, and the response depends on the strain and the environmental conditions (Miracle and Serra, 1989). It has been observed that sudden changes in salinity and temperature may cause immobilization of *B. plicatilis* (Øie and Olsen, 1993).

As food quantity and quality, salinity, temperature and other environmental factors influence the population development of rotifers, manipulation of these parameters could diminish their reproduction rate and consequently their growth performance, resulting in a sub-optimal rotifer culture.

Special attention should be given to bacterial microflora. Both beneficial and detrimental bacteria develop in a rotifer culture. Some *Pseudomonas* and *Acinetobacter* strains improve the growth performance of rotifers and while *Flavobacteria* and others are listed as growth-repressing bacteria (Maeda and Hino, 1991). Yu *et al.* (1990) reported that *Vibrio alginolyticus* Y5 (2.5×10^4 CFU ml^{-1}) caused a decrease of the growth rate in rotifer populations. Vitamin B_{12} producing bacteria, on the other

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hand, support the growth of rotifers (Yu *et al.* 1988). Bacteria are furthermore responsible for the high level of ammonia, recycling part of the organic matter, creating deficiencies in the food supply; and eventually as a possible cause of diseases (Coves *et al.*, 1990). The main purpose of the present study was to investigate whether the culture conditions, i.e. the dietary regime and the salinity may influence the physiological performance of rotifers and to what extent the latter could be evaluated in a challenge test with a pathogen, *Vibrio anguillarum* TR27, isolated from a rotifer culture.

The rotifers *B. plicatilis* (L type) used in this study were obtained from a stock culture maintained on an algal diet (*Chlorella* sp.) at 25° C, 25 g l^{-1} salinity and a light intensity of 3000 lux. For each experiment, the rotifers were transferred to 1000 ml glass cones. The culture was performed in diluted seawater (25 g l^{-1} salinity) filtered on a 0.45 µm capsule membrane filter, disinfected with sodium hypochlorite (5 mg l^{-1}) and neutralized with sodium thiosulphate before the beginning of the experiments. The initial density of the rotifers varied between 100 and 250 rotifers ml^{-1}. The rotifers were adapted to a self-emulsifying yeast-based diet (Culture Selco® (CS), INVE Aquaculture, Belgium) during two reproductive cycles. The normal dietary regime for the rotifer culture was adapted from Lavens *et al.* (1994) (feeding regime as a function of rotifer density) and reduced to 45% for the sub-optimal cultures. The experiments consisted of three consecutive reproduction cycles. Each culture cycle consisted of a 3 day culture period, after which the water was renewed before starting the next cycle.

After the acclimation period of 6 days (two reproduction cycles) the *V. anguillarum* strain TR27 (10^8–10^9 CFU ml^{-1}) was added to the culture medium and a final concentration of 10^6–10^7 CFU ml was obtained. The *V. anguillarum* strain TR27 was isolated from a rotifer culture in a commercial hatchery and stored in liquid nitrogen. Before being used the bacterial strain was grown on marine agar (MA) (Difco Co. Ltd, USA) at 25° C for 24 h and checked for purity before being collected with a sterile swab and suspended in 10 ml of a saline solution (pure sodium chlorate dissolved in deionized water) (0.85%). To assess bacterial numbers, the density of the suspension was measured by means of a photospectrometer (550 nm) and a dilution of the suspension was plated on marine agar. In the last experiment, samples from rotifer cultures were taken to trace back the *V. anguillarum* strain 24 h after bacterial inoculation. The samples were plated out on VAM (*V. anguillarum* medium) (Alsina *et al.*, 1994) and TCBS (Thiosulphate Citrate Bile Sulphate) medium (Difco Co. Ltd, USA). The *V. anguillarum* strain TR27 appeared to belong to serotype 06. It was impossible to trace back this strain from the media by means of a serological test (Mono-Va, Bionor Aqua, Norway) because serotype 06 cannot be detected by this test. In order to avoid mortality due to an abrupt salinity increase, a saline solution (pure sodium chlorate dissolved in deionized water) of 220 g l^{-1} salinity was added to the rotifer medium (25 g l^{-1}) so that salinity increased at a rate of 2 g l^{-1} per 30 min (final concentration of 35 g l^{-1}). The experiments were performed with three replicates for each treatment and each experiment was repeated three times in different periods under the same conditions. For both dietary regimes (optimal and sub-optimal rotifer cultures), control treatments were set up without addition of bacteria and/or saline solution. The rotifer densities were counted daily.

At the end of a culture cycle (3 days) the rotifer growth rate was estimated using the following formula (Øie *et al.*, 1994):

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

where: μ = specific growth rate (SGR), N_0 = initial rotifer density, N_t = rotifer density at day t and t = culture period in days.

Growth rates of the rotifers in the experiments were subjected to a one-way ANOVA and Tukey's multiple range test was applied to detect significant differences

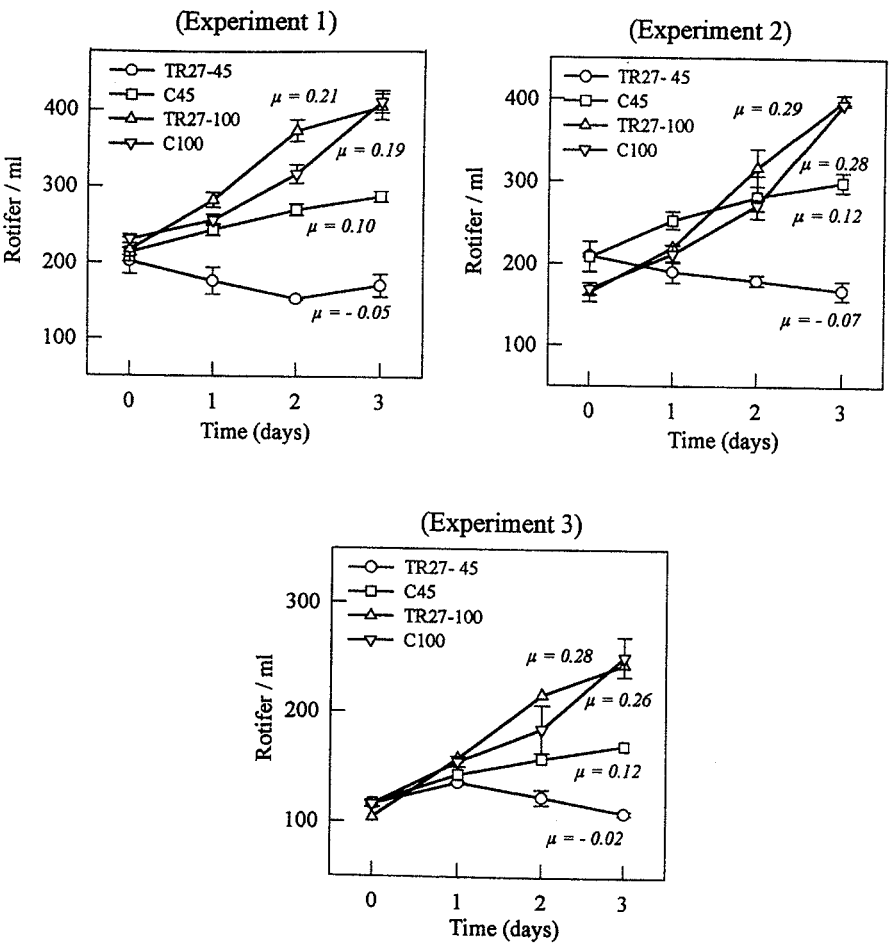

FIG. 1. Mean values and SD of the rotifer density after infection with *V. anguillarum* TR27 for three repeated experiments in time. C100: control fed 100% CS; C45: control fed 45% CS; TR27–100: 100% CS infected with TR27; TR27–45; 45% CS infected with TR27. (μ = Specific Growth Rate.)

among means ($P < 0.05$). Homoscedasticity of variances was controlled by Barlett's test. In the case of non-homogeneity of variance, growth data was transformed to satisfy assumptions of ANOVA.

The growth rate of the rotifers in the optimal cultures ranged between 0.2–0.43 in the first and the second reproduction cycles in optimal conditions but reached only 0.09–0.17 in the sub-optimal cultures. Significant differences ($p < 0.05$) were detected among the growth rate of the optimal (100% CS) and the sub-optimal cultures

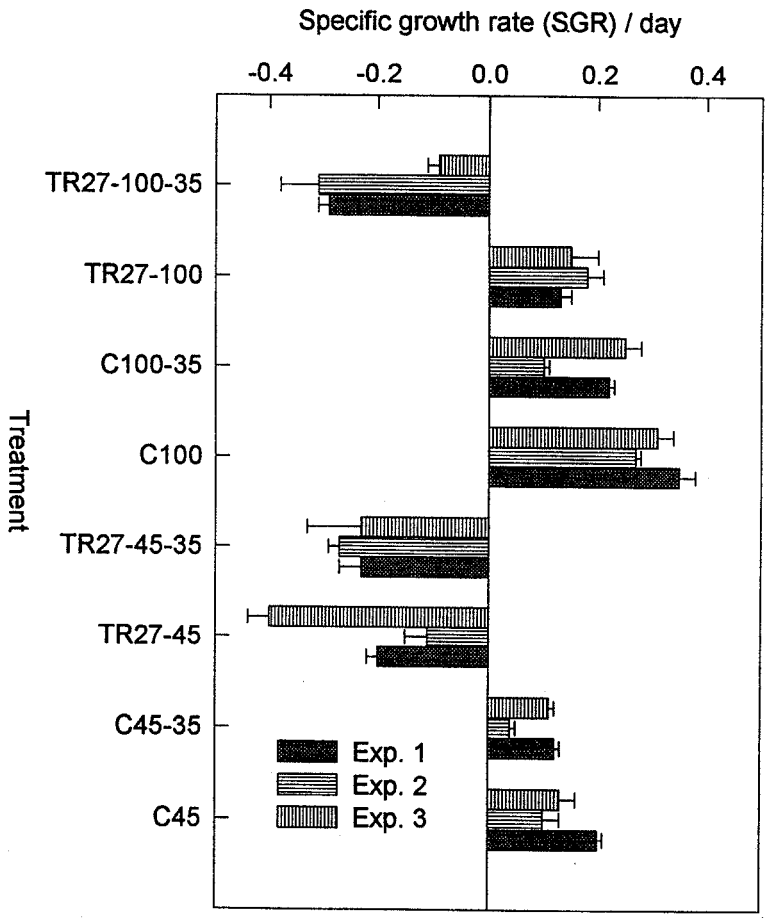

FIG. 2. Mean values and SD of the specific growth rate (SGR) of the rotifers after infection with *V. anguillarum* TR27 and addition of saline solution for three repeated experiments in time. C100: control fed 100% CS; C45: control fed 45% CS; C100–35 control fed 100% CS in 35 g l^{-1} salinity; C45–35 control fed 45% CS in 35 g l^{-1} salinity; TR27–100: 100% CS infected with TR27; TR27–45: 45% CS infected with TR27; TR27–100–35: 100% CS infected with TR27 in 35 g l^{-1} salinity; TR27–45–35: 45% CS infected with TR27 in 35 g l^{-1} salinity.

(45% CS). The growth rate was also significantly ($P < 0.05$) affected by addition of bacterial strain TR27 and saline solution to the culture medium. In the case of the sub-optimal culture, the insufficient supply of food resulted in a lower growth rate. Hirayama and Kusano (1972) reported that inadequate food supply causes rapid decrease in the population since the females are not able to produce offspring with enough energy reserves.

The addition of *V. anguillarum* strain TR27 (10^6–10^7 CFU ml^{-1}) resulted in a negative growth rate in the sub-optimal cultures in the three consecutive experiments (Fig. 1) while the optimal cultures were not affected. The negative growth rate in the sub-optimal cultures exposed to the bacterial strain could result from a changed ratio in the number of bacteria per food intake. An analogous observation was made by Nicolas *et al.* (1989) who observed that in the presence of poor quality micro-algae, *Pavlova lutheri*, rotifers grazed more on bacteria in order to obtain the nutrients lacking in algal food. He concluded that a better adapted food meeting the rotifer needs could decrease waste and grazing. Skjermo and Vadstein (1993) demonstrated that the grazing of rotifers on bacteria is strongly influenced by the physiological condition of the animals and that at low food concentration more bacteria are ingested. Vadstein *et al.* (1993) pointed out that the increasing retention of bacteria by animals with poor physiological status may be an adaptation to conditions with inadequate food supply.

At the increased salinity, the addition of *V. anguillarum* caused a negative growth rate in both the optimal and the sub-optimal culture, which was not the case in the control treatment without *V. anguillarum* TR27. It is known that food intake is depressed at high salinities (Hirayama and Ogawa, 1972; Yamasaki and Hirata, 1985). According to Lebedeva and Orlenko (1995) salinity appreciably affects the rate of maturation, and total lifespan. They concluded that at high salinity the coefficient of food assimilation of the rotifer is low. In this way, a higher salinity resulting in a lower food intake and/or assimilation, may have caused stress conditions and reduced the resistance to bacterial infection by *V. anguillarum* TR27 (Fig. 2).

A sub-optimal and optimal rotifer culture procedure was developed allowing reproducible results in terms of growth rate among replicates and between repeated experiments. Only under sub-optimal or stressing culture conditions the growth rate of rotifers could be affected by undesirable bacteria. The technique of experimental infection can be applied as a tool to evaluate the condition of rotifers.

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International study on *Artemia*. LIII. Morphological study of *Artemia* with emphasis to Old World strains. I. Bisexual populations

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Abstract

A detailed morphological and allometrical study was performed with adult males and females of eleven bisexual populations of brine shrimp *Artemia*. Multivariate procedures, discriminant and cluster analysis, allowed to separate and group together populations which exhibit great genetic similarities. The eleven populations studied form four distinct groups: the *A. franciscana* group, the *A. tunisiana* group, the *A. urmiana* group and a broader group which includes Eastern Old World populations. Scanning electron microscopy revealed differences in the male genital organs of an *A. tunisiana* population by lacking a medial protuberance in the base of the penes while the pattern of the ectodermal ridges of the brood pouch of *A. urmiana* markedly differed from the other populations studied.

Introduction

The brine shrimp *Artemia* is a cosmopolitan Anostracan crustacean. The genus is a complex of species and superspecies defined by the criterion of reproductive isolation (Browne & Bowen, 1991). The means to approach the various populations taxonomically and to classify them into biological species are the study of cytogenetics, anatomical characters and electrophoretic data (Beardmore & Abreu-Grobois, 1983).

Many scientists are considering the genus *Artemia* as a complex of sibling species although many studies show that there are morphological differences among the individuals of different species (Amat, 1980; Hontoria & Amat, 1992a,b; Pilla, 1992; Triantaphyllidis et al., 1995). Comparative morphological characterization has mostly been performed on females. Recently, Pilla (1992) and Pilla & Beardmore (1994) showed that male morphometric characters can be at least as informative.

It will soon be possible to extend our present knowlegde on the biogeography and evolution of the genus as several new strains from Asia are becoming available for study, especially from the People's Republic of China (P.R. China), Kazakhstan and Iran. In this paper we perform a morphological study of *Artemia* populations that belong to well-described species and compare them with populations that are taxonomically still not well characterized. The use of multivariate procedures has been employed to test the efficiency of these methods and create a data matrix helpful to classify future samples of unknown origin or those taxonomically problematic.