Cellulases and Their Biotechnological Applications

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Abstract

For a long-range solution to the global issues of energy, chemical and food, the most abundant, renewable and sustainable bioresource cellulose could be a feasible solution. The depolymerisation of cellulose by a group of enzyme cellulases could potentially lead to the development of various value-added products. Due to their immense potential, cellulases are involved in various industrial and biotechnological applications related to pulp and paper, textile, fuel and other organic chemical synthesis industries. However, to further economise the cellulase production, extensive research is being carried out using various approaches including genetic manipulation and process engineering. In this chapter, a brief overview of cellulases and their potential applications are being discussed.

Keywords

Cellulase • Cellulose • Biotechnology • Pulp and paper industry • Textile industry • Fuel industry

Introduction

Cellulose is one of the most abundant, renewable and sustainable source of feedstock, which can be utilised for the development of various value-added products (Kuhad and Singh 1993, 2007; Kung et al. 1997; Kuhad et al. 2011; Gao et al. 2008). The annual production of cellulose has been estimated to be approximately 15×10^{12} t per year of the total biomass produced through photosynthesis. Structurally, cellulose is a fibrous, insoluble and a major crystalline polysaccharide constituent of plant cell walls, composed of repeating cellobiose units linked by β-1,4-glucosidic bonds (Jagtap and Rao 2005).

In nature, cellulose is used as a food source by a wide variety of organisms including fungi, bacteria, plants and protists as well as a wide range of invertebrate animals, such as insects, crustaceans, annelids, mollusks and nematodes (Watanabe and Tokuda 2001; Davison and Blaxter 2005).

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The complete cellulase system includes exo-β-1, 4-glucanases (EC 3.2.1.91), endo-β-1,4-glucanases (EC3.2.1.4) and β-1,4-glucosidase (EC 3.2.1.21) (Wilson and Irwin 1999). These enzymatic components act sequentially in a synergistic system to facilitate the breakdown of cellulose and the subsequent biological conversion to a utilisable energy source, glucose (Beguin and Aubert 1994).

Due to their immense potential, cellulases have been used in various industrial and technological applications. These include use of cellulases in deinking of paper waste, paper industry, textile industry, biopolishing and biostoning of denim jeans, food and feed industry, sugar and oligosaccharides production, biofuel production and production of other value-added commodities.

In recent years, fundamental and applied researches on cellulase enzyme have not only generated significant scientific knowledge but also have revealed their enormous potential in biotechnology, making significant advances towards the production and alteration technology of cellulase enzyme using several biotechnological approaches. A brief overview about cellulases, cellulolytic microbial strain improvement and their various biotechnological applications has been provided here.

Structure of Cellulose

Cellulose is a glucan polymer of D-glucopyranose units, which are linked together by β-1,4-glucosidic bonds. The cellulose has an average degree of polymerisation (DP) of at least 9,000–10,000 and possibly as high as 15,000. An average DP of 10,000 would correspond to a linear chain length of approximately 5 μm in wood. An approximate molecular weight for cellulose ranges from about 10,000 to 150,000 Da. Anhydrocellobiose is the repeating unit of cellulose. Coupling of adjacent cellulose chains and sheets of cellulose by hydrogen bonds and van der Waals forces results in a parallel alignment and a crystalline structure with straight, stable supramolecular fibres of great tensile strength and low accessibility (Demain et al. 2005; Nishiyama et al. 2003; Notley et al. 2004; Zhang and Lynd 2004). The cellulose molecule is very stable, with a half-life of 5–8 million years for β-glucosidic bond cleavage at 25°C. There are several types of cellulose in wood: crystalline and noncrystalline and accessible and non-accessible. Most wood-derived cellulose is highly crystalline and may contain as much as 65% crystalline regions. The remaining portion has a lower packing density and is referred to as amorphous cellulose. Accessible and non-accessible refer to the availability of the cellulose to water, microorganisms, etc. The surfaces of crystalline cellulose are accessible but the rest of the crystalline cellulose is non-accessible, whereas most of the noncrystalline cellulose is accessible but part of the noncrystalline cellulose is so covered with both hemicelluloses and lignin that it becomes non-accessible. Concepts of accessible and non-accessible cellulose are very important in moisture sorption, pulping, chemical modification, extractions and interactions with microorganisms.

Cellulases and Their Mechanism

Cellulases are generally defined as enzymes which hydrolyse the β-1, 4 glycosidic bonds within the chain that comprise the cellulose polymer. Fungal and bacterial cellulases significantly differ in their structure and functions. Fungal cellulases are composed of a carbohydrate-binding module (CBM) at the C-terminal joined by a short polylinker region to the catalytic domain at the N-terminal. The CBM is comprised of approximately 35 amino acid residues, and the linker region is a highly glycosylated region unusually rich in serine, threonine and proline amino acid residues (Divine et al. 1988). This linker region is also the site of proteolytic cleavage accomplished by several general serine proteases. Broadly, there are three types of cellulases:

1. Endoglucanase or carboxymethyl cellulase (E.C. 3.2.1.4)
2. Exoglucanase or cellobiohydrolase or filter paper cellulase (E.C. 3.2.1.91)
3. β-glucosidase or cellobiase (E.C. 3.2.1.21)

Unlike noncomplexed fungal cellulase, anaerobic bacteria possess complexed cellulase systems,

called cellulosomes (Doi and Tamaru 2001; Demain et al. 2005). The functional unit of cellulosome is scaffoldin, which contains cohesins, a cellulose-binding domain (CBD) or CBM; a dockerin, X modules of unknown function and an S-layer homology (SLH) module (Doi and Kosugi 2004). The cohesins are modules made up of ~150 amino acid residues and usually present as tandem repeats in scaffoldins. It has been demonstrated that the cohesins specifically show the interaction to the noncatalytic dockerin modules identified in cellulosomal complex (B´eguin et al. 1990, 1994; Ding et al. 2008; Fontes and Gilbert 2010). While dockerins consist of approximately 70 amino acids containing two duplicated segments (~22 amino acid residues). Dockerins are usually present in a single copy at the C terminus of cellulosomal enzymes. The first 12 amino acid residues in each segment resemble the calcium-binding loop of EF-hand motifs (helix-loop-helix motif) in which the calcium-binding residues, aspartate or asparagine, are highly conserved (Fontes and Gilbert 2010). These enzymatic subunits are bound to the scaffoldin through the interaction of the cohesins and dockerins to form the cellulosomes. The arrangement of the modules on the scaffoldin subunit and the specificity of the cohesin(s) and/or dockerin for their modular counterpart dictate the overall architecture of the cellulosome. This interaction (cohesion- dockerin) is species specific, i.e. the dockerins that are found in *Clostridium cellulolyticum* cellulosomal enzymes do not show interaction with the cohesins that are found in *C. thermocellum* and vice versa (Pages et al. 1997). Moreover, both cohesins and dockerins are highly homologous within the same species, and the residues directly involved in protein: protein recognition are highly conserved within a species.

Mechanism of Cellulases

As discussed in previous section, the structure and function of fungal and bacterial cellulases are quite different. The fungal cellulase system contains three major enzyme components: endoglucanase, cellobiohydrolase and β-glucosidase. The exoglucanase acts on the reducing ends of the cellulose chain and release cellobiose as the end product; endoglucanase randomly attacks the internal o-glycosidic bonds, resulting in glucan chains of different lengths; and the β-glucosidases act specifically on the β-cellobiose disaccharides and produce glucose (Beguin and Aubert 1994; Kuhad et al. 1997, 2010a, b, c) (Fig. 6.1).

There is a high degree of synergy between cellobiohydrolases (exoglucanases) and endoglucanases, which is required for the efficient hydrolysis of cellulose (Din et al. 1994; Teeri et al. 1998; Boraston et al. 2004; Gupta et al. 2009). The products of endoglucanases and cellobiohydrolases, which are cellodextrans and cellobiose, respectively, are inhibitory to the enzyme's activity. Thus, efficient cellulose hydrolysis requires the presence of β-glucosidases which cleaves the final glycosidic bonds producing glucose (end product). Typically, cellobiose and cellodextrins are taken up by the microorganism and internally cleaved via cellodextrin phosphorylases or cellobiose phosphorylases to create glucose monophosphate, which is energetically favoured. Some bacteria also produce intra- or extracellular β-glucosidases to cleave cellobiose and cellodextrins and produce glucose to be taken up by or assimilated by the cell. Mechanism of cellulose degradation by aerobic bacteria is similar to that of aerobic fungi, but it is clear that anaerobic bacteria operate a different system.

The major difference between fungal enzymes and cellulosomal enzymes is that the fungal enzymes usually contain a CBM for guiding the catalytic domain to the substrate, whereas the cellulosomal enzymes carry a dockerin domain that incorporates the enzyme into the cellulosome complex. Otherwise, both the free and cellulosomal enzymes contain very similar types of catalytic domains (Bayer et al. 2004).

The cellulosomes contain substrate-binding sites, which bind the cellulosome tightly to the substrate and concentrate the hydrolytic enzymes to specific sites (Doi 2008). CBMs play a key role in the deconstruction of complex insoluble composites exemplified by the plant cell wall. Initial studies by Bayer and their colleagues (1998) showed that the CBD contained a planar

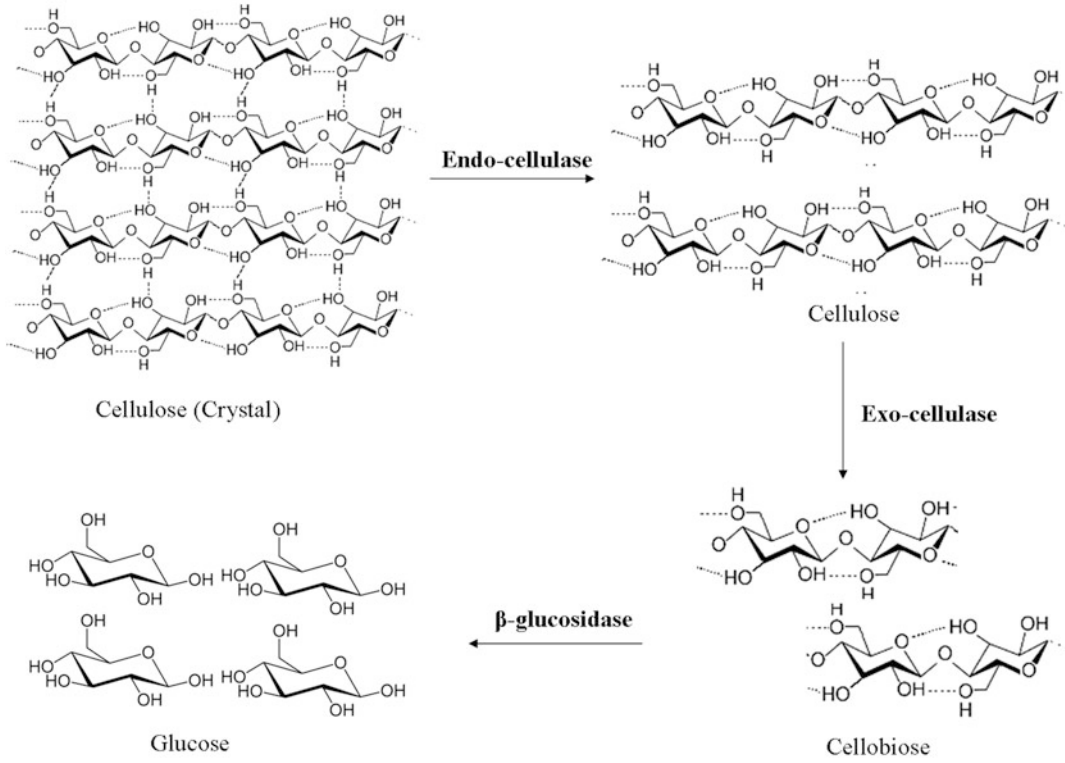

Fig. 6.1 Schematic representation of enzymatic hydrolysis of cellulose

configuration that interacted with the cellulose and involved the amino acids tryptophan, aspartic acid, histidine, tyrosine and arginine in binding the scaffoldin protein to cellulose. Upon binding to the substrate, the cellulosome complex undergoes a supramolecular reorganisation so that the cellulosomal subunits redistribute to interact with the different target substrates. For this purpose, the various cellulosomal enzymes include different types of CBMs from different families that exhibit appropriate specificities that complement the action of the parent enzyme (Bayers et al. 2004). The presence of a large variety of cellulosomal enzymes allows the cellulosome to degrade a wide variety of lignocellulosic materials (Maki et al. 2009).

Mechanistically, the reactions catalysed by all cellulases are suggested to involve general acid–base catalysis by a carboxylate pair at the enzyme active site, though different in structure. One residue acts as a general acid and protonates the oxygen of the o-glycosidic bond; at the same time, the other residue acts as a nucleophile. Depending on the distance between the two carboxylic groups, either inverting (~10 Å distances) or retaining (~5 Å-distances) mechanisms are observed in cellulases. Moreover, the involvement of multiple enzymes with a wide range of substrate specificities enables constant enzymatic actions on lignocellulosics.

Sources of Cellulases

Exploitation of cellulose depends on their efficient microbial degradation. A broad spectrum of cellulolytic microorganisms mainly fungi and bacteria have been identified over the years (Kuhad and Singh 2007). Moreover, the genetic material recovered directly from environmental samples has also shown the potential to exploit the novel cellulases trapped in the genomes of unculturable microbes. Different sources of cellulases are briefly described in this section.

Table 6.1 Cellulase-producing fungi

Microorganism	Reference	Microorganism	Reference
Acremonium cellulolyticus	Fang et al. (2008)	*Paecilomyces inflatus*	Kluczek-Turpeinen et al. (2007)
Agaricus arvensis	Jeya et al. (2010)	*Penicillium echinulatum*	Camassola and Dillon (2009)
Aspergillus niger NIAB 280	Hanif et al. (2004)	*Penicillium decumbens*	Sun et al. (2008)
Aspergillus terreus M11	Gao et al. (2008)	*Penicillium brasilianum*	Jorgensen and Olsson (2006)
Daldinia eschscholzii	Karnchanatat et al. (2008)	*Pleurotus ostreatus*	Membrillo et al. (2008)
Humicola grisea	Mello-De-Sousa et al. (2011)	*Phlebia gigantea*	Niranjane et al. (2007)
Lentinus tigrinus	Lechner and Papinutti (2006)	*Piromyces communis*	Kim et al. (2008)
Melanocarpus sp.	Kaur et al. (2006)	*Sclerotium rolfsii*	Ludwig and Haltrich (2003)
Monascus purpureus	Daroit et al. (2007)	*Scytalidium thermophilum*	Kaur et al. (2006)
Myceliophthora sp.	Badhan et al. (2007)	*Thermoascus aurantiacus*	Leite et al. (2008)
Mucor circinelloides	Saha (2004)	*Trichoderma atroviride*	Kovacs et al. (2008)
Neocallimastix frontalis	Srinivasan et al. (2001)	*Trichoderma reesei* RUT 30	Juhasz et al. (2005)
Orpinomyces sp.	Hodrova et al. (1998)		

Cellulases from Fungi

Fungi are well-known agents of decomposition of organic matter in general and of cellulosic substrate in particular; however, it is still unclear how broadly and deeply cellulolytic capability extends through the fungal world. Cellulase-producing fungi are widespread among fungi and include species from the ascomycetes (*Trichoderma reesei*), basidiomycetes (*Fomitopsis palustris*) and also few anaerobic species (*Orpinomyces* sp.) (Kuhad et al. 1994; Hodrova et al. 1998; Wyk et al. 2000; Srinivasan et al. 2001; Leite et al. 2008). Few cellulase-producing fungi are listed in Table 6.1.

Among fungi, soft rot are the best known for producing cellulases, and among them, *Trichoderma* has been the best characterised (Juhasz et al. 2005; Wen et al. 2005; Kovacs et al. 2008). Other well-known cellulase-producing soft rots are *Aspergillus niger, Fusarium oxysporum, Neurospora crassa,* etc. (Kuhad et al. 1997; Daroit et al. 2007; Gao et al. 2008; Sun et al. 2008). Besides soft rots, brown rot and white rot fungi are also actively involved in the cellulose degradation; however, both of these classes of fungi degrade wood by distinctly different mechanisms (Kuhad et al. 1994). Brown rot fungi depolymerise cellulose rapidly during the early decay of wood, and one reason may be the lack of exoglucanase (Kuhad et al. 1997). However, there are also few contrasting reports of exoglucanases-producing microbes. Recently, Deswal and coworkers (2011) have reported a brown rot fungus *Fomitopsis* sp. RCK 2010 having a good amount of all the three enzymes and have also shown the hydrolysis efficiency of pretreated lignocellulosic substrates. Besides *Fomitopsis* sp., other well-known cellulase-producing brown rots are *Poria placenta, Lenzites trabea, Coniophora puteana* and *Tyromyces palustris*. The cellulase-producing ability in white rots is heterogeneous. These microbes are most commonly known for lignin degradation. The common examples of cellulase-producing white rots are *Phanerochaete chrysosporium, Sporotrichum thermophile* and *Trametes versicolor*.

Anaerobic fungi also play a key role in the degradation of plant cell wall materials. They have the ability to degrade plant cellulose because they can produce an array of all the cellulolytic enzymes. Anaerobic fungi can only degrade the structural polysaccharides but cannot utilise the lignin moieties. Among anaerobic fungi, the most studied are *Neocallimastix frontalis* (Srinivasan et al. 2001), *Piromyces* (*Piromonas*) *communis* (Kim et al. 2008), *Orpinomyces* sp. (Hodrova et al. 1998), etc.

Table 6.2 Cellulase-producing bacteria

Source	Reference	Source	Reference
Acinetobacter junii F6-02	Lo et al. (2010)	*Butyrivibrio fibrisolvens A 46*	Hazlewood et al. (1990)
Anoxybacillus sp. *527*	Liang et al. (2009)	*Cellulomonas ANS-NS2*	Lo et al. (2009)
Acinetobacter anitratus	Ekperigin (2007)	*Cellulomonas biazotea*	Rajoka and Malik (1997)
Bacillus subtilis	Heck et al. (2002) and Kim et al. (2009)	*Clostridium thermocellum*	Chinn et al. (2008) and Dharmagadda et al. (2010)
Bacillus subtilis CBTTK 106	Krishna (1999)	*Clostridium cellulolyticum*	Desvaux et al. (2000)
Bacillus pumilus EB3	Ariffin et al. (2008)	*Clostridium acetobutylium*	Sabathe et al. (2002)
Bacillus amyloliquefaciens DL-3	Lee et al. (2008)	*Clostridium papyrosolvens*	Thirumale et al. (2001)
Bacillus licheniformis	Bischoff et al. (2006)	*Eubacterium cellulosolvens*	Moon and Anderson (2001)
Bacillus sp. *AC-1*	Li et al. (2008)	*Fibrobacter succinogenes S 85*	Bera-Maillet et al. (2009)
Bacillus sp. *DUSELR 13*	Rastogi et al. (2010)	*Geobacillus* sp. *WSUCF1*	Rastogi et al. (2010)
Bacillus circulans	Hakamada et al. (2002)	*Paenibacillus curdlanolyticus*	Waeonukul et al. (2009)
Bacillus flexus	Trivedi et al. (2011)	*Salinivibrio* sp. *NTU-05*	Wang et al. (2009)
Bacteroides sp.*P-1*	Ponpium et al. (2000)	*Ruminococcus albus F-40*	Ohara et al. (2000)

Bacteria

Cellulolytic bacteria often produce cellulases in small amounts, and degradation of cellulose seems to take place by a cluster of multienzyme complexes, which are difficult to disrupt without the loss of total activity (Kuhad et al. 1997; Doi 2008). Most of the bacterial cellulolytic enzymes are reported from *Bacillus* (Lee et al. 2008; Ariffin et al. 2008; Rastogi et al. 2010), *Acinetobacter* (Ekperigin et al. 2007; Lo et al. 2010), *Cellulomonas* (Rajoka and Malik 1997; Lo et al. 2009) and *Clostridium* (Chinn et al. 2008; Desvanux et al. 2000; Dharmagadda et al. 2010). Typically, aerobic bacteria play predominant roles in natural systems, accounting for 90–95% of bacterial cellulose degradation, the remaining 10% or less is degraded by diverse bacteria under anaerobic conditions (Carere et al. 2008). In addition to these, rumen bacteria have also shown to be producers of cellulase enzymes that can degrade structural components of cell walls (Kuhad et al. 1994). Among these, *Fibrobacter succinogenes* (Bera-Maillet et al. 2009) and *Ruminococcus albus* (Ohara et al. 2000) are most extensively studied. Recently, cellulolytic activity has been reported from thermophilic bacteria *Anoxybacillus* sp. (Liang et al. 2009), *Bacillus* sp. (Rastogi et al. 2010), *Geobacillus* sp. (Rastogi et al. 2010) and *Bacteroides* sp. (Ponpium et al. 2000). The list of few cellulase-producing bacteria is shown in Table 6.2.

Metagenomic Cellulolytic Genes

In addition to the culturable microbes, several metagenomic studies have also been carried out for the isolation of cellulase gene from various environmental samples (Ferrer et al. 2005; Palackal et al. 2007; Duan et al. 2009; Liu et al. 2009; Shedova et al. 2009; Wang et al. 2009). Ferrer et al. (2005) isolated seven new clones encoding β-1, 4-endoglucanase activity from cow rumen. Pottkamper et al. (2009) identified three novel cellulases that can degrade cellulose even in the presence of ionic liquids. Duan and coworkers (2009) isolated a novel endoglucanase C67-1, gene from buffalo rumen, which is very stable under both acidic (up to pH 3.5) and alkaline (up to pH 10.5) conditions. In another report, an endoglucanase Umcel5G, derived from rabbit cecum, was isolated which has the property to hydrolyse a wide range of substrates (Feng et al. 2007). Few studies on isolation of cellulase gene from metagenomic approaches are listed in Table 6.3.

Table 6.3 List of metagenomic sources of cellulases

Cellulase type	Source	Library type	Insert (kb)	Substrate	Reference
Endoglucanase	Anaerobic digester	Plasmid	12-Feb	CMC,MUC	Healy et al. (1995)
Endoglucanase	Lake sediment	λ phage	10-Feb	CMC	Rees et al. (2003)
Endoglucanase	Soil	Cosmid	25–40	CMC	Voget et al. (2003)
Endoglucanase	Lake sediment	λ phage	2.0–5.5	CMC	Grant et al. (2004)
Endoglucanase	Cow rumen	λ phage	5.5	OBR-HEC	Ferrer et al. (2005)
β-glucosidase	Soils from wetland	Fosmid	35	MUC	Kim et al. (2007)
Endoglucanase	Rumen fluid	λ phage	3	Dye-linked azo-xylan	Palackal et al. (2007)
Endoglucanase/β-glucosidase	Rabbit cecum	Cosmid	35.1	CMC,MUC, EH-FAC	Feng et al. (2007)
Endoglucanase	Hindgut of higher termite	Fosmid and plasmid	–	PASC	Warnecke et al. (2007)
Endoglucanase	Soil	Fosmid	–	CMC	Kim et al. (2008)
Endoglucanase/β-glucosidase	Soil, rumen	λ phage	5.3	CMC,MUC, EH-FAC	Wang et al. (2009)
Endoglucanase	Cow rumen	Plasmid	15	CMC	Shedova et al. (2009)
Endoglucanase/β-glucosidase	Compost	Cosmid	33	CMC	Pang et al. (2009)
Endoglucanase/β-glucosidase	Buffalo rumen	Cosmid	35	CMC,MUC, EH-FAC	Duan et al. (2009)
Endoglucanase/β-glucosidase/ Cellodextrinase					
Endoglucanase	Buffalo rumen	Cosmid	46.1	MUC	Liu et al. (2009)
β-glucosidase	Alkaline polluted soil	Plasmid	3.5	EH-FAC	Jiang et al. (2009)
Endoglucanase	Aquatic community and soil	Cosmid	–	CMC	Pottka¨mper et al. (2009)
β-glucosidase	Sludge	Cosmid	35	EH-FAC	Jiang et al. (2010)
Endoglucanase	Pot soil	Fosmid	40	CMC	Sita (2010)

Industrial Application of Cellulases

Cellulases have biotechnological potential in various industries, including food, brewery and wine, industrial waste to chemical feedstock, animal feed, textile and laundry, pulp and paper and agriculture, as well as in research and development of single-cell protein (Poutanen 1997; Bhat and Bhat 1997; Bajpai 1999; Bergqvist et al. 2005; Bamforth 2009; Kuhad et al. 2011).

Role of Cellulases in Food Industry

Cellulases play a prominent role in extraction of juice from a wide range of fruits and vegetables (Humpf and Schrier 1991; Sreenath et al. 1994; Bhat 2000; Bergqvist et al. 2005; Kuhad et al. 2011) (Table 6.4). Cellulases are used not only to improve the cloud stability and texture of nectars and purees but also to decrease their viscosity (Grassin and Fauquembergue 1996; Bhat 2000; Hui 2006). Cellulases are also used for food colouring agents production and in the extraction of olive oil and carotenoids (Grohman and Baldwin 1992; Faveri et al. 2008; Belitz et al. 2009). Moreover, cellulase is also used to alter the sensory properties of fruits and vegetables, by increasing their aroma and volatile characteristics (Humpf and Schrier 1991; Krammer et al. 1991; Dauty 1995; Bhat 2000; Hui 2006).

Role of Cellulases in Beer Industry

Beer brewing involves malting of the barley in a malt house followed by the preparation and fermentation of the wort in the brewery. Malting depends mainly on germination of seed, which initiates the biosynthesis and activation of

Table 6.4 Role of cellulases in food biotechnology

S. no.	Function	Application	Reference
1.	Hydrolysis of cell wall components; decreasing the viscosity and maintaining the texture of fruit juice	Improvement in pressing and extraction of juice from fruits and oil from olives; releasing flavour, enzymes, proteins, polysaccharides, starch and agar	Galante et al. (1998), Bergqvist et al. (2005), and Kuhad et al. (2011)
2.	Infusion of pectinase and glucosidase for easy peeling/firming of fruits and vegetables	Alteration of the sensory properties of fruits and vegetables	Krammer et al. (1991)
3.	Partial or complete hydrolysis of cell wall polysaccharides and substituted celluloses	Improvement in soaking efficiency; homogeneous water absorption by cereals; the nutritive quality of fermented foods; the rehydrability of dried vegetables and soups; the production of oligosaccharides as functional food ingredients and low-calorie food substituents and biomass conversion; extract of olive oil, Purees	Beguin and Aubert (1994), Bhat and Bhat (1997), Cinar (2005), and Faveri et al. (2008)
4.	Hydrolysis of arabinoxylan and starch	Separation and isolation of starch and gluten from wheat flour	Bhat (2000)
5.	Release of antioxidants from fruit and vegetable pomace	Controlling coronary heart disease and atherosclerosis; reducing food spoilage	Bhat (2000)

amylases, carboxypeptidase and cellulases which act in synergy under optimal conditions to produce high-quality malt. Therefore, the addition of cellulases is known to improve not only the beer qualities but also their overall production efficiency (Galante et al. 1998).

Role of Cellulases in Animal Feed Industry

Cellulases have a wide range of potential applications in animal feeding (Lewis et al. 1996; Bhat 2000; Knowlaton et al. 2007; Pariza and Cook 2010). Cellulases are the main class of enzymes used in monogastric feed and ruminant feed (Graham and Balnave 1995; Lewis et al. 1996; Kung et al. 1997). They can be used either to eliminate anti-nutritional factors present in raw materials or to degrade certain cereal components in order to improve the nutritional value of feed.

Role of Cellulases in Textile and Laundry Industry

The cellulases in textile industry are most commonly used for biostoning, biopolishing and biofinishing (Kirk et al. 2002; Lima et al. 2005; Ibrahima et al. 2010). The advantages of using cellulase-based biostoning are less labour-intensive, worn look, reduce damage, and create the possibility to automate the process (Galante et al. 1998; Pazarlioglu et al. 2005). While during biopolishing, the cellulases act on small fibre ends that protrude from the fabric surface, where the mechanical action removes these fibres and polishes the fabrics (Sukumaran et al. 2005). The cellulases remove short fibres and surface fuzziness, smoothen the appearance, remove the soil, improve colour brightness and increase hydrophilicity and moisture absorbance (Sukumaran et al. 2005; http://www.mapsenzymes.wm/enzymes_detergent.asp).

Role of Cellulases in Pulp and Paper Industry

Cellulase has been used in the pulp and paper industry for various purposes. The effect of enzymatic modification of coarse mechanical pulp using cellulase led to significant energy saving (Pere et al. 1996). Cellulases have also been used for the modification of fibre properties to improve drainage, beatability and runnability of the paper industry (Noe et al. 1986; Pommier et al. 1989, 1990). The cellulases have also been observed to

be the most effective for recycling the waste papers from books, magazines and newspaper which could have value addition via deinking and reuse of fibre either in manufacturing of newspaper or ethanol production (Kuhad et al. 2010a, b, c). The main advantage of enzymatic deinking is the avoidance of the use of alkali. Deinking, using enzymes at acidic pH, also prevents the alkaline yellowing, simplifies the deinking process, changes the ink particle size distribution and reduces the environmental pollution (Kirk et al. 2002; Kuhad et al. 2010a, b, c; Liu et al. 2010). In addition, the enzymatic deinking improves the fibre brightness, strength properties, pulp freeness and cleanliness as well as reduces fine particles in the pulp (Liu et al. 2009; Kuhad et al. 2010a, b, c).

Role of Cellulases in Agriculture Industry

Many cellulolytic fungi such as *Trichoderma* sp., *Geocladium* sp., *Chaetomium* sp. and *Penicillium* sp. are known to facilitate enhanced seed germination, rapid plant growth and flowering and increased crop yields (Bailey and Lumsden 1998; Harman and Bjorkman 1998; Bhat 2000; Fontaine et al. 2004; Wei et al. 2009). β-1,3-glucanase from *T. harzianum* CECT 2413 induced morphological changes such as hyphal tip swelling, leakage of cytoplasm and the formation of numerous septae and inhibited the growth of *Rhizopus solani* and *Fusarium* sp. (Benitez et al. 1998). Besides, they are also capable of degrading the cell wall of plant pathogens and controlling the plant disease. Cellulase is also used to improve soil quality and reduce dependence on mineral fertilisers (Escobar and Hue 2008; Han and He 2010).

Role of Cellulases in R&D Industries

Cellulases and related enzymes can also be used as potential tools for generating new strains capable of producing high levels of enzymes of commercial interest. Mixture of cellulases and other enzymes results in the solubilisation of fungal or plant cell wall to produce protoplast (Beguin and Aubert 1994). Cellulose-binding domains (CBD) of cellulases, which function normally when fused to heterologous proteins, have been successfully used either as an affinity tag for the purification of proteins or immobilisation of fusion proteins (Assouline et al. 1993; Greenwood et al. 1992; Tomme et al. 1995). Similarly, using the scaffoldin CBD of the *C. thermocellum* cellulosome, a novel affinity column was prepared for the purification of antibodies (Bayer et al. 1995).

Role of Cellulases in Biofuel Industry

A potential application of cellulases is the conversion of cellulosic material to glucose and other fermentable sugars, which in turn can be, used as microbial substrate for the production of single-cell protein or fermentation products like ethanol (Sukumaran et al. 2005; Kuhad et al. 2010a, b, c). Production of ethanol from renewable resources via fermentation represents an important process for production of alternative fuels (Sukumaran et al. 2005; Kuhad et al. 2010a, b, c, 2011). Ethanol has a unique combination of attributes including low life-cycle greenhouse gas emissions, a high level of sustainability, and seamless integration into the existing transport system with potential to have a large-scale impact (Ward and Singh 2002; Gupta et al. 2009; Kuhad et al. 2010a, b, c).

Cellulase in Pharmaceutical Industries

Since humans poorly digest cellulose fibre, taking a digestive enzyme product, like digestin, that contains cellulase enzymes could be important for healthy cells. Fungal hemicellulase and cellulase enzyme system helps in rapid hydrolysis of cellulose, hemicellulose and beta-glucan polymers in food. The gummy substances take up a lot of water and swell up to about ten times, thus hindering the action of enzymes on other biomolecules (http:/www.expresspharmaonline.com/20041028/biochemicals01.html).

Table 6.5 Applications of plant cell-wall-degrading enzymes and cellulolytic microorganisms in research and development as well as in agriculture

S. no.	Function	Application	Reference
1.	Solubilisation of plant or fungal cell walls	Production of plant or fungal protoplasts, hybrid and mutant strains	Beguin and Aubert (1994)
2.	Inhibition of spore germination, germ tube elongation and fungal growth	Biocontrol of plant pathogens and diseases	Lorito et al. (1994), Benitez et al. (1998), and Harman and Kubicek (1998)
3.	Affinity tag, affinity systems, conjugation and gene fusion	Affinity purification, immobilisation and fusion of proteins, enzymes and antibodies; production of hybrid molecules for various applications	Bayer et al. (1995)
4.	Exogenous cellulase accelerated decomposition of cellulose in soil	Soil fertility, plant growth	Han and He (2010)

Strategies for Cellulase Improvement

The use of cellulases for various applications demands their cost-effective production. Therefore, to improve cellulases titer and their ratios, various approaches like mutagenesis, genetic engineering and protein engineering have been used.

Mutation is one of the most commonly used approaches for the cellulase improvement (Durand et al. 1988; Anwar et al. 1996; Chand et al. 2005; Adsul et al. 2007). There are several reports where mutagenised strains have shown better properties over their parent strain. Chand et al. (2005) gave ETBr and 1-methyl −3-nitro-1-nitrosoguanidine treatment to *A. niger,* and the resultant strain *A. niger* CMV5-A10 exhibited twofold enhanced cellulase production. Similarly Adsul et al. (2007) increased the cellulase production twofold from EMS and UV-mutated *P. janthinellum* NCIM 1171. Though mutagenesis has improved the cellulase quality, but the instability of mutants due to reversion remains a big hurdle.

Moreover, cloning and expression of both bacterial and fungal cellulase genes in various hosts have also been attempted to improve the cellulase production (Kataeva et al. 1999; Abdeev et al. 2003; Park et al. 2005; Hong et al. 2009; Mekoo et al. 2010) (Table 6.5). Cloning of cellulases (endoglucanase and cellobiohydrolase) from *Clostridium* has been reported by several workers (Shima et al. 1989; Wang et al. 1993). A hyperthermophile cellulase from *Pyrococcus horikoshii* was successfully cloned and overexpressed in the *B. brevis* host vector system and enhanced the cellulase production by 20-fold (Kashima and Udaka 2004). Similarly, Park and coworkers (2005) have cloned a thermostable exoglucanase gene from *Streptomyces* sp. M23 in *S. lividans* TK-24 which was stable up to 100°C. In another report, Li et al. (2008) cloned a thermostable endoglucanase gene from *B. subtilis* in *E. coli* successfully with threefold increase in activity. While recently a novel, acid-tolerant endoglucanase from *Martelella mediterranea* a marine bacterium cloned and expressed in *E. coli* with unchanged properties (Table 6.6).

Similar to bacterial cellulases, cloning of fungal cellulases and expression in appropriate host have also been carried out since long (Table 6.5). Hamada and Hirohashi (2000) successfully cloned and characterised the exocellulase gene from white rot fungus *Irpex lacteus* using northern hybridisation. Haakana et al. (2004) cloned three genes (two endoglucanase and one CBH) from *Melanocarpus albomyces* and expressed in *T. reesei* under the control of the *T. reesei* CBHI promoter increasing the production level several times. While Hong et al. (2007) reported cloning of thermostable β-glucosidase from *T. aurantiacus* and expressed the β-glucosidase gene in *Pichia pastoris* and as a result, they developed recombinant yeast strain able to utilise cellobiose as a carbon source. Further, to improve the β-glucosidase yield and total cellulase activity of *T. reesei*,

Table 6.6 List of some recombinant cellulase-producing microorganisms

Microorganisms	Type	Cloning/expression vector	Cloning host	Reference
A. Bacteria				
Pectobacterium chrysanthemi	Glycosyl hydrolase	pBluescript II SK +	*E. coli*	Cho et al. (2002)
Sinorhizobium meliloti	CMCase	pUC 18, pet 22b	*E. coli*	Michaud et al. (2002)
Clostridium thermocellum	Endoglucanase	E35S-L-lic B	*Tobacco*	Abdeev et al. (2003)
Bacillus licheniformis	Endoglucanase	pBluescript SK(+)	*E. coli*	Liu et al. (2004)
Xylella fastidiosa	Endoglucanase	pet 20(b)	*E. coli*	Wulff et al. (2006)
Pseudomonas DY 3	–	pGEMT	*E. coli*	Zeng et al. (2006)
Cytophaga hutchinsonii	Endoglucanase	pGEM/pet 28 a	*E. coli XLB- Gold*	Louime et al. (2007)
Bacillus subtilis	Endocellulase	pGEMT/pet 28 a	*E. coli*	Li et al. (2008)
Myxobacter sp. *AL-1*	Cellobiohydrolase	pCR-Blunt II-TOPO	*E. coli*	Ramírez et al. (2008)
Bacillus subtilis	Cel L 15, Cel L73	pet 28 a	*E. coli*	Li et al. (2009)
Caldicellulosiruptor saccharolyticus	β-glucosidase	pet 28 a	*E. coli ER 2566*	Hong et al. (2009)
Martelella mediterranea	Endoglucanase	pUC 18/pGEX-6p-1	*E. coli*	Dong et al. (2010)
B. Fungi				
Thermoascus aurantiacus	CBH	λgt10 vector	*S. cerevisiae*	Hong et al. (2003)
Aspergillus aculeatus	Cellobiohydrolase		*A. oryzae*	Kanamasa et al. (2003)
Thermobifida fusca	Endoglucanase	pIJ699	*S. lividans*	Posta et al. (2004)
Melanocarpus albomyces	Endoglucanase	pALK1231	*T. reesei*	Haakana et al. (2004)
Talaromyces emersonii	β-glucosidase	lGEM-11	*E. coli*	Collins et al. (2007)
Penicillium chrysogenum	CBH	pGEM-T vector	*E. coli*	HOU et al. (2007)
Thermoascus aurantiacus	β-glucosidase	pPICZαvector	*P. pastoris*	Hong et al. (2007)
Irpex lacteus	Cellobiohydrolase	pUC119/PT7-Blue	*E. coli*	Toda et al. (2008)
Rhizopus stolonifer	CMCase	–	*E. coli*	Tang et al. (2009)
Chaetomium thermophilum	Cellobiohydrolase	–	*P. pastoris*	Li et al. (2008)
Penicillium sp.	Endoglucanase	pJAL721	*A. oryzae*	Krogh et al. (2009)
Neocallimastix sp.		pCT/pTRW10	*Lactococcus lactis*	Ozkose et al. (2009)
Penicillium echinulatum	Endoglucanase	pPIC9	*P. pastoris*	Rubini et al. (2009)
Oenococcus oeni	Phosphoglucosidase	pet 14 b	*E. coli*	Capaldo et al. (2011)
Penicillium decumbens	Endoglucanase	pMD18-T/ pAJ401	*S. cerevisiae*	Xiao- Min et al. (2010)
Trichoderma reesei	CBH,	pMI519	*Ashbya gossypii*	Ribeiro et al. (2010)
Trichoderma reesei	β-glucosidase, CBH	pMD18-T	*T. reesei*	Zhang et al. (2010)
Penicillium occitanis	CBH	*pMOSblue T-vector*	*Penicillium occitanis*	Bhiri et al. (2010)

extracellular β-glucosidase was overexpressed under the control of the modified four-copy CBHI promoter (Zhang et al. 2010).

In addition to genetic engineering strategies, protein-engineering approaches have also been used to improve cellulase quality. Escover-Kousen et al. (2004) observed 40% increase in cellulase activity on amorphous cellulose or soluble cellulose using integration of computer modelling and site-directed mutagenesis. Moreover, by combining 2 CBDs, one from *T. reesei* and other from *C. stercorarium,* Mahadeven et al. (2008) increased the activity by 14–18 folds. Recently, Scott et al. (2010) modified the linker peptides of cellulase to reduce its binding to lignin for enhanced cellulose hydrolysis.

Conclusion

In a world with a rapidly increasing population and approaching exhaustion of many natural resources, enzyme technology offers a great potential for many industries to help meet the challenges they will face in years to come. As outlined above, cellulases are used in several different industrial products and processes, and new areas of application are constantly being added. The use of recombinant gene technology has further improved manufacturing processes and enabled the commercialisation of enzymes that could previously not be produced. Furthermore, the latest developments within modern biotechnology, introducing protein engineering and directed evolution, have further revolutionised the development of industrial enzymes, which are opening new avenues for utilisation of various agrowastes as a source of renewable resources and could solve the problem of waste management as well.

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