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10TH HARDWOOD CONFERENCE PROCEEDINGS

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A comparison of the wood decay abilities of common white-rot fungi from the Carpathian Basin

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ABSTRACT

Saprotrophic white-rot (WR) fungi play an essential role in the carbon cycle of forest ecosystems by efficiently breaking down and mineralizing all lignocellulosic substances in the forests using a wide variety of extracellular hydrolytic and oxidative wood-degrading enzymes. Here we compared the plant cell wall degrading enzyme (PCWDE) profiles of regularly occurring *Armillaria* species with those of the most common WR fungi, including two saprotrophic (*Dichomitus squalens*, *Phanerochaete chrysosporium*) and two pathogenic (*Ganoderma lucidum*, *Heterobasidion annosum*) species from the Carpathian Basin. Our results show that genes related to lignocellulose, crystalline cellulose and pectin degradation are highly prominent in *Armillaria* species compared to the other coexisting WR species. The data is consistent with a possible prevailing role of armillarioids in forest communities in lignocellulose degradation and assertive invasive, pathogenic activities.

INTRODUCTION

White-rot (WR) fungi, using various hydrolases, esterases, lyases and oxidative enzymes, are capable of efficiently degrading all wood-composing major biopolymers, including the highly recalcitrant lignin, crystalline cellulose and cellulose-bound xylan, and also all of the more accessible, regular plant cell wall components, cellulose, hemicellulose and pectin (Riley *et al.*, 2014; Couturier *et al.*, 2018; Miyauchi *et al.*, 2020a).

Wood-decomposing fungi use a wide range of plant cell wall-degrading enzymes (PCWDEs) which belong to carbohydrate-active enzymes (CAZymes). CAZymes cover over 300 protein families classified into six major classes based on their amino acid sequence similarity, protein domain structures and enzymatic mechanisms. Three of the six classes, glycoside hydrolases (GHs), carbohydrate esterases (CEs), and polysaccharide lyases (PLs), represent degradative enzymes. Two additional classes, the auxiliary activity enzymes (AAs), typically through their redox-active contributions, and other modular proteins carrying specific carbohydrate-binding domains (CBMs), help condition GH, CE, and PL enzymes to access complex carbohydrates, thereby harnessing them to partake in various PCWDE activities. (Cantarel *et al.*, 2009; Levasseur *et al.*, 2013).

We aimed to compare the full genomic PCWDE repertoires of four regularly occurring *Armillaria* species (*A. cepistipes*, *A. gallica*, *A. mellea* and *A. ostoyae*) to four common basidiomycete WR fungi, representing pathogenic (*Ganoderma lucidum*, *Heterobasidion annosum*) and saprotrophic (*Dichomitus squalens*, *Phanerochaete chrysosporium*) species from the Carpathian Basin.

The four *Armillaria* species have distinct habitat preferences, with *A. ostoyae* and *A. cepistipes* invading coniferous trees, while *A. mellea* and *A. gallica* preferably inhabiting deciduous forests. Regarding their pathogenicity potential, *A. mellea* and *A. ostoyae* are facultative necrotrophic species acting as primary pathogens, while *A. gallica* and *A. cepistipes* are considered as opportunistic pathogens invading weakened, compromised trees and forests (Kedves *et al.*, 2021). *Armillaria* species are reliable indicators of an incipient forest decline, and as destructive WR fungi, they can further damage and destroy the weakening trees. Regarding the other common WR species, *P. chrysosporium*, the first basidiomycete whose genome

was sequenced, is a saprotrophic WR fungus known as a very efficient lignin degrader of both hardwood and softwood trees (Singh and Chen, 2008). The second saprotrophic WR species, *D. squalens*, is an efficient saprotroph found mainly on softwoods but it can also grow on hardwoods (Kowalczyk et al., 2019). Finally, regarding the two pathogenic WR species, *G. lucidum* is a widely distributed pathogen of both softwoods and hardwoods (Grinn-Gofroń *et al.*, 2021), while *H. annosum* is recognised as a severe pathogen of coniferous trees (Brūna *et al.*, 2021).

Here we focus on contrasting the CAZyme profiles of *Armillaria* species with those of the other common WR fungi. Therein, we aim to identify the genome-level traits behind the superbly efficient lignocellulose degrading abilities of the armillarioids and to shed light on the distinctive pectin-degrading arsenals, as possible pathogenic or virulence-related activities that make virulent *Armillaria* species a severe threat to a wide range of tree species.

EXPERIMENTAL METHODS

The amino acid sequences of *A. mellea*, *A. gallica*, *A. cepistipes*, *A. ostoyae*, *G. lucidum*, *D. squalens*, *H. annosum*, and *P. chrysosporium* were downloaded from the JGI Mycocosm (<https://sfamjournals.onlinelibrary.wiley.com/doi/epdf/10.1111/1462-2920.14416/mycocosm.jgi.doe.gov/mycocosm/home>) website (Table 1).

Table 1: List of the fungal species used for the comparative genomics analyses

Organism	JGI name	Number of Proteins
<i>Armillaria cepistipes</i>	Armillaria cepistipes B5	23460
<i>Armillaria ostoyae</i>	Armillaria ostoyae C18/9	22705
<i>Armillaria gallica</i>	Armillaria gallica 21-2 v1.0	25704
<i>Armillaria mellea</i>	Armillaria mellea ELDO17	15 646
<i>Heterobasidion annosum</i>	Heterobasidion annosum v2.0	13 405
<i>Ganoderma lucidum</i>	Ganoderma sp. 10597 SS1 v1.0	12 910
<i>Dichomitus squalens</i>	Dichomitus squalens CBS464.89 v1.0	15 295
<i>Phanerochaete chrysosporium</i>	Phanerochaete chrysosporium RP-78 v2.2	13 602

To perform CAZyme annotation of the downloaded amino acid sequences, we used dbcan2 (<http://cys.bios.niu.edu/dbCAN2>) (Zhang *et al.*, 2018) which utilizes three cutting edge tools such as diamond BLAST, HMMER (pattern search using hidden Markov model) and hotpep (homology to peptide) to predict CAZymes using the latest CAZyme database. The CAZymes were further classified into subcategories based on their functionality, as described by Miyauchi *et al.* (2020b).

RESULTS AND DISCUSSION

We analyzed 142727 proteins and, in total, identified 2638 (*A. cepistipes*: 417, *A. gallica*: 472, *A. mellea*: 420, *A. ostoyae*: 424, *D. squalens*: 246, *G. lucidum*: 276, *H. annosum*: 191, *P. chrysosporium*: 192) CAZyme candidates, and found a dominance of PCW numbers over FCWs in all fungal species (Fig 1).

Regarding lignin degradation, which in the first place involves high-oxidation potential class II peroxidases (PODs), *Armillaria* species possess a significantly higher number of genes (Fig 2), indicating that the lignocellulose-decomposing and lignin-mineralizing potential is indeed overwhelming in all *Armillaria* species independently of host or habitat specialization (Sipos *et al.*, 2017). Besides PODs (AA2), numerous other enzymes capable of degrading or modifying lignin and lignocellulose are classified under Auxiliary Activities (AA families). Laccases, one of the AA1 subfamilies (AA1_1) capable of cleaving lignin bonds, and other AA class enzymes, AA3_2 and AA7, were far more prominent in *Armillaria* species than in the other WR fungi we analyzed (Fig 2). AA3_2 genes depict secreted aryl-alcohol oxidases, which, by producing hydrogen peroxide, stimulate lignocellulose degradation in cooperation with the peroxidases. AA7 genes represent oligosaccharide flavo-oxidases which may transfer electrons to an AA9 lytic polysaccharide monooxygenase (LPMO) and facilitate cellulose degradation independently from exogenous reductants (Haddad Momeni *et al.*, 2021). Haddad Momeni *et al.* also found AA7 counts highly abundant in pathogenic fungal species, suggesting its role in virulence for *Armillaria* species.

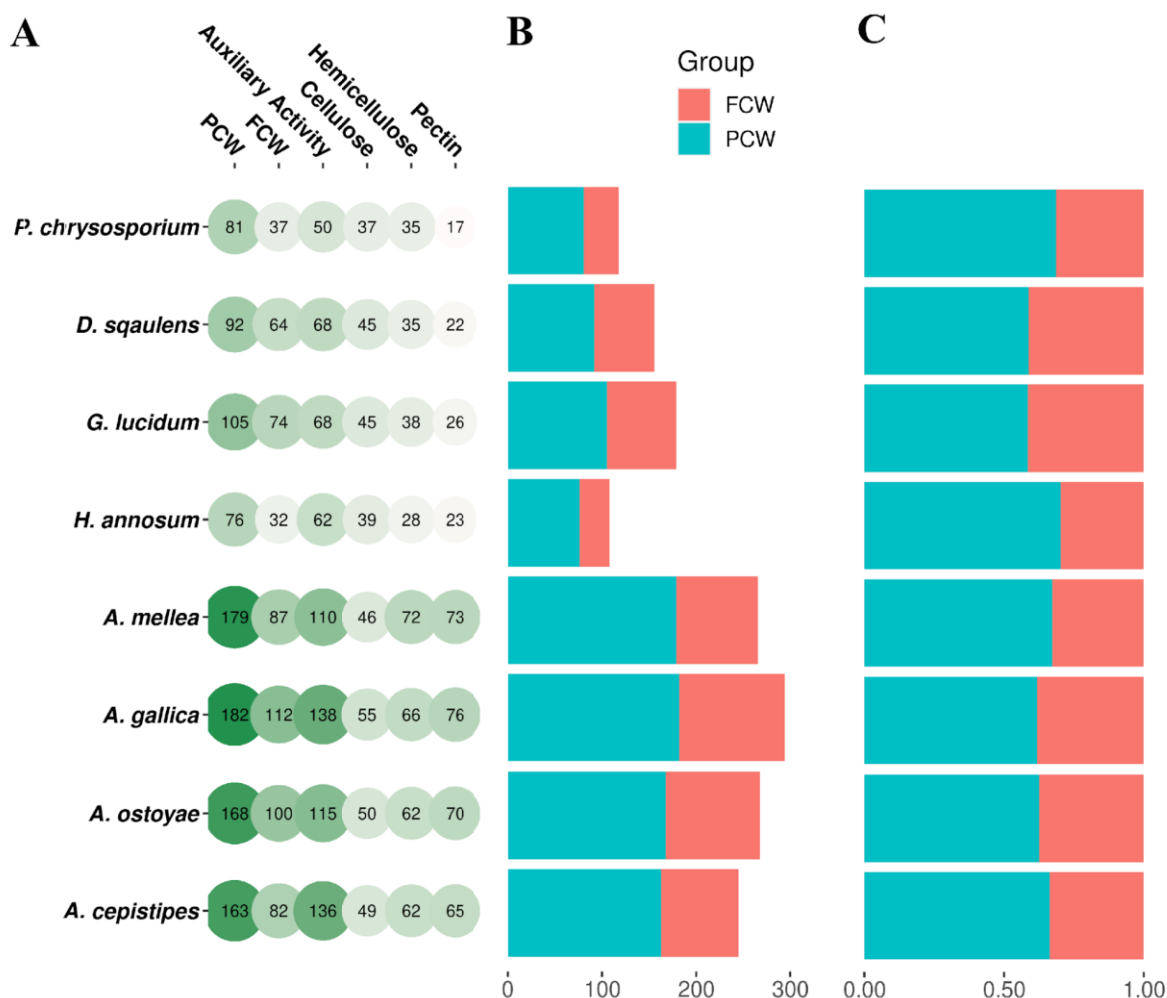


Figure 1: Count distribution of major CAZyme categories (PCW and FCW), classified according to Miyauchi *et al.* (2020a). PCW refers to those groups of enzymes potentially involved in plant cell wall degradation and FCW depicts the fungal cell wall-associated enzymes. A) Species-wise count distribution of PCW, FCW, Auxiliary activity (including lignin-related activities), Cellulose, Hemicellulose and Pectin degrading CAZymes. B) PCW and FCW refer to the total counts in each fungal genome. C) PCW and FCW counts indicate their ratios in each fungal genome

We found that the AA7 gene repertoire was exclusive and highly expanded in *Armillaria* species in contrast to the other WR species analyzed. Interestingly, although AA7 genes seemed prevalent in phytopathogenic fungi (Momeni *et al.*, 2021), they were absent from the genome of *H. annosum*, a severe WR pathogen of coniferous forests.

CBM families are involved in aiding the other groups of CAZymes in accessing plant polysaccharides. *Armillaria* species showed increased counts of xylan, chitin and pectin binding CBMs including CBM5, CBM13, CBM50 and CBM67 families (Fig. 2). Several carbohydrate esterases (CEs), which hydrolyze polysaccharides by de-O or de-N acylation, were also expanded in armillarioids compared to other fungal species. The most prominently represented CE4 and CE8 families are involved in hemicellulose and pectin hydrolyzation (Andlar *et al.*, 2018; Benoit *et al.*, 2012).

Glycoside hydrolases (GHs) refer to the enzymes that catalyze the hydrolysis of the glycosidic linkage of glycosides. PCW-degrading glycoside hydrolases GH1 and GH28 were represented with the highest gene numbers in the armillarioids (Fig 2). The GH28 family was already reported by Sprockett *et al.* (2011) to be expanded in necrotrophic fungal species, and GH28 enzymes might also significantly contribute to the necrotrophic lifestyle of pathogenic *Armillaria* species. Other virulence factor candidates, the pectin degrading PL1 and PL3 genes, were already reported to be overrepresented in *Armillaria* genomes (Sipos

et al. 2017). Therefore, the presence of an extensive repertoire of pectin-modifying and -degrading enzymes could profoundly contribute to the plant invasive, necrotrophic behavior of the armillarioid species.

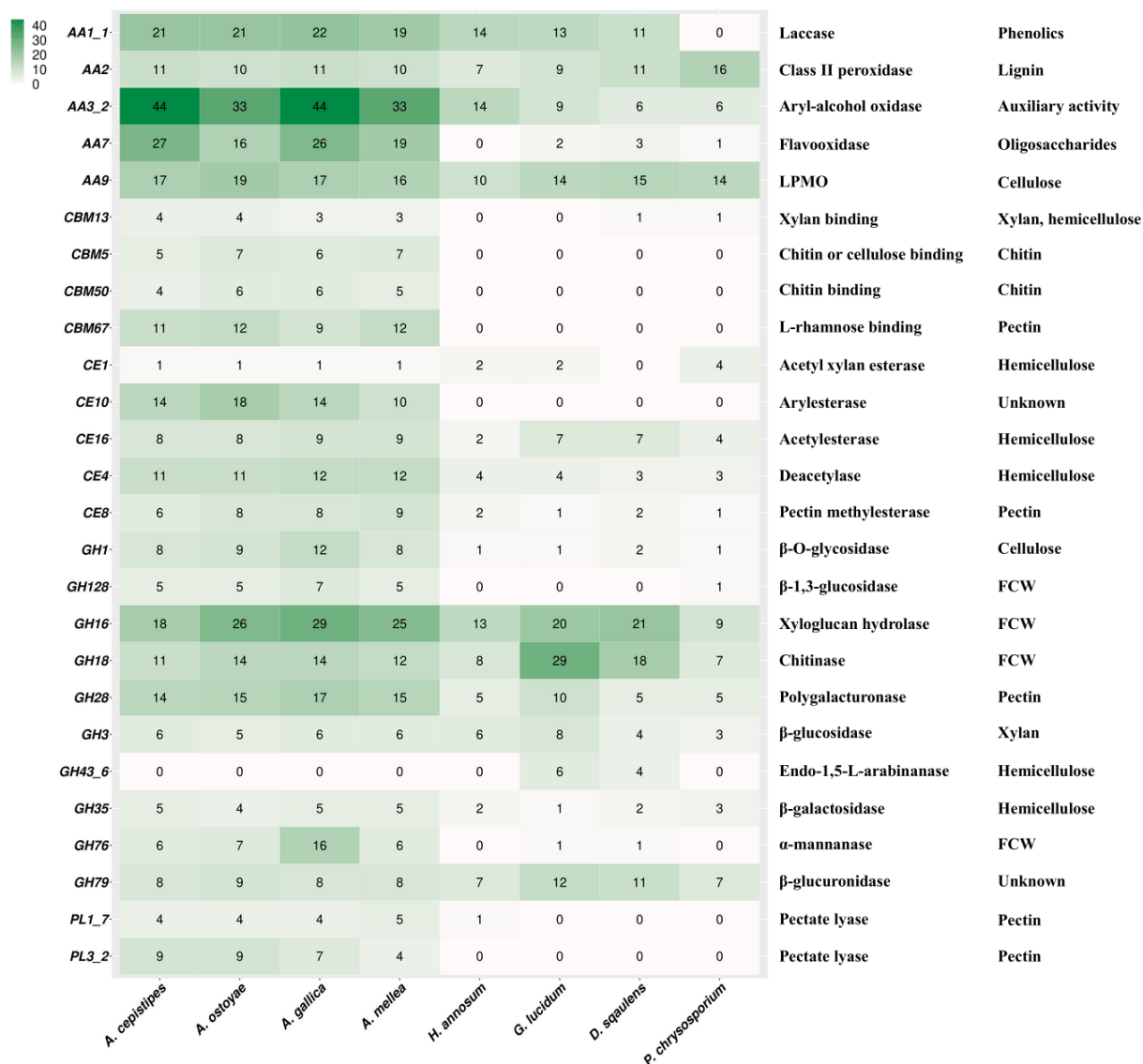


Figure 2: Distinguishing CAZyme class counts for eight WR species. AA: auxiliary activity, CBM: carbohydrate-binding module, CE: carbohydrate esterases, GH: glycoside hydrolases and PL: polysaccharide lyases

CONCLUSIONS

We compared the plant cell wall biopolymer-degrading CAZyme profiles in the four most common white-rot fungi of the Carpathian Basin with those of the coexisting *Armillaria* species. We found that armillarioids have a much richer arsenal of lignin-, pectin-, cellulose-, and hemicellulose-degrading enzymes. The large repertoires of lignocellulose-degrading enzymes may well contribute to the distinctive wood-decaying potential of the armillarioids and make them suitable candidates for applications in commercial areas such as biomass processing for biofuel production (Abraham and Puri, 2020). In addition, the extracellular enzymes produced by armillarioid species belonging to auxiliary activities (AA), besides degrading lignin and crystalline cellulose, can efficiently decompose all other phenolics and terpene-related compounds produced in plant tissues (Andlar et al., 2018).

The expanded repertoire of certain PCWDE families, including lignocellulose- and pectin-degrading enzymes, not common in other WR fungi, may well contribute to the overall impact of *Armillaria* species

as efficient saprotrophic wood decayers and underground survivors, as well as severe necrotrophic forest pathogens.

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