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CRISPR-CAS9-MEDIATED ENHANCEMENT OF HEAT TOLERANCE AND BIOFUEL PRODUCTIVITY IN MICROALGAE: A REVIEW

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Abstract

The increasing demand for sustainable and renewable energy sources has intensified interest in microalgae as promising feedstocks for biofuel production. Microalgae possess several advantages, including rapid growth rate, high lipid accumulation, efficient carbon dioxide utilization, and the ability to grow in diverse environmental conditions. However, elevated temperature remains a major challenge affecting algal biomass productivity, photosynthetic efficiency, lipid biosynthesis, and overall industrial performance. Heat stress induces oxidative damage, disrupts metabolic pathways, and reduces biofuel yield, thereby limiting the commercial feasibility of algal biofuel systems. Recent advances in genetic engineering, particularly CRISPR-Cas9 genome editing technology, have provided new opportunities for improving thermotolerance and metabolic efficiency in microalgae. CRISPR-Cas9 enables precise modification of genes associated with heat shock proteins, antioxidant defense systems, photosynthesis, carbon fixation, membrane stability, and lipid metabolism. In addition, the integration of synthetic biology, systems biology, and multiomics approaches has accelerated the development of stress-resilient algal strains with enhanced biofuel productivity. This review discusses the impact of heat stress on algal systems, recent developments in CRISPR-mediated algal biotechnology, important genetic targets associated with thermal adaptation, and the role of genome editing in improving biofuel production. Furthermore, the review highlights current challenges, prospects, and the potential of CRISPR-engineered thermotolerant microalgae for sustainable renewable energy applications.

Keywords: *CRISPR-Cas9; Microalgae; Heat tolerance; Biofuel production; Genetic engineering; Lipid biosynthesis*

1. Introduction

As the world increasingly calls for sustainable and renewable energy resources, the study of alternative, environmentally-friendly sources to fossil fuels has gained in momentum. Fossil fuels like petroleum, coal, and natural gas still make up a major part of greenhouse gas emissions, climate change, and environmental degradation. Biofuels produced from biological sources have been identified as viable and sustainable energy sources in this context, as they can help to lower carbon emissions and reliance on non-renewable fuels. As of today, microalgae are receiving significant research and development focus among the different biofuel feedstocks tested due to their fast growth rate, large capacity to accumulate lipids and tolerance to a variety of environmental conditions [1,2].

Microalgae are considered to be the most efficient photosynthetic microorganisms, which can be used to transform sun energy and carbon dioxide in the atmosphere to valuable biomolecules like lipids, carbohydrates, proteins and pigments. These properties make them ideal for use in biodiesel and other biofuels. Microalgae offer a number of benefits when compared to traditional terrestrial biofuel crops such as biomass production, land usage efficiency, and the ability to cultivate in wastewater, or saline environments, where competition for food is not a concern [3,4]. Additionally, the development of algal biotechnology has broadened the application of microalgae from biofuel production to other uses such as wastewater treatment, carbon sequestration, pharmaceuticals, nutraceuticals, and industrial bioproduct synthesis [5].

Although there is a huge potential in the production of algal biofuel on a large scale, there are several environmental and physiological restrictions associated with it. Of these, heat stress is a significant problem that impacts both algal growth and photosynthetic efficiency, in addition to its impact on lipid metabolism and overall biomass productivity. High temperatures may cause oxidative stress, stabilize membranes, decrease enzyme functions, and decrease energy-rich metabolite accumulation which are needed for biofuel production. Concerns about the thermal stability of algal strains being grown in industrial outdoor systems have also been exacerbated with climate change and warming up of the planet [4]. Therefore, the development of thermotolerant algal strains is a crucial research target to enhance algal biofuel production systems, in terms of economic viability and sustainability.

Recent developments in genetic modification and synthetic biology have enabled new possibilities to increase the stress resistance and metabolic efficiency in microalgae. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 genome editing system has proven to be one of the most precise and potent technologies for targeted genetic manipulation among these technologies. CRISPR-Cas9 allows researchers to make site-specific changes within the genome, helping to regulate genes related to heat tolerance, lipid biosynthesis, photosynthetic performance and stress adaptation [2,6]. CRISPR-Cas9 has the advantages of higher precision, lower cost, higher efficiency, and multiplex genome editing compared with the traditional genetic engineering method.

In recent years, the use of CRISPR-Cas9 technology in microalgal biotechnology has been growing exponentially. The success of the editing of genes for metabolic engineering, stress responses, and production of industrial biomolecules in algal species has been shown in several studies [1,5]. Genome editing methods also offer great potential to boost lipid content, thermal stress, and biomass production under stress [3]. Moreover, the synthetic biology and CRISPR-mediated engineering has boosted the development of next-generation algal strains that can be used for sustainable biofuel production and environmental applications [6].

The purpose of this review is to critically analyze the use of genome editing techniques using CRISPR-Cas9 in the improvement of algal strains for biofuel application with focus on heat tolerance. The review highlights the importance of algae as feedstocks for renewable biofuels, the effect of heat stress on algal systems, and advances in algal biotechnology using the CRISPR system. In addition, key genes for thermal adaptation are identified and the potential of genome engineering strategies to increase the productivity of biofuels and the sustainability of algal cultivation systems is discussed..

2. Algae as Biofuel Producers

2.1 Microalgae as Renewable Energy Sources

Amongst all the renewable feedstock, microalgae are one of the most promising sources for sustainable biofuel production because of their high photosynthetic efficiency, high growth rate and biochemical versatility. In addition, microalgae could be grown in non-arable areas and saline or wastewater sites, which reduces the competitive use of food crops and freshwater resources as compared to traditional biofuel crops (e.g., soybean, corn, and palm oil plants) [7,8]. They also play a crucial role in mitigating greenhouse gas emissions and the global carbon neutrality program because they are able to generate energy-rich biomolecules by photosynthesis using atmospheric carbon dioxide. Therefore, microalgae have become a subject of great interest as environmentally sustainable alternatives to next-generation bioenergy production systems. Due to its biochemical composition, microalgae are very well suited for obtaining biodiesel, bioethanol, bio-gas and bio-hydrogen. Several species of algae have the ability to store large amounts of lipids and carbohydrates in specific nutrient and environmental conditions.

The above metabolites can be effectively transesterified, fermented and anaerobically digested to produce renewable fuels [9,10]. Furthermore, microalgae outperform terrestrial biofuel crops in terms of cultivation time, as it takes much less time to grow and has a higher biomass productivity, with biomass harvested continuously and increased yield per unit area [11]. The species Chlamydomonas reinhardtii, Nannochloropsis, Scenedesmus and Chlorella are extensively studied for their biofuel potential due to their adaptability and high lipid accumulation ability. During the last few years, significant progress has been achieved in algal biotechnology and synthetic biology, which have spurred the further exploration of microalgae as biofactories. Algal metabolism has been engineered for better biofuel production and resistance to stress by optimizing metabolic pathways and using synthetic biology interventions [8,9]. Furthermore, the use of the CRISPR-Cas system in genetic engineering of algae has greatly improved the accuracy and efficiency of algal strain development strategies [13]. These technological advancements are likely to be critical to the ability to address some of the current productivity constraints of algal farming systems at an industrial scale.

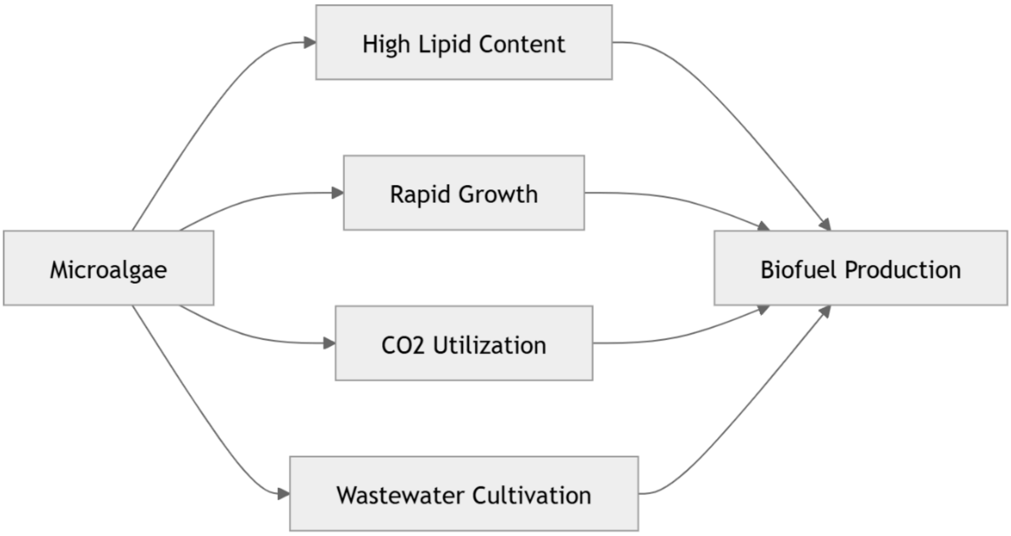

Figure 1. Advantages of microalgae for biofuel production.

2.2 Lipid Accumulation and Biomass Productivity

A key property of microalgae that is critical for biodiesel applications is lipid accumulation. Many species of microalgae are capable of storing higher amount of neutral lipids, especially triacylglycerols, which can be used as the main raw materials for the biodiesel production process under favorable environmental conditions and/or stress conditions [4,10]. In algal cells, the pathways for lipid biosynthesis are greatly affected by nutrient deprivation, light intensity, salinity and temperature. In particular, nitrogen starvation has been extensively reported to increase lipid storage, due to the re-routine of cellular carbon flux to lipid storage production versus protein synthesis.

In recent years, it has been shown that the productivity of lipids in microalgae can be significantly increased by metabolic engineering techniques, by targeting genes involved in the fatty acid biosynthesis and carbon metabolism [5,13]. One of the crucial challenges for industrial biofuel production is to develop GM strains that can produce large amounts of lipids in environmental stress. The progress in the field of synthetic biology and genome editing using CRISPR has paved the way for specific modifications in lipid metabolic pathways, thus enhancing not only the quantity, but also the quality of lipids for industrial uses [8].

In addition to lipid accumulation, the biomass productivity is another important parameter that affects the economic viability of algal biofuel production. The high biomass yield directly translates to higher production of biofuels and better industrial scalability. In large-scale culture systems, however, photosynthetic inefficiencies, under-exploitation of light and environmental stress are sometimes a constraint on biomass production. To overcome these drawbacks, researchers have tried to find variations on light-harvesting antenna systems that can maximize photosynthetic efficiency and biomass productivity in microalgae [15]. The ability of the shortened light-harvesting antenna complexes to increase the light penetration in dense algal culture has been demonstrated, which we believe leads to an improvement in photosynthetic performance and overall biomass production.

2.3 Advantages of Algae Over Conventional Biofuel Crops

Microalgae offer various benefits over traditional biofuel feedstocks from terrestrial sources, making them a promising option for sustainable energy generation. Their major benefit is that they have much higher biomass productivity and growth rate than traditional oilseed crops. For microalgae, the production of lipids is significantly higher per hectare with a relatively smaller cultivation area [12]. Additionally, unlike first generation biofuel crops, they can be grown on non-agricultural land, thereby alleviating the potential environmental impact of deforestation, land degradation and food insecurity often raised with first generation biofuel crops.

The other benefit of microalgae is that they are versatile and adaptable to the environment, with multifunctional industrial applications. Numerous algal species are capable of growing thrivingly in wastewater, industrial effluents and saline environments, and at the same time play a role in the removal of nutrients and carbon dioxide sequestration [14]. These dual functionalities are useful in building integrated bioremediation-biorefineries that can solve environmental pollution problems and produce renewable energy. The incorporation of wastewater treatment with algal biofuel production also contributes to circular bioeconomy models and sustainable resource management strategies.

However, the commercialization of algal biofuels still has several technical and economic challenges, such as cultivation expenses, efficiency of harvesting, and environmental stress management [11]. Hence, there is a growing interest in the cultivation of algal strains that are genetically modified for improved growth capacity, stress resistance and metabolic efficiency. Combining the more advanced genome editing technologies, including CRISPR-Cas9 systems, is likely to enable the creation of robust algal strains that will be able to maintain high biofuel production under variable environmental conditions [7,13].

Table 1. Major Microalgal Species and Their Biofuel Potential

Microalgal Species	Major Characteristics	Biofuel Potential	Industrial Significance	References
Chlamydomonas reinhardtii	Fast growth, well-studied genetic model	Biodiesel and biohydrogen production	Widely used in genetic engineering and CRISPR studies	[3,9]
***Nannochloropsis* spp.**	High lipid accumulation capacity	Biodiesel production	Commercially important for lipid-based biofuels	[4,10]
***Scenedesmus* spp.**	High biomass productivity and stress tolerance	Biodiesel and biogas production	Suitable for wastewater treatment and biofuel applications	[12,14]
***Chlorella* spp.**	Rapid growth and efficient CO_2 fixation	Biodiesel and bioethanol production	Frequently used in industrial algal cultivation systems	[8,11]
***Spirulina* spp.**	High protein content and environmental adaptability	Bioethanol and biogas production	Used in nutraceutical and environmental applications	[17,23]
Parachlorella kessleri	Efficient lipid biosynthesis	Biodiesel production	Important species for CRISPR-mediated genome editing studies	[31]
Fistulifera solaris	Oleaginous diatom with high oil productivity	Advanced biodiesel production	Potential industrial strain for metabolic engineering	[41]
Cyanobacteria	Efficient photosynthesis and carbon fixation	Biohydrogen and bioethanol production	Important in synthetic biology and carbon capture systems	[22,25]

3. Heat Stress in Algal Systems

3.1 Physiological Effects of Heat Stress

Temperature is one of the most important environmental factors influencing growth, metabolism and productivity of algae. High temperature causes damage to the membrane structure, enzyme function and photosynthetic activity, which decreases the growth of biomass and lipid production [16]. Additionally, heat stress affects PSII, reduces chlorophyll, and impairs carbon fixation, thereby reducing biofuel productivity [15,16]. When exposed to thermal stress, microalgae trigger protective mechanisms, such as heat shock proteins, antioxidant systems etc. But these defenses can be overwhelmed by high temperatures over long periods leading to irreversible cellular damage [18]. So, enhancing algal thermotolerance is crucial for sustainable algal cultivation at large scales.

3.2 Oxidative Stress and Reactive Oxygen Species

Reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxide are produced as a result of heat stress, causing damage to proteins, lipids and nucleic acids [20]. High levels of ROS disrupt the stability of the membranes, photosynthesis and lipid metabolism, which impacts negatively on algal growth and biodiesel production [17]. Researchers have been interested in improving the antioxidant defence systems via genetic engineering strategies in order to decrease the oxidative damage [18,20]. Gene manipulation of antioxidant enzymes, like superoxide dismutase and catalase, can enhance the resistance to stress and stability of the cells of microalgae.

3.3 Impact of Elevated Temperature on Lipid Metabolism and Biofuel Production

Heat stress has a significant impact on lipid metabolism and biodiesel productivity of microalgae. High temperatures affect the composition and unsaturation of the lipids in membranes and affect pathways of lipid biosynthesis [20]. While moderate stress can induce the formation of lipids, most of the time, heat stress leads to reduced biomass and lipid productivity [10]. Strategies to increase the capability of algae to perform photosynthesis, carbon fixation, and stress tolerance have been improved in recent years by advances in bioengineering and in the field of synthetic biology [16,18]. Furthermore, light-driven synthetic biology and cyanobacterial cell factory systems have been proven to have potential for enhancing production of renewable biofuels [22]. The developments are useful for the emerging application of thermotolerant microalgae in sustainable energy and environmental uses [21,23].

Table 2. Effects of Heat Stress on Microalgal Physiology and Biofuel Production

Heat Stress Factor	Physiological Effect on Microalgae	Impact on Biofuel Production	References

Elevated temperature	Disruption of cellular metabolism	Reduced biomass productivity	[15,16]
Oxidative stress	Excessive production of reactive oxygen species (ROS)	Damage to proteins, lipids, and nucleic acids	[17,20]
Photosystem damage	Reduction in photosynthetic efficiency and chlorophyll content	Lower carbon fixation and lipid synthesis	[15,16]
Membrane instability	Altered membrane fluidity and permeability	Impaired nutrient transport and metabolic activity	[20]
Enzyme denaturation	Loss of enzymatic activity under thermal stress	Reduced metabolic efficiency	[18]
Lipid metabolism disruption	Changes in fatty acid composition and lipid biosynthesis	Decreased biodiesel yield and quality	[10,20]
Environmental fluctuations	Combined stress from light, salinity, and nutrients	Reduced industrial cultivation stability	[21,23]
ROS accumulation	Cellular oxidative damage	Lower growth and biofuel productivity	[17,20]

4. CRISPR-Cas9 Technology in Algal Biotechnology

4.1 Mechanism of CRISPR-Cas9 Gene Editing

CRISPR-Cas9 is a very powerful genome editing technique which allows the precise modification of target genes via RNA-guided DNA cleavage. It is composed primarily of a guide RNA and Cas9 nuclease that form a complex that introduces genetic change at a specific site [24]. CRISPR-Cas9 has many advantages over traditional genetic engineering techniques such as precision, low expense, and the ability to edit several genes at once [27]. In recent years, there have been significant advances in the use of CRISPR beyond gene knockout, such as transcriptional regulation and metabolic engineering [24]. These developments have spurred algal biotechnology research for enhanced lipid production, stress resistance, and biofuel production [13].

4.2 Delivery Systems and Genetic Transformation in Microalgae

Delivery of CRISPR components into algal cells is still a challenge due to the hard-to penetrate cell wall and species-specific structures within the cell. To enhance the efficiency of genome editing, various transformation techniques have been developed such as electroporation, particle bombardment, Agrobacterium-mediated transformation, and nanoparticle-assisted delivery [7]. CRISPR delivery systems using nanoparticles have demonstrated great promise for improving the efficiency of transformation without causing harm to the cells [7]. Transformation systems for the other industrially important alga *Parachlorella kessleri* have also been developed with successful transformation of this species using CRISPR [31].

4.3 Applications of CRISPR-Cas9 in Metabolic Engineering of Microalgae

CRISPR-Cas9 has greatly enhanced metabolic engineering approaches in microalgae for biofuel and bioproduct production. Genome editing can be used to precisely regulate pathways involved in lipid metabolism, carbon fixation and photosynthesis to improve biomass productivity and stress tolerance [26,27]. The biofactories are increasingly being developed for use by microalgae in the production of biodiesel, pigments, proteins and other industry chemicals [29]. Multiomics strategies and the use of CRISPR as a genetic engineering tool have led to better identification of genetic targets for biofuel production, carbon sequestration, and wastewater treatment [25,26]. The advances in sustainable algal biorefineries and renewable energy systems have also been enhanced by synthetic biology approaches [28]. The uses of CRISPR have also been extended to the wider field of renewable fuel and industrial biotechnology such as the conversion of lignocellulosic biomass and microbial biofuels [32,33]. Yet, problems like off-target mutations, low homologous recombination efficiency, and regulatory issues still persist in large-scale commercialization of genome-edited algal strains [18,30].

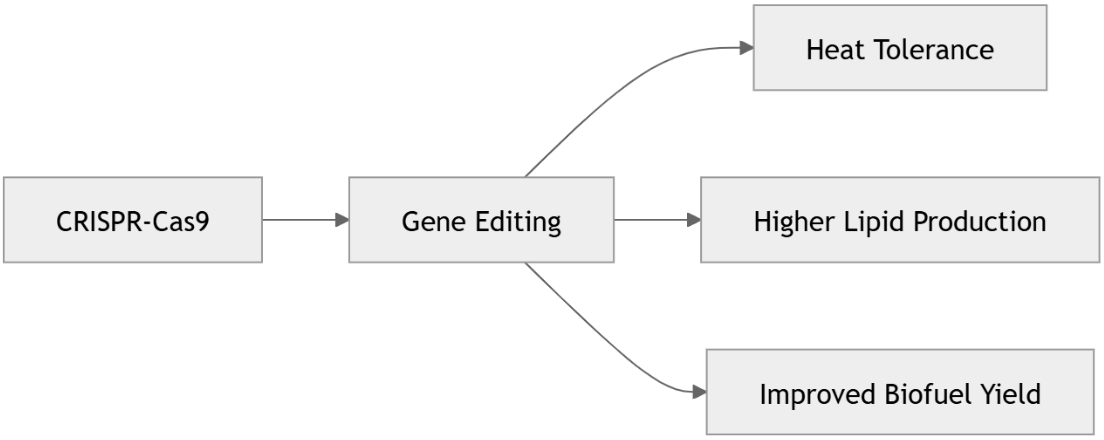

Figure 2. Role of CRISPR-Cas9 in improving algal biofuel productivity.

5. Genetic Targets for Heat Tolerance

5.1 Heat Shock Proteins and Thermal Adaptation

Under thermal stress, algal cells are sensitive to heat-induced damage of proteins and HSPs are molecular chaperones that prevent protein aggregation and stabilize them under heat stress. High temperatures break open proteins and slow down the metabolic processes, thus compromising cellular function. Microalgae employ proteins like HSP70 and HSP90 to activate the heat shock response pathway to counteract these effects [38]. The regulation of heat shock proteins using CRISPR-Cas9 is a promising approach to enhance thermotolerance in microalgae. The advancement of synthetic biology and programmable cell engineering also helps the development of algal strains that are able to maintain their productivity under changing environmental conditions [39].

5.2 Antioxidant Defense Systems and Oxidative Stress Regulation

The generation of Reactive Oxygen Species (ROS) in Heat Stress leads to the formation of oxidative stress, which damages proteins, lipids and nucleic acids. Algal cells are protected from oxidative stress by antioxidant enzymes, including superoxide dismutase, catalase and ascorbate peroxidase [20]. Antioxidant pathway modification by genetic engineering could be used to enhance thermotolerance and cellular stability in microalgae. With the development of advanced genetic tools and stress-resilient microbial systems, the application of biotechnology in sustainable biofuel production has been further strengthened [42,43].

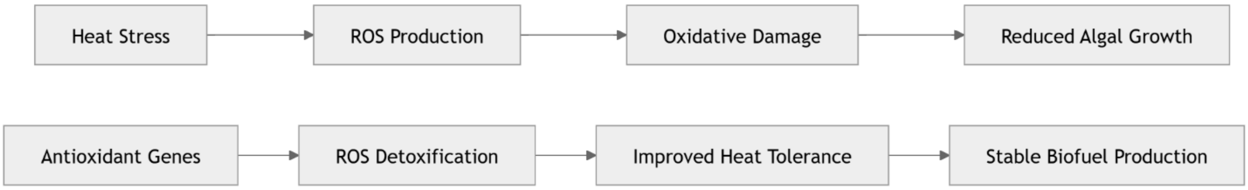

Figure 3. Antioxidant defence response under heat stress in microalgae.

5.3 Photosynthetic Stability and Carbon Fixation Pathways

Photosynthesis is highly sensitive to heat stress and high temperatures can decrease the rate of carbon fixation and biomass productivity of microalgae. Thus, there are several genes that are crucial for the stability of the photosystems, carbon metabolism and light-harvesting complexes, which are therefore good targets for CRISPR-mediated engineering [16]. Engineering of photosynthetic pathways and carbon fixation system can enhance thermal tolerance and carbon sequestration efficiency of algae [25,40]. Furthermore, the expression of transgenic genes in oleaginous microalgae has been optimized for enhanced production of biofuel precursors with the aid of advanced expression systems [41].

5.4 Membrane Stability and Lipid Metabolism Under Heat Stress

High temperatures have an impact on membrane stability and lipid metabolism, as they change the fluidity state of membranes and the fatty acid composition. Microalgae begin to change their lipid profiles in order to preserve cellular functions under stress conditions [20]. In fact, oleaginous microalgae have the potential to be better heat-tolerant and more productive in biodiesel production when the pathways of lipid metabolism are manipulated by CRISPR [36]. There has been further development of algal genome editing and mutation-induced strain improvement for the development of thermotolerant and industrially robust algal strains in recent years [34,35,37].

Table 3. Major Genetic Targets Associated with Heat Tolerance in Microalgae

Genetic Target	Biological Function	Role in Heat Tolerance	Potential CRISPR Application	References
Heat shock proteins (HSP70, HSP90)	Protein folding and stabilization	Protects cells from thermal damage	Upregulation of stress-response genes	[38,39]
Superoxide dismutase (SOD)	Reactive oxygen species detoxification	Reduces oxidative stress	Enhancement of antioxidant defense pathways	[20,42]
Catalase (CAT)	Breakdown of hydrogen peroxide	Maintains cellular redox balance	Improved oxidative stress tolerance	[20,43]
Ascorbate peroxidase (APX)	Removal of reactive oxygen species	Protects photosynthetic machinery	Regulation of antioxidant enzyme expression	[20,42]
Photosystem-related genes	Photosynthetic stability	Maintains photosynthetic efficiency under heat stress	Optimization of photosynthetic pathways	[16,25]
Carbon fixation genes	CO_2 assimilation and metabolism	Supports biomass productivity during stress	Enhancement of carbon sequestration efficiency	[25,40]

Fatty acid biosynthesis genes	Lipid synthesis and storage	Maintains membrane stability and biodiesel production	Increased lipid accumulation	[20,36]
Membrane stability genes	Regulation of membrane fluidity	Protects cellular integrity under elevated temperatures	Improved thermotolerance	[20,37]
Stress signaling genes	Cellular stress-response regulation	Coordinates adaptation to environmental stress	Multiplex genome editing for stress adaptation	[38,39]
Transgene expression systems	Enhanced metabolic regulation	Improves expression of stress-related pathways	Optimization of biofuel-related traits	[41]

6. CRISPR-Mediated Enhancement of Biofuel Productivity

6.1 Enhancement of Lipid Biosynthesis Through Genome Editing

CRISPR-Cas9 has greatly facilitated metabolic engineering approaches to increase lipid content of microalgae for biodiesel production. Modification of the genes involved in fatty acid synthesis and storage selectively could lead to an increase in neutral lipid without compromising biomass productivity [5]. The changes are crucial for the economic development of algal biorefineries. Recent developments in synthetic biology and multiomics methods have aided in the identification of genes involved in lipid metabolism and stress adaptation [45]. Engineered algal strains are created with a newly discovered technology known as CRISPR to regulate these pathways, so that more triacylglycerol is produced and precursors of the biodiesel are accumulated. Dynamic control of metabolic pathways under environmental stress conditions are further supported by synthetic biology approaches [46].

6.2 Improvement of Photosynthetic Efficiency and Biomass Productivity

One of the key parameters that affects the growth rate in biomass and productivity of biofuels from microalgae is photosynthetic efficiency. The use of CRISPR-Cas9 for engineering photosynthetic pathways has been explored for solar energy conversion and biomass production [16]. In algae, researchers have been targeting the light-harvesting complexes, the electron transport pathways, and carbon fixation systems to improve photosynthesis processes [49]. These changes enhance the production of biomass, accumulation of lipids, and overall biofuel production. Systems biology and genome editing have also led to the development of programmable algal cells with optimized metabolic functions [39,45].

Table 4. CRISPR-Mediated Strategies for Improving Photosynthetic Efficiency and Biomass Productivity in Microalgae

Target Area	CRISPR-Mediated Strategy	Expected Outcome	Relevance to Biofuel Production	References
Light-harvesting complexes	Modification of antenna size	Improved light distribution in dense cultures	Higher biomass productivity	[16,49]
Photosystem stability	Editing genes linked to photosystem protection	Reduced heat-related photosynthetic damage	Stable growth under stress	[16]
Electron transport pathways	Regulation of electron flow genes	Improved energy conversion efficiency	Enhanced biomass generation	[45,49]
Carbon fixation pathways	Targeted editing of carbon metabolism genes	Increased CO_2 assimilation	Greater lipid and biomass yield	[39,45]
Stress-response genes	Multiplex editing of heat and oxidative stress pathways	Improved thermal resilience	Consistent productivity in outdoor systems	[38,48]
Metabolic regulation	Integration with systems biology tools	Optimized carbon partitioning	Improved biofuel precursor production	[45,46]

6.3 Development of Thermotolerant and Industrially Robust Algal Strains

Thermal stress plays a major role in the growth, photosynthesis and lipid biosynthesis of algae and therefore development of thermotolerant algae strains is crucial for industrial biofuel production. CRISPR-Cas9 is a useful tool for gene modification of heat tolerance and metabolic stability genes [38]. In recent years, genome editing is feasible in several algal species with the help of transformation systems based on the CRISPR approach [31,44]. Furthermore, in recent years, the genes linked to heat tolerance and stress adaptation have been identified with the help of random mutagenesis and omics technologies [47,48]. These strategies can help develop high-performing thermotolerant algal strains.

6.4 CRISPR and Sustainable Algal Biorefineries

The evolution of sustainable algal bio-refineries for CCS, wastewater treatment, and renewable biofuel production has been boosted by the CRISPR-Cas9 technology [14,25]. Value added bio-products, like bioethanol, pigments, proteins and nutraceutical compounds, can also be produced by engineered microalgae [46]. The microbial biomass valorization also contributes to the advancement of algal biomass towards renewable fuels and industrial biochemicals [50]. In summary, the combination of genome engineering using CRISPR and sustainable bioprocessing methods can contribute to the creation of effective and eco-friendly algal biofuel systems.

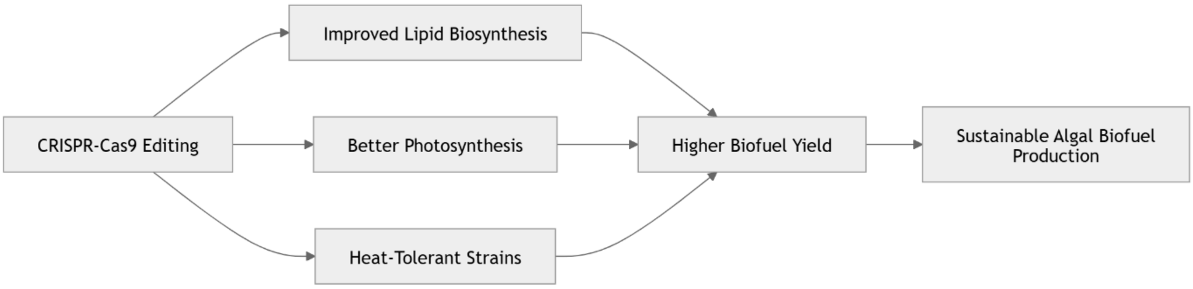

Figure 4. CRISPR-Cas9-mediated enhancement of algal biofuel productivity.

7. Challenges and Limitations

Although the potential uses of CRISPR-Cas9 in enhancing heat tolerance and biofuel production in microalgae are promising, some challenges remain before it can be applied to industry on a large scale. The problem with this is the low transformation efficiency of many algal species, which is attributed to the differences in the cell wall and cellular structure of the algal species. Such differences present a challenge in the establishment of standard genome editing protocols for various microalgal strains.

One further restriction is the possibility of off-target mutations in which off-target genomic areas can be edited along with the targeted gene. These changes may lead to metabolic pathway disruption, photosynthetic and lipid biosynthesis, which in turn, influence the stability of the strain and its industrial performance. Thus, careful design of guide RNA and molecular validation are crucial before commercial use of the edited algal strains.

Algae thermotolerance is also a polygenetic trait involving several genes that are linked to antioxidant defense, membrane stability, photosynthesis and lipid metabolism. Thermotolerance in outdoor culture conditions where algae are subjected to several environmental stressors may require editing of just a few genes. Also, some modifications which could increase the tolerance to stress might lead to lower growth rate or to less biomass productivity, which would be a challenge in maximizing survival and maximizing biofuel production.

However, commercialisation of CRISPR modified algae is further hampered by regulatory, ecological and economic issues. Uncontrolled release of genetically modified organisms (GMOs) into the environment can have environmental consequences, and differences in national regulations lead to uncertainty in industrial approval. Furthermore, the production cost of algal biofuel is still high with the costs of cultivation, harvesting and downstream processing. Commercialization of strain improvement via CRISPR technology therefore requires the development of efficient cultivation methods, as well as sustainable biorefinery approaches.

In summary, problems like transformation barriers, off-target effects, complicated stress-response pathways, environmental issues and high production expenses must be solved before the widespread use of CRISPR-engineered thermotolerant algae to produce sustainable biofuels can be achieved.

8. Future Perspectives

CRISPR-Cas9-mediated algal biotechnology has proven to be a very promising strategy for boosting heat tolerance, metabolic efficiency, and biofuel productivity of microalgae. With the advent of novel, engineered algal systems for sustainable bioenergy production, new applications are anticipated as the synthetic and systems biology, metabolic engineering, and genome editing technologies have rapidly advanced. The next challenge is likely to be multiplex genome editing strategies that will be able to simultaneously regulate several genes involved in heat tolerance, lipid biosynthesis, photosynthetic efficiency, carbon fixation, and oxidative stress management. These types of engineered whole genome manipulation techniques could help generate highly resilient algal strains that produce high biomass and biofuel yields in variable environments.

There is also the possibility that the integration of artificial intelligence, machine learning, and computational metabolic modelling will help to accelerate further algal biotechnology research. These technologies can be used to help identify important genetic targets associated with thermal adaptation, metabolic regulation and stress-response pathways. Furthermore, advanced multiomics technologies, such as transcriptomics, proteomics, metabolomics and epigenomics, could offer a greater understanding of complex cellular responses to heat stress and environmental challenges. This information can be used to inform accurate engineering steps through CRISPR to increase algal growth and biofuel production.

Next generation genome editing technologies like CRISPR-Cas12, CRISPR-Cas13, base editing and prime editing systems are also expected to make advances in the future. These sophisticated platforms could provide more precise editing, efficiency and fewer off-target mutations than traditional CRISPR-Cas9 methods. Furthermore, new limitations on transformation efficiency and species-specific applicability of microalgae could be addressed by the development of novel nanoparticle mediated delivery systems and ribonucleoprotein mediated transformation techniques.

Finally, integration with sustainable bioeconomy and biorefinery solutions with engineered microalgae is one of the key future directions. The thermotolerant algal strains with efficient carbon capture, wastewater treatment, and the production of renewable biofuels could play a significant role in environmental sustainability and climate change mitigation. Integrating algal cultivation with the use of industrial, CO_2 and domestic wastewater treatment systems can also help with the economic viability and utilization of resources.

While all these are positive strides, effective commercialization of algal biofuels produced with CRISPR technologies will need more rigorous biosafety standards, ecological risk assessment, and public acceptance of genome edited organisms. Yet, the quick pace of progress in algal genome engineering suggests that algal biotechnology based on CRISPR technology has great potential to contribute to future renewable energy systems and sustainable low carbon bioeconomy projects.

9. Conclusion

Microalgae are attractive renewable feedstocks for sustainable biofuel production because of their high carbon dioxide assimilation, high lipid content and fast growth. But high temperatures have a negative impact in biomass productivity, photosynthesis and lipid biosynthesis in large-scale cultivation systems. Algal biofuels are not industrially viable due to their susceptibility to oxidative damage and disruption from heat stress. CRISPR-Cas9 genome editing has proven to be a powerful method to increase the thermotolerance and metabolic efficiency in microalgae. Genetic engineering of algae to target heat-shock proteins, antioxidant defense, photosynthesis, carbon fixation, membrane stability and lipid metabolism could help the algae to resist stressors and boost biofuel production in the face of stress. In addition, the use of synthetic biology and multiomics has helped to rapidly optimise algal strains for renewable energy. Although these developments have taken place, there are still several hurdles to overcome, such as low transformation efficiency, off-target mutations, regulatory issues and high production costs. Further research should thus be directed towards enhancing the precision of editing, designing efficient delivery systems and towards sustainable large-scale crop cultivation strategies. In summary, the CRISPR-Cas9 approach to enhancing heat tolerance in algal strains has great promise in the development of next-generation biofuels and in helping to meet world sustainability and low-carbon energy targets.

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