

Plant Terpenes and Lignin as Natural Cosubstrates in Biodegradation of Polychlorinated Biphenyls (PCBs) and Polycyclic Aromatic Hydrocarbons (PAHs)

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Abstract The objective of this minireview is to examine how cometabolic biodegradation of polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) might be affected by plant terpenes and lignins as natural substrates abundant in nature. The topics covered, hence, are environmental significance of PCBs and PAHs, nature and distribution of plant terpenes and lignin, structural and metabolic similarities of the natural compounds to PCBs and PAHs, and possible roles of the natural substrates in inducing the biodegradative pathways of PCBs and PAHs

Keywords PCBs, PAHs, terpenes, lignin, biodegradation, bioremediation, induction, RT-PCR

PROPERTIES AND ENVIRONMENTAL SIGNIFICANCE OF PCBs AND PAHs

PCBs are synthetic organic chemicals that are comprised of a biphenyl backbone with 1 to 10 chlorine radicals and have been used widely since they were first introduced in 1929 in making such compounds including pesticides dielectric fluids in capacitors and transformers, hydraulic fluids, solvent extenders, flame retardants, plasticizers and cutting oils due to their low chemical reactivity, heat stability, nonflammability and high electrical resistance. Several hundred million pounds of PCBs are known to be released into the environment until now, where they became persistent by adsorbing into organic compounds in soil and sediments due to their hydrophobicity, resulting in the accumulation in a variety of biota including humans. PCBs are strictly regulated because of their potential toxicity (e.g., mutagenicity and endocrine disruption). PCBs are generally recalcitrant but decontamination of PCBs in the environment may be possible through combustion, photolysis and microbial degradation. PCBs, primarily accumulated in sediments, can be shielded from photolysis and incomplete combustion of PCBs can generate polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDFs). PCDFs are extremely toxic to certain species of laboratory animals, particularly guinea pigs [1]. Microbial degradation, however, holds the greatest promise for an inexpensive way of decontaminating polluted sites with PCBs.

PAHs are compounds composed of two or more fused aromatic rings that belong to a class of aromatic hydrocarbons consisting of three or more fused benzene rings in linear, angular and cluster arrangements. PAHs are usually formed during coal gasification and liquification processes and improper waste incineration practices. PAHs are widespread environmental pollutants that have been found to have toxic, mutagenic, and carcinogenic properties [2]. PAHs have low solubility in water, high melting and boiling points, and low vapor pressure. Solubility decreases, melting and boiling points increase, and vapor pressure decreases with increasing molecular weight. Anthropogenic PAHs affect living organisms through their toxicity. The mechanism of toxicity for PAHs seems to be through interference with cellular membrane function and enzyme systems associated with cell membrane. Human exposure to PAHs can occur through the inhalation of tobacco smoke and polluted air [3], ingestion of contaminated foods and exposure to environmental contaminants in soil and water [4]. Destruction of PAHs, however, is possible given microbial degradative activities were developed throughout evolutionary periods of time.

PROPERTIES OF PLANT TERPENES AND LIGNIN, AND THEIR SIGNIFICANCE

Terpenes

Monoterpenes are naturally occurring compounds produced by plants and animals. The majority of these compounds are unsaturated hydrocarbons (C₁₀), but derivative some of their oxygenated derivatives such as alcohols, ketones, and carboxylic acids are known as

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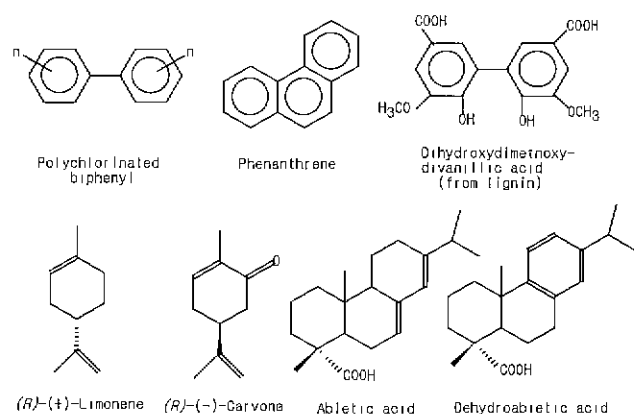


Fig. 1. Structural similarities between polychlorinated biphenyl/polycyclic hydrocarbon and plant terpenes/lignin as natural substrates

monoterpenoids [5]. Monoterpenes are branched-chain C₁₀ hydrocarbons comprised of two isoprene units and are widely distributed in nature with more than 400 naturally occurring monoterpenes identified [6]. *R*-(+)-limonene can be obtained from orange peel oil up to 50,000 ton a year [7], and its allylic oxidation yields *cis/trans*-carveol and carvone. *S*-(+)-carvone (caraway-like flavour) and *R*-(-)-carvone (spearmint-like flavour) are important aroma compounds for use in foods and beverages (Fig. 1). Resin acids, including dehydroabietic acid (DhA), are diterpenoids which are a minor component of wood (Fig. 1). These naturally occurring diterpenoids are believed to cause much of the toxicity to fish, being pulp mill effluents. In overall, terpenes holds a potential interest practical applications especially in the fragrance and flavour industries, as well as in the pharmaceutical and chemical industries [8].

Lignin

Lignin is one of the most abundant aromatic compounds in the biosphere and is the most abundant aromatic components of vascular plants. It is composed of various phenylpropanes having guaiacyl, syringyl *p*-hydroxyphenyl and biphenyl nuclei linked and polymerized through a variety of nonhydroxyl stable C-C and C-O-C bonds [9]. The structure of lignin is based on the phenyl propanoid unit, which consists of an aromatic ring and a 3-C side chain. Three precursors of lignin are synapyl, coniferyl, and coumaryl alcohol, formed from cinnamyl alcohol. Lignin is not formed by a specific enzyme but in a chemical reaction involving phenols and free radicals. It is formed as an encrusting material on the cellulose and hemicellulose matrix, and is not arranged in a specific order. The structure of biphenyl nuclei in lignin is dihydroxy-dimethoxy divanillic acid (DDVA; Fig. 1)

ECOLOGY OF TERPENE AND LIGNIN DEGRADERS AND THEIR METABOLISM

Ecology

An extensive screening of bacteria able to grow on carveone or limonene as the sole carbon source yielded many Gram positive strains from environmental sources including freshwater sediments, and some of these were able to utilize limonene, carveone and their derivatives. Seven strains of the class *Proteobacteria* utilizing dehydroabietic acid were able to use a mixture of 12- and 14-chlorodehydroabietic acid (Cl-DhA) [10]. Consistent with the ability of certain bacteria to use DhA as the sole organic substrate, a report indicates that aerobic biological treatment systems are capable of effectively removing resin acids [10]. Recently, it has been suggested that monoterpenes play an important role in altering nitrogen and carbon cycling in forest soils [11]. Lignin degradation in salt marsh ecosystems is an important biogeochemical process due to high primary productivity in such ecosystems and the abundance of vascular-plant-derived lignocellulosic material [12]. In a salt marsh, bacteria mediate most of the lignin degradation [13]. Recently, in a study conducted to assess the degradation potential of marine bacteria for pulp mill wastes rich in lignin-related compounds, two numerically important bacteria were isolated from the wastes [14]. One of the isolates did not utilize natural polymers, but could grow on a variety of monosaccharides, alcohols, and amino acids. It also utilized methanol and aromatic compounds (phenol, benzoate, *p*-hydroxy benzoate, and vanillate). Complete degradation of lignin in natural environments involves various microorganisms, including fungi and bacteria [15]. Although most researches on the biochemistry and genetics of lignin degradation have focused their attention on fungi, bacteria are also capable of solubilizing, transforming, and mineralizing a variety of lignin preparations and lignin-like polymers and monomers [16].

Metabolism and Degradative Pathways of Terpenes and Lignin

Monooxygenase from *Pseudomonas fluorescens* NCIMB 11671 grown on α -pinene as a sole carbon and energy source catalyzed NADH-linked oxygenation of α -pinene to the corresponding epoxide [17]. The absorption spectrum of the enzyme was typical of a flavoprotein. In this study, the deduced amino acid sequence data revealed a region with strong homology to the σ 54-dependent promoters, and a region close to the C-terminus showed a striking similarity to Rieske-type-iron-sulphur centers. Furthermore, data banks were searched for conserved sequences for the open reading frame (ORF) of α -pinene monooxygenase. This search identified quite extended similarities with chlorobiphenyl dioxygenase from *Pseudomonas pseudoalcali-*

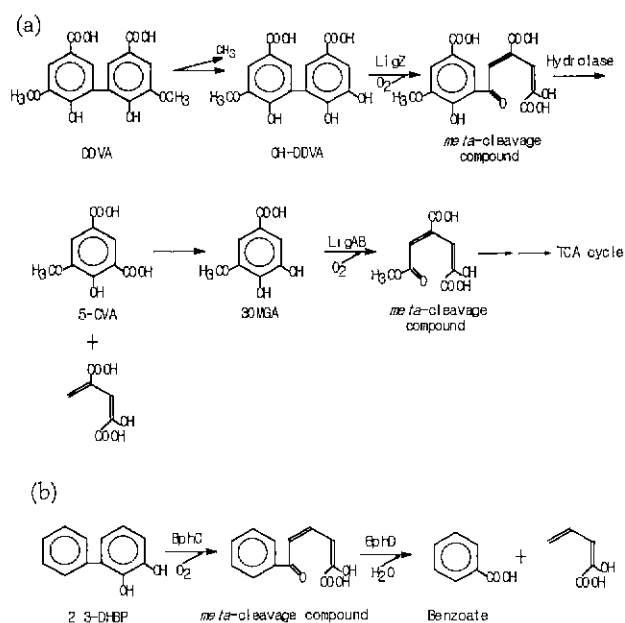


Fig. 2. Proposed metabolic pathway for dihydroxy-dimethoxydivanillic acid (DDVA) by *Sphingomonas paucimobilis* SYK-6 (a) Pathway for degradation of 2,3-dihydroxybiphenyl (2,3-DHBP) to benzoate by PCB-degraders (b) [21]

genes and toluate 1,2-dioxygenase (*xylX*) from *Pseudomonas putida* plasmid pWW0, indicating that there may be an evolutionary relationship between α -pinene monooxygenase and biphenyl dioxygenase and other monoaromatic dioxygenases. Two representative strains (DhA-33 and DhA-35) able to grow on DhA were isolated from a sequencing batch reactor to treat a process stream from a paper mill [19]. They were most closely related to *Sphingomonas yanoikuyae* and *Zoogloea ramigera*, respectively. Both isolates could also grow in other abietanes, i.e., abietic and palustric acids. *Pseudomonas abietaniphila* BKME-9 could, however, degrade DhA via ring hydroxylation by a novel dioxygenase. The *ditA1*, *ditA2*, and *ditA3* genes, which encode the α and β subunits of oxygenase and ferredoxin of diterpenoid dioxygenase, respectively, were isolated and sequenced [22].

Lignin is composed of various intermolecular linkages between phenylpropanes and guaiacyl, syringyl, *p*-hydroxyphenyl, and biphenyl nuclei. Analysis of the metabolic pathway has shown that the dimeric lignin compounds (e.g., DDVA) are degraded to protocatechuate or 3OMGA (3-*O*-methylgallic acid) and that these compounds are cleaved by protocatechuate 4,5-dioxygenase encoded by *ligAB*. Among the dimeric lignin compounds, the degradation of β -aryl ether and the biphenyl structure is the most important, because β -aryl ether is most abundant in lignin (50%) and the biphenyl structure is so stable that its decomposition is rate-limiting in lignin degradation [20]. A proposed metabolic pathway for DDVA was shown and com-

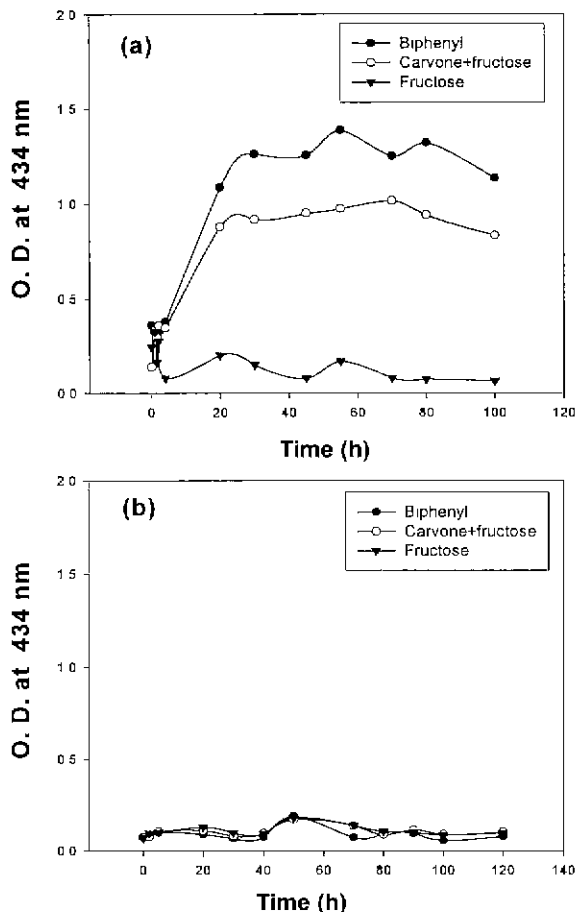


Fig. 3. Time course of accumulation of *meta* ring fission product from 4,4'-dichlorobiphenyl (DCBp) by the resting cells of *Arthrobacter* sp. B1B (a) and *Alicyclopes eutrophus* H850 (b) grown on biphenyl, carvone+fructose, and fructose [23]

pared with the degradative pathway of 2,3-dihydroxybiphenyl which is an oxidation product of biphenyl (or PCBs) [21] (Fig. 2).

THE POSSIBLE COMETABOLISM OF PCBs AND PAHS WHEN INDUCING OF THE DEGRADATIVE PATHWAY BY TERPENES OR LIGNIN

Induction by Terpenes

Search for a soluble and nontoxic inducer in PCB bioremediation has led to the hypothesis that plant terpenes may be the "natural" substrates for biphenyl biodegradation enzymes, or for their ancestors, since biphenyl is not naturally abundant [18] and structural similarities exist between plant terpenes and biphenyl (Fig. 1). This hypothesis leads to an interesting question of whether the diterpenoid dioxygenase would be ancestral to biphenyl dioxygenase [22]. *P. abietaniphila*

BKME-9 could not grow on biphenyl as a sole organic substrate [10]. However, it might be interesting to test the possible cometabolism of DhA and biphenyl by BKME-9 or the transformation of biphenyl by the enzyme encoded by the cloned gene *ditA*. A structure-function analysis of potential inducers of PCB biodegradation by *Arthrobacter* sp. strain B1B suggested that isoprenoids were able to induce PCB degradation, with the most potent inducer being an aromatic isoprenoid (*p*-cymene) much resembling the aromatic region of the DhA molecule [10] (Fig. 1). The authors' recent experiment revealed that a monoterpene, carvone, could induce the PCB degradative pathway in *Arthrobacter* sp. B1B and *Alcaligenes eutrophus* H850 [23] (Fig. 3).

Induction by Lignin

A diverse group of fungi have the ability to nonspecifically degrade a wide range of PAHs, some of them degrading lignin (ligninolytic). White rot fungi are ligninolytic and are known to degrade PAHs including the potent carcinogen benzo[a]pyrene and to detoxify PAH-polluted soils and sediments. A battery of extracellular enzymes responsible for the degradation of lignin, lignin peroxidases (LiPs), manganese peroxidases (MnPs) and laccases, is believed to be involved in the degradation of PAHs. Laccases are blue copper oxidases that catalyze one-electron of organic substrates coupled with the four-electron reduction of molecular oxygen to water. Although laccases exhibit relaxed substrate specificity, they may be less important than LiPs and MnPs in the oxidation of PAHs. The best-studied white rot fungus, *Phanerochaete chrysosporium*, catalyzes one-electron oxidation of PAHs to produce unstable aryl cation radicals. Different LiP isozymes exhibit different kinetic properties. Until now, 10 structural genes for LiP isozymes, designated *lipA* through *lipJ*, have been found. The expression of 10 *lip* genes in anthracene-transforming soil cultures of *P. chrysosporium* has been examined by mRNA extraction from soil and by the quantification of *lip* mRNA by competitive reverse transcriptase-PCR (RT-PCR) [24]. In this study, the expression of the 10 *lip* genes was found to be regulated differently. The oxidation of anthracene proceeded constantly throughout the 25-day course of the experiment, suggesting that this compound is oxidized by several LiP isozymes. The ability to metabolize various lignin-related compounds and to cause limited alterations of modified and native lignins has been reported for certain actinomycetes and nonenteric, nonfilamentous bacterial species. An enteric bacterial isolate, *Serratia marcescens*, was able to oxidize the lignin-related monomer vanillin, and to metabolize solvent fractionated Kraft pine lignin [25]. *Sphingomonas paucimobilis* SYK-6 is able to degrade a wide variety of dimeric lignin compounds, including β -aryl ether, biphenyl, and diarylpropane. Here, PCA (protocatechuate) is the key intermediate metabolite in the lignin degradation pathway in *S. paucimobilis* SYK-6, and the PCA metabolic pathway plays a key role in lignin degradation by this

strain [26]. To our knowledge, there have been little reports regarding lignin-degrading bacteria that can also cometabolize PAHs to date. Their existence in nature, however, remains to be uncovered.

PERSPECTIVES

Alternative natural substrates, such as terpenes [18, 23] and flavonoids [27], have been shown to enhance PCB degradation to an equal or greater extent than biphenyl. The mechanisms by which natural plant products enhance degradation of PCBs are unclear. However, it may be likely that terpenes and lignin (and/or their metabolites) can induce PCB-degradative pathway. This assumption is based on the structural similarities between plant terpenes or lignin (and their metabolic products) and biphenyl (and its metabolites). The use of plant terpenes or lignin as an inducer for PCB or PAH degradative pathway has clear advantages: good bioavailability, compatibility (nontoxicity) in natural environments, and their ubiquity in the environment.

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