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Review Article

A Narrative Review on Biofuel Production Using Gene Editing Technology

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Abstract

This review highlights the advantages and utilizes various biotechnologies to improve the biofuel production of bacteria, algae, fungi, and higher plants through the manipulation of their genetic content using the CRISPR Cas9 gene editing technology. CRISPR-Cas 9, or protein clusters of regularly interspaced short palindromic repeats, is the most basic and effective tool so far of any for gene editing in specific locations within the genome. Biofuel diversification improved with gene knockout techniques with the CRISPR-Cas9 mechanism. CRISPR-Cas9 also become the preferred technique to alter the organism's metabolic pathways and genome for the production of industrial biofuel. It continues to analyze the contribution of microorganisms to biofuel production as well as the techniques of genome editing to improve the production of certain substances, including genetically modified algae, yeast, and bacteria for the improvement of production. Because of the unending increase in the demand for fuel and the global challenge of warming, the need for such biofuel production has a reason. The review provides a summary of the recent trends in the extent of research carried out in this area in relation to the genetic engineering techniques used.

Keywords: Biofuels; Gene Editing; Crispr; Bacteria

Introduction

The 20th century saw an unprecedented rise in the use of oil products, and this is likely to escalate shortly. There is a great need for fuel and it is the backbone of manufacturing, power generation, and transport systems. As indicated by the recent relative changes in consumption patterns of oil's by-products, several socio-economic and ecological challenges have arisen. It is justifiable to say that the crises based on the inflation of fossil fuels and the other climatic changes indicate that there is a need for clean and renewable fuels that can sustain [1]. As it is thought that the development of environmentally friendly and biodegradable new fuels would replace the use of fossil fuels [2]. Production of biofuels from biomass is a low-cost and environmentally sustainable method to address the challenge of dwindling fossil fuel resources. These interchangeable and inexhaustible fuel sources, like biodiesel and bioethanol, have been of great concern to industries, governments, and researchers due to their incredible benefits [3]. Lignocellulosic biomass, which is not intended for food production, provides reliable renewable energy. Biomass such as poplar, sunflower, and jatropha refers to the biofuel crops of the lignocellulosic kind. Their geographical distribution and abundance make them a favorable biomass source for biofuels. Because of these reasons, they can be produced and utilized without the limitation of carving them as food sources, as is the case with first-generation feedstocks [4,5]. On the contrary, there are several technological and scientific hurdles to addressing the biofuels sector using lignocellulosic biomass as feedstock.

Many strains of microorganisms have been demonstrated to produce biofuels during fermentation. One of the widely used yeasts for large-scale ethanol production from simple sugars is *Saccharomyces cerevisiae*. Some of the notable strains used for fermentation include *Clostridium thermosaccharolyticum, Zymomonas mobilis, Thermoanaerobacter mathranii, C. thermohydrosulfuricum, T. brockii, and T. ethanolicus.* The improvement of the existing microbial strains for biofuel production appears to be assisted by the discipline known as site-specific genome editing, which is cutting-edge in the field of genomics. In the native microorganisms, the changes are made site-specifically, such as performing knocking down, knocking out, and knocking in of the genes, of which genetic engineering is often used to apply these changes to the organisms.

In contrast to traditional genetic engineering, which requires first cutting the gene to be manipulated, altering the gene outside of the host and putting it back inside the organism, or transforming the organism with a new gene to change specific characteristics of the organism [6], The site-specific genome editing techniques RNA-guided Endonuclease-Mediated (REM) and Modified Endonuclease-Mediated (MEM) have recently been employed for enhancement of strains. One such example is the REM technique of genetic warfare and a general genetic alteration tool – the CRISPR-Cas9 system in humans; the Cas9 protein associated with CRISPR technology is a nuclease complexed with a guide RNA that leads it to a particular

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DNA base. This approach of genome manipulation where Cas9 protein is delivered into the cell with gRNA, has been considered a revolutionary technique in the field of biology and has several creative uses in bioenergy production [7].

The genomes of various microorganisms, especially bacteria, yeast, filamentous fungi, and algae, have been altered using this advanced tool known as CRISPR/Cas9 technology [8]. The Cas9 protein encoded by the developed CRISPR/Cas9 systems has made the method of gene editing more versatile and easier to use. In order to integrate all these advances to enhance the production of biofuels, the present study explores biofuel-producing organisms, particularly their CRISPR/Cas9 genome editing applications in microbes.

Microbial Role in Biofuel Production

The production of bioenergy derives from a number of microorganisms belonging to various categories, such as algae and yeast, filamentous fungi, and bacteria, among others. Possible microbial strains exist in all important steps of the bioconversion process, pretreatment, hydrolysis, and fermentation, which are all required in the production of biofuel. The case of fungi like *Pleurotus florida, Phanerochaete chrysosporium, and Ceriporiopsis subvermispora* for the value addition of microbes shares the raw material (celluloses and hemicelluloses) for further processes [9]. This is why it is known that bacteria of *Clostridium, Bacillus, Cellulomonas, Ruminococcus, Bacteroides, Erwinia, Thermomonospora, Acetovibrio, Streptomyces, and Microbispora* genera express hydrolytic enzyme activity. Enzymatic hydrolysis of lignocellulosic substrates has been reported to be carried out by a number of fungal genera, including *Trichoderma, Schizophyllum, Sclerotium, Aspergillus, Fusarium, and Humicola, Schizophillum, and Penicillium* [10]. It has been documented that several groups of bacteria, yeasts, and filamentous fungi can, under particular conditions, ferment sugars into ethanol. It is also reported that different types of microorganisms, among which only a few are utilized in the industry for their high aerobic capacity strains, produce ethanol to different degrees. This is mainly due to its robustness and appropriateness, allowing for the yeast S. cerevisiae to be the most popular yeast strain applied for industrial ethanol fermentation [11].

Genome Editing and CRISPR-Cas9

Genome editing, also known as gene editing, is a series of scientific techniques that enables the modification of an organism's DNA. These technologies make it possible to add, delete, or modify genetic material at particular locations in the genome. Genome engineering is a very successful method for introducing desirable features into a single organism. This method alters the native genome in a highly specific manner for the increased synthesis of a certain metabolite, changing the physiological features of a particular bacterium [12]. This method allows for the introduction, deletion, and up-or-down-regulation of a gene at a particular location within an organism. This procedure did not involve traditional gene separation, in vitro engineering, and subsequent re-transfer to the host cell to alter the physiological characteristics of that individual [13]. There are two methods for genome engineering: (i) REM engineering and (ii) MEM engineering. Genome engineering using the CRISPR/CRISPR-associated protein 9 (Cas9) system is REM-based [14].

In contrast, MEM-based genome engineering uses the Transcription Activator-Like Effector Nucleases (TALENs) system [15] and the Zinc Finger Nucleases (ZFNs) system [16]. All of these genome engineering techniques have completely transformed the biological sciences and allied disciplines of study. In contrast, the CRISPR/Cas9 system has emerged as a promising approach to address the limitations of ZFNs and TALENs. ZFNs and TALENs are limited due to a lack of effective delivery vehicles, off-target effects, toxicity, and low efficiency.

A well-known example is CRISPR-Cas9, which stands for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9. The CRISPR-Cas9 system has generated great attention in the scientific community because it is faster, less expensive, more accurate, and more potent than current genome editing approaches.

CRISPR-Cas9 was developed from a naturally occurring genome editing mechanism that bacteria use as an immunological response. When bacteria become infected with viruses, the viruses' DNA fragments are captured and inserted into the bacteria's own DNA in a certain pattern to form CRISPR arrays, which are a type of segment. In order to "remember" the viruses, the bacteria can use CRISPR arrays (or closely related ones). The bacteria create RNA segments from the CRISPR arrays that recognize and bind to particular sections of the viruses' DNA in the event that they retaliate and strike. In order to deactivate the virus, the bacteria use Cas9 or a related enzyme to split the DNA.

The two scientists, Doudna and Charpentier, have significantly contributed to understanding the CRISPR/Cas9 system's mechanism. They claimed that in order to defend themselves from phage attack, bacteria transcribe spacer sequences and palindromic repeats into a lengthy RNA molecule, which is then cut into pieces (referred to as crRNAs) by trans-activating RNA (tracrRNA) and protein Cas9 [17]. Subsequently, it was found that tracrRNA and crRNA could be combined to form a single guide RNA (sgRNA), which could then be organized into a powerful tool for locating and cleaving specific DNA sections with the help of the Cas9 nuclease. CrRNA carries a sequence that combines with tracrRNA to form a hairpin loop-like structure, which enables the Cas9 enzyme to cleave DNA sequence by recognizing crRNA as a guide. As a result, it provides the capability of gene editing. Either non-homologous end-joining (NHEJ) or homology-directed repair is used to reunite the cleaved DNA (HDR). A specific DNA sequence is inserted using a DNA repair template to produce the desired outcome [18].

Genetic Engineering of Microbial Cells using CRISPR/Cas9 for Increased Biofuel Production

In the near future, new ground-breaking technologies are expected to enable researchers to fully use microbial cells for enhanced biofuel production [19]. To achieve these objectives, CRISPR/Cas9-mediated site-directed mutagenesis is required to enhance the metabolic capacity of the microbial cells. Recent studies have shown that CRISPR/Cas9-mediated genome engineering enhanced the biofuel tolerance, inhibitor tolerance, and thermotolerance of the microbial cells, as well as modifications in cellulose and hemicellulose, which can increase the generation of biofuels.

Biofuel Production using Genetically Engineered Algae

Researchers have been trying to find the best way to maximize biodiesel and bioethanol production from phototrophic algae since the 1970s. Algae are incredibly slimy, small organisms that, like phototrophic organisms (plants), depend on sunshine, carbon dioxide, and other vital nutrients like phosphorus and nitrogen for energy. A wide range of biocomponents, including protein, fat, carbohydrates, antioxidants, and pigments, are found in microalgae. Due to its biochemical components, microalgae can be used to make biofuels like bioethanol and biodiesel. With time, the algae stopped growing or replicating and became lethargic. Its biological system begins producing fatty lipids during this latent stage [20,21].

The main benefits of using algae to produce biofuels include low energy input requirements, the use of fertilizer (as in first-generation biofuel), the ability to produce bioethanol and biodiesel using microalgae, and the ability to combine microalgal biomass with wastewater treatment (second-generation biofuel) for increased biofuel production. Abiotic stress conditions such as salinity, nutrition depletion, and replenishment, heat stress, phytochromes, UV radiation, light, etc., are some of the difficulties with employing microalgae [23].

Numerous microalgal species, including *Chlorella sp. NC64A, Micromonas pusilla, Phaeodactylum tricornutum, Ostreococcus tauri, Nannochloropsis oceanica, Chlamydomonas reinhardtii, Volvox carteri, Aureococcus anophagefferens, Dunaliella salina, and Botryococcus braunii UTEX 572,* have been sequenced [20,23]. Scientists used transgenic microalgae that were altered by targeting accountable genes using forward or reverse techniques and screening for random knockout libraries to address the problems associated with abiotic stress [20]. Scientists studied the 20 factors affecting transcription to control the generation of lipids in algae. By using the CRISPR-Cas system, 18 transcription factors were eliminated, which caused the algae to double their lipid production [23].

A team of researchers from the University of California has used CRISPR to weaken particular genes, increasing the production of lipids in algae by twofold. Two companies, Synthetic Genomics, and Exxon Mobil, are working together on studies to advance and improve the production of biofuels. By 2025, it is predicted that the research will be able to produce 10,000 barrels of algae used to make biofuels [23].

Biofuel Production using Genetically Engineered Yeast

One of the most common organisms used in manufacturing for the creation of alcohol and bread is yeast. Yeast gets stressed out throughout the bioproduction recycling process because it produces too many toxic proteins or metabolites. Pre-treatment of chemicals is one of the bioproduction processes used to speed up the conversion of cellulose into sugars; nevertheless, these chemicals are extremely poisonous to yeast [23]. The fermentation of sugars into biofuels is done with the help of yeast. When producing biofuels, scientists have used the CRISPR-Cas system to shield yeast against the hazardous and damaging effects of chemicals. In order to make yeast resistant (i.e., tolerant) to chemicals used in pre-treatments, scientists employed CRISPR to make two changes to the one gene responsible.

Biofuel Production using Genetically Engineered Bacteria

For the fermentation of biogas, acetogenic microorganisms like *Clostridium autoethanogenum* are used. These organisms are used in industries to manufacture ethanol on a large Scale. Prior to the development of CRISPR as a tool for gene editing, the commercial exploitation of this microbe was extremely difficult due to the lack of knowledge regarding the biochemical mechanisms of acetogenic microbes and the lack of a gene editing technique to investigate some particular genes responsible for the production of bioethanol. CRISPR has recently increased the effectiveness of gene knockout in these organisms.

Conclusion

The scientific community has developed reliable techniques for the efficient and sustainable production of an alternative fuel source due to the depletion of fossil fuel resources and growing environmental concerns**.** In order to increase the efficiency of microbial cells for the production of biofuel. The use of the CRISPR/Cas system to adapt these organisms to produce large quantities while overcoming the toxicity of chemicals and abiotic stress during commercial production is, therefore, a promising option to address these environmental issues, cut greenhouse gas emissions, and stop global warming. This study emphasizes the recent advancement in CRISPR/Cas9-mediated genome editing of microbial cells for the increased production of biofuels.

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