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Study of Cellulose Degrading enzyme Cellulase Produced by Cellulolytic fungus

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ABSTRACT

The rapid depletion of fossil fuel reserves has raised concerns among experts throughout the world.The environmental effect of fossil fuels and the continuous energy crisis have prompted researchers to investigate alternate energy sources. Lignocellulosic biomass could serve as an appropriate replacement to fossil fuels. It is composed of lignin, cellulose, and hemicellulose, with cellulose and hemicellulose being the most readily available and unexplored renewable resource. To deal with these challenges and minimize environmental impact, a green solution based on the cellulase enzyme complex has been provided. Cellulase enzymes generated by cellulolytic fungus, including endoglucanase, cellobiohydrolase, and β-glucosidase, convert insoluble cellulose into monomeric glucose subunits. They are utilized in a variety of sectors, including food, agriculture, paper and pulp, textiles and laundry, beverages, pharmaceuticals, medicine, and biofuel production.These enzymes have played a significant role in waste management across the world, which is a significant issue for developing countries. Cellulolytic fungi are the primary decomposers of plant litter in the soil. This decomposition process releases nutrients including carbon, nitrogen, and phosphorus, making them available for use by plants and other creatures in the soil food web. Cellulolytic fungi contribute to soil structure by digesting cellulose-rich plant residue.

Introduction

Complex enzymes like cellulase and amylase are highly desirable for application in industry.Cellulolytic enzymes are crucial to the natural biodegradation processes that occur when bacteria, actinomycetes, protozoa, and cellulolytic fungus successfully break down plant lignocellulosic materials. However, it is commonly recognized that fungi are the agents that break down organic matter in general and cellulosic substrate in particular. (Lynd et al.,2002)The use of lignocellulosic biomass has great promise for meeting global energy,chemical, and material needs in a sustainable and renewable way.(Supply and B. T. A.,2005)Following previous work on the hydrolysis of lignocellulosic material, it is easy to hydrolyze lignocellulosic material and agricultural waste materials that include both cellulose and starch to fermentable sugars using a combination of amylases and cellulases. (Shambe et al.,1985) Both starch and cellulose are composed of 1,4 connected o-glucopyranose units; however, starch has α D-(1-4) links and cellulose has β D-(1-4) connections. As such, they are hydrolyzed by distinct enzymes and have radically different conformations.(St Pierre et al.,1980)Thick cell walls, mostly made of polysaccharides and the aromatic polymer lignin, are present in all plant cells. Plant cells are extremely resistant to disease assault thanks to the complex composite structures that these polymers have evolved into. (Somerville et al.,2004)Cell wall synthesis is the process by which complex enzymatic machinery synthesizes cellulose, which plays a crucial structural role in plants. (Somerville and Chris ,2006)There are numerous unanswered concerns regarding the enzymatic machinery involved in the production of plant cell walls, despite extensive study in this area. (Somerville et al.,2004)On a dry weight basis, cellulose, hemicellulose, and lignin make up around 20–50%,15–35%, and 10–30% of plant cell walls, respectively (Pauly et al.,2008).Cellulose is the most prevalent biological substance on Earth because it is the most frequent polymer found in terrestrial plants. Comparing cellulose to other polysaccharides found in plant cell walls, it is also the carbohydrate polymer that is most resistant to catalytic breakdown.(Himmel et al.,2007) The most prevalent biological substance in the world, cellulose, is recycled in large part by fungi that exhibit cellulolytic activity. All plant material undergoes this bio deterioration throughout the decaying process. In this process, a complex of three major classes of activity—i) endoglucanase, (ii) exoglucanase (cellobiohydrolase), and (iii) ß-glucosidase—converts the cellulose macromolecule, a ß-1,4-linked glucose polymer that is typically found in close association with lignin, into soluble glucose monomers from an insoluble polymer. (Gong et al.,1979)Endoglucanase randomly targets the interior β1-4 glycosidic linkages of cellulose molecules. Exo-glucanases split non-reducing ends of cellulose, releasing cellobiose units, while β-glucosidase cleaves cellobiose Into glucose . (Jampala et al.,2017)The enzyme complex known as cellulase works in concert to convert cellulose into glucose. It might serve as a substitute for the cellulose's acid hydrolysis. (Wood et al.,1972)In addition to being important for natural ecosystems, the conversion of cellulose via cellobiose to glucose has immense biotechnological potential. It offers a way to use any type of cellulosic material as the main source of ethanol and glucose, two organic feed ingredients. Whereas the latter is a source of artificial organic compounds, the former provides edible biomass. (Radford et al.,1996)Among the most plentiful renewable resources are cellulose and insoluble starch, which may be processed into usable products in an environmentally responsible manner with the help of enzymes.(Mandels et al.,1969) Asresult, several researchers have tried to improve the effectiveness of enzymes derived from natural sources. Additionally, it has sparked widespread interest in the low-cost, easily accessible enzymatic synthesis from microbial sources. Since fungal amylase and cellulase can be regulated and their end products should be easier to manipulate than those found in higher eukaryotic sources, a great deal of study has been done on them. (Pečiulytė and Dalė,2007)Owing to its immense use across diverse sectors, cellulase enzyme now holds the third place in the worldwide enzyme industry share (≈15%) behind amylase (≈25%) and protease (≈18%).(Sajith et al.,2016)Several sectors, including food, biofuel, agriculture, pharmaceuticals, pulp and paper, fermentation, textiles, and agriculture, employ this enzyme. The animal feed industry began employing cellulase enzyme in the early 1980s, then the food sector (Shambe et al.,1985).

Fungal cellulase system:-

Fungal cellulases are less complicated and free than anaerobic bacteria having cellulosomes.They include endoglucanases, exoglucanases, and β-glucosidases, all of which are glycoproteinecous molecules with various forms. (Bhat et al.,1997).Soft rot fungi are well-known for creating cellulases, with Trichoderma reesi being the most well studied. Other known soft rots that produce cellulase include Aspergillus niger, Fusarium oxysporum, and Neurospora crassa. (Kovács et al.,2008, Wen et al., 2005, Juhasz et al.,2005, Gao et al.,2008).Trichoderma reesi produces eight endoglucanases, two exoglucanases known as cellobiohydrolases (CBH I and CBH II), and seven β-glucosidases, making it the most researched fungal cellulase. (Sukumaran,et al.,2005)CBH I, a fungal enzyme, breaks down oligomers at their reducing ends and is essential for breaking down crystalline cellulose (CD and CBD). CBH II, a kind of fungal cellulase, has a kink on its surface resembling a small tunnel with subsites capable of cohering one monomeric unit. It attacks cellulose at non-reducing ends(Sukumaran,et al.,2017) .Anaerobic fungi help degrade plant cell walls. They are capable of breaking down plant cellulose. They can create all types of cellulolytic enzymes. Anaerobic fungus can only breakdown structural polysaccharides and cannot use lignin moieties. The most researched anaerobic fungi are Neocallimastix frontalis, Piromyces communis, etc.(Srinivasan et al.,2001, Kim et al.,2008)

Biotechnological application of cellulases:-

Cellulases have biotechnological applications in sectors such as food, brewing, and wine, as well as in the conversion of industrial waste into chemicals,textile and laundry, pulp and paper, animal feed, agriculture, and research into single-cell protein.

1.Role of Cellulases in Biofuel Industry:-

Cellulases are most commonly used to produce value-added compounds from widelyavailable lignocellulosic waste, making it a successful commercial application. Cellulases primarily produce biochemicals from cellulosic waste. The use of this renewable supply of cellulose to create industrially .The availability of relevant chemicals is limited due to the high cost of enzymes. The scientific community aims to overcome this impediment byscreening new cellulases and improving current enzymes to match industrial demands. Grape fruit peel waste was converted into fermentable sugars using a mixture of cellulases and pectinases.(Wilkins et al.,2007) Cellulases may convert cellulosic material into glucose and fermentable sugars, which can be utilized as a microbial substrate for producing single-cell protein or ethanol. (Kuhad et al.,2010).Microbial cellulases may produce ethanol from textile waste, making it an alternative biomass source.(Jeihanipour et al.,2009)Ethanol has several benefits, including low life-cycle greenhouse gas emissions, high sustainability, and smooth integration into current transportation systems. It has the potential to make a significant effect. (Ward et al.,2002, Gupta et al.,2009)

2.Use of cellulase in laundry and textile biotechnology:-

The cellulases in textile industry are most commonly used for biostoning, biopolishing and bio finishing .Cellulase, ideally neutral and endoglucanase rich removing extra dye from denim textiles may be softened without causing fiber damage, resulting in biostoning and the manufacturing of high quality, ecologically friendly washing powder wereas cellulase, ideally acidic and endoglucanase rich excess microfibrils are removed from the surface of cotton and non denim textiles, resulting in biopolishing.Endoglucanase-rich cellulases produce superior biofinishing outcomes. (Galante et al., 1998, Godfrey et al.,1996, Uhlig et al.,1998).The advantages of using cellulase-based biostoning are less labour-intensive, worn look, reduce damage, and create the possibility to automate the process .(Pazarlioglu et al.,2005)

3.Pulp and paper industry:-

Since the 1980s, enzymes have been used to overcome limitations of traditional pulp recycling processes. Cellulases have been effectively utilized to deink and remove pollution particles from paper without harming its brightness or strength.Mechanical pulping, which involves grinding and purifying woody materials, is generally related with excessive energy use. Bio-pulping, which uses cellulases and other enzymes, is a sustainable and energy- efficient method. Refining can produce microscopic pulp particles, reducing the rate of drainage during papermaking.(Mai et al.,2004)Enzymatic de-inking offers benefits such as pulp-free, clean appearance, increased brightness and fiber strength, and reduced environmental impact. (Pleach et al.,2003,lee et al.,2007) Cellulase has been utilized in the pulp and paper industry for a variety of applications. Enzymatic modification of coarse mechanical pulp using cellulase resulted in significant energy savings. Cellulases are the most effective way to recycle waste paper from books, magazines, and newspapers. They may add value by deinking and reusing the fiber for newspaper manufacturing or ethanol generation. (Kuhad et al.,2010, Noe et al.,1986,Pere et al.,1996).

4. Animal feed industry:-

Enzymes play a key role in treating animal diets by removing anti-nutritional elements included invegetables and grains.Ruminants have a more complex forage diet containing cellulose, hemicellulose, pectin, and lignin compared to chickens and pigs that eat cereal. Enzyme preparations with high amounts of cellulase, hemicellulase, and pectinase enhance the nutritional content of forages.(Graham et al.,1995, Kung et al.,1997, Lewis etal.,1996).Cellulases accelerate fiber digestion, influencing ruminants' normal gastrointestinal processes and leading to increased ability to digest nutrients .(Murad et al.,2009, Murad et al.,2010)Pretreating forage with cellulases improves milk production and nutritional accessibility in dairy cows, leading to improved animal health.(Juturu et al.,2014).

5 .Food industry:

Cellulases are widely used in food biotechnology for juice extraction, nectar viscosity reduction, puree concentration, sensory enhancement, olive oil extraction, and bakery product quality improvement. Cellulases are used to improve the cloud stability and texture of nectars and purees, as well as reduce their viscosity. Fruit juices are cloudy due to the presence of polysaccharides such as cellulose, hemicelluloses, lignin, pectin, starch, metals, proteins, and tannins. The commercial enzyme preparation 'Rapidasepomaliq', which contains cellulases,hemicellulases, and pectinases from Trichoderma and Aspergillus, has been used at an industrial scale. These enzymes were known as macerating enzymes .Extraction of olive oil requires crushing and grinding olives, transferring the paste through decanters, and centrifugation at high speeds to obtain the oil. Olivex, a high pectinase enzyme with low levels of cellulase and hemicellulase derived from A. aculeatus, was used commercially to improve olive oil extraction. (Bhat et al.,2000, Efrati et al.,2013, Kuhad et al.,2010, Singh et al.,2013, Vaillant et al.,2001, Grassin et al.,1996).Exogenous glucanases and related polysaccharidases can enhance the overall quality of beer and wine. Beer is made by malting barley in a malt house and fermenting the wort in a brewery, while wine is made by extracting juice from grapes and fermenting it with yeast. Malting relies on seed germination to activate enzymes such as amylases, carboxypeptidase, and cellulases. These enzymes work together to make high-quality malt under ideal conditions. Adding cellulases improves both beer quality and manufacturing efficiency. Wine production relies on three external enzymes: pectinases, b-glucanases, and hemicellulases. (Galante et al.,1998)

6 .Agriculture Industry

Cellulolytic fungi including Trichoderma sp., Geocladium sp., Chaetomium sp. And Penicillium sp. improve seed germination, plant growth, and agricultural yields. They havebeen used to create protoplasts for plants and fungi. These enzymes improve soil fertility andquality.Certain fungi have cellulases and enzymes that can break down plant diseases' cell walls and reduce illness. Trichoderma harzianum strain P1 b-1,3-glucanase and N-acetyl-glucosaminidase were shown to limit the spore germination and germ tube elongation of B.cinerea in a synergistic manner.(Bailey et al., 1998, Harman et al.,1998, Lorito et al.,1994).

Conclusion

In conclusion, the study of cellulase produced by cellulolytic fungi has demonstrated the enzymes' potential in various industrial applications. Continued research and development in this field are likely to contribute significantly to advancements in sustainable technologies and bioprocesses.

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