Hemicellulases from *Penicillium* and *Talaromyces* for lignocellulosic biomass valorization:- A review

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Abstract

The term hemicellulose groups different polysaccharides with heterogeneous structures, mannans, xyloglucans, mixed-linkage β-glucans and xylans, which differ in their backbone and branches, and in the type and distribution of glycosidic linkages. The enzymatic degradation of these complex polymers requires the concerted action of multiple hemicellulases and auxiliary enzymes. Most commercial enzymes are produced by *Trichoderma* and *Aspergillus* species, but recent studies have disclosed *Penicillium* and *Talaromyces* as promising sources of hemicellulases. In this review, we summarize the current knowledge on the hemicellulolytic system of these genera, and the role of hemicellulases in the disruption and synthesis of glycosidic bonds. In both cases, the enzymes from *Penicillium* and *Talaromyces* represent an interesting alternative for valorization of lignocellulosic biomass in the current framework of circular economy.

Keywords: fungi, enzymes, plant polysaccharides, applications

1. Introduction

Monosaccharides are covalently linked to each other or to other molecules through glycosidic bonds. These linkages are among the most stable in nature, but glycosyl hydrolases (GHs) accelerate their breakdown by a factor of 10^{17} compared to the rate of

spontaneous autohydrolysis. That is the reason why these enzymes, also called glycosidases, are considered one of the most efficient biocatalysts available (Rye and Withers, 2000). On the other hand, the huge diversity of natural carbohydrates shows its counterpart in the high number of known GH-type enzymes. According to the Enzyme Commission (EC) number, that classifies enzymes based on the reaction they catalyze, GH activities comprise from EC 3.2.1.1 to EC 3.2.1.212. The enzymes active on carbohydrates are also classified in the CAZy database, organized in families (currently, 168) of structurally related proteins that degrade or create glycosidic bonds (http://www.cazy.org/Glycoside-Hydrolases.html).

The huge variety of GHs allows their use in plenty of industrial applications, and the current market field of glycosidases includes, at least, the paper, food, textile and energy sectors (Polaina and MacCabe, 2007). The most common application for these enzymes during the last decade can be found within the energy industry, specifically to produce second generation (2G) bioethanol as an alternative to fossil fuels and first-generation bioethanol. Lignocellulosic ethanol is obtained by hydrolysis of cellulose and hemicellulose to their corresponding monosaccharides, essentially glucose and xylose, which are then fermented to ethanol (Sweeney and Xu, 2012). Robust and efficient GHs are needed to depolymerize plant cell wall polysaccharides, and most research efforts have been devoted to cellulose deconstruction. However, hemicellulose is now considered a valuable part of plant biomass (Gírio *et al.*, 2010) whose exploitation is mandatory in a circular economy framework (Ubando *et al.*, 2020).

What we know as hemicellulose actually comprises a heterogeneous series of polysaccharides that differ in their backbone, branches, type and distribution of glycosidic linkages (Scheller and Ulvskov, 2010; Ebringerová, 2006). They are usually

classified in four types: i) xylans, branched heteropolysaccharides composed of a β-(1,4)-xylopyranose backbone with different side chains; ii) mannans or galactomannans, partially branched polymers with a β-(1,4)-mannopyranose main chain; iii) xyloglucans, with a cellulose-like backbone of β-(1,4)-glucopyranose and side chains of α-xylopyranose; and iv) mixed-linkage β-glucans, formed by a linear backbone of β-1,3 and β-1,4-glucopyranose units. Xylans are the most abundant hemicellulose in cereals and hardwoods (angiosperms), whereas galactomannan and galactoglucomannan are the main components of softwoods (gymnosperms) and glucomannan predominates in some hardwoods (Polizeli *et al.*, 2005).

Depolymerization of hemicellulose can be achieved either physicochemically or biologically using hemicellulases, but enzymatic approaches are considered more sustainable because they are performed under mild conditions and do not generate toxic byproducts. In any case, methods and conditions should be chosen and optimized based on the configuration selected for the process (Moreno *et al.*, 2015).

The primary function of hemicellulases is to degrade hemicellulose. Although this statement may seem obvious, many hemicellulases catalyze not only the breakage but also the synthesis of glycosidic bonds. Both activities, hydrolysis and synthesis, are simply different outcomes of the catalytic mechanism proposed by Daniel Koshland for retaining glycosidases (Koshland, 1953). According to this classical rationale, glycosidases can be classified as inverting or retaining attending to the anomeric configuration of the reaction product. Inverting glycosidases operate in a single step through two acidic amino acids (Asp or Glu), one of them acting as a general base catalyst (carboxylate anion in the side chain) and the other one as a general acid catalyst (protonated carboxylic acid in the side chain). When water gets into the active site, it is

deprotonated by the carboxylate (basic catalyst), allowing its attack to the anomeric carbon. This attack is assisted by the acid catalyst, which transfers a proton to the released aglycon. In this single mechanism, the initially deprotonated catalyst gets a proton and vice versa while, at the same time, a saccharide product, whose anomeric configuration is opposite to that of the initial substrate, is released.

Retaining enzymes also require the participation of two catalytic carboxyl groups (Asp or Glu), but catalysis occurs through two well separated steps and, because of that, it receives the name of double displacement mechanism. In the first step, carboxylate acts as a nucleophile, attacking the anomeric center of the substrate with the catalytic assistance of the so-called catalytic acid/base residue, which donates a proton to the released aglycon. This step ends with the formation of an enzyme-substrate intermediate that is disrupted in the subsequent phase, in which an external nucleophile, as water, performs a second nucleophilic attack on the anomeric carbon of the substrate. This new attack is also assisted by the second carboxylic residue, now acting as a general base catalyst that receives a proton from the nucleophile to activate it. According to this mechanism, the released hemiacetal keeps the anomeric configuration of the initial carbohydrate (Rempel and Withers, 2008). Interestingly, if the external nucleophile is any compound other than water, a new glycosidic bond is formed linking the carbohydrate from the enzyme-substrate intermediate to the acceptor nucleophile. This process receives the name of transglycosylation (see section 4), and it can be potentially catalyzed by any retaining glycosidase.

Bacteria and fungi are the main producers of hemicellulases in nature. Despite the fact that the use of prokaryotes entails certain advantages over eukaryotic sources, i.e. easier genetic manipulation and scale-up culturing (Terpe, 2006), most bacterial

glycosidases are intracellular or periplasmic. Hence, commercial hemicellulases usually come from filamentous fungi, as they usually enter in the secretion pathway releasing high levels of extracellular proteins (Polizeli *et al.*, 2005). The enzymes of a wide variety of ascomycetes have been extensively applied as core biocatalysts in lignocellulosic biorefineries (Ferreira *et al.*, 2016), in particular species from *Trichoderma,* whose enzymes are used at the industrial level. *Trichoderma reesei,* renamed as *Hypocrea jecorina* (Martinez *et al.*, 2008), is the main source of commercial cellulases and xylanases. However, *Aspergillus* species also produce high levels of endoxylanases and β-xylosidases, as well as thermostable isoforms of these enzymes (de Vries and Visser, 2001), and several species from *Penicillium* and *Talaromyces* have emerged as promising sources of hemicellulases with great biotechnological potential (Chávez *et al.*, 2006). In this review, we analyze the current state of the research related with the hemicellulolytic system of these species, and their associated biotechnological applications.

2. *Penicillium* and *Talaromyces* as producers of hemicellulases

Penicillium and *Talaromyces* are two closely related genera of ascomycetes that comprise more than 350 and 88 fungal species, respectively (Visagie *et al.*, 2014; Yilmaz *et al.*, 2014). These organisms, widely distributed in nature, live usually as saprophytes or plant pathogens, and many of their species have been postulated as excellent enzyme producers for plant biomass applications. Their cellulases were recently reviewed (Vaishnav *et al.*, 2018), but many specimens produce the enzymatic arsenal required to degrade the heterogeneous plant hemicelluloses (Lee *et al.*, 2012; Yoon *et al.*, 2007;Chávez *et al.,* 2006). The secretion of xylanases, feruloyl esterases,β-xylosidases and arabinofuranosidases by several species of *Talaromyces* as a response to different

lignocellulosic inducers has been reported (Goyari *et al.*, 2015, Tuohy *et al.*, 1993). Besides, some strains like *T. emersonii* and *Talaromyces thermophilus* produce thermostable endoxylanases, β-xylosidases and xylanolytic auxiliary enzymes (Ben Romdhane *et al.*, 2010, Guerfali *et al.*, 2009), which is interesting from an industrial perspective. In fact, the Chinese industry uses *P. oxalicum* (renamed as *Penicillium decumbens*) for large-scale production of lignocellulosic biomass-degrading enzymes. The reason is that this species secretes enzymatic preparations with better balance of (hemi-) cellulolytic activities than *T. reesei* (Liu *et al.*, 2013a), since its genome encodes a remarkably higher number of CAZymes for hydrolyzing cellulose and xylans (Liao *et al.*, 2015). However, strains like *Talaromyces cellulolyticus* (previously *Acremonium cellulolyticus*) and *Talaromyces amestolkiae* also secrete a battery of GH, including hemicellulases (de Eugenio *et al.*, 2017; Inoue *et al.*, 2014), and possess more of genes encoding CAZymes than the reported in *T. reesei* or *P. oxalicum* (Table 1), which suggests their wide potential.

However, despite the number and potential of most of the native enzymes from *Penicillium* and *Talaromyces,* they are not usually applied in industry because large-scale processes are yet to be established to produce adequate amounts and combinations of proteins in a short time. The development of massive DNA sequencing and new bioinformatics tools allows mining an increasing number fungal genomes, which makes possible to look for novel xylanases with improved catalytic properties. The heterologous production and protein engineering of suitable enzymes can provide tailor-made biocatalysts to exploit lignocellulosic materials, producing valuable compounds. In this sense, most of the enzymes characterized from *Penicillium* and

Talaromyces species come from new isolates or are putative proteins selected from fungal genomes.

3. Hemicellulose-degrading enzymes

The secretion of different hemicellulose-degrading enzymes is usually dependent on the presence of their corresponding substrate in the medium. The size of the polysaccharides prevents their direct import into the cells, but the corresponding degradation products can access the intracellular environment acting as inducers.

Thus, the most accepted model involves the existence of certain constitutive endoxylanases and mannanases which, despite of being produced at low level, are necessary to start the hydrolysis, since the oligosaccharides released induce the expression of the whole xylanolytic system (Collins *et al.*, 2005).

Table 2 summarizes the occurrence of hemicellulolytic enzymes in *Penicillium* or *Talaromyces* species, according to CAZy database. Their characteristics and catalytic properties are described below.

3.1. Enzymes active on xylan

Xylan, present in dicotyledons and cereal grains, constitutes the second most abundant type of polysaccharide in the biosphere and is the main hemicellulose (Ebringerova *et al.*, 2005; Scheller and Ulvskov, 2010). Structurally, xylan consists of a backbone of β-1,4-linked D-xylopyranosyl units, frequently acetylated and highly branched by short side chains of arabinose or glucuronic acid, depending on the plant source. Attending to the nature of the branches, xylans are usually classified as glucuronoxylans, with residues of α-D-glucuronic or 4-*O*-methyl-D-glucuronic acid, glucuronoarabinoxylans, and arabinoxylans, depending on the proportion of arabinose and uronic acid (Fig. 1A).

Xylan breakdown requires the concerted action of multiple glycosidases and auxiliary enzymes (Fig. 1A). The main catalysts implicated in degradation are endo-β-1,4-xylanases (EC 3.2.1.8) and β-xylosidases (EC 3.2.1.37), usually acting together with α-L-arabinofuranosidases (EC 3.2.1.55), xylan α-1,2-glucuronidases (EC 3.2.1.131), α-glucuronidases (EC 3.2.1.139), acetylxylan esterases (3.1.1.72), feruloyl esterases (EC 3.1.1.73) (Shallom and Shoham, 2003) and reducing-end xylose-releasing exo-oligoxylanases (REX, EC 3.2.1.156) (Honda and Kitaoka, 2004). However, the whole enzyme battery may not be present in all organisms and enzymes may be produced at different levels, being some of them scarce or residual.

3.1.1. Endo-β-1,4-xylanases

Fungal xylanases are produced in the presence of any lignocellulosic residue (Beg *et al*., 2001), but their maximum expression is usually achieved in cultures with xylan or substrates that contain this polysaccharide. The endoxylanase activity is widely distributed among the CAZy families, with representatives in GH families 3, 5, 8, 9, 10, 11, 12, 16, 26, 30, 43, 44, 51, 62, 98, and 141. Only those from families 8, 43 and 98 work according to an inverting mechanism, and the most studied endoxylanases are retaining enzymes belonging to families 10 and 11 (Nordberg Karlsson *et al.*, 2018; Polizeli *et al.*, 2005).

GH10 xylanases are more versatile and efficient than their GH11 counterparts when applied to the hydrolysis of heteroxylans, which has been related to their capacity for hydrolyzing β-1,4 linkages between xylopyranose residues located in the surroundings of xylan substituents, as methyl-glucuronic or acetic acids. Similarly, they are much less affected by the (uncommon) presence of β-1,3 linkages in the main chain (Biely *et al.*, 1997). Currently, the CAZy database includes 358 characterized GH10 enzymes, many of

which are xylanases produced by *Penicillium* or *Talaromyces* strains. GH10 endoxylanases from species like *Penicillium canescens*, *P. oxalicum*, *Penicillium chrysogenum*, or *Penicillium simplicissimum* were reviewed by Chávez *et al.* (2006). Since then, the inventory of GH10 xylanases has grown, including those from *T. cellulolyticus* (Kishishita *et al.*, 2014), *T. funiculosus* (Furniss *et al.*, 2005) or *Talaromyces leycettanus* (Wang *et al.*, 2016), and *Penicillium pinophilum,* which combine thermostability and tolerance to acid pH (Cai et al., 2011).

On the contrary, GH11 endoxylanases are very selective, hydrolyzing exclusively β-1,4 linkages. They are strongly inhibited by the proximity of ramifications (Biely *et al.*, 1997), although they act better than GH10 xylanases on insoluble substrates. Most of the 266 enzymes included in GH11 are endo-β-1,4-xylanases, as i.e. *P. oxalicum* (Yin et al., 2013), *Penicillium citrinum* (Tanaka et al., 2005), *Penicillium sclerotiorum* (Knob and Carmona, 2010), *Penicillium glabrum* (Knob *et al.*, 2013), *T. cellulolyticus* (Watanabe *et al.*, 2014) or *T. amestolkiae* (Nieto-Domínguez *et al.*, 2017b).

The endoxylanases from other families are less studied. Family GH30 groups endo-enzymes specialized in glucuronoxylans' degradation (Urbanikova et al., 2011), with a reported member in *T. celullolyticus* (Nakamichi et al., 2019). Interestingly, despite GH5 being one of the largest categories in CaZy, to date no *Penicillium* or *Talaromyces* endoxylanase is known to belong to it, and the same occurs in families 43 and 51, which contain few examples of characterized endoxylanases.

3.1.2. β-xylosidases

Exo-1,4-β-D-xylosidases, generally known as β-xylosidases, have a central role in hydrolysis of xylan together with endoxylanases, releasing xylose monomers from the non-reducing end of small oligosaccharides. In CAZy, these enzymes are distributed

among the GH families 1, 2, 3, 5, 10, 30, 39, 43, 51, 52, 54, 116 and 120, and all of them, except those included in GH43, are retaining enzymes. The majority of β-xylosidases from *Talaromyces* and *Penicillium* belong to families GH3, GH43, and GH39 (Knob *et al.*, 2010; Kousar *et al.*, 2013), and the most studied and commercially applied are classified in the two first groups. GH3 β-xylosidases have been characterized in *P. sclerotium* (Knob and Carmona, 2010), *T. emersonii* (Rasmussen *et al.*, 2006), *T. cellulolyticus* (Kanna *et al.*, 2011), and *T. amestolkiae* (Nieto-Domínguez *et al.*, 2015), and GH43 in *Penicillium herquei* (Ito *et al.*, 2003), *P. oxalicum* (Ye *et al.*, 2017) and *T. purpurogenum* (Ravanal *et al.*, 2010). However, despite the small number of enzymes characterized in these genera, many genes of GH3 or GH43 β-xylosidases have been annotated, which shows their abundance. At the catalytic level, GH3 and GH43 β-xylosidases from *Talaromyces* and *Penicillium* are outstanding for their high substrate affinity (Liu *et al.*, 2013b; Nieto-Domínguez *et al.*, 2015) and frequently show α-L-arabinofuranosidase side activity (Nieto-Domínguez *et al.*, 2015). Considering these traits, future research should focus on GH3 and GH43 β-xylosidases.

GH39 enzymes with exo-1,4-β-D-xylosidase activity are barely explored, and most of the identified enzymes are from prokaryotic organisms. However, putative GH39 enzymes from *Penicillium rubens*, *T. marneffei*, and *Talaromyces rugulosus* have been annotated, suggesting that they are also represented among these ascomycetes.

3.1.3. Accessory enzymes

The heterogeneous nature of xylans requires the participation of additional activities to achieve full hydrolysis. These enzymes will remove branches and substituents in the different types of xylans, and not all of them are glycosyl hydrolases.

a) α-L-Arabinofuranosidases. These exo-enzymes hydrolyze the linkage between an α-L-arabinofuranosyl substituent and a xylose unit of the backbone (Poria *et al.*, 2020). They act from the non-reducing end of their target linkages, releasing arabinofuranose units. GH families 2, 3, 5, 10, 39, 43, 51, 54, 62 include characterized enzymes with this activity, sharing groups with endoxylanases and β-xylosidases (Juturu and Wu, 2013). The α-L-arabinofuranosidases from ascomycetes are mainly ubicated in families GH43, 51, 54, and 62, and only GH51 and GH54 include retaining enzymes. Family GH51 comprises the largest number of characterized α-L-arabinofuranosidases, with one from *P. chrysogenum,* one from *T. purpurogenus*, and several annotated putative enzymes from both genera are awaiting to be analyzed. Families GH54 and 62 are poorly known. They contain, respectively, 19 and 24 characterized members, with more occurrence of bacterial than fungal α-L-arabinofuranosidases. However, *P. chrysogenum* (Sakamoto and Kawasaki, 2003), *T. purpurogenus* (Ravanal and Eyzaguirre, 2015) and *P. oxalicum* (Hu *et al.*, 2018) produce these types of enzymes. As a general rule, it is considered that members of the GH62 family are less active on synthetic substrates, such as *p*NP-α-L-arabinofuranoside, than those belonging to families 51 or 54. However, a proper assessment of these differences in specificity requires more biocatalysts to be characterized.

b) Xylan α-1,2-glucuronidases and α-glucuronidases. These biocatalysts hydrolyze the glycosidic bond between glucuronic or 4-*O*-methylglucuronic acid and the xylopyranose units of the xylan main chain. The first group hydrolyzes exclusively α-1,2 linkages in xylan whereas α-glucuronidases show a wide substrate range. These enzymes are found in families GH4 (only bacterial enzymes), GH67 and GH115. Family GH67 contains 26 characterized enzymes whose activity is not directed to the whole polymer,

but against glucuronoxylan fragments released by the action of endoxylanases. Their targets are the glucuronic acid substituents attached to the xylopyranose residue of the non-reducing end (Biely *et al.*, 1997). On the contrary, the recently created family GH115 groups a small number of xylan α-1,2-glucuronidases and α-glucuronidases that act directly on the polysaccharide, hydrolyzing 4-*O*-metilglucuronic substituents at any position of the chain (Juturu and Wu, 2014).

The information on the fungal enzymes included in these categories is quite limited. The only GH67 α-glucuronidase characterized in *Penicillium* and *Talaromyces* is produced by *Penicillium aurantiogriseum* and was patented by Novozymes (Hansen *et al.*, 2009). The studies on enzymes from these groups will probably expand in the future, since they are essential to eliminate xylan ramifications, and putative α-glucuronidases have been discovered in *P. rubens*, *T. marneffei*, or *T. rugulosus*.

c) Acetylxylan esterases. These xylanolytic enzymes, that catalyze the hydrolysis of ester linkages between a xylopyranose residue and its acetyl substituent, belong to the carbohydrate esterases (CE) superfamily. Their synergy with endoxylanases and β-xylosidases for xylan hydrolysis was demonstrated by Kosugi *et al.* (2002). In CAZy, they are classified in families CE1, 2, 4, 5, 6, 7 and 12 (Juturu and Wu, 2014), with most fungal acetylxylan esterases included in families CE1 and 5 (Kameshwar and Qin, 2018; Liu *et al.*, 2013a). CE1 acetylxylan esterases are produced by *P. purpurogenum* (Gordillo *et al.*, 2006), *T. emersonii* (Waters *et al.*, 2012) or *P. chrysogenum* (Yang *et al.*, 2017). Regarding CE5 acetylxylan esterases, their production has only been reported for *P. aurantiogriseum* and *T. purpurogenus* (Mardones *et al.*, 2018).

d) Feruloyl esterases hydrolyze ferulic acid residues esterified to certain arabinoses in the side chains of xylan (Oliveira *et al.*, 2019), and are grouped exclusively in family

CE1. Although the classical definition of this group refers only to ferulic acid, feruloyl esterases comprise enzymes able to release other types of phenolic acids. The first feruloyl esterase reported in *Penicillium* or *Talaromyces* was described in *P. funiculosum* (Kroon *et al.*, 2000) but they have been found in other species such as *P. rubens*, *Penicillium brasilianum* or *T. stipitatus* (Dilokpimol *et al.*, 2016).

e) REX are exo-oligoxylanases inactive against polymeric xylan that exert their action from the reducing-end of xylooligosaccharides of at least three residues, releasing xylose and xylobiose as final products (Juturu and Wu, 2014). All characterized REX enzymes belong to family GH8 and have a bacterial origin, and there are currently no genes annotated with this activity in ascomycetes.

The pool of enzymes described above work concerted and synergistically on these complex substrates. Since xylans are highly branched heteropolysaccharides, GH11 endoxylanases find few available targets and release high-molecular mass products, which are substrates for GH10 xylanases. As the hydrolytic process advances, the accessory enzymes reduce the branching of the polymer, facilitating the action of GH11 endoxylanases that can now produce shorter xylooligosaccharides. At this point, REX enzymes can participate in the process, hydrolyzing XOS into xylose and xylobiose, and β-xylosidases complete the hydrolysis by releasing the monosaccharide.

3.2 Mannans

Mannans are also heterogeneous polymers, sometimes strongly associated with other components of plant cell walls, and are the second most abundant hemicellulose (Malgas *et al.*, 2015). Structurally, these polysaccharides consist of a linear backbone of either β-1,4-linked D-mannose or D-mannose and D-glucose in glucomannans (Fig. 1B). The last polymers have mannose to glucose ratio around 3:1 and have high

polymerization degree. Acetylations and galactose side chains can be present, and when galactose content is higher than 5% (w/w) the resulting polymer is known as galactoglucomannan.

Mannan-degrading enzymes (Fig. 1B) include GHs and carbohydrate esterases, being the key enzymes β-mannanases (EC 3.2.1.78), β-mannosidases (EC 3.2.1.25) and β-glucosidases (EC 3.2.1.21). Other auxiliary enzymes acting on branches are α-galactosidases (EC 3.2.1.22) and acetylxylan esterases (EC 3.2.1.72).

3.2.1. β-Mannanases

These enzymes are endo-hydrolases cleaving random or specific bonds between D-mannoses in the polymer backbone, generating new ends. They belong to families GH5, GH26 and GH113, and most fungal mannanases described up to date are classified in subfamily GH5-7. These biocatalysts act on substrates larger than trimers, yielding different oligomers (usually from dimers to tetramers). Their activity on galactogluco- and galactomannans is conditioned by the content and distribution of galactose side chains throughout the polymer since they can´t act near a branching point. GH5 and GH113 mannanases have been determined to catalyze transglycosylation reactions under appropriate conditions. Within the biocatalysts active on plant mannan, β-mannanases are the most studied in *Talaromyces* and *Penicillium*, and several GH5 enzymes of outstanding thermostability have been heterologously produced in *Pichia pastoris* (Liu *et al.*, 2019).

β-Mannosidases

These exo-enzymes release D-mannose or D-glucose from non-reducing ends of products released by mannanases and are crucial to complete mannan hydrolysis. They are grouped in families GH1 and GH2, displaying a retaining reaction mechanism

(Chauhan and Gupta, 2017). Despite fungi (usually *Aspergillii*) are common sources of mannosidases (Kurakake *et al.*, 2006), a single representative of this group is known in *Penicillium* and *Talaromyces*: a GH2 β-mannosidase produced by *T. cellulolyticus* grown using glucomannans as carbon source (Uechi *et al.*, 2020).

β-Glucosidases

The role of these enzymes in mannan hydrolysis is to release D-glucose units from the non-reducing ends of the oligomers produced by the action of β-mannanases. β-glucosidases are well-known cellulolytic enzymes and some *Penicillium* and *Talaromyces* species have been reported as excellent sources of these biocatalysts. However, it remains unknown if the same enzymes involved in cellulose hydrolysis would be active on mannans. The dual capacity of degrading cellulose and mannans has not been studied for most of the analyzed β-glucosidases. Whether or not the same enzymes are responsible for the hydrolysis of mannan and glucose oligomers or are produced under the same stimuli is yet to be ascertained.

3.2.4. Accessory enzymes

As occurred with xylans, several enzymes that act on mannans' branches are required for completing hydrolysis:

a) α-Galactosidases are exo-hydrolases releasing α-1,6-linked terminal galactose molecules from galactooligosaccharides and other substrates (Linares *et al.*, 2020). Fungal α-galactosidases are mostly grouped in family GH27, and show higher substrate specificity than their bacterial counterparts, spread in families GH4, 27 and 36. Notably, several GH27 α-galactosidase isoenzymes with remarkably different substrate specificity have been characterized in *P. subrubescens* and *P. simplicissimum* (Linares *et al.*, 2020;

Luonteri *et al.*, 1998), which can be explained by the release of different galactose-branched mannooligomers by the action of endo-mannanases.

b) Acetylmannan esterases specifically deacetylate mannan oligomers Hitherto, no enzymes with these characteristics have been found in *Penicillium* and *Talaromyces*, although acetylxylan esterases are common in fungal genomes.

3.3. Xyloglucans

The percentage and composition of xyloglucan varies depending on the vegetal source, with the highest representation in dicotyledonous angiosperms. The polysaccharide has a backbone of β-1,4 D-glucose decorated with D-xylose at positions *O*-6. The distribution and the nature of the side chains provides a broad structural diversity, as they can also bear β-L-arabinose or β-D-galactose and α-L-fucose (Fig. 2A). In addition, the xyloglucan backbone may display two main conformations, XXXG or XXGG, where X is a xylosylated glucose and G a non-substituted glucopyranose. Additional side chains of galactose, fucose or arabinose can be found, increasing the structural variability of the polymer (Ebringerova *et al.*, 2005). Due to this heterogeneous nature, a battery of enzymes is necessary to accomplish its full degradation (Fig. 2A). The main catalysts involved are xyloglucan-active endoglucanases (EC 3.2.1.151), α-xylosidases (EC 3.2.1.177) and β-glucosidases (EC 3.2.1.21), meanwhile α-L-fucosidase (EC 3.2.1.51) and α-galactosidase (EC 3.2.1.22) can also act as accessory hydrolases.

3.3.1 Xyloglucan-active endoglucanases

Also known as generic xyloglucanases (Matsuzawa *et al.*, 2016), these enzymes are responsible for cleaving the backbone of xylose-substituted glucoses. True xyloglucan endoglucanases are usually highly specific but, depending on the degree of substitution

of the polymer and the specificity of the enzymes, the main chain could also be attacked by endo-β-1,4-glucanases, usually involved in cellulose metabolism (Sinitsyna *et al.*, 2010). Xyloglucanases belong mostly to GH families 12 and 74 (Rashmi and Siddalingamurthy, 2018), and most putative glycosidases from *Penicillium* and *Talaromyces* active on xyloglucan are included in the first group. GH12 enzymes act on non-branched glucose units, preferably on hexamers and longer oligomers, and have a retaining mechanism. The two xyloglucanases produced by *P. canescens* are among the few studied in depth. One of them is a GH12 specific for xyloglucan, and the other a GH74 that also degrades carboxymethyl cellulose and β-glucan to a lesser extent (Sinitsyna *et al.*, 2010). In addition, a GH12 xyloglucanase from *P. oxalicum* was recombinantly produced in *P. pastoris*, showing similar properties to the GH12 enzyme from *P. canescens* (Xian *et al.*, 2016).

3.3.2 α-Xylosidases

Following in importance, α-xylosidases release xylose units attached to the glucose backbone (Matsuzawa *et al.*, 2020). They belong to family GH31, that contains few characterized enzymes, mostly with α-glucosidase activity (de Vries *et al.*, 2005). In *Penicillium* or *Talaromyces*, an intracellular enzyme from *Penicillium wortmannii* (renamed as *Talaromyces wortmannii*) was reported to act over *p*NP-α-D-xylopyranoside and xyloglucan oligosaccharides (Matsuo *et al.*, 1996), and 36 GH31 enzymes from these genera remain without assigned function in CAZy, representing attractive sources of potential new α-xylosidases.

3.3.3 β-Glucosidases

Since the backbone of xyloglucans is made of glucose, the third type of enzymatic activity involved in their degradation is β-glucosidase. However, even though the role of

these enzymes in cellulose depolymerization is fully established, little is known about their activity on xyloglucans. A particular type of GH3 β-glucosidase, that releases isoprimeverose (D-xylopyranose-(1,6)-D-glucopyranose) from the non-reducing ends of oligoxyloglucans, was described in *A. oryzae* (Matsuzawa *et al.*, 2016). Similar enzymes have been found in many other fungi and bacteria, suggesting that they could be widely distributed, but no information on oligoxyloglucan β-glucosidases from *Penicillium* or *Talaromyces* species has been reported so far.

3.3.4. Accessory enzymes

Debranching of L-fucose and D-galactose residues is carried out by α-fucosidases (EC 3.2.1.51) belonging to families GH29 and GH95 and β-galactosidases (EC 3.2.1.23) from GH2 and GH35 (Sims *et al.*, 1997), although the information on these enzymes is scarce. *Penicillium multicolor* produces an α-fucosidase that showed to be active on the disaccharide α-L-Fuc-(1,2)-D-Gal, which is similar to the linkages in xyloglucan (Ajisaka *et al.*, 1998). However, this enzyme has been used only for transglycosylation, and there are no further studies on its biochemistry, structure, or physiology. The role of β-galactosidases in xyloglucan degradation is only suggested based on the existence of enzymes of broad substrate specificity in *A. niger* (Gamauf *et al.*, 2007) but such versatility has not been reported for the β-galactosidases characterized from *Penicillium* and *Talaromyces* species.

3.4. Mixed-linkage glucans

These linear polysaccharides are found in endospermic cell walls of some cereals associated with cellulose microfibrils, being especially abundant in oat and barley grains (Ebringerova *et al.*, 2005). They are usually composed of short β-1,4-glucose regions (GGG-GGGG) discontinued by β-1,3-linked glucose (Fig. 2B). Lichenan, a particular

mixed-linkage glucan from *Cetraria islandica,* has 82% of 1,3-linked cellotriosyl and cellotetraosyl units and longer cellulose-like (18%) segments. Although structurally similar to cellulose, these glucans are soluble, since the homogeneous distribution of β-1,3 links disturbs the linear orientation of glucose fibrils (Chaari and Chaabouni, 2019).

The main enzymes involved in their depolymerization are the lichenases (EC 3.2.1.73), that cleave β-1,4 bonds next to β-1,3-glucose and are inactive on β-1,4-glucans (Fig. 2B). Microbial β-1,3-1,4-glucanases are classified in family GH16, and *Penicillium* and *Talaromyces* species are good producers of lichenases with interesting properties. GH16 lichenases active only on barley glucan and lichenan have been purified from the thermophilic fungus *T. emersonii* (Murray *et al.*, 2001) and *Penicillium occitanis* (Chaari *et al.*, 2014).

It is worth to note again that the hydrolysis of the different types of cellulosic and hemicellulosic biomass is an interconnected process for saprophytic fungi. This allows, for instance, the repurposing of enzymes described as cellulases (EC 3.2.1.4) or laminarinases (EC 3.2.1.39) for the hydrolysis of mixed-linkage glucans (Fig. 2B). These polysaccharides have been described as very powerful cellulase inducers for *Talaromyces* and *Penicillium* species, which consistently exhibit a high potential for their degradation. Some cellulases display even higher specific activity towards laminarin and barley glucan than on carboxymethyl cellulose, as TlCel5A from *T. leycettanus* (Gu *et al.*, 2019). Most likely, this broad specificity of cellulases may arise from the structural similarity of both polymers, as observed in chitosan-degrading proteins (Xia *et al.*, 2008). Endoglucanases from *T. emersonii* and *T. amestolkiae* may belong to this nonspecific group, since they are active on soluble β-1,4- and β-1,3;1,4-linked glucans and lichenan

(Méndez-Líter *et al.*, 2018; Zhao *et al.*, 2014), and some β-glucosidases could participate in degradation of mixed-linkage oligosaccharides.

3.4. Novel hemicellulases

Over the last decade, novel types of polysaccharide-degrading enzymes have been identified. Some of them, originally considered as hydrolases and included in GH61 or CBM33 families, were later identified as oxidoreductases (Vaaje-Kolstad *et al.*, 2010) and renamed as lytic polysaccharides monooxygenases (LPMOs).

These enzymes are now included in the CAZy database as auxiliary activities (AA), and have been usually related to cellulose deconstruction. However, some LPMOs of families AA9, 10, and 14 have proved to degrade hemicelluloses, like xylan or xyloglucan (Agger *et al.*, 2014). Few of them have been characterized to date, but the number of potential LPMO genes detected in fungal genomes has increased exponentially in recent years and most enzymes are being currently studied.

The vast majority of eukaryotic LPMOs are classified in the AA9 family, counting 596 genes annotated and up to 30 characterized enzymes, according to CAZy database. Since *Penicillium* and *Talaromyces* secrete efficient enzymes for lignocellulose degradation, they are expected to be LPMO producers. Putative genes have been annotated in *P. oxalicum*, *Penicillium parvum*, *P. rubens*, *T. marneffei* or *T. rugulosus* (Gene Bank Data Base), as well as in the genome of *Talaromyces piceae* (He *et al.*, 2017) and *T. amestolkiae* (de Eugenio *et al.*, 2017). However, these enzymes may not be active on hemicellulosic polysaccharides, as occurred with the LPMO from *Penicillium verruculosum* (Semenova *et al.*, 2020).

Family AA14 comprises only two known xylan-degrading LPMOs, isolated from *Pycnoporus coccineus* (Couturier *et al.*, 2018). Other 25 eukaryotic sequences have been

uploaded to the CAZy database, 14 of them from *T. rugulosus* genome. In any case, much remains to be investigated in this group of enzymes.

4. Transglycosylation reactions and protein engineering for synthetic enzymes

As already mentioned, some retaining glycosidases catalyze the production of glycosides by transglycosylation. The synthesis of specific glycosidic bonds is still a technical challenge. On the one hand, chemical methods have low selectivity and require laborious procedures and hazardous reagents. On the other hand, the use of glycosyltransferases, the conventional enzymatic approach, is hampered by their low stability and the high cost of the sugar donors (Danby and Withers, 2016). In this scenario, transglycosylation represents a promising alternative since retaining glycosidases are robust biocatalysts that operate in a wide range of cost-effective substrates. Consequently, a considerable research effort has been focused on the identification of novel retaining glycosidases able to synthesize high yields of valuable glycosides. This quest has encompassed all kingdoms of life, including lignocellulolytic fungi, which are particularly interesting due to the huge potential of lignocellulose as raw material for applications involving transglycosylation.

One of the first reported examples was a β-xylosidase from *P. wortmannii* (Deleyn *et al.*, 1985), that catalyzed the synthesis of alkyl-xylosides using alkan-ols as acceptors. Equivalent reactions have been reported to be catalyzed by the β-xylosidases S1 from *Penicillium herquei* IF0 4674 (Ito *et al.*, 2003) and BxTW1 from *T. amestolkiae* (Nieto-Domínguez *et al.*, 2015). It should be noticed that alkan-ols are considered interesting transglycosylation acceptors for xylanases, since alkyl-xylosides can be used as green surfactants. Interestingly, nature has provided some examples of glycosyl hydrolases with a transglycosylation/hydrolysis balance remarkably displaced to the synthesis of

new glycosidic bonds. These enzymes, exclusively found in plants, are called transglycosylases and comprise a few activities, among which only xyloglucan-endotransglycosylase is considered hemicellulasic. The potential use of these enzymes as models to engineer the transglycosylation/hydrolysis ratio in other glycosidases prompted the identification of transglycosylases. However, the structural particularities underlying their special properties seem subtle and hard to predict, and are poorly understood (Bissaro *et al.*, 2015). Conversely, the approaches based on rational engineering of the catalytic residues of glycosidases to improve their synthetic capacities have proven to be more successful. In this area, professor Stephen Withers has led the field by designing the two main classes of mutant glycosyl hydrolases: glycosynthases (Mackenzie *et al.*, 1998) and thioglycoligases (Jahn *et al.*, 2003). In the case of glycosynthases, the residue responsible for the nucleophilic attack is replaced by a catalytically inert amino acid; hence the mutant is not able to catalyze the first step of the double displacement. Because of this, glycosynthases require the addition of an activated donor, mimicking the enzyme-substrate intermediate. This intermediate is conventionally attacked by the nucleophilic acceptor and by this way a non-hydrolysable product is obtained. Regarding thioglycoligases, they are obtained by replacing the catalytic acid/base. Without its assistance, mutants need a donor with a good leaving group, like dinitrophenyl sugars, to succeed in the formation of the enzyme-substrate intermediate, and then, a strong nucleophile to disrupt it. Thioglycoligases offer an effective method for synthesizing *S*-glycosidic linkages instead of *O*-glycosidic, because of their capacity for transferring a carbohydrate to thiosugars (strong nucleophiles). The obtained *S*-glycosides are inhibitors of wild-type GHs, displaying a wide variety of applications (Hancock *et al.*, 2006).

Despite the increasing number of reported glycosynthases and thioglycoligases (Danby and Withers, 2016), to the best of our knowledge there is only one example of engineering hemicellulases from *Talaromyces* or *Penicillium*, which is the recently published thioglycoligase derived from the β-xylosidase BxTW1 (Nieto-Domínguez *et al.*, 2020). However, this single example has meant a groundbreaking development for the thioglycoligase strategy. The engineered enzyme has expanded remarkably the potential acceptors for these mutants, forming *O*-, *N*-, *S*- and *Se*-glycosides, sugar esters and phosphoesters, with moderate to high yields. Among the plethora of new glycoconjugates attainable with this biocatalyst, the ones derived from flavonoids are particularly attractive, since the pharmacological activities of these polyphenols, may be improved by glycosylation (Nieto-Domínguez *et al.*, 2017a)

5. Biotechnological applications for valorization of lignocellulosic biomass

Considering the variety of substrates for GHs, their catalytic mechanisms, and the great number of reported enzymes, it is not surprising that they are among the most industrially-produced catalysts. Hemicellulases comprise interesting enzymes with relevant applications in the production of 2G bioethanol. Nevertheless, lignocellulosic biomass has a huge potential to meet global demands for raw materials in different industries and is used to obtain prebiotic oligosaccharides (Nieto-Dominguez *et al.*, 2017), chemicals, proteins, biomaterials (Witzler *et al.*, 2018), biohydrogen and biogas (Mulat *et al.*, 2018). Some of the most remarkable applications of hemicellulases produced by *Penicillium* or *Talaromyces* strains will be discussed below.

5.1. Saccharification of lignocellulosic biomass

The enzymatic degradation of hemicelluloses during saccharification of plant biomass facilitates the accessibility of cellulases to their catalytic targets, positively

impacting the total release of simple sugars. For this reason, commercial cellulolytic cocktails like Rapidase Pomaliq (Gist-Brocades), Celluclast 1.5 L (Novozymes), or ClarexML, include also different amounts of xylanases (Hang and Woodams, 2001). Other hemicellulase complexes like Cellic HTec® (Novozymes), Multifect Xylanase® MX (Genencor) or Accellerase®XY (DuPont) have been used together with cellulases for saccharification of several lignocellulosic materials, enhancing the yield of reducing sugars (Ibarra-Díaz et al., 2020; Qing and Wyman, 2011). Cellic HTec2 and Accelerase XY were also tested for degradation of commercial beechwood xylan, producing 74% and 55.8% hydrolysis, respectively (Bibra et al., 2018).

Similarly, different hemicellulases from *Penicillium* and *Talaromyces* species have also been evaluated for these purposes. Many of them are thermostable (Maalej-Achouri *et al.*, 2009) or stable at acidic pHs (Knob and Carmona, 2010; Shibata *et al.*, 2017), which is interesting because substrates can be acid-pretreated.

The use of non-commercial hemicellulases from these genera to improve saccharification of plant residues has been reported for the endoxylanase Xyl10A from *T. cellulolyticus*, applied for enzymatic hydrolysis of pretreated corn stover (Inoue *et al.*, 2014) and the xylanases produced by *Penicillium* sp. SS1 (Bajaj *et al.*, 2011) or *Penicillium* sp. KSM-F532 (Shibata *et al.*, 2017) tested in degradation of sugarcane bagasse, orange peel, wheat bran or rice straw. Furthermore, β-xylosidases are essential in this process, as they complete xylan degradation to xylose. For example, the β-xylosidase from *T. thermophilus* was used with Ultraflo, a cellulolytic cocktail from Novozymes, for vinasse saccharification (Rasmussen *et al.,* 2006), and an alkali-tolerant GH43 β-xylosidase from *P. oxalicum* was reported to promote the full breakage of xylan corn stover (Ye *et al.*, 2017).

For bioconversion of branched hemicellulose, α-L-arabinofuranosidases have a synergistic effect with other xylan-degrading enzymes (Poria *et al.*, 2020). The saccharification yields of alkali-extracted arabinoxylan and exploded rice straw increased by combining xylanases and cellulases with the α-L-arabinofuranosidases from *T. thermophilus* or *Penicillium* sp. LYG 0704 (Lee *et al.*, 2011). The hydrolysis of alkali-pretreated cornstalk and bran with the bifunctional xylanase/cellulase *Tc*Xyn10A and the α-L-arabinofuranosidase from *T. leycettanus* also showed the synergistic effect of these enzymes (Liu *et al.*, 2019).

The acetylxylan esterases break linkages between lignin and hemicellulose, which can be used to pretreat lignocellulose. For example, the bifunctional acetyl xylan esterase/arabinofuranosidase rPcAxe from *P. chrysogenum* was used with endoxylanases to debranch xylan corn stover (Yang *et al.*, 2017).

Broad substrate specificity mannanases are interesting for bioethanol production and other industrial applications (Chauhan and Gupta, 2017), and most commercial cellulolytic cocktails contain low levels of mannanases. In this sense, supplementation of a commercial cocktail with a β-mannanase-rich crude from *Penicillium* sp. improved the saccharification of steam-exploded wood of *Pinus radiata* (Cameron *et al.*, 2015).

Xyloglucan-degrading enzymes are also useful for complete degradation of hemicelluloses (Rashmi and Siddalingamurthy, 2018) and, due to the structural proximity of xyloglucan and cellulose, they have been traditionally applied to pretreat lignocellulosic wastes to improve the accessibility of cellulases (Benko *et al.*, 2008).

5.2 Production of oligosaccharides from hemicelluloses

Prebiotics are defined as ‘‘non-digestible (by the host) food ingredients that have a beneficial effect through their selective metabolism in the intestinal tract” (Gibson *et*

al., 2004). Such effect is usually interpreted in terms of promoting the growth of probiotic microorganisms in the lower gastrointestinal tract, which has been associated to an increasing number of health benefits (Pandey *et al.*, 2015). When referring to hemicelluloses, the main prebiotics produced are xylooligosaccharides (XOS) and mannooligosaccharides (MOS) derived from the use of xylanases and mannanases. *Penicillium* and *Talaromyces* species are attractive producers of these catalysts, and their enzymatic firepower has been used to obtain XOS and MOS.

XOS are soluble oligosaccharides formed by 2-10 xylopyranose units linked by β-1,4 glycosidic bonds, and are produced by xylan degradation using chemical, enzymatic or chemo-enzymatic methods. Consequently, a substantial research effort has been focused on the screening and testing of different xylanases for XOS production and new studies are released every year. Available reports show a variety of approaches including direct use of native endoxylanases, engineered proteins, or combination of different organisms. Moreover, the efficient XynC from *P. funiculosum* was heterologously produced in *A. nidulans* to take advantage of the high secretion levels of this host, using the recombinant xylanase for XOS production (Menezes *et al.*, 2018).

From the perspective of the xylan substrate, corncob is one of the preferred raw materials for XOS production (Driss *et al.*, 2014; Wu *et al.*, 2019), although endoxylanases from *Penicillium* and *Talaromyces* have been successfully applied to others as sugarcane bagasse (Goldbeck *et al.*, 2016) or rice husk (Menezes *et al.*, 2018). It is worth mentioning that a XOS mixture produced from birchwood xylan using XynM from *T. amestolkiae* stimulates the growth of beneficial microbiota and prevents the development of potential pathogenic bacteria (Nieto-Dominguez *et al.*, 2017). Commercial preparations containing fungal xylanases have also been effectively used

for XOS production, as Rapidase Pomaliq (Yoon et al., 2006), and NS22083 and NS22002 from Novozymes (Bhatia et al., 2020).

In the same way, endo-mannanases hydrolyze mannan releasing soluble MOS, mainly composed of 2-10 β-1,4-mannopyranose units. MOS are considered prebiotics associated to growth promotion of *Lactobacilli* and *Bifidobacteria* and to a series of health benefits such as anti-obesity, anti-neoplastic, anti-allergic or immunomodulatory effects (Suryawanshi and Kango, 2021). In this context, several endo-mannanases from *Penicillium* and *Talaromyces* species have been evaluated for production of MOS. *P. occitanis* secretes three β-mannanases, tested to produce MOS from locust bean gum and ivory nut mannan (Blibech *et al.*, 2011), and seems to be a valuable source of endoxylanases, including enzymes with uncommon properties (Driss *et al.*, 2014). Konjac flour was treated with a thermoresistant mannanase from *T. cellulolyticus* for MOS production (Yang *et al.*, 2019), and an alkali-tolerant β-mannanase from *Penicillium freii* F63, expressed in *P. pastoris,* was assayed for degradation of locust bean gum, guar gum and konjac flour (Wang *et al.*, 2012).

Other oligosaccharides are derived from the enzymatic hydrolysis of mixed-linkage glucans, that are often wrongly grouped as β-glucan oligosaccharides. Little is known about these oligosaccharides, however, some studies indicate that they act as prebiotics, stimulating the growth of *Bifidobacterium* and *Lactobacillus* species (Barreteau *et al.*, 2006). In this regard, some endo-β-1,3-1,4-glucanases from *Talaromyces* and *Penicillium* have been used for the production of oligosaccharide mixtures from hydrolysis of lichenan (Driss *et al.*, 2014; Murray *et al.*, 2001).

5.3 Other applications

Hydroxycinnamic acids (ferulic acid and its derivatives) exhibit biological activities relevant to the pharmaceutical industry (Gopalan *et al.*, 2015). Among the vast applications of hemicellulases, the uses given to feruloyl esterases to release hydroxycinnamic acids from lignocellulosic wastes are outstanding. The occurrence of these enzymes in *Penicillium* or *Talaromyces* is much more common than in other groups of xylanases. Three potential feruloyl esterases were identified in *T. stipitatus*, and one of them released ferulic acid from wheat bran and sugar beet pulp (Garcia-Conesa *et al.*, 2004), and the enzyme from *T. cellulolyticus* liberated ferulic and *p*-coumaric acids from hydrothermally-treated rice husk and other bagasses (Watanabe *et al.*, 2015). Similarly, Kroon *et al.* (2000) reported that the feruloyl esterase from *P. funiculosum* released 98% of ferulic acid from wheat bran, working synergistically with a xylanase.

Pulp biobleaching represents the major application of xylanases worldwide. Conventional papermaking processes require the use of a huge quantity of hazardous chemicals. The use of these enzymes facilitates chemical bleaching, increases the lignin extraction from kraft pulps and reduces chlorine compounds, resulting in important environmental benefits (Bajpai, 2004). The commercial xylanase preparation Ecopulp TX-200A from *T. reesei* (AB Enzymes Oy, Finland) (Shatalov and Pereira, 2007) and Pulzyme HC (Novozymes) (Skals et al., 2008) have been successfully used in this process. In the case of enzymes from *Talaromyces* and *Penicillium*, the action of *T. thermophilus* xylanases reduced chlorine consumption by 40% during bleaching of kraft pulp (Maalej-Achouri et al., 2012) and that from *Penicillium crustosum* was very effective in the bleaching of *Eucalyptus* kraft pulp (De Sousa Silva et al., 2016), contributing in both cases to more environmentally-friendly processes. On the other hand, commercial

xylanases cocktails, as Ultraflo L from *Humicola insolens* (Novozymes) have been used for deinking in the recycling paper (Ibarra et al., 2012).

Hemicellulases also have applications in the food industry. Since arabinoxylan is one of the main components of wheat flour, the addition of xylanases could improve the rheological properties of bread like softness, extensibility, and elasticity (Bhardwaj *et al.*, 2019). Driss *et al.* (2014) showed that an endoxylanase produced by *P. occitanis* Pol6 improved the flavor, taste, softness, and overall acceptability of the bread.

On the other hand, lichenases are used in breweries, and their application in valorization of lignocellulose residues has also been explored. For example, a thermostable endo-β-glucanase from *T. emersonii* generated oligosaccharides of different length from barley-β glucan (Murray *et al.*, 2001), and the β-glucanase from *P. pinophilum* (GH16 family) showed the highest specific activity reported towards this same substrate and activity against lichenan and laminarin (Chen *et al.*, 2012).

6. Research needs and perspectives

New and robust hemicellulases are still required for efficient and green exploitation of lignocellulosic biomass, the most abundant renewable resource on Earth. To this end, nature provides a vast portfolio of microbial enzymes, as those secreted by *Penicillium* and *Talaromyces* species,whose full potential still awaits to be unleashed. Recent advances in “omics” sciences, including the accessibility to exponentially growing metagenomic and genomic data or the development of high-throughput proteomics facilitate the identification of novel biocatalysts. However, wild-type enzymes often lack the characteristics that make them applicable in industrial processes, such as thermal and pH stability or tolerance to inhibitors or denaturing factors. In this regard, the advances in protein engineering approaches, especially directed evolution and rational

mutagenesis have boosted the tailored design of novel biocatalysts with improved properties. Despite numerous advances, the cost of enzyme production is still a bottleneck hampering the industrial use of hemicellulases. Protein immobilization techniques could enhance the cost-effectiveness of theses enzymes increasing their catalytic activity and operational stability. In addition, more suitable hosts in terms of recombinant protein yields are being developed by metabolic engineers.

Future trends to widen the spectrum of potential applications of hemicellulases should focus on aspects as the design of cascade reactions and their repurposing for non-natural functions, for exploitation of plant biomass using sustainable bioprocesses.

7. Conclusion

In the last decade, *Penicillium* and *Talaromyces* have shown to be promising sources of hemicellulases. The results summarized in this review highlight the potential of these fungi, not only as known producers of degradative enzymes, but also as niches to search for novel biocatalysts, natural or redesigned, that allow to overcome the challenge of the selective synthesis of glycoside bonds. Despite the scarcity of hemicellulases characterized from these genera, fungal genomes mining facilitates the discovery of novel and robust catalysts, with improved catalytic capacities, for treatment of lignocellulose biomass.

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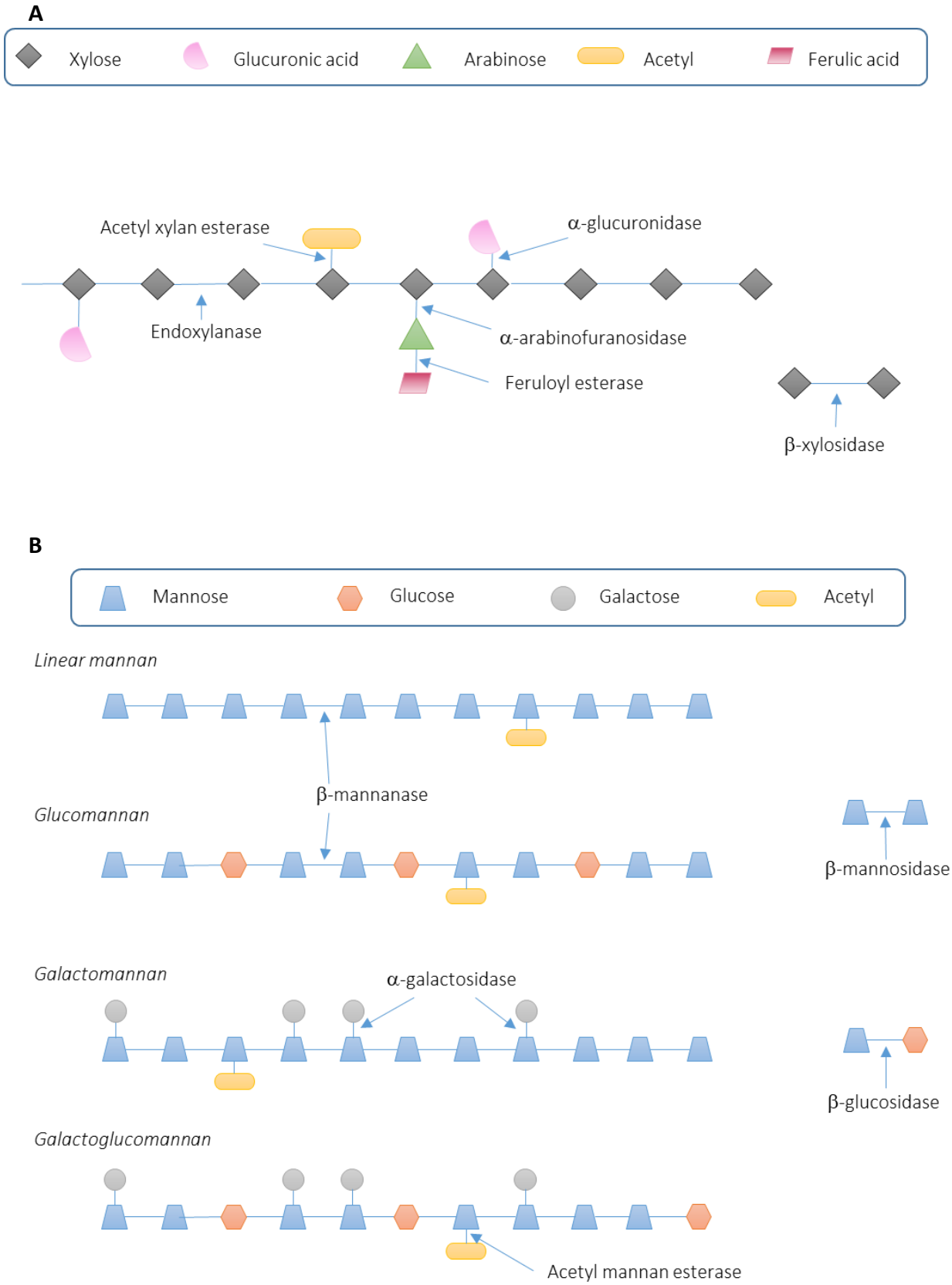
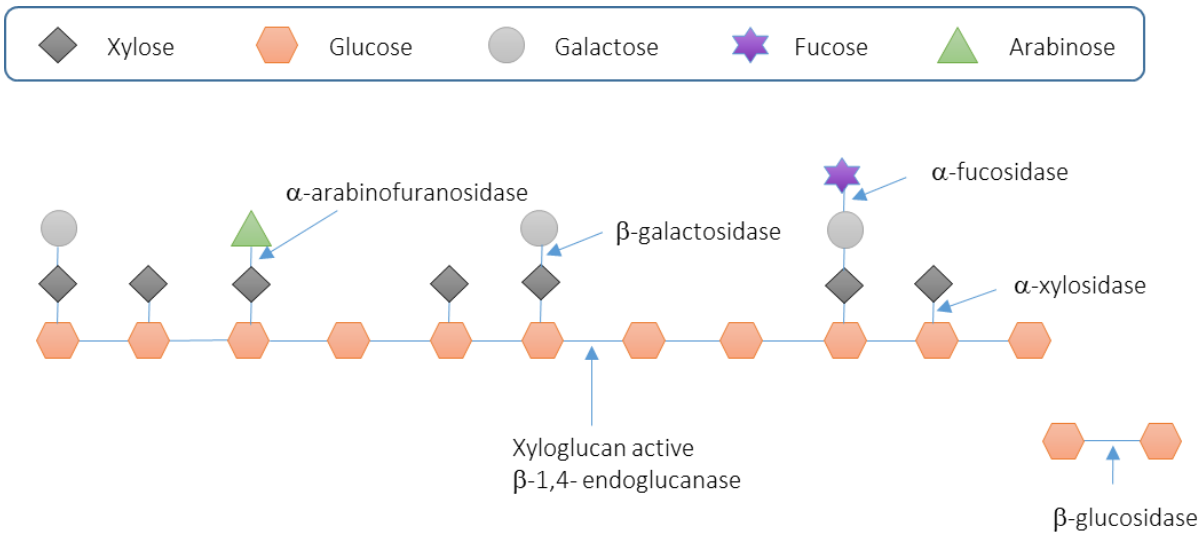


Fig. 1. Basic model and degrading enzymes of xylan (A) and mannan (B). Arrows point at bonds hydrolyzed by the corresponding enzymes.

A



B

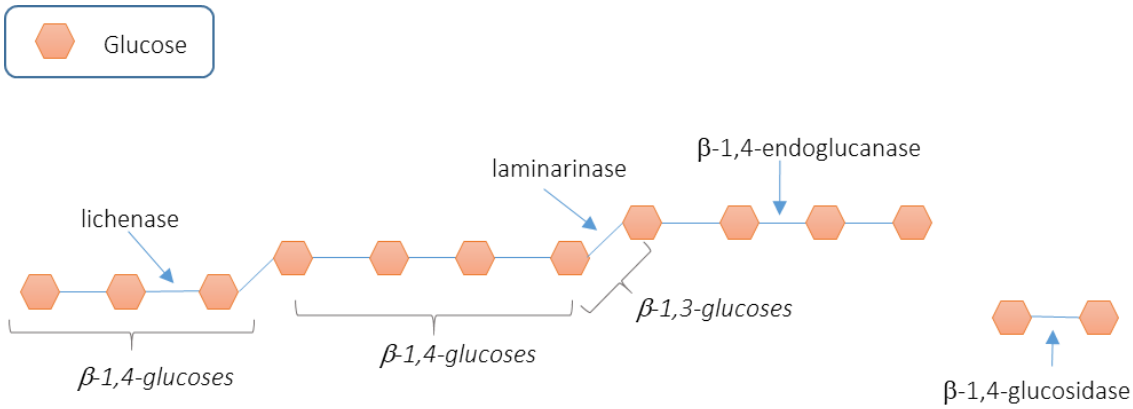


Fig. 2. Basic model and degrading enzymes of xyloglucan (A) and mixed-linkage glucans (B). Arrows point at bonds hydrolyzed by the corresponding enzymes. Curly brackets show β-1,3- and β-1,4-regions in mixed-linkage glucans.

Table 1. Analysis of total CAZymes and the main genes encoding hemicellulolytic enzymes in the genomes of H. jecorina, P. oxalicum, T. cellulolyticus, and T. amestolkiae. The information was obtained from studies performed by Hakkinen et al., 2012, Liu et al., 2013, Fujii et al., 2015, de Eugenio et al., 2017, and complemented with the last additions to the CAZy database.

Activity	**Family**	***Trichoderma reseei***	***Penicillium oxalicum***	***Talaromyces cellulolyticus***	***Talaromyces amestolkiae***
		Encoded enzymes			
Endoxylanase	GH10	1	4	2	1
	GH11	3	5	7	9
	GH30	2	2	3	6
β-Xylosidase	GH3	1	4	1	4
	GH39	1	0	0	2
β-Xylosidase/α-L-arabinofuranosidase	GH43	2	12	22	16
α-L-Arabinofuranosidase	GH54	2	1	1	6
	GH62	1	2	1	3
α-Glucuronidase	GH67	1	2	2	1
	GH115	1	0	0	1
β-mannase, xyloglucannase, lichenase, endoxylanase.	GH5	8	13	12	14
Lichenases	GH16	0	4	0	2
AXE and feruloyl esterases	CE1	0	3	1	1
	CE5	3	1	1	3
Xyloglucanase	GH74	1	0	0	3
LPMO	AA9/AA14	2	2	0	1
TOTAL GENES		**228**	**371**	**249**	**325**

Table 2. Summary of the occurrence of hemicellulolytic enzymes in *Penicillium* or *Talaromyces* species, according to CAZy database.

GH or CE Family	Enzymes characterized		Genes identified		Described activities
	Total	In *Penicillium* or *Talaromyces*	In eukaryotic organisms	In *Penicillium* or *Talaromyces*	
GH 1	344	2	1910	15	β-mannosidase
GH 2	179	2	523	24	β-mannosidase
GH 3	313	10	1752	78	β-xylosidase
GH 5	567	15	2786	88	β-mannanase, xyloglucan-specific endo-β-1,4-glucanase, lichenase, endoxylanase
GH 8	76	0	12	0	Reducing-end xylanase
GH 9	1297	0	170	0	Laminarinase
GH 10	358	11	550	30	Endoxylanase
GH 11	266	18	557	40	Endoxylanase
GH 12	83	2	243	11	Xyloglucan endoglucanase
GH 16	220	3	3749	57	Lichenase, endo-1,3(4)-β-glucanase
GH 26	76	0	51	0	β-mannase
GH 27	70	3	480	16	α-1,4-galactosidase
GH 29	40	0	182	2	α-fucosidase
GH 30	39	3	145	13	Endoxylanase
GH 35	75	5	829	22	β-1,4-galactosidase
GH 36	76	2	334	6	α-1,4-galactosidase
GH 39	21	0	70	4	β-xylosidase
GH 43	183	6	708	48	α-L-arabinofuranosidase, β-xylosidase
GH 51	83	3	244	14	α-L-arabinofuranosidase
GH 54	19	6	73	21	α-L-arabinofuranosidase, β-xylosidase
GH 62	24	4	85	10	α-L-arabinofuranosidase
GH 67	26	1	45	5	α-glucuronidases
GH 74	33	0	31	1	Xyloglucanase
GH 95	11	0	75	2	α-fucosidase
GH 115	8	0	87	0	α-glucuronidases
CE 1	37	3	180	14	Feruloyl esterase, acetylxylan esterase
CE 5	18	2	389	16	Acetylxylan esterase