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REVIEW

Bacteria and lignin degradation

Jing LI, Hongli YUAN, Jinshui YANG (✉)

State key Laboratory of Agrobiotechnology, Key Laboratory of Agro-Microbial Resource and Application, Ministry of Agriculture, Center of Biomass Engineering, College of Biological Sciences, China Agricultural University, Beijing 100193, China

Abstract Lignin is both the most abundant aromatic (phenolic) polymer and the second most abundant raw material. It is degraded and modified by bacteria in the natural world, and bacteria seem to play a leading role in decomposing lignin in aquatic ecosystems. Lignin-degrading bacteria approach the polymer by mechanisms such as tunneling, erosion, and cavitation. With the advantages of immense environmental adaptability and biochemical versatility, bacteria deserve to be studied for their ligninolytic potential.

Keywords lignin degradation, bacteria, genetic manipulation

1 Introduction

Lignin is a major component of plant materials and is the most abundant aromatic substance present in the biosphere by far. As an integral cell wall constituent, lignin provides plants strength and resistance (Argyropoulos and Menachem, 1997). Moreover, lignin participates in water transport in plants and forms a barrier against microbial destruction by protecting the readily assimilable polysaccharides (Monties and Fukushima, 2001). Chemically, lignin is a heterogeneous, optically inactive polymer, consisting of phenylpropanoid interunits linked by different types of covalent bonds (e.g. aryl-ether, arylaryl, and carbon-carbon bonds) (Brunow, 2001). β-O-4-linked ethers, as the predominant structures, make up about half the total, followed by phenylcoumarans, resinols, and various minor subunits (Fig. 1). It has been widely accepted that lignin is racemic (Ralph et al., 1999). Therefore, even a simple β-O-4-linked dimmer that contains two asymmetric carbons exists as four stereoisomers. Because the number of isomers increases geometrically with the number of subunits, lignin presents a complex and non-repeating three-dimensional surface. Formed by lignin's various bond types and their heterogeneity, an irregular noncrystalline network of the plant cell wall is very resistant to microbial degradation.

In biosphere, a wide variety of species are involved in lignin biodegradation, including fungi, plants, animals and also bacteria (Poulos et al., 1993; Perestelo et al., 1996; Modi et al., 1998; Nagarathnamma et al., 1999). Among them, white rot fungi have attracted widespread attention because of their powerful lignin-degrading enzymatic systems (Hatakka, 1994). On the other hand, the stability of fungi are not good in practical treatment under extreme environmental and substrate conditions, such as higher pH, oxygen limitation, and high extraction and lignin concentrations (Daniel and Nilsson, 1998). Bacteria are worthy of being studied for their ligninolytic potential due to their immense environmental adaptability and biochemical versatility (Chandra et al., 2007). However, details of the scale and method by which such breakdown takes place are limited. There is a wide range of examples where actinomycetes and other bacteria have been identified as lignocellulose-degrading microorganisms. These strains come from a great variety of aerobic and anaerobic sources, including compost soil, terrestrial environments, and aquatic ecosystems (Tabak et al., 1959; Crawford et al., 1973; Gibson et al., 1973; Fukuzumi and Katayama, 1977; Haider et al., 1978; Forney and Reddy, 1980; Antai and Crawford, 1981; Janshekar and Fiechter, 1982; McCarthy, 1987; Srinivasan and Cary, 1987; Smith and Ratledge, 1989; Winter et al., 1991; Nilsson and Daniel, 1992; Ajit et al., 1994; Rob et al., 1996; Ruttiman et al., 1998; Björdal et al., 1999; Watanabe et al., 2003; Ko et al., 2007; Yang et al., 2007).

2 Bacteria of lignin degradation

Much research has been done on the breakdown of wood by fungi and the enzymes produced by fungi, while the degradation of wood cell walls by bacteria was not ascertained until the 1980s. Although physiological and taxonomic affiliations of lignin-degrading bacteria are not

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E-mail: yangjsh1999@163.com

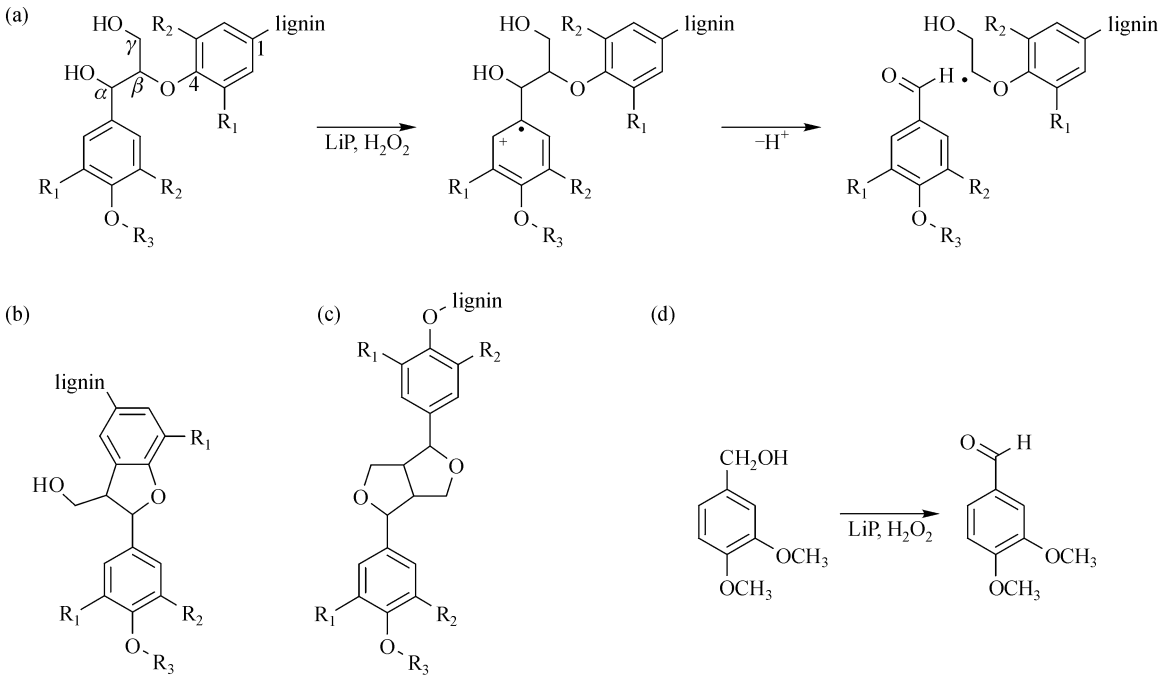


Fig. 1 Chemical structures and reactions discussed in the text. (a) The principal α-O-4 structure of lignin and the pathway for its Cα-Cβ cleavage by LiP. (b) A phenylcoumaran lignin structure. (c) A resinol lignin structure. (d) LiP-catalyzed oxidation of the fungal metabolite veratryl alcohol. Gymnosperms contain lignins in which most subunits have $R_1 = OCH_3$ and $R_2 = H$. Angiosperm lignins also contain these structures but have, in addition, subunits in which $R_1 = OCH_3$ and $R_2 = OCH_3$. Grass lignins contain both types of structures but have, in addition, some subunits in which $R_1 = H$ and $R_2 = H$. These nonmethoxylated lignin structures are more difficult to oxidize than those that contain one or two methoxyl groups. In the predominating nonphenolic structures of lignin, R_3 = lignin, whereas $R_3 = H$ in the minor phenolic structures. (Hammel and Cullen, 2008)

well known, three main morphological forms of cell wall degradation have been discovered: tunneling, erosion, and cavitation (Blanchette, 1995). Tunneling bacteria are rare in waterlogged wood because they appear to require a good supply of oxygen. They produce minute tunnels to migrate through the cell wall. Erosion bacteria are responsible for the predominant form of degradation in waterlogged archaeological wood, since they seem to tolerate near-anaerobic or fully anoxic environments (Kim et al., 1996; Björdal et al., 1999). Erosion bacteria are typically rod-shaped, and they attack the wall from the lumen into the secondary walls singly or in small groups (Holt, 1983). Cavitation bacteria, presenting in the wood cell lumen, apparently utilize products derived from the activities of wood degraders (Singh et al., 1990).

2.1 Aerobic bacteria

Phylogenetically and taxonomically, bacteria of several genera, including *Alcaligenes*, *Arthrobacter*, *Nocardia*, *Pseudomonas*, and *Strepomyces*, have been found degrading single ring aromatic substrates (Mahadevan, 1991). Radioactive methods are usually used in quantitative lignin degradation studies, while, with the disadvantage of rough estimation, non-isotopic methods are usually restricted to qualitative lignin degradation studies (Crawford et al., 1980). The radioactive lignins play an important role in the initial steps of studies on lignin degradation. After microbial degradation, the fate of the ^{14}C-label could be measured by using ^{14}C-labelled lignins, i.e. in mineralizing lignin, in solubilizing lignin, in forming $^{14}CO_2$, etc. (Crawford et al., 1980).

The data from early determinations with non-isotopical experiments was disappointing, thus radioactive methods began to be used widely for their superiority. *Nocardia* was reported to have released between 4% and 7% $^{14}CO_2$ from ^{14}C-ring labeled maize lignin or synthetic lignins in 15 days, and 6% to 15% of the label was present in the side chains and methoxyl groups of the same substrates (Trojanowski et al., 1997), whereas *Pseudomonas* set free only 1% $^{14}CO_2$, irrespective of the location of the label (Kaplan and Hartenstein, 1980). Distinctly, *Bacillus megaterium* mineralized 12% of ^{14}C-side chain labeled spruce lignin, but only 0.3% of ^{14}C-ring dehydrogenative polymerizates (DHPs) in 20 days (Robinson and Crawford, 1978). Later, *Nocardia*, *Pseudomonas*, and *Corynebacterium* strains were reported to degrade 1.0%–10% of four different lignin preparations, as measured with a spectrophotometer. In this case, the lignin was unlabelled, so the value may overestimate the actual degradation (Janshekar and Fiechter, 1982). Kerr et al. (1983) reported that *Arthrobacter* sp. KB-1, cultured on four lignin prepara-

tions from peanut hulls, mineralized 2.9% of the lignin component of *S. alternifolia* (^{14}C-lignin) lignocellulose in 10 days. (^{14}C)DHP, (O^{14}CH$_3$)DHP, and (^{14}C-ring)DHP can be mineralized by resting cells of *Xanthomonas* sp., wherein about 30% of the label of substrates had evolved as $^{14}CO_2$ after 20 days, and the oxidation of the methoxyl groups was slightly faster than that of the rings and the side chains (Kern, 1984). Oxygen was a necessity in the degradation of DHPs, and the degradability in pure oxygen was equal to that in air. The label was not only evolved as $^{14}CO_2$, but also incorporated into RNA, DNA, and protein by *Xanthomonas* sp., indicating that the bacterium utilized synthetic lignin as a carbon source (Archana and Mahadevan, 2002). Kern and Kirk (1987) further characterized the lignin degrading activity of *Xanthomonas* sp. Using ^{14}C-methylated spruce lignin plus five DHP preparations of different molecular sizes, degrees of methylation, and locations of the label, the bacterium merely degraded substrates of molecular weight up to 1000 daltons, apparently.

Tunneling bacteria, mentioned above, are also capable of mineralizing synthetic lignins. Separate strains can reach values of $^{14}CO_2$ evolution of 11%, 10.4%, and 6.0% from ring-, side chain-, and methoxyl labeled lignins, respectively, although these bacteria have not been identified yet. Actinomycetes have been the subject of the greatest research effort in this field (McCarthy, 1987). Crawford gained the first evidence of lignin mineralization by *Streptomyces* strains and demonstrated up to 3.5% oxidation to $^{14}CO_2$ of (^{14}C-β) lignin-labeled maple lignocellulose after 41 days. The ability of *Streptomyces* to attack lignin was confirmed later by Phelan et al. (1979) and by Crawford et al. (1982), who showed that these microorganisms mineralize side chain- and ring-labeled lignins. Moreover, they bring about decay of the glucan component of lignocellulose.

2.2 Anaerobic bacteria

It is not only in aerobic conditions, but also in anaerobic conditions that the ^{14}C-labeled lignin could be degraded by bacteria. The microorganisms causing the decay have not been characterized. However, both $^{14}CO_2$ and $^{14}CH_4$ have been detected. It is assumed that they might consist primarily of bacterial consortia (Holt and Jones, 1983). After anaerobic incubation with various microbial samples, no conversion of alkyl-, aryl-, or methoxyl ^{14}C-labeled DHPs to $^{14}CO_2$ plus $^{14}CH_4$ was found (Hackett et al., 1977). However, Zeikus et al. (1982) found that with ^{14}C-Kraft lignin and highly depolymerized synthetic ring-U-^{14}C-labeled lignin, the degradation occurred under anaerobic conditions. After 16 days of anaerobic incubation of synthetic lignins of low MW with microbial samples from lake sediments, about 15% of the lignins were converted to $^{14}CO_2$ plus $^{14}CH_4$, while the higher MW component remained intact.

Under anaerobic conditions, the conversion of radiolabeled lignin substrates to gaseous products is very slow. Benner et al. (1984) found that the degradation of ring-U-^{14}C-labelled DHPs by two mesophilic microbial populations was only 1.8% and 3.7%, after about 10 months. Thermophilic bacteria decomposed 4% of the labeled synthetic lignin to $^{14}CO_2$ plus $^{14}CH_4$ in 60 days. However, the mineralization of pine lignin was below 2.7%. It was found that at least in some anaerobic cultures, the labeled gases may evolve from non-lignin contaminants present in the substrates or from low MW lignin structures released abiotically (Kirk and Farrell, 1987). Furthermore, degradation of lignin by bacteria in natural anoxic environments may be slow but significant.

3 Enzymes of lignin degradation

Lignin, with a highly complex and relatively random structure, is tenaciously resistant to degradation (Ralph, 2005). Depending on the degree of crystallization of the lignin, enzymes can cleave the various specific bonds within lignin but vary in effectiveness, and each enzyme is specific to a particular chemical bond. Lignin's wide varieties of chemical bonds make specific cleavage by the active site of an enzyme difficult, and it requires many enzymes, each with a specific active site, for degradation.

3.1 Enzyme of aerobes

There are two major groups of intracellular enzymes of aerobes involved in lignin degradation under aerobic conditions: peroxidases and phenol oxidases. Both enzymes are glycosylated, which could increase the stability of the enzymes (Nie et al., 1999).

3.1.1 Peroxidases

Peroxidases (EC 1.11.1.7) are haem-containing enzymes that catalyze a number of oxidative reactions and hydroxylations, using hydrogen peroxide (H_2O_2) as the electron acceptor (Ralph, 2005). Peroxidases exist in bacteria, fungi, plants, and animals. In consideration of the sequence similarity and structural divergence, they are viewed as belonging to a super-family consisting of three major classes (Welinder, 1992): mitochondrial yeast cytochrome c peroxidase, chloroplast and cytosol ascorbate peroxidases, and gene duplicated bacterial peroxidase (class I); secretory fungal peroxidases (class II); classical, secretory plant peroxidases (class III).

Peroxidases are enzymes defined as oxidoreductases using hydroperoxides as electron acceptor, and are able to catalyze the oxidation of a large variety of substrates such as phenol, aromatic amines, and other compounds such as alkyl peroxides and aromatic peracids (Sjoblad and Bollag, 1981). Most of them have a common catalytic cycle (Everse et al., 1991):

$$\text{Native peroxidase}(Fe^{3+}) + H_2O_2 \rightarrow \text{Compound} - I + H_2O \quad (1a)$$

$$\text{Compound} - I + AH_2 \rightarrow \text{Compound} - II + AH^* \quad (1b)$$

$$\text{Compound} - II + AH_2 \rightarrow \text{Native peroxidase}(Fe^{3+}) + AH^* + H_2O \quad (1c)$$

In the first reaction (1a), there is a two-electron oxidation of the ferriheme prosthetic group of the native peroxidase by H_2O_2 (or organic hydroperoxides). Compound-I (oxidation state + 5) is an intermediate derived from the first reaction, consisting of oxyferryl iron (Fe^{4+}= O) and a porphyrin π cation radical. In the following reaction (1b), compound-I is reduced by the first electron donor AH_2, receives one electron, and forms compound-II (oxidation state + 4). Then compound-II accepts an additional electron from AH_2 in the third step (1c), whereby the enzyme is returned to its native resting state, ferriperoxidase. During the peroxidase reaction, different oxidation products are formed, depending on the various natures of the substrates. Electron donors such as aromatic amines and phenolic compounds are oxidized to free radicals, AH* (reactions (1b) and (1c)) (Dunford and Adeniran, 1986). When reaction (1a) occurs on an electrode surface, compound-I can be directly reduced into ferriperoxidase by a heterogeneous electron transfer (ET) which comes from the electrode material, instead of redox mediators. The aim of these approaches is to lead to a reduction current correlated with the concentration of peroxide in the solution. At high concentrations of peroxide, the peroxidases turn into an enzymatically inactive form, denoted as compound-III (oxidation state + 6) (Adeiran and Lambeir, 1989).

Because lignin peroxidase can catalyze the oxidation of substrates with a reduction potential greater than 1.3 volts, the range of its substrates is extremely broad. The enzymes have been proven to utilize lignin monomers, dimers, and trimers as well as polycyclic aromatic compounds as substrates (Haemmerli et al., 1986). Side-chain fragmentation (C-C cleavage) caused by the radicals (compounds I and II) results in the breakdown of the lignin polymer (Evans and Fuchs, 1988). They can also catalyze lipid peroxidative pathways, which are capable of oxidizing substrates such as lignin-model dimers and polycyclic aromatic hydrocarbons.

3.1.2 Phenol oxidases

Phenol oxidases (PO) are excreted mainly by microorganisms. They use oxygen as the final electron acceptor to catalyze the oxidation of recalcitrant aromatic compounds such as lignin into more readily available substrates (Cullen and Kersten, 1996). Many studies have found that purified PO are involved in the biodegradation and detoxification processes of some aromatic pollutants (Criquet et al., 2000; Farnet et al., 2004). Moreover, these enzymatic activities can be inhibited or induced by various xenobiotic compounds, such as heavy metals (Baldrian and Gabriel, 2002; Tuomela et al., 2005). Therefore, PO immobilized on supports are increasingly applied to investigate water and soil pollution in conjunction with bioremediation (Durán and Esposito, 2000; Gianfreda and Rao, 2004; Novotny et al., 2004). Based on substrate specificity, the phenol oxidases can be divided into laccases and polyphenol oxidases.

3.1.2.1 Laccase

Laccase, as a cuproprotein, is a member of a small group of enzyme, denominated blue oxidases (Karam and Nicell, 1997). Laccase (E.C. 1.10.3.2, *p*-benzenediol: oxygen oxidoreductase), an oxidoreductase, is able to catalyze the oxidation of various aromatic compounds (particularly phenols) with the reduction of oxygen to water (Fig. 2) (Thurston, 1994; Karam and Nicell, 1997). Four copper atoms, exhibited in laccases in general, play important roles in enzyme catalytic mechanisms. According to specific spectroscopic and functional characteristics, copper atoms are located in different binding sites and are classified into three types (Yaropolov et al., 1994; Xu, 1996; Durán, 1997; McMillin and Eggleston, 1997).

The molecular weight of laccase is quite large (MW 70000) (Bourbonnais et al., 1997), which makes it impossible to penetrate deep into wood. Moreover, due to its rather low-redox potential (0.5–0.8 V), laccase is unable to oxidize non-phenolic (C_4-etherified) lignin units with a high-redox potential (> 1.5 V) (Galli and Gentili, 2004). In view of these limitations, laccase alone can only oxidize phenolic lignin units (Sjöström, 1993) at the substrate surface. Therefore, laccase is often used with an oxidation mediator, a small molecule that is able to extend the effect of laccase to non-phenolic lignin units and to overcome the accessibility problem (Galli and Gentili, 2004). The mediator, called LMS, is first oxidized by laccase and then diffuses into the cell wall to oxidize lignin, which is inaccessible to laccase (Fig. 3). Laccase-mediated oxidation of non-phenolic lignin units can follow an electron transfer, a radical hydrogen atom transfer, or an ionic mechanism, depending on the mediator (Barreca et al., 2004). Several organic and inorganic compounds, such as thiol and phenol aromatic derivatives, *N*-hydroxy compounds and ferrocyanide, have been reported as effective mediators for the above-mentioned purposes (Susana and José, 2006). Claus et al. (2002) found that the LMS strengthened dye decolorization and some dyes resistant to laccase degradation were decolorized.

Laccase is not only widely distributed in higher plants (Mayer and Harel, 1979) and fungi (Xu, 1996; Karam and Nicell, 1997), but also found in some bacterial strains of

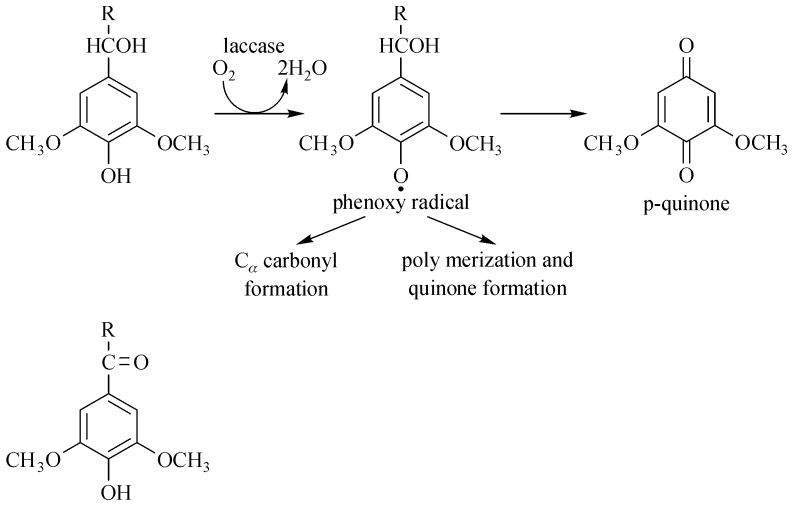


Fig. 2 Typical reaction of laccase: phenol oxidation. It shows a typical laccase reaction, where a phenol undergoes a one electron oxidation to form a free radical. This active oxygen species can be transformed into a quinone in a second oxidation step. The quinone as the free radical product can undergo polymerization. (Adapted from Rosana et al., 2002)

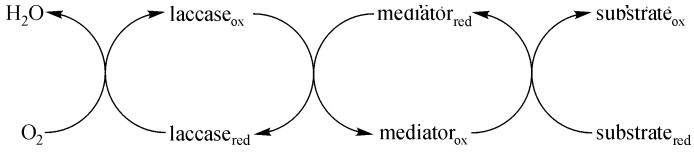


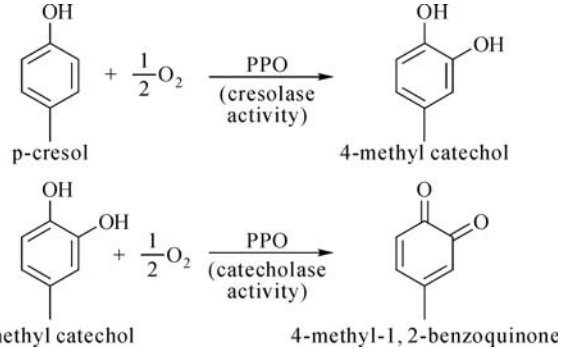
Fig. 3 The redox cycle of laccase-catalyzed oxidation of the LMS. (Adapted from Petri and Andreas, 2008)

Azospirillum lipoferum (Givaudan et al., 1993) and *Alteromonas* sp. (Sanchez-Amat and Solano, 1997). Recently, following exhaustive BLAST searches of the non-redundant protein sequence database (http://www.ncbi.nlm.nih.gov/index.html) and unfinished microbial genomes (http://www.tigr.org), using fungal laccase sequences as queries, Alexandre and Zhulin (2000) found that several sequences of microbial proteins showed a significant similarity to fungal laccases (Table 1). Sequences with significant similarity to fungal laccases throughout the entire length were taken for further analysis. It was proved that laccases, soluble and excreted in bacteria, are widespread in bacteria.

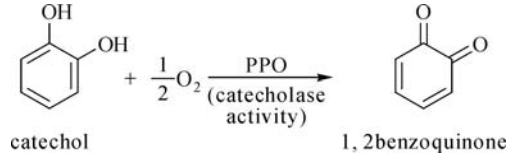
3.1.2.2 Polyphenol oxidases

Polyphenol oxidases or tyrosinases (PPO), containing a dinuclear copper center, are able to insert oxygen in a position *ortho*- to an existing hydroxyl group in an aromatic ring with the concomitant oxidation of the diphenol to the corresponding quinone (Alfred, 2006). The structure of the active site of the enzyme is highly conserved, in which copper is bound by six or seven histidine residues and a single cysteine residue (Alfred, 2006). Shahriar et al. (2007) found that the reactions catalyzed by PPO were as follows. All of the reactions utilize molecular oxygen.

a) Reaction with monophenols (e.g. *p*-cresol):



b) Reaction with diphenols (e.g. catechol):



c) Reaction with triphenols (e.g. pyrogallol):

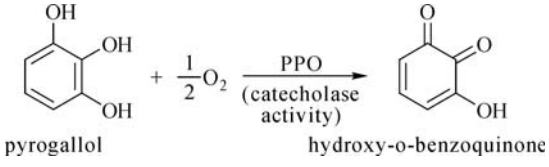


Table 1 Putative bacterial laccases

species	protein name[a]	protein sequence analysis				
		similarity to laccase[b]	length/aa	signal peptide	TM[c]	copper-binding sites[d]
Mycobacterium tuber culosum	Rv0846c_2916905	3e–36	504	–	–	+
Escherichia coli	PcoA_1073341	1e–29	605	+	–	+
Caulobacter crescentus	Contig_122[e]	6e–29	ND	ND	–	+
Pseudomonas syringae	CopA_116921	8e–28	609	+	–	+
Bordetella pertussis	Contig_449[e]	9e–28	591	+	–	+
Xanthomonas campestris	CopA_1073083	3e–26	635	+	–	+
Pseudomonas aeruginosa	Contig_52[e]	4e–26	ND	ND	–	+
Mycobacterium avium	Contig_982[e]	1e–22	ND	ND	–	+
Pseudomonas putida	CumA_4580028	2e–22	459	+	–	+
Rhodobacter capsulatus	3128288	1e–16	491	+	–	+
Yersinia pestis	Contig_768e	5e–15	ND	ND	–	+
Campylobacter jejuni	Contig_1[e]	3e–12	512	+	–	+
Escherichia coli	YacK_2506227	9e–09	516	+	–	+
Aquifex aeolicus	Sufl_2983586	1e–07	527	+	–	+

[a]Protein name and GenBank identification numbers are given (separated by underscores). [b]E (expected) value (Ref. 7) for the closest hit with a fungal laccase. [c]TM, the presence of transmembrane regions according to hydrophobicity analysis. [d]Conservation of copper-binding sites can be viewed at www.llu.edu/llu/medicine/micro/laccase/. [e]TiGR preliminary identification numbers. Abbreviation: ND, not determined. (Adapted from Alexandre and Zhulin, 2000)

Apparently, the enzyme extensively exists in bacteria, fungi, plants and animals (Alfred, 2006). *In vitro* studies have proven that polyphenol oxidase is involved in the degradation of natural phenols with more complex structures such as anthocyanins and flavanols (Finger, 1994). However, these compounds cannot be oxidized by the enzymes directly but by the quinones formed by polyphenol oxidase from catechol and catechin (Jiménez and García-Carmona, 1999).

3.2 Enzymes of anaerobes

A wide variety of aromatic compounds, including phenols related to lignin, have been found to be biodegradable in strict anaerobic conditions (Balba and Evans, 1977; Evans, 1977). In aerobic bacteria, molecular oxygen is used as a common cosubstrate for key enzymes of aromatic metabolism. In contrast, in anaerobic bacteria, all oxygen-dependent reactions are replaced by a set of alternative enzymatic processes. Using nitrate, sulfate or Fe(III) as terminal electron acceptors, anaerobic bacteria comprise organisms with anaerobic respiratory chains. It is suggested that some of the enzymatic processes involved in the degradation of phenol by anaerobes uniquely exist in the aromatic metabolism of anaerobic bacteria (Matthias and Georg, 2005).

The initial steps of anaerobic phenol metabolism are composed of the following reactions (Fig. 4):

Two different enzymes are involved in the carboxylation of phenol: a phenylphosphate synthase that transforms phenol with ATP into phenylphosphate, forming AMP and phosphate (Schmeling et al., 2004) and a phenylphosphate carboxylase that transforms phenylphosphate with CO_2 into 4-hydroxybenzoate and phosphate, using Mn as the metal cofactor (Lack et al., 1991; Schühle and Fuchs, 2004).

By a specific AMP-forming carboxylic acidcoenzyme A ligase, 4-hydroxybenzoate is first activated to 4-hydro-

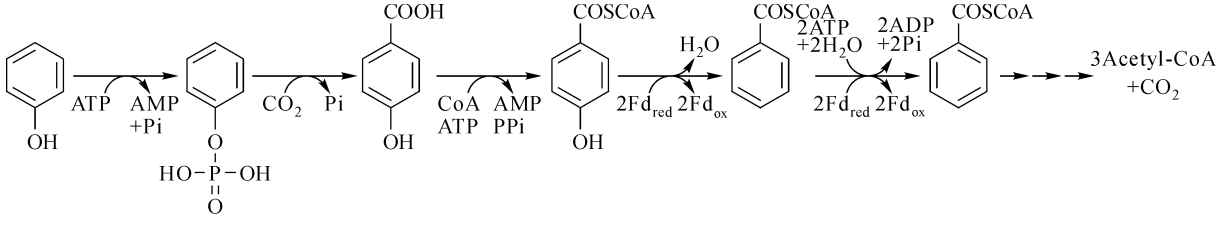


Fig. 4 Enzymatic reactions involved in anaerobic phenol metabolism. Reactions catalyzed by (1) phenylphosphate synthase, (2) phenylphosphate carboxylase, (3) 4-hydroxybenzoate CoA ligase, (4) 4-hydroxybenzoyl-CoA reductase, (5) benzoyl-CoA reductase and (6) enzymes involved in modified β-oxidation reactions (Adapted from Matthias and Georg, 2005).

xybenzoyl-CoA (Biegert et al., 1993; Gibson et al., 1994). Then the phenolic coenzyme A ester is dehydroxylated to benzoyl-CoA by 4-hydroxybenzoyl-CoA reductase (Brackmann and Fuchs, 1993).

Benzoyl-CoA is reduced to a non-aromatic, cyclic dienoyl-CoA compound by benzoyl-CoA reductase (Boll et al., 2000). According to the hydrolyzation of two molecules of ATP, benzoyl-CoA reductase couples aromatic ring dearomatization to a stoichiometric hydrolysis (Boll and Fuchs, 1995; Boll et al., 1997). Then the product is further converted to three molecules of acetyl-CoA and one molecule of CO_2.

4 Genetic manipulation of lignin degradation bacteria

The genes encoding protocatechuate 3, 4-dioxygenase from a marine isolate from *Bacillus subtilis* have been successfully cloned and expressed (Gold et al., 1998). The enzymes produced by the bacteria can catalyze the cleavage of interunit linkages, according to bacteria cultured on model dimmers (Archana and Mahadevan, 2002).

Some researchers constructed genomic libraries of ligninolytic strains to clone specific genes. The advantage of this strategy is that it allows cloning of genes, which code for enzymes that might not have been isolated (Vicuna, 1988). *Bacillus* sp., isolated from decaying coir, is able to utilize 0.25% indulin (kraft pine lignin) as the sole carbon source. The genomic library of *Bacillus* sp. has been constructed into the high copy number plasmid vector pUC19. The two clones of MBA5 and MBA23 were selected based on the ability of utilizing indulin (Archana and Mahadevan, 2002).

5 Conclusions

Lignin degradation involves multiple biochemical reactions. Though the role of fungal lignin peroxidases has been well established only in recent years, the study of bacterial lignin degradation has become increasingly important. Bacteria of several genera such as *Alcaligenes*, *Arthrobacter*, *Nocardia*, *Pseudomonas*, and *Strepomyces* readily degrade single ring aromatic substrates. The genes involved in lignin degradation have been cloned and expressed.

However, the involute chemical structure of lignin requires that the enzymes be highly versatile, and they use free radicals as a means to attack lignin, which makes them able to break down a very wide range of other compounds. Furthermore, the enzymes involved in lignin degradation in bacteria do not seem to closely resemble those of the better understood fungi, which have limited the use of bioinformatics in the identification of potential lignin degrading enzymes.

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