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Cross-mating of euryhaline rotifer *Brachionus plicatilis* strains as a means to develop useful strains for larval fish food

Tomonari Kotani [a,*], Kazuhiko Ihara [b], Atsushi Hagiwara [c]

[a] *Department of Marine Biotechnology, Faculty of Life Science and Biotechnology, Fukuyama University, 452-10 Ohama, Innoshima, Hiroshima 722-2101, Japan*
[b] *Faculty of Fisheries, Nagasaki University, Bunkyo 1-14, Nagasaki 852-8521, Japan*
[c] *Graduate School of Science and Technology, Nagasaki University, Bunkyo 1-14, Nagasaki 852-8521, Japan*

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Abstract

In the field of live food science, newly inbred strains resulting from cross-mating are of interest, especially if these strains have valuable characteristics, such as high fecundity or suitable size for the mouth of larvae. We conducted cross-mating trials using Japanese and German strains of *Brachionus plicatilis* and reproductive parameters were characterized and compared among their progenies. Two hybrid strains A and B were obtained from the cross-mating between a Japanese female and German male, and between a German female and Japanese male, respectively. Percent mictic female production and fertilization in both hybrid strains were lower (0%), compared with the parental strains (16.7–78.4%). Strain A did not reproduce sexually, but was capable of asexual reproduction. Higher population growth was observed in the hybrid strains within crosses relative to parents. The population growth rates of parental strains were 0.31, while those of hybrid strains ranged from 0.35 to 0.37. Lorica length of hybrid strains was intermediate between the two parental strains. Using the cross-mating technique, it may be possible to produce new rotifer strains with phenotypes useful to aquaculturists.

Keywords: Rotifer; *Brachionus plicatilis*; Cross-mating; Hybrids; Biometry; Reproduction; Survival

1. Introduction

Euryhaline rotifers of the genus *Brachionus* are important live foods in larval rearing of marine fishes and crustaceans. They are cyclical parthenogens and can produce fertilized eggs in mictic generations. The progeny from fertilized eggs can be phenotypically different from the parental strain (Hagiwara and Hino, 1989, 1990). It is possible that the phenotype of rotifers from fertilized eggs produced from cross-mating between rotifer strains is different from that of the parental strains. Fu et al. (1993) performed a crossing experiment with three *B. plicatilis* and four *B. rotundiformis* strains. Fertilized eggs were produced within each species, but were not between species. Fertilized eggs also were produced among strains within a species, although the fertilization rates among strains were lower than those within each strain (Fu et al., 1993). The biological characters of the hybrid strains produced from cross-matings have not yet been characterized.

* Corresponding author. Tel.: +81 845 24 2933; fax: +81 845 24 3449.
E-mail address: tkotani@ma.fuma.fukuyama-u.ac.jp (T. Kotani).

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Recently, some new methods of rotifer culture, e.g. continuous culture and high-density culture, are being applied in aquaculture and it was suggested that these methods may require rotifers with appropriate reproductive characters (Koiso, 2000). In larval rearing, it is important to select the most appropriate rotifer strain for the species and growth characteristics of the cultured fish (Sugimoto, 1989). However, the reproductive character of many rotifer strains has not been investigated thoroughly and rotifer strains with known origin and reproductive characteristics are usually not stocked in many hatcheries (Kuwada and Hino, 2001). Rotifer mass culture should use strains with high productivity, stability, and production quality (Hagiwara et al., 2001). Therefore, in order to raise the efficiency of rotifer mass production, rotifer strains with superior biological characters are required. However even the most basic cross-mating schemes have not been applied to rotifers to improve their characteristics for aquaculture (Kuwada and Hino, 2001). Therefore, the evaluation of the biological characters of hybrid rotifer strains and the preservation of these strains is important.

In this study, we aimed to produce fertilized eggs from cross-mating among rotifer strains genetically distinct from each other, and to investigate some biological characteristics of the hybrid strains.

2. Materials and methods

We used two parental strains from Japan and Germany. The Japanese strain has been cultured for many years in Tokyo University after collection from an eel culture pond. A subculture has been maintained as NH1L strain (Hagiwara et al., 1994) at Nagasaki University since 1990. The German strain was collected in Schlei-Fjord in 1988 and cultured in Nagasaki University. The Japanese and German strains are genetically isolated geographically and their genetic distinctiveness was demonstrated by differentiation of genetic markers (Fu et al., 1991a,b). Each strain was established by cloning from one individual. The crossing procedure employed in this study was reported previously (Fu et al., 1993). Before tests, rotifers from the two parental strains were cultured in 250 ml flasks at 25 °C and salinity of 22 psu by diluting natural seawater with distilled water. Rotifers were fed with $1–2\times10^7$ cells/ml of the microalgae *Nannochloropsis oculata* daily to maintain the population in exponential growth stage. The *N. oculata* was cultured in medium containing KW21 (Dai-ichi Seimou Co. Ltd.), and it was harvested in exponential growth phase and concentrated by centrifugation.

For each strain, fifty newborn males and 100 amictic female eggs whose embryos have actively moving cilia were collected from the pre-culture. Both males and amictic eggs were randomly placed in a petri dish with 5 ml seawater (22 psu) and incubated in total darkness at 25 °C, and fed with 7×10^6 cells/ml of *N. oculata*. One set of crosses used Japanese males and German females and a second set used German males and Japanese females. On the second and third day of culture, *N. oculata* was replenished in the culture medium. In order to avoid mating between offspring produced by the parental females, newly mature amictic females and unfertilized mictic females were counted and removed whenever they were found over 7 days. The fertilized mictic females were transferred to a new culture medium. The fertilized eggs were laid on the bottom and were removed. Each hatchling from resting eggs was moved to new medium and individually cultured as a clone. Two hybrid clones (A and B) from each cross were selected randomly. The biological character of these four hybrid clones and two parental strains was investigated.

2.1. Biometry

The experimental procedure was the same as described in a previous study (Fu et al., 1991a). Amictic eggs were collected from each culture in exponential growth stage. In order to collect eggs, rotifers were transferred to a small amount of seawater in a 30 ml screw-capped glass-bottle. Bottles were vortexed, knocking the eggs from the females. Amictic eggs were collected and cultured in 5 ml of seawater with 7×10^6 cells/mL of *N. oculata* in a petri-dish at 25 °C in the dark. After 1 h, neonates were moved to a screw-capped glass-bottle including 5 ml of seawater with 7×10^6 cells/ml of *N. oculata*. After 48 h from the start of the culture, adults produced eggs and offspring. These eggs were collected and were again cultured in *N. oculata* suspension. Offspring hatching from those eggs, after 48 h of culture, were carrying their first amictic eggs. They were used for morphological measurements. In order to make measurements of the lorica (especially spines on the lorica) easy, the animals were fixed with the vapor of acetic acid. The body sizes were measured under a digital microscope (VH-8000, Keyence Corp.). Twenty individuals were randomly picked from each strain. Length and width of the lorica of each individual were measured. A one-way ANOVA and Tukey's test was performed to compare the body size among strains.

2.2. Reproductive characteristics — batch culture

Ten rotifers carrying their first amictic eggs of each strain were inoculated to 10 ml of 17 psu diluted