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REVIEW
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# Bioprospecting thermostable cellulosomes for efficient biofuel production from lignocellulosic biomass

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**Abstract**

The adverse climatic conditions due to continuous use of fossil-derived fuels are the driving factors for the development of renewable sources of energy. Current biofuel research focuses mainly on lignocellulosic biomass (LCB) such as agricultural, industrial and municipal solid wastes due to their abundance and renewability. Although many mesophilic cellulolytic microorganisms have been reported, efficient and economical bioconversion to simple sugars is still a challenge. Thermostable cellulolytic enzymes play an indispensible role in degradation of the complex polymeric structure of LCB into fermentable sugar stream due to their higher flexibility with respect to process configurations and better specific activity than the mesophilic enzymes. In some anaerobic thermophilic/thermotolerant microorganisms, few cellulases are organized as unique multifunctional enzyme complex, called the cellulosome. The use of cellulosomal multienzyme complexes for saccharification seems to be a promising and cost-effective alternative for complete breakdown of cellulosic biomass. This paper aims to explore and review the important findings in cellulosomics and forward the path for new cutting-edge opportunities in the success of biorefineries. Herein, we summarize the protein structure, regulatory mechanisms and their expression in the host cells. Furthermore, we discuss the recent advances in specific strategies used to design new multifunctional cellulosomal enzymes, which can function as lignocellulosic biocatalysts and evaluate the roadblocks in the yield and stability of such designer thermozymes with overall progress in lignocellulose-based biorefinery.

**Keywords:** Cellulose hydrolysis, Cellulosomes, Scaffoldin, Nanomachines, Synergy

## Background

The progressive rise in energy crisis with the depletion of fossil-derived fuels has led to the search for other substitutive sources of energy, which are renewable, sustainable, economical, environment friendly and convenient. Energy from renewable biomass can be exploited as a potential alternative to fossil-derived fuels (Arora et al. 2015).

Biofuel production by biological conversion of lignocellulose is rising as a promising strategy, which can be considered as cost-effective, environmentally sustainable and alternative to non-renewables (Behera et al. 2014). The biochemical conversion of lignocellulosic biomass (LCB) comprises three main steps, viz. pretreatment, saccharification and fermentation. Many researchers have reported the production of biofuels using LCB (Kumar et al. 2010; Arora et al. 2014). However, despite the extensive research on bioconversion of cellulosic material, there are certain technological barriers. One of the major challenges is the efficient enzymatic hydrolysis of recalcitrant LCB (Stern et al. 2014). The breakdown of cellulose requires very high synergistic interactions among the cellulose-degrading enzymes (Behera et al. 2013). Commercial extraction of fermentable sugars from lignocellulosic material can be accomplished by the use of cellulases. However, the production of cellulases at the industrial level remains very challenging due to high cost. The high cost of cellulase production is attributed

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to several factors including the use of costly inducers such as cellulose and lactose and controlling the process by the dynamics of the induction-repression mode (Sukumaran et al. 2005). Appropriate amount of glucose is required in the medium for meeting the physiological needs of the cell and prevention of feedback inhibition of cellulases, which further requires expensive monitoring systems (Ju and Afolabi 1999). Another challenge for cellulase production is the development of bioprocess with cost-effective titer of cellulase using cheap media (Shahriarinour et al. 2011). Moreover, the problems with many cellulase-producing microorganisms are requirement of special culturing conditions and low product yield with secretion of some unwanted products (Lambertz et al. 2014). Further, saccharification with the conventional mesophilic microorganisms remains challenging due to high enzyme loading to meet the industrial needs, long incubation period and lesser mass transfer at lower temperatures (Stern et al. 2014; Rollin et al. 2011).

Recent studies mainly focus on the use of thermophilic/thermotolerant microorganisms and thermozymes due to their cost-effective catalytic process (Arora et al. 2014). Thermozymes are reported to be more stable at various environmental stresses with higher specific activities, longer half-lives, substrate solubility and more diffusion rates, thereby aiding in the process economy (Anbar et al. 2012; Wu and Arnold 2013). Mostly, anaerobic thermophilic microorganisms possess highly efficient enzymes that are structured in multienzyme complexes known as cellulosomes (Fontes and Gilbert 2010). Cellulosomal enzymes work synergistically without any inducer requirement and show very high level of regulation without being repressed by the product (Yamada et al. 2013).

Herein, the architecture and assembly of various subunits of cellulosomes along with gene regulation are discussed. This would provide a comprehensive update of the physiology of cellulosome-producing microorganisms. Furthermore, specific strategies in the construction of designer cellulosomes are discussed. The aim of this review article is to explore the latest advances in cellulosomics and their indispensible role in the biomass-based refineries.

## Thermostable cellulase family

The tightly packed structures of cellulose and its cross-linking with hemicellulose and lignin make the process of cellulose degradation very slow and costly (Behera et al. 2014). Hydrogen bonding between the chains makes the structure of lignocellulosic biomass very rigid and recalcitrant (Yamada et al. 2013). Thermostable cellulases are characterized by hydrophobic protein core and polarity at the surface; compact structure with lesser internal voids; higher content of proline and lesser content of asparagines, glutamine, methionine and cysteine; increased level of H-bonding, isoelectric points and salt bridges (Taylor and Vaisman 2010; Li et al. 2011).

The cellulolytic enzymes are categorized into three main groups: endoglucanases, exoglucanases and β-glucosidases with scientific nomenclature as (E.C. 3.2.1.4), (E.C. 3.2.1.176) and (E.C. 3.2.1.21), respectively (Garvey et al. 2013; Juturu and Wu 2014). The cleavage of internal bonds of the cellulose chain is catalyzed by endoglucanases (endo-1,4-β-glucanases) that catalyze randomly, while the ends of the chain are cleaved by the exoglucanases (exo-1,4-β-glucanases), which release cellobiose. Further, the breakdown of bonds of cello-oligosaccharides and cellobiose is catalyzed by β-glucosidases which release the glucose units (Kumar et al. 2008; Prasetyo et al. 2011).

Cellulase activity is mostly found in *Clostridiales* (anaerobic) and *Actinomycetales* (aerobic), respectively. Both aerobic and anaerobic microorganisms have different modes of action as discussed below.

### Cellulose hydrolysis by aerobic microorganisms

Aerobic microorganisms do not adhere to cellulose; instead, they secrete extracellular enzymes which initiate the hydrolysis resulting in the formation of cellodextrins. These cellodextrins are then transferred inside the cell where they are oxidized to carbon dioxide and water (Lynd et al. 2002; Horn et al. 2012).

### Cellulose hydrolysis by anaerobic microorganisms

Anaerobic bacteria adhere to the cellulose fibers by a unique structure known as cellulosome and then release cellulolytic enzymes which cause hydrolysis of cellulose to cellodextrins. Adhesion to cellulose can also occur via fimbriae, pili or carbohydrate epitopes of bacterial glycocalyx (Sadhu and Maiti 2013). Some of the hydrolyzed molecules enter into the cell where they undergo fermentation. The products of fermentation are used for cross-feeding non-cellulolytic bacterium. Other cellodextrin molecules formed by hydrolysis are effluxed out of the cells and are used by other non-adherent cellulolytic bacterium. *Clostridium thermocellum* is a highly efficient bacterium known for degradation of cellulose with the help of its cellulosome (Lamed et al. 1983).

## Cellulosomes: futuristic approach for cellulose degradation

Extremely efficient nanomachines, known as cellulosomes, are produced by many anaerobic and ruminal microorganisms to degrade the complex polysaccharides of plant cell wall and crystalline cellulose (Hyeon et al. 2013). Such supramolecular complexes were first discovered in thermophilic bacterium *C. thermocellum.*

Cellulosomes are multienzyme complexes held together by the high affinity of interaction among its subunits (Gefen et al. 2012). Major functions of cellulosomes include a high rate of substrate uptake, tight and specific interaction with the substrate, synergistic activity and processivity of the enzymes (Desvaux 2005). Cellulosomic complexes have been investigated in many other species including *Clostridium acetobutylicum*, *C. josui*, *C. cellulovorans*, *C. papyrosolvens*, *C. cellulolyticum*, *C. clariflavum*, *Bacteroides cellulosolvens*, *Ruminococcus albus*, *R. flavefaciens* and *Acetivibrio cellulolyticus* (Cho et al. 2010; Hemme et al. 2010; Karpol et al. 2013; Smith and Bayer 2013; Ichikawa et al. 2014).

The complex architecture of cellulosomes enables various enzymes attached to act in a coordinated way and increase the efficiency of the enzymes through synergistic interactions (Morais et al. 2010; Chanal et al. 2011). The enzymes in the cellulosomes are positioned in close proximity, so that the whole system can act synergistically for depolymerisation of cellulose (Hyeon et al. 2011; Mazzoli 2012).

The numbers of cellulosomal enzymes vary from species to species in different microorganisms with different catalytic mechanisms and substrate specificities (Morais et al. 2010; Bayer et al. 2008; Doi 2008). In case of polycellulosomes, more than 100 subunits have been reported (Bras et al. 2012). The assembly of various cellulosomal proteins has been exclusively studied in *C. thermocellum* (Belaich et al. 1997; Bayer et al. 2004; Ding et al. 2012).

The hydrolysis of the polymers of plant cell wall depends upon the extent of interaction between various subunits of cellulosomes (Tamaru et al. 2010). The synergistic interaction between various subunits of cellulosomal enzymes with the substrate results in the formation of cellobiose from cellulose, thereby leading to efficient degradation of complex biomass (Fontes and Gilbert 2010).

## Cellulosome structure and assembly

Cellulosomes are well-organized nanomachines playing an elementary function in the breakdown of complex polymers of cell wall. The cellulosome is composed of both structural and catalytic subunits consisting of scaffoldin, cohesion and dockerin, carbohydrate-binding module (CBM), surface layer homology domain and catalytic enzymes (Mazzoli et al. 2012). The assembly of different components of cellulosomes on the surface of the cell is shown in Fig. 1. These subunits are held together by highly specific type I and type II interactions between the cohesins and the dockerins (Bayer et al. 2004). Type I interactions are the interactions between dockerins and scaffoldin cohesions. In addition to this, scaffoldin dockerins interact with cohesins on the cell surface. Such interactions are termed as type II interactions (Smith and Bayer 2013). Different components of cellulosomes are discussed below.

### Scaffoldin

Scaffoldin subunit is an anchoring protein which has a dual function of incorporating various enzymatic subunits through the tight interactions between the cohesin and dockerin moieties and binding to the substrate with the help of carbohydrate-binding module (CBM) (Mazzoli et al. 2012; Himmel et al. 2010). In some microorganisms, multiple scaffoldins have been reported where a primary scaffoldin is anchored by a secondary scaffoldin subunit for amplifying the catalytic moieties onto the surface (Doi 2008).

### Cohesin and dockerin

The particular sequence of cohesin and dockerin represents the signature sequences of the cellulosomal protein (Shoham et al. 1999). The assembly of whole cellulosomal subunit depends upon the affinity of interaction between dockerin domains having duplicated sequences and cohesin domains containing repeated sequences, as this interaction provides the most favorable arrangement for the enzymes to act efficiently on the substrate (Jeon et al. 2012). The heterogenous nature of cellulosomes is due to the variability in the interaction of cohesin–dockerin moieties with difference in the amount of cohesin repeats, nature of enzymes bound to the scaffolding protein and species-specific variations (Bomble et al. 2011; Koukiekolo et al. 2005; Doi and Kosugi 2004; Fierobe et al. 2005).

Dockerins are symmetrical molecules usually present at the C-terminus with two duplicated segments of approximately 22 amino acids (Fontes and Gilbert 2010). Calcium plays a very critical role in the functioning and stability of dockerin molecules (Karpol et al. 2013). Each of the duplicated segments contains calcium-binding residues (asparagines or aspartate) which are highly conserved in the first 12 residues (Fontes and Gilbert 2010). The tight interaction between dockerin and cohesin is dependent upon calcium, which binds with the dockerin and brings about folding and conformational changes (Adams et al. 2005). Chen et al. (2014) investigated the nuclear magnetic resonance (NMR)-derived structure of dockerin module from Cel48S of *C. thermocellum* and concluded that the dockerin moiety does not undergo structural alterations induced by cohesin, but assumes a calcium-dependent cohesin-primed conformation.

### Carbohydrate-binding module

A carbohydrate-binding module (CBM), belonging to family 3a, is present on the non-catalytic scaffolding protein

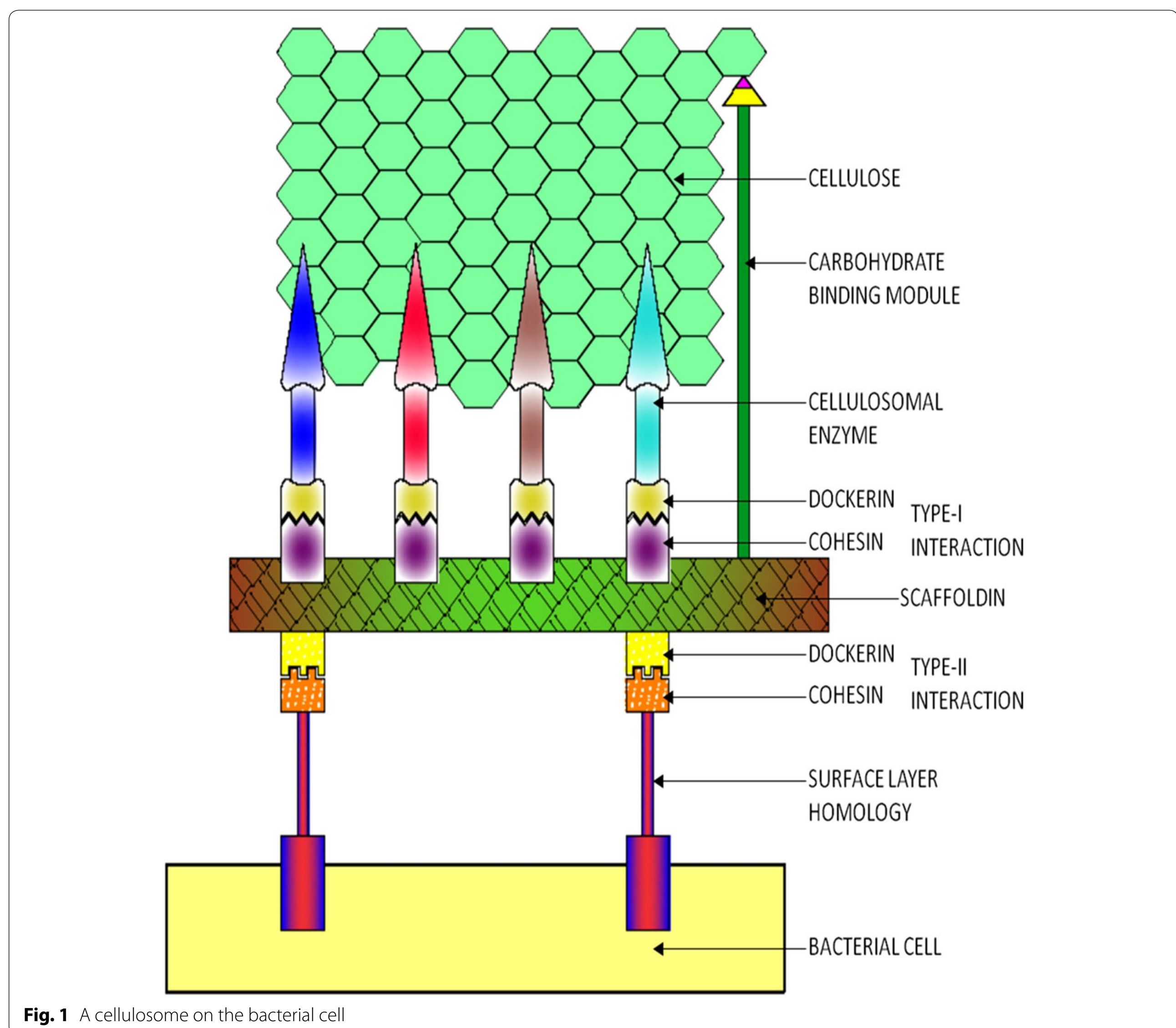


**Fig. 1** A cellulosome on the bacterial cell

and aids in the recognition, attachment and effective degradation of cellulose by disrupting the crystal surface at the solid–liquid interface (Ichikawa et al. 2014; Lavan et al. 2009). This domain is non-catalytic by nature, but it plays a significant role in the enzymatic activity of the cellulosomal enzymes. The binding of CBM with the cellulosic substrate nullifies the hydrogen bonding and brings the cellulosomal enzymes close to the cellulose, thus making the breakdown more efficient compared to the free enzymes (Lynd et al. 2002; Tamaru et al. 2000; Caspi et al. 2008).

CBMs have been categorized into three different categories according to their substrate range, viz. type A, type B and type C which bind to insoluble polysaccharide surfaces, soluble glycan chains and smaller saccharides, respectively. Several researchers have reported various modifications in the activity and composition of various subunits of cellulosomes to increase the interaction between the target substrate with one or more CBMs (Elkins et al. 2010; Raman et al. 2009). The nature of amino acids present at the CBM determines the specificity of binding with the substrate. For example, the presence of polar and aromatic amino acids in CBM (of CBM3a family) of CbpA protein allows the preferential binding of *C. cellulovorans* to crystalline cellulose rather than amorphous cellulose (Boraston et al. 2004; Yaniv et al. 2012).

#### Surface layer homology domain

The anchoring of cellulosome to the cell surface is made by surface layer homology (SLH) domain through

non-covalent interactions (Bayer et al. 2008; Tamaru et al. 2000). The SLH forms a protein layer outside of the cell wall, which is highly conserved in the cellulosomal bacteria (Desvaux et al. 2006). The scaffolding protein CbpA contains repeated domains with bacterial SLH homology-related anchoring functions in *C. cellulovorans* (Tamaru et al. 2000).

### Cellulosomal enzymes

The catalytic domains, i.e., cellulosomal enzymes are assembled by their dockerin moieties linked to cohesin domains of scaffoldin by calcium dependent interactions (Cho et al. 2010; Ding et al. 2008). Localization of broad substrate range enzymes and their close proximity for efficient formation of enzyme–substrate complex are the significant factors for increasing the efficiency of the cellulosomes. Cellulosomes are known to have better enzymatic activity as compared to free enzymes because of the close proximity of various cellulases which act synergistically (Blanchette et al. 2012; Krauss et al. 2012; You et al. 2012a). However, the mechanism of protein assembly and organization of various catalytic enzymes in the cellulosomes is partially known (Wilson 2011). The complex architecture of the cellulosomal complex helps to minimize the diffusion of several smaller saccharides and facilitate their easy and fast uptake by glycosidases for complete hydrolysis (Vodovnik and Logar 2010). The cellulosomal microorganisms can vary the composition of various catalytic subunits according to the substrate available to them (Cho et al. 2010; Blouzard et al. 2010; Tsai et al. 2010). A variety of cellulosomes with variable composition can be assembled on a single species with various enzymes bound to the scaffolding protein (Bomble et al. 2011).

## Cellulosomal gene clusters and their regulation

The mechanism of gene expression has been widely studied in *C. thermocellum*. The genes encoding the cellulolytic enzymes are generally clustered on the chromosomes in cellulosome-producing microorganisms (Lynd et al. 2002). The expression of cellulosomal genes is mediated by a set of anti-σ factors and their cognate alternative-σ factors present in the genome of *C. thermocellum* (Nataf et al. 2010). Anti-σ factors are multimodular proteins present with intracellular domain for σ factor, transmembrane domain and an extracellular domain having CBM or other sugar-binding modules. The binding of CBM to the substrate triggers a change in the conformation of anti-σ factors, thereby releasing the alternative-σ factors bound to it. This released alternative-σ factor finally initiates the transcription of cellulosomal genes by interacting with RNA polymerase (Nataf et al. 2010).

An equivalent set of alternative and anti-σ factors have been reported in *Bacteroides thetaiotaomicron* and *Acidothermus cellulolyticus* (Nataf et al. 2010). But till date, very little information has been available about the molecular mechanism of cellulosomal genes in other species and efforts are being made to understand the physiology and expression of these genes in other species for their successful exploitation in biorefineries.

## Challenges of cellulosomes

The current research on biofuel production deals with the technological barriers of economical conversion of lignocellulosic biomass to fermentable sugars. However, the high cost of the enzymes is the driving force for the search of other substitutes for efficient hydrolysis of the complex biomass. Moreover, the production of cellulosomes in native microorganisms is inadequate for industrial-scale support (Stern et al. 2014). The efficient enzymatic hydrolysis is dependent on the set of enzymes on the cellulosome, with the most effective enzyme composition being dependent on the respective substrate (Blouzard et al. 2010; Chundawat et al. 2011). Another obstacle is difficult and costly cultivation of native cellulosome-producing bacteria due to requirement of anaerobic conditions (Lambertz et al. 2014).

In view of this, efforts are being made to artificially construct the cellulosomes to depolymerize the cellulosic substrate to simple fermentable sugars for biofuel production. Moreover, with increase in understanding the physiology and mechanism of gene regulation of cellulosomes, efforts are being made for the expression of cellulosomal complexes in recombinant microorganisms (Nataf et al. 2010). During the past few years, efforts have been made for the construction of "designer cellulosomes", i.e., artificial complexes that could efficiently depolymerize cellulose. The construction of designer cellulosomes is very challenging, but this technology can be efficiently improved and utilized in the biorefineries (Fontes and Gilbert 2010).

## Alternative-designer cellulosomes

The concept of designer cellulosomes was first suggested by Bayer et al. (1994) and the first attempt of construction and utility was accomplished by Fierobe et al. (2001). Designer cellulosomes are artificially constructed complexes of enzymes, generally equipped with a dockerin module that interacts with cohesin module with high specificity and affinity and degrades the polymeric cellulose efficiently (Vazana et al. 2012). The arrangement of different cellulosomal subunits enables controlling the composition and spatial constraints, thereby increasing the efficiency of the whole system (Bayer et al. 2007). Hence, the disruption of the chain in the cellulose is the

rate-limiting step rather than the cleavage of the glycosidic bond, thereby rendering the substrate easily accessible to enzyme for the hydrolysis.

Designer cellulosomes have been constructed using different approaches such as construction of a shortened scaffoldin with cohesins of corresponding specificity, construction of chimeric scaffoldin with different cohesins and addition of dockerin moieties to enzymes (Ding et al. 2008). Apart from the cellulosomal enzymes, various free enzymes from *Thermobifida fusca* and *Neocallimastix patriciarum* can also be exploited for the construction of cellulosomes (Caspi et al. 2008). A CBM domain is constructed on the artificial scaffoldin, which serves as the harboring backbone. The construction and various components of designer cellulosomes are shown in Table 1.

In the design of chimeric scaffoldin, the arrangement of different cohesin subunits and distance between them are maintained by the linker segments (Vazana et al. 2012; Hendrix et al. 2013). The linker regions are accountable for the plasticity and catalytic efficiency of the cellulosomes by enabling the spatial arrangement of the catalytic modules to adjust according to the substrate and allow the enzymes to act synergistically (Garcia-Alvarez et al. 2011; Molinier et al. 2011). The amino acid residues in the linker segments can vary from 5 to 8 residues as in *C. cellulolyticum*, *C. acetobutylicum*, *C. cellulovorans* and *C. josui* to 20–40 residues as in *B. cellulosolvens*, *R. flavefaciens, A. cellulolyticus* and *C. thermocellum* (Bayer et al. 2009). Vazana et al. (2013) investigated the spatial organization of the scaffoldin subunit along with their effects on hydrolysis of cellulose by designing trivalent designer scaffoldins with CBM and three divergent cohesin modules and concluded that long intermodular linkers provide better flexibility and spatial positioning of the attached enzyme in the cellulosome complex. Molinier et al. (2011) investigated the association between the conformational flexibility and the inter-modular linkers of scaffold protein of cellulosomes and reported that specific inter-cohesins sequence or distance was not required to induce proximal synergy.

Construction of cohesin domains in the designer cellulosome from various species allows incorporation of the desired dockerin-bearing enzymes, which could interact synergistically with efficient hydrolysis of the complex substrate (Morais et al. 2010; Caspi et al. 2008; Vazana et al. 2010).

Another important aspect in the construction of designer cellulosomes is the surface display of cellulosomal proteins. Anchoring of cellulosomal enzymes on the surface protects them from the proteases and thermal degradation of the host cells (Wieczorek and Martin 2010), which makes such cellulosomes highly desirable in recombinant microbes (Hasunuma and Kondo 2012). Surface display also promotes synergism and specific activity in different enzymes attached due to very close proximity (Schwarz 2001) and reduces the amount of total enzyme added to the bioreactor (Matano et al. 2012a). Moreover, surface display aids in higher yield due to prevention of irreversible desorption of the enzyme from its substrate (Bayer et al. 1994; Schwarz 2001; Matano et al. 2012b; Tanaka and Kondo 2015). The surface-displayed enzymes work synergistically to hydrolyze cellulose to cello-oligosaccharides and then to glucose, which is very close to the cell surface and is immediately taken up by the fermenting microorganism instead of diffusing out into the medium, thereby increasing the yield (Yamada et al. 2013). Since the fermentable sugars released are easily transported inside the cells for subsequent metabolic pathways, the level of glucose in the medium remains low, thereby decreasing the chances of contamination. Several mechanisms for the surface display of protein include covalent interactions with long-chain fatty acids and covalent or non-covalent interactions with the cell wall through linkage to the cell membrane or via transmembrane domains (Desvaux et al. 2009). The microorganisms can also be modified to secrete the cellulosomal components, which cannot be anchored on the cell surface (Fan et al. 2012; Goyal et al. 2011; Kim et al. 2013; Tsai et al. 2009).

However, apart from various advantages, surface display also suffers from few shortcomings. The amount of cellulosomal enzymes to be displayed is dependent on the available surface area (Yamada et al. 2013). Also, the unifunctional surface-displayed cellulosomes are not capable of 2-D diffusion (Tanaka and Kondo 2015). Apart from this, the cohesin–dockerin interactions are species specific, which restricts their utilization over a narrow range. Another problem with the surface-displayed cellulosomes is the effective cellulosomal assembly over the cell surface and higher specific activity of the enzymes.

Keeping in view these weaknesses, many researchers have worked on few modifications in surface-displayed cellulosomes to improve their potential. Wen et al. (Wen et al. 2010) spatially distributed the minicellulosomal complexes on the yeast cell surface for 2-D diffusion and reported an ethanol yield of 0.31 g $g^{-1}$ on phosphoric acid-swollen cellulose (PASC) consumed. To overcome the narrow range of species-specific interactions, a combination ratio of each cell type in the consortium may be optimized, where each cell type displays an effective overall cellulosome assembly in the synthetic consortium. Tsai et al. (2010) optimized the mixing ratio of different populations and constructed a synthetic consortium expressing different components of cellulosomes for the conversion of amorphous (phosphoric

**Table 1 Construction of synthetic cellulosomes**

| New component/cellulosome | Scaffoldin | Enzymes/(source) | Mode of construction | Substrate | Saccharification rate | References |
|---|---|---|---|---|---|---|
| Chimeric CBM3-CglT | *C. thermocellum* scaffoldin | β-glucosidase (CglT)/(*T. brockii*) | Fusion of CBM3 from scaffolding protein Cip A into N-terminal region of CglT | Rice straw | 70 % | Waeonukul et al. (2013) |
| Nanocluster | CdSe–ZnS core–shell quantum dots QDs | Cel A/(*C. thermocellum*); Cel E/(*C. cellulolyticum*) | Metal affinity between core–shell QDs and polyhistidine tag | n.d. | n.d. | Tsai et al. (2013) |
| Mini-cellulosome | Mini-scaffoldin (mini-Cip A) | Endoglucanase/(*B. subtilis*); processive endoglucanase/(*C. thermocellum*); cellobiohydrolase/(*C. phytofermentans*) | High-affinity interaction between three cohesins | Avicel | n.d. | You et al. (2012b) |
| Nanocluster | Polystyrene nanospheres | Cellulase/(*T. viride*) | EDC and sulfo-NHS coupling chemistry | n.d. | n.d. | Blanchette et al. (2012) |
| Hybrid scaffoldin (Scaf 4) | Parental scaffoldin Scaf 4 protein | Cel48F/(–); Cel9G/(–) | Length and composition of inter-module linker connecting *C. thermocellum* and *C. cellulolyticum* cohesin modified | Crystalline cellulose | 2-fold increase | Molinier et al. (2011) |
| Functional minicellulosome in *Corynebacterium glutamicum* | Mini CbpA | Chimeric endoglucanase/(*C. cellulovorans*) | Fusion of endoglucanase E from *C. thermocellum* with endoglucanase from *C. cellulovorans* | CMC | 2.8-fold increase | Hyeon et al. (2011) |
| Scaffoldin-linked exoglucanase | SP1 scaffoldin protein | Cel6B exoglucanase/(*T. fusca*) | Fusion of chimeric form of enzyme to a cellulosomal dockerin module | n.d. | n.d. | Morais et al. (2010) |
| Rosettasome | Group II chaperonins from the hyperthermoacidophilic archaeon *S. shibatae* | Cel9B, Cel9k, Cel9R, Cel48S/(*C. thermocellum*) | Fusion of cohesin module from *C. thermocellum* to circular permutant of rosettasome subunit | n.d. | n.d. | Mitsuzawa et al. (2009) |
| Designer nanosome | Chimeric scaffoldin | Cel A, Cel F/(*C. cellulolyticum*) | Cohesion–dockerin interaction | n.d. | n.d. | Fierobe et al. (2001) |

*n.d.* Not defined

acid-swollen) cellulose to ethanol with 93 % of maximum theoretical yield. A 4.2-fold increase in the hydrolysis of phosphoric acid-swollen cellulose (PASC) was reported with a tetravalent cellulosome expressed on the surface of the yeast cell as compared to the free enzymes (Tsai et al. 2013). Ito et al. (2009) developed recombinant yeast having a designer minicellulosome system displayed on its surface by assembling different components of the cellulosomal system *C. cellulovorans*, *T. reesei* and *A. aculeatus* for the hydrolysis of β-glucan. Similar studies were reported by Yanase et al. (2010a, b) for the surface display of cellulolytic enzymes in *K. marxianus* on β-glucan for ethanol production. You et al. (2012a) reported that efficient digestion of low- accessibility cellulose in the pretreated biomass could be raised up to 90 % by constructing synthetic cellulosomes. Further, type I and type II interactions may be exploited for effective cellulosomal assembly. Fan et al. (2012) utilized type I and type II cohesin–dockerin interactions to construct minicellulosomes and mediate the anchoring of cellulosomes onto the cell surface, respectively, which allowed the complete regulation of cellulosome assembly, their attachment to cell surface and insertion of catalytic units. For improvement in the effective cellulosomal assembly and higher specific activity of cellulosomal enzymes, many structural changes in the enzymes have been incorporated. Suzuki et al. (2012) reported deglycosylation of cellulosomal enzymes, which enhances the cellulosomal assembly in *S. cerevisiae*. Matsuoka et al. (2014) reported that deletion of MNN2 increased the surface-displayed beta-glucosidase and endoglucanase activity by 1.6-fold and 1.9 fold, respectively, than that of the wild-type isolate.

However, synthetic cellulosomes constructed so far are less active than their natural counterparts. Construction of better complexes is required with efficient catalytic activity than the wild cellulosomes. Thus, more efforts are being made to construct broad substrate-range minicellulosomes (Fontes and Gilbert 2010) and multiple scaffoldin units in larger cellulosomes (Fan et al. 2012) for biofuel production at the industrial scale (Alper and Stephanopoulos 2009).

## Applications of cellulosomes

The future of cellulosomics is very immense and bright. Cellulosomes can be applied to various fields including biorefineries, bioadsorption, bioremediation, production of useful chemicals and as biosensors. Cellulosomes can play a major role in the biorefineries for the depolymerization of complex biomass (Nakashima et al. 2011). Goyal et al. (2011) constructed a minicellulosome by developing a yeast consortium for display of scaffolding protein and secretion of dockerin-fused enzymes for efficient bioethanol production. Bioadsorption of rare and essential metal ions like tungstate and molybdate have also been reported for adsorption and resource recovery (Nishitani et al. 2010; Kuroda et al. 2012). Another field of their application is bioremediation, where they are used as pollutant removers (Fukuda et al. 2010; Kuroda and Ueda 2010; Kuroda and Ueda 2011). Degradation of many toxic compounds using decontaminating enzymes fused with cellulose-binding CBMs has been reported, which enabled a single-step purification and immobilization of fusion proteins into different cellulosic materials (Xu et al. 2002). Cellulosomal systems armed on the cell surface can also be utilized for production of various chemicals like ethyl hexanoate (Su et al. 2010), isoflavone aglycones (Kaya et al. 2008), carnosine (Inaba et al. 2010), chitosan and alginate oligosaccharides (Fukuda et al. 2007; Liu et al. 2009).

Based on the highly specific cohesin–dockerin interactions, cellulosomes can be used to develop single or multi-target biosensors (Jeon et al. 2012). Moreover, engineered CBMs fused with antibody-binding domains can be used as biosensors for sensing pathogens, biomarkers and environmental pollutants (Hussack et al. 2009). Another application of engineered CBM is to characterize native and engineered carbohydrate biomaterials by using various fluorescent techniques (Gourlay et al. 2012; Gao et al. 2014). Apart from this, CBMs can serve as attractive affinity tags and, hence, used in the production and purification of antibodies, peptides and enzymes (Hyeon et al. 2011, 2012; Ramos et al. 2010; Wan et al. 2011). Ramos et al. (2013) have reported the production and purification of recombinant antimicrobial peptides in *Escherichia coli* by using CBM.

Apart from this, many researchers have also reported other applications of cellulosomal enzymes including removal of bacterial biofilms (Juturu and Wu 2014); enhancement of mechanical and physical properties of paper (Cadena et al. 2010; Shi et al. 2014); in textile, detergent and food processing (Juturu and Wu 2014; Karmakar and Ray 2011; Kuhad et al. 2011; Oliveira et al. 2015).

## Future prospects

The utilization of lignocellulosic feedstocks as an alternative to fossil-derived fuels for biofuel production aids in a cleaner and green environment. Construction of better cellulosomal proteins to efficiently hydrolyze cellulose to fermentable sugars for the production of bioethanol is under progress. The recombinant microorganisms with designer cellulosomal enzymes would convert the lignocellulosic biomass to simple sugars in the biorefineries.

**Abbreviations**

CBM: carbohydrate-binding module; LCB: lignocellulosic biomass; NMR: nuclear magnetic resonance; PASC: phosphoric acid swollen cellulose; SLH: surface layer homology.

**Authors' contributions**

RA wrote this manuscript. SB and NKS contributed general advice. SK edited the manuscript. All authors read and approved the final manuscript.

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**Compliance with ethical guidelines**

**Competing interests**

The authors declare that they have no competing interests.

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