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Monitoring of the physiological status as the heat stress tolerance in the rotifer: a novel test for health diagnosis and ecological monitoring

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Abstract — To develop a simple method to evaluate physiological condition of the rotifer *Brachionus plicatilis*, important species as a live-food for aquaculture, we examined the heat stress tolerance using a household microwave oven. The rotifers were previously acclimated at each of 15°C, 20°C and 35°C, and exposed to heat treatment at every 10°C with the highest at 45°C. Subsequently, the animals were returned to the original temperature, and survival rates were recorded until 24 hours after the treatment. Under the 45°C-treatment, the animals acclimated at 15°C showed significantly lower survival rate (29%) than those at 25°C and 35°C with 75% and 84%, respectively. In contrast, under the treatments below 35°C, the survival rates were constant around 80% regardless of the acclimation temperature. These results suggest that the heat stress tolerance can be examined by the microwave oven, and the method will be useful at hatcheries for stable mass culture of the rotifer.

Keywords: *Brachionus plicatilis*, heat stress, live food, mass culture

Introduction

Euryhaline rotifers *Brachionus plicatilis* and *B. rotundiformis* have been used worldwide as a live food organism for marine fish larvae (Hagiwara et al. 2001). To date, mass culture technique of the rotifers has been improved, achieving extremely high-density at 20,000 individuals/mL (Yoshimura et al. 1996). In addition to the high-density mass culture, a continuous method also has been employed which requires relatively lesser labor and cost. Both the two methods are commonly carried out under eutrophic condition with commercial dietary microalgae, typically *Nannochloropsis oculata*. Because a shortage of food supply suppresses an individual's reproductive activity (Yoshinaga et al. 2000), resulting in low population growth rate (Yoshinaga et al. 2001a), continuous food supply is essential for the rotifer's mass production.

Under the eutrophic condition, the rotifer population is surrounded by unstable environment, which can cause problems concerning an unexpected stagnant growth and collapse of the population. So far, various environmental factors such as food quantity (Yoshinaga et al., 2000), water quality (Yu and Hirayama, 1986), bacterial flora (Hagiwara et al. 1994), contaminating zooplankton (Hagiwara et al. 1995) have been suggested to affect the population growth rate of the rotifers. Because multiple factors simultaneously affect the rotifer population, both directly and indirectly with variable intensities, optimization of culture condition is difficult, especially for long-term culture. Indeed, the success of the rotifer's mass culture often relies on a personal skill from the long experience at each hatchery.

Monitoring the rotifers' physiological condition is a key factor to establish the stable mass culture. Hitherto, various methods have been developed such as swimming speed and egg ratio (Korstad et al. 1995), enzyme activity (de Araujo et al. 1998, 2000), hyper-saline tolerance (Koiso and Hino, 1999), and starvation tolerance (Yoshinaga et al. 2001b). However, these methods require labor and cost, and thus not feasible to routinely carry out at hatcheries.

Heat stress induces the expression of a group of proteins, called heat shock protein (HSP) or stress protein, functions as molecular chaperones to assist the refolding of denatured proteins. Therefore, such responses to various stresses determine the physiological condition of organisms in various environments. Indeed, expression of single species of stress protein (HSP70) has been utilized for environmental monitoring in various invertebrate and vertebrate species (reviewed by Mukhopadhyay et al. 2003). Kaneko et al. (2001) have observed a change in the *hsp*70 transcript during population growth of the rotifer, suggesting that HSP70 is a candidate molecule to monitor the physiological condition of the species' population dynamics. Therefore, in this study, we have developed a simple method to evaluate the rotifer's physiological condition. Our method is based on evidences that (1) an ability to persist against various stressors tightly links with the physiological condition, and (2) against various environmental stimuli, organisms respond by relatively simple mechanism such as an expression of stress proteins at cellular level (Diller, 2006). Accordingly, in this study we have examined the heat stress tolerance as a representative index of physiological condition of the rotifers under various temperatures.

Materials and Methods

We used the Ishikawa strain of the rotifer *B. plicatilis* (Yoshinaga et al., 1999). The Ishikawa strain reproduces by absolute parthenogenesis in our culture system. The rotifers were cultured in sterilized artificial seawater of salinity at 16 ppt. Purchased dietary microalgae *N. oculata* (Nikkai center, Machida) was rinsed with the artificial seawater, and fed to the rotifer *ad libitum*. The experimental animals were acclimated at each of three temperatures of 15°C, 25°C and 35°C prior to the experiments.

A microwave oven (rating, 1000 Watt; Sharp, model RE-5N) was employed to quickly warm up the seawater medium. Water volumes were adjusted for constant duration of warming up to various temperatures between 10°C and 30°C. In our system, the warming time was constant at 100 seconds, and water volumes were 240 mL, 130 mL and 60 mL for 10°C, 20°C and 30°C increasing treatments, respectively. Water was stirred every 10 seconds to equally warm up the media.

Twenty animals were inoculated into seawater media of certain volumes, and subjected to the treatment by the microwave oven. The death of an individual was defined when cilia movement was not observed, and the timing of death was determined as the median between the two observations before and after death. Experiments were repeated for 3 to 5 times independently

Results

Water temperatures warmed up by the microwave oven were stable throughout the study (Fig. 1a). Regardless of the acclimation temperature between 15°C to 35°C, survival rates after Δ10°C and Δ20°C heat treatments were high at around 0.8 (Fig. 1b). In contrast, when the rotifers acclimated at 15°C were exposed to Δ30°C treatment (45°C), the survival rate was significantly lower than those of the other treatments (ANOVA and Fisher's PLSD post-hoc test, both $P<0.001$, $n=20$). The survival rate did not differ between

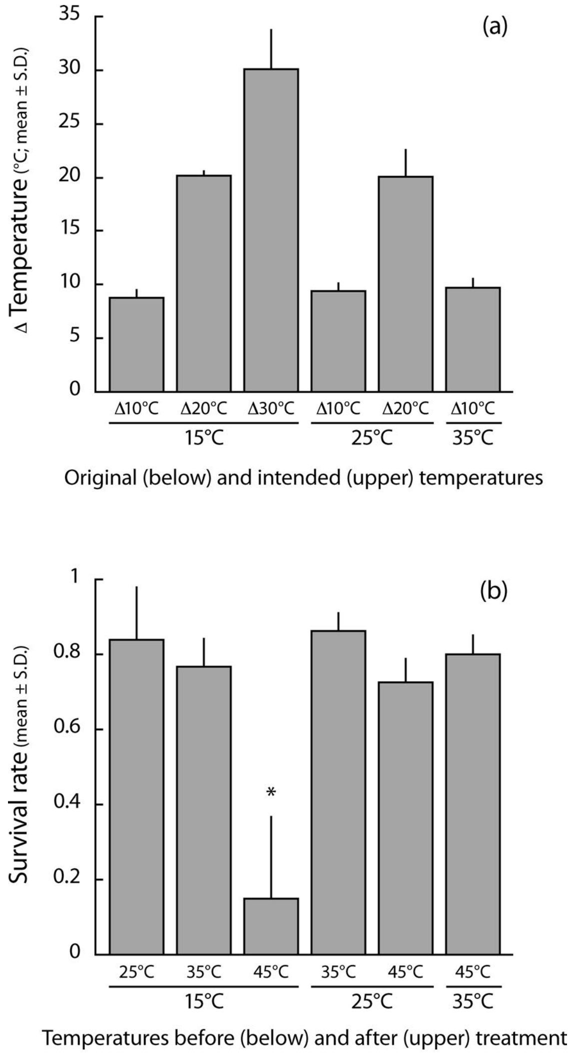

Figure 1. Water temperature after microwave oven treatment (**a**) and survival rate of the rotifer by a heat-stress tolerance test (**b**). **a**, The seawater media at 15°C, 25°C or 35°C were warmed up for Δ10–30°C by the microwave oven. **b**, Rotifers acclimated at either 15°C, 25°C and 35°C were subjected to the heat treatment at every 10°C with the upper limit of 45°C. After the treatment, animals showed high survival rate except the group acclimated at 15°C with Δ30°C treatment ($P<0.001$; PLSD test, $n=20$).

just after and 24 hours after the heat treatment, and successful hatch was observed from amictic eggs that were laid during the heat treatment (results not shown).

Discussion

The rotifers acclimated at 25°C and 35°C persisted at 45°C, while those at 15°C did not. These findings are interesting because the highest temperature at which zooplankton can persist has been estimated to be around 45°C (Taylor and Mahoney 1988). *B. plicatilis* is eurythermal species, and our results suggest that the species has an ability to survive at extreme temperature if previously acclimated at higher temperature.

In this study, we have aimed to develop a simple test to evaluate the physiological status of the rotifer. At each hatchery, the microwave oven condition should be considered prior to the test, but the method is quite simple and consistent. Application at each hatchery will prove the usefulness of our method, and an accumulation of the results will be important to establish the stable mass culture of the rotifer. In addition, our method would be useful for ecological monitoring not only in the rotifer but also in the other zooplankton species inhabit in coastal waters.

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