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FUNGAL CELLULASE; PRODUCTION AND APPLICATIONS: MINIREVIEW

Amer Ahmed
Department of Biochemistry and Biotechnology, Faculty of Science, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
aa.biotechiub@gmail.com

Aasia Bibi
Department of Chemistry, Faculty of Science, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
aasiabibi40@yahoo.com

Abstract

Cellulose is the most abundant biomaterial derived from the living organisms on the earth; plant is the major contributor to the cellulose pool present in the biosphere. Cellulose is used in variety of applications ranging from nanomaterials to biofuel production. For biofuel production, cellulose has first to be broken-down into its building blocks; β-D-glucosyl unit which subsequently can be fermented to different product such as ethanol, acetic acids, among others. Cellulase is the enzymatic system, which degrades cellulose chains to glucose monomers. Cellulase is a group of three enzymes endoglucanase, exoglucanases and β-glucosidases which act together to hydrolyze cellulose to glucose units. Cellulases are found in bacteria, fungi, plants, and some animals. Fungi are the preferred source of cellulase for industrial applications since they secrete large quantities of cellulase to culture medium. Despite a remarkable number of fungi found to produce cellulase enzymes, few have been extensively investigated because they produce large quantities of these enzymes extracellularly. In this mini-review, the production of cellulase from fungi and the parameters affecting cellulase production are discussed briefly on *light of recent publications. Furthermore, potential applications of cellulase enzymes are highlighted.*

Keywords

Fungi, Cellulase, Production, Optimization, RSM, Application, Biofuel

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1. Introduction

Cellulose is the most abundant biomaterial derived from the living organisms on the earth. Plant is the major contributor to the cellulose pool in the biosphere being synthesized through the process of photosynthesis. Thus it is the major constituent of plant biomass followed by hemicellulose and lignin (Nidhi et al. 2017, Saxena et al. 2009). Chemically, cellulose consists of β-D-glucopyranoside units that are linked together via β-D-glucosyl bonds (Ahmed et al. 2017b). Despite the fact that cellulose potentially can be used in wide range of applications from nanomaterials to biofuel production, majority of cellulose, annual production estimated 1.5 x 10^{12} tons, is being wasted (Al-Kharousi et al. 2015). For the cellulose to be utilized in various industrial applications it needs first to be converted into its building blocks (Glucose) by the hydrolysis of β-D-(1,4) glucosidic linkages. Naturally, cellulose degradation is mediated by an enzymatic system referred to as cellulases. Cellulase is a group of three individual enzymes namely endoglucanase (endo-1,4-β-D-glucanase (EG), EC 3.2.1.4), cellobiohydrolase (exo-1,4-β-D-glucanase (CBH), EC 3.2.1.91), and β-glucosidase (1,4-β-D-glucosidase (BG), EC 3.2.1.21) (Dashtban et al. 2010). These enzymes work synergistically to degrade cellulose to glucose units which can then be used in various biotechnological applications such as textile, paper and pulp industry, laundry industry, biofuel production and amino acids synthesis (Ahmed et al. 2017b, Imran et al. 2016, Sun and Cheng 2002). Microorganisms are the major contributor to cellulose degradation and carbon recycling (Lynd et al. 2002). Cellulases are synthesized by bacteria, fungi, plants and some animals, and anaerobic microorganisms are known to produce single discrete cellulase system known as cellulosome, more powerful and efficient system for cellulose degradation. Cellulosome, since its discovery, has been worked out and reviewed by many experts (Ahmed et al. 2017a, Artzi et al. 2017, Doi and Kosugi 2004, Zhang Xiao-Zhou and Zhang 2013). For the last three decades cellulase enzymes have been the focus of many research groups which aim at its production, characterizations, engineering, and applications in various industries. The annual sale of cellulase reached up 8% of total enzyme markets and is expected to exceed the protease market in the future (Horn et al. 2012). Currently many research agencies are focusing on cellulase production for biomass conversion and biofuel production because the cost of cellulases is the major obstacle in biomass hydrolysis and industrialization. Fungi are a crucial contributors to cellulose decomposers, accounting for 80% of the cellulose breakdown in nature particularly forest ecosystem where fungi play a significant roles in biomass decomposition. Fungal species known to degrade cellulose encompass members of the *Ascomycota* (Hernandez et al. 2018, Timo et al. 2017), *Basidiomycota* (Baldrian and Valaskova 2008), and *chytrids* encountered in the rumen of some animals. Remarkably, aerobic fungi are known to secrete large quantities of extracellular cellulase making them preferable for industry comparably to anaerobic fungi which are known to synthesize multi-enzyme complexes, cellulosome, bound to cell surface making its recovery ultimately difficult (Imran et al. 2016, Quiroz-Castañeda and Folch-Mallol 2013). Cellulases have been produced and characterized from different aerobic fungi such as *Aspergillus* (Bansal et al. 2012), *Trichoderma* (Ellilä et al. 2017), *Penicillium* (Prasanna et al. 2016), among others. The present mini-review aims to briefly discuss the production of fungal cellulases with focus on the parameters affecting their production on light of recent publications. It will also discuss the various potential applications of cellulases in various industrial sectors.

2. Production of Cellulases

Enzymes production is the first crucial step in enzyme technology which needs to be paid much attention since it determines the economic feasibility of the process. Considerable number of cellulase has been produced from fungal species such as *Aspergillus ornatus* (Toor and Ilyas 2014), *Penicillium sp.* (Picart et al. 2007, Prasanna et al. 2016), *Aspergillus terreus* MS105 (Sohail et al. 2016), *Aspergillus terreus* M1 (Gao et al. 2008), *Aspergillus niger* and *Rhizopus sp.* (Santos et al. 2016), *Aspergillus niger* (Baig and Saleem 2012), *Trichoderma longibrachiatum* (Pachauri et al. 2017), *Beauveria Bassiana* (Petlamul et al. 2017), among many others.

2.1 Factor Affecting Cellulase Production

Several fermentation conditions play fundamental roles on cellulases production, among which fermentation method, carbon source, nitrogen source, pH, temperature, salt/metal ions effect, incubation time, aerations, and fungal species (Norouzian 2008, Okoye et al. 2013, Saini et al. 2017).

2.1.1 Fermentation Method

Fungal cellulases have been produced through solid state fermentation (SSF) and submerged fermentation (SmF). In SSF, the fungal species is grown on one or more solid substrate such as rice straw, wheat bran, corn husk, cassava cake, or sugar cane bagasse without or very low water content. The grown microorganism utilized the solid substrate steadily and slowly thus under SSF condition, the microorganism can be grown for long period of time, for instance, for several days (Ahmed et al. 2017). The high productivity, cheap substrate utilization, low energy requirement are the advantages of SSF. Moreover, under SSF conditions, there is minimal water output and lacking of foam up which make it economically feasible (Faisal and Benjamin 2016). SSF shortcomings are limited to heat generation and lack of knowledge on automation (Ahmed et al. 2017, Shweta 2015, Soccol et al. 2017). SSF has been utilized for cellulase production from several fungal species such as *lichtheimia romosa* (Garcia et al. 2015), *Phaffomycetaceae* (Cerda et al. 2017), Dipodascaceae (Cerda et al. 2017), *Trichoderma citrinoviride* AUKAR04 (Periyasamy et al. 2017), *Humicola insolens* MTCC 1433 (Singla and Taggar 2017), among many others. On the other hand, in SMF, free flowing liquid like molasses and or broths supplemented with different nutrients is used to cultivate of microorganisms. The enzymes including cellulase and metabolic byproducts are secreted into fermentation medium and medium supplements or nutrients are rapidly utilized and a continuous supply with is needed. SMF has several advantages such as simplicity of sterilization, heat and mass transfer, process monitoring (pH, temperature, and soluble molecules) and automation, and extraction and recovery of enzymes and bioactives (Ahmed et al. 2017). Several cellulase enzymes have been produced by SMF from different fungal species including *Aspergillus flavus* (Gomathi et al. 2012), *Aspergillus Niger* FC-1(Jiang et al. 2013), *Aspergillus niger* (Reddy et al. 2015), among many others.

2.1.2 Carbon Source

Carbon source is the major factor affecting the cellulases production, attributing to the fact that cellulases are inducible enzymes that are expressed by cells in response to different carbon source present in the fermentation medium (Saini et al. 2017, Zhang Y. et al. 2017). For instance, optimal cellulase production from *Hypocrea jecorina* QM6a, QM9414, and RUTC-30 was attained in medium containing microcrystalline celluloses as the sole carbon source (Dashtban et al. 2011). *Penicillium sp*. produced the highest cellulases activity on lactose containing media among different carbon sources tested such as sarbose, maltose, sucrose, lactose, dextrose, galactose, cellobiose, and CMC (Prasanna et al. 2016). Expression of different cellulase isoforms in response to carbon source has also been reported (Amore et al. 2013). For examples, *Aspergillus terreus* expressed four endoglucanase (EG) isoforms in presence of rice straw as solid substrate or corn cobs liquid substrate. Similarly, supplementation of fructose and cellobiose to corn cobs medium up-regulates at least one of EG while adding mannitol, ethanol and glycerol selectively suppressed the expression of three EG isoforms. Similarly four isoforms of β-glucosidase (βG) was expressed in presence of corn cob containing medium and addition of glucose, cellobiose, mannitol, fructose, sucrose or glycerol repressed of one or more βG isoforms (Nazir et al. 2010). *Aspergillus fumigatus* Z5 grown on culture media containing glucose, avicel, and rice straw secreted 61, 125, and 152 proteins, respectively. Proteomic analysis suggested that glycoside hydrolases including cellulases, hemicellulases were overexpressed on rice straw and avicel containing media compared to glucose used as carbon sources (Liu et al. 2013). The molecular mechanisms by which the expression of these different isoforms are regulated and different carbon sources influence the quantity and isoforms expression are not well established which hampered genetic engineering of these fungi for industrial purpose (Coradetti et al. 2012). Thus understanding the mechanisms of cellulase expression hold a critical significance for enhancement of cellulase enzymes production and have been investigated in *Aspergillus* and *Trichoderma* (Gautam et al. 2011). These fungi produce extracellular cellulase enzymes when they are grown on media containing plant polymers, or short oligosaccharides as an energy source, and when cultivated on media containing easily metabolizable sugar such as glucose, the expression of these enzymes is repressed. Carbon catabolite repression is considered the most acceptable mechanism to repress cellulase production when grown on easily metabolizable sugars (Antonella et al. 2013).

Recently Zhang *et al* published a study demonstrated that *Rhizopus stolonifera* host a gene which encode for cellobiose synthetase (CBS) to synthesize cellobiose from uridine diphosphate glucose (UDPG). CBS was found to play a fundamental role in expression of cellulase gene through the induction of the cellobiose-responsive regulators CLR1 and CLR2 and thus inducing the transcription of cellulase genes. The author suggested that minimal constitutive expression of cellulase may be driven by cellobiose synthesized by CBS from carbohydrate metabolites (Zhang Y. et al. 2017).

2.1.2 Nitrogen Source

Another important factor affect protein secretion in fungi is nitrogen. Different nitrogen source can be included in fermentation medium for cellulase production. Among organic nitrogen source that's can be used are peptone, yeast or beef extract, tryptone or soybean meal. Inorganic nitrogen source like ammonium sulphate, ammonium chloride, ammonium hydrogen phosphate can also be used as a nitrogen source (Ahmed et al. 2017c, Kachlishvili et al. 2006). Optimum cellulase activity was achieved from *Penicillium* sp. when cultivated on yeast extract containing medium (Prasanna et al. 2016). *Trichoderma reesei* showed optimum production of cellulase when cultivated on *Parthenium* biomass containing ammonium molybdate, peptone or yeast extracts as nitrogen source (Saini et al. 2017).

2.1.3 pH and Temperature

Optimization of parameters such as pH and temperature is also of crucial significance for enzyme production since these physicochemical parameter affect the growth of microorganism hence the bioactives production. The optimal cellulase production from *Penicillium* sp was attained on Czapek-Dox medium at pH 5.0 and 30˚C (Prasanna et al. 2016). Similarly, optimum production of celllulase by *Aspergillus tubingensis* KY615746 was achieved at pH 4, and temperature of 30 °C (El-Nahrawy et al. 2017).

2.1.4 Incubation Time

Myceliophthora heterothallica produced the highest endoglucanase on SSF containing wheat bran or sugarcane occurred at 192 hours and on SmF containing cardboard at 168 hours (Teixeira da Silva et al. 2016). Optimal production of carboxymethylcellulase (CMCase) from *Aspergillus hortai* under SMF was achieved after 96 h (El-Hadi et al. 2014).

2.2 Statistical Approach for Optimization of Cellulase Production

Optimization of cellulases production is critical process for efficient and cost-effective cellulase production. Traditionally optimization of cellulase production is carried out by employing One Variable at A time (OVAT) approach. OVAT involves varying one parameter at a time keeping other factors constant. OVAT is regarded as a laborious technique, time consuming and misleading approach because these parameters are independent and OVAT tend to ignore the interactions between them, in addition to extensive time needed to perform large number of experiments.

Statistical approaches such as Surface Response Methodology and Plackett–Burman Design is an efficient approach employed for optimization of fermentation parameters (Shajahan et al. , Singh et al. 2014). Several studies have employed statistical methods for optimization of cellulase production. Cellulase production from *Trichoderma reesei* was optimized using Plackett-Burman design of 9 nutrients for their influence on cellulase secretion using Response Surface Methodology (RSM). The study demonstrated that the optimal concentration of avicel, soybean cake flour, KH_2PO_4, and $CoCl_2{\cdot}6H_2O$ for cellulase production were 25.30 g/l, 23.53 g/l, 4.90 g/L, and 0.95 g/l, respectively (Saravanan et al. 2012). In another study, using statistical Full Factorial Design (FFD), optimal cellulase production from *Penicillium funiculosum* ATCC11797 was achieved on culture media containing avicel (10 g/l) as carbon source, urea (1.2 g/l), yeast extract (1.0 g/l), KH_2PO_4 (6.0 g/l), and $MgSO_4{\cdot}7H_2O$ (1.2 g/l) with an agitation speed of 220 rpm and aeration rate of 0.6 vvm. These conditions resulted in activities of 508 U/l for FPase, 9,204 U/l for endoglucanase, and 2,395 U/l for β-glucosidase which are 3.6-9.5 times higher than production using non-optimized conditions (de Albuquerque de Carvalho et al. 2014). Cellulase production from *Trichoderma reesei* RUT C-30 was optimized employing a two stage statistical design namely Fractional Factorial Design and Response Surface Box Behnken Design on wheat bran and cellulose under SSF. This approach resulted in a 3.2-fold increase in CMCase production to 959.53 IU/gDS (Idris et al. 2017). The statistical approach for optimization of fermentation conditions are considered efficient because the interaction of multiple variables are taken into consideration and the number of experiments needed to be performed are reduced to minimum (Ahmed et al. 2017, Shajahan et al. 2017, Singh et al. 2014).

3. Application of Cellulases

Cellulases, over many decades, are used in various industrial applications, securing the third rank among enzymes annual sale and expected to exceed the protease in the near future (Menendez et al. 2015). Cellulase enzymes have got tremendous applications in different industries including biofuel production, paper and pulp industry, detergent industries, animal feeds among others.

3.1 Biomass Hydrolysis and Biofuel Production

Cellulase along with other enzymes is used in the hydrolysis of biomass into sugar and other chemicals. Sugar either hexoses or pentoses are then fermented to bioethanol or other fuel.

With the rapid increase in world population accompanied by increase demand of energy, depletion of fossil fuel, and enhanced greenhouse effect from traditional fuel, there is crucial need to develop or search for cheap, renewable and sustainable sources of energy. Thus cellulases involves in biofuel productions and minimization of energy crisis and environmental pollution (Horn et al. 2012, Sharada et al. 2014). However, the bioconversion of pretreated cellulose-based materials at the industrial level into fermentable sugars employ a mixture of enzymes for complete hydrolysis, of which the cost is very high, making biorefining processes economically unfeasible. Thus the search of biocatalysts such as cellulases with novel properties exemplified by high thermostabilty, acidophilicity and high solvent tolerance could help to overcome the cost hurdles. Cellulases application in biomass hydrolysis and biofuel productions is currently the subject of numerous studies supported by different agencies across the world (Budihal et al. 2016, Srivastava et al. 2015).

3.2 Paper and Pulp Industry

Cellulases are used in the paper and pulp industry which has expanded significantly in the last decades from 320 to 395 million tons (Przybysz Buzała et al. 2016). Pulping process can be achieved either through mechanical or biomechanical manners. Mechanical pulping such as refining and grinding of the woody raw material results in pulps containing high content of fines, bulk, and stiffness. On the other hand, biomechanical pulping employing enzymes such as cellulases results in around 20–40% energy savings during refining making the process economically feasible and significantly improved hand-sheet strength properties (Demuner et al. 2011, Sharada et al. 2014). It has also been reported that addition of cellulases enhanced the bleachability of softwood kraft pulp and improve the final brightness score comparable to that of xylanase treatment (Kuhad et al. 2011).

3.3 Waste Management

Cellulase can be used in waste management. For instance, cellulases are used in the conversion of cellulosic municipal solid wastes to desirable chemicals and energy. Cellulases benefits in minimizing the effect of cellulose waste on our environment and driving the conversion of the pollutants to an alternative source of energy and chemicals thus displacing our growing dependence on fossil fuels (Bayer et al. 2007, Gautam et al. 2011, Kuhad et al. 2011a).

3.4 Animal Feed Industry

Cellulase has a great potential to be used in the animal feeds industry. Cellulase can be used in the pretreatment of agricultural silage and grain feed to enhance nutritional value and performance of animals (Kuhad et al. 2011). Similarly, addition of cellulase, along with other enzymes, can eliminate anti-nutritional factors present in the feed grains such as arabinoxylans, cellulose, dextrins, inulin, lignin, pectins, β-glucan, and oligosaccharides by degrading them. This in turn enhances the nutritional value and improves animal's health and performance (Asmare 2014, Murad and Azzaz 2010, Sharada et al. 2014).

3.5 Laundry and Detergent Industry

Cellulases are also used in the laundry and detergent industry which is one of the most popular markets for enzymes sale accounting for 20-30%, with lipase and proteases are major enzymatic component. An innovative approach recently adopted in this industry is the use of alkaline cellulases, protease and lipase results in a crucial improvement of color brightness and dirt removal from the cotton blend garments (Juturu and Wu 2014, Olsen and Falholt 1998).

3.6 Textile Industry

The most successful and popular application of cellulases is textile industry. Cellulases are used in textile wet processing such as finishing of cellulose-based textiles, biostoning of jeans and biopolishing of cotton and other cellulosic fabrics in order to improve hand and appearance (Arja 2007, Duran and Duran 2000, Juturu and Wu 2014).

3.7 Wine and Beverage Industry

Cellulase enzymes along with glucanase can be used to improve both quality and yields of the fermented products such as wine and beverages. For examples, during wine production, cellulase, pectinases, glucanases, and hemicellulases are used to improve color extraction, skin maceration, must clarification, filtration, and finally the wine quality and stability. Addition of β-glucosidases can increase the aroma of wines by hydrolyzing glycosylated precursors into their aglycones and glucose (Araujo et al. 2008, Kuhad et al. 2011).

3.8 Other Applications

Cellulases have also been applied in agriculture where they are used to hydrolyze the cell wall of plant pathogens thus controlling the plant infection and diseases. Many cellulolytic fungi including *Trichoderma* sp., *Geocladium* sp., *Chaetomium* sp., and *Penicillium* sp. are known to play a key role in agriculture by enhancing the seed germination, rapid plant growth and flowering, improved root system and increased crop yields (Behera et al. 2016, Kuhad et al.

2011). Cellulases have also been used for the improvement of the soil quality (Phitsuwan et al. 2013). In addition, cellulases are used in food processing during fruit and vegetable juices manufacturing to improve extraction (Sharada et al. 2014, Zhang Xiao-Zhou and Zhang 2013). Furthermore, applications of cellulases along with macerating enzymes has been found to increase extraction of olive oil under cold processing conditions and to improve its antioxidants and vitamin E contents (Aliakbarian et al. 2011, Sharma et al. 2015). Moreover, humans is known to poorly digest cellulose fiber and taking a digestive enzyme product containing cellulases like Digestin help to relieve digestive problems such as malabsorption (Gurung et al. 2013, Sharada et al. 2014). Finally, an interest in applying cellulases enzymes in chemical analysis such as diagnostic and food analysis has been considered (Li et al. 2012).

4. Conclusion

The increase demand of energy and natural products combines with increase in the demand of industrial enzymes such as cellulases being critical enzymes in degradation of biomass and biofuel production. The major hurdle in the production of biofuel and other products from biomass is the lack of efficient economically feasible cellulase. Microorganisms represent a part of the solution of this problem because they can produce a robust set of enzymes to degrade biomass i.e., cellulase, hemicellulase and lignin degrading enzymes. Fungi have the advantage over other microbes because of their ability to secrete large quantities of biomass-degrading enzymes when grown on cheap substrates. Although remarkable work has been performed on production, characterization of cellulases from different fungi, future studies should focus on manipulation of cellulase research by gene and protein engineering to enhance the efficiency of biomass degradation and bioconversion. Application of statistical methods for optimizations of cellulase production on SmC and SSF from fungi is another frontier in cellulase research. Similarly, understanding transcription regulation of cellulase and other biomass degrading enzymes is significant to designing the best culture conditions for production of the best enzymes.

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