Algal Research 16 (2016) 12–19

Contents lists available at ScienceDirect

Algal Research

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

Transcriptome-based global analysis of gene expression in response to carbon dioxide deprivation in the green algae *Chlorella pyrenoidosa*

Jianhua Fan *, Hui Xu, Yuanguang Li *

State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, PR China

ARTICLE INFO

Article history:
Received 20 November 2015
Received in revised form 17 February 2016
Accepted 29 February 2016
Available online xxxx

Keywords:
Chlorella
CO_2
Growth
Carbon concentrating mechanism
Gene expression

ABSTRACT

Microalgae can tolerate different CO_2 concentrations and its mechanism of rapid CO_2 fixation is key to understanding the relationship between intracellular substance and energy conversion, as well as to optimize the production of microalgae bioproducts. The present study conducted a transcriptome-based analysis of gene expression of an industrial *Chlorella pyrenoidosa* strain, FACHB-9, with high oil production ability under different CO_2 concentrations. Transcriptome-based gene expression analysis indicated the redirection of central metabolism under CO_2 deprivation. The C3 pathway served as the main metabolic pathway for *C. pyrenoidosa*, which was subjected to a high CO_2 environment in the present study. Similar to C4 plants wherein limited CO_2 activates CO_2-concentrating mechanism to compensate for the low activity of RuBisCO in the Calvin cycle, *C. pyrenoidosa* undergoes CO_2 compensation by active transport to ensure sufficient amounts of Ci for its growth and metabolism. Comparative analysis has allowed the identification of several candidate genes for further strain improvement. These genes encode proteins that might be CAs, as indicated by its localization to the cell membrane or chloroplast membrane, or act as Ci transporters that assist Ci transmembrane transportation. Certain types of ABC transport proteins and CCCH-type zinc finger proteins also showed significant changes in gene expression. These findings indicate that these may be promising targets for functional and genetic modification studies. The results not only reveal the significance of the mechanism of carbon sequestration to eukaryotic green algae, but also provide a basis for further construction of the energy microalgae cell factory with high efficiency carbon biofixation capacity.

© 2016 Elsevier B.V. All rights reserved.

1. Background

Microalgal energy has recently become a research topic of interest. Previous studies have shown that the growth of photoautotrophic microalgae for biodiesel production involves large-scale assimilation of industrial wastes and gases [1,2], thereby resulting in carbon emission reduction, which may not only significantly decrease its cost of cultivation, but also would benefit from the Clean Development Mechanism (CDM) [3]. In addition, the developmental process of utilizing microalgal energy may resolve issues relating to energy crisis and protection of national security. However, although the prospect of microalgae carbon dioxide (CO_2) sequestration is currently unclear, research studies conducted around the world have mainly focused on the initial stages of its development, and the bottleneck of lacking mature technologies often leads to costly experimental procedures [3,4].

Current studies on carbon sequestration in microalgae mainly focus on the transmission and absorption of CO_2 [5], seed selection and breeding [6], metabolic mechanism of photosynthetic carbon fixation and molecular modification [7,8], high efficiency cultivation and its effect of reducing emissions [9], and post-processing and comprehensive utilization of biomass [10]. Because our understanding of intracellular photosynthesis is limited, current studies on carbon sequestration mechanisms as well as the genetic background of high-performance industrial algae have been mostly conducted in terms of process optimization, whereas the potential of photosynthetic carbon fixation by microalgae has not been fully explored [11]. This problem has thus prompted scientists to perform studies on the optimization and evaluation of the carbon sequestration process, as well as on system integration, despite the lack of theoretical guidance in the underlying mechanisms of energy production in microalgae [12,13].

Therefore, it is necessary to systematically study the mechanisms underlying photosynthesis, carbon sequestration, and other metabolic processes in microalgae to provide theoretical and experimental bases for improving its photosynthetic efficiency accelerating its growth, and in improving its production of intracellular substances such as lipids, proteins, and pigments by using techniques in genetic engineering and systems biology [8,14–16]. Microalgae can tolerate different concentrations of CO_2 and its mechanism of rapid CO_2 fixation is key to understanding the relationship between intracellular substances and energy conversion, as well as in optimizing the production of

* Corresponding authors at: 130 Meilong Road, 301 Mail box, Shanghai 200237, PR China.
E-mail addresses: jhfan@ecust.edu.cn (J. Fan), ygli@ecust.edu.cn (Y. Li).

http://dx.doi.org/10.1016/j.algal.2016.02.032
2211-9264/© 2016 Elsevier B.V. All rights reserved.

microalgal bioproducts [14,17]. The elucidation of the mechanism underlying rapid CO_2 fixation also serves as the principle behind the development of a large-scale, efficient, and low-cost microalgal carbon sequestration technology.

Most microalgae have evolved a CO_2-concentrating mechanism (CCM) [18]. The concentration of CO_2 in water is relatively very low, and microalgae have evolved a set of mechanisms to adapt to this particular environmental condition. Researchers have earlier determined that the CCM can change the affinity of microalgae to inorganic carbon to increase CO_2 levels at the RuBisCO carbon fixation site, which in turn increases the rate of photosynthesis and decreases the rate of light respiration [19]. However, this CCM is often downregulated in a high-CO_2 concentration environment [17,18]. This phenomenon thus improves the efficiency of light energy whereas the ability of microalgae to tolerate high-intensity light is reduced. Therefore, CCM is an important regulatory component of photosynthesis, especially for C4 plants and aquatic photosynthetic organisms [20].

The transport of inorganic carbon is a process that depends on the energy generated during algal photosynthesis. Hence, CCM is also vulnerable to various environmental factors such as light, temperature, CO_2 concentration, and nutrient levels [20]. In green algae, CO_2 concentration is one of the most important factors that regulate CCM, and high CO_2 concentrations (≥1%) significantly inhibit the activity of carbonic anhydrase and the formation of CCM [17]. Fang and Brueggeman studied the effects of CO_2 concentration in relation to changes in the intracellular global gene expression network of *Chlamydomonas* by transcriptome sequencing, and determined that approximately 25% of its genes are differentially expressed, including most CCM key genes and regulatory factors [8]. Furthermore, research studies have also shown that the accumulation of RuBisCO and carbonic anhydrase in pyrenoids is strongly correlated to the CO_2 concentration of the culture environment, and that pyrenoid plays an important role in the CCM [21]. However, current investigations mainly focus on the model species, *Chlamydomonas reinhardtii*, and the universality of CCM in unicellular green algae remains unknown.

The microalgal species of interest of the present study is *Chlorella pyrenoidosa*, which differs from the general laboratory species. This species can efficiently produce proteins, chlorophyll, lutein, and chlorella growth factors [22,23]. By using a novel technique developed in our laboratory, which we have called sequential heterotrophy dilution photoinduction cultivation strategy, high-density and high-quality cultivation of *C. pyrenoidosa* biomass was achieved, which is considered to be of high industrial application value [24]. Moreover, it can rapidly fix CO_2 and gather >50% of oil by manipulating the culture conditions, as well as can be used in large-scale cultivation outdoors [14,22]. Based on these properties, *C. pyrenoidosa* has been regarded as the model species for industrial development of microalgal energy and carbon sequestration.

The present study conducted a transcriptome-based analysis of gene expression of an industrial *C. pyrenoidosa* strain, FACHB-9, with high oil production ability under different CO_2 concentrations. The results not only reveal the mechanism underlying carbon sequestration in eukaryotic green algae, but also provide a basis for the development of the microalgal energy factories with high-efficiency carbon biofixation capacity.

2. Materials and methods

2.1. Algal strains and culture conditions

C. pyrenoidosa (FACHB-9) was obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB-Collection, Wuhan, China) and purified aseptically for the subsequent studies. The cells were grown at 30 °C in air-lift column photobioreactors (60 cm high and 5 cm diameter) containing 800 mL of BBM medium. Irradiance was approximately 150 μmol/m^2/s under continuous illumination using white fluorescent lamps. The photobioreactors were aerated with 5% CO_2 (v/v, high-CO_2, HC) or with ambient air (low-CO_2, LC), at a flow rate of 0.3 L/min. Four group experiments supplemented with 20 mM HEPES buffer solution (pH 7.2) were cultured with bubbling of 5% CO_2 for the first 5 days, and then two groups were transferred to air conditioned settings until the end of the cultivation. Biomass concentration was measured by spectrophotometer (optical density: 680 nm) and dry cell weight (DCW).

2.2. RNA isolation, library preparation, and sequencing

The cells enter a logarithmic phase after three to seven days of inoculation. All culture samples were collected from the bioreactors at 0, 1, 4, and 24 h after shifting from HC to LC conditions, and then immediately centrifuged at 5000*g* for 5 min at 4 °C. The supernatant was discarded and the centrifuge tube was immersed in liquid nitrogen to freeze the cellular pellet for later processing. Total RNA was isolated using TRIzol (Invitrogen, USA) and then treated with RNAase-free DNase I (Takara, Japan). The RNA solutions were stored at −80 °C for subsequent library preparation and qPCR detection.

RNA quality was assessed using the Agilent 2100 Bioanalyzer. Libraries were constructed from about 2 μg of RNA using Illumina's TruSeq RNA Sample Preparation Kit. The average insert size of each library was about 155 bp. The standard Illumina protocol was used in library construction. Subsequently, the mRNA-seq libraries were sequenced on an Illumina HiSeq 2000 platform and 100-bp paired-end reads were generated.

2.3. Transcriptome mapping, annotation, and differential expression analysis

Default parameters were used to pass reads using the Illumina quality control pipeline. After removal of adapters, poly-N strands, and low-quality reads, all filtered reads were examined by FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) to confirm data quality. The processed sequence files were then mapped to the assembled genome (The *C. pyrenoidosa* whole-genome sequencing project (PRJNA171991) has been completed and deposited to the DDBJ/EMBL/GenBank under the accession number ANZC00000000) by using Bowtie 2 and TopHat 2, with a tolerance of up to two mismatches and three indels. The mRNA-seq raw data are available at the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE69816.

The general isogenes (unique transcripts) were compared with the NCBI non-redundant protein database and eggNOG (evolutionary genealogy of genes: non-supervised Orthologous Groups) using the Blast algorithm with a cut-off E-value $\leq 10^{-5}$. Gene ontology (GO) classification of each gene model was carried out using Blast2go software and Kyoto Encyclopedia of Genes and Genomes (KEGG) classification was performed using KASS and KEGG automatic annotation sever, respectively.

For each of the mRNA-seq datasets, the transcript relative abundance (TRA) was expressed as the number of reads that were aligned to the annotated gene models using the HTSeq method and then normalized to the reads per kilobase of exon model per million mapped fragments (RPKM) values. Then, differential expression analysis of the samples was conducted using the DEGseq method, with a threshold q-value of <0.005. Fold expression changes between different time points were calculated using TRA $\log_2$ ratios. Genes were regarded as differentially expressed when these showed at least a 2-fold change and ≤5% false discovery rate.

2.4. Quantitative real-time PCR validation

To verify the results of mRNA-seq analysis, the expression levels of the selected genes were validated by using standard qRT-PCR methods. First-strand cDNA synthesis and qRT-PCR were performed using the

Download English Version:

https://daneshyari.com/en/article/8086965

Download Persian Version:

https://daneshyari.com/article/8086965

Daneshyari.com