Review Article

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Advancements in Strain Development and Genetic Engineering for Cellulase Production: A Systematic Review

Navneet Sharma*, Deepali Aggarwal and Sheelendra Mangal Bhatt
School of Life Science and Technology, IIMT University, Meerut, UP, India
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*navneet.sh15@gmail.com

ABSTRACT

Strains, defined as subtypes or genetic variants of microorganisms, are selectively developed at an industrial scale to enhance the production of cellulases and other metabolites. These strains, bacterial or fungal, are genetically engineered to improve traits such as rapid growth, genetic stability, productivity, and ease of cultivation. Genetic modifications are typically aimed at increasing or altering gene expression to maximize output. Cellulases are enzymes that catalyze the breakdown of cellulose into simpler sugars, enabling its conversion into biofuels and other value-added products. In particular, bacterial cellulose produced by Acetobacteraceae is recognized for its purity, biocompatibility, and natural origin, making it highly suitable for applications in material science. To further enhance cellulase yields, researchers identify and utilize strains capable of high productivity under low-nitrogen conditions. Recombinant DNA technology is employed to sequence genomes, introduce genetic constructs, and express target proteins in host cells. In fungal systems, advanced approaches such as the Reconstruction of Expression Regulatory Network (REXRN) have been applied to fine-tune gene expression and enhance the production of cellulase enzymes involved in cellulose degradation. Together, these strategies support the development of high-performance microbial strains for industrial biotechnology applications.

Keywords: Strain; Biofuel; Cellulase; Biomass; Strain selection; Genetic engineering.

1. INTRODUCTION

Cellulase is an enzyme used in biofuel production due to its ability to catalyze the breakdown of cellulose or cellulose-based materials (Kubicek *et al.* 1993). Biofuel is a versatile and eco-friendly energy source that has obtained widespread recognition and adoption in recent years. Biofuels are preferred over fossil fuels due to their tendency to utilize or decompose organic matter, such as crops, agricultural residues, or cellulosic wastes (Lübeck and Lübeck 2018). One of the key advantages of biofuels is their renewable nature. Unlike fossil fuels, which are finite and non-renewable, biofuels can be continually produced as long as there is a steady supply of organic matter. This makes them a valuable resource in the quest for long-term energy sustainability (Shoji *et al.* 2007). Furthermore, biofuels are considered more environment-friendly compared to fossil fuels. When burned, biofuels release significantly less quantity of carbon dioxide (CO2) and other greenhouse gases into the atmosphere. Reducing carbon emissions helps mitigate climate change and lowers overall emissions across many industries (Tubb and Seba, 2021).

Biofuels have diverse and wide-ranging applications. In the transportation sector, they can be used as a direct replacement for gasoline and diesel fuels. They can be blended with conventional fuels or used in pure form, depending on the engine and infrastructure compatibility (Wang *et al.* 2020) making them an attractive option for reducing emissions from cars, trucks, and even airplanes. In addition, biofuels have the potential to generate electricity and heat homes. Biomass power plants can convert organic matter into biogas or bio-oil, which can then be used to produce electricity through combustion or other conversion processes. Additionally, biofuels can be used in residential heating systems, providing a renewable and eco-friendly alternative to heating systems comprised of fossil fuels.

The production and use of biofuels have gained significant attention and investment in recent years (Bergquist *et al.* 2002). Governments, industries, and research institutions are actively exploring ways to increase the efficiency and utility of biofuel production processes, which include advancements in feedstock cultivation, conversion technologies, and distribution infrastructure.

However, it is essential to note that the production of biofuels is not without challenges. The cultivation of crops for biofuel production can compete with food production and lead to deforestation or

increased water usage (Hansen *et al*. 2021). Therefore, it is crucial to ensure that biofuel production is carried out sustainably and responsibly, considering environmental, social, and economic factors. Hence, biofuels offer a renewable and environmentally friendly alternative to fossil fuels. With their lower carbon emissions and versatile applications, biofuels have the potential to play a significant role in reducing greenhouse gas emissions and combating climate change.

2. LITERATURE REVIEW

2.1 Importance of Cellulase

Cellulose is abundant in various feedstocks, such as found in agricultural residues, crop residues, plants, and animal waste, making it a beneficial source for biofuel production. However, due to the complex and complicated structure of cellulose, it is very difficult to convert it into usable sugars for fermentation. This is where cellulase comes in. It acts as a catalyst, breaking down the cellulose into simpler sugars, such as glucose and xylose, through the hydrolysis process. These sugars can then be utilized by microorganisms, e.g., yeast or bacteria, to produce biofuels like ethanol. The hydrolytic conversion of cellulose into sugars is very important for the economic viability of biofuel production, as it determines the overall productivity and cost-effectiveness of the process. One of the advantages of using cellulase in biofuel production is its high efficiency in converting cellulose into usable sugars. Cellulose is converted into its constituent sugars due to the hydrolytic action of the cellulase enzyme. This efficiency allows for a high productivity of sugars, which can then be utilized in biofuels. Furthermore, cellulase is compatible with various feedstocks, making it a versatile enzyme in biofuel production. It can be used with different plant materials, including agricultural residues, forestry, or animal wastes. This flexibility in feedstock selection allows for the usage of multiple biomass resources, reducing the dependence on specific crops and promoting sustainable biofuel production (Bischof *et al.* 2016).

Another significant advantage of cellulase is its renewable nature. It can be produced from renewable sources, such as microorganisms or genetically modified plants, making it an environmentally friendly option for biofuel production. By utilizing renewable sources for cellulase production, the overall carbon footprint of biofuel production can be reduced. Hence, cellulase and nano-cellulase play an important role in the production of biofuels by efficiently converting cellulose into usable sugars. Its compatibility with various feedstocks and renewable nature makes it an attractive option for sustainable biofuel production. By utilizing cellulase, biofuel production processes can become more efficient and environmentally friendly, contributing to the development of a greener energy sector (Malik and Javed, 2024).

2.2 Application of Cellulase

Cellulase, an enzyme that catalyzes the breakdown of cellulose into smaller sugar molecules, finds wide-ranging applications in various industries. One prominent application of cellulose is in the biofuel industry, where it is utilized to convert cellulose-rich biomass into bio-ethanol. The most useful strategy is the enzymatic hydrolysis of cellulosic biomass, which gives more specificity, stereoselectivity, and greater conversion (Mumtaz *et al.* 2022) This enzymatic process, known as cellulolysis, enables the efficient extraction of sugars from cellulose, which can then be utilized and distilled to manufacture bio-ethanol. Another significant application of cellulose is in the textile industry, where it is employed to enhance the softness and texture of fabrics. Cellulase enzyme is used in the process of bio-finishing, which involves treating textiles with cellulose to remove surface fuzz and protruding fibers. This treatment results in smoother and more comfortable fabrics, improving the overall quality of the end product. Furthermore, cellulase has proven to be very valuable for the production of animal feedstocks (Sun and Su, 2019). By breaking down cellulose present in plant-based feed ingredients, cellulase enzymes aid in the release of nutrients that are otherwise inaccessible to animals. This enhances the digestibility and nutritional value of the feed, leading to improved animal health and productivity.

In summary, the application of cellulase extends beyond the biofuel, textile, and animal feed industries (Maheshwari *et al.* 2000) Its versatility and ability to break down cellulose make it a valuable tool in various other sectors, including food and beverage, paper and pulp, and waste management. As research and technology continue to advance, the potential applications of cellulase, presented in Table 1, are likely to expand, further contributing to sustainable and efficient processes in numerous fields.

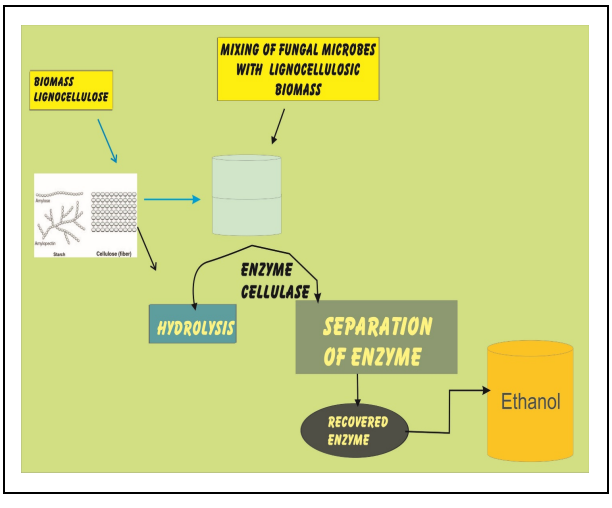


Fig. 1: Conversion of lignocellulose biomass into cellulase (Arya *et al.* 2021)

Table 1. Industrial application of cellulase

Industrial Application	Function	Reference
Biofuel Industry	Cellulase is utilized to convert cellulose-rich biomass into bio-ethanol.	(Rajendran *et al.* 2015)
Textile Industry	Treating textiles with cellulose to remove surface fuzz and protruding fibers.	(Dong *et al.* 2020)
Food-making and the Alcoholic Beverage Industry	Enhancing the texture and durability, and stability of certain food products.	(Jan *et al.* 2024)
The Paper and Pulp Industry	Separating the ink and other contaminants from the fibers, and allowing the production of high-quality recycled paper.	(Vinković *et al.* 2023)
Pharmaceutical Industry	In drug delivery systems, as well as in the treatment of certain medical conditions, such as cellulitis, a bacterial skin infection.	(Khan *et al.* 2024)
Biofuel Industry	Nanoparticle-based biofuel production.	(Mumtaz *et al.* 2022)
Fertilizer Industry	Synergistic effect of alginate lyase and cellulase on seaweed bioconversion.	(Sun and Su, 2019)

Cellulase finds its use in the food-making and alcoholic beverage industry to enhance the texture, durability, and stability of certain food products. For example, it can be added to fruit juices to decompose the pectin present in the fruit, resulting in a more tasty juice. Cellulase can also be utilized in the production of wine and beer to enhance the extraction of flavors and improve the clarity of the final product. In the paper and pulp industry, it is used in the process of paper recycling; by breaking down the cellulose fibers in waste paper, cellulase enzymes help to separate the ink and other contaminants from the fibers, allowing for the production of high-quality recycled paper. Cellulase also plays a role in waste management, for the decomposition of organic and inorganic wastes (Selbmann *et al.* 2013).

By breaking down cellulose-rich materials like agricultural waste, food processing waste, and yard waste, cellulase enzymes aid in the decomposition process, accelerating the breakdown of organic matter and reducing the volume of waste. In addition, cellulase has potential applications in the pharmaceutical and medical fields. Research is being conducted to explore the use of cellulase in drug delivery systems, as well as in the treatment of certain medical conditions such as cellulitis, a bacterial skin infection.

In a nutshell, cellulase is a useful enzyme with versatile applications in many industries. Its ability to break down cellulose into smaller sugar molecules makes it a valuable tool for biofuel production, textile enhancement, animal feed production, food and beverage processing, paper recycling, waste management, and pharmaceutical and medical applications (Arntzen *et al.* 2020).

2.3 Strain Selection for High Cellulase Production

Differentiating the strain selection process to enhance cellulase production is of paramount importance for several reasons. Firstly, cellulase materials are derived from plant biomass, such as agricultural residues, plant and animal waste materials, and forestry residues. These materials have immense potential as commercial alternatives to fossil fuels and traditional materials like plastics. However, the efficient conversion of plant biomass into cellulase-based materials involves the use of microorganisms, specifically cellulolytic strains, which can effectively break down cellulose into its constituent sugars. Filamentous fungi can also be used as host strains to produce cellulose (Xiang *et al.* 2023). The selection of the right strain is crucial because different microorganisms possess varying capabilities to degrade cellulose. Some strains may have higher cellulolytic activity, while others may have better tolerance to harsh conditions or inhibitors present in the biomass. By differentiating the strain selection process, researchers can identify and isolate strains that exhibit superior cellulolytic activity, robustness, and adaptability to specific feedstock or process conditions. Secondly, the strain selection process can also be employed to enhance the yield of specific cellulase-based materials having desired properties (Brar *et al.* 2019). For example, certain strains may produce cellulases or other enzymes that are more efficient in breaking down cellulose into sugars, leading to higher yields of fermentable sugars for biofuel

production. Other strains may produce enzymes with unique characteristics such as thermo-stability or tolerance to high concentrations of inhibitors, which can be advantageous in industrial-scale processes. A strain must be genetically stable, metabolically capable, less prone to mutation, and capable of growing under specific pH and temperature conditions. It should not be degraded by the presence of proteolytic enzymes (Roth *et al.* 2023).

Furthermore, strain selection can also be employed to improve the overall efficiency and application of cellulase material production. By selecting strains that have faster growth rates or higher productivity, the time and resources required for large-scale production can be significantly reduced. This can lead to lower production costs and make cellulase materials more competitive with traditional materials. Identifying and isolating strains with superior cellulolytic activity, adaptability, and desired properties can contribute to the development of a more economic and useful process (Ntana *et al.* 2020). In addition, the strain selection process can also play an important role in ensuring the sustainability of cellulase material production. By selecting strains that have a less contaminating impact, such as those that produce fewer by-products or require less energy for growth and metabolism, the overall carbon emission of the production process can be minimized. This is particularly important in the process of climate change and reducing greenhouse gas emissions. Moreover, differentiating strain selection processes can also contribute to the development of a diverse and resilient bio-economy. By exploring and utilizing a wide range of microorganisms, researchers can tap into the vast genetic diversity present in nature and discover novel strains with unique capabilities. This not only enhances the production of cellulase materials but also opens up opportunities for the discovery of new biological products using biotechnological applications. Precisely, the strain selection process plays a crucial role in enhancing the production of cellulase materials.

2.4 Strain Development

The development of strain for the production of high yields of cellulase-based material has been a subject of significant interest and research in recent years. Researchers have recognized the importance of developing strains that are capable of efficiently producing large quantities of cellulase material. These strains are specifically engineered to optimize the production process and increase the yield of cellulase material. By focusing on strain development, scientists aim to overcome the limitations and challenges associated with traditional methods of cellulase material production. Strain development involves identifying and modifying specific genes and metabolic pathways in the strain to enhance its ability to convert plant biomass into cellulose material. By improving the strain's ability to degrade complex carbohydrates, e.g., cellulose and hemicellulose, into simpler sugars, scientists can significantly increase the yield of cellulase material. Furthermore, strain development focuses on improving the strain's tolerance to harsh conditions and inhibitory compounds that are present in the production process. This is crucial as it allows effective conversion of plant biomass into cellulose-based material, even in challenging environments. By enhancing the strain's capability to work at high temperatures, acidic pH, and toxic byproducts, researchers can ensure a more robust and reliable production process. Cellulase material can be used as a feedstock for biofuel production, providing a greener alternative to traditional fossil fuels (Daba *et al.* 2021). In addition, it can be used in bio-refineries to give a wide range of valuable products that include organic chemicals, materials, and biological polymers. Through their efforts, researchers are striving to achieve higher yields of cellulase material, ultimately leading to a more economical and environment-friendly approach (Hassan *et al.* 2019).

2.5 High Cellulase Production

Strain enhancement involves modifying the genetic makeup of the microorganisms to augment their ability to degrade and convert cellulose biomass into desired products. One approach to strain enhancement is genetic engineering, which involves introducing specific genes into the microorganisms to enhance their cellulolytic activity (Méndez-Líter *et al.* 2021). For example, genes encoding cellulases, enzymes that break down cellulose, can be introduced to increase the efficiency of biomass degradation. Similarly, genes encoding enzymes involved in the various metabolic pathways can be used to enhance the yield of desired products (Amara *et al.* 2023). Another approach to strain enhancement is directed evolution, which involves the growth of microorganisms in a controlled environment and selecting them for desirable characteristic traits over multiple generations. This process can lead to the emergence of strains with improved cellulolytic activity and product yield. Directed evolution can be achieved through techniques such as mutagenesis, where the microorganisms are exposed to mutagens to induce genetic variations, and screening or selection, where the strains with desired traits are identified and isolated. In addition to genetic engineering and directed evolution, other strategies can be employed to enhance the productivity of cellulase (Nevalainen *et al.* 2005). One approach is to optimize the growth conditions, such as temperature, pH, and nutrient availability, to create an environment conducive to the microorganisms' growth and productivity. Furthermore, process engineering techniques, such as reactor design and optimization, can be employed to improve the efficiency of biomass conversion and product recovery. Overall, enhancing the productivity of cellulase through strain enhancement is a

promising avenue for increasing the production of biofuels and various biochemical products (Kolasa *et al.* 2014). By harnessing the power of genetic engineering, directed evolution, and process optimization, researchers and engineers can gain the importance of cellulase biomass as a renewable and environmentally friendly resource. Fermentation optimization and reactor design can be used to improve the efficiency of biomass conversion and product recovery.

Through a process of continuous cultivation and selection, microorganisms can evolve to adapt to specific growth conditions and improve their productivity. In fungi, the presence of the enzyme β-glycosidase that acts especially on the cellobiose disaccharides produces glucose (Periyasamy *et al.* 2023). This can involve subjecting the microorganisms to high cellulose concentrations, harsh environmental conditions, or other stressors to select strains with improved cellulose utilization and product formation (Lopes *et al.* 2018). By combining different microorganisms in a co-culture system, researchers can take advantage of their complementary metabolic capabilities to enhance the production of cellulase materials. For example, one microorganism may specialize in cellulose degradation, while another may be more efficient in converting the resulting sugars into target products (Raimbault 1998). By improving the efficiency and yield of microorganisms involved in cellulose utilization, researchers aim to unlock the full potential of cellulase materials as sustainable alternatives to fossil-based products (Kalyanpur, 2002).

3. GENETIC ENGINEERING OF FUNGAL/MICROBIAL CELLS FOR HIGH CELLULASE PRODUCTION

Genetic engineering involves the careful manipulation and modification of an organism's genes, allowing scientists to create new genetic combinations that may not occur naturally. Genetic manipulation of fungal or microbial cells to enhance cellulase production has become a major area of research in the field of biotechnology and human welfare. Certain discoveries and inventions have been made that use fungi for high cellulose production (Barzee *et al.* 2021). By modifying the genetic makeup of these cells, scientists aim to optimize their ability to produce cellulose, ultimately increasing efficiency and yield (Labrou 2021). The process of genetic engineering involves introducing specific genes or altering existing ones in fungal or microbial cells (Mayer *et al*. 2020).

These genetic modifications can enhance the enzymes responsible for cellulose production, improve the metabolic pathways involved in cellulose synthesis, or increase the overall efficiency of cellulose production. By manipulating the genetic makeup of these cells, scientists can fine-tune their ability to produce cellulose, leading to higher yields and improved quality (Meyer *et al.* 2021). Genetic manipulation of fungal or microbial cells offers a promising solution by enhancing their ability to break down and convert cellulose into biofuels. In addition to biofuels, genetic manipulation of fungal or microbial cells for enhanced cellulase production can also lead to the production of other useful products derived from cellulose. For example, in the production of bio-plastics, cellulose is used as a substrate, which is easily degradable, causing less pollution in the environment as compared to traditional plastics.

Genetic manipulation of fungal or microbial cells to enhance cellulase production holds great potential for advancing the development of sustainable and renewable sources of biofuels as well as other valuable products derived from cellulose (Agarwal *et al.* 2021). Genetic engineering is used to genetically modify microorganisms to produce large amounts of metabolites, which can range from therapeutic proteins to highly specialized ones. The genetic composition of the organisms is the most crucial piece of information that is needed. Selecting the target areas for genetic modifications is made simpler by understanding the functions and sequencing of the entire genome. Effective techniques to deliver guided genetic modifications into an organism are needed for targeted strain engineering, which tends to increase the production of cellulose in fungi by metabolic engineering methods. The primary obstacle to obtaining a sufficient number of transformants through homologous integration or expression cassette deletion has long been the relatively low efficiency of gene targeting. This is primarily because of the complexity brought about by the introns present in the corresponding genes, as well as the complexity due to glycosylation.

It is observed that filamentous fungi produce a wide range of metabolites in sizable amounts. Their capacity to proliferate on less expensive substrates has rendered them possible suppliers of metabolites for use in industrial settings. Plasmids that incorporate into the genomes of the majority of filamentous fungi are used to convert them, giving the fungal transformants stability. Therefore, there is a great deal of potential to use filamentous fungi as hosts for recombinant DNA. However, non-homologous recombination in fungi is not without difficulties. DNA repair using a non-homologous end-joining (NHEJ) mechanism was done by deactivation of its components (Guangtao *et al.* 2009).

Aspergillus and Trichoderma reesei strains are used as hosts for the expression of genes of fungal and non-fungal origins; however, for an increase in cellulose production, heterologous gene expression is preferred, and the component ratios to boost efficiency are focused. To ensure that cellulolytic fungi can accurately respond to carbon sources in their surroundings, repression and induction mechanisms concurrently control the synthesis

of cellulolytic enzymes. In Penicillium oxalicum and other cellulolytic fungi, despite the identification and genetic engineering of numerous proteins, especially transcription regulatory factors accountable for these processes, it remains difficult to modify a single target for cellulase production. T. reesei is the best-producing strain for cellulases; however, its genome has fewer genes encoding cellulases and hemicellulases than any other fungal genome that has been sequenced (Martinez *et al.* 2008). Therefore, the cause and mechanism of the elevated secretion level are yet unknown. It has been noted that the regulatory gene cre1 mediates the carbon catabolite suppression of the filamentous fungal T. reesei's cellulase and hemicellulase genes. The gene, such as cre1, was either eliminated or substituted with a shortened mutant form, cre1-1, which was found in a RUT-C30 mutant strain and used for an increase in the production of the enzyme. When they were cultivated in fermentation conditions that induced the hydrolase genes, they also displayed increased amounts of hydrolytic enzymes. The results of this investigation showed that, in both inducing and non-inducing environments, cre1 functions as a gene modulator for the genes expressing cellulose and hemicellulose. In the mutant strains carrying the cre1 and cre1-1 genes, no phenotypic differences were seen, showing that the cre1-1 gene functions as a null allele. Hence, cre1 was observed to be a target gene for increased enzyme production in T. ressei (Nakari-Setälä *et al.* 2009). To increase the production of lignocellulolytic enzymes, genetic modification of P. oxalicum was done systematically.

CLR-2, which acts as a cellulose degradation regulator, is a transcription factor domain-containing protein present in fungi. 135 of the 212 genes in Neurospora crassa that responded to avicel were discovered to be CLR-1/CLR-2 regulated (Coradetti *et al.* 2012). Since CLR-B also has its homolog in P. oxalicum, it is conserved in ascomycete fungi and is necessary for triggering cellulase expression. Genes are also over-expressed with the promoter gpdA from Aspergillus nidulans to enhance the constitutive first target level of CLR-B. The presence of major intracellular β-glucosidase also negatively impacts the induction of cellulases and xylanases. Additionally, when the preferred carbon sources were present, the production of cellulases and hemicellulases was inhibited.

Several screening methods were used for the comparative examination of "RE 10", the wild type, and JU-A10-T. The results showed that RE 10 had very high cellulolytic capability and more cellulase expression and secretion as compared to the wild type (Yao *et al.* 2015). It is surprising to note that the mutant's cellulolytic capacity was at par with the P. oxalicum mutant from the industrial strain.

To increase cellulase expression, a fungal strain was generated that is capable of overexpressing positive regulatory genes and knocking down negative regulatory genes. P. oxalicum mutant (JU-A10-T) has been the foundation of China's industrial-scale cellulase enzyme preparations for the past 20 years. Its genome was recently sequenced, which has given researchers important new information to mine for components that are essential for biomass conversion (Liu *et al.* 2013b). Additionally, a portion of the mechanism governing the transcription control, inducer delivery, and signal transduction of P. oxylum's cellulase gene expression has been discovered. Through the use of gene disruption analysis, three main cello-dextrin transporters—CdtC, 470 transcription factors, in addition to 15 new and 4 important transcriptional regulators, have a single-gene deletion library generated. They have been recognized, defined, and used as cellulase expression regulatory networks (Li *et al.* 2020).

To genetically modify fungi for an increase in the production of cellulose and other products, a novel technology called Reconstruction of Expression Regulatory Network (REXRN) has been developed. Mutant RE 10 generated using REXRN showed a considerable increase in cellulase and hemicellulase production as compared to the commercial strain JU-A10-T (Yao *et al.* 2015).

(Yao *et al.* 2015). reported a systematic over-expression investigation of nine genes encoding β-glucosidase in wild-type strain 114-2 of P. oxalicum using both constitutive and inducible promoters, by over-expression of β-glucosidase (BGL) BGL1, BGL4, or BGL5. EG It was possible to obtain high-yielding β-glucosidase producers, with many-fold yields. BHs are usually glycosylated like other fungal enzymes. As a rule, catalytic domains (CD) contain the N-glycosylation, whereas peptide linkers, rich in Ser and Thr residues, are heavily decorated with O-linked glycans (Payne *et al.* 2013). To protect against protease activity, an O-linked glycan in a linker may be preferred (Beckam *et al.* 2012; Greene *et al.* 2015).

According to another study, the native enzyme's activity on cellulose was greater as compared to an over-N-glycosylated recombinant CBH I of T. reesei (rTrCel7A) found and expressed in Aspergillus niger (Jeoh *et al.* 2008). Later, the same group of scientists demonstrated that site-directed mutagenesis could be used to create a new N-glycosylation site in recombinant Cel7A of Penicillium funiculosum (rPfCel7A) or remove an existing one in rTrCel7A, which shows a tremendous increase in the enzyme's activity against crystalline cellulose (Adney *et al.* 2009).

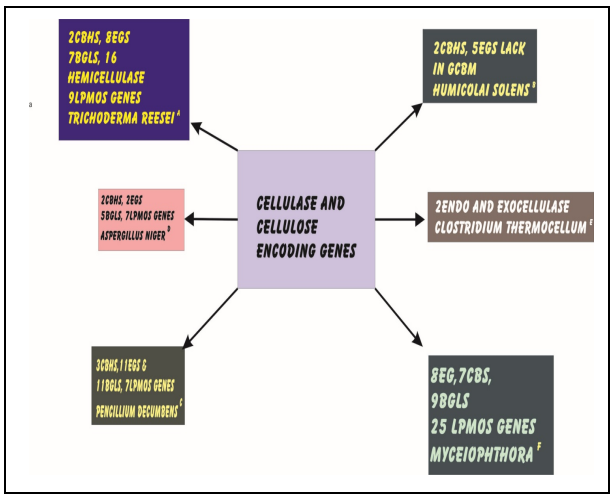


Fig. 2: Cellulase-cellulose encoding genes present in different organisms ([A]Törrönen *et al.* 1994; [B]Schülein, 1997; [C]Liu *et al.* 2013b; [D]Singhania *et al.* 2011; [E]Bayer *et al.* 1998; [F]Karnaouri *et al.* 2014)

The gpdA promoter found in Aspergillus nidulans and Pencillium oxalicum Depression and induction amplified (knock out technique), augmentation of the synthesis of cellulase. Yao and associates (2015) discussed penicillium RUT-C-30 Trichoderma reesei Transformation mediated by Agrobacterium, Enhanced activity of β-glucosidase.

Using this method to increase the specific activity of recombinant CBH I from Penicillium verruculosum (rPvCel7A) that was expressed in Penicillium canescens, (Gusakov *et al.* 2017) proved that the N-linked glycans were involved in intricate interactions with a polymeric substrate in catalysis, which was in line with earlier research (Dotsenko *et al.* 2016).

Using site-directed mutagenesis methods, (Gusakov *et al.* 2017) showed that in the CD of the recombinant P. verruculosum CBH II (rPvCel6A) enzyme, N-linked glycans represented a significant portion of the catalytic activity involved in the process of the enzyme. These data, based on earlier findings in the highly active CBH IIb (MtCel6B) from Myceliophthora thermophila (also known as Chrysosporium lucknowense), suggest that the N-glycan influence mechanism appears to be common in both GH6 and GH7 processive CBHs.

Gene inactivation and promoter substitution methods have been used to increase the production of cellulolytic enzymes. It is also recommended that the promoter of the cellobiohydrolase I gene (CBH I) be powerful and highly inducible. Additionally, T. reesei is using this promoter for eukaryotic heterologous protein expression (Uusitalo *et al.* 1991). Genetic engineering was used to change the percentage of distinct cellulases produced by the hypercellulolytic T. reesei mutant strain.

A cDNA modified in vitro to be incapable of coding for active enzymes was also used to inactivate the main cellobiohydrolase gene.. As a result, strains with higher concentrations of a particular EG or strains devoid of the primary cellobio-hydrolase were produced (Harkki *et al.* 1991).

4. CONCLUSION

Improving the efficiency and yield of microorganisms involved in the process enables the usage of eco-friendly, more energy-generating, and renewable bio-fuels as alternatives to traditional fossil fuels. This not only reduces our dependence on fossil resources but is also responsible for climate change by reducing the release of harmful gases into the environment. Additionally, strain enhancement techniques can be further optimized and tailored to specific applications, opening up new possibilities for the production of innovative and environmentally friendly products. Industrial strains that produce cellulase have been effectively used for strain improvement through mutation; however, the procedure is extremely unpredictable and time-consuming. Effective targeted changes have been made possible by the identification of key cellulase-producing fungal strains by whole genome sequencing and the understanding of their genetic roles. Strong commercial strains have been genetically engineered to develop a single strain having the capability to produce cellulase with high biomass saccharification potency. For that, targeted modifications at promoter, transcriptional factor, catabolite repressor, and enzyme gene sequences such as BGL, endoglucanase (EG), cellobiohydrolase CBH, auxiliary enzymes, and xylanase can be done. Research needs to be driven in the direction of the economic production of second-generation biofuels.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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