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Detection of intracellular neutral lipid content in the marine microalgae *Prorocentrum micans* and *Phaeodactylum tricornutum* using Nile red and BODIPY 505/515

**Songcui Wu · Baoyu Zhang · Aiyou Huang · Li Huan ·
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Abstract Diatoms and dinoflagellates not only have extensive distribution and a huge biomass in marine ecosystems, but also have high lipid accumulation in nature or after physiological and genetic modification, which indicates that these organisms may be optimal candidate algal strains for biodiesel production. In this study, we determined the content of intracellular neutral lipids (triacylglycerol [TAG]) in the dinoflagellate *Prorocentrum micans* and in the diatom *Phaeodactylum tricornutum* using NR and BODIPY 505/515 staining. The freshwater green alga *Scenedesmus obliquus* was used as a control. Optimum concentrations of 1.000 and 1.500 $\mu g\ mL^{-1}$ were determined for neutral lipid Nile red (NR) staining in *P. micans* and *P. tricornutum*. Unlike NR staining, the optimal concentrations of BODIPY 505/515 staining in *P. micans* and *P. tricornutum* were lower, at 0.100 and 0.075 $\mu g\ mL^{-1}$, respectively. High correlation coefficients of $R^2=0.990$ and $R^2=0.989$ were obtained for *P. micans* and *P. tricornutum* intracellular neutral lipid content and the relative fluorescence intensity with NR staining, while the reference alga, *S. obliquus*, had a relatively low correlation coefficient of $R^2=0.908$ when stained with NR. The neutral lipid content determined by thin-layer chromatography-flame ionization detector matched the analytical data from fluorescence measurements. These results indicated that NR and BODIPY 505/515 staining can be used as an excellent high-throughput approach to screen marine diatoms and dinoflagellates.

Keywords Marine microalga · BODIPY 505/515 · Nile red · Neutral lipid · Triacylglycerol · Biodiesel

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Introduction

Microalgae, as an ideal feedstock for biodiesel production, have attracted worldwide attention, due to their high biomass, photosynthetic efficiency and lipid productivity (Hu et al. 2008). Screening excellent algal strains is the key to developing biodiesel production from microalgae, which is dependent on high-throughput selected methods. Although current methods, such as solvent extraction, gravimetric means, thin-layer chromatography (TLC), high performance liquid chromatography (HPLC) and gas chromatography (GC), can be used to quantitatively or qualitatively analyze cellular lipids effectively, these conventional methods are generally time intense and material consuming (De la Hoz Siegler et al. 2012; Chen et al. 2009), and thus unsuitable for high-throughput screening purposes or small batch cultured experiments. As a result, increased attention has been focused on the use of lipophilic fluorescence probes (Nile red [NR] and BODOPY 505/515) to determine intracellular lipid contents, as this fluorescent method requires only a small sample volume and a short analysis time. Moreover, NR, as a common fluorescent dye, has been widely used in microalgal lipid quantification (Cooksey et al. 1987; McGinnis et al. 1997; de la Jara et al. 2003; Liu et al. 2008; Yu et al. 2009; Chen et al. 2009) especially used in green algae (Huang et al. 2009; Chen et al. 2009; Cooksey et al. 1987), and there are also many reports about the two model diatoms *Phaeodactylum*

tricornutum and *Thalassiosira pseudonana* (Yu et al. 2009; Gardner et al. 2012; Wong and Franz 2013). Meanwhile, BODPY 505/515, the new lipophilic neutral fluorophore, has high fluorescent quantum yields, great fluorescent performance and good photo stability (Cooper et al. 2010), which make it promising for wide application. And it has already been used for lipid detection in some green algae (Govender et al. 2012; Xu et al. 2012; Velmurugan et al. 2013). However, the application of this method in the determination of the lipid contents in other microalgal species like dinoflagellates and diatoms are relative rare and still requires further investigation. Furthermore, the fluorescent method is usually used to monitor freshwater algal lipid, therefore its suitability for determining marine algal lipid contents also requires future research.

With the exception of green algae, other eukaryotic microalgae such as diatoms and dinoflagellates also have potential as a biodiesel feedstock. In particular, diatoms, which are major plankton species, are extensively distributed in both freshwater and seawater. Due to their high biomass and species diversity, it has been suggested that diatoms may create around 20 % of the earth's primary productivity (Falkowski et al. 1998; Field et al. 1998). Dinoflagellates are also widely distributed worldwide and are mainly marine. When light intensity and temperature in nature are optimal, both diatoms and dinoflagellates can grow very quickly and cause harmful algal blooms resulting in an enormous biomass. Although it is difficult to achieve the biomass obtained in nature when diatoms and dinoflagellates are cultured in the laboratory, under laboratory conditions there is still the potential for high biomass production once their rapid growth mechanism is understood. In addition, due to the shortage of freshwater resources on land, the development of marine resources is vital. Diatoms and dinoflagellates are abundant in the ocean and as important marine plankton they will be the focus of marine resources for biodiesel research in the future. Therefore, high-throughput screening of potential oleaginous marine diatom and flagellate strains is promising in biodiesel production.

Although there are research on diatoms and some other microalgae species, as far as we known, reports on dinoflagellates neutral lipid research are relative rare. In the present study, we evaluated intracellular lipid contents of the dinoflagellate *Prorocenteum micans* and the diatom *Phaeodactylum tricornutum* using both NR and BODIPY 505/515 staining as NR method can be a reference. The freshwater green alga *Scenedesmus obliquus* was used as a control. A systematic comparison of the applicability of the two fluorescent methods for diatoms and dinoflagellates was conducted in the present study, which will provide some practical applications for high-throughput selection of marine diatoms and dinoflagellates for future biodiesel research.

Material and methods

Scenedesmus obliquus, a freshwater green alga, was obtained from the Yellow River, China. Under normal cultivation conditions, *S. obliquus* was grown in sterilized BG11 freshwater medium. In nitrogen deprived conditions, the alga was cultured in BG 11 with 100 mg L^{-1} NaNO$_3$. The marine algae *Prorocentrum micans* and *Phaeodactylum tricornutum* were screened from the East China Sea and were cultivated in sterilized f/2-Si medium as described by Guillard and Ryther (1962). When the cultures were under nitrogen deprivation, NaNO$_3$ was not added to the f/2-Si medium. Each strain was maintained in triplicate in 500-mL flasks with 300 mL medium and grown at 20 ± 1°C under a light intensity of 100 μmol photons m^{-2} s^{-1} in a 12:12 h light–dark (L/D) cycle.

Staining of algal cells with conventional staining method

Before the fluorescence determination, we set a pre-test to verify if the conventional staining methods (NR and BODIPY 505/515) were adapted for the three algal cells. Algal cultures were cultivated for 12 days and stained according to the conventional staining methods for microalgae (Cooksey et al. 1987; McGinnis et al. 1997; Liu et al. 2008; Govender et al. 2012; Xu et al. 2012).

Nile red fluorescence determination of neutral lipid

In the pre-test, the conventional NR staining method was found ineffective for the thick-walled green alga *S. obliquus*. DMSO was added in the algal cultures as a carrier solvent to promoted NR permeability. DMSO concentration, incubation time and temperature were optimized according to the improved NR staining method of Chen et al. (2009).

NR (9-(diethyl amino)benzo[*a*]phenoxazin-5(5*H*)-one; Sigma-Aldrich) was prepared as a stock solution of 0.100 mg mL^{-1} in acetone. The three microalgal cultures were diluted to a defined cell concentration of 1×10^6 cells mL^{-1} before staining. All defined cell concentration cultures were stained using a concentration ranging from 0.100 to 3.000 μg mL^{-1} of NR-acetone solution. After 7 min incubation in darkness, the suspensions were analyzed using a fluorescence spectrophotometer (HITCHI F-4500) with excitation and emission wavelengths of 480 and 570 nm, respectively.

Optimization of BODIPY 505/515 fluorescence method

BODIPY 505/515 (4,4-difluro-1,3,5,7-tetramethyl-4-bora-3*a*,4adiaza-*s*-indacene; Invitrogen Molecular Probes, USA) was dissolved in DMSO as a stock solution at a concentration of 0.500 mg mL^{-1} and stored in a dark bottle away from light. Pre-treatments for BODIPY 505/515 staining of three algae were performed similar to the NR staining. To determine the

optimal BODIPY 505/515 concentration, a range of concentrations from 0.005 to 0.200 $\mu g \ mL^{-1}$ were added to the suspension (1×10^6 cells mL^{-1}) to achieve final concentrations of 0.005, 0.025, 0.050, 0.075, 0.100 0.125, 0.150, 0.175 and 0.200 $\mu g \ mL^{-1}$. The excitation and emission wavelengths of the spectrofluorometer (HITACHI F-4500) for monitoring BODIPY 505/515 fluorescence were 488 and 510 nm, respectively.

The relative fluorescence intensity per milliliter of defined cell concentration suspension was achieved after subtraction of auto-fluorescence of algal cells and self-fluorescence of BODIPY 505/515 and NR from the spectral peak value reading at 510 and 570 nm, respectively.

Thin-layer chromatography-flame ionization detector investigation of neutral lipids

To confirm the results of the fluorescence method for the evaluation of intracellular neutral lipid quantification, conventional TLC-FID was used as a control. Lipids were extracted using a modified chloroform–methanol system according to Bligh and Dyer (1959). The extract was evaporated with N_2 flow to remove solvents and was resolved by adding 50 μL chloroform. Then, 8 μL residua, as the stock solution, was spotted three times on chromarod, and then developed in the mobile phase I involved benzene: chloroform: acetic acid (150: 60: 2, v/v/v). After dryness, redeveloped in mobile phase II consisted of benzene: n-hexane (1:1, v/v), followed by evaporating the solvent at oven under 70°C for 3 min, and then detected by a flame ionization detector. Further accurate quantitative analysis of the triacylglycerol (TAG) content was obtained from the detailed data afforded by the TLC-FID (IATROSCAN MK6/MK6s) by determination of the specific peak position of TAG via retention time and calculating the peak area of TAG on the chromatogram. The standard used in the chromatography experiments was glycerin trioleate.

Growth and neutral lipid accumulation in the three algal species under normal and nitrogen deprived cultivation

Each strain was maintained in normal and nitrogen deficient medium, respectively. Algal growth was measured by cell numbers and the optical density (OD) values at optical wavelength of P. tricornutum (730 nm) (Satoh et al. 2001; Tanaka et al. 2005; Kitao et al. 2008; Sakaguchi et al. 2011), P. micans (678 nm) (Sorokin 1973) and S. obliquus (650 nm) (Zhang et al. 1997; Wei et al. 2010; Tang et al. 2011; Devi et al. 2012) using a spectrophotometer (UV-1800). A hemocytometer was used to count algal cell numbers. Neutral lipid content in the three algae was measured by NR and BODIPY 505/515 staining. Algal growth and neutral lipid accumulation were determined on days 3, 5, 7, 9 and 11. Samples were taken at

the same stage in the L/D cycle, which were always after 8 h of light treatment.

Comparison of lipid content determination by NR, BODIPY 505/515 fluorescent methods and TLC-FID

A comparison of the two fluorescent measurements and conventional TLC-FID was conducted to verify whether the fluorescent methods were effective in determining the neutral lipid content in marine diatoms and dinoflagellates. The cells for lipid determination were obtained from 12-day nitrogen deprived cultures of the three algae. Three replicates were used for both fluorescent measurement and thin-layer column chromatography determination.

Microscopic observation of stained algal cells

The stained algal cells were observed by laser confocal microscopy, using a Carl Zeiss microscope. The 40× objective was selected to view stained algal intracellular lipid droplet distribution under blue light excitation. Photographs were taken using Zen 2010 software.

Statistical analysis

The t test was used to identify data of any significance within the treatments, and the Pearson test was applied to analyze the correlation of the data derived from the experimental measurements. In each case, a P value <0.05 was considered significant.

Results

Optimization of staining of algal cells with Nile red and BODIPY 505/515

According to pre-experiment, the S. obliquus suspension was added to 10 % DMSO and placed in a water bath for 15 min at 40°C before staining, while P. tricornutum and P. micans were stained without pre-treatment. As the concentration of NR increased, a significant difference in the fluorescence intensity of the defined cell concentration cultures was observed. P. tricornutum reached a maximum of 28.50±5.29 (a.u.) at 1.500 $\mu g \ mL^{-1}$ (Fig. 1b) and P. micans reached a maximum fluorescence intensity of 589.21±17.7 (a.u.) at 1.000 $\mu g \ mL^{-1}$ (Fig. 1c), while the maximum fluorescence intensity of S. obliquus was 17.45±0.98 (a.u.) at 1.000 $\mu g \ mL^{-1}$ (Fig. 1a). In addition, the dye uptake characteristics also differed in the three algae species.

As shown in Fig. 2, the fluorescence intensity of the three algae stained with BODIPY 505/515 showed a maximum level of 363.2±23.3 (a.u.) at 0.075 $\mu g \ mL^{-1}$ in P. tricornutum (Fig. 2b) cells with increased concentration of BODIPY 505/
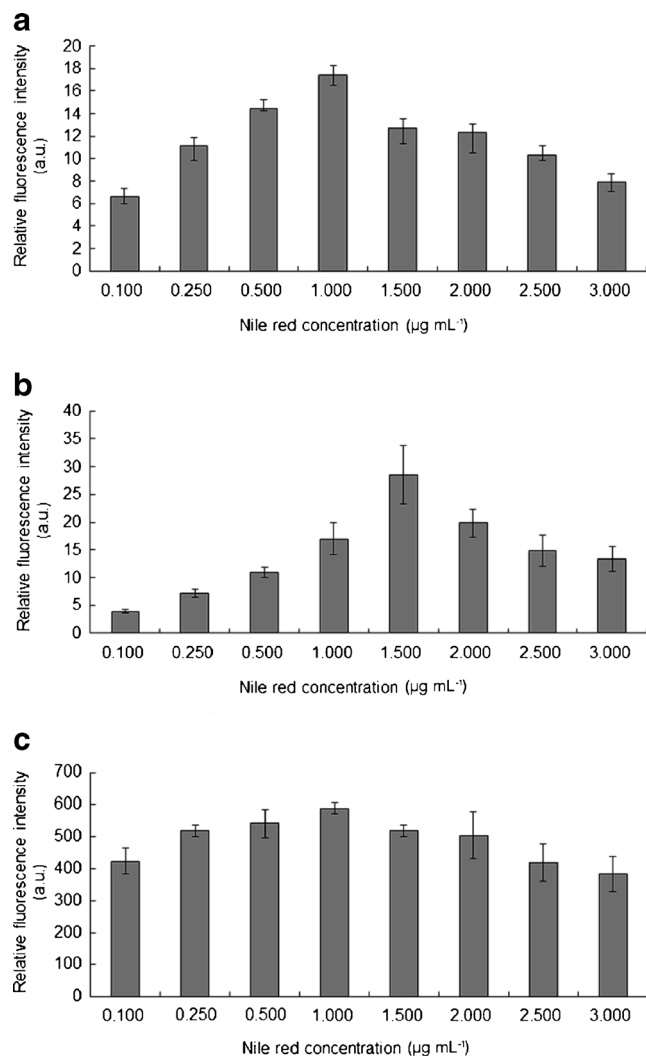
Fig. 1 Effects of staining concentration on fluorescence intensity of NR used for **a** *S. obliquus*, **b** *P. tricornutum*, and **c** *P. micans*. Samples were incubated in darkness for 7 min at room temperature before viewing. The excitation and emission wavelengths used for fluorescence determination were 480 and 570 nm, respectively. Data are expressed as a mean±SD ($n=3$)

Fig. 2 Effects of staining concentration on the fluorescence intensity of BODIPY 505/515⁻ stained cells in **a** *S. obliquus*, **b** *P. tricornutum*, and **c** *P. micans*. Samples were incubated in darkness for 7 min at room temperature before viewing. The excitation and emission wavelengths used for fluorescence determination were 488 and 510 nm, respectively. Data are expressed as a mean±SD ($n=3$)

515, while *S. obliquus* (Fig. 2a) and *P. micans* (Fig. 2c) reached a maximum fluorescence intensity of 374.1±1.15 (a.u.) and 341.1±19.8 (a.u.) at 0.100 μg mL^{-1}, respectively. The optimal concentration of BODIPY 505/515 for the three microalgae was lower than that required for NR staining which required a minimum concentration of 1.000 μg mL^{-1}. For BODIPY 505/515 staining, *S. obliquus* also required similar pre-measurements to those taken using NR staining. The time required to stain algal cells with BODIPY 505/515 was short, at just required 2–7 min, while NR staining needed at least 7 min.

Effects of nitrogen deprived of algal growth and lipid accumulation

Figure 3 shows the growth of the three samples cultured under normal and nitrogen deprived conditions during 12 days
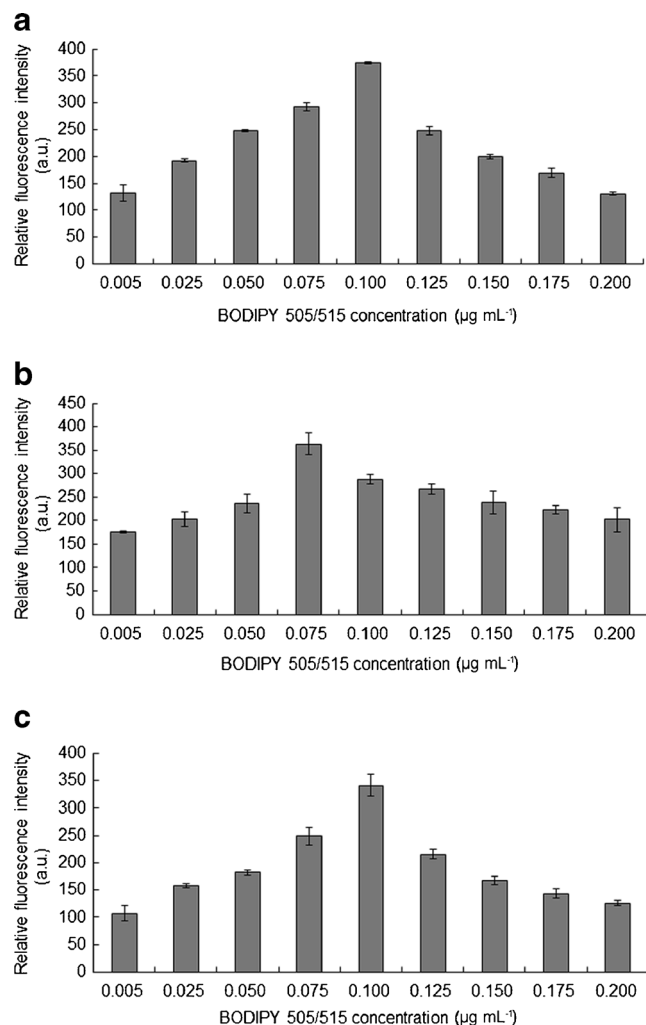
of cultivation. *P. micans* grew slowly and accumulated less biomass whether in normal or nitrogen deprived medium. The neutral lipid content changes under normal and nitrogen deprived cultivation are shown in Fig. 4, and were determined by NR and BODIPY 505/515 on days 3, 5, 7, 9 and 11. The neutral lipid content of the three samples under normal cultivation conditions, decreased from day 3 to day 5, and after 5 days cultivation lipid started to accumulate. When samples were maintained in nitrogen deprived medium the neutral lipid content increased from days 3 to 7, reaching a maximum on day 7 (Fig. 5), and then gradually decreased. In nitrogen deprived medium, neutral lipid storage in the three algae was higher than that in normal medium as their cell division was suppressed and a greater amount of carbon was available for lipid storage (Roessler 1990; Sukenik and Wahnon 1991). Studies have shown that
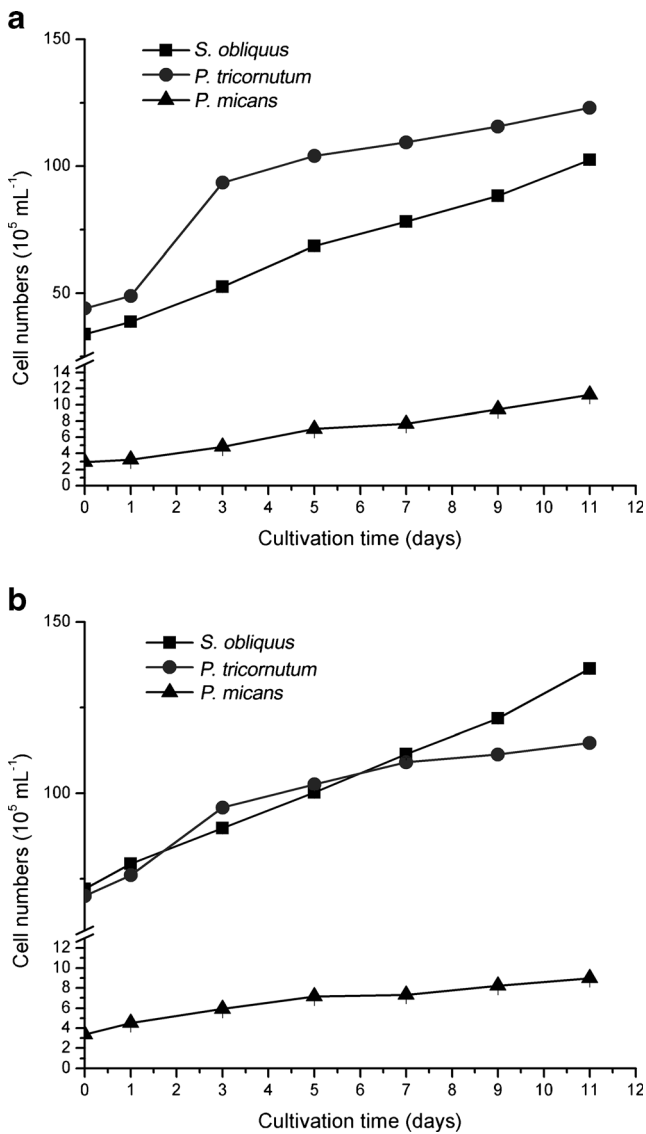
Fig. 3 Growth of *P. tricornutum*, *S. obliquus* and *P. micans* under normal (**a**) and nitrogen deprived (**b**) cultivation conditions. Errors bars represent standard deviation ($n=3$)

Fig. 4 Neutral lipid content of *P. tricornutum*, *S. obliquus* and *P. micans* were determined by NR (**a**) and BODIPY 505/515 (**b**) staining under normal cultivation. The optimal dye concentration was used for each sample. Data are expressed as a mean±SD ($n=3$)

low temperature, high light intensity and nitrogen deprivation can influence lipid accumulation, however, of these stress factors, and nitrogen deprivation has the greatest effect on lipid accumulation (Li et al. 2010).

Comparison of lipid content by TLC-FID, NR and BODIPY 505/515 fluorescence

According to Chen et al. (2009) and Xu et al. (2012), fluorescent dyes (NR and BODIPY 505/515) combine with intracellular TAG specifically and do not combine with other intracellular components, so that the higher fluorescence intensity of the alga corresponds to higher neutral lipid content in the cell. Figure 5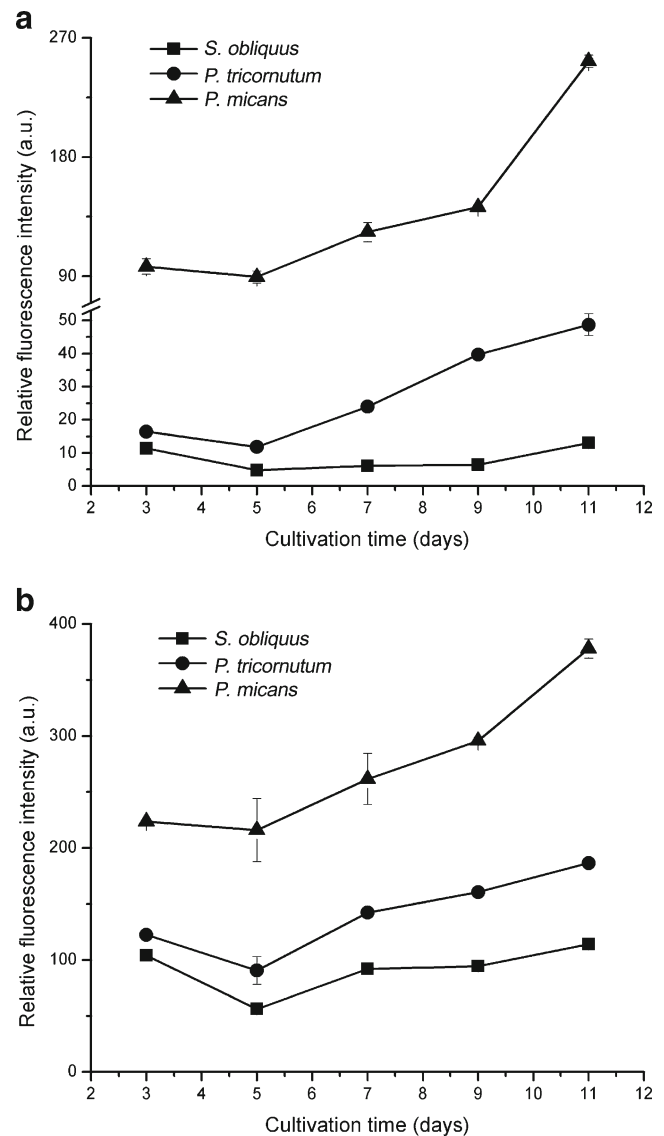a and b shows that in nitrogen deprived medium, the neutral lipid content of *P. tricornutum* and *P. micans* first increased, achieved a maximum on day 7, and then started to decrease. Whether stained with NR or BODIPY 505/515 at the optimal dye concentration, similar trends were obtained from the analytical data of fluorescence intensity in the three algae. Moreover, *P. micans* had the highest relative fluorescence intensity, followed by *P. tricornutum*, and *S. obliquus* had the lowest value, and the relative fluorescence intensity of BODIPY 505/515 staining of the three organisms was higher than NR staining.

The TLC-FID data are shown in Fig. 5c, in which changes in TAG content relative to time for *P. micans*, *P. tricornutum* and *S. obliquus* matched the analytical data from fluorescent measurement. The TAG content of dry weight (w/w, %)
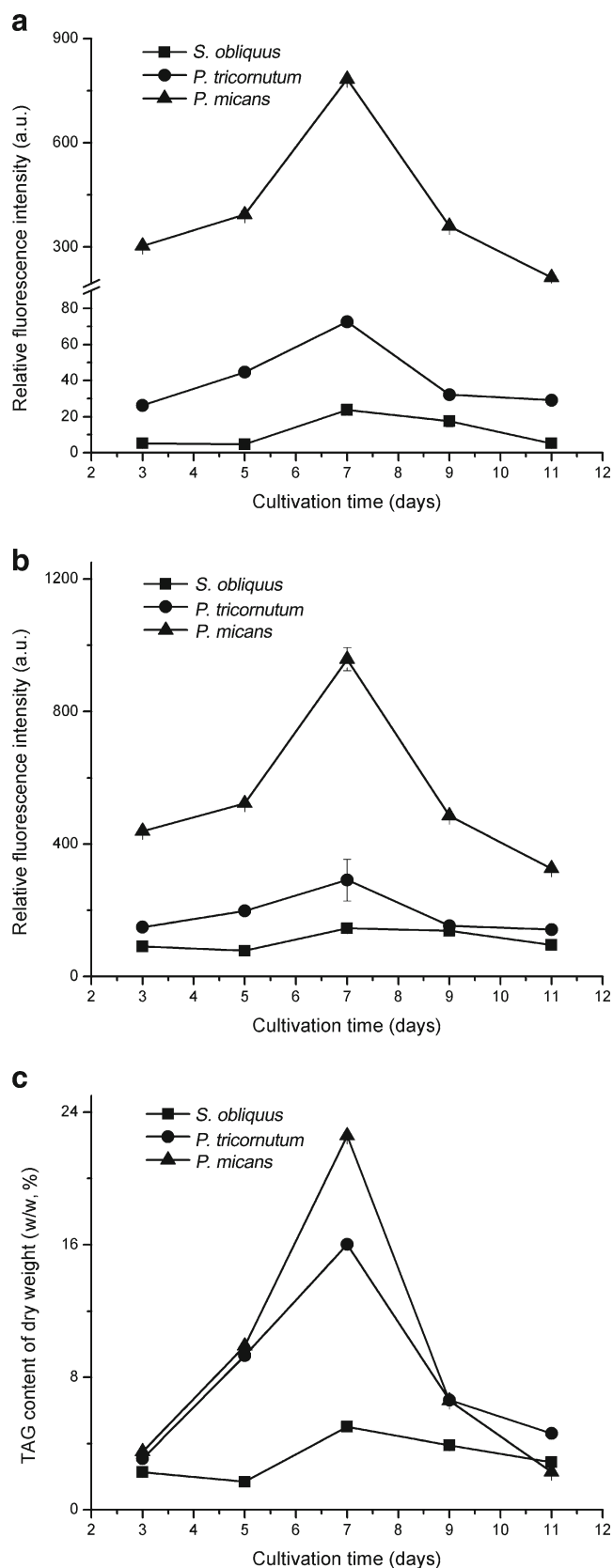
Fig. 5 Neutral lipid content changes of *P. tricornutum*, *P. micans* and *S. obliquus* on the set of nitrogen deprived cultivation were determined by NR staining (a), BODIPY 505/515 fluorescent method (b) and TLC-FID (c). Data are expressed as a mean±SD (n=3)

(Fig. 5c) increased initially, achieved a maximum of 22.58 and 16.03 % for *P. micans* and *P. tricornutum* on day 7, respectively, and then decreased. These results indicate that NR and BODIPY 505/515 staining are effective for the determination of neutral lipid in marine diatoms and dinoflagellates.

Correlation between lipid fluorescence intensity and neutral lipid content

Cooksey et al. (1987) found cellular fluorescence of NR stained cells and gravimetrically or chromatographically determined lipid were linearly correlated. In order to quantify the intracellular neutral lipid by relative fluorescence intensity, a correlation between the fluorescence intensity and cell neutral lipid content was obtained from each sample using both staining methods in our study. The linear regression equations for *P. micans*, *P. tricornutum* and *S. obliquus* are discussed in the following paragraphs.

For *P. micans*, the linear relationship between relative fluorescence intensity derived from NR stained cells and neutral lipid content can be expressed as: $y = 0.0368x - 6.1195$, $R^2 = 0.990$ ($P < 0.05$) (Fig. 6e), while the linear regression equation for BODIPY 505/515 staining was $y = 0.0333x - 9.2061$, $R^2 = 0.987$ ($P < 0.05$) (Fig. 6f).

For *P. tricornutum*, the correlation between NR relative fluorescence intensity and neutral lipid content can be described as $y = 0.2639x - 2.8728$, $R^2 = 0.989$ ($P < 0.05$) (Fig. 6c); when stained with BODIPY 505/515, the regression equation was expressed as $y = 0.0794x - 6.8918$, $R^2 = 0.987$ ($P < 0.05$) (Fig. 6d).

As the reference, *S. obliquus* also yielded a linear relationship between its NR relative fluorescence intensity and neutral lipid content, which can be expressed as $y = 0.1425x + 1.5454$, $R^2 = 0.908$ ($P < 0.05$) (Fig. 6a), while the linear regression equation for BODIPY 505/515 relative fluorescence intensity and neutral lipid content of *S. obliquus* was $y = 0.0424x - 1.4975$, $R^2 = 0.935$ ($P < 0.05$) (Fig. 6b). The correlation coefficient was relatively low.

In these equations, y represents the neutral lipid content of dry weight (w/w, %) and x represents the relative fluorescence intensity derived from the NR or BODIPY 505/515 analytical data. The variability of fluorescence measurements and the fluorescence background of the dye may be major factors which affected the linearity of fluorescence.

In terms of the regression equation, microalgal cellular neutral lipid content can be monitored by NR fluorescence and BODIPY 505/515 fluorescence analyzed by spectrofluorometry during microalgal growth. Compared to traditional gravimetric lipid analysis, the fluorescent methods were convenient and rapid.
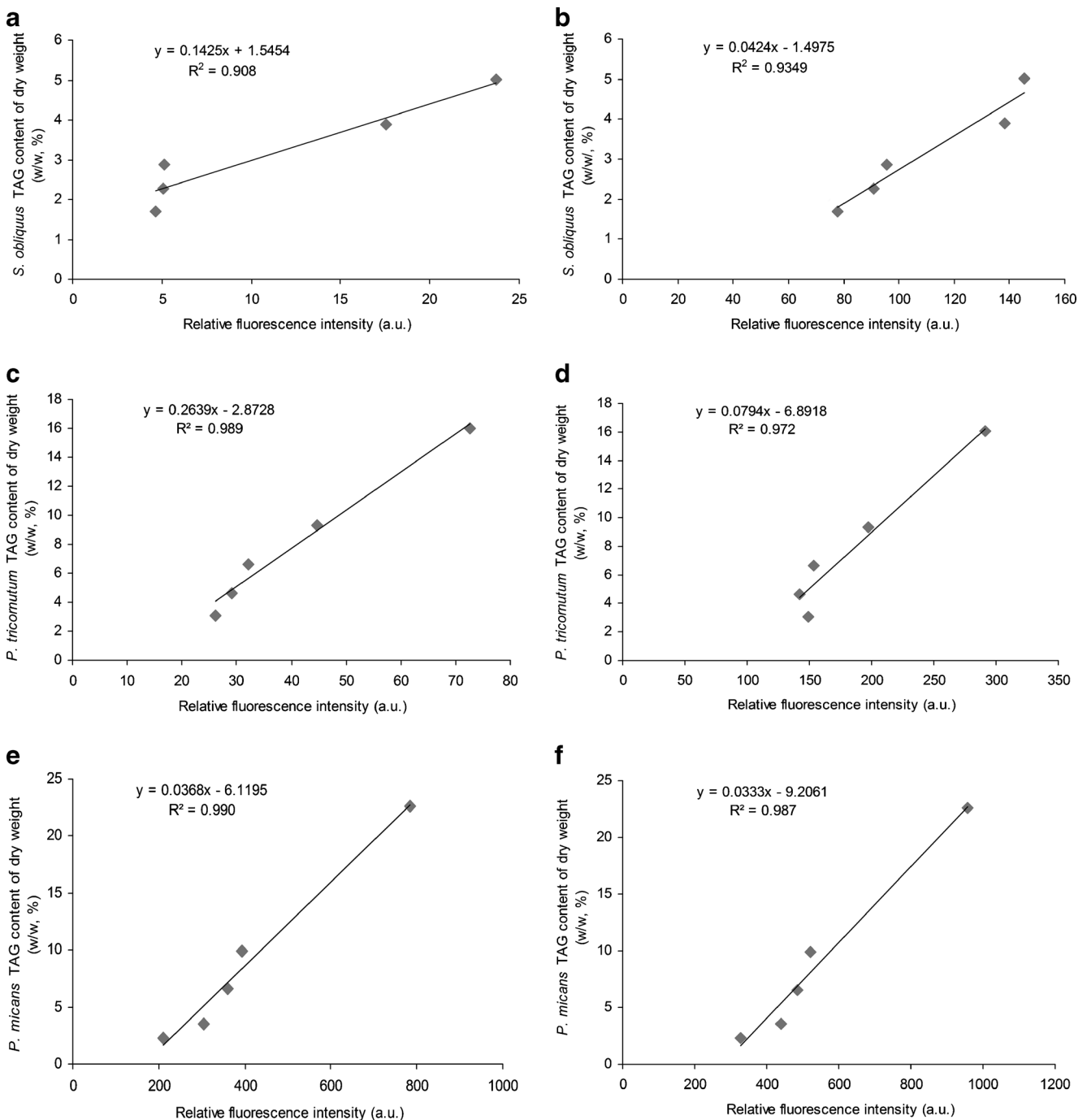
Fig. 6 Linear correlation between cellular fluorescence of stained cells and intracellular neutral lipid content of the three algae. Correlation of NR stained cells and neutral lipid content of **a** *S. obliquus*, **c** *P. tricornutum* and **e** *P. micans*. Fluorescence of BODIPY 505/515 stained cells response to neutral lipid content of **b** *S. obliquus*, **d** *P. tricornutum* and **f** *P. micans*

Examination of stain uptake by algal cells

Morphology of the three algae was observed by fluorescence microscopy excited by blue light. When stained with BODIPY 505/515, the lipid droplets in *P. tricornutum*, *P. micans* and *S. obliquus* cells showed green fluorescence.

When stained with NR, algal neutral lipid bodies showed characteristic orange fluorescence. The red color was due to chlorophyll auto-fluorescence. Confocal microscopic images seen in Fig. 7 show the distribution and volume changes of the neutral lipid droplets in the three algal cells. Both BODIPY 505/515 and NR were specific for intracellular neutral lipid
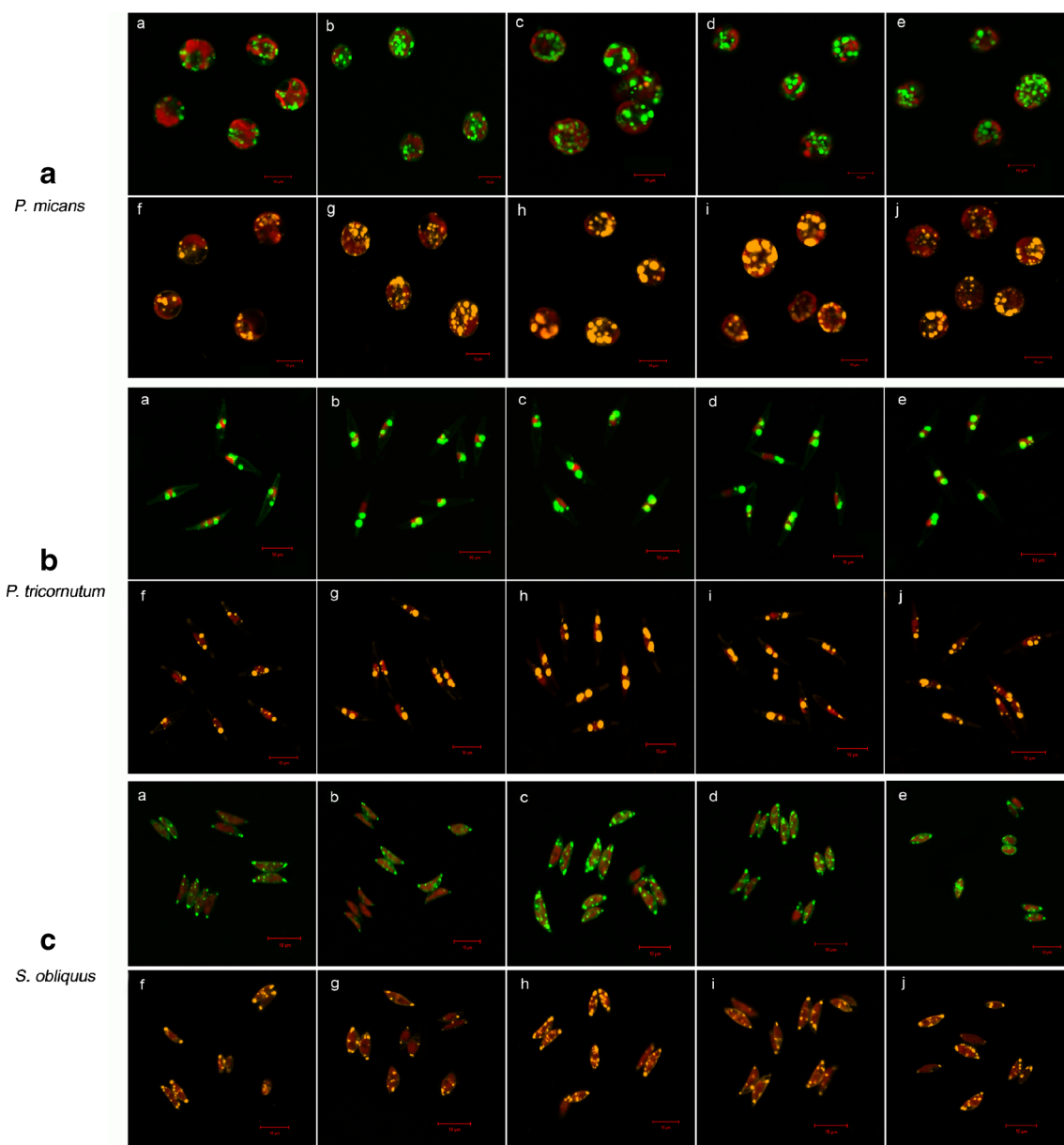
Fig. 7 Morphological changes of lipid droplets in *P. micans* (**a**), *P. tricornutum* (**b**) and *S. obliquus* (**c**) relative to cultivation times under nitrogen deprivation. Algal cells were stained by BODIPY 505/515 (a–e) and NR (f–j), and cultured for 3 days (a, f), 5 days (b, g), 7 days (c, h), 9 days (d, i), 11 days (e, j). Scale bar = 10 μm

bodies and the fluorescence did not quickly bleach during fluorescence microscopy observation, these results indicated both dyes possess good fluorescence photo-stability.

Discussion

Phaeodactylum tricornumtum, *P. micans* and *S. obliquus* are three main eukaryotic microalgal species which belong to different classes and have different evolutionary history. *P. micans* belongs to Prorocentraceae, has an un-intact cell wall

structure which is covered with patterned holes and crackles, and due to its particular cell wall structure two dyes can easily penetrate into the cell and combine with the lipid droplets. The diatom *P. tricornutum* belongs to the class Bacillariophyceae, and it has a unique cell wall with a regular pattern of small perforated pores thus allowing excellent permeability. Of these three algae species, the green alga *S. obliquus* has the most advanced evolutionary position and the most complete thick cell wall structure. When *S. obliquus* suspensions were stained with NR and BODIPY 505/515, and even after the use of DMSO it was difficult for both BODIPY505/515 and NR to

permeate into the algal cell. This indicated that the variation in cell wall structure and composition of the three algae species may cause different fluorescence characteristic. This view is consistent with the point that the intracellular NR fluorescence varies with different algae due to the structure and composition of the cell wall as described by Gao et al. (2008) and Chen et al. (2009). Furthermore, according to Brennan et al. (2012), some thick cell-walled green algae (e.g., *Nannochloropsis oculata*) are naturally difficult to stain using BODIPY 505/515, and solvent pre-treatments are required. These studies show that the cell wall structure and composition can influence the uptake of both fluorescence dyes. However, Cooper et al. (2010) and Govender et al. (2012) proposed that the cell wall structure and composition did not have an effect on BODIPY505/515 permeability. Although the algal strains they used for BODIPY 505/515 determination were all green algae, the different species may be differed in fluorescence characteristics. Compared with green algae, diatoms and dinoflagellates are more suitable for fluorescent measurement, as they have no pre-treatment requirements. Hence, NR and BODIPY505/515 staining can be used as a high-throughput approach to screen marine diatoms and dinoflagellates.

Consistent with the staining results, apparent linear relationships between relative fluorescence intensity of NR staining and neutral lipid content of *P. micans* and *P. tricornutum* were achieved with high correlation coefficients (*P. micans*: $R^2=0.990$; *P. tricornutum*: $R^2=0.989$). In contrast, the correlation coefficient (R^2)=0.908 ($P<0.05$) between the fluorescence intensity and the neutral lipid content obtained from *S. obliquus* using NR staining was lower. Although previous studies showed that the relative fluorescence intensity of NR stained cells and algal lipid content demonstrated a linear relationship with a high correlation coefficient of $R^2=0.99$ in *Chlorella vulgaris* (Liu et al. 2008; Chen et al. 2009; Huang et al. 2009) and $R^2=0.97$ in *Chlorella saccharophila* (Isleten-Hosoglu et al. 2012), the correlation coefficient differed in *S. obliquus*, *C. vulgaris* and *C. saccharophila*, which may be related to the complexity and the integrity of the cell wall composition and structure of these *Chlorella* spp. The dinoflagellate *P. micans* may be a potential oleaginous microalga for biodiesel production as it showed excellent staining results and a high correlation coefficient between the fluorescence intensity and neutral lipid content.

Dinoflagellates can bloom in nature. However, as shown in Fig. 3, the growth of *P. micans* was extremely slow, although it had high lipid production, only a small amount of biodiesel would be created due to low biomass accumulation during laboratory cultivation. With the increasing number of harmful algal blooms, thorough research into the causative algae and the mechanism involved has been carried out, however, this research was focused on ecological parameters. As the two major red-tide algae, diatoms and dinoflagellates are plentiful in marine ecosystems. Their growth that can be manually

controlled under laboratory conditions as *Chlorella* sp. and *Spirulina* sp., once their growth mechanisms are fully examined in the laboratory, will become possible. Both diatoms and dinoflagellates may be optimal candidate algae strains for biodiesel production.

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