Improvement of rotifer as the new food item in larviculture†

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In larviculture, rotifers (*Brachionus plicatilis* sense strict) are generally used as the initial food source.[1] The lorica length of rotifers is distributed in the range of 170–320 μm in individuals carrying amictic eggs. The next food item *Artemia* nauplii has a body length of 400–1,000 μm. Although the proper management of feed size and density associated with fish larval growth is needed,[2] there is a size gap between rotifers and *Artemia* nauplii. No feed items to bridge the size gap of 320–400 μm have been developed in the food scheme of fish larvae.

To improve the mass mortality and abnormal development of fish larvae during the period of growth corresponding to the size gap, this study applied carbon and argon heavy-ion-beam irradiation in mutation breeding[3,4] to select rotifer mutants with larger lorica sizes.

In our previous study, the Notojima strain, known as the largest rotifer strain in Japan, was irradiated with carbon (C) and argon (Ar) ion beams at different doses. We developed a screening method with the lorica length as an indicator and established 56 large mutants (Table 1).[5] In this study, we measured the population growth rate as the parameter of good bait. The population growth rate of each large mutant line was observed with 5-mL cultures. For each line, every five individuals were inoculated into 6 wells containing 5 mL of fresh culture medium (18 practical salinity units) with Chlorella. The total number of rotifers in each well was counted after 5 days. The population growth rate of each well was calculated with the following equation: population growth rate = ln (total population/5 individuals)/5 days). The average values of each large mutant line were calculated using data obtained with six replications and standardized using the control group.

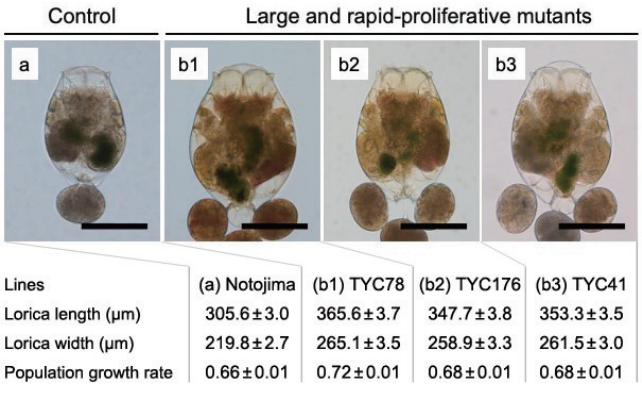

Fig. 1. Photograph of rotifer lines with proliferative difference and the control. (a) Control rotifer. (b1-3) Large and proliferative mutants with higher population growth rates than the control. Values are mean ± standard errors, and images of rotifer lines are of individuals with the average lorica lengths. Scale bars represent 200 μm.

Table 1. Frequency of large and rapid-proliferative mutants

Heavy ion	Dose (Gy)	Irradiated rotifer (No.)	Active proliferation		Large mutants		Rapid proliferation	
			Lines (No.)	Frequency (%)	Lines (No.)	Frequency (%)	Lines (No.)	Frequency (%)
C ion	100	240	195	81.3	10	5.1	3	1.5
	150	250	183	76.3	6	3.3	2	1.1
	200	504	391	77.6	13	3.3	6	1.5
	300	504	321	63.7	13	4.0	2	0.6
	400	240	98	40.8	9	9.2	1	1.0
	600	240	60	25.0	1	1.7	0	0.0
Ar ion	25	216	197	91.2	1	0.5	0	0.0
	50	216	145	67.1	2	1.4	1	0.7
	75	216	140	64.8	1	0.7	0	0.0
	100	216	84	38.9	0	0.0	0	0.0
	150	216	17	7.9	0	0.0	0	0.0
Total		3048	1831	60.1	56	3.1	15	0.8

The following 3 mutant lines showed a significantly higher population growth rate than the control: TYA41 (Steel's multiple comparison tests, $P < 0.05$), TYC78, and TYC176 ($P < 0.01$) (Fig. 1). The large mutant lines showed an equal or higher growth rate than the control are categorized as the rapid proliferation in Table 1. The frequency of appearance was calculated using the mutant lines showing active proliferation after irradiation: mutant frequency (%) = (number of rapid proliferation)/(number of active proliferation) × 100. The highest frequency was observed at 100 and 200 Gy with the C-ion beam. For TYC78 produced at 200 Gy, we decided that a C-ion beam of 200 Gy was the optimal irradiation condition to establish a large rotifer. Similarly, the highest frequency was observed at 50 Gy with the Ar-ion beam, and TYA41 was selected from the same condition. This was also determined to be the optimum irradiation dose. Thus, these three mutants are suitable to solve the feed-size gap between rotifers and *Artemia* nauplii and should improve the marine fish larviculture system.

Food shortages due to population growth and increased consumption are a major global concern, and countries around the world are searching for ways to increase food production. The enlarged rotifers obtained in this study could potentially provide a stable supply of larger rotifers at low cost, enhancing aquaculture.

† Condensed from the article in Biosci. Biotech. and Biochem. (DOI: 10.1093/bbb/zbaa094) (2021)

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