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Review

Antifungal activities of wood extractives

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ABSTRACT

Extractives are non-structural wood molecules that represent a minor fraction in wood. However, they are source of diverse molecules putatively bioactive. Inhibition of fungal growth is one of the most interesting properties of wood extractives in a context of wood preservation, crop protection or medical treatments. The antifungal effect of molecules isolated from wood extractives has been mainly attributed to various mechanisms such as metal and free radical scavenging activity, direct interaction with enzymes, disruption of membrane integrity and perturbation of ionic homeostasis. Lignolytic fungi, which are microorganisms adapted to wood substrates, have developed various strategies to protect themselves against this toxicity. A better knowledge of these strategies could help both developing new systems for extractive removal in biomass valorization processes and using these molecules as antifungal agents.

1. Introduction

Wood is an abundant renewable material composed of three valuable polymers, lignin, cellulose and hemicellulose. Most applications focus on the valorization of cellulose and hemicellulose as a resource for energy and fuel production (FitzPatrick *et al.*, 2010). Lignin, an alkyl-aromatic polymer comprising 15–30 % of biomass, is typically underused in selective conversion processes because of its intrinsic heterogeneity, which presents technical challenges for producing lignin-derived chemicals or biofuels (Beckham *et al.*, 2016). In nature, microorganisms have adapted to use this complex substrate as a carbon source. In particular, white-rot fungi are the only organisms capable of completely mineralizing all components of wood, by secreting a complex enzymatic system to breakdown both cellulose and lignin. This enzymatic system is composed of Carbohydrate-Active Enzymes (CAZYmes) that modify and breakdown oligo- and poly-saccharides, and peroxidases, laccases, and additional oxidative enzymes generating aromatic radicals that cleave diverse lignin ether linkages through non-enzymatic reactions (Martínez *et al.*, 2005). From an ecological point of view, these fungi are thus key actors in carbon cycling in the biosphere. Meta-analyses revealed that wood traits such as wood nitrogen and phosphorus concentrations, C:N ratio, lignin content, wood density and maximum tree height and diameter, are important drivers of variation in wood decomposition rates by microorganisms, mainly by governing fungal community

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and nutritional strategies (Pietsch *et al.*, 2014; Schilling *et al.*, 2015; van Geffen *et al.*, 2010; Weedon *et al.*, 2009; Yamashita *et al.*, 2015). Moreover, wood decomposability could also be driven by the chemical composition of lignin and hemicelluloses, and the variability of extractives. The latter are non-cell wall small molecules that can be removed from wood by solvents. These compounds can be classified in 2 main groups: (1) aliphatic and alicyclic compounds (terpenes and terpenoids (including resin acids and steroids), esters of fatty acids, fatty acids and alcohols, alkanes) and (2) phenolic compounds (simple phenols, stilbenes, lignans, isoflavones, condensed tannins, flavonoids and hydrolyzable tannins) (Stenius, 2000). The composition of extractives in wood varies widely from species to species, and it depends on which part of the tree was used for the isolation, the geographical origin, and the season of sampling (Doussot *et al.*, 2002; Kebbi-Benkeder *et al.*, 2015; Prida and Puech, 2006). Moreover, the total amount of extractives in a given species depends on growth conditions like carbon dioxide concentrations or temperature (Kilpeläinen *et al.*, 2005). Chemical extractives contents (organic, aqueous, tannic and phenolic) are generally higher in the heartwood, the more colored part of wood that is also more resistant to fungal attack (Schultz and Nicholas, 2000). Heartwood has been analyzed both by time-of-flight secondary ion mass spectrometry (TOF-SIMS) imaging and gas chromatography-mass spectrometry in *Cryptomeria japonica*. The results showed that the predominant constituent of heartwood extractives was ferruginol, a diterpene phenol, that was almost evenly distributed in heartwood tissue (in the tracheid cell walls, in the cell walls of the axial parenchyma cells and ray parenchyma cells, and also inside these parenchyma cells) (Imai *et al.*, 2005). High amount of extractives, even more than in heartwood, have been detected in knotwood. This could be due to branch departure that constitutes potential weakness subjected to biotic and abiotic injuries, and to the natural ability of trees to self-prune, since species with high flavonoid content are generally bad pruning species (Kebbi-Benkeder *et al.*, 2015). Extractives are synthetized by the tree during its life to protect itself against biotic and abiotic attack. While remaining within the dead wood, these molecules may be important controllers of decomposition rates. Indeed a close correlation has been established between wood durability and extractive content and diversity (Kirker *et al.*, 2016; Pometti *et al.*, 2010). In accordance, some fungal species as *Coniophora puteana*, *Heterobasidion annosum*, *Phellinus sulphurascens*, and *Phellinus weirii* were able to grow on and decay leached western red cedar (WRC) wood more readily than non-leached WRC. This observation has been correlated to the amount of extractive compounds, since leached wood contained ~80 % less extractives than non-leached WRC (Chedgy *et al.*, 2009).

In this review, we describe the antifungal activities of wood extractives and the systems developed by wood decay fungi to bypass the inhibitory activity of these molecules.

2. Antifungal activity of wood extractives

The antifungal effect of some molecules found in wood extractives has been demonstrated at length in the literature. Some examples are shown in Table 1. Their bioactivity has been attributed to various mechanisms such as metal and free radical scavenging activity, direct interaction with enzymes, disruption of membrane integrity and perturbation of ionic homeostasis (Fig. 1).

Inhibition of the degradative capabilities of rot fungi

Metal and free radical scavenging

The metal chelation property of wood extractives could drastically impact wood degradation activities of fungi, which use haem-containing class II peroxidases and copper-dependent laccases in the case of white-rot fungi, and Fenton-mediated radical production in the case of brown-rot fungi (Pollegioni *et al.*, 2015). Troponoids are a family of compounds that possess antifungal activity both on white and brown-rot fungi, through the binding of ferric or ferrous iron to form $[Fe(trop)_3]$ or $[Fe(trop)_2]$ precipitates respectively, and the inhibition of Fe(III) reduction avoiding initiation of the Fenton reaction (Diouf *et al.*, 2002; Yen *et al.*, 2008). Β-thujaplicin, one of the most known troponoids reduces the activity of laccase, but also other metal-containing enzymes as tyrosinase and lipoxygenase (Poma *et al.*, 1999; Suzuki *et al.*, 2000).

Besides metal scavenging, wood extractives can scavenge reactive oxygen species that are required for the enzymatic or oxidative process of wood decay. Indeed, lignocellulolytic enzymes are too large to penetrate into undecayed wood cell walls; therefore, they generate radical intermediates responsible for local lignin attack. Lignans and flavonoids have a high antioxidative potency and/or radical scavenging capacity (Willför *et al.*, 2003). For example, the flavonoids 3,4′,7,8-tetrahydroxyflavanone and teracacidin have antifungal activities, *in vitro* radical scavenging activity and are able to inhibit laccase activity on syringaldazine. The kinetics studies of laccase activity with teracacidin as an inhibitor suggest that teracacidin might not act directly on the active site of laccase, but instead inhibit the radical reaction after laccase forms a complex with syringaldazine (Mihara *et al.*, 2005). Another study showed that phenol-rich extracts of different red maple (*Acer rubrum* L.) tissues possess high radical scavenging capacities against superoxide anion hydroxyl radical, peroxyl radical, hypochlorite ion, hydrogen peroxide and nitric oxide. In particular, stem bark extracts, which yielded the higher content of total phenol among the studied tissues, were the most efficient in radical scavenging (Royer *et al.*, 2011).

Direct binding onto wood degradative enzymes

As described above, laccase activity can be reduced by the low availability of metal and reactive oxygen species due to extractive chelation. Moreover, docking analyses showed the ability of medicarpin, a phytoalexin identified in *Dalbergia congestiflora* heartwood, to inhibit *Trametes versicolor* laccase (PDB ID: 1GYC) by directly binding (1) to the T1 site of the protein, reducing bonding with lignin components, (2) at the O_2 channel site preventing O_2 reduction and (3) at the H_2O channel site blocking the release of water molecules (Martínez-Sotres *et al.*, 2015, 2012). Few examples concern the inhibition of lignolytic peroxidases, which seem not to be affected. Indeed, phenolic load showed an inhibitory effect on laccase, while it was not