Lignocellulolytic and Chitinolytic Glycoside Hydrolases: Structure, Catalytic Mechanism, Directed Evolution and Industrial Implementation

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Abstract

Lignocellulolytic and chitinolytic glycoside hydrolases are commonly used enzymes in commercial processes and thus are well documented for their vital role in agriculture, textile, paper, food, biofuel and healthcare sectors. Ongoing research is targeted to further enhance the applicability of these vital enzymes in the above mentioned sectors. In this regard, an up-to-date knowledge of enzymatic structure, catalytic mechanism and several modern approaches like recombinant technology, chemical and enzymatic pre-treatment, enzyme immobilization and directed evolution for the improvement of enzymes production, activity and stability is highly required. This review provides an up-to-date information and discussion on the various lignocellulolytic and chitinolytic glycoside hydrolases regarding the vital aspects of the enzymes *viz*. catalytic mechanism, structural features, directed evolution approaches, current applications and under research potential applications.

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Introduction

Lignocellulose and chitin biomass are the top two naturally most abundant polymeric biomass on earth (Bar-On et al. 2018). Due to the renewable nature, both of them are highly explored for the generation of wide range of value-added products and bioenergy generation. The conversion of these lignocellulosic and chitin containing biomass has been carried out by various technologies involving chemical and enzymatic means (Ngernyuang et al. 2018; Yadav 2017). However, enzymatic conversion is always preferred due to environmental safety and highly specific product development. In this regard, lignocellulolytic and chitinolytic glycoside hydrolases (GH) has been of prime interest for the scientific and industrial community due to their specific natural affinity towards lignocellulose and chitin, respectively. The lignocellulolytic GH are well documented for their application in, food, beverages, animal feed, laundry, textile, paper and pulp as well as in agriculture and biofuel generation (Isikgor and Becer 2015). Whereas, chitinolytic GH has shown their potential applicability in healthcare, agriculture, and food industries (Patil et al. 2000). These vast range of applications has resulted in enormous demand of lignocellulolytic and chitinolytic GH production with improved characteristics. In order to achieve cost-effective and industrial-scale production of these enzymes a lot of techniques have been developed. These include exploration of novel sources for enzyme production, utilization of chemical and molecular techniques to boost the production and properties as well as genetic manipulation in order to fulfil the requirement of industries i.e. high activity and productivity over a wide range of pH and temperature along with eco and cost-effective production (Liu and Kokare 2017). In the present study, detailed discussion is included about various lignocellulolytic and chitinolytic GH in term of their structure, occurrence, catalytic mechanism, directed evolution strategies and various developed as well as under developing industrial applications.

Glycoside Hydrolases

GH are the group of widely spread enzymes existing in all living organisms with an exception of some Achaeans and unicellular parasitic eukaryotes (Naumoff 2011). GH catalyzes the hydrolysis of glycosidic bond between two or more carbohydrates or between a carbohydrate and a non-carbohydrate moiety. In order to enhance the ease of study and understanding, the GH were classified on various basis like substrate specificity, mode of action and amino acid sequence similarities (Henrissat 1991). GH classification on the basis of substrate specificity is simplest one and is the basis of recommendation of the International Union of Biochemistry and

Molecular Biology (IUBMB). The IUBMB classification of GH is expressed as EC 3.2.1.x, where x signifies the substrate specificity as well as molecular mechanism or type of linkages in some cases. However, the intrinsic problem associated with substrate specificity based classification is in accommodating the enzymes that can act upon several substrates. Additionally, the classification is also unable to reflect about the 3D structural features of these enzymes. Another extensively used GH classification is on the basis of mode of action i.e. *exo* (attacks specifically at one of the termini of polymer chain) or '*endo*' (attacks randomly within the polymer chain) (Davies and Henrissat 1995). Although the dissimilarity assessment on the basis of mode of action is a powerful mean of classification but the distinction measurement of many enzymes with processive or multiple-attack nature is extremely challenging. The drawbacks of the conventional classification system leads to the development of amino acid sequence similarities based classification. The amino acid sequence similarities based classification is based on the fact that there is direct relationship between sequence and folding similarities and hence, it provides structural, evolutionary and mechanistic information of enzymes (Henrissat 1991). According to the Carbohydrate-Active Enzyme database (CAZy), GH are divided in 156 family with 5,99,654 modules or in 18 clans on the basis of conserved protein folds (Henrissat and Bairoch 1996) (www.cazy.org). The clans reflects about a group of families with significant similarity in their tertiary structure, catalytic residues and mechanisms.

Catalytic mechanism: GH catalyzes the hydrolysis of glycosidic bond by the involvement of two amino acid residues of the enzymes, among which one be the proton donor commonly glutamate or aspartate while the other be a nucleophile or general base residue like glutamic or aspartic acid (Davies and Henrissat 1995). These catalytic residues spatial position are accountable for the hydrolysis via overall retention or overall inversion of the anomeric configuration. In case of some enzymes the catalytic nucleophile are also replaced by the acetamido group at C-2 of the substrate (Terwisscha van Scheltinga et al. 1995). In GH, the inversion of anomeric configuration takes place through single-nucleophilic substitution whereas, the retention occurs due to double-displacement mechanism involving covalent glycosyl-enzyme intermediate (Ardèvol and Rovira 2015). During the retention, first glycosylation takes place in which one residue act as nucleophile that attacks on the anomeric centre to displace the aglycon resulting to the formation of glycosyl enzyme intermediate (Koshland 1953). Simultaneously, the other residue acts as an acid catalyst and protonates the glycosidic oxygen upon bond disruption. Following the second step of deglycosylation, the GH is hydrolysed by water and the other residue serves as base catalyst and deprotonate the water molecules (Koshland 1953). GH families 18, 20, 25, 56, 84 and 85 contains enzymes with no catalytic nucleophile and hence the intramolecular nucleophile is provided through the neighbouring group participation mechanism by 2-acetamide groups that results in the formation of an oxazolinium ion intermediate (Terwisscha van Scheltinga et al. 1995; Vocadlo and Withers 2005). However, the GH of families 33, 34 and 143 uses a tryosine as a catalytic nucleophile which has to be activated by an adjacent base residue (Ndeh et al. 2017; van Aalten et al. 2001).

Directed Evolution of Enzymes

Enzymes based biocatalysis has emerged as one of the most promising and widely accepted means over the chemical processes for product generation (Turner 2009). Nowadays, enzymes are used in detergent, textile, pulp and paper, food and cosmetic industries (Cherry and Fidantsef 2003). These rapid increasing industrial applicability of enzymes are possible due to the day to day biotechnological advancements made in the field of recombinant DNA technology, fermentation techniques and directed evolution. Among these the directed evolution has promptly emerged as a prevailing approach for refining the characteristics of enzymes in a targeted manner. It can be explained as an artificial approach to mimic and speed up the natural evolutionary process in laboratory with the help of molecular biology techniques (Turner 2009). Directed evolution can alter enzymes to act in new reaction environments, optimize their catalytic activity towards substrates and enable them to catalyze new chemical reactions. The basic concept of directed evolution has shown in Fig. 1. The major steps in the directed evolution of enzymes, includes identification of preliminary state protein, modification of its gene, an expression and

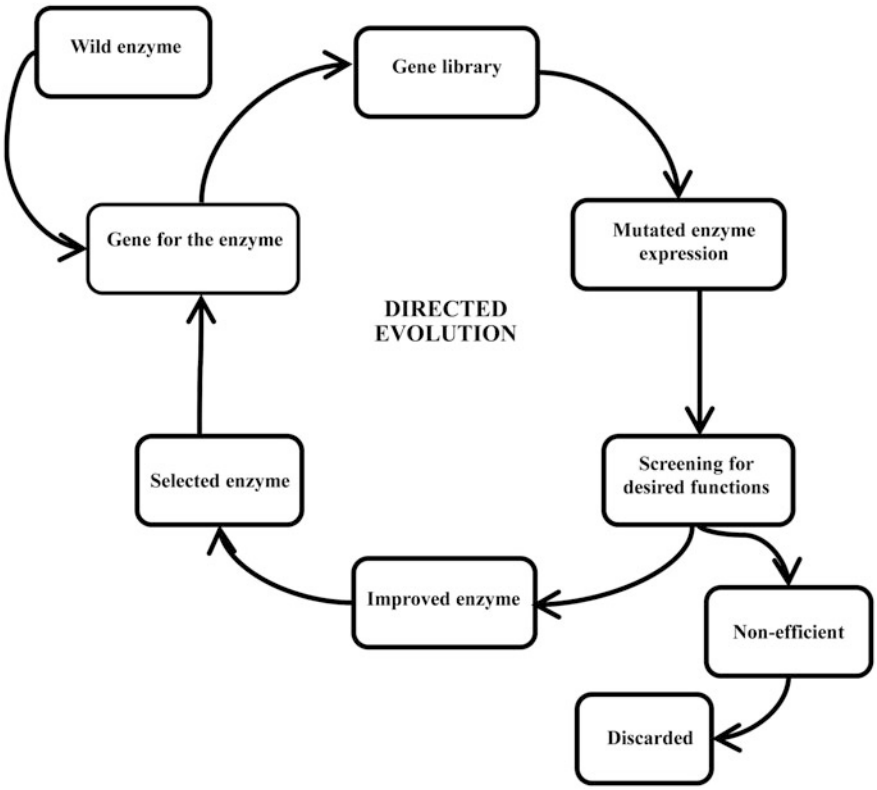

Fig. 1 Steps involved in the directed evolution of enzymes

screening strategy, re-modification, re-screening, and so on till an acceptable performance level in term of enzymatic activity, binding affinity or specificity is achieved (Turner 2003).

Lignocellulolytic Glycoside Hydrolases

Lignocelluloses, the major structural components of all plants is the most abundant organic compound present on the earth. About 10 to 50 $\times$ 10^9 tons of lignocelluloses are produced annually that is approximately 50% of the world's total biomass (Kuhad and Singh 1993). Lignocelluloses consist mainly cellulose (35–50%), hemicellulose (20–35%) and lignin (10–25%) which in different species, tissues and according to the maturity of plant cell wall (Isikgor and Becer 2015). Apart from them lignocelluloses also contain proteins, oils, and ash in less amount. Cellulose is a semi-crystalline, linear syndiotactic polymer of D-glucopyranose subunits linked together through intra- and intermolecular β-(1 → 4)–glycosidic bonds and provides strength to the material (Menon and Rao 2012). While, hemicellulose comprises of shorter, branched polymer chains of various C_6 and C_5 sugars that function as glue around and between the cellulose bundles. The C_6 sugar mainly contains glucose together with mannose and galactose while, C_5 sugars includes xylose and arabinose (Lange 2007). The next major contributor of lignocellulosic biomass is lignin which is a complex amorphous tri-dimensional polymer of propyl-phenol that is embedded in and bound to the hemicellulose and provides rigidity to the structure (Andlar et al. 2018). The detail structure of lignocellulose and their enzymatic degradation can be easily understand by Fig. 2. The enormous amount of lignocelluloses upon degradation has shown a wide range of applicability in various industries like biofuels, paper and pulp, agriculture, food etc. (Malherbe and Cloete 2002). The conversion of biomass into value-added products has become so effective and popular due to the practice of different lignocellulolytic enzymes i.e. pectinases, cellulases, hemicellulases and ligninases (Arevalo-Gallegos et al. 2017). The tremendous research success in the field of lignocellulolytic GH has resulted into several patents, some of recent patents publications are summarised in Table 2. As the present study is centered on GH, thus, the authors will focus only on cellulases and hemicellulases which are the members of GH.

Cellulases

Cellulases belongs to family 5, 6, 7, 9, 45 and 48 of GH and it catalyzes the endohydrolysis of (1 → 4)-β-D-glucosidic linkages (www.cazy.org). Cellulases mainly consist of three group of enzymes i.e. endo-(1,4)-β-D-glucanase (EC 3.2.1.4) which randomly attacks on the internal O-glycosidic bonds leading to the generation of glucan chains with various lengths, second is exo-(1,4)-β-D-glucanase (EC 3.2.1.91) that acts on the ends of cellulose chain releasing β-cellobiose and the third one is β-glucosidases (EC 3.2.1.21) which shows specific

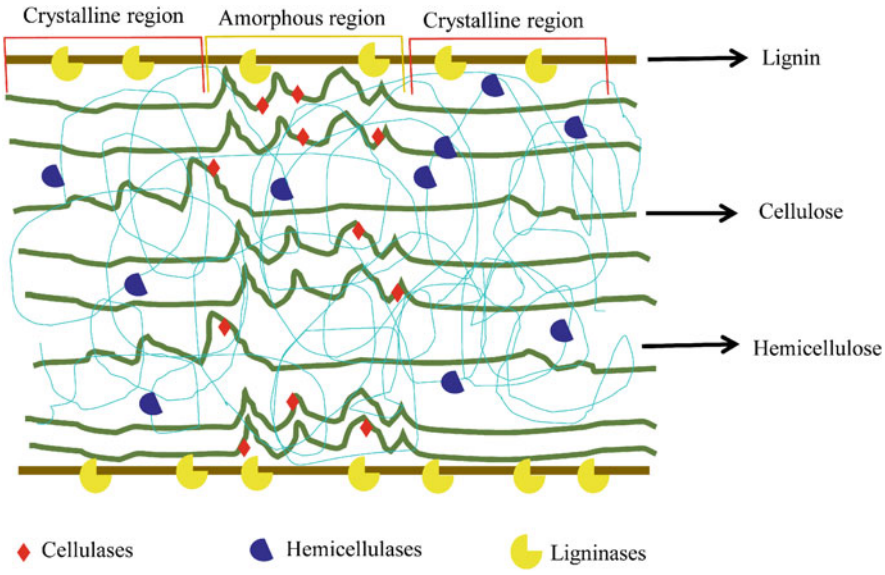

Fig. 2 Lignocellulose structure and enzymatic degradation

affinity towards the β-cellobiose disaccharides and generates glucose (Kuhad et al. 2011). The catalysis of GH family 5 and 7 cellulases follows retaining mechanism while GH family 6, 9, 45 and 48 shows inverting mechanism. Commercial production of cellulases has been in practice since last three decades. Several fungal genera like *Aspergillus* (Devi and Kumar 2017), *Fusarium* (Ramanathan et al. 2010), *Penicillium* (Jung et al. 2015), *Trichoderma* (Ellilä et al. 2017) has been reported to be high cellulolytic abilities. In case of bacteria *Bacillus* (Shajahan et al. 2017), *Pseudomonas* (Gautam et al. 2010), *Clostridium* (Xiong et al. 2018) has been reported to be produced cellulases on higher amount.

Hemicellulases

Hemicellulases are a diverse group of enzymes that hydrolyze hemicelluloses and are grouped in family 1, 2, 3, 4, 5, 10, 11, 26, 27, 39, 43, 53, 62, 67 and 116 of GH on the basis of amino acid sequence similarities (www.cazy.org). The GH families 43 and 67 of hemicellulases shows inverting mechanism for catalysis while, rest all the families with hemicelluloses follow retaining mechanism for catalysis. However, hemicellulases of GH family 62 catalysis mechanism is still not clear. These GH families mainly contains hemicelluloses *viz.* endo-β-1,4-xylanase, exo-β-1,4-xylosidase, α-L-arabinofuranosidase, α-glucuronidase, α-galactosidase, endo-β-1,4-mannanase, exo-β-1,4-mannosidase and β-glucosidase (Shallom and Shoham 2003). Hemicellulases are mainly reported to be produced from *Aspergillus* (Tallapragada and Venkatesh 2017), *Trichoderma* (Inoue et al. 2016), *Thermomyces*

(Gramany et al. 2016), *Streptomyces* (Phuengmaung et al. 2018), *Bacillus* (Tariq et al. 2018) etc.

Advancement in the Directed Evolution of Cellulases and Hemicellulases

Cellulases and hemicellulases have well established significant commercial applications in textile industries *viz*. as biopolishing agents, in the pilling of fabrics, in the reduction of fuzz, and substituting in stonewash (Mojsov 2011; Schülein 2000). But, from the last three decades the research is concentrated on the degradation of lignocellulosic biomass for development of value-added products or the generation of biofuels (Mansour et al. 2016). However, these highly anticipated applications of converted lignocellulosic biomass has not yet been economically feasible. The major interruption for the development of an economical process is the cost and less efficiency of lignocellulose-degrading enzymes. In this regard, various bioprocess development strategies like exploration of higher lignocellulolytic enzyme producers, optimization of production media and conditions, biological or chemical pretreatment of the biomass and molecular means for high enzymes expression along with computational approach have been developed and checked for its efficiency to convert into industrial scale (Koutinas et al. 2012). However, all traditional approaches have not resulted into much success. So, the current scientific community is focusing on the employment of directed evolution along with other traditional developed techniques in order to enhance the enzyme production level as well as to improve its catalytic activity by altering their natural molecular and physical behaviors (Table 1) (Guo et al. 2018).

Diogo et al. (2015), developed a chimeric hemicellulase and reported three fold enhancement in the xylose production and improved thermotolerance as shift of optimum temperature was from 35 to 50 °C. The developed chimeric enzyme was the molecular fusion of two enzymes i.e. GH11 endo-1,4-β-xylanase and GH43 β-xylosidase from *Bacillus subtilis* in order to alter the substrate cleavage rate. Similarly, GH family 26 β-mannanase from *Bacillus sp*. MK-2 was altered through random mutagenesis in *B. subtilis* WB800 and the three positive mutants namely K291E, Q112R and L211I were selected on the basis of improved specific activities (Zhang et al. 2019). The single acid substitution in K291E was resulted into the 3.5 fold increment in catalytic efficiency while, the mutants Q112R (200%) and L211I (80%) showed increase in catalytic efficiency towards konjac glucomannan. In a study, Goedegebuur et al. (2017) reported improved thermal stability of cellobiohydrolase of GH family 7 from *Hypocrea jecorina* through the employment of directed evolution. By the directed evolution a variant FCA398 was developed and it was exhibiting 10.4 °C enhancement in T_m with a 44-fold increased half-life compared to the wild-type enzyme. Similarly, thermal stability was enhanced by improving the activity α-L-arabinofuranosidase of *Geobacillus vulcani* GS90 through directed evolution carried by one round error-prone PCR (Sürmeli et al. 2018). In the study, the selected enzyme variants GvAbf L307S and GvAbf Q90H/

Table 1 Recent directed evolved lignocellulolytic and chitinolytic glycoside hydrolases with improved properties and potential applications

Enzyme	Enzyme source	Technique used	Improved properties	Proposed application	References
Chitinase	*Bacillus subtilis* WB600	Site-directed mutagenesis	Expression level and specific activity	COS production	Pan et al. (2019)
β-Mannanase	*Bacillus sp.* MK-2	Random mutagenesis	Catalytic efficiency and thermal stability	Industry competent β-mannanase	Zhang et al. (2019)
Endo-β-1,4-glucanase	*Streptomyces sp.* G12	Random mutagenesis	Hydrolytic activity	Bioconversion of lignocellulosic biomass	Cecchini et al. (2018)
Chitinase	Serratia marcescens B4A	Site-directed mutagenesis	Kinetic and thermal stability	Suitable for industrial and biotechnological use	Emruzi et al. (2018)
Xylanase	*Aspergillus niger* ATCC1015	Site-directed mutagenesis	Catalytic activity and thermostability	Potentiality for industrial application	Wu et al. (2018)
Xylanase	*Bacillus circulans*	Random mutagenesis	Enzyme activity and thermal stability towards alkaline pH	For pulping and bleaching processes	Shah et al. (2018)
Chitosanase	*Bacillus sp.* MN	Rolling-circle PCR	Substrate specificity	Partially acetylated CHS COS production	Regel et al. (2018)
Cellobiohydrolase	*Hypocrea jecorina*	Site-directed mutagenesis	Thermal stability	Conversion of cellulosic biomass to fermentable sugars	Goedegebuur et al. (2017)
Chitosanase	*Renibacterium sp.* QD1	Site-directed mutagenesis	Thermostability	Hydrolysis of CHS to produce COS	Han et al. (2017)
Xylanase β-xylosidase	*T. reesei* ATCC66589	Disparity mutagenesis	Enzyme activity	Disparity mutagenesis for improving enzyme activity	Watanabe et al. (2019)

L307S showed 2.5-fold increase in the specific activities compared to the α-L-arabinofuranosidase of *G. vulcani* GS90. Irfan et al. (2018), developed six mutants (R81P, H82E, W185P, D186E, W185P/D186E and H82E/W185P/D186E) through site-directed mutagenesis by interchanging the xylanase producing residue of *G. thermodenitrificans* C5 with proline and glutamic acid. Both the mutant and wild type enzymes were expressed in *E. coli* BL21 and upon comparing to wild type (control) the mutant, H82E/W185P/D186E showed increment in half-life for thermal inactivation i.e. 13 times at 60 °C, 15 times at 65 °C, 9 times at 70 °C and 5 times at 75 °C. Due to the incredible growing exploration in the field of directed evolution of lignocellulolytic GH, the economical utilization of lignocellulosic biomass can be attained in the near future and hence there is also need of alike attention towards other naturally abundant biomass such as chitin containing waste.

Chitinolytic Glycoside Hydrolases

Chitin is the second most ubiquitous natural structural polymer after cellulose which is mainly present in the exoskeletons of arthropods, crustaceans, mollusks as well as in the cell wall of fungi and algae (Kumar et al. 2018d). Chitin is mainly produced from the seafood processing industries and is posing a serious environmental concern to the coastal areas (Yadav et al. 2019a). Chitin is an ordered crystalline microfibrils made up of β (1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucose units and is found in three forms i.e. α, β and γ (Dutta et al. 2004). Among these α-chitin is the predominant and is formed by the antiparallel organization of microfibrils. However, sheets are present in antiparallel organization in β-chitin. In γ-chitin sheets are in both parallel as well as antiparallel fashion (Rinaudo 2006). Alike lignocellulosic biomass, the chitin has enormous applicability upon its transformation into derivatives with novel and improved properties i.e. chitosan (CHS), chitooligosaccharides (COS) and *N*-acetylglucosamine (GlcNAc) (Kumar et al. 2018a). CHS is the deacetylated form of chitin and is composed of α (1 → 4)-linked 2-amino-2-deoxy-β-D-glucopyranose. The vital properties of chitin and its derivatives that boosts its necessity of exploitation for human wellbeing *viz*. mucoadhesive, hemostatic, antimicrobial, antioxidant, antitumor, biodegradable and biocompatible (Younes and Rinaudo 2015). The chitin containing biomass can be converted into the valuable products through the chitinolytic enzymes *viz*. chitinases, chitosanases, chitin deacetylases and *N*-acetyl glucosaminidases (NAG). The cleavage action pattern of different chitinolytic enzymes has been diagrammatically represented through Fig. 3. The majority of chitinolytic enzymes are placed in GH class of CAZy database except chitin deacetylase (EC 3.5.1.41) which is grouped in family 4 of carbohydrate esterase class of CAZy database. There are a lot of research work has done related to the chitinolytic GH and many of them has patented. Some of the related patent publications with claimed application are discussed in Table 2.

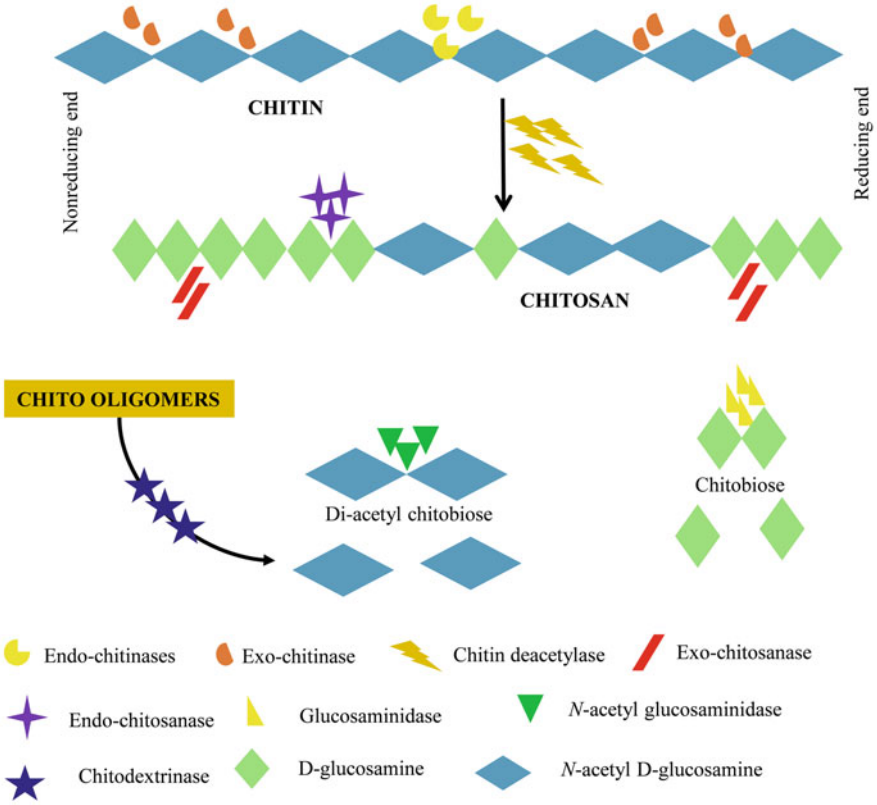

Fig. 3 Action pattern of chitinolytic enzymes

Chitinases

Chitinases (EC 3.2.1.14) are GH with specific affinity towards chitin and it randomly hydrolyse glycosidic linkages in the chitin and chitodextrins in a non-progressive manner to generate low molecular weight chitooligomers and free ends on which exochitinases and exochitodextrinases can act (www.cazy.org). On the basis of mode of action chitinases can be either endochitinases or exochitinases (Kumar et al. 2018b). The endochitinases randomly cleave the chitin chain at internal sites, generating soluble low molecular mass chitooligomers *viz.* chitobiose, chitotriose and chitotetraose (Hamid et al. 2013). However exochitinases contains two major enzymes groups: chitobiosidases (EC 3.2.1.29), involved in the catalysis of progressive release of di-acetylchitobiose starting at the non-reducing end of the chitin chain, and 1–4-β-glucosaminidases (EC 3.2.1.30), splitting the oligomeric products of endochitinases and chitobiosidases with the formation of GlcNAc monomers (Hamid et al. 2013). Chitinases has been mainly placed into family 18 and 19 of GH in the CAZy classification (Henrissat and Bairoch 1996). Family 18 chitinases

Table 2 Recent patent publication related to lignocellulolytic and chitinolytic glycoside hydrolases

Title	Patent/publication no.	Lignocellulolytic/ chitinolytic GH involved	Application claimed	Reference
Method of producing nanofibrillar cellulose with high absorptivity to fat and cholate	US9149064B2	Xylanase Cellulase	Enhanced fat and cholate absorbing capacity	Zhao et al. (2015)
Chitinase-3-like protein 1 as a biomarker of recovery from kidney injury	US2014/0200184A1	Chitinase-3-like 1/Brp-39/YKL-40	Biomarker for kidney injury and its reparative response	Elias et al. (2014)
Liquid detergent composition comprising cellulosic polymers and cellulase	US2018/006621A1	Cellulase	Formulation of liquid detergent for laundry	Pickering et al. (2018)
Insecticidal chitinase protein its encoding nucleotide and application thereof	US10006014B2	Chitinase	In insect control	Singh et al. (2018)
Preparation of a baked products comprising fibers treated by a cellulase	US20190029272A1	Cellulase Xylanase Cellobiohydrolase	Bakery products with improved anti-staling properties	Niemann (2019)
Method and system for preparing pulp for paper with grass straws as raw materials	US20180105851A1	Hemicellulases	Pulp preparation from grass straws	Sun and Zhongyu (2018)
Process for treating wastewater	US201900399932A1	Xylanase Cellulase Hemicellulase	Sludge treatment	Flannery (2019)
Cosmetic use of chitinase-type proteins	US9926587B2	Chitinase-type protein (YKL-40)	Stimulate terminal epithelial differentiation	Bernard and Donovan (2018)

follows the retention mechanism for catalysis while the family 19 chitinases shows inverting mechanism for catalysis. Family 18 chitinases utilizes *N*-acetamido carbonyl oxygen (i.e. through the neighboring group participation) as nucleophile in the double-displacement reaction instead of the common enzyme derived nucleophile. Family 19 chitinases contains chitinases of class I, II, and IV whereas, the family 18 contains chitinases of class III and V (Patil et al. 2000). Chitinases are found in a varied organisms *viz*. bacteria, fungi, yeasts, plants, actinomycetes, arthropods, and humans. The chitinases from microbial sources are of prime concern due to greater possibilities of converting them into commercial-scale production. In this regard, some chitinases producing fungi like *Thermomyces lanuginose* (Zhang et al. 2015b), *Aspergillus niveus* (Alves et al. 2018), *Humicola grisea* (Kumar et al. 2017), *T. viride* (Omumasaba et al. 2001) and bacteria such as *Paenibacillus barengoltzii* (Yang et al. 2016), *Serratia marcescens* (Horn et al. 2006), *B. pumilus* (Rishad et al. 2016) can be consider for industrial-scale production.

Chitosanases

Chitosanases (EC 3.2.1.132) are the GH that catalyzes the endohydrolysis of β-(1 → 4) linkages between D-glucosamine (GlcN) residues of partly acetylated CHS from its reducing end. However, there are also exo-β-D-glucosaminidase (EC 3.2.1.165) that attack CHS from its non-reducing end. Chitosanases are placed into families 5, 8, 46, 75 and 80 of GH on the basis of amino acid sequence (Lombard et al. 2014). However, on the basis of specificity of cleavage positions for the partly acetylated CHS, chitosanases are grouped into three subclasses i.e. subclass I (act on both GlcN-GlcN and GlcNAc-GlcN linkages), subclass II (split only GlcN-GlcN linkages) and subclass III (cleave both GlcN-GlcN as well as GlcN-GlcNAc linkages) (Thadathil and Velappan 2014). Families 8, 46, 75, and 80 chitosanases followed inverting mechanism for catalysis while, only family 5 chitosanases has been reported to use retaining mechanism for catalysis (www.cazy.org). Generally the molecular mass of chitosanases are present in the range of 20–75 kDa. However, the molecular mass of chitosanases from *A. fumigatus* KH-94 has been reported to be 108 kDa (Kim et al. 1998). Mostly, chitosanases are produced from bacteria, fungi, cyanobacteria and plants. Recently, chitosanases are reported to be produced from bacteria like *B. mojavensis* (Liaqat et al. 2018), *P. macerans* (Doan et al. 2018), *Pseudoalteromonas* sp. (Zhou et al. 2019) and from fungi like *Penicillium* sp. (Aktuganov et al. 2019), *Gongronella butkeri* (Seki et al. 2018), *Aspergillus* sp. (Zhang et al. 2015a).

N-Acetyl Glucosaminidases

NAG (EC 3.2.1.96) are the GH that catalyzes the endohydrolysis of *N,N″*-diacetylchitobiosyl units. In this reaction one GlcNAc residue remains attached to the protein and the rest of oligosaccharides are released in an intact manner.

However, β-*N*-acetylhexosaminidase (EC 3.2.1.52) hydrolyzes the terminal non-reducing *N*-acetyl hexosamine residues. On the basis of amino acid sequence similarities NAG are placed into families 3, 20, 73, 84 and 85 of GH in CAZy database (Lombard et al. 2014). The GH families 3, 20, 84 and 85 follows retaining mechanism for catalysis whereas, the family 73 catalytic mechanism is not clear. NAG is present in various tissues in human body and helps in breaking chemical bonds of glycosides and amino sugars that forms the structural components of several tissues and NAG also serve vital role in the degradation and disposal of many parts of cell (Wen and Kellum 2012). NAG has been detected in a range of bacteria, fungi, insects, plants and animals. Recently, NAG has been reported from bacteria like *Streptomyces alfalfa* (Lv et al. 2019), *B. subtilis* (Nayyab et al. 2017), *Corynebacterium glutamicum* (Matano et al. 2016) and from fungi like *A. versicolor* (Bojarová et al. 2019), *T. reesei* (Chen et al. 2015).

Update on the Directed Evolution of Chitinolytic Glycoside Hydrolases

Chitinolytic GH has been well explored for their applications in the conversion of chitin and CHS into their oligomers and monomers that has a huge potential applicability a wide range of sectors *viz*. medicine, agriculture, wastewater treatment, food and cosmetics. Apart from the polymer degradation ability chitinolytic GH also has a lot of application in agriculture in fighting against phytopathogens and various insects (Kumar et al. 2018c, d). In order to strive the demand there is much more want of developing processes having enhanced level of chitinolytic enzyme production with better efficiency in terms of catalytic power, temperature stability and wide range of pH stability. In this regard, a lot of notable work has been done and still ongoing with addition like finding of novel strains, improving fermentation conditions, effective pretreatment strategies and recombinant DNA technology, but still the desired level of output has not achieved (Devi and Kumar 2017; Gramany et al. 2016; Inoue et al. 2016). Recently, the combination of these conventional techniques along with the directed evolution approaches are promising a lot for the commercial scale production of chitinolytic GH and their valuable bioactive oligomers and monomers (Table 1) (Abdul Manas et al. 2018). The secretion efficiency and thermal stability of chitosanaseA from *Mitsuaria chitosanitabida* 3001 was significantly enhanced through the directed evolution and reported 1.5 fold increment in secretion efficiency and 17% (at 50 °C) enhancement in thermal stability as compared to the wild –type chitosanaseA (Yun et al. 2006). The study used inactive chitosanaseA mutant gene (G151D) in order to perform mutation through an error-prone PCR technique and the gene that restored chitosanase activity were selected. Fan et al. (2007), reported an enhancement in the catalytic ability of chitinase produced from *Beauveria* bassiana through error prone PCR and DNA shuffling. The amino acid alterations were performed outside the two putative substrate binding sites and the catalytic region namely SXGG and DXXD XDXE in the Bbchit1 gene. DNA shuffling technique was also used to bring

betterment in pH performance and activity with two chitosanase gene from *B. cereus* KNUC51 and *B. cereus* KNUC55 (Park and Ghim 2009). The DNA shuffled products i.e. YM18 and YM20 were reported to enhance chitosanase specific activity as compared to the native up to 250% and 350%, respectively. The study also reported that the shuffle product MY20 was exhibiting a shift in the optimal pH level from 5.5 to 6.5. Similarly, the properties of chitinase from *B. licheniformis* were improved through the directed evolution carried out through error prone PCR and DNS shuffling (Songsiriritthigul et al. 2009). The study reported 2.7 and 2.3 fold increment in the average catalytic efficiency at pH 3.0 and 6.0, respectively. Yu and Xu (2012), reported 1.8 fold increased endochitinase activity towards 2-nitrophenyl-*N*-acetyl-β-D-glucosaminide and 3.5 fold increment in endochitinase activity towards colloidal chitin through an error prone PCR directed evolution. The thermostability of chitinase produced from *S. marcescens* B4A was improved by the site-directed mutagenesis of G191V (Emruzi et al. 2018). The study suggested an increase in thermostability of 5 and 15 fold at 50 and 60 °C respectively with a decrease in the K_m and V_{max} of about 1.3 and three fold, respectively. Recently, a study suggested that the N-terminal sequence is essential for the optimum temperature, pH stability, thermostability and catalytic efficiency of chitosanase CsnA from *Renibacterium* sp. QD1 (Han et al. 2018). The used extra 7-residue *N*-terminal sequence was not from the regular secondary structure in chitosanase. The above-mentioned studies suggests the possibilities directed evolution for the improvement of chitinolytic GH in term of their catalytic efficiency, thermostability and pH stability. But, these works are in their initial stage in concern of commercial level output so, there is a lot of improvement and studies required.

Application in Industries

The lignocellulolytic and chitinolytic GH have shown immense applicability in a wide range of sectors (Sarrouh et al. 2012). Lignocellulolytic GH are utilized commonly in paper and pulp, textile and laundry, beverages and biofuel industries while the chitinolytic GH has more explored for medicine, agriculture and food industries (Fig. 4). At present development of techniques for the generation of biofuel from the naturally available biomass is one of the prime concern of the scientific community due to the rapid utilization of the conventional fossils fuels. The lignocellulolytic and chitinolytic GH possess specific affinity towards the most abundant biomasses of the earth and hence are widely applied in the value-added product generation. There are some success achieved in the form of commercial product development which are presented through Table 3 with their application. In the forthcoming section of the study, authors are going to discuss about the various utilization of the lignocellulolytic as well as the chitinolytic GH in different industries.

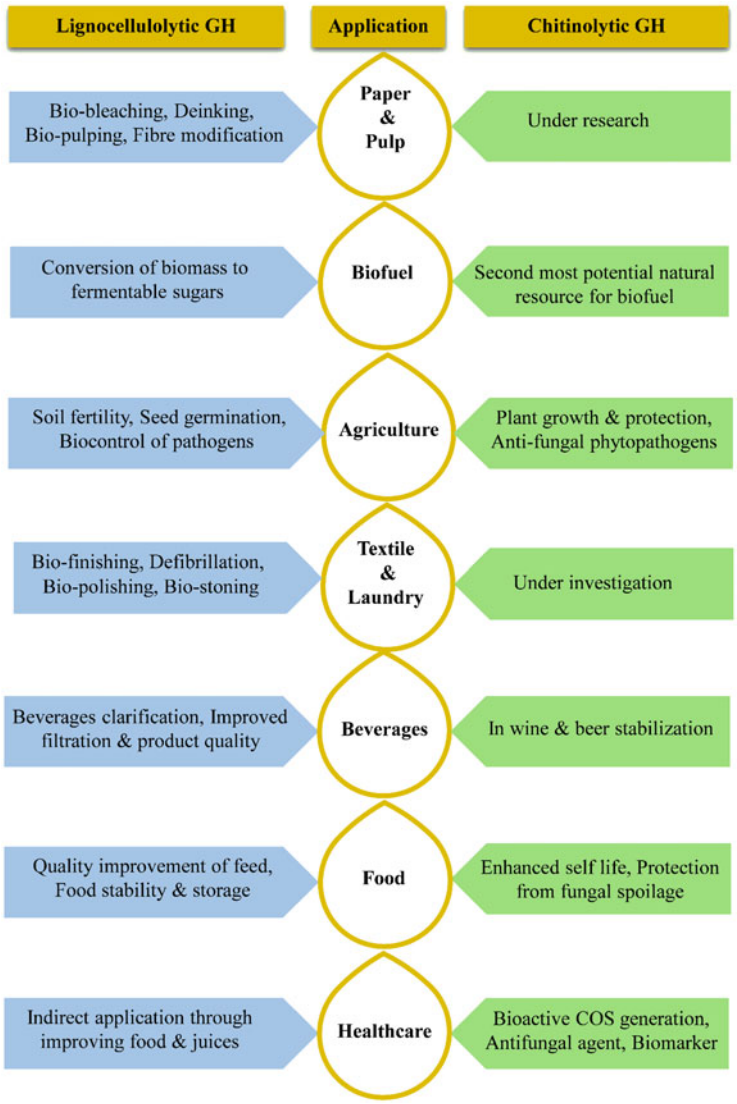

Fig. 4 Application of lignocellulolytic and chitinolytic glycoside hydrolases

Paper and Pulp

Lignocellulolytic GH have been tremendously used in the pulp and paper industries due to their vast range of applicability *viz*. de-inking, pulping, bleaching, fibers modification, debarking, pulp fiber characterization, and drainage improvement (Imran et al. 2019; Singh et al. 2016). These enzymes has imparted crucial role in lowering the overall production cost of paper with improved qualities. The

Table 3 Commercially available lignocellulolytic and chitinolytic glycoside hydrolases and their application

Product name	Enzymes	Source	Application
Celluclast®	Cellulase	*Trichoderma ressei*	Enhanced conversion of cellulosic materials into fermentable sugars
Cellic®CTec2	Cellulase β-glucosidases Hemicellulase	Not reported	Degrade cellulose to fermentable sugar Cellulosic ethanol production
Accellerase®TRIO™	Exoglucanase Endoglucanase Hemicellulase β-glucosidases	*T. ressei*	Accelerates biomass conversion processes
Cellic®HTec2	Endoxylanase Cellulase	Not reported	Boost conversion of hemicellulose to fermentable sugar Cellulosic ethanol generation
Fibrezyme®G4	Cellulase	*Myceliophthora thermophila*	Reduces energy requirement and increase production rate for pulp and paper industries
AlternaFuel®CMAX™	Cellulase β-glucosidase Hemicellulase Arabinase	*M. thermophila*	Degradation of lignocellulosic biomass Effective in both acidic and neutral processes
Chitinase (by Sigma-Aldrich, USA and Megazyme, Ireland)	Chitinase	*Streptomyces griseus* *Clostridium thermocellum* *T. viride* *S. marcescens*	For research only
Chitosanase (by Sigma-Aldrich, USA)	Chitosanase	*S. griseus*	For research only

utilization of lignocellulolytic GH in paper and pulp industries have attained their commercial position around two decades ago due to the remarkable research in this field. Thus there we are describing only about the latest improvements made in the paper and pulp industries related to lignocellulolytic GH. Buzała et al. (2016), investigated the effect of xylanases on kraft pulp and reported saving of refining energy without reducing the paper characteristics. The study used enzyme preparation made up of xylanase from *T. lanuginosus*, cellulase from *Aspergillus* sp., and a multienzyme preparation NS-22086 which contained the both enzymes and reported shorter time for freeness (30°SR) with greater water retention value. The modern industrial enzymatic process are also targeting on the recycling of enzyme as some amount of active enzyme are left after the process can be used in order to reduce the production cost. Wang et al. (2016), demonstrated the use of fresh cellulase addition in recycling cellulase for the industrial level enzymatic treatment of the kraft-based dissolving pulp. The study also reported recovery of about 48.8–35.1% of cellulase

activity from the filtered liquor in five recycle rounds and the recovered cellulase were can be reused in the treatment of pulp. Similarly, some combined process like involvement of pulp fractionation and cellulase treatment of each fraction has also investigated (Duan et al. 2017). The combined approach at a given viscosity resulted into lower polydispersity index (3.71 vs 4.98) with a greater fock reactivity (85.6 vs 76.3%) compared to the cellulase treatment alone. Recently, scientific community is also paying its key attention on the paper waste management and their reusability techniques development. Jain et al. (2017), showed the utilization of thermostable cellulase from *Thermoascus aurantiacus* RCKK for the hydrolysis of office waste paper, algal pulp (*Gracillaria verulosa*) and biologically treated wheat straw at 60° with release of significant amount of substrate i.e. 830 mg/ml, 285 mg/g and 260 mg/g, respectively. The formulation of cellulase and xylanase from *Escherichia coli* SD5 was found to be promoting deinking efficiency and paper pulp modification with reduction of kappa number and hexenuronic acid (Kumar et al. 2018e). Kaschuk and Frollini (2018), investigated the impact of different cellulosic material properties like average molecular mass, crystallinity index, and hemicelluloses content on the efficiency of the enzyme derived conversion of cellulose to glucose. The study suggested highest yield for the conversion of 88% in case of sisal pulp, followed by 64% for microcrystalline cellulose and 52% conversion for filter paper. Recently, significant reduction in the refining energy with improved fiber freeness was achieved at lab as well as plant scale (Tripathi et al. 2019). The study reported reduction in refining energy of enzymatic treated pulp by 29.3% in lab scale as well as 20% in plant scale trials. The remarkable research progress in the field of lignocellulosic GH has resulted in the low cost and better quality production in pulp and paper industries. However, there is still need of attention on the utilization of other natural polymer like chitin and their degrading enzymes in these industries.

Textile and Laundry

Lignocellulolytic GH has gained attention in textile and laundry from last two decades due to their ability of modifying cellulosic fibers in desired manner as well as capacity of enhancing fabric quality. Nowadays, these enzymes are best-known for their bio-stoning and bio-polishing applications. They applicability has widely accepted in washing powders because of their capability of enhancing detergent performance and in removal of small, fuzzy fibrils from fabric surfaces in order to improve the appearance and color brightness (Bhat 2000). Battan et al. (2012), evaluated the impact of thermostable xylanase from *B. pumilus* ASH in the processing of textiles and found 0.9% higher whiteness values of micro poly fabrics after bioscouring with the xylanase as compared to the chemical scoured fabrics. Similarly, the bioscouring of flax fibers through the cellulase-free xylano-pectinolytic enzymes was carried out and resulted in the 1.84% sugar release, 4% weight loss, 19.46% increase in brightness along with 8.2% whiteness enhancement (Kaur et al. 2016). The study also suggested that the enzymatic pretreatment of flax fibers reduced the scouring chemical consumption up to 70% with 30% reduction in

the bleaching chemicals as compared to the control flax fiber. Gumel et al. (2018), extracted cellulase from *Aspergillus niger* by using pineapple peel as the substrate and the produced cellulase was applied to cotton fabrics at different concentration at pH 5.5 and temperature 55 °C. The study reported that the cellulase was able to remove staple fibers from the fabrics leading to glabrous appearance and soft touch as well as the better dye uptake. Xylanases from *T. longibrachiatum* KT693225 has been reported to be highly effective in wet processing stages in textile industries like desizing, bioscouring and biofinishing (El Aty et al. 2018). Although, the chitinolytic GH has not reported to have direct utilization in textile and laundry but, the deacetylated form of chitin (i.e. CHS) has gained tremendous applicability in textile industry due to its antimicrobial activity.

Biofuel

Lignocellulose being the most abundant renewable organic material is considered as the most promising alternate of the conventional petroleum fuel. A lot of attempts has been carried out in the conversion of the enormous biomass into biofuel (Himmel et al. 2007; Yadav et al. 2019b). In this regard, lignocellulolytic GH are proposing to play major role due to their specific affinity towards the lignocellulosic biomass. The enzyme derived energy production always has an upper hand on the fossils fuels in term of environmental management. The utilization of lignocellulolytic GH obtained from fungi are highly explored for biofuel production (Srivastava et al. 2018). For the enhanced biofuel production strategies are developed in order to reduce enzyme dose. Liu et al. (2016), engineered a cellulose adherent *Saccharomyces cerevisiae* and it was capable of directly producing ethanol from rice straw with 40% less enzyme dose. The developed strain showed clear cell-to-cellulose adhesion with a tearing pattern of cellulose degradation resulting into the enhanced hydrolysis efficiency. Another study demonstrated the improved efficiency of bioethanol production from *Eichhornia crassipes* by the mean of statistical optimization (Das et al. 2016). The study reported two fold increase in ethanol production compared to the unoptimized condition. Yarbrough et al. (2017), compared the capability for coproduction of nanocellulose and fermentable sugars by the utilization of two different enzyme system. The first cellulase enzyme system was the "free enzyme" system of *Trichoderma reesei* and second one was a complex multifunctional enzymes produced from *Caldicellulosiruptor bescii*. The study also suggested the complex enzyme system better than the free enzyme system in term of total cellulose conversion, sugar production, and nanocellulose generation (Yarbrough et al. 2017). Gil and Maupoey (2018), developed in integrated design for the simultaneous saccharification and fermentable of pineapple waste for the enhanced ethanol generation as well as extraction of bromelain. The study reported increase in ethanol production ($4.7 \pm 0.3\%$ v/v) by direct fermentation, ($5.4 \pm 0.1\%$ v/v) through simultaneous saccharification and fermentation and ($4.9 \pm 0.4\%$ v/v) from saccharification and fermentation of the solid waste. β-xylosidase from *Aspergillus niger* has also been reported to have potential of bioethanol generation from

lignocellulose as the hydrolysis of pretreated straw at 70 °C by the lignocellulosic enzyme cocktail resulted in 19-fold increment in xylose level after 6 h (Boyce and Walsh 2018). Enzyme immobilization on various matrix has also been explored to enhance the enzyme efficiency for biofuel production. Cellulase from *Bacillus subtilis* UniMAPKB01 was immobilized on a multi-walled carbon nanotubes and the immobilized enzyme was capable of producing 0.129 mg/0.5 ml of glucose which serves as the precursor for bioethanol production (Naresh et al. 2018). The chemical pretreatment of grasses like vetiver grass and switchgrass has shown enormous potentiality of biofuel production. Subsamran et al. (2019), reported 90 FPU/ml of fermentable sugar from enzymatic hydrolysis with yield of 21.10 and 5.85 g/l of bioethanol production from the 1% (w/v) NaOH and 0.5% (v/v) H2SO4 pretreatment of vetiver grass, respectively at 121 °C for 60 min. Recently, switchgrass was pretreated with (3 g/l) acetic acid and the simultaneous saccharification and fermentation approach was employed by the help of *Clostridium saccharoperbutylacetonicum* N1–4 to produced 8.6 g/l butanol. There are some success in the conversion of biofuel production from lignocellulosic biomass through lignocellulolytic GH but still it is lacking behind in term of cost efficiency and produced biofuel application related engineering. Nowadays, researchers are also paying attention the chitinous biomass for energy generation but research in this field in its primitive due to the vast applicability of chitin oligomers and chitin derived products in the field of medicine and agriculture.

Agriculture

The lignocellulolytic GH has been extensively exploited for their use in agriculture and reported to have application in enhancing soil fertility, promoting cell growth, improvement of seed germination and protection, biocontrol of pathogens and diseases, extraction of bioactive compounds, production of protoplast (Phitsuwan et al. 2013). The utilization of plant cell wall disrupting lignocellulolytic GH for the extraction of novel natural bioactive compounds from plants with plenty of beneficial properties has been extensively reviewed (Puri et al. 2012). Chamani et al. (2012), evaluated different cellulase and pectinase enzymes treatments on the production of protoplast and its viability in *Lilium ledebeourii* and reported the significant effect of cellulase treatment on the protoplast production. The cellulase at 4% level was the most effective treatment with 3.71×10^5 protoplast/g FW. Lignocellulolytic GH producing microorganisms has been well documented for their application in the biological control of phytopathogenic fungi. Cellulase from *T. harzianum* Th22 was reported to stimulate 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxzzin-3(4H)-one (DIMBOA) as well as the defense-related gene expression in maize root against *Fusarium graminearum* (Saravanakumar et al. 2018). The study suggested that the cellulase gene *Thph1* and *Thph2* were accountable for the biocontrol of *F. graminearum* and its associated plant diseases by activating jasmonate acid, ethylene, systemic acquired resistance, DIMBOA and plant innate immunity-related gene expression in the maize root. Recently, cellulase has also

been reported to increase the endophytism of *Metarhizium brunneum* CB15 in potato plants (Krell et al. 2018). The beads treated with cellulase and inactivated baker's yeast was reported to upsurge mycelial progress by 13.6% with a shift from mycelial growth to spore formation i.e. maximum numbers of $2.5 \times 10^8 \pm 6.1 \times 10^7$ per bead (Krell et al. 2018).

Similarly, chitinolytic GH has been also well investigated in agriculture sector for their widespread employment as an agent of plant growth and protector. The chitinolytic GH have natural affinity towards the hydrolysis of chitin, which is the major component of cell wall of fungi. The intrinsic property of chitinolytic GH made it the most demanding agent to fight against the phytopathogens and helps in improving the crop cultivation and productivity (Kumar et al. 2018c). Chitinases has reported to be effective in controlling crop diseases like rot, blight, rust, spot and wilt diseases of various crop plants as well in the development of resistance against plant diseases (Sharma et al. 2011). Awad et al. (2017), investigated partial pure fungal exochitinase on mortality of *Galleria mellonella*, *Spadoptera littoralis* and *Agrotis ipsilon* and it was reported to be 92, 86.67 and 65.67%, respectively. A transgenic tomato was developed *via in vitro* and *in planta* transformation technique with enhanced salt and drought tolerance through the expression of osmotin-like protein and *Chi11* genes (Kumar et al. 2016). Insect chitinase gene, *CsCht10* was reported to control *Chilo suppressalis* Walker which is one of the most destructive pest for rice crop (Zhao et al. 2018). Cheng et al. (2017), cloned an endochitinases, VDECH from *Verticillium dahlia* strain Vd080 that showed high thermostability. The study also suggested that VDECH was able to triggered plant defense responses with hypersensitive response, oxidative burst, and elicited increased expression of defense-related genes in *Arabidopsis* as well as in cotton. Chitinase I gene from barley was expressed in *E. coli* Rosetta strain under the control of T7 promoter in pET 30a vector and the expressed chitinase was more effective as compared to control against phytopathogenic fungi like *Alternaria solani*, *Fusarium spp*., *Rhizoctonia solani* and *V. dahlia* (Toufiq et al. 2018). Recently, chitinase and chitinolytic GH derived CHS and COS based products has been developed in the form of biopesticides and biofertilizers by various companies and their industrial-scale production and field trials are in progress that reflects the possibilities of presence of such products in market in near future.

Beverages

Lignocellulolytic GH are extensively used in the preparation of beverages products like wine, beer, and fruits as well as vegetables juices (Bhat 2000). The GH application improves the primary fermentation and extraction processes along with enhancing the quality of the products. Moreover, they are also known for parting significant role in the advancement of clarification and aroma of beverages (Kaur and Gill 2019). Recently, a lot of successful attempts has been made for the utilization of GH enzymes in winemaking from grapes (Gao et al. 2019). Several lignocellulolytic GH enzymes are also utilized in the processing of fruit and vegetables and in the

improving the quality of the processed juices (Kumar 2015; Toushik et al. 2017). Zhao et al. (2013), reported the production of thermophilic xylanase from *Achatomium* sp. Xz-8 with catalytic efficiency of 3710 ml/s/mg. The produced xylanase showed improved filtration of 20.24% and 38.50% when combined with the commercial β-glucanase under the stimulated mashing conditions which was comparable to Ultraflo, Novozymes. Recently, a magnetic biocatalyst of pectinase and cellulase were prepared and compared to the glutaraldehyde-activated magnetite in term of magnetic properties, immobilization parameters, stability and grape juice clarification (Dal Magro et al. 2018). The study suggested about the increased possibility of recovery of biocatalysts (i.e. cellulase, 33.4%) applicable in juice industries when the magnetic technology was integrated with enzyme technology. However, the chitinolytic GH has been less studied in the field of beverages industries but, the chitinolytic GH derived products of chitin has been reported to be applicability in providing stability and production to the beverages (Rocha et al. 2017; Yang et al. 2017).

Food

The lignocellulolytic and chitinolytic GH has a wide range of application in food industries *viz*. quality improvement, extraction, clarification, stability and protection. Lignocellulolytic GH has been extensively explored for their function utilization in fruits and vegetables processing industries (Toushik et al. 2017). Cellulase and pectinase treated *Ecklonia cava* extract showed anti-obesity effects in C57BL/6 N mice with high-fat diet induced obesity (Kim et al. 2018). The anti-obesity effects were monitored through evaluating change in body weight, fat, serum lipid levels and lipogenic enzymes levels and the results of the study suggested that the *Eckloni cava* supplementation reduces high-fat diet induced obesity and following metabolic disorders (Kim et al. 2018). Cellulase and hemicellulase has also been applied for the enhanced utilization of the leftover processed by-products i.e. okara from the soymilk and tofu production (Vong et al. 2017). The total nutritional value of okara was achieved through the sequential saccharification by Celluclast® (cellulase) and Viscozyme® L (cellulase and hemicellulase), followed by *Yarrowia lipolytica* derived fermentation. The resulted okara was reported to have higher antioxidant activity with enhanced volume of total amino acids and ferulic acid. Lignocellulolytic GH has also been well documented for their valuable role in improving animal feed. The impact of xylanases on the nutrient digestibility and gut microbiota of growing pigs were investigated in a study conducted by Zhang et al. (2018). The study found that xylanase C from *Bacillus subtilis* more effective when applied to wheat-based diets whereas, xylanase A from *Fusarium verticilliodes* was showing better result with corn-based diets. Similarly, xylanases were reported to improve digestibility of dietary fibers in the stomach and hindgut along with the enhancement in the energy status of pigs fed based on wheat diets (Abelilla and Stein 2018). Xylanases and other lignocellulolytic GH has been investigated in the quality and nutritive value enhancement of wheat and other

grains used for poultry industries. Nourmohammadi et al. (2018), reported the better result of wheat-based feed diet from xylanase supplementation on male broilers in the terms of growth performance, energetic efficiencies, nitrogen balance and energy partitioning. The chitinolytic GH are more linked with the preservation of food mainly against post-harvest spoilage causing fungal pathogens like *Monilima spp*., *Botrytis cinerea*, *Penicillium expansum* (da Silva 2019). Chitinases with vicilins and lectins from legume flours were reported to have antifungal activity which resulted into the enhanced shelf-life of wheat bread (Rizzello et al. 2017). Moreover, the degraded enzymatic products of chitin i.e. CHS and COS are widely applied in the food packing industries due to their bioactive properties and improving physical qualities of the packing materials (Dutta et al. 2009).

Healthcare

Lignocellulolytic GH and chitinolytic GH has shown immense potential in the healthcare of human beings. They are reported to have a wide range of biomedical applications. Although the lignocellulolytic GH are not directly applicable for maintaining human healthcare. However, they are indirectly involved in promoting human health through their valuable application in juice, alcohol, tobacco, bakery, poultry, piggery and fishery (Kunamneni 2016). In contrast, the chitinolytic GH has huge role in human welfare directly as well as indirect manner. Indirectly, chitinolytic GH helps in the generation of bioactive CHS and COS, which are well known for their well-established applications in tissue engineering, drug delivery, wound healing, and as nutraceuticals (Kumar et al. 2019). However, chitinolytic GH has also direct involvement in human healthcare *viz*. as antifungal agent and as biomarker for cancer and lung diseases (Nagpure et al. 2014). In this regard, the role of human chitinases and chitinase-like proteins to serve as indicators for inflammation and cancer has been well studied (Kzhyshkowska et al. 2007). Chitinase present in human beings are considered as to play crucial role in the establishment of type 2 innate immunity. Vannella et al. (2016), showed the role of acidic mammalian chitinase in initiating protective type 2 responses to gastrointestinal nematodes *Nippostrongylus brasilliensis* and *Heligmosomoides polygyrus* through using acidic mammalian chitinase deficient mice. Similarly, Cohen et al. (2017) reported that cancer-associated fibroblasts derived chitinase 3-like 1 facilitated tumor progression growth and imitated type-2 immunity. The study, also reported signaling axis between fibroblasts, cancer cells and immune cells in breast tumors. Chitinase 3 like 1 was also explored for its pathological role in promoting mimicry formation leading to tumor cell-mediated vascularization and it was found that the chitinase 3-like 1 expression was correlated with the formation of tumor cell-associated vascular channels in the absence of endothelial cells (Ngernyuang et al. 2018). Xing et al. (2017), showed that chitinase 3-like 1 secreted by peritumoral macrophages could serve as a suitable biomarker for the forecast of esophageal squamous cell carcinoma. Seibold et al. (2008), reported that the chitinase activity in the lung was due to the activity of chitotriosidase with the presence of acidic

mammalian chitinase in inactive form. The study also suggested that the chitinase activity trends to reduce in subjects with asthma however, there was high level of chitinase activity detected in the habitual smokers that resulted in the upregulation of chitotriosidase gene expression in macrophages. Moreover, chitinolytic GH obtained from microbial sources also has been explored for their potential application in controlling human pathogenic bacteria and fungi but the research is still in its primitive stage (Allonsius et al. 2019). Despite of a lot of research work done, still there is need of more study in order to understand the role and applicability of chitinases and other chitinolytic GH present in human beings and also to measure the impact of chitinolytic GH enzymes when applied from external sources on the complex human system.

Conclusions and Future Prospects

Although lignocellulolytic and chitinolytic GH has been well explored for their potential applications but still their industrial level utilization has been achieved in limited sectors. Lignocellulolytic GH has achieved their competent utilization in the industries like paper and pulp, textiles, detergents, and beverages. On the other hand chitinolytic GH has proved their applicability in food, agriculture and healthcare. However, still there is a lot of work is required in the utilization of these GH for the cost-effective generation of bioenergy from the two most naturally abundant renewable biomass of the earth in the form of lignocellulose and chitin. There is also a lot of work and studies required for the application of lignocellulosic and chitinolytic GH for the biomedical industries. At present researchers are paying a lot of attention on these two most demanding area so, we can hope economical and highly effective solution in the near future.

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