

PRODUCTION OF LIGNOCELLULOLYTIC ENZYMES BY MUSHROOMS

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ABSTRACT

Biopolymers contained within or derived from plant biomass form by far the largest pool of soil carbon. The decomposition of lignocellulose in the soil environment thus attracts considerable attention. Lignocellulose is composed mainly of the polysaccharidic polymers cellulose and hemicelluloses, and the polyphenolic polymer lignin. During transformation in soils, humic substances (humus, humic and fulvic acids) are formed from both lignocellulose and structural components of microbial decomposers. This is achieved through the concerted action of lignocellulose-degrading enzymes, whose activity is regulated by soil properties, land use and the identity of their microbial producers. Soil fungi seem to be the most important players in lignocellulose transformation processes due to their ability to attack both polysaccharides and polyphenols in the soil organic matter. While some basic concepts of regulation of enzymatic activity have been outlined, questions regarding enzyme production and diversity at the molecular level are just recently being addressed. Moreover, current results show that the ability to decompose lignin and cellulose is not restricted to saprotrophic basidiomycetes and ascomycetes but that the contribution of ectomycorrhizal fungi can be important. From a practical viewpoint, the ability to produce lignocellulose-decomposing enzymes by mushrooms is important for their cultivation, efficient substrate use, high yield and production of value-added products from lignocellulosic agrowastes.

Keywords: Lignin; Cellulose; Decomposition; Enzymes

INTRODUCTION

Since the biopolymers contained within lignocellulose form by far the largest pool of soil carbon and represent the most important input of organic material into soils, its decomposition attracts considerable attention. Nowadays, lignocellulose transformation is in a focus of biotechnology efforts aiming at a cost-efficient production of bioethanol, edible or medicinal mushrooms or other value-added products.

The lignocellulose is composed mainly of the polysaccharides cellulose and hemicelluloses and the three dimensional polyphenolic polymer of lignin. During transformation, lignocellulose is incorporated into fungal biomass or serves as an energy source while its remains are transformed into humic substances. It is important to note that while polysaccharides are resources for both carbon and energy acquisition by soil microorganisms, the degradation of lignin and probably also humic substances does not provide enough energy to support its decomposition and it thus does not have the primary nutritional role. The degradation of lignin and cellulose has been the topic of several recent reviews [1-4] but the degradation of other lignocellulose components, namely the pectins and hemicellulose, has only recently attracted attention [5].

RESULTS AND DISCUSSION

Lignin-modifying enzymes. Lignin is a branched three dimensional polymer of phenylpropane units. Due to the variety of chemical bonds and the complexity of its structure, lignin represents the most recalcitrant component of lignocellulose. The ligninolytic systems of basidiomycetous fungi consist of oxidases, peroxidases and hydrogen peroxide-producing enzymes. Ligninolytic oxidase – laccase – oxidizes its substrates using molecular oxygen, while the peroxidases need hydrogen peroxide provided by auxilliary enzymes.

Lignin peroxidase (EC 1.11.1.14) and manganese peroxidase (MnP; EC 1.11.1.13) are able to cleave the lignin polymer and to ultimately perform lignin mineralization [3, 6] while laccase (phenoloxidase, polyphenol oxidase, EC 1.10.3.2) can oxidise phenolic compounds including lignin and its derivatives. However, although it might be involved in some lignin transformation pathways, the enzyme alone can not cleave or mineralize lignin or humic compounds [7, 8]. In addition, lignin can also be attacked by fungal peroxygenases reported from the mushroom *Agrocybe aegerita* and several others [9]. Recently, the evidence increases that nonenzymatic, oxidative pathways are also involved in the decomposition of lignin [2]. This is supported by the fact that there is only a weak relationship between lignin mineralization and production of ligninolytic oxidases and peroxidases [10-12].

Laccase is the most frequently measured oxidative enzyme in soils, a target of several past studies [13, 14]. However, laccase is the enzyme of multiple roles spanning from interspecific interactions over defence against the toxicity of phenols or heavy metals up to fruiting processes and morphogenesis [7, 15, 16]. However, due to the inability to transform lignin, its ecological role in lignocellulose decomposition turnover seems to be generally largely overestimated.

As mentioned above, ligninolytic enzymes also need for their action enzymes providing hydrogen peroxide – most typically the aryl alcohol oxidase or glyoxal oxidase. These enzymes have not been assayed in lignocellulosic substrates very often, but at least one of them, aryl alcohol oxidase, was detected in cultures of litter-decomposing basidiomycetes [17].

Since humic substances carry structural similarities to lignin, ligninolytic enzymes are probably the most important in the degradation of soil humic substances [6, 11, 12]. The producers of the most important enzyme, Mn-peroxidase, the litter-decomposing basidiomycetes, are thus thought to play a major role in the transformation of these compounds [18]. However, due to the heterogeneous nature of humic substances, also horseradish peroxidase, β -glucosidase and Mn^{3+} or H_2O_2 are able to cleave or decolorize them as well as the radical-producing systems involved in polysaccharide degradation [2, 19].

Polysaccharide hydrolases. Cellulose is the main polymeric component of most lignocellulosic materials and represents the most abundant polysaccharide on Earth. The chemical composition is simple: it consists of D-glucose residues linked by β -1,4-glycosidic bonds to form linear polymeric chains of several thousand glucose residues. Cellulose contains both highly crystalline regions where individual chains are linked to each other, and less-ordered amorphous regions. The degradation of crystalline regions is much slower than that of the amorphous ones and some microorganisms are able to attack only amorphous cellulose [2]. A typical system for efficient cellulose decomposition includes endo-type hydrolases (endo-1,4- β -glucanases, EC 3.2.1.4), exo-type hydrolases (cellobiohydrolases [CBH] EC 3.2.1.4) and 1,4- β -glucosidases (EC 3.2.1.21), the complementary activities acting synergistically. Typical cellulolytic systems of saprotrophic cellulose-degrading fungi (e.g. the cord-forming or wood-rotting basidiomycetes) consist of multiple enzymes of all the three above groups. Cellobiohydrolases are produced with specificity for either the reducing or non-reducing ends of cellulose polymer [2, 20].

Hemicelluloses are low molecular mass linear or branched polymers usually containing various sugar units including mannose, galactose, xylose and glucose [5]. Xylans, consisting of xylose units, and glucomannans, consisting of glucose and mannose units, are the main hemicelluloses of angiosperm and conifer trees, respectively, while other lignocellulosic materials may additionally contain considerable amounts of arabinogalactans and galactans [1]. Enzymatic decomposition of hemicelluloses requires a set of hydrolytic enzymes reflecting the structural variability of the substrate. Hemicellulose hydrolysis proceeds through the concerted action of endo-type enzymes, side-group cleaving enzymes and exotype enzymes as well as enzymes cleaving the main chain substituents, e.g. the acetyl groups in xylans. Cleavage ultimately results in the liberation of monomeric sugars and acetic acid.

The hemicellulases most typically found among fungi are endo-1,4- β -xylanase (EC 3.2.1.8) and 1,4- β -xylosidase (EC 3.2.1.37), but several other enzymes are known to be produced by saprotrophic soil fungi, including endomannanases, β -mannosidases, galactosidases, arabinosidases and acetyl esterases as well as debranching enzymes [11, 21-23]. The decomposition of hemicellulose is not limited by its physical structure but rather by the diversity of chemical composition and intramolecular bonding. Many cellulases and hemicellulases have been recently demonstrated to have a broad and overlapping substrate specificity, so that it is not always simple to link a specific enzyme with a target substrate [2]. Polysaccharides in wood or other lignocellulosic materials have also demonstrated to be degraded by non-enzymatic radical-producing systems based on cellobiohydrolase, quinone cycling or small glycopeptides. Their role in the soil is probably more limited in litter or straw than in wood, but cellobiose dehydrogenase has already been demonstrated in several soil or litter-associated fungi [2].

Genes encoding lignocellulose-degrading enzymes in the *Pleurotus ostreatus* genome. Recent efforts led to the analysis of full genome sequence of the lignocellulolytic fungus, *Pleurotus ostreatus*. The results confirm previous studies reporting the production of a complex complement of extracellular enzymes by this fungus [15, 24], showing that its genome contains genes encoding various enzymes active on cellulose, hemicelluloses and lignin (Table 1). Especially the ligninolytic system is highly functionally redundant in this white-rot fungus.

Table 1: Predicted gene models in the genome of the saprotrophic basidiomycete *Pleurotus ostreatus* PC15 (<http://genome.jgi-psf.org>).

EC number models	Enzyme	Predicted gene
3.2.1.1	α -amylase	6
3.2.1.4	cellobiohydrolase	1
3.2.1.15	polygalacturonase	3
3.2.1.20	α -glucosidase	9
3.2.1.21	β -glucosidase	13
3.2.1.23	β -galactosidase	3
3.2.1.24	α -mannosidase	1
3.2.1.25	β -mannosidase	3
3.2.1.26	β -fructofuranosidase	1
3.2.1.58	glucan 1,3- β -glucosidase	8
3.2.1.67	galacturan 1,4- α -galacturonidase	1
3.2.1.X	unspecified glycosylhydrolases	>5
1.10.3.2	laccase	12
1.11.1.X	haem peroxidases	9

CONCLUSIONS

Fungi seem to be the most important players in lignocellulose transformation processes under environmental conditions due to their ability to attack both polysaccharides and polyphenols in the soil organic matter. While some basic concepts of regulation of enzymatic activity have been outlined, questions regarding enzyme production and diversity at the molecular level are just recently being asked [25]. Moreover, current results show that the ability to decompose lignin and cellulose is not restricted to saprotrophic basidiomycetes and ascomycetes but that the contribution of ectomycorrhizal fungi can also be important [26]. From a practical viewpoint, the ability to produce lignocellulose-decomposing enzymes by mushrooms is important for their cultivation, efficient substrate use, high yield and production of value-added products from lignocellulosic agro-wastes, and future research will hopefully provide better insight into the relationships among fungal enzyme production, substrate transformation and production of desired products.

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