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Nutritional Value of *Candida utilis* for Rotifer and Larval Flounder *Paralichthys olivaceus*

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Baker's yeast, *Saccharomyces cerevisiae*, has been widely used as a food organism for rotifers used in the larval production of marine fish. However, the nutritional value of the yeast is relatively poor compared with that of the marine alga *Chlorella*. We examined the nutritional value of another yeast, *Candida utilis*, and whether its food value could be increased through manipulation such as a cell wall treatment. *Candida utilis* and *S. cerevisiae* and their manipulated varieties were assessed with regard to the growth and nutrition of the rotifer *Brachionus plicatilis*. Larvae of the flounder *Paralichthys olivaceus* were cultured with rotifers fed on the yeast species, and the dietary value of the rotifers for the larvae was examined. Rotifers that were fed *C. utilis* grew faster than those provided with *S. cerevisiae*. Rotifers grew slightly faster on manipulated yeast than on non-manipulated yeast varieties. Of the two yeast species, *C. utilis* had better dietary value for rotifers. Flounder larvae cultured with rotifers that had fed on *C. utilis* displayed better growth and survival (%) than did those cultured with rotifers that had fed on *S. cerevisiae*. Although the manipulated variety of *C. utilis* was better than the non-manipulated variety in terms of rotifer growth, the flounder larvae survived (%) and grew better when they were fed rotifers that had eaten non-manipulated *C. utilis*. However, the nutritional value of this yeast species was still lower than that of *Chlorella*.

Key words: *Brachionus plicatilis*, *Candida utilis*, Manipulated yeast, *Paralichthys olivaceus* larvae, *Saccharomyces cerevisiae*

Introduction

Yeast is rich in protein, minerals, and vitamins (Brown et al., 1996; Olvera-Novoa et al., 2002). As a live food for rotifers, yeast is a more economical option than microalgae because yeast has a shorter generation time and will grow in lower-cost culture mediums (Nell, 1993). Yeast is also an inexpensive dietary supplement to replace fishmeal in cultured fish diets (Takii et al., 1999; Akiyama et al., 2001; Muzinic et al., 2004). Nevertheless, the use of yeast in aquaculture has been limited by the poor digestibility caused by its double-bond cell wall with thick inner layer (Farkas, 1985; Coutteau et al., 1990) and by its imbalanced essential amino acid composition (Mahnken et al., 1980; Davies and Wareham, 1988; Olvera-Novoa et al., 2002).

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The yeast most commonly used in rotifer culture is *Saccharomyces cerevisiae*, commonly known as baker's yeast. However, this yeast has lower contents of highly unsaturated fatty acids (HUFA) than the marine microalga *Chlorella* (a representative microalgal food of rotifers). As a result, the survival (%) and growth of fish larvae that fed on rotifers cultured primarily with baker's yeast were low (Watanabe et al., 1980; Cho et al., 2001). It is clear that the nutritional quality of rotifers for larval fish depends on the diet of the rotifers (Evjemo and Olsen, 1997; Castell et al., 2001; Nghia et al., 2001). Rotifers that are fed ω-yeast (made from baker's yeast and fish oil; Kitajima et al., 1980; Hossain et al., 1989) or emulsions of marine oil (Chu et al., 1982; Numaguchi and Nell, 1991; Sorgeloos, 1998) tend to be fed to fish larvae, even though they may be lower in dietary value than *Chlorella*-fed rotifers (Watanabe et al.,

1989; Coutteau et al., 1998). Yeast is used primarily for economic reasons because the mass culturing of *Chlorella* is expensive and difficult, and the cultures undergo frequent sudden crashes.

Consequently, there is a demand for new species of yeast to substitute for baker's yeast or *Chlorella*. Although many alternative approaches have focused on enriching the food value of yeast for rotifers by using cuttlefish liver oil or commercial products such as SELCO® (INVE Aquaculture Nutrition, Ogden, UT, USA) and AlgaMac (Biomarine®; Aquafauna Biomarine, Inc., Hawthorn, CA, USA) (Kitajima et al., 1980; Watanabe et al., 1983; Coutteau et al., 1998; Cho et al., 2001), and on the manipulation of yeast species for better nutrition for shellfish larvae (Coutteau et al., 1990; Coutteau et al., 1994; Nell et al., 1996), almost all of these studies have still focused on baker's yeast. We compared the marine yeast *Candida utilis* to baker's yeast (*S. cerevisiae*) as related to the mass culturing of the rotifer *Brachionus plicatilis* and the dietary value of rotifers fed *C. utilis* to *Paralichtys olivaceus* larvae

Materials and Methods

We used two species of yeast, *Candida utilis* (ATCC 9950, from the American Type Culture Collection) and baker's yeast, *Saccharomyces cerevisiae* (DBY 747 from the Cell and Molecular Biology Lab., Seoul National University, Korea), and their manipulated varieties. Yeasts were cultured in a homogenizer using the methods by Moon and Kim (1998), and the manipulated yeast was prepared by a chemical treatment (Kim and Chung, 2001). First, the yeast was suspended at a concentration of 200 mg wet weight/mL in a sterilized solution containing 1 M Na_2-EDTA and 0.2 M Tris-buffer, and then vortexed. After adding and mixing 0.3 M 2-mercaptoethanol, the yeast was incubated at 30℃ for 1 hr. Finally, the manipulated yeast was obtained as a type of protoplastic cell by centrifugation at 5,000 rpm for 5 min after washing the incubated yeast in sterilized distilled water.

To compare the growth of the *Brachionus plicatilis* rotifers fed the yeasts only, the rotifers were cultured in 100-mL flasks at an initial density of 20 ind./mL in 15 g/L filtered seawater at 25°C with low aeration and continuous light (ca. 3,000 lux). One individual rotifer was fed daily with approximately 20×10^4 yeast cells (Kim et al., 2000). The daily density/mL of the rotifers in each flask was calculated at the same time, and the specific growth rate (SGR, r) per day was calculated by the equation developed by Guillard and Ryther (1962):

$$r=3.322\times(\log (N_1/N_0))/(t_1-t_0),$$

where N is number of rotifers, and t is time. This experiment was conducted in triplicate.

For nutrient analysis of the rotifers fed different diets, rotifers were cultured in a 2-ton capacity tank using the same method described above and fed manipulated or non-manipulated *C. utilis*. The rotifers were also fed *S. cerevisiae* or the marine microalga *Chlorella ellipsoidea* (KMCC-20, from the Korea Marine Microalgae Culture Center) as the control groups. Rotifers at maximum density were harvested using a 50-μm sieve and stored in a freezer at -80°C until analysis. Crude proteins in samples were estimated by the Kjehldahl method (Strickland and Parsons, 1972). Crude lipids were determined by ether extraction. Ash content was determined after combusting the samples (AOAC, 1984). Amino acid analysis was performed by the ninhydrin detection method using an Amino Acid Analyser S433 (Sykam, Gliching, Germany) with 4 mm×150 mm column size 570 nm and 440 nm absorbance, 0.25 mL/min reagent flow rate, 0.45 mL/min buffer flow rate, 120°C reactor temperature, 15 m reactor size, and 65 min analysis time.

For fatty acid analysis, lipids were determined as by Folch et al. (1957). Fatty acid methyl esters were analyzed using gas chromatography (HP 5890, Hewlett-Packard, Palo Alto, CA, USA) with a flame ionization detector equipped with a HP-INNOWax capillary column (30 m×0.32 mm, i.d., film thickness 0.5 μm, Hewlett-Packard). Injector and detector temperatures were 250°C and 270°C, respectively. The column temperature was programmed from 170°C to 225°C at a rate of 1°C/min. Helium was used as the carrier gas. Fatty acids were identified by comparison with known standards. All nutrient analyses were performed in duplicate.

Fertilized eggs of the flounder *Paralichtys olivaceus* were hatched in a 1-ton tank at 21.5±0.5°C. Two days after hatching, 500 larvae were placed in each 200-L tank of filtered seawater in triplicate and were maintained at 21.5±0.5°C with low aeration. Rotifers cultured on *C. ellipsoidea*, baker's yeast, *C. utilis*, or manipulated *C. utilis* were rinsed with filtered seawater and supplied to larval tanks at a density of 10 ind./mL/day. Each day, 20% of the water was changed in each larval tank, and the bottom detritus was removed. Larvae survival (%) and growth were measured for 12 days.

The nutritional value of *C. utilis* for flounder larvae was also tested by the enrichment technique. Two hours before they were supplied to the fish larvae, the rotifers cultured with *C. ellipsoidea* were enriched with a density of 20×10^4 cells of baker's yeast or *C. utilis* (manipulated or non-manipulated), or starved (as a control). Rotifers were then distributed to the larvae as previously described, and the survival (%) and growth of the larvae were measured for 13 days. The larval experiment was conducted in triplicate.

Statistical analyses of all data were performed using Statix 4.0 analytical software (St. Paul, MN, USA). Significant differences among mean values of different treatments were determined by one-way analysis of variance (ANOVA) at a significance level of 5%.

Results

Rotifers that were fed *Candida utilis* and manipulated *C. utilis* reached the greatest densities of 67 and 68 ind./mL on the sixth and fifth days of the experiment, respectively; there were significant differences between the two yeast groups in terms of rotifer growth (Table 1). In contrast, rotifers that were fed baker's yeast (*Saccharomyces cerevisidae*) and manipulated baker's yeast had significantly lower maximum densities, only 37 and 31 ind./mL, respectively. In the case of *C. utilis*, rotifers that were fed the manipulated yeast variety reached maximum density one day earlier than those that were fed the non-manipulated variety. Rotifers that were fed manipulated yeasts tended to have higher SGR compared with those that were fed non-manipulated yeasts. Rotifers that fed on manipulated *C. utilis* had significantly higher SGR than those that fed on the non-manipulated variety. This result indicates that *C. utilis* was better than baker's yeast for rotifer growth.

With regard to nutrient composition, rotifers that were fed *Chlorella* had the highest protein (50.4%) and lipid (10.3%) contents, whereas those that were fed baker's yeast had the lowest protein (39.1%) and lipid (4.1%) contents. The nutritional values of rotifers that fed on manipulated and non-manipulated *C. utilis* were similar and higher than those of rotifers that fed on baker's yeast (Table 2).

In terms of total amino acids, rotifers that were fed *Chlorella* contained the greatest amount (49.5%), and those that were fed baker's yeast had the least (38.4%). Rotifers that were fed non-manipulated *C. utilis* had 46.9% of the total amino acids, which was slightly higher than the value for rotifers that were fed the manipulated variety (44.1%). Rotifers that were fed *C. utilis* had 23.4% of essential amino acids (EAA), a value similar to that of rotifers that were fed *Chlorella* (24.7%); rotifers that were fed baker's yeast had the lowest EAA value (18.6%). The ratio of EAA to non-essential amino acids (NEAA) was 1.0 in rotifers that were fed either *Chlorella* or non-

Table 1. Growth of rotifers fed on yeast for seven days (initial density, 20 ind./mL)

Day	BY	MB	CU	MC
1	24 ± 1.61^a	25 ± 0.58^a	24 ± 0.76^a	25 ± 1.32^a
2	35 ± 1.61^b	26 ± 0.58^c	40 ± 2.29^a	39 ± 2.02^a
3	31 ± 2.78^b	31 ± 1.53^b	40 ± 3.21^a	43 ± 1.32^a
4	33 ± 2.75^c	26 ± 0.29^d	48 ± 0.29^b	55 ± 1.61^a
5	37 ± 1.89^c	20 ± 1.44^d	59 ± 1.00^b	68 ± 1.04^a
6	33 ± 1.50^c	12 ± 0.87^d	67 ± 0.76^a	57 ± 0.50^b
7	36 ± 1.26^b	10 ± 0.58^c	55 ± 1.50^a	38 ± 1.32^b
SGR	0.17 ± 0.0151^d	0.21 ± 0.0237^c	0.29 ± 0.0027^b	0.35 ± 0.0044^a

Values (means) in the same rank with different superscripts are significantly different ($P < 0.05$).
BY, baker's yeast; MB, manipulated baker's yeast; CU, *Candida utilis*; MC, manipulated *C. utilis*.
SGR, specific growth rate of the rotifers, from day 1 to the day of the highest density.
$[3.322\times(\log(N_1/N_0))/(t_1-t_0)$, where N = individual density and t = time].

Table 2. Proximate analyses of rotifers that were fed *Chlorella* and yeasts (unit: %, in dry matter base)

	CHL	BY	CU	MC
Crude protein	50.4 ± 1.00^a	39.1 ± 0.96^c	47.3 ± 1.13^b	45.2 ± 0.59^b
Crude lipid	10.3 ± 0.74^a	4.1 ± 0.44^b	4.8 ± 0.37^b	4.7 ± 0.35^b
Crude ash	7.5 ± 0.68^a	6.8 ± 0.27^a	6.5 ± 0.24^a	6.3 ± 0.30^a
Moisture	80.5 ± 0.92^b	81.2 ± 0.28^{ab}	82.3 ± 0.27^a	81.5 ± 0.58^{ab}

CHL, rotifers that were fed *Chlorella ellipsoidea*; BY, rotifers that were fed baker's yeast; CU, rotifers that were fed *Candida utilis*; MC, rotifers that were fed manipulated *C. utilis*.

Table 3. Amino acid composition of rotifers that were fed *Chlorella* and yeasts (unit: % dry weight)

Amino acid	CHL	BY	CU	MC
Arginine	3.71±0.08 [a]	3.27±0.08 [a]	3.48±0.31 [a]	3.54±0.10 [a]
Histidine	3.26±0.13 [a]	2.33±0.08 [c]	3.10±0.08 [ab]	2.83±0.08 [b]
Isoleucine	2.74±0.20 [a]	2.09±0.01 [c]	2.62±0.11 [ab]	2.31±0.14 [bc]
Leucine	2.75±0.30 [a]	2.07±0.02 [b]	2.62±0.06 [a]	2.41±0.11 [ab]
Lysine	3.07±0.01 [a]	2.22±0.13 [c]	2.96±0.14 [ab]	2.67±0.18 [b]
Methionine	3.27±0.27 [a]	2.06±0.11 [b]	2.98±0.30 [a]	2.35±0.05 [b]
Phenylalanine	3.47±0.41 [a]	2.71±0.21 [b]	3.30±0.13 [ab]	3.04±0.17 [ab]
Threonine	2.46±0.18 [a]	1.76±0.14 [b]	2.38±0.17 [a]	2.24±0.07 [a]
Valine	2.46±0.33 [a]	2.10±0.06 [a]	2.34±0.21 [a]	2.27±0.24 [a]
Cysteine	5.21±0.18 [a]	4.98±0.30 [a]	4.81±0.08 [a]	4.94±0.10 [a]
Tyrosine	3.81±0.19 [a]	2.85±0.04 [b]	3.62±0.04 [a]	3.67±0.27 [a]
Alanine	1.56±0.47 [a]	1.21±0.14	1.50±0.18 [a]	1.41±0.07 [a]
Aspartic acid	2.80±0.17 [a]	1.98±0.00 [b]	2.66±0.26	2.46±0.44 [ab]
Glutamic acid	3.08±0.25 [a]	2.34±0.28 [b]	2.94±0.21 [ab]	2.68±0.18 [ab]
Glycine	2.42±0.07 [a]	1.37±0.11 [b]	2.30±0.13 [a]	2.13±0.23 [a]
Proline	0.88±0.09 [a]	0.87±0.11 [a]	0.83±0.06 [a]	0.82±0.05 [a]
Serine	2.18±0.20 [a]	1.82±0.07 [b]	2.10±0.08 [ab]	2.01±0.10 [ab]
NH_3	0.36±0.03 [a]	0.33±0.08 [a]	0.33±0.31 [a]	0.32±0.03 [a]
Total	49.49 [a]	38.35 [d]	46.87 [b]	44.10 [c]
EAA	24.73 [a]	18.56 [c]	23.44 [a]	21.39 [b]
NEAA	24.76 [a]	19.85 [d]	23.43 [b]	22.71 [c]
EAA/NEAA	1.00	0.94	1.00	0.94

CHL, rotifers that were fed *Chlorella ellipsoidea*; BY, rotifers that were fed baker's yeast; CU, rotifers that were fed *Candida utilis*; MC, rotifers that were fed manipulated *C. utilis*; EAA, essential amino acids; NEAA, non-essential amino acids.

manipulated *C. utilis*, whereas the ratio was 0.94 in rotifers that were fed either manipulated *C. utilis* or baker's yeast (Table 3).

Rotifers that were fed *Chlorella* also had the highest fatty acid content, 25.8% of which was eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). In contrast, rotifers that fed on baker's yeast had the lowest fatty acid content (2.2%). Although the total fatty acid components of rotifers that were fed either *C. utilis* (93.9%) or *Chlorella* (97.4%) were similar, the sum of EPA and DHA levels was much lower in rotifers that were fed the yeast (4.8%) than in those that were fed *Chlorella* (25.8%; Table 4). Rotifers that were fed manipulated *C. utilis* had lower total fatty acid content (including EPA+DHA) than those fed the non-manipulated variety. Arachidonic acid (ARC, 20:4n6) was highest in *Chlorella*-fed rotifers (3.9%) and lowest in those fed with baker's yeast (0.3%)

Flounder larvae that were provided with *Chlorella*-fed rotifers had the highest survivorship (37.1%), significantly higher than that of the other treatment groups. Larvae that were supplied with rotifers fed either the manipulated or non-manipulated *C. utilis* had significantly higher survivorship (32.4 and 34.9%, respectively) than larvae supplied with rotifers fed baker's yeast; these latter larvae had the lowest survivorship (29.8%). No significant differences were observed in the survivorship of larvae that were supplied with rotifers that had been fed non-manipulated and manipulated varieties of *C. utilis*. Similar tendencies were observed among treatment groups in terms of larval growth (total length; Table 5). In this case, no significant differences were found between larvae that were provided with rotifers fed *Chlorella* or *C. utilis*.

Flounder larvae that fed on rotifers enriched with either manipulated or non-manipulated *C. utilis* varieties showed higher survivorship (38-39%) and longer size in total length (6.3-6.5 mm) than did larvae that were supplied with rotifers that had either been fed baker's yeast or starved for 2 hr (Table 6). No significant differences in survivorship or growth were observed between the larvae that were supplied with rotifers that had fed on the manipulated or non-manipulated *C. utilis* varieties.

Discussion

The thick cell walls of yeast can be indigestible by marine larvae (Watanabe et al., 1980). Research into manipulation of the yeast cell wall has attempted to overcome this problem (Coutteau et al., 1990; Moon

Table 4. Fatty acid composition of rotifers that were fed *Chlorella* and yeasts (unit: area %)

Fatty acids	CHL	BY	CU	MC
$C_{14:0}$	0.84±0.08 ^{c}	1.50±0.10 ^{c}	4.44±0.11 ^{b}	8.75±0.54 ^{a}
$C_{16:0}$	19.15±0.40 ^{b}	7.97±0.16 ^{d}	23.60±1.29 ^{a}	14.67±0.62 ^{c}
$C_{18:0}$	2.91±0.11 ^{b}	4.67±0.17 ^{a}	4.95±0.04	2.50±0.06 ^{c}
$C_{14:1n5}$	0.42±0.03 ^{c}	0.31±0.01 ^{c}	0.73±0.06 ^{b}	1.54±0.17 ^{a}
$C_{16:1n7}$	16.07±0.72 ^{b}	24.21±1.02	14.94±0.78 ^{b}	15.02±0.85 ^{b}
$C_{18:1n9}$	12.58±2.29 ^{d}	38.87±0.34	25.79±1.17 ^{b}	19.25±0.44 ^{c}
$C_{18:2n6}$	6.67±0.23 ^{a}	4.88±0.45 ^{b}	7.17±0.13 ^{a}	4.76±0.11 ^{b}
$C_{18:3n3}$	0.07±0.00 ^{c}	1.45±0.30 ^{ab}	1.06±0.25 ^{bc}	2.15±0.14 ^{a}
$C_{20:1n9}$	2.76±0.04 ^{a}	0.60±0.03 ^{b}	0.73±0.08 ^{b}	0.80±0.16 ^{b}
$C_{20:2n6}$	0.72±0.04 ^{b}	0.27±0.01 ^{c}	0.29±0.04 ^{c}	1.55±0.04 ^{a}
$C_{20:3n3}$	0.22±0.01 ^{c}	0.66±0.07 ^{a}	0.46±0.03 ^{b}	0.38±0.01 ^{b}
$C_{20:4n6}$	3.87±0.01 ^{a}	0.26±0.03 ^{d}	0.83±0.03 ^{c}	1.63±0.04 ^{b}
$C_{20:5n3}$	18.46±0.71 ^{a}	1.11±0.07 ^{b}	1.00±0.04 ^{b}	0.56±0.06 ^{b}
$C_{22:2}$	0.53±0.01 ^{c}	0.68±0.03 ^{c}	1.40±0.11 ^{b}	2.95±0.13 ^{a}
$C_{22:4n6}$	1.98±0.13 ^{b}	2.35±0.03 ^{a}	0.39±0.03 ^{c}	2.56±0.18 ^{a}
$C_{22:5n6}$	2.00±0.06 ^{b}	0.70±0.03 ^{d}	2.32±0.08 ^{a}	1.67±0.04 ^{c}
$C_{22:5n3}$	0.83±0.04 ^{a}	0.69±0.01 ^{b}	-	0.63±0.08 ^{b}
$C_{22:6n3}$	7.32±0.30 ^{a}	1.12±0.13 ^{d}	3.81±0.10 ^{b}	3.09±0.13 ^{c}
Total	97.4 ^{a}	92.3 ^{a}	93.91 ^{a}	84.46 ^{b}
Other	2.60 ^{d}	8.70 ^{b}	6.09 ^{c}	15.54 ^{a}
EPA+DHA	25.78 ^{a}	2.23 ^{d}	4.81 ^{b}	3.65 ^{c}

CHL, rotifers that were fed *Chlorella ellipsoidea*; BY, rotifers that were fed baker's yeast; CU, rotifers that were fed *Candida utilis*; MC, rotifers that were fed manipulated *C. utilis*; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Table 5. Survivorship and total length of larval flounder *Paralichthys olivaceus* that were supplied with rotifers that had been fed *Chlorella* and yeasts for twelve days (initial length, 2.85 ± 10 mm)

	CHL	BY	CU	MC
Survival (%)	37.1 ± 2.0^{a}	29.8 ± 1.1^{c}	34.9 ± 1.0^{b}	32.4 ± 1.5^{b}
Total length (mm)	6.1 ± 0.5^{a}	5.3 ± 0.3^{b}	5.8 ± 0.3^{ab}	5.6 ± 0.3^{b}

Values (means) in the same rank with different superscripts are significantly different ($P < 0.05$); CHL, Larvae that were supplied with rotifers that had been fed *Chlorella ellipsoidea*; BY, Larvae that were supplied with rotifers that had been fed baker's yeast; CU, Larvae that were supplied with rotifers that had been fed *Candida utilis*; MC, Larvae that were supplied with rotifers that had been fed manipulated *C. utilis*.

Table 6. Survivorship and growth in total length of larval flounder *Paralichthys olivaceus* that were supplied with rotifers that had been enriched with various yeasts (initial total length, 2.85 ± 10 mm)

	ECU	EMC	EBY	Control
Survival (%)	38.5 ± 2.4^{a}	39.6 ± 2.2^{a}	32.5 ± 2.6^{b}	31.7 ± 3.2^{b}
Total length (mm)	6.5 ± 0.29^{a}	6.3 ± 0.3^{a}	5.9 ± 0.3^{b}	5.6 ± 0.3^{b}

Values (means) in the same rank with different superscripts are significantly different ($P < 0.05$); ECU, Larvae that were supplied with rotifers that had been enriched with *Candida utilis* for 2 hr; EMC, Larvae that were supplied with rotifers that had been enriched with manipulated *C. utilis* for 2 hr; EBY, Larvae that were supplied with rotifers that had been enriched with baker's yeast for 2 hr; Control, Larvae that were supplied with rotifers that had been starved for 2 hr.

et al., 1996; Kim and Chung, 2001). However, most studies of yeast for aquaculture have involved baker's yeast, *Saccharomyces cerevisiae*.

We have shown that *Candida utilis* is superior to *S. cerevisiae* in terms of rotifer growth. Rotifers that were fed manipulated yeast varieties showed significantly higher SGR and reached maximum culture density earlier than did those that were fed non-manipulated varieties. In a study of yeast strains, Moon et al. (1996) found that the SGR and biomass yield for *C. utilis* under optimum pH and temperature were much higher than the values for *S. cerevisiae*. In a study of the use of *Candida* sp. and baker's yeast in combination with *Chlorella* for the mass culture of

rotifers, the rotifers that were fed *Candida* sp. grew significantly faster than rotifers that were fed baker's yeast (James et al., 1987). *Chlorella*-fed rotifers had the highest protein content, and those that were fed baker's yeast had the lowest. The protein content of rotifers that were fed *C. utilis* was higher than that of rotifers that fed on baker's yeast but still lower than that of rotifers fed on *Chlorella*. The protein content of rotifers that were fed on manipulated varieties tended to be higher than that of rotifers fed on non-manipulated varieties. Similar results were observed among diets in terms of crude protein content and total and essential amino acids. These findings indicate that the manipulated yeasts enhance the growth rates of rotifers due to their improved digestibility. However, the removal of the cell walls reduced the nutritional value of the manipulated yeasts relative to the non-manipulated varieties. In our study, rotifers that were fed manipulated *C. utilis* showed reduced levels of methionine and essential amino acids compared with rotifers that were fed non-manipulated varieties. Previous studies have reported that methionine deficiencies and imbalanced essential amino acids in yeast substituted for fish meal protein reduced the efficiency of fish growth and feed utilization (Mahnken et al., 1980; Davies and Wareham, 1988; Olvera-Novoa et al., 2002).

In our study, *Chlorella*-fed rotifers were always more nutritious than yeast-fed rotifers. Among yeast-fed rotifers, those that were fed baker's yeast performed the worst. The differences in lipid content between *Chlorella*-fed and yeast-fed rotifers were particularly high in comparison with the differences in protein content. The crude lipid content of *Chlorella*-fed rotifers was much greater than that of yeast-fed rotifers. These results agree with previous findings (Maruyama et al., 1988). The lipid content was similar between rotifers that were fed manipulated and non-manipulated *C. utilis*. The levels of EPA and DHA, which are two very important fatty acids for marine larvae (Watanabe, 1993; Coutteau et al., 1998; Nghia et al., 2001), were very low in yeast-fed rotifers (2.2-4.8%) relative to those in *Chlorella*-fed rotifers (25.8%). Levels of EPA and DHA were higher in rotifers that were fed non-manipulated *C. utilis* (4.8%) than in rotifers that were fed the manipulated variety (3.7%). Rotifers fed *C. utilis* or baker's yeast had similar EPA levels (1.0-1.1%). However, DHA levels were three times higher in rotifers fed *C. utilis* than in those fed baker's yeast.

Rotifers fed yeast varieties (0.6-1.1%) had much lower EPA levels than rotifers fed *Chlorella* (18.5%), but DHA levels in rotifers fed *Chlorella* were only about two times higher than levels in rotifers fed *C. utilis* (3.8%). Arachidonic acid, ARC (20:4n6) is also an important essential fatty acid for active transport of ions and osmoregulation in marine fish (Praag et al., 1987; Castell et al., 1994; Castell et al., 2001). In this study, rotifers fed *Chlorella* had the highest ARC levels (3.9%); however, rotifers fed *C. utilis* showed higher levels of ARC than rotifers fed baker's yeast.

The survivorship of larval flounders was signify-cantly higher when larvae were supplied with rotifers that had been fed *C. utilis* rather than baker's yeast, but both yeasts were inferior to *Chlorella*. Although the difference in larval survivorship was not significant, rotifers that were fed non-manipulated *C. utilis* tended to promote higher growth and survivorship in larvae relative to those that were fed the manipulated variety. Larval flounder that fed on rotifers enriched with different yeasts showed patterns of survivorship and growth similar to those of previous experiments. Larvae that fed on rotifers that had been starved for 2 hr showed the lowest survivorship and growth rates, indicating that the nutritional value of rotifers decreases sharply when starved (Frolov and Pankov, 1992).

Lee and Kim (2001) found that the production of *C. utilis* on molasses compared favorably in terms of price with the purchase of commercial yeasts. Manipulated yeasts can be maintained fairly well (up to 71% survival) when stored for 3 weeks at 4°C (Kim and Chung, 2001).

We have shown that *C. utilis* provides a significantly better diet than baker's yeast for rotifers that are cultured to feed larval fish. The manipulation of the cell wall of this yeast reduces the nutritional value of the rotifer. Therefore, we recommend that non-manipulated *C. utilis* be used instead of baker's yeast in rotifer culture.

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