MICROBIOLOGY

Characterization of a green microalga UTEX 2219-4: Effects of photosynthesis and osmotic stress on oil body formation

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ABSTRACT. Microalgae are an important potential feedstock for biodiesel production. Understanding the physiology of lipid biosynthesis in microalgae is pivotal to their management in aquaculture. A freshwater green microalgal strain, UTEX 2219-4, was isolated from UTEX 2219, which was reported to contain two strains. Its ITS sequences were closely related to those in the family Scenedesmaceae. Nitrogen starvation, salt stress and osmotic stress greatly enhanced lipid biosynthesis in this strain, while a combination of nitrogen deficiency and osmotic stress produced the most dramatic effects. Chloroplasts condensed and photosynthesis efficiency declined about 50% after 3 days of nitrogen starvation. Chlorophyll degradation followed the same trend but was more severe than the reduction of photosynthesis efficiency. Oil body formation was not observed in the cells kept in the dark under nitrogen starvation, suggesting that photosynthesis, rather than autophagy, is the main player in oil body formation. Under non-saturating light intensity levels coupled with nitrogen starvation, the oil body formation under 200 μmol/m²s light intensity was more efficient than that under 100 μmol/m²s. DCMU blocked both photosynthesis and oil body formation, again suggesting that photosynthesis, rather than autophagy, provides the energy for oil body formation during nitrogen starvation.

Keywords: Autophagy; Microalgae; Oil body; Osmotic stress; Photosynthesis.

INTRODUCTION

Seeking renewable feedstock for bioenergy production is imperative considering the current problems of excessive carbon dioxide emission and petroleum depletion. Biodiesel is one of the better choices among varieties of bioenergy, and microalgae are claimed to be the best crop for biodiesel production (Chisti, 2008; Hu et al., 2008). Most green microalgae produce starch under normal conditions. Upon exposure to nutritional stress, such as nitrogen or phosphate starvation, they start to synthesize triacylglycerol which is stored in their oil bodies (Shifrin and Chisholm, 1981). Isolation of new microalgal species that can adapt to tropical or subtropical environments with high growth rates and high lipid contents is pivotal to algal biodiesel production. Understanding the physiology of oil body formation in green microalgae is essential for effective aquaculture management and for the development of the microalgal biodiesel industry.

Oil contents of green microalgae increase significantly in a few days of nitrogen starvation treatment (Wang et al., 2009; Li et al., 2010; Moellering and Benning, 2010). Although the sensing and signaling process is still not clear, a nitrate transporter is proposed to function as a nitrate sensor in *Arabidopsis* (Ho et al., 2009). In nitrogen-deficient conditions, many organisms undergo autophagy to recycle part of the cytoplasm including organelles, which is a self-degrading process common in eukaryotes that provides needed energy and raw materials for cellular repair (Bassham et al., 2006; Yorimitsu and Klionsky, 2005). In *Chlamydomonas*, autophagy is also induced by nitrogen starvation and other stress (Perez-Perez et al., 2010). Whether the regained energy and raw materials are solely for life support or can be converted to storage lipids is unknown. Under nitrogen starvation, biosynthesis of amino and nucleic acid is arrested, but the light reactions of photosynthesis are still active. The cells have to prevent electron accumulation in the thylakoid membrane to minimize free radical production. The primary product of carbon fixation reactions is triose phosphate, which can be used for either starch or lipid biosynthesis. Biosynthesis of the two kinds of macromolecules does not require ni-

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trogen, thus they are good destinations for photosynthetic energy. However, it appears that lipid biosynthesis is more favored by oleaginous green microalga than starch under nitrogen starvation. No conclusive reasons for this phenomenon have been identified. According to our present results, there is apparently a mechanism(s) that alters the carbon partitioning of the cells to lipid biosynthesis upon exposure to nitrogen deficient conditions and osmotic stress. This mechanism(s) is useful from the perspective of biodiesel production because this switch(s) may control oil yield in microalgae.

Here we report the isolation and characterization of a green microalga UTEX 2219-4, that was misplaced in the genus *Chlorella* under the collection number UTEX 2219. UTEX 2219-4 is more closely related to species in the family Scenedesmaceae than to others. We found that the energy for lipid biosynthesis in these cells during nitrogen starvation came mostly from photosynthesis rather than from autophagy, and that osmotic stress presumably enhanced this lipid biosynthesis by further altering carbon partitioning towards lipid biosynthesis. The reason why oleaginous green microalgae are in favor of producing lipids rather than starch under nitrogen starvation is discussed.

MATERIALS AND METHODS

Microalgae and growth conditions

The freshwater green microalga UTEX 2219, under the registration name *Chlorella minutissima,* was purchased from the Culture Collection of Algae at the University of Texas at Austin (UTEX). It is noted in the UTEX web that this collection contains two strains (Kessler and Huss, 1992), (http://web.biosci.utexas.edu/utex/algaeDetail.aspx?algaeID=4682). We isolated one strain from this collection by growing single colonies and sequencing their rDNAs and internal transcribed spacers (ITS). This strain was designated as UTEX 2219-4. These single species cells were grown in a modified Bold 3N medium containing 4.4 mM $NaNO_3$, 0.17 mM $CaCl_2$, 0.3 mM $MgSO_4$, 0.22 mM K_2HPO_4, 0.65 mM KH_2PO_4, 0.43 mM NaCl, and the same levels of metals and vitamins described in Table 2 of Berges et al. (2001) on an orbital shaker at 150 rpm under continuous cool white light at room temperature. Light intensities are specified in the Results.

DNA extraction

Microalgal cells were harvested by centrifugation at 4000 rpm (Eppendorf 5810 R), 25°C for 5 min. The cells were broken by a beadbeater in 0.5 mL lysis buffer containing 0.3 M NaCl, 50 mM Tris-HCl (pH 8), 20 mM EDTA, 0.34 mM N-Lauroylsarcosine Sodium, and 1.75 M urea. This mixture was incubated at 65°C for 10 min, and spun at 13,200 rpm (Eppendorf 5415 R), 25°C for 5 min. The supernatant was transferred to a new tube, and 0.5 mL of phenol/chloroform (1:1) was added to this tube. After mixing to denature proteins, the tube was spun

again under the above conditions. The upper phase was transferred to a new tube, and an equal volume of isopropanol was mixed into the supernatant. After incubation at room temperature for 10 min, this tube was spun again under the above conditions. The supernatant was discarded, and the pellet was rinsed with 70% ethanol twice and dried in the air. The pellet was dissolved in 50 μL of TE buffer containing RNase.

PCR amplification and sequencing of rDNA

The 18 S rDNA was amplified by PCR using the primers: 18S forward-"TTTCTGCCCTATCAACTTTCGATG" and 18S reverse-"TACAAAGGGCAGGGACGTAAT". The ITS1-5.8 S-ITS2 region rDNA was amplified using the primers: ITS forward2: "CTAGAGGAAGGAGAAGTCGTAACA" and ITS reverse2: "TGATATGCTTAAGTTCAGCGG". The PCR products amplified by the two pairs of primers were single bands on the agarose gels. DNA sequencing was carried out by the dideoxy method using the primers above.

Phylogenetic analysis

Thirteen ITS2 sequences were aligned using the software Mega 4.1 (Tamura et al., 2007). Well-aligned regions were selected from each sequence, and used for phylogenetic analysis. There were a total of 178 positions in the final dataset. The Neighbor-Joining Method was used to construct the tree in Figure 1. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches.

Lipid extraction and oil body staining

Total lipids were extracted based on the methods described in Bligh and Dyer, 1959; and Chiu et al., 2008. Cells were harvested by centrifugation and smashed in 1 ml methanol chloroform mix (v/v = 2/1) using 0.5 g glass beads (Sigma G-9268, 425-600 μm) in a mini beadbeater. Cell debris was removed using centrifugation. The supernatant was transferred to a glass tube, and 1 mL chloroform and 1 mL 1% NaCl solution were added to this tube. These components were mixed well then centrifuged at 10,000 rpm for 5 min to allow phase separation. The chloroform phase was transferred to a new glass tube, blow-dried using nitrogen air and weighted. Oil bodies in live cells were stained by the fluorescence dye Nile Red (1 mg/mL in acetone) and imaged using a Nikon Eclipse 80i fluorescence microscope.

Measurement of photosynthesis efficiency and chlorophyll content

Photosynthesis efficiency was determined based on oxygen evolution rates. Ten mL of algal cells at 4×10^7 cell/mL density were gently harvested using centrifugation. These cells were resuspended in fresh medium with or without 0.68 M sorbitol as indicated in the results. These cells were kept in the dark for 10 min to deplete oxygen,

which was measured by a Clark-type oxygen electrode (Hansatech Inc. England). NaHCO$_3$ solution was then added to the sample to reach 3 mM, and the oxygen evolution rate of these cells was measured for 10 min under 100 μmol/m^{2}s light intensity at room temperature. DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) was purchased from the Sigma company, USA (Cat. # D-2425). Chlorophyll of the cells was extracted using 80% acetone. After mixing by vortex for 20 min, the sample was stored at 4°C overnight. The debris was pelleted by centrifugation, and absorbance at 645 and 663 nm of the supernatant was measured by a spectrophotometer. Chlorophyll content was calculated by the equation: Total chlorophyll (μg/mL) = 20.2(A$_{645}$) + 8.02(A$_{663}$); Chlorophyll a (μg/mL) = 12.7(A$_{663}$) - 2.69(A$_{645}$); Chlorophyll b (μg/mL) = 22.9(A$_{645}$) - 4.68(A$_{663}$).

Transmission Electron Microscopy (TEM)

The algal cells were fixed in 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.0 at 4°C for 4 hours. After three 20 min buffer rinses, the samples were postfixed in 1% OsO$_4$ in the same buffer for 4 hours at room temperature and then rinsed in three 20 min changes of buffer. Samples were dehydrated in an acetone series, embedded in Spurr's resin, and sectioned with a Lecia Reichert Ultracut S or Lecia EM UC6 ultramicrotome. The ultra-thin sections (70-90 nm) were stained with uranyl acetate and lead citrate. A Philips CM 100 transmission electron microscope at 80 KV was used for viewing.

RESULTS AND DISCUSSION

UTEX 2219-4 is closely related to members in Scenedesmaceae

The ITS1-5.8S-ITS2 and the 18S rDNA regions of UTEX 2219-4 were amplified and sequenced (GenBank accession numbers HQ218939 and HQ218940, respectively), and the ITS sequences were used to BLAST the GenBank. The results showed that the most closely related species to this green alga are members of the Scenedesmaceae family rather than the Chlorellaceae family, which comprises *Chlorella*. The ITS2 sequences of 12 closely related species representing 12 genera were aligned, and a phylogenetic tree was constructed based on the alignment using the Neighbor-Joining method. As shown in Figure 1, UTEX 2219-4 is most closely related to *Neodesmus danubialis*, which is a species in the Scenedesmaceae. Of the 12 species, two are mosses (*Plagiomnuim* and *Imbribryum*), one is a member of the Haematococcaceae family (*Haematococcus*) and the remaining are within Scenedesmaceae.

It is not surprising to find that mosses are closely related to green algae, it has already been well accepted that mosses are descendents of a green algal species. On the other hand, one might wonder how the UTEX 2219-4, that is supposed to be a *Chlorella* species, can be closely related to members belonging to Scenedesmaceae and Haematococcaceae, rather than to Chlorellaceae. The taxonomy of green algae has been found increasingly inaccurate as more microalgal genomes are sequenced. For example, *Chlorella vulgaris* C-169 has been renamed *Coccomyxa* sp. C-169 after the Joint Genome Institute sequenced its genome (http://genome.jgi-psf.org/Chlvu1/Chlvu1.home.html). Therefore, there is a need to regroup microalgal taxonomy based on molecular markers.

UTEX 2219-4 contained both starch granules and oil bodies after three days of nitrogen starvation

Most green microalgae produce starch as an energy reservoir under normal conditions. In nitrogen-deficient conditions, they start to accumulate triacylglycerol, which is stored in oil bodies (Moellering and Benning, 2010; Wang et al., 2009; Li et al., 2010). A few possible energy and carbon sources for the triacylglycerol biosynthesis at this
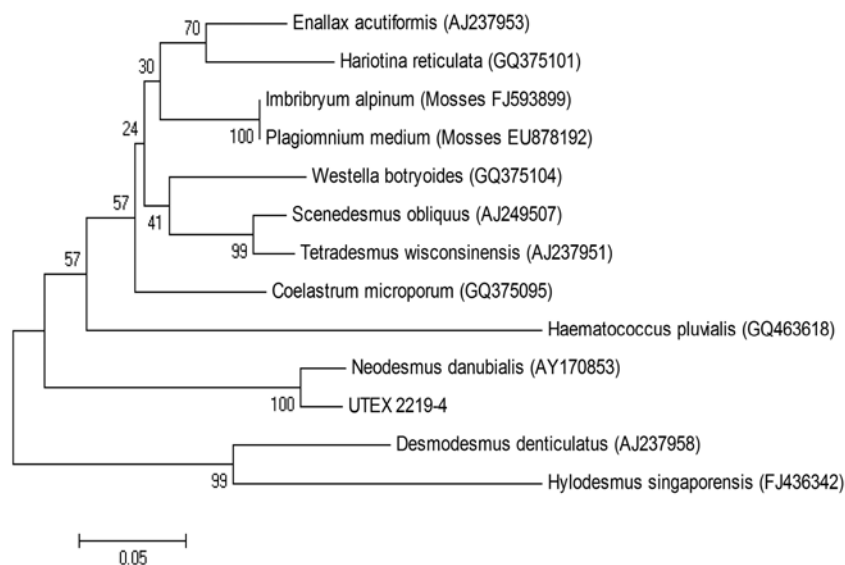

Figure 1. Phylogenetic analysis of UTEX 2219-4 and its closely related species based on ITS2 sequences in GenBank. ITS1 and ITS2 sequences of UTEX 2219-4 were used to BLAST in GenBank, and the 12 most closely related species, representing 12 genera, were selected for phylogenetic analysis. This tree was constructed based on the well-aligned regions in ITS2 sequences of each species. This tree showed that UTEX 2219-4 is closely related to species in the family Scenedesmaceae rather than to Chlorellaceae species. The numbers in the parenthesis are the accession numbers of the respective ITS sequences in GenBank. The scale bar measures the distance between species. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches.

stage include photosynthesis, autophagy and the degradation of chloroplast constituents such as their membrane, pigments, pyrenoid, and starch granules. Autophagy is a common phenomenon among eukaryotes when they are exposed to nitrogen-deficient conditions. Part of the cytoplasm, sometimes including organelles, is transported into lysosomes or vacuoles for recycling (Chen et al., 2009). To gain more insight into the physiological changes before and after nitrogen starvation stress, the subcellular structures of UTEX 2219-4 were examined by transmission electron microscopy. As shown in Figure 2, the cell had a relaxed chloroplast with a low quantity of starch prior to nitrogen starvation, compared to the cell after 3 days of nitrogen starvation, which showed a more condensed chloroplast with oil bodies and significant amount of starch granules. The condensed chloroplast suggests damage due to nitrogen starvation. The increased quantity

of starch granules, and its co-existence with oil bodies in these cells, suggests that carbon sources in these cells during nitrogen starvation were not only allocated to storage lipid production but also to starch biosynthesis. This phenomenon leaves room for carbon partitioning manipulation in the cells.

It was reported very recently that wild type *Chlamydomonas* has the same phenomenon (Li et al., 2010). In wild type *Chlamydomonas*, a large quantity of starch granules was observed after two days of nitrogen starvation. Upon blocking its starch biosynthesis pathway, the mutant *Chlamydomonas* produced storage lipids almost exclusively. Thus, it seems whether a green microalga is oleaginous depends on the energy partitioning between starch and lipid biosynthesis when under nitrogen starvation.

Osmotic stress enhances lipid biosynthesis in both with or without nitrogen source in microalgal cells

An insignificant ratio of UTEX 2219-4 cells contained very low quantities of oil bodies before nitrogen starvation treatment. When these cells were shifted to the nitrogen starvation conditions for 3 days, their lipid content increased about 50% as shown in Table 1A, compared to the lipid level before nitrogen starvation. When the cells were treated with nitrogen starvation coupled with 0.34 M (2%) NaCl or KCl salt stress, their lipid contents increased by 80% and 66%, respectively. To our surprise, when the cells were treated with nitrogen starvation coupled with 0.68 M sorbitol, which has the same osmolarity as 0.34M NaCl and KCl, their cell lipid content increased by 150% after 3 days. We wondered whether osmotic stress alone has the same effect. As shown in Table 1B, when non-starved cells were treated in 0.68 M sorbitol for 3 days, their lipid content reached 33% of the biomass. This lipid level was higher than those cells nitrogen-starved for 3 days, and
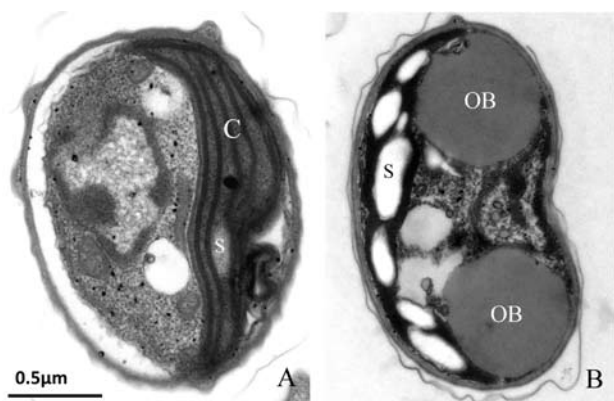

Figure 2. TEM of UTEX 2219-4 before and after nitrogen starvation. These algal cells were grown in modified Bold 3N medium under continuous 200 μmol/m² s light intensity until OD_{682} = 1. (A) Before nitrogen starvation, (B) After three days of nitrogen starvation. C: Chloroplast; S: Starch granule; OB: Oil body.

Table 1. Lipid contents of UTEX 2219-4 in various conditions. These algal cells were grown in Bold 3N medium under continuous 100 μmol/m² s light intensity until OD_{682} = 0.5, and subjected to different treatments.

A. These cells were nitrogen-starved for 3 days, with or without salt or sorbitol stress. Mean ± SD with three repeats.

Culture condition	Lipid (mg/L)	Biomass (dry weight, mg/L)	Lipid content (% of dry weight)
Before nitrogen starvation	19 ± 1.15	113 ± 5.77	16.53 ± 1.60
Nitrogen starvation for 3 d	33 ± 4.16	133 ± 5.77	24.95 ± 2.04
Nitrogen starvation with 0.34M NaCl for 3 d	40 ± 3.46	133 ± 11.55	30.08 ± 2.55
Nitrogen starvation with 0.34M KCl for 3 d	39 ± 3.06	143 ± 15.28	27.58 ± 2.78
Nitrogen starvation with 0.68M Sorbitol for 3 d	53 ± 3.06	126 ± 11.55	42.30 ± 4.09

B. These cells were grown in normal conditions until OD_{682} = 0.5. Sorbitol solution was added to reach 0.68 M to create osmotic stress. Equal volume of water was added into the control. The treatment was lasted for 3 days. Mean ± SD with three repeats.

Culture condition	Lipid (mg/L)	Biomass (dry weight, mg/L)	Lipid content (% of dry weight)
Normal conditions (control)	22 ± 0.53	117 ± 5.57	18.48 ± 0.51
0.68M Sorbitol stress for 3 d	40 ± 1.67	121 ± 6.11	32.88 ± 1.39

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comparable to the cells under nitrogen starvation coupled with 0.34 M salt stress.

It is well known that nitrogen starvation enhances lipid biosynthesis in green microalgae (Shifrin and Chisholm, 1981; Wang et al., 2009; Li et al., 2010; Moellering and Benning, 2010). The effects of osmotic stress on oil body formation, on the other hand, are less studied. In the case of *Dunaliella* in which beta-carotene biosynthesis is enhanced by salt/osmotic stress, the beta-carotene droplets are formed within chloroplasts (Lamer set al., 2008). To our knowledge, this is the first report that osmotic stress enhances oil body formation in microalgae. How the signals of nitrogen starvation or osmotic stress are sensed and executed are still elusive. Nitrogen starvation is one important cue for terrestrial plants to switch from a vegetative to a reproductive stage. When terrestrial flowering plants face nitrogen deficiency, their leaves turn yellow starting from

the oldest ones, and the nutrients in the leaves are mobilized to young leaves, flowers and eventually to seeds, which are best able to survive harsh environments. There are two scenarios that try to explain why green microalgae produce lipids when under nitrogen starvation. The first theorizes that more lipids in the cells increases buoyancy, so that it is easier for the cells to drift to new niches. The second proposes that when microalgal cells are undergoing nitrogen starvation, they are not able to consume ATP and NADPH by synthesizing amino or nucleic acids. One way to quench the electrons in the thylakoid membrane is to synthesize lipids, which do not require nitrogen. However, it is equally true that starch synthesis does not require nitrogen. But by doing so, cell buoyancy will be decreased. Actually the two hypotheses are not mutually exclusive and can be combined into one. We propose that lipid production in green microalgae is an adaptation to cope with
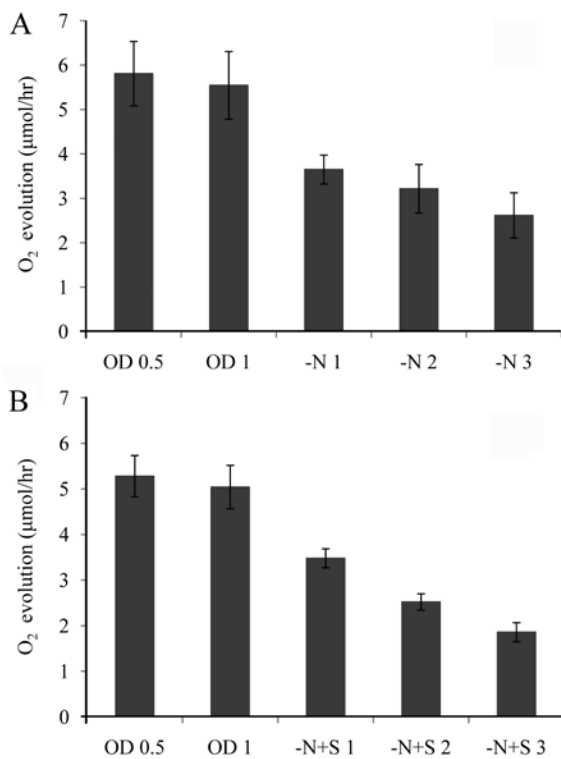

Figure 3. Photosynthesis efficiency of UTEX 2219-4 in various conditions. These algal cells were grown in modified Bold 3N medium under continuous 200 μmol/m² s light intensity until OD_{682} reached 0.5 or 1, when photosynthesis efficiency was measured. At OD_{682} = 1, cells were further subjected to nitrogen starvation (-N) or nitrogen starvation plus 0.68 M sorbitol (-N+S) for different days under the same light intensity. (A) Column OD0.5, OD1, -N1, -N2, and -N3 represent photosynthesis efficiency of cells at OD_{682} = 0.5, 1, and cells at OD_{682} = 1 stressed by nitrogen starvation for 1, 2, and 3 days, respectively. (B) Column OD0.5, OD1, -N+S1, -N+S2, and -N+S3 represent photosynthesis efficiency of cells at OD_{682} = 0.5, 1, and cells at OD_{682} =1 stressed by nitrogen starvation coupled with 0.68M sorbitol for 1, 2, and 3 days, respectively. The error bars represent standard deviations from three repeats.
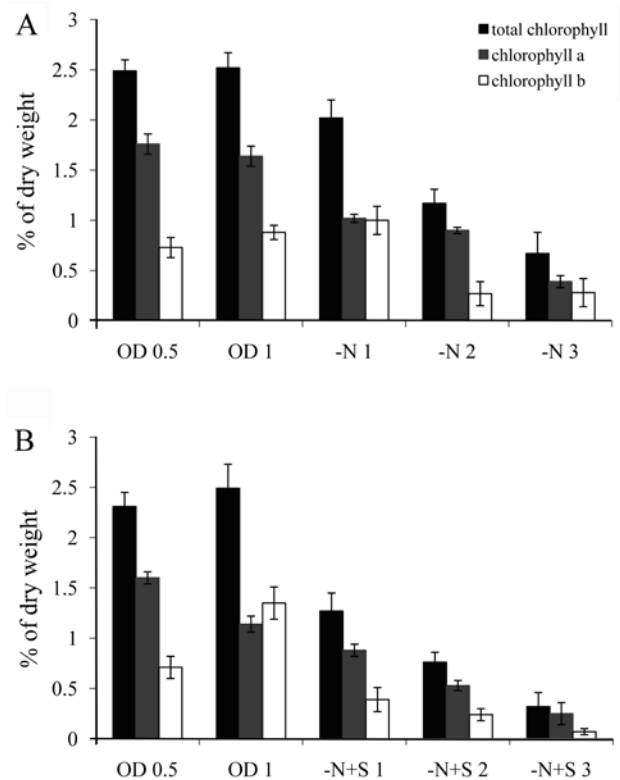

Figure 4. Chlorophyll content of UTEX 2219-4 under various conditions. These algal cells were grown in modified Bold 3N medium under continuous 200 μmol/m² s light intensity until OD_{682} reached 0.5 or 1 when chlorophyll content was measured. At OD_{682} = 1, cells were further subjected to nitrogen starvation (-N) or nitrogen starvation plus 0.68 M sorbitol (-N+S) for different days under the same light intensity. (A) Column groups OD0.5, OD1, -N1, -N2, and -N3 represent chlorophyll contents of cells at OD_{682} = 0.5, 1, and cells at OD_{682} = 1 stressed by nitrogen starvation for 1, 2, and 3 days, respectively. (B) Column groups OD0.5, OD1, -N+S1, -N+S2, and -N+S3 represent chlorophyll contents of cells at OD_{682} = 0.5, 1, and cells at OD_{682} = 1 stressed by nitrogen starvation with 0.68M sorbitol for 1, 2, and 3 days, respectively. The error bars represent standard deviations from three repeats.

starvation, because lipid biosynthesis is not only a way to quench electrons in the chloroplast but also to adjust buoyancy of the cells that contain starch, so that their probability of drifting away from a harsh environment is enhanced, especially for those microalga without flagellates.

Osmotic stress imposes a water availability problem within the cells, which impairs physiological and biochemical activities. Abilities to escape from or adapt to osmotic stress determine the success of a species. In terrestrial plants, many biochemical modifications are adopted to cope with osmotic stress such as drought and high salt levels. One adaptation is the re-enforcement of the wax layer that covers aerial parts and suberin in the roots to prevent water loss. Under osmotic stress, expression of genes involved in very-long-chain fatty acids biosynthesis are upregulated to provide the precursors for wax and suberin biosynthesis (Lee et al., 2009). Apparently, an increase in lipid biosynthesis in microalgal cells under osmotic stress also increases their probability of drifting to new waters. It is interesting to see the differences in physiological functions between terrestrial plant and microalgal lipid usage when under osmotic stress.

Photosynthesis efficiency decreased in nitrogen-starved microalgal cells

Since lipid biosynthesis in the cells was enhanced during nitrogen starvation, we further investigated changes in

their photosynthetic efficiency before and during nitrogen starvation. Photosynthetic efficiency and total chlorophyll content of these cells at $OD_{682} = 0.5$ or 1 was about the same (Figures 3 and 4, respectively). When these cells at $OD_{682} = 1$ were subjected to either nitrogen starvation or nitrogen starvation coupled with 0.68 M osmotic stress, photosynthetic efficiency declined about 35% on the first day. The efficiency declined further, but at a slower pace, on the second and third days, The efficiency of the cells under double stress conditions declined more than that under nitrogen starvation alone. Total chlorophyll content followed a similar trend but its reduction was more severe than that of photosynthetic efficiency.

It is intriguing to consider how, if the energy and carbon sources for enhanced lipid biosynthesis were from photosynthesis, the double stressed cells could produce more lipids than the cells under nitrogen starvation alone, especially since the photosynthetic efficiency was lower in the double stressed cells. This suggests that there may be an energy source other than photosynthesis involved in lipid biosynthesis, or that carbon partitioning in the downstream of photosynthesis was further shifted in the double stressed cells. As mentioned above, autophagy is a common self-degrading process that occurs in eukaryotic cells undergoing nitrogen starvation. One might assume in this case, that autophagy supplies energy and a carbon source for lipid biosynthesis. However, we did not observe
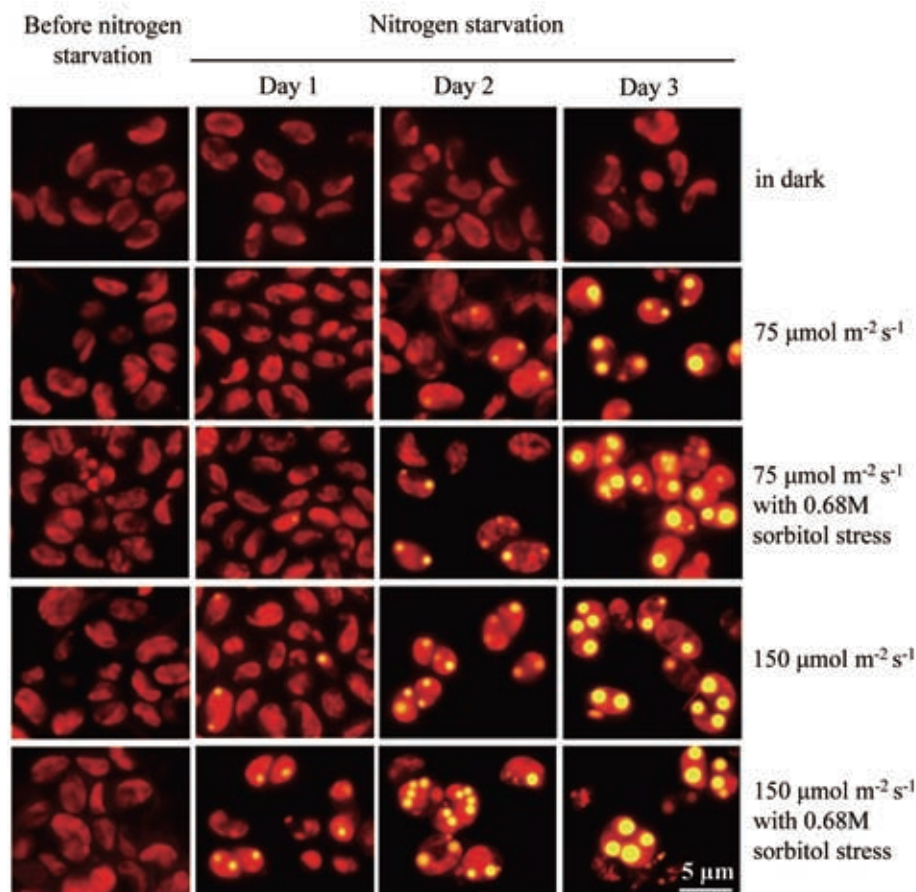

Figure 5. Oil body quantity of UTEX 2219-4 in four conditions stained by Nile Red. The yellow fluorescence is from oil bodies and the red from chloroplasts. These algal cells were grown in continuous 100 μmol/m^2 s or 200 μmol/m^2 s in modified Bold 3N medium until $OD_{682} = 0.5$, followed by nitrogen starvation for three days with or without 0.68 M sorbitol stress. The higher oil body formation efficiency in both groups under 200 μmol/m^2 s compared to their respective 100 μmol/m^2 s counterparts suggests that photosynthesis, rather than autophagy, plays the major role in oil formation when algal cells under nitrogen starvation.
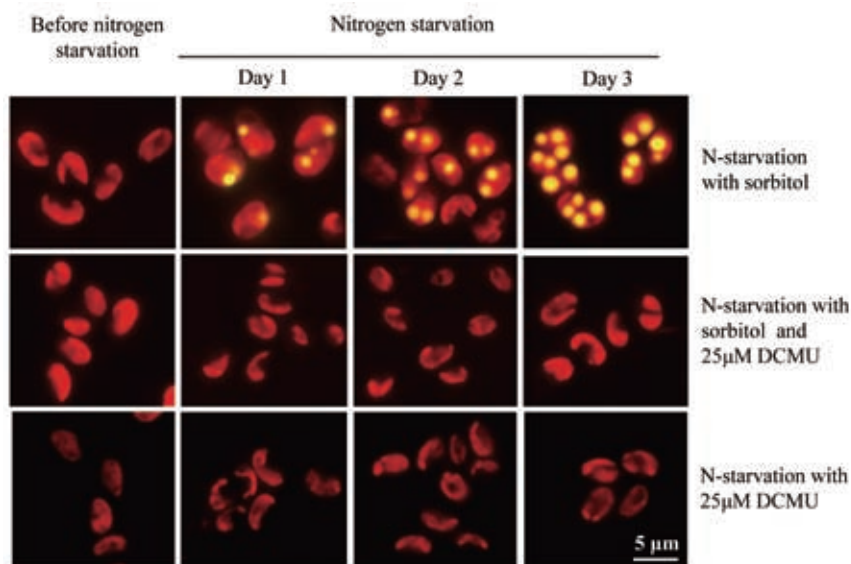
Figure 6. DCMU blocks oil body formation. The yellow fluorescence is from oil bodies and the red from chloroplasts. These algal cells were grown in modified Bold 3N medium under continuous 200 $\mu mol/m^2 s$ light intensity until OD_{682} = 1, followed by nitrogen starvation with or without 0.68 M sorbitol and 25 μM DCMU for three days. Since DCMU blocks the electron transport chain in the chloroplasts and no oil body is observed in the treated cells, photosynthesis, rather than autophagy, provides most of the energy for triacylglycerol biosynthesis when algal cells are under nitrogen starvation.

autophagy playing a significant role in the enhanced lipid biosynthesis (see below). The explanation of carbon partitioning change is thus more favored than is autophagy. We propose that, when nitrogen starvation is coupled with osmotic stress, that the energy and carbon source for biosynthesis is shifted from starch to lipid, resulting in a higher lipid content in the double stressed cells than in the nitrogen starved ones.

Energy for lipid biosynthesis in green microalgae under nitrogen starvation is mostly from photosynthesis

We did not observe oil body formation in nitrogen-starved algal cells kept in the dark (Figure 5), which suggests that autophagy does contribute much if any energy to oil body formation. If it is true that the energy for microalgal oil body formation under nitrogen starvation is derived from photosynthesis, it is expected that, under below saturation level of light intensity for photosynthesis, oil body quantity in the cells under nitrogen starvation would have a positive correlation to light intensity. As expected, the oil quantity of cells starved under 100 $\mu mol/m^2 s$ was significantly lower than that of cells starved under 200 $\mu mol/m^2 s$ starting on Day 2, as shown in Figure 5. The same pattern was observed for the nitrogen-starved cells under 100 $\mu mol/m^2 s$ and 200 $\mu mol/m^2 s$ with osmotic stress starting from Day 1. The results support our hypothesis that photosynthesis supplies most of the energy for oil body formation in green microalgae under nitrogen starvation.

To further verify this hypothesis, DCMU was used to block electron transport in the chloroplast to examine oil body formation. As shown in Figure 6, 25 μM DCMU completely suppressed oil body formation under nitrogen starvation with or without osmotic stress. DCMU competes against plastoquinone for a site in the D1 protein of the PSII. Once DCMU binds to this site, electron transport is blocked, effectively shutting down photosynthesis (Trebst, 2007). Twenty-five μM DCMU was selected

based on the minimal concentration necessary to suppress oxygen evolution of the cells. The cells stayed green and looked normal under the microscope through their 3 days of treatment.

Our results show that the energy required for lipid biosynthesis under nitrogen starvation is mostly derived from photosynthesis and not other processes such as autophagy or chloroplast constituent degradation, and that carbon partitioning downstream photosynthesis is an important factor for efficient oil body formation in green microalgae.

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綠藻 UTEX 2219-4 之研究：光合作用與滲透壓逆境對於油粒體之形成的影響

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　　微藻是生質柴油的重要潛在來源之一。瞭解微藻的脂質合成之調控機制有助於生質柴油的生產管理。我們從 UTEX 2219 中分離出一株淡水綠藻並命名為 UTEX 2219-4。以它的 ITS 序列做親緣關係研究，結果顯示這株綠藻與 Scenedesmaceae 科中物種較為相近。缺氮、高鹽及滲透壓逆境使 UTEX 2219-4 的脂質含量快速上升，而以缺氮及滲透壓逆境併用之效果最好。光合作用效率及葉綠素含量在缺氮下快速下降。在缺氮及黑暗中，UTEX 2219-4 沒有油粒體形成。在缺氮及不飽和照度下，UTEX 2219-4 的油粒體在 200 μmol/m²s 之下多於在 100 μmol/m²s 下，而 DCMU 阻斷了油粒體的形成。以上結果顯示生成油粒體的能源主要來自光合作用，而非從自噬作用的生理調節而來。

關鍵詞：自噬作用；微藻；油粒體；滲透壓逆境；光合作用。