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CELLULASE AND ITS ROLE IN INDUSTRIES: A REVIEW

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ABSTRACT

Cellulose is the most abundant polymer present in various agricultural wastes. These wastes can be converted into useful products by degradation of cellulose present inside these wastes. The conversion is catalysed by cellulase enzyme. This review describes the bacterial cellulase enzyme including their classification, isolation, production and applications. In this, we have mentioned the isolation and screening techniques of bacterial cellulase along with some other microbial species, which are having potential of producing cellulase enzyme. It also discusses the submerged and solid-state production of cellulase enzyme along with applications in textile, paper, production of biofuel, food and animal feed industry, oil extraction, brewing, and agriculture. Now a days, industrial enzymes achieved a great place due to their easy production from cheap agricultural wastes together with their magnificent role in above mentioned industries. These features attract the industries, so that they can make their products economically valuable and also improve the protocols accordingly.

Keywords: Cellulase; Fermentation; Isolation and screening; Industrial applications; Eco-friendly (agrowastes).

INTRODUCTION

The most remarkable feature of enzymes is to convert complex and ecological degradative process into more feasible and environmentally friendly form. Detailed knowledge of various enzymes like their occurrence and roles lead to the evolution of novel biological processes. The perpetual utilization of enzymes from several years due to their specificity in industries, metabolism and biochemical processes concomitantly known as "enzyme technology". Cellulase is one of the most popular inducible enzymes synthesized by various microorganisms such as bacteria and fungi. Cellulase enzymes are becoming the main focus of research these days due to their role and applications in various industries. Cellulase enzymes help in the breakdown of cellulose polysaccharides which is present inside the plant cell wall along with xylan and pectin. Cellulose is a glucose polymer of anhydro-D-glucose units linked by 1, 4-β-D-glucoside bonds, which makes a major part of plant cell wall. Cellulose and hemicellulose consist nearly 40-60% of plant cell wall materials (Mccann and Carpita 2008). Cellulose can be converted easily into its monomeric form by biological means *i.e.* by cellulase enzymes. Different microorganisms have the ability into produce cellulase for the conversion of cellulose polymer (one of the most abundant polymers on earth) to glucose monomer (Saha *et al*., 2006). So, it is important to develop a cost-effective strategy, which can utilize the low-cost carbon sources such as agricultural wastes (Shanmugapriya *et al*., 2012).

Cellulase comprises of three different enzymes *i.e.* endoglucanases, also called 1,4-β-D-glucan-4-glucanohydrolase or carboxymethyl cellulase (EC 3.2.1.4); exoglucanases 1, 4-β-D-Glucan cellobiohydrolases (cellobiohydrolases) (EC 3.2.1.91) or 1, 4-β-D-oligoglucan cellobiohydrolases (also known as cellodextrinases) (EC 3.2.1.74) and β-glucosidases or β-D-glucoside glucohydrolases (EC 3.2.1.21) (Shankar and Isaiarasu 2011). All three enzymes act in consecutive manner to catalyse the cellulose. Various fungal cellulase has been reported in literatures but bacterial cellulase have high growth rate in comparison to fungal form. So,

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therefore more research has been focussed on bacterial isolate, which can produce high cellulase activity along with high thermo and pH stability. Bacteria and fungi have been known to produce enzymes freely in solution, but some other microorganisms have also been known to produce cell-bound enzymes with multi-protein complexes known as cellulosomes (Shaikh *et al*., 2013). Firstly, cellulosome was recognized in thermophilic spore-forming *Clostridium thermocellum* (Maki *et al*., 2011). Cellulase production rely on various growth parameters such as inoculum size, pH, temperature, aeration, growth and incubation time (Immanuel *et al*., 2006). It also depends on several activators and inhibitors (Muhammad *et al*., 2012). Due to the potential role of cellulase in academic along with research in industries have increased their demand commercially from last several years (Singh *et al*., 2007). Therefore, there is need to develop new methodology for their screening and production in order to attain their major goal in industries.

So, we can say that cellulose is the most abundant polymer present in various agricultural wastes. These wastes can be converted into useful products by degradation of cellulose present inside these wastes. The conversion is catalysed by cellulase enzyme. This review describes the bacterial cellulase enzyme including their classification, isolation, production and applications. In this we have mentioned the various isolation and screening techniques of bacterial cellulase along with some other microbial species having potential of producing cellulase enzyme. It also discusses the submerged and solid-state production of cellulase enzyme along with applications in textile, paper, production of biofuel, food and animal feed industry, oil extraction, brewing, and agriculture. Now a days, industrial enzymes achieved a great place due to their easy production from cheap agricultural wastes together with their magnificent role in above mentioned industries. These features attract the industries, so that they can make their products economically valuable and also improve the protocols accordingly.

Classification of Cellulase

Cellulase production from different microorganisms and detailed analysis of cellulosomes helps to classify the cellulase on the basis of their mode of action (Sadhu and Maitri 2013).

Endoglucanases (EC 3.2.1.4)

Endoglucanses cut at internal bond of cellulose polymer and release the various monosaccharides units of unequal length.

Exoglucanase (EC 3.2.1.91)

Exoglucanases attacks at the exposed end of cellulose chain produced after the attack of endoglucanse and results in formation of various disaccharides or tri-tetrasaccharide units.

Exoglucanases (EC 3.2.1.74)

This is another form of exoglucanases, which act on the reducing end of cellulose polysaccharides and produces various disaccharide units.

β - Glucosidases (EC 3.2.1.21)

It cleaves the non-reducing end of cellulose after the attack of exoglucanses and generates several monomeric units.

Mechanism of cellulose degradation

As it is clear from the action of above-mentioned enzymes that the conversion of cellulose to glucose, involving the attack of endoglucanases and exoglucanases, these two enzymes decreases the length of polymeric cellulose and produces various di-tetra saccharides units. The final step is performed by β-glucosidase that converts these saccharide units into monomeric glucose units (Hasunuma *et al*. 2013) as shown in Fig.1.

Cellulase enzyme producing microorganisms

Various bacterial and fungal strains have been reported in literature due to their ability to produce cellulase (Abdel-Raheem and Shearer 2002). Several bacterial species, such as *Bacillus, Cellulomonas, Cellovibrio, Micrococcus, Pseudomonas* and *Sporocytophaga* (Immanuel *et al*. 2006) have been well documented. Various *Bacillus* species such as, *B. agardherans, B. circulans* and *B. subtilis* have also reported (Li *et al*. 2006; Korpole *et al*. 2011; Bai *et al*. 2012). *Bacillus* species like *B. circulans* have ability to produce endoglucanase (Hakamada *et al*. 2002). Similarly, *Bacillus amyoliquefaciens* (Lee *et al*. 2008) and *Bacillus thuringiensis* (Lin *et al*. 2012) have been reported to produce cellulase. Some aerobic and anaerobic cellulase producing bacteria are shown in Table (Kuhad *et al*. 2011).

Screening and Isolation of Bacterial Cellulase

For the screening of bacterial cellulase, soil samples of different areas have to be collected. Serial dilutions of soil samples were prepared and spread over on the various cellulose nutrient media plates containing 0.5% peptone, 0.3% beef extract, 1.5% agar and 1% cellulose respectively. The plates will be incubated at 37°C for 48 h, after the incubation time period plates were evaluated for the presence of colonies. These colonies are further used for the preparation of replica plates under the same conditions (Varghese *et al*. 2017). After the growth of bacterial colonies, these plates were submerged with 0.5% congo red solution and further destained with 1 M NaCl for 20 minutes and 20-30 minutes respectively. The colonies of cellulase producing bacteria will evaluated by measuring the zone of hydrolysis around the bacterial colonies (Gessesse and Gashe1997). The above-mentioned protocol will repeated again to get the pure cellulase producing colonies. This is the quantitative screening, where the bacterial colonies were utilised for the production of cellulase enzymes under different physiological conditions and various physiochemical parameters are also optimized in order to get the maximum production of enzyme.

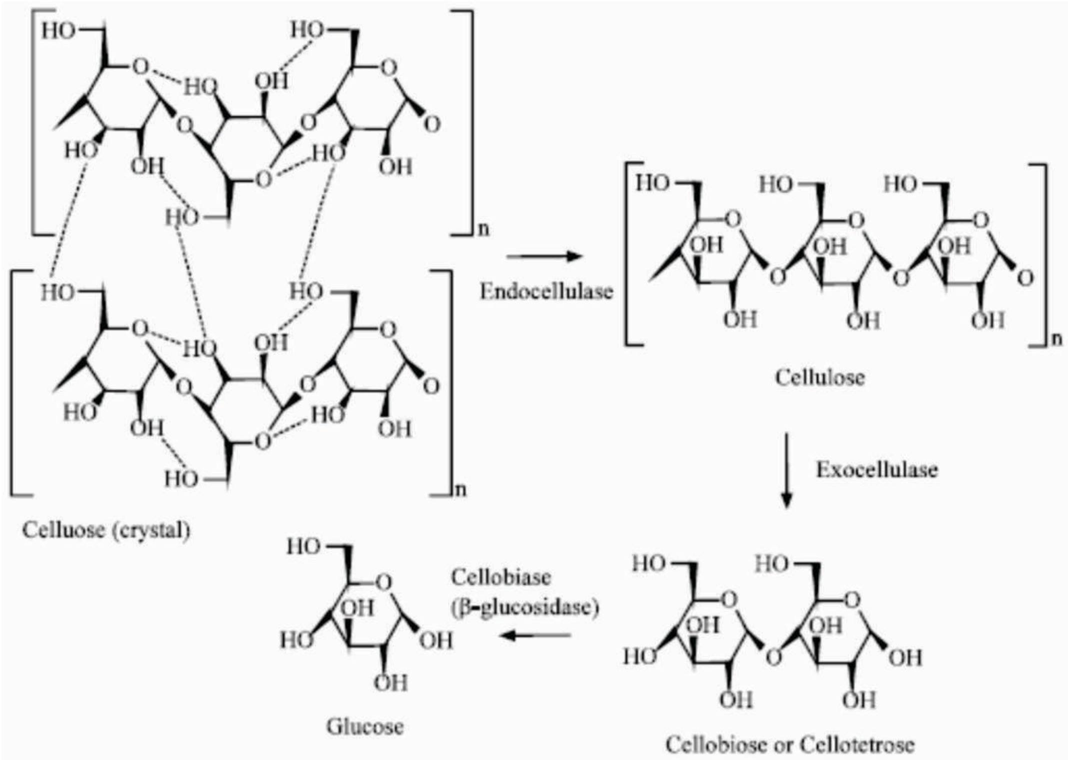

Fig. 1: Mechanism of cellulase enzyme.

Table 1: Some cellulase producing Bacteria.

Cellulase Producing Bacteria	
Aerobic bacteria	**Anaerobic bacteria**
Acinetobacter junii; A. amitratus; Acidothermus cellulolyticus; Anoxybacillus sp.; Bacillus subtilis; B. pumilus; B.amyloliquefaciens; B. licheniformis; B. circulan; B. flexus; Bacteriodes sp.; Cellulomonas biazotea; Cellvibrio gilvus;Eubacterium cellulosolvens; Geobacillus sp.; Microbispora bispora; Paenibacillus curdlanolyticus; Pseudomonascellulosa; Salinivibrio sp.; Rhodothermus marinus	*Acetivibrio cellulolyticus; Butyrivibrio fibrisolvens; Clostridium thermocellum; C. cellulolyticum; C. acetobutylium;C.papyrosolvens; Fibrobacter succinogenes; Ruminococcus albus*

Cellulase Production using Fermentation

Production of enzymes using fermentation is one of the most popular method. Fermentation involves the use of microorganisms for converting the complex polysaccharides into their respective monomeric form. Fermentation techniques used for the cultivation of enzymes comprises of two different form (Table 2) i.e Submerged fermentation (SmF) and Solid-state fermentation (SSF). Optimum growth conditions required by several microorganisms are not same, every microbe needs some specific growth parameters for maximum enzyme production. These conditions have to be optimized for attaining maximum enzyme titre and this is achieved by either 'one-variable-at-a-time' or statistical approach (Pandiyan *et al*.2014).

Submerged Fermentation (SmF)

SmF uses a liquid medium for growing the microbes. All the essential nutrients were dissolved in this liquid broth for the production of enzyme by the microbe. It is the easy method with simple handling. This method is widely used for purification purpose (Subramaniyam *et al*. 2012).

Solid-State Fermentation (SSF)

SSF uses solid substrates such as wheat bran, bagasse, paddy straw and other agricultural wastes with very low moisture content. All the substrates used in this fermentation can be reused again (Babu and Satyanarayana 1996).

Applications of Cellulase

Initially cellulase enzymes were used for biological conversion of biomass and various cellulose containing materials, which are thought to be the waste of agricultural residue. But now, these waste materials are used for maximal production of cellulase enzyme for their utilization in textile industry, paper industry, food and animal feed industry, oil extraction, brewing, agriculture and biofuel production.

Textile industry

Cellulase have been used in textile industries for the bio-stoning of various denim clothes to produce softness and faded look to the jeans. During this process, cellulase break down the small fibers and produces a well-furnished fabric. Conventionally, pumice stone are used for this purpose. The advantage of using cellulase over pumice stone is less damage to the fiber and also environmentally safe (Saranraj *et al*. 2012).

Paper and Pulp industry

Cellulase alone or in combination with xylanase have been used for biopulping or biobleaching to produce a paper with improved physicochemical properties such as burst factor, tear factor, tensile strength and pulp freeness. The use of enzyme also helps to reduce the amount of toxic chemicals used for bleaching. Cellulase also used for deinking of paper by partial hydrolysis of carbohydrate molecules present in paper (Kuhad *et al*. 2010).

Food and Animal Feed Industry

Cellulases have been used widely in food and feed industries. In food industries cellulase is used for production of fruit and vegetable juices. It was done by enzymatic extraction and clarification of juices. In brewery they are also used for the improvement of barley malting.

Table 2: Production of bacterial cellulase enzyme under different fermentation conditions.

Bacterial sp.	Substrate used	Fermentation process	References
Bacillus sp. AC-1	CMC	SmF	Li *et al*., 2006
Anoxybacillus flavithermus [2] EHP[2]	CMC	SmF	Ibrahim and Ahmed 2007
Cellulomonas cellulans	Paddy straw	SmF	Mishra *et al*., 2007
Clostridium thermocellum	Cellulose & paper pulp	SSF and SmF	Zhuang *et al*., 2007
Bacillus amyloliquefaciens	Rice hull	SmF	Lee *et al*., 2008
Bacillus sp. NZ	Agricultural residues	SSF	Nizamudeen and Bajaj 2009
B. subtilis KO	Molasses	SmF	Shabeb *et al*., 2010
Anoxybacillus sp.527	Crystalline cellulose	SmF	Liang *et al*., 2010
B. subtilis A-53	Rice bran	SmF	Lee *et al*., 2010
Bacillus sp. LFC	CMC	SmF	Korpole *et al*., 2011
B. subtilis	CMC	SmF	Deka *et al*., 2011
Microbacterium sp. MTCC 10047	CMC	SmF	Sadhu *et al*., 2011
B. cereus	Palm Kernel Cake	SSF	Lah *et al*., 2012
B. licheniformis MVS1 and MVS3	CMC, Filter paper	SSF	Acharya and Chaudhary 2012
Bacillus subtilis BS05	Sugarcane baggase	SmF	Irfan *et al*., 2012

Bacillus amyloliquefaciens SS35	CMC	SmF	Singh *et al*., 2014
Bacillus sp. SMIA-2	Sugarcane bagasse & corn steep liquor	SmF	Ladeira *et al*., 2015
Brucella and *B. licheniformis*	Maltose and CMC	SmF	Behera *et al*., 2016
B. licheniformis RT-17	Sugarcane bagasse	SmF	Tariq *et al*., 2018
Paenibacillus polymyxa ND24	Sugarcane bagasse	SmF	Bohra *et al*., 2018

Cellulases along with other enzymes have been also used to improve the nutritional value of forages used as animal feed. This is done by the pre-treatment of agricultural fodder and grain feed by removing antinutritional substances (Imran *et al*. 2016).

Oil Extraction

Cellulase have been employed for extraction and clarification of olive oil. Use of enzyme increases the amount and antioxidants of oil. Enzyme treatment also decreases the rancidity and viscosity of the oil (Kuhad *et al*. 2010).

Brewing Industry

Microbial enzymes help in the fermentation process of several beverages such as beers and wines. Enzyme treatment increases the quality and final quantity off mentation products. An improved cocktail of enzymes like cellulase with pectinase can be used to enhance the production of brewing industries (Bamforth 2009).

Agriculture industry

Enzymes also have potential applications in agricultural industries for increasing the crop growth along with treatment of various crop diseases. Cellulases improve the fertility of soil. Various agricultural residues such as addition of straw into soil have the capability to improve the quality of soil and decreases the need of fertilizers (Escobar and Hue 2008). So, cellulase can be used for rapid decaying of straw to increase the quality of soil (Han and He 2010).

Production of Biofuel

Production of various biofuels such as bioethanol and biobutanol, is the only solution to decrease the use of various non-renewable fossil fuels. These fossils produce lot of problems in the environment. Bioethanol can be produced by direct fermentation of simple sugars (called first-generation ethanol) or from saccharification of complex sugars to simple ones, followed by fermentation (called second-generation ethanol). Use of agrowastes such as sugarcane bagasse, corncob, cashew apple bagasse, rice straw, banana stem, pineapple peel for bioethanol production make these wastes valuable and also don't tamper our environment (Siqueira *et al*. 2020).

CONCLUSION

The main idea for bioprocessing of agroresidues is entirely depends upon cellulase and cellulase producing microorganisms. Commercially, cellulase enzymes are produced worldwide and are also being utilised in food, animal feed, agriculture, paper, and textile industrial applications. Enhancement in cellulase enzyme titre using novel biotechnological techniques such as protein or metabolic engineering will make the future of enzyme-based industries along with production of thermostable or pH stable cellulase enzymes.

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