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Selection of Suitable Species of *Chlorella*, *Nannochloris*, and *Nannochloropsis* in High- and Low-Temperature Seasons for Mass Culture of the Rotifer *Brachionus plicatilis*

Jean Hee Bae and Sung Bum Hur*

Department of Marine Bio-materials and Aquaculture, Pukyong National University, Busan 608-737, Korea

Abstract

To find seasonally optimal microalgae for mass culture of the rotifer *Brachionus plicatilis*, the growth rates of 12 microalgal species (two marine *Chlorella* spp., five marine *Nannochloris* spp., two marine *Nannochloropsis* spp., one estuarine *Nannochloropsis* sp., and two estuarine *Chlorella* spp.) were compared at 25°C at 15 psu and 30 psu. Among these, six species showing high growth rates were chosen and examined again at high (30°C and 32°C) and low (10°C) temperatures. Their amino and fatty acids and the dietary value of the rotifers that fed on each microalgal species were examined. *Nannochloris* sp. (KMMCC-119) and *Chlorella vulgaris* (KMMCC-120) showed the highest growth rates at temperatures over 30°C and at 10°C, respectively. The growth rate of *Nannochloris* was higher than those of *Chlorella* and *Nannochloropsis* at high temperatures, but lower than those of the latter at low temperatures. The growth rate of rotifers fed on *Nannochloropsis* was highest and that of those fed on *Chlorella* was lowest. Levels of eicosapentaenoic acid and docosahexaenoic acid were highest in *Nannochloropsis* and lowest in *Nannochloris*. However, total amino acid content was highest in *Nannochloris* and lowest in *Chlorella*. In conclusion, *Nannochloropsis* sp. (KMMCC-33) was the best microalgal species for the mass culture of the rotifer. However, during high- or low-temperature seasons in which *Nannochloropsis* does not grow well, *Nannochloris* spp. (KMMCC-119, 395) and *C. vulgaris* (KMMCC-120) would adequately replace *Nannochloropsis* sp. (KMMCC-33).

Key words: *Brachionus plicatilis*, *Chlorella*, *Nannochloris*, *Nannochloropsis*, Rotifer

Introduction

The rotifer *Brachionus plicatilis* is prevalently used as an early larval food for the seed production of seawater fish, as it is small, with low motility, and is suitable for high-density culture. Since the rotifer was first used as feed for *Pagrus major* in Japan (Fukusho, 1989), microalgae and yeast have been widely used as feed in the mass production of the rotifer.

Examples of commonly used microalgae and yeast include the microalgae *Nannochloropsis* (Fukusho, 1989; Kostopoulou and Vadstein, 2007; Ferreira et al., 2009), *Chlorella* (Maruyama et al., 1997; Cabrera et al., 2005; Zhou et al., 2009), and *Nannochloris* (Witt et al., 1981; Cabrera and Hur, 2001, Cho et al., 2007), and the yeasts bread yeast *Saccharomyces cerevisiae* (Gilberto and Mazzola, 1981; Sarma et al., 2002; Wang et al., 2009) and marine yeast *Candida utilis* (Kim et al., 2005).

Yeasts are more economical than microalgae, but contain insufficient amounts of unsaturated fatty acids, which are essential for the growth of rotifers (Watanabe et al., 1980; Kim et al., 2009). In an attempt to supplement the shortcomings of yeast, ω-yeast was developed using bread yeast infused with squid oil. However, this had lower nutritional value than microalgae and caused water pollution (Hirayama and Funa-

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***Corresponding Author**

E-mail: hurs@pknu.ac.kr

moto, 1983). Thus, in spite of the high costs incurred on the mass culture of rotifers, live microalgae are still preferred by hatcheries (Hur, 1991; Borowitzka, 1997).

Chlorella, *Nannochloris*, and *Nannochloropsis* have been well-known to be easily mass-cultured and to have high contents of protein or unsaturated fatty acids (Chini Zittelli et al., 1999; Hu and Gao, 2003; Cho et al., 2007). However, they are also at high risk of sudden mortality at temperatures over 30°C, and their low growth rates in water temperatures under 10°C are problematic (Fukusho et al., 1985; Hur, 1991).

Therefore, this study aimed to identify a new species among *Chlorella*, *Nannochloris*, and *Nannochloropsis* that would be specifically suitable for mass culture as rotifer feed during seasons of high and low temperature.

Materials and Methods

Culture of microalgae

The microalgae used in this study, from the Korea Marine Microalgae Culture Center (KMMCC), included nine marine species and three estuarine species. These consisted of four kinds of *Chlorella*, five kinds of *Nannochloris*, and three kinds of *Nannochloropsis* (Table 1). Their growth rates were observed and compared to each other.

Microalgae in the log phase stage were inoculated in 100 mL of f/2 culture medium (Guillard and Ryther, 1962) in a 250-mL Ehrenmeyer flask at a density of 100×10^4 cells/mL. Microalgae were cultured in standing water at 25°C and 15 and 30 psu, with continuous light of 100 μmol m^{-2} s^{-1}, three times during 7 days. The salinity was adjusted by the mixture of filtered seawater and distilled water.

To measure growth rate, cells were counted by hemacytometer regularly, four times a day. The specific growth rate (SGR) was calculated according to Guillard (1973) [SGR = $3.322 \times \log(N_1/N_0)/t$, where t is culture days after inoculation, and N_0 and N_1 are cell density after inoculation or t days, respectively].

Table 1. List of microalgal species used in the study

Habitat	KMMCC No.	Species	Size (μm)	Sampling area
Marine	79	*Chlorella salina*	2.4 ± 0.50	China
	95	*Chlorella vulgaris*	2.1 ± 0.60	Deukryang Bay
	58	*Nannochloris* sp.	3.1 ± 1.22	Gamcheon Bay
	16	*Nannochloris oculata*	2.1 ± 0.60	UTEX1998
	117	*Nannochloris* sp.	1.9 ± 0.63	Hwajinpo
	119	*Nannochloris* sp.	1.8 ± 0.62	Deukryang Bay
	395	*Nannochloris* sp.	2.3 ± 0.43	Puan
	33	*Nannochloropsis* sp.	2.8 ± 0.95	Dadaepo
	13	*Nannochloropsis oceanica*	1.9 ± 0.63	Japan
Estuarine	120	*Chlorella vulgaris*	2.3 ± 0.47	Hwajinpo
	137	*Chlorella* sp.	2.4 ± 0.68	Nacdong River
	327	*Nannochloropsis* sp.	2.3 ± 0.91	Sooyoung Bay

KMMCC, Korea Marine Microalgae Culture Center; UTEX, The Culture Collection of Algae at the University of Texas at Austin.

Growth of six kinds of microalgae at high and low temperature

Six kinds of microalgae that showed high growth rates in the aforementioned experiment were cultured at high temperatures of 30°C and 32°C and a low temperature of 10°C at 15 psu with 100 μmol m^{-2} s^{-1} of continuous lighting, namely, *Nannochloropsis* sp. (KMMCC-33), *Nannochloropsis oceanica* (KMMCC-13), *Nannochloris oculata* (KMMCC-16), *Nannochloris* sp. (KMMCC-119), *Nannochloris* sp. (KMMCC-395), and *Chlorella vulgaris* (KMMCC-120). Their SGR was examined using the previously described method for 10 days.

The adequacy of the six microalgae as rotifer feed

The rotifer *Brachionus plicatilis* (R-4, L-type), provided by the Culture Collection of Useful Marine Plankton (CCUMP) at Pukyung National University in Korea, was used in this study. The amount of microalgae fed daily to an individual rotifer was in accordance with algal cell volume as follows. For the smallest cells, *N. oculata*, *Nannochloris* sp. (KMMCC-119), and *Nannochloris* sp. (KMMCC-395), 30×10^4 cells were provided as feed. For *N. oceanica* and *Nannochloropsis* sp. (KMMCC-33), 22×10^4 cells were fed. For the largest cells, *C. vulgaris* (KMMCC-120), 15×10^4 cells were supplied. The rotifer was inoculated at 10 individuals/mL in 100 mL of a 250-mL Ehrenmeyer flask and cultured in standing water at 26°C and 15 psu under continuous lighting of 60 μmol m^{-2} s^{-1}. Culture was conducted in triplicate during 5 days. One milliliter of each culture group was arbitrarily drawn and placed in Lugol's solution and the number of rotifers in 1 mL was counted under a stereoscopic microscope three times a day. The SGR of the rotifers was calculated by the method described above.

Analysis of the nutritional content of the microalgae and rotifers

The amino and fatty acids of the six selected microalgae and the rotifers fed on three selected microalgae, which induced high growth rates in rotifers, were analyzed. They were cultured by the method described above. At the end of the log phase of growth, they were harvested and kept at -80°C until analysis.

For the analysis of amino acids, 20 mg of sample infused with 15 mL of 6 N HCl was heated, sealed, and hydrolyzed at 110°C for 24 h. The sample was then filtered and dried to

remove HCl. Then 25 mL of the sample was set by sodium dilution buffer (pH 2.2) and a portion of the sample was analyzed by the ninhydrin method using S433 (Sykam, Fürstenfeldbruck, Germany). Conditions of the analysis were as follows: column size, 4 mm × 150 mm; absorbance level, 570 nm and 440 nm; reagent flow rate, 0.25 mL/min; buffer flow rate, 0.45 mL/min; reactor temperature, 120°C; reactor size, 15 m; and analysis time, 65 min.

For the analysis of fatty acids, 20 mg of sample in a 15-mL flask was added to 2 mL of 10% BF_3-methanol. Nitrogen was added to the sample and heated at 85°C for an hour and a half to draw methyl ester (Morrison and Smith, 1964; Budge, 1999). Cooled to 30-40°C, the sample was combined with water and hexane to draw fatty acids separately. The extracted fatty acids were analyzed with a HP GC 6890 Plus installed with a HP autosampler (Agilent Technologies, Santa Clara, CA, USA). The GLC used in this analysis was the DB-225 (20 m × 0.1 mm, i.d., 0.1 μm film thickness; J&W Scientific, Agilent Technologies, Santa Clara, CA, USA). Conditions of the analysis were as follows: column temperature levels, 60-195°C (25°C/min); temperature conditions, 195-205°C (3°C/min), 205-230°C (8°C min); injector, 250°C; detector, 250°C; and carrier gas used, He (60 cm/s). Fatty acids were identified by comparison with known standards.

Statistical analysis

The results of this study were analyzed by one-way ANOVA, and Duncan's multiple range test (Duncan, 1955) was applied for the significance level ($P < 0.05$). The SPSS version 17 (SPSS Inc., Chicago, IL, USA) program was used for all statistical analyses.

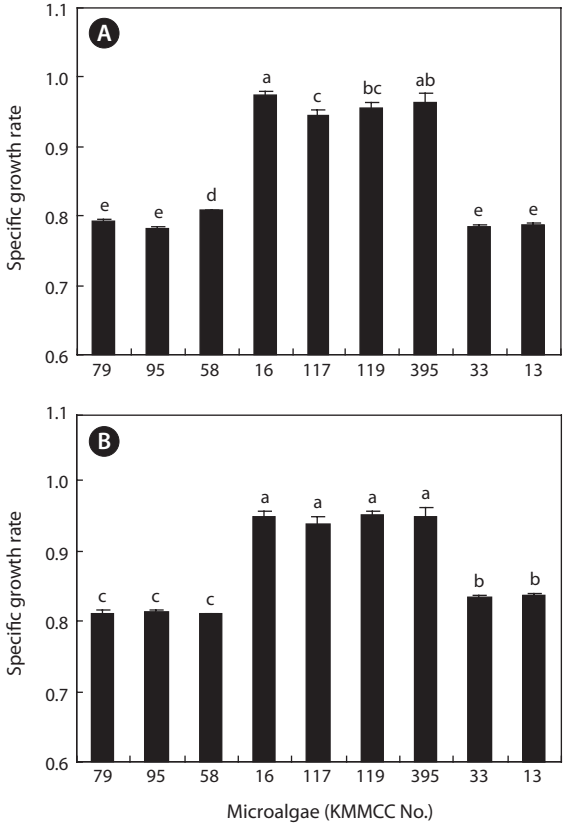

Fig. 1. Specific growth rate of nine marine microalgal species at 30 psu (A) and 15 psu (B), 25°C and 100 μmol m^{-2} s^{-1} (KMMCC, Korea Marine Microalgae Culture Center; KMMCC-79, *Chlorella salina*; KMMCC-95, *C. vulgaris*; KMMCC-58, *Nannochloris* sp.; KMMCC-16, *N. oculta*; KMMCC-117, *Nannochloris* sp.; KMMCC-119, *Nannochloris* sp.; KMMCC-395, *Nannochloris* sp.; KMMCC-33, *Nannochloropsis* sp.; KMMCC-13, *N. oceanica*). Different letters on the bar mean significantly difference ($P < 0.05$).

Results

Growth of marine microalgae

After 7 days culture of the nine marine microalgae at 30 psu and 15 psu, results (Fig. 1) indicated that *N. oculata* and *Nannochloris*. sp. (KMMCC-395) in 30 psu showed the highest growth rates of 0.9734 and 0.9640, respectively (highest cell densities, 11,229 × 10^4 cells/mL and 10,733 × 10^4 cells/mL, respectively). The growth rates of *C. salina* and *C. vulgaris* at 0.7912 and 0.7812 were not significantly different from each other. The growth rates of *N. oceanica* and *Nannochloropsis*. sp. at 0.7866 and 0.7842 were as low as those of *Chlorella*, while *Nannochloris* showed a significantly higher growth rate than those of *Chlorella* and *Nannochloropsis* ($P < 0.05$). The growth rate of *Nannochloris* sp. (KMMCC-58) was significantly lower than those of the other four kinds of *Nannochloris* ($P < 0.05$).

At 15 psu, the growths of *N. oculata* and the three kinds of *Nannochloris* (KMMCC-117, 119, and 395) were highest, in the range of 0.9393 and 0.9504 (highest cell density, 9,718 × 10^4 cells/mL to 10,145 × 10^4 cells/mL). Conversely, *Nannochloris* sp. (KMMCC-58) showed a significantly lower growth rate than other strains, similar to that of *Chlorella*. *N. oceanica* and *Nannochloropsis* sp., the two kinds of *Nannochloropsis*, showed significantly lower growth rates compared to the four kinds of *Nannochloris*, but significantly higher growth rates than *Chlorella* ($P < 0.05$). The growth rates of *Chlorella* and *Nannochloropsis* tended to be higher at 15 psu than at 30 psu. However, the four kinds of *Nannochloris*, except for *Nannochloris* sp. (KMMCC-58), showed slightly higher growth rates at 30 psu.

Growth of estuarine microalgae

At 30 and 15 psu, the growth rates of *C. vulgaris* (KMMCC-120) were 0.8495 and 0.8601 (6,166 × 10^4 cells/mL and 6,491 × 10^4 cells/mL), respectively, indicating the sig-

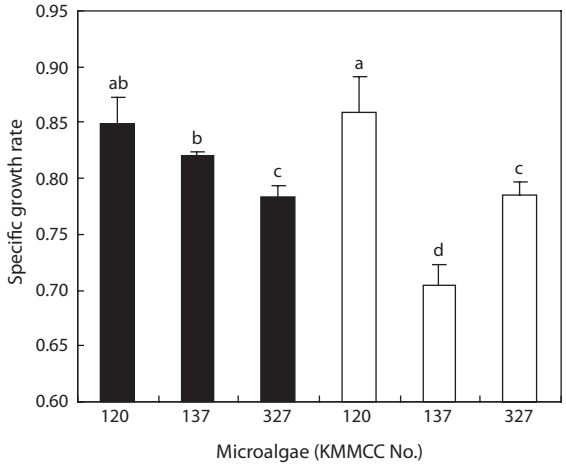

Fig. 2. Specific growth rate of three estuarine microalgal species at 30 psu (black bar) and 15 psu (white bar), 25°C and 100 μmol $m^{-2} s^{-1}$ (KMMCC, Korea Marine Microalgae Culture Center; KMMCC-120, *Chlorella vulgaris*; KMMCC-137, *Chlorella* sp.; KMMCC-327, *Nannochloropsis* sp.). Different letters on the bar mean significantly difference ($P < 0.05$).

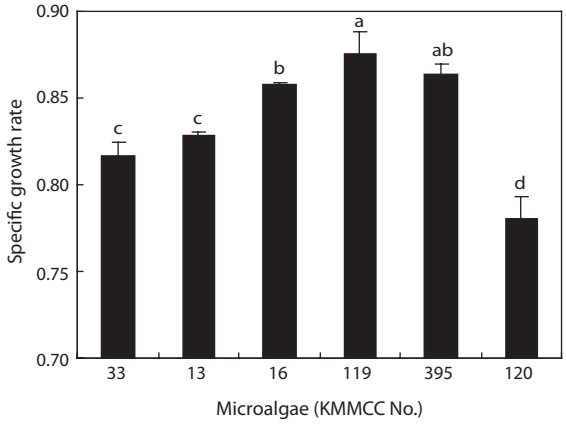

Fig. 3. Specific growth rate of six microalgal species at 15 psu, 25°C, 100 μmol $m^{-2} s^{-1}$ (KMMCC, Korea Marine Microalgae Culture Center; KMMCC-33, *Nannochloropsis* sp.; KMMCC-13, *N. oceanic*; KMMCC-16, *Nannochoris oculata*; KMMCC-119, *Nannochoris* sp.; KMMCC-395, *Nannochoris* sp.; KMMCC-120, *Chlorella vulgaris*). Different letters on the bar mean significantly difference ($P < 0.05$).

nificantly highest rate ($P < 0.05$) (Fig. 2). The growth rates of *Chlorella* sp. (KMMCC-137) and *Nannochloropsis* sp. (KMMCC-327) were in the range of 0.7781-0.8204, which were lower than that of *C. vulgaris* (KMMCC-120). The growth rate of *Chlorella* sp. (KMMCC-137) was significantly higher at 30 psu than at 15 psu. As a result, *Chlorella* sp. (KMMCC-137) was distinguished as a marine microalga. The growth rates of the other two kinds of *Chlorella* indicated no significant difference according to salinity ($P < 0.05$).

In contrast to the estuarine *C. vulgaris* (KMMCC-120), which showed a higher growth rate than marine *Chlorella* (KMMCC-79 and 95), *Chlorella* sp. (KMMCC-137) and *Nannochloropsis* sp. (KMMCC-327) showed similar growth rates to those of marine microalgae.

Analyses of the nutritional content and growth of six selected microalgae, including *Nannochloropsis*, *Nannochloris*, and *Chlorella*

The growth and nutritional content of the marine microalgae *N. oceanica*, *Nannochloropsis* sp. (KMMCC-33), *N. oculata*, *Nannochloris* sp. (KMMCC-119), and *Nannochloris* sp. (KMMCC-395) and the estuarine *C. vulgaris* (KMMCC-120) were studied.

Growth rates under the same culture conditions, 15 psu, 25°C, and 100 μmol $m^{-2} s^{-1}$, are shown in Fig. 3. Among the six kinds of microalgae, the growth rate of *Nannochloris* sp. (KMMCC-119) was highest at 0.8753 (highest cell density, $6,987 \times 10^4$ cells/mL). The growth rates of the two kinds of *Nannochloropsis* were significantly higher than that of *Chlorella* and lower than that of *Nannochloris* ($P < 0.05$). In addition, the growth rate of estuarine *C. vulgaris* (KMMCC-120), 0.7807 (highest cell density, $4,416 \times 10^4$ cells/mL), was the lowest compared to those of marine microalgae ($P < 0.05$).

Nannochloris sp. (KMMCC-395) contained the highest percentage amino acid content at 72.97% and *C. vulgaris* (KMMCC-120) the lowest at 45.72% (Table 2). The amino acid contents of *N. oceanica* and *Nannochloris* sp. (KMMCC-119) were lower than that of *Nannochloris* sp. (KMMCC-395) and

Table 2. Amino acid composition (%) of six microalgal species

Amino acid	KMMCC No.					
	33	13	16	119	395	120
Arginine	3.16	3.33	2.14	3.14	4.29	2.59
Histidine	1.12	1.31	1.51	1.76	2.52	1.42
Isoleucine	2.15	2.72	1.59	2.48	3.13	2.18
Leucine	4.07	5.06	3.56	4.76	6.38	4.28
Lysine	3.07	3.66	2.75	3.40	4.61	2.73
Phenylalanine	2.74	2.97	3.18	3.25	4.29	2.55
Threonine	2.22	2.62	2.11	2.54	3.42	2.04
Valine	2.93	3.39	2.71	3.10	3.87	2.60
Alanine	3.08	3.61	3.80	5.21	6.20	3.94
Aspartic acid	4.39	5.02	4.81	5.59	7.49	5.02
Glutamic acid	6.96	8.12	7.95	9.36	13.05	6.77
Glycine	2.59	3.23	2.58	3.04	4.08	2.87
Proline	7.29	6.40	3.37	3.36	3.89	2.70
Serine	1.93	2.16	1.96	2.37	3.10	2.33
Tyrosine	1.84	1.98	3.01	2.05	2.68	1.70
Total	49.54	55.58	47.03	55.41	72.97	45.72
EAA	21.46	25.06	19.55	24.43	32.51	20.39
NEAA	28.08	30.52	27.48	30.98	40.49	25.33
EAA/NEAA	0.76	0.82	0.71	0.79	0.80	0.80

KMMCC, Korea Marine Microalgae Culture Center ; KMMCC-33, *Nannochloropsis* sp.; KMMCC-13, *N. oceanic*; KMMCC-16, *Nannochoris oculata*; KMMCC-119, *Nannochoris* sp.; KMMCC-395, *Nannochoris* sp.; KMMCC-120, *Chlorella vulgaris*; EAA, essential amino acid; NEAA, non-essential amino acid.

higher than that of *C. vulgaris*. Among nonessential amino acids, glutamine and leucine contents were high. Essential amino acids were highest in *Nannochloris* sp. (KMMCC-395) at 32.51%.

Nannochloropsis, *Nannochloris*, and *Chlorella* indicated high contents of fatty acids at ratios of 14:0, 15:1, 16:0, and 16:1 (Table 3). The contents of polyunsaturated fatty acid, PUFA, in *Nannochloropsis* sp. (KMMCC-33) and *Nannochloris* sp. (KMMCC-119) were highest at 40.68% and 39.63%, respectively. The content of eicosapentaenoic acid (EPA, 20:5n-3) in *Nannochloropsis* sp., 34.88%, was high compared to that in *Nannochloris* sp. (KMMCC-119), which was the lowest at 0.35%. The contents of docosahexaenoic acid (DHA, 22:6n-3) in *Nannochloropsis* sp. (KMMCC-33) and *Nannochloris* sp. (KMMCC-119) were as low as 0.29% and 0.02%, respectively. The content of EPA+DHA in *Nannochloropsis* sp. (KMMCC-33), 35.17%, was the highest and in *Nannochloris* sp. (KMMCC-119), 0.37%, the lowest.

Table 3. Fatty acid composition (%) of six microalgal species

Fatty acid	KMMCC No.					
	33	13	16	119	395	120
8:0	0.41	0.20	0.66	0.16	0.09	0.40
10:0	0.26	0.20	-	0.16	-	-
11:0	0.07	-	0.45	0.04	0.19	0.21
12:0	0.29	0.30	-	0.74	-	-
13:0	0.87	0.30	0.42	0.04	0.22	0.23
14:0	4.28	4.20	3.61	4.80	3.50	2.25
14:1	0.64	0.20	1.23	0.48	0.36	1.20
15:0	0.29	-	0.68	0.59	0.27	0.37
15:1	0.19	-	1.42	0.12	0.40	0.61
16:0	17.49	24.30	9.24	26.83	15.40	10.96
16:1	25.75	19.00	1.54	5.33	8.69	3.04
17:0	0.18	-	6.97	0.28	1.18	7.55
17:1	0.74	-	16.14	8.48	3.24	11.66
18:0	0.19	0.60	1.10	2.08	2.99	0.99
18:1n9	3.31	10.20	6.23	10.14	29.59	5.64
18:2n9	4.50	5.70	1.29	15.41	5.73	2.924
18:3	-	0.20	-	23.63	-	-
18:3n6	0.16	-	14.28	0.017	0.11	22.32
18:3n9	0.16	-	-	-	4.87	-
20:0	0.06	-	32.26	0.02	8.21	24.64
20:1	0.05	-	-	0.02	4.12	0.94
20:2	0.05	-	1.47	0.08	1.66	-
20:3	0.16	0.90	-	0.04	0.11	-
20:4	0.38	3.10	-	0.04	-	-
21:0	4.07	-	-	-	0.71	0.34
20:5n-3	34.88	19.20	0.38	0.35	6.28	3.74
22:0	-	-	-	0.03	-	-
22:1	0.02	-	0.635	0.02	-	-
22:2	0.10	-	-	0.04	2.09	-
24:0	0.03	-	-	0.02	-	-
22:6n-3	0.29	-	-	0.02	-	-
others	-	11.30	-	-	-	-
Total	100	100	100	100	100	100
Saturated	28.49	30.10	55.39	35.75	32.76	47.94
Monounsaturated	30.70	29.40	27.19	27.59	46.40	23.09
Polyunsaturated	40.68	29.10	17.42	39.63	20.85	29.98
EPA + DHA	35.17	19.20	0.38	0.37	6.28	3.74

KMMCC, Korea Marine Microalgae Culture Center ; KMMCC-33, *Nannochloropsis* sp.; KMMCC-13, *N. oceanic*; KMMCC-16, *Nannochoris oculata*; KMMCC-119, *Nannochoris* sp.; KMMCC-395, *Nannochoris* sp.; KMMCC-120, *Chlorella vulgaris*; EPA, eicosapentaenoic acid; DHA, docosah`exaenoic acid.

Growth of *Nannochloropsis*, *Nannochloris*, and *Chlorella* in high and low water temperatures

The growth rates of six kinds of *Nannochloropsis*, *Nannochloris*, and *Chlorella* at high (30°C and 32°C) and low (10°C) temperatures are shown in Fig. 4. At 30°C, *Nannochloris* sp. (KMMCC-119) and *N. oculata* showed the highest cell density within 9-10 days of culture at 7,950 × 10^4 cells/mL and 7,951 × 10^4 cells/mL, respectively. The growth rate of *N. oceanica* was 2,991 × 10^4 cells/mL up to the sixth day of culture, but thereafter, the growth rate rapidly decreased. At 32°C, *Nannochloris* spp. (KMMCC-119 and 395) showed the highest cell density among the microalgae at 6,475 × 10^4 cells/mL and 5,932 × 10^4 cells/mL, respectively. In comparison to the other microalgae, however, *N. oceanica* showed a much lower cell density, as it did at 30°C.

The growth rates of *N. oculata* and *Nannochloris* sp. (KMMCC-119) at 30°C were significantly the highest: 0.6313 and 0.6281, respectively. Conversely, *N. oceanica* showed the lowest growth rate: 0.3483 ($P < 0.05$). At 32°C, growth rates significantly differed according to the individual strain. The growth rate of *Nannochloris* sp. (KMMCC-119), 0.6017, was the highest and that of *N. oceanica*, 0.1521, was the lowest. In high water temperatures of 30°C and 32°C, *Nannochloris* had a significantly higher growth rate than *Chlorella* and *Nannochloropsis* ($P < 0.05$).

At 10°C, the growth rate of *C. vulgaris* (KMMCC-120) was significantly the highest at 0.5052 (highest cell density, 3,316 × 10^4 cells/mL) ($P < 0.05$). Growth rates of the other marine microalgae were low, in the range of 0.0109-0.3303 (highest cell density, 107-986 × 10^4 cells/mL). The growth rate of *N. oculata* in particular was significantly the lowest at 0.0109 (highest cell density, 107 × 10^4 cells/mL).

Dietary value of the six kinds of *Nannochloropsis*, *Nannochloris*, and *Chlorella* microalgae as feed for rotifers

The growth rates of rotifers fed on the six kinds of microalgae are shown in Fig. 5. In 5 days of culture, the growth rates of rotifers fed on *Nannochloropsis* sp. (KMMCC-33) and *N. oceanica* were significantly higher than those of the other experimental groups at 0.6806 (highest density, 301 individuals/mL) and 0.6605 (highest density, 272 individual/mL), respectively ($P < 0.05$). *Nannochloris* sp. (KMMCC-395) and

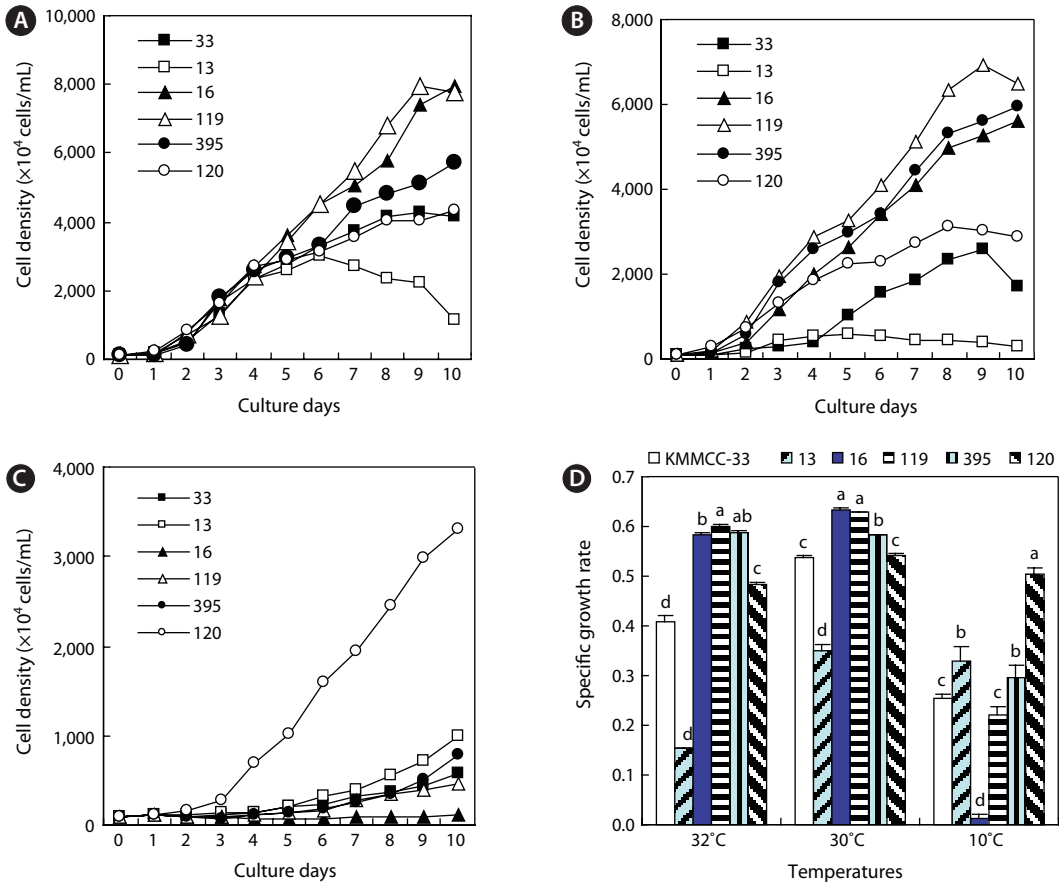

Fig. 4. Cell density of six microalgal species at 30°C (A), 32°C (B) and 10°C (C) and specific growth rate (D) under 15 psu and 100 μmol $m^{-2} s^{-1}$ (KMMCC, Korea Marine Microalgae Culture Center; KMMCC-33, *Nannochloropsis* sp.; KMMCC-13, *N. oceanic*; KMMCC-16, *Nannochoris oculata*; KMMCC-119, *Nannochoris* sp.; KMMCC-395, *Nannochoris* sp.; KMMCC-120, *Chlorella vulgaris*). Different letters on the bar in each temperature mean significantly difference ($P < 0.05$).

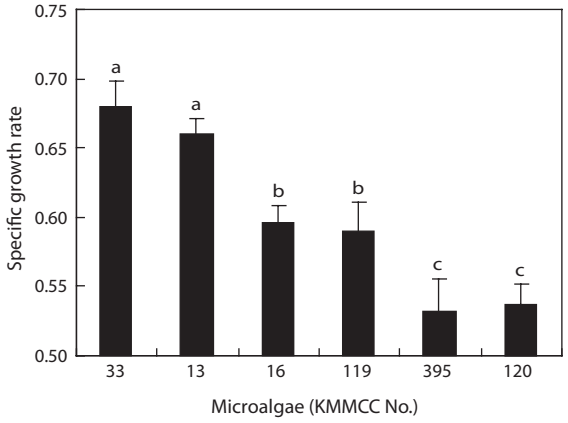

Fig. 5. Specific growth rate of *Brachionus plicatilis* fed different microalgal species (KMMCC, Korea Marine Microalgae Culture Center; KMMCC-33, *Nannochloropsis* sp.; KMMCC-13, *N. oceanic*; KMMCC-16, *Nannochoris oculata*; KMMCC-119, *Nannochoris* sp.; KMMCC-395, *Nannochoris* sp.; KMMCC-120, *Chlorella vulgaris*). Different letters on the bar mean significantly difference ($P < 0.05$).

C. vulgaris (KMMCC-120) showed the lowest growth rates at 0.5332 and 0.5376 ($P < 0.05$), respectively. *Nannochloris* spp. (KMMCC-16 and 119) indicated lower growth rates than *Nannochloropsis* sp. (KMMCC-33) and *N. oceanica*, but higher growth rates than *C. vulgaris* (KMMCC-120) and *Nannochloris* sp. (KMMCC-395) ($P < 0.05$).

Nannochloris sp. (KMMCC-119), with a high growth rate at high temperature, *C. vulgaris* (KMMCC-120), with a high growth rate at low temperature, and *Nannochloropsis* sp. (KMMCC-33), as a nutritious feed for rotifers, were cultured in three separate groups. The amino acid contents of these groups were analyzed (Table 4). The kinds of amino acids in the three experimental groups were similar to each other. The content of total amino acids in rotifers fed on *C. vulgaris* (KMMCC-120) was the highest at 57.15%, and on *Nannochloropsis* sp. (KMMCC-33) was the lowest at 50.54%. Rotifers showed relatively high contents of leucine and lysine among essential amino acids and of glutamine and aspartate among nonessential amino acids.

For the contents of fatty acids in rotifers fed on the afore-

mentioned three kinds of microalgae (Table 5), those fed on *C. vulgaris* (KMMCC-120) contained 41.62% of C18:2n9 compared to 11.0% and 24.60% in *Nannochloropsis* sp. (KMMCC-33) and *Nannochloris* sp. (KMMCC-119), respectively. The contents of C16:0 in all three experimental groups were similar, in the range of 12.63-15.94%. The total content of PUFA in rotifers fed on *C. vulgaris* (KMMCC-120) was the highest at 63.51%. The content of EPA was highest in the *Nannochloropsis* sp. (KMMCC-33) group at 15.27%, and the content of DHA in rotifers fed on *C. vulgaris* (KMMCC-120) was the highest at 9.39%.

Discussion

The growth of rotifers depends on the kind of microalgae used as feed (Hirayama et al., 1979; Cho et al., 2007). Various kinds of microalgae as feed for rotifers have been reported, with *Nannochloropsis*, *Nannochloris*, and *Chlorella*, which are highly nutritious and suitable for high density culture, being the most widely used in mass culture.

One of the obstacles to the mass culture of rotifers comes from difficulties in the outdoor mass culture of microalgae during certain seasons. In summer, sudden cell mortality often occurs, and in the winter, the cell growth rates tend to be very low. Thus, further developing microalgae that are highly adaptive to conditions in the two aforementioned seasons is essential (Watanabe et al., 1978; James and Abu-Rezeq, 1988; Hur, 1991).

This study aimed to identify microalgae among *Nannochloropsis*, *Nannochloris*, and *Chlorella*, which are specifically adaptive to high- and low-temperature seasons in Korea. The optimal salinity for the culture of rotifers was 15 psu (Miracle and Serra, 1989; Kim et al., 2005). Since marine microalgae are also euryhaline, their growth rates were compared at salinities of 15 and 30 psu.

Nine kinds of marine microalgae showed similar growth rates to each other at 15 and 30 psu. Their growth, however, exhibited slight differences according to microalgal type

Table 4. Amino acid composition (%) of *Brachionus plicatilis* fed different microalgal species

Amino acid	KMMCC No.		
	33	119	120
Arginine	3.48	4.16	3.99
Histidine	1.24	1.39	1.37
Isoleucine	2.54	2.86	3.02
Leucine	4.08	4.5	4.83
Lysine	4.09	4.44	4.34
Phenylalanine	3.17	2.95	3.2
Threonine	2.24	2.21	2.59
Valine	3.10	3.58	3.67
Alanine	2.32	2.77	3.13
Aspartic acid	5.47	6.01	5.97
Glutamic acid	7.69	8.03	8.85
Glycine	2.03	2.45	2.57
Proline	3.87	3.85	4.08
Serine	2.68	2.72	3.10
Tyrosine	2.54	2.09	2.44
Total	50.54	54.01	57.15
EAA	23.94	26.09	27.01
NEAA	26.6	27.92	30.14
EAA/NEAA	0.90	0.93	0.90

KMMCC, Korea Marine Microalgae Culture Center ; KMMCC-33, *Nannochloropsis* sp.; KMMCC-119, *Nannochoris* sp.; KMMCC-120, *Chlorella vulgaris*; EAA, essential amino acid; NEAA, non-essential amino acid.

Table 5. Fatty acid composition (%) of *Brachionus plicatilis* fed different microalgal species

Amino acid	KMMCC No.		
	33	119	120
8:0	0.07	0.05	0.05
10:0	0.06	0.03	-
11:0	-	-	-
12:0	0.40	0.14	0.06
13:0	0.47	0.54	0.55
14:0	4.49	2.94	2.27
14:1	0.40	0.85	-
15:0	0.56	0.56	-
15:1	-	0.17	-
16:0	14.32	15.94	12.63
16:1	16.14	9.26	8.35
17:0	0.49	0.47	0.51
17:1	0.80	0.63	0.26
18:0	3.06	3.29	2.98
18:1n9	1.85	1.38	-
18:2n9	11.0	24.60	41.62
18:3n6	3.96	7.92	4.16
18:3n9	0.81	0.18	-
20:0	-	7.38	0.68
20:1	0.77	2.08	3.75
20:2	1.36	2.08	2.43
20:3	0.55	0.83	0.18
20:4	-	1.26	0.12
21:0	3.67	2.39	1.13
20:5n-3	15.27	9.27	5.61
22:0	-	0.37	0.30
22:1	-	0.92	1.49
22:2	11.12	-	-
24:0	5.11	3.14	1.51
22:6n-3	3.29	0.80	9.39
Total	100	100	100
Saturated	32.70	37.24	22.67
Monounsaturated	19.96	15.29	13.85
Polyunsaturated	47.36	46.94	63.51
EPA + DHA	18.56	10.07	15.00

KMMCC, Korea Marine Microalgae Culture Center ; KMMCC-33, *Nannochloropsis* sp.; KMMCC-119, *Nannochoris* sp.; KMMCC-120, *Chlorella vulgaris*; vulgaris; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

and salinity. Three estuarine microalgae also showed similar growth rates at 15 and 30 psu. Based on these results, the 12 kinds of microalgae studied in this research can be inferred to be suitable as feed for rotifers because they were euryhaline.

The growth rates of the six kinds of microalgae cultured at 30°C and 32°C for the high-temperature experiments were highest for *Nannochloris* and lowest for *Nannochloropsis*. The growth rate of *Chlorella* was much lower than that of *Nannochloris*, but higher than that of *Nannochloropsis*. In terms of the highest cell density at 30°C and 32°C, *Nannochloris* showed a 25% decrease in growth rate, while *Chlorella* and *Nannochloropsis* exhibited 40% and 35% decreases in their growth rates, respectively. These results highlight the fact that the two aforementioned microalgae are more prone than *Nannochloris* to mortality at temperature levels over 30°C.

Among the three kinds of *Nannochloris*, the growth rates of *Nannochloris* sp. (KMMCC-119) collected at Deukryang Bay and imported *N. oculata* (UTEX, 1998) were the highest at 30°C. The growth rate of *Nannochloris* sp. (KMMCC-395) collected at Puan was significantly lower ($P < 0.05$). At 32°C, however, the growth rate of *Nannochloris* sp. (KMMCC-119) was the highest and that of *N. oculata* was the lowest. *Nannochloris* sp. (KMMCC-395) showed similar growth at both 32°C and 30°C. Such growth traits can be explained by *Nannochloris* sp. (KMMCC-395) being adaptive to higher temperatures, as it originated from salt ponds in Puan.

At 10°C, for the low-temperature experiment, *N. oculata* and *Nannochloris* sp. (KMMCC-119), which were highly vital at high temperature, exhibited the lowest growth rate. However, the growth of *C. vulgaris* (KMMCC-120), which was isolated from brackish water, was significantly higher than those of the other microalgae, which were from marine water ($P < 0.05$). Thus, it is considered suitable for mass culture in low-temperature seasons.

At high temperature, *N. oculata* was found to have a higher growth rate than *Chlorella ellipsoidea* or *Nannochloropsis salina* (James et al., 1989; Hur, 1991). *Phaeodactylum tricornutum*, which belongs to the Bacillariophyceae, shows a higher growth rate than *C. ellipsoidea* at low temperature. However, it is also reported to be inadequate as a rotifer feed because its dietary value is lower than that of *C. ellipsoidea* (Hur, 1991; Cho et al., 2007).

The essential amino acid contents of most microalgae are influenced by factors including the intensity of lighting (Thompson et al., 1990; Brown et al., 1997), temperature (James et al., 1989; Thompson et al., 1992), pH (Guckert and Cooksey, 1990), culture medium (Wikfors et al., 1984), and harvesting times (Brown et al., 1997; Pernet et al., 2003). In this study, the fatty acid contents of the two kinds of *Nannochloropsis*, 16:0 and 16:1, were high, which is consistent with reports by Hodgson et al. (1991) and Volkman et al. (1993). Whyte and Nagata (1990) reported that the main components of fatty acids in the marine *Chlorella saccharophila* were 16:0, 16:1n7, and 18:1n9. However, estuarine *C. vulgaris* (KMMCC-120) differed from marine *C. saccharophila* in its main component of fatty acids 17:1 and 20:0.

With regard to EPA and DHA, Volkman et al. (1993) reported that *Nannochloropsis* sp. cultured at 20°C contained no DHA and 16.1-28.2% EPA. The result of the present study, on microalgae cultured at 25°C, slightly differs from that of Volkman et al. (1993). Conversely, the report of James et al. (1989) on *Nannochloropsis* cultured at 25°C containing 0.4% of DHA is similar to the result of the present study.

The fatty acid contents of rotifers fed on *Nannochloropsis* sp. (KMMCC-33), *Nannochloris* sp. (KMMCC-119), and *C. vulgaris* (KMMCC-120) were 16:0, 16:1, and 18:2, respectively, which were similar to the contents of fatty acids in the aforementioned microalgae. The growth rate of rotifers fed on *Nannochloropsis* sp. (KMMCC-33), which had the highest EPA content, was high and its content of EPA was also high. Thus, the nutritional content of a feed can be concluded to directly affect the rotifer (Scott and Middleton, 1979; Ben-Amotz et al., 1987; Frolov et al., 1991).

Nannochloris spp. (KMMCC-119 and 395), isolated from Korean coastal waters, showed higher growth rates at 30°C and 32°C than the foreign species *N. oculata* (UTEX, 1998). Estuarine *C. vulgaris* (KMMCC-120) showed a high growth rate at 10°C, at which temperature most microalgae hardly survived. However, their effectiveness as rotifer feed was lower than that of *Nannochloropsis* because of their low EPA and DHA contents.

In conclusion, *Nannochloropsis* sp. (KMMCC-33) is the best choice for the mass culture of rotifers. *Nannochloris* spp. (KMMCC-119 and 395) and estuarine *C. vulgaris* (KMMCC-120) seem the most adequate species to replace *Nannochloropsis* sp. (KMMCC-33) during high- and low-temperature seasons, respectively.

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