Fungal Enzymatic Degradation of Cellulose

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Abstract In nature, filamentous fungi are potent degraders of cellulose as they are able to produce a high number and broad variety of cellulases with complementary catalytic activities. These enzymes include notably classical glycoside hydrolase activities, i.e., endoglucanases, cellobiohydrolases, and β-glucosidases. Oxidative enzymes are also involved in cellulose deconstruction, such as the newly discovered lytic polysaccharide monooxygenases (LPMOs), and auxiliary nonenzymatic proteins are involved in substrate targeting and loosening. In this chapter, the actions of the enzymatic partners are described, as well as their kinetics and the interactions between cellulases and with non cellulase enzymes (i.e., synergism). Because recalcitrant cellulose is still a challenge to date, strategies to discover new efficient biocatalysts from fungal biodiversity are also presented here.

Keywords Cellulose · Cellulase · Fungi · Sugar oxidation · Saccharification · Synergies · Auxiliary activities

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1 Introduction

Lignocellulosic biomass is the largest renewable source of carbohydrates on Earth and cellulose is its main component. Cellulose is a homopolymer of β-1,4 linked glucose, organized in linear microfibrils that form very recalcitrant crystalline-like structures. In the plant cell wall, cellulose is tightly intermeshed with the other components, hemicellulose, lignin and pectin, making the whole structure extremely recalcitrant to microbial attack.

In the past decades, the deconstruction of the plant cell wall has become a major challenge for many industrial applications, including production of biofuels, biomaterials, and high value products. In particular, the access to cellulose and its hydrolysis into monomers and oligomers is still a bottleneck that has been mobilizing research efforts.

In nature, microorganisms are potent degraders of lignocellulose which they use as energy source. In particular, filamentous fungi play a key role in recycling nutrients in forest ecosystems. They are extremely well adapted for the degradation of biomass and as such are able to produce a high number and broad variety of enzymes with complementary catalytic activities to degrade cellulose-rich materials (Couturier et al. 2012; Sigoillot et al. 2012). Such enzymes include the classical glycoside hydrolases, namely, endoglucanases, cellobiohydrolases, and β-glucosidases, as well as oxidative enzymes, among which cellobiodehydrogenases and the newly discovered lytic polysaccharide monooxygenases (LPMOs). Filamentous fungi have adopted different strategies to perform efficient degradation of cellulosic biomass.

2 Enzymes Involved in Cellulose Degradation

2.1 The CAZy Classification

Enzymes involved in carbohydrate deconstruction are grouped in the carbohydrate-active enzyme (CAZy) classification based on comparison of their amino acid sequence, three-dimensional structure and catalytic mechanism [www.cazy.org; www.cazypedia.org; (Lombard et al. 2014)]. The CAZy database gathers the enzymes involved in the modification of carbohydrates into several groups, Glycoside Hydrolases (GH) that cleave glycosidic bonds, Glycosyl Transferases (GT) which form new glycosidic bonds, Polysaccharide Lyases (PL) which cleave uronic acid-containing polysaccharide chains, Carbohydrate Esterases (CE) that allow deacylation of polysaccharide chains (Henrissat et al. 1991). Auxiliary Activity (AA) enzymes have been added more recently (Levasseur et al. 2013). Most of AA enzymes are oxidoreductases acting on lignin and carbohydrates and among them four families have been recently described as LPMOs. Finally, Carbohydrate-Binding Modules (CBM) are noncatalytic modules appended to

enzymes which are involved in substrate targeting. In October 2015, the CAZy database included 135 GH families, 16 CE families, 13 AA families, and 71 CBM families.

2.2 The Classical Cellulose-Acting Enzymes

Historically, a system of three complementary enzymatic activities has been described as being in charge of cellulose degradation: endoglucanases, cellobiohydrolases, and β-glucosidases (For a review see Payne et al. 2015; Fig. 1). They are able to hydrolyze the β-1,4 covalent bonds that connect glucose units in the cellulose chains and act synergistically with different specificities. Accordingly, their structural organization and catalytic mechanisms allow for the accommodation of corresponding substrates.

Endoglucanases (EG, endo-1,4 β D-glucanases, EC 3.2.1.4) randomly cleave β-1,4 bonds in amorphous areas of cellulose chains and generate new reducing and nonreducing ends. They are classified in several CAZy families, namely, GH5, GH6, GH7, GH9, GH12, GH44, GH45, and GH74. Endoglucanases display a variety of structures, such as β jelly roll as *Aspergillus niger* family GH12 *An*EglA (1KS4, Khademi et al. 2002) or $(\beta/\alpha)_8$ barrel as *Trichoderma reesei Tr*Cel5A (3QR3, Lee et al. 2011) as well as two possible catalytic mechanisms with retention of configuration or with inversion of configuration (Davies and Henrissat 1995). However, to accommodate cellulose chains, endo-acting cellulase structures have in common a large cleft containing the catalytic amino acids (Davies and Henrissat 1995).

Cellobiohydrolases (CBHs, cellulose 1,4-β-cellobiosidases, EC 3.2.1.91), are processive enzymes which release cellobiose from either reducing (GH7 CBHs) or nonreducing ends (GH6 CBHs) of cellulose fragments released by endoglucanases. GH6 CBHs display an inverting catalytic mechanism, whereas GH7 CBHs use a

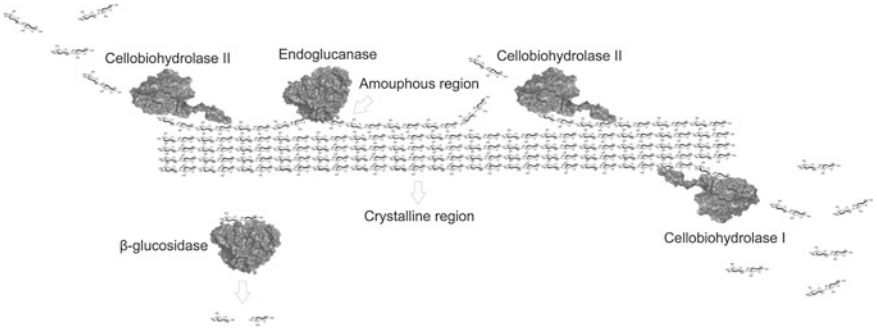

Fig. 1 Illustration depicting the hydrolysis of cellulosic materials using endoglucanases, exoglucanases, and β-glucosidases

retaining mechanism. Three-dimensional structure examples include the Basidiomycetes *Coprinopsis cinerea* Cel6A and Cel6C CBHs (3VOG and 3A64, respectively, Tamura et al. 2012) and *Phanerochaete chrysosporium Pc*Cel7D (1GPI, Munoz et al. 2001). CBHs harbor cleft- or tunnel-bearing structures which allow the enzyme to slide on cellulose chain for the next cleavage while the product is being released.

β-glucosidases are the third partner of the cellulase system and catalyze the cleavage of cellobiose or cello-oligomers into glucose. They are characterized by a pocket-containing topology that allows optimal detection of the nonreducing extremity and leads to the cleavage of a single sugar unit. Because of this topology, β-glucosidases are nonprocessive enzymes, since the substrate has to be released after each cleavage event to allow the new glucose unit to exit the pocket. In the CAZy database, β-glucosidases are grouped in families GH1 and GH3. A few fungal β-glucosidase structures have been solved, among which the ones of *T. reesei*, the family GH1 *Tr*Bgl2 (3AHY) and family GH3 *Hj*Cel3A (3ZYZ), the latter being the most abundant β-glucosidase in *T. reesei* enzyme cocktails (Jeng et al. 2011; Karkehabadi et al. 2014). Both exoglucanases and β-glucosidases are strongly inhibited by their reaction products cellobiose and glucose, respectively (Teugjas and Väljamäe 2013).

2.3 *Oxidative Enzymes Involved in Cellulose Deconstruction*

Complementary to their typical hydrolytic cellulases, fungi have developed oxidative degradation enzymes. These enzymes have been recently identified and described as LPMO enzymes (Quinlan et al. 2011; Vaaje-Kolstad et al. 2010; Harris et al. 2010). LPMOs are classified into four auxiliary activity (AA) families, AA9 (formerly GH61), AA10 (formerly CBM33), AA11, and AA13 of the Carbohydrate-Active enZyme database (CAZy; http://www.cazy.org; Levasseur et al. 2013). The AA10 family contains mainly enzymes of bacterial and viral origin that cleave cellulose and chitin mostly at the C1 position (Forsberg et al. 2011; Hemsworth et al. 2013). The LPMOs classified in the AA11 and AA13 families, respectively, cleave chitin and starch and share important structural features with the two previously characterized families (Vu et al. 2014a; Leggio et al. 2015; Hemsworth et al. 2014). This section will focus mainly on the AA9 family containing only fungal LPMOs active on lignocellulose although much of what is known about the fungal cellulolytic LPMOs is likely applicable across the LPMO superfamily.

In fungi, these enzymes have first been classified into the GH61 family after one member of the family, *T. reesei* EGL4, was reported displaying a weak endoglucanase activity (Saloheimo et al. 1997; Karlsson et al. 2001). However, there was described as "weak endoglucanases" as the activity was several orders of magnitude lower than what had been observed in other endoglucanases. In 2008, the first

reported structure of the *T. reseei* Cel61B (Karkehabadi et al. 2008) suggested another activity for those enzymes. Its structure closely resembles to the CBP21 protein (AA10 formerly CBM33), a chitin-binding protein from the bacterium *Serratia marcescens*. This enzyme was obtained few years earlier and had been proposed to enhance chitin degradation through a non-catalytic mechanism (Vaaje-Kolstad et al. 2005).

In the last few years, GH61 have drawn increasing attention because of their «stimulating» effect on cellulase cocktails for biomass conversion (Harris et al. 2010). Other structure and biochemical study have revealed their oxidative mechanism, described their active site and highlighted some important structural features (Quinlan et al. 2011; Harris et al. 2010; Vu et al. 2014b; Kittl et al. 2012; Beeson et al. 2012; Li et al. 2012; Phillips et al. 2011). In August 2015, family AA9 includes 301 members among which 7 members have had their three-dimensional structure solved (Karkehabadi et al. 2008; Wu et al. 2013; Quinlan et al. 2011; Harris et al. 2010; Borisova et al. 2015; Li et al. 2012). These analyses revealed a structural β-sandwich fold of typically 8–10 β-strands with a flat surface where binding with the substrate occurs mostly via stacking interactions with planar aromatic residues. A type II copper ion exposed at the surface is coordinated a "histidine brace" formed by two highly conserved histidine residues, one of which corresponds to the N-terminal histidine, and one tyrosine (Langston et al. 2011; Li et al. 2012). Fungal AA9 LPMOs are secreted enzymes and can contain post-translational modifications. One of the most unusual is the methylation of the N-terminal histidine at the imidazole Nε. This modification is found only in fungal LPMOs and its role is unclear and still under debate.

The oxidation of glucose units has been described mostly at the C1 or C4 position (Beeson et al. 2012; Phillips et al. 2011; Bennati-Granier et al. 2015; Li et al. 2012; Vu et al. 2014b), but a few studies suggested oxidation of the C6 position as well (Bey et al. 2013; Quinlan et al. 2011). AA9 LPMOs are classified into three groups, depending on their regioselective mode of action: type 1 LPMOs will oxidize at C1 and release soluble oligosaccharides with an aldonic acid at their reducing end; type 2 LPMOs will oxidize at C4 and release ketoaldose at the nonreducing end; and type 3 will oxidize at both C1 and C4 and release a mixture of alodnic acid and ketoaldose. AA9 LPMOs require a reducing cofactor for activity, such as ascorbic acid (Forsberg et al. 2011; Quinlan et al. 2011), fragment of lignins (Dimarogona et al. 2012), or enzymes like the cellobiose dehydrogenase (CDH) (Langston et al. 2011; Bey et al. 2013; Phillips et al. 2011). CDHs and AA9 LPMOs are often cosecreted in fungal cultures (Poidevin et al. 2014; Navarro et al. 2014). A clear indication of the synergy was obtained when it was shown that the combination of *Thermoascus auranticus* AA9 and *Humicola isolens* CDH greatly enhanced cellulose degradation (Langston et al. 2011).

Although no structural complex with their substrates is available, binding of the substrate may occur via aromatic-carbohydrate interactions. Indeed, some aromatic residues on the substrate-binding surface are conserved and the spacing matches the spacing between glucose subunits in cellulose (Harris et al. 2010; Li et al. 2012; Wu et al. 2013). Some structural differences have been observed among the different

AA9 LPMOs characterized. More AA9 LPMO members need to be characterized in order to identify the molecular determinants involved in their substrate specificity. For instance, two AA9 LPMOs have been recently shown to act on soluble cello-oligosaccharides, i.e., *Nc*LPMO9c and *Pa*LPMO9H (Isaksen et al. 2014; Bennati-Granier et al. 2015).

2.4 Ancillary Proteins

2.4.1 Carbohydrate-Binding Modules

Cellulolytic enzymes can be associated with non-catalytic modules among which the CBM are an important group (for an extensive review, see Várnai et al. 2014). CBMs play a role for substrate targeting and binding and often increase the overall catalytic activity of the enzyme especially on crystalline substrates. Based on their topology, CBMs have been grouped in three structural and functional groups by Boraston et al. (2004): type-A or surface-binding CBMs, type-B or glycan-chain binding CBMs, and type-C, or small sugar binding CBMs. Cellulose-acting enzymes are typically associated with type-A CBMs, which present a flat surface exposing aromatic residues allowing the interaction with cellulose chains. Another classification of CBMs is found in the CAZy database, in which CBMs are classified based on structure and binding specificity (www.cazy.org; Lombard et al. 2014). Among the 71 CBM families, three families gather CBMs identified in fungal cellulases that show binding to cellulose: CBM1, CBM6, and CBM63. CBM1 family comprises most of the modules associated with fungal cellulose-acting enzymes. These CBMs are approximately 40 residues long, and can be located either at the N- or C-terminus of the catalytic module, alone or in a multimodular organization (Guillén et al. 2010).

In fungal genomes, the number of identified CBM1 modules varies, from none in some brown-rot fungi such as *Postia placenta* and *Fomitopsis pinicola* to more than 30 in some white-rot species such as *Phanerochaete chrysosporium* and *Bjerkandera adusta*. In white-rot fungi, the distribution of CBM1s among the different families of cellulases is heterogeneous, with some families such as GH7 CBH and AA9 LPMOs being often found as single modules, whereas GH5 endoglucanase and GH6 CBH are associated with CBM1s. In brown-rot fungi, the cellulose degradation system does not rely on cellulases and accordingly the number of associated CBM1s is also smaller, with most GH5 endoglucanases and AA9 LPMOs being found as single domains.

2.4.2 Expansins

Expansins are another type of non-catalytic proteins that can play a role in cellulose degradation (For a recent review, see Liu et al. 2015). The presumed mechanism is

a disruption of hydrogen bonding between cellulose microfibrils or between cellulose and other cell wall polysaccharides leading to an enhanced accessibility of cellulases to cellulose chains (Saloheimo et al. 2002; McQueen-Mason and Cosgrove 1994). The expansin-like protein from *T. reesei*, *Tr*Swo1, revealed a capability for disruption of cellulose fibers in cotton or filter paper without yielding any detectable reducing sugars (Saloheimo et al. 2002). The resolution of its structure revealed that *Tr*Swo1 has a CBM1 N-terminal, a linker region, and an expansin-like C-terminal domain (20 % identity), which in expansins are similar in structure and sequence to the catalytic site of family GH45 (Saloheimo et al. 2002). More recently, Andberg et al. (2015) proved the hydrolytic activity of *Tr*Swo1. The mode of action of this enzyme is similar to both endo- and exoglucanases. Hence, *Tr*Swo1 could reduce the viscosity of reaction environments containing barley β-glucan, hydroxyethyl cellulose, and carboxymethyl cellulose (or typical endoglucanases substrates) at a consistency of 1 %. On the other hand, when the composition of barley β-glucan hydrolysates were investigated, cellobiose was the main reaction product with no evidence for intermediates, while for a typical endoglucanase (Cel5A), cellopentaose and cellohexaose were predominantly released. *Tr*Swo1 presented a limited activity on barley β-glucan, since only 1.2 % of dry mass was solubilized in either 15 s or 24 h of hydrolysis. It was suggested that the *Tr*Swo1 mode of action involved an initial attack in the middle of cellulose chain and a subsequent processive action along the chain releasing cellobiose. Hydrolysis of barley β-glucan was probably stopped when *Tr*Swo1 came across a β-(1→3) glycosidic bond and possibly stayed permanently bound at this substrate site. Expansin-related proteins have been identified in both Basidiomycetes and Ascomycetes and a few have been characterized from *Aspergillus fumigatus* (Chen et al. 2010), *B. adusta* (Quiroz-Castañeda et al. 2011), *Schizophyllum commune* (Tovar-Herrera et al. 2015). Studies have investigated the activity of expansins and expansin-like proteins in cellulase cocktails and they revealed that expansins enhance cellulose degradation. For instance, Gourlay et al. (2013) observed that the SWOI addition on a steam pretreated corn stover promoted cellulose and hemicellulose solubilisation primarily to their corresponding oligomers. The authors attributed this nonhydrolytic effect to the release of preexisting oligomers that were bound to the substrate surface, but small concentrations of glucose and xylose were also released in the substrate hydrolysate.

3 Strategies to Improve Cellulose Degradation

3.1 Combination and Synergism of Cellulolytic Enzymes

Although the combination of enzymes from the different families (glycoside hydrolases and oxidases) is theoretically enough to carry out complete conversion of cellulose into monomers, complex kinetics, cellulose crystallinity as well as

product inhibition leads to a limited degradation efficiency in practice. The kinetics of cellulose degradation by the different enzymatic partners are complex and many models have been proposed to understand the activity of cellulases on cellulose over the course of degradation. Bansal et al. (2009), have summarized in a review the sequence of steps involved in cellulose degradation from the adsorption of endoglucanase and cellobiohydrolase onto their substrate to detachment from the chain and hydrolysis of cellobiose into glucose by beta-glucosidases. In this sequence of events, many factors impact kinetics, causing an overall decreasing rate of reaction over time. Nonproductive binding of cellulases on other components of lignocellulosic substrates has been extensively studied, by FPLC (Gao et al. 2014), colorimetric quantification (Guo et al. 2014), or quartz crystal microbalance (Rahikainen et al. 2013) such as nonproductive cellulase binding, enzyme deactivation, and mostly substrate depletion, and product inhibition. Substrate cristallinity is also cited as a factor for decreasing reaction rate, since amorphous regions are hydrolysed first and the more crystalline regions remain as recalcitrant, resulting in an increase of crystalline fraction of cellulose over time (Chen et al. 2007). This model is called the two-phase substrate model and reflects the physical complexity of the cellulose, which affect both accessibility and reactivity. For an extensive review of models and parameters involved in cellulose degradation kinetics, see Bansal et al. (2009).

Cellulolytic enzymes with different specificities exhibit synergistic action on fibers, simultaneous action of multiple enzyme components resulting in a significantly higher hydrolysis yield than the sum of the hydrolysis yields of the individual enzyme components. This phenomenon called synergism has been described more than twenty years ago. Endo–exo and exo-exo synergisms have been distinguished. An example of endo-exo synergy in fungal cellulases occurs between *T. reesei* endoglucanase *Tr*Cel5A (EGII) and its cellobiohydrolase partner *Tr*Cel7A (CBHI) (Medve et al. 1998). Different types of CBHs working together can also lead to a synergetic degradation of cellulose, such as TrCel7A (CBHI) working on the reducing end of cellulose chains and TrCel6A (CBHII) which acts on nonreducing ends. Real-time visualization of crystalline cellulose degradation by *T. reesei* CBHs was performed using high-speed atomic force microscopy (Igarashi et al. 2011). TrCel7A molecules were observed to slide unidirectionally along the crystalline cellulose surface but at one point exhibited collective halting analogous to a traffic jam. Changing the crystalline polymorphic form of cellulose by means of an ammonia treatment increased the apparent number of accessible lanes on the crystalline surface and consequently the number of moving cellulase molecules. Treatment of this bulky crystalline cellulose simultaneously or separately with TrCel6A resulted in a remarkable increase in the proportion of mobile enzyme molecules on the surface.

While endoglucanases increase the available sites for exoglucanases, β-glucosidases decrease the exoglucanase inhibition by converting cellobiose into glucose (Wood 1985). Multiple factors affect the synergy between cellulases. These include the specific activity of the enzymes, the ratio between them, the enzyme loading and the chemical composition and structure of cellulosic substrates.

Cellulolytic glycoside hydrolases also exhibit strong synergism with their oxidative counterparts, LPMOs. This synergy was first described in 2010, when the actual effect of LPMO was still under question (Harris et al. 2010), but their addition to a cellulase cocktail allowed to significantly decrease enzyme loadings to hydrolyse cellulosic biomass. AA9 LPMOs seem to exhibit activity on both amorphous and crystalline cellulose, whereas endoglucanases presents no activity against the latter. This might be the reason why AA9 LPMOs display a high synergy with cellulases and may provide half of the enzymatic loading required for hydrolysis (Harris et al. 2010, 2014).

3.2 *Fungal Accessory Enzymes*

Another type of synergy involving cellulases and noncellulolytic enzymes can increase fungal degradation of cellulose: because of the complex structure of lignocellulose, efficient cellulose degradation also depends on accessory activities to allow access for cellulases to cellulosic fibers.

Cellulose microfibrils are associated with some hemicelluloses which hampers access for cellulolytic enzymes. In particular, whereas arabinoxylan and (1,3)(1,4)-β-glucan do not interact strongly with cellulose (Mikkelsen et al. 2015), other components of the cell wall such as softwood mannan has been suggested being tightly associated with cellulose fibrils (Åkerholm and Salmén 2001). Accordingly, addition of mannanases to *T. reesei* cellulolytic cocktails led to a synergistic effect and an increase of glucose release from nonpretreated softwood in saccharification assays (Couturier et al. 2011). Such synergies have also been described between pectinases and cellulases (Zhang et al. 2013) for the hydrolysis of steam-exploded hemp and confirms the spatial contacts between pectin and cellulose that have been described in primary cell wall in several studies (Cosgrove 2014; Wang et al. 2015). Xylanases (Hu et al. 2011) and a xyloglucanase (Benko et al. 2008) have demonstrated a synergistic effect when employed in combination with cellulases on specific substrates, leading to improved conversion of cellulose compared to cellulases alone. Gao et al. (2011) demonstrated that, by adding endoxylanases to a cellulolytic enzyme system, the glucose release from AFEX (ammonia fiber expansion) pretreated corn stover increased from 56 to 83 % after 24 h hydrolysis. Selig et al. (2008) achieved an 84 % improvement in the enzymatic hydrolysis of hot water pretreated corn stover by adding an endoxylanase, a ferulic acid esterase and an acetyl xylan esterase to a the cellobiohydrolase Cel7A. These authors also observed that the resulting synergistic effect is more evident when low Cel7A loadings are used.

Tabka et al. (2006) studied the effects of adding xylanases, feruloyl esterases, and laccases on the hydrolysis of dilute sulphuric acid impregnated steam-exploded wheat straw. The addition of hemicellulases caused an enhancement in the substrate glucose yield. On the other hand, the addition of laccase promoted the cleavage of covalent bonds in lignin, showing a negative effect that was associated to the

inhibition of cellulases by the accumulation of phenolic compounds in the substrate hydrolysates.

4 Perspectives in Cellulose Hydrolysis Through the Exploration of Fungal Biodiversity

The most studied cellulolytic system to date is probably that of the ascomycete fungus *T. reesei*, largely used in industry and engineered for decades to be used in biomass hydrolysis applications. *T. reesei* genome was sequenced in 2008 (Martinez et al. 2008) and revealed a relatively reduced set of GHs in general and of cellulases in particular, with 5 endoglucanase genes, 2 cellobiohydrolase genes, and 15 β-glucosidase genes. Only 3 LPMO genes of family AA9 are also encoded by *T. reesei* genome. Despite the presence of all types of cellulase activities, *T. reesei* enzyme cocktails are not able to achieve a complete degradation of cellulose. In the past few years, the search for novel CAZymes has been expanded to the exploration of fungal strains from tropical forests (Berrin et al. 2012), marine environment (Arfi et al. 2013) and pathogens (Couturier et al. 2012). For example, the maize pathogen *Ustilago maydis* was identified as a good source of enzymes for improvement of *T. reesei* cellulolytic capabilities. *U. maydis* genome revealed one of the smallest sets of genes that encode for CAZymes with only 95 glycoside hydrolases (Kämper et al. 2006), but investigation of its secretome highlighted a significant fraction of putative oxidoreductases that are potentially involved in the depolymerisation of lignocellulose. The authors suggested that *U. maydis* oxidoreductases could participate in the depolymerisation of lignocellulose via the formation of highly reactive oxidants.

Classically, cellulolytic systems of white-rot and brown-rot fungi have been opposed. Cellulose degradation of white-rot mostly rely on GH6, GH7, LPMOs, and numerous CBM1s allowing anchoring of catalytic modules on crystalline cellulose. On the other hand, brown-rot fungi system barely contains cellulases and mostly use nonenzymatic processes based on hydroxyl radical produced by Fenton reaction (Martinez et al. 2009). However, recent work suggests that the separation of brown-rot and white-rot fungi in two distinct groups might have been an oversimplification and that some fungi display intermediate modes of action (Floudas et al. 2012, 2015; Riley et al. 2014).

A wealth of fungal genomics and postgenomics (transcriptomics and secretomics) information has been generated in the last few years. More than 265 fungal genomes (more than 90 corresponding to basidiomycetes) are publically available. These studies constitute a solid basis to identify the main players involved in the degradation of cellulosic biomass through comparative–omic studies. Complex portfolios of fungal enzymes are secreted in response to environment and growth substrates. The study of fungal secretomes from the scope of their different lignocellulosic biomass degradation strategies and lifestyles would facilitate their use in

the treatment of lignocellulose as carbon feedstock for biofuel production and further biorefinery processes (Alfaro et al. 2014).

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