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Original Article

Impacts of Nitrogen Starvation on Expression Profiles of Involving Genes in Triacylglycerol Biosynthesis in *Chlamydomonas reinhardtii*: Towards Microalgal Systems Biotechnology

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ABSTRACT

Objective: *Chlamydomonas reinhardtii*, the most commonly studied model organism in microalgal kingdom, is known to be a rich lipid producer. **Methods:** Here, a naturally isolated *C. reinhardtii* strain was cultivated phototrophically at Erlenmeyer scale in BG11 culture medium. After reaching to stationary growth phase, a nitrogen limited regime with different nitrogen concentrations was applied. **Results:** Quantitative real time PCR analysis of *DGTT1* and *DGTT2* genes involved in triacylglycerol accumulation confirmed an increased level of expression for *DGTT1* (21.05-fold increase) in nitrogen starvation compared with the nitrogen rich conditions. Besides, *DGTT2* transcript levels showed a slight decrease in nitrogen deprivation condition (0.61-fold). **Conclusion:** The results opened a new way to direct microalgal systems biotechnology towards maximization of triacylglycerol production for green energy production.

Key words: *Chlamydomonas reinhardtii*, Expression profile, Nitrogen starvation, Systems biotechnology, Triacylglycerol.

Key message: The expression profiles of *DGTT1* and *DGGT2* genes change during nitrogen starvation in *C. reinhardti*.

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INTRODUCTION

In recent years, with rising prices for fossil fuels, apprehensions about the completion of a variety of energy resources and global concerns about environmental pollution caused by fossil fuels utilization, the global needs for clean and renewable sources of energy, is demanded more than ever.[1] Among the various alternative energy sources, exploitation of eukaryotic microalgae, as a renewable and affordable fuel source, seems very feasible, and nowadays many studies have been conducted by researchers all around the world.[2]

Microalgae are fast growing organisms with high contents of lipids which make them reproducible and reliable sources for biodiesel production. Nevertheless, the biggest problem in the way of their use, is their poor lipid productivity and yield and the final costs of biodiesel production. Therefore, approaches to increase the lipid productivity and reducing the final costs seems critical.[3]

Changes in environmental conditions such as temperature, light intensity and number of minerals in the medium, such as adding iron or urea nitrogen or phosphorus to culture medium or lowering its amounts makes up the bulk of microalgae lipid content in the form of triacylglycerol (TAG) stores.[4] TAG is obtained from a combination of three fatty acids esterified with glycerol. All eukaryotic organisms can synthesize TAGs, and the main constitute of the lipid contents in vegetable oils and animal fats is considered to be TAG.[5] Nevertheless, the details of the molecular mechanisms involved in TAG accumulation phenomenon is still in its infancy. Although, the eukaryotic microalga *Chlamydomonas reinhardtii* has not been regarded as a biodiesel producer in industrial scale, it has been considered as the preferred molecular model organisms over other species of microalgae.[6] Besides, due to its available complete genome sequence[7], it has been regarded as an attractive model to study many biological phenomena such as starch or lipid metabolism, energy carrier synthesis, photosynthesis, or cellular responses to nutrient starvation. *C. reinhardtii* is capable of phototrophic or heterotrophic metabolism and shows a high responsive capability against the environmental stressors in the form of metabolic changes. However, the details of TAG metabolism in *C. reinhardtii*, has been merely studied. On the other hand, the TAG production pathway is a vital part to optimize a robust process for microalgal biodiesel production.

Two distinct acyltransferases, diacylglycerol transferases (DGAT) and phospholipid: diacylglycerol acyltransferase (PDAT) are involved in TAG biosynthesis in different organisms.[8] After complete genome sequencing of *C. reinhardtii* microalga[7], it was discovered that there is only one type 1 DGAT (DGAT1) in its genome, whilst five different encoding gene are responsible for type 2 DGAT (*DGTT1* to *DGTT5*).[9]

Some studies have been dealt with the mechanisms involved in TAG production during nutrient depletion in *C. reinhardtii*.[10,11] Nevertheless, there is still a controversy in the results of previous studies and the exact role of each acyltransferase enzyme in TAG accumulation phenomena is still unveiled.

Here we investigated the expression profile of *DGTT1* and *DGTT2* genes during nitrogen limitation in naturally isolated *C. reinhardtii* strain. Beside its growth kinetics, biomass and lipid production and productivity and TAG content of lipids in presence of different nitrogen levels were also examined. The molecular detailed of TAG accumulation phenomena might be useful to increase the biosynthesis of TAG, improve the lipid productivity and reduce the operating costs of microalgal biodiesel production. Besides, the results of this study could be used for *C. reinhardtii* systems biotechnology towards overproduction of TAG and other valuable bioactive compounds.

MATERIALS AND METHODS

Strain and culture medium

The C. reinhardtii (Chlorophyta, Chlorophyceae) strain obtained from the Microalgal Culture Collection of Shiraz University of Medical Sciences (MCCS), Shiraz, Iran was cultivated in sterile BG11 culture medium composed of (per liter): $NaNO_3$ (1.5 g); K_2HPO_4 (0.04 g); $MgSO_4.7H_2O$ (0.075 g); $CaCl_2.2H_2O$ (0.036 g); citric acid (0.006 g); ferric ammonium citrate (0.006 g); Na_2EDTA (0.001 g); Na_2CO_3 (0.02 g) and the trace element solution (10 mL) (H_3BO_3 (2.86 mg L^{-1}); $MnCl_2.4H_2O$ (1.81 g L^{-1}); $ZnSO_4.7H_2O$ (0.222 g L^{-1}); $Na_2MoO_4.2H_2O$ (0.39 g L^{-1}); $CuSO_4.7H_2O$ (0.079 g L^{-1}); $Co(NO_3)_2.6H_2O$ (0.0494 g L^{-1})).[12] After examining the uniformity and possible contamination in the obtained microalgal sample, the growth study of *C. reinhardtii* was observed for 21 days. The experiment performed in 500 mL Erlenmeyer flasks containing 200 mL of culture media. The flasks were incubated at 25 °C in an orbital incubator shaker (PECO, Iran). The cultivation conditions for 96h were set at the agitation rate of 130 rpm and the light intensity of 60 mol m^{-2} s^{-1} to obtain the final cell concentration of about 30×10^4 cell mL^{-1}. After 10 days of cultivation, this primary seed culture was employed as a standard inoculum for further studies in shake flask experiment.

Cultivation conditions

Two modes of cultivation including normal and nitrogen starved conditions. In normal cultivation mode, the pre-cultured *C. reinhardtii* cells were transferred to three distinct 500 mL Erlenmeyers containing 200 mL of fresh BG11 medium. The cultivation procedure was continued for another 11 days under previously described conditions. In nitrogen starvation mode, *C. reinhardtii* cells from previous section was initially centrifuged at 3000×*g*, 20ºC for 5 min. The separated microalgal cells were washed using an isotonic normal saline solution (0.09 g NaCl L^{-1}) twice to remove the possible cell debris. The obtained pellet was then resuspended in 200 mL of nitrogen starved medium (containing the BG11 medium without $NaNO_3$). The starvation experiment was also exploited in triplicate and the mean values for the observed trends were reported.

RNA extraction procedure

The microalgal cells were centrifuged at 3500 rpm, 4ºC for 5 min and then the total RNA content was isolated using a commercially available RNA extraction kit (catalog number: RN7713C) (RNX™-PLUS buffer provided by SinaClon company, Tehran, Iran) according to the provided protocol by manufacturer. Briefly, 1 mL of the ice cold RNA™-PLUS solution and 1 mL of the microalgal supernatant were transferred to a poly propylene tube. It was vortexed for 10 sec and then incubated for 5 min at room temperature. 200 µL of chloroform was as added to the mixture and mixed for 15 sec. It was then incubated on ice for 5 min at 0 ºC. Next it was centrifuged at 12000 rpm at 4ºC for 15 min. The obtained aqueous phase was transferred to a new 1.5 mL RNase-free tube and an equal volume of isopropanol was added to it. It was gently mixed and incubated on ice for 15 min at 0 ºC. The mixture was centrifuged at 12000 rpm at 4 ºC for 15 min. The obtained supernatant was discarded and ethanol (75%, 1 mL) was added to the tube. It was vortexed for 10 sec to dislodge the pellet. It was then centrifuged at 7500 rpm and 4 ºC for 8 min. The supernatant was removed and the pellet was remained (5 min) till drying at room temperature. The resulted pellet was dissolved in DEPC treated water (50 µL). To improve the dissolving process, the test tube was placed in55 ºC water bath for 10 min. The purified total RNA was quantified using spectrophotometrically (Pico Drop P200, Alfa Biotech, Glasgow, UK). The prepared RNA was then treated with DNase I (RNase-free) kit (E. C. 3.1.21.1, catalog number: AM2222) (Thermo Fischer Scientific, Massachusetts, USA) to reduce the possible genomic DNA contents. To synthesize the first strand cDNA sequence, RevertAid Reverse Transcriptase (200 U μL^{-1}) (catalog number: EP0441) (Thermo Fischer Scientific, Massachusetts, USA) was employed with oligo dT primers in the final volume of 20 µL.

Primer design

AlleleID® software version 7.83 for windows, PREMIER Biosoft, Palo Alto, CA, USA was used for designing the target primers for *18S rRNA* gene (FJ864686.1) from another *C. reinhardtii* strain[13] as an internal control gene, *DGTT1* (KC788199) and *DGTT2* (KC788200) genes.[9] The designed forward and reverse primers for three mentioned genes with their amplification length and other properties are presented in Table 1.

Real time PCR protocol

Real-time PCR experiment was performed using MiniOpticon thermal cycler system (Bio-Rad Laboratories Inc., Hercules, CA, US) with Opticon Monitor™ analysis software control. The reaction was exploited in a total volume of 20 µL. In each tube, 1 µL of cDNA, 10 µL SYBR® Premix Ex Taq ™ II (TaKaRa, Japan) and 4 pmol of each designed primer was used. The employed real-time PCR cycle were optimized at 94°C for 2 min as initial denaturation step, followed by 40 cycles of amplification at 94°C for 10 sec, differing respect to the annealing temperature (Ta) for each employed primer for 15 and 30 secs of extension (72°C). Besides, two different negative controls including a reaction without reverse transcriptase enzyme and another without cDNA template were also examined. At the end of PCR reaction, the amplified products were used to analyze the dissociation curves. Moreover, it was confirmed that there is only a PCR product for each PCR reaction. Triplicate experiments were performed for each sample. To determine the relative fold of quantification, adopted DDCT method[14] was employed. The observed differences at a level of 5% was regarded as significant. GraphPad prism version 6.00 (GraphPad Software, La Jolla California, USA) was employed for the statistical analysis.

Table 1: The sequence of employed primers for real-time PCR amplification, its melting temperature (Tm) and its resulted product size

Primer	Primer Length (bp)	Tm (ºC)	Amplicon length	Sequence
DGTT1 F	23	56.52	996	ACATAATAGAGGTACAGCAGGAA
DGTT1 R	20	64.07	996	CGCTGGCCATCGTGCAATGA
DGTT2 F	20	56.55	975	ATGGCGATTGATAAAGCACC
DGTT2 R	20	62.23	975	TCAGCTGATGACCAGCGGTC
18S rRNA F	25	57.31	148	GTCAGAGGTGAAATTCTTGGATTTA
18S rRNA R	21	62.44	148	AGGGCAGGGACGTAATCAACG

Compositional analysis

To determine the growth profile of the studied microalga, dry cell weight method was employed. 10 mL of each cultures medium was aseptically transferred to a test tube and centrifuged for 5 min at 10000 *g* and 4 °C. The obtained *C. reinhardtii* pellets were washed twice with an isotonic normal saline solution (0.09 g NaCl L^{-1}). To remove the possible impurities, it was centrifuged again. The final microalgal pellets were dried at 95 °C overnight. The total lipid contents of *C. reinhardtii* was extracted using a previously adopted protocol with some modifications.[15] Briefly, 1 g of biomass was boiled for 2 min in isopropanol followed by homogenization in chloroform-methanol (1:2) mixture with butylated hydroxytoluene (BHT) (0.05% w/v) as antioxidant. Then a mixture of water and chloroform (0.8 and 5 mL respectively), enriched by an aqueous solution of potassium chloride (0.88 w/v) was added. After vigorous shaking, the obtained solvent phase was collected and then dried under nitrogen gas. To investigate the total lipid content of *C. reinhardtii*, the gravimetric method was utilized.

After cell lysis using the rapid heating and cooling method, TAGs were measured using cooled-blank method with an assay kindly provided by Taylor L. Weiss, Department of Biology, Washington University, Saint Louis, US (personal communication)., It includes three 1.5 mL tubes each containing final volumes of 400 µL. Distilled water (400 µL) was used as blank. The glycerol standard solution was 2.5 mg mL^{-1} equivalent triolein concentration (catalog number: G7793, Sigma-Aldrich, Saint Louis, MO, US). 380 µL of above mentioned glycerol standard solution was mixed with 20 µL distilled water before application. The sample cuvette contained 20 µL of the extracted cell lysate and 380 µL of a commercially available serum triglyceride determination kit (catalog number: TR0100, Sigma-Aldrich, Saint Louis, MO, US). The described tubes were gently mixed and then centrifuged at 12000 rpm, 4 °C for 2 min. after removing the cell debris, 350 µL of each solution was used for TAG determination assay using spectrophotometrically at 520 nm. The experiment was performed in triplicate and the mean values were used to calculate the triolein concentration using the below equation (Eq. 1):

$$\text{Triolein conc.} = \frac{(\text{Abs}_{\text{sample reaction}} - \text{Abs}_{\text{sample blank}})}{(\text{Abs}_{\text{standard reaction}} - \text{Abs}_{\text{standard blank}})} \times \left(\frac{\text{Standard conc.}}{\text{Dilution factor}}\right) \quad \text{(Eq. 1)}$$

in which the dilution factor was regarded as 5.

RESULTS AND DISCUSSION

Cell growth, biomass and growth kinetics

The microalgal growing pattern was observed in normal and nitrogen limitation conditions during 28 days of cultivation composed of 10 days of normal cultivation and another 12 days of cultivation in a fresh culture medium with the same composition (N_1) or 12 days of nitrogen limitation (N_0, N_2 and N_3) mode. The employed method for growth measurement was dry cell weight method in which the sampling was performed every two days Figure 1. *C. reinhardtii* cells displayed a typical sigmoidal growth pattern in both studied cultures. During first six days of cultivation, the microalgal cells remained in their lag phase, and entered to the logarithmic phase of growth from the 6th day to the 25th day of cultivation followed by the stationary and death phases from 25th to the 28th day. The microalgal cells grew faster in normal cultivation mode than in the nitrogen limited conditions. The maximum obtained dry cell weight (X_{max}) and the biomass productivity (P_{max}) were found to be 1.548 g L^{-1} and 0.085 g L^{-1} d^{-1} (N_0); 5.115 g L^{-1} and 0.232 g L^{-1} d^{-1} (N_1); 3.127 g L^{-1} and 0.207 g L^{-1} d^{-1} (N_2) and 1.861 g L^{-1} and 0.113 g L^{-1} d^{-1} (N_3) Table 2. On the other word, nitrogen limitation caused a noticeable reduction in biomass production up 30.26% (N_0); 61.13% (N_2) and 36.38% (N_3) compared with the normal cultivation mode (N_1). The observed phenomenon in biomass production trends might be attributed to the unfavored physiologic circumstances due to lowered levels of nitrogen in the culture medium. The same results have been reported by other researchers studying the effects of nitrogen depletion on biomass production and growth rates in *C. reinhardtii*.[16,17]

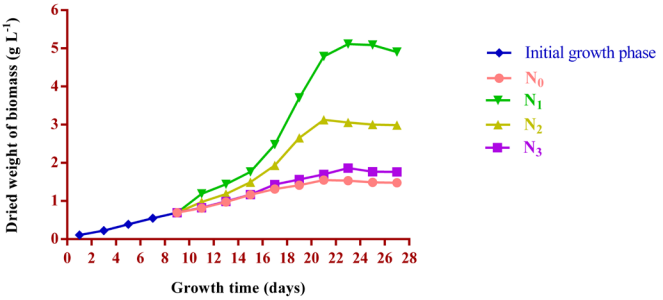

Figure 1: Growth rate of *C. reinhardtii* under different concentrations of nitrogen in shake flask scale during 28 days of study using dry cell weight method.

Table 2: The growth parameters, cell number, maximum biomass and lipid productivities in the studied *C. reinhardtii* strain under different nitrogen concentrations during 28 days of study

Cultivation mode	Growth parameters*		Productivity	
	X_{max}	μ_{max}	P_{max}	L_{max}
N_0 (0 g L^{-1})	1.548	0.017	0.085	0.021
N_1 (1.5 g L^{-1})	5.115	0.092	0.232	0.110
N_2 (1.0 g L^{-1})	3.127	0.066	0.207	0.075
N_3 (0.5 g L^{-1})	1.861	0.035	0.113	0.093

*X_{max} = maximum biomass concentration (g L^{-1}); μ_{max} = maximum specific growth rate (d^{-1}); P_{max} = maximum biomass productivity (g L^{-1} d^{-1}); L_{max} = maximum lipid productivity (g L^{-1} d^{-1}); N_0 = cultivation at 0 g L^{-1} nitrogen; N_1 = cultivation at 1.5 g L^{-1} nitrogen; N_2 = cultivation at 1.0 g L^{-1} nitrogen; N_3 = cultivation at 0.5 g L^{-1} nitrogen.

Lipid production and productivity

Moreover, the influences of different nitrogen levels on lipid production and productivity was also examined Figure 2 a-d. As presented in Figure 2a, the accumulated lipids in the normal cultivation conditions (N_1) was reached to 1.337 g L^1. The maximum lipid productivity in this cultivation was reached up to 0.110 g L^{-1} d^{-1}. On the other word, the total lipid content in N_1 medium was 26.14% in the total produced biomass. At the end of cultivation process, the final obtained lipid production from three different nitrogen depleted media reached to 0.849 g L^{-1} (N_2) Figure 2b, 0.635 g L^{-1} (N_3) Figure 2c and 0.783 g L^{-1} (N_0) Figure 2d. The reported values were equal to 28.05% (N_2), 34.12% (N_3) and 42.07% (N_0). In this context, the observed lipid productivity values were noticed to be 0.021 g L^{-1} d^{-1}, 0.075 g L^{-1} d^{-1}, and 0.093 g L^{-1} d^{-1}, at N_0 (0 g L^{-1}), N_2 (1.0 g L^{-1}) and N_3 (0.5 g L^{-1}), respectively. On the other words, nitrogen depletion, caused a noticeable decrease in the lipid productivity trends.

The observed results from growth kinetics and productivity studies in *C. reinhardtii* strain were in agreement with the provide results by other researchers.[18,19] It could be concluded that higher amounts of lipid accumulation were occurred in nitrogen limited conditions. The obtained data implied the standing contribution of nitrogen concentrations in biomass biosynthesis pathways. However, concerning the lipid production and productivity trends, it was indicated that nitrogen depletion

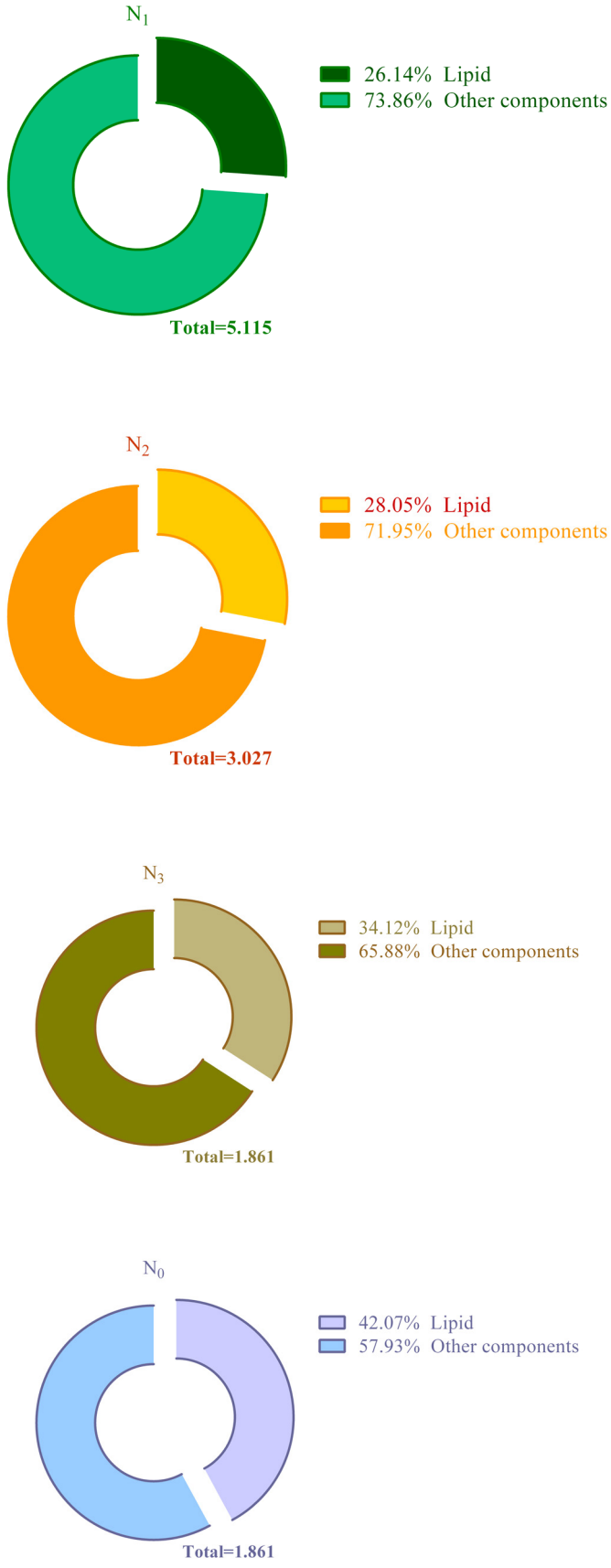

Figure 2: The total lipid content of *C. reinhardtii* under different concentrations of nitrogen (a-d) (% w/w) in the final obtained biomass.

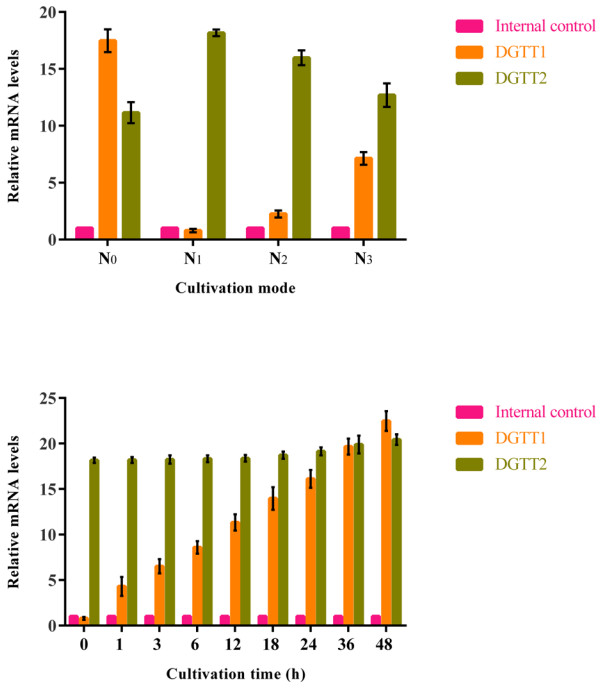

Figure 3: *DGTT1* and *DGTT2* gene expression in a) different times, b) different concentrations.

strategy could be considered as a powerful parameter to maximize the lipid production yields. Besides, *C. reinhardtii* prefers to accumulate biomass in nitrogen rich medium instead of increasing its lipid contents. The same trends have been observed in other *C. reinhardtii* strains.[16]

qRT-PCR analysis of DGTT1 and DGTT2 genes

To investigate the effects of nitrogen starvation on the expression levels of two major genes involved in TAG accumulation in *C. reinhardtii* microalga, qRT-PCR analysis was performed. To accomplish a successful real time PCR analysis, the employed primers are considered as a critical point. Due to its great sensitivity and robust performance, we chose AlleleID 7.5 software to design the requested primers. A previously sequenced ribosomal gene (18S rRNA) from another *C. reinhardtii* strain was selected as the internal control gene to normalize the observed data.

Figure 3a displays the obtained results from quantitative analysis of *DGTT1* and *DGTT2* expression profiles during normal and nitrogen starved conditions. The expression levels for *DGTT1* transcript showed a significant increase (21.05, 7.76 and 2.45-fold increase than N_1, N_2 and N_3, respectively) in nitrogen depleted medium (N_0) in comparison with its values in nitrogen containing media. On the other hand, the *DGTT2* transcript in N_0 exhibited 0.61, 0.70 and 0.88-fold decrease compared with N_1, N_2 and N_3, culture media respectively.

The statistical analysis (Kruskal-Wallis analysis with Tukey's post hoc analysis) proved the significant influence of nitrogen starvation on the relative expression of *DGTT1* (*p*-value: 0.0189) and *DGTT2* (*p*-value: 0.0168). Culture medium without nitrogen (0 g $NaNO_3$) showed the highest *DGTT1* expression. Besides, a gradual increase was observed in *DGTT1* expression pattern from nitrogen starved to culture media with higher nitrogen concentration.

In nitrogen starvation condition, *DGTT1* upregulation was happened within the first three hour and was kept in its elevated amounts during cultivation up to 36h in comparison with the normal cultivation condi-

tions with a normal decrease during 48h of study Figure 3b. There was not a significant difference ($p \leq 0.05$) for *DGTT2*.

Until now, some researchers have been dealt with the effects of nitrogen starvation on the physiology and metabolic pathways in *C. reinhardtii*.[20]

The results of previous studies have shown that the relative expression profiles of *DGTT1* and *DGTT2* genes differs under nitrogen, iron, sulfur and phosphorous.[21,22]

Using qRT-PCR analysis, we showed that nitrogen starvation of a naturally isolated *C. reinhardtii* strain results to high expression levels of *DGTT1* over observed levels from other nitrogen containing media. Remarkably, though the total produced lipid content was higher in nitrogen starved strain, but the total obtained biomass (dry cell weight) was lesser than nitrogen rich conditions. This phenomenon indicates that nitrogen deprivation might divert the cellular resources towards lipid production instead of biomass biosynthesis. Recently, omics-based approaches [23] and metabolic flux analysis data [24] has been stablished for *C. reinhardtii*. The results of the current study could be helpful in researches concerning systems biology and biotechnology of this microalga.

Triacylglycerol content of lipids

In optimized physiologic conditions, *C. reinhardtii* tends to synthetize fatty acids mainly for esterification towards glycerol-based membrane lipids.[5] On the other hand, during deprivation conditions, the lipid biosynthetic pathway alters towards neutral lipid accumulation such as TAGs. TAGs, are considered as storage molecules which will quickly degraded in optimal conditions to produce the demanded cellular energy.[25] Among the five major enzymes involved in TAG biosynthesis, *DGTT1* and *DGTT3* are known to be only active in nutrient rich media. On the other hand, *DGTT2*, *DGTT4* and *DGTT5* have not a distinct contribution in TAG biosynthesis during normal conditions. During different nutrient starvation, such as zinc, iron, phosphorus, sulfur and nitrogen, DGTT1 and DGAT1 levels exhibit a significant increase. Besides, it has been shown that *DGTT2* displays a great role in TAG accumulation during nitrogen or phosphorus starvation.[26]

The total content of TAGs from the obtained lipids were quantified and compared in for different cultivation modes Figure 4. The obtained data show an increasing trend towards TAG accumulation in lower nitrogen levels. In nitrogen rich, medium (N_1) only 0.186 g L^{-1} of the total produced lipids were TAG (14%). In nitrogen, limited media (N_2) and (N_3) the maximum amounts of 0.175 g L^{-1} and 0.164 g L^{-1} TAGs was observed which were equal to 21% and 26% of the total obtained lipids, respectively.

Microalgae are a good choice for fatty acids accumulation.[27] Noticeably, the highest amount of TAG accumulation (0.299 g L^{-1}, 0.38%) was reported in nitrogen starved medium (N_0).

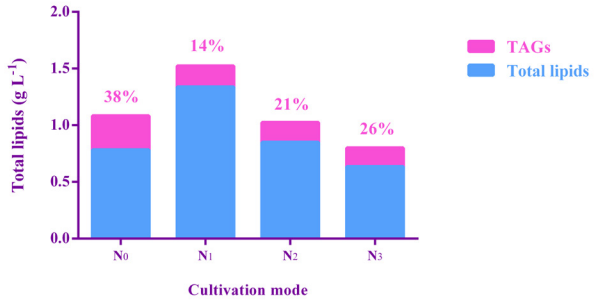

Figure 4: Total triacylglycerol (TAG) concentration (the upper segment of each column) obtained from four different nitrogen levels compared with the total produced lipid (the whole column). The presented results are the calculated mean values from triplicate experiments.

In despite of several advantages over other competitive resources such as microbes or plants, biodiesel production from microalgae have some shortcomings such as lower lipid production and productivities.[28] In this study, we showed the applicability of using nutrient stress strategy to redirect the microalgal metabolic pathways towards non-polar lipids and mainly TAGs. Real-time reverse-transcription PCR (qRT-PCR) analysis of this strain confirmed high expression level of *DGTT1* over the level observed in the control strain.

The precise mechanisms involved in the overexpression of metabolic pathways for TAG biosynthesis are not clearly determined. The results of the current study could shed light on the exact metabolic and biochemical changes due to nitrogen deficiency. Besides, the results of this study could suggest potential metabolic networks for genetic or metabolic engineering and for better understanding of the *C. reinhardtii* systems biotechnology for biomass and lipid production.

Notably, in this study a maximum level of 42.07% for lipid accumulation was shown in a robust *C. reinhardtii* strain which warrants further optimization and scale-up studies for large scale lipid and biomass production.

CONCLUSION

The obtained results, deciphered the metabolic and biochemical response of a naturally isolated strain of *C. reinhardtii* to nitrogen limitation conditions. Besides the quantitative changes in *DGTT1* and *DGGT2* expression profiles was determined and elucidated new targets for metabolic engineering of *C. reinhardtii* for TAG overexpression and other valuable bioactive compounds. Furthermore, the results demonstrated the ability of *C. reinhardtii* to produce TAGs for green energy production and other purposes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS USED

DGAT: Diacylglycerol transferase; **PDAT:** Phospholipid: diacylglycerol acyltransferase; **qRT-PCR:** Quantitative real-time polymerase chain reaction; **TAG:** Triacylglycerol.

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