Article Id
AL04333

CRISPR/CAS MEDIATED ADVANCES IN GENE EDITING TOOLS IN BIOFUEL PRODUCTION

[1]Haralakal Keerthi Kumari*, [1]Swati and [1]Chethan, T

Email

keerthihk2283@gmail.com

[1]University of Agricultural Sciences, GKVK, Bangalore-65, India

One of the main causes of greenhouse gas (GHG) emissions is the consumption of fossil fuels. A potential substitute for fossil fuels in the transportation, power generating and heating sectors is biofuel. Fossil fuels can be combined with biofuel or utilized as a substitute for them. When biofuels are burned, substantially less greenhouse gas is released into the atmosphere than when traditional fuels are used (Lapuerta *et al*., 2017). Therefore, the use of biofuel can aid in the prevention of environmental degradation.

Biofuels can be divided into four categories based on the feedstock: first, second, third, and fourth generation biofuels (FGBs). The biofuels of the first generation, and second generation which are derived from sugar, starch and biofuel crops have the fears about competition for arable land and raw minerals Algal-based third-generation biofuels have garnered significant interest because of their high yield, ability to assimilate carbon dioxide (CO_2) and ease of processing. For improved biofuel production, fourth generation biofuel is derived from genetically modified algal biomasses and other microorganisms.

Among gene editing, tools the clustered regularly interspaced short palindromic repeats-CRISPR-associated proteins (CRISPR-Cas) system, an RNA-guided immune system in bacteria and archaea, is the most modern weapon in the genetic engineering armory. The manipulation of several aspects of the production of biofuels is made easier by this straightforward but accurate tool.

Basic Components of CRISPR/Cas and Their Function

A single-guide RNA (sgRNA) and an RNA-guided Cas9 endonuclease are the two primary parts of the CRISPR-Cas9 system. Each of the two nuclease domains that the Cas9 protein comprises, HNH and RuvC, cleaves one strand of the target double-stranded DNA.

The combination of tracrRNA and crRNA is simplified in to single-guide RNAs (sgRNAs). Together, the sgRNA and Cas9 nuclease generate the Cas9 ribonucleoprotein (RNP), which can attach to and break the targeted DNA. Moreover, the binding of the Cas9 protein to the target DNA requires the presence of a protospacer adjacent motif (PAM) sequence (Ran *et al.*,2013).

Limitations in Biofuel Production

a) Feedback inhibition
b) Affects the survivability of microorganisms.
c) Temperature fluctuations
d) Selection of a single microbe for targeted co-fermentation
e) less capacity to use substrates.
f) Availability of less feed stock

CRISPR/CAS Mediated Engineering Strategies in Biofuel Production

1. Strain Improvement: CRISPR/Cas9-mediated site-directed mutagenesis is required to achieve high level of biofuel production by enhancing the metabolic performance of the microbial cells.
2. Metabolic Engineering: Using genome editing technologies, targeted changes to certain amino acids can modify the specificities of cellulases.
3. Gene Knockouts and Knock-ins: CRISPRi-based knockdown is inducible and reversible, which enables the temporal and dynamic regulation of interested genes.
4. Gene silencing: Tuning gene repression is helpful because some genes are extremely sensitive to knockdown and many genes of interest are expected to be expressed under tight control.
5. Gene regulation: Mechanistically, dCas enzymes repress transcription by preventing the binding of RNA polymerase or, if targeted to open reading frames, by interfering with transcription elongation.

Advances in CRISPR/CAS Tools to Address the Challenges

1. a. Cas9n-mediated single-nick generation and HR:

Homology-directed repair is another way to fix DNA lesions when a homologous template is present. In the study performed by Xu *et al*. (2015) to mutate the *pyrF* gene by small

DNA deletion, a homologous donor template with a length of 2 kb carrying a 23-bp deletion in the middle was designed and cloned it into pCas9-pyrF and pCas9n-pyrF, generating all-in-one pCas9-pyrF donor and pCas9n-pyrF-donor plasmids.

b. CRISPR-Cas12

A compact enzyme called C2c1 facilitates packing into vectors, which may contribute to the simpler and quicker transfection process. Cpf1(Cas12) just needs crRNA, but C2c1 also needs a tracrRNA, in contrast to Cas9. The Cpf1 system's growing number of guide-RNAs and small size contribute to improved flexibility and expand its use in multiplex genome editing. Here is an example where the Cas 12a enzyme is used to cut/delete the highly expressed gene that is *pyrF* responsible for orotate phosphoribosyl transferase in *Clostridium ljungdahlii.* Ran *et al*., (2019) have succeeded in deletion of that gene and conclude that CRISPR/Cas12 can be used as a novel tool in modifying *Clostridium ljungdahlii,* which is a good producer of biofuel.

2. **Base Editing:** Base editing is a precise gene-editing technique that allows the direct conversion of one DNA base pair into another without inducing double-strand breaks. This method can reduce off-target effects and has the potential to be more accurate than traditional CRISPR-Cas methods.

3. **Prime Editing:** Prime editing is a relatively new technique that allows for the precise editing of specific DNA sequences without causing double-strand breaks. It uses a catalytically impaired Cas9 protein and a specially engineered reverse transcriptase to introduce changes at the target site.

4. **CRISPR Interference (CRISPRi) and CRISPR Activation (CRISPRa):** enable the regulation of gene expression without making permanent changes to the DNA sequence. These tools can be used to fine-tune the expression of genes involved in biofuel production pathways. The RuvC and HNH nuclease domains are present in the Cas9 protein. Double-stranded DNA is cleaved by these two regions. The Cas9 protein loses its endonuclease activity when the H840A and D10A mutations are incorporated; this is known as deactivated Cas9 (dCas9). While it is not able to cut DNA, the dCas9 protein is a great RNA-guided DNA binding protein. After being tested in *E. coli* for its capacity to selectively suppress gene expression, the CRISPR/dCas9 system gave rise to the CRISPR interference (CRISPRi) method.

5. **Multiplexed CRISPR Systems:** simultaneous editing of multiple genes, which is crucial for optimizing biofuel production pathways. Researchers are developing CRISPR tools that allow the editing of multiple genes in a coordinated manner.

6. **Synthetic Biology Approaches:** CRISPR technologies are often combined with synthetic biology approaches to design and engineer microorganisms for enhanced biofuel production. This includes the construction of synthetic gene circuits and the optimization of metabolic pathways.

7. **High-Throughput Screening:** used to identify genes and pathways that can be targeted to improve biofuel production efficiency. This involves systematically editing genes in a high-throughput manner to assess their impact on the desired traits.

How Advanced Tools Increase Precision and Efficiency

- Enables targeted editing without generating double-stranded DNA breaks.
- Avoids undesirable indels.
- Helps to overcome Cas9 toxicity.
- Truncation of metabolic pathways
- Precise insertions of nucleotides
- Gene inactivation without knocking out.

Conclusion

- To deal with the current problem of global energy demand, effective measures are required.
- The CRISPR-Cas gene editing technique can boost non model organism's biofuel output.
- Gene suppression or inactivation using CRISPRi redirects metabolic flow to biofuel production pathways.
- CRISPR-Cas genome editing may improve substrate utilization, fermentation inhibitor tolerance and biomass breakdown cellulases and hemicellulases.

References

Alok, A., Sandhya, D., Jogam, P., Rodrigues, V., Bhati, K. K., Sharma, H. AND Kumar, J. (2020). The rise of the CRISPR/Cpf1 system for efficient genome editing in plants. *Frontiers of plant science*, 11,264-268.

Lapuerta, M., Barba, J., Sediako, A.D., Kholghy, M.R. AND Thomson, M. J., 2017, Morphological analysis of soot agglomerates from biodiesel surrogates in a coflow burner. *Journal of aerosol science*, 111:65–74.

Ran, F. A., Hsu, P. D., Wright, J., Agarwala, V. AND Scott, D. A., 2013, Genome engineering using the CRISPR-Cas9 system. *Nat. Protoc.*, 8: 2281–2308.

Ran, Z., Yanqiang, L., Huan, Z., Changsheng, C., Jin, W., Weihong, J. AND Yang, G., 2019, CRISPR-Cas12a-mediated gene deletion and regulation in *Clostridium ljungdahlii* and its application in carbon flux redirection in synthesis gas fermentation. *ACS Synthetic Biology*, 8(10): 2270–2279

Xu, T., Li, Y., Shi, Z., Hemme, C. L., Li, Y, Zhu, Y., Van Nostrand, J. D., He, Z., Zhou, J. 2015. Efficient genome editing in *Clostridium cellulolyticum* via CRISPR-Cas9 nickase. *Applied and Environmental Microbiology*, 81:4423–4431.