WHAT BASIDIOMYCETE GENOMES TEACH US ABOUT WOOD BIODEGRADATION MECHANISMS

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ABSTRACT

Considerable confusion existed for years about the mechanisms ultimately responsible for wood biodegradation by basidiomycetes. Interest in wood decay, as a biotechnological model for the sustainable production of fuels and chemicals, led to sequencing of the first basidiomycete genomes. Massive sequencing tools extended the number of white-rot and brown-rot fungal genomes available, enabling us to establish general conclusions on the biodegradation mechanisms involved in each of them. Lignin degradation by white-rot fungi appears always associated with the presence of high redox-potential peroxidase genes in their genomes. Such genes are absent from all the brown-rot fungal genomes. Moreover, polysaccharide degradation by the latter fungi is not associated with an expansion of genes encoding carbohydrate-acting enzymes. This fact, together with the presence of genes encoding peroxide-generating and Fe^{3+}- and quinone-reducing enzymes, supports the chemical depolymerization of wood polysaccharides via Fenton chemistry in the case of brown rot. The genomic analyses also outlined the evolutionary history of the different peroxidase families in wood-rotting basidiomycetes, in particular fixing the origin of lignin-degrading peroxidases at the end of the Carboniferous period, which coincides with the end of coal formation via accumulation of undecayed plant biomass in the soil.

I. FIRST BASIDIOMYCETE GENOMES

The increasing availability of basidiomycete genomes (transcriptomes and secretomes) provides answers to several questions concerning the biodegradation mechanisms of wood-rotting fungi. Most of these genomes were sequenced at the Joint Genome Institute of the US Department of Energy with the purpose of understanding fungal decay of wood, for the biotechnological production of fuels and other chemicals from renewable plant feedstocks. Basidiomycetes have developed two main degradation strategies resulting in so-called white-rot and brown-rot decay of wood (because of the color of the transformed material) [1]. Chemical analysis showed that white-rot fungi remove wood lignin, simultaneously or preferentially with respect to polysaccharides, while brown-rot fungi remove polysaccharides preferentially to leave a modified lignin-rich residue [2]. Simultaneously, using simple and polymeric model compounds, it was shown that white-rot fungi are able to mineralize lignin, which in nature protects wood polysaccharides against hydrolysis by most cellulolytic organisms (brown-rot excluded).

In addition to the so-called ligninolytic peroxidases discovered in the 80's, a variety of other oxidative enzymes (including laccases, different peroxidases, oxidases and other oxidoreductases), as well as redox mediators (such as phenolic and nonphenolic aromatic compounds from fungal metabolism or lignin degradation, lipid and oxygen radicals, metal cations, etc) have been associated with fungal degradation of lignin in a process that was defined as an "enzymatic combustion" [3], in contrast to polysaccharide degradation, which is seen as a generally well understood, largely hydrolytic process. This puzzling situation, already emphasized in some early reviews [4], persisted for more than 20 years until the development of the first genome sequencing platforms.

Phanerochaete chrysosporium, the model white-rot fungus for many years, was the first basidiomycete whose genome was sequenced in 2004 [5]. A few years later, the first brown-rot fungal genome, transcriptome and secretome of *Postia placenta* was available [6] and their comparison with the corresponding *P. chrysosporium* genome [5], transcriptome and secretome [7] provided interesting information on the wood biodegradation mechanisms by these two model white-rot and brown-rot basidiomycetes. This early period of sequencing of individual wood-rotting fungal genomes was completed with the genomes of *Ceriporiopsis subvermispora* [8], a selective lignin degrader, and also those of *Pleurotus ostreatus* [9] and *Agaricus bisporus* [10], which are two representative lignin-degrading agarics that grow on wood and soil, respectively (note that all the above typical lignin-

degrading fungi are polypores). More recently, larger sequencing projects were developed, where the initial conclusions obtained from the genomes of the first fungi sequenced were extended and completed using the genomes of a variety of white-rot and brown-rot basidiomycetes, as reported by Floudas et al. [11], who analyzed 31 ascomycete and basidiomycete genomes.

II. DIVERGENT WHITE-ROT AND BROWN-ROT DEGRADATION MECHANISMS

To obtain general conclusions on the mechanisms of wood degradation, we comparatively analyzed thirteen white-rot and seven brown rot fungal genomes [9,11-13]. Among the different oxidative enzymes mentioned above, genes for ligninolytic peroxidases - the so-called lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase (VP) enzymes - appear in all the white-rot fungal genomes but are absent from all the brown-rot fungal genomes, whereas laccases, oxidases and other oxidoreductase families are present in both white-rot and brown-rot fungal genomes.

Among the above peroxidases, MnPs are present in all white rot fungi, being accompanied by LiPs, VPs or both, depending on the type of fungus. LiP is able to degrade nonphenolic lignin model dimers and nonphenolic synthetic lignin (the latter in the presence of the fungal metabolite veratryl alcohol) [14,15] and the same capabilities have been recently shown for VP [16]. On the other hand, MnP, and also VP, oxidize Mn^{2+} to Mn^{3+}, which can: i) initiate lipid peroxidation reactions resulting in lignin degradation by reactive lipid radicals; or ii) oxidize minor phenolic units in lignin or lignin-derived phenols [17]. None of the above peroxidases are found in brown-rot fungi, whose genomes include only a few genes encoding so-called generic peroxidases (GPs), which are characterized by a low redox-potential that limits them to oxidation of phenolic substrates (and some dyes). The specific presence of genes encoding LiP, VP and MnP in white-rot fungal genomes, and their frequent duplication giving rise to numerous isoenzymes, strongly support their involvement in the attack on lignin characteristic of wood-rotting basidiomycetes.

However, the brown-rot fungal genomes not only lack the genes for ligninolytic peroxidases, in agreement with the lifestyle described above that only includes limited modification of lignin [2], but also contain a much smaller number of genes for carbohydrate-acting enzymes (CAZYs) as compared with white-rot fungi. In particular, cellulolytic enzymes that contain cellulose-binding domains are unexpectedly rare. This fact, together with the presence and expression of genes for H_2O_2-generating oxidases and Fe^{3+}- and quinone-reducing enzymes, indicates that polysaccharide attack by brown-rot fungi is largely non-enzymatic, proceeding via Fenton chemistry ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\cdot$) [6]. That is, not only lignin depolymerization by white-rot fungi but also polysaccharide depolymerization by brown-rot fungi depend heavily on oxidative processes.

Although a few white-rot fungal species degrade lignin preferentially with respect to polysaccharides (selective decay pattern), most white-rot fungal species degrade polysaccharides simultaneously with lignin [18]. This fact, together with the Fenton-based mechanism for brown-rot decay described above, justifies the apparent paradox raised by the presence of more, and more diverse, genes for carbohydrate-degrading enzymes in the genomes of white-rot fungi (known for their ability to attack lignin) than in those of brown-rot fungi (known for their ability to degrade polysaccharides). The above applies not only to genes encoding carbohydrate hydrolases but also to the recently discovered lytic polysaccharide monooxygenase genes, involved in the initial oxidative attack on recalcitrant carbohydrates [19], which are more abundant in white-rot than in brown-rot fungal genomes [11,20].

A common characteristic of white-rot and brown-rot fungal genomes, and the corresponding transcriptomes and secretomes, is the presence, duplication and expression of oxidase genes responsible for H_2O_2 generation. Interestingly, brown-rot fungi produce methanol oxidase that uses methanol from lignin demethylation for reducing O_2 to H_2O_2, while white-rot fungal oxidases use both carbohydrate-derived (such as glyoxal oxidase and pyranose oxidase) and lignin-derived (such as aryl-alcohol oxidase) reducing substrates. Therefore, considering the main (limiting) reactions as shown by genomic analyses, white-rot and brown-rot decay of wood by basidiomycetes can be seen as an H_2O_2-dependent oxidation (of lignin and polysaccharides, respectively) catalyzed by two different iron species, Fe^{3+} in peroxidase heme cofactors and free Fe^{2+}, respectively.

Concerning laccases, the presence of the corresponding genes is not the determinant factor for white-rot or brown-rot fungal lifestyles since they are widespread in basidiomycete, and also ascomycete, genomes. Therefore, their contribution to lignocellulose biodegradation would differ according to the wood decay pattern and fungal species. In white-rot decay they would oxidize the minor phenolic units in lignin, and the lignin-derived phenols (contributing to similar reactions catalyzed by the MnPs characteristic of white-rot fungi) and it has been suggested that some of the (stable) phenoxy radicals formed could also be involved in lignin degradation as natural redox mediators [21]. In brown-rot species they could contribute

to an alternative mechanism for both Fe^{3+} reduction and H_2O_2 generation via quinone redox-cycling [22]. A similar process was first reported in white-rot fungi [23], although the analysis of fungal genomes does not provide evidence for the involvement of Fenton chemistry in the attack on lignin by these species.

III. WHITE-ROT FUNGI: ORIGIN AND EVOLUTION OF LIGNINOLYTIC PEROXIDASES

The comparative analyses of fungal genomes reveals that not only the number but also the diversity of lignin-degrading peroxidases expanded in the lineage leading to the ancestor of wood-rotting basidiomycetes, which is reconstructed as a white-rot species, and then contracted in parallel lineages leading to brown-rot and mycorrhizal species [11]. The appearance of white-rot decay, made possible by peroxidase evolution leading to the first lignin-degrading enzymes, might have coincided with the sharp decrease in the rate of organic carbon burial around the end of the Carboniferous period, as shown by molecular clock analyses.

Moreover, ancestral state reconstructions [11,12] have shown that an ancestral non-ligninolytic GP (related to those found in ascomycetes and plants) gave rise to the first MnP, by incorporating a Mn^{2+}-oxidation site formed by three acidic residues located near one of the heme propionates. In the subsequent evolutionary history of basidiomycete peroxidases, the ancestral MnPs apparently gave rise at least twice to the first VPs, by incorporating a lignin degradation site that consists of a radical-forming exposed tryptophan that can abstract electrons from the bulky lignin polymer and transfer them to the activated heme cofactor. The last step in ligninolytic peroxidase evolution was the loss of the Mn-oxidation site, conserved in all VPs, thus giving rise to the first LiPs, which represent the most efficient lignin-degrading peroxidases. Interestingly, the last transition occurred only in the evolution of basidiomycetes from the order Polyporales, while VP remained as the only lignin-degrading enzyme in the order Agaricales.

The above evolution of basidiomycete peroxidases followed the evolution of lignin and precursor polymers in land plants, whose functions include protection of polysaccharides from biodegradation. Accordingly, the absence, from the middle Devonian to late Carboniferous periods, of white-rot fungi that can efficiently mineralize these recalcitrant aromatic polymers resulted in the massive accumulation of dead woody and nonwoody plant biomass (from Paleozoic seed ferns, lycophytes, equisetophytes, progymnosperms, and early gymnosperms mainly represented by Cordaitales species). This accumulation generated large coal deposits and also a simultaneous reduction of the very high carbon dioxide content in the primitive atmosphere. This largely irreversible carbon dioxide fixation process by primitive land plants stopped at the end of the Carboniferous period with the evolution of white-rot fungi equipped with lignin-degrading peroxidases, thus enabling the balanced recycling of carbon currently known on earth.

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