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Review

Thermostable cellulases: Current status and perspectives

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ABSTRACT

It is envisaged that the utilization of lignocellulosi mass for etha on for transport sector, would make cellulases the most demanded industrial The greatest p al of cellulolytic enzymes lies in ethanol production from biomass by enzymati ydroly cellulose but low thermostability and low titer of cellulase production resulting into high cost of the enzym ch is the major set-back. A number of research groups are working on cellulase to im thermostability to be able to perform hydrolysis at elevated temperatures which would eventual crease the efficiency of cellulose hydrolysis. The technologies developed from lignocellulosic biomass via ce ose hydrolysis mise environmental and economical sustainability in the long run along with non-depende on nonrenew energy source. This review deals with the important sources of thermostable cellulases, anism, its lation, strategies to enhance the thermostability further with respect to its importance for bio s.

1. Introduction

In recent years there has been remarkable ap ension out environmental safety which led to the green bio sses d nts mainly to substitute most of the unsafe and flat g ch processes. Hence, these initiatives raise e dema enzymes in several industrial applications. Enzy e biocataly d can be advantageous for industrial appli ns ey rarely re ire toxic metal ions for its function, hen creating th ssibility to use more environmental friendly proc g (Comfort et a 004).

Moreover, the require t of en mes that are more stable at elevated temperature, mo ive an bstrate-specific enzymes are high in demand (Singh et al, Tr ishnav al, 2018). Thermostable enzymes offer rob catalys rnativ s are able to withstand the often relativel ndition in strial processing. Use and development olecul iology te ques, permitting genetic analysis and gene fer f inant production, led to dramatically increased acti the of thermostable enzymes during the 1990's. This a imulated isolation of a number of microbes from thermal environm in order to access enzymes that could significantly increase th window for enzymatic bioprocess operations.

At present time when the technology is moving at a fast pace towards biorefinery, the enzymes plays an utmost important role in bioconversion of biomass. There is a renewed interest in commercial utilization of lignocellulosic biomass which is considered to be the only foreseeable source of energy (Sukumaran et al., 2005, Lynd et al., 2002), and it has been projected based on a carbohydrate-based economy that the future of mankind would be highly dependent on utilization of these biomass. Environmental concerns on utilizing fossil fuels and its depletion led to the development on alternative and renewable sources of energy. Lignocellulosic biomass can be utilized to generate bioethanol via enzymatic route to be utilized as transportation fuel.

Cellulases are the second largest industrial enzyme by dollar volume which is going on increasing with the increased demand for various industrial applications such as detergent industry, textile industry, paper processing industry, animal feed industry and fruit juice industry. Though there are several potential industrial applications of cellulases, but the importance of lignocellulosic ethanol has brought cellulases in the main frontier (Sukumaran et al., 2005, Singhania et al., 2010). It is envisaged that cellulases may become the largest volume industrial enzyme if ethanol from lignocellulosic biomass through enzymatic route becomes a major transportation fuel. The commercial potential of using cellulases lies in its efficiency of converting lignocellulosic biomass into glucose through enzymatic hydrolysis which can be utilized to generate a number of value added products such as ethanol.

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Cellulose (β-1,4-linked glucose units) is the most abundant polysaccharide on the biosphere representing most promising raw material for bioconversion with the average annual production estimated at 4×10^9 tons (Yin et al., 2010). Only limited numbers of organisms have the ability to carry out its complete decomposition due to the recalcitrance of its glycosidic linkages (Lynd et al., 2002, Rastogi et al., 2010) requiring a complex enzyme cellulase, consisting of different components acting in a synergistic manner so as to hydrolyze cellulose completely.

2. Mode of action

Microbial cellulase systems can be regarded into complexed or non-complexed. Hydrolysis of insoluble cellulose mainly requires the extracellular cellulases secretion by the organism. The cellulase systems comprised of either extracellular or cell-linked enzymes and they are belonging to different classes or categorized based on their structural characteristics and mode of actions.

Cellulases are hydrolyzing enzymes hydrolyze the β-1, 4-D-glucan bonds in cellulose to yield glucose, cellobiose and oligosaccharides. Several microorganisms are known as potential producers of cellulases. Cellulases have defined based on substrate specificity and structure in different enzyme classifications. However, mainly three types of cellulases are involved in the hydrolysis of native cellulose for example, cellobiohydrolase (CBH), endo-β-1, 4-glucanase (EG) and β-glucosidase (BGL) (Schulein, 1988). There are numerous enzymes within these classifications; however, the most studied fungus for cellulase production *Trichoderma reesei* produces two CBH components, eight EG components and seven β-glucosidases (Aro et al., 2005). This is the one which is most studied multiple-enzyme-complex comprising together endoglucanase (EG), cellobiohydrolase (CBH) and β-glucosi (BGL). For effective and accomplished cellulose hydrolysis, synerg c action of these three cellulase components is essential. EG forms n in the cellulose polymer thus opening the reducing and non-reduci ends. Moreover, cellobiohydrolase (CBH) acts on th ing an non-reducing ends to release cellobiose and cello-o saccha e units, whereas β-glucosidases (BGL) ultimately chop cello to free the glucose and completing the hydrolysis (kun complete cellulase system containing EG H and components, therefore acts synergistically to conve ystalline cel into glucose.

Most of the cellulases exhibit a specific domain structure, (1) catalytic domain (CD) and (2) ulose-binding ain (CBD) or carbohydrate binding module M) which is usually nected through a peptide linker (Ohmiya d., 199 kka et al., 2000). The catalytic domain comprises the ti e where s the CBDs domain contribute in binding to cellulo

3. Thermo e cel ases for el applications

Generall drolysis of cellulose occurs at 40–50° which is usua garded as slow rates of hydrolysis and are characterized with lo d of sugars with incomplete hydrolysis and are sensitive to microbi contamination. These limitations could be resolved by using thermostable enzymes produced by thermophilic/thermotolerant microorganism. Thermostable cellulases have the number of commercial applications, as the paper processing industries; which are always interested in such type of cellulases that can withstand higher temperatures. In addition, one of the most important applications of thermostable cellulase is in the bioconversion of cellulosic biomass into the fermentable sugars for biofuels production at elevated temperature, due to the fact that cellulose swells well at higher temperature thereby supporting the higher reaction rate which definitely would cause economic utilization of cellulase. Thus, thermophilic cellulases are desirable in such applications since their activity at higher temperatures offer several benefits such as reduced hydrolysis times (Viikari et al., 2007), decreased risk of contamination (Abdel Benat et al., 2010), facilitated recovery of volatile products such as ethanol (Taylor et al., 2009), and lower costs for cooling after thermal pretreatment (Turner et al., 2007, Yeoman et al., 2010). All above benefits result in increasing the economic utilization of cellulases; which is one of the major costs adding component to the bioethanol technology (Singhania et al., 2010, Vaishnav et al., 2018).

Thermostable enzymes from thermophillic microorganisms have attracted attention from researchers since last three decades; however, the interest in thermophiles and the way proteins are able to function at elevated temperatures actually started as early as in the 1960's (Brock and Freeze, 1969). Microroganisms that hav optimal growth temperature as above 55 °C are regarded as th (Brock, 1986). It is dominated by bacterial species and o w eukar are known to grow above this temperature, as som gi grow in temperature range 50–55 °C (Maheshwari et a 2000) ther the roorganisms that can grow at and above C are kn as h thermophiles (Kristjansson and Stetter, 1). Hyp thermo pecies are dominated by archaea with f cepti such as *Th motoga* and *Aquifex* (Stetter, 1996).

3.1. Thermostab ulase from b

Cellulases are usu more stable than general enzymes in functioning latively high peratures (Chang et al., 2016). Both fungi and both have bee xploited for cellulase production. Till last fe ears, the emphasis was placed only on fungal cellulases because of it bility to se large amount of less complex cellulases extracel ly. It was ed and produced via recombinant DNA technology in a g bacterial host. However, recently the shift has been observed ds the bacterial cellulases, because of robust bacterial th, its capability of survival in harsh conditions of bioconversion stability and presence of multi-enzyme complexes which provides increased function and synergy.

In general, two types of cellulase systems exists: one type consists of extracellular cellulases in filamentous fungi and in aerobic bacteria that act synergistically to degrade cellulose, while the second type is an enzyme complex called the "cellulosome," in anaerobic bacteria such as *Clostridium thermocellum* which consists of a non-enzymatic scaffolding protein associated with various enzymatic subunits that act synergistically to degrade cellulose and hemicelluloses (Mathew et al., 2008). Non-complexed cellulase systems are more common and are currently most exploited for industrial applications. However, the hydrolysis of cellulose by the bacteria (bacterial cellulase) has several features such as: more stability, increased specific activity and facilitated mass transfer (Viikari et al., 2007, Maki et al., 2009, Rastogi et al., 2010). Therefore, thermophilic cellulose-degrading bacteria have been isolated from various environments such as soil (Abdel-Fattah et al., 2007, Lee et al., 2008, Assareh et al., 2012), compost systems (Lu et al., 2005, Mayendea et al., 2006, Ng et al., 2009) and hot springs (Zhao et al., 2015; Potprommanee et al., 2017). Thermophilic bacteria belonging to the strains *Bacillus*, *Geobacillus*, *Caldibacillus*, *Acidothermus*, *Caldocellum* and *Clostridium* are known to produce thermostable cellulases as given in Table 1 (Rastogi et al., 2010, Zambare et al., 2011).

3.2. Thermostable cellulase from fungi

A number of thermophilic fungi have been isolated and studied in recent years such *Acremonium thermophilum*, *Chaetomium thermophilum*, *Humicola grisea*, *Humicola insolens*, *Melanocarpus albomyces*, *Talaromyces emersonii*, etc., and the cellulases produced from these fungi have been purified and characterized at both structural and functional level. Purified thermophilic fungal cellulases have been characterized in terms of their molecular weight, optimal pH, optimal temperature, thermostability, and glycosylation. The molecular weight of thermophilic fungal cellulase exhibits a wide range (30–250 kDa) with

Table 1
Showing different thermostable cellulases from various microorganisms.

Microorganisms	Name of enzyme	Thermostability at temperature	References
Bacillus	BsCel5A	70% activity at 75 °C for 30 min or even less	Santos et al. (2012)
Geobacillus sp. 70PC53	GsCelA	70% activity at 75 °C after 4 h	Ng et al. (2009)
Geobacillus sp. HTA426	CMCase	Stable at 50–70 °C for 5 h	Potprommanee et al. (2017)
Bacillus sp. SR22	Endoglucanase, Bc22Cel	30% of the activity retained at 80 °C at high salt molarity (1.5 M NaCl)	Dos Santos et al. (2018)
Acremonium hermophilum	Cel7a	Optimal pH 60 °C	Voutilainen et al. (2008)
Chaetomium thermophilum	Cel7a	Optimal pH 65 °C	Voutilainen et al. (2008)
Humicola grisea	Egl2	80% residual activity for 10 min at 75 °C	Takashima et al. (1999)
Chaetomium thermophilum	Cbh3	Half-life period 45 min at 70 °C	Li et al. (2009)
Humicola grisea	*egl3*	75% residual activity for10 min at 80 °C	Takashima et al. (1999)
Humicola grisea	*egl4*	75% residual activity for10 min at 80 °C	[illegible]ima et al. (1999)
Humicola grisea var *thermoidea*	*Egl* and cbh1	Stable for 10 min at 60 °C and 55 °C respectively	[illegible] et al. (1996)
Humicola insolens	*cbhII*	*T*1/2: 95 min at 63 °C	Heinze[illegible] al. (2009a,b)
Talaromyces emersonii	*cel3a*	*T*1/2: 62 min at 65 °C	Murray e[illegible]2004)
Talaromyces emersonii	*cel7*	*T*1/2: 68 min at 80 °C	Grassick et[illegible]2007)
Talaromyces emersonii	*cel7A*	*T*1/2: 30 min at 70 °C	[illegible]outilaine[illegible]al. (2010)
Thermoascus aurantiacus	*Cbh1*	80% residual activity for 60 min at 65 °C	[illegible]g et[illegible]2003a)
Thermoascus aurantiacus	*egl*	stable for 60 min at 70 °C	[illegible]. (2003b)
Thermoascus aurantiacus	*bgl1*	70% residual activity for 60 min at 60 °C	Ho[illegible]t al. (2007)

carbohydrate contents varying between 2 and 50%. Usually, thermophilic fungal cellulases are single polypeptides although it has been reported that some beta-glucosidases are dimeric (Mamma et al., 2004). Optimal pH and temperature are similar for the majority of the purified cellulases from thermophilic fungi which are quite similar to cellulases produced from mesophilic fungi too. Thermophilic fungal cellulases are active in the pH range 4.0–7.0 and exhibit maximum activity at 50–80 °C (Table 1). In addition, they exhibit remarkable thermal stability and are stable at 60 °C with longer half-lives at 70, 80, and 90 °C than those from other mesophilic fungi. The structural characteri[illegible] revealing the increased stability of thermophilic proteins have[illegible] studied more extensively in thermophilic bacteria and hyperther[illegible] philic archaea (Pack and Yoo, 2004, Trivedi et al., 2006) in comparis[illegible] to fungi. Hence, the understanding of the nature an[illegible]anism [illegible] thermostability of proteins from thermophilic fungi[illegible]lati[illegible]poor in[illegible] comparison with thermophilic proteins from the[illegible]philic b[illegible]eria and hyperthermophilic archaea. Till date there is [illegible]mm[illegible] terminants for protein thermostability est[illegible]hed [illegible]and hence it has been proposed that there is more th[illegible]one contrib[illegible]responsible for protein thermostability. A recent [illegible]suggested th[illegible]increase in ion pairs on the protein surface [illegible] a st[illegible]r hydrophobic interior are the major factors supporti[illegible]increased th[illegible]stability in proteins (Taylor and Vaisman, 2010)[illegible]nce, further cha[illegible]ization of amino acid residues and its ro[illegible] therm[illegible]ability is necessary for comprehensive understanding [illegible]most[illegible]ty of cellulases in thermophilic fungi.

3.3. Therme[illegible]e celli[illegible]e from m[illegible]nome

The trad[illegible]pr[illegible] to identify and exploit these enzymes is culture-depe[illegible]t, which greatly restricts the accessible diversity of thermophile-deriv[illegible]atural products. The relatively small percentage of microorganisms t[illegible]can be readily cultured under laboratory conditions has led to the development of novel culture methods and the adoption of culture-independent methods (Ferrer et al., 2007, Lewin et al., 2013). However, the application of novel cultivation strategies is slow and will not enable cultivation of the extant diversity of microbial genomes from extreme environments, necessitating new approaches to exploit natural products from as-yet uncultured microbes for industrial application (Daniel, 2004). The use of a culture-independent metagenomics approach permits access to microbial genomes and their biologically active molecules through isolation of DNA from environmental microbes followed by direct sequencing or cloning DNA to generate a metagenomics library (Handelsman, 2004, Banik and Brady, 2010). The library can then be screened by both sequence-based and function-based m[illegible]ods for nat[illegible]ro[illegible] discovery (Delmont et al., 2011, Milshte[illegible]2014).

Even tho[illegible]sev[illegible]ethodological biases have been recognized, a metagenomic approach[illegible]shown to be effective in discovery of enzym[illegible]novel activiti[illegible]ilson and Piel, 2013; Milshteyn et al., 20[illegible]. The first published example of a functional metagenomic appr[illegible]h for enzym[illegible]discovery was the cloning of cellulases from "zoo-ol[illegible]ries" (Heal[illegible]al., 1995). Hereafter, metagenomics approaches hav[illegible]n conti[illegible]ously used to discover many novel carbohydrate-active [illegible]CAZymes) from soil (Jiang et al., 2009; Nacke et al., 2012), cow rumens (Pope et al., 2010), sediments (Klippel et al.,2014), [illegible]ical reactors (Mewis et al., 2013) and aquatic environments [illegible]ebu[illegible]t et al., 2011, Martin et al., 2014). It has been hypothesized that carbohydrate-degrading enzymes would be encoded in the metagenomes of microorganisms populating oil reservoirs as it is given in the history that petroleum reserves are formed from phytoplankton and would have evolved enhanced thermal stability (Tissot and Welte, 1978). Identification of novel thermostable cellulases could be a vital step in improving economic feasibility of cellulosic biofuel production because processes such as simultaneous saccharification and fermentation (SSF) or separated hydrolysis and fermentation (SHF) are high temperature operations, which necessitates thermostable cellulase. Lewin et al. (2014) revealed that an oil reservoir microbial assemblage harbored novel metagenomic diversity and could be explored for thermostable cellulases and other CAZymes using a combination of function-and sequence-based methods, demonstrating the strength of hybrid screening approaches. A novel thermostable archaeal cellulases that are stable up to at least 80 °C was obtained. One of the cellulase candidates, was demonstrated to be a multi-module, thermostable archaeal enzyme with high activity on different cellulose substrates, producing cellobiose and glucose in a single enzyme reaction (Lewin et al., 2014). Jensen et al. (2018) explored a thermostable enzyme for cellulose processing from the metagenome of a thermostable microbial community derived from rice straw inoculated with compost and incubated at 55 °C. This led them to express and characterize a 45 kDa two domain thermostable bacterial cellulase comprised of a GH6 domain and a C-terminal CBM2domain. They presented the functional and structural characteristics of this enzyme, calledmgCel6A, and assess its potential for use in high-temperature industrial degradation of sulfite pulped lignocellulosic biomass (Norway spruce). Hence with the advent of novel techniques in metagenomic, this approach can be exploited to obtain a potent thermostable cellulase for industrial application.

4. Structure of thermostable cellulase

Modular structure is a common characteristic of non-complex cellulases. Typically, cello biohydrolases and endocellulases are composed of four regions as signal peptide that mediates secretion, a cellulose-binding domain (CBD) for anchorage to the substrate, a hinge region (linker) rich in Ser, Thr and Pro residues, and a catalytic domain (CD) responsible for the hydrolysis of the substrate. The mature proteins are *O*- and *N*-glycosylated in the hinge region and the CDs, respectively. *T. emersonii* CBHII is characterized by a modular structure (Murray et al., 2003) whereas CBH1 from the same fungus consists solely of a catalytic domain (Grassick et al., 2004). Similarly, *Chaetomium thermophilum* CBH1 and CBH2 consist of a typical CBD, a linker, and a catalytic domain. In contrast, CBH3 only comprises a catalytic domain and lacks a CBD and a hinge region (Li et al., 2009). Thus, variations between cellulases within the same mechanistic class have been observed. Fungal CBDs are composed of less than 40 amino acid residues which interact with cellulose through a flat or platform-like hydrophobic binding site formed by three conserved aromatic residues. The binding site is thought to be complementary to the flat surfaces presented by cellulose crystals (Hashimoto, 2006, Shoseyov et al., 2006). The (110) faces of the cellulose crystalline micro-fibrils have been proposed as the putative CBD binding site (Dagel et al., 2011). This arrangement enables the gluco-pyranoside rings of cellulose to be fully exposed and available for hydrophobic interactions. For the efficient hydrolysis of crystalline cellulose by these enzymes, the tight binding to cellulose mediated by the CBD is necessary which was demonstrated by deletion of the CBDs from *T. reesei* Cel7A and Cel6A and *H. grisea*CBH1 greatly reducing enzymatic activity toward crystalline cellulose (Takashima et al., 1998). Substitution of the three conserved aromatic residues (W494, W520, and, Y521) in *H. grisea* CBH1 CBD with other amino acids (G, F or W) has demonstrated the importance of these residues in the interdependency of high activity of *H. grisea* CBH1 on crystalline cellulose and high cellulose-binding ability (Takashima et al., 200). Several thermophilic fungal cellulases have been studied for its 3D structures. Li et al. (2011) has reviewed different 3D structures of thermophilic cellulases belonging to different glycosyl hydrolase classes such as 5, 6, 7, 12 and 45.

5. Strategies for improved thermostability

Even though the thermostable cellulases are available, the prospective to increase their thermostability further would be advantageous for industrial applications. Mutation is an accepted strategy for changes to be made at genetic level. However, the screening of large number of mutants is tedious, still most accepted method for improvement. Improvement of *Talbomyces* Cel7B has been pursued by error-prone PCR. 9 positive mutant clones were screened from 14,600 random clones by arobotic high-throughput thermostability screening method (Voutilainen et al., 2007). Two positive thermostable mutants, Ala30Thr and Ser, showed improvements in unfolding temperatures (T_m) by 1.5 and 3.5 °C, respectively. In addition, the optimum temperature on a soluble substrate for the Ala30Thr mutant was improved by 5 °C. The amino acid alterations are located in the β-strands furthest away from the active site tunnel of the Cel7B enzyme, which could improve protein packing. Hence, random mutagenesis was employed as successful strategy for improving thermostability, however as mentioned earlier too, the screening is a challenge. Recently, Cel7A cellobiohydrolase from the thermophilic fungus *T. emersonii* was engineered using rational mutagenesis to improve its thermostability and activity (Voutilainen et al., 2010). Additional disulfide bridges were introduced into the catalytic module of Cel7A. Three mutants demonstrated improved thermostability reflected by an improvement in avicel hydrolysis efficiency at 75 °C. Structural analysis of *H. grisea* Cel12A, a thermostable endoglucanase, has revealed three unusual free cysteines in the enzyme: Cys175, Cys206, and Cys216. Subsequently, the following Cel12A mutants were constructed by site-directed mutagenesis: Cys175Gly, Cys206Pro, and Cys216Val. It was found that the three free cysteines play a significant role in modulating the stability of the enzyme (Sandgren et al., 2005). More specifically, mutation of Cys206 to Pro and Cys216 to Val caused a reduction in the *T*m of 9.1 and 5.5 °C, respectively, compared to the wild-type enzyme. Moreover, when the free Cys175was mutated to a Gly, the *T*m of the enzyme was increased by 1.3 °C. It has been reported that endoglucanases are characterized by variations in amino acid compositions resulting in fold-specific thermostability (Yennamalli et al., 2011) thus providing new strategies for improvement of thermostability.

SCHEMA uses protein structure data to generate new purpose-specific sequences that minimize structure disruption when they are recombined in chimeric proteins. Chang et al. (2016) have shown the probable mechanism of thermostability comparing two cellulases each from *Bacillus* and *Geobacillus* bacteria. These cellulases are characterized by unique thermostability and are potentially useful in the biofuel and animal feed industries. The group identified a cellulase, GsCelA, from thermophilic *Geobacillus* sp. 70PC53, which is much more thermostable than its *Bacillus* homolog, BsCelA. Thus, these two cellulases provide a pair of structures ideal for investigating the mechanism regarding how these enzymes can retain activity at high temperatures. Chang et al. (2016), have applied the SCHEMA non-contiguous recombination algorithm as a novel tool, which assigns protein sequences into blocks for domain swapping in a way that lessens structural disruption, to generate a set of chimeric proteins derived from the recombination of GsCelA and BsCel5A. Analyzing the activity and thermostability of this designed library set, which requires only a limited number of chimeras by SCHEMA calculations, revealed that one of the blocks primarily contribute to the higher thermostability of GsCelA. Hence, the thermostable chimeric cellulase containing this block when assayed against swollen avicel showed significantly higher activity at 75 °C and higher thermostability compared to the parental enzymes. With further structural determinations and mutagenesis analyses, a 310 helix was identified as being responsible for the improved thermostability of this block. It was also found that in the presence of ionic calcium and crown ether (CR), the chimeric cellulase retained 40% residual activity even after heat treatment at 90 °C. Thus combining crystal structure determinations and structure-guided SCHEMA recombination, the mechanism responsible for the high thermostability enzymes could be determined and a novel recombinant enzyme with significantly higher activity could be generated. SCHEMA has been employed to create thermostable fungal cellulases (Heinzelman et al., 2009a,b). The high resolution of *H. insolens* CBHII as a template for SCHEMA yielded a collection of highly thermostable CBHII chimeras. Using the computer-generated sequences, a total of 31 new cellulase genes were synthesized and expressed in *Saccharomyces cerevisiae*; each of these cellulases was found to be more stable than the most stable parent cellulase from *H. insolens*, as measured either by half-life of inactivation at 63 °C or by *T*1/2. These findings demonstrated the value of using structure-guided recombination to discover important sequence-function relationships for efficient generation of highly stable cellulases Chang et al. (2016).

6. Mechanism of thermostability

In *Sulfolobus solfataricus* a small DNA binding protein, Sso7d, not only imparts thermostability to the DNA but also promotes the annealing of complementary strands above the melting point and the ATPase-dependent rescue of the aggregated proteins (Ciaramella et al., 2002). Thermophiles are reported to have a zigzag structure of surface layer proteins which are thermostable and resist denaturation and proteolysis as well (Kumar and Nussinov, 2001). Chaperones are produced by these organisms which help to refold the proteins to their native form and restore their functions (Laksanalamai and Robb, 2004; Singh et al., 2010). Besides the above strategies, thermophilic bacteria,

actinomycetes and archaea tolerate high temperatures by increased electrostatic, hydrophobic and disulfide interactions in their proteins (Ladenstein and Ren, 2006, Pebone et al., 2008). Certain thermophilic enzymes are stabilized by certain conformational changes (Fitter, 2003). However, certain metals, inorganic salts and substrate molecules are also reported to impart the thermostability (Vieille and Zeikus, 2001). Based on the thermal behavior of these enzymes, the Equilibrium Model has been described to reveal the effect of temperature on enzyme activity by reversible active-inactive transition states (Daniel et al., 2008). Due to the increasing demand of highly thermostable industrial enzymes, certain computational algorithms and bioinformatic tools have been designed, which can predict protein rigidity and stability. Protein stabilization can be carried out by site-directed mutagenesis, and gene shuffling (Hayashi et al., 2001). The GsCelA enzyme belongs to a particular group of *Geobacillus*. The recombinant GsCelA expressed in *E. coli* exhibited ten-fold greater specific activity than the commercially available endo-glucanase from *Trichoderma reesei* and uniquely retained its activity after long-term heating and low pH treatments (Ng et al., 2009). The amino acid sequence of GsCelA indicates it is a member of the glycoside hydrolase GH5 family of cellulases but shares only 53.1% similarity with other members in this group (Ng et al., 2009). In contrast to its full-length sequence, the catalytic core of GsCelA has 60% homology with that of BsCel5A from *Bacillus subtilis* 168. BsCel5A, another cellulase belonging to the GH5 enzymes, is the major endoglucanase in *Bacillus*. BsCel5A from different *Bacillus subtilis* strains have been cloned and characterized for their application in biofuel production (Meng et al., 2014, Santos et al., 2012). BsCel5A is also a thermostable enzyme, though it is not as tolerant at high temperatures as GsCelA, retaining 70% of its optimal activity after incubation at 75 °C for 30 min or less. A TIM-barrel $(\alpha/\beta)8$ catalytic domain and a β-sheet cellulose binding module (CBM3) shown to be present in the cellulase BsCel5A (Santos et al., 2012).

7. Regulation of thermostable cellulases

Regulation of cellulases in thermophilic fungi quite similar to those of mesophilic fungi (Li et al., 2011) such *richoderma reesei* in which the regulation has been studied in detail. Other complexed cellulase systems for example cellulosome intrinsic to anaerobic bacteria. Cellulosomes usually bulges on the terial cell wall and abide stable enzyme comp s. Cellulosidial cellulo c system has been thoroughly studied in the most co hensively way in *C. thermocellum*. Cellulosome of *C. thermocellum* us comprising a non-catalytic protein *cipA* with erse catalytic modu and exhibit exo- and endo glucanase a ies. The composition of the cellulosome differs with respect to o nism. Fundamental understanding upon cellulase systems and control regulation is vital for potential enzyme ion an si engineering strategies for best component binati to achie ximum biomass hydrolysis.

Aerob ngi an teria have mainly a non–complexed cellulase systems, in h s of the cellulase system are excreted. Classic examp f this type of cellulase system in reported in *Trichoderma reesei* s fungus produces two exoglucanases marked as CBHI and CBHII; mo ver, nearly eight endoglucanases named as EGI to EGVIII, and seven β-glucosidases-denoted as BGI to BGVII. In thermophilic fungi also multiple forms of cellulases are produced similar to mesophilic fungi (Maheshwari et al., 2000). *Humicola insolens* also known to have non-complexed cellulase system homologues to *T. reesei* and comprises as a minimum seven cellulases. *Thermobifida* bacterium also produces whole components of the cellulase system comprising exo- and endo-glucanases. For example, in *Humicola grisea* four CBH has been reported from family 7 whereas, in *Aspergillus niger*, a mesophilic fungus, two CBH are known. Observed multiplicity of cellulolytic enzymes (Soni and Soni, 2010) could be the result of genetic redundancy or may be the outcome of differential posttranslational and/or post-secretion processing (Maheshwari et al., 2000).

Cellulases are secreted as inducible enzymes thus the regulation of its production is exceptionally controlled by mechanisms of activation and repression. Cellulase genes of *T. reesei* are regulated extremely synchronized. Thermophilic fungi are also known to possess inducer/repressor system for cellulase regulation in which cellulases are induced by the presence of cellulose (Maheshwari et al., 2000). The secretion of cellulases are induced by the cellulose or other oligosaccharide products whereas it is suppressed with the enough availability of utilizable sugars (Sukumaran et al., 2005). Sophorose (disaccharide) is subsequently regarded to be the best possible inducer especially for the *Trichoderma* cellulase system, it was recommended that the inducer is formed via trans-glycosylation of basal expression of β-glucosidase. Likewise, oxidized products of cellulose hy as cellobiose, δ-cellobiose-1-5 lactone etc. may also as ind of cellulase (Mandels et al., 1962). Another popul ducer utilize commercial cellulase production is lactose, which ighly ex ted as it is cheaper. However, mechanism lactose fo cell e induction is not fully understood, but i anticipated th bly levels of intracellular galactose-1-ph ate m be regula ng the signaling for expression. Glucose m diat se repr on overrides the induction of cellulase sy ; howe le-rep on might be carrying out via an induction chanism occ ough glucose trans-glycosylation.

The promoter of t l cellulases mainly comprising binding sites not onl *CREI* catab repressor protein but also for transcription ctivators of cellulas expression proteins II (*ACE II*), also the C T sequence which binds general transcriptional activator comp s denoted a *HAP*' proteins. Moreover, *ACEII* binds to the *cbh1* pr ers in *T. r i*, hence it is assumed to regulate the *cbh1*, *cbh2*, *egl1*, *egl2* e ssion. Likewise, *Ace1* gene also yields a transcription factor a *EII* which has its binding sites at *cbh1* promoter; however it performs as a cellulase repressor. Hypothetically, Glucose repression of cellulase expression system in *T. reesei* is carrying out through *CRE1* catabolite repressor protein. Moreover, the promoter region of four genes in *T. reesei* such as *cbh1*, *cbh2*, *eg1* and *eg2* has *CRE1* binding sites demonstrating the controlled regulation of above genes via (carbon) catabolite repression. Just as *cre1* in *T. reesei* which is a regulatory gene sequence expressing a negatively affecting transcription factor inhibiting cellulase transcription in presence of glucose; CRE1 genes from two thermophilic fungi (*Talaromyces emersonii*, *Thermoascus aquaticus*) have also been reported. Though the full repertoire of transcription factors influencing cellulase gene expression in thermophilic fungi has not been described completely as in *T reesei*, but the potential regulatory element consensus sequences have been identified in the 5′ upstream regions of thermophilic fungal cellulase genes (Murray et al., 2003, Grassick et al., 2004, Collins et al., 2007, Pocas-Fonseca et al., 2000, Soni and Soni, 2010, Voutilainen et al., 2008).

Such information's offer better comprehension about biochemistry of cellulase for controlled gene regulation, however it is still uncertain how the related genes are being regulated and which transcriptional activator activate the promoter of cellulase. Though, considerable research in this area is being undertaken and anticipating that applied exploitation of the growing knowledge and interventions on genetics of cellulolytic microbes can improve cellulase production and related technology.

8. Heterologous expression

With the advent of genetic manipulation, it is possible to obtain thermostable enzymes from mesophilic host strain. The genes can be cloned and expressed into a host to give thermostable enzyme. Cellulases are glycosyl hydrolases classified into families 1, 3, 5, 6, 7, 8, 9, 10, 12, 16, 44, 45, 48, 51, and 61 (http://www.cazy.org/). Thermophilic fungal cellulases are found in families 1, 3, 5, 6, 7, 12, and 45 (Li et al., 2011). Based on literature, Li et al. (2011) gave a summary of

about 50 genes encoding thermostable fungal cellulases which were cloned, expressed and analyzed. Thus, the production technologies for thermostable cellulases would be similar to the existing one for mesophilic cellulase as the host are usually mesophilic fungi.

Cellulase genes from thermophilic fungi have been cloned and expressed successfully in host organisms such as *E. coli*, yeast and filamentous fungi. *T. reesei* was employed as host organism to express a gene encoding a beta-glucosidase of *T. emersonii* and as a result the recombinant secreted cellulase contained 17 potential N-glycosylation sites in its functionally active form (Murray et al., 2004). Majority of recombinant cellulase expressed in yeast and filamentous fungi are glycosylated (Takashima et al., 1999, Li et al., 2009). Importantly, the glycosylation of cellulases could contribute further to the improvement of their thermostability as it has been previously reported (Meldgaard and Svendsen, 1994). The extent of glycosylation depends on the culture condition and the type of strain as well (Mamma et al., 2004). However, extensive glycosylation in recombinant enzymes could lead to reduced activity and increased non-productive binding on cellulose (Jeoh et al., 2008). Hence, it is a trade-off and need to analyze the extent of glycosylation which is optimum for recombinant enzymes.

9. Conclusions and perspectives

Development of potential thermostable cellulases would play a major role in materializing the vision of eco-friendly lignocellulosic ethanol technology into a reality. Thermostability is the most desired trait of any industrial enzyme which allows fast rate of reaction at elevated temperature thereby decreasing the usage of enzyme. Although the commercial lignocellulosic ethanol production began in different parts of the world, still continuous research is desirable to improve thermostability of the cellulase and its production f proving cost, specific activity and substrate specificity to achieve b techno-economic feasibility. Thermostable cellulases from bacte fungal and even from metagenome would be useful. Successful attemp made towards bringing the desired changes in the p ear pro mising for improving thermostability of cel e si icantly. Thermostable cellulase would allow to operate t ocess o drolysis at elevated temperature which would event lly rate or efficiency of hydrolysis of biomas sulting conomic feasibility of technology.

Thus, while remarkable develop ts been made cellulases research for improving technological poten nd considerable successes have been claimed to h hieved, but t remains that still we do not have 'efficient rmostable cellulase in the market for biomass hydrolysis. It s es the d of long way to go on cellulases research.

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References

Abdel-Banat, B.M., Hoshida, H., Ano, A., Nonklang, S., Akada, R., 2010. High-temperature fermentation: how can processes for ethanol production at high temperatures become superior to the traditional process using mesophilic yeast? Appl. Microbiol. Biotechnol. 85 (4), 861–867.

Abdel-Fattah, Y.R., El-Helow, E.R., Ghanem, K.M., Lotfy, W.A., 2007. Application of factorial designs for optimization of avicelase production by a thermophilic *Geobacillus* isolate. Res. J. Microbiol. 2, 13–23.

Aro, N., Pakula, T., Penttila, M., 2005. Transcriptional regulation of plant cell wall degradation by filamentous fungi. FEMS Microbiol. Rev. 29, 719–739.

Assareh, R., Zahiri, H.S., Noghabi, K.A., Aminzadeh, S., Khaniki, G.B., 2012. Characterization of the newly isolated *Geobacillus sp.* T1, the efficient cellulase-producer on untreated barley and wheat straws. Bioresour. Technol. 120, 99–105.

Banik, J.J., Brady, S.F., 2010. Recent application of metagenomic approaches toward the discovery of antimicrobials and other bioactive small molecules. Curr. Opin. Microbiol. 13 (5), 603–609.

Brock, T.D., 1986. Introduction, an overview of the thermophiles. In: Brock, T.D. (Ed.), Thermophiles: general, Molecular and Applied Microbiology. John Wiley & Sons, New York, pp. 1–16.

Brock, T.D., Freeze, H., 1969. *Thermus aquaticus* gen. n. and sp. n., a nonsporulating extreme thermophile. J. Bacteriol. 98, 289–297.

Chang, C.J., Lee, C.C., Chan, Y.T., Trudeau, D.L., Wu, M.H., Tsai, C.H., Yu, S.M., Ho, T.H.D., Wang, A.H.J., Hsiao, C.D., Arnold, F.H., C 016. Exploring the mechanism responsible for cellulase thermostal y s guided recombination. PLoS One 11 (3), e0147485.

Ciaramella, M., Pisani, F.M., Rossi, M., 2002. M lar biology of e nophiles: recent progress on the hyperthermophilic archaeon bus. Anton. I venhoek 81, 85–97.

Collins, C.M., Murray, P.G., Denman, 07. Molecu ing a pression analysis of two distinct β-glucosidase g bg1 and aven1, w fferent biological roles from the thermophilic phytic s *Talaron emersonii*. Mycol. Res. 111 (7), 840–849.

Comfort, D.A., Chhabra, S Con hou, C.J ing, K.L., Johnson, M.R., Jones, K.L., Sehgal Kelly, R 004. Str biocatalysis with hyperthermophilic nes. Green C 45 5.

Dagel, D.J., Liu, Y t, L.L., Luo, Y., M.E., Qi, Xu., Zeng, Y., Ding, S.Y., Smith, S., 2 in s aging of single bohydrate-binding modules on cellulose microfibrils. Phy. C 115 (4), 635–641.

Daniel, R.M., Danson, M.J., L l, R., Lee, C.K., Peterson, M.E., 2008. The effect of te on enzyme acti ew insights and their implications. Extremophiles 61–59.

Da R., 2004. The soil metagenome–a rich resource for the discovery of novel natural oducts. Curr. Biotechnol. 15 (3), 199–204.

De t, T.O., Malan C., Prestat, E., Larose, C., Monier, J.M., Simonet, P., Vogel, 2011. Met omic mining for microbiologists. ISME J. 5 (12), 1837–1843.

Dos S as, B.O., de França, A.F.J., Gorlach-Lira, K., Velasques, J., Migh Santos, E.A., 2018. A new salt-tolerant thermostable cellulase from a marine *Bacillus* Sp. Strain J. Microbiol. Biotechnol. 28 (7), 1078–1085.

M., Golyshina, O., Beloqui, A., Golyshin, P.N., 2007. Mining enzymes from extremophiles. Curr. Opin. Microbiol. 10 (3), 207–214.

tter, J., 2003. A measure of conformational entropy change during thermal protein unfolding using neutron spectroscopy. Biophy J. 84 (6), 3924–3930.

Grassick, P.G., Murray, R., Thompson, R., Collins, C.M., Byrnes, L., Birrane, G., Higgins, T.M., Tuohy, M.G., 2004. Three-dimensional structure of a thermostable native cellobiohydrolase, CBH IB, and molecular characterization of the cel7 gene from the filamentous fungus *Talaromyces emersonii*. Eur. J. Biochem. 271 (22), 4495–4506.

Handelsman, J., 2004. Metagenomics: application of genomics to uncultured microorganisms. Microbiol. Mol. Biol. Rev. 68 (4), 669–685.

Hashimoto, H., 2006. Recent structural studies of carbohydrate binding modules. Cell. Mol. Life Sci. 63 (24), 2954–2967.

Hayashi, K., Ying, L., Singh, S.P., Kaneko, S., Nirasawa, S., Shimonishi, S., Kawata, Y., Imoto, T., Kitaoka, M., 2001. Improving enzyme characteristics by gene shuffling: application to β-glucosidase. J. Mol. catalysis B: Enzyamatic 11, 811–816.

Healy, F.G., Ray, R.M., Aldrich, H.C., Wilkie, A.C., Ingram, L.O., Shanmugam, K.T., 1995. Direct isolation of functional genes encoding cellulases from the microbial consortia in a thermophilic, anaerobic digester maintained on lignocellulose. Appl. Microbiol. Biotechnol. 43 (4), 667–674.

Heinzelman, P., Snow, C.D., Smith, M.A., Yu, X., Kannan, A., Boulware, K., Villalobos, A., Govindarajan, S., Minshull, J., Arnold, F.H., 2009a. SCHEMA recombination of a fungal cellulase uncovers a single mutation that contributes markedly to stability. J. Biol Chem. 284 (29), 26229–26233.

Heinzelman, P., Snow, C.D., Wu, I., Nguyen, C., Villalobos, A., Govindarajan, S., Minshull, J., Arnold, F.H., 2009b. A family of thermostable fungal cellulases created by structure-guided recombination. Proc. Natl. Acad. Sci. U S A. 106 (14), 5610–5615.

Hong, J., Tamaki, H., Kumagai, H., 2007. Cloning and functional expression of thermostable β-glucosidase gene from Thermo*ascus aurantiacus*. Appl. Microbiol. Biotechnol. 73 (6), 1331–1339.

Hong, J., Tamaki, H., Yamamoto, K., Kumagai, H., 2003a. Cloning of a gene encoding thermostable cellobiohydrolase from *Thermoascus aurantiacus* and its expression in yeast. Appl. Microbiol. Biotechnol. 63 (1), 42–50.

Hong, J., Tamaki, H., Yamamoto, K., Kumagai, H., 2003b. Cloning of a gene encoding a thermo-stable endo-β-1,4-glucanase from *Thermoascus aurantiacus* and its expression in yeast. Biotechnol. Lett. 25 (8), 657–661.

Jensen, M.S., Fredriksen, L., MacKenzie, A.K., Pope, P.B., Leiros, I., Chylenski, P., Williamson, A.K., Christopeit, T., Østby, H., Vaaje-Kolstad, G., Eijsink, V.G.H., 2018. Discovery and characterization of a thermostable two-domain GH6 endoglucanase from a compost metagenome. PLoS One 13 (5), e0197862.

Jeoh, T., Michener, W., Himmel, M.E., Decker, S.R., Adney, W.S., 2008. Implications of cellobiohydrolase glycosylation for use in biomass conversion. Biotechnol. Biofuels 1 (10).

Jiang, C., Ma, G., Li, S., Hu, T., Che, Z., Shen, P., Yan, B., Wu, B., 2009. Characterization of a novel β-glucosidase-like activity from a soil metagenome. J. Microbiol. 47 (5), 542–548.

Klippel, B., Sahm, K., Basner, A., Wiebusch, S., John, P., Lorenz, U., Peters, A., Abe, F., Takahashi, K., Kaiser, O., Goesmann, A., Jaenicke, S., Grote, R., Horikoshi, K., Antranikian, G., 2014. Carbohydrate-active enzymes identified by metagenomic analysis of deep-sea sediment bacteria. Extremophiles 18 (5), 853–863.
Kristjansson, J.K., Stetter, K.O., 1992. Thermophilic bacteria. In: Kristjansson, J.K. (Ed.), Thermophilic bacteria. CRC Press Inc, London, pp. 1–18.
Kumar, S., Nussinov, R., 2001. How do thermophilic proteins deal with heat? Cell. Mol. Life Sci. 58, 1216–1233.
Ladenstein, R., Ren, B., 2006. Protein disulfides and protein disulfide oxidoreductases in hyperthermophiles. Fed. Eur. Biochem. Soc. J. 273 (18), 4170–4185.
Laksanalamai, P., Robb, F.T., 2004. Small heat shock proteins from extremophiles. Extremophiles 8, 1–11.
Lee, Y.J., Kim, B.K., Lee, B.H., Jo, K.I., Lee, N.K., Chung, C.H., Lee, Y.C., Lee, J.W., 2008. Purification and characterization of cellulase produced by *Bacillus amyoliquefaciens* DL-3 utilizing rice hull. Bioresour. Technol. 99, 378–386.
Lewin, A., Johansen, J., Wentzel, A., Kotlar, H.K., Drablos, F., Valla, S., 2014. The microbial communities in two apparently physically separated deep subsurface oil reservoirs show extensive DNA sequence similarities. Environ. Microbiol. 16 (2), 545–558.
Lewin, A., Wentzel, A., Valla, S., 2013. Metagenomics of microbial life in extreme temperature environments. Curr. Opin. Biotechnol. 24 (3), 516–525.
Li, D.C., Li, A.N., Papageorgiou, C., 2011. Cellulases from thermophilic fungi: recent insights and biotechnological potential. Enz. Res. https://doi.org/10.4061/2011/308730.
Li, Y.L., Li, H., Li, A.N., Li, D.C., 2009. Cloning of a gene encoding thermostable cellobiohydrolase from the thermophilic fungus *Chaetomium thermophilum* and its expression in *Pichia pastoris*. J. Appl. Microbiol. 106 (6), 1867–1875.
Lu, W.J., Wang, H.T., Yang, S.J., Wang, Z.C., Nie, Y.F., 2005. Isolation and characterization of mesophilic cellulose degrading bacteria from flower stalks-vegetable waste co-composting system. J. Gen. Appl. Microbiol. 51, 353–360.
Lynd, L.R., Weimer, P.J., van Zyl, W.H., Pretorius, I.S., 2002. Microbial cellulose utilization: fundamentals and biotechnology. Microbiol. Mol. Biol. Rev. 66, 506–577.
Maheshwari, R., Bharadwaj, G., Bhat, M.K., 2000. Thermophilic fungi: their physiology and enzymes. Microbiol. Mol. Biol. Rev. 64, 461–488.
Maki, M., Leung, K.T., Qui, W., 2009. The prospects of cellulase-producing bacteria from the bioconversion of lignocellulosic biomass. Int J Biol Sci. 5 (5), 500–516.
Mamma, D., Hatzinikolaou, D.G., Christakopoulos, P., 2004. Biochemical and catalytic properties of two intracellular beta-glucosidases from the fungus *Penicillium decumbens* active on flavonoid glucosides. J. Mol. Cat. B: Enz. 27 (4–6), 183–190.
Mandels, M., Parrish, F.W., Reese, E.T., 1962. Sophorose as an inducer of cellula[se] *Trichoderma reesei*. J. Bacteriol. 83, 400–408.
Martin, M., Biver, S., Steels, S., Barbeyron, T., Jam, M., Portetelle, D., Michel, G., Vandenbol, M., 2014. Identification and characterization of a halotolerant, cold-active marine endo-β-1,4-glucanase by using functional metagenomics of seaweed-associated microbiota. Appl. Environ. Microbiol. 80 (16), 4958–4[...] research on fungal cellulases for lignocellulose degradation. CSIR [...]07.
Mayendea, L., Wilhelmia, B.S., Pletschke, B.I., 2006. Cellul[...]CMCases [...] polyphenol oxidases from thermophilic Bacillus spp. isolated [...] com[...] Biochem. 8, 2963–2966.
Meldgaard, M., Svendsen, I., 1994. Different effect[...] glycosyl[...] the thermostability of highly homologous bacterial (1,[...]-β-glucanases [...] from yeast. Microbiology 140 (1), 159–166.
Meng, F., Ma, L., Ji, S., Yang, W., Cao, B., 2[...] isola[...] characterization of *Bacillus subtilis* strain BY-3, a thermophilic [...] efficient cel[...] producing bacterium on untreated plant biomass. Lett Ap[...] icrobiol. 59 (3), [...]12.
Mewis, K., Armstrong, Z., Song, Y[...] aldwin, S.A., Withers, [...] Hallam, S.J., 2013. Biomining active cellulases [...] a minin[...] oremediation system. J. Biotechnol. 167 (4), 462–471.
Milshteyn, A., Schneider, J.S., [...] S [...]14. Minin[...] the metabiome: identifying novel natural products from microb[...] munities. [...] Biol. 21 (9), 1211–1223.
Murray, P., Aro, N., [...] Grass [...] Pentt[...], Saloheimo, M., Tuohy, M., 2004. Expression in [...] *reesei* a[...] ization of a thermostable family 3 β-glucosidas[...] the m[...] rately ther[...] ic fungus *Talaromyces emersonii*. Protein Exp. Pu[...] (2), 24[...]7.
Murray, P.G., [...]as, C[...] Tuohy, M.G., 2003. Molecular cloning, transcriptional, [...]ression anal[...] of the first cellulase gene (cbh2), encoding cellobiohydrolase [...] m the moderately thermophilic fungus *Talaromyces emersonii* and structure pre[...] of the gene product. Biochem. Biophy. Res. Commun. 301 (2), 280–286.
Nacke, H., Engelhaupt, M., [...]rady, S., Fischer, C., Tautzt, J., Daniel, R., 2012. Identification and characterization of novel cellulolytic and hemicellulolytic genes and enzymes derived from German grassland soil metagenomes. Biotechnol. Lett. 34 (4), 663–675.
Ng, I.S., Li, C.W., Yeh, Y.F., Chen, P.T., Chir, J.L., Ma, C.H., Yu, S.M., Ho, T.H., Tong, C.G., 2009. A novel endo-glucanase from the thermophilic bacterium *Geobacillus sp.* 70PC53 with high activity and stability over a broad range of temperatures. Extremophiles 13 (3), 425–435.
Ohmiya, K., Sakka, K., Karita, S., Kimura, T., 1997. Structure of cellulases and their application. Gene Rev. 14, 365–414.
Pack, S.P., Yoo, Y.J., 2004. Protein thermostability: structure-based difference of amino acid between thermophilic and mesophilic proteins. J. Biotechnol. 111 (3), 269–277.
Pebone, E., Limauro, D., Bartolucci, S., 2008. The machinery for oxidative protein folding in thermophiles. Antioxid Redox Signal. 10 (1), 157–169.
Pocas-Fonseca, M.J., Silva-Pereira, I., Rocha, B.B., Azevedo, M.D.O., 2000. Substrate-dependent differential expression of *humicola grisea* var. thermoidea cellobiohydrolase genes. Can. J. Microbiol. 46 (8), 749–752.
Pope, P.B., Denman, S.E., Jones, M., Tringe, S.G., Barry, K., Malfatti, S.A., McHardy, A.C., Cheng, J.F., Hugenholtz, P., McSweeney, C.S., Morrison, M., 2010. Adaptation to herbivory by the Tammar wallaby includes bacterial and glycoside hydrolase profiles different from other herbivores. Proc. Natl. Acad. Sci. U.S.A. 107 (33), 14793–14798.
Potprommanee, L., Wang, X.Q., Han, Y.J., Nyobe, D., Peng, Y.P., Huang, Q., Liu, J.Y., Liao, Y.L., Chang, K.L., 2017. Characterization of a thermophilic cellulase from *Geobacillus sp.* HTA426, an efficient cellulase producer on alkali pretreated of lignocellulosic biomass. PLoS One 12 (4).
Rastogi, G., Bhalla, A., Adhikari, A., Bischoff, K.M., Hughes, S.R., Christopher, L.P., Sani, R.K., 2010. Characterization of thermostable cellulases produced by *Bacillus* and *Geobacillus* strains. Bioresour. Technol. 101 (22), 8798–8806.
Rebuffet, E., Groisillier, A., Thompson, A., Jeudy, A., Barbeyron, T., Czjzek, M., Michel, G., 2011. Discovery and structural characterization of a novel glycosidase family of marine origin. Environ. Microbiol. 13 (5), 1253–1270.
Sakka, K., Kimura, T., Karita, S., Ohmiya, K., 2000. M[...]eding of cellulolytic microbes, plants, and animals for biomass utiliz[...] J. B[...]oeng. 90, 227–233.
Sandgren, M., Stahlberg, J., Mitchinson, C., 2005[...]uctural and [...]emical studies of GH family 12 cellulases: improved therma[...]lity, and ligand [...]plexes. Prog. Biophy. Mol. Biol. 89 (3), 246–291.
Santos, C.R., Paiva, J.H., Sforca, M.L., [...] J.L., [...] R.Z., Co[...], Akao, P.K., Hoffmam, Z.B., Meza, A.N., Smet[...].H., Nogue[...], Po[...]pov, I., Xavier-Neto, J., Squina, F.M., Ward, [...]tuller, R[...] Zeri, A[...]mi, M.T., 2012. Dissecting structure-functio[...]lity rel[...]ships of a [...]ostable GH5-CBM3 cellulase from *Bacillus subt[...]* Bio[...] J. 441 (1), 95–104.
Schulein, M., 1988. Cellul[...]s of [...] *reesei*. In[...] Wood, W.A., Abelson, J.N. (Eds.), Methods in [...]nology v[...]0. Acade[...]ress, New York, pp. 234–242.
Shoseyov, O., Shani[...]evy, I., 2006. [...]ydr[...]binding modules: biochemical properties and [...]plications. Mic[...]ol. Biol. Rev. 70 (2), 283–295.
Singh, A., Adsu[...] Va[...] N., Mathur, A[...]ghania, R.R., 2017. Improved cellulase production [...] *Penicilli[...]thinellum* mutant. Indian J. Exp. Biol. 55, 436–440.
Singh, S.P., Purohit, M.K., Ao[...] S., Kitaoka, M., Hayashi, K., 2010. Effect of growth te[...], induction and [...]lar chaperones on the solubilization of over-ex[...]ed cellobiose phosphoryla[...] from *Cellvibrio gilvus* under *in-vivo* conditions. [...]otechnol. Bioproc. Eng. 15 (2), 273–276.
Si[...]nia, R.R., Suk[...]an, R.K., Patel, A.K., Larroche, C., Pandey, A., 2010. [...]vancement an[...]nparative profiles in the production technologies using solid-[...]nd submer[...]fermentation for microbial cellulases. Enzyme Microb. Technol. 4[...]549.
Soni, S.K[...]2010. Regulation of cellulase synthesis in *Chaetomium erraticum*. Bioresourses 5 (1), 81–98.
[...]K O., 1996. Hyperthermophilic prokaryotes. FEMS Microbiol. Rev. 18, 149–158.
[...]R.K., Singhania, R.R., Pandey, A., 2005. Microbial cellulases – production, applications and challenges. J. Sci. Ind. Res. 64, 832–844.
Takashima, S., Iikura, H., Nakamura, A., Hidaka, M., Masaki, H., Uozumi, T., 1998. Isolation of the gene and characterization of the enzymatic properties of a major exoglucanase of *Humicola grisea* without a cellulose-binding domain. J. Biochem. 124 (4), 717–725.
Takashima, S., Iikura, H., Nakamura, A., Hidaka, M., Masaki, H., Uozumi, T., 1999. Comparison of gene structures and enzymatic properties between two endoglucanases from *Humicola grisea*. J. Biotechnol. 67 (2–3), 85–97.
Takashima, S., Nakamura, A., Hidaka, M., Masaki, H., Uozumi, T., 1996. Cloning, sequencing, and expression of the cellulase genes of *Humicola grisea* var. thermoidea. J. Biotechnol. 50 (2–3), 137–147.
Takashima, S., Ohno, M., Hidaka, M., Nakamura, A., Masaki, H., Uozumi, T., 2007. Correlation between cellulose binding and activity of cellulose-binding domain mutants of Humicola grisea cellobiohydrolase 1. FEBS Lett. 581 (30), 5891–5896.
Taylor, M.P., Eley, K.L., Martin, S., Tuffin, M.I., Burton, S.G., Cowan, D.A., 2009. Thermophilic ethanologenesis: future prospects for second-generation bioethanol production. Trends Biotechnol. 27 (7), 398–405.
Taylor, T.J., Vaisman, I.I., 2010. Discrimination of thermophilic and mesophilic proteins. BMC Struct. Biol. 10 article S5.
Tissot, B.P., Welte, D.H., 1978. Evolution of the Biosphere, Petroleum Formation and Occurrence: A New Approach to Oil and Gas Exploration. Springer, Berlin, pp. 14–20.
Trivedi, S., Gehlot, H.S., Rao, S.R., 2006. Protein thermostability in Archaea and Eubacteria. Gen. Mol. Res. 5 (4), 816–827.
Turner, P., Mamo, G., Karlsson, E.N., 2007. Potential and utilization of thermophiles and thermostable enzymes in biorefining. Microb. Cell Fact. 6, 9.
Vaishnav, N., Singh, A., Adsul, M., Simranjeet, K., Mathur, A., Puri, S.K., Singhania, R.R., 2018. Penicillium: the next emerging champion for cellulase production. Bioresour. Technol. Rep. https://doi.org/10.1016/j.biteb.2018.04.003.
Vieille, C., Zeikus, G., 2001. Hyperthermophilic enzymes: sources, uses, and molecular mechanisms for thermostability. Microbiol. Mol. Biol. Rev. 65 (1), 1–43.
Viikari, L., Alapuranen, M., Puranen, T., Vehmaanpera, J., Siika-Aho, M., 2007. Thermostable enzymes in lignocellulose hydrolysis. Adv. Biochem. Eng. Biotechnol. 108, 121–145.
Voutilainen, S.P., Boer, H., Linder, M.B., Puranen, T., Rouvinen, J., Vehmaanpera, J., Koivula, A., 2007. Heterologous expression of *Melanocarpus albomyces* cellobiohydrolase Cel7B, and random mutagenesis to improve its thermostability. Enz. Microb. Technol. 41 (3), 234–243.
Voutilainen, S.P., Murray, P.G., Tuohy, M.G., Koivula, A., 2010. Expression of *Talaromyces emersonii* cellobiohydrolase Cel7A in Saccharomyces cerevisiae and rational mutagenesis to improve its thermostability and activity. Protein Eng. Des. Sel. 23 (2), 69–79.
Voutilainen, S.P., Puranen, T., Siika-Aho, M., Lappalainen, A., Alapuranen, M., Kallio, J., Hooman, S., Viikari, L., Vehmaanperä, J., Koivula, A., 2008. Cloning, expression, and

characterization of novel thermostable family7 cellobiohydrolases. Biotechnol. Bioeng. 101, 515–528.
Welcome to the Carbohydrate-Active Enzymes Database. http://www.cazy.org/.
Wilson, M.C., Piel, J., 2013. Metagenomic approaches for exploiting uncultivated bacteria as a resource for novel biosynthetic enzymology. Chem. Biol. 20 (5), 636–647.
Yennamalli, R.M., Rader, A.J., Wolt, J.D., Sen, T.Z., 2011. Thermostability in endoglucanases is fold-specific. BMC Struc. Biol. 11 Article ID: 10.
Yeoman, C.J., Han, Y., Dodd, D., Schroeder, C.M., Mackie, R.I., Cann, I.K., 2010. Thermostable enzymes as biocatalysts in the biofuel industry. Adv. Appl. Microbiol. 70, 1–55.
Yin, Y.J., Lin, H.H., Xiao, Z.R., 2010. Purification and characterization of a cellulase from *Bacillus subtilis* YJ1. J. Mar. Sci. Technol. 18 (3), 466–471.
Zambare, V.P., Bhalla, A., Muthukumarappan, K., Sani, R.K., Christopher, L.P., 2011. Bioprocessing of agricultural residues to ethanol utilizing a cellulolytic extremophile. Extremophiles 15 (5), 611–618.
Zhao, C., Lu, X., Deng, Y., Huang, Y., Liu, B., 2015. Purification and characterization of thermostable cellulase from consortium XM70 in terrestrial hot spring with sugarcane bagasse. Tropical J. Pharm. Res. 14 (4), 591–598.