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Cellulases of Bacterial Origin and their Applications: A Review

Agrawal Shweta

Sanghvi Institute of Management and Science, Indore 452010, India

Abstract: ***The cellulosic biomass in the form of agricultural waste, is a renewable and abundant resource with great potential for bioconversion to value-added bioproducts . It can be degraded by cellulase produced by cellulolytic bacteria to more useful products, such as single-cell protein for use in animal feeds or ethanol for use as a fuel or chemical feedstock.. This enzyme has various industrial applications and therefore, there is resurgence in utilization of biomass for fuel production employing cellulases and hence forth in obtaining better yields and novel activities. Improving the economics of such processes will involve cost reduction in cellulase production which may be achieved by better bioprocesses and genetic improvement of cellulase producers to yield more of the enzyme. The review discusses application of cellulose and the types of cellulolytic bacteria.***

Keywords: Biofuel, Cellulase, Endoglucanase, Lignocellulose, Bacteria

1. Introduction

Cellulose is the major structural component of higher plants and is amongst the most abundant of organic molecules-about 7 x 10^{11} tonnes on our earth. Additionally, it is a renewable, chiefly unexploited and inexpensive resource with 4 X 10^{10} tonnes of annual production, as a result of photosynthesis (Coughlan, 1985). Agricultural residues for instance leaves, stems, and stalks from sources such as sugarcane bagasse, corn stover, corn fiber, rice straw, rice hulls, woody crops, and forest residues are a great source of lignocellulosic biomass. Above and beyond, these there are multiple sources of lignocellulosic waste from industrial and agricultural processes, like; citrus peel waste, coconut biomass, sawdust, paper pulp, industrial waste, municipal cellulosic solid waste and paper mill sludge. To add, dedicated energy crops for biofuels could include perennial grasses such as Switch grass and other forage feed stocks such as Miscanthus, Elephant grass, Bermuda grass, etc (Sadhu & Maiti, 2013).

A promising strategy for proficient utilization of this renewable resource is the microbial hydrolysis of lignocellulosic waste and fermentation of the resulting reducing sugars for production of desired metabolites or biofuel (Sukumaran et al., 2005). Contrastingly, cellulose is almost always found associated with other compounds, such as hemicelluloses, lignin and other polysaccharides, which further obscure its bioconversion. Therefore, there is dire need to develop cheaper and efficient pretreatment methods for exposing the cellulose in biomass easily accessible to cellulolytic attack (Robson and Chambliss, 1989).

Cellulases add up to 8% of the worldwide industrial enzyme demands. The cellulase market is anticipated to increase considerably with cellulases being used to hydrolyze pretreated cellulosic material to sugars, which can be fermented to bioethanol and biobased products on large scales. The cellulase market has been projected to be as high as US $ 400million per year, in the United States. In the period 2004 -2014 a boost of about 100 %, in the utilization of cellulase as a specialty enzyme, is anticipated. The biotechnology companies Genencor International and Novozymes Biotech have reported technological innovations resulting in the reduced cellulase cost for the cellulose-to-ethanol process, from US$ 5.40 per gallon of ethanol to approximately 20 cents per gallon of ethanol (Sadhu & Maiti, 2013).

2. Breakdown of Cellulose by Cellulases

Hydrolysis of cellulose by the enzyme cellulase involves hydrolysis of the glycosidic bonds connecting the β-D-glucosyl residues of the cellulose. The general architecture of cellulases features two discrete globular domains: a catalytic domain, accountable for the hydrolysis reaction itself and a cellulose-binding domain, with no catalytic activity, nevertheless enhancing adsorption of the enzyme onto insoluble macromolecular cellulose. In the native enzymes the two domains are connected together by a linker peptide (Fig. 3) (Sukumaran et al., 2005).

Mechanistically, cellulase is a family of 3 groups of enzymes, endo-(1,4)-β-D-glucanase (EC 3.2.1.4), exo-(1,4)-β-D-glucanase (EC 3.2.1.91) and β-glucosidases (EC 3.2.1.21). The exoglucanase (CBH) acts on the terminals of the cellulose chain and releases β-cellobiose as the end product; endoglucanase (EG) randomly attacks the internal O-glycosidic bonds producing glucan chains of different lengths and the β-glycosidases act specifically on the β-cellobiose disaccharides and produces glucose units.

Although the mechanism of cellulose degradation by aerobic bacteria is similar to that of aerobic fungi but anaerobic bacteria operate on different system. Cellulosomes located on the cell surface mediate adherence of anaerobic cellulolytic bacterial cells to the substrate, which thereafter undergo a supramolecular reorganization, so that the cellulosomal subunits redistribute to interact with the various target substrates (Kuhad et al., 2011 and Sukumaran et al., 2005).

2.1 Microorganisms producing Cellulases

Cellulolytic microbes are usually carbohydrate degraders and are by and large unable to use proteins or lipids as energy sources for growth. Cellulolytic microbes notably the bacteria *Cellulomonas* and *Cytophaga* can utilize a variety of other carbohydrates in addition to cellulose, while the anaerobic celluloytic species have a restricted carbohydrate range, limited to cellulose and or its hydrolytic products (Sukumaran et al., 2005) some of the crucial cellulose producing genera are discussed here:

2.2 Clostridia species

The members of this genus are strictly anaerobic to aerotolerant sporeforming bacilli found in soil as well as in normal intestinal flora of man and animals. Both gram-positive and gram-negative species exist in this genus (Wells et al, 1996). Various clostridia, including *Clostridium thermocellum*, *Clostridium cellulolyticum*, and *Clostridium stercorarium* produce cellulases. *Clostridium thermocellum* is a thermophilic anaerobe which possesses one of the best-characterized bacterial cellulase systems (Robson and Chambliss, 1989). *Clostridium acetobutylicum* and *Clostridium beijerinckii* are the most commonly used strains for converting hemicellulose sugars to butanol (Mussatto and Teixeira, 2010).

2.3 Cellulomonas species

The members of this genus are Gram-variable, mesophilic and nonsporeforming rods with high G + C content (72%). These organisms are capable of using crystalline celluloses for growth and produce extracellular cellulases. The cellulolytic species of the Cellulomonas genus include *C. fimi*, *C. uda*, *C. flavigena* and *C. fermentans*.

2.4 Bacillus species

Members of the Bacillaceae are Gram- positive, endospore-forming bacteria which are known to secrete an extensive array of enzymes, including amylases, proteases, β-glucanases, and hemicellulases. Thus far, these organisms have been found to produce only endo-β-1,4-glucanases and have not been capable of extensively degrading crystalline cellulose. The cellulolytic enzymes of *Bacillus* species are almost entirely extracellular and soluble in form. A variety of species of this genus, including strains of *B. subtilis*, *B. polymyxa*, *B. licheniformis* and *B. cereus*, secrete cellulases.

2.5 Thermomonospora species

The member s of this actinomycete genus are thermophilic, aerobic. A variety of species, including strains of *T. fusca* and *T. curvata,* secrete cellulases. Concurrent with growth, these organisms produce highly active extracellular cellulases that can degrade both amorphous and crystalline cellulose and that do not appear to be present in a complex. As yet, mainly endoacting β-1,4-glucanases have been reported and these appear to vary widely in their ability to hydrolyse the various cellulosic substrates (Robson and Chambliss, 1989).

2.6 Streptomyces species

Actinomycetes, are amongst one of the several acknowledged cellulase producers and has attracted extensive research attention due to their probable application in the recovery of fermentable sugars from cellulose that can be of benefitted to human consumption and to the ease of their growth. Streptomycetes are the largest and well-studied group of actinomycetes (El-sersy et al., 2010). Streptomycetes are Gram-positive, and have genomes with high GC content (Madigan et al., 2005). Three mesophilic species *Streptomyces flavogriseus*, *Streptomyces lividans*, *Streptomyces thermodiastaticus* (Crawford and Coy, 1972), *Streptomyces olivochromogenes* have shown cellulose production during growth on various substrates (MacKenzie et al., 1987)

2.7 Ruminococcus species

A predominant genus of rumen bacteria is Ruminococcus, some species of which can ferment highly ordered cellulose fibers. Reportedly, production of cellulase by *R. albus* and *R. flavefaeiens* is constitutive during exponential and stationary growth phases (Robson and Chambliss, 1989). *Ruminococcus albus*, *Ruminococcus flavefaciens* are also characterized for production of cellulosome-like complexes. Cellobiohydrolase activity is also apparently associated with the *Ruminococcus flavefaciens* cellulase complexes (Gilbert and Hazlewood,1993).

2.8 Bacteroides species

The mesophilic anaerobe *Bacteroides succinogenes* is another example of a highly cellulolytic rumen organism. Cellulase production in it also appears to be constitutive and is not repressed by glucose or cellobiose (Robson and Chambliss, 1989). The organism also produced a cell-bound cellobiase. *Bacteroides cellulosolvens* is characterized for producing cellulases with high specific activity but they do not produce high enzyme titres (Sun and Cheng, 2002).

2.9 Erwinia species

Members of this phyto- pathogenic genus are known to secrete a wide variety of hydrolytic enzymes, including pectinases, proteases and cellulases, which aid in the breakdown of plant cell walls. Currently, the best characterized Erwinia cellulolytic system is that of *E. chrysanthemi*, the causal agent of soft rot disease of plants .

2.10 Acetivibrio species

Acetivibrio cellulolyticus can use only cellulose, cellobiose and salicin for growth and therefore differs from most cellulolytic organisms. This Gram-negative mesophilic, anaerobic bacterium was relatively recently isolated from sewage sludge and was found to produce a largely soluble extracellular cellulase system capable of degrading crystalline forms of cellulose (Robson and Chambliss, 1989).

3. Applications of Cellulases

Several decades back, cellulases were initially investigated for the bioconversion of biomass which later gave way to research in the industrial applications of the enzyme in food, animal feed, textiles and detergents and in the paper industry. With the scarcity of fossil fuels and the growing need to find alternative sources for renewable energy and fuels, there is a rejuvenation of the interest in the bioconversion of lignocellulosic biomass using cellulases and other enzymes. There are numerous fields where the technologies and products using cellulases have reached the stage where these enzymes have become indispensable. Table 1 lists industrial applications of cellulose enzyme:

Table 1: Industrial applications of cellulose enzyme (Sukumaran et al., 2005 and Kuhad et al., 2011).

Industry	Applications
Textile Industry	• replacing the use of pumice stones for biostoning and release of excess indigo dye of denim garments to provide softness and faded look, , • digesting off the protruding fiber ends from the fabric for a better finish. • softening, defibrillation and processes for providing localized variation in the colour density of fibers
Laundry and Detergents	• improved cleanliness and color brightness and dirt removal • removal of rough protuberances in cotton fabrics; • antiredeposition of ink particles
Food and Animal Feed	• extraction and clarification of fruit and vegetable juices, • extraction of olive oil • enhanced malting of barley in beer manufacturing, • carotenoid extraction during the production of food colours. • enhancement in the nutritive quality of forages improving the feed conversion ratio and/or increasing the digestibility of a cereal-based feed. • better texture and quality of bakery products • increased viscosity of fruit purees; • increased texture, flavor and volatile properties of fruits and vegetables • controlled bitterness of citrus fruits. • release of the antioxidants from fruit and vegetable pomace.
Pulp and Paper Industry	• modification of the coarse mechanical pulp and hand sheet strength properties by biomechanical pulping • deinking of and removal of coating and toners from recycled fibers improving drainage and runnability of paper mills. • bio- characterization of pulp fibers • production of easily biodegradable cardboard • production of soft paper, paper towels and sanitary paper • removal of adhered paper • reduced energy requirement; reduced chlorine requirement; improved fiber brightness and pulp freeness and cleanliness.
Biofuel	• production of microbial substrates for the production of single cell proteins and lipids • production of fermentation products like ethanol, solvents, organic acids. • production of energy-rich animal feed and with improved nutritional quality ruminant performance, digestion and absorption • preservation of high quality fodder
Agriculture	• Control of plant pathogens and disease • generation of plant and fungal protoplasts • enhanced seed germination and improved root system • enhanced plant growth and flowering • enhanced soil quality and reduced dependence on mineral fertilizers
Fermentation	• increased malting and mashing; • improved pressing and color extraction from grapes • improved primary fermentation and quality of beer • enhanced must clarification and aroma of wines • superior viscosity, filtration rate and wine stability
Others	• reduction in biomass waste • production of hybrid molecules • production of designer cellulosomes.

4. Concluding Remarks

Microbial cellulases are now commercially producing by numerous industries globally and are being extensively used in variety of industries. These biological aspects of processing of cellulosic biomass become the essence of future researches involving cellulases and cellulolytic microorganisms. The problems which demand attention is not limited only to cellulase production, but involves a concerted effort in understanding the vital physiology of cellulolytic microbes and the implementation of this knowledge coupled with engineering principles to attain enhanced processing and utilization of this most profuse natural resource.

5. Future Prospects

The facets open to consideration in future include technologies for pre-treatment of cellulosic materials to facilitate microbial attack, processes for cost effective production of cellulases, treatment of biomass for production of hydrolytic products, which can then serve as substrates for downstream fermentative production of valuable metabolites, organism development by metabolic engineering. Improvements in cellulase activities or imparting of desired features to enzymes by protein engineering are probably additional areas where cellulase research has to advance.

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Author Profile

Ms. Shweta Agrawal is Assistant Professor at the Sanghvi Institute of Management and Science in Indore, India. She has a teaching and research experience of 9 years. Her research interests include microbiological bioremediation and biodegradation with deep insight into the enzymatic systems and metabolic pathways involved in bioremediation and biodegradation of pollutants.