Algal Research 16 (2016) 54–62

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Comparative transcriptome analysis reveals a potential photosynthate partitioning mechanism between lipid and starch biosynthetic pathways in green microalgae

Wen-Chi Chang [a], Han-Qin Zheng [a], Ching-Nen Nathan Chen [b,c,*]

[a] Institute of Tropical Plant Sciences, National Cheng Kung University, Tainan 701, Taiwan
[b] Department of Oceanography, National Sun Yat-sen University, Kaohsiung 804, Taiwan
[c] Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan

ARTICLE INFO

Article history:
Received 13 October 2015
Received in revised form 25 February 2016
Accepted 1 March 2016
Available online xxxx

Keywords:
Microalgae
Biodiesel
Lipid biosynthesis
Starch biosynthesis
Photosynthate partitioning
Environmental stress

ABSTRACT

Comparative transcriptome analysis provides a tool to top-view regulations of biochemical pathways in the cells and predicts the key enzymes by comparing gene expression changes in different conditions. Transcriptomes of a green microalga *Neodesmus* sp. UTEX 2219-4 treated under different stresses favorable or unfavorable for lipid biosynthesis were sequenced and compared to elucidate the photosynthate partitioning mechanism between fatty acid and starch biosynthesis pathways. This microalga contains 20,556 isotigs, and 8387 of these have homologs in the Non-Redundant (NR) database. The fatty acid and starch biosynthetic pathways of this microalga were reconstructed. The expression levels of the isotigs related to these pathways and involved in photosynthesis were calculated. The photosynthetic light reaction genes were down-regulated but electron carriers were up-regulated under osmotic and salt stresses, suggesting that ATP production was supported by cyclic electron transport in these conditions. In lipid producing conditions, expression of multiple isotigs in the fatty acid biosynthesis pathway was up-regulated, indicating that overexpression of acetyl-CoA carboxylase alone was not sufficient to enhance lipid biosynthesis. Results from this study suggested that triose phosphate isomerase is the key enzyme regulating photosynthate partitioning between fatty acid and starch biosyntheses in green microalgae.

1. Introduction

The burning of fossil fuels at an ever-increasing rate over the last 200 years has caused excessive carbon dioxide release into the atmosphere, which is one of the major causes of global climate change. This has led to growing interest in alternative energy sources, one of which is microalgal biodiesel, which has a number of advantages over other options. For example, biodiesel is a liquid fuel with a high energy density that is suitable for long distance truck and aviation purposes, something that does not appear to be true for electricity or ethanol. Second, the cultivation of microalgae can utilize municipal wastewater as a source of fertilizer [1,5], which could ameliorate one form of environmental pollution that contributes to red tides and dead zones in the sea. In terms of reducing air pollution, the high concentration of carbon dioxide produced by industry can be directed into microalgal cultivation and used as a carbon source. Third, microalgal yields are much higher than those of traditional crops [4], although significant amount of energy is required for the operations in order to reach high productivity (personal unpublished results). Nonetheless, the side products of microalgal biodiesel production such as proteins, starch, cellulose, natural pigments and antioxidants, glycerol and oxygen are valuable in various applications. Fourth, cultivation of microalgae can use non-arable and marginal land, thus avoiding competition with the food supply.

Green microalgae are examined by numerous microalgal biodiesel studies due to their high productivities. However, most green microalgae synthesize and store starch in their cells as an energy reservoir, and only a small fraction of these species store lipids in the significant amounts needed to be used as feedstock for biodiesel production. These so-called "oleaginous microalgae" still accumulate starch to different degrees. In the perspective of biodiesel production, it would be ideal to eliminate starch biosynthesis in oleaginous microalgae and direct the photosynthate to fatty acid biosynthesis, since the biosynthesis of both molecules use the same precursor triose phosphate that is produced by photosynthesis.

While two strategies of genetic manipulation have been examined in order to enhance lipid yields in microalgae, they have not provided satisfactory results. The first strategy is over-expression of acetyl-CoA carboxylase (ACCase). ACCase is considered the key enzyme in the fatty acid biosynthesis pathway, which is in the upstream of triacylglycerol biosynthesis. This enzyme, a single polypeptide with multi-functional domains in eukaryotes, converts acetyl-CoA into malonyl-CoA. The

* Corresponding author at: Department of Oceanography, National Sun Yat-sen University, Kaohsiung 804, Taiwan.
E-mail address: nathanc@mail.nsysu.edu.tw (C.-N.N. Chen).

http://dx.doi.org/10.1016/j.algal.2016.03.007
2211-9264/

modified molecule malonyl-ACP is required for the fatty acid elongation process. However, over-expression of ACCase in *Cyclotella cryptica* and *Navicula saprophila* did not increase lipid yields significantly in the transgenic lines [6,21]. The second strategy is to block starch biosynthesis in microalgae, and a few starchless mutants have been isolated in *Chlamydomonas* to achieve this [22]. These mutants were examined whether their lipid contents were higher than those in the wild-type strains in the manipulated conditions [15,16,24]. Initially, judged based on biomass, significant higher lipid contents were reported in these mutants. However, in a more careful study based on cell number and in comparisons with their progenitor strains, these mutants did not show greater lipid contents per cell [22].

Although these results are discouraging, they do not rule out the possibility of a photosynthate partitioning mechanism in green microalgae. Actually this mechanism is plausible because regulation of photosynthate flow between the starch and fatty acid biosyntheses is required to cope with various physiological and environmental conditions. Obviously, a comprehensive analysis of the gene expression of these pathways in both lipid-producing and starch-producing conditions can help understand the regulation mechanism. In such a comparative study, bottlenecks and regulation points in the pathways can be predicted.

Prediction of key regulation points in the cells is a function of transcriptome analysis. Coupled with the oil production physiology of *Neodesmus* sp. UTEX 2219-4 reported in a previous work [23], this microalga provided a chance for an insightful comparative transcriptome analysis to detect the key enzyme(s) in the pathways. The results of these analyses in the current study revealed a potential regulation point in the upstream of the fatty acid and starch biosynthetic pathways, and its role can be tested straightforwardly in the future because it is encoded by a single gene in this genome.

2. Material and methods

2.1. Microalga and cultivation conditions

The green microalga *Neodesmus* sp. UTEX 2219-4 was isolated from UTEX collection No. 2219 which was reported as containing two strains [13,23]. The medium used for single colony isolation and liquid cultivation was 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, and 0.43 mM NaCl [19], and the same levels of minerals described in Table 2 of Berges et al. [2]. These cells in 300 mL medium were grown in 1-L flasks on an orbital shaker at 150 rpm and 28 °C, and continuously illuminated in 200 μmol m^{-2} s^{-1} using T5 cool white fluorescent lamps. For the stress treatments, cells at $OD_{682} = 0.5$ (grown from initial $OD_{682} = 0.1$) were harvested using centrifugation and resuspended in the stress media. Osmotic stress was imposed by using 0.68 M sorbitol, and salt stress by using 0.34 M NaCl. Nitrogen starvation treatment was conducted by resuspending the cells in the medium without addition of nitrogen source. These cells were treated in the specified stress conditions on an orbital shaker for three and six hours before their total RNAs were isolated. Equal quantities of total RNAs from the two time points of each stress treatment were pooled for transcriptome sequencing.

2.2. RNA extraction, pyrosequencing, contig assembly and annotation

Microalgal cells were smashed by a mini-beadbeater and total RNA was extracted using a guanidine method [3]. Thirty micrograms of total RNA was subjected to mRNA isolation using Dynabeads mRNA Purification Kit (Invitrogen Life Technologies) followed by an RNA fragmentation treatment (RNA fragmentation reagents, Ambion Applied Biosystems). Double strand cDNAs were synthesized based on the fragmented RNA using cDNA Synthesis System (Roche Diagnostics) and random primers. The double strand cDNAs were ligated with the 454 adaptors to prepare the sequencing libraries using the GS Rapid Library Preparation Kit (Roche Diagnostics) according to the manufacturer's protocol. The libraries were clonally amplified by emulsion PCR to enrich the fragments followed by a pyrosequencing reaction run on the GS FLX platform with the Titanium Chemistry, according to the manufacturer's instructions (Roche Diagnostics). Sequencing reads of the four samples (normal conditions, nitrogen starvation, osmotic stress and salt stress treatments) were pooled and assembled by the GS De Novo Assembler version 2.7 using cDNA/transcriptome options.

Open reading frames of isotigs and contigs were predicted by the GS De Novo Assembler. Sequences with translated peptide shorter than 70 amino acids and singletons shorter than 210 nucleotides were discarded. For functional annotation of proteins, the translated amino acid sequences were compared to the non-redundant (NR) protein database of NCBI and UniRef50 using BLASTp and BLASTx algorithms with a cut-off E-value $\leq 10^{-6}$ (ver. 2.2.25).

2.3. Unique sequence mapping and expression level calculation

Expression levels of unique transcripts and singletons were calculated by mapping reads of each sample to the pool of transcripts and singletons using the BLAT program [12] with default parameters, and the identity cut-off line between a read and a transcript was set at 70%. The pslReps program was used to select the best mapping results. The abundance of each transcript and singleton was presented as reads per million transcripts (RPM).

2.4. Gene ontology (GO) analysis and cross-species functional similarity analysis (top-hit)

The GO terms of annotated unique sequences were analyzed by comparing them to the UniProt database [25]. Unique sequences of *Neodesmus* sp. UTEX 2219-4 were compared with those of other microalgal species using the BLAST program. The criteria of similarity analysis were the same as those for the transcript annotations. Peptide sequences from various microalgal species were downloaded from the Joint Genome Institute, Department of Energy, USA (http://www.jgi.doe.gov).

2.5. KEGG metabolic pathway analysis

KEGG metabolic pathway maps were used to demonstrate gene expression regulation of *Neodesmus* sp. UTEX 2219-4 treated in stress conditions. After KEGG ortholog ID annotation, the unique sequences were mapped onto KEGG pathways. When several transcripts were mapped to the same ortholog ID (transcript group), a Z-test was used to remove transcripts with outlier values among the transcript group (Z-score higher than 2 or less than −2). The gene expression value in the pathway maps was obtained by averaging the expression values of the remaining transcripts.

2.6. Construction of a web-based database for the transcriptome survey

A web-based database, AlgaePath, was constructed using the transcriptome datasets for users to identify transcript abundance and pathway information of *Neodesmus* sp. UTEX 2219-4 [26]. This database can be accessed online at http://algaepath.itps.ncku.edu.tw.

3. Results and discussion

3.1. Sequencing, de novo assembly and annotation of an oleaginous microalga transcriptome

The lipid content of the green microalga *Neodesmus* sp. UTEX 2219-4 increased from 17% to 42% in dry biomass when the cells were treated in osmotic stress coupled with nitrogen starvation for three days. Osmotic stress or nitrogen starvation alone had the same effects, although