BIOCHEMICAL FEATURES INFLUENCING MUSHROOM-SUBSTRATE COMPATIBILITY

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ABSTRACT

The capacity of mushroom fungi to utilise the wide range of lignocellulosic substrates adopted for cultivation depends in large part on the production of hydrolytic and oxidative enzymes (cellulases, hemicellulases and ligninases) that degrade the major component macromolecules (cellulose, hemicellulose, lignin). The proportion of these macromolecules in different lignocellulosic materials, and the ability of a given mushroom species to degrade them, can vary significantly. In particular, although the cellulose and hemicellulose components are readily solubilised by the fungal hyphae to provide nutrients for growth, considerable variation exists in the ability of a given species to degrade the more complex and recalcitrant lignin element. This, in turn, is dependent on the different combinations of ligninases (e.g. lignin peroxidases, manganese peroxidases, laccases) produced by the fungus. Thus, while *Volvariella volvacea* is a prolific producer of key cellulolytic enzymes (endoglucanases, cellobiohydrolases, β-glucosidases), among the recognized ligninolytic enzymes it appears able to synthesize only laccases. This is reflected in the ability of the straw mushroom to grow well on cotton wastes consisting largely of cellulose but not on more "woody" materials such as sawdust. Conversely, mushroom species able to synthesize ligninolytic peroxidases (*Lentinula edodes*, *Pleurotus* spp.) can grow on a wider spectrum of lignocellulosic wastes. Also linked to mushroom-substrate compatibility is the enzymic capacity of the former to detoxify harmful compounds (e.g. phenolics) often present in the latter. Using specific examples, this presentation will detail key biochemical features involved in lignocellulose degradation and in the neutralisation of toxic constituents, and will correlate these features to mushroom-substrate compatibility.

Keywords: substrate compatibility, cellulases, hemicellulases, ligninolytic enzymes, *Volvariella volvacea, Lentinula edodes*, *Pleurotus*

INTRODUCTION

Vast amounts of lignocellulosic waste which is the major structural component of woody and non-woody plants, are generated annually through the agricultural, forestry and food processing industries. Edible mushroom cultivation represents an economically-viable system for converting these wastes into an added-value commodity for use either as a nutritious food source or as a source of 'mushroom nutriceuticals' [1]. Consequently, a wide range of lignocellulosic residues have been adopted for this purpose (Table 1).

Table 1. Examples of lignocellulosic wastes used for mushroom cultivation

Cereal straws	Sawdust
Bagasse	Corn cobs
Wood pulp	Oil palm waste
Cotton wastes	Coconut husks
Coffee grounds	Banana leaves
Water hyacinth	Tree bark

Since mushrooms are unable to use solar energy, they synthesize a wide range of extracellular enzymes that allow them to degrade complex organic macromolecules present in the growth substrate into soluble compounds that can be absorbed by the fungus for nutrition ("nutritive absorption"). Both fungal and substrate-associated factors will determine the ability of a given mushroom species to grow and fruit on a particular lignocellulosic material.

Plant cell walls are composed of three major constituents: cellulose, hemicellulose and lignin. Lignin is particularly difficult to biodegrade, and reduces the bioavailability of the other cell wall constituents. Therefore, a key fungal-associated determinant will be the mushroom's capacity to synthesize the hydrolytic and oxidative enzymes required to degrade these macromolecules. Cellulose is a linear polymer of β-1,4-linked anhydrous glucose units, and the basic repeating unit is cellobiose. The chains are packed into arrays called microfibrils that contain both crystalline (ordered) and amorphous

(less-ordered) regions. The simplicity of the cellulosic structure, using repeated identical bonds, means that only a small number of enzymes are required to degrade this material. Hemicelluloses (xylans and mannans) are branched polymers of xylose, arabinose, galactose, mannose and glucose with various side-chain substituents (e.g. galactose, acetyl), attached by different glycosidic linkages. Hemicelluloses bind bundles of cellulose fibrils to form microfibrils, which enhance the stability of the cell wall. They also cross-link with lignin, creating a complex web of bonds which provide structural strength, but also challenge microbial degradation. Lignin is a complex polymer of phenylpropane units (*p*-coumaryl, coniferyl, sinapyl), which are cross-linked to each other with a variety of different chemical bonds (Fig. 1). Gymnosperm lignins are composed mainly of coniferyl alcohol units, angiosperm lignins of approximately equal amounts of coniferyl and sinapyl residues, and grass lignins of approximately equal amounts of all three cinnamyl alcohols [2].

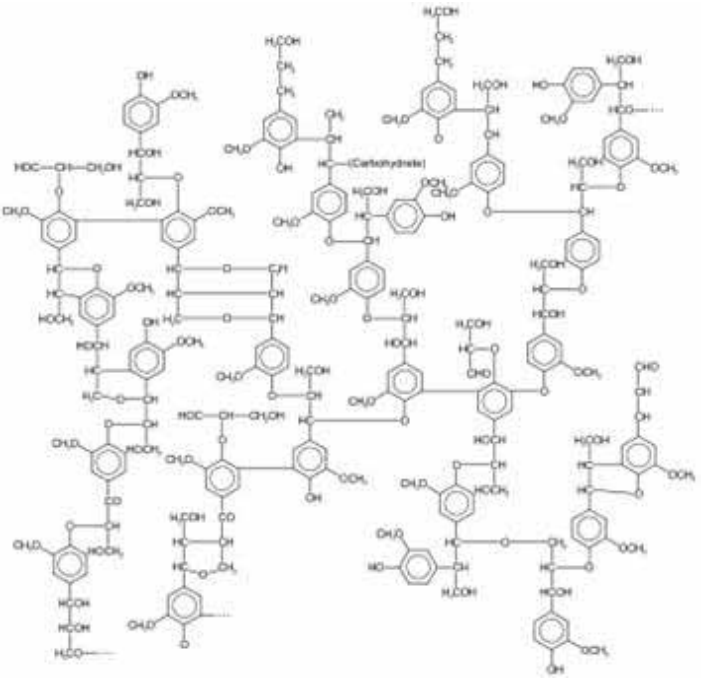

Figure 1. Representative section of the lignin macromolecule

Since lignin is the most recalcitrant component of plant cell walls, the higher the proportion of lignin the lower the bioavailability of the substrate. The effect of lignin on the bioavailability of other cell wall components is thought to be largely a physical restriction, with lignin molecules reducing the surface area available to enzymatic penetration and activity [3]. The distribution of cellulose, hemicellulose and lignin in some materials adopted for mushroom cultivation is shown in Table 2.

Table 2. Cellulose, hemicellulose and lignin content of some representative materials used for mushroom cultivation

Residue	Lignin (%)	Cellulose (%)	Hemicellulose (%)
Wheat straw	18.0	30.5	28.4
Barley straw	11.0	48.0	21.0
Rice straw	12.5	32.1	24.0
Cotton straw	15.0	42.0	12.0
Cottonseed hulls	24.0	49.0	17.0
Bagasse	18.9	33.4	30.0
Peanut hulls	23.0	42.0	9.0
Ground corn cobs	7.0	28.0	55.0
Soy bean hulls	2.0	48.0	17.0

Enzymes involved in the degradation of lignocellulosic wastes

Three classes of enzymes catalyse the degradation of the major macromolecular components of lignocellulose, namely cellulases, hemicellulases and ligninases. Conversion of cellulose to glucose requires the activity of three hydrolytic enzymes: endoglucanases (EC 3.2.1.4) (EGs) that attack amorphous regions in the cellulose chain, cellobiohydrolases (EC 3.2.1.91) (CBHs) that release cellobiose units and cello-oligomers, and β-glucosidases (EC 3.2.1.21) (BGLs) that hydrolyse cellobiose and cello-oligomers to glucose. EGs and CBHs are located extracellularly, while three types of β-glucosidase have been reported, i.e. extracellular, intracellular and plasma membrane-bound. Complete breakdown of hemicellulose requires

endo-1,4- β-xylanases (EC 3.2.1.8) and endo-1,4- β-mannanases, which attack the backbone chains of xylans and mannans generating non-substituted or branched xylo- and manno-oligosaccharides, respectively. The main chain substituents are released by the corresponding glycosidases and esterases. Extracellular enzymes involved in lignin transformation are lignin peroxidases (EC 1.11.1.14) (LiPs), manganese peroxidases (EC 1.11.1.13) (MnPs), versatile peroxidases (EC 1.11.1.16) (VP, hybrid peroxidase, polyvalent peroxidase) [4] and laccases (EC 1.10.3.2). LiPs oxidizes nonphenolic lignin substructures by abstracting one electron and generating cation radicals that are then decomposed chemically [5]. MnPs oxidize Mn (II) to Mn (III) which then oxidizes phenolic rings to phenoxyl radicals which lead to decomposition [6]. VPs, although catalyzing the oxidation of Mn^{2+} to Mn^{3+} by H_2O_2, differ from the above enzymes by their manganese-independent activity enabling them to oxidize substituted phenols by a mechanism similar to that reported for LiP. Laccases oxidized phenols to phenoxyl radicals as well as catalyzing other reactions in the presence of mediators [7]. Based on the enzyme production patterns of a range of lignin-transforming fungi, three categories have been proposed: (i) lignin-manganese peroxidase group (ii) manganese peroxidase-laccase group and (iii) lignin peroxidase-laccase group [8]. The most efficient lignin degraders, based on the release of $^{14}CO_2$ from ^{14}C-[Ring]-labelled synthetic lignin (DHP), fall into the first group. Many of the most selective lignin-degrading fungi belong to the second, although they have only moderate to good [^{14}C]DHP mineralizing ability. The lignin peroxidase-laccase fungi degrade [^{14}C]DHP relatively poorly [8].

Lignocellulose degradation by cultivated mushroom species

Lignocellulose degradation by several edible fungi species and, in some cases, associated enzyme production and corresponding gene expression, has been well documented including *Pleurotus* species [4, 9, 10], *Agaricus bisporus* [11], *Lentinula* (*Lentinus*) *edodes* [12-14], *Grifola frondosa* [15-17] and *Volvariella volvacea* [18, 19]. Camarero *et al.* [4] reported the secretion of two peroxidases in lignocellulose cultures of *Pleurotus eryngii*, a manganese-dependent peroxidase similar to that first described in *Phanerochaete chrysosporium* [6], and a second protein (PS1) that catalyzed both the oxidation of Mn (II) to Mn (III) by H_2O_2 and oxidized substituted phenols by a manganese-independent activity similar to that reported for LiP. Addition of Mn (II) to cotton stalks enhanced the preferential degradation of lignin by *Pleurotus ostreatus* (oyster mushroom) [20]. Releasing Mn (II) suppression of a versatile peroxidase by over-expression of the MnP4 gene (one of the nine members of the MnP gene family that constitutes part of the ligninolytic system of *P. ostreatus*) improved its ligninolytic functionality [21]. Conversely, inactivation of the versatile peroxidase-encoding gene (*mnp2*) resulted in reduced lignin degradation by this fungus [22]. Recent sequencing of the *P. ostreatus*genome has revealed the absence of LiPs and the presence of three VPs and six MnPs [23].

Lignin peroxidase activity was not detected in the compost extracts used to cultivate *A. bisporus*. However, the correlation between manganese peroxidase and laccase activities and the decomposition of radio labelled lignin and synthetic lignins by *A. bisporus* suggested significant roles for these two enzymes in lignin degradation by this fungus [11].

Unlike the aforementioned mushrooms, *V. volvacea* appears to lack key enzymes required to degrade the lignin component of highly lignified substrates [18]. This may account in part for the relatively low biological efficiency (i.e. conversion of growth substrate into mushroom fruit bodies) of the straw mushroom, about 10% on rice straw but up to 40% following the introduction of high cellulose cotton waste 'composts' [24]. Of the recognized lignin-modifying enzymes, only laccase production is well-documented although the *V. volvacea* genome contains two putative MnP and two VP

Table 3. Key fungal enzymes catalyzing degradation of the main components of lignocellulose

Lignocellulose component	Key enzymes
Cellulose	Endo-1,4-glucanases Cellobiohydrolases â-Glucosidases
Hemicellulose	Endo-1,4-â-xylanases Endo-1,4-â-mannases â-Xylosidases â-Mannosidases Side-chain-releasing glycosidases p-Coumaryl/feruloylesterases
Lignin	Manganese-dependent peroxidases Versatile peroxidases Lignin peroxidases Laccases

encoding genes but no LiP gene sequences [25]. Interestingly, eleven genes (*vv-lac1* to *vv-lac11*) encoding laccase homologues were identified, five of which were identical to laccase genes reported in previous studies [26, 27]. However, the straw mushroom is equipped with the key enzymes necessary to hydrolyse cellulose; i.e. EG, CBH and BGL. Several different isoforms of these enzymes are synthesized when *V. volvacea* is grown in the presence of a cellulosic substrate in both submerged culture in the laboratory, and in solid-state cultivation systems representative of those used for industrial cultivation [19, 28-30]. The key fungal enzymes catalyzing degradation of the main components of lignocellulose are listed in Table 3.

A B C

Figure 2 (A-C). Cultivation of edible mushrooms on lignocellulose

A: *Lentinula edodes* on artificial sawdust logs, B: *Pleurotus eryngii* on a mixture of corncobs and sawdust, C: *Volvariella volvacea* on cotton waste.

Detoxification of phenolic residues in lignocellulosic substrates used for mushroom cultivation

Lignocellulosic wastes of the type used for mushroom cultivation often contain phenolic monomers [31], some of which are reported to inhibit fungal growth [32] and the hydrolytic enzymes that catalyze the breakdown of cellulose and hemicellulose [33, 34]. In addition to a direct role in lignin transformation, laccase may also function in the polymerization of toxic phenols, and possibly other highly reactive species generated during lignin breakdown, from which the fungal mycelium must be protected [35]. This may explain the specific induction of laccase proteins by phenols and assign functionality to the numerous laccase isoforms identified in *V. volvacea* (Fig. 3) and many other fungi.

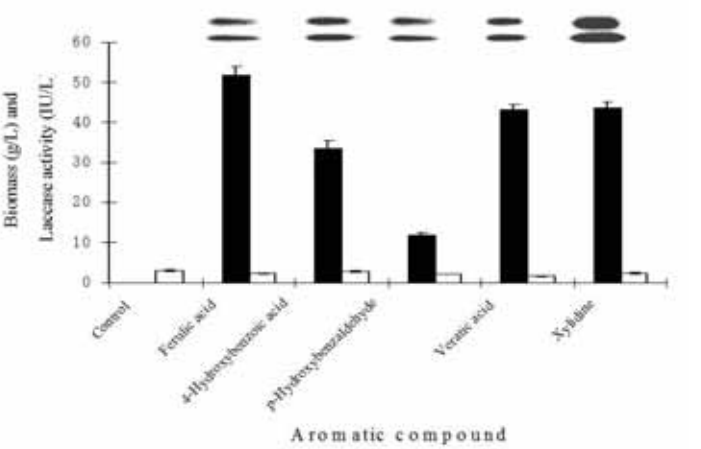

Figure 3. Induction of *Volvariella volvacea* laccase isoforms by aromatic compounds

A direct relationship has been established between lignocellulolytic enzyme profiles and the capacity of an individual mushroom species to grow and fruit on a particular lignocellulosic substrate. More information about the ligninolytic systems of other cultivated mushrooms, combined with the astute observations and fine-tuning of growers, will inevitably lead to greater mushroom-substrate compatibility and increased fruit body yields.

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