Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Fungal enzymes for environmental management

Ursula Kües

Fungal ligninolytic enzymes have broad biotechnological applications. Particularly laccases and certain fungal class II peroxidases from white-rot basidiomycetes are considered in degradation of persistent organic pollutants. Promising processes with reusable immobilized laccases in special reactors have been developed up to pilot scale for degradation of pollutants in water. Bioremediation of chemically complex soils with their large indigenous microbial communities is more difficult. Living fungi and their enzymes are employed. Bioaugmentation, introduction of for example white-rots for enzyme production into a polluted soil, and biostimulation of suitable resident organisms by nutritional manipulations are strategies in degradation of pollutants in soil. Bioaugmentation has been successfully implemented on small scale for soils in biobeds and for specific materials such as olive mill wastes.

Address
Department of Molecular Wood Biotechnology and Technical Mycology, Büsgen-Institute, University of Göttingen, Büsgenweg 2, Götttingen D-37077, Germany

Corresponding author: Kües, Ursula (ukuees@gwdg.de)

Current Opinion in Biotechnology 2015, **33**:268–278

This review comes from a themed issue on **Environmental biotechnology**

Edited by **Spiros N Agathos** and **Nico Boon**

For a complete overview see the Issue and the Editorial

Available online 9th April 2015

http://dx.doi.org/10.1016/j.copbio.2015.03.006

Introduction

Saprotrophic fungi have crucial roles in ecosystem functioning. Primarily, they facilitate organic matter decomposition and nutrient recycling in favor of own and other organisms growth and can have additional indirect effects on above-ground and below-ground ecology and species composition [1,2]. Lignocellulose from plant cell walls with its three main components cellulose, hemicellulose and lignin represents the largest organic renewable resource on earth but it is also most recalcitrant to degradation. This is due to the structure of the cell wall microfibrils in which the elementary cellulose fibrils are coated and cross-linked by hemicellulose matrices and in which the lignin shelter is then covalently linked to the hemicellulose. It is thus the hydrophobic lignin that protects the cell walls from humidity and microbial degradation [3–5]. Specific basidiomycete fungi can enzymatically attack all the polymers in the complex-structured lignocellulose. The appearance of such white-rot fungi million years ago allowed for the first time massively the fast nutrient recycling from wood required for new plant growth, with evolutionary impact on plant diversification. Concomitantly with the innovation of fungal lignocellulolytic enzyme machineries, the Carboniferous period had found its end. Land plants were not anymore simply buried and chemically transformed to coal but instead could become effectually decomposed into their components [6••].

Based on the ability to degrade lignin along or not with cellulose and hemicellulose, wood decay has traditionally been divided into white rot and brown rot mainly exerted by basidiomycetes and soft rot mainly performed by ascomycetes. As already indicated, the white-rots have the unique enzymatic abilities to selectively or simultaneously attack the persistent lignin to free the fermentable polycarbohydrates for enzymatic decomposition [7,8]. In brown rot, lignin is attacked by Fenton chemistry and chemically modified into a brown oxidized form which allows access of enzymes to the cellulose for oxidative depolymerisation [9,10]. Poorly understood soft rot with partial enzymatic degradation of cell wall polysaccharides and slight alterations of lignin can occur under high wood moisture content [11].

Typically, lignin degradation by white rots involves highly specialized class II peroxidases (PODs) with high-oxidation potential [7,8]. However, recent evaluation of the decay modes together with the genomes of the basidiomycetes *Botryobasidium botryosum*, *Jaapia argillaceae*, *Cylindrobasidium torrendii* and *Schizophyllum commune* suggests that forms of white rot exist independent of any PODs. Decay modes show features of in between white and brown rot and of soft rot [12••,13•]. In contrast, litter decomposing fungi might be best adopted to humic substances by expanding numbers of genes for specific types of enzymes, for example genes for heme-thiolate peroxidases, but these have also retained some enzymatic ability for white rot [14••]. Loss or reductions of genes for similar groups of enzymes lead in the basidiomycetes on a number of occasions from white to brown rot and also to mycorrhizal lifestyles, respectively [6••,10,15]. Increasing evidence supports that various mycorrhizal fungi have the abilities to act as occasional litter decomposers [17]. The mycorrhizal *Paxillus involutus* for instance has been shown to apply a trimmed brown rot mechanism with Fenton chemistry to plant litter [18,19], and *Cortinarius* species exhibit high peroxidase activity in soil for decomposition

Box 1 Laccases (EC 1.10.3.2; *p*-diphenol oxygen oxidoreductases) are multi-copper-oxidases with their true biological functions and natural substrates little understood and known. Most fungal laccases are extracellular enzymes. In essence, these enzymes are biochemically characterized on artificial substrates. Laccases have a broad substrate range and act with low specificity on *o*-phenols and *p*-phenols and often also on aminophenols and phenylenediamines under transfer of four electrons from organic substrate to molecular oxygen. Importantly, the substrate range can become broaden and the kinetics of reactions enhanced by laccase-mediator-systems (LMSs) acting in a chain of electron transfers in which a compound is oxidized by the enzyme and the oxidized form then mediates the oxidation of a substrate that may not be a factual target of the enzyme (Figure 2).

Peroxidases (EC 1.11.x; donor:hydrogen-peroxide oxidoreductases) comprise different superfamilies of phenoloxidases that use H_2O_2 or organic hydrogen peroxide as electron accepting cosubstrates. Main fungal high-redox class II peroxidases involved in biodegradation of lignocellulose with an exceptional broad organic and also inorganic substrate range are secreted heme-containing lignin peroxidases (LiPs; EC 1.11.1.14), manganese peroxidases (MnPs; EC 1.11.1.13), and versatile peroxidases (VPs; EC 1.11.1.16). Another family of largely unclarified biological functions but of high biotechnological interest for degradation of recalcitrant compounds presents dye-decolorizing peroxidases (DyPs; EC 1.11.1.19). DyPs are bifunctional enzymes with oxidative and hydrolytic activities on phenolic and non-phenolic organic compounds, some of which, for example some recalcitrant textile dyes and *p*-nitrophenol, are poorly accepted by other peroxidases. Halogenating chloroperoxidases (CPOs; EC 1.11.1.10) and unspecific or aromatic peroxygenases (UPOs/APOs; EC 1.11.2.1) belong to the heme-thiolate peroxidase (HTPs; haloperoxidases) superfamily. HTPs transfer peroxide-oxygen to substrate molecules. Among, UPOs have exceptionally broad reaction competences on a wide variety of substrates on which they perform various reactions including aromatic peroxygenation, double-bond epoxidation, hydroxylation of aliphatic compounds, ether cleavage, sulfoxidation, N-oxidation, bromide oxidation and more.

Tyrosinases (EC 1.14.18.1; monophenol monooxygenases; phenolases; monophenol, *o*-diphenol:oxygen oxidoreductases; L-tyrosine,L-dopa:oxygen oxidoreductases) are type III copper proteins. Upon binding of molecular oxygen, tyrosinases catalyze *o*-hydroxylation of monophenols (monophenolase reaction cycle, reaction 1) to generate as intermediates *o*-diphenols that are subsequently oxidized into reactive *o*-quinones (diphenolase reaction cycle, reaction 2). Tyrosinases are cytosolic enzymes that participate in pigment synthesis such as melanin. Best known for applications in biotechnology is *Agaricus bisporus* tyrosinase (mushroom tyrosinase) causing in its host mushroom browning.

P450 cytochrome monooxidases (EC 1.14.14.1; unspecific monooxygenases; flavoprotein-linked monooxygenases; P450s; CYPs) are intracellular heme-thiolate-containing oxidoreductases acting on a wide range of substrates in stereo-selective and regio-selective manner under consumption of O_2. Activated by a reduced heme iron, these enzymes add one atom of molecular oxygen to a substrate, usually by a hydroxylation reaction. However, various other reactions such as epoxidation, sulfoxidation, dealkylation and more can also occur. P450-catalyzed reactions require NAD(P)H as donors for electrons to be transferred via a flavoprotein or ferredoxin to the second oxygen atom from a cleaved O_2 molecule. Members of the highly diverged and functionally very diverse P450 superfamily have essential roles in biosynthetic pathways of specific primary and secondary metabolites, others act in metabolization of xenobiotics.

Glutathione transferases (EC 2.5.1.18; glutathione S-transferases; glutathione conjugating enzymes; GSTs) catalyze the nucleophilic attack by reduced glutathione (GSH) of an electrophilic carbon, nitrogen or sulfur atom in non-polar compounds. Conjugation of GSH to the electrophilic substrates makes the substrates more water-soluble. GSTs are intracellular enzymes present in different subcellular compartments. They have a broad substrate specificity and act in detoxification of various structurally different endogenous toxic metabolites, superoxide radicals and exogenous toxic chemicals. In fungi, there are at least eight distinct classes of GSTs (GTT1, GTT2, Ure2p, MAK16, EFb1, GSTFuA, GSTO, GHR).

of organic matter [16]. Mycorrhizal and typical saprotrophic species tend to be found distinctly in separate soil areas, along with specific functions in the rhizosphere and in the soil, respectively. Endophyte implies localization within plant tissues (endosphere) but such species also assemble in zones inhabited by typical saprotrophs [20,21]. Soil pH values as one parameter can determine whether soil-borne fungi colonize roots and tend toward an endophytic lifestyle of no harm to the host [22]. Under certain conditions, endophytes may change into pathogens [23], pathogens on one plant might be mycorrhizal on another [24,25], and litter and wood decay fungi may also have mycorrhizal properties [10,26]. There is apparently much continuum possible between the different lifestyles and situations of fungi in the soil. To verify such versatility, the soil-borne organisms will appoint and express to need different sets of enzymes.

Enzymes that break down cellulose, hemicellulose and lignin are over-arching called cellulases, hemicellulases and lignin-modifying enzymes (LMEs), respectively. By sequence, catalytic mechanism and enzymatic specificity, these enzymes divide into multiple families and subfamilies, the constantly expanding information on which is compiled in the knowledge-based CAZy database together with information on enzymes with auxiliary activities [27,28•]. Enzymes in lignocellulose degradation are commonly extracellular, which is compulsory by the large molecule sizes of the envisaged substrates. Larger polymers are broken down into smaller fragments and finally into individual molecule units that might be taken up into the cells for eventual metabolic use [8] or for further detoxification by the xenome, that is the protein machineries for detection, transport and metabolism of xenobiotics [29••]. Detoxification pathways of the xenome are constituted among others of multigenic families of intracellular cytochrome P450 monooxygenases and glutathione transferases, respectively (Box 1). These superfamilies of enzymes are particularly highly expanded in wood degraders (in white-rots and brown-rots) and in plant litter decay species but also to some extent in symbiotic species. Among other functional roles they have in primary and secondary metabolism, the enzymes likely diverged in different species to deal with the multiple harmful lignin metabolites and related compounds in humus generation and with the countless plant defense metabolites soil fungi are confronted with in nature [29••,30,31].

There are multiple purposes in biotechnology as where ligninolytic enzymes [5,32–35] and enzymes for