Transcriptomics and Proteomics Studies for Biofuel Production in Microalgae: An Overview

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ABSTRACT

With increasing concerns about the global crude oil consumption, sustainable alternative fuels from renewable resources are gaining more and more attention. In the recent push of phycological research, microalgae have emerged as one of the most sustainable lipid-based biofuel feedstocks. However, their intrinsic genetic potential is usually not sufficient to enable significantly high biofuel productivity. In this regard, understanding the genes and metabolic pathways through omics studies leading to improved bio-product yield is crucial. Integrative omics analysis is a powerful biotechnology-based approach allowing abundant access to varied metabolic pathways and has facilitated understanding of the complete system of microalgae. These techniques assist us to acquire a large volume of data from a single experiment. There are several reports on the significant use of various omics techniques to upgrade the strains of microalgae for improving the microalgal biofuel production. Omics approaches that have helped in the enhancement of the cellular lipid content, are highlighted in this article. This review summarizes the current knowledge about omics studies of microalgae intending to increase the production of biofuel.

Key words: Algomics; Biofuel; Microalgae; Proteomics; Transcriptomics

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Introduction

Sustainable source of energy is a need of the present time as depleting fossil fuel supplies, escalating fuel prices, geopolitical issues and growing concern about the effects of climate change have compelled us to think about alternate sources of energy. To date, many alternative renewable energy sources, such as solar, wind, wave and tidal, have been postulated and investigated to replace fossil fuels (Brar et al., 2021). Unfortunately, none of these alternatives can compete with fossil fuels in terms of their energy efficiency, cost-effectiveness and reliability (Brar et al., 2021). However, after the discovery of 3rd generation biofuel it was thought that this could be a revolution in the field of search for alternative fuels. Biofuels, which are generated from diverse biological sources such as vegetable oil, food grains, animal fat, or lipids from yeast, bacteria, or algae, have established a niche as a substitute for fossil fuels (Hegde et al., 2015; Singh et al., 2020).

Among these sources, microalgae have been recognized as a promising biofuel alternative and a prospective third-generation feedstock for biofuel (Correa et al., 2021). The efficiency of microalgae to generate biofuel is remarkable due to their relatively high lipid content, fast growth rate, and the ability to thrive in unfavourable conditions (Li et al., 2008). It has been demonstrated that the oil yielding capacity of microalgae is 25 times greater than that of the traditional biofuel feedstock such as palm oil (Brar et al., 2021). However, because of poor biomass yield of microalgal cells cultivated in photoautotrophic mode and high harvesting costs, the application of microalgae as a source of biofuel remains in its infancy. The challenges can be resolved by using tailored microalgal strains that can effectively produce boosted biomass with enhanced levels of lipid content for efficient biofuel generation (Shahid et al., 2020). The advantages of upgraded microalgal strain can be understood by some current developments and applications. Genetic transformation and optimization of growth conditions approach have been recognized as a promising tool for enhancing the production of biomass and lipid in algal cells (Sirohi et al., 2021). Nonetheless, despite the efficiency of genetic manipulation and optimization of

culture conditions in several microalgae species, such as *Auxenochlorella pyrenoidosa* (H. Chick) Molinari & Calvo-Pérez*, Chlorella sorokiniana* Shihira & R.W. Krauss 1965*, Chlorella vulgaris* Beijerinck 1890, *Chlamydomonas reinhardtii* P.A. Dangeard, nom. cons. 1888*, Phaeodactylum tricornutum* Bohlin 1898 and *Scenedesmus obliquus* (Turpin) Kützing 1833, only 35–89% (of dry weight) of lipid content have been attained and the highest percentages resulted from stress conditions (Rodolfi et al., 2009; Přibyl et al., 2012; Niu et al., 2013; Breuer et al., 2014). This illustrates that new biotechnological and genetic approaches must be employed in order to make better use of microalgae by enhancing the lipid accumulation and productivity (Tamayo-Ordóñez et al., 2017).

Omics studies for algae known as 'algomics' (Mishra et al., 2019), is beneficial for studying the metabolic functions and identifying the probable solutions for the development of algae biorefineries (Sirohi et al., 2021). In this framework, advances in next-generation sequencing (NGS) technology have enabled the quick assembly of microalgal genomic sequences (Radakovits et al., 2010; Tirichine and Bowler, 2011), which give data for using omics technology to analyse the metabolism of value-added biomolecules (Guarnieri et al., 2011; Tran et al., 2016). Omics platforms have been applied to acquire a comprehensive knowledge of algal fatty acid biosynthetic pathways to ameliorate the production of lipid (Patade et al., 2018; Salama et al., 2019). Numerous microalgal species, such as *Chlamydomonas reinhardtii*, *Phaeodactylum tricornutum*, *Ostreococcus tauri*, *Chlorella* sp. NC64A, *Volvox carteri*, *Botryococcus braunii*, *Nannochloropsis oceanica*, *Dunaliella salina* and *Ostreococcus lucimarinus* have been comprehensively sequenced, and databases are accessible to strengthen the functional annotation of genes that are involved in lipid metabolism (Benmoussa, 2016; Salama et al., 2019).

Various omics approaches like genomics, trans-criptomics, proteomics and metabolomics generate massive amounts of data that may be interpreted using different software and have appeared significant in developing the improved microalgal strains with upgraded biomass and lipid production for biofuel generation (Bhattacharjya et al., 2021; Chakdar et al., 2021a). The majority of omics-based research is focused on identification of the gene targets that increase lipid biosynthesis in microalgae (Rawat et al., 2021). Furthermore, significant progress has been achieved

in the direction of gene overexpression or deletion in various microalgal strains with great potential for biofuel generation (Lin et al., 2013) as shown in Figure 1. For instance, Transcriptome analysis of *Chlamydomonas reinhardtii* demonstrated increased lipid accumulation with considerable up-regulation of lysophosphatidic acid acyltransferase and glycerol-3-phosphate dehydrogenase,
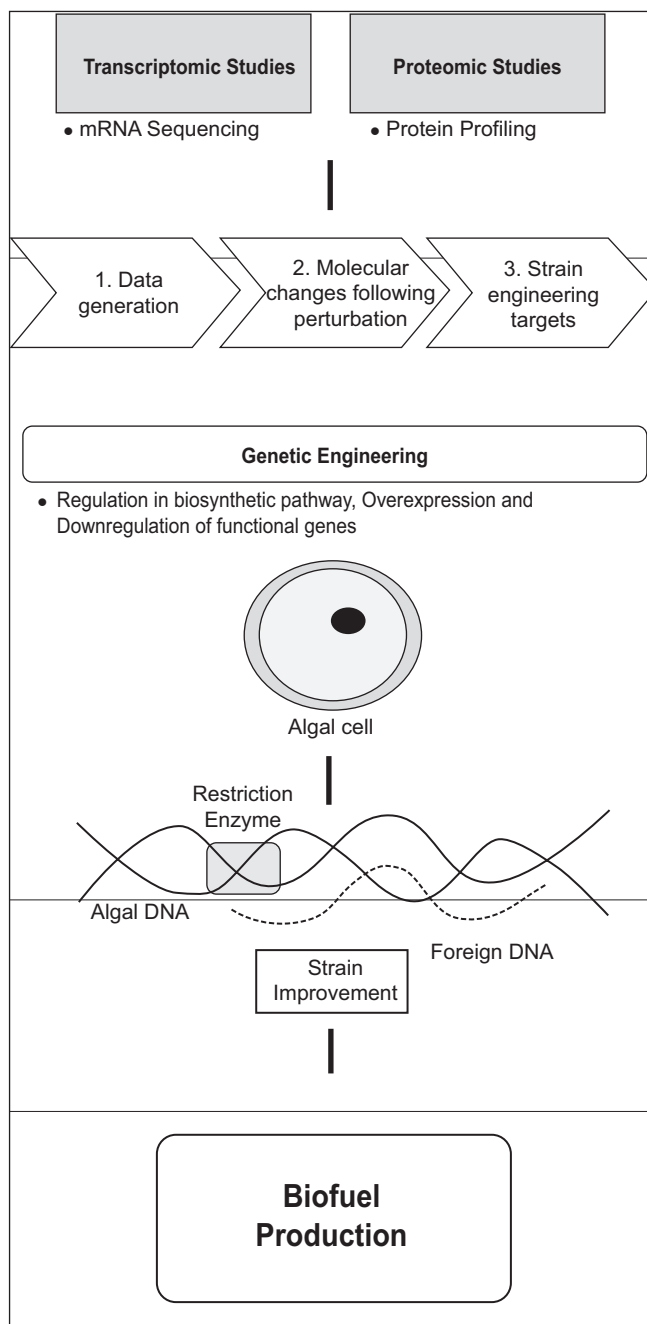
Figure 1. Illustration of transcriptomics and proteomics approaches towards microalgal strain improvement for biofuel production (adapted and modified from Rai et al. 2016 and Bharadwaj et al. 2019).

indicating a positive link between gene transcription and intracellular lipid accumulation (Lv et al., 2013). Similarly, acetyl-CoA synthetase (ACS) gene in *C. reinhardtii* was over-expressed which led to the rise in lipid content by 6-folds compared to its natural form (Rengel et al., 2018). Malonyl-CoA and ACS are two important enzymes that catalyze the formation of acetyl-CoA from acetate, a key molecule in lipid biosynthesis (Sirohi et al., 2021). Lipid production in *Phaeodactylum tricornutum* has been enhanced by the overproduction of D GAT-2 (Diglyceride acyl transferase) gene (Niu et al., 2013).

Another study reported that overexpression of the glycerol-3-phosphate acyltransferase 2 enzyme isoform improves lipid content while lowering protein and carbohydrate levels in *P. tricornutum* (Wang et al., 2020). Furthermore, an increment in the proportion of shorter chain fatty acids in *P. tricornutum*, have been noted through heterologous expression of two thioesterases responsible for synthesizing lauric acid and myristic acid (Radakovits et al., 2011). The acyl-acyl carrier protein (ACP) thioesterases are known to be specific for short chain fatty acids and incorporation of up to 75–90% of short length fatty acids into the triacylglycerides (TAG) was recorded. Hence, it can be concluded that the overexpression of thioesterases is a significant strategy to boost biofuel production in microalgae (Radakovits et al., 2011; Brar et al., 2021).

According to the literature analysis outlined above, several metabolic pathways in algae may be modified or altered using-omics approach to improve the production of commercially valuable products such as biofuel. The application of algomics will give a better knowledge of how microalgal cell components and their metabolism can be possibly used to incorporate into high quality production, therewith making microalgal biorefinery idea viable and sustainable. This study addresses the role of transcriptomics and proteomics towards improving microalgae efficiency for biofuel production.

Transcriptomics

Transcriptomics is a molecular technique that generates information about gene expression and the functional characteristics of an organism since it embodies the entire set of transcripts in a cell (Shah et al., 2019). Microalgal transcriptomic studies mainly focuses on identifying differentially expressed transcripts in diverse physiological stress conditions, and hence provides a more comprehensive view than the genomic strategy (Rai et al., 2016). Transcriptomic sequence databases include only transcribed genomic regions, excluding information on post-transcriptional regulation and untranscribed genomic regions (e.g. promoters, introns), hence simplifying eukaryotic genetic analyses (Molnár et al., 2012; Shah et al., 2019). Transcriptomic studies can also help in the reconstruction of metabolic pathways (Molnár et al., 2012). The transcriptome of *Chlorella minutissima* UTEX2341 revealed the presence of enzymes involved in TAG biosynthesis, such as 3-ketoacyl-ACP synthase, malonyl-CoA-ACP transacylase, and 3-ketoacyl-ACP reductase, indicating that it provides both valuable information and comprehensive molecular pathways in terms of genetic resources (Yu et al., 2016). Fundamental studies on transcriptomics of *Dunaliella tertiolecta*, a potential feedstock for biofuel, were conducted by Rismani-Yazdi et al., to discover the enzymes involved in the biosynthesis of TAG, fatty acids, and starch (Rismani-Yazdi et al., 2011). Subsequent analyses demonstrated global similarity in lipid and starch biosynthesis pathways and enzymes among *D. tertiolecta*, *Chlamydomonas reinhardtii* and higher plants (Durrett et al., 2008; Rismani-Yazdi et al., 2011).

Transcriptomic analysis provides an in-depth knowledge of gene function with genetic alteration and through the analysis of RNA transcripts of microalgae for effective biofuel production (Mishra et al., 2019). Furthermore, transcriptomic study (sequencing and annotation) has helped in finding genes involved in lipid biosynthesis in *Chlorella minutissima*, for biodiesel production (Yu et al., 2016). In a report, 50% increase in fatty acid methyl ester (FAME) was observed in *Chlorella vulgaris* under nitrogen deficient conditions via transcriptomic analysis (Guarnieri et al., 2011). Transcriptomic analyses in *Nannochloropsis oceanic* under nitrogen deprived conditions revealed that seven putative diacylglycerol acyltransferase (DGAT) genes were upregulated, whereas six were downregulated (Li et al., 2014). Transcriptome analysis together with lipidomic study in *C. reinhardtii* led to the identification of genes involved in TAG biosynthesis pathway (DAG acyltransferase DGTT1 and phospholipase A2 homolog) under heat stress (Légeret et al., 2016). Functional annotation of *Botryococcus braunii* race B (green microalga), revealed several operational biological pathways (Molnár et al., 2012). Transcriptomic data from another alga, *Neochloris oleoabundans*,

exhibited a five-fold rise in triglyceride synthesis under nitrogen-limiting circumstances (Rismani-Yazdi et al., 2012). Data on the genes and biosynthetic pathways involved in lipid/TAG production in *N. oleoabundans* were acquired by quantifying gene expression under various nitrogen concentrations and assemblage of transcriptome. This new repository of knowledge may be exploited for further study and strain engineering for lipid accumulation and will permit metabolic engineering to assist in the production of renewable liquid fuel (Rismani-Yazdi et al., 2012).Transcriptomic studies can accelerate the progress towards using microalgae for biofuel production as shown in Table 1. These transcriptomic evaluations provide background information for genomic level alteration in algae to improve distinct production capability potential or stress tolerance (Sirohi et al., 2021).

Proteomics

Genomics and transcriptomics give information about a cell's genetic complexity and expression patterns, whereas proteomics explores protein-protein interactions and protein expression. Proteomics seeks to discover post-translational modifications, protein arrangements in multiprotein complexes, and their distribution in cells (Salama et al., 2019; Carrasco-Reinado et al., 2022). Proteomics technologies have improved markedly over the years from recognising proteins to quantifying them (Rai et al., 2016). It is a potent technique that has been employed to understand the function of proteins in the formation of biomolecules, particularly in response to stress (Carrasco-Reinado et al., 2022). Proteomics also helps in the identification of proteins that play significant roles in lipid accumulation and provides vital information about the genes to be modified for the increment of lipid biosynthesis. High-performance liquid chromatography (HPLC), Tandem mass spectrometry, 2D-gel electrophoresis, liquid chromatography–mass spectrometry (LC–MS), and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) are some frequently used techniques to examine post-translational modifications, identify proteins involved in the biosynthesis processes and for quantitative analysis (Liska et al., 2004; Shahid et al., 2020; Chakdar et al., 2021b; Carrasco-Reinado et al., 2022). Proteomics has assisted in gaining knowledge about how protein expression can be downregulated or upregulated at translational and post-translational level during varying stress conditions which influence TAG accumulation in the cells (Anand et al., 2018; Arora et al., 2018). Oleaginous microalgae yield a substantial amount of triacylglycerol (TAG) and fatty acids, which may serve as a potential feedstock for the synthesis of biofuels and other bio-products (Sirohi et al., 2021). Proteome database gives an insight into microalgal mechanisms for the accumulation of lipid. Xue et al. examined the function of malic enzymes in the accumulation of lipids in *Phaeodactylum tricornutum*. Their findings showed that overexpression of malic enzyme in transgenic *P. tricornutum* resulted in a 2.5-fold increase in total lipid content (Xue et al., 2015). In proteomic study by Tran et al. (2016) results indicated 60% FAME in *Nannochloropsis oculata* development under nitrogen deprivation, and the nitrogen from protein and pigment degradation was channelled predominantly into fatty acid biosynthesis (Tran et al., 2016). Guarnieri et al. (2013a) adopted a proteomics-based approach to

Table.1. Omics approach for the production of biofuel in microalgae

Algal Species	Omics approach	Condition	Target Product	Ref.
Auxenochlorella protothecoides	Transcriptomics and proteomics	Effect of nitrogen, phosphorus and temperature, and oil accumulation	Biofuel	(Park and Choi, 2018; Xing et al., 2018)
Botryococcus braunii	Transcriptomics	N_2 deprivation, high salt, cobalt enrichment, $NaHCO_3$, salicylic acid, and acetic acid	Hydrocarbons and biofuel	(Park and Choi, 2018)
Nannochloropsis gaditana	Transcriptomics and proteomics	Nitrogen alterations and light intensity regimes	Biofuel	(Ajjawi et al., 2017; Shah et al., 2017)
Nannochloropsis gaditana	Transcriptomics	Different light regimes	Biofuel	(Patelou et al., 2020)
Chlorella vulgaris	Proteomics	Nitrogen deprivation	TAG and lipid	(Guarnieri et al., 2013b)
Picochlorum sp.	Transcriptomics	High temperature and salinity stress	Biofuel	(Kumar et al., 2020)
Dunaliella salina	Transcriptomics and Proteomics	Nitrogen depletion, oxidative stress, arsenate, salinity, and high bicarbonate ion level	Biofuel and glycerol	(Kumar et al., 2020)
Fistulifera solaris	Proteomics	Nutrient deplete and replete conditions	Biofuel	(Nonoyama et al., 2019)

investigate the process and strain engineering targets in *Chlorella vulgaris* for excessive production of lipids necessary for improved biofuel production (Guarnieri et al., 2013a). A thorough analysis of this microalga's proteome in the presence and absence of nitrogen indicated prospective targets for the strain improvement to boost the lipid content. This was explained by the fact that nitrogen deprivation in *C. vulgaris* promotes lipid accumulation (Guarnieri et al., 2013a). Proteomics together with transcriptomics may offer insights into microalgae and provide a platform needed for the strain enhancement (Table 1).

Future Prospects

Omics approaches appear to be promising for modifying metabolic pathways in microalgae in order to increase the production of industrially significant products. The current review study examines algomics, which can have a significant impact on lipid content in algae for biofuel generation. Numerous studies have successfully carried out transcriptomics and proteomics analysis to identify the microalgal genes showing differential expression and enzymes involved in the metabolic pathways of lipid accumulation. Moreover, substantial shifts in the biochemical flux caused by external factors can affect the physiology and metabolic pathways of algae, which are reflected in their transcriptomic and proteomic analysis. Transcriptomics, for example, can help in the reconstruction of intrinsic metabolic pathways in microalgae to improve fatty acid biosynthesis. Expression of proteins in response to stress, using proteomics, has facilitated the overproduction of TAG/lipids in microalgae. A thorough knowledge of changes in transcriptome, and proteome could assist in determining lipid accumulation mechanisms. Despite recent advances in research, various challenges still need to be addressed in order to make the production of microalgal-derived biofuel a cost-effective process. Access to more transcriptomes and proteomes for microalgae is necessary to aid the identification of novel genes and associated gene products in the biochemical pathways that can be linked for optimum production of biofuel. High biofuel productivity could be attained by concurrent analysis of different 'omics' strategies and their interactions. In conclusion, for the cost-competitive production of biofuels, a thorough knowledge of microalgal metabolism is crucial, to genetically engineer microalgal strains competent for yielding high lipid content.

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