Plant wastes and sustainable refineries: What can we learn from fungi?

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Abstract

The valorization of plant wastes allows access to renewable carbon feedstocks without increasing the demand for plant biomass production. Plant wastes are the non-edible residues and waste streams from agriculture, agroindustry and forestry. The chemical diversity and recalcitrance to degradation of such wastes challenge our ability to transform and valorize these resources into value-added compounds. Fungi that thrive on plant tissues have gained a huge diversity of enzymatic toolkits for the finely-tuned degradation of glycan and lignin polymers. Our knowledge on the enzymatic systems developed by fungi now guides innovations for plant waste bioprocessing. Here, we provide an overview of the most recent findings in the hydrolytic and oxidative systems used by fungi for the degradation of recalcitrant plant polymers. We present recent promising success in applying fungal enzymes or fungal fermentations on plant wastes, and discuss the forthcoming developments that could reinforce fungal biotechnology entering a variety of industrial applications.

Introduction

Plant biomass is considered as an interesting alternative to fossil fuels since it is an abundant source of renewable carbon, from which biofuels, biochemicals, and biomaterials can be produced with overall reduction in greenhouse gas (GHG) emissions (e.g. (Vera et al., 2020)). However, the use of plant biomass as a sustainable carbon source can only be envisioned if (i) it does not impact the land use for feed and food production, (ii) it ensures the preservation of natural areas, and (iii) it does not depend on the long-distance transport of raw biomass. As a consequence, the nonedible residues and wastes from agriculture (e.g. straws, husks), food processing (e.g. seed-oil press cakes, brewer's spent grain, sugar beet pulp) and forestry (e.g. leaf litters, wood logs, barks, wood sawdust) are privileged renewable resources. When a valorization route is possible, such plant residues are not considered anymore as plant "wastes" but as "co-products" that are further used in biorefinery cascade processing. The challenge then lies in our ability to process plant wastes with wide chemical diversity and strong recalcitrance to degradation. The recalcitrance of lignocellulose is mainly due to the intertwining of cellulose microfibrils and lignin polymers (Harris & Stone, 2008). In this context, the microorganisms that possess enzymatic arsenals to modify and degrade plant cell wall polymers can inspire the design of bioprocesses for the recovery of lignin and glycans and their derivatives for downstream production of bioenergy, biobased polymers and biochemicals (Stichnothe et al., 2020; Kumar & Verma, 2020), (Figure 1).

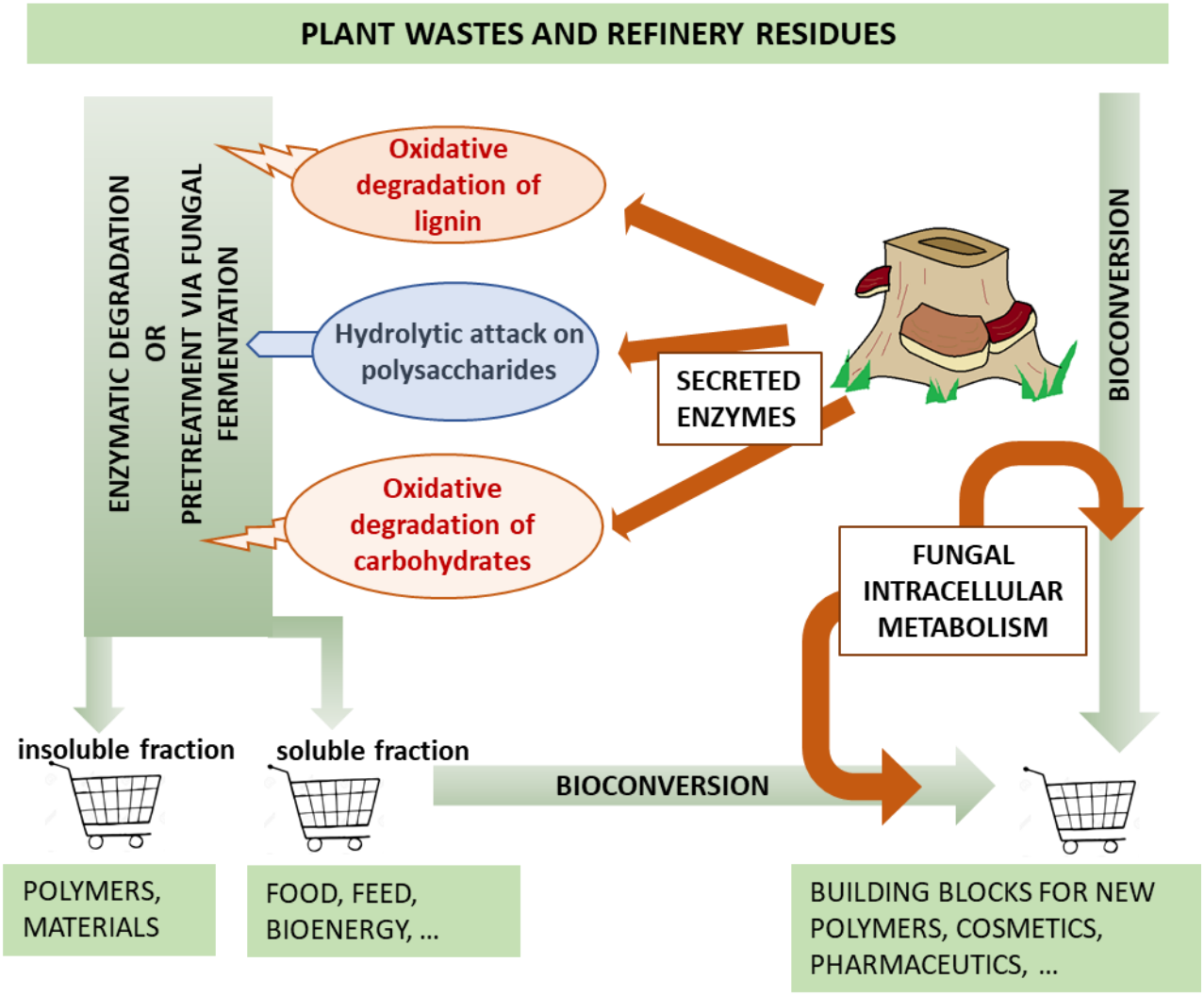

Figure 1. Overview on the utility of fungi for the production of bio-based molecules from plant wastes and refinery residues. After treatment with fungal enzymes or pretreatment via fungal fermentation, the insoluble cellulose- or lignin-rich fractions are used for the synthesis of polymers or materials. The released soluble molecules might be used in the food, feed or bioenergy sectors, or further converted in value-added molecules via the fungal intracellular metabolism.

For about 750 million years, the fungi living on plant tissues have co-evolved with plants and acquired a large diversity of plant cell wall-degrading or -modifying enzymes. Some have become the predominant source of enzymes currently used on an industrial scale for biomass transformation (Van Den Brink & De Vries, 2011). For example, *Trichoderma reesei*, a fungus that was first isolated on American soldiers' uniforms during World War II in the Solomon Islands, efficiently degrades cellulose. This species has undergone intensive strain engineering to maximize the production yields of cellulolytic enzymes and the effectiveness of enzyme cocktails dedicated to biomass degradation, notably by upgrading, through synthetic biology, with enzymes from other biomass-degrading fungi (Seidl & Seiboth, 2010; Berrin et al., 2014). In recent years, consistent efforts have aimed at strengthening our understanding of the fungal enzymes that target recalcitrant plant polymers. These studies have highlighted the richness of the enzymatic arsenals secreted by fungi with different lifestyles or different substrate preferences.

In this review, we explore how the fungal biodiversity inspires the design of new biocatalysts to help the transition towards a bioeconomy and the valorization of plant wastes. We review recent findings on the fungal enzymatic systems dedicated to lignocellulose degradation, and promising approaches to transform plant wastes into valuable bioproducts.

1. Adaptation of the fungal enzymatic arsenals to land plant polysaccharides

Fungi are heterotrophs that use extracellular digestion to degrade the organic matter they use as a source of carbon and energy. The enzymatic machineries they secrete to access carbon vary widely based on the plant tissues they colonize and the type of association they establish, from symbiosis to pathogenicity and saprotrophy. In the past few years, the discovery of new fungal plant cell wall-degrading enzymes has largely benefited from the enormous amount of newly produced genomic data (Grigoriev et al., 2014; Muggia et al., 2020*) and the generation of reliable bioinformatic tools for their functional annotation (Drula et al., 2021).

The earliest land plants, as well as their closest relatives, the streptophyte algae, contained cellulose and pectin in their cell walls and lacked lignin (Popper et al., 2011). Accordingly, early diverging fungi did possess pectinase- and cellulase-encoding genes (Lange et al., 2019), as part of the ancestral fungal toolkit for breaking down plant cell wall (Douzery et al., 2004; Zimmer et al., 2007; Parfrey et al., 2011). Later, pectinase genes have undergone rapid duplications in organisms that adopted a plant-based nutrition (Chang et al., 2015). The evolutive adaptation of fungi to the plant polysaccharides paralleled the acquisition of a wide panel of glycoside hydrolases and polysaccharide lyases that match the diversity of the glycan bonds encountered in cellulose, hemicellulose and pectin polymers (Table 1) and a suite of auxiliary enzymes that are not directly active on the polymers but contribute to the degradation (Hage & Rosso, 2021*)). For instance, GH131 glucanases, which cleave β-1,3 and β-1,4 glucans, and improve the release of glucose monomers from wood fibers, appeared in fungi and were later transferred to plant parasitic oomycetes via lateral gene transfert (Anasontzis et al., 2019). Overall, the fungal enzymes that target plant cell wall glycans are distributed in 75 families of carbohydrate-active enzymes (CAZymes) as classified in the CAZy database (http://www.cazy.org/; Drula et al., 2021).

Table 1. Fungal carbohydrate-active enzymes (CAZymes) active on plant-cell walls, and their reported enzymatic activities. AA: auxiliary activity; CE: carbohydrate esterase; CRO: copper radical oxidase; GH: glycoside hydrolase; PL: Polysaccharide Lyases; POD: Class II peroxidase. Adapted from Hage H. & Rosso M.N., 2021.

Substrate	Enzymatic activity	CAZy family
Lignin	Laccase	AA1_1 (CRO)
	Manganese peroxidase	AA2 (POD)
	Lignin peroxidase	
	Versatile peroxidase	
Hemicellulose	Endo-1,4-β-xylanase	GH5_22, GH8, GH10, GH11, GH30_7
	xyloglucanase	GH74, GH44
	Endo-β-1,4-mannanase	GH5_1, GH5_7, GH26, GH113, GH134
	Endo-α-1,5-arabinanase	GH93
	β-1,3-glucanase	GH16
	α-L-arabinofuranosidase	GH43, GH51, GH54, GH62
	β-Glucuronidase	GH115, GH2
	α-1,2-glucuronidase	GH67, GH115
	β-mannosidase	GH2, GH5_2
	β-Galactosidase	GH35, GH53
	α-Galactosidase	GH27, GH36
	β-xylosidase	GH52, GH54, GH120, GH30_1, GH39
	α-L-fucosidase	GH29, GH95, GH141
	Acetylxylan esterase	CE1, CE2, CE3, CE4, CE6, CE16
	Cutinase	CE5
	Glucuronyl methyl esterase	CE15
	Lytic polysaccharide monooxygenase	AA14
Cellulose	Endoglucanase	GH5,4, GH5_5, GH12, GH45, GH74, GH131
	cellobiohydrolase	GH6, GH7, GH5_1, GH48
	β-Glucosidase	GH1, GH3, GH30_1, GH5_7, GH5_22
	Lytic polysaccharide monooxygenase	AA9, AA16
Pectin	Polygalacturonases	GH28, GH78
	β-glucuronyl hydrolase	GH88, GH105
	α-L-rhamnosidase	GH78, GH106
	β-1,4-galactanase	GH53
	Polygalacturonate lyase	PL1, PL3, PL9
	Rhamnogalacturonan lyas	PL4, PL11, PL26
	Rhamnogalacturonan acetylesterase	CE12
	Pectin methylesterase	CE8
Cutin	Cutinase	CE5

The orchestration of the secretion of these enzymes by the fungi contributes to the efficacy of deconstruction of the plant cell wall polymers and the release of assimilable saccharides. The concomitant secretion of endoglucanases and cellobiohydrolases allows exo-endo synergy in which endoglucanases create new chain ends for exoglucanases (Henrissat et al., 1985). In wood decay fungi from the Trametes group, we observed the concomitant production of sets of enzymes active

on cellulose and on the backbone and side chains of hemicelluloses. For example, during growth on wheat straw or woody substrates, these fungi simultaneously produce CAZymes that target β-1,4-glucans (AA9, GH3, GH5, GH6, GH7, GH45 and GH131), β-1,4-xylans (GH10 and GH11 xylanases), and the bounds between xylan and arabinose (GH43, GH51 and GH62 arabinofuranosidases), the glucuronoyl groups (GH115 glucuronidase), the acetyl groups (CE4 and CE16 acetyl esterases) or the 4-O-methyl glucuronoyl groups (CE15 glucuronoyl esterases) (Miyauchi et al., 2016, 2017, 2020). Besides, these fungi also rely on a subset of these enzymes being fused to a CBM1 carbohydrate binding module, that favors access of the catalytic module to insoluble substrates such as crystalline cellulose (Lehtiö et al., 2003; Fong et al., 2016). It is proposed that the concomitant secretion of a wide panel of enzymes allows for co-operativity and synergism between the enzymes that target the backbones and the side chains of complex polysaccharides.

2. Oxidative enzymes active on recalcitrant polymers

In addition to hydrolytic enzymatic systems, the most efficient plant biomass degraders use finely tuned oxidative degradation of plant cell wall polymers, mediated both by chemical and enzymatic attack (Figure 2). During the chemical attack, a panel of oxido-reductases and ferric reductases contribute to the production of hydrogen peroxide, which is the source for the generation of reactive oxygen species through the Fenton reaction ($Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + H_2O + HO^{\bullet}$), leading to non-specific cleavage of the polymers at the vicinity of the fungal hyphae. This chemical attack is predominant in fungi that produce brown rot during wood decay, and was observed in the initial steps of wood decay, allowing the loosening of the plant cell wall before the secretion of hydrolytic enzymes (Zhang et al., 2019).

On the contrary in fungi that produce white rot, two classes of enzymes, the ligninolytic class II peroxidases and the lytic polysaccharide monooxygenases (LPMOs) drive the oxidative cleavage of lignin and cristalline cellulose, respectively. Again, several studies suggest that the oxidative degradation is predominant at earlier degradation stages, presumably to uncover the polysaccharides and amorphous cellulose while preserving other enzymes from oxidative damage (Martínez et al., 2005; Eastwood et al., 2011 ; Navarro et al., 2014; Zhang et al., 2016).

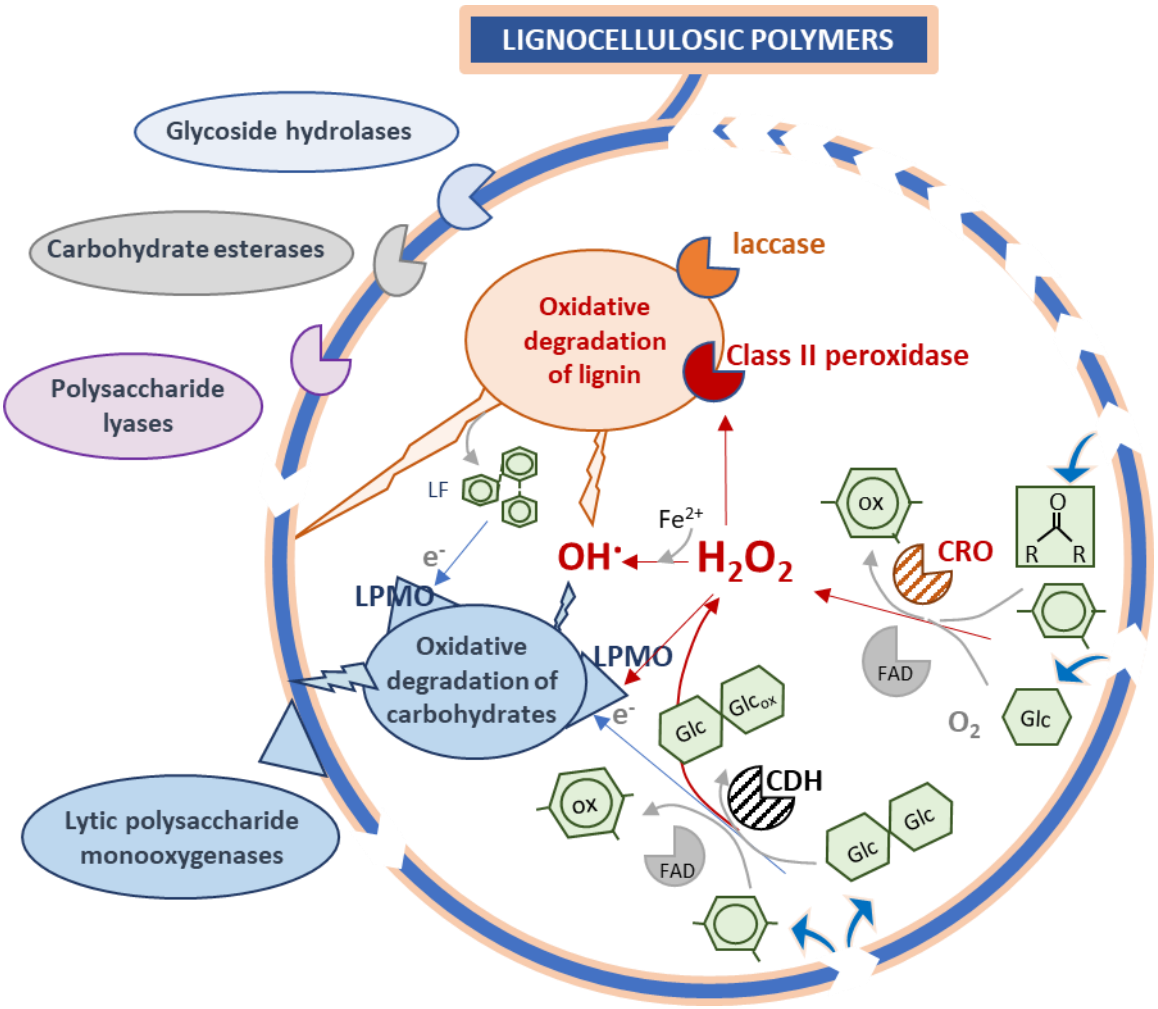

Figure 2. Overview of the extracellular enzymatic systems used by saprotroph fungi to degrade recalcitrant plant polymers. In the course of the degradation, several lignin- and saccharide-derived compounds (in green) are released. These compounds are the substrates for cellobiose dehydrogenase (CDH), copper radical oxidases (CRO) and FAD dependent oxidoreductases that fuel the oxidative enzymatic network via electron transfer to LPMOs or the production of hydrogen peroxide (H_2O_2). H_2O_2 plays a central role in the oxidative degradation of lignocellulose polymers, as a co-substrate for lytic polysaccharide monooxygenases (LPMO) and ligninolytic class II peroxidases, and as a substrate for the formation of the hydroxyl radical via the Fenton reaction (red arrows). Glc: glycans; LF: lignin fragments.

Ligninolytic class II peroxidases (CAZy family AA2) have contributed to the adaptation of fungi to woody substrates. These enzyme, which are only found in white-rot fungi, arose from generic peroxidases with low redox potential (Floudas et al., 2012; Mathé et al., 2019). Recent findings have further shown striking correlations between lignin composition modifications in land plants and the appearance of new ligninolytic class II peroxidases in saprotroph fungi. The lignin of angiosperm trees is composed of G and S phenylpropane units and is chemically more complex than the lignin of gymnosperm trees (composed of G units). The resurrection of ancestral enzymes showed that the modification of lignin composition in angiosperms drove a fascinating coevolution of fungal

manganese peroxidases (MnP) to versatile peroxidases (VP), in which a surface tryptophan confers the ability to abstract electrons from non-phenolic lignin moieties (Ayuso-Fernández et al., 2018**). Finally, lignin peroxidases (LiP) appeared by convergent evolution from different VP ancestral genes (Ayuso-Fernández et al., 2018), possibly contributing to the adaptation of the fungi to diverse ecological niches and plant substrates (buried wood, decayed wood, leaf litter and grass litter; Ruiz-Dueñas et al., 2021*). The ligninolytic class II peroxidases display broad substrate specificity on low redox-potential substrates (MnP), on non-phenolic aromatic compounds (LiP), or both (VP; (Martínez et al., 2018). These enzymes are of primordial relevance for the valorization of plant wastes, since lignin acts as a barrier that protects cellulose and hemicelluloses from the enzymatic attack. The availability of a panel of ligninolytic enzymes is one key to cope with the modifications in lignin physicochemical properties during plant biomass processing or pre-treatments (Bugg & Rahmanpour, 2015; Linde et al., 2021; Daou et al., 2021; de Eugenio et al., 2021).

Laccases also contribute to the degradation of lignin and have for long been identified as biocatalysts of high relevance for the production of lignocellulose-derived phenolics and aromatics as building blocks for the chemical synthesis of high-value products (Kües, 2015). Laccases are multicopper oxidases with high redox potential, which catalyze the non-specific oxidation of phenolic compounds (Christopher et al., 2014; Sousa et al., 2021). The spectrum of substrates for laccases is widened by chemical mediators released from the biomass, such as acetosyringone, syringaldehyde or *p*-coumaric acid. These redox mediators act as electron shuttles, promoting the oxidation of complex bulky substrates and/or of substrates with higher redox potential than the enzymes themselves. One current challenge in the use of laccases for lignocellulose deconstruction is that the same enzymes also catalyze the re-polymerization of the oxidation products (e.g. Steinmetz et al., 2020*).

The LPMOs, first discovered in chitinolytic bacteria (Vaaje-Kolstad et al., 2010), are abundantly secreted by fungi (Berrin et al., 2017). Fungal LPMOs may target cellulose, xylan, starch, pectin or chitin. LPMOs coordinate a single copper co-factor and use the co-substrate, O_2 or H_2O_2, to reduce Cu(II) to Cu(I) and abstract a hydrogen atom at the C1 and/or C4 position of the glycoside bonds at the surface of polysaccharides. The oxidation leads to the formation of a lactone, which is spontaneously hydrolyzed to an aldonic acid (oxidation at the C1 position) or a 4-gemdiol-aldose (oxidation at the C4 position) (Tandrup et al., 2018). The current model proposes that LPMOs are active at the surface of cellulose microfibrils to relax the crystalline structure and generate polysaccharide chain extremities that are accessible to cellobiohydrolases. Their biological importance is most probably broader as some LPMOs are also active on amorphous polymers and oligosaccharides. LPMOs have been added to existing commercial enzymatic cocktails to boost the recovery of carbohydrates from the plant biomass (Johansen, 2016). Importantly, new LPMO families are regularly identified from fungi, that enlarge the array of enzymatic toolkits to boost the recovery of saccharides from plant biomass (Couturier et al., 2018*; Filiatrault-Chastel et al., 2019).

Besides the secretion of oxidative enzymes, fungi are of valuable interest to understand the overall chemical and enzymatic systems that allow oxidase transition to the reduced active state, or that generate the H_2O_2 co-substrate required for ligninolytic class II peroxidases and LPMOs during lignocellulose attack. The synergistic and regulatory interplay between fungal ligninolytic peroxidases and H_2O_2-generating glyoxal oxidases was first proposed in the wood decay fungus *Phanerochaete chrysosporium* (Kersten & Cullen, 2014), and further demonstrated in other fungi (Daou & Faulds, 2017). The current model proposes that glyoxal oxidases are secreted in the reduced inactive form and are activated in the presence of a peroxidase and a peroxidase substrate or a strong oxidant. Glyoxal oxidases subsequently use O_2 as co-substrate to oxidize the aldehydes generated during

lignin and carbohydrate degradation, and generate the H_2O_2 required for ligninolytic peroxidase activity (Daou & Faulds, 2017). As indicated above, LPMOs require the reduction of the copper Cu(II) to Cu(I) to reach the active state. A wide range of electron donors can fuel LPMOs, including compounds released from the biomass as a result of the activity of other secreted enzymes (e.g. gallic acid, phenols, oxidized sugars and lignin derivatives) or fungal secondary metabolites (phenols, reduced quinone derivatives). Several secreted enzymes can also serve as direct electron donors for LPMOs, such as cellobiose dehydrogenases, oligosaccharide dehydrogenases or pyrroloquinoline-quinone-dependent pyranose dehydrogenases, or generate the H_2O_2 required for the peroxygenase reaction catalyzed by LPMOs, such as FAD-dependent oligosaccharide oxidases/dehydrogenases (reviewed in Manavalan et al., 2021*; Haddad Momeni et al., 2021*). By modulating the availability of the H_2O_2 co-substrate, the H_2O_2-consuming ligninolytic class II peroxidases might further contribute to the tuning of LPMO activity at the vicinity of the hyphae (reviewed in Bissaro et al., 2018**; Várnai et al., 2021).

Overall, these recent findings highlight the complexity of the enzymatic systems that allow efficient deconstruction of lignocellulose polymers by fungi. On the one hand, bunches of hydrolases, esterases and lyases that target the diverse glycan bonds are globally secreted simultaneously by fungi. On the other hand, the oxidative degradation of the plant cell wall polymers involves a finely tuned interplay between the oxidases that target lignin, cellulose or hemicelluloses and the dehydrogenases and copper radical oxidases that are required for oxidase activity (Vieira Manclaro et al., 2022). H_2O_2 and redox mediators, resulting either from the Fenton reaction or from enzymatic activities further tune the activity and recycling of the oxidases.

3. Plant waste valorization via fungal bioconversion and fungal enzymes

Fungi can contribute in different ways to plant waste valorization. First, the fungal enzymes can be produced in heterologous systems and applied directly on the plant wastes. This strategy allows the design of enzymatic cocktails that might be customized in order to correspond to the cellulose, hemicelluloses, pectin and lignin composition of the targeted substrates. To reduce the cost of enzyme production, the fungi can be directly grown on the wastes. In such cases, the wastes represent a cheap carbon source for the fungal growth with concomitant secretion of the enzymes of interest. The cultures may be done in submerged fermentation or solid-state fermentation. Submerged cultures of model fungi have been used for a while and still undergo improvements in enzyme production yields. For example, co-cultures of fungal strains that produce different sets of enzymes have shown some promising results, although efforts are still necessary to understand the underpinning molecular mechanisms that induce enzyme production by the fungi in these conditions (Sperandio & Filho, 2021). Recently, solid-state fermentation has attracted interest because it allows the production of reasonable yields of enzymes from non-model fungi (including basidiomycete fungi) at reduced cost, with low water and energy demand (reviewed in Leite et al., 2021*). Recent efforts on the production of fungal enzymes by solid-state fermentation have shown successful outcomes for a variety of enzymes, such as ligninolytic peroxidases (Sosa-Martínez et al., 2021), mannanases (Favaro et al., 2020), or laccases (Gupta & Jana, 2019; Wang et al., 2019). Besides, this strategy was also successfully used for the production of fungal metabolites, such as organic acids, which are important platform molecules with applications in a wide range of markets, such as food, plastics, coating, or cosmetics (e.g. Liaud et al., 2014; Jiménez-Quero et al., 2020).

Alternatively, plant wastes undergo bioprocessing, via fungal fermentation or enzyme treatment, for the release of the plant compounds that can be further processed into valuable chemicals. As an example, the pre-treatment of wheat straw, by fermentation with a ligninolytic fungus, significantly

improved the yields in released saccharides and the overall methane production yield after anaerobic yeast fermentation (Rouches et al., 2018). In another study, a two-step bioconversion process was used on rapeseed meal to convert the abundant sinapate esters into canolol, a compound with antioxidant, antimutagenic and anticarcinogenic properties. Rapeseed meals were first treated with a fungal feruloyl esterase to release free sinapic acid and then, the recovered sinapic acid was converted into canolol by the basidiomycete *Neolentinus lepideus* (Odinot et al., 2017; Laguna et al., 2019). Several other applications are emerging from plant waste bioprocessing using fungal enzymes, such as the production of polymer building blocks from plant-derived 5-hydroxymethylfurfural (HMF) (Daou et al., 2019 ; Birmingham et al., 2021) or the oxidation of plant derived alcohols into aldehydes for the flavors and flagrance industry (Ribeaucourt et al., 2021a,2021b).

Other recent advances in plant waste bioconversion are coming from lignin valorization. Lignin is an abundant feedstock issued from biomass refinery and the pulp/paper industries, which attracts interest for conversion into biofuels and biochemicals, owing to its high carbon-to-oxygen ratio and rich aromatic skeleton. One envisioned strategy is a two-step process, in which extracellular microbial enzymes generate the lignin-derived aromatics that are subsequently converted to value-added bioproducts through microbial metabolism. The ability of fungi to degrade native and technical lignin is yet unrivaled, and the bacterial species are usually slower at degrading lignin than fungal species, probably because of limited activity of the bacterial peroxidases on non-phenolic aromatic compounds (Linde et al., 2021**). However, several bacteria have shown the ability to change lignin-derived aromatic compounds into intermediary products, such as protocatechuic acid and catechol. Such bacteria can be used as chassis for metabolic engineering aimed at the conversion of lignin-derived aromatics in bio-based products, such as polyhydroxyalkanoates (PHAs), microbial lipids, vanillin, and muconic acids (Iram et al., 2021). Whether fungi do use lignin as a carbon source for their metabolism (e.g. Daou et al., 2021) has been a matter of debate until a recent systems biology approach demonstrated the ability of two wood decayers to channel lignin degradation products towards central metabolism via acetyl-CoA and succinyl-CoA intermediates. The proposed pathway is homologous to the bacterial aromatic catabolic pathway and involves oxidative decarboxylase and hydroxylase activities preceding aromatic ring-opening by a dioxygenase activity (del Cerro et al., 2021**).

Conclusion and perspectives

In the context of a pressing need for developing a sustainable bioeconomy, fungal biodiversity is a precious reservoir of enzymatic systems and metabolic pathways of high potential for the production of biochemicals and biomaterials derived from recalcitrant plant wastes. The wastes derived from agriculture, forestry or the agroindustry are then considered as co-products, whose valorization is expected to reduce the demand for fossil fuel while preserving land usage for food, feed and natural areas. A wealth of enzymes that cleave lignocellulose polymers have been identified in the fungal kingdom. Yet, the landscape of fungal lignocellulose degrading enzymes remains largely untapped. Some of the enzymes classified in the CAZy database still call for functional characterization, and new fungal CAZymes are still being discovered (e.g. Couturier et al., 2018; Filiatrault-Chastel et al., 2019).

Although the cost for enzyme production is still a limiting factor for the treatment of large volumes of biomass or the production of compounds of modest economic value, the use of plant wastes as a carbon source for the microorganisms that synthetize the enzymes lowers the ultimate cost for the production while reducing the waste burden from the environment. The use of filamentous fungi for plant waste bioconversion at industrial scales suffers from limited growth rate as compared to other

industrial microorganisms. However, fungi have the potential for combining plant polymer degradation in the extracellular space and the release of lignocellulose-derived molecules to intracellular metabolic pathways and their bioconversion in value-added molecules.

Biotechnological developments are underway to improve fungal strains, for example with the addition of optimized biosynthesis gene clusters within the genome, or the adjustment of metabolic pathways via CRISPR-Cas9/Cas12a-based genome manipulation (Asemoloye et al., 2021). Such developments may, in the near future, solve current limitations in using filamentous fungi at industrial scale.

After decades of works on industrial workhorses such as *T. reesei*, it is now admitted that a single fungus cannot outperform for all applications, and that the compounds released during extracellular digestion, including the toxic compounds, have to be sustained by the fungus, via adapted intracellular metabolic pathways. As an example, fungi that efficiently degrade lignin have to cope with toxic lignin-derived aromatic compounds. In the bioenergy area, fungi used for biomass pretreatment prior to yeast fermentation and ethanol production should alleviate the accumulation of microbial inhibitory compounds (Hahn-Hägerdal et al., 2007). In this context, the screening of fungal collections can guide the identification of the most suitable strains in dedicated applications (e.g. Zhou et al., 2015; Grandmontagne et al., 2021; Navarro et al., 2021). Our knowledge on the evolutive adaptations of fungi to specific plant hosts or substrates can guide the selection of targeted fungal taxonomic groups to optimize the outcome of the screenings. For example, the study of ligninolytic class II peroxidases in saprotrophic agaricomycetes and their evolution in wood and litter degraders has highlighted fungal species with rich gene repertoires and strong ligninolytic activities (Hage et al., 2021; Ruiz-Dueñas et al., 2021).

New research developments are rising from the discovery that co-cultures of different fungal strains may increase the production of enzymes (Baldrian, 2004) or allow for synergistic effects between enzymes produced by the different strains (Sugano et al., 2021). However, the implementation of such strategies will require a better understanding of the molecular interactions between the fungal strains.

Beyond organic wastes, fungi and their enzymatic systems dedicated to lignocellulose degradation are nowadays envisioned as relevant tools for the breakdown of synthetic molecules. The activity of fungal oxidases on aromatic substrates has stimulated the search for fungi able to degrade synthetic dyes, which have shown promising results (Eichlerová & Baldrian, 2020; Navarro et al., 2021). Recalcitrant lignocellulose polymers also share chemical properties (high molecular weight, hydrophobicity, insolubility) with artificial polymers and plastics, making fungi appealing clues for the bioremediation or recycling of these artificial wastes (Purohit et al., 2020; Daly et al., 2021). Although the use of fungi and their enzymes for plant waste valorization still faces technical challenges and cost effectiveness pitfalls to reach industrial scale, fungi will undoubtedly contribute to the transition from fossil carbon overexploitation to a bioeconomy centered on renewable carbon.

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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Ligninolytic peroxidases are restricted to a class of saprotroph basidiomycete fungi named Agaricomycetes. In this fungi, the ability to degrade lignin was associated with the appearance of Class II peroxidases that used diffusible metal cations to attack its phenolic moiety (manganese peroxidases). The authors show here that these enzymes would have acquired the ability to degrade nonphenolic lignin by using a tryptophanyl radical interacting with the bulky polymer at the surface of the enzyme. Most strikingly, using ancestral enzyme resurrection, they show that the surface tryptophan improved the enzyme activity on lignin and its stability at acidic pH. They propose that the evolution of ligninolytic peroxidases was most probably related to changes in the lignin composition of angiosperms as compared to gymnosperms.

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The authors provide an exhaustive overview of current knowledge on LPMOs, their catalytic properties and redox partners. They discuss the monooxygenase (O_2-based) and peroxygenase (H_2O_2-based) reaction paradigms currently asserted for LPMOs. They propose H_2O_2 could be a central regulatory metabolite in biomass conversion, and discuss how the balance between H_2O_2-producing and -consuming systems could contribute to the regulation of LPMO activity. Finally, they explore how our understanding of natural biomass conversion by fungi translates into the design of better industrial biorefining processes.

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Using comparative genomics, the authors identified a new family of fungal lytic polysaccharide monooxygenases (LPMOs) prevalent among saprotroph fungi with strong ability to degrade wood. They demonstrate that LPMOs classified in the AA14 family of the CAZy database are active on xylans. They show that the addition of these enzymes to currently available enzymatic coktails substantially increases the efficiency of saccharide extraction from wood.

Daly, P., Cai, F., Kubicek, C. P., Jiang, S., Grujic, M., Rahimi, M. J., Sheteiwy, M. S., Giles, R., Riaz, A., de Vries, R. P., Akcapinar, G. B., Wei, L., & Druzhinina, I. S. (2021). From lignocellulose to plastics : Knowledge transfer on the degradation approaches by fungi. In *Biotechnology Advances* (Vol. 50). https://doi.org/10.1016/j.biotechadv.2021.107770

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Whether wood decay fungi that degrade lignin are able to utilize lignin as a carbon source has been till now a matter of debate. By using a combination of ^{13}C-isotopic labeling, in silico genome analysis, proteomic, transcriptomic and metabolomic approaches, the authors show that two model white-rot fungi utilize poplar-derived aromatic compounds as a carbon source. They identify the intracellular dioxygenase enzymes able to cleave aromatic rings and propose the downstream catabolic steps towards central carbon metabolism. This work will undoubtedly have huge impact

on our capacity to use fungal enzymes and fungal fermentation for the valorization of lignin-derived compounds.

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Lombard, V., Guigliarelli, B., Biaso, F., Haon, M., Grisel, S., Henrissat, B., Welner, D. H., Master, E. R., Berrin, J. G., & Abou Hachem, M. (2021). Discovery of fungal oligosaccharide-oxidising flavo-enzymes with previously unknown substrates, redox-activity profiles and interplay with LPMOs. *Nature Communications*, *12*(1). https://doi.org/10.1038/s41467-021-22372-0

By using a combination of phylogenetics, enzyme biochemistry and structure-function analyses, the authors expand our knowledge on flavo-enzymes from the AA7 CAZy family. They provide the structure features that explain the distinction between oxidase and dehydrogenase members of the AA7 family, and show that AA7 dehydrogenases can potentiate LPMO activity by direct transfer of electrons. The demonstration of oligosaccharide-oxidizing activity expands the substrate range of fungal AA7s and the perspectives for the design of functional and efficient AA7-LPMO systems targeting recalcitrant biomass.

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This review describes current developments in the production of lignocellulolytic enzymes by solid state fermentation. It shows the advantages of solid-state fermentation for the production of enzymes with high stability at different temperatures and pH, improving their applicability in industrial settings. It presents the current limitations in large-scale productions and proposes future trends in SSF bioreactor design to achieve enzyme production with low environmental impact and low cost.

Liaud, N., Giniés, C., Navarro, D., Fabre, N., Crapart, S., Gimbert, I. H.-, Levasseur, A., Raouche, S., & Sigoillot, J.-C. (2014). Exploring fungal biodiversity : organic acid production by 66 strains of filamentous fungi. *Fungal Biology and Biotechnology*, *1*(1). https://doi.org/10.1186/s40694-014-0001-z

**Linde, D., Ayuso-Fernández, I., Laloux, M., Aguiar-Cervera, J. E., de Lacey, A. L., Ruiz-Dueñas, F. J., & Martínez, A. T. (2021). Comparing ligninolytic capabilities of bacterial and fungal dye-decolorizing peroxidases and class-ii peroxidase-catalases. *International Journal of Molecular Sciences*, *22*(5). https://doi.org/10.3390/ijms22052629

Recently, several reports on the ligninolytic activity of bacteria have emerged in the scientific literature. In bacteria, the ligninolytic capabilities are attributed to dye-decolorizing peroxidases (DyPs). However, the use of diverse conditions and substrates for the enzymatic assays made it difficult to get a clear view on the efficiency of bacterial and fungal DyPs and to compare them with fungal Class II peroxidases. In this work, two bacterial DyPs, one fungal DyP, and two class-II fungal peroxidases, a VP and a LiP, were comparatively studied. Using monomeric and dimeric model compounds as substrates, the authors observed sharp differences in the oxidation of nonphenolic aromatics, with LiP showing the highest catalytic efficiencies. The authors observed no activity for the bacterial DyPs on non-phenolic models. This work now allows the selection of efficient enzymes for the production of aromatic compounds from technical lignin and leverage the economic sustainability of bio-refineries.

*Manavalan, T., Stepnov, A. A., Hegnar, O. A., & Eijsink, V. G. H. (2021). Sugar oxidoreductases and LPMOs – two sides of the same polysaccharide degradation story ? In *Carbohydrate Research* (Vol. 505). https://doi.org/10.1016/j.carres.2021.108350

The efficiency of LPMOs on recalcitrant polymers has recently stimulated research on the overall

redox processes that regulate their activity. In this review, the authors expose the enzymatic and abiotic processess that impact the reducing power, and the hydrogen peroxide and oxygen availability at the vicinity of the enzyme. They describe the various electron sources so far identified that reduce LPMOs to the active form, and discuss the roles of the fungal oxidoreductases that might fuel their activity *in vivo* and *in vitro*.

Martínez, A. T., Camarero, S., Ruiz-Dueñas, F. J., & Martínez, M. J. (2018). Chapter 8 : Biological Lignin Degradation. In *RSC Energy and Environment Series* (Vols. 2018-January, Issue 19). https://doi.org/10.1039/9781788010351-00199

Martínez, Á. T., Speranza, M., Ruiz-Dueñas, F. J., Ferreira, P., Camarero, S., Guillén, F., Martínez, M. J., Gutiérrez, A., & Del Río, J. C. (2005). Biodegradation of lignocellulosics : Microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *International Microbiology*, *8*(3).

Mathé, C., Fawal, N., Roux, C., & Dunand, C. (2019). In silico definition of new ligninolytic peroxidase sub-classes in fungi and putative relation to fungal life style. *Scientific Reports*, *9*(1). https ://doi.org/10.1038/s41598-019-56774-4

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Miyauchi, S., Navarro, D., Grigoriev, I. V., Lipzen, A., Riley, R., Chevret, D., Grisel, S., Berrin, J.-G., Henrissat, B., & Rosso, M.-N. (2016). Visual comparative omics of fungi for plant biomass deconstruction. *Frontiers in Microbiology*, *7*(AUG). https://doi.org/10.3389/fmicb.2016.01335

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*Muggia, L., Ametrano, C. G., Sterflinger, K., & Tesei, D. (2020). An overview of genomics, phylogenomics and proteomics approaches in ascomycota. In *Life* (Vol. 10, Issue 12). https://doi.org/10.3390/life10120356

The authors provide an exhaustive overview of the major advances in fungal genomics, and the recent technological advances that provide access to yet untapped phylogenetic taxa or environmental samples. They describe how the expansion of genomic data supports phylogenomic and proteomic studies, and enhance our understanding on the fungal evolution and fungal biological functions. They report several examples selected from plant and animal opportunistic and pathogenic, extremophilic/polyextremotolerant filamentous and yeast-like fungi, as well as lichenized fungi.

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Navarro, David, Chaduli, D., Taussac, S., Lesage-Meessen, L., Grisel, S., Haon, M., Callac, P., Courtecuisse, R., Decock, C., Dupont, J., Richard-Forget, F., Fournier, J., Guinberteau, J., Lechat, C., Moreau, P. A., Pinson-Gadais, L., Rivoire, B., Sage, L., Welti, S., … Favel, A. (2021). Large-scale phenotyping of 1,000 fungal strains for the degradation of non-natural, industrial compounds. *Communications Biology*, *4*(1). https://doi.org/10.1038/s42003-021-02401-w

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*Ruiz-Dueñas, F. J., Barrasa, J. M., Sánchez-García, M., Camarero, S., Miyauchi, S., Serrano, A., Linde, D., Babiker, R., Drula, E., Ayuso-Fernández, I., Pacheco, R., Padilla, G., Ferreira, P., Barriuso, J., Kellner, H., Castanera, R., Alfaro, M., Ramírez, L., Pisabarro, A. G., … Martínez, A. T. (2021). Genomic Analysis Enlightens Agaricales Lifestyle Evolution and Increasing Peroxidase Diversity. *Molecular Biology and Evolution*, *38*(4). https://doi.org/10.1093/molbev/msaa301

Agaricales are a group of saprotroph basidiomycetes with diverse substrate preferences (leaf litter, grass litter, wood, decayed wood). Using in-depth phylogenetic analysis of lignilolytic peroxidases, the authors show that the sequence diversity of these enzymes is higher than previously reported, and propose that differences in the protein sequences may acount for different activities on lignins with diverse chemical compositions.

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In practice, the fractionation of lignin and technical lignin is impaired by repolymerization reactions (i.e. lignin condensation) that lead to low monomer yields and create modified lignin structures that are highly recalcitrant to further degradation. Here the authors propose a membrane filtration system to extract the reaction products produced during the laccase treatment of an industrial lignin stream. They provide the proof-of-concept demonstrating that *in-situ* membrane extraction of the reactive fragments of lignin from the bulk medium can be useful against detrimental repolymerization reactions. This work provides a promising perspective on the valorization of lignin-rich industrial wastes.

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