ers GENOMICS

Your connection to CRISPR/Cas9

GENOME MODIFICATION:

A NEW PARADIGM FOR BIOFUEL

There is a huge demand for fuel due to its consumption in transportation, energy generation and for industrial purposes. Recently, the demand for petroleum-based fuel has resulted in a number of economic and environmental concerns and attentive efforts are needed to encourage the emerging alternative fuels (Gowen and Fong, 2011). Biofuels, produced from biomass, render an environmentally clement and cost-efficient solution for fossil fuel impoverishment. These substitutive and inexhaustible sources of fuel as biodiesel and bioethanol have captivated increasing attention from industry, decision-makers, and scientists because of their valuable advantages (Xing et al., 2012).

BIOFUEL

Biofuel is the combustible fuel obtained from biomass and are characterised as either first or second generation biofuels. First generation biofuels are unprocessed organic materials such as wood chips & pellets used for cooking or generating electricity. Second generation biofuels are processed and liquified and include ethanol and biodiesel.

Biofuel is produced either through a fermentation process or a chemical reaction. The type of biofuel produced depends on the processing of the biomass. For example, ethanol is a by-product of the fermentation of plant sugars whereas biodiesel is produced as a result of a chemical reaction between greases with alcohol.

ROLE OF MICROORGANISMS IN MODERN BIOFUEL PRODUCTION:

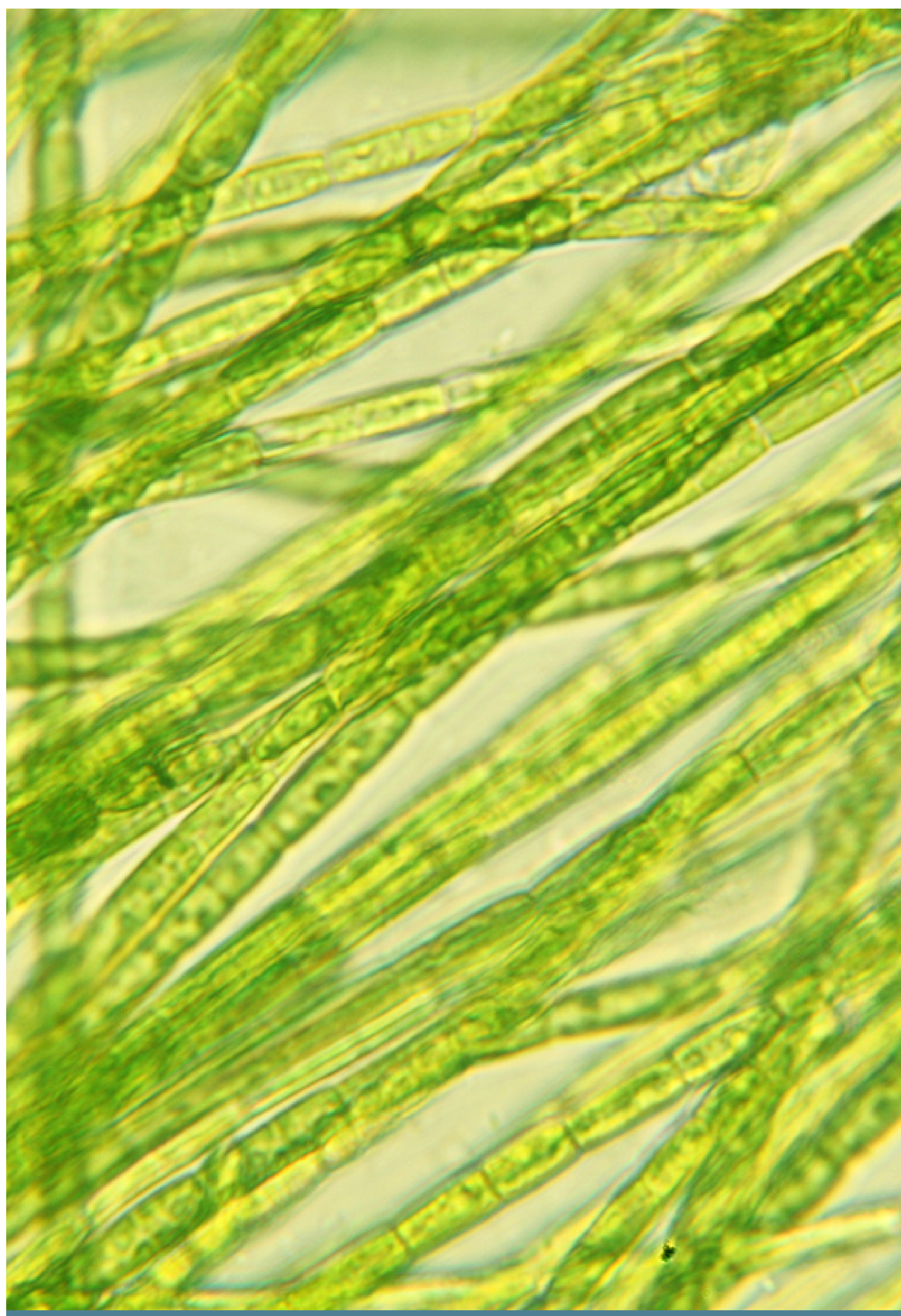
The microorganisms of various groups such as bacteria, yeast, and filamentous fungi play a pivotal role in the production of biofuels. All major stages of bioconversion including pre-treatment, hydrolysis, and fermentation can involve microbial strains for efficient biofuel production.

Pre-treatment:
Microbe-mediated pre-treatment of feedstock results in the disintegration of structural components and exposes the chemical constituents (i.e., celluloses and hemicellulases) for downstream processing (Chandel et al., 2013; Zabed et al., 2016).

Hydrolysis:
Various microbe enzymes (such as cellulases, hemicellulases and many more) are responsible for enzymatic hydrolysis and are utilized in hydrolysate production for the fermentation process (Sun and Cheng, 2002). Various bacterial and fungal genera have been reported to produce these hydrolytic enzymes. Several bacterial species (such as Clostridium, Bacillus, Cellulomonas & Ruminococcus have been reported used for hydrolytic enzyme production.

Fermentation:
Similarly, various microorganisms including bacteria, yeast, and filamentous fungi have the potential to convert monomeric sugars into ethanol under anaerobic conditions. It has been reported that different microorganisms have some potential for ethanol production but only a few microbial strains with high ethanol production potential are utilized on a commercial scale. The yeast S. cerevisiae is most commonly employed for ethanol production on an industrial scale due to its robust and well-suited nature (Galbe and Zacchi, 2002; Sarris and Papanikolaou, 2016).

GENOME MODIFICATION



Genome editing is an efficient tool to introduce desirable traits into a single organism. This process alters the native genome in a very precise manner to change the physiological characteristics of an individual microbe for the enhanced production of a particular metabolite (Ulaganathan et al., 2017). CRISPR is a remarkable genome editing tool that has been revolutionizing research in every industry, and bioenergy is no exception. By using this approach, a gene can be introduced, deleted, modified, or simply up or down regulated within an organism.

CRISPR USED TO GENERATE BIOFUEL FROM ALGAE

A broad and varied collection of photosynthetic eukaryotic organisms are collectively referred to as algae. Similar to plants, these creatures also require carbon dioxide, sunlight, and other nutrients like nitrogen and phosphorus to exist. They begin to conserve energy if you put them on an restricted nutrition and deprive them of vital nutrients. Instead of expanding and proliferating, they slow down, enter a latent state, and begin to accumulate fatty lipids.
Scientists have been working to double the amount of biodiesel that can be produced from phototropic algae, and CRISPR has made this possible.

Using CRISPR to modify the genes, a group of scientists from California have discovered a method to double the lipid production in algae. Twenty transcription factors that control lipid synthesis in algae have been discovered by researchers. They were able to double the synthesis of lipids in algae by using CRISPR to eliminate 18 of these. (Ajjawi et al. 2017)

CRISPR PROTECTS YEAST FROM DAMAGE IN BIOFUEL PRODUCTION

Powerful model organisms like yeast are frequently utilized in industrial production. However, during the bioproduction cycle, the stress of excessive protein or metabolite creation affects the organisms. Yeast may also be harmed by pre-treatment chemicals that are intended to hasten the breakdown of cellulose into sugars. (Higgins et al., 2018)

In the fermentation of sugars into biofuels, yeast is crucial. Now, using CRISPR, scientists have discovered a method to shield yeast from harm throughout the biofuel manufacturing process. A single gene was altered twice by the research team, making yeast resistant to certain pre-treatment chemicals. (Higgins et al., 2018)

CRISPR ACTS AS A GENE-EDITING TOOL FOR ACETOGENIC BACTERIA

For gas fermentation, the acetogenic bacterium Clostridium autoethanogenum is commonly employed for its efficiency. This microscopic bacterium can also be utilized to produce ethanol on a large scale. However, because scientists only have a basic understanding of acetogenic bacteria and due to to few efficient genetic tools and high-throughput engineering platforms available, commercializing them has been difficult. CRISPR has recently demonstrated enhanced gene-deletion effectiveness in this bacteria, making it a practical tool for designing this organism. (Xia et al., 2020)

CONCLUDING REMARKS

Greenhouse gases are released during the combustion of fossil fuels and is one of the leading causal factors of global warming. An alternate source of energy is desperately needed and energy biotechnology offers a promising solution. Currently, bioenergy can meet only 10% of the world's energy demand. Expanded use of energy biotechnology is needed to produce commercially viable amounts biofuels and CRISPR has already proven that it can help. With further application of CRISPR technology and more and more companies investing in CRISPR-based bioenergy research, significant and meaningful progress toward ending the world's energy crisis is within our reach.

REFERENCES:

Ajjawi, I. et al. (2017) "Lipid production in Nannochloropsis Gaditana is doubled by decreasing expression of a single transcriptional regulator," Nature Biotechnology, 35(7), pp. 647–652. Available at: https://doi.org/10.1038/nbt.3865.

Chandel, A.K. et al. (2013) "Ultra-structural mapping of sugarcane bagasse after oxalic acid fiber expansion (OAFEX) and ethanol production by Candida Shehatae and saccharomyces cerevisiae," Biotechnology for Biofuels, 6(1), p. 4. Available at: https://doi.org/10.1186/1754-6834-6-4.

Galbe, M. and Zacchi, G. (2002) "A review of the production of ethanol from Softwood," Applied Microbiology and Biotechnology, 59(6), pp. 618–628. Available at: https://doi.org/10.1007/s00253-002-1058-9.

Gowen, C.M. and Fong, S.S. (2011) "Applications of systems biology towards microbial fuel production," Trends in Microbiology, 19(10), pp. 516–524. Available at: https://doi.org/10.1016/j.tim.2011.07.005.

Higgins, D.A. et al. (2018) "Natural variation in the multidrug efflux pump sge1 underlies ionic liquid tolerance in yeast," Genetics, 210(1), pp. 219–234. Available at: https://doi.org/10.1534/genetics.118.301161.

Sarris, D. and Papanikolaou, S. (2016) "Biotechnological production of ethanol: Biochemistry, processes and technologies," Engineering in Life Sciences, 16(4), pp. 307–329. Available at: https://doi.org/10.1002/elsc.201400199.

Sun, Y. and Cheng, J. (2002) "Hydrolysis of lignocellulosic materials for ethanol production: A Review," Bioresource Technology, 83(1), pp. 1–11. Available at: https://doi.org/10.1016/s0960-8524(01)00212-7.

Ulaganathan, K. et al. (2017) "Genome engineering for breaking barriers in lignocellulosic bioethanol production," Renewable and Sustainable Energy Reviews, 74, pp. 1080–1107. Available at: https://doi.org/10.1016/j.rser.2017.01.028.

Xia, P.-F. et al. (2020) "Reprogramming acetogenic bacteria with CRISPR-targeted base editing via deamination," ACS Synthetic Biology, 9(8), pp. 2162–2171. Available at: https://doi.org/10.1021/acssynbio.0c00226.

You, Y. et al. (2017) "Enhancement of ethanol production from green liquor–ethanol-pretreated sugarcane bagasse by glucose–xylose cofermentation at high solid loadings with mixed saccharomyces cerevisiae strains," Biotechnology for Biofuels, 10(1). Available at: https://doi.org/10.1186/s13068-017-0771-7.

Zabed, H. et al. (2016) "Fuel ethanol production from lignocellulosic biomass: An overview on feedstocks and technological approaches," Renewable and Sustainable Energy Reviews, 66, pp. 751–774. Available at: https://doi.org/10.1016/j.rser.2016.08.038.