

between in vivo and in vitro thermal resistance. Therefore, the use of primary culture cells and free cells from a caudal fin clip could be useful for evaluating thermal resistance traits in selective breeding of fish.

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

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Introduction

Monogonont rotifer is an excellent model organism for speciation study. Cross-mating experiments have been conducted on euryhaline *Brachionus* strains in order to determine their species boundaries (Snell, 1989; Fu et al., 1993; Gomez and Serra, 1995; Hagiwara et al., 1995). In the field of live food science, newly inbred strains resulting from cross-mating are of interest, especially if these strains have valuable characteristics.

Materials and methods

We conducted cross-mating trials using Tokyo and German strains of *Brachionus plicatilis*. Reproductive parameters were characterized and compared among parental Tokyo and German strains, and their progenies. Two progeny strains A and B were obtained from the cross-mating between Tokyo ♀ and German ♂, and between German ♀ and Tokyo ♂, respectively.

Results and Discussion

Percent mictic female production and fertilization in progeny strains were lower,

compared with parental strains. Strain A did not induce sexual reproduction. Higher population growth was achieved in newly produced strains after inbreeding (Table 1). The population growth rates of parental strains were 0.31, while those of progeny strains ranged from 0.35 to 0.37. Lorica length of progeny strains was intermediate of that the two parental strains (Table 1).

It is possible to manipulate reproductive characteristics of rotifers by regulating its life cycle. For example, by preserving a resting egg under low temperature (5°C) and hatching thereafter, its derived rotifer strain shows higher rate of population growth (Hagiwara and Hino, 1989). In other study, Hagiwara et al. (2004) found that a rotifer strain with higher rate of sexual reproduction is obtained by culturing a resting egg hatchling under starved condition.

By combining these methods with cross-mating technique, it is possible to obtain suitable rotifer strains that would satisfy the demand of aquaculturists, who conduct larval rearing of different fish species under variable environment.

Table 1. Population growth and lorica size of parental rotifer strains and those produced by cross-mating

Rotifer strain	Specific population growth rate (r)	Lorica size (μm)
Tokyo	0.314	304 \pm 18
Germany	0.307	265 \pm 14
Tokyo ♀ x German ♂	0.368	280 \pm 12
German ♀ x Tokyo ♂	0.352	292 \pm 17

References

- Fu Y., Hagiwara A., Hirayama K., 1993. Crossing between seven strains of the rotifer *Brachionus plicatilis*. Nippon Suisan Gakkaishi 59: 2009-2016.
- Gomez A., Serra M., 1995. Behavioral reproductive isolation among sympatric strains of *Brachionus plicatilis* Muller 1786: insights into the status of this taxonomic species. Hydrobiologia 313/314: 111-119.
- Hagiwara A., Hino A., 1989. Effect of incubation and preservation on resting egg hatching

and mixis in the derived clones of the rotifer *Brachionus plicatilis*. *Hydrobiologia* 186/187: 415-421.

Hagiwara A., Kadota Y., Hino A. 2004. Maternal effect by stem females in *Brachionus plicatilis*: effect of starvation on mixis induction in offspring. *Hydrobiologia* (in press)

Hagiwara A., Kotani T., Snell T. W., Assava-Aree M., Hirayama K. 1995. Morphology, reproduction, genetics and mating behavior of small, tropical marine rotifer *Brachionus* strains (Rotifera). *J. Exp. Mar. Biol. Ecol.* 194: 25-37.

Snell T. W., 1989. Systematics, reproductive isolation and species boundaries in monogonont rotifers. *Hydrobiologia* 186/187: 299-310.

The analysis of immune responses of a novel CC-chemokine gene from Japanese flounder *Paralichthys olivaceus*.

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Tomoya Kono, Riichi Kusuda, Eijiro Kawahara and Masahiro Sakai.

A novel CC-chemokine gene was isolated from the Japanese flounder *Paralichthys olivaceus* by expressed sequence tag analysis. The function of this CC-chemokine gene was studied by DNA injection. To investigate the immune responses to the CC-chemokine, a plasmid construct containing the novel CC-chemokine protein expressed in serum on 1, 3 and 5 days after plasmid injection were estimated by ELISA. CC-chemokine gene injection increased the migration of phagocytic cells. Macrophage functions such as production of superoxide anion and phagocytosis were also stimulated by this gene injection. Thus, this gene from Japanese flounder has functional similarities to that of a mammalian CC-chemokine gene.