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## Degradation of plant cell wall polymers

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### Introduction

Processes of natural bioremediation of lignocellulose involve a range of organisms, but predominantly fungi (Hammel, 1997). Laboratory studies on the degradation of lignocellulose, including wood, straw, and cereal grains, have focused mainly on a few fungal species that grow well in the laboratory and can be readily manipulated in liquid culture to express enzymes of academic interest. Our current understanding of the mechanism of lignocellulose degradation stems from such studies. Although some of these enzymes have economic potential in a range of industries, for example pulp and paper manufacture and the detergent industry, it is frequently expensive and uneconomic to use them for bioremediation of pollutants in soils and water columns. In the successful commercial bioremediation processes developed, whole organisms have been used in preference to their isolated enzymes (Lamar & Dietrich, 1992; Bogan & Lamar, 1999; Jerger & Woodhull, 1999).

Most fungi are robust organisms and are generally more tolerant to high concentrations of polluting chemicals than are bacteria, which explains why fungi have been investigated extensively since the mid-1980s for their bioremediation capacities. However, the species investigated have been primarily those studied extensively under laboratory conditions, which may not necessarily represent the ideal organisms for bioremediation. Fungi in little-explored forests of the world, for example tropical forests, may yet prove to have even better bioremediation capabilities than the temperate organisms currently studied, exhibiting more tolerance to temperature and specialist environments. This chapter discusses the current state of knowledge on the degradation of lignocellulose and how this relates with the ecology of lignocellulolytic fungi. This knowledge is important to modify and enhance the mechanisms of degradation of industrial pollutants such as chlorophenols, nitrophenols and polyaromatic hydrocarbons by these fungi.

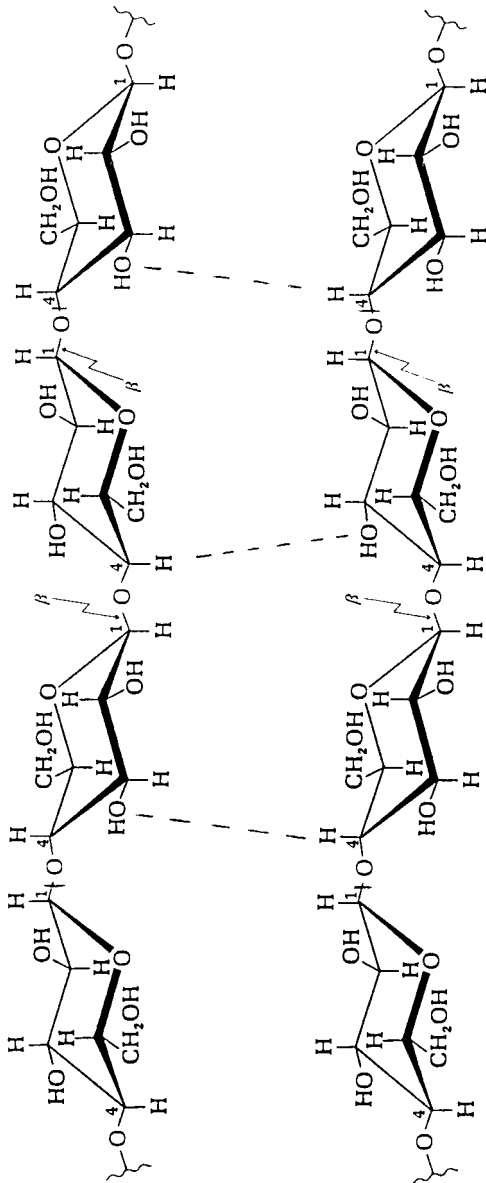


Fig. 1.1. Structure of cellulose formed from  $\beta$ -1,4-linked cellobiose units, with hydrogen bonding between parallel chains.

### Structure and function of plant cell walls

Plant cell walls offer several benefits to the growing and mature plant. They provide an exo-skeleton giving rigidity and support, enabling the water column to reach the plant apex, and they serve as a protective barrier against predators and pathogens. As the wall is an extracytoplasmic product, it was not considered to be a living part of the cell (Newcomb, 1980), although this view is now challenged and many metabolic processes are now known to occur within the cell wall structure for maintenance and in response to attack by pathogens (Dey, Brownleader & Harborne, 1997a). Every cell of a plant is surrounded by a primary wall that undergoes plastic extension as the cell grows. Some cells such as those of the parenchyma keep a primary wall throughout their lifespan. The primary wall is composed of cellulose fibrils, hemicellulose and protein with large amounts of pectin forming a viscous matrix that cements the wall together. The precise molecular composition varies between cell types, tissues and plant species, although an approximate dry weight ratio would be 30% cellulose, 25% hemicellulose, 35% pectin, and 10% protein (Taiz & Zeiger, 1991).

Cellulose is formed by polymerization of D-glucose molecules linked in the  $\beta$ -1,4 position, resulting in flat, linear chains (Fig. 1.1). Hydrogen bonding between chains leads to the formation of a microfibril up to 3 nm in diameter. These crystalline microfibrils are laid down in different orientations within the primary wall, providing the structural support for the wall. The surrounding wall matrix is composed of hemicellulose, protein and pectin in which the microfibrils are embedded. Hemicelluloses are mixed polymers of different neutral and acidic polysaccharides. They adhere to the surface of the cellulose microfibrils by hydrogen bonding, through OH groups on the sugars, and enhance the strength of the cell wall. Pectic polysaccharides are covalently bound to the hemicelluloses. The protein components of the primary cell wall are hydroxyproline-rich glycoproteins, named extensins, that are involved in cell wall architecture and plant disease resistance (Brownleader *et al.*, 1996; Dey *et al.*, 1997b). As some cell types mature, a secondary cell wall is deposited between the primary wall and the plasmalemma that is more dense than primary walls and binds less water. Wood contains cell walls of the greatest maturity of all plant cell types, with several layers (S1, S2, S3) in the secondary wall (Fig. 1.2). These are composed of cellulose, hemicellulose and lignin but with less pectin than primary cell walls. An approximate ratio of these components would be 35% cellulose, 25% hemicellulose including pectin,

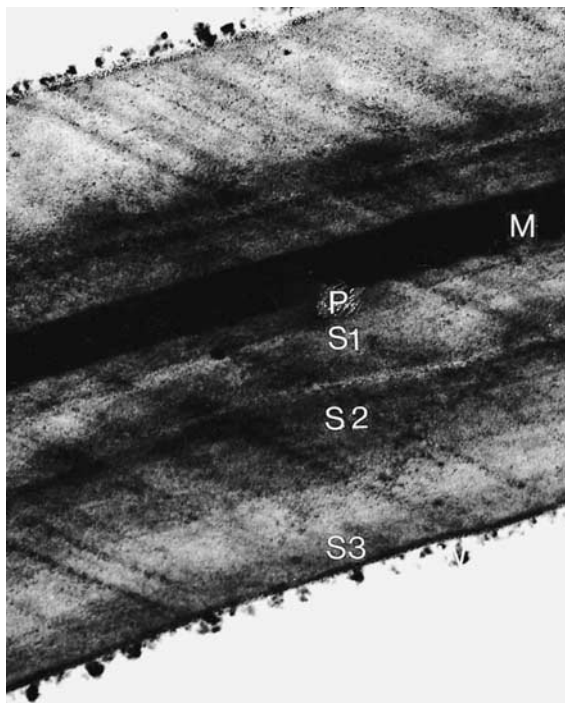


Fig. 1.2. Transmission electron micrograph of an ultra-thin section of beech wood showing the wood cell wall structure. M, middle lamella; P, primary wall layer; S1, S2 and S3, secondary wall layers. Magnification  $\times 28\,000$ .

and up to 35% lignin, depending on the plant species. The cellulose microfibrils are orientated at different angles in each layer of the three secondary wall layers to provide increased strength. Lignin gives rigidity to the wall (Cowling & Kirk, 1976; Montgomery, 1982). Strength is related to these structural components, particularly the orientation and crystallinity of the cellulose microfibrils (Preston, 1974), whereas toughness is a reflection of the elastic component of the cell wall, giving an indication of the potential for plasticity (Lucas *et al.*, 1995).

Lignin is a three-dimensional aromatic polymer that surrounds the microfibrils, with some covalent attachment to the hemicellulose. It is composed of up to three monomeric units of cinnamyl alcohols: coumaryl alcohol, mainly confined to grasses; coniferyl alcohol, the major monomeric unit in gymnosperm wood; and sinapyl alcohol, predominant in angiosperm wood (Fig. 1.3). Polymerization of these monomers is by a free radical reaction catalysed by peroxidase. This results in a variety of bonds

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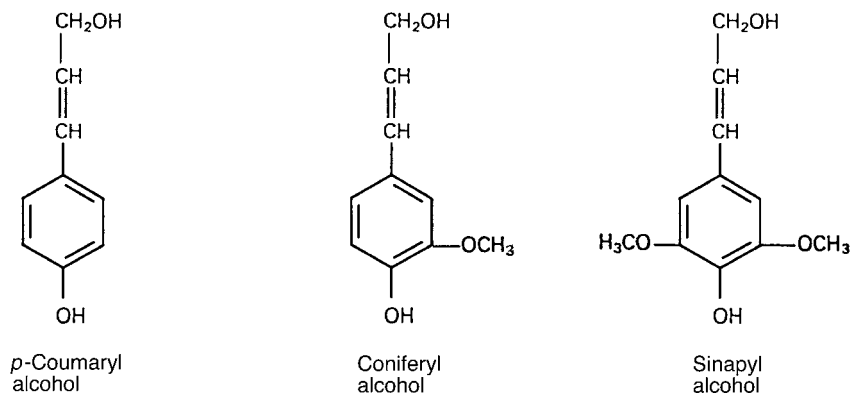


Fig. 1.3. Monomeric phenylpropanoid units that polymerize to form lignin.

in the polymer, with  $\beta$ -O-4 bonds between the C<sub>4</sub> carbon of the aromatic ring and the  $\beta$ -carbon of the side chain being the predominant bond linkage. Other common bonds are  $\alpha$ -O-4, C<sub>3</sub> or C<sub>5</sub> aryl-O-4 linkages with some biphenyl linkages. Substitution on the aromatic rings of the monomers determines the type of lignin as syringyl (made from coniferyl and sinapyl alcohols) or guaiacyl lignin (made from only coniferyl alcohol) (Adler, 1977). Side chains of lignin are composed of cinnamyl alcohols, aldehydes and hydroxylated substitutions on the  $\alpha$ - and  $\beta$ -carbons. It is the high proportion of ether bonds in the polymer, from the methoxy groups and polymerizing bonds, that gives lignin its unique structure and properties as a strong resistant polymer. It has been found that the polysaccharides in the cell wall can influence the structure of lignin during synthesis. The motion of coniferyl alcohol (one of the lignin monomers) and its oligomers near a cellulose surface can change the course of dehydrogenation polymerization into lignin, as electrostatic forces restrict the motion of the monomer and oligomers (Houtman & Atalla, 1995). This is consistent with experimental observations of the lignin-polysaccharide alignment in cell walls as observed by Raman microprobe studies (Atalla & Agarwal, 1985).

Hemicelluloses in the secondary wood wall vary between plant species, with xylan the predominant polymer. The simple  $\beta$ -1,4-linked-D-xylopyranosyl main chain carries a variable number of neutral or uronic acid monosaccharide substituents, or short oligosaccharide side chains (Fig. 1.4). Hardwood (angiosperms) xylans are primarily of the glucuroxylan type, while softwood (gymnosperms) xylans are glucuronoarabinoxylans (Joseleau, Comtat & Ruel, 1992). The structure

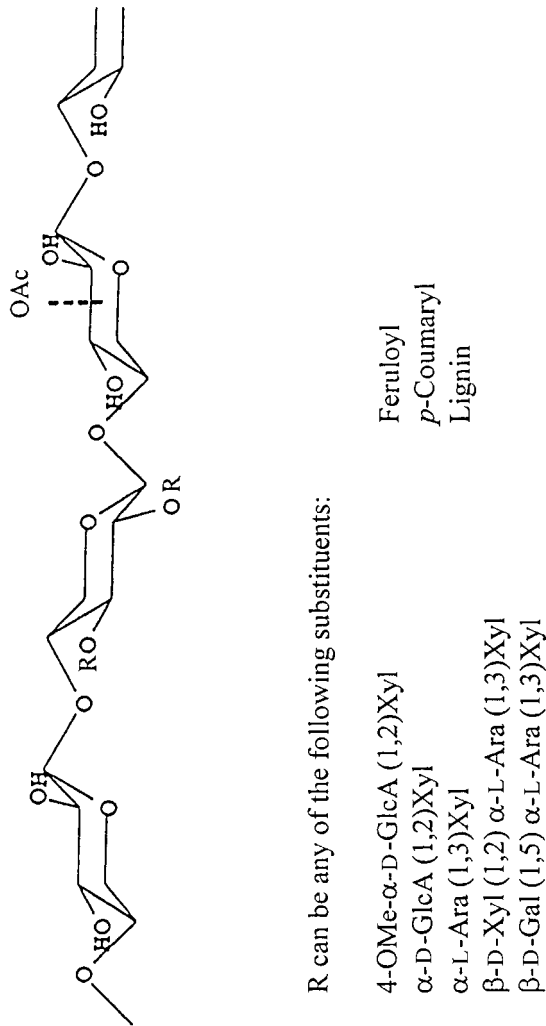


Fig. 1.4. Basic structure of xylan. Me, methyl; GlcA, gluconic acids; Xyl, xylose; Ara, arabinose; Gal, galactose.

of xylans in the wood cell wall is difficult to characterize but it is clear that many covalent linkages occur with other wall polymers, as complete extraction of xylan from wood requires drastic alkaline conditions. Stable lignin–xylan complexes remain in wood pulp after Kraft pulping, and may involve carbon–carbon bonding while other bonds forming acetals or glycosides include oxygen. Other cross-linking polymers in the cell wall are ferulic and *p*-coumaric acids, providing important structural components. Dehydrodiferulate oligosaccharide esters have been extracted from wheat bran but not the free dehydrodiferulate acids, indicating that cross-linkages are formed with hemicellulose components (Kroon *et al.*, 1999).

The secondary wood cell wall structure has been visualized using transmission electron microscopy, revealing distinct layers in the secondary wall (Fig. 1.2). The dense wall structure makes the cells impenetrable to micro-organisms without prior degradation of the wall polymers. The size of the pores within the wood cell wall, and hence water-holding capacity of the wall, is low; permeability studies with indicator molecules and dyes indicate that molecules above 2000 Da are unable to penetrate (Cowling, 1975; Srebotnik, Messner & Foisner, 1988; Flournoy, Kirk & Highley, 1991). In wheat cell walls, pores with radii of 1.5–3 nm (measured by gas adsorption) predominate, which are below the size that would allow free penetration by degrading enzymes (Chesson, Gardner & Wood, 1997). Casual predators and pathogens are deterred from establishing an ecological niche in such substrates.

### **The ecophysiology of lignin degradation**

Most reviews of lignocellulose degradation have focused on the mechanisms of the process rather than the ecophysiology of the organisms involved. The Basidiomycota and Ascomycota, mostly in the orders ‘Aphyllorphorales’, Agaricales and Sphaeriales, are considered by Cooke & Rayner (1984) to be responsible for decomposition of a high proportion of the annual terrestrial production of 100 gigatonnes of lignocellulose-rich plant cell wall material, of which lignin alone accounts for 20 gigatonnes (Kirk & Fenn, 1982). The basis of most studies on lignocellulose-degrading fungi has been economic rather than ecological, with focus on the applied aspects of lignin decomposition, including biodeterioration, bioremediation and bioconversion. This has led to an overemphasis on a few fungi as model organisms, particularly in the study of lignin decomposition, without any attempt to decide if they represent the spectrum of lignin-degrading systems in the fungi as a whole. Awareness and understanding of a

wider number of species with good potential for economic use will lead to improvements in bioremediation technology.

Another gap in our knowledge of ligninolytic fungi is that not only have comparatively few taxa been studied but nearly all of them originate from the northern temperate forest and taiga biomes. This is in spite of the fact that the biodiversity of decomposer fungi is much higher in tropical ecosystems, especially tropical forest. In tropical forest, 74% of the primary production is deposited as woody litter and 8% as small litter, 10–35 tonnes litter ha<sup>-1</sup> yr<sup>-1</sup>, illustrating the enormous quantities of lignocellulose processed by decomposer fungi and termites in tropical forest (Swift, Heal & Anderson, 1976). The tropical forest biome contains 400–450 gigatonnes of plant biomass compared with 120–150 gigatonnes for temperate and boreal forest biomes (Dixon *et al.*, 1994). It is estimated that there are three times more taxa of higher fungi in tropical ecosystems than in other forest ecosystems, of which a much higher proportion are decomposers (Hedger, 1985). In spite of this, the isolation and screening of wood- and litter-decomposing fungi from tropical forests has yet to be systematically commenced (Lodge & Cantrell, 1995).

### *Lignocellulose degradation by fungi used in bioconversion of lignocellulose wastes*

Most world mushroom production is from *Agaricus bisporus*, *Pleurotus ostreatus*, and *Lentinula edodes* and related species (Stamets & Chilton, 1983; Stamets, 1993), all grown on a range of substrates prepared from lignocellulose wastes such as straw and sawdust. These taxa have been widely used in physiological studies of cellulose and lignin decomposition in order to determine the role of lignocellulolytic systems in bioconversion of lignocellulose wastes to fruit bodies. Detailed studies on the ligninases of these taxa provided the early understanding of the ligninase systems (Wood, 1980; Kirk & Farrell, 1987; Hatakka, 1994). However, surprisingly little is known of the ecophysiology of the mycelia of these fungi in their natural environments: soil and litter in the case of *Agaricus* spp. and wood in the case of *Pleurotus* spp. and *L. edodes*. These are very different resources, and the published contrasts in the enzyme systems of these two groups of cultivated fungi may be related to the autecology of their mycelia (Wood, Matcham & Fermor, 1988). However there are no *in vivo* studies of lignocellulose degradation by these fungi. When used for bioremediation, it is the fungal mycelia and not their fruit bodies that transform lignocellulosic wastes and transform aromatic pollutants.



***The ecology of wood-rotting fungi***

Another source of isolates for the study of lignin decomposition has been the higher fungi, which cause significant economic losses to the timber industry. Pathogens of trees are an obvious example and include fungi such as *Armillaria* spp. and *Heterobasidion annosum*, where pathogenicity includes white rot exploitation of the lignocellulose resource by mycelia of these fungi (Stenlid & Redfern, 1998). Their ligninolytic systems have been studied (Asiegbu *et al.*, 1998; Rehman & Thurston 1992), findings showing that their role in pathogenicity is much less important than in the subsequent phases of saprotrophic exploitation and inoculum production (Rishbeth, 1979).

Decay of timber in-service has also yielded information on the lignocellulose-degrading enzymes produced by fungi like *Serpula lacrymans*, *Lenzites trabea*, and *Fibroporia vaillantii*. These basidiomycetes are all brown rot fungi, a physiological group that probably coevolved with the Coniferales in the northern taiga and temperate forests (Watling, 1982) and which are important because most in-service timber in Europe and the USA is softwood. Few studies have been made of these economically important fungi in the natural environment. It is salutary to realise that *S. lacrymans*, the dry rot fungus, although the subject of many papers on the nature of its mode of decomposition of cellulose (Montgomery, 1982; Kleman-Leyer *et al.*, 1992), has never been found outside the built environment, although recent studies indicate that it may have its origins in North Indian forests (White *et al.*, 1997). Another well-studied wood-degrading taxon little known outside the laboratory is the white rot thermophilic basidiomycete *Phanerochaete chrysosporium*, which was first considered as a problem in the 1970s in self-heating wood chip piles in its anamorphic state, *Sporotrichum pulverulentum* (Burdsall, 1981). Although this fungus has been the subject of many investigations of cellulases and ligninases because of their potential in bioremediation (Johnsrud, 1988), its natural 'niche' remains unknown.

***The ecology of *Trametes versicolor* and the dynamics of wood decay***

Up to the early 1980s, most detailed studies on lignin-degrading enzymes were on the 'economically important' fungi discussed above. However, since then the search for ligninolytic systems has been extended to include species of little economic importance but of applied potential because of

their rate of growth and high enzymic activity. The most obvious example is *Trametes* (*Coriolus*) *versicolor*, the ligninases of which were first studied by Dodson *et al.* (1987), which causes white rot decay of broad-leaved tree species in temperate forest ecosystems. This fungus has been widely used in bioremediation programmes and characterization of its ligninases is now well understood, providing information that can be related to its ecophysiology in the natural environment. A good example is the regulatory effect of nitrogen on ligninolytic enzyme expression, a reflection of the inductive effect of low nitrogen levels (C:N 200:1 to 1000:1) found in wood (Swift 1982; Leatham & Kirk, 1983).

Unfortunately, laboratory results of this type have led to the simplistic view that 'success' of fungi in the natural environment can be simply related to the physiology of their mycelia in culture. It might be assumed that active ligninases and cellulases and fast mycelial growth in culture can explain the ubiquity of *T. versicolor* in broad-leaved forest. Fortunately, studies on the ligninases of this fungus coincided with studies on the population dynamics of communities of wood decay fungi, including *T. versicolor* (Rayner & Todd, 1979). The fungus causes a rapid white rot invasion of moribund or fallen trees of species such as birch, beech and oak. Rayner & Webber (1985) have shown that the outcome of primary resource capture by fungi like *T. versicolor* is a result of mechanisms that operate in the early stages of colonization. Early phases of expansion are by a rapidly extending mycelium, which utilizes free sugars in the wood of the tree. Entry into broken or cut ends of the wood from the spore rain means that an individual mycelium is usually restricted to an elongated form because of the faster rates of expansion of mycelia along vessels and tracheids. Following this resource capture, contact between mycelia of genetically distinct individuals of *T. versicolor*, and with mycelia of other species of wood-rotting fungi, results in combative behaviour. *T. versicolor* is typical of early colonizers of wood, an assemblage of fungi characterized by Cooke & Rayner (1984) as disturbance tolerant, with a combative mycelial strategy and active lignocellulose exploitation. The wood volume retained by the mycelium is covered by a melanized pseudosclerotial plate, resulting in a mosaic of individuals – the 'spalted' wood of the turners. White rot exploitation of the wood within these volumes by lignocellulolytic enzymes produced by the mycelia may then take place for a number of years. However, the initial phase of occupation and retention of the resource has little to do with the lignocellulolytic potential of the fungus. Comparison of lignocellulose decomposition by common white rot competitors of *T. versicolor*, for example *Stereum hirsutum* and *Hy-*