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β-1,3-Glucan biodegradation and utilization: Enzyme discovery, engineering, and industrial applications

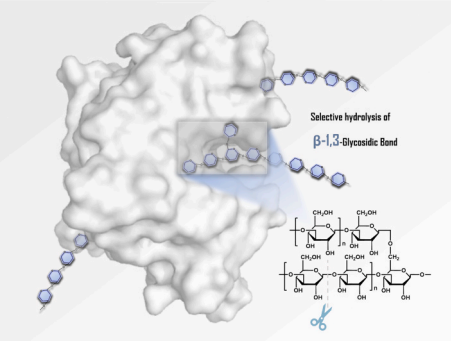
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HIGHLIGHTS

- •*β*-1,3-Glucan bioconversion supports sustainable biomass valorization.
- •Structural and mechanistic features define *β*-1,3-glucanase diversity.
- •Discovery, production, and engineering strategies shape enzyme performance.
- •Agriculture, fermentation, biorefining, and environmental uses show broad potential.
- •High-throughput screening and AI-guided design drive future enzyme innovation.

GRAPHICAL ABSTRACT



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ABSTRACT

β-1,3-Glucans are structurally diverse polysaccharides widely distributed in marine algae, fungi, and plants, and their enzymatic depolymerization is important for carbon turnover, biomass processing, and polysaccharide valorization. *β*-1,3-Glucanases are diverse glycoside hydrolases (GHs) that cleave *β*-1,3-glycosidic linkages and enable the biological transformation and industrial utilization of these polymers. These enzymes provide a mechanistic and technological bridge between *β*-1,3-glucan metabolism in nature and the development of sustainable processes for biomass valorization and functional product generation. This review summarizes recent advances in *β*-1,3-glucan enzymatic bioconversion by integrating GH family classification, structural diversity, catalytic mechanisms, enzyme discovery, molecular engineering, production optimization, and emerging applications. Particular attention is given to how variations in fold architecture, active-site organization, and carbohydrate-binding modules (CBMs) collectively determine substrate recognition, catalytic mode, and product specificity toward structurally distinct *β*-1,3-glucans such as laminarin, curdlan, callose, and fungal cell-wall glucans. For an application-oriented perspective, we further discuss how sequence-guided discovery, structure-

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based engineering, and heterologous production can be integrated to improve thermostability, catalytic efficiency, substrate adaptability, product control, and scalable enzyme production. Applications in agriculture, fermentation processing, biomass refining, functional oligosaccharide production, and environmental management are also discussed. Finally, we outline remaining challenges and emerging opportunities, particularly regarding the molecular basis of substrate recognition and product specificity, as well as artificial intelligence (AI) assisted predictive design of customized β-1,3-glucanases for next-generation polysaccharide biotechnology.

1. Introduction

β-1,3-glucans are a class of biological macromolecules composed of D-glucopyranose monomers linked by β-1,3-glycosidic bonds. In nature, β-1,3-glucans exist in diverse forms, including laminarin, curdlan, callose, and fungal cell wall glucans, which are widely distributed across algal, fungal, and plant systems (Caseiro et al., 2022). In marine ecosystems, laminarin acts as a major storage polysaccharide in most microalgae, accounting for up to 35 % dry weight of some species and representing an important component of marine carbon flux (Zhang et al., 2024; O'Sullivan et al., 2010). In fungi, β-1,3-glucan is a core structural component of their cell walls, contributing to mechanical strength and osmotic tolerance through cross-linking with chitin, mannan, and other components (Shree et al., 2024; Ruiz-Herrera and Ortiz-Castellanos, 2019). In higher plants, β-1,3-glucan mainly occurs in the form of callose and participates in growth, development, and responses to pathogens and environmental stresses (German et al., 2023).

Given the widespread occurrence and important biological roles of β-1,3-glucans, enzymes involved in their degradation have attracted considerable attention. β-1,3-Glucanases are enzymes belonging to glycoside hydrolase (GH) families that specifically hydrolyze β-1,3-glycosidic bonds, and participate in a range of biological processes across bacteria (Cheng et al., 2009; Cheng et al., 2013; Plakys et al., 2022), fungi (El-Shora et al., 2021; Wang et al., 2025), and plants (Osuki et al., 2016; Thimmapuram et al., 2001; Alderkamp et al., 2007; Hehemann et al., 2010; Labourel et al., 2014; Unfried et al., 2018; Bäumgen et al., 2021). As key enzymes involved in the degradation of fungal cell walls and certain algal polysaccharides, β-1,3-glucanases play vital roles in microbial metabolism and plant immunity (Umemoto et al., 1997; Huang et al., 2004; Perrot et al., 2022; Liu et al., 2023; Orozco-Mosqueda et al., 2023). Importantly, the functional diversity of β-1,3-glucanases is not solely determined by catalytic residues but arises from the interplay between substrate architecture and enzyme molecular organization. Differences in GH family, fold architecture, catalytic-site topology, and carbohydrate-binding modules (CBMs) collectively determine substrate recognition, catalytic mode, product distribution, and application suitability (Zhao et al., 2013; Lombard et al., 2014). Despite this functional versatility, naturally occurring β-1,3-glucanases often exhibit insufficient thermostability, limited activity toward insoluble or highly branched substrates, poorly controlled product profiles, and inadequate production efficiency, thereby limiting their large-scale utilization.

In recent years, with the rapid advancement of tools such as genomics and bioinformatics, research on β-1,3-glucanases has gradually shifted from single-enzyme characterization toward a systematic engineering approach encompassing discovery, modification, production, and application (Maris et al., 2020; Zhang et al., 2024; Salazar et al., 2001). Previous reviews have provided valuable summaries of β-1,3-glucanases classification, catalytic mechanisms, and specific application areas (Caseiro et al., 2022; Jiang et al., 2024; Roncero et al., 2019). Building on this foundation, an integrated consideration of substrate organization, substrate recognition, catalytic behavior, and rational engineering toward application would help researchers develop a more comprehensive and mechanistic understanding of β-1,3-glucanases.

In this review, we provide an integrated perspective on β-1,3-glucanases by linking substrate structure, enzyme architecture, engineering strategies, and application performance. We summarize their structural and catalytic diversity, highlighting how GH-family-specific folds, active-site configurations, and CBMs determine substrate recognition, catalytic behavior, and product specificity. We then examine recent advances in enzyme discovery, molecular engineering, heterologous production, and emerging applications, emphasizing strategies to overcome limitations in stability, substrate adaptability, and industrial performance. Finally, we summarize major applications and future perspectives, with particular attention to AI-assisted design of customized β-1,3-glucanases for next-generation polysaccharide biotechnology.

2. Classification and structural basis of functional diversity

2.1. Structural diversity and catalytic classification

β-1,3-Glucanases perform distinctly different physiological functions in nature, and this functional diversity is directly reflected in the heterogeneity of their molecular structures. From a sequence- and structure-based perspective, β-1,3-glucanases are distributed across multiple GH families (Zhao et al., 2013; Lombard et al., 2014). Although these enzymes catalyze the hydrolysis of the same β-1,3-glycosidic linkage, each GH family possesses distinct fold architectures and catalytic-site organizations that collectively determine substrate recognition, catalytic mechanism, product specificity, and ultimately their suitability for different biological and industrial applications (Fig. 1A).

In addition to GH family classification, β-1,3-glucanases are also commonly grouped according to their modes of action into *endo*-β-1,3-glucanases and *exo*-β-1,3-glucanases. Exo-β-1,3-glucanases (EC 3.2.1.58) cleave at the terminal ends of macromolecular sugar chains, yielding primarily glucose as the hydrolysis product. Endo-β-1,3-glucanases (EC 3.2.1.39) randomly cleave internal β-1,3-glycosidic linkages within macromolecular glucan chains, thereby generating low-molecular-weight β-1,3-glucan oligosaccharides (Fontaine et al., 1997; Fibriansah et al., 2007; Fontaine et al., 1997). Exo-acting β-1,3-glucanases are mainly distributed in GH3, GH5, GH17, GH55, GH128, and GH132, whereas *endo*-acting enzymes in GH5, GH16, GH17, GH55, GH64, GH81, GH128, GH152, GH157, and GH158 (Fig. 1B). These two classification frameworks are complementary: GH families reflect evolutionary and structural relationships, whereas endo/exo classification denotes the mode of glycosidic bond cleavage.

At the catalytic level, the hydrolysis of β-1,3-glycosidic bonds by β-1,3-glucanase adopts two modes: the retention and inversion mechanisms (Fig. 1C). Enzymes with a retention mechanism typically possess two key catalytic residues (e.g., Glu or Asp). One residue acts as a general acid to donate a proton, promoting glycosidic bond cleavage and forming an enzyme-glycoside intermediate. The other residue subsequently directs a water molecule to attack the intermediate, generating the hydrolysis product while retaining the substrate's stereochemistry. Enzymes with an inversion mechanism feature two catalytic residues acting synergistically as an acid and a base, directly hydrolyzing the substrate into products and causing a reversal of configuration (Davies and Henrissat, 1995).

Although GH-family classification, endo/exo cleavage mode, and catalytic mechanism describe different aspects of β-1,3-glucanase diversity, they are complementary frameworks linking enzyme evolution, hydrolysis patterns, and catalytic chemistry (Zhao et al., 2013; Lombard et al., 2014). However, enzymes within the same GH family may still exhibit distinct substrate preferences and product profiles due to

differences in active-site architecture and substrate-binding topology (Fontaine et al., 1997; Fibriansah et al., 2007; Fontaine et al., 1997; Davies and Henrissat, 1995), as discussed in detail in the following section.

2.2. Structural basis of substrate recognition

Structural diversity is a major determinant of substrate recognition and catalytic specificity among β-1,3-glucanases. As summarized in Table 1, β-1,3-glucanases are distributed across multiple GH families. These structural variations provide the basis for the functional diversity of β-1,3-glucanases toward different β-1,3-glucan substrates. Among these GH families, GH16, GH17, and GH64 represent the most extensively studied *endo*-β-1,3-glucanase families and collectively illustrate the structural basis underlying substrate recognition (Davies and Henrissat, 1995; Qin et al., 2015; Krishnan et al., 2019; Ferrer, 2006; Calloni et al., 2023). Although these enzymes catalyze the hydrolysis of the same β-1,3-glycosidic linkage, their fold architectures, catalytic-groove geometries, and substrate-binding subsites differ considerably, resulting in distinct substrate preferences and catalytic behaviors (Fig. 1A).

The GH16 family enzymes exhibit a typical jelly-roll-like β-sheet structure with a narrow, curved catalytic groove. This architecture favors the accommodation of extended, relatively linear β-1,3-glucan chains through continuous hydrogen-bond networks and aromatic stacking interactions along the substrate-binding cleft. Consequently,

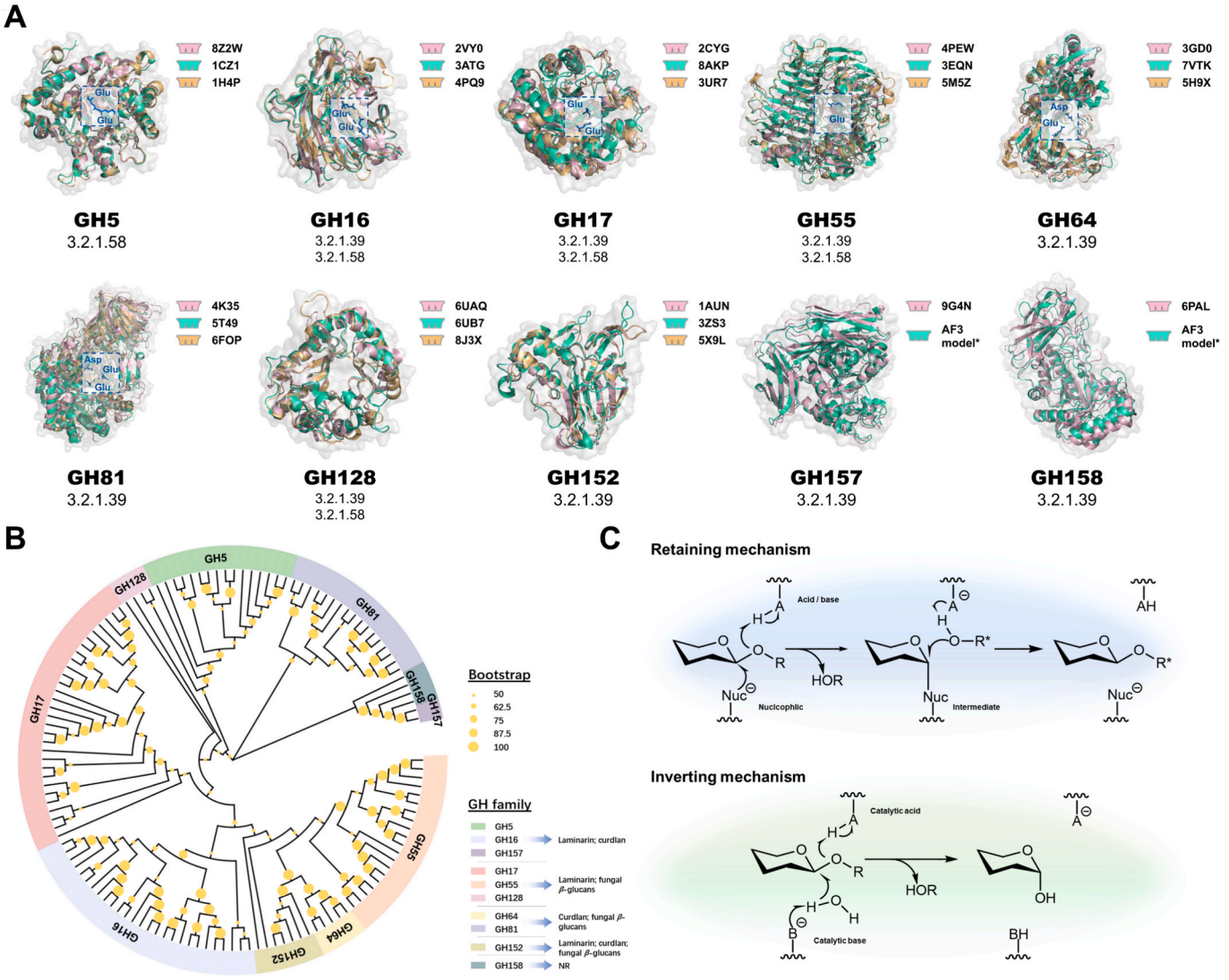


Fig. 1. Structural diversity, evolutionary relationships, and catalytic mechanisms of β-1,3-glucanases. (A) Structural superimposition of representative β-1,3-glucanases across various GH families, illustrating the diversity of protein folds, catalytic-site organizations, and active-site architectures underlying functional differentiation. Catalytic residues are depicted as stick models, except GH128, GH152, GH157, and GH158, for which experimentally validated catalytic-site information remains limited or incomplete. The EC numbers are provided below each GH family, representing *endo*-acting (EC 3.2.1.39) or *exo*-acting (EC 3.2.1.58) cleavage mode. Experimentally resolved structures are labeled with Protein Data Bank (PDB) identifiers, whereas AlphaFold-predicted models are indicated separately for GH157 and GH158 due to limited structural information. Detailed enzyme information is provided in Table S1. (B) Phylogenetic distribution and evolutionary relationships of representative β-1,3-glucanases. The phylogenetic tree was constructed using the maximum likelihood method based on representative β-1,3-glucanase sequences, with different colors indicating GH families and representative substrate preferences annotated to highlight the relationship between evolutionary divergence and functional specialization. For GH158, substrate information is not available (NA) due to limited existing reports. Detailed sequence information is provided in Table S2. (C) Typical catalytic mechanisms of β-1,3-glucanases: Retentive catalysis first cleaves the glycosidic bond to form an intermediate, which is then hydrolyzed to yield the product while preserving the substrate's stereochemistry. Inversional catalysis directly hydrolyzes the substrate into the product, causing a configuration inversion.

Table 1
Comparative structural and functional characteristics of representative β-1,3-glucanase GH families.

GH family	Hydrolysis Mode	Typical Structure	Active-site topology	Typical substrate	Representative source
GH5	Mainly exo	$(\beta/\alpha)_8$ barrel fold	Pocket or cleft-like active site; retaining catalytic machinery	Laminarin; curdlan	Fungi (*Saccharomyces* sp., *Candida* sp.); bacteria
GH16	Mainly endo	β-jelly-roll fold	Narrow, curved catalytic groove	Laminarin; curdlan	Bacteria (*Zobellia* sp., *Streptomyces* sp., *Paenibacillus* sp., *Nocardiopsis* sp.)
GH17	Endo and exo	$(\beta/\alpha)_8$ barrel fold	Catalytic groove with family-dependent side-pocket features	Laminarin; fungal β-glucans; callose	Plants (*Arabidopsis* sp.); fungi
GH55	Endo and exo	Two β-helical domains	Extended cleft or tunnel-like substrate-binding site	Laminarin; fungal β-glucans	Fungi (*Phanerochaete* sp., *Lentinula* sp.)
GH64	Mainly endo	$(\alpha/\alpha)_6$-barrel fold	Wide crescent-shaped groove	Curdlan; yeast β-glucan; fungal β-glucans	Bacteria (*Streptomyces* sp., *Paenibacillus* sp.)
GH81	Mainly endo	β-jelly-roll fold	Open cleft adapted for β-1,3-glucan quaternary/triple-helical recognition	Curdlan; fungal β-glucans	Bacteria (*Paenibacillus* sp., *Bacillus* sp.); fungi (*Rhizomucor* sp., *Trichoderma* sp.)
GH128	Endo and exo	(α/β)-barrel scaffold	Pocket-shaped active site	Laminarin; fungal β-glucans	Fungi (*Lentinula* sp., *Cryptococcus* sp., *Blastomyces* sp.)
GH152	Mainly endo	β-jelly-roll fold	NA*	Laminarin; curdlan; fungal β-glucans	Fungi (*Lentinula* sp.)
GH157	Mainly endo	$(\beta/\alpha)_8$ barrel fold	NA	Laminarin; curdlan	Bacteria (*Bacteroides* sp.)
GH158	Mainly endo	$(\beta/\alpha)_8$ barrel fold	NA	NA	Bacteria (*Victivallis* sp., *Bacteroides* sp.)

* NA, not available.

GH16 enzymes exhibit high catalytic efficiency toward linear substrates such as laminarin and curdlan, but show reduced activity toward mixed-linkage β-1,3/1,4-glucans or highly branched glucans because β-1,6-linked side chains introduce steric hindrance that interferes with productive substrate binding (Davies and Henrissat, 1995).

GH17 family enzymes primarily originate from plants, which possess a $(\beta/\alpha)_8$ barrel fold together with relatively open substrate-binding grooves and auxiliary side pockets (Qin et al., 2015). These structural features provide sufficient space to accommodate β-1,6-linked branches without severe steric clashes, enabling GH17 enzymes to recognize branched fungal glucans and callose more efficiently than GH16 enzymes. Such differences explain their important physiological roles in plant cell-wall remodeling and defense responses.

GH64 family enzymes are commonly found in bacteria and fungi, featuring a $(\alpha/\alpha)_6$-barrel structure with a highly stable conformation. Their substrate-binding pockets are relatively open, accommodating both β-1,3 main chains and complex β-1,6 branches (Krishnan et al., 2019; Ferrer, 2006; Calloni et al., 2023). This enlarged substrate-binding cleft readily accommodates triple-helical curdlan as well as highly cross-linked fungal β-glucans. Unlike the relatively narrow groove of GH16 enzymes, the broader catalytic cleft of GH64 enzymes permits simultaneous accommodation of multiple glucosyl residues and β-1,6 branches, thereby facilitating hydrolysis of structurally complex substrates such as yeast cell wall glucans (Table 1).

Substrate-binding topology also influences catalytic mode beyond the contribution of catalytic residues. Open catalytic grooves generally favor *endo*-type cleavage by accommodating multiple glucan subsites for internal bond hydrolysis, whereas narrow grooves or tunnel-like active sites restrict substrate positioning and promote *exo*-type cleavage with glucose release. Therefore, active-site geometry links substrate recognition with hydrolysis patterns. Collectively, fold architecture, groove geometry, aromatic interactions, hydrogen-bond networks, and branch accommodation determine substrate specificity, catalytic efficiency, and product distribution, providing a structural basis for rational enzyme engineering and application-oriented selection (Davies and Henrissat, 1995; Qin et al., 2015; Krishnan et al., 2019; Ferrer, 2006; Calloni et al., 2023).

2.3. Active-site architecture and product specificity

Although substrate recognition determines whether β-1,3-glucans can be efficiently hydrolyzed, the hydrolysis products ultimately determine the industrial value of individual β-1,3-glucanases. Endo-β-1,3-glucanases generally generate β-glucooligosaccharides with different degrees of polymerization, whereas *exo*-β-1,3-glucanases progressively release glucose from chain termini (Fontaine et al., 1997; Fibriansah et al., 2007; Fontaine et al., 1997). Consequently, product specificity forms an essential link between enzyme structure and downstream applications, including functional oligosaccharide production, biomass saccharification, and biofuel generation.

Product distribution is primarily governed by the organization of substrate-binding subsites within the catalytic groove rather than catalytic chemistry alone. Endo-acting enzymes containing open catalytic clefts generally possess multiple sugar-binding subsites, allowing substrates to bind in different registers and generating heterogeneous oligosaccharides with various chain lengths (Davies and Henrissat, 1995; Yang et al., 2020). In contrast, enzymes possessing well-defined substrate-binding pockets or tunnel-like active sites usually produce more uniform hydrolysis products because substrate positioning is more strictly constrained (Davies and Henrissat, 1995; Qin et al., 2015; Krishnan et al., 2019; Ferrer, 2006; Calloni et al., 2023; Yang et al., 2020). Representative examples clearly illustrate this relationship. For example, some GH64 β-1,3-glucanases, such as SpGlu64A, have been reported to preferentially produce laminaripentaose because their wide catalytic groove accommodates a defined number of glucose residues while steric constraints near the reducing-end subsites prevent further hydrolysis (Ma et al., 2024). In contrast, many GH16 *endo*-β-1,3-glucanases generate heterogeneous β-glucooligosaccharides because their relatively open catalytic groove permits cleavage at multiple internal positions (Labourel et al., 2014; Yang et al., 2020). Exo-acting members of GH5 can continuously release glucose through sequential hydrolysis, making them fundamentally different from oligosaccharide-producing *endo*-glucanases (Bäumgen et al., 2021).

In addition to active-site architecture, substrate structural complexity also influences enzyme specificity. Enzymes acting on linear β-1,3-glucans generally produce relatively uniform oligosaccharides, whereas enzymes capable of accommodating β-1,6-branched glucans often generate more heterogeneous products because branched substrates can adopt multiple binding modes within the catalytic groove (Davies and Henrissat, 1995; Qin et al., 2015; Krishnan et al., 2019; Ferrer, 2006; Calloni et al., 2023). Consequently, branch accommodation represents another important structural determinant of product specificity.

2.4. CBM-mediated substrate binding and catalytic performance

Catalytic chemistry alone cannot fully explain the substrate specificity of β-1,3-glucanases. For insoluble, highly ordered, or heavily cross-

linked glucans, productive substrate binding is equally critical. CBMs can anchor enzyme molecules to the substrate surface through hydrophobic interactions and hydrogen bonds, increasing local substrate concentration and facilitating the capture of substrate chains by the enzyme active site (Cheng et al., 2009; Plakys et al., 2022).

Different CBM families exhibit distinct substrate-binding preferences and consequently play different functional roles during β-1,3-glucan degradation (Table 2). CBM4-containing modules generally exhibit broad affinity toward both soluble and less ordered β-1,3-glucans through extended carbohydrate-binding surfaces, thereby improving enzyme accessibility to linear substrates such as laminarin (Cheng et al., 2009). In contrast, CBM6, CBM56, and CBM81 preferentially enhance interactions with insoluble or highly ordered β-glucans, including curdlan and fungal cell-wall glucans, through larger aromatic binding platforms or extended substrate-binding grooves (Ma et al., 2024; Jia et al., 2021; Wei et al., 2025). These structural differences enable CBMs to selectively improve enzyme performance toward substrates with distinct physical properties rather than universally enhancing catalytic activity.

Previous work has revealed that PsLam81A of the GH81 family from *Paenibacillus* sp. contains a CBM56 module. Its removal significantly reduces the enzyme's ability to hydrolyze the linear insoluble glucan curdlan, while having a minor impact on its activity toward soluble substrates (Plakys et al., 2022). Likewise, fusion of CBM56 to the GH64 enzyme SpGlu64A significantly increased hydrolysis of insoluble β-1,3-glucans (Ma et al., 2024), whereas CBM4-containing LamA exhibited improved degradation of both laminarin and insoluble β-1,3-glucans through enhanced substrate binding (Cheng et al., 2009). These observations collectively indicate that the contribution of CBMs depends strongly on substrate architecture, crystallinity, and solubility.

Current studies suggest several general principles for CBM engineering. CBM fusion is most effective for enhancing degradation of insoluble or highly crystalline substrates but provides limited benefit for soluble glucans. Moreover, selecting CBM families according to substrate properties is generally more important than increasing the number of binding modules, while linker composition, domain orientation, and compatibility between catalytic and binding modules also substantially influence enzyme performance (Cheng et al., 2009; Plakys et al., 2022; Ma et al., 2024; Jia et al., 2021; Wei et al., 2025; Tamashiro et al., 2012). Therefore, rational combination of catalytic domains and CBMs represents an effective strategy for tailoring β-1,3-glucanases to specific industrial substrates.

3. Screening and discovery strategies for β-1,3-glucanases

3.1. Genomics- and bioinformatics-driven discovery

By utilizing genomic and metagenomic sequence data, bioinformatics approaches have become central tools for identifying potential β-1,3-glucanase genes at the sequence level (Qin et al., 2023) (Fig. 2, PART I). Researchers often utilize databases such as CAZy to screen for sequences highly homologous to known β-1,3-glucanases through sequence alignment, thereby predicting their family classification. For example, a gene designated GluH, encoding a GH16 *endo*-β-1,3-glucanase, was identified from the genome of an Arctic deep-sea bacterium *Hymenobacter siberiensis*. This enzyme exhibited distinct cold adaptation properties, maintaining over 40 % of its maximum activity at 25 °C (Kim et al., 2024). Similarly, Burkhardt *et al.* identified two novel GH16 family *endo*-β-1,3-glucanases, FLamA and FLamB, from hot spring environmental metagenomes. These enzymes displayed maximum activity toward laminarin at 90 °C and pH 6.5, demonstrating remarkable thermal stability (Burkhardt et al., 2019). These studies highlight the effectiveness of sequence-based mining in expanding the functional diversity of β-1,3-glucanases across extreme environments.

Hidden Markov models (HMMs) have also been widely applied in the discovery and functional characterization of novel members within the β-1,3-glucanase family. Researchers frequently utilize known β-1,3-glucanase domains to identify potential homologous enzyme genes. For example, HMM-based analysis identified 58 GH17 β-1,3-glucanases in the *Lycium barbarum* genome (Zhang et al., 2024) and facilitated automated classification of newly identified GH16 enzymes into the CAZy database (Viborg et al., 2019). In addition, sequence-guided functional annotation has enabled the discovery of previously unclassified β-1,3-glucanases, such as the thermotolerant enzyme EngU from volcanic soil metagenomes, thereby expanding the known diversity of GH families (Angelov et al., 2017).

Compared with phenotype-based screening, sequence-driven approaches offer unparalleled throughput and access to uncultured microorganisms, but they cannot directly verify catalytic activity or substrate specificity (Qin et al., 2023). Consequently, experimental validation remains indispensable for confirming enzyme function and identifying candidates with industrially relevant properties.

3.2. Function-based phenotype screening

Direct isolation of enzyme-producing microorganisms, genes from environmental samples, or strains based on enzymatic activity phenotypes is also a classical and still highly effective strategy for discovering β-1,3-glucanases. The central principle of this approach is to link an

Table 2
Functional comparison and engineering implications of representative CBMs.

CBM family	Representative enzyme	Preferred substrate	Primary function	Modular design implication	Reference
CBM4 and CBM9	LamA from *Paenibacillus* sp.	Laminarin	Enhances hydrolysis of insoluble β-1,3-glucans	Suitable for broadening substrate range toward soluble β-glucans	(Cheng et al., 2009)
CBM6	CBM6E from *Saccharophagus degradans*	Curdlan, insoluble β-1,3-glucans	Improves interaction with insoluble β-glucans	Useful for improving accessibility to linear β-1,3-glucan chains	(Jia et al., 2021)
CBM56	PsLam81A from *Paenibacillus* sp.	Curdlan, insoluble β-1,3-glucans	Enhances hydrolysis of insoluble β-1,3-glucans, limited effect on branched β-glucans	Preferred for engineering enzymes targeting recalcitrant linear β-1,3-glucan substrates	(Plakys et al., 2022)
CBM56	SpGlu64A from *Streptomyces pratensis*	Curdlan, insoluble β-1,3-glucans	Enhances adsorption and hydrolysis of insoluble curdlan	Preferred for engineering enzymes toward recalcitrant curdlan or fungal cell-wall glucans	(Ma et al., 2024)
CBM81 and CBM43	BsGlc157A from *Bacteroides* sp.	Curdlan, insoluble β-1,3-glucans	Enhances recognition of ordered β-1,3-glucan substrates	Promising for targeting crystalline or triple-helical β-1,3-glucans	(Wei et al., 2025)
CBM13	Endo-β-1,3-glucanase from *Cellulosimicrobium cellulans*	Laminarin	Enhances substrate affinity through cooperative β-glucan recognition	Suitable for improving binding affinity toward soluble β-1,3-glucans and oligosaccharides	(Tamashiro et al., 2012)

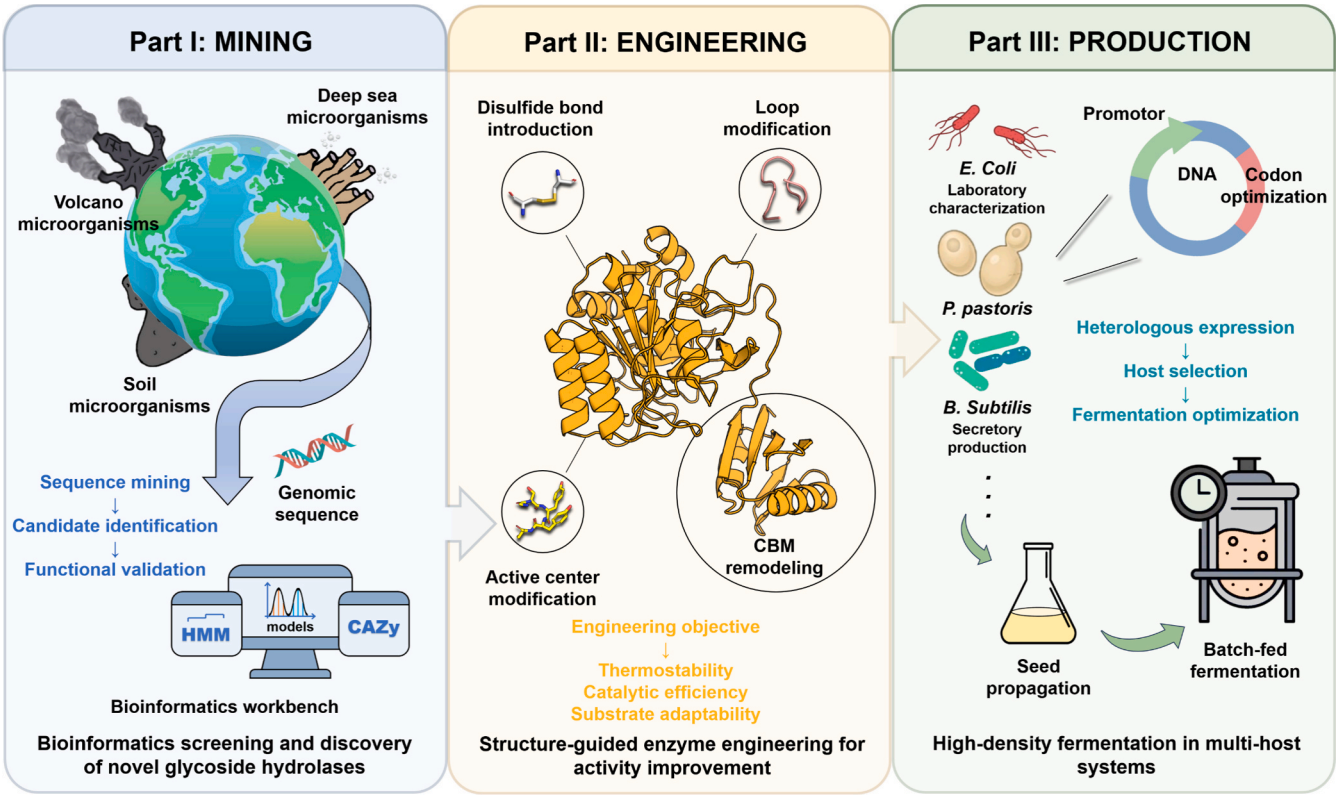


Fig. 2. Schematic overview of he biotechnological development of β-1,3-glucanases. The workflow illustrates the transition from natural enzyme discovery to industrial biocatalyst development, including (I) sequence- and function-based mining of novel β-1,3-glucanases from diverse microbial resources using genomic information, bioinformatics tools, and functional validation; (II) structure-guided enzyme engineering targeting key performance parameters, including thermostability, catalytic efficiency, and substrate adaptability through active-site modification, structural rigidification, and CBM remodeling; and (III) heterologous expression in suitable microbial hosts, coupled with process optimization and fermentation scale-up to enable efficient and scalable enzyme production.

observable phenotype with the hydrolysis of a target substrate, thereby enabling the recovery of enzymes or microorganisms with β-1,3-glucan-degrading activity from large sample populations or clonal libraries.

The traditional agar plate screening method involves culturing strains on media containing specific substrates and identifying colonies that form hydrolytic zones. After inoculating a mixture of environmental bacterial strains, the plates are incubated, and β-1,3-glucanase-producing strains can be readily identified by observing clear hydrolytic zones through staining. For example, a *Paenibacillus* sp. bacterium was isolated from soil using yeast cell wall as the screening substrate. Through halo-based activity assays and liquid chromatography-mass spectrometry (LC-MS) analysis, the authors identified the extracellular glucanase sequence and cloned the GH81 family endoglucanase PsLam81A. This enzyme contains the CBM56 accessory module and exhibits efficient hydrolytic activity toward the linear β-1,3-glucan substrate curdlan (Plakys et al., 2022).

Collectively, sequence-guided prediction and function-based validation provide complementary approaches for expanding the discovery of novel β-1,3-glucanases by combining broad candidate identification with experimental confirmation of catalytic performance (Plakys et al., 2022; Qin et al., 2023; Ramada et al., 2010). The characteristics of the currently available discovery strategies are comparatively summarized in Table 3, highlighting their respective advantages, limitations, and suitable application scopes.

Table 3
β-1,3-glucanase discovery and screening.

Strategy category	Method	Advantages	Limitations	Suitable application scope	References
Computational-based discovery	BLAST-based homology search	High sequence diversity coverage; fast annotation	Cannot confirm enzymatic activity; biased toward known families	Discovery of homologous β-1,3-glucanases	(Qin et al., 2023)
	HMM profile search	Sensitive detection of remote homologs; family classification	Limited functional prediction; depends on training dataset	Identification of novel GH families	(Zhang et al., 2024; Viborg et al., 2019; Angelov et al., 2017)
Computational-based engineering	Protein engineering-guided in silico screening	Enables de novo prediction and prioritization	Limited by training data	Candidate prioritization and design	(Liu et al., 2026)
Experimental-based screening	Agar plate screening	Direct visualization of hydrolysis; simple and low-cost	Low throughput; substrate bias; semi-quantitative	Environmental isolates and microbial screening	(Plakys et al., 2022)
	Droplet microfluidics screening	High throughput; highly sensitive	High instrumentation cost; technical complexity	Directed evolution and protein engineering	(Huang, 2009; Ladeveze et al., 2023; Samlali et al., 2022)

4. Molecular engineering strategies for β-1,3-Glucanases

4.1. Engineering thermostability via structural rigidification

Thermal stability is one of the primary engineering objectives for β-1,3-glucanases because industrial bioprocesses often operate under elevated temperatures. Structural rigidification, including disulfide bond engineering, loop optimization, hydrophobic interaction enhancement and electrostatic network remodeling, has therefore become an effective strategy for improving enzyme stability.

Disulfide bonds, as one of the most crucial covalent constraints in proteins, connect two spatially adjacent cysteine residues. These covalent bonds restrict the conformational flexibility of enzyme molecules, thereby reducing the probability of unfolding at elevated temperatures. In β-1,3-glucanase engineering, introducing disulfide bonds or modifying loop regions within the enzyme molecule represents a key enzyme engineering strategy for enhancing thermal stability through structural rigidification. For instance, Feng *et al.* resolved the crystal structure of a thermophilic β-1,3-glucanase from the GH16 family, isolated from a compost microbial metagenome, revealing two naturally occurring disulfide bonds. Through site-directed mutagenesis of these disulfide bonds (C160G and C180I), the enzyme's optimum temperature decreased from 75 °C to 70 °C, confirming the contribution of disulfide bonds to thermal stability. Interestingly, disruption of the disulfide bond in the C160G mutant paradoxically enhanced enzyme activity (Feng et al., 2021). This result suggests that while improving enzyme stability is desirable, it must be balanced against flexibility to avoid the detrimental effects of excessive rigidity on activity (Feng et al., 2021; Qin et al., 2017).

Loop regions likewise play important roles in substrate entry, positioning, and catalytic stability in β-1,3-glucanases. During engineering, the non-essential flexibility of the loop is often reduced through methods such as proline substitution to enhance the overall structural rigidity. Taking the study of GH16 β-1,3-glucanase from *Alkalihalobacillus clausii* as an example, combinatorial mutagenesis significantly increased the melting temperature (T_m) and enhanced catalytic efficiency. Molecular dynamics studies revealed that this improvement stemmed from mutations that increased the overall structural rigidity of the enzyme molecule, thereby achieving synergistic optimization of stability and catalytic efficiency (Zhang et al., 2024).

Besides covalent constraints, strengthening hydrophobic interactions and electrostatic networks further improves enzyme robustness. Semi-rational engineering of hydrophobic residues near the substrate entrance enhanced substrate accessibility and catalytic efficiency, whereas introduction of additional salt bridges has been proposed as another effective strategy for improving protein stability (Zhang et al., 2024; Yu et al., 2025). Likewise, the naturally occurring salt-bridge network in the thermophilic β-1,3-glucanase from *Pyrococcus furiosus* contributes to remarkable structural stability even under strongly denaturing conditions, illustrating the importance of electrostatic interactions in maintaining enzyme integrity (Chiaraluce et al., 2005).

Although structural rigidification generally improves thermal stability, its effects on catalytic activity are enzyme-dependent. Disruption of a GH16 disulfide bond enhanced activity despite reduced stability, whereas loop rigidification improved both properties (Feng et al., 2021; Zhang et al., 2024). These findings indicate that thermostability is controlled by multiple structural determinants, and rational engineering should balance rigidity and catalytic flexibility rather than maximize structural constraints.

4.2. Optimizing catalytic efficiency through active site reconstruction

The conformation of an enzyme's active site directly determines its substrate recognition and catalytic efficiency. By (semi-)rationally designing the spatial arrangement of key residues surrounding the active site, the synergistic interaction between the enzyme and its substrate can be significantly enhanced. Accordingly, precise optimization of active-site architecture has become a core strategy for improving the catalytic performance of engineered β-1,3-glucanases.

By analyzing the crystal structure of SpGlu64A, a GH64 family β-1,3-glucanase from *Streptomyces pratensis*, it was revealed that residues Tyr161, Asn163, and His393 in its catalytic groove influence the enzyme's substrate-binding process. Upon introducing a targeted mutation to replace tyrosine 161 with the slightly smaller and more hydrophobic phenylalanine (Y161F), steric hindrance in the catalytic groove was reduced, thus leading to an approximately 1.7-fold increase in specific activity. This demonstrates that appropriate relaxation of active-site constraints can facilitate substrate binding and product release, thereby mitigating the inhibitory effects caused by product accumulation (Jia et al., 2021). Furthermore, based on structural comparisons and molecular dynamics simulations, the engineering of β-1,3-glucanase BglS27 involved replacing Asn54 near the active site with Trp. This modification enhanced substrate binding stability and facilitated nucleophilic attack by the catalytic residue, resulting in a 3.6-fold increase in specific activity and significantly improved degradation efficiency for the target substrate (Gao et al., 2023).

Although both strategies improved catalytic efficiency, their mechanisms differed. In SpGlu64A, activity enhancement resulted from reduced steric hindrance in the catalytic groove, whereas BglS27 benefited from strengthened substrate binding (Jia et al., 2021; Gao et al., 2023). These findings indicate that similar engineering goals may rely on distinct structural mechanisms and cannot be directly transferred across enzymes without considering substrate-binding architecture and catalytic dynamics.

4.3. Improving substrate compatibility through CBM engineering

Many β-1,3-glucanases naturally contain non-catalytic CBMs, whose modular combinations profoundly influence enzyme performance. Although CBMs do not directly catalyze glycosidic bond cleavage, their affinity for specific polysaccharide interfaces is often a key determinant of overall hydrolytic efficiency. Their principal role is to enhance the effective proximity of the enzyme to the substrate surface (Table 4).

For insoluble substrates such as curdlan or spore walls, enzymes lacking CBMs often exhibit extremely low degradation rates due to their limited adsorption capacity. For example, the GH16 family β-1,3-glucanase PglA from *Paenibacillus* sp. comprises an *N*-terminal leader peptide, a catalytic domain, and a *C*-terminal domain. Ternary mutation studies indicated that removal of the *C*-terminal domain improved the optimal temperature and thermal stability, suggesting that this region partially compromises the enzyme's heat resistance. However, the intact enzyme exhibited stronger binding and hydrolysis capabilities toward insoluble β-1,3-glucan than the truncated variant, indicating that the *C*-terminal domain functions as a CBM and enhances the enzyme's adsorption to the substrate (Cheng et al., 2013). Furthermore, since CBMs from different sources exhibit specific substrate preferences, enzyme activity toward particular substrates can be tailored through module replacement. In the aforementioned modification of SpGlu64A, the Y161F mutation, followed by fusion with the CBM56 domain, increased the enzyme's total activity by 2.63-fold. The CBM56 domain specifically enhanced the adsorption of insoluble β-1,3-glucan substrates (Jia et al., 2021). A similar CBM synergistic effect was also demonstrated in BglF from *Nocardiopsis* sp., which naturally lacks a CBM module. After fusion of the *C*-terminus, an additional domain possessing CBM functionality, the resulting enzyme exhibited significantly enhanced binding capacity and hydrolytic activity toward insoluble β-1,3-glucans such as curdlan (Jia et al., 2021; Koizumi et al., 2009).

Compared with active-site engineering, CBM engineering mainly improves substrate accessibility and is highly substrate-dependent. CBM fusion can enhance the degradation of insoluble β-glucans, whereas CBM truncation may improve thermal stability at the expense of substrate binding and hydrolytic efficiency, highlighting the trade-off between

Table 4
Representative molecular engineering strategies for improving the performance of β-1,3-glucanases.

Engineering objective	Enzyme (GH family)	Source	Main substrate	Optimum T/pH	Specific activity (U/mg)	Engineering strategy	Representative engineering outcome	Preliminary Conclusions	References
Stability	Actglu (GH16)	Metatranscriptomic data	Curdlan	75 °C/pH 5.5	146.9 (WT); 171.4 (C160G)	Disulfide bond engineering (C160G/C180I)	17 % Activity increase; T_{opt} decreased to 70 °C	Activity-stability trade-off	(Feng et al., 2021)
Stability & Activity	ULam111 (GH16)	*Flavobacterium* sp. UMI-01	Laminarin, Curdlan	30 °C/pH 6.0	360 (laminarin A); 366 (curdlan)	Calcium-binding motif engineering (H12E/N14D); disulfide bond mutation (C31S/C34S)	Activity retained at 40–50 °C	Multiple determinants of thermostability	(Qin et al., 2017)
						Active-site mutation (F212W)	Altered substrate recognition	Residue-dependent substrate specificity	
Stability & Activity	AC-GA (GH16)	*A. clausii* KSM-K16	Curdlan	75 ℃/pH 6.5	124.88 % of WT	Semi-rational design; combinational mutation (S52T/M120L)	T_m increased by 4.39 ℃, and k_{cat}/K_m increased by 81.5 %	Activity–stability balance	(Zhang et al., 2024)
Stability	pfLamA (GH16)	*P. furiosus*	Laminarin	80 ℃/pH 6.5	NA*	Ca^{2+}-dependent structural stabilization analysis	Enhanced tertiary structure under denaturing conditions	Ca^{2+}-dependent stabilization	(Chiaraluce et al., 2005)
Activity & Substrate compatibility	SpGlu64A (GH64)	*S. pratensis*	Curdlan	50 ℃/pH 7.5	83.1 (WT); 188.1 (CBM56, Y161F)	Active-site mutation (Y161F) and CBM56 fusion	2.26-Fold activity increase	Structure-dependent catalysis; CBM mainly benefits insoluble substrates	(Jia et al., 2021)
Activity	BglS27 (GH16)	*Streptomyces* sp. S27	Laminarin	65 ℃/pH 5.5	249 (WT); 902 (N54W)	Active-site mutation (N54W)	3.6-Fold higher activity; 5.5-fold higher catalytic efficiency	Enhanced substrate binding	(Gao et al., 2023)
Substrate compatibility	LamA (GH16)	*Paenibacillus* sp.	Laminarin, curdlan, pachyman	50 ℃/pH 5.5–6.0	NA	CBM4_9 repeats and Fa5/8C fusion	Enhanced hydrolysis of insoluble β-1,3-glucans	Substrate-dependent CBM effects	(Cheng et al., 2009)
Substrate compatibility	BglF (GH16)	*Nocardiopsis* sp. F96	Pachyman, curdlan	60 ℃/pH 9.0	NA	CAD fusion	Improved binding and hydrolysis of insoluble β-1,3-glucans	Substrate-specific CBM function	(Koizumi et al., 2009)

* NA, not available.

stability and substrate accessibility (Cheng et al., 2013; Jia et al., 2021; Koizumi et al., 2009). Variations in substrates and assay conditions among studies further highlight the need for standardized evaluation using industrially relevant substrates.

5. Production and expression optimization of β-1,3-Glucanase

Currently, microorganisms reported to produce β-1,3-glucanase include species of the genera *Trichoderma*. *Trichoderma reesei* is known to secrete three extracellular enzymes, one of which is β-1,3-glucanase, contributing to the disruption of pathogenic fungal cell walls. *Trichoderma harzianum* can also naturally produce extracellular β-1,3-glucanase (Bamforth, 1980; Budiarti et al., 2009; Thodore and Panda, 1994; de la Cruz et al., 1995). Hong *et al.* purified a β-1,3-glucanase from the fermentation broth of *Paenibacillus barengoltzii* and demonstrated that the enzyme could disrupt the cell-wall structures of the growing mycelia of phytopathogenic fungi *Pythium aphanidermatum* and *Rhizoctonic solani* AG-4 (Hong and Meng, 2003). However, the native production levels of β-1,3-glucanase in wild-type strains struggles to meet the growing demands of industrial applications.

To improve enzyme yield, heterologous expression systems have been widely developed. *E. coli* is the preferred host for rapid protein expression and biochemical characterization (Shi et al., 2010; Wang et al., 2021; Li et al., 2020), whereas *Pichia pastoris*, *Bacillus subtilis*, *Yarrowia lipolytica*, and filamentous fungi provide superior extracellular secretion and are therefore more suitable for industrial enzyme production. In particular, multi-copy integration in *P. pastoris* further enhances recombinant protein yields (Zhang et al., 2019; Xu et al., 2006). As summarized in Table 5, selection of an appropriate host should consider secretion capacity, production cost, and industrial scalability in addition to recombinant enzyme activity.

Enhancing transcription and translation efficiency is one of the key strategies for improving the heterologous expression of β-1,3-glucanase. Codon optimization is currently a widely used and highly effective approach. Gao *et al.* engineered a fungal-derived β-1,3-glucanase gene to exhibit *Saccharomyces cerevisiae* codon preference, significantly enhancing its expression level in yeast (Gao et al., 2021). Promoter selection is another crucial determinant of expression efficiency. In the same study, the expression-driving effects of three promoters (AOX1, GAP, and FLD1) on β-1,3-glucanase in *Pichia pastoris* GS115 were compared. The GAP promoter exhibited significantly higher sustained expression capacity than other promoters (Gao et al., 2021); enabling recombinant enzyme expression activity to reach a maximum of 49.64 U/mL.

Beyond genetic optimization, culture medium optimization also plays a critical role in improving expression efficiency. Peng *et al.* investigated the β-1,3-glucanase EXG1 from the marine yeast *Williopsis saturnus* and expressed this enzyme in *Y. lipolytica*, a host with strong secretory capacity. Using PPB medium, a nutritionally balanced formulation optimized for secretory protein accumulation, they achieved significant expression of the target enzyme. Additionally, transformant screening led to the identification of a recombinant strain with significantly elevated production, in which extracellular β-1,3-glucanase activity increased from 1.6 U/mL in the wild-type strain to 16 U/mL, corresponding to an approximately tenfold improvement (Peng et al., 2011).

Optimizing fermentation conditions can further enhance recombinant β-1,3-glucanase production at the process level. For example, when an *endo*-β-1,3-glucanase gene from *T. harzianum* was expressed in *P. pastoris* KM71 in a 7 L bioreactor, the recombinant enzyme achieved a peak activity of approximately 198.57 U/mL after 118 h of induction. This improvement was achieved through optimization of key fermentation parameters, including an initial pH of 6.0, an inoculation size of 2.5 %, methanol induction at around 1.0 %, and an induction temperature of 30 °C, together with fed-batch cultivation and controlled methanol induction in the bioreactor. This level significantly exceeded the results from unoptimized small-scale cultures, demonstrating that expression levels can be increased several-fold through adjustments to fermentation parameters and production scale (Gao et al., 2021). These strategies based on the combined optimization of host and process conditions provide a feasible engineering approach for the industrial-scale production of high-yield β-1,3-glucanase (Fig. 2, PART III).

Different expression hosts exhibit distinct advantages for β-1,3-glucanase production. *E. coli* is widely used for rapid protein expression and biochemical characterization because of its fast growth and simple genetic manipulation, whereas *P. pastoris*, *Y. lipolytica*, and Aspergillus oryzae are more suitable for large-scale production owing to their superior extracellular secretion capacities (Zhang et al., 2019; Xu et al.,

Table 5
Representative heterologous expression systems for β-1,3-glucanases and their characteristics.

Enzyme	Source	Expression host	Substrate	Specific activity (U/mg)	Induction strategy/cost	Secretion capability	Main advantages	Major limitations	References
BglS27	*Streptomyces* sp.	*E. coli* BL21 (DE3)	laminarin	236	IPTG/high	Poor	Fast growth; low cost; easy engineering	Poor secretion; limited industrial enzyme production	(Shi et al., 2010)
AcGluA	*Archangium* sp.	*E. coli* BL21 (DE3)	laminarin	112.3	IPTG/high	Poor	Fast growth; low cost; easy engineering	Poor secretion; limited industrial enzyme production	(Wang et al., 2021)
Mbbgl	*Macrotermes barneyi*	*E. coli* BL21 (DE3)	laminarin	16.7	IPTG/high	Poor	Fast growth; low cost; easy engineering	Poor secretion; limited industrial enzyme production	(Li et al., 2020)
BGN	*T. harzianum*	*P. pastoris* GS115	curdlan	292.56	Methanol/low	Excellent	High secretion; scalable fermentation	Methanol induction; glycosylation	(Gao et al., 2021)
EXG1	*P. pastoris*	*P. pastoris* X-33	laminarin	70	Methanol/low	Excellent	High secretion; scalable fermentation	Methanol induction; glycosylation	(Xu et al., 2006)
ExgA	*A. oryzae*	*P. pastoris* X-33	laminarin	289	Methanol/low	Excellent	High secretion; scalable fermentation	Methanol induction; glycosylation	(Boonvitthya et al., 2012)
WsEXG1	*W. saturnus*	*Y. lipolytica* Po1h	laminarin	511	Constitutive/ none	Excellent	Strong secretion; industrial potential	Fewer expression systems available	(Peng et al., 2011)
EgCel17A	*Euglena gracilis*	*A. oryzae*	laminarin	40.5	Constitutive/ none	Excellent	Excellent secretion; industrial enzyme producer	Longer fermentation; difficult morphology control	(Takeda et al., 2015)

*IPTG, Isopropyl β-D-1-thiogalactopyranoside.

2006). Therefore, selecting an appropriate expression system requires balancing enzyme yield, secretion efficiency, production cost, and downstream processing according to specific demands (Zhang et al., 2019; Xu et al., 2006; Gao et al., 2021; Peng et al., 2011; Gao et al., 2021).

6. Tailored applications of β-1,3-Glucanases

6.1. Enhancing agricultural efficiency and plant protection

β-1,3-Glucan is a key component of the cell walls of many plant pathogenic fungi. Accordingly, β-1,3-glucanase can directly hydrolyze pathogen cell wall glucans, thereby restricting their invasion and spread, and offering considerable potential for crop protection (Fig. 3, left panel, Table 6). The induction mechanism and functional characteristics of β-1,3-glucanase provide a controllable molecular intervention pathway for green agriculture. Beneficial rhizosphere microorganisms can protect plants against diseases by inducing resistance or enhancing tolerance to infection. They significantly increase the expression and activity of multiple defense-related enzymes within plants, including β-1,3-glucanase, thereby promoting the reduction of fungal lesions and strengthening disease resistance (Orozco-Mosqueda et al., 2023; Neale et al., 1990; van Loon et al., 1998; Raupach and Kloepper, 2000; Karthikeyan et al., 2006; Gu et al., 2024). Likewise, transgenic expression of β-1,3-glucanase genes from tobacco or legumes has significantly enhanced resistance to rice blast, gray mold, wilt, and downy mildew in several crops (Mohammadizadeh-Heydari et al., 2024; Lin et al., 1995; Kavanagh and Spillane, 1995; Yu et al., 2023). Overexpression of β-1,3-glucanase in tobacco also inhibited the growth of *Fusarium poae*, *Rhizoctonia solani*, and *Alternaria solani*, demonstrating its potential for broad-spectrum disease control (Rajninec et al., 2021). In contrast, some fungal pathogens, such as *Colletotrichum graminicola*, exploit β-1,3-glucanase as a virulence factor to suppress host immune responses and facilitate infection (Gu et al., 2024). Together, these findings indicate that β-1,3-glucanases can play distinct roles depending on their biological origin, acting either as a plant defense factor or as a virulence-associated effector in fungal pathogens, highlighting their diverse roles in plant–microbe interactions (Lavanya et al., 2022).

6.2. Fermentation processing and high-value utilization of biological resources

β-1,3-Glucanase is commonly employed in fermentation broth processing and cell wall disruption. Enzymatic cell lysis represents one of the mild yet highly efficient methods compared to chemical treatment and physical disruption. The combined use of β-1,3-glucanase with protease and chitinase can accelerate yeast cell lysis while improving extraction efficiency and product purity (Monreal et al., 1967; Rodriguez-Pena et al., 2013). The primary active components in the commercially available cell wall-lysing enzymes Zymolyase and Lyticase are β-1,3-glucanases. These enzymes demonstrate effective cell wall removal against a variety of fungi and can be utilized for protoplast preparation, protein extraction, and molecular procedures such as DNA and RNA recovery (Scott and Schekman, 1980; Carvalho et al., 2021). Ionut *et al.* found that adding β-1,3-glucanase to the spent brewer's yeast extraction process significantly increased yeast extract yield, which substantially reduced resource consumption and waste generation (Avramia and Amariei, 2021) (Fig. 3, middle panel).

Additionally, studies indicate that β-1,3-glucanase can hydrolyze β-1,3-glycosidic bonds in polysaccharides such as laminarin and curdlan from brown algae, thereby depolymerizing complex biomass into fermentable sugars and bioactive oligosaccharides. These products can ultimately serve as intermediates for the production of high-value-added biofuels and bio-based chemicals. β-1,3-Glucanase can also be applied in fungal removal and biofilm control during wastewater treatment. It effectively disperses biofilms formed by *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei*. When adding β-1,3-glucanase 24 h after biofilm initiation, biofilm formation was significantly suppressed, indicating its potential for controlling fungal communities in environmental treatment applications (Tan et al., 2017; Tan et al., 2018) (Fig. 3, right

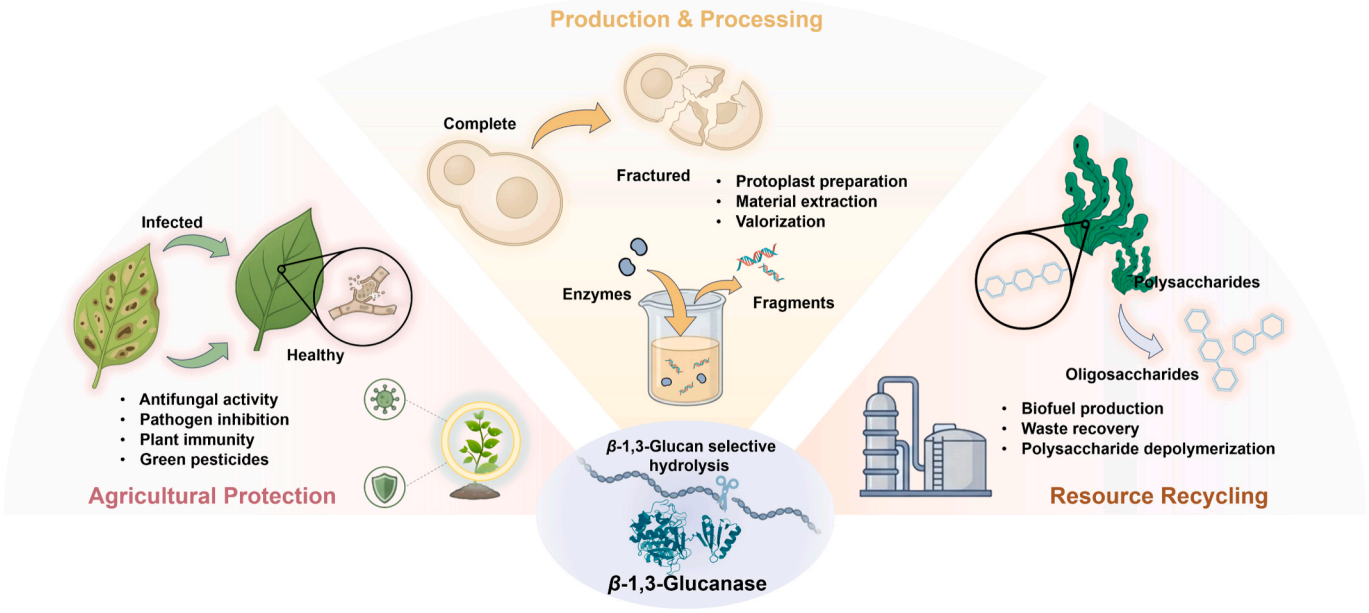


Fig. 3. Application landscape of β-1,3-glucanases in biotechnology and bioprocessing. By selectively degrading β-1,3-glucan, β-1,3-glucanases demonstrate significant application value in agricultural protection, cell processing, preparation of functional components, and recovery of polysaccharide resources. The figure illustrates the application landscape of β-1,3-glucanases in biotechnology and bioprocessing, including agricultural protection, where β-1,3-glucanases contribute to antifungal activity by degrading fungal cell-wall glucans, suppressing pathogen growth, and enhancing plant defense responses (Left panel); production and processing, where selective β-1,3-glucan hydrolysis enables fungal cell-wall disruption, protoplast preparation, and recovery of intracellular components (Middle panel); and resource recycling, where enzymatic depolymerization of β-1,3-glucan-rich biomass generates oligosaccharides and fermentable products for biofuel production, waste valorization, and high-value bioproduct generation (Right panel).

Table 6
Application performance and characteristics of β-1,3-glucanases.

Application	Main Source	Validation level	Representative performance	Key enzyme requirements	References
Agriculture/ Plant protection	Mainly GH17, GH64 and GH81, e.g., PsLam81A and plant β-1,3-glucanases	In vitro; in planta; transgenic validation	Enhanced resistance to rice blast, gray mold, wilt and downy mildew; inhibition of *F. poae*, *R. solani* and *A. solani*; induction of plant defense responses	Broad substrate specificity; activity toward fungal cell-wall glucans; environmental stability	(van Loon et al., 1998; Raupach and Kloepper, 2000; Karthikeyan et al., 2006; Gu et al., 2024; Mohammadizadeh-Heydari et al., 2024; Lin et al., 1995)
Cell lysis/ Fermentation	Mainly GH64 and GH81, e.g., Zymolyase and Lyticase	Commercial application; industrial validation	Efficient yeast cell-wall degradation; improved protoplast preparation; enhanced protein, DNA and RNA extraction	High activity toward branched β-glucans; efficient extracellular secretion	(Monreal et al., 1967; Rodriguez-Pena et al., 2013; Scott and Schekman, 1980; Carvalho et al., 2021)
Biomass refining	Mainly GH16 and GH81	In vitro; laboratory validation	Hydrolysis of laminarin and curdlan into fermentable sugars and bioactive β-glucooligosaccharides for biofuel and biochemical production	High catalytic activity; thermostability; activity toward insoluble substrates	(Yang et al., 2020; Wargacki et al., 2012; Enquist-Newman et al., 2013; Wang et al., 2016; Kim et al., 2018)
Environmental biotechnology	Mainly GH64 and GH81	In vitro; laboratory validation	Significant inhibition of biofilm formation by *C. tropicalis*, *C. parapsilosis* and *C. krusei*	Environmental tolerance; long operational stability	(Tan et al., 2017; Tan et al., 2018)

panel).

7. Artificial Intelligence (AI)-assisted predictive design and remaining challenges

AI-assisted enzyme design provides a promising strategy to transform β-1,3-glucanase engineering from empirical optimization toward predictive and application-oriented design. Recent studies have provided initial evidence for the feasibility of AI-guided optimization of β-1,3-glucanases. Liu et al. recently developed an AI-assisted computational workflow for engineering the thermostability of the GH17 *endo*-β-1,3-glucanase RmBgt17A. By integrating ProteinMPNN-based sequence design, structure prediction, and molecular dynamics simulation, beneficial variants were identified and experimentally validated, with one designed variant achieving simultaneous improvement in thermostability and hydrolytic activity (Liu et al., 2026). This study represents an important first step toward applying AI-guided approaches to β-1,3-glucanase engineering, although current efforts remain largely focused on optimizing individual properties such as thermal stability.

Future AI-assisted β-1,3-glucanase engineering is expected to move beyond single-property optimization toward application-oriented enzyme design. By integrating sequence information, structural

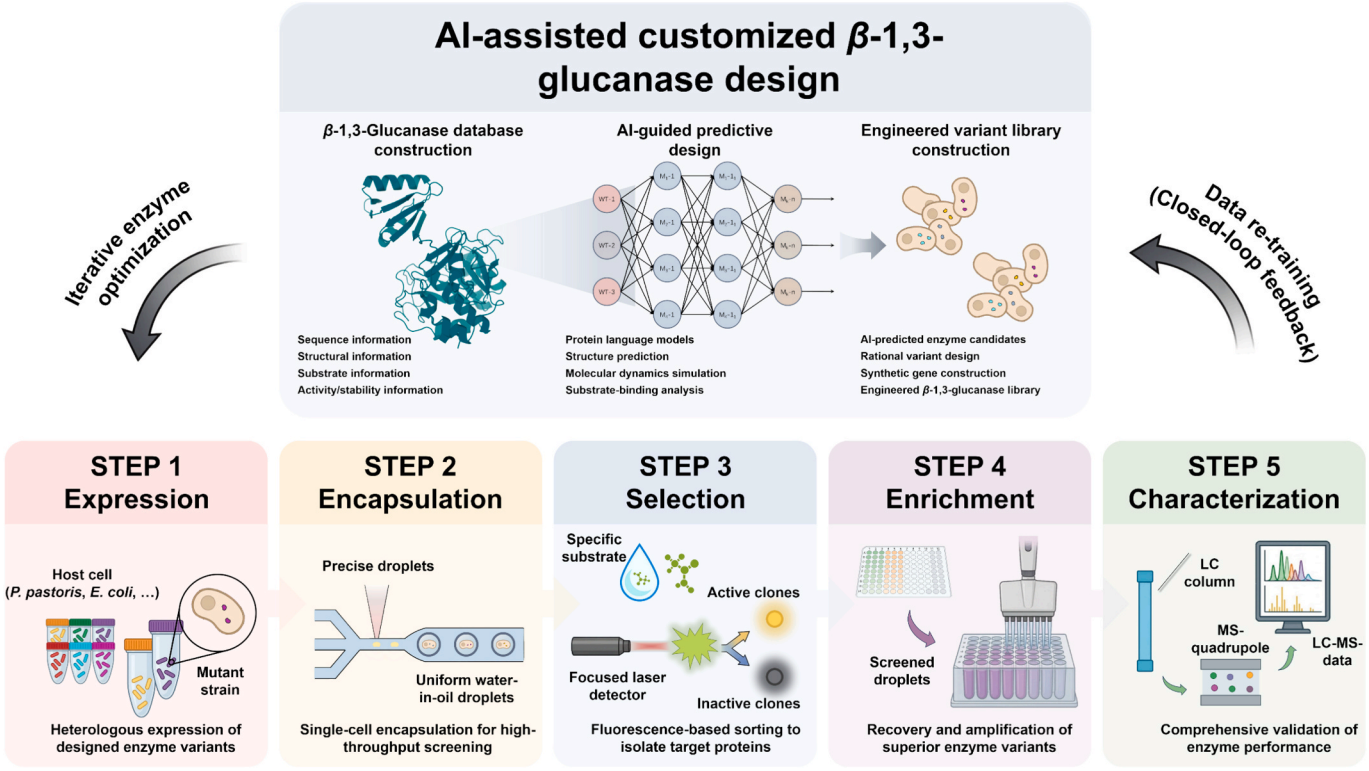


Fig. 4. AI-assisted predictive design and optimization framework for customized β-1,3-glucanases. The workflow integrates multidimensional β-1,3-glucanase databases containing sequence, structural, substrate, activity, and stability information with AI-guided predictive models for enzyme optimization. AI-based approaches enable the identification of candidate variants through sequence analysis, structural prediction, molecular dynamics simulation, and substrate-binding evaluation, followed by rational variant design and engineered enzyme library construction. The resulting variants are evaluated through heterologous expression, high-throughput activity screening, and comprehensive characterization, including kinetic analysis, LC-MS based product profiling, and functional validation. This framework provides a future strategy for developing β-1,3-glucanases with enhanced thermostability, improved activity toward insoluble or highly branched substrates, and controlled product specificity.

features, substrate specificity, and stability-related parameters, AI-guided models combined with high-throughput screening and experimental validation may accelerate the development of β-1,3-glucanases with tailored catalytic properties (Fig. 4). Furthermore, controlling product specificity represents another major challenge, as hydrolysis outcomes are determined by the dynamic interactions among substrate architecture, active-site topology, and enzyme conformational flexibility (Yang et al., 2020; Jia et al., 2021). These advances may ultimately enable the design of enzymes with improved activity toward insoluble or highly branched substrates, controlled production of specific β-glucooligosaccharides, and novel substrate preferences.

High-throughput functional screening will be an essential component of this AI-driven optimization cycle. Fluorescent substrates, such as 4-methylumbelliferyl β-1,3-linked glucooligosaccharides, have provided valuable tools for β-1,3-glucanase activity detection. In addition, droplet-based microfluidic platforms have enabled ultrahigh-throughput screening of carbohydrate-active enzymes by coupling enzymatic reactions with fluorescence or absorbance-based signals (Huang, 2009; Ladeveze et al., 2023; Samlali et al., 2022). Nevertheless, most existing screening systems rely on soluble substrates or indirect signal conversion, whereas industrially relevant substrates such as curdlan and fungal cell-wall glucans are insoluble and structurally heterogeneous. Therefore, developing reliable fluorescence-based screening strategies that directly reflect activity toward insoluble or highly ordered β-1,3-glucans remains a major challenge. Integration of AI prediction, engineered variant libraries, advanced screening platforms, and functional validation will be critical for accelerating the development of next-generation β-1,3-glucanases for industrial bioconversion.

8. Conclusion

β-1,3-Glucanases constitute a structurally diverse and functionally versatile group of GH enzymes with broad biotechnological potential. Systematic analysis of their molecular characteristics and family distribution reveals that distinct structural folds and active site configurations directly determine their substrate recognition patterns, catalytic mechanisms, and environmental adaptability (Zhao et al., 2013). In recent years, continuous discovery of novel β-1,3-glucanases has significantly expanded the functional boundaries of this enzyme class. Moreover, integrated mining strategies combining sequence-based analysis with phenotype-based functional screening have provided effective approaches for identifying atypical family members and previously uncharacterized enzymes.

In terms of engineering, researchers have achieved synergistic improvements in both the stability and catalytic efficiency of β-1,3-glucanases, demonstrating that (semi-)rational design is an effective approach to overcoming the performance limitations of natural enzymes. Meanwhile, at the expression and production levels, progress in host system selection, codon optimization, promoter and signal peptide engineering, and fermentation conditions optimization has steadily improved the yield of β-1,3-glucanase and driven it from laboratory-scale production to industrial-scale manufacturing (Thodore and Panda, 1994; de la Cruz et al., 1995). At the application level, β-1,3-glucanases have demonstrated clear value in multiple fields, including plant protection, fermentation processing, biomass valorization, and environmental treatment (Rajninec et al., 2021; Monreal et al., 1967; Rodriguez-Pena et al., 2013).

Despite these advances, important challenges remain. The molecular mechanisms underlying substrate recognition, branch tolerance, CBM-mediated interactions, and product specificity remain incompletely understood, limiting rational β-1,3-glucanase design (Calloni et al., 2023; Yang et al., 2020; Ma et al., 2024; Jia et al., 2021). Future engineering should move beyond single-parameter optimization toward multi-objective strategies integrating catalytic efficiency, substrate accessibility, product distribution, and stability (Angelov et al., 2017; Ramada et al., 2010; Liu et al., 2026; Huang, 2009; Ladeveze et al., 2023; Samlali et al., 2022; Feng et al., 2021). In particular, combining AI-assisted predictive design with structural analysis, molecular dynamics, high-throughput screening, and experimental feedback will accelerate the development of customized β-1,3-glucanases with improved activity toward insoluble or highly branched substrates and controlled product profiles.

CRediT authorship contribution statement

Ke Fang: Writing – original draft, Formal analysis, Conceptualization. **Liangju Yuan:** Writing – review & editing, Writing – original draft, Project administration. **Yujing Cui:** Validation, Data curation. **Xianliang Zheng:** Resources, Project administration, Funding acquisition. **Ruishan Jia:** Project administration, Funding acquisition, Formal analysis. **Kun Liu:** Writing – review & editing, Supervision, Funding acquisition. **Shengying Li:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Ethics declaration

The author(s) declare(s) that the study does not involve humans nor animal subjects.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2026.135884.

Data availability

The sequences used for phylogenetic analysis and structural comparison, along with their corresponding GenBank accession numbers, are listed in the supplementary information. The structural models are from the PDB and are publicly available from the relevant database.

References

Caseiro, C., Dias, J.N.R., de Andrade Fontes, C.M.G., Bule, P., 2022. From cancer therapy to winemaking: the molecular structure and applications of β-glucans and β-1,3-glucanases. Int. J. Mol. Sci. 23 (6), 3156.

Zhang, Y.S., Zhang, Y.Q., Zhao, X.M., Liu, X.L., Qin, Q.L., Liu, N.H., Xu, F., Chen, X.L., Zhang, Y.Z., Li, P.Y., 2024. Metagenomic insights into the dynamic degradation of brown algal polysaccharides by kelp-associated microbiota. Appl. Environ. Microbiol. 90 (2), e0202523.

O'Sullivan, L., Murphy, B., McLoughlin, P., Duggan, P., Lawlor, P.G., Hughes, H., Gardiner, G.E., 2010. Prebiotics from marine macroalgae for human and animal health applications. Mar. Drugs 8 (7), 2038–2064.

Shree, A., Pal, S., Verma, P.K., 2024. Structural diversification of fungal cell wall in response to the stress signaling and remodeling during fungal pathogenesis. Physiol. Mol. Biol. Plants 30 (5), 733–747.

Ruiz-Herrera, J., Ortiz-Castellanos, L., 2019. Cell wall glucans of fungi. A review. Cell Surf. 5, 100022.

German, L., Yeshvekar, R., Benitez-Alfonso, Y., 2023. Callose metabolism and the regulation of cell walls and plasmodesmata during plant mutualistic and pathogenic interactions. Plant Cell Environ. 46 (2), 391–404.
Cheng, Y.M., Hong, T.Y., Liu, C.C., Meng, M., 2009. Cloning and functional characterization of a complex endo-β-1,3-glucanase from *Paenibacillus* sp. Appl. Microbiol. Biotechnol. 81 (6), 1051–1061.
Cheng, R., Chen, J., Yu, X., Wang, Y., Wang, S., Zhang, J., 2013. Recombinant production and characterization of full-length and truncated β-1,3-glucanase PglA from *Paenibacillus* sp. S09. BMC Biotech. 13, 105.
Plakys, G., Gasparavičiūtė, R., Vaitekūnas, J., Rutkienė, R., Meškys, R., 2022. Characterization of *Paenibacillus* sp. GKG endo-β-1,3-glucanase, a member of family 81 glycoside hydrolases. Microorganisms 10 (10), 1930.
El-Shora, H.M., El-Sharkawy, R.M., Khateb, A.M., Darwish, D.B., 2021. Production and immobilization of β-glucanase from Aspergillus niger with its applications in bioethanol production and biocontrol of phytopathogenic fungi. Sci. Rep. 11 (1), 21000.
Wang, N., Lin, Q., Wang, Z., Shi, H., Gao, Y., Zen, J., Lou, K., Huo, X., 2025. Multi-omics analysis of *Trichoderma reesei* mutant with high glucanase activity. Sci. Rep. 15 (1), 25184.
Osuki, K. i.; Hashimoto, S.; Suzuki, A.; Araragi, M.; Takahara, A.; Kurosawa, M.; Kucho, K. i.; Higashi, S.; Abe, M.; Uchiumi, T., Gene expression and localization of a β-1,3-glucanase of Lotus japonicus. J. Plant Res. 2016, 129 (4), 749-758.
Thimmapuram, J., Ko, T.S., Korban, S.S., 2001. Characterization and expression of β-1,3-glucanase genes in peach. Mol. Genet. Genomics 265 (3), 469–479.
Alderkamp, A.C., Van Rijssel, M., Bolhuis, H., 2007. Characterization of marine bacteria and the activity of their enzyme systems involved in degradation of the algal storage glucan laminarin. FEMS Microbiol. Ecol. 59 (1), 108–117.
Hehemann, J.H., Correc, G., Barbeyron, T., Helbert, W., Czjzek, M., Michel, G., 2010. Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. Nature 464 (7290), 908–912.
Labourel, A., Jam, M., Jeudy, A., Hehemann, J.H., Czjzek, M., Michel, G., 2014. The β-Glucanase ZgLamA from *Zobellia galactanivorans* evolved a bent active site adapted for efficient degradation of algal laminarin. J. Biol. Chem. 289 (4), 2027–2042.
Unfried, F., Becker, S., Robb, C.S., Hehemann, J.H., Markert, S., Heiden, S.E., Hinzke, T., Becher, D., Reintjes, G., Krüger, K., Avcı, B., Kappelmann, L., Hahnke, R.L., Fischer, T., Harder, J., Teeling, H., Fuchs, B., Barbeyron, T., Amann, R.I., Schweder, T., 2018. Adaptive mechanisms that provide competitive advantages to marine bacteroidetes during microalgal blooms. ISME J. 12 (12), 2894–2906.
Bäumgen, M., Dutschei, T., Bornscheuer, U.T., 2021. Marine polysaccharides: Occurrence, enzymatic degradation and utilization. Chembiochem 22 (13), 2247–2256.
Umemoto, N., Kakitani, M., Iwamatsu, A., Yoshikawa, M., Yamaoka, N., Ishida, I., 1997. The structure and function of a soybean β-glucan-elicitor-binding protein. P. Natl. Acad. Sci. USA 94 (3), 1029–1034.
Huang, G.L., Mei, X.Y., Liu, M.X., Liu, T.C., 2004. Synthesis, (1→3)-β-D-glucanase-binding ability and phytoalexin-elicitor activity of (R)-2,3-epoxypropyl (1→3)-β-D-pentaglucoside. Bioorg. Med. Chem. Lett. 14 (24), 6027–6029.
Perrot, T., Pauly, M., Ramírez, V., 2022. Emerging roles of β-glucanases in plant development and adaptative responses. Plants 11 (9), 1119.
Liu, H., Lu, X., Li, M., Lun, Z., Yan, X., Yin, C., Yuan, G., Wang, X., Liu, N., Liu, D., Wu, M., Luo, Z., Zhang, Y., Bhadauria, V., Yang, J., Talbot, N.J., Peng, Y.-L., 2023. Plant immunity suppression by an exo-β-1,3-glucanase and an elongation factor 1α of the rice blast fungus. Nat. Commun. 14 (1), 5491.
Orozco-Mosqueda, M.d.C., Fadiji, A.E., Babalola, O.O., Santoyo, G., Bacterial elicitors of the plant immune system: An overview and the way forward. Plant Stress 2023, 7, 100138.
Zhao, Z., Liu, H., Wang, C., Xu, J.R., 2013. Comparative analysis of fungal genomes reveals different plant cell wall degrading capacity in fungi. BMC Genomics 14, 274.
Lombard, V., Golaconda Ramulu, H., Drula, E., Coutinho, P.M., Henrissat, B., 2014. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res. 42 (1), 490–495.
Maris, K.; Purkan.; Sri, S.; Afaf, B., A novel 1,3-B-glucanase gene from the metagenomic expression library of Achatina fulica's digestive gland. Iran. J. Pharm. Res. 2020, 19 (3), 483-493.
Zhang, Z., Yu, S., Gu, J., Tan, F., Wan, S., Gao, H., Qin, Z., 2024. Biochemical characterization of a β-1,3-glucanase from *Bacteroidetes* sp. having transglycosylase activity suitable to synthesize β-glucooligosaccharides. J. Agric. Food Chem. 72 (49), 27294–27303.
Salazar, O., Molitor, J., Lienqueo, M.E., Asenjo, J.A., 2001. Overproduction, purification, and characterization of β-1,3-glucanase type II in *Escherichia coli*. Protein Expr. Purif. 23 (2), 219–225.
Jiang, Y., Chang, Z.P., Xu, Y., Zhan, X.B., Wang, Y.Y., Gao, M.J., 2024. Advances in molecular enzymology of β-1,3-glucanases: a comprehensive review. Int. J. Biol. Macromol. 279 (3), 135349.
Roncero, C., Vázquez de Aldana, C.R., Glucanases and Chitinases. Curr. Top. Microbiol. 2019, 425, 131-166.
Fontaine, T., Hartland, R.P., Beauvais, A., Diaquin, M., Latge, J.P., 1997. Purification and characterization of an endo-1,3-beta-glucanase from Aspergillus fumigatus. Eur. J. Biochem. 243 (1–2), 315–321.
Fibriansah, G., Masuda, S., Koizumi, N., Nakamura, S., Kumasaka, T., 2007. The 1.3 a crystal structure of a novel endo-beta-1,3-glucanase of glycoside hydrolase family 16 from alkaliphilic *Nocardiopsis* sp. strain F96. Proteins 69 (3), 683–690.
Fontaine, T., Hartland, R.P., Diaquin, M., Simenel, C., Latge, J.P., 1997. Differential patterns of activity displayed by two exo-beta-1,3-glucanases associated with the Aspergillus fumigatus cell wall. J. Bacteriol. 179 (10), 3154–3163.
Davies, G., Henrissat, B., 1995. Structures and mechanisms of glycosyl hydrolases. Structure 3 (9), 853–859.
Qin, Z., Yan, Q., Lei, J., Yang, S., Jiang, Z., Wu, S., 2015. The first crystal structure of a glycoside hydrolase family 17 β-1,3-glucanosyltransferase displays a unique catalytic cleft. Acta Crystallogr. D 71 (8), 1714–1724.
Krishnan, B., Srivastava, S.S., Sankeshi, V., Garg, R., Srivastava, S., Sankaranarayanan, R., Sharma, Y., 2019. $\beta\gamma$-Crystallination endows a novel bacterial glycoside hydrolase 64 with Ca($^{2+}$)-dependent activity modulation. J. Bacteriol. 201 (23), e00392–e00419.
Ferrer, P., 2006. Revisiting the *Cellulosimicrobium cellulans* yeast-lytic beta-1,3-glucanases toolbox: a review. Microb. Cell Fact. 5, 10.
Calloni, R.D., Muchut, R.J., Garay, A.S., Arias, D.G., Iglesias, A.A., Guerrero, S.A., 2023. Functional and structural characterization of an endo-β-1,3-glucanase from *Euglena gracilis*. Biochimie 208, 117–128.
Yang, J., Xu, Y., Miyakawa, T., Long, L., Tanokura, M., Zhou, N.-Y., 2020. Molecular basis for substrate recognition and catalysis by a marine bacterial laminarinase. Appl. Environ. Microbiol. 86 (23), e01796–e01820.
Ma, J., Jiang, Z., Yan, Q., Lv, A., Li, Y., Yang, S., 2024. Structural and functional analysis of SpGlu64A: a novel glycoside hydrolase family 64 laminaripentaose-producing β-1,3-glucanase from *Streptomyces pratensis*. FEBS J. 291 (9), 2009–2022.
Jia, X., Wang, C., Du, X., Peng, H., Liu, L., Xiao, Y., He, C., 2021. Specific hydrolysis of curdlan with a novel glycoside hydrolase family 128 β-1,3-endoglucanase containing a carbohydrate-binding module. Carbohydr. Polym. 253, 117276.
Wei, Y., Luo, S., Wei, X., Chen, Z., Dong, L., Zhu, L., Zhang, D., Gao, H., Qin, Z., 2025. Mutational analysis and application of a multidomain GH157 family endo-β-1,3-glucanase from *Bacteroides* sp. M27. J. Agric. Food Chem. 73 (28), 17855–17866.
Tamashiro, T., Tanabe, Y., Ikura, T., Ito, N., Oda, M., 2012. Critical roles of Asp270 and Trp273 in the α-repeat of the carbohydrate-binding module of endo-1,3-β-glucanase for laminarin-binding avidity. Glycoconj. J. 29, 77–85.
Qin, Z., Yu, S., Zhang, K., Wei, X., Li, J., Zhang, Z., Wan, S., Gao, H., 2023. Characterization of a glycoside hydrolase family 157 endo-β-1,3-glucanase that displays antifungal activity against phytopathogens. J. Agric. Food Chem. 71 (27), 10383–10392.
Kim, D.Y., Lee, Y.M., Lee, J.S., Chung, C.W., Son, K.H., 2024. Novel, cold-adapted D-laminaribiose- and D-glucose-releasing GH16 endo-β-1,3-glucanase from *Hymenobacter siberiensis* PAMC 29290, a psychrotolerant bacterium from Arctic marine sediment. Front. Microbiol. 15, 1470106.
Burkhardt, C., Schäfers, C., Claren, J., Schirrmacher, G., Antranikian, G., 2019. Comparative analysis and biochemical characterization of two endo-β-1,3-glucanases from the thermophilic bacterium *Fervidobacterium* sp. Catalysts 9 (10), 830.
Zhang, X., Zheng, P., Wen, X., Bei, Z., 2024. Comprehensive analysis of β-1,3-glucanase genes in wolfberry and their implications in pollen development. Plants (basel) 14 (1), 52.
Viborg, A.H., Terrapon, N., Lombard, V., Michel, G., Czjzek, M., Henrissat, B., Brumer, H., 2019. A subfamily roadmap of the evolutionarily diverse glycoside hydrolase family 16 (GH16). J. Biol. Chem. 294 (44), 15973–15986.
Angelov, A., Pham, V.T.T., Ubelacker, M., Brady, S., Leis, B., Pill, N., Brolle, J., Mechelke, M., Moerch, M., Henrissat, B., Liebl, W., 2017. A metagenome-derived thermostable β-glucanase with an unusual module architecture which defines the new glycoside hydrolase family GH148. Sci. Rep. 7 (1), 17306.
Ramada, M.H., Lopes, F.A., Ulhoa, C.J., Silva Rdo, N., 2010. Optimized microplate beta-1,3-glucanase assay system for Trichoderma sp. screening. J. Microbiol. Methods 81 (1), 6–10.
Liu, R.M., Li, S.M., Zhang, H.T., Qiu, J., Liu, Y.H., Liu, H.J., Li, J.M., Zheng, P., 2026. AI-guided thermal stabilization of endo-β-1,3-glucanase. Chinese Chem. Lett. 112729.
Huang, G., 2009. Synthesis and stability assay of 4-methylumbelliferyl (1→3)-beta-D-pentaglucoside. J. Enzym. Inhib. Med. Ch. 24 (2), 453–456.
Ladeveze, S., Zurek, P.J., Kaminski, T.S., Emond, S., Hollfelder, F., 2023. Versatile product detection via coupled assays for ultrahigh-throughput screening of carbohydrate-active enzymes in microfluidic droplets. ACS Catal. 13 (15), 10232–10243.
Samlali, K., Alves, C.L., Jezernik, M., Shih, S.C.C., 2022. Droplet digital microfluidic system for screening filamentous fungi based on enzymatic activity. Microsyst. Nanoeng. 8, 123.
Feng, J., Xu, S., Feng, R., Kovalevsky, A., Zhang, X., Liu, D., Wan, Q., 2021. Identification and structural analysis of a thermophilic β-1,3-glucanase from compost. Bioresour. Bioprocess. 8 (1), 102.
Qin, H.M., Miyakawa, T., Inoue, A., Nakamura, A., Nishiyama, R., Ojima, T., Tanokura, M., 2017. Laminarinase from *Flavobacterium* sp. reveals the structural basis of thermostability and substrate specificity. Sci. Rep. 7 (1), 11425.
Zhang, Y., Zhang, T., Miao, M., 2024. Semi-rational design in simultaneous improvement of thermostability and activity of β-1,3-glucanase from *Alkalihalobacillus clausii* KSMK16. Int. J. Biol. Macromol. 283 (2), 137779.
Yu, X., Hu, Y., Li, Q., Lv, Y., Tang, H., Wen, L., Cheng, Y., Chen, Z., Zhang, T., Wu, H., 2025. Overview of various protein engineering strategies to improve the catalytic activity, thermostability, and acid/base stability of beta-glucanase. Int. J. Biol. Macromol. 308 (3), 142685.
Chiaraluce, R., Gianese, G., Angelaccio, S., Florio, R., van Lieshout, J.F., van der Oost, J., Consalvi, V., 2005. Calcium-induced tertiary structure modifications of endo-beta-1,3-glucanase from *Pyrococcus furiosus* in 7.9 M guanidinium chloride. Biochem. J 386 (3), 515–524.
Gao, F., Liu, X., Dong, R., Liao, M., Qin, X., Wang, X., Zhang, W., Huang, H., Luo, H., Yao, B., Wang, Y., Tu, T., 2023. Exploring the antifungal mechanism of β-1,3-glucanase for effectively inhibiting the food contamination by Aspergillus flavus and Aspergillus fumigatus. LWT 187, 115342.

Koizumi, N., Masuda, S., Maeda, K., Isoda, Y., Yatsunami, R., Fukui, T., Nakamura, S., 2009. Additional carbohydrate-binding modules enhance the insoluble substrate-hydrolytic activity of beta-1,3-glucanase from alkaliphilic *Nocardiopsis* sp. F96. Biosci. Biotech. Bioch. 73 (5), 1078–1082.

Bamforth, C.W., 1980. The adaptability, purification and properties of exo-beta 1,3-glucanase from the fungus *Trichoderma reesei*. Biochem. J 191 (3), 863–866.

Budiarti, S.W., Widyastuti, S.M., Margino, S., 2009. Purification and characterization of à-1,3-glucanase from the antagonistic fungus *Trichoderma reesei*. HAYATI J. Biosci. 16 (3), 115–119.

Thodore, K., Panda, T., 1994. Production of *β*-1,3-glucanase from *Trichoderma harzianum* in surface and submerged culture processes and its role on protoplast generation from *Trichoderma reesei* mycelium. Bioprocess Eng. 10 (4), 161–166.

de la Cruz, J., Pintor-Toro, J.A., Benitez, T., Llobell, A., Romero, L.C., 1995. A novel endo-beta-1,3-glucanase, BGN13.1, involved in the mycoparasitism of *Trichoderma harzianum*. J. Bacteriol. 177 (23), 6937–6945.

Hong, T.Y., Meng, M., 2003. Biochemical characterization and antifungal activity of an endo-1,3-beta-glucanase of *Paenibacillus* sp. isolated from garden soil. Appl. Microbiol. Biotechnol. 61 (5–6), 472–478.

Shi, P., Yao, G., Yang, P., Li, N., Luo, H., Bai, Y., Wang, Y., Yao, B., 2010. Cloning, characterization, and antifungal activity of an endo-1,3-beta-D-glucanase from *Streptomyces* sp. S27. Appl. Microbiol. Biotechnol. 85 (5), 1483–1490.

Wang, Y., Li, D., Dong, C., Zhao, Y., Zhang, L., Yang, F., Ye, X., Huang, Y., Li, Z., Cui, Z., 2021. Heterologous expression and characterization of a novel glycoside hydrolase family 55 *β*-1,3-glucanase, AcGluA, from *Archangium* sp. strain AC19. Appl. Microbiol. Biotechnol. 105 (18), 6793–6803.

Li, J., Cao, C., Jiang, Y., Huang, Q., Shen, Y., Ni, J., 2020. A novel digestive GH16 *β*-1,3 (4)-glucanase from the fungus-growing termite *Macrotermes barneyi*. Appl. Biochem. Biotechnol. 192 (4), 1284–1297.

Zhang, S.B., Zhang, W.J., Zhai, H.C., Lv, Y.Y., Cai, J.P., Jia, F., Wang, J.S., Hu, Y.S., 2019. Expression of a wheat *β*-1,3-glucanase in *Pichia pastoris* and its inhibitory effect on fungi commonly associated with wheat kernel. Protein Expr. Purif. 154, 134–139.

Xu, Z., Shih, M.C., Poulton, J.E., 2006. An extracellular exo-beta-(1,3)-glucanase from *Pichia pastoris*: Purification, characterization, molecular cloning, and functional expression. Protein Expr. Purif. 47 (1), 118–127.

Gao, M., Yang, G., Li, F., Wang, Z., Hu, X., Jiang, Y., Yan, J., Li, Z., Zhan, X., 2021. Efficient endo-*β*-1,3-glucanase expression in *Pichia pastoris* for co-culture with *Agrobacterium* sp. for direct curdlan oligosaccharide production. Int. J. Biol. Macromol. 182, 1611–1617.

Peng, Y., Liu, G.L., Yu, X.J., Wang, X.H., Jing, L., Chi, Z.M., 2011. Cloning of exo-*β*-1,3-glucanase gene from a marine yeast *Williopsis saturnus* and its overexpression in *Yarrowia lipolytica*. Mar. Biotechnol. (N.Y.) 13 (2), 193–204.

Gao, M.J., Yan, J.J., Zhao, Y., Zhu, L., Yang, G.S., Zhan, X.B., 2021. Expression of a thermostable *β*-1,3-glucanase from *Trichoderma harzianum* in *Pichia pastoris* and use in oligoglucosides hydrolysis. Process Biochem. 107, 74–82.

Boonvitthya, N., Tanapong, P., Kanngan, P., Burapatana, V., Chulalaksananukul, W., 2012. Cloning and expression of the Aspergillus oryzae glucan 1,3-beta-glucosidase a (exgA) in *Pichia pastoris*. Biotechnol. Lett 34 (10), 1937–1943.

Takeda, T., Nakano, Y., Takahashi, M., Konno, N., Sakamoto, Y., Arashida, R., Marukawa, Y., Yoshida, E., Ishikawa, T., Suzuki, K., 2015. Identification and enzymatic characterization of an endo-1,3-*β*-glucanase from *Euglena gracilis*. Phytochemistry 116, 21–27.

Neale, A.D., Wahleithner, J.A., Lund, M., Bonnett, H.T., Kelly, A., Meeks-Wagner, D.R., Peacock, W.J., Dennis, E.S., 1990. Chitinase, beta-1,3-glucanase, osmotin, and extensin are expressed in tobacco explants during flower formation. Plant Cell 2 (7), 673–684.

van Loon, L.C., Bakker, P.A., Pieterse, C.M., 1998. Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 36, 453–483.

Raupach, G.S., Kloepper, J.W., 2000. Biocontrol of cucumber diseases in the field by plant growth-promoting rhizobacteria with and without methyl bromide fumigation. Plant Dis. 84 (10), 1073–1075.

Karthikeyan, M., Radhika, K., Mathiyazhagan, S., Bhaskaran, R., Samiyappan, R., Velazhahan, R., 2006. Induction of phenolics and defense-related enzymes in coconut (*Cocos nucifera* L.) roots treated with biocontrol agents. Braz. J. Plant Physiol. 18 (3), 367–377.

Gu, X., Cao, Z., Li, Z., Yu, H., Liu, W., 2024. Plant immunity suppression by an *β*-1,3-glucanase of the maize anthracnose pathogen *Colletotrichum graminicola*. BMC Plant Biol. 24 (1), 339.

Mohammadizadeh-Heydari, N., Tohidfar, M., Maleki Zanjani, B., Mohsenpour, M., Ghanbari Moheb Seraj, R., Esmaeilzadeh-Salestani, K., 2024. Co-overexpression of chitinase and *β*-1,3-glucanase significantly enhanced the resistance of iranian wheat cultivars to *Fusarium*. BMC Biotech. 24 (1), 35.

Lin, W., Anuratha, C.S., Datta, K., Potrykus, I., Muthukrishnan, S., Datta, S.K., 1995. Genetic engineering of rice for resistance to sheath blight. Nat. Biotechnol. 13 (7), 686–691.

Kavanagh, T.A., Spillane, C., 1995. Strategies for engineering virus resistance in transgenic plants. Euphytica 85 (1–3), 149–158.

Yu, Y., Wang, S., Xu, C., Xiang, L., Huang, W., Zhang, X., Tian, B., Mao, C., Li, T., Wang, S., 2023. The *β*-1,3-glucanase degrades callose at plasmodesmata to facilitate the transport of the ribonucleoprotein complex in Pyrus betulaefolia. Int. J. Mol. Sci. 24 (9), 8051.

Rajninec, M., Fratrikova, M., Boszoradova, E., Jopcik, M., Bauer, M., Libantova, J., 2021. Basic *β*-1,3-glucanase from Drosera binata exhibits antifungal potential in transgenic tobacco plants. Plants (basel) 10 (8), 1747.

Lavanya, S.N., Niranjan-Raj, S., Jadimurthy, R., Sudarsan, S., Srivastava, R., Tarasatyavati, C., Rajashekara, H., Gupta, V.K., Nayaka, S.C., 2022. Immunity elicitors for induced resistance against the downy mildew pathogen in pearl millet. Sci. Rep. 12 (1), 4078.

Monreal, J., De Uruburu, F., Villanueva, J.R., 1967. Lytic action of beta-(1-3)-glucanase on yeast cells. J. Bacteriol. 94 (1), 241–244.

Rodriguez-Pena, J.M., Diez-Muniz, S., Bermejo, C., Nombela, C., Arroyo, J., 2013. Activation of the yeast cell wall integrity MAPK pathway by zymolyase depends on protease and glucanase activities and requires the mucin-like protein Hkr1 but not Msb2. FEBS Lett. 587 (22), 3675–3680.

Scott, J.H., Schekman, R., 1980. Lyticase: endoglucanase and protease activities that act together in yeast cell lysis. J. Bacteriol. 142 (2), 414–423.

Carvalho, V.S.D., Gomez-Delgado, L., Curto, M.A., Moreno, M.B., Perez, P., Ribas, J.C., Cortes, J.C.G., 2021. Analysis and application of a suite of recombinant endo-*β*(1,3)-D-glucanases for studying fungal cell walls. Microb. Cell Fact. 20 (1), 126.

Avramia, I., Amariei, S., 2021. Spent brewer's yeast as a source of insoluble *β*-glucans. Int. J. Mol. Sci. 22 (2), 825.

Tan, Y., Leonhard, M., Ma, S., Moser, D., Schneider-Stickler, B., 2017. Dispersal of single and mixed non-albicans *Candida* species biofilms by *β*-1,3-glucanase in vitro. Microb. Pathog. 113, 342–347.

Tan, Y., Ma, S., Leonhard, M., Moser, D., Schneider-Stickler, B., 2018. *β*-1,3-glucanase disrupts biofilm formation and increases antifungal susceptibility of *Candida albicans* DAY185. Int. J. Biol. Macromol. 108, 942–946.

Wargacki, A.J., Leonard, E., Win, M.N., Regitsky, D.D., Santos, C.N.S., Kim, P.B., Cooper, S.R., Raisner, R.M., Herman, A., Sivitz, A.B., Lakshmanaswamy, A., Kashiyama, Y., Baker, D., Yoshikuni, Y., 2012. An engineered microbial platform for direct biofuel production from brown macroalgae. Science 335 (6066), 308–313.

Enquist-Newman, M., Faust, A.M.E., Bravo, D.D., Santos, C.N.S., Raisner, R.M., Hanel, A., Sarvabhowman, P., Le, C., Regitsky, D.D., Cooper, S.R., Peereboom, L., Clark, A., Martinez, Y., Goldsmith, J., Cho, M.Y., Donohoue, P.D., Luo, L., Lamberson, B., Tamrakar, P., Kim, E.J., Villari, J.L., Gill, A., Tripathi, S.A., Karamchedu, P., Paredes, C.J., Rajgarhia, V., Kotlar, H.K., Bailey, R.B., Miller, D.J., Ohler, N.L., Swimmer, C., Yoshikuni, Y., 2013. Efficient ethanol production from brown macroalgae sugars by a synthetic yeast platform. Nature 505 (7482), 239–243.

Wang, D., Kim, D.H., Kim, K.H., 2016. Effective production of fermentable sugars from brown macroalgae biomass. Appl. Microbiol. Biotechnol. 100 (22), 9439–9450.

Kim, D.H., Kim, D.H., Lee, S.H., Kim, K.H., 2018. A novel *β*-glucosidase from *Saccharophagus degradans* 2-40T for the efficient hydrolysis of laminarin from brown macroalgae. Biotechnol. Biofuels 11, 64.