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Microbial Activity on the Degradation of Lignocellulosic Polysaccharides

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Abstract: In present world there is an increase in demand for organic waste disposal to minimize pollution and maximize resource recovery. Several workers from various parts of the world have reported successful conversion of waste materials to useful compost. Lignocellulose comprises three different polymer types: lignin, hemicellulose and cellulose. Bioconversion of lignocellulosic material through microbial enzyme to produce fermentable sugars has been given serious consideration and continuous research and development activities has been carried out in laboratories around the world. This article highlights the significant research findings and reviews the state of the art in this very important area of biotechnology.

Key words: Polysaccharides, cellulose, hemicellulose, lignin

Introduction

Lignocellulosic wastes are abundantly available in the form of cereal crop residues, sugar cane bagasse and forestry wastes and the principal components of these wastes are cellulose, hemicellulose and lignin. Cellulose is a polymer of glucose while hemicellulose is predominantly composed of xylans which, on hydrolysis, yield the pentose sugar and xylose. Both these sugars can be fermented by suitable microorganisms to produce ethanol (Srinivasan, 1992, Chidthaisong *et al*., 1999). Wood is not only the most stable of bioproducts; its sheer quantity is one of the major factors determining the shape and character of most natural environments. The polymers that make up lignocellulose give strength to plant materials and are the dominant component of biomass on the land surface. Their stability makes degradation difficult. The bulk of nearly all land plants consist of leaf material and of the plant's structural support, varying from a slender wheat stem to the immense trunk of a noble forest tree. All these structures require mechanical strength and this is provided by lignocellulose, the main component of the cell walls of plant tissue and constituting 90% of the dry weight of the plant cell. So ubiquitous, lignocellulose is the principal form of fixed carbon in the terrestrial biosphere. The wood is very great resistance to degradation, particularly if it is kept dry. This resistance depends upon the extraordinary complexity of woody materials and the array of enzymes required for their biodegradation. These are the simplest of four big classes of biochemicals (a subset of organic molecules) involved in life.

Carbohydrates are the basic energy sources and stores for all organisms, and also commonly structural. The simplest carbohydrates are the monomers called sugars, known as monosaccharides. Glucose and fructose are two well known ones because they make up the disaccharide sucrose that constitutes household sugar. Moreover, another sugar called lactose, made of glucose and galactose; and maltose, in beer, is two glucose units. These are six carbon sugars, in the form of a ring with hydroxyl (-OH) groups on most of the carbons and a ketone or aldehyde group (C=O and H-C=O). Another group of important sugars are the five carbon sugars. So when these monomers are linked together in chains to make polymers, or polysaccharides, they can form a storage system for energy (Chidthaisong *et al*., 1999). This linkage is done by removing water at two nearby hydroxyls, a chemical reaction called condensation or synthesis or dehydration. All the polymers considered are formed by condensation with loss of water (sometimes called metabolic water). The reverse reaction is called hydrolysis, meaning splitting by water. In animals the major polymer is glycogen, a highly branched structure of mostly glucose sub-units. Plants have starch, a polymer of glucose that is not branched (Srinivasan, 1992). These polymers can also be structural: cellulose is the material forming the rigid cell walls of plants, which is polymers of starch cross-linked into sheets by hydrogen bonds, made possible by the -OH groups. In animals there is chitin which does something similar in the exoskeletons of crustaceans, insects, and many other animals.

The Components of Lignocellulose: Lignocellulose comprises three different polymer types: lignin, hemicellulose and cellulose. Indeed, its extraordinary properties result directly from its being a mixture of polymers with different physical and chemical properties.

Cellulose: Cellulose at first sight appears to be a very uninteresting molecule. The simplest sort of polymer, it is made up only of glucose units in unbranched chains, each composed of thousands of sugar units with all the bonds between the glucose residues of the same sort. This simplicity is deceptive: the regularity of the cellulose structure allows individual chains to align with others and constitute a microfibril so regular in its structure that it has crystal-like properties. The packing of cellulose chains into fibrillar structures (microfibrils are bundled into macroscopic fibers) not only gives woody materials their strength but also makes cellulose much more difficult to degrade than similar polymers that do not form ordered structures. The exact structure of cellulose is critical to its properties; if the orientation of the bonds between glucose residues is changed from B- (as in cellulose) to A- (as in amylose, a component of starch), the resulting polymer has much less tendency to form ordered structures and, indeed, is rapidly degraded by a single enzyme in our own saliva. Bioconversion or enzymatic hydrolysis of cellulose has been regarded as the ultimate choice in view of the specificity as well as the simpler operating conditions for producing glucose.

Hemicellulose: Although traditionally called "hemicellulose," this second component of lignocellulose is misnamed. Hemicelluloses are mixtures of polymers made up of sugars (mostly not glucose) and sugar derivatives; the polymers may be branched and comprise different types of unit. A major component of lignocellulose in many types of plant (including trees, cereals and other grasses) is xylan. Xylan has a backbone of five-carbon sugar units (many of them acetylated), with side chains of sugar derivatives and is highly charged by virtue of acidic groups (sugar acids). Other hemicelluloses contain other sugars but, like the xylans, are all unordered, branched and charged. Hemicelluloses provide a matrix in which cellulose fibers are embedded to form the layered structure of plant cell walls. The strength of lignocellulose depends on this matrix for its water-holding capacity because dehydrated cellulose fibrils have almost no

mechanical strength. On dehydration, wood shrinks dramatically and can be easily broken by hand. Remarkably, this loss of strength on dehydration is reversible - a property exploited in the timber industry where wood is dried in kilns to a suitable water content for use in construction, rather than having to wait for it to "season" as was done traditionally. Hemicelluloses are always important components of plant cell walls and in some lignified tissues are the predominant material (Table 1).

Lignin: Lignin, the third component present in lignocellulose, is the Earth's most abundant aromatic polymer and the most unusual of the lignocellulose polymers which is more recalcitrant to biodegradation. Delignification of lignocellulose by suitable chemical treatments is an essential step before it can be considered suitable for bioconversion to fermentable sugars or liquid fuels. Cellulose chains, sometimes thousands of glucose residues long, are dwarfed by lignin which forms a molecular network in the plant cell wall such that the whole of the plant conceivably contains a continuously connected lignin molecule. It is almost impossible to describe lignin structure in exact chemical terms because it has no stereochemical regularity. This results from the way it is assembled and has profound consequences for its degradation. Lignin is made up of units called lignols, each of which is an aryl propanol composed of an aromatic ring and a three-carbon chain. The lignols are structurally very closely related to the amino acids phenylalanine and tyrosine from which they are ultimately derived. Although lignin structure includes bonds between aryl propane units of great variety, for our purposes it is important to notice only that there are large numbers of carbon-carbon bonds and ether linkages, neither of which are easily susceptible to hydrolytic cleavage mechanisms. Special bonds link lignin to hemicelluloses. Lignin is more hydrophobic than the hemicelluloses and cellulose. It is likely that its structure, along with that of the hemicelluloses, contributes to the strength of the plant cell wall but this is probably not its main function. The distinctive and useful property of lignin is that it is so difficult to degrade; it therefore forms a protective layer preventing cell wall degradation by pathogens. The resistance of lignin and the crystal-like structure of cellulose fibrils are the two great problems to be overcome in the degradation of lignocellulose.

Microbial Hydrolysis of Polysaccharide: Microorganisms are efficient degraders of starch, chitin and the polysaccharides in plant cell walls. Attempts to purify hydrolases led to the realization that a microorganism may produce a multiplicity of enzymes, referred to as a system, for the efficient utilization of a polysaccharide. In order to fully characterize a particular enzyme, it must be obtained free of the other components of a system (Glazer and Nikaido, 1995). Quite often, this proves to be very difficult because of the complexity of a system. This realization led to the cloning of the genes encoding them as an approach to eliminating other components. More than 400 such genes have been cloned and sequenced and the enzymes they encode have been grouped into more than 50 families of related amino acid sequences (Bok *et al*., 1998; Ichi-ishi *et al*., 1998). The enzyme systems revealed in this manner are complex on two quite different levels. First, many of the individual enzymes are complex, as they are modular proteins comprising one or more catalytic domains linked to ancillary domains that often include one or more substrate-binding domains. Second, the systems are complex, comprising from a few to 20 or more enzymes, all of which hydrolyze a particular substrate. Systems for the hydrolysis of plant cell walls usually contain more components than systems for the hydrolysis of starch and chitin because the cell walls contain several polysaccharides. In general, the systems produced by different microorganisms for the hydrolysis of a particular polysaccharide comprise similar enzymes from the same families. Chemistry is moving into a new era in which renewable resources and starting materials such as D-glucose will likely be prominent features of industrial chemical manufacture. The keys to this progress are the design, development and use of microbial biocatalysts. Aromatic biosynthesis serves as a paradigm for how biocatalysts can be manipulated to achieve the yield, rate and purity criteria central to chemical manufacture. A disproportionate amount of the metabolic carbon flow of the biocatalyst must first be directed into the common pathway of aromatic amino acid biosynthesis. The inability of individual enzymes to convert their substrate to product fast enough to avoid substrate accumulation further impedes carbon flow through the common pathway.

Table 1: Lignocellulose composition (%)

Plants	Cellulose	Hemicellulose	Lignin
Grasses	25-40	25-50	10-30
Softwoods	45-50	25-35	25-35
Hardwoods	45-55	24-40	18-25

Enzymes have become exceedingly valuable tools in organic synthesis as the reactions they catalyze generally proceed under mild conditions and in high stereo- and regioselectivity. Advances in microbiology and genetic engineering have greatly increased the availability of various enzymes. One of the most useful applications of enzyme-catalyzed chemical transformations is in the synthesis of water-soluble, polyfunctional organic molecules such as carbohydrates. As the pivotal roles that carbohydrates play in biological processes become more evident, access to these compounds becomes increasingly important. Science has long recognized the ubiquitously occurring deoxysugars as a novel and important class of carbohydrate, by virtue of the variety of potent and intriguing biological activities they exhibit. The study of the biosynthesis of these naturally vital molecules at a molecular level has received a great deal of attention in recent years, whether it be the well-established study of deoxyribonudeotide biosynthesis via ribonucleotide reductase or newer areas that include 3,6-dideoxyhexose construction and O antigen variation, as well as the emerging scrutiny of the biosynthesis of deoxysugar ligands of antibiotics and cardiac glycosides. All of the 2,6-dideoxy sugars contained within the structure of chromomycin A3 are derived from D-glucose. Enzyme assays were used to confirm the presence of hexokinase, phosphoglucomutase, UDPG pyrophosphorylase (UDPGP), and UDPG oxidoreductase (UDPGO), all of which are involved in the pathway of glucose activation and conversion into 2,6-dideoxyhexoses during chromomycin biosynthesis. Levels of the four enzymes in *Streptomyces* spp. cell extracts were correlated with the production of chromomycins (Espinosa *et al*., 1999 and Liu and Rosazza, 1998). The pathway of sugar activation in *Streptomyces* spp. involves glucose 6-phosphorylation by hexokinase, isomerization to G-1-P catalyzed by phosphoglucomutase, synthesis of UDPG catalyzed by UDPGP, and formation of UDP-4-keto-6-deoxyglucose by UDPGO (Bok *et al*., 1998; Ichi-ishi *et al*., 1998; Espinosa *et al*., 1999; Fernández *et al*., 1998; Liu and Rosazza, 1998).

The way a white rot fungus manages to integrate the degradation of lignin, cellulose and hemicellulose when degrading natural material is unknown. Just how the white rot fungi (and for that matter the brown rotters) avoid damage from the radical species they generate, and why some organisms produce such a profusion of ligninolytic isoenzymes, are issues that remain to be explored. The lignin-degrading systems of white rotters are among the most effective biological systems for removing some of the worst organic pollutants devised by humans - the polyaromatic hydrocarbons and halogenated aromatics. It has been found that white-rot

fungi have been adjudged most promising for bioconversion of lignocellulosic into animal feed by solid state fermentation because of their ability to colonize and cause preferential delignification of native lignocellulose-like straw (Yadav *et al*., 1988; Zadrazil, 1980; Zadrazil 1985; Meevootisom *et al*., 1984).

Microbial Break down of Cellulose: Lignocellulose, or for that matter, pure cellulose (as produced by the cotton plant), are very large molecules and completely insoluble materials so the first step(s) in their degradation by environmental microbes must be extracellular. No microbial cell can absorb molecular complexes of the size of a cellulose fiber, still less that of lignin molecules. Lignocellulose breakdown is catalyzed by secreted enzymes which constitute a major fraction of the extracellular proteins in soil and compost systems. The enzymatic machinery for degrading cellulose, hemicellulose and lignin is possessed only by microorganisms. It is true that plants must contain enzymes for the occasional cleavage of their own cell wall materials to allow a whole range of structural changes during development, but neither higher plants nor animals can achieve the large-scale depolymerization of any of the lignocellulose polymers. Although animals do have a role in lignocellulose degradation in some environments, it is a mechanical one: ruminants and numerous soil earthworms and many arthropods chew up plant material into tiny fragments, greatly increasing the surface area available for attack and hence the rate of degradation by microbial enzymes. Cellulase is a multicomponent enzyme system comprised of at least three major enzymes, viz., endoglucanase (Cx, carboxymethylcellulase), exoglucanase (C1, cellobiohydrolase) and beta-glucosidase (cellobiose). For complete hydrolysis of soil cellulose, the synergistic action of all the three components is necessary and the final product will be glucose (Srinivasan, 1992; Laurent *et al*., 2000; Stratmann *et al*., 1999; Volff and Altenbuchner, 2000). It is also well known that endoglucanase acts to bring about an initial breakdown of the cellulose molecule while the exoglucanase acts from the non-reducing end to remove cellobiose units. The beta-glucosidase completes the hydrolysis of cellobiose to glucose (Hicks *et al*., 1994). Microbial sources for cellulase production have been investigated in detail. While many bacteria, such as *Cellulomonas*, or fungi, such as *Chaetomium*, can attack and consume cellulose as a metabolic source, cell-free multicomponent cellulase enzyme secretion (extracellular) in significant quantities to be of practical value is restricted to a few fungal strains (Srinivasan, 1992).

Bacteria: The environments in which lignocellulosic material can accumulate may be well aerated (aerobic) or essentially oxygen-free (anaerobic); both anaerobic and aerobic cellulose degraders have evolved. *Clostridium thermocellum* is an example of an anaerobic bacterial cellulose degrader. Originally isolated from compost, which becomes both anaerobic and very hot if not regularly mixed, this bacterium grows best at around 60°C. Its complement of cellulolytic proteins illustrates most of the range of enzymatic activities that appear to be required in order to degrade cellulose: endo-beta--1,4-glucanase which cleaves bonds apparently at random in a cellulose chain ("endo-" enzymes attack anywhere in the molecule, "exo-" enzymes only at the ends) - surprisingly, the organism has 15 separate genes for endogluconases; exoglucanases and cellobiohydrolases which cleave the terminal glucose and cellobiose (two glucose at a time), respectively, from a chain, and can do so only from one end; beta-glucosidase enzymes which cleave single glucose residues from small glucose polymers just a few units long (Ichi-ishi *et al*., 1998). No one of these enzymes on its own is much good at breaking down undamaged cellulose fibers. They work in concert, which helps to explain why the *C. thermocellum* cellulolytic system is so elaborate. Held together by a scaffolding protein, this complex of catalytic activities (cellulosome) is ideally arranged to work cooperatively: it can be anchored to the substrate (the cellulose fiber) without the need for every component to have its own cellulose-binding domain. Because the cellulosome also binds to the *C. thermocellum* cell wall, the bacterium is anchored to the substrate in exactly the place where its nutrient is being solubilized. But this does not explain all the complexity - why are components of the hemicellulose-degrading machinery (xylanases) included and why are so many different endoglucanases involved (Ichi-ishi *et al*., 1998).

For aerobic bacteria like *Cellulomonas fimi*, there is presently no evidence for a cellulosome but the proteins are nevertheless surprisingly complicated. Multiple versions of some catalytic functions are present and many of the components are multi-domain proteins. While all have a cellulose-binding as well as a catalytic domain, some also embody other (apparently non-catalytic) domains of unknown function. A further point of interest is the presence of an enzyme hydrolyzing both cellulose and xylan, another hint that cellulose and hemicellulose degradation are closely integrated processes. The anaerobic fungi inhabiting the rumen of cows, sheep and other ruminants are the most recently discovered major group of cellulolytic microbes. They are amongst the most powerfully cellulolytic of all organisms; the enzymes they use have properties similar to the bacterial examples but are even more complicated. Some of these fungal cellulases have two or more catalytic domains, one an endocellulase and another a xylanase. Non-catalytic domains of several different types are found, including one for cellulose-binding, as well as xylan-binding domains and others of unknown function. The presence of cellulosomes is uncertain. Recently, a new methods are given for the production of cellodextrins by the TFA-catalyzed hydrolysis of cellulose and for the subsequent analytical and preparative high performance liquid chromatography of these useful oligosaccharides (Hicks *et al*., 1994).

Fungus: These fungi share the rumen environment with many other microbes. As understanding of the structure of cellulolytic proteins has grown, it turns out that the cellulases of anaerobic fungi are more closely related to those of anaerobic bacteria than to those of aerobic fungi. This suggests that, as the rumen evolved, progenitors of the anaerobic fungi acquired cellulase genes from bacteria, an example of natural horizontal gene transfer which has probably often occurred during evolution in microbial communities. Many cellulolytic aerobic fungi are plant pathogens or symbionts that live cooperatively with their plant hosts. Their cellulose-degrading abilities are probably used not primarily for nutrition but to make holes in the plant cell wall as an aid to infection. By contrast, other fungi are devastating lignocellulose and cellulose degraders. *Trichoderma reesei* has an extensively studied aerobic cellulolytic system. Biochemical analysis of the enzymes secreted by cellulolytic organisms is extremely complicated because: (I) many enzymatically similar proteins can be produced; (ii) they are often only produced in the presence of cellulose - to which they bind tightly - so they are difficult to detect and purify; and (iii) the culture fluid in which they occur typically also contains proteases and glycosidases which can modify part of the cellulase molecules, making it appear that there are more proteins being produced than in fact are present (Berghem and Petterson, 1983; Nogawa *et al*., 1999). A commercial cellulase preparation using a *Penicillium funiculosum* has been developed in the UK while a basidiomycete, *Irpex lacteus*, has been the source of a commercial cellulase in Japan called "Driselase" (Srinivasan, 1992).

The cellulases of *T. reesei* include four endoglucanases, similar in catalytic activity to their bacterial counterparts, and two cellobiohydrolases. During growth on cellulose, two endoglucanases and the two cellobiohydrolases predominate. It is this mixture of enzymes (which can be dissociated from the cellulose by a biochemical trick) that is sold as a commercial product. If you have bought "stone washed" jeans in the last few years, they were almost certainly treated with *T. reesei* cellulases rather than being put in a washing machine with stones from the beach. The *T. reesei* cellulases all have a cellulose-binding domain so they are strongly bound to crystalline cellulose. There is evidence (but not yet certainty) that the cellulose-binding domain can help to disrupt the ordered structure of a cellulose microfibril, thus making a cellulose chain more accessible to the hydrolytic (chain-cleaving) activity of the enzyme. The favored explanation for this plethora of cellulases is that the enzymes together can more readily degrade the polymer than any individual enzyme can on its own. Thus, the rate at which crystalline cellulose is hydrolyzed by a mixture of a cellobiohydrolase and an endoglucanase would be expected to be greater than the sum of the rates of the two enzymes acting in separate reactions (Fuglsang *et al.*, 2000). This is indeed what happens, but strangely some mixtures of pairs of cellobiohydrolases or pairs of endoglucanases are also synergistic, which lacks obvious mechanistic explanation. *T. reesei* and its close relatives among the ascomycetes (the "soft rot fungi" - see box above) rapidly degrade pure cellulose and cellulose in plant tissue that is not heavily lignified but attack the cellulose in woody plant material poorly if at all. The "white rot" lignin-degrading fungi, as we shall shortly see, are often powerful cellulose degraders as well and employ more or less the same complement of enzymes as *T. reesei*. (Reese *et al.*, 1950). There is, however, another small but important group - the "brown rotters," all basidiomycete fungi related to mushrooms, puff balls and brackets. The best known are the "cellar fungus" and *Serpula lacrymans*, the cause of dry rot of building timbers causing £150 million of damage to buildings every year in the U.K. alone. A number of other fungi such as *T. koningii* (Wood and McCare, 1972), *Sporotrichum thermophila* (Coutts and Smith, 1976), *Chaetomium cellulolyticum* (Chahal and Hawksworth, 1976) and *Myceliophthora thermophila* (Sen *et al.*, 1981) have been reported to possess cellulolytic activity.

These fungi are able to degrade the cellulose in wood without degrading lignin, although they may cleave or modify it here and there. The wood is converted to a dark brown fragile material with loss of strength occurring more quickly than loss of weight. The organisms secrete endoglucanases but whether they also secrete cellobiohydrolases is still obscure. In addition, cellobiose dehydrogenase is produced, a puzzling enzyme: because it catalyzes several different reactions, it is difficult to determine which ones are important in natural wood decay. One of the processes yields iron (III) and hydrogen peroxide, reactants which can form the hydroxyl radical HO, which is the most reactive free radical species produced by biological systems and might explain the rapid fragmentation of cellulose fibrils by these fungi.

Hemicellulose Degradation: Like cellulose, the hemicelluloses, the smallest of the lignocellulose polymers, are amenable to hydrolytic cleavage; however, for the degradation of the whole range of hemicellulose structures, a complement of some 24 enzymes is required (Srinivasan, 1992). Most of them have now been described from at least one microbe but a full description of the complete complement, and of the genes encoding them, is not yet available for any single organism. Most is known about the enzymes degrading xylan (which is the other major polysaccharide component in the plant biomass), the endoxylanases and xylosidases (Moracci *et al.*, 2000). Hemicellulose degradation is a complex process because the enzymes are typically produced as multiple isoenzymes (enzymes with apparently the same specific catalytic function existing in more than one physical form that differ from each other in such properties as optimum pH); some have cellulose-binding domains, some are part of a multi-domain protein in which other domains degrade cellulose while, in some organisms, xylanase production is more strongly increased by the presence of cellulose than of xylan. The reasons for this variety are not understood. Enzymatic hydrolysis of xylan by xylanases yield xylose, which is also potentially capable of being fermented to ethanol. Potential yeast strains, such as *Pachysolen tannophilus* and *Candida shehatae*, are being intensively investigated, but so far no practical technology for xylose fermentation to ethanol has emerged.

Lignin Degradation: The biological destruction of lignin requires oxygen. Thus lignin degradation simply does not occur in some natural environments. For example, in certain bogs, plant communities continually deposit dead material which, over many thousands of years, accumulates as peat because in waterlogged (and hence oxygen-depleted) acidic environments, lignin is not degraded. It is not known if less acidic anaerobic environments will allow lignin degradation but, in any event, it is very slow (Kirk and Farrell, 1987). Lignin is probably degraded by some bacteria although none of them has been isolated and grown in laboratory culture. Many filamentous bacteria (actinomycetes) can clearly modify lignin (this is a major feature of compost formation) but they cannot convert the bulk of lignin to carbon dioxide - that is, they cannot mineralize it. The only organisms shown to be able to mineralize a substantial fraction of lignin are all basidiomycetes - the white rot fungi. When they degrade wood or leaf litter, mineralization of the lignin component results in bleaching to a whiter color than undamaged wood - hence the name. Since degradation of lignin by white rotters is an oxidative process, scientists have long searched for oxidative lignolytic enzymes. Such enzymes are of three types and not all white rot fungi use all of them to the same extent. Difficult to unravel, the process remains the least well understood major biochemical process (Gold and Alic, 1993). Remember that the natural substrate is an insoluble polymer of extraordinary complexity, mixed in its natural state with two other types of polymer, all making it very difficult to relate the natural process to laboratory studies. The three categories of enzyme are laccase, lignin peroxidase and manganese peroxidase.

Lignin is not a metabolizable substrate and it has been shown that considerable energy from associated carbohydrates in the lignocellulose has to be expended to obtain even a very limited lignin breakdown (Srinivasan, 1992). Some bacteria and actinomycetes have been shown to possess the capability requisite for lignin degradation, but the group that has greatest potential are the white-rot fungi belonging to the basidiomycetes. Considerable research work on the isolation and characterization of ligninase from white-rot fungi, in particular *Phanerochaete chrysosporium (=Sporotrichum pulverulentum)*. Laccase has been known as a fungal product for over a hundred years but confusion about the nature of its substrates and mode of action led to a search for alternative enzymes to explain lignin breakdown. For the last 20 years, attention has focused on a fungus called *Phanerochaete chrysosporium* because it is a good wood degrader, grows rapidly and well in laboratory liquid culture and appears to produce no laccase. During the 1980s, two new enzymes were described from this fungus- (I) lignin peroxidase and (ii) manganese peroxidase. They are remarkably similar in structure and both oxidize substrates at the expense of hydrogen peroxide reduction. These peroxidase, particularly the lignin peroxidase (the more strongly oxidizing of the two),

can cleave a wide range of model compounds incorporating the types of bond found in lignin. They are good candidates for lignin degradation although it has been difficult to obtain conclusive proof *in vitro*. *P. chrysosporium* produces a great profusion of the enzymes. Eight different lignin peroxidase and four different manganese peroxidase isoenzymes, some of them, in turn, encoded by a whole family of related genes.

The discovery of lignin peroxidase and manganese peroxidase was a turning point in understanding lignin breakdown, not only because enzymes were described which could cleave most if not all the bonds linking lignin units, but also because their properties, particularly those of manganese peroxidase, led to new ideas about lignin degradation. Trivalent manganese [Mn(III)] of manganese peroxidase is a strong oxidizing agent which reacts with lignin leading to bond cleavage. Although the manganese ion is chelated to a small organic acid and somewhat stabilized, the oxidizing reagent is very small compared with an enzyme molecule and can diffuse into the lignin matrix, attacking bonds quite inaccessible to the active sites of the large proteinaceous lignin and manganese peroxidase. Thus, manganese peroxidase functions not by degrading lignin directly but by generating a diffusible mediating substrate. It is likely that similar mechanisms operate both for lignin peroxidase and laccase.

Although lignocellulosic materials are one of the most abundant resources available o earth, their structural resistance to chemical and microbial attack often poses problems in their degradation. Lignocellulose biotechnology in the present state of the art is far from a realistic, commercially attractive and technologically viable proposition. It would appear that intensive research, including application of molecular biology and recombinant DNA techniques, will be necessary to achieve the "dramatic breakthrough" for making lignocellulose biotechnology a viable proposition in future.

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