J Appl Phycol (2017) 29:2789–2800
DOI 10.1007/s10811-017-1167-6

Lipid accumulation and anti-rotifer robustness of microalgal strains isolated from Eastern China

Cheng Yuan[1] · Yan-Lin Zheng[2,3] · Wan-Lu Zhang[1] · Ru He[1] · Yong Fan[1] · Guang-Rong Hu[1] · Fu-Li Li[1]

Received: 2 February 2017 / Revised and accepted: 9 May 2017 / Published online: 30 May 2017

Abstract Large-scale production of biofuels and other high-value products from microalgae focuses on strain selection with desired characteristics such as high lipid productivity and robustness. However, microalgae often suffer severe biomass losses due to contamination that comes in the form of herbivores, especially rotifers. In this study, we found that the total lipid content of 54 microalgal strains from Eastern China ranged from 12.6 to 59.9% of dry cell weight (dw), with an average of 35.4% dw. Lipid productivity of these microalgal strains varied from 0.04 to 0.14 g L^{-1} day^{-1}, and 15 showed lipid production rates over 0.10 g L^{-1} day^{-1}. The fatty acid profiles of 34 microalgal strains with lipid content over 40% dw were analyzed by gas chromatography. We found that the amount of C18:1 increased with increasing total lipid content, whereas C14:0 and C16:0 showed a negative correlation to the total amount of lipids. An experimental rotifer-microalgae predator-prey model was constructed, and the anti-rotifer robustness of these microalgal strains was evaluated. We found that *Didymogenes* sp. HN-4 can depress the growth of rotifers significantly. This study demonstrates the biodiversity of the native Chinese microalgae in lipid productivity and robustness, which lays the groundwork for mass algal culture.

Keywords Biodiesel · Microalgae · Lipid production · Fatty acid profile · Anti-rotifer

Cheng Yuan and Yan-Lin Zheng are contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s10811-017-1167-6) contains supplementary material, which is available to authorized users.

✉ Guang-Rong Hu
hugr@qibebt.ac.cn

✉ Fu-Li Li
lifl@qibebt.ac.cn

[1] Key Laboratory of Biofuels, Shandong Provincial Key Laboratory of Energy Genetics, and Qingdao Engineering Laboratory of Single Cell Oil, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, No.189, Song Ling Road, Qingdao 266101, People's Republic of China

[2] College of Mathematics and Systems Science, Shandong University of Science and Technology, Qingdao 266590, People's Republic of China

[3] College of Electrical Engineering and Automation, Shandong University of Science and Technology, Qingdao 266590, People's Republic of China

Introduction

Increasing energy demands and decreasing reserves of fossil fuels are becoming some of the most urgent challenges facing humanity (Vasudevan and Briggs 2008; Xin et al. 2010). Therefore, developing renewable energy is an important worldwide energy policy (Najafi et al. 2011). Recently, renewable fuels such as biodiesel and bioethanol have attracted attention. Biodiesel derived from animal and terrestrial plant triacylglycerols (TAGs) can be used directly by existing diesel engines, but their yield is not enough to meet energy demands (Chisti 2008; Hu et al. 2015). Microalgae have a simple cellular structure, have high photosynthetic efficiency and lipid content, and are abundant in aquatic and terrestrial environments throughout the world. Some species of microalgae can produce large quantities of lipids as a storage product, regularly achieving 50 to 60% of their dry cell weight (dw) (Griffiths and Harrison 2009). Microalgae have a higher biomass and lipid productivity than conventional terrestrial crops and can grow in wastewater (Hu et al. 2013; Fon Sing et al. 2013; Mar

et al. 2016). Furthermore, microalgae can fix carbon from CO_2-rich flue gas emissions and some produce valuable co-products (de Morais and Costa 2007; Chiu et al. 2009; Saadaoui et al. 2016).

The economic feasibility of microalgae cultivation greatly depends on strain selection, photo-bioreactor design, and culture strategies for mass production (U.S. DOE (2010)). It is prudent to select strains from the local environment for culture in local conditions (Jimenez-Perez et al. 2004; Mata et al. 2010). High biomass productivity is important both for lipid productivity and the ability to compete with contaminating strains (Fig. 1). A high lipid content can reduce the cost of extraction and purification per unit of product (Borowitzka 1992). Other characteristics such as cell size, tolerance to CO_2, heterotrophic or mixotrophic growth capabilities, lipid class and fatty acid composition, and types of co-products produced can also influence the cost of microalgal biodiesel production in mass-scale cultures (Grobbelaar 2000; Liu et al. 2012; Borowitzka 2013; Guo et al. 2016;).

On the other hand, when cultivated in outdoor mass culture systems, e.g., raceway ponds or panels, microalgae often suffer severe biomass losses due to contamination with herbivores (i.e., cladocerans, copepods, and rotifers), bacteria, fungi, viruses, and competing microalgae (Smith and Crews 2014). Among these, rotifers can destroy microalgae cells in as little as one night by directly eating microalgae cells or causing them to congregate and precipitate (Yoshida et al. 2004). Over the past few decades, rotifers have been thought to be an excellent eukaryotic model organism for the study of the eco-evolutionary dynamics between zooplankton and phytoplankton (Meng et al. 2014, 2015; Meng and Wang 2016). Recently, with the increasing interest in oleaginous microalgae for biofuel production, rotifer contamination has attracted increasing attention. To date, several pesticides extracted from plants have been used to control rotifer blooms in algae cultivation systems (Huang et al. 2014a, 2014b, 2017), but microalgal resistance to rotifers has not been reported. Therefore, it is necessary to investigate microalgal robustness, especially in terms of the ability to resist rotifers (Day et al. 2012), taking the outdoor environment for producing microalgal biofuel in mass culture systems into account (Stephens et al. 2010). Recently, an ecology-based strategy has been proposed, for example, by growing in closed photo-bioreactor or at extreme condition to avoid contamination (Mooij et al. 2015). In order to evaluate the biodiversity of microalgae in terms of lipid productivity and anti-rotifer robustness for biodiesel production in China, 54 microalgal strains were isolated from Eastern China, their lipid productivities and fatty acid profiles were measured, and the anti-rotifer robustness of these microalgal strains was evaluated. New native microalgal species with high lipid productivity for biodiesel production were screened for

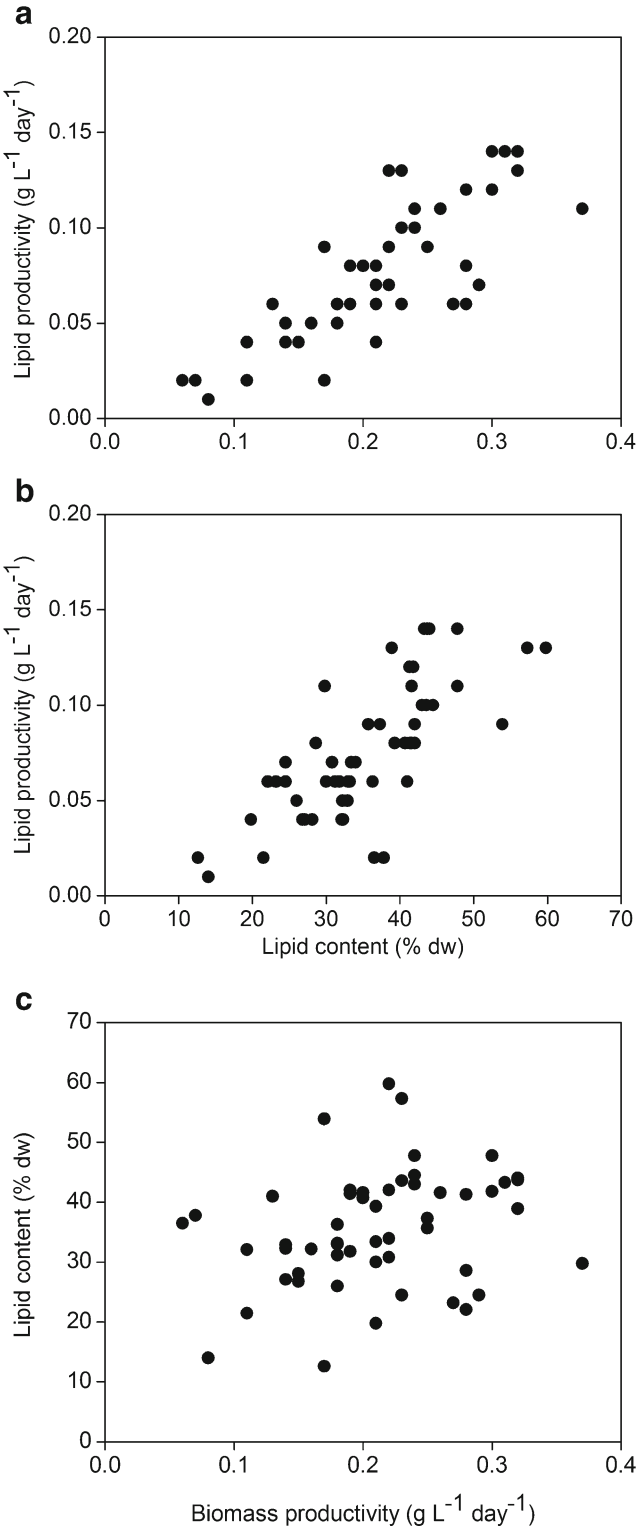

Fig. 1 Correlation analysis of the relationship between lipid productivity and biomass productivity (**a**), lipid productivity and lipid content (**b**), and the interaction of lipid content and biomass productivity (**c**)

future large-scale cultivation by artificial complex algal communities.

Materials and methods

Strains and culture conditions

Microalgal strains were isolated from various bodies of water including ponds and rivers in Shandong Province, China, from 2008 to 2010. The strains were identified by their 18S rRNA genes or *ITS* sequence and by morphology. The accession numbers for the 18S rRNA genes or *ITS* genes of the strains reported in this study are KF689546 to KF689592 (Table S1). The strains were cultured in BG11 medium (Rippka et al. 1979) or TAP medium (Gorman and Levine 1965) and were maintained at 25 ± 1 °C under continuous illumination provided by daylight fluorescent tubes at 70–80 μmol PAR photons m^{-2} s^{-1}. The nervonic acid-producing *Mychonaste afer* HSO-3-1 and anti-rotifer *Didymogenes* sp. HN-4 have been preserved in the China General Microbiological Culture Collection Center (*CGMCC*) with the identifying codes CGMCC No. 4654 and CGMCC No. 8401, respectively.

Cultivation and induction of lipid synthesis in bubbled-columns

Microalgae cultivated in log-phase growth in Erlenmeyer flasks were transferred to 500-mL glass bubbled-columns (4.1 cm in diameter) containing 250 mL of BG11 medium with 4.4 mM $NaNO_3$ at 1:6 (*v*/*v*) dilution to keep the original OD_{750} value around 0.2. The columns were maintained at 25 ± 1 °C and were bubbled with a sterile gas comprised of air supplemented with 2% (*v*/*v*) CO_2. Continuous artificial illumination of 300 ± 20 μmol PAR photons m^{-2} s^{-1} was provided by daylight fluorescent tubes on one side. The cells were harvested after 13 days. All experiments were performed in duplicate.

The optical density of the cultures was determined by OD_{750} using a UV/VIS spectrophotometer (2600, UNICO, China). Culture growth was also estimated by measuring the dw. Five milliliter of algal suspension was sampled every 12 h and filtered through a pre-weighed 0.8-μm microporous filter paper. The filter paper was oven-dried overnight at 105 °C. The difference between the final weight and the weight before filtration was the dw.

Lipid extraction and fatty acid analysis

Microalgal cells were harvested by centrifugation at 8000 rpm for 10 min. The cell pellets were then lyophilized and pulverized. The total lipids of the algal cells were extracted with a modified chloroform–methanol–water system (Bligh and Dyer 1959). In a 10-mL tube, 30–40 mg dry algae powder, 4 mL chloroform, and 2 mL methanol were added and shaken for 10 s to dissolve the algae powder. The tube was incubated at 200 rpm for 12 h at 30 °C and was then centrifuged for 10 min at 3500 rpm. Then 6 mL of supernatant was collected and transferred to another tube and 2 mL of methanol and 3.6 mL deionized water were added to give a final ratio of chloroform–methanol–water of 10:10:9. The tube was centrifuged at 3500 rpm for 5 min, and the chloroform layer was transferred into a pre-weighed tube (w_1) and dried for 30 min under N_2 at 60 °C. Then, it was dried under a vacuum (Lantian DZF-6050, Hangzhou, China) for 1.5 h at 60 °C and was weighed (w_2). The lipid weight was calculated by subtracting w_1 from w_2.

Methyl esters from the microalgae lipids were formed by 2% H_2SO_4-methanol solution at 85 °C for 2.5 h. An analysis of fatty acid methyl esters was performed with a gas chromatograph (Varian 450-GC, Varian, USA) using a fused column (CP-WAX58, 25 m × 0.25 mm). The flow of carried gas was pure nitrogen, and 1 μL methyl ester sample solution was injected for analysis. The split ratio was 1:30. The column temperature was maintained at 100 °C for 2 min, elevated to 250 °C at a rate of 10 °C min^{-1}, and maintained for a further 3 min. The injector temperature was set at 250 °C. The flame-ionization detector temperature was set at 280 °C. Fatty acids were identified by comparing the retention time obtained to those of known fatty acid methyl ester mixtures of C:8 to C:24 standards from Sigma-Aldrich, USA.

The anti-rotifer ability of different microalgae strains

The *Rotaria* species used in study was collected from an open pond of microalgal culture system in Pingdu, Qingdao, China. It belongs to the bdelloid rotifers and is an asexual oviparous microorganism (Fontaneto et al. 2007). Their eggs are oval with a diameter of ~10 μm, and the adults are 100–300 μm long (Fig. 5a). A typical *Rotaria* sp. has a brain, a stomach, and an excretory system, but no circulatory system. *Rotaria* prey on microalgae cells by beating cilia, which create water currents that bring the microalgae cells into the rotifer mouth.

Anti-rotifer experiments testing microalgal resistance to rotifers were conducted in a 24-well microplate with 2.0–2.2 mL microalgae solution in each well (the initial density of the microalgae strains was about 3.0×10^6 cells mL^{-1}, $n = 3$ wells per strain), and the rotifers were dispensed into the wells (the initial rotifer density was 20–30 rotifers mL^{-1}). Afterwards, the resulting microalgal strains with anti-rotifer

Table 1 Lipid content, biomass and lipid productivity of microalgal strains isolated from different sites in East China

Species	Biomass yield ($g\ L^{-1}$)[a]	Biomass productivity ($g\ L^{-1}\ day^{-1}$)	Lipid content (% dw)[a]	Lipid productivity ($g\ L^{-1}\ day^{-1}$)
Chlamydomonas sp. XZL-4	1.49 ± 0.02	0.11	32.1 ± 1.50	0.04
Chlamydomonas sp. YQJ-1	0.81 ± 0.34	0.06	36.5 ± 1.89	0.02
Chlorella sp. HSO-2-3	1.93 ± 0.11	0.15	26.8 ± 0.00	0.04
Chlorella sp. SGH-6	0.86 ± 0.00	0.07	37.8 ± 1.10	0.02
Chlorella sp. XJH-1-1	2.84 ± 0.12	0.22	42.0 ± 1.73	0.09
Chlorella sp. DMY-2-2	2.99 ± 0.05	0.23	57.3 ± 6.13	0.13
Chlorella sp. DXH-2-1	1.84 ± 0.06	0.14	27.1 ± 5.44	0.04
Chlorella sp. QQ-4-1	1.47 ± 0.00	0.15	28.1 ± 0.12	0.04
Chlorella sp. RZ-2	0.77 ± 0.44	0.08	14.0 ± 4.42	0.01
Chlorella sp. SYSK-4-1	3.06 ± 0.08	0.24	44.5 ± 0.59	0.10
Chlorella sp. SZK-3	2.31 ± 0.06	0.18	33.0 ± 0.64	0.06
Chlorella sp. XFZ-1-1	3.27 ± 0.10	0.25	37.3 ± 1.78	0.09
Chlorella sp. XZL-1	2.53 ± 0.00	0.21	30.0 ± 0.17	0.06
Chlorella sp. ZSLD-2-1	2.34 ± 0.16	0.18	31.2 ± 2.43	0.06
Desmodesmus sp. DZL-4	2.05 ± 0.39	0.16	32.2 ± 1.65	0.05
Desmodesmus sp. QXY-6-3	3.75 ± 0.44	0.29	24.5 ± 0.96	0.07
Desmodesmus sp. RZ-5	3.46 ± 0.12	0.27	23.2 ± 3.53	0.06
Desmodesmus sp. BSH-4-1	3.03 ± 0.02	0.23	43.6 ± 0.73	0.10
Desmodesmus sp. DW-4-1	2.93 ± 0.12	0.21	19.8 ± 2.17	0.04
Desmodesmus sp. IR-7	3.65 ± 0.01	0.28	41.3 ± 1.65	0.12
Desmodesmus sp. NCP-1-2	2.71 ± 0.13	0.23	24.5 ± 2.85	0.06
Desmodesmus sp. QQ-1	2.81 ± 0.91	0.22	30.8 ± 1.14	0.07
Desmodesmus sp. SC-2-2	2.99 ± 0.21	0.25	35.7 ± 1.73	0.09
Desmodesmus sp. SGH-3	3.93 ± 0.00	0.30	47.8 ± 0.48	0.14
Desmodesmus sp. XFZ-4-1	4.09 ± 0.19	0.32	44.0 ± 1.12	0.14
Desmodesmus sp. XGR-1-1	2.65 ± 0.30	0.20	41.6 ± 0.05	0.08
Desmodesmus sp. XJH-2-2	2.32 ± 0.25	0.18	36.3 ± 1.69	0.06
Micractinium sp. HSD-2-1	2.26 ± 0.15	0.17	12.6 ± 2.04	0.02
Micractinium sp. IR-4	1.45 ± 0.40	0.11	21.5 ± 2.17	0.02
Micractinium sp. DZL-5-1	2.53 ± 0.20	0.19	41.4 ± 2.06	0.08
Micractinium sp. ZSLT-11–2	1.78 ± 0.27	0.14	32.3 ± 1.82	0.04
Mychonastes afer HSO-3-1	2.23 ± 0.17	0.17	53.9 ± 0.49	0.09
M. homosphaera XFZ-2-1	2.54 ± 0.01	0.20	40.7 ± 0.59	0.08
Scenedesmus abundans BZ-3-3	2.81 ± 0.54	0.22	34.0 ± 5.52	0.07
Scenedesmus obliquus HSD-3-1	2.46 ± 0.12	0.19	31.8 ± 1.13	0.06
Scenedesmus obliquus LSSK-4-1	1.74 ± 0.06	0.13	41.0 ± 3.39	0.06
Scenedesmus obliquus RZ-4-1	3.87 ± 0.00	0.30	41.8 ± 5.12	0.12
Scenedesmus obliquus SC-2-1	4.20 ± 0.07	0.32	38.9 ± 0.17	0.13
Scenedesmus obliquus SGH-5	3.92 ± 0.36	0.28	28.6 ± 1.20	0.08
Scenedesmus obliquus XL-4-2	3.16 ± 0.04	0.24	43.0 ± 0.89	0.10
Scenedesmus obliquus ZSLC-11–1	2.34 ± 0.54	0.18	33.2 ± 2.05	0.06
Scenedesmus sp. ASU	2.45 ± 0.11	0.19	42.0 ± 0.56	0.08
Scenedesmus sp. HSD-4-1	1.85 ± 0.17	0.14	32.9 ± 2.75	0.05
Scenedesmus sp. HSD-5-3	3.07 ± 0.10	0.24	47.8 ± 1.69	0.11
Scenedesmus sp. KTX-2	4.84 ± 0.07	0.37	29.8 ± 2.41	0.11
Scenedesmus sp. NCP-2-1	3.42 ± 0.13	0.26	41.6 ± 2.85	0.11
Scenedesmus sp. BZ-2-2	2.76 ± 0.79	0.21	33.4 ± 3.44	0.07
Scenedesmus obliquus DHD-3	4.06 ± 0.10	0.31	43.3 ± 3.39	0.14

Table 1 (continued)

Species	Biomass yield ($g\ L^{-1}$)[a]	Biomass productivity ($g\ L^{-1}\ day^{-1}$)	Lipid content (% dw)[a]	Lipid productivity ($g\ L^{-1}\ day^{-1}$)
Scenedesmus sp. HD-5	2.69 ± 0.16	0.21	39.3 ± 2.09	0.08
Scenedesmus sp. RZ-1-1	4.12 ± 0.02	0.32	43.7 ± 3.80	0.14
Scenedesmus sp. SGH-5-1	2.51 ± 0.42	0.18	26.0 ± 1.06	0.05
Scenedesmus sp. ZSLT-8-2	3.66 ± 0.01	0.28	22.1 ± 0.18	0.06
Selenastrum sp. XL-3-3	2.87 ± 0.02	0.22	59.8 ± 3.80	0.13
Didymogenes sp. HN-4	4.44 ± 0.25	0.28	46.4 ± 3.18	0.13

[a] All experiments were conducted at least three times

abilities were subjected to an anti-rotifer assay in flasks. The density of microalgal cells and rotifers for each well was checked every 2 days during the culture period of 8 days. Microalgal cell density was determined with globulimeters, and rotifer density was counted using a plankton counter. The rotifer morphology was observed and recorded with an inverted microscope (Olympus IX51, Japan). The intrinsic rate of increase (γ) of the rotifer populations was calculated with the following formula (Schalk et al. 2007; Song et al. 2009; Zhang et al. 2013;): $\gamma = (\ln N_t - \ln N_0)/T$, where T is the duration of the assay in days and N_0 and N_t are the rotifer densities at the initial day and the eighth day, respectively.

After cultivation for 6 days in TAP medium, *Didymogenes* sp. HN-4 cells were harvested by centrifugation, and the supernatant was collected. Afterwards, the supernatant was filtered through a 0.45-μm microporous filter paper to remove microalgae cells. Rotifers (*Rataria* sp.) and *Chlorella* sp. XZL-1 (included as rotifer food) were co-cultured in the cell-free filtrate of *Didymogenes* sp. HN-4, and the population density of the rotifers was recorded every 2 days.

Statistical analysis

Two-tailed paired t tests were applied to ascertain significant differences using SPSS 16.0, and the level of statistical significance was set at $P < 0.05$.

Results

Lipid content and algae biomass of 54 microalgal strains

The lipid content and biomass of 54 microalgal strains were determined under laboratory conditions. These microalgae were isolated from different bodies of water such as ponds and rivers from East China (Fig. S1, Table S1). The lipid content of the microalgal strains ranged from 12.6 to 59.9% dw, with an average of 35.4% dw. Forty-one microalgal strains had a lipid content over 30% under the culture conditions (Table 1). *Selenastrum* sp. XL-3-3 had the highest lipid content of 59.8% dw. Eighteen microalgal strains had a biomass yield over 3 $g\ L^{-1}$ (Table 1). The maximum dw biomass yield of these green microalgae was 4.84 $g\ L^{-1}$. The maximum dw biomass productivity of these green microalgae was 0.37 $g\ L^{-1}\ day^{-1}$. Fifteen microalgal strains had lipid production rates over 0.10 $g\ L^{-1}\ day^{-1}$.

Differences in lipid content and biomass between microalgal species

The average lipid content of 5 microalgal species including 49 strains isolated from different sites is summarized in Fig. 2. Different microalgal species showed significant differences in lipid content and biomass. The

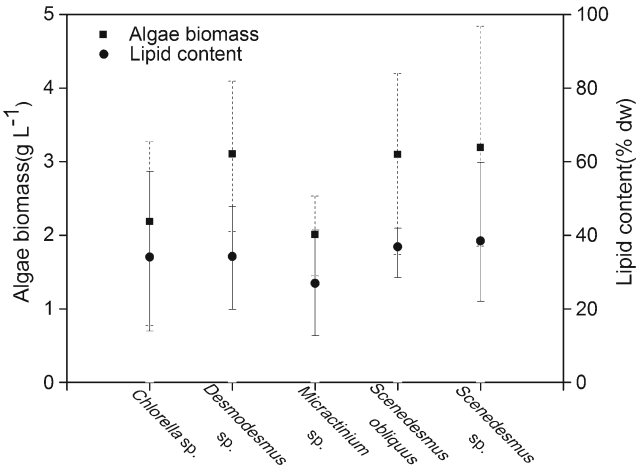

Fig. 2 Mean lipid content and microalgae biomass for 5 species isolated from different sites and cultured under controlled environmental conditions. The cultures were initially supplied with 4.4 mM $NaNO_3$ and were harvested at the stationary phase. There were at least three replicates for every isolate. The *error bars* show the minimum and maximum recorded values for each species

highest lipid content in these microalgal species was *Scenedesmus* sp., which had an average lipid content of 38.4% dw. The lipid content of the other four species including *Micractinium* sp., *Chlorella* sp., *Desmodesmus* sp., and *Scenedesmus obliquus* ranged from 12.6 to 41.4, 14.0 to 57.3, 19.8 to 47.8, and 28.6 to 41.8% dw, respectively. This variability showed that there were differences in the lipid content in different strains even within the same algal species. Thus, in future studies, it is necessary to test the lipid content and fatty acid profile of each additional or novel isolate.

Desmodesmus sp. and *Scenedesmus* sp. isolated from different sites had high average algae biomass yields of 3.10 and 3.19 g L^{-1}, respectively. The algal biomass yields of *Desmodesmus* sp. ranged from 2.32 to 4.09 and from 1.85 to 4.84 g L^{-1} for *Scenedesmus* sp. *Micractinium* sp. had lower algal biomass yields from 1.45 to 2.53 g L^{-1}, with an average biomass yield of 2.01 g L^{-1}. *Scenedesmus* sp. KTX-2, isolated from a pond, had the highest biomass yield of 4.84 g L^{-1}.

Studies have reported the lipid content and biomass productivity of numerous microalgal species (Chisti 2007; Griffiths and Harrison 2009; Mata et al. 2010). Mata et al. (2010) suggested that *Chlorella* may be a good option for biodiesel production. In our research, the average lipid content of *Chlorella* sp. (34.1% dw) was similar to that of other species, but the average biomass yield (2.18 g L^{-1}) was lower. However, *Scenedesmus* spp. might be a good option due to its higher average lipid content (38.4% dw) and biomass yield (3.19 g L^{-1}). Only taking the lipid content and biomass yield into consideration, *Scenedesmus* sp. KYX used in the present study has the potential to be one of the best candidates for future biofuel production. Nevertheless, whether a microalgal strain should be selected as a promising feedstock for biodiesel production also depends on other factors, such as its growth rate in the outdoor environment and its growth ability under specific environmental conditions.

Maximum specific growth rate and doubling time

The specific growth rate and doubling time of 12 microalgal strains with a lipid content over 40% dw were determined (Table 2). The doubling time of these green algae was 6 to 31 h, with an average of 16 h. Griffiths and Harrison (2009) surveyed the literature and reported that the average doubling time for freshwater algae is 20 h. Rapid growth is important both for overall productivity and the ability to compete with contaminating strains (U.S. DOE (2010)). In this study, *S. obliquus* RZ-4-1 and XL-4-2 had the shortest doubling times of 6.3 and 6.2 h, respectively. The doubling time of *Selensatrum* sp. XL-3-3 was 9.3 h. It should be noted that these three microalgae were all isolated from ponds. *Selensatrum* sp. XL-3-3 and *S. obliquus* XL-4-2 were isolated from Qingdao, Shandong, in August 2009 and *S. obliquus* RZ-4-1 was isolated from Rizhao, Shandong, in December 2009. *Desmodesmus* sp. XFZ-4-1 had a long doubling time of 31.4 h.

The average doubling time of *S. obliquus* was the shortest among these isolates, and the average lipid content and biomass yield of *S. obliquus* were 36.9% and 3.09 g L^{-1} dw, respectively (Fig. 2). The short doubling time, high average lipid content, and biomass productivity of *S. obliquus* made it

Table 2 Maximum specific growth rate (μ_{max}, h^{-1}), lipid content (% dw), lipid productivity (g L^{-1} day^{-1}), and doubling time (T_d, h) for 13 microalgal strains with lipid content over 40% dw in bubbled-column bioreactor

Algae strains	Lipid content (% dw)[a]	Lipid productivity (g L^{-1} day^{-1})	μ_{max} (h^{-1})	T_d (h)
Desmodesmus sp. IR-7	41.3 ± 1.65	0.12	0.066	12.27
Chlorella sp. XJH-1-1	42.0 ± 1.73	0.09	0.082	17.83
Micractinium sp. DZL-5-1	38.4 ± 2.06	0.16	0.069	16.81
Desmodesmus sp. XGR-1-1	41.6 ± 0.05	0.08	0.040	16.81
Desmodesmus sp. XFZ-4-1	44.0 ± 1.12	0.14	0.053	31.35
Scenedesmus sp. HSD-5-3	47.8 ± 0.10	0.11	0.072	13.87
Scenedesmus obliquus RZ-4-1	41.8 ± 5.12	0.12	0.159	6.27
Desmodesmus sp. BSH-4-1	43.6 ± 0.02	0.10	0.037	27.36
Selenastrum sp. XL-3-3	59.8 ± 0.02	0.13	0.107	9.31
Scenedesmus obliquus XL-4-2	43.0 ± 0.89	0.10	0.162	6.16
Scenedesmus obliquus DHD-3	43.3 ± 0.10	0.14	0.060	16.62
Mychonastes afer HSO-3-1	53.9 ± 0.49	0.09	0.077	12.93
Chlorella sp. DMY-2-2	57.3 ± 6.13	0.13	0.053	18.89

[a] All experiments were conducted at least three times

a good option for further mass-scale cultivation in outdoor environments.

Fatty acid composition

The fatty acid compositions of the 34 microalgal strains with a lipid content over 30% dw are shown in Table 3 (Fig. S2). The major component of the fatty acid composition was C18:1, which accounted for more than half of the total fatty acid content in 14 strains. The second major fatty acid was C16:0. In all species tested, C14:0 to C20:0 were detected. C22:0 was detected in most of the samples except *Chlorella* sp. XZL-1, *Micractinium* sp. ZSLT-11-2, *Chlorella* sp. XJH-1-1, *S. obliquus* SGH-5, and *Desmodesmus* sp. IR-7. C24:0 was only detected in 12 microalgal strains.

The C18:1, C16:0, and C14:0 content showed significant correlations with the total amount of lipids ($P < 0.05$) (Fig. 3). According to positive Pearson correlation analysis, we found that the amount of C18:1

Table 3 Fatty acid methyl esters (FAMEs) and fatty acid compositions of 34 microalgal strains with the lipid content over 30% dw

Algal strains	FAMEs yield (mg g^{-1})	Fatty acid composition (relative content %)										
		14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	22:0	22:1	24:0
Scenedesmus obliquus XL-4-2	607	0.06	18.9	1.10	5.00	51.7	13.21	9.49	0.26	0.18	0.13	ND
Desmodesmus sp. XGR-1-1	433	0.07	26.2	1.04	10.88	42.3	7.31	10.50	1.61	0.19	ND	ND
Scenedesmus sp. HSD-5-3	521	0.07	21.0	0.10	5.42	61.7	8.70	2.57	0.15	0.11	0.17	ND
Chlorella sp. DMY-2-2	586	0.08	17.2	0.47	4.23	55.8	9.95	11.92	0.17	0.08	0.08	ND
Scenedesmus sp. RZ-1-1	417	0.08	20.0	1.16	7.17	56.1	9.89	4.52	0.59	0.25	0.27	ND
Scenedesmus obliquus LSSK-4-1	557	0.09	13.1	0.31	3.42	65.7	10.58	4.77	0.32	0.56	0.46	0.70
Micractinium sp. DZL-5-1	613	0.10	18.4	1.02	4.27	34.8	8.13	2.19	0.23	0.07	0.11	ND
Scenedesmus obliquus RZ-4-1	427	0.10	19.6	1.34	5.49	50.7	14.95	6.91	0.45	0.21	0.23	ND
Desmodesmus sp. BSH-4-1	591	0.11	30.6	2.84	5.18	47.8	7.56	4.59	0.49	0.27	0.25	0.30
Chlorella sp. XFZ-1-1	451	0.57	27.1	3.71	2.22	58.7	33.41	14.02	0.62	0.57	0.22	ND
Desmodesmus sp. XFZ-4-1	369	0.13	18.3	1.24	5.75	52.0	14.99	6.60	0.53	0.21	0.22	ND
Scenedesmus sp. DHD-3	419	0.13	19.3	2.44	3.59	53.4	12.17	7.76	0.59	0.18	0.47	ND
Chlorella sp. SYSK-4-1	451	0.14	26.8	1.07	10.31	35.8	9.47	14.75	1.38	0.13	0.08	0.09
Mychonastes afer HSO-3-1	221	0.16	14.4	5.74	5.36	62.6	5.41	4.61	0.25	0.19	0.69	0.54
Scenedesmus obliquus SC-2-1	339	0.17	26.3	1.35	4.81	50.1	14.22	4.23	0.15	0.17	0.08	0.05
Desmodesmus sp. XJH-2-2	404	0.30	33.0	1.94	6.79	45.8	4.23	6.60	0.90	0.18	ND	0.19
Scenedesmus abundans BZ-3-3	543	0.19	36.9	1.41	6.82	41.7	7.06	5.13	0.66	0.15	ND	ND
Selenastrum sp. XL-3-3	275	0.20	17.8	0.13	5.30	63.9	5.02	5.88	0.19	0.28	0.22	1.08
Scenedesmus obliquus ZSLC-11-1	770	0.22	22.4	1.31	3.92	52.0	11.81	7.90	0.20	0.10	0.11	ND
Scenedesmus sp. HD-5	341	0.24	36.1	1.27	5.74	41.8	9.07	4.77	0.53	0.12	0.35	ND
Scenedesmus sp. BZ-2-2	275	0.24	23.2	0.73	4.98	52.6	9.54	8.34	0.17	0.19	ND	ND
Mychonastes homosphaera XFZ-2-1	526	0.25	18.7	2.80	5.95	51.5	16.78	2.72	0.34	0.02	0.38	0.60
Chlorella sp. XZL-1	280	0.25	25.0	0.63	1.55	19.2	43.23	9.95	0.18	ND	ND	ND
Desmodesmus sp. SC-2-2	319	0.32	34.2	2.58	5.93	47.4	3.96	3.89	0.61	0.47	0.43	0.25
Micractinium sp. ZSLT-11-2	403	0.35	16.0	0.47	2.04	50.4	27.68	2.78	0.34	ND	ND	ND
Chlorella sp. XJH-1-1	202	0.35	37.0	1.42	7.69	35.6	7.44	9.18	1.28	ND	ND	ND
Chlorella sp. ZSLD-2-1	322	0.36	27.6	1.84	5.13	52.3	6.39	5.42	0.38	0.31	ND	0.28
Chlamydomonas sp. YQJ-1	397	0.37	33.7	0.74	3.67	10.7	38.01	11.46	0.78	0.39	ND	0.24
Scenedesmus obliquus SGH-5	490	0.37	24.4	2.00	4.22	40.7	22.42	5.65	0.20	ND	ND	ND
Desmodesmus sp. SGH-3	688	0.38	30.9	0.13	10.29	52.4	1.17	0.64	2.29	0.15	0.93	0.73
Chlamydomonas sp. XZL-4	417	0.38	28.1	0.35	2.62	9.8	40.26	17.71	0.45	0.18	ND	0.20
Desmodesmus sp. DZL-4	511	0.70	37.0	3.12	4.98	44.2	6.08	2.57	0.66	0.21	0.44	ND
Desmodesmus sp. IR-7	332	0.73	34.9	0.07	5.60	34.8	9.56	13.43	0.91	ND	ND	ND
Desmodesmus sp. QQ-1	430	0.73	39.1	3.29	5.26	46.7	6.43	2.71	0.70	0.22	0.46	ND

ND not detected

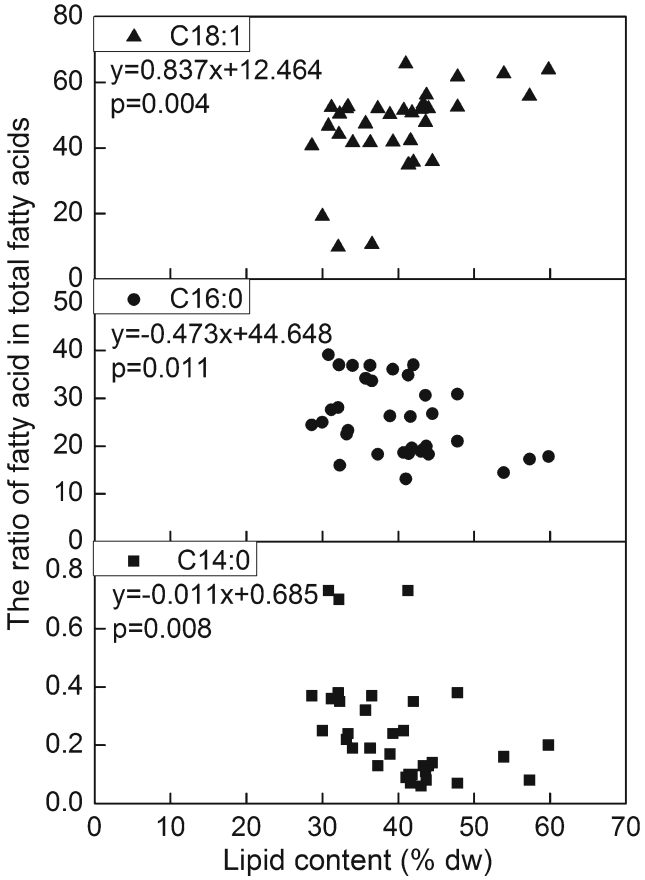

Fig. 3 Correlation analysis of relative fatty acid composition (C18:1, C16:0, and C14:0 and lipid content of dry cell weight)

increased with the accumulation of total lipid. On the contrary, C14:0 and C16:0 showed negative correlations to the amount of total lipid. In these green algal strains, the C18 fatty acid content ranged from 60 to 80% of the total fatty acids. The C18 methyl ester content of *Desmodesmus* sp. XL-1-1 reached 637 mg g^{-1} of the total lipid content. In *Scenedesmus* sp. IR-7 and *Chlorella* sp. XJH-1-1, C16:0 reached 34.8 and 37.0% of the total fatty acids, respectively. Biodiesel quality is determined by fatty acid composition, and C18 fatty acids including oleic acid, octadecadienoic acid, and octadecanoic acid are appropriate for biodiesel (Xu et al. 2006). Ramos et al. reported that the presence of monounsaturated compounds (C18:1, C20:1, and C22:1) gave a high cetane number to the biodiesels (Ramos et al. 2009). The rich C18 methyl ester content of *Desmodesmus* sp. XL-1-1 showed its potential for biodiesel production.

In *Chlorella* sp. XFZ-2-1, *Selenastrum* sp. XL-3-3, *Mychonastes homosphaera* LSSK-4-1, and *Mychonastes afer* HSO-3-1, fatty acid composition analysis indicated the presence of nervonic acid (C24:1), which has been shown to have great potential in medical applications. The nervonic acid content of *M. afer* HSO-3-1 was 3.8% of the total fatty acid content (Yuan et al. 2011).

Anti-rotifer robustness of naturally occurring microalgae

Using an experimental model of a rotifer-microalgae predator-prey system in 24-well microplates, 34 microalgal strains were tested for their anti-rotifer ability. According to data from field investigations on pilot-cultivation system of microalgae, the microalgae biomass suffers serious losses, up to 90%, if the number of rotifers per milliliter exceeds 1000. On the other hand, a small amount of biomass loss is observed when the rotifer density does not exceed 200 rotifers mL^{-1}. Thus, if the rotifer density was lower than 200 rotifers mL^{-1} and/or the intrinsic rate (r) was less than 0.22 on the eighth day of culturing in the cell suspension of a certain microalgae strain, that strain was considered to have anti-rotifer ability.

The characteristics of 34 microalgae strains and the growth rates of rotifers in corresponding algal suspensions are summarized in Table 4. The highest rotifer growth rate of 0.532 day^{-1} was observed in a suspension of the strain *Chlamydomonas reinhardtii* XZL-4. Based on the threshold suggested above, 5 microalgal strains including *Chlorella* sp. ZSLD-2-1, *S. obliquus* SGH-5, *S. obliquus* DHD-3, *Selenastrum* sp. XL-3-3, and *Didymogenes* sp. HN-4 were able to suppress the growth of rotifers, and the lowest rotifer growth rate, 0.128 day^{-1}, was achieved in the cell suspension of strain HN-4. There was no significant relationship between the feeding preference of rotifers and the microalgal taxon.

The presence of rotifers leads to a strong selection for anti-rotifer defenses by microalgae prey, some avoiding or deterring rotifers due to their morphology, some by providing inadequate nutrition to support rotifer growth or reproduction, and some by producing chemical compounds that cause rotifer paralysis, death, or reproductive disorder (Yoshida et al. 2003, 2004; Miyazaki et al. 2005; Kubanek et al. 2007). In the genus *Scenedesmus*, the cellular aggregation of *S. obliquus* SGH-5 and *S. obliquus* DHD-3 were able to deter rotifer swallowing (Fig. S1). We noted that *Didymogenes* sp. HN-4 is a unicellular green alga with cells of 3–4 μm in diameter, a size which should have been easily devoured by the rotifers. However, rotifer growth was inhibited in the first 6 days in cell suspension of *Didymogenes* sp. HN-4. After cultivation for 6 days in TAP medium, the HN-4 cells were harvested by centrifugation, and the supernatant was collected and filtered to remove the microalgae cells. When the rotifers (*Rataria* sp.) and *Chlorella* sp. XZL-1 (as rotifer food)

Table 4 Characteristics of microalgae strains and intrinsic growth rate of rotifers in corresponding algal suspensions

Algal strains	Biomass loss (%)	Cell size (μm)	Flagella	Cells aggregation	Rotifer density (rotifers mL^{-1})	Rotifer intrinsic growth rate γ (day^{-1})
Chlorella sp. SZK-3	68.0	5–9	No	No	160	0.232
Chlorella sp. ZSLD-2-1	28.0	6–8	No	No	120	0.196
Chlorella sp. QQ-4-1	96.0	6–7	No	No	395	0.322
Chlorella sp. SGH-6	83.7	5–9	No	No	240	0.287
Chlorella sp. XZL-1	91.0	4–6	No	No	1000	0.461
Chlamydomonas reinhardtii XZL-4	91.0	6–7	Yes	No	1760	0.532
Desmodesmus sp. BSH-4-1	76.5	7–9	No	No	215	0.246
Desmodesmus sp. XFZ-4-1	48.3	6–10	No	No	235	0.257
Desmodesmus sp. XJH-2-2	85.0	6–10	No	No	267	0.3
Desmodesmus sp. SC-2-2	39.0	8–10	No	No	395	0.345
Chlamydomonas sp. YQJ-1	80.0	7–9	Yes	No	1200	0.462
Desmodesmus sp. QQ-1	ND	8–11	No	No	200	0.237
Desmodesmus sp. DW-4-1	20.0	9–11	No	No	180	0.247
Desmodesmus sp. RZ-5	42.0	6–8	No	No	390	0.343
Desmodesmus sp. NCP-1-2	2.3	5–8	No	No	250	0.265
Desmodesmus sp. SGH-3	52.5	7–10	No	No	435	0.362
Mychonastes afer HSO-3-1	86.9	4–6	No	No	495	0.378
Micractinium sp. HSD-2-1	94.6	5	No	No	520	0.384
Micractinium sp. DZL-5-1	73.4	5	No	No	210	0.271
Scenedesmus sp. ZSLT-8-2	49.5	7–12	No	2–4 cells	220	0.262
Scenedesmus obliquus LSSK-4-1	78.0	6–9	No	2–4 cells	180	0.247
Scenedesmus obliquus XL-4-2	80.9	5–7	No	2–4 cells	290	0.284
Scenedesmus obliquus HSD-4-1	57.0	6–8	No	2–4 cells	546	0.385
Scenedesmus obliquus SGH-5	49.0	7–8	No	2–4 cells	135	0.211
Scenedesmus sp. RZ-1-1	13.0	7–10	No	2–4 cells	310	0.292
Scenedesmus obliquus RZ-4-1	55.3	8–12	No	2–4 cells	160	0.232
Scenedesmus obliquus DHD-3	22.0	7–10	No	2–4 cells	105	0.179
Scenedesmus sp. KTX-2	8.0	6–8	No	2–4 cells	175	0.243
Scenedesmus obliquus ZSLC-11–1	27.0	8–10	No	2–4 cells	255	0.29
Scenedesmus obliquus SC-2-1	57.0	5–7	No	2–4 cells	360	0.333
Scenedesmus sp. HD-5	48.0	6–8	No	2–4 cells	300	0.316
Selenastrum sp. XL-3-3	9.0	4–6	No	No	140	0.193
Desmodesmus sp. XGR-1-1	80.0	6–9	No	No	355	0.309
Didymogenes sp. HN-4	23.2	5–7	No	No	130	0.128

ND not detected

were co-cultured in the cell-free filtrate of *Didymogenes* sp. HN-4, rotifer growth was still suppressed (Fig. 4a), whereas the alga *Chlorella* sp. XZL-1 grew normally with a concentration of 4–6 × 10^7 cells mL^{-1}. The sterilized supernatant of strain HN-4 did not affect rotifer growth in the presence of *Chlorella* sp. XZL-1. In addition, the rotifers and eggs in the 100 and 75% filtrates were significantly lower than those in the control (fresh TAP), indicating that the oviposition of adult rotifers and/or the hatching rate of eggs were suppressed (Fig. 4b). Furthermore, in the presence of a cell suspension or filtrate of HN-4, the rotifer *Rataria* sp. showed abnormal morphology and moved slowly (Fig. 5b).

Discussion

Rodolfi et al. (2009) reported that lipid-rich strains in general show lower biomass productivity. In this study, no significant correlation between lipid content and biomass productivity

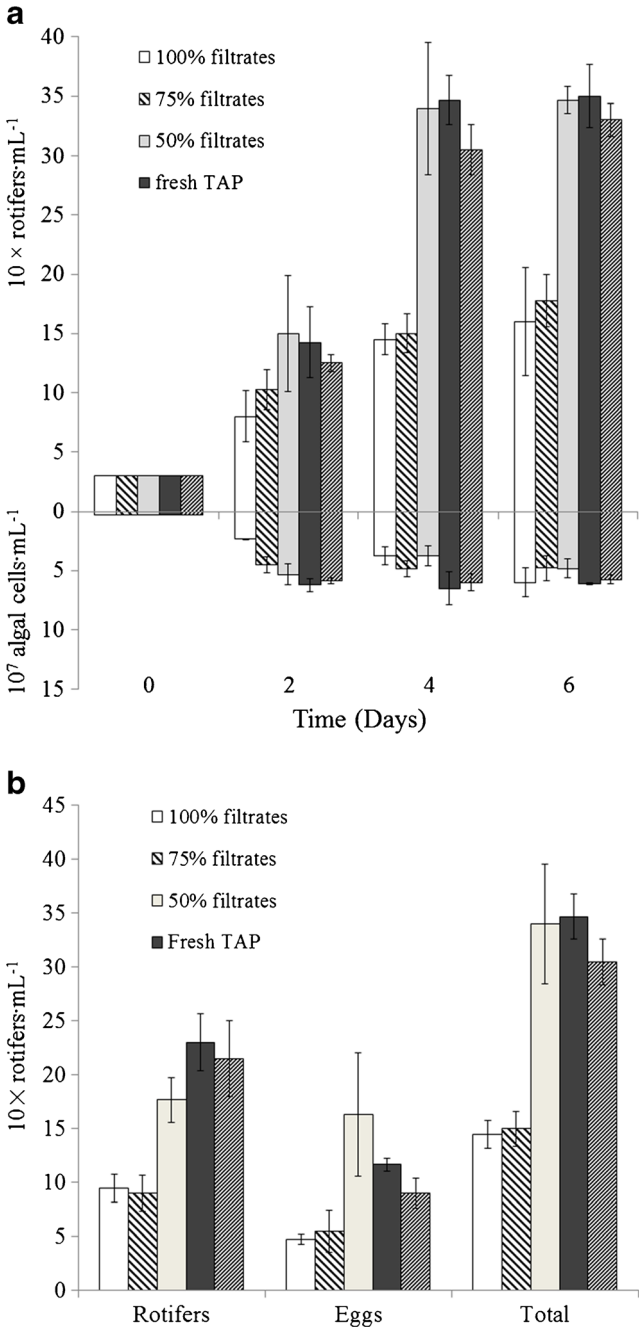

Fig. 4 Chemical defense analysis of *Didymogenes* sp. HN-4 against rotifer grazing. **a** The density of rotifers and *Chlorella* sp. XZL-1 were determined when they were cultivated in flasks containing different proportions of unsterile cell-free filtrates from a suspension of *Didymogenes* sp. HN-4 grown in TAP medium and sterilized filtrates of strain HN-4 by autoclaving. *Chlorella* sp. XZL-1 was co-cultured as rotifer food. **b** The density of rotifer adults and eggs at the fourth day under the same culture conditions as above. All experiments replicated at least three times

was observed (Fig. 1). Griffiths and Harrison (2009) summarized the literature on microalgal growth rates, lipid content, and lipid productivity for 55 microalgal species including 17 Chlorophyta, 11 Bacillariophyta, and 5 Cyanobacteria, and indicated the importance of lipid productivity over lipid content and growth rates when selecting species for biodiesel production. Microalgae often accumulate lipids under nutrient limitations such as nitrogen and phosphorus depletion and under other culture stress conditions relating to light intensity and temperature (Courchesne et al. 2009; Li et al. 2010). In this study, all microalgal strains were cultured in BG11 medium with 4.4 mM $NaNO_3$ (25% of normal BG11) under batch culture conditions with the aim of inducing lipid synthesis in the stationary phase. For microalgal strains with similar lipid content, we suggest that biomass productivity should be a primary factor used to determine which microalgae may produce biodiesel under nitrogen-deficient conditions.

All plant and microalgae cells produce fatty acids from acetyl-CoA by a common pathway localized in plastids. Although a small portion of the newly synthesized acyl chains is used for lipid synthesis within the plastid (the prokaryotic pathway), a major portion is exported into the cytosol for glycerolipid assembly at the endoplasmic reticulum (ER) or other sites (the eukaryotic pathway). In the plastids, fatty acids are synthesized from acetyl-CoA in a three-step process (Cagliari et al. 2011). In brief, the plastidial pathway of fatty acid biosynthesis consists of two enzyme systems: acetyl-CoA carboxylase and fatty acid synthase (Ohlrogge and Jaworski 1997). Among them, the KAS I enzyme catalyzes the condensation reaction of 4:0-ACP with malonyl-ACP, giving rise to 14:0-ACP and 16:0-ACP (Tai and Jaworski 1993). In our study, the amount of C18:1 increased with increasing total lipid content, but C14:0 and C16:0 showed negative correlations to the amount of total lipid. We inferred that the enzyme which catalyzes the reactions from 14:0-ACP and 16:0-ACP is a rate-limiting enzyme in lipid synthesis for low lipid-level strains.

Considering the rotifer growth in *Didymogenes* sp. HN-4 culture and cell-free filtrates, respectively (Fig. 4), we postulated that *Didymogenes* sp. HN-4 produced and secreted certain chemical compounds to defend itself against rotifer grazing, and these compounds were sensitive to high temperature or pressure. Several red tide dinoflagellates such as *Karenia brevis* and *Heterocapsa circularisquama* have been reported to show chemical defenses against marine zooplankton such as rotifer grazing by producing the toxic compounds brevetoxins or photosensitizing hemolytic toxin, which has a similar structure as the pyropheophorbide-α methyl ester (Kubanek et al. 2007; Miyazaki et al. 2005). Marine diatoms like *Skeletonema costatum* and *Pseudonitzschia delicatissium* inhibit the reproduction of zooplankton such as copepods by decreasing their egg fecundity and hatching success, and three aldehydes that are responsible for this activity have been characterized (Miralto et al. 1999).

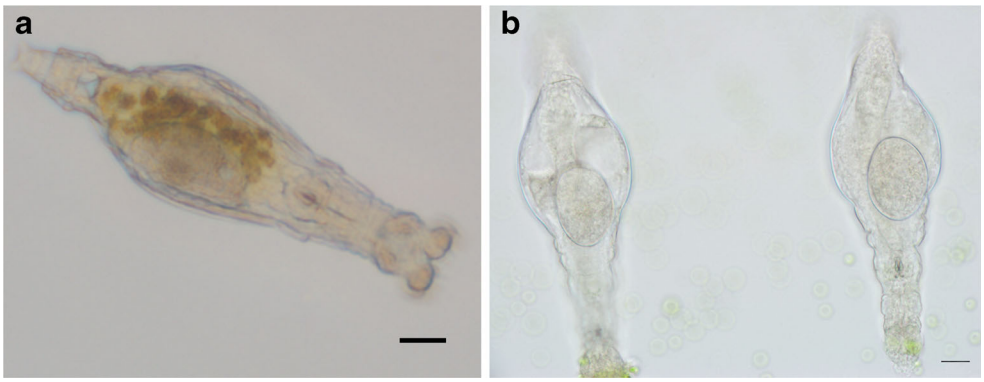

Fig. 5 Normal morphology of rotifers when fed with *Chlorella* sp. XZL-1 (**a**) and abnormal morphology of rotifers when cultivated in the presence of the filtrate of *Didymogenes* sp. HN-4 grown in TAP medium (**b**). The *scale* in (**a**) and (**b**) is 20 μm

In this study, the rotifer morphology changed in the presence of *Didymogenes* sp. HN-4, but the mechanism remains to be further investigated.

In the past, the industrial sector and scientific community have paid more attention to individual so-called productive microalgal strains, but neglected mismatches between the productive strain and the cultivation system, especially in outdoor environments, which has hampered the large-scale production of microalgal feedstock (Mooij et al. 2015). Recently, Hamilton et al. (2014) proposed the use of a polyculture system utilizing complex algal communities to solve the problem of algal pond crashes and to increase biomass production. They recommended that designed algal communities should be constructed according to niche differentiation that is complementary and/or different abiotic and biotic resistance of the local cultivation environments. Algal communities are not constructed randomly, but require exact strain assemblages based on the ecological principles of niche partitioning and complementarity. In this work, we not only demonstrated biodiversity of characteristics that are desirable, such as biomass, lipid production, fatty acid profile, and anti-rotifer robustness, in native Chinese microalgae, but also quantified these attributes, which benefits the future construction of complex algal communities.

In conclusion, in this study the lipid productivity, fatty acid composition, and anti-rotifers robustness of microalgal species isolated from Eastern China were investigated. Different strains belonging to the same species showed great differences in lipid content, indicating the necessity to test the lipid content and profile for each additional or novel strain. *Scenedesmus* sp. KTX-2 is a good option for biodiesel production due to its high lipid content (38.4% dw), biomass yield (3.19 g L^{-1}), and morphological defense against rotifers. The presence of the valuable co-product nervonic acid (C24:1) in microalgal strains indicates a potential for the medical application of microalgae. *Didymogenes* sp. HN-4, which can depress the growth of rotifers, was selected based on a rotifer-microalgae predator-prey system. This work demonstrates the biodiversity of desired productive characteristics and the robustness of microalgae isolated from Eastern China, which lays the groundwork for mass algal culture and future studies on the economics of algal biodiesel production through ecology-based principles.

Acknowledgements This study was supported by the National Science Foundation of China (U1232126), the National High Technology Research and Development Program of China (2014AA022003), the China Postdoctoral Science Foundation (2016M602162), and the Scientific Research Foundation of Shandong University of Science and Technology for Recruited Talents (2015RCJJ054) and was carried out in the framework of the Boeing-QIBEBT Joint Research Laboratory for Sustainable Aviation Biofuels Collaboration Agreement.

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