Int Aquat Res (2022) 14:81-94
https://doi.org/10.22034/IAR.2022.1954594.1255

REVIEW

Overexpressing key enzymes in lipogenesis to boost microalgae cellular oil content for biofuel production, A mini-review

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Received: 09 March 2022 / Accepted: 12 June 2022 / Published online: 15 June 2022

Abstract Rapid population growth globally and urbanization have caused a significant drop in fossil fuel feedstocks, pushing countries to seek alternative sources. Microalgae are a feasible biofuel feedstock due to their high photosynthetic efficiency, which gives various potential benefits for the environmentally friendly biofuel production process, besides the high biomass productivity strains required for a long-term integrated platform. Consequently, modifying lipid metabolic pathways to increase lipid production in microalgae cells consider a viable strategy. Overexpression and transformation of key genes involved in lipid biosynthesis are regarded to be crucial methods for increasing lipid production. Whereas transformation and gene editing are two approaches affecting these alterations, that have been sufficiently established in microalgae, transforming these strains into a very adaptive stage for successfully designing ways to produce biofuel. The current mini-review focuses on potential strategies for improving microalgae metabolic engineering, specifically lipogenesis, by regulated overexpression of native genes or transgenes such as acetyl coenzyme-A carboxylase, diacylglycerol acyltransferase, and malic enzyme genes, as well as current limitations and gaps and future directions.

Keywords Metabolic engineering . Gene transfer . Biofuel . Acetyl coenzyme-A carboxylase . Diacylglycerol acyltransferase

Introduction

Energy security is among the vital difficulties that have been extensively considered and discussed in the current decades; due to the worldwide increase in energy requests and the crisis of fossil fuel reduction (Cobos et al. 2017; Hopkins et al. 2019). The petroleum diminution may be attributed to urbanization, industrialization, and the overgrowth of the world population (Bharathiraja et al. 2015; Elsayed et al. 2017). Moreover, petroleum fuels are currently excavated from restricted sources that are centered in a specific region of the world. This gives superiority to countries owning these resources, while others will shortly be suffering from energy crises (Sharma and Singh 2017; Shin et al. 2018). Furthermore, fossil fuel combustion produces; CO_2, CO, black carbon, nitrogen oxides, methane, sulfur dioxide, and volatile organic

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compounds, that consider the main sources of global warming (Oda et al. 2018; Mousavi et al. 2019). Consequently, the above critical issues, looking for fossil fuel alternative sources is an urgent necessity, that relies on cheap and renewable sources has received a lot of consideration currently.

Biofuel production from microalgae is considered a promising source, due to microalgae having a high growth rate and replication, high efficacy at photosynthesis, high lipid content, and ability to stand in an extensive range of environments (Ogburn and Vogt 2017; Bharadwaj et al. 2020). Moreover, microalgae consume CO_2 from the atmosphere, which alleviates greenhouse gas, also microalgae consider a source of numerous biofuel types as; biodiesel, bioethanol, and biohydrogen (Huang et al. 2010; Mishra et al. 2018; Hussein et al. 2022). Microalgal lipids are considered an applicable, sustainable exporter of biofuel and other hydrocarbons with industrial applications. Subsequently, researchers sought for optimizing lipid production by genetic modification (Golden et al. 1987; Singh et al. 2022).

Genetic engineering currently acquired extra considerations due to new and powerful genetic tools exist, and the genome can be modified to our necessity, more accurately than previously (Fajardo et al. 2019; Bharadwaj et al. 2020). Whereas great interests have been devoted to use genetic engineering approaches to get desired traits, such as triggering lipid accumulation in different microalgae species. Over the past decade, noteworthy developments have been completed in the capability to modify the genomes to enhance lipid accumulation through genetic modification. Among these methods are: [a] raising the speed of group CoA and carboxylic acid synthesis; [b] suppression of lipases and ß-oxidation enzymes to block degradation; [c] overexpression of enzymes responsible for triacylglycerols (TAG) synthesis in Kennedy pathway; [d] modification of specified thioesterase and desaturases to control fatty acid chain size and saturation (Saini et al. 2019; Eungrasamee et al. 2019).

In the provided mini-review, we conducted a summary survey for most studies that exclusively covered the lipid improvement in microalgae by overexpressing acetyl coenzyme-A carboxylase (ACC), diacylglycerol acyltransferase (DGAT), and malic enzyme (ME) genes, specifically from the engineering process of the gene, plasmid, transformation method, and achieving results.

Overview of engineered microalgae

In the last decades, genetically modified microalgae have got considerable consideration as promising catalysts for the transfer of CO_2 into lipid the backbone of the microalgae biofuel (Chi et al. 2008). Genetic engineering methods especially transformation have been quite recognized in microalgae, that convert these microorganisms to an extremely manageable platform to confirm effective biosynthetic paths for biofuel production (Radakovits et al. 2010). The method of DNA transformation involving methods to insert external DNA and effectual markers and plasmids has been established well to reduce the biofuel industry cost through increasing microalgae lipid production (Chen et al. 2014). Several DNA transformation procedures for example electroporation, gene guns, glass beads, and *Agrobacterium* have been formed to insert DNA in microalgae cells (Nazari and Raheb 2015).

Transformation techniques in microalgae cells

Electroporation is a well-recognized technique to transfer external DNA into a host. However, this technique utilizes an electrical current within the microalgae membrane causing a temporary hole creation, that simplifies the uptake of foreign genes (Venegas-Calerón et al. 2010; Bolhassani et al. 2014). However, utilizing the electroporation technique needs a complicated apparatus that works by an electric current to insert the external DNA into the cell. In addition, several optimization actions and applying a cell wall lysis are required (Coll 2006). Furthermore, additional techniques appropriate for algae nuclear transformation include using “Silicon Carbide Whiskers” in which DNA may be introduced into microalgae strains by agitating the cells in the presence of glass beads and polyethylene glycol (Te and Miller 1998; Dunahay et al. 1997). Gene guns are currently also considered the most effective technique for the transport of external genes into the host microalgal cell utilizing the microcarrier particles (Heiser 1992). Microcarrier atoms either one of gold or tungsten, are covered with a plasmid including foreign DNA and fired up at microalgal colonies cultivated on agar plates (Kindle 1990), but unfortunately, it is not available at all research labs especially in Egypt because of its high cost. Besides using polyethylene glycol during sonication (Jarvis and

Brown 1991), and culturing with *Agrobacterium tumefaciens* (Kumar et al. 2004).

Currently, the transformation of a gene depending on using polymer nanoparticles via biodegradable non-viral delivery carriers as chitosan nanoparticle is a significant method (Abdel-Razik et al. 2017). Chitosan is a carbohydrate polymer that significantly has been utilized in DNA delivery (Raftery et al. 2013). Polymer carbohydrate nanoparticles have numerous advantages to be used as a gene carrier; applicability to use with any type of cells, preventing transgenic silencing through nanoparticles complex managing plasmid DNA (pDNA) copies, and multigene insertion can be accomplished easily by nanoparticles without reliant on the outdated construction technique of multifaceted carrier (Fu et al. 2012; Abdel-Razik et al. 2017). One of the nanoparticles polymer chitosan that possesses unique characteristics includes biodegradability, biocompatibility, low immunogenicity, and low cytotoxicity (Jiang et al. 2008), making them useful for gene transport (Huang et al. 2020). The foundation of functional amino groups in the chitosan structure facilitates an electrostatic interaction between chitosan nanoparticles and negatively charged pDNA (Duceppe and Tabrizian 2010; Fathy et al. 2020). Chitosan nanoparticles and pDNA complex show high stability, which prevents DNA degradation before introduction into the cellular cytosol (Abdel-Razik et al. 2017).

Biofuel demanding and fossil fuel alternative

Biofuel production from microalgae cells, which are prokaryotic or eukaryotic photosynthetic organisms, is considered a vital source because of the high rate of growth and duplication, easily of scale-up, highly effective at photosynthesis, high lipid content, and could stand a wide range of environments as salt and wastewater, in addition to large scale microalgal culture doesn't require cultivable land, variety in adaptation to environmental fluctuations and harvesting methods when compared with traditional oil crops (Ogburn and Vogt 2017; Bharadwaj et al. 2020). Microalgal biofuel is considered applicable and sustainable with marketable applications. Whereas lipid bodies function in microalgal cells is hypothetical to be greatly more intricate than serving as a carbon stock, a source of fatty acids, but also required for membrane synthesis and remodeling (Murphy 2001).

Microalgae photosynthesis consumes CO_2 to regenerate microalgal biomass that could be used to yield commercially valued products (Baek et al. 2016). Alleviation of greenhouse gas by microalgae is taken into regard, further environmentally friendly and advantaged than chemical or physical CO_2 elimination (Zhang et al. 2009; Srivastava et al. 2020). Also, they are likewise a foundation of extremely valued molecules for example vitamins, proteins, polyunsaturated fatty acids, pigments, and carbohydrates (Brodie et al. 2017; Koutra et al. 2018). Those components give several uses in fertilizers, nutrition, medical, and cosmetic industries owing to their several biological activities as; antioxidant, anticancer, antihypertension, immunomodulatory, and prevention of cardiovascular problems that have been utilized for thousands of years by many populations (Keeling et al. 2014; Saini et al. 2019; Zhang et al. 2020). The crucial challenge in microalgal biofuel is to discover the promising technique to improve lipid productivity during continuing their rapid growth.

Microalgae lipid production optimization

Certain microalgae species produce lipid up to 50% of their biomass, which consider better than other sources for biofuel (Fig. 1) (Hu et al. 2008; Sun et al. 2019). Even though microalgae are qualified for accumulating great amounts of lipid, this is often related to external stress that compromises biomass productivity (Wang et al. 2009; Zhou et al. 2018). One of the core challenges is to discover the technique that will allow microalgae to increase lipid productivity, during continuing to grow quickly and robustly.

Lipids are usually considered the maximum valuable fraction of microalgal biomass. Different types of lipids are found in microalgae, but TAG is the backbone of biofuel production (Chia et al. 2018). TAG biosynthesis in microalgae similar to advanced plants is a complicated process. Whereas, *Chlamydomonas reinhardtii* is predestined to have 113 genes implicated in lipogenesis (Li-Beisson et al. 2015; Wase et al. 2018), while *Phaeodactylum tricornutum* has 106 genes (Wase et al. 2018; Mühlroth et al. 2013). The Kennedy pathway is the principal metabolic pathway in microalgae and is referred to as acyl-CoA-dependent TAG biosynthesis. A popularized scheme for the synthesis of TAG in eukaryotic microalgae (Fig. 2) is composed of three major steps; [a] acetyl-CoA carboxylation to form malonyl-CoA, the obligating stage

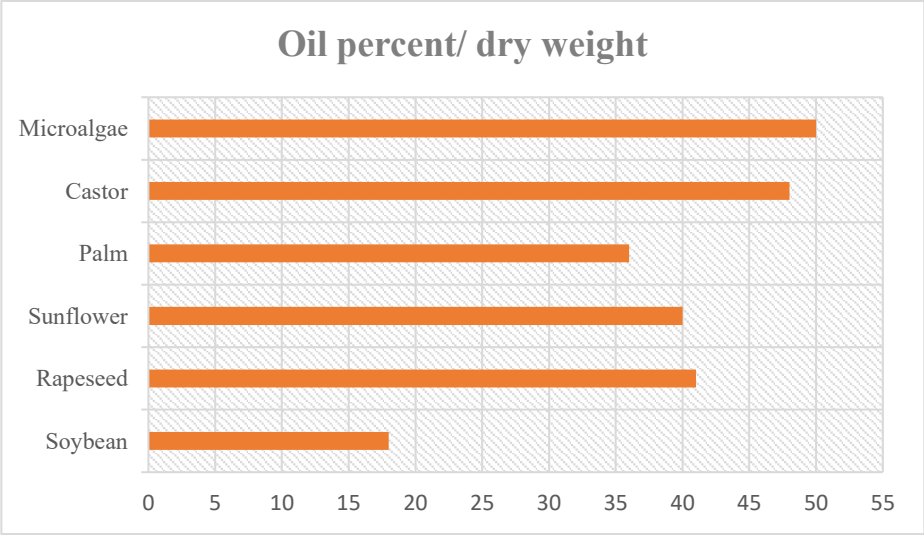

Fig. 1 Microalgae oil content compared with other biofuel feedstocks, adopted from (Chisti 2007; Elsayed 2017)

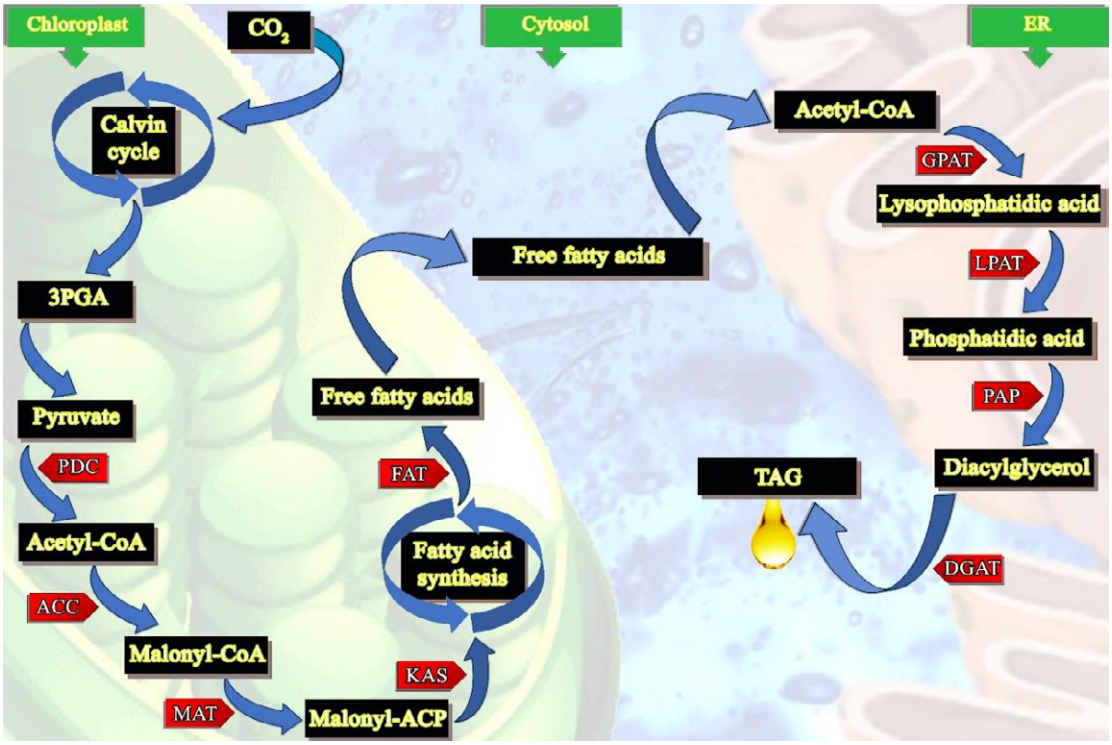

Fig. 2 Simplified diagram of TAG biosynthesis in microalgae. CoA: Coenzyme A-carboxylase, 3PGA: 3-Phosphoglycerate, ACP: Acyl-carrier protein, PDC: Pyruvate dehydrogenase complex, KAS: 3-Ketoacyl-ACP synthase, GPAT: Glycerol-3-phosphate acyl-transferase, FAT: Fatty acyl-ACP thioesterase, DGAT: Diacylglycerol acyltransferase, LPAT: Lyso-phosphatidic acid acyltransferase, PAP: Phosphatidic acid phosphatase, TAG: Triacylglycerols.

for fatty acid biosynthesis, [b] acyl chain elongation, and [c] TAG formation (Nobusawa et al. 2017). The biological pathway for lipid synthesis in blue-green algae (Fig. 3) resembles that of prokaryotes.

Microalgae metabolic engineering

Genetic engineering of microalgae has developed owing to the unlimited variety of products that can be manufactured from its cell biomass. Confronting an ambiguous future with food and energy deficiencies

Fig. 3 Simplified diagram of fatty acid synthesis in *Synechocystis* sp. ACC: acatyl-CoA carboxylase, fabD: malonyl coenzyme A-acyl carrier protein transacylase ACP: Acyl-carrier protein, plsX, plsY, plsC, putative phosphate acyl-transferases, fabB: β-Ketoacyl-ACP synthase I, fabF: β-Ketoacyl-ACP synthase II, fabH: β-Ketoacyl-ACP-synthase III, fabG: β-Ketoacyl-ACP reductase, fabZ: β-Hydroxyacy-ACP dehydrase, fabI: Enoyl-ACP reductase 1, adopted from (Fathy et al. 2021).

and increasing climate change, microalgal engineering has turned into one of the best critical roads for such emerging difficulties that are faced today (Baek et al. 2016; Daboussi et al. 2014; Poliner et al. 2020). Microalgae are prospective sources for future biofuel and various valued natural metabolites. Some researchers seek for optimizing the lipid enrichment and the lipid bioactive compound composition by genetic manipulation (Fathy et al. 2021). Some genetically modified strains have been produced to resolve challenging problems and accomplish economic practicality by genetic engineering (Hamed 2016). In synthetic biology, the elementary gene manipulation procedure rests the same, containing host choice, gene selection, plasmid building, transformation methods, selection system, and DNA editing tools. In microalgae, genetic engineering, transformation, and selection techniques are crucial steps to achieve the objective (Bharadwaj et al. 2020; Ng et al. 2017).

Great interests have been devoted to applying genetic engineering approaches to get desired traits, such as triggering lipid accretion in different microalgae species. Through the past decade, noteworthy developments have been achieved in the capability to modify genomes to enhance TAG accumulation through genetic modification. Among these methods is the overexpression of the following enzymes.

Acetyl coenzyme-A carboxylase gene (ACC)

ACC assembles the first obligated stage in fatty acid synthesis through producing malonyl-CoA from carboxylation acetyl-CoA, and fatty acid synthase complexes were identified (Radakovits et al. 2010; Fathy et al. 2021). Besides, the ACC enzyme enters carbon sugar metabolism which makes an irreversible reaction that transforms acetate to acetyl-CoA (Kumari et al. 1995; Russ et al. 2012).

Table 1 Summarizing the literature data depends on the ACC gene to enhance lipid production

Gene	Donor	Acceptor	Change	Plasmid	Transformation method	Reference
ACC	*Cyclotella cryptica*	*Cyclotella cryptica* and *Navicula saprophila*	2 to 3-folds in ACC activity. But no change in lipid content	pACCNPT10 and pACCNPT 5.1	Gene gun	(Dunahay et al. 1995; Sheehan et al. 1998)
ACC	*E. coli*	*Dunaliella bardawil*	ACC was enhanced by 15.8%. But the lipid content did not significantly increase	pGEX-6P-1-accA	Electroporation, and gene gun	(Ren 2009)
ACC	*E. coli* CFT073	*Schizochytrium* sp. TIO1101	Transformant fatty acid increased by 11.3%	pBluescript II SK (+)	Electroporation	(Yan et al. 2013)
ACC2	C. *reinhardtii*	C. *reinhardtii*	TAG accumulation increased by 2.4-folds	Phyco105-CrACS2	Glass-beads	(Rengel et al. 2018)
accDA CB	*Synechocystis* sp. PCC 6803	*Synechocystis* sp. PCC 6803	Lipid content after day four from growth improved to 2.3-folds higher unsaturated lipid content which reduced after that.	pEERM	Natural transformation	(Eungrasamee et al. 2019)
accA	*E. coli* DH5α	*Synechocystis* sp. PAK13	Increased total lipid content by 3.6-folds	pLUG	Chitosan nanoparticles	(Fathy et al. 2021)

Here we survey all data that fall in our hands about microalgae ACC overexpressing from the Web of Science and ProQuest. Table.1 collects this data chronologically, the first trial to engineer microalgal TAG biosynthetic pathways was done by Dunahay et al. (1995) to rise lipid production in the diatom *Navicula saprophila* and *Cyclotella cryptica* through overexpressing the native ACC of *Cyclotella cryptica*. However, ACC activity increased by 2 to 3-folds in the transformant but failed to increase lipid content. In addition to Ren (2009) modified *Dunaliella bardawil* by transferring the ACC gene from the *E.coli* but the result indicated no significant enhancement in the lipid content of the transformant strain. However, Yan et al. (2013) modified *Schizochytrium* sp. by cloning the ACC gene from *E. coli*. The transferred ACC gene was effectively integrated and expressed and exhibited a higher fatty acid proportion by 1.11-fold. Furthermore, Rengel et al. (2018) transformed *Chlamydomonas reinhardtii* with a native ACC gene, which leads the transformant to show 6-folds higher levels of ACC activity and 2.4-folds higher accumulation of TAG. Eungrasamee et al. (2019) engineered *Synechocystis sp.* by four ACC subunits a, b, c, and d from wild strains that improved total lipid content after day four from growth to 32%. Furthermore, Fathy et al. (2021) manipulated *Synechocystis* sp. PAK 13 by accA from *E. coli* DH5α and improved lipid content to 3.6-folds higher than wild strains.

Although ACC is responsible for the first stage of fatty acid biosynthesis in the chloroplast, there is little research that uses it as a key to up-regulate lipid production. Whereas few articles have been examined it since 1995, this may be attributable to the ACC being a complicated enzyme, such as in *E. coli*, which comprises four subunits (Kondo et al. 1991).

Diacylglycerol acyltransferase (DGAT)

DGAT enzymes catalyze the ending and crucial step in the TAG construction by moving an acyl group from acyl-CoA to diacylglycerol to produce TAG (Li et al. 2016). Table.2 collects this data chronologically, However, articles that utilized *Phaeodactylum tricornutum* as a DGAT enzyme gene donor were; Guihéneuf et al. (2011) cloned DGAT gene in *Saccharomyces cerevisiae* that increased TAG biosynthesis and leads to increased DGAT activity expression to 190-folds under nitrogen starvation. Also, Cui et al. (2013) transferred the DGAT gene to *S. cerevisiae,* which led to the formation of lipid droplets. Moreover, Niu et al. (2013) modified *Phaeodactylum tricornutum* by cloning the native DGAT gene, which showed a high expression of transformed DGAT that boosted fatty acids, and the TAG content up to 35%. Additionally, Dinamarca et al. (2017) modified *Phaeodactylum tricornutum* by native DGAT gene and detected that the transformant has 2-folds higher total lipid. Moreover, Han et al. (2019) transferred the DGAT gene into *Nannochloropsis oceanica*, which considerably enriched the total lipid contents by 28% to 46% and TAG

Table 2 Summarizing the published data on using DGATs to enhance lipid accumulation

Gene	Donor	Acceptor	Change	Plasmid	Transformation method	Reference
DGAT1	*Phaeodactylum tricornutum*	*S. cerevisiae*	The lipid body formed by successful restoration of TAG biosynthesis in the deficient phenotype by PtDGAT1 increased to a maximum of 190-folds	pYES2 and pGEM-T	Polyethylene glycol/lithium acetate	(Guihéneuf et al. 2011)
G3PDH, GPAT, LPAAT, PAP, and DGAT	*Saccharomyces cerevisiae* INVSC1 and *Yarrowia lipolytica* Po1g	*C. minutissima* UTEX 2219	Lipid content increased to 2-folds	pAlgae	Electroporation	(Hsieh et al. 2012)
DGAT2	*C. reinhardtii* CC3491	*C. reinhardtii* CC3491	No change in lipid content	pJR38 and pGenD	Glass bead method	(La Russa et al. 2012)
DGAT3	*P. tricornutum*	*Saccharomyces cerevisiae* SCY62	Nile Red confirmed the formation of oil bodies	pYES-PtDGAT3	Polyethylene glycol/ lithium acetate method	(Cui et al. 2013)
DGAT2	*Chlamydomonas strain* CC-503	*S. cerevisiae* BY4742	TAG content increased to 9-folds	pCH078	-	(Hung et al. 2013)
DGAT2	*Phaeodactylum tricornutum* CCMP2561	*Phaeodactylum tricornutum* CCMP2561	Neutral lipid content increased by 35%	pHY18	Electroporation	(Niu et al. 2013)
DGAT4	*Chlamydomonas reinhardtii* CC-408	*Chlamydomonas reinhardtii* CC-408	TAG content increased to 29-folds	pMD20	Electroporation	(Iwai et al. 2014)
DGAT2	*Neochloris oleoabundans* UTEX 1185	*S. cerevisiae*	Nile Red confirmed the formation of TAG	pYES2	Transformation kit (Invitrogen, USA)	(Chungjatupornchai and Watcharawipas 2015)
DGAT1	*Chlamydomonas reinhardtii*	*Scenedesmus obliquus* CPC2	Lipid content increased by 2-folds	pCAMBIA 1301	Electroporation	(Chen et al. 2016)
DGAT2	*Nannochloropsis oceanica* CCAP 849/10	*Nannochloropsis oceanica* CCAP 849/10	Neutral lipid content increased by 69%	pNa03	Electroporation	(Li et al. 2016)
DGAT1	*Chlorella ellipsoidea*	*Saccharomyces cerevisiae*	Fatty acid increased by 142%	pYES2.0	-	(Guo et al. 2017)
		Arabidopsis sp.	Improved oil content by 8-37%	pCAMBIA2301-CeDGAT1	Agrobacterium tumefaciens	
		Brassica napus	Improved oil content by 12-18%.		Agrobacterium tumefaciens	
DGAT2	*Neochloris oleoabundans* UTEX 1185	*Neochloris oleoabundans strain* UTEX 1185	TAG content increased to 3.2-folds	pAR-DGAT2 and pB2-DGAT2	Electroporation	(Klaitong et al. 2017)
DGAT2	*Phaeodactylum tricornutum*	*Phaeodactylum tricornutum*	Lipid content increased to 2-folds	pBle_Dgat2D	Microparticle bombardment	(Dinamarca et al. 2017)
DGAT1	*Nannochloropsis oceanica* IMET1	*S. cerevisiae*	TAG increasing showed considerable in cells	pYES2-CT 2017	S.c. Easy Comp Transformation Kit (Invitrogen)	(Wei et al. 2017)
		N. oceanica	TAG increased by 1.25-1.44-folds	Te RNAi vector construction for NoDGAT1A	Electroporation	
		Chlamydomonas strain UVM4	No change	pOpt_Clover_Hyg	Glass beads	
		Yeast strain H1266	TAG increased to 53%	pYES2.1 TOPO®	pYES2.1 TOPO® TA Expression Kit	
DGAT5	*Nannochloropsis oceanica* CCMP1779	*Nannochloropsis oceanica* CCMP1779	Led to a strong increase of TAG content in lipid extracts under N-replete conditions	pnoc gfp dggt5pro vector	Electroporation	(Zienkiewicz et al. 2017)
		Arabidopsis thaliana	TAG content increased to 3-folds	pEarleyGate 101 vector	*A. tumefaciens*	
		Tobacco	Fatty acids and TAG to 2-folds	pEarleyGate 101 vector	*A. tumefaciens*	

Table 2 Continued

DGAT2	*N. oceanica*	*N. oceanica*	Polyunsaturated fatty acid increased to 11.2-folds	pXJ433	Electroporation	(Xin et al. 2017)
DGAT2 (a and b)	*Phaeodactylum tricornutum*	*Nannochloropsis oceanica*	Total lipid contents increased by 28.49% - 46.05% and TAG by 37.72% - 55.47%	pCB801	Electroporation	(Han et al. 2019)

by 37% to 55.47%.

Other studies depending on *Nannochloropsis oceanica* as a gene donor are presented; Li et al. (2016) overexpressed native DGAT in *Nannochloropsis oceanica,* which increased TAG biosynthesis significantly by 69% in the transformant. Furthermore, Wei et al. (2017) cloned the DGAT gene to yeast strain cells that exposed a significant TAG proliferation. Despite expression in *Chlamydomonas reinhardtii* did not affect TAG enhancement, but in *Nannochloropsis oceanica* DGAT overexpression enhanced TAG by 1.25 to 1.44-fold. Also, Zienkiewicz et al. (2017) used the DGAT gene and modified yeast to boost TAG accumulation up to 53% of overall lipid. While cloning the DGAT gene in *Arabidopsis thaliana* increase TAG in the plant leaves by 3-folds, and *Tobacco transient* expression in leaves is caused by a doubling of fatty acids associated with TAG. In addition, Xin et al. (2017) modified *Nannochloropsis oceanica* by native DGAT that created a different fraction of saturated fatty acid, monounsaturated fatty acids, and polyunsaturated fatty acid in TAG varied by 1.3, 3.7, and 11.2 folds.

Moreover, other studies that utilized *Chlamydomonas reinhardtii* as a DGAT gene donor are presented; La Russa et al. (2012) cloned native DGAT of *Chlamydomonas reinhardtii* unfortunately, it was no significant shift in the accumulative lipid contents was detected. Additionally, Hung et al. (2013) investigated that DGAT enhanced TAG synthesis in *S. cerevisiae* by 9-folds. Furthermore, Iwai et al. (2014) cloned native DGAT of *Chlamydomonas reinhardtii*, the transformant intensely improved TAG accumulation to 29-folds. Moreover, Chen et al. (2016) modified *Scenedesmus obliquus* by overexpression DGAT gene, that enhanced lipid substance in the transformant up to 2-folds.

On the other hand, other research reports depended on *Neochloris oleoabundans* as a DGAT gene donor; Chungjatupornchai and Watcharawipas (2015) transformed *Saccharomyces cerevisiae* by DGAT, which led to lipid development and TAG synthesis. Further, Klaitong et al. (2017) engineered *Neochloris oleoabundans* by native DGAT that led to total lipid yield amplified to 3.2-folds; while TAG productivity improved to 4.3-folds. Additionally, Guo et al. (2017) cloned the DGAT gene of *Chlorella ellipsoidea* into yeast that considerably improved fatty acid production by 142%, also in *Arabidopsis thaliana* and *Brassica napus* noticeably improved oil content by 8-37% and 12-18% respectively. Although amplification of total lipid stock in seeds was up to 25-50% in each of the transformant *Arabidopsis* and *B. napus*. While Hsieh et al. (2012) cloned DGAT from *Yarrowia lipolytica* into *Chlorella minutissima* that its overexpression with other genes led to the enhanced lipid production of the transformant *Chlorella minutissima* up to 2-folds.

A plethora of articles depends on using DGAT genes to boost lipid accumulation which starts in 2011 with around 17 reports. Most of them utilized *Phaeodactylum tricornutum* as a gene donor in five studies, while *Chlamydomonas reinhardtii* and *Nannochloropsis oceanica* both of them used in four studies, all these studies succeed in enhancing lipid content.

The malic enzyme (ME)

ME has been detected to be engaged in various metabolic pathways, including photosynthesis lipogenesis, and energy metabolism (Courchesne et al. 2009; Tsuchida et al. 2001). Whereas ME works as biocatalysts on the irreversible oxidative decarboxylation of malate to produce pyruvate and NADPH (Vongsangnak et al. 2012).

The results of Talebi et al. (2014) after engineered *Dunaliella salina* by cloning the ME gene from *Chlamydomonas reinhardtii* achieved 12% proliferation in lipid content. While, Xue et al. (2015) cloned native *Phaeodactylum tricornutum* ME, considerably improved total lipid production by 2.5-folds. Moreover, Xue et al. (2016) transformed *Chlorella pyrenoidosa* by ME gene of *Phaeodactylum tricornutum* that improved TAG to 3.2-folds, and total lipid content amplified to 40.9%. In addition, Yan et al. (2019) genet-

Table 3 Summarizing the published data used ME to enhance lipid productivity

Gene	Donor	Acceptor	Change	Plasmid	Transformation method	Reference
ME-AccD	*C. reinhardtii*	*D. salina* 19/18	Lipid content increased to 12%	pGH-ME-AccD	Bombardment	(Talebi et al. 2014)
ME	*Phaeodactylum tricornutum* CCMP251	*Phaeodactylum tricornutum* CCMP2561	Total lipid content increased by 2.5-folds	pHY-PtME	Electroporation	(Xue et al. 2015)
ME	*P. tricornutum*	*Chlorella pyrenoidosa*	Neutral lipid content increased by 3.2-folds	pHY11	Electroporation	(Xue et al. 2016)
ME	*Chlorella protothecoides*	*Chlorella protothecoides*	Lipid content increased by 2.8-folds	pBI121	Microparticle bombardment	(Yan et al. 2019)
ME2	*Chlamydomonas reinhardtii*	*Chlamydomonas reinhardtii*	The fatty acid and lipid content were increased by 23.4 and 19.9% respectively	pAR::ME2	Electroporation	(Kim et al. 2019)

ically transformed *Chlorella protothecoides* by cloning native ME that improved lipid content by 2.8-folds. Also, Kim et al. (2019) modified *Chlamydomonas reinhardtii* by overexpression of malic enzyme isoform 2 (ME2) resulting in a 2-3 folds rise in mRNA level and up to 23.4 and 19.9% increase in fatty acid and lipid content, respectively all data are represented in (Table.3) chronologically.

Other engineered enzymes involved in the lipogenesis

Glycerol-3-phosphate acyltransferase (GPAT) is the precarious enzyme that catalyzes the first step of TAG formation. Therefore, Niu et al. (2016) overexpressed the native GPAT of *Phaeodactylum tricornutum,* that improved TAG content by 2-folds. In addition, Duarte-Coello et al. (2019) transferred the GPAT gene from *Chlamydomonas reinhardtii* to *S. cerevisiae* which enhanced GPAT activity.

Furthermore, Peng et al. (2014) overexpressed the native delta 5-desaturase that considers rate-limiting enzymes in the production of polyunsaturated fatty acids, gene in *Phaeodactylum tricornutum* improved TAG contents to 65% in the transformant. Whereas, Kaye et al. (2015) overexpressed native delta 12-desaturase in *Nannochloropsis oceanica* considerably changed the composition of total lipids. Moreover, Osada et al. (2017) cloned the native glucose-6-phosphate dehydrogenase gene that encodes NADPH production in *Fistulifera solaris* which increased lipid productivity by 1.5-fold (Table.4) collect these data in detail.

Conclusion and future prospectives

Microalgae are considered more popular as a source of biofuel and metabolic engineering appears to be an essential component in enhancing lipid productivity in microalgae. The lipid content of microalgae used in biofuel production is affected by environmental conditions. Furthermore, in optimal conditions, certain microalgae species have a high rate of lipid productivity, which is the inverse of what occurs in outdoor settings. However, over the last decade, research has resulted in a dramatic advance in our ability to regulate gene expression in microalgae. In addition to genetic transformation and screening techniques, the development of methods to improve lipid buildup will be supported. One of the most essential aspects of enhancing microalgae metabolic engineering is that it can eventually lead to sustainable biomass and biofuel production. Therefore, numerous studies on the overexpression or deletion of lipogenesis-related enzymes have been done. The ACC gene created more acetyl-CoA, whereas the ME gene produced more pyruvate and NAPDH, which enhanced fatty acid synthesis. Consequently, endoplasmic reticulum enzymes that up-regulate the downstream process as a result of DGAT gene overexpression consume acyl-CoA intermediates quickly and accelerate TAG production. Although the bulk of them are believed to have produced positive results, more scaled-up data is required to obtain a valid judgment about stability and large-scale commercial production.

Future studies are possible to modify key enzymes such as Rubisco using CRISPR technology and a

Table 4 Summarizing the published data used PEPC, GPAT, Desaturase, and G6PD enzymes increasing lipid

Gene	Donor	Acceptor	Change	Plasmid	Transformation method	Reference
Glycerol-3-phosphate acyltransferase (GPAT)	*P. tricornutum*	*P. tricornutum*	Lipid content increased two folds	pHY29	Electroporation	(Niu et al. 2016)
Glycerol-3-phosphate acyltransferase (GPAT)	*Chlamydomonas reinhardtii* CC-125	*Saccharomyces cerevisiae* BY4742	Enhanced GPAT activity	psGC	Yeastmaker™ Yeast Transformation Kit (Clontech)	(Duarte-Coello et al. 2019)
Δ*5 desaturase gene in P. tricornutum (PtD5b)*	*Phaeodactylum tricornutum* CCMP2561	*Phaeodactylum tricornutum* CCMP2561	EPA exposed a proliferation of 58% in modified microalgae. Also, neutral lipid contents improved to 65% in the modified one.	pHY18	Electroporation	(Peng et al. 2014)
Δ12-desaturase (NoD12)	*Nannochloropsis oceanica* CCMP1779	*Nannochloropsis oceanica* CCMP1779	Phosphatidylethanolamine (PE), from 9.3% to 2.1%, in diacyl glycerol trimethyl homoserine (DGTS), from 4.4% to 1.1%, and in phosphatidylinositol (PI), from 12.7% to 8.2% compared to the wild type.	pSelect100-NoD12	Electroporation	(Kaye et al. 2015)
glucose-6-phosphate dehydrogenase (G6PD)	*Fistulifera solaris* JPCC DA0580	*Fistulifera solaris* JPCC DA0580	G6PD increased lipid productivity by 1.5-fold.	-	Microparticle bombardment	(Osada et al. 2017)

rational design. However, because this is fresh information, more research is needed to address issues such as off-target mutations and CRISPR-Cas9 delivery techniques to improve gene editing efficiency. Additionally, microalgae have a diverse species range, whereas algal research has primarily focused on a few strains. More research on additional potential strains is needed for future use besides utilizing new tools in gene transferee.

Competing interests The authors declare that they have no conflict of interest.

Funding This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors contributions All authors contributed equally, and finally, read and approved the final manuscript.

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