

Review Article

The Role of Molecular Tools in Microalgal Strain Improvement: Current Status and Future Perspectives

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Abstract

Microalgae are microscopic, typically single-celled photosynthetic organisms found in freshwater, marine, and even terrestrial environments. Microalgae are crucial to global ecosystems because they are primary producers, forming the base of the aquatic food chain and producing about half of the Earth's oxygen through photosynthesis. Microalgae are vital sustainable feedstocks with applications spanning food, feed, biofuels, and high-value bioproducts. However, their industrial-scale use faces challenges due to the limited robustness and productivity of wild-type strains. Recent advancements in molecular tools and genetic engineering have ushered in a new era for microalgal strain improvement. Molecular tools, including genetic engineering, random mutagenesis, and advanced selection methods such as fluorescence-activated cell sorting (FACS), constitute powerful approaches for microalgal strain improvement. These tools enable precise genome modifications, creation of tailor-made phenotypes, and selection of mutants with enhanced productivity and stress tolerance. The scope of this review encompasses the diverse molecular techniques employed in strain optimization covering forward and reverse genetics, site-directed mutagenesis, adaptive laboratory evolution, and non-GMO random mutagenesis. The significance lies in overcoming bottlenecks in microalgal commercialization by improving strain performance and enabling sustainable bioproduct generation. This article aims to synthesize current advancements, critically analyze the integration of these molecular tools with high-throughput technologies, discuss regulatory considerations, and outline future perspectives for accelerating microalgal strain development to meet industrial and environmental demands.

Keywords

Microalgae, Strain Improvement, Molecular Tools, Genomic Editing, Genetic Engineering

1. Introduction

Microalgae are photosynthetic microorganisms widely recognized as natural biofactories due to their ability to efficiently convert sunlight, CO₂, and nutrients into biomass rich in valuable compounds such as proteins, carbohydrates, lipids, pigments, vitamins, and bioactive molecules [1, 2]. Microalgae are valuable as sustainable feedstock for food, feed, bio-

fuels, pharmaceuticals, and other bioproducts, but their commercial use is limited by strains that lack sufficient productivity and stress tolerance [3]. Molecular and genetic engineering tools offer precise methods to modify microalgal genomes to create strains with desired traits such as higher lipid content, stress resistance, and tailored bioproduct syn-

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thesis [4, 5].

Current approaches include both classical methods like random mutagenesis combined with high-throughput selection techniques (e.g., fluorescence-activated cell sorting) and advanced genome editing technologies such as CRISPR-Cas9, TALENs, and zinc finger nucleases [6]. These tools enable forward and reverse genetics to identify gene functions and introduce targeted mutations, which help in generating improved algal strains with specific characteristics [4, 7]. The integration of omics data (genomics, transcriptomics, proteomics) guides rational strain design and accelerates development [5].

Despite progress, challenges remain with optimization of transformation methods, regulatory frameworks, and biosafety concerns related to genetically modified microalgae [8]. Future perspectives emphasize combining molecular tools with adaptive laboratory evolution and synthetic biology to fast-track strain improvement and enable cost-effective, scalable production of microalgal bio-products [4, 7, 9].

Molecular tools play a transformative role in microalgal strain improvement by enabling precise genetic modifications and high-throughput selection, which are essential for overcoming production limitations and unlocking the full biotechnological potential of microalgae [7]. This review article aims to serve as a valuable resource for researchers, biotechnologists, and industrial stakeholders by critically evaluating the current status of molecular tools in microalgal strain improvement and providing informed outlooks on how these tools can be harnessed to unlock the full biotechnological potential of microalgae.

2. Overview of Molecular Tools

Molecular tools for microalgal strain improvement encompass a range of techniques designed to modify the genetic makeup of microalgae to enhance desirable traits such as productivity, stress tolerance, and metabolite yield [5, 9]. These tools broadly fall into two categories: random mutagenesis and targeted genetic engineering.

Random mutagenesis involves exposing microalgae to physical (e.g., UV-C radiation) or chemical mutagens to induce random mutations across the genome [10, 11]. These classical forward genetics approach generates diverse mutants without introducing foreign DNA, allowing selection of improved strains based on desired phenotypes [5]. Combining random mutagenesis with high-throughput screening methods like fluorescence-activated cell sorting (FACS) accelerates identification of promising mutants with enhanced lipid production or growth rates [12].

Genetic transformation methods such as biolistic particle bombardment and electroporation are common techniques to introduce foreign DNA into microalgal cells despite challenges posed by thick cell walls [9]. Biolistics uses micro-projectiles (tungsten or gold particles) to physically deliver DNA, usable for nuclear and organelle transformation, while

electroporation applies electrical pulses to permeabilize cell membranes for DNA uptake [13].

Genome editing technologies represent advanced molecular tools enabling precise, site-directed modifications [14]. Engineered nucleases such as zinc-finger nucleases (ZFNs), TALENs, and especially CRISPR-Cas9 are widely used to induce targeted double-strand breaks in DNA, triggering repair mechanisms that can disrupt, insert, or replace genes [15, 16]. CRISPR-Cas9 has gained prominence due to its simplicity, versatility, and ability to perform multiplex editing [15]. However, editing efficiency varies across species, and challenges remain regarding stable and transient expression systems to avoid off-target effects.

Additionally, omics approaches (genomics, transcriptomics, proteomics, metabolomics) guide molecular tool application by identifying candidate genes and regulatory pathways for targeted improvement [17, 18]. Integration with adaptive laboratory evolution and high-throughput mutant libraries further enhances strain development pipelines [5].

3. Current Status of Molecular Tools in Microalgae

The current status of molecular tools in microalgae demonstrates remarkable achievements, particularly through CRISPR/Cas-based genome editing, coupled with transcriptional regulation and omics integration, although species-specific challenges remain significant [19, 20]. Since the first application of CRISPR/Cas9 in *Nannochloropsis* species in 2016, targeted gene knockouts have effectively improved traits such as lipid productivity without compromising growth or CO₂ fixation [21]. For instance, attenuation of the transcription regulator *ZnCys* in *Nannochloropsis gaditana* doubled lipid production while maintaining robust growth, highlighting CRISPR's potential to enhance microalgal bio-product synthesis [19]. Beyond gene knockouts, CRISPR-based transcriptional regulators (CRISPRa/i) have enabled precise modulation of gene expression without DNA cleavage; for example, activation of DNA methyltransferase genes through *dCas9-VP64* in *Nannochloropsis* enhanced photosynthesis and growth, pointing to the promise of reversible and fine-tuned metabolic control [19, 22].

Gene silencing techniques, such as RNA interference and CRISPR interference, further complement editing by down-regulating target genes, broadening the toolbox for optimization of metabolic pathways [23, 24]. Omics technologies encompassing genomics, transcriptomics, proteomics, and lipidomics play an indispensable role by identifying critical target genes and non-essential genomic regions, thus guiding rational genome editing and pathway engineering for improved strain performance [25].

Transformation methods, including electroporation, particle bombardment, *Agrobacterium*-mediated delivery, and glass bead agitation, have been adapted for different micro-

algal species [5]. However, species-specific barriers like complex cell wall architecture and strong genetic silencing mechanisms often impede transformation efficiency and stable expression of editing components [26]. Recent advances such as engineered nuclear localization signals improve Cas9 nuclear import, doubling editing efficiency and reducing cytotoxicity in industrial strains. Furthermore, delivery of Cas9 ribonucleoproteins (RNPs) enables transient expression and markerless editing, addressing regulatory concerns related to transgenic DNA presence [20].

Despite these accomplishments, CRISPR editing efficiencies vary widely across species, and high-throughput mutant screening remains a bottleneck [27]. Model organisms like *Chlamydomonas reinhardtii* benefit from well-established protocols with editing efficiencies ranging from 10% to nearly 70%, while non-model, industrially relevant microalgae still require customized solutions due to genetic and cellular diversity [20]. Emerging CRISPR variants such as Cas12a and advances in multiplex editing further expand the scope of precise genome modifications, augmenting the potential to develop microalgal strains tailored for biofuels, nutraceuticals, and environmental remediation [25].

Instantly, CRISPR/Cas-based genome editing, transcriptional regulation systems, and omics-guided pathway engineering have collectively revolutionized microalgal strain improvement [28]. While species-specific challenges in transformation and gene delivery persist, ongoing innovations in molecular tools and synthetic biology promise to overcome these hurdles, accelerating the development of robust, high-yield microalgal strains optimized for sustainable biotechnological applications [29].

4. Applications and Impact of Molecular Tools on Traits

Molecular tools have profoundly impacted the enhancement of key traits in microalgae, driving significant advances in lipid production, growth performance, and the biosynthesis of high-value compounds with broad environmental and industrial implications [19]. Targeted genome editing and transcriptional regulation have been successfully employed to increase lipid accumulation, a crucial factor for improving biofuel yields [7]. For example, CRISPR/Cas-mediated knockout or downregulation of genes involved in lipid degradation pathways or competing metabolic routes has led to microalgal strains with substantially higher lipid content without compromising cell viability, thereby advancing the feasibility of sustainable biodiesel production [19]. Concurrently, improvements in growth rates and photosynthetic efficiency have also been achieved through genetic modifications targeting photosynthetic apparatus components and regulatory factors. Enhanced photosynthetic capacity not only accelerates biomass accumulation but also improves carbon fixation, underscoring the role of molecular tools in boosting

overall productivity [30].

Beyond biofuels, molecular engineering has enabled the tailored production of diverse high-value compounds such as pigments (carotenoids, phycobiliproteins), proteins, vitamins, and pharmaceuticals [31]. Through pathway engineering and overexpression of key biosynthetic genes, microalgae have been transformed into efficient biofactories for nutraceuticals and therapeutic agents, expanding their commercial potential [32]. These advancements contribute to more cost-effective and scalable production processes compared to traditional extraction from terrestrial plants [33].

Moreover, the environmental and industrial implications of molecularly improved microalgal strains are substantial [34]. Enhanced carbon capture through improved photosynthesis aids in mitigating greenhouse gas emissions, while strains engineered for resilience to abiotic stressors support more robust cultivation under variable conditions [35]. This increases the sustainability and economic viability of large-scale microalgal biorefineries. Additionally, molecular tools facilitate the development of algae-based wastewater treatment technologies by enabling strains to tolerate and metabolize pollutants effectively [36].

Instantly, the application of molecular tools has enabled the precise enhancement of microalgal traits critical for biofuel production, growth optimization, and the biosynthesis of valuable biomolecules, with far-reaching benefits for sustainable industry and environmental management. These innovations continue to push microalgae closer to realizing their full potential as versatile, eco-friendly bioresource platforms.

5. Current Limitations and Challenges

Current limitations and challenges in employing molecular tools for microalgal strain improvement primarily revolve around low transformation efficiencies and species-specific barriers [37]. The rigid and diverse cell wall structures of many microalgal species impede efficient delivery of genetic material, making transformation methods such as electroporation, biolistic bombardment, and *Agrobacterium*-mediated transformation highly variable in success depending on the species and condition [32, 38]. Achieving high rates of targeted genetic modification remains difficult, particularly because homologous recombination is a key mechanism for precise gene knock-in is inherently low in microalgae [19]. Although advances such as co-delivery of Cas9-gRNA ribonucleoprotein complexes with DNA repair templates and optimization of homology arm lengths have improved knock-in efficiencies to around 15% in *Chlamydomonas reinhardtii*, these results are still species-dependent and not universally reproducible [39].

Off-target effects pose another challenge, as unintended edits can disrupt non-target genes, demanding improved specificity of CRISPR components and comprehensive screening strategies [40]. Furthermore, the presence of powerful gene silencing sys-

tems in some microalgae can reduce the expression and effectiveness of introduced genetic constructs, complicating stable genome editing [41]. Regulatory concerns also present substantial hurdles; the biosafety of genetically modified microalgae must be rigorously assessed, especially in the context of environmental release and commercial scaling, with policies varying widely across jurisdictions. Public acceptance and containment measures add further complexity [42].

From an economic and scalability perspective, the current molecular techniques often require sophisticated equipment and labor-intensive protocols that limit cost-effectiveness for large-scale applications [43]. Scaling up genetically engineered microalgal cultivation involves overcoming cultivation stresses, maintaining genetic stability, and achieving consistent high yield under industrial conditions, which remain ongoing challenges [32]. Therefore, despite significant progress, these biological, technical, regulatory, and economic barriers collectively constrain the rapid and widespread deployment of molecularly improved microalgal strains for commercial biofuels, bioproducts, and environmental solutions.

6. Future Perspectives

Future perspectives for molecular tools in microalgal strain improvement are highly promising, driven by emerging technologies and interdisciplinary integration. Next-generation genome editing platforms, including base editors and prime editors, offer unprecedented precision by enabling targeted nucleotide changes without inducing double-strand breaks, thus minimizing off-target effects and genomic instability [44]. Multiplexed genome editing, where multiple genes are simultaneously targeted, is expected to accelerate the engineering of complex traits like enhanced lipid biosynthesis or stress tolerance [45]. Advances in delivery systems, such as nanoparticle-mediated transfer and improved ribonucleoprotein complexes, aim to overcome species barriers and increase transformation efficiencies while reducing transgene integration, facilitating markerless and minimal genome modifications [46]. This minimal genome engineering approach holds the potential to streamline algal genomes by removing non-essential elements, thereby optimizing cellular resources for desired bioproduct synthesis.

Fine-tuned metabolic control via CRISPR activation and interference (CRISPRa/i) systems promises dynamic regulation of gene expression, allowing real-time adaptation of microalgal metabolism to environmental conditions or production demands without permanent genome disruption [19]. Integration of these molecular tools with artificial intelligence (AI) and machine learning is poised to revolutionize strain design by enabling predictive modeling of gene networks and phenotype outcomes, guiding rational, data-driven modifications and high-throughput screening strategies.

The regulatory landscape is also expected to evolve in response to advances in genome editing, with potential for clearer guidelines and risk assessments that distinguish ge-

nome-edited strains from traditional genetically modified organisms, thus facilitating their acceptance and commercialization [47]. Coupled with these regulatory improvements, the ongoing reduction in bioprocessing costs and increased demand for sustainable bio-based products indicate strong commercial potential for molecularly enhanced microalgal strains [7]. Collectively, these advancements will accelerate the development of robust, high-yielding, and environmentally resilient microalgae tailored for applications in biofuels, nutraceuticals, pharmaceuticals, and environmental management, marking a new era in microalgal biotechnology.

7. Conclusion

Molecular tools have played a transformative role in advancing microalgal strain improvement, unlocking new possibilities for enhancing productivity, stress tolerance, and the biosynthesis of valuable compounds essential for sustainable biotechnology. Key achievements such as precise genome editing with CRISPR/Cas systems, transcriptional regulation via CRISPRa/i, and the integration of multi-omics approaches have significantly accelerated the development of robust microalgal strains tailored for biofuel production, nutraceuticals, and environmental applications. Despite these successes, challenges including low transformation efficiencies, off-target effects, species-specific barriers, and regulatory complexities continue to limit the full exploitation and commercialization of genetically improved microalgae. Looking ahead, emerging next-generation genome editing technologies, improved delivery methods, and computational tools like artificial intelligence promise to overcome current limitations by enabling sophisticated, multiplexed, and fine-tuned metabolic engineering. As regulatory frameworks evolve and bioprocessing becomes more cost-efficient, the potential impact of molecularly enhanced microalgae on sustainable bioeconomies is substantial, offering eco-friendly alternatives for renewable energy, medicine, and environmental stewardship. Ultimately, continued innovation and interdisciplinary collaboration will be vital to realizing the full promise of molecular tools in shaping the future of microalgal biotechnology.

Abbreviations

AI	Artificial Intelligence
CO ₂	Carbon Dioxide
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DCAS9	Dead Cas9 Enzyme
DNA	Deoxyribonucleic Acid
FACS	Fluorescence-activated Cell Sorting
GRNA	Guiding Ribonucleic Acid
RNA	Ribonucleic Acid
RNPS	Ribonucleoproteins

TALENS Transcription Activator-like Effector Nucleases
 UV-C Ultraviolet C
 ZFNS Zinc-Finger Nucleases

Author Contributions

Alebachew Molla: Conceptualization and framing, Comprehensive literature collection and critical analysis, Writing the original draft, Reviewing and editing, Providing expert interpretation.

Gedif Meseret: Reviewing and editing, Providing expert interpretation. The authors read and approved the final manuscript.

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Conflicts of Interest

The author declares no conflicts of interest.

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