Bioresource Technology 245 (2017) 1352–1361

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Review

Genetic modification: A tool for enhancing beta-glucosidase producti for biofuel application

Reeta Rani Singhania [a], Anil Kumar Patel [b,*], Ashok Pandey [c], Edgard Ganansou [d]

[a] DBT-IOC Advanced Bio-Energy Research Centre, Indian Oil Corporation; R&D Centre, Sector-13, Faridabad 121007, India
[b] Department of Molecular Biosciences and Bioengineering, University of Hawaii at Manoa, Honolulu 96822, HI, United State
[c] Center of Innovative and Applied Bioprocessing, Sector 81, Knowledge City PO Manauli, SAS Nagar, Mohali 140306, Punjab, I
[d] Bioenergy and Energy Planning Research Group, École Polytechnique Fédérale de Lausanne, CH-1015 Lausanne, Switz d

HIGHLIGHTS

- Beta glucosidase is the rate limiting enzyme in cellulase complex.
- Fungal cellulases are usually deficient of optimum BGL.
- Genetic modification is capable of producing high titers of BGL with desired pro ties.
- GT BGL is highly desired for high density glucose conversion from cellulose.

ARTICLE INFO

Article history:
Received 28 March 2017
Received in revised form 17 May 2017
Accepted 19 May 2017
Available online 21 May 2017

Keywords:
Beta-glucosidase
Biofuel
Biomass
Bioethanol
Cellulase
Genetic engineering

ABSTRACT

Beta-glucosidase (BGL) rate nzyme for cellulose hydrolysis as it acts in the final step of lignocellulosic mass con to convert cellobiose into glucose, the final end product. Most of the fungal strai cellula roduction are deficient in BGL hence BGL is supplemented into cellulases to have fficien iomass ersion. Genetic engineering has enabled strain modification to produce BG imally h desired operties to be employed for biofuel applications. It has been cloned either dire to lacking BGL or into another expression system, to be overexpressed so as to e blen to BGL deficient cellulases. In this article, role of genetic engineering to overcome BGL limitations in ellulase cocktail and its significance for biofuel applications has been critically reviewed.

Contents

* Corresponding author.
E-mail addresses: anilkpatel22@gmail.com, patelani@hawaii.edu (A.K. Patel).

http://dx.doi.org/10.1016/j.biortech.2017.05.126
0960-8524/

1. Introduction

Lignocellulosic biomass is the most abundant and ubiquitous material available to be utilized for the benefits of mankind. It is mainly composed of cellulose, lignin and hemicelluloses and the amount varies from 30–50%, 20–30% and 20–40% respectively. Cellulose is the major component and is a linear polymer of glucose linked by β-1,4 linkage. It is acted upon by cellulases which consist of three major components *viz.* endoglucanases (EC 3.2.1.4), exoglucanases (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21) (Singhania et al., 2013; Mohanram et al., 2013). These enzymes act in perfect synergism and tight regulation under natural conditions and convert the cellulose polymer into its monomers (Singhania et al., 2010). Fig. 1 shows all the components of cellulase acting together to hydrolyze crystalline cellulose. Endoglucanases act randomly along the chain length, thereby producing new sites to be attacked by exoglucanases (cellobiohydrolases). LPMOs are non-hydrolytic proteins, which help cellulase components to access cellulose fibers by producing nicks in between the chain. Exoglucanases hydrolyse cellulose polymers at the terminal reducing or non-reducing ends, producing mainly cellobioses along with small oligosaccharides. The liberated cellobioses are acted by β-glucosidases (BGL), which convert into glucose. Thus BGL is the rate-limiting enzyme in cellulose bio-conversion (Singhania et al., 2013) and determines the rate of forward reactions. Cellobiose accumulation inhibits the exoglucanase activity and reducing the overall saccharification rate, therefore, its conversion into glucose via BGL is nec... Moreover accumulated glucose reversibly inhibits the ... activity and thus the regulatory mechanism for BGL is qui... edious. The... BGL is most important in biomass hydrolysis, a... st of the ...roorganisms producing cellulases are BG... eficient ... t there ... some other components also produce ... which coul... cellulase efficiency significantly such ... xylan... . About ... 40% of biomass is hemicellulose, which a... a ...sical barrier and limits the accessibility of the cell... es t... ose (Ve... ets and Dahman, 2011). Blending of he... ellulases ... cial... ylanases in the enzymatic cocktails sh... ynergistic ... in cellulase activities and increases ...erall ... harification efficiency (Hu et al., 2011).

Lignin is another ... ponent in the biomass, which is the most rec... t to digestio... d is responsible for protection of bio-m... s from microbial attacks. It restricts cellulase to access cellu-l... es. Lignin de... adation could be also possible by enzymes like l... ase but the... enzymes are quite slow and hence pretreatment o... mass is ... quired to remove lignin and to make cellulose amo... e acted on by cellulases. It has been rightly considered as one of the biggest barrier for economic bioconversion of ... ss (Saini et al., 2016).

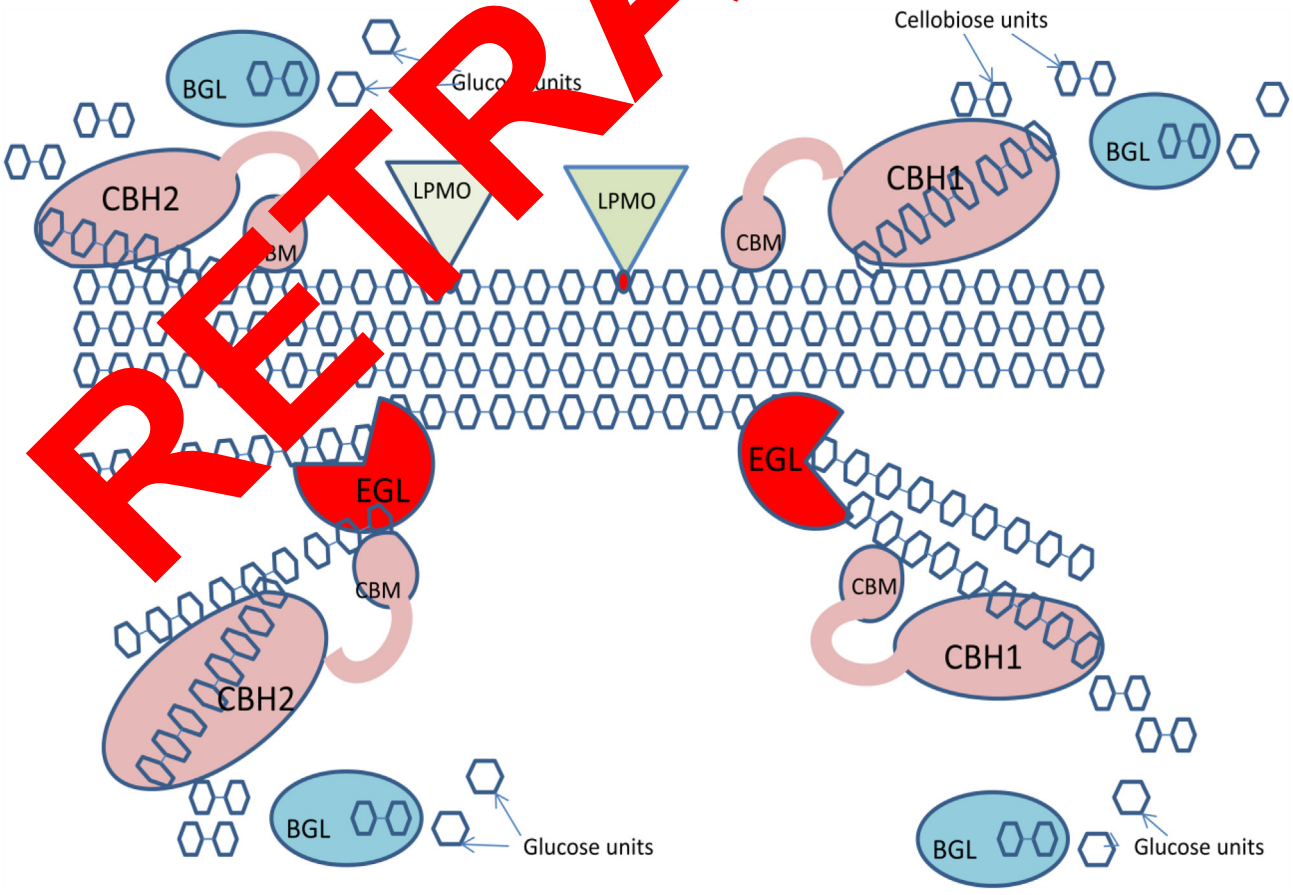

Fig. 1. Cellulase component acting in synergism to hydrolyze cellulose chain.

The understanding of lignocellulose matrix structure and conversion mechanism of hydrolytic and accessory enzymes (*e.g.*, xylanases and β-glucosidases) can provide significant insights on the performance of cellulase cocktails for enhancing saccharification efficiency (Hu et al., 2011; Zimbardi et al., 2013). Therefore, screening of novel microorganisms and bioprospecting in search of superior enzymes is an ambitious project that needs targeted approach, thus resulted superior enzymes may act synergistically with existing commercial cellulases for increasing its hydrolytic efficiency. There is a commercial need of a complex and coherent enzyme cocktail, which may act synergistically to unlock the complexity of lignocellulose polymer and convert it into fermentable sugars to the greater extent possible. These enzymes are the major costly inputs in the overall process and bringing down the cost of enzymatic saccharification is a current challenge. Supplementation of BGL blended cellulase is an effective strategy for maximizing saccharification efficiency. BGL has wider applications and great significance in biomass to bioethanol production.

2. Significance of BGL

BGL is well-characterized, biologically important enzyme that catalyzes the transfer of glycosyl group between oxygen nucleophiles. This transfer reaction results in the hydrolysis of β-glucosidic linkage occurs between carbohydrate residues in aryl-amino-, or alkyl-β-D-glucosides, cyanogenic-glucosides, short chain oligosaccharides and disaccharides under physiological conditions. This also exhibits, synthesis of glycosyl bond between different molecules under defined conditions. Various BGLs are found all domains of living organisms, where they play essential ro in the removal of non-reducing terminal glucosyl residues fro saccharides and glycosides. BGLs function in glycolipid and exoge nous glycoside metabolism in animals, defense, cell nifica- tion, cell wall β-glucan turnover, phytohormon ctiva , and release of aromatic compounds in plants, and mass c version in microorganisms. These functions lead BGL to and industrial applications (Cairns and E 2010), ecent years, there has been considerable attention GLs due t ir potential roles in various biotechnologic es such as rolysis of isoflavone glucosides, productio of fue anol from lignocellulosic biomass, release of a atic compo from flavorless precursor etc., The physiol al roles of BGL are erse depended on its occurrence in biol al syst and its location. For example, cellulose hydrolysis and la duction occurs due to transglycosylation activity of BGL h alolytes glucan synthesis by BGL during cell wall ment me metabolism, fruit ripening and defense chan s in p whereas BGL is involved in the hydro s of gl syl ceramides in humans and mammals. Hence, BGL vi es in human's results into Gaucher's disease non-neuropathic lysosomal storage disorder (Lieberman et al. 07). Moreover, hydrolysis of soybean isoflavone glycosides is a portant application of BGL in industries.

In recent decade, due to the advent of biofuel program from biomass via enzymatic route, BGL has acquired center position being the rate-limiting component in the enzyme cocktail. BGL is the rate-limiting enzyme because it is responsible for the final step of lignocellulose conversion of cellobiose and/or short cellodextrins into glucose (Singhania et al., 2013). *Trichoderma reseei* produced cellulases containing endoglucanases (18%), cellobiohydrolases (72%) and β-glucosidases (<1%) (Margeot et al., 2009). The β-glucosidase from *Aspergillus niger* is generally used to complement the cellulolytic cocktail of *T. reseei* (Del Pozo et al., 2012). Hence, β-glucosidases supplementation in the enzymatic cocktails is inevitable for formulating an efficient cellulolytic cocktail. Various studies showed, enzymatic mixture augmentation with β-glucosidases or microbes with greater β-glucosidase potential have resulted an increase in sugar yields (Del Pozo et al., 2012; Sukumaran et al., 2009).

The β-glucosidase proportion is meager in most of the commercial cellulases, which results accumulation of cellobioses and cellooligosaccharides in the medium and causes catabolite repression on endoglucanase and cellobiohydrolase (Wang et al., 2013). Enzyme giants such as Novozymes have come up with series of cellulase cocktail specifically for biomass hydrolysis, the first of being celluclast, which is different from CellicCtec2 (the advanced version) in being deficient of β-glucosidase. Though Novozyme keep on improving its cocktail and came u licCtec3 which is superior to even CellicCtec2 as has l report be supplemented with additional auxiliary en s. The cell es producing microorganisms produce BGL at di t capacit and there are different isoforms of BGL d their ession vels varied depending on media comp tion o physi tions, which induces BGL isoforms pr tion (ghania et , 2011; Rajasree et al., 2013). Each BGL ma ferent roperties, however in the crude sample th are an d toge and separate analysis is possible only purificatio s of BGL is usually done using artificia e in the fer tation broth.

3. BGL is

ormation is lacking about the interaction of BGL with their sub ates, especi with respect to the aglycone moiety, which form basis o emendous diversity in terms of substrate range and is for subtle differences in substrate specificity. Usually BGL activities are measured using artificial substrates such PG (para nitrophenyl beta-D-glucopyranoside) or methyl yl beta-D-glucoside (MUG). BGL activities are also measured with its real substrate cellobiose. Most of the BGL are likely to show high catalytic activity and high km against artificial substrate *e.g.,* pNPG and MUG but not with cellobiose. Kinetics of the BGL depends on the configuration of its substrate, however cellobiose requires a conformational change for catalysis. BGL has a very rigid structure in S1 substrate binding site which accommodate first glucose of cellobiose but the second glucose of cellobiose may change the conformation using rotation of the σ-bond of the glucoside so as to fit in the substrate binding site (Nam et al., 2010). Though, it is not needed in case of pNPG where nitrophenol follows the same binding patterns as of glucose. This is the reason behind low Kcat/Km of BGL towards cellobiose than the substrate pNPG. Hence, it is necessary to investigate potentialities of BGL with cellobiose rather than pNPG or MUG for accurate accountability of the natural process.

Single strain can produce multiple BGLs depending on the nutritional and physical parameters (Singhania et al., 2011). Different BGLs may belong to different glycosyl hydrolase (GH) families, as they differ in their properties and structures. Studying the BGL classification based on their structure is very important to gain insights on their functionality.

4. Classification of BGLs

BGLs have been classified into glycoside hydrolase (GH) families GH1, GH3, GH5, GH9, and GH30, based on their amino acid sequences, while other β-glucosidases remained to be classified. An alternative classification system has been developed for glycoside hydrolases based on amino acid sequence and structural similarity (Henrissat, 1991; Henrissat and Davies, 1997; Cantarel et al., 2009). In this system, those enzymes with overall amino acid sequence similarity and well conserved sequence motifs are grouped into the same family. The families that have similar

catalytic domain structures and conserved catalytic amino acids, suggestive of a common ancestry and catalytic mechanism, are grouped into clans (Henrissat and Davies, 1997; Cantarel et al., 2009). Of these clans, GH-A has the largest number of families, and it includes the β-glucosidase-containing families GH1, GH5, and GH30, which consists of proteins with (β/α) 8-barrel structures. In contrast, the active site of GH3 enzymes comprises two domains, while GH9 enzymes have (α/α) 6 barrel structures. GH1 includes the largest number of characterized β-glucosidases. The mechanism by which GH1 enzymes recognize and hydrolyzing substrates with different specificities remains an area of intense study (Cairns and Esen, 2010). GH1 BGLs have shown several desirable properties required for biofuel applications. One of the most important properties for BGL is its glucose tolerance capacity, which decides its importance in cellulase cocktail and it is the main factor required to drive the reaction forward at higher glucose concentration.

5. Specific traits of BGL

5.1. Glucose tolerance

Product inhibition of BGL by glucose is considered to be a limiting step in enzymatic hydrolysis of biomass, especially when higher sugar yields are required. Mostly the BGL's are subjected to feedback inhibition by its own product; glucose which results into bulk addition of BGL into cellulase cocktail to achieve desired yield during hydrolysis of biomass resulting in increased cost of enzymes. To circumvent this issue glucose tolerant BGLs are r[illegible]st. Glucose tolerance of BGL is a significant desirable property capable of decreasing the cellulolytic enzyme loads during [illegible]o-cellulose hydrolysis needed to attain acceptable yields, thus re[illegible]ing the costs of bioethanol production (Singhania [illegible] 2013).

Remarkably, some BGL belongs to the GH1 [illegible]mily exhib[illegible] unusual properties, being tolerant to or eve[illegible]stimula[illegible] by high[illegible] glucose concentrations. Glucose tolerance [illegible]GL [illegible]es coupled to a stimulatory effect of gluco[illegible]n st[illegible] degrad[illegible]on in a given range of glucose concentr[illegible]ns (up to [illegible]). This property seems to be exclusive to so[illegible]cosyl hydr[illegible] family 1 (GH1) (Henrissat, 1991; Canta[illegible] et a[illegible]009). Furthe[illegible]more, the family GH3 β-glucosidases [illegible] not know[illegible] be glucose tolerant as are inhibited by gluc[illegible]. However, the[illegible]re reports where GH3 β-glucosidases ha[illegible] shown[illegible]lucose toler[illegible]nce up to 400 mM glucose (Ramani et [illegible]015)[illegible]e limitation can also be tackled by removing glucose w[illegible]s produ[illegible], for which technologies such as memb[illegible] filters[illegible]uld h[illegible]sed to separate monomers of sugars s[illegible]to [illegible]d feed[illegible]ibition. Simultaneous saccharificatio[illegible]d ferm[illegible]ation cou[illegible] also be an answer to this limitation; wi[illegible] gluc[illegible]rted to ethanol simultaneously hence glucose re[illegible] always [illegible] lower concentration. Thus feedback inhibition of [illegible] is a major challenge to circumvent, when we need glucose to[illegible]t BGLs.

5.1.1. Structural basis for glucose tolerance in BGLs family GH1

The structural basis for the glucose tolerance and stimulation of BGLs is still elusive. Elucidation of the mechanisms of glucose tolerance and stimulation of the GH1 BGL would definitely be crucial to improve their application through enzyme engineering. As suggested by enzymatic assays; BGL stimulation is regulated either through an allosteric effect by glucose binding to a secondary site (Zanoelo et al., 2004; Souza et al., 2010) or by kinetic modulation of enzyme turnover associated with trans-glycosylation (Uchima et al., 2011, 2013). Yang et al. (2015) also compared the primary and tertiary structures of two GH1 β-glucosidases with distinct glucose dependence. They mutated some putative glucose-dependence relevant sites to investigate their exact roles. Both biochemical and structural characterization of the mutants suggested that some sites at the entrance and middle of the substrate channel regulate the effects of glucose, and the relative binding affinity/preference of these sites to glucose modulates the glucose dependence. Structural bases for the effects of glucose on GH1 and GH3 BGL remained unclear (Liu et al., 2011). To address mechanism, first crystal structure of glucose stimulated fungal BGL was studied at native and glucose complexed forms, revealed the shape and electrostatic properties of the entrance to the active site (including +2 subsite), mainly determine the glucose tolerance. The aromatic Trp168 and the aliphati[illegible]are conserved in glucose-tolerant GH1 enzymes and [illegible]ribute t[illegible]ieving enzyme inhibition by imposing constraint[illegible]he +2 subs[illegible]hat limit the access of glucose to the 1 subsi[illegible]e. Th[illegible]e structur[illegible]eterminants for glucose tolerance in BGLs[illegible]ghlights [illegible]mpor[illegible]ce of the steric constraints imposed by t[illegible]nique active [illegible]ance and the +2 subsite which limit gl[illegible]se acc[illegible]to the 1 [illegible]bsite (de Giuseppe et al., 2014). The ge[illegible]f [illegible] active [illegible]e pocket may support a secondary catal[illegible] transg[illegible]sylatio[illegible]eaction using glucose as acceptor. It is [illegible]greement w[illegible]he[illegible]mulatory mechanism previously dem[illegible]for GH1 β-g[illegible]osidase isolated from a microbial metag[illegible]nomic [illegible]ple (Uchima et al., 2011, 2013). This GH1-family [illegible]zyme (Hen[illegible] 1991; Cantarel et al., 2009) presents sh[illegible]proximately tw[illegible]d activation at 50 and 100 mM concen-t[illegible]ions of glucose or xylose, respectively, and maintains full or [illegible]her activity [illegible] to 450 mM glucose or 730 mM xylose (Souza e[illegible] 2013). In [illegible]tudy Liu et al. (2011) showed that 184th residue of [illegible]el BG[illegible]ily 1 could be responsible for its glucose tolerance which[illegible]roved based on site directed mutagenesis studies, [illegible]here mutant strain exhibited better glucose tolerance level from [illegible]ompared to 14.9 in the wild one (Liu et al., 2011). Moreover, *H. insolens* BGL showed high catalytic efficiency for cellobiose hydrolysis and good thermal stability, thus being a very attractive biocatalyst for industrial use in plant-biomass saccharification processes (Souza et al., 2010). The GH1 family β-glucosidases are 10–1000-fold more glucose tolerant than GH3 BGLs, and comparative structural analysis shows a clear correlation between active-site accessibility and glucose tolerance. The active site of GH1 BGLs is located in a deep and narrow cavity, which is in contrast to the shallow pocket in the GH3 famLily BGLs. These findings shed light on the molecular basis for glucose tolerance and indicate that GH1 BGLs are more suitable than GH3 BGLs for biotechnological applications involving lignocellulosic biomass saccharification (de Giuseppe et al., 2014). The homology modeling of BGL from *Bacillus subtilis* which was cloned and expressed into *E. coli* revealed that protein has 19α-helices and 4 β-sheets and adopted (β/α)8 TIM barrel structure (Chamoli et al., 2016). Substrate docking and LigPlot analysis depicted the amino acids of active site involved in hydrogen bonding and hydrophobic interactions with substrates.

Structure-based protein-engineering methods are proven strategies to obtain improved enzymes (Lundemo et al., 2013), and de Giuseppe et al. (2014) have provided valuable information to guide rational approaches aiming to integrate glucose tolerance/activation with other desirable properties to generate catalytically efficient BGL for biofuel production and other industrial applications. It also provides fresh insight into the current understanding of the properties and mechanisms of GH1 β-glycosidases and related enzymes that modulate their activity via feedback control mechanisms (Yang et al., 2015).

5.2. Inhibition and induction by glucose

BGL commonly gets inhibited by glucose, its own end product but it gets stimulated also by glucose. Uchima et al. (2011) reported an unusual finding that one of the BGL from *Neotermes*

koshunensis (lower termite) successfully expressed in *Aspergillus oryzae*, was stimulated by 1.3-fold when glucose was present in the reaction mixture at a concentration of 200 mM. The BGL from *Thermoanaerobacterium aotearoense* cloned and expressed in *E. coli* induced at 50–250 mM glucose concentration and tolerant to glucose inhibition with K*i* of 800 mM glucose concentration (Yang et al., 2016). These characteristics to stimulate enzyme activity by glucose make it of great interest for biotechnological applications, especially for bioethanol production (Uchima et al., 2011). Kuusk and Väljamäe (2017) have analyzed different reaction schemes using the catalysis by retaining BGLs as a model. They found that simple competition of inhibitor with nonproductive substrate binding could account for enzyme activation by inhibitor without involving any allosteric effects. The transglycosylation of inhibitor also explains the inhibitor effect on enzyme activation. For both mechanisms, the activation caused through *k*cat increase due to increasing inhibitor concentration, while *k*cat/Km are always decreased. Therefore, the activation by inhibitors was more pronounced at high substrate concentrations. Possible contribution of these mechanisms in inhibitor-dependent enzyme activation has been demonstrated based on the rate-limiting glycosidic bond hydrolysis, also particular glucose-unit-binding subsites interaction (Kuusk and Väljamäe, 2017). Transglycosylation is another unique feature of BGL, showing synthetic activity along with hydrolase activity.

5.3. Transglycosylation

BGL normally catalyzes the hydrolysis of glycosidic linkages in disaccharide or glucose substituted molecules, and also depic unique property of transglycosylation under conditions that fav a back reaction that can be applied to promote the synthesis oligosaccharides. Transglycosylation occurs at high product (glu cose) concentration (Bohlin et al., 2013). Oligosa s play numerous roles in biological systems such as m cular cognition in intercellular communication and sign ransdu on and have shown considerable potential as therapeut Du ied roles of β-glucosidases in nature, the satile mes can be of use in several synthetic reactions a eviewed by tia et al. (2002). In recent time transglycosyl acity of β-g sidase was found to be very beneficial or cel induction among fungi. There are reports that f al BGL when ed to a high concentration of glucose swit to syntheses acti and generate oligosaccharides such ellobi sophorose, etc., which are known as inducers and ce ngi to produce cellulases. This property has been explore had be eported as economical ways of genera ulase ce which are otherwise quite costly and ad the c all cost e enzyme production process making it conomi Transglycosylation could also be a reason for inhibitio usually thought to be a feedback inhibition by wn product glucose. Bohlin et al. (2013) concluded in his stud t the hydrolytic slowdown at high substrate concentrations sole elies on an increased flow through the transglycosylation pathway and not an inhibition that delay the catalytic cycle. The experimental data was modeled to obtain kinetic parameters for both hydrolysis and transglycosylation which would be useful for the evaluation of BGLs for industrial use.

Study on structures of BGL has enabled researchers to alter its properties at DNA level to our benefits. Jeng et al. (2012) has mutated to attacking nucleophile in β-glucosidase to convert it into a glycosynthase for the synthesis of oligosaccharides. Several amino acids, including the two catalytic glutamates, of a BGL from *Neotermes koshunensis* were mutated in order to elucidate the detailed mechanism of hydrolysis and transglycosylation. It was revealed from structural studies that Glu193 not only acts as the catalytic acid/base but also plays an important role in controlling substrate entry and product release. It was found that new gluco-conjugated products were lodged in the active-site pocket in the crystal structures of the mutant, implying that mutation enhanced transglycosylation activity. It appears to be suitable for development as a useful tool for the enzymatic synthesis of numerous therapeutic oligosaccharides via the transglycosylation reaction (Jeng et al., 2012). This finding highlights an innovative way to further improve β-glucosidases by genetic modification for the enzymatic synthesis of oligosaccharides to be useful for enzyme production.

In this review, we focus mainly on the enhancement of β-glucosidases production by genetic mod nd its significance for unlocking the trapped sugar om con lignocellulosic polymers, ultimately resulting hanced sug to ethanol yield.

6. Genetic modification

Genetic modifica is the p popular tool which enables to combi ultiple de le t s into a single organism. It can be used t eer microb igh metabolite production which could fr mple protei s to highly specific therapeutic proteins. However, the cases may not be as simple as it sound se of the in t complexity of the organisms itself or t metabolites needed to be expressed. Cloning and expressing pr yotic gene bacterial systems are comparatively easier tha he eukary gene which are more complex and have intr nd exon its genomic sequences. Efforts are needed in the di tain engineered BGL with glucose tolerant propties along with the search of glucose tolerant native BGL source. genes from a large number of bacterial, mold, yeast, plant, an al systems have been cloned and expressed in both *E. coli* nd eukaryotic hosts such as *S. cerevisiae*, *Pichia stipitis* and filamentous fungi. The complexity of the genetic engineering might e dealt elsewhere; rather than going deep into the process, we shall discuss that how researchers have employed this powerful tool for altering BGL producing capacity of microorganisms.

Though exogenous supplementation of β-glucosidase overcomes the inhibition, the high cost and low availability of β-glucosidase turns the ethanol production very expensive (Ramani et al., 2015). If the heterologous enzyme can be produced in the host microorganisms producing other components, it becomes more economical as separate production and mixing at optimal ratio is not required saving time and energy. Cloning and overexpression of BGL resulted in BGL rich cellulase production which improved hydrolysis efficiencies when acted towards biomass (Yang et al., 2016). Thus cloning and overexpression of β-glucosidases is highly desirable for increasing cellulase efficiency resulting in the cost economics of the saccharification of the biomass.

It has been proposed since last few decades that if desired gene for BGL can be cloned and expressed in heterologous strain, it would be very beneficial much more than generating cocktail by mixing enzymes outside. Although BGL with desirable properties whose expression level is low in its parent strain; cloned and expressed strongly in another host will also be advantageous. Several thermotolerant BGLs from bacteria have been cloned and expressed in *E. coli*. Table 1 gives an account of rBGL its source, host and properties which is an updated version of Singhania et al. (2013).

6.1. Bacterial rBGL

Bacteria are also known for cellulolytic activities but most of their enzymes are produced intracellularly unlike fungi which

Table 1
Properties of recombinant BGL.

Source	Host organism	Properties of recombinant BGL	References
Caldicellulosiruptor saccharolyticus DSM 8903	*E. coli*	Optimum activity at pH 5.5and 70 °C for p-nitrophenyl (pNP)-β-D-glucopyranoside. The half-lives of the enzyme at 60, 70, and 80 °C were 250, 24.3, and 0.4 h, respectively.	Hong et al. (2009)
Bacillus subtilis	*E. coli*	Specific activity of 54.04 U/mg, Optimum pH 6.0 and temperature 60 °C, Ki for glucose 1.9 mM, Kcat/Km against p-nitrophenyl-β-d-glucopyranoside (292.53 mM^{-1} s^{-1})	Chamoli et al. (2016)
Thermoanaerobacterium aotearoense	*E. coli*	pH 6.0 and temperature 60 °C, specific activity 256.3 U/mg wet cells, km and Vmax of 25.45 mM and 740.5 U/mg, respectively against cellobiose, Ki 800 mM against glucose and activated at 50–250 mM of glucose solution	Yang et al. (2016)
A metagenomic library of rumen cattle feed		highest sp activity of 2.5 × 103 U/mg for pNPG, km 0.309 mmol/L and Vmax 7.292 μmol/min, Optimum pH 5.0, temp 38 °C	Li et al. (2014)
Marine microbial metagenomic library		Half Ki 1000 mM and get activated at glucose concentration < 400 mM, belong to GH[illegible] and sta[illegible] at high cations and NaCl concentrations	[illegible]ang et al. (2010)
Compost		Glucose tolerance at 1000 mM and possess transglycosylation activity	[illegible]iyama et al. ([illegible]3)
Neotermes koshunensis	*Aspergillus oryzae*	rBGL stimulated by 1.3-fold in presence of 200 mM glucose. km and Vm[illegible]ere 0.7[illegible]and 16 U/mg. Active at pH 5.0–9.0.	[illegible]ima et al. [illegible]011)
Penicillium funiculosum NCL1	*Pichia pastoris*	rBGL showed optimal activity at pH 5.0 and 60 °C, high substrate [illegible]ersion r[illegible]f 2,083 [illegible] min/mg with cellobiose shows tolerance up to 400 mM glucose	Ramani et al. (2015)
Neocallimastix patriciarum	*Pichia pastoris*	GH family 3, 34.5 U/mg against cellobiose, optimally active [illegible] 40 [illegible].0. it was [illegible]tter in saccharification than Novozyme 188	Chen et al. (2012).
Paecilomyces thermophila	*Pitchia pasrtoris*	GH family 3, 274.4 U/ml, optimal pH 6 and temp 60 °C	Yan et al. (2012)
Saccharomycopsis fibuligera	*S. cerevisae*	1.02 IU/mg	Shen et al., (2008)
Talaromyces emersonii	*T. reesei*	GH family 3, thermostable active at 71.5 °C, Vmax [illegible]12 IU/m[illegible] [illegible]254 mM for glucose	Murray et al. (2004)
Periconia sp.	*Pitchia pasrtoris*	Thermotolerant BGL, optimal activity at [illegible] at pH 5–7	Harnpicharnchai et al. (2009)
Penicillium oxalicum (wild type)	*Penicillium oxalicum* 11–13 and 14–32 mutant strain	BGL1 over-expressing mutant I1-13 p[illegible]cularly improved the β-glucosidase activity at a factor of 65-folds, resulting in levels of up to [illegible]U/ml. The mu[illegible]l4-32 with over-expression of BGL4 achieved the highest glucose yield in [illegible]accharificati[illegible]f delignified corncob residue at only 25% enzyme load compared to the pare[illegible]rain RF	Yao et al. (2015)
Penicillium decumnens	*T. reesei*	Six-to-eightfold more B[illegible]tivity than th[illegible]	Ma et al. (2011)
Aspergillus niger	*T. reesei*	5.3 IU/ml (106) times [illegible]ative BGL	Wang and Xia, (2011)
Aspergillus fumigatus	*Pitchia pasrtoris*	Active at pH 6.0 and at 6[illegible] with [illegible]vity of about 100 IU/mg	Liu et al. (2012)
Periconia sp.	*T. reesei QM9414*	10.5-fold BGL activity incr[illegible]d [illegible] 2.2 to 23.9 IU/mg, thermotolerant and active inacidic pH	Dashtban and Qin, (2012)
Fervibacterium islandicum	*E. coli*	GH fam[illegible], th[illegible]stable	Jabbour et al. (2012)
Chaetomium thermophilum	*Pitchia pasrtoris*	O[illegible]lly acti[illegible]H 5.0 and [illegible]0 °C	Xu et al. (2011)
Aspergillus aculateus	*T. reesei*	[illegible]0 U/m[illegible]ist cellobiose	Nakazawa et al. (2012)

produces extracellular prot[illegible]. There are [illegible]rts on thermotolerant BGL from bacteria ([illegible]g et al., 2016; [illegible]hamoli et al., 2016) which are produced i[illegible]cellul[illegible] with desirable properties. The *bgl* gene from thes[illegible]rain[illegible] were cloned, overexpressed and resulted in enhanced [illegible]y as w[illegible]as improved properties. Yang et al. (2[illegible]oned [illegible]seq[illegible]ced the *bgl* gene from *Thermoanaerob[illegible]rium[illegible]tearoen[illegible]* BGL belongs to the GH1 with the can[illegible]al glyc[illegible]e hydrolas[illegible] family 1 (GH1) (β/α)8 TIM barrel fold. The [illegible]ifi[illegible]ed relatively high temperature and pH stability [illegible] its highest activity respectively at 60 °C and 6.0 proving the fa[illegible]hat most of the thermostable BGL belongs to GH family 1. The [illegible]plementation of the purified BGL to commercial cellulase resulted in about 20% enhancement in saccharification efficiency while hydrolyzing sugarcane bagasse (Yang et al., 2016). These properties of the purified BGL should have important practical implication in its potential applications for better industrial production of glucose or bioethanol started from lignocellulosic biomass. Another glucose tolerant thermostable BGL was cloned from *Bacillus subtilis* in *E. coli* and overexpressed (Chamoli et al., 2016). The enzyme retained more than 85% of maximum activity after 1 h preincubation at 60 °C and exhibited high tolerance against detergents and organic solvents. A rBGL from *Caldicellulosiruptor saccharolyticus* DSM 8903 with a specific activity of 13 U/mg hydrolyzed cello-oligosaccharides from n = 2 to 5 as substrates using 4 mM sugars to glucose at 70 °C within 16 h at 3 U/mg of enzyme, which is significant (Hong et al., 2009). A novel cellulase recycling method has been developed using a combination of cellulosomes of *Clostridium thermocellum* and *Thermoanaerobacter brockii* β-glucosidase (Waeonukul et al., 2013). The efficient BGL secretion with exploration of structural and functional relationship offer vistas for large scale production and various industrial applications.

Secretion of recombinant protein into the medium using the fungal system, offers some advantages over intracellular production by *E. coli*. The *E. coli* system has a drawback in that the formation of disulfide bonds is hampered by a highly reducing environment in the cytoplasm. In contrast, carrier protein-mediated secretion of recombinant protein in fungi would allow the correct folding of the protein with the aid of chaperones and oxidizing environment in the lumen of the endoplasmic reticulum, thereby increasing the possibility to obtain a protein harboring the native structure (Uchima et al., 2011).

6.2. Eukaryotic rBGL (fungi and yeasts)

Filamentous fungi are known to be a good BGL producer and a number of BGL from glycosyl hydrolase family 3 and 1 have been purified and characterized from various fungi. In spite of bringing good producers of the enzyme, reports on cloning of BGL from fungi are relatively low. This is mostly due to the complexities

associated with the presence of introns in their genes and due to complexities associated with glycosylation. Filamentous fungi are known to produce a broad range of metabolites at significant quantities. Their ability to grow on cheaper substrate have made them potential source of metabolites for industrial applications. Knowledge of fungal genetics has accumulated since years of research and industrial applications. Along with the ability to secrete proteins they can perform posttranslational modification such as glycosylation and disulfidation (Wang et al., 2005). Most of the filamentous fungi are transformed with plasmid which integrates into their genome and thereby providing stability to the fungal transformants. Thus filamentous fungi have tremendous potential to be employed as hosts for recombinant DNA. Genus *Aspergillus* and strain *Trichoderma reesei* have been used as hosts for expressing several genes of fungal as well as nonfungal origin.

Since, *T. reesei* produces the other components in sufficient quantity; it would be of high value to engineer *T. reesei* with gene of BGL having desirable properties such as thermostability and acidic pH tolerant; which can produce sufficient amount of BGL leading to better biomass hydrolysis rate. Engineering *T. reesei* in such a way that it produces the whole suit of saccharifying enzymes including BGL could be one of the approaches to reduce the processing cost.

Yeasts have been also well exploited both for the source of *bgl* gene as well as the host organisms to express fungal BGL. The *bgl* 2 gene from *T. reesei* along with alpha-galactosidase gene was cloned into *S. cerevisae* and expressed both the enzyme extracellularly (Zhang et al., 2012). Production of cellulosic ethanol was demonstrated by expressing heterologous endoglucanase and BGL gene from *Clostridium thermocellum* and *S. fibuligera*, respectively in *S. cerevisae* (Jeon et al., 2009). An industrial ethanol producing strain *S. cerevisae* have been constructed which could produce ethanol directly from microcrystalline cellulose by simultaneous saccharification with 88% of the theoretical yield of ethanol.

Pichia pastoris, due to its exceptional high cellular protein production capacity has been an ideal gene expression for industrial enzyme production and was exploited for heterologous protein expression. *Pichia* is known to possess β-glucosidase producing ability and has been used commercially as a host clone and express recombinant protein from fungi (Ramani et al., 2015; Chen et al., 2012). Thermostable native *bgl* gene from *A. fumigatus* were cloned and expressed in *P. pastoris* stable at 70 °C, at pH 4.0–7.0 which proves its suitability for lignocellulose hydrolysis (Liu et al., 2012). Ramani et al. (2015) have cloned cDNA encoding BGL from *Penicillium funiculosum* NCIM by consensus RTPCR. Though there are reports on cloning of fungal GH3 protein in *Pichia* but very few reports are there on overexpression. It was also tolerant to glucose concentration up to 400 mM. Thus, it was a suitable for an efficient cellulase cocktail, however GH1 BGLs are not glucose tolerant. It exhibited twofold increase in glucose yield when supplemented with crude cellulase of *T. reesei* Rut-C30 in cellulose hydrolysis (Ramani et al., 2015) proving it a thermo- and glucose tolerant BGL and a potential supplement for commercial cellulase in cellulose hydrolysis and thereby assures profitability in bioethanol production.

Chen et al. (2012) also engineered *Pichia pastoris* for expressing a cDNA encoding BGL isolated from the buffalo rumen fungus *Neocallimastix patriciarum*. It was found to be better than the commercial BGL, Novozym188 and saccharification efficiency was increased (Chen et al., 2012). Lee et al. (2013) engineered a *Saccharomyces cerevisiae* strain expressing a cellodextrin transporter and an intracellular β-glucosidase from *Neurospora crassa*. This engineered strain could be employed for simultaneous saccharification and fermentation with enhanced yield at reduced costs. Protein expressions could be highly elevated by cloning the gene under the control of a strong promoter and cellobiohydrolase 1 (cbh1) is such a strong promoter. The cbh1 has been the widely used promoter in eukaryotic system for expression of different protein and has been extensively used for heterologous gene expression constructs in *T. reesei* which is commonly used in commercial enzyme preparation (Celluclast) with enhanced BGL and filter paper activity (Wang and Xia, 2011; Zhang et al., 2010). However there are reports that cbh1 and cbh2 (cel6a) promoters which are known to be very strong, were found to reduce overall cellulase activity (Rahman et al., 2009a). Use of xyn3 promoter to drive expression of *T. reesei* BGL1 (Cel3a) was recently reported greater BGL activity than the parent strain without significant in total cellulase activity (Rahman et al., 2009b). Recently, recombinant *T. reesei* was constructed with *A. aculeateus* BGL gene that expressed under the control of xyn1 promoter, capable of saccharification of pretreated cellulosic biomass. Recombinant strain expressed 25-fold higher BGL than that of endogenous *T. reesei* and 63-fold higher than that of the parent strain, *A. aculeateus* (Nakazawa et al., 2012). Li et al. (2015) used a strong constitutive promoter of gene *pde_02864* encoding ribosomal protein S8, which was used to over-express three β-glucosidases: BGL1, BGL4, and BGL5 of *Penicillium* (Yao et al., 2016). It could be interesting to have hyper BGL producing gene with desired properties cloned in filamentous fungi which is already producing other the components of cellulase, which in turn will help to make the process economic.

In the last 2 years, *Penicillium oxalicum* mutant (JU-A10-T) produces cellulase enzyme preparations at industrial scale in China (Liu et al., 2013). Recently, its genome has been sequenced, providing valuable information to mine novel components that play major roles in biomass saccharification (Liu et al., 2013b). The Reconstruction of Expression Regulatory Network (REXRN) technology has been developed as a new strategy to engineer fungi that enhance cellulase and protein production. One mutant in particular (RE-10 from REXRN) displayed a drastic increase in cellulase and hemicellulase production, and produced even higher values compared to the industrial strain JU-A10-T (Yao et al., 2015). However, the β-glucosidase has not yet been improved to the same level as other inducible cellulases. This makes further improvement of its β-glucosidase activity necessary. Yao et al. (2016) have presented a systematic over-expression analysis of nine β-glucosidase encoding genes in the wild-type strain of *P. oxalicum*. All mutants showed significantly enhanced levels of β-glucosidase at transcriptional, protein, and activity levels. Furthermore, the inducible promoter from *bgl2* was used to conditionally over-express the β-glucosidases BGL1 and BGL4. They over-expressed BGL1, BGL4, or BGL5 using both constitutive as well as inducible promoter. High yielding β-glucosidase producers were obtained and the yields were elevated from 2–65-fold. Surprisingly, this induced expression strategy has improved expression efficiency significantly. In addition, they substantially reduced the enzyme loads in the saccharification of a natural lignocellulose material delignified corncob residue. The mutants with over-expression of BGL4 achieved 75% reduction in enzyme load compared to the parental strain (Yao et al., 2016).

6.3. Metagenomic rBGL

Thus, metagenomic approach allows us to clone the microbial genome directly from the environmental samples into the culturable bacteria. Complex microbial community analysis coupled with various environmental habitats revealed that only a small proportion of microorganisms surviving on earth can be cultivated in vitro, while majority of them remain unculturable (Vartoukian et al., 2010). Several BGLs have been obtained via metagenomic strategies (Bao et al., 2012). As our interest is only the gene

producing BGL and not the microorganism, the gene can be isolated, cloned and expressed. These widespread unculturable unexplored microbes hold diverse group of biocatalysts that are stable and active under different extreme conditions (Fernández-Arrojo et al., 2010), which could be exploited for benefits of mankind. Metagenomics is one of the key strategies for discovering diverse biocatalysts encoded in nature (Jiang et al., 2011). This approach has been successfully used to identify biocatalysts with desired activities by applying low-throughput function-based screening of environmental DNA clone libraries (Steele et al., 2009). Even after the advances of third generation sequencing; complete genes availability from metagenomic assemblies is limited (Pareek et al., 2011). Congregated mining of the genetic information of β-glucosidase was explored across different environments including hydrothermal spring (Schröder et al., 2014), wetlands (Nam et al., 2008), sludge (Jiang et al., 2011), pulp wastewater (Yang et al., 2013), gut and rumen (Wang et al., 2012; Bao et al., 2012; Zhu et al., 2011; Healy et al., 1995), anaerobic digesters (Healy et al., 1995) and compost (Uchiyama et al., 2013). Moreover, some detailed biochemical and molecular descriptions of metagenome-derived β-glucosidases have been exposed to display their unique characteristics. Reports demonstrates metagenome derived BGL are being stable and active at wide range of pH and extreme temperature as 105 °C (Jiang et al., 2011; Schröder et al., 2014). The Bgl1D derived from alkaline polluted soil is remarkably stable across a broad range of pH (5.5–10.5) and is not affected by high concentration of metal ions (Jiang et al., 2011). A thermostable glycosyl hydrolase 1 family BGL from archeal origin, isolated from hydrothermal spring, was active even at 105 °C and also performed well in the presence of different reagents and solvents (Sch… et al. 2014). Another thermostable BGL was isolated from the … tional screening of a metagenomic library from termite gut w…h showed its highest activity at 90 °C (Wang et al., 2012). One uni… BGL with very high glucose tolerance ability of up… mM w… obtained from compost and it showed high… ansgl…osylatio… activity by generating sophorose, laminar…se, cell…ose, and gentiobiose (Uchiyama et al., 2013). It could … e… date for obtaining high glucose concen…tion in… ydrolysate to convert into bio-ethanol as well as… enerate ind…s for cellulase production. Similar glucos…e was exh…d by the BGL derived from marine meta…genom… retaining 50% of its activity even at 1000 mM … glucose (Fang … al., 2010). Interestingly, it was activated … glucose at concen…tions lower than 400 mM. This enzym… as also…ive at high NaCl concentration signifying its origin … m…e environment. Nam et al. (2008) attempted successfully … ngineer… protein extracted from wetland in … enha…stab…y and activity. All of these selected B… prote… show …ess protein similarity with the sequen… availabl… database which proves novelty.

There … fe… GLs but novel ones which have been reported fro… etagenomic origin. Li et al. (2014) isolated a new BGL from a me…nomic library of cattle rumen feeding with *Miscanthus sinensis* b…e function-based screening, belonging to glycoside hydrolase family 3 (GH3). It was recombinantly expressed, purified and biochemically characterized and was activated by glucose at the concentration lower than 40 mM. These new entries from unculturable microbial systems aid to incorporate novel insights into the structure-function relationship and substrate recognition of β-glucosidase and reflect its versatile biotechnological applications.

6.4. Miscellaneous

There is a report where the three functional β-glucosidase genes were obtained from the bacterium *Clostridium cellulovorans* (CcBglA), the fungus *Trichoderma reesei* (TrBgl2), and the termite *Neotermes koshunensis* (NkBgl) with the crystal structures of *CcBglA*, *TrBgl2* and *NkBgl* and expressed in *E. coli* at a level of 70–250 mg/l (Jeng et al., 2011). The overall structures of these enzymes were similar to those belonging to the β-retaining GH1, which have a classical (α/β)8 TIM barrel fold. Each contains a slot-like active site cleft and a more variable outer opening, related to its function in processing different lengths of β-1,4-linked glucose derivatives. The two essential glutamate residues for hydrolysis are spatially conserved in the active site. According to Jeng et al. (2011) *CcBglA* exhibits the highest potential for use in the process of lignocellulosics ethanol production, especially for the processes of SSF and SSCF due to its h…me activity at a moderate reaction temperature ne… neutral p… The structural and functional properties of these …e β-glucos…es from various biological sources open im…porta…enues for …actical implications (Jeng et al., 2011).

Use of all these tech…es co…d elim…nwanted cost in ethanol production si… cellul…ic enzy…s account for 40–50% of the operation…e…s (Xu e…al., 2009). Thus, genes responsible for e…ression …fficient…d versatile β-glucosidase can be transfe… to more in…i… tractable and robust organisms to en…e…e saccharifi…on of the complex biomass polysacch…de.

7. …nclusion

…BGL finds … spot among cellulases in biomass hydrolysis n…ly becaus… ommercially produced cellulases are BGL defici… …-…ate-limiting component. Several strategies were adopted … circumvent BGL deficiency in cellulase cocktail *e.g.*, …rologous blending and genetic modification. BGL from metage… offers excellent advantages, as they are active even at 105 °C. Heterologous over-expression of BGL was successful for blending and preparing cocktail. Cloning and over-expressions under strong promoter are promising strategy for over-producing BGL. BGL tolerance to high glucose and temperature has great significance in biomass bioconversion. BGL structure elucidation enabled targeted modification by site-specific mutation, which could be highly specific.

Acknowledgement

Author RRS acknowledge Department of Biotechnology, Govt. of India, for DBT – Bioscience Energy Overseas Fellowship.

References

Bao, L., Huang, Q., Chang, L., Sun, Q., Zhou, J., Lu, H., 2012. Cloning and characterization of two β-glucosidase/xylosidase enzymes from yak rumen metagenome. Appl. Biochem. Biotechnol. 166, 72–86.
Bhatia, Y., Mishra, S., Bisaria, V.S., 2002. Microbial beta-glucoidases: cloning, properties and applications. Crit. Rev. Biotechnol. 22, 375–407.
Bohlin, C., Praestgaard, E., Baumann, M.J., Borch, K., Praestgaard, J., Monrad, R.N., Westh, P., 2013. A comparative study of hydrolysis and transglycosylation activities of fungal β-glucosidases. Appl. Microbiol. Biotechnol. 97 (1), 159–169.
Cairns, J.R.K., Esen, A., 2010. Β-Glucosidases. Cell. Mol. Life Sci. 67, 3389–3405.
Cantarel, B.L., Coutinho, P.M., Rancurel, C., Bernard, T., Lombard, V., Henrissat, B., 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for glycogenomics. Nucleic Acids Res. 37, D233–D238.
Chamoli, S., Kumar, P., Navani, N.K., Verma, A.K., 2016. Secretory expression, characterization and docking study of glucose-tolerant -glucosidase from *B. subtilis*. Int. J. Biol. Macromol. 85, 425–433.
Chen, H.L., Chen, Y.C., Lu, M.Y., Chang, J.J., Wang, H.T., Ke, H.M., Wang, T.Y., Ruan, S. K., Wang, T.Y., Hung, K.Y., Cho, H.Y., Lin, W.T., Shih, M.C., Li, W.H., 2012. A highly efficient β-glucosidase from the buffalo rumen fungus *Neocallimastix patriciarum* W5. Biotechnol. Biofuels 5, 24–33.
Dashtban, M., Qin, W., 2012. Overexpression of an exotic thermotolerant beta-glucosidase in *Trichoderma reesei* and its significant increase in cellulolytic activity and saccharification of barley straw. Microb. Cell. Fact. 1, 63–77.
de Giuseppe, P.O., Souza, T.de A., Souza, F.H., Zanphorlin, L.M., Machado, C.B., Ward, R.J., Jorge, J.A., Furriel Rdos, P., Murakami, M.T., 2014. Structural basis for

glucose tolerance in GH1 β-glucosidases. Acta. Crystallogr. D Biol. Crystallogr. 70, 1631–1639.
Del Pozo, M.V., Fernández-Arrojo, L., Gil-Martínez, J., Montesinos, A., Chernikova, T. N., Nechitaylo, T.Y., Waliszek, A., Tortajada, M., Rojas, A., Huws, S.A., Golyshina, O.V., Newbold, C.J., Polaina, J., Ferrer, M., Golyshin, P.N., 2012. Microbial β-glucosidases from cow rumen metagenome enhance the saccharification of lignocellulose in combination with commercial cellulase cocktail. Biotechnol. Biofuels 5, 73–85.
Fang, Z., Fang, W., Liu, J., Hong, Y., Peng, H., Zhang, X., Sun, B., Xiao, Y., 2010. Cloning and characterization of a beta-glucosidase from marine microbial metagenome with excellent glucose tolerance. J. Microbiol. Biotechnol. 20, 1351–1358.
Fernández-Arrojo, L., Guazzaroni, M.E., López-Cortés, N., Beloqui, A., Ferrer, M., 2010. Metagenomic era for biocatalyst identification. Curr. Opin. Biotechnol. 21, 725–733.
Harnpicharnchai, P., Champreda, V., Sornlake, W., Eurwilaichitr, L., 2009. A thermotolerant beta-glucosidase isolated from an endophytic fungi, *Periconia sp.*, with a possible use for biomass conversion to sugars. Protein Expr. Purif. 67, 61–69.
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 lignocelluloses. Appl. Microbiol. Biotechnol. 43, 667–674.
Henrissat, B., 1991. A classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem. J. 280, 309–316.
Henrissat, B., Davies, G., 1997. Structural and sequence-based classification of glycosyl hydrolases. Curr. Opin. Struct. Biol. 7, 637–644.
Hong, M.R., Kim, Y.S., Park, C.S., Lee, J.K., Kim, Y.S., Oh, D.K., 2009. Characterization of a recombinant beta-glucosidase from the thermophilic bacterium *Caldicellulosiruptor saccharolyticus*. J. Biosci. Bioeng. 108 (1), 36–40.
Hu, J., Arantes, V., Saddler, J.N., 2011. The enhancement of enzymatic hydrolysis of lignocellulosic substrates by the addition of accessory enzymes such as xylanase: is it an additive or synergistic effect. Biotechnol. Biofuels 4, 36–48.
Jabbour, D., Klippel, B., Antranikian, G., 2012. A novel thermostable and glucosetolerant beta-glucosidase from *Fervibacterium islandicum*. Appl. Biochem. Biotechnol. 93, 1947–1956.
Jeng, W.Y., Lin, C.T., Chang, W.J., Liu, C.I., Wang, A.H., 2012. High-resolution structures of *Neotermes koshunensis* b-glucosidase mutants provide insights into the catalytic mechanism and the synthesis of glucoconjugates. Acta. Cryst. D68, 829–838.
Jeng, W.Y., Wang, N.C., Lin, M.H., Lin, C.T., Liaw, Y.C., Chang, W.J., Liu, C.I., Liang, Wang, A.H., 2011. Structural and functional analysis of three β-glucosid from bacterium *Clostridium cellulovorans*, fungus *Trichoderma reesei* and term *Neotermes koshunensis*. J. Struct. Biol. 173 (1), 46–56.
Jeon, E., Hyeon, J.E., Suh, D.J., Suh, Y.W., Kim, S.W., Song, K.H. O., 2009. Production of cellulosic ethanol in Saccharomyces cere logous expressing *Clostridium thermocellum* endoglucanase *Saccha ycopsis fibuligera* beta-glucosidase genes. Mol. Cell. 28, 369–3
Jiang, C., Li, S.X., Luo, F.F., Jin, K., Wang, Q., Hao, Z.Y., Wu, hao Shen, P.H., Tang, X.L., Wu, B., 2011. Biochemical cha cteri two nov glucosidase genes by metagenome expressio ing. Biore Technol. 102, 3272–3278.
Kuusk, S., Väljamäe, P., 2017. When subst its and inhibi stivates: implications of β-glucosidases. Biotec Biotf 7.
Lieberman, R.L., Wustman, B.A., Huertas, P., Powe Jr, Pine, C.W., Khanna, R., Schlossmacher, M.G., Ringe, etsko, G.A., Structure of acid betaglucosidase with pharm ogical chaperone des insight into Gaucher disease. Nat. Chem 3, 101 07.
Lee, W.H., Nan, H., Kim, H Y.S., Simultaneous saccharification and fermentation by engineer yces cerevisiae without supplementing extracellular β-glucosidase. J nol. 167 322.
Li, Y., Liu, N., Yang hao, F. Y., Ti Lu, X., 2014. Cloning and characterizati β-Gluco a metagenomic library of Rumen of cattle fe with *M anthus sin* BMC Biotechnol. 14, 85–93.
Li, Z., Yao, G R., Gao, an, Q., Liu, M., ang, P., Liu, G., Qin, Y., Song, X., Zhong, Y., Fang, and dose-controlled regulation of cellulase gene expres *cillium cum*. PLoS Genet. 11, e1005509.
Liu, D., Zhang, g, X., Zhang, Z., Song, S., Miao, Y., Shen, Q., 2012. Characterization thermostable beta-glucosidase from *Aspergillus fumigatus* Z5, and it tional expression in *Pichia pastoris* X33. Microb. Cell. Fact. 11, 25–39.
Liu, G., Zhang, L., Qin, Y., Zou, G., Li, Z., Yan, X., Wei, X., Chen, M., Chen, L., Zheng, K., Zhang, J., Ma, L., Li, J., Liu, R., Xu, H., Bao, X., Fang, X., Wang, L., Zhong, Y., Liu, W., Zheng, H., Wang, S., Wang, C., Xun, L., Zhao, G.P., Wang, T., Zhou, Z., Qu, Y., 2013a. Long-term strain improvements accumulate mutations in regulatory elements responsible for hyper-production of cellulolytic enzymes. Sci. Rep. 3, 1569–1575.
Liu, G., Zhang, L., Wei, X., Zou, G., Qin, Y., Ma, L., Li, J., Zheng, H., Wang, S., Wang, C., Xun, L., Zhao, G.P., Zhou, Z., Qu, Y., 2013b. Genomic and secretomic analyses reveal unique features of the lignocellulolytic enzyme system of *Penicillium decumbens*. PLoS ONE.
Liu, J., Zhang, X., Fang, Z., Fang, W., Peng, H., Xiao, Y., 2011. The 184th residue of beta-glucosidase Bgl1B plays an important role in glucose tolerance. J. Biosci. Bioeng. 112, 447–450.
Lundemo, P., Adlercreutz, P., Karlsson, E.N., 2013. Improved transferase/hydrolase ratio through rational design of a family 1 β-glucosidase from *Thermotoga neapolitana*. Appl. Environ. Microbiol. 79 (11), 3400–3405.
Ma, L., Zhang, J., Zou, G., Wang, C., Zhou, Z., 2011. Improvement of cellulase activity in *Trichoderma reesei* by heterologous expression of a beta-glucosidase gene from *Penicillium decumbens*. Enzyme Microb. Technol. 49, 366–371.
Margeot, A., Hahn-Hagerdal, B., Edlund, M., Slade, R., Monot, F., 2009. New improvements for lignocellulosic ethanol. Curr. Opin. Biotechnol. 20, 372–380.
Mohanram, S., Amat, D., Choudhary, J., Arora, A., Nain, L., 2013. Novel perspectives for evolving enzyme cocktails for lignocellulose hydrolysis in biorefineries. Sus. Chem. Proc. 1, 15–26.
Murray, P., Aro, N., Collins, C., Grassick, A., Penttila, M., Saloheimo, M., Tuohy, M., 2004. Expression in Trichoderma reesei and characterisation of a thermostable family 3 beta-glucosidase from the moderately thermophilic fungus *Talaromyces emersonii*. Protein Expr. Purif. 38, 248–257.
Nakazawa, H., Kawai, T., Ida, N., Shida, Y., Kobayashi, Y., Okada, H., Tani, S., Sumitani, J., Kawaguchi, T., Morikawa, Y., Ogasawara1, W., 2012. Construction of a recombinant Trichoderma reesei strain expressing Aspergillus a betaglucosidase 1 for efficient biomass conversion. Biotechnol. Bioen
Nam, K.H., Kim, S.J., Kim, M.Y., Kim, J.H., Yeo, Y e, C.M., Ju , Hwang, K.Y., 2008. Crystal structure of engineered beta sidase from a metagenome. Proteins 73, 788–793.
Nam, K.H., Sung, M.N., Hwang, K.Y., 20 Struc insights in he substrate recognition properties of β-gluco e. Biochem hys. commun. 391, 1131–1135.
Pareek, C.S., Smoczynski, R., Tre , 2011 encing ologies and genome sequencing. J. Appl. Genet 3–4
Rahman, Z., Shida, Y., Furu wa, Okada Ogasawara, W., Morikawa, Y., 2009a. Evaluatio d chara tion of oderma reesei cellulase and xylanase promote ppl. Microbi echnol. 82, 899–908.
Rahman, Z., Shida, awa, T., Suzuk da, H., Ogasawara, W., Morikawa, Y., 2009b. A ati Trichoderma r cellulase and xylanase promoters through hom logous bination for enhanced production of extracellular beta-glucosidase I. Biosci chnol. Biochem. 73, 1083–1089.
Rajasree athew, G.M., A., Sukumaran, R.K., 2013. Highly glucose t t β-glucosidase from spergillus unguis: NII 08123 for enhanced olysis of biomass. J. Ind. Microbiol. Biotechnol. http://dx.doi.org/10.1007/295-013-129
Ran G., Meera, B itha, C., Rajendhran, J., Gunasekaran, P., 2015. Molecular c and exp ion of thermostable glucose-tolerant β-glucosidase of *Pe a fun m* NCL1 in *Pichia pastoris* and its characterization. J. Ind. Micro nol. 42 (4), 553–565.
ni, J.K., Patel, A.K., Adsul, M., Singhania, R.R., 2016. Cellulase adsorption on lignin: dblock for economic hydrolysis of biomass. Renewable Energy 98, 29–42.
S lleuche, S., Blank, S., Antranikian, G., 2014. Characterization of a heat-active archaeal β-glucosidase from a hydrothermal spring metagenome. Enzyme Microb. Technol. 57, 48–54.
Shen, Y., Zhang, Y., Ma, T., Bao, X., Du, F., Zhuang, G., Qu, Y., 2008. Simultaneous saccharification and fermentation of acid-pretreated corncobs with a recombinant *Saccharomyces cerevisiae* expressing beta-glucosidase. Bioresour. Technol. 99, 5099–5103.
Singhania, R.R., Sukumarana, R.K., Patel, A.K., Larroche, C., Pandey, A., 2010. Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. Enzyme Microb. Technol. 46, 541–549.
Singhania, R.R., Sukumaran, R.K., Rajasree, K.P., Mathew, A., Gottumukkala, L., Pandey, A., 2011. Properties of a major β-glucosidase-BGL1 from *Aspergillus niger* NII-08121 expressed differentially in response to carbon sources. Process Biochem. 46 (7), 1521–1524.
Singhania, R.R., Patel, A.K., Sukumaran, R.K., Larroche, C., Pandey, A., 2013. Role and significance of beta-glucosidases in the hydrolysis of cellulose for bioethanol production. Bioresour. Technol. 127, 500–507.
Souza, F.H.M., Nascimento, C.V., Rosa, J.C., Masui, D.C., Leone, F.A., Jorge, J.A., Furriel, R.P.M., 2010. Purification and biochemical characterization of a mycelial glucose- and xylose-stimulated beta-glucosidase from the thermophilic fungus *Humicola insolens*. Process Biochem. 45, 272–278.
Souza, F.H.M., Inocentes, R.F., Ward, R.J., Jorge, J.A., Furriel, R.P.M., 2013. Glucose and xylose stimulation of a β-glucosidase from the thermophilic fungus *Humicola insolens*: A kinetic and biophysical study. J. Mol. Catal. B-Enzym. 94, 119–128.
Steele, H.L., Jaeger, K.E., Daniel, R., Streit, W.R., 2009. Advances in recovery of novel biocatalysts from metagenomes. J. Mol. Microbiol. Biotechnol. 16, 25–37.
Sukumaran, R.K., Singhania, R.R., Mathew, G.M., Pandey, A., 2009. Cellulase production using biomass feed stock and its application in lignocelluloses saccharification for bioethanol production. Renewable Energy 34, 421–424.
Uchima, C.A., Tokuda, G., Watanabe, H., Kitamoto, K., Arioka, M., 2011. Heterologous expression and characterization of a glucose-stimulated β-glucosidase from the termite *Neotermes koshunensis* in *Aspergillus oryzae*. Appl. Microbiol. Biotechnol. 89, 1761–1771.
Uchima, C.A., Tokuda, G., Watanabe, H., Kitamoto, K., Arioka, M., 2013. A novel glucose-tolerant β-glucosidase from the salivary gland of the termite Nasutitermes takasagoensis. J. Gen. Appl. Microbiol. 59, 141–145.
Uchiyama, T., Miyazaki, K., Yaoi, K., 2013. Characterization of a novel β-glucosidase from a compost microbial metagenome with strong transglycosylation activity. J. Biol. Chem. 288, 18325–18334.
Vartoukian, S.R., Palmer, R.M., Wade, W.G., 2010. Strategies for culture of 'unculturable' bacteria. FEMS Microbiol. Lett. 309, 1–7.
Volynets, B., Dahman, Y., 2011. Assessment of pretreatments and enzymatic hydrolysis of wheat straw as a sugar source for bioprocess industry. Int. J. Eng. Environ. 2, 427–446.

Waeonukul, R., Kosugi, A., Prawitwong, P., Deng, L., Tachaapaikoon, C., Pason, P., Ratanakhanokchai, K., Saito, M., Mori, Y., 2013. Novel cellulase recycling method using acombination of *Clostridium thermocellum* cellulosomes and *Thermoanaerobacter brockii* β-glucosidase. Bioresour. Technol. 130, 424–430.

Wang, L., Ridgway, D., Gu, T., Moo-Young, M., 2005. Bioprocessing strategies to improve heterologous protein production in filamentous fungal fermentations. Biotechnol. Adv. 23, 115–129.

Wang, B., Xia, L., 2011. High efficient expression of cellobiase gene from *Aspergillus niger* in the cells of *Trichoderma reesei*. Bioresour. Technol. 102, 4568–4572.

Wang, Q., Qian, C., Zhang, X.Z., Liu, N., Yan, X., Zhou, Z., 2012. Characterization of a novel thermostable β-glucosidase from a metagenomic library of termite gut. Enzyme Microb. Technol. 51, 319–324.

Wang, S., Liu, G., Wang, J., Yu, J., Huang, B., Xing, M., 2013. Enhancing cellulase production in *Trichoderma reesei* RUT C30 through combined manipulation of activating and repressing genes. J. Ind. Microbiol. Biotechnol. 40 (6), 633–641.

Xu, Q., Singh, A., Himmel, M.E., 2009. Perspectives and new directions for the production of bioethanol using consolidated bioprocessing of lignocellulose. Curr. Opin. Biotechnol. 20, 364–371.

Xu, R., Teng, F., Zhang, C., Li, D., 2011. Cloning of a gene encoding beta-glucosidase from *Chaetomium thermophilum* CT2 and its expression in Pichia pastoris. J. Mol. Microbiol. Biotechnol. 20, 16–23.

Yan, Q., Hua, C., Yang, S., Li, Y., Jiang, Z., 2012. High level expression of extracellular secretion of a beta-glucosidase gene (PtBglu3) from *Paecilomyces thermophila* in *Pichia pastoris*. Protein Expr. Purif. 84, 64–72.

Yang, C., Niu, Y., Li, C., Zhu, D., Wang, W., Liu, X., Cheng, B., Ma, C., Xu, P., 2013. Characterization of a novel metagenome-derived 6-phospho-β-glucosidase from black liquor sediment. Appl. Environ. Microbiol. 79, 2121–2127.

Yang, F., Yang, X., Li, Z., DU, C., Wang, J., Li, S., 2016. Overexpression and characterization of a glucose-tolerant β-glucosidase from *T. aotearoense* with high specific activity for cellobiose. Appl. Environ. Microbiol. 99 (21), 8903–8915.

Yang, Y., Zhang, X., Yin, Q., Fang, W., Fang, Z., Wang, X., Zhang, X., Xiao, Y., 2015. A mechanism of glucose tolerance and stimulation of GH1 β-glucosidases. Sci. Rep. 25 (5), 17296–17307.

Yao, G., Wu, R., Qinbiao Kan, Q., Gao, L., Liu, M., Yang, P., Du, J., Li, Z., Qu, Y., 2016. Production of a high-efficiency cellulase complex via β-glucosidase engineering in Penicillium oxalicum. Biotechnol Biofuels 9, 78.

Yao, G., Li, Z., Gao, L., Wu, R., Kan, Q., Liu, G., Qu, Y., 2015. Redesigning the regulatory pathway to enhance cellulase production in *Penicillium oxalicum*. Biotechnol. Biofuels 8, 71–86.

Zanoelo, F.F., Polizeli, M.L., Terenzi, H.F., Jorge, J.A., 2004. Beta-glucosidase activity from the thermophilic fungus *Scytalidium thermophilum* is stimulated by glucose and xylose. FEMS Microbiol. Lett. 240, 137–143.

Zhang, J., Zhong, Y., Zhao, X., Wang, T., 2010. Deve[illegible] of the cellulolytic fungus *Trichoderma reesei* strain with enhanced [illegible]e and filter paper activity using strong artificial cellobiohy[illegible]se 1 prom[illegible]ioresour. Technol. 101, 9815–9818.

Zhang, L., Guo, Z.P., Ding, Z.Y., Wang, Z.X.[illegible] G.Y., 2012. [illegible]truction of the industrial ethanol-producing str[illegible] of *Sacc[illegible]ces cerev[illegible]* able to ferment cellobiose and melibiose. Appl[illegible]chem. Mic[illegible] 48, [illegible]221.

Zhu, L., Wu, Q., Dai, J., Zhang, S[illegible], F., 201[illegible]. Evide[illegible]lose metabolism by the giant panda gut mic[illegible]me. Pro[illegible]l. Acad. [illegible]08, 17714–17719.

Zimbardi, A.L., Sehn, C., [illegible] L.F[illegible]uza, F.H., Masui, D.C., Nozawa, M.S., Guimarães, L.H., Jor[illegible] J.A.[illegible]P., 2013. [illegible]mization of β-Glucosidase, β-Xylosidase and X[illegible]ase Pro[illegible]n by *Coll[illegible]chum graminicola* under solid-state fermenta[illegible] and applicat[illegible] ray[illegible]garcane trash saccharification. Int. J. Mol. Sci. [illegible]-2902.