

Algomics for the Development of a Sustainable Microalgae Biorefinery

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Abstract

Biofuels production from biomass is a sustainable alternative to fossil fuel. Biomass is a renewable source comparatively to an expected depleted fossil fuel. Microalgae are known by its potential to grow on any land where none other crop can grow, can produce biomass all year long. The biomass could be used to produce biofuels and byproducts without competing with food. Unfortunately, microalgae biorefinery is not commercially viable. High-value byproducts could contribute in the biorefinery feasibility. There is a need to improve their yield and quality. Algomics is the application of genomic and post-genomic approaches and considered powerful tools for a better understanding of algae cells metabolism and physiology. Then, the data would be used to develop sustainable strategies for oil and byproducts yield and quality improvement and a profitable microalgae industry.

Keywords: Microalgae; Biomass; Biorefinery; Biodiesel; Algomics; Metabolomics

Introduction

Many advantages are associated with replacing fossil fuel by biodiesel. It is produced from renewable biomass. It can be continuously produced comparatively to an expected depletion of fossil fuel. Biodiesel is a biodegradable and non-toxic product, and safe to be shipped. The use of biodiesel is associated by a significant reduction of greenhouse gases, carbon dioxide, and sulfur dioxide in air emission. It reduces also asthma-causing soot, and can be produced domestically [1]. Consumers in the US used over 2 billion gallons of biodiesel in 2015. This record consumption reduced 18.2 million metric tons of carbon dioxide production in the US (www.EPA.GOV). Many land crops biomass such as soybean, corn, rapeseeds and sugarcane can be used to produce biodiesel. Moreover, the demand on biofuels resulted in an increase in using land crops for energy production. US fuels companies reported producing 2.09 billion gallons of biodiesel in 2015 [2]. The use of land crops for renewable energy and the global population growth are major challenging concerns of the global food and energy security. These data showed a continuous increase of biofuels production. It started to partially divert land crops from their original uses as human food and animal feed to nonconventional fuels production. A non-competitive to food and sustainable bioenergy source could be more feasible and globally accepted.

The production of biodiesel from waste cooking oil and animal fat is an environmentally friendly process, and avoids city waste treatment and handling. However, this alternative process cannot significantly affect the total biofuels production and will not be able to respond to the global biofuel demand. The only crop that could use salt, brackish wastewater, and be produced on non-arable land, and could be source of food and renewable energy is microalgae. This fabulous micro-crop has the potential to yield biomass 50 times more than any other land crop biomass. Moreover, it converts CO₂ and H₂O with sunlight into chemical energy, and yields high oil content sometimes more than 50% of the dry weight. Microalgae biomass is produced yearly. Hu et al. reported an extensive review on microalgae for biofuel [3].

The challenge is how to make microalgae biorefinery profitable and sustainable. High-value micro-crop cell components could make microalgae biorefinery commercially feasible. A better understanding of high-value microalgae cell components and lipids metabolism could be integrated into yield and quality improvement programs [4]. Algomics is the application of omics technologies to study algae

including genomics, transcriptomics, proteomics and metabolomics are tools that could be used to further cell metabolism knowledge within a short time than few decades before [5-7].

Genomics

Genomics became a powerful tool after the development of advanced automatic sequencing technology during the last two decades. It is complemented by bioinformatics to assemble DNA sequences and analyze gene structure and expression. The human genome research projects demonstrated the power of omics in general and genomics in particular. Both scientists and clinicians can study the role and even interaction between different genetic factors to develop therapeutic strategies. In the case of microalgae, a full genome sequencing of strains with potential commercial applications will enable scientists to use other omics technologies for a better understanding of high-value cell components and lipids metabolism and the overall cell behavior under stress and non-stress condition. Many strains genomes were recently sequenced including *Botryococcus braunii* Showa, *Botryococcus braunii* UTEX 572, *Chlorella* sp. NC64A, *Chlamydomonas reinhardtii*, *Coccomyxa* sp. C-169, *Dunaliella salina*, *Ostreococcus tauri*, *Micromonas pusilla* and *Nannochloropsis oceanica* (<http://genome.jgi.doe.gov>). Comparative genomic analysis contributed in the understanding of some mechanism of microalgae adaptation to extreme environmental conditions [8]. Homologous recombination is another tool to study functional genomics [9]. They were able to generate knockouts of nitrate (NR) and nitrite (NiR) reductase genes in *Nannochloropsis* sp. in one single transformation step. This result provided evidence that the strain is haploid. *Nannochloropsis* sp is a microalgae strain known by its high growth rate. The use of this genetic transformation technique on *Nannochloropsis* sp could enable scientists to explore further microalgae functional genomics and develop a realistic knowledge

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for future gene manipulation and establish different procedures in the biorefinery to improve the yield and quality of high-value byproducts and oil. Chemical genomics is another way to characterize bioactive compounds specific trafficking pathways via endomembrane trafficking system [10].

Post-genomics

To achieve targeted goals, post-genomics tools could complement the genomics data. Many post-genomics studies were performed on strains with sequenced genome. However, the *de novo* approach developed by Guamieri et al. could be performed on strains with unsequenced genome [11]. It is a tool to bypass the genome sequencing step and is discussed below.

Transcriptomics

Transcriptomics is involved after the transcription of genes to RNA. It is the study of a single or group of genes expression dynamic. In case of uncharacterized genes, Aoki et al. developed a new gene coexpression database for microalgae (<http://alcodb.jp>) [12]. The gene coexpression analysis is powerful when there are some uncharacterized genes to be profiled and analyzed. It is based on the similarity of gene expression profiles and in the case of expressed genes “functionally and coordinately related”. The database was developed using green *Chlamydomonas reinhardtii* and red *Cyanidioschyzon merolae*. *C. reinhardtii* is generally used as the model green algae in post-genomics analysis.

The *de novo* transcriptome approach reported by Guamieri et al. [11] started by transcriptome sequencing of *Chlorella vulgaris* UTEX 395, followed by a comparative global transcriptomic and proteomic study. A combination of available software and new bioinformatics tools they developed were performed for transcriptome sequencing data assembly. It offers the opportunity to perform a comprehensive system biology investigation of microalgae species with unsequenced genome.

Proteomics and Metabolomics

Proteomics and metabolomics are involved after the translation of mRNA to protein. Both omics could be used to identify peptides and their profiling. The post-transcriptional regulation of microalgae cells reaction to environmental changes could be investigated using proteomics and metabolomics approaches. It is well known that photosynthesis efficiency is one of the bottlenecks in the physiology and metabolism of any photoautotrophic cell. It could affect the growth rate, byproducts and lipids yield. In this regard, Battchikova et al. reported a proteomics review on cyanobacterial photosynthesis process [13]. A study reported by Nguyen et al. targeting purified oil bodies of *C. reinhardtii* under nitrogen deprivation by proteomic profiling showed a number of proteins involved in vesicle trafficking and transport [14].

Comparative proteomic analysis is another efficient way to understand the cell metabolism and identify key proteins involved biosynthesis pathways and trafficking systems [15]. Garnier et al. performed a comparative proteomic analysis on wild and mutant *Tisochrysis lutea* strains under two metabolic conditions. Proteomes analysis showed a differential expression of proteins between both strains under nitrogen starvation conditions [16]. Identified proteins demonstrated the influence of nitrogen starvation on lipids metabolism, carbohydrate catabolism and carbon homeostasis. Proteomics results could be complemented with an overall transcriptomic study to understand genes expression regulation. The procedure helps targeting

genes of interest to manipulate yield and quality of lipids accumulated in oil bodies of microalgae cells.

Metabolomics is another approach to complement genomics and other post-genomics technologies for gaining insights into functional biology systems. The metabolomics investigation could be performed on proteins and non-proteins with specific role in metabolites biosynthesis pathways. Generally, there are many metabolomics techniques including protein and metabolites profiling, specific target analysis and metabolic fingerprinting. Since there is diversity in cell metabolites and their physicochemical properties, the metabolic analysis could require more than one approach. There is a range of highly sophisticated and sensitive technologies available to identify peptides and metabolites and their dynamic in the overall cell metabolism.

Although lipids metabolism network is complex, it should be one of the major targets using proteomics and metabolomics. Lipidomics is an approach to understand the process of fatty acid synthesis and lipids accumulation oil bodies in the microalgae cells, and then develop strategies to improve the yield and quality. For example, a link between the dynamic metabolic profiles obtained by the metabolomic study and the improvement of lipids biosynthesis during microalgal cultivation [17]. The study was performed on *Chlamydomonas sp.* JSC4 using different growth condition including nitrogen depletion and light intensity, CE/MS metabolite analysis and ¹³C-labeling for the profiling and turnover.

Conclusion

Omics and bioinformatics are highly advanced techniques in the study of algae cells could be performed in short time. It is a unique opportunity to carryout large-scale studies and to develop a realistic understanding of cell physiology and metabolism. Algomics data would provide an understanding of oil and high value metabolites biosynthesis pathways under stress and non-stress conditions. This further knowledge could be used to manipulate the cell factory and improve oil and high-value products yield and quality [18,19]. There is a review on recent advances made in algal genetic manipulation [20], and there are many research centers and University facilities that offer services in this regard. The outcome would be the development of commercially feasible microalgae biorefineries and boost the microalgae industry.

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