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REVIEW ARTICLE

CRISPR/Cas genome editing in microalgae: Revolutionizing biotechnology through precision genetic engineering

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Abstract

Microalgae biotechnology has witnessed a significant transformation with the advent of CRISPR/Cas genome editing technologies, which enabled precise gene alterations that were unattainable using conventional methods. Traditional transformation techniques have advanced to CRISPR-based platforms, mainly due to significant developments in delivery methods like as systems based on nanoparticles and RNP (ribonucleoprotein complexes), which overcame species-dependent barriers and demonstrated the innovative influence of CRISPR technology on genetic engineering of microalgal systems over traditional methods. Effective metabolic engineering advancements for improved pigment, lipid and biomolecule synthesis are crucial approaches. For this, comprehensive analyses of gene deletions or knockouts, overexpression and regulatory changes from model organisms like *Chlamydomonas reinhardtii* to economically important organisms like *Nannochloropsis* are important. This study highlights elevated expression of biosynthetic genes and suppression of competitive pathways, along with significant developments in multiplexed genome editing, which enable coordinated pathway alterations by precisely targeting many genes. Despite novel approaches, existing constraints like as off-target consequences, regulatory barriers and editing efficiency constraints are critically evaluated for enhancement of CRISPR technology in microalgal modification. Revolutionizing developments such as optogenetics-CRISPR inclusion, pan-genomic genetic modifications of microalgal populations and AI-driven autonomous genome engineering will establish engineering microalgae as leading platforms for sustainable biotechnology.

Keywords: *Chlamydomonas*; CRISPR/Cas; metabolic engineering; microalgae; *Nannochloropsis*

Introduction

Microalgae is one of the most promising platforms for sustainable biotechnology applications. It is having distinctive advantages including rapid growth rates, high photosynthetic efficiency and the ability to produce wide range of valuable compounds through complex metabolic networks. These photosynthetic microorganisms have emerged as multipurpose bio factories capable of synthesizing biofuels, pharmaceuticals, nutraceuticals and industrial chemicals while simultaneously addressing environmental challenges such as carbon dioxide (CO_2) mitigation.

The molecular basis of microalgae's biotechnological potential lies in their metabolic diversity and genetic plasticity. One important regulatory point for lipid biosynthesis is the *ACCase* gene family, which codes for acetyl-CoA carboxylase (1). *ACCase1* is usually elevated during nitrogen shortage to increase the generation of fatty acids, while *ACCase2* preserves basal metabolism. Similarly, the diacylglycerol acyltransferase (*DGAT)* genes represent critical bottlenecks in triacylglycerol synthesis, with *DGAT1* expression often limiting in high-lipid production scenarios (2). *DGAT* enzymes catalyse the final step of triacylglycerol (*TAG*) biosynthesis by acylating diacylglycerol to form *TAG*, making them inherently rate-limiting in lipid accumulation pathways. In multiple eukaryotic microalgae and yeast, *DGAT1* expression levels directly correlate with *TAG* yields, confirming its bottleneck role (3, 4).

The intricate regulatory networks controlling microalgal metabolism have been uncovered by recent developments in systems biology. The *NRR1* transcription factor in *Chlamydomonas* acts as a master regulator during nitrogen deprivation, simultaneously upregulating genes involved in lipid synthesis (*GPAT, LPAAT, PAP*) while downregulating chlorophyll biosynthesis genes (*CHLM, CHLN*) (5). This coordinated regulation demonstrates the molecular machinery that CRISPR technology can precisely modify. Microalgae are regarded as valuable resources for sustainable production of biofuels, high-value chemicals and environmental applications due to their rapid growth, carbon dioxide fixation and diverse metabolic pathways (6). However, CRISPR technology in microalgae faces challenges such as low editing efficiency, difficulties in delivering CRISPR

components and limited genetic tools for precise regulation (7, 8). This review focuses on recent advances addressing these barriers to advance microalgal biotechnology.

CRISPR/Cas systems: Principles and mechanisms

The RNA-guided DNA cleavage mechanism of CRISPR/Cas9 ensures high editing accuracy. gRNA (guide RNA) interaction causes conformational changes in the Cas9 protein, aligning the HNH and RuvC domains for precise DNA double-strand break (9). Target availability for *SpCas9* is controlled by the PAM (protospacer adjacent motif) sequence NGG. The total number of PAM locations differs significantly throughout microalgal genomes, ranging from about one for every 8 bp in AT-rich sections to one for every 16 bp in GC-rich sequences (10).

The mechanism of DNA cleavage involves the formation of an R-loop structure where the gRNA replaces one strand of the target DNA, allowing base-pairing with the complementary strand. The HNH domain cleaves the complementary strand whereas the RuvC domain cleaves the non-complementary strand, resulting in a blunt-ended double-strand break approximately 3 base pairs upstream of the PAM sequence (11). The precision of this cleavage mechanism has made Cas9 particularly significant for applications requiring predictable cut sites in microalgal genomes.

Cas12a and alternative CRISPR systems

Beyond the globally used Cas9 system, alternative CRISPR nucleases have significantly expanded the toolkit for microalgal genome editing. Cas12a (formerly Cpf1) provided several advantages over Cas9, including the requirement for a different PAM sequence (TTTV), which increases target site availability in AT-rich microalgal genomes (12). The distinct cleavage pattern of Cas12a produces staggered cuts with 4-5 nucleotide overhangs, which can facilitate more efficient homology-directed repair (HDR) in certain applications.

Cas12a exhibits improved specificity when compared to Cas9, with reduced off-target activity attributed to its requirement for a longer spacer sequence (typically 20-25 nucleotides versus 17-20 for Cas9) and different DNA binding mechanisms (13). In microalgal applications, this enhanced specificity is valuable where full off-target screening might be difficult and genomic resources may be limited.

Though their use in microalgae is still restricted because of their lower cutting efficiency, the creation of miniature CRISPR systems, including Cas12f1 (previously Cas14), has created new opportunities for applications with size restrictions (14). These compact systems could be useful for delivery systems with payload restrictions or multiplexed applications. Without causing permanent genomic modifications, Cas13 systems, which target RNA instead of DNA, have demonstrated potential for gene silencing applications in microalgae. Targeting RNA enables transient and reversible modulation of gene expression without altering genomic DNA, thereby preserving genetic integrity and avoiding heritable off-target mutations; it allows dosage-dependent knockdown for precise phenotypic tuning; it bypasses DNA repair pathways, reducing cytotoxicity and unintended genomic alterations; and it supports multiplexed, rapid deployment to interrogate multiple transcripts simultaneously for dynamic functional studies in microalgae (15). Cas13 is very appealing for study essential genes or producing conditional knockdown phenotypes due to the reversible nature of RNA targeting (16).

Base editing and prime editing systems

The development of base editing systems has revolutionized precision genome editing by enabling targeted nucleotide substitutions without creating double-strand breaks. Cytosine base editors (CBEs) and adenine base editors (ABEs) have been successfully implemented in several microalgae species, specifically *Synechococcus elongatus*, *Chlamydomonas reinhardtii* allowing for the introduction of point mutations, correction of deleterious mutations or creation of stop codons (17).

Base editing applications in microalgae have demonstrated value for adjusting enzyme activities through targeted amino acid substitutions. Protein engineering efforts have been accelerated and advanced structure-function studies in microalgal systems have been made possible by the capacity to generate libraries of point mutants at certain locations (18).

Prime editing represents the newest advancement in precision genome editing, enabling targeted insertions, deletions and substitutions without requiring DSBs or donor templates. Although primary editing is still in its early stages for microalgal applications, it provides unprecedented flexibility for precisely modifying genes (18).

Evolution of genetic engineering in microalgae

Traditional genetic modification approaches

Since the 1980s, genetic engineering has evolved significantly, from basic transformation techniques to advanced CRISPR-based systems. Physical disruption techniques were used in the early stages of microalgal transformation. The first successful transformation achieved in *Chlamydomonas reinhardtii* using glass bead agitation in the presence of DNA and polyethylene glycol (PEG) (19). Although transformation efficiency remained around 10 % with significant cell mortality, this pioneering work made *Chlamydomonas* the foundational model organism (20) as it combines the genetic tractability of yeast with the photosynthetic machinery of plants, featuring a haploid lifecycle for immediate mutant phenotyping and rapid genetic mapping via tetrad analysis. It supports both photoautotrophic and heterotrophic growth, enabling versatile metabolic studies and benefits from a fully sequenced genome with efficient transformation and CRISPR/Cas editing tools for nuclear and organellar loci (21).

Electroporation has emerged as the most controlled physical method, utilizing electrical pulses to produce transient membrane pores for DNA uptake. Despite being a breakthrough, early electroporation techniques required extensive species-specific optimization of voltage, pulse duration and buffer composition, with variable success across different microalgae taxa (22). Although they offered gentler options, chemical transformation techniques utilizing PEG-mediated uptake and calcium chloride treatment had low efficiency and time-consuming protocols.

The transformation of recalcitrant species, especially marine diatoms with strong silicate cell walls, was achieved using particle bombardment (biolistic). This approach involved coating gold particles with DNA and accelerating them into target cells using compressed gas, allowing transformation independent of cell wall composition (23). However, biolistics frequently led to cellular damage and intricate, multi-copy integration patterns, complicating downstream characterization and strain stability.

Through the bacterial T-DNA machinery, Agrobacterium-mediated transformation was adopted from plant systems which offered more consistent integration patterns. Many marine and extremophile microalgae such as *Nannochloropsis sp*, *Arctic Chlorella sp.* proved incompatible with this biological approach, despite its success in some species (24).

Critical limitations of traditional methods included (i) random DNA integration that could disrupt essential genes and unpredictable expression patterns, (ii) position effects that could cause transgene expression to vary depending on chromosomal context. Furthermore, limitations includes the need for selectable markers raising regulatory concerns, low transformation efficiency that requires extensive screening; and the challenge of obtaining marker-free strains essential for commercial applications (25, 26).

Transition to CRISPR technology

The advent of CRISPR/Cas systems marked a paradigm shift in microalgal genetic engineering, addressing fundamental limitations of traditional approaches. The first successful CRISPR applications in *Chlamydomonas reinhardtii* demonstrated targeted gene knockout with unprecedented precision, achieving editing efficiencies exceeding 70 % compared to < 10 % for traditional. The necessity for costly screening programs, which are a feature of random integration systems, was eliminated by this significant increase in efficiency and precision.

CRISPR technology provided several revolutionary advantages, including DNA-free delivery via RNP complexes that addressed regulatory concerns regarding transgenic organisms, multiplexing capabilities that allowed for the simultaneous editing of multiple genes and precision targeting through programmable guide RNAs that allowed modification of specific genomic loci (27, 28). Beyond simple nucleases, a variety of CRISPR tools have been developed, such as base editing systems for accurate nucleotide substitutions without double-strand breaks, CRISPR interference (CRISPRi) for reversible gene silencing and CRISPR activation (CRISPRa) for targeted upregulation (17, 29).

CRISPR's broad applicability across microalgae taxa was proved by its rapid species expansion. Effective protocols for industrially viable species such as *Nannochloropsis oceanica*, *Chlorella vulgaris*, *Dunaliella salina and Phaeodactylum tricornutum* were developed after success with *Chlamydomonas*, each requiring species-specific optimization of guide RNA design, delivery methods and culture conditions While CRISPR-Cas genome editing has been extensively applied to model microalgae such as *Chlamydomonas reinhardtii* and *Nannochloropsis* species, other industrially relevant taxa like *Scenedesmus* and *Isochrysis* remain largely underexplored. These genera hold substantial promise due to their robust growth, lipid productivity and suitability for large-scale cultivation; however, few studies have reported successful CRISPR-mediated genome modifications in these species. The scarcity of genomic resources, complex cell wall structures and species-specific transformation challenges contribute to limited CRISPR data. Expanding gene-editing efforts to these underutilized but commercially important microalgae is critical for unlocking their full biotechnological potential (7, 30). Expanding applications beyond model organisms required the development of computational techniques for species-specific guide RNA creation and off-target prediction (31).

Delivery methods for CRISPR components in microalgae

Physical and chemical transformation techniques

Successful application of CRISPR mainly depends on effective delivery strategies that overcome the structural barriers posed by diverse microalgal cell walls varying from silica-based frustules in diatoms to cellulose structures in green algae (32). Electroporation remains the most widely adopted method, with new protocols tailored for RNP delivery producing better outcomes than plasmid -based strategies. To increase cell survival, contemporary systems use exponential decay generators with optimal voltage (1-5 kV), capacitance (10-50 µF) and specialized low-conductivity buffers that contain protective chemicals like trehalose (33).

Microinjection provides the most direct delivery approach, which precise control over dosage and subcellular localization. Although labour-intensive, recent developments in microfluidic systems and femtosecond laser-assisted injection have enhanced throughput and made conventional microinjection applicable to smaller species that were previously inaccessible (34).

Chemical transformation methods have been improved for CRISPR applications, with PEG-calcium chloride protocols optimized for RNP delivery. Extended incubation protocols at reduced temperatures (4-15 °C for 30-60 min) have improved transformation efficiency while maintaining the integrity of the RNP complex (35).

By employing species-specific cellulase and pectinase enzyme cocktails, protoplast-based delivery technique disintegrates cell wall barriers through enzymatic breakdown. Though it involves more regeneration phases, this method was shown to be very successful for recalcitrant species and generates high efficiency for transformation (25).

Cell penetrating peptides (CPPs) represent a novel biological approach utilizing peptides like TAT and pVEC to facilitate membrane translocation. Editing efficiency (2-3 fold) is increased through enhanced nuclear targeting of Cas proteins. This was attained with recent improvement of nuclear localization signals especially those obtained from plant pathogen-based sequences (36, 37).

Advanced nanoparticle-based delivery systems

Nanoparticle-based delivery represents the cutting-edge of CRISPR delivery technology, which provides enhanced stability, regulated release and reduced cellular toxicity (38). Biocompatible vehicles that can encapsulate plasmid DNA and RNP complexes while protecting them from degradation are lipid-based nanocarriers, such as liposomes and solid lipid nanoparticles. Ionizable lipid nanoparticles, based on successful mRNA vaccine formulations, utilize pH-sensitive lipids that become cationic under acidic conditions, facilitating cytoplasmic release and endosomal escape (39).

Polymeric nanoparticle systems provide remarkable versatility through adjustable physicochemical properties. For both DNA and RNP complexes, poly lactic-co-glycolic acid (PLGA) nanoparticles provide biodegradable carriers with controllable release kinetics, achieving > 80% encapsulation efficiency. While dendrimeric carriers enable precise control over size, charge and surface functionalization chitosan-based systems offer cationic delivery with inherent mucoadhesive properties (40, 41).

Inorganic nanoparticle systems include mesoporous silica nanoparticles (MSNs) offer high surface areas and tuneable pore sizes; gold nanoparticles offer remarkable stability and surface functionalization capabilities and magnetic nanoparticles enabling targeted delivery through external magnetic fields (42, 43). Smart delivery systems allow stimuli-responsive components enabling triggered release in response to light exposure, enzyme activity, or pH variations. These systems offer unprecedented spatial and temporal control over CRISPR component delivery, with applications ranging from optically controlled editing to targeted cellular responses (44).

Species-specific considerations and optimization

Species-specific traits must be carefully considered while choosing a delivery technique for CRISPR-mediated transformation. Because of their adapted membrane compositions, marine microalgae frequently need greater field strengths for electroporation, whereas halophilic species like *Dunaliella* require specific buffer systems that can withstand high salt levels (45). While green algae with cellulose walls react favourably to enzymatic pretreatment or protoplast-based techniques, diatoms with silicate frustules benefit from particle bombardment or nanoparticle-based procedures that can pass through rigid cell walls.

Culture conditions significantly influence delivery efficiency, with logarithmic phase cells typically exhibiting higher transformation rates. Species-specific adjustments are needed for pre-treatment techniques that include cell wall-weakening enzymes and cell density optimization, which are usually 10^6-10^8 cells/ml (46). Comparative analysis of these techniques is provided in Table 1.

Multiple delivery methods have been developed for introducing CRISPR components into microalgae, including electroporation, microinjection, PEG-calcium chloride-mediated transformation, species-specific enzymatic digestion with cellulase and pectinase, protoplast-based delivery, cell-penetrating peptides and nanoparticle-based systems. Electroporation and microinjection typically provide moderate to high transformation efficiencies, but face challenges related to scalability and cell viability. PEG-calcium chloride and protoplast-based approaches are labor-intensive, species-specific and limited to strains amenable to cell wall removal. Enzymatic treatments facilitate increased permeability but require species-specific optimization. While cell-penetrating peptides offer low cytotoxicity, their large-scale applicability remains under development. Nanoparticle-based delivery methods hold promise for targeted, less toxic delivery; however, their high production costs and scalability issues currently limit industrial adoption. Selecting an optimal delivery method necessitates balancing transformation efficiency, cost, scalability and cell viability, prompting ongoing refinement for commercial microalgal genome editing applications (38, 47).

Applications of CRISPR in microalgal metabolic engineering

Lipid production enhancement through targeted gene modification

Metabolic engineering in microalgae has redefined the production of high value compounds through CRISPR technology by targeted alteration that markedly elevates the production of commercially important biomolecules. This precision approach has overcome many limitations of traditional metabolic engineering, allowing researchers to create optimized production strains with unprecedented efficiency and predictability.

Triacylglycerol biosynthesis pathway engineering: For the enrichment Multiple regulatory points in the triacylglycerol biosynthesis (*TAG*) pathway have been targeted for the enrichment of lipid accumulation using CRISPR- mediated metabolic engineering. *DGAT1* , an enzyme represents the rate-limiting step in TAG synthesis in most of the microalgae. Overexpression studies using CRISPR-mediated promoter engineering have shown that upregulation of *DGAT1* (3-5-fold increase in transcript levels) can enhance lipid content by 40-60 % in *Nannochloropsis* species (48).

Conversely, targeted knockout of *DGAT2*, which competes with *DGAT1* for the same substrate, has proven effective in redirecting metabolic flux. In *Chlamydomonas*, complete *DGAT2* knockdown produced a 2.5-fold increase in TAG accumulation while regulating cellular viability, indicating the accuracy that CRISPR editing can achieve (49).

The *PDAT* (phospholipid: diacylglycerol acyltransferase) gene family provides an alternative pathway for TAG synthesis. CRISPR-mediated overexpression of *PDAT1* (8-fold transcript increase) in *Phaeodactylum tricornutum* resulted in enhancement

Table 1. Comparative analysis of genetic modification techniques in microalgal systems

Transformation approach	Success rate	Targeting specificity	Merits	Major constraints	References
Conventional approaches					
Electroporation	0.1-5 %	Non-specific	Broad applicability, easier protocol	Poor transformation rates, elevated cell mortality	(46)
Particle bombardment	0.01-1 %	Non-specific	Effective for resistant cell walls	Multiple random insertions, variable outcomes	(78)
Chemical-mediated (PEG/ Calcium chloride)	0.05-2 %	Non-specific	Reduced cellular stress	Extremely poor efficiency	(51)
Agrobacterium-mediated	1-10 %	Partially directed	Superior to physical methods	Restricted strain compatibility	(51)
Site-Specific approaches					
Zinc finger nucleases	5-20 %	Site-directed	Targeted methodology	Complex protein engineering, expensive	(52)
TALEN systems	10-30 %	Site-directed	Enhanced flexibility over ZFNs	Complicated assembly, bulky proteins	
CRISPR/Cas9 (plasmid-based)	30-80 %	Highly precise	Streamlined design, economical	Potential off-site effects, PAM constraints	(27, 53)
CRISPR/Cas9 ribonucleoprotein	40-85 %	Highly precise	Selection marker-independent, fewer off-targets	Protein degradation issues, delivery obstacles	(28, 70)
CRISPR/Cas12a	25-70 %	Highly precise	Alternative PAM recognition, improved accuracy	Reduced efficiency relative to Cas9	(12, 13)
Base editing	15-60 %	Single base resolution	Eliminates double-strand breaks	Restricted editing range	(17, 18)
Prime editing	5-40 %	Ultra-precise	Eliminates donor template requirement	Reduced efficiency, complex guide design	(18)

of lipid yields while simultaneously reducing phospholipid content, indicating successful metabolic remodelling (50).

Fatty acid catabolism pathway disruption: Targeted disruption of β-oxidation genes has emerged as a powerful strategy for enhancing lipid accumulation. A reliable method for enhancing fat accumulation is the targeted disruption of β-oxidation genes. Knockout of the *ACX1* gene, which encodes acyl-CoA oxidase (the first enzyme in β-oxidation), has been accomplished in several species. The *ACX1* gene encoding acyl-CoA oxidase, the first enzyme in β-oxidation, has been successfully knocked out in multiple species. During nitrogen rich conditions, knockout of *ACX1* in Chlamydomonas resulted in 3-fold increase in cellular lipid content due to elimination of 90 % of fatty acid degradation activity (51).

ACAT (acetyl-CoA C-acetyltransferase) knockout experiment *TAG* accumulation can be increased by preventing stored lipids from being metabolized during recovery from stress conditions by eliminating this key β-oxidation enzyme. Following a complete *ACAT* omission in *Nannochloropsis* retained a high lipid content (> 50 % dry weight) which is a notable improvement over wild-type strain (52).

The *LACS* (long-chain acyl-CoA synthetase) gene family regulates fatty acid activation for β-oxidation. Multiplexed knockout of *LACS1, LACS2* and *LACS4* in *Chlamydomonas* reduced fatty acid catabolism by 85 %, with the triple knockout strain accumulating lipids continuously during cultivation rather than only under stress conditions (27).

Glycerolipid biosynthesis regulation: The first step in glycerolipid synthesis is catalysed by *GPAT* (glycerol-3-phosphate acyltransferase). Expression of *GPAT* in *Chlorella vulgaris* was elevated sixfold because of CRISPR-mediated *GPAT* promoter editing which added more transcription factor binding sites, improving the organism's overall ability for lipid biosynthesis (53).

A further significant control point is the *LPAAT* (lysophosphatidic acid acyltransferase) family. By using CRISPR-mediated integration of additional gene copies, *LPAAT1* overexpression resulted in 4-fold increase in enzyme activity and 45 % higher lipid content in engineered *Dunaliella salina* strains (54).

In *TAG* synthesis, *PAP* (phosphatidic acid phosphatase) generates diacylglycerol, the intermediate precursor, from phosphatidic acid by removing the phosphate group. *DGAT1* upregulation and targeted overexpression i.e., 5-fold transcript increase, resulted in synergistic effects thereby increasing exceeding 70 % dry weight in optimized *Nannochloropsis* strains (55).

Carotenoid and pigment pathway engineering

Astaxanthin production enhancement: β-carotene undergoes multiple enzymatic modifications sequentially as part of the astaxanthin biosynthesis pathway. Two enzymes namely *BKT* (β -carotene ketolase) and *CHY* (β-carotene hydroxylase) controls astaxanthin accumulation. Astaxanthin production was increased to 8 % dry weight in *Haematococcus pluvialis* by CRISPR-mediated *BKT* overexpression (12-fold increase), which was a 4-fold improvement over wild-type strains (30).

Strains with simultaneous 6-fold upregulation of *CHY* and overexpression of *BKT* were able to produce astaxanthin rapidly under adverse conditions. During high-intensity cultivation, the modified strains maintained cellular viability while accumulating astaxanthin at twice the rate of control strains. Since *LCYE* (lycopene ε-cyclase) and *LCYB* (lycopene β-cyclase) compete for the same lycopene substrate, α-carotene is produced instead of β-carotene. The α-carotene branch pathway flow was reduced by targeted *LCYE* knockout, which reallocated 100 % of lycopene into the synthesis of β-carotene and astaxanthin (56).

Zeaxanthin and lutein optimization: *ZEP* (zeaxanthin epoxidase), a carotenoid biosynthetic enzyme, reduces zeaxanthin buildup by converting zeaxanthin to violaxanthin. Complete knockout of *ZEP* in Chlamydomonas resulted in zeaxanthin concentrations reaching 2.5 % dry weight, with engineered strains showing enhanced photoprotection under high-light conditions (57).

Reverse reaction of violaxanthin back to zeaxanthin was catalysed by *VDE* (violaxanthin de-epoxidase) The overexpression (8-fold increase) of *VDE* enhances zeaxanthin recovery rates followed by light stress, which makes strains better adapted to varying light conditions typical of outdoor cultivation (58).

LCYE activity is essential for lutein biosynthesis, producing α-carotene, which is subsequently hydroxylated by *CHY*. In targeted knockout backgrounds, balanced overexpression of *LCYE* and *CHY* (4-fold and 6-fold, respectively) removed the competing carotenoid branches, resulting in lutein purities that surpassed 85 % of total carotenoids (59).

Phycoerythrin and phycobiliprotein enhancement: Both apoprotein and chromophore generation are intricately regulated in the process of phytobiliprotein biosynthesis. *PEB* genes regulate the synthesis of phycoerythrobilin chromophores, while *PEAA* and *PEAB* encode the α and β subunits of phycoerythrin. *PEAA* (5-fold), *PEAB* (4-fold) and *PEBA* (6-fold) were all co-upregulated by CRISPR in *Porphyridium cruentum*, resulting in phycoerythrin concentrations that reached 15 % of total cellular protein. Unbalanced apoprotein buildup, which generally occurs with single-gene overexpression strategies, was prevented by the synchronized expression.

By cleaving heme to biliverdin, *HO1* (heme oxygenase) initiates the biosynthesis of chromophores. Overall phycobiliprotein production was found to be rate-limited by *HO1* overexpression (3-fold), with knockout strains showing total pigment synthesis elimination despite normal apoprotein levels.

Protein and pharmaceutical production systems

Recombinant protein expression optimization: The generation of recombinant proteins in microalgae has been transformed by CRISPR-based integration at identified safe-harbouring loci. In Chlamydomonas, the *PSA1* locus consistently shows high-level expression; integrated transgenes exhibit expression that is 5-10 times higher than that of random integration sites.

Recombinant protein expression was 8-fold higher with *HSP70A* promoter engineering via CRISPR-mediated enhancer elements addition than with native promoter strength. Heat-inducible properties of the modified promoter allowed for the development of controllable protein production systems. The stability of recombinant proteins has increased because of the knockout of competing proteases. *FtsH* protease knockout offered further defence against protein degradation, while *CLPP* (ATP-dependent Clp protease) knockout in Chlamydomonas

tripled the stability of recombinant antibodies.

Glycosylation pathway modification: Because microalgal N-linked glycosylation is different from mammalian patterns, it frequently needs to be modified to produce therapeutic proteins. Humanization has focused on *ALG* genes that regulate the initial stages of glycosylation. Through targeted *ALG3* knockout followed by human *ALG3* knock-in replacement, glycosylation patterns more closely resembling mammalian profiles were produced (60, 61). *OST* (oligosaccharyl transferase) subunit modifications that involve precise amino acid changes employing base editing have fine-tuned glycosylation efficiency. For recombinant proteins, specific mutations in *OST1* (*D234N, K456R*) increased N-glycosylation site occupancy from 60-85 %.

Secretion pathway enhancement: Microalgae's protein secretion efficiency is controlled by genes involved in the SEC pathway. While *SEC62* knockout eliminated competing translation initiation that reduced secretion efficiency, *SEC61* overexpression (4-fold) enhanced the translocation of recombinant proteins into the endoplasmic reticulum. Signal peptide recognition was improved by optimizing the components of the signal recognition particle (SRP) through targeted mutation. In comparison to wild-type systems, the *SRP54* subunit modification (*L145F* mutation) resulted in a 2.5-fold increase in the secretion of proteins that are difficult-to-express.

Microalgae have gained attention as sustainable platforms for producing therapeutic proteins and vaccines due to their rapid growth, genetic tractability and low production costs. Notable examples include the expression of monoclonal antibodies, vaccine antigens and antimicrobial peptides in *Chlamydomonas reinhardtii* and *Nannochloropsis* species. Optimizing cultivation conditions such as light intensity, CO_2 supply, mixing and nutrient availability in photobioreactors is crucial for maximizing microalgal growth and bioproduct yield. Dynamic control of CO_2 concentration, for example, using pulsed gas supply, can reduce inhibitory effects and significantly enhance biomass productivity. These process-level optimizations complement molecular improvements and are essential for successful scale-up of microalgae-based production systems (62). These systems offer safe, scalable production free from human pathogens, although optimization of expression and post-translational modifications remains a challenge. Microalgal-derived vaccine candidates targeting diseases like malaria, influenza and COVID-19 highlight their pharmaceutical potential, pending improvements in yield and regulatory approval (63, 64). Various applications are illustrated in Fig. 1.

Metabolic flux redirection and pathway integration

Central carbon metabolism modifications

A key branch point between glycolysis and the oxidative pentose phosphate pathway is *PGI* (phosphoglucose isomerase). While maintaining sufficient glycolytic flux for energy production, partial PGI knockdown (60 % reduction in activity) shifted carbon flux toward NADPH generation fostering enhanced fatty acid biosynthesis (65).

Glycolytic flux rates are controlled by phosphofructokinase (*PFK*) regulation. Carbon fixation and energy generation under high light conditions were accelerated by CRISPR-mediated *PFK* promoter alteration that eliminated transcriptional repressor binding sites, increasing enzyme expression 3-fold.

The Rubisco complex requires balanced expression of large (*RBCL*) and small (*RBCS*) subunits. In engineered *Synechococcus* strains, *RBCS* overexpression (2-fold) elevated Rubisco assembly efficiency by 40 %, enhancing CO_2 fixation rates and overall productivity (2).

Cofactor and energy metabolism

Optimization of NADH dehydrogenase complex through *NUO* gene modifications increased the efficiency of respiratory electron transport efficiency. Coordinate overexpression of *NUO1* and *NUO2* (3-fold each) maintained metabolism during diurnal cultivation cycles and enhanced cellular energy status during dark periods (5).

FNR (ferredoxin-NADP+ reductase) links photosynthetic electron transport to NADPH production for biosynthesis. overexpression of *FNR* (4-fold) increased the availability of NADPH for fatty acid synthesis, particularly effective when combined with modifications in lipid pathway (66).

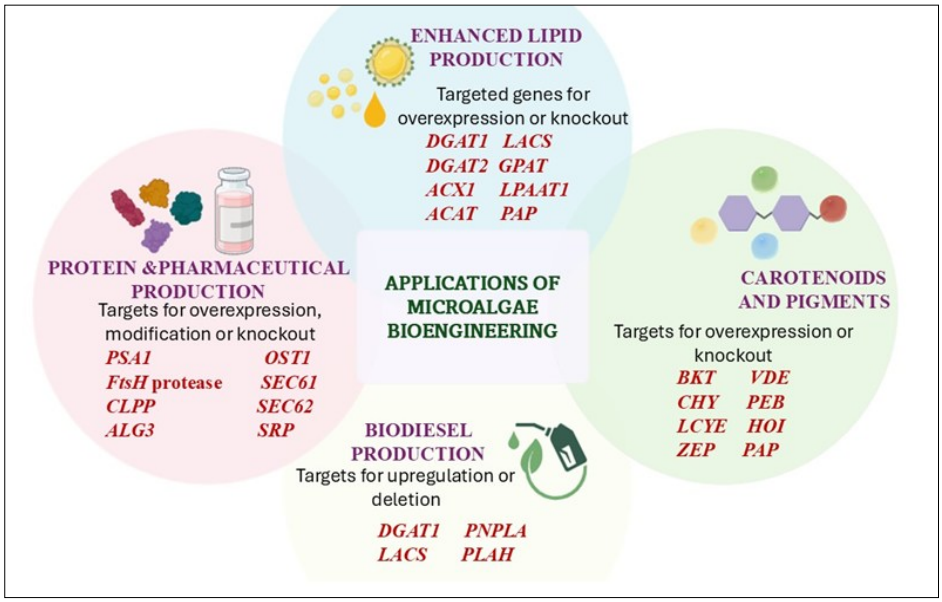

Fig. 1. Applications of microalgae bioengineering.
Some elements of this figure were created with BioRender.com

Pyruvate dehydrogenase activity and central metabolism are affected by *THI* genes that control thiamine production. While *THI1* overexpression enhanced pyruvate utilization efficiency by 25 %, *THI4* knockout created thiamine auxotrophs that could be used for biocontainment (67).

Chlamydomonas reinhardtii: The model platform

Comprehensive metabolic engineering

Twelve genes were altered at the same time throughout several paths in a highly modern project of *Chlamydomonas* engineering. To eradicate lipid catabolism *ACX1, LACS1* and were made functionless by knocking out (28). Also, starch synthase (*STA6*) was made functionless by knocking out to stop starch buildup, *RBCS* was suppressed (80 % reduction) to lessen fixation of carbon battle and *NRR1* was continuously induced using promoter alteration (68).

This extensive editing produced exceptional outcomes: under typical growing environment, the lipid concentration increased to 75 % of its dry weight, which is a 6-fold enhancement over the wild races. Instead of undergoing stress induction, this means the modified strain regularly deposited lipids while sustaining 85 % of wild growth rates (69).

Novel biocontainment systems

Chlamydomonas biological containment using CRISPR required the development of key gene dependencies. Spermidine auxotrophs that were produced via spermidine synthase (*SPDS*) deletion are unable to thrive without external supplementation. By involving both amino acids for development, the double auxotroph produced additional containment strategies when combined with *ARG7* knockout (arginine auxotroph) (28).

Protochlorophyllide oxidoreductase A(*PORA*) alteration was used in enhanced containment to determine light wavelength sensitivity. The enzyme has spectral demands that were altered by precise amino acid variations (*W590F, L434I*), which made the strain centered on LED wavelengths that are unavailable in natural conditions (70).

Nannochloropsis: Industrial scale applications

Coordinated lipid pathway optimization

The development of strains appropriate for the massive production of biodiesel was the key objective of commercial *nanochloropsis* engineering. The complete strategy aimed to eliminate conflicting paths and improve the production of lipids. *DGAT1* was recreated and combined at two different loci, resulting in an eight-fold spike in overall expression, whereas *ACCase1* was increased by five times through promoter manipulation (71).

To inhibit fatty acid stimulation for β-oxidation, all 4 *LACS* genes were deleted by knocking out at the same time. To stop lipid deterioration, patatin-like phospholipase (*PNPLA*) and phospholipase A2 (*PLAH*) were also removed. Through base editing, the fatty acid makeup of the Stearoyl-ACP desaturase was changed, resulting in an increase in oleic acid levels from 15-45% for better biodiesel characteristics (72).

With adjusted fatty acid profiles and a 68 % lipid content by dry weight, the final altered strain maintained uniform manufacturing properties across 50 culture cycles and displayed enhanced cold-flow qualities that are crucial for biodiesel usage (45).

Stress tolerance engineering

There is a need for increased tolerance for stress in external agriculture. Genes associated to the *HSP70* family were consistently overexpressed; increased heat shock resistance from 38 - 45 °C maximum surviving temperature was achieved by *HSP70A* - 6 fold, *HSP70B* 4-fold and *HSP70C*3-fold (36).

The four-fold overexpression of Na+/H+ antiporter (*NHX*) enhanced resistance to salt, allowing for growing saltwater with lower freshwater needs. The modified strains continued 80 % of their ideal growth rates while sustaining salt levels up to 4 % NaCl when paired with pyrroline-5-carboxylate synthase(*P5C5*) overexpression for proline buildup (46).

Multiplexed engineering in *Phaeodactylum tricornutum*

Engineering of sgRNA was categorized into two main steps: optimal sgRNA design and evaluation of the selected sgRNAs. sgRNAs were designed using web tools such as CRISPOR and CRISPR-P 2.0 (73), which analysed factors including on-target scores, off-target impact predictions by assessing mismatches, presence of restriction sites, GC content and the location of sgRNA within the coding sequence (CDS) or untranslated region (UTR). The designed sgRNAs were then evaluated for stable secondary structure formation, possible off-target sites and thermodynamic parameters such as enthalpy and entropy values. The secondary structure of sgRNAs was predicted using RNA fold (74) and their activity was confirmed by assessing optimal structural features (75). Off-target potential and enthalpy of the selected sgRNAs were subsequently evaluated using Cas-OFFinder (76) and IDT OLIGOANALYSER, respectively, to determine their specificity and stability for targeted editing. Advanced bioinformatic tools like SMAP design streamline multiplex CRISPR workflows by integrating multiplex PCR primer and gRNA design for high-throughput screening. SMAP design rapidly generates specific, compatible primers and gRNAs across multiple loci, enhancing design accuracy and reducing turn around time. Validated across multiple species, this tool supports efficient mutation detection and improves reproducibility, making it especially useful for complex genomes such as those of microalgae (77).

Coordinated metabolic network modification

To increase the synthesis of fucoxanthin, 15 genes in *P. tricornutum* were concurrently modified in an advanced multiplexed CRISPR engineering study project work. To improve precursor supply, the pathway for the biosynthesis of carotenoid was completely rearranged. Phytoene synthase (*PSY*) was overexpressed eight times, phytoene desaturase (*PDS*) six times and ζ-carotene desaturase (*ZDS*) four times (67).

To get rid of the conflicting β-carotene and α-carotene branches, *LCYB* and *LCYE* were both deleted by knocking out. To stop fucoxanthin deterioration, *VDE* and *ZEP* were removed, while fucoxanthin synthase (*FCS*) was overexpressed ten times. To enhance the accessibility of isoprenoid precursors, geranylgeranyl pyrophosphate synthase (*GGPPS*) was also overexpressed three times (78).

Despite fucoxanthin accounting for 89 % of total carotenoids, the multiplexed strain's dry weight fucoxanthin amounts were 4.2 %, up from 0.3 % in the wild. Industrial viability was proved by the maintenance output consistency throughout 30 culture cycles (26).

Silicon metabolism and frustule engineering

The metabolism of silicon in *P. tricornutum* was altered to lower silica needs without compromising cell structure. Polyamine-dependent methylated polyamine oxidase (*PDMPO*), which is involved in silica incorporation, was primarily deleted by knocking out (by 50 %) whereas silicon transporter (*SIT*) expression was lowered by 70 % using CRISPRi (26). Callose synthase (*CAL*) was upregulated by 4-fold and cellulose synthase (*CEL*) was overexpressed by 6-fold as part of compensatory cell wall thickening. In media deprived of silicon, the altered strain grew properly while retaining the structure and ability to generate lipids. Species-Specific CRISPR implementation strategies of various species are given in Table 2.

Conclusion and future perspective

The integration of CRISPR-Cas mediated genome editing technology and microalgal research has contributed to biotechnological innovation and sustainable solutions to global challenges. This study reveals that CRISPR has made significant attempts in model organisms like *Chlamydomonas reinhardtii* and many *Nannochloropsis* species. But factors like species-specific optimization and different transformation efficiencies across many microalgal strains are vital challenges influencing the efficiency. The successful modification of enhanced lipid-producing strains for biofuels, enhanced protein expression systems and stress-tolerant variants demonstrates the potential of this technology. Genome-edited microalgae exhibit high potential for industrial applications ranging from sustainable biofuel production to the synthesis of high-value pharmaceutical compounds and environmental remediation through enhanced CO_2 capture. In the future, this field is moving towards more advanced approaches including multiplexed editing strategies and the integration of synthetic biology principles, while expanding attempts to make non-model but industrially relevant species more feasible to genetic modification. Improved focus on reduction of off-target effects and delivery method refinements will lead to better efficacy of this technology. However, commercialization has many regulatory constraints that change significantly between different countries and regions, which requires proper scientific communication between scientists, policymakers and industry stakeholders for acceptance and implementation of this technology. Beyond these regulatory considerations, socio-economic implications must be addressed, including equitable access to technology, intellectual property rights that may influence innovation and market dynamics and the need for fair benefit-sharing with all stakeholders. Potential environmental biosafety risks also warrant careful evaluation and management, particularly regarding unintended ecological impacts, horizontal gene transfer and containment of modified strains in natural environments. Despite the existing technical challenges, the combination of CRISPR technology with understanding of microalgal biology positions these microscopic organisms as key players in developing sustainable solutions for environmental, energy and biotechnology applications that could help address some of the most vital global challenges.

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Authors' contributions

MSS and CK contributed to the original manuscript writing and editing. RR led the conceptualization and manuscript drafting. SV, RS, RA and MR, provided critical review and editing of the manuscript. All authors read and approved the final manuscript.

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References

1. Ma Z, Cheah WY, Ng I-S, Chang J-S, Zhao M, Show PL. Microalgae-based biotechnological sequestration of carbon dioxide for net zero emissions. Trends Biotechnol. 2022;40(12):1439-53. https://doi.org/10.1016/j.tibtech.2022.09.002
2. Tarafdar A, Sowmya G, Yogeshwari K, Rattu G, Negi T, Awasthi MK,

Table 2. Species-specific CRISPR implementation strategies in microalgae

Microalgal species	Delivery strategy	Cas system	Transformation success	Major constrains	References
Chlamydomonas reinhardtii	Electroporation/ ribonucleoprotein	Cas9, Cas12a, base editors	70-85 %	Cell wall degradation necessity	(28, 68, 79)
Nannochloropsis oceanica	Electroporation/ RNP/ direct injection	Cas9, Cas12a	45-70 %	Marine environment adaptation, robust cell envelope	(69, 71)
Nannochloropsis salina	RNP delivery/ episomal vectors	Cas9	40-65 %	Halotolerance optimization challenges	(70, 80)
Chlorella vulgaris	Electroporation/ protoplast methods	Cas9	35-60 %	Cell wall impedance, regeneration difficulties	(33, 81)
Phaeodactylum tricornutum	Electroporation/ biolistic/ RNP	Cas9, multiplexed systems	25-50 %	Intricate cell wall architecture, limited molecular resources	(82, 83)
Dunaliella salina	Electroporation/ polyethylene glycol	Cas9	30-55 %	Extreme halophilic conditions	(45)

et al. Environmental pollution mitigation through utilization of carbon dioxide by microalgae. Environ Pollut. 2023;328:121623. https://doi.org/10.1016/j.envpol.2023.121623

3. Yen CL, Stone SJ, Koliwad S, Harris C, Farese RV Jr. Thematic review series: glycerolipids. DGAT enzymes and triacylglycerol biosynthesis. J Lipid Res. 2008;49(11):2283-301. https://doi.org/10.1194/jlr.R800018-JLR200

4. Vingiani GM, De Luca P, Ianora A, Dobson ADW, Lauritano C. Microalgal enzymes with biotechnological applications. Mar Drugs. 2019;17(8):459. https://doi.org/10.3390/md17080459

5. Liang M-H, Wang L, Wang Q, Zhu J, Jiang J-G. High-value bioproducts from microalgae: strategies and progress. Crit Rev Food Sci Nutr. 2019;59(15):2423-41. https://doi.org/10.1080/10408398.2018.1455030

6. Wijffels RH, Barbosa MJ. An outlook on microalgal biofuels. Science. 2010;329(5993):796-9. https://doi.org/10.1126/science.1189003

7. Dhokane D, Shaikh A, Yadav A, Giri N, Bandyopadhyay A, Dasgupta S, et al. CRISPR-based bioengineering in microalgae for production of industrially important biomolecules. Front Bioeng Biotechnol. 2023;11:1267826. https://doi.org/10.3389/fbioe.2023.1267826

8. Baek K, Kim DH, Jeong J, Sim SJ, Melis A, Kim J-S, et al. DNA-free two-gene knockout in *Chlamydomonas reinhardtii* via CRISPR-Cas9 ribonucleoproteins. Sci Rep. 2016;6:30620. https://doi.org/10.1038/srep30620

9. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816-21. https://doi.org/10.1126/science.1225829

10. Anders C, Niewoehner O, Duerst A, Jinek M. Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. Nature. 2014;513(7519):569-73. https://doi.org/10.1038/nature13579

11. Nishimasu H, Ran FA, Hsu PD, Konermann S, Shehata SI, Dohmae N, et al. Crystal structure of Cas9 in complex with guide RNA and target DNA. Cell. 2014;156(5):935-49. https://doi.org/10.1016/j.cell.2014.02.001

12. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell. 2015;163(3):759-71. https://doi.org/10.1016/j.cell.2015.09.038

13. Kim D, Kim S, Kim S, Park J, Kim J-S. Genome-wide target specificities of CRISPR-Cas9 nucleases revealed by multiplex Digenome-seq. Genome Res. 2016;26(3):406-15. https://doi.org/10.1101/gr.199588.115

14. Karvelis T, Bigelyte G, Young JK, Hou Z, Zedaveinyte R, Budre K, et al. PAM recognition by miniature CRISPR-Cas12f nucleases triggers programmable double-stranded DNA target cleavage. Nucleic Acids Res. 2020;48(9):5016-23. https://doi.org/10.1093/nar/gkaa208

15. Montagud-Martínez R, Márquez-Costa R, Heras-Hernández M, Dolcemascolo R, Rodrigo G. On the ever-growing functional versatility of the CRISPR-Cas13 system. Microb Biotechnol. 2024;17(2):e14418. https://doi.org/10.1111/1751-7915.14418

16. Abudayyeh OO, Gootenberg JS, Konermann S, Joung J, Slaymaker IM, Cox DB, et al. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. Science. 2016;353(6299):aaf5573. https://doi.org/10.1126/science.aaf5573

17. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature. 2016;533(7603):420-4. https://doi.org/10.1038/nature17946

18. Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019;576(7785):149-57. https://doi.org/10.1038/s41586-019-1711-4

19. Kindle KL. High-frequency nuclear transformation of *Chlamydomonas reinhardtii*. Proc Natl Acad Sci USA. 1990;87(3):1228-32. https://doi.org/10.1073/pnas.87.3.1228

20. Debuchy R, Purton S, Rochaix J-D. The argininosuccinate lyase gene of *Chlamydomonas reinhardtii*: an important tool for nuclear transformation and for correlating the genetic and molecular maps of the ARG7 locus. EMBO J. 1989;8(10):2803-9. https://doi.org/10.1002/j.1460-2075.1989.tb08426.x

21. Marshall WF. *Chlamydomonas* as a model system to study cilia and flagella using genetics, biochemistry and microscopy. Front Cell Dev Biol. 2024;12:1412641. https://doi.org/10.3389/fcell.2024.1412641

22. Shimogawara K, Fujiwara S, Grossman A, Usuda H. High-efficiency transformation of *Chlamydomonas reinhardtii* by electroporation. Genetics. 1998;148(4):1821-8. https://doi.org/10.1093/genetics/148.4.1821

23. León-Bañares R, González-Ballester D, Galván A, Fernández E. Transgenic microalgae as green cell-factories. Trends Biotechnol. 2004;22(1):45-52. https://doi.org/10.1016/j.tibtech.2003.11.003

24. Kumar M, Jeon J, Choi J, Kim S-R. Rapid and efficient genetic transformation of the green microalga *Chlorella vulgaris*. J Appl Phycol. 2018;30(3):1735-45. https://doi.org/10.1007/s10811-018-1396-3

25. Fischer N, Rochaix J-D. The flanking regions of PsaD drive efficient gene expression in the nucleus of the green alga *Chlamydomonas reinhardtii*. Mol Genet Genomics. 2001;265(5):888-94. https://doi.org/10.1007/s004380100485

26. Hallmann A. Advances in genetic engineering of microalgae. In: Grand challenges in algae biotechnology. Springer; 2020. p. 159-221. https://doi.org/10.1007/978-3-030-25233-5_5

27. Zhang Y-T, Jiang J-Y, Shi T-Q, Sun X-M, Zhao Q-Y, Huang H, et al. Application of the CRISPR/Cas system for genome editing in microalgae. Appl Microbiol Biotechnol. 2019;103(8):3239-48. https://doi.org/10.1007/s00253-019-09726-x

28. Baek K, Kim DH, Jeong J, Sim SJ, Melis A, Kim J-S, et al. DNA-free two-gene knockout in *Chlamydomonas reinhardtii* via CRISPR-Cas9 ribonucleoproteins. Sci Rep. 2016;6(1):30620. https://doi.org/10.1038/srep30620

29. Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell. 2013;152(5):1173-83. https://doi.org/10.1016/j.cell.2013.02.022

30. Jeong B-r, Jang J, Jin E. Genome engineering via gene editing technologies in microalgae. Bioresour Technol. 2023;373:128701. https://doi.org/10.1016/j.biortech.2023.128701

31. Doench JG, Fusi N, Sullender M, Hegde M, Vaimberg EW, Donovan KF, et al. Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. Nat Biotechnol. 2016;34(2):184-91. https://doi.org/10.1038/nbt.3437

32. Gutiérrez S, Lauersen KJ. Gene delivery technologies with applications in microalgal genetic engineering. Biology. 2021;10(4):265. https://doi.org/10.3390/biology10040265

33. Kim J, Chang KS, Lee S, Jin E. Establishment of a genome editing tool using CRISPR-Cas9 in *Chlorella vulgaris* UTEX395. Int J Mol Sci. 2021;22(2):480. https://doi.org/10.3390/ijms22020480

34. Zhang S, Shen J, Li D, Cheng Y. Strategies in the delivery of Cas9 ribonucleoprotein for CRISPR/Cas9 genome editing. Theranostics. 2021;11(2):614. https://doi.org/10.7150/thno.47007

35. Nomura T, Inoue K, Uehara-Yamaguchi Y, Yamada K, Iwata O, Suzuki K, et al. Highly efficient transgene-free targeted mutagenesis and single-stranded oligodeoxynucleotide-mediated precise knock-in in the industrial microalga *Euglena gracilis* using Cas9 ribonucleoproteins. Plant Biotechnol J. 2019;17(11):2032. https://doi.org/10.1111/pbi.13174

36. Kang S, Jeon S, Kim S, Chang YK, Kim Y-C. Development of a pVEC peptide-based ribonucleoprotein (RNP) delivery system for genome

editing using CRISPR/Cas9 in *Chlamydomonas reinhardtii*. Sci Rep. 2020;10(1):22158. https://doi.org/10.1038/s41598-020-78968-x

37. Le TT, Tran Q-G, Park S-B, Yoon HR, Choi D-Y, Cho D-H, et al. Efficient secretory production of recombinant proteins in microalgae using an exogenous signal peptide. Front Microbiol. 2025;16:1603204. https://doi.org/10.3389/fmicb.2025.1603204
38. Feng S, Xie X, Liu J, Li A, Wang Q, Guo D, et al. A potential paradigm in CRISPR/Cas systems delivery: at the crossroad of microalgal gene editing and algal-mediated nanoparticles. J Nanobiotechnol. 2023;21(1):370. https://doi.org/10.1186/s12951-023-02139-z
39. Kulkarni JA, Cullis PR, Van Der Meel R. Lipid nanoparticles enabling gene therapies: from concepts to clinical utility. Nucleic Acid Ther. 2018;28(3):146-57. https://doi.org/10.1089/nat.2018.0721
40. Liang J, Yan H, Wang X, Zhou Y, Gao X, Puligundla P, et al. Encapsulation of epigallocatechin gallate in zein/chitosan nanoparticles for controlled applications in food systems. Food Chem. 2017;231:19-24. https://doi.org/10.1016/j.foodchem.2017.02.106
41. Katas H, Alpar HO. Development and characterisation of chitosan nanoparticles for siRNA delivery. J Control Release. 2006;115(2):216-25. https://doi.org/10.1016/j.jconrel.2006.07.021
42. Slowing II, Vivero-Escoto JL, Wu C-W, Lin VS-Y. Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers. Adv Drug Deliv Rev. 2008;60(11):1278-88. https://doi.org/10.1016/j.addr.2008.03.012
43. Plank C, Anton M, Rudolph C, Rosenecker J, Krötz F. Enhancing and targeting nucleic acid delivery by magnetic force. Expert Opin Biol Ther. 2003;3(5):745-58. https://doi.org/10.1517/14712598.3.5.745
44. Kloxin AM, Kasko AM, Salinas CN, Anseth KS. Photodegradable hydrogels for dynamic tuning of physical and chemical properties. Science. 2009;324(5923):59-63. https://doi.org/10.1126/science.1169494
45. Feng S, Hu L, Zhang Q, Zhang F, Du J, Liang G, et al. CRISPR/Cas technology promotes the various application of *Dunaliella salina* system. Appl Microbiol Biotechnol. 2020;104(20):8621-30. https://doi.org/10.1007/s00253-020-10892-6
46. Suttangkakul A, Sirikhachornkit A, Juntawong P, Puangtame W, Chomtong T, Srifa S, et al. Evaluation of strategies for improving the transgene expression in an oleaginous microalga *Scenedesmus acutus*. BMC Biotechnol. 2019;19(1):4. https://doi.org/10.1186/s12896-018-0497-z
47. Patel VK, Das A, Kumari R, Kajla S. Recent progress and challenges in CRISPR-Cas9 engineered algae and cyanobacteria. Algal Res. 2023;71:103068. https://doi.org/10.1016/j.algal.2023.103068
48. Vavitsas K, Kugler A, Satta A, Hatzinikolaou DG, Lindblad P, Fewer DP, et al. Doing synthetic biology with photosynthetic microorganisms. Physiol Plant. 2021;173(2):624-38. https://doi.org/10.1111/ppl.13455
49. Einhaus A, Krieger A, Köhne L, Rautengarten B, Jacobebbinghaus N, Saudhof M, et al. Genome editing of epigenetic transgene silencing in *Chlamydomonas reinhardtii*. Trends Biotechnol. 2025. https://doi.org/10.1016/j.tibtech.2025.04.019
50. Jagadevan S, Banerjee A, Banerjee C, Guria C, Tiwari R, Baweja M, et al. Recent developments in synthetic biology and metabolic engineering in microalgae towards biofuel production. Biotechnol Biofuels. 2018;11(1):185. https://doi.org/10.1186/s13068-018-1181-1
51. Fajardo C, Carrasco-Reinado R, Escobar-Niño A, Fernández-Acero FJ. Microalgae: molecular biology, proteomics and applications. Prime Arch Mol Biol. 2020;1:45. https://doi.org/10.37247/pamb.2020.1.45
52. Kurita T, Iwai M, Moroi K, Okazaki K, Nomura S, Saito F, et al. Genome editing with removable TALEN vectors harboring a yeast centromere and autonomous replication sequence in oleaginous microalga. Sci Rep. 2022;12(1):2480. https://doi.org/10.1038/s41598-022-06495-y
53. Patel VK, Soni N, Prasad V, Sapre A, Dasgupta S, Bhadra B. CRISPR-Cas9 system for genome engineering of photosynthetic microalgae. Mol Biotechnol. 2019;61(8):541-61. https://doi.org/10.1007/s12033-019-00185-3
54. Naduthodi MIS, Barbosa MJ, van der Oost J. Progress of CRISPR-Cas based genome editing in photosynthetic microbes. Biotechnol J. 2018;13(9):1700591. https://doi.org/10.1002/biot.201700591
55. Lin JY, Lin WR, Ng IS. CRISPRa/i with Adaptive Single Guide Assisted Regulation DNA (ASGARD) mediated control of *Chlorella sorokiniana* to enhance lipid and protein production. Biotechnol J. 2022;17(10):2100514. https://doi.org/10.1002/biot.202100514
56. Kneip JS, Kniepkamp N, Jang J, Mortaro MG, Jin E, Kruse O, et al. CRISPR/Cas9-mediated knockout of the lycopene ε-cyclase for efficient astaxanthin production in the green microalga *Chlamydomonas reinhardtii*. Plants. 2024;13(10):1393. https://doi.org/10.3390/plants13101393
57. Song I, Kim J, Baek K, Choi Y, Shin B, Jin E. The generation of metabolic changes for the production of high-purity zeaxanthin mediated by CRISPR-Cas9 in *Chlamydomonas reinhardtii*. Microb Cell Fact. 2020;19(1):220. https://doi.org/10.1186/s12934-020-01480-4
58. Li C, Pan Y, Yin W, Liu J, Hu H. A key gene, violaxanthin de-epoxidase-like 1, enhances fucoxanthin accumulation in *Phaeodactylum tricornutum*. Biotechnol Biofuels Bioprod. 2024;17(1):49. https://doi.org/10.1186/s13068-024-02496-3
59. Tokunaga S, Morimoto D, Koyama T, Kubo Y, Shiroi M, Ohara K, et al. Enhanced lutein production in *Chlamydomonas reinhardtii* by overexpression of the lycopene epsilon cyclase gene. Appl Biochem Biotechnol. 2021;193(6):1967-78. https://doi.org/10.1007/s12010-021-03524-w
60. Mathieu-Rivet E, Mati-Baouche N, Walet-Balieu ML, Lerouge P, Bardor M. N- and O-glycosylation pathways in the microalgae polyphyletic group. Front Plant Sci. 2020;11:609993. https://doi.org/10.3389/fpls.2020.609993
61. Mathieu-Rivet E, Kiefer-Meyer MC, Vanier G, Ovide C, Burel C, Lerouge P, et al. Protein N-glycosylation in eukaryotic microalgae and its impact on the production of nuclear expressed biopharmaceuticals. Front Plant Sci. 2014;5:359. https://doi.org/10.3389/fpls.2014.00359
62. He L, Subramanian VR, Tang YJ. Experimental analysis and model-based optimization of microalgae growth in photo-bioreactors using flue gas. Biomass Bioenergy. 2012;41:131-8. https://doi.org/10.1016/j.biombioe.2012.02.025
63. Kumar G, Shekh A, Jakhu S, Sharma Y, Kapoor R, Sharma TR. Bioengineering of microalgae: recent advances, perspectives and regulatory challenges for industrial application. Front Bioeng Biotechnol. 2020;8:914. https://doi.org/10.3389/fbioe.2020.00914
64. Banerjee A, Ward V. Production of recombinant and therapeutic proteins in microalgae. Curr Opin Biotechnol. 2022;78:102784. https://doi.org/10.1016/j.copbio.2022.102784
65. Maeda Y, Yoshino T, Matsunaga T, Matsumoto M, Tanaka T. Marine microalgae for production of biofuels and chemicals. Curr Opin Biotechnol. 2018;50:111-20. https://doi.org/10.1016/j.copbio.2017.11.018
66. Ahmad Kamal AH, Mohd Hamidi NF, Zakaria MF, Ahmad A, Harun MR, Chandra Segaran T, et al. Genetically engineered microalgae for enhanced bioactive compounds. Discover Appl Sci. 2024;6(9):482. https://doi.org/10.1007/s42452-024-06116-5
67. Hao X, Chen W, Amato A, Jouhet J, Maréchal E, Moog D, et al. Multiplexed CRISPR/Cas9 editing of the long-chain acyl-CoA synthetase family in the diatom *Phaeodactylum tricornutum* reveals that mitochondrial ptACSL3 is involved in the synthesis of storage lipids. New Phytol. 2022;233(4):1797-812. https://doi.org/10.1111/nph.17911
68. Shin S-E, Lim J-M, Koh HG, Kim EK, Kang NK, Jeon S, et al. CRISPR/

Cas9-induced knockout and knock-in mutations in *Chlamydomonas reinhardtii*. Sci Rep. 2016;6(1):27810. https://doi.org/10.1038/srep27810

69. Wang Q, Lu Y, Xin Y, Wei L, Huang S, Xu J. Genome editing of model oleaginous microalgae *Nannochloropsis* spp. by CRISPR/Cas9. Plant J. 2016;88(6):1071-81. https://doi.org/10.1111/tpj.13307
70. Poliner E, Takeuchi T, Du Z-Y, Benning C, Farré EM. Nontransgenic marker-free gene disruption by an episomal CRISPR system in the oleaginous microalga *Nannochloropsis oceanica* CCMP1779. ACS Synth Biol. 2018;7(4):962-8. https://doi.org/10.1021/acssynbio.7b00362
71. Naduthodi MIS, Claassens NJ, D'Adamo S, van der Oost J, Barbosa MJ. Synthetic biology approaches to enhance microalgal productivity. Trends Biotechnol. 2021;39(10):1019-36. https://doi.org/10.1016/j.tibtech.2020.12.010
72. Kim Z-H, Kim K, Park H, Lee CS, Nam SW, Yim KJ, et al. Enhanced fatty acid productivity by *Parachlorella* sp., a freshwater microalga, via adaptive laboratory evolution under salt stress. Biotechnol Bioprocess Eng. 2021;26(2):223-31. https://doi.org/10.1007/s12257-020-0001-1
73. Liu H, Ding Y, Zhou Y, Jin W, Xie K, Chen L-L. CRISPR-P 2.0: an improved CRISPR-Cas9 tool for genome editing in plants. Mol Plant. 2017;10(3):530-2. https://doi.org/10.1016/j.molp.2017.01.003
74. Lorenz R, Bernhart SH, Höner zu Siederdissen C, Tafer H, Flamm C, Stadler PF, et al. ViennaRNA package 2.0. Algorithms Mol Biol. 2011;6(1):26. https://doi.org/10.1186/1748-7188-6-26
75. Heinlein M. RNA tagging. Springer; 2020. https://doi.org/10.1007/978-1-0716-0712-1
76. Bae S, Park J, Kim J-S. Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. Bioinformatics. 2014;30(10):1473-5. https://doi.org/10.1093/bioinformatics/btu048
77. Develtere W, Waegneer E, Debray K, De Saeger J, Van Glabeke S, Maere S, et al. SMAP design: a multiplex PCR amplicon and gRNA design tool to screen for natural and CRISPR-induced genetic variation. Nucleic Acids Res. 2023;51(7):e37. https://doi.org/10.1093/nar/gkad036
78. Doron L, Segal Na, Shapira M. Transgene expression in microalgae-from tools to applications. Front Plant Sci. 2016;7:505. https://doi.org/10.3389/fpls.2016.00505
79. Ghribi M, Nouemssi SB, Meddeb-Mouelhi F, Desgagné-Penix I. Genome editing by CRISPR-Cas: a game change in the genetic manipulation of *Chlamydomonas*. Life. 2020;10(11):295. https://doi.org/10.3390/life10110295
80. Ryu AJ. Engineering of *Nannochloropsis salina* for enhanced lipid biosynthesis by high-throughput screening and genome editing. 2020.
81. Lin W-R, Ng I-S. Development of CRISPR/Cas9 system in *Chlorella vulgaris* FSP-E to enhance lipid accumulation. Enzyme Microb Technol. 2020;133:109458. https://doi.org/10.1016/j.enzmictec.2019.109458
82. Daboussi F, Leduc S, Maréchal A, Dubois G, Guyot V, Perez-Michaut C, et al. Genome engineering empowers the diatom *Phaeodactylum tricornutum* for biotechnology. Nat Commun. 2014;5(1):3831. https://doi.org/10.1038/ncomms4831
83. Hao X, Chen W, Amato A, Jouhet J, Maréchal E, Moog D, et al. Multiplexed CRISPR/Cas9 editing of the long-chain acyl-CoA synthetase family in the diatom *Phaeodactylum tricornutum* reveals that mitochondrial ptACSL3 is involved in the synthesis of storage lipids. New Phytol. 2022;233(4):1797-812. https://doi.org/10.1111/nph.17911

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