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Chapter 7

THE POTENTIAL ROLE OF ENZYMATIC CATALYSIS AND METABOLIC ENGINEERING IN LIGNIN VALORIZATION

***Wenya Wang*[1,2,*], *Chen Shi*[1] *and Robert J. Linhardt*[3]**
[1]College of Life Science and Technology,
Beijing University of Chemical Technology, Beijing, China
[2]State Key Laboratory of Pulp and Paper Engineering,
South China University of Technology, Guangzhou, China
[3]Departments of Chemistry and Chemical Biology,
Chemical and Biological Engineering,
Center for Biotechnology and Interdisciplinary Studies,
Rensselaer Polytechnic Institute, Troy, NY, US

ABSTRACT

Lignin, the second richest biomass, is considered to be the potential resource of high value-added chemicals with the rapid development of lignocellulose biorefinery. During the past decades, physical and chemical methods have been used widely to degrade lignin into chemicals; however, they are lagged by several challenges, such as the fewer yields of low-weight molecules, higher energy consumption and non-specific cleavage. A large number of metabolic pathways in organism have been discovered for the lignin degradation and bioconversion, which was consist of lignolytic enzymes including radical lignolytic enzymes and non-radical lignolytic enzymes. The metabolic engineering of non-radical lignolytic enzymes shows a promising value for the conversion of lignin into aromatic chemicals or other high value-added chemicals. In the present mini-review, recent developments on enzyme catalysis and metabolic engineering of lignin valorizaiton will be

* Corresponding Author Email: wangwy@mail.buct.edu.cn.

summarized and discussed, including already discovered non-radical lignolytic enzymes, their metabolic pathways and molecular mechanism for lignin conversion, their recent application in lignin biorefinery and the possible combination of bio-catalyst and physical/chemical methods for lignin refinery.

Keywords: lignin valorization, non-radical lignolytic enzymes, enzyme catalysis, metabolic engineering, high value-added chemicals

INTRODUCTION

Lignin is a major component of lignocellulose and is also the most abundant aromatic polymer on earth. Lignin has a highly branched, three-dimensional, poly-phenolic structure that includes three phenylpropane units, namely *p*-coumaryl, coniferyl and sinapyl, joined by ether and C–C linkages. While lignin's structural heterogeneity and poly-phenolic composition results in its inherent stability and recalcitrance, lignin can ultimately be converted into CO_2 by microorganisms (bacteria, fungi, actinomycetes, etc.), maintaining the carbon balance in nature. The biological degradation of lignin by microorganisms is a complex process, involving the extracellular oxidative degradation of natural lignin into low-molecular weight aromatics, followed by their intracellular metabolic degradation, and their bioconversion as carbon and energy sources. The enzymes involved in these processes can be classified, on the basis of their reaction mechanism, as radical-dependent and non-radical lignolytic enzymes [1, 2], which constitute the metabolic pathways of lignin degradation in nature. Generally, the radical-dependent lignolytic enzymes are secreted extracellularly to produce the free radicals that degrade lignin into low-molecular-weight products. These radical-dependent lignolytic enzymes have been studied extensively over the past few decades, and have been thoroughly reviewed [3, 4]. With the rapid development of lignocellulose biorefinery, the intracellular metabolic degradation and bioconversion of lignins with a low-molecular weight aromatics has provided a new approach for the lignin volarization.

THE BIO-DEGRADATION OF LIGNIN-DERIVED AROMATIC

Currently, the detailed metabolic pathways, transportation and regulation of lignin-derived aromatics are mainly associated with bacterial resources. Corresponding research into fungi have lagged behind, and the metabolic pathways for aromatics in fungi have been mostly proposed on the basis of discovered intermediates. Early studies indicated that the production of ligninolytic enzymes in fungi occurred during secondary metabolism and were mainly triggered by limited nutrient levels, including carbon and nitrogen limitations

[5, 6]. These studies suggest that the large scale production of ligninolytic enzymes in fungi is not directly related to the catabolism of aromatics [7]. Thus, the metabolism and regulation of lignin-derived aromatics in fungi is far more complicated than those in bacteria.

Lignin is a highly branched three-dimensional poly-phenolic structure, consisting of *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units. In addition, *β-O*-4 linkages account for 40% to 70% of chemical bonds in natural lignin. Consequently, the metabolism of lignin-derived aromatics would necessarily involve enzymatic hydrolysis of *β-O*-4 linkages and the metabolic pathways of lignin derivatives from G-/S-/H-type units.

The Enzymatic Degradation of β -Ethers

It has been discovered that enzymes acting on *β*-ethers can cleave *β-O*-4 bonds in lignin model compounds. The *β*-etherase system of *Sphingobium* sp. SYK-6 is most well-known among these enzymes. The degradation of *β-O*-4 bond by *Sphingobium* sp. SYK-6 includes C_α dehydrogenase (LigD, LigL, LigN, LigO), etherase enzyme (LigE, LigF, LigP) and glutathione-lyase (LigG) (Figure 1). The genes encoding these three kinds of enzymes have a number of family members, which have been reviewed by Wang [8] and Kamimura [9]. The mining of these three kinds of enzymes was undertaken for the National Center for Biotechnology Information (NCBI) by Kamimura and coworkers to identify additional *β*-ether enzymes [9]. The *β*-etherase and C_α-dehydrogenase gene orthologs are mainly distributed in *Sphingomonadaceae* with the exception of LigE orthologs that are somewhat widely distributed in *α*-proteobacteria, in which *Sphingomonadaceae* represents 53%. The orthologs of the glutathione-removing enzyme gene were mainly distributed in *α*-proteobacteria, however, the *Nu* class of Glutathione S-transferase (GST) from *Novosphingobium* sp. MBES04 (GST3), with stereospecificity for both R and S substrates, is distributed in α-proteobacteria, *β*-proteobacteria and *γ*-proteobacteria [9]. These results indicate that in nature *Sphingomonadaceae* is specialized for *β*-aryl ether catabolism, which is in agreement with the reports of the Bugg and coworkers [10]. The different family members of the *β*-etherase systems show a variety of pH optima, thermal stabilities and substrate specificities. In addition to their enantioselective differences, the chemical functionality of their substrates can significantly impact the activity of these enzymes. These functionalities include groups on the aromatic rings, linkages between two aromatic rings, groups at C_α, the side chain of C_β, and the positions of methoxy groups on the aromatic rings. In particular, the presence of a hydroxyl group at the C_α position of lignin model compounds makes the *β-O*-4 bond resistant to the *β*-etherase. When the group at this C_α position is replaced by carbonyl, the *β*-etherase can effectively cleave the *β*-ether bond linking the aromatic groups in dimeric compounds [11, 12].

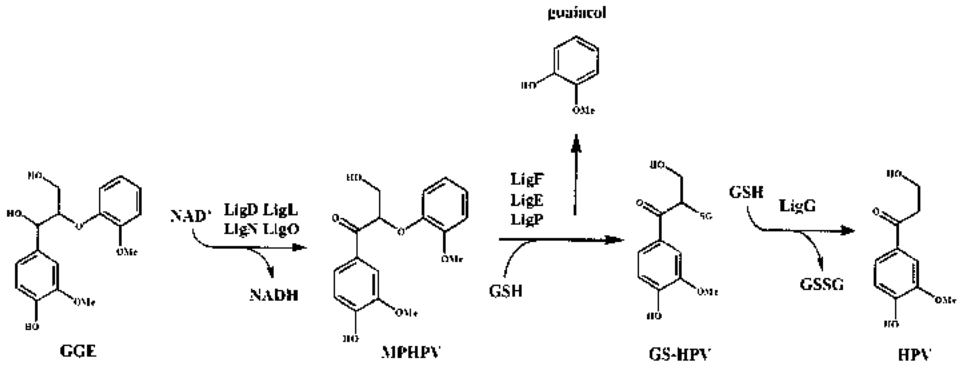

Figure 1. Reaction procedure of the LigDFG enzyme system. GGE, guaiacylglycerol-β-guaniacyl ether; MPHPV, α-(2-methoxyphenoxy)- β-hydroxypropiovanillone; GS-HPV, α-glutathionyl-β-hydroxypropiovanillone; and HPV, β-hydroxypropiovanillone.

The large number of gene family members identified might have evolved to adapt to the environment and the intrinsic heterogeneity of lignin in order to effectively utilize lignin as a carbon and an energy source. However, from the point of industrial application, the presence of a large number of enzymes makes a process unduly complex. Thus, it is important to study the catalytic mechanisms of these enzymes to discover the enzymes that act on a broad range of substrates. Recently, the X-ray crystal structures of β-ether degrading enzymes have been reported [13-15] and these will undoubtedly be used in combination with gene mining and protein engineering to promote studies on β-ether degrading enzymes.

The Metabolic Pathways of Lignin Derivatives from G-/S-/H-Units

In practice, natural lignin can be depolymerized into a large number of chemical products after physical, chemical or biological treatment. The type and number of these chemical products are dependent on the operating conditions. In the present paper, the metabolic pathways of depolymerizing products only focus on the aromatic slurry. The metabolic pathways of *p*-coumaric acid (pCA), ferulic acid (FA) and sinapic acid (SA) have been studied extensively. G-/S-/H- units and many lignin-derived aromatics share the common chemical structure with pCA, FA and SA. Consequently, the metabolic pathways of pCA, FA and SA have been chosen to introduce the catabolic pathways for lignin-derived aromatics.

*p*CA and FA can be catabolized to protocatechuate (PCA) or catechol (CA) through CoA-dependent β-oxidation and CoA-dependent non-β-oxidation pathways (Figure 2A). In the CoA-dependent β-oxidation pathway, CoA is first catalytically added, followed by double bond hydrolysis, further oxidation and sulfurolysis to remove the ethyl group. *p*CA

and FA are eventually hydrolyzed to *p*-hydroxybenzoic acid (*p*HBA) and vanillic acid, respectivcly [8, 16, 17] (Figure $2B_1$, B_5). The CoA-dependent non-β-oxidation pathway is similar to the β-oxidation pathway with the exception that in the CoA-dependent β-oxidation pathway further thiolysis and oxidation were not observed after the first removal of acetyl-CoA [16, 18] (Figure $2B_2$, B_6).

Additionally, there is also a side chain reduction pathway in the metabolism of pCA and FA. In the plants *Glechoma* sp. and *Vanilla* sp., a CoA-independent pathway produces *p*HBA from *p*HA, which proposed to involve one reductase (*p*HBALS) and one decarboxylase (*p*HBALS) (Figure $2B_7$) [16, 19]. In *Corynebacterium glutamicum*, FA is reduced by aromatic reductase (RE) to form dihydroferulic acid and decarboxylase (DCL) catalyzes dihydroferulic acid to produce acetic acid and vanillic acid. This pathway is generally present in bacteria and fungi grown under anaerobic conditions [8]. In addition, FA can also be reduced to coniferyl alcohol, which is further degraded to vanillic acid [20, 21] (Figure $2B_4$).

Notably, FA can also be degraded by non-oxidative decarboxylation [8, 22, 23], which has not been observed in pCA catabolism. FA, in the non-oxidative decarboxylation pathway, is catalytically decarboxylated to vanillin and vanillic acid (Figure $2B_3$), which has been found in *Fusarium solani* (Mart) Sacc., [24] *Bacillus coagulans* [25], and *Bacillus cereus* strain PN24 [26].

Most catabolic microorganisms for *p*CA and FA cannot degrade SA, which indicates the adverse impact of aromatic methoxyl groups on SA catabolism.[27] The initial step from SA to syringic acid is proposed to be catalyzed by radical lignolytic enzymes (laccases) [27, 28] or non-radical lignolytic enzymes (decarboxylases) [29, 30] (Figure 2A). No genes corresponding to the decarboxylases involved in non-radical catalysis have yet been reported. SA is transformed into syringic acid through the removal of two carbon atoms from its side chain. In *Sphingomonas paucimobilis* SYK-6, syringic acid is *O*-demethylated by a tetrahydrofolate-dependent *O*-demethylase (DesA) to produce 3-*O*-methylgallate (3MGA), and then the 3MGA is *O*-demethylated by another *O*-demethylase (LigM) to generate gallic acid. The aromatic ring of 3MGA and gallic acid can be cleaved by dioxygenase, moving into the TCA cycle [31-33]. There is currently no evidence to support the transformation of syringic acid to PCA or CA prior to ring opening.

THE TRANSPORTATION OF LIGNIN DERIVATIVES FROM G-/S-/H-UNITS AND THEIR METABOLIC REGULATION

The genes involved in aromatic metabolism are usually physically assembled in operons or in clusters within bacteria. Around the aromatic metabolizing genes there are always transporting genes responsible for the uptake of the aromatic substrates and

regulating genes, which act as *trans*-elements to regulate the aromatic metabolism. Metabolizing genes, transporting genes, and regulating genes have developed to efficiently assimilate the substrate and catabolize it for carbon and energy demands.

In bacteria, there are four transporting systems for the uptake of lignin-derived aromatic compounds: ATP-binding cassette (ABC) transporters, major facilitator superfamily (MFS) transporters, a tripartite ATP-independent periplasmic (TRAP) transporter, and an ion transporter (IT) superfamily. The four transporting systems show signs of binding affinity towards *p*CA, FA, SA or their derivatives [9].

ABC transporters are widely distributed and are well-studied transporters. They can transport an enormous variety of substrates, ranging from small ions to large organic molecules [34]. When *Enterobacter lignolyticus* SCF1 and *Bacillus ligniniphilus L1* was cultivated in media with lignin as sole carbon source, the production of ABC transporters increased [35], demonstrating that the ABC transporters were involved in the assimilation of lignin-derived aromatics. A fluorescence thermal shift-based assay indicated that the component of ABC transporters can interact with *p*CA, FA and SA with different binding affinities [36]. In *Rhodopseudomonas palustris*, the co-crystallization of ABC transporter (CouP) and FA indicates that H-bond interactions occur between the 4-OH group of the aromatic ring and His309/Gln305 and also between the carboxyl group on the FA side chain and Arg197, Ser222, and Thr102 [37]. CouT in *R. jostii* RHA1 is believed to be a MFS transporter for *p*-hydroxycinnamate. At the mRNA level, *cou*T is upregulated in a strain cultivated on FA and pCA. The growth status indicates that pCA is better than FA as the growth substrate, reflecting the substrate specificity of *couT* [38]. In addition, there are also porins, substrate-specific channels or TonB-dependent receptors at the outer membrane of Gram-negative bacteria, involved in the uptake of aromatics [9]. There are not direct reports of their involvement in the transport of pCA, FA, and SA.

The metabolizing genes of aromatics are usually clustered in the operon, around which regulating genes were recruited to manipulate the metabolic pathway and transportation for the effective catabolism. While the regulators of many aromatic monomers have been discovered, no regulators of lignin-derived biaryls have been reported. The FerC from *Sphingobium* sp. SYK-6 recognizes the thioester product of *p*CA, FA, and SA as effectors [9]. This suggests that FerC and its cognate FA catabolic regulon in SYK-6 are involved in the metabolism of lignin derivatives from G-/S-/H-type units. Other reported regulators only bind a portion of feruloyl-CoA, *p*-coumaroyl-CoA and sinapoyl-CoA. For example, CouR from *R. jostii* RHA1 and HcaR from *Acinetobacter* sp. ADP1 recognizes *p*-coumaroyl-CoA and feruloyl-CoA as effectors [39, 40], and CouR from *R. palustris* CGA009 and FerR from *P. fluorescens* BF13 bind *p*-coumaroyl-CoA and feruloyl-CoA, respectively [41, 42]. In addition to the regulators of the metabolic pathways of *p*CA, FA, and SA, the regulating genes for protocatechuate [9], vanillate [9], benzoate [43], and

catechol [44], the intermediates of *p*CA, FA, and SA catabolism, have also been discovered. This suggests that the regulation mechanism of lignin-derived aromatics is a complicated system involving substrate competition, the integration of different metabolic pathways, and environmental factors. In *Pseudomonas putida*, the catabolite repressor (Crc) binds to and inhibits the translation of *ben*R mRNAs, and *ben*R encodes the transcriptional activator inducing the expression of benzoate degradation genes. Further research indicates that Crc can also bind to the translation initiation regions of the mRNA of several structural genes in benzoate degradation and the benzoate transporter gene, which suggests that Crc may also control benzoate degradation and uptake. All this evidence demonstrates that Crc can regulate benzoate metabolism at multi-tier levels, including uptake, induction and degradation [45]. The catA2 gene in *Pseudomonas putida* mt-2 works as a enzymatic safety valve for excess of catechol, the intermediate of benzoate catabolism, to alleviate the toxicity of catechol. The catA2 gene is located downstream of the *ben* operon and the *ben* operon is regulated by the benR. CatA is in the *cat* operon for the normal catechol catabolism, which is activated by carR [44]. PcaY is an aromatic acid chemoreceptor in *P. putida* F1, which belongs to the *pca* operon under the control of PcaR. PcaK, a transporter protein, is also in the *pca* operon. Data suggests that PcaK facilitates the uptake of 4-hydroxybenzoate, resulting in the increase of pcaY expression. Consequently, the chemotaxis, transport, and metabolism of aromatic compounds are integrated by PcaR in *P. putida* [46]. Recent studies show the existence of an aerobic-anaerobic metabolism switch [43] and a regulator coupled with dioxygenase [47] in aromatic catabolism. This provides many potential tools for increasing lignin volarization with microbiology by metabolic engineering.

BIOTRANSFORMATION FOR THE PRODUCTION OF CHEMICALS FROM DEPOLYMERIZED LIGNIN AND LIGNIN MODEL COMPOUNDS

There are two approaches for the biotransformation of depolymerized lignin or lignin model compounds into chemicals. The first is *in vitro* enzymatic conversion and the second is *in vivo* metabolic conversion. Given that 40% to 70% of the chemical linkages in lignin are β-O-4, researchers have tried to degrade the lignin using a β-etherase system to obtain value-added chemicals or chemical precursors for the further metabolic engineering (Table 1). However, improving degradation efficiency is a big challenge for *in vitro* enzymatic catalysis. Moreover, although some value-added chemicals have been produced from lignin using microorganisms, most reports still rely on lignin model compounds to study the metabolic conversion *in vivo*.

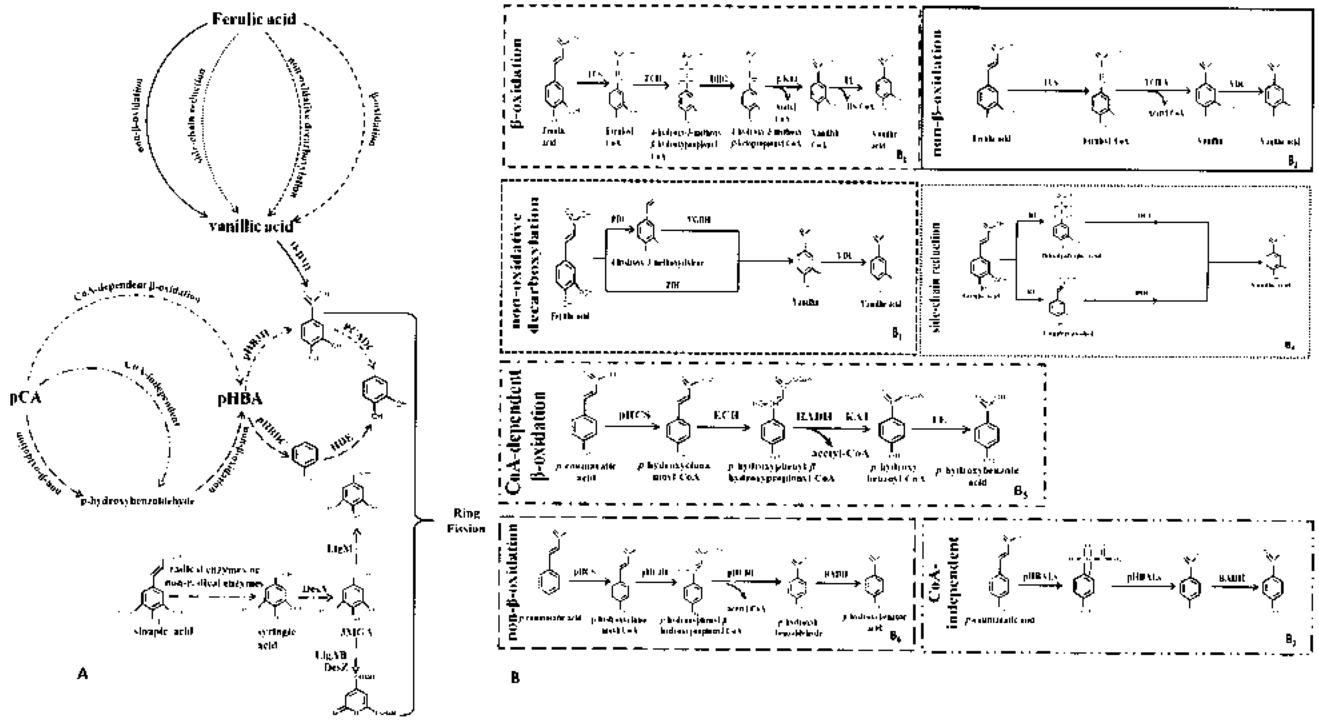

Figure 2. Metabolic pathways of p-coumaric acid, ferulic acid and sinapic acid. (A) The brief outline of metabolic pathways; (B) Detailed metabolic pathways. BADH, benzaldehdye dehydrogenase; ECH, enoyl-CoA hydratase; HADH, 3-hydroxyacyl-CoA dehydrogenase; HDE, hydroxylase; pHBALS, p-hydroxybenzaldehydesynthase; pHCHL, p-hydroxycinnamoyl-CoA hydratase/lyase; pHBDC, p-hydroxybenzoate decarboxylase; pHB3H, p-hydroxybenzoate-3-hydroxylase; pHCS, p-hydroxycinnamoyl-CoA synthetase; KAT, 3-ketoacyl-CoA thiolase; PCADC, protocatechuate deccarboxylase ; DCL, decarboxylase; DHG, dehydrogenase; ECH, enoyl-CoA-hydratase; ECH/A, enoyl-CoA-hydratasc/aldolase; FCS, feruloyl-CoA synthelase; FDC, ferulic acid decarboxylasc; β-KTE, β-keto thiolase; O-DML, O-demethylase; PCADC, protocatechuate deccarboxylase; POE, phenol oxidase; RE, reducase; TE, thiolase; VD, vanillin decarboxylase; VGDH, 4-vinylguaiaol dehydrogenase ; 3MGA, 3-O-methylgallate; PDC, pyrone-4,6-dicarboxylic acid.

Table 1. The enzymatic conversion *in vitro* of natural lignin

Lignin resource	Involved enzymes	Process	The reacting conditions	Products and yield	The organic solution for lignin dissolution	Reference
softwood/hardwood alkali-lignin and bagasse organosolv-lignin	LigD/ligF/ligG/AVR	One pot	0.1mM ammounium acetate buffer (pH 9.0); NAD^+; GSH	Guaiacol/Ferulic acid/Eugenol/ Acetovanillone/Vanillin (Softwood); Guaiacol/Vanillin (Hardwood); No products detected(Organosolv-lignin)[a]	-------	[1]
C-MWL and E-MWL[b]	SDR3/ SDR55/ GST4/ GST5/ GST3	One pot	0.1M TAPS(pH 8.5); NAD^+; GSH; 15°C	GHP from C-MWL (2.4 ± 0.005 wt % [24 ± 0.05 mg g-1 lignin]); GHP/SHP from E- MWL (1.9 ± 0.012 and 4.7 ± 0.025 wt % [19 ± 0.12 and 47 ± 0.25 mg g-1 lignin], respectively)	*N,N*-dimethylformamide	[48]
OrganoCat lignin from beech	Laccase/LigE/LigF-NA/LigG-TD	Three steps	Setp I: violuric acid; 0.1M sodium acetate buffer (pH 5); [EMIM] [EtSO4]; room temperature; 250 rpm; 5 days. Setp II: 50 mM glycine/NaOH buffer (pH 9.5); DMSO (25%); GSH;25°C; overnight. Setp III: DMSO in step II was diluted to 10% with water; 20°C; overnight.	Oliy faction (coniferylaldehyde, guaiacyl unies, syringyl units and oligomeric species); 12.5 wt% yield.	DMSO	[49]
HP lignin and MCS lignin[c]	LigD/LigN/LigE/LigF /NaGSTNU (or LigG)/ AvGR	One pot	25 mM Tris buffer (pH 8.0); DMSO (2.0%); NAD^+; GSH; room temperature; 4 hours	1.0mM HPS from HP lignin (12.5 wt% yield); 0.4 mM HPV, 0.1 mM HPS and trien from MCS lignin (5% yield (HPV + HPS))[d]	DMSO	[50]

a: Yield referred to Reiter[1].

b: Milled lignin from *Cryptomeria japonica* (C-MWL) and milled lignin from *Eucalyptus globulus* (E-MWL).

c: HP - the high-syringyl hybrid poplar; MCS - maize corn stover.

d: HPV- hydroxypropiovanillone; HPS- hydroxypropiosyringone.

Enzymatic Conversion *in Vitro*

Given the complexity of lignin structures, the large scale depolymerization lignin, into value-added functional aromatic compounds, is difficult using chemical or physical treatment [2]. Enzymatic methods, particularly ones using non-radical lignolytic enzymes, represent an alternative approach for lignin valorization under environmentally-friendly and substrate-specific conditions [2].

The cleavage of natural lignin with the β-etherase system was first carried out by Reiter [1] (Table 1). The β-etherase system included LigD/F/G and AvGR, and softwood/hardwood alkali-lignin and bagasse organosolv-lignin were selected as substrates. GPC analysis showed that softwood and hardwood alkali-lignin were only slightly degraded, but this did not occur with bagasse-organosolv lignin even after 7 days of treatment [1]. Subsequently, Picart prepared a fluorescently labeled synthetic lignin model (DHP-MUAV), a multiple polymer of coniferyl alcohol and α-*O*-(β-methylumbelliferyl) acetovanillone (MUAV), to assess whether β-etherases could cleave β-*O*-4 aryl ether linkages present in lignin-like polymers. The results showed that DHP-MUAV was converted into different fragments of smaller mass, suggesting that it was possible for the β-etherase to catalyze cleavage of lignin-like polymers [12]. Afterwards, three different research groups [48-50] reported the enzymatic degradation of natural lignin with improved β-etherase systems (Table 1). The highest yield of enzymatic degradation was 12.5 wt% and different depolymerization products were obtained (Table 1). Based on data from the *in vitro* enzymatic conversion, the efficiency of β-etherase systems towards natural lignin or lignin-like polymers was far lower than towards lignin model dimers. This might result from the problems of enzymatic enantioselectivity, substrate availability, or the inhibitory effect of lignin on enzyme activity [51]. Consequently, more research is still required to understand the mechanism of β-etherase catalysis and the interaction between these enzymes and lignin. In addition, from the view of industrial application, the yield of enzyme-hydrolyzing products still needs to be improved. Further studies need to be carried out on the β-etherase system, such as mining or engineering enzymes to broaden their range of substrates and improve their efficiency, simplifying the process, reducing its cost.

Metabolic Conversion *in Vivo*

The "funneling pathway" includes both the upper and lower pathways, present in many microorganisms, for the metabolism of aromatic compounds [52] (Figure 3). In the upper pathways, aromatic molecules are catabolized into several conserved intermediates, including catechol (CA) in bacteria and protocatechuate (PCA) in most fungi and some bacteria [52]. In the lower pathways, the aromatic rings of these conserved intermediates

are cleaved by dioxygenases, producing ring-opened species that are metabolized through the β-ketoadipate pathway into the TCA cycle [52, 53].

Recently, researchers have used metabolic engineering to explore the biotransformation of lignin and lignin-derived monomers into different chemicals for the purpose of lignin valorization (Table 2). These target chemicals include aromatics, ring-opened chemicals, TCA cycle chemicals, lipids and lipid derived polymers (polyhydroxyalkanoate, PHA). These chemicals are distributed within upper pathways, lower pathways, TCA cycle, and lipid synthesis pathways (Figure 3).

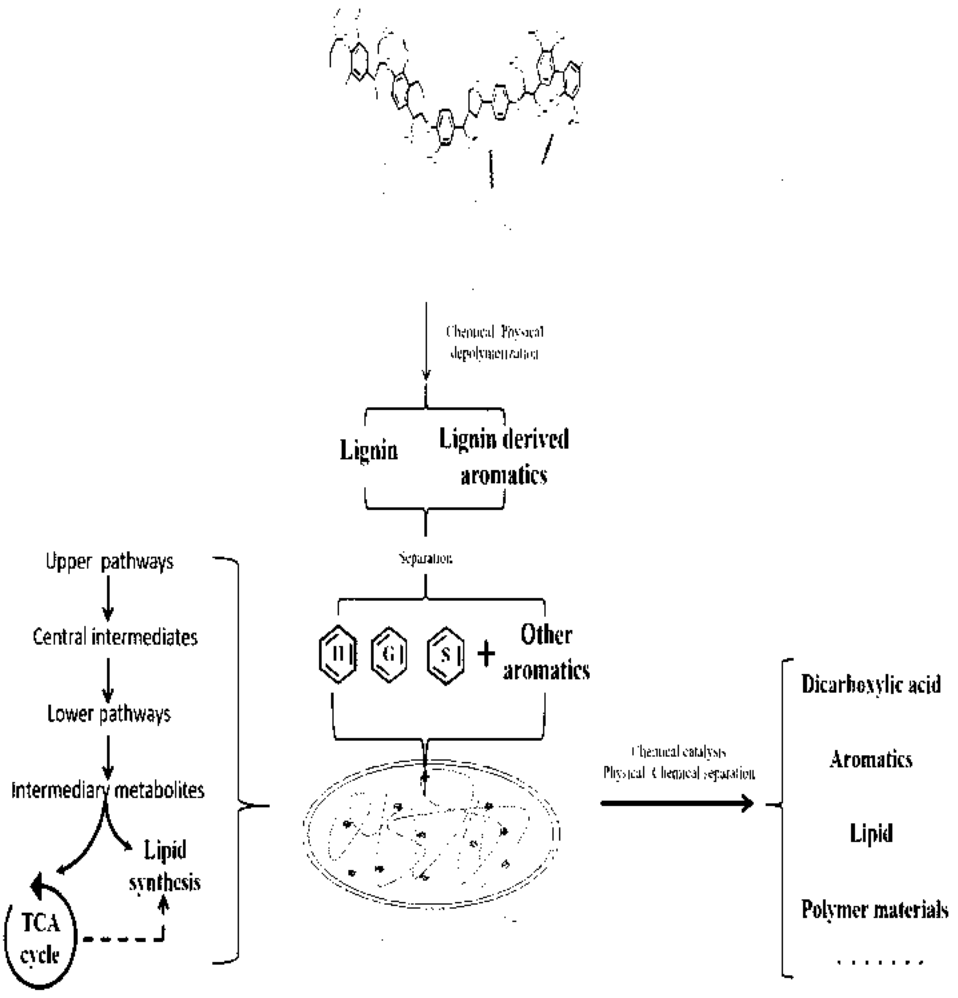

Figure 3. Lignin valorization by combining enzymatic catalysis and metabolic engineering and using a multi-disciplinary approach.

Table 2. The reported chemicals biotranformed from lignin or lignin model compounds

Molecule	Substrate	Host	Reference
Pyruvate, Lactate	benzoate and *p*-coumarate	*P. pudita* KT2440	[74]
β-Ketoadipic acid, Muconolactone	protocatechuic acid	*P. pudita* KT2440	[58-59]
Muconic acid	catechol, *p*-coumarate, benzoate, ferulate, guaiacol, vanillin, lignin	*E. coli*, *E. coli* XL-1 Blue, *P. pudita* KT2440, *Sphingobium* sp. SYK-6, *Amycolatopsis* sp. ATCC 39116	[61-68, 97]
Picolinic acid, HMS[a]	catechol	*Pseudomonas putida* (arvilla) mt-2 ATCC 23973	[70]
3-Carboxy-muconate	vanillin	*Escherichia coli* BL21-Gold (DE3)	[60]
Benzoic/ p-hydroxybenzoic acid	corn stover hydrolysate	*Rhodopseudomonas palustris*	[57]
2,5-PDCA, 2,4-PDCA[b]	wheat straw lignocellulose	*Rhodococcus jostii RHA1*	[71]
Fatty acid, PHA	alkaline pretreated lignin, organosolv lignin, kraft lignin	*Aspergillus fumigatus, P. pudita KT2440 and A514, Cupriavidus basilensis B-8, Pandoraea sp. ISTKB*	[53, 76, 83-85]
Lipid	4-hydroxybenzoic acid, resorcinol, vanillic acid, lignin, sorghum biomass	*Rhodococcus opacus DSM 1069 and PD630, Rhodococcus jostii RHA1 VanA-, Trichosporon oleaginosus, Cunninghamella echinulata FR3*	[77-82]
Vanillin	eugenol, isoeugenol, ferulic acid, vanillic acid, lignin	*E. coli, Pseudomonas putida, Rhodococcus, Pycnoporous*	[55-56, 101-104]
PDC[c]	protocatechuate	*P. putida PpY1100*	[69]
Succinic acid	DHP (Synthetic Lignin)	*Phanerochaete chrysosporium*	[75]

Note: a. 2-Hydroxymuconic semialdehyde (HMS); b. Pyridine 2,5-dicarboxylic acid (2,5-PDCA) Pyridine 2,4-dicarboxylic acid (2,4-PDCA); and c. 2-Pyrone-4,6-dicarboxylic acid (PDC).

Over the past several decades, various biotechnology-based approaches have been developed for the production of vanillin. A number of specialized microorganisms have been used to produce vanillin from aromatic molecules such as eugenol, isoeugenol, FA, vanillic acid [8, 54, 55]. Lignin has also been studied as a starting material for the biocatalytic production of vanillin. When a vanillin-dehydrogenase deletion strain of *R. jostii* RHA1 was grown for 144 h on pretreated wheat straw lignocellulose, it was found to accumulate vanillin with yields of up to 96 mg/L [56]. In addition, ferulate catabolic pathways and *β*-aryl ether cleavage produces vanillin as an intermediate metabolite within different lignin degradation pathways, providing valuable approaches for the bio-production of vanillin from lignin. When *Rhodopseudomonas palustris* is cultivated in corn stover hydrolyzate, the bioconversion of aromatics can be observed. The deletion of *badE* gene of *R. palustris*, encoding benzoyl-CoA reductase, results in the accumulation of benzoic acid from aromatics in hydrolyzate and the deletion of *hbaBCD* gene, encoding 4-hydroxybenzoyl-CoA reductase, accumulates 4-hydroxybenzoic acid. The concentration

of both benzoates was at more than 1 mM. It is interesting that the *R. palustris* can only utilize the aromatics in lignocellulose hydrolyzate, while leaving sugars unaltered. Thus, this approach provides an effective method to remove bio-toxic factors from biomass hydrolyzate before further biotransformation [57].

Dicarboxylic acid and its derivatives originate from the cleavage of aromatic ring in central intermediates. Currently dicarboxylic acid and its derivatives account for most kinds of microbial biotransformation products coming from lignin or lignin model compounds. These chemicals include β-ketoadipic acid [58], muconotactone [58, 59], 3-carboxy-muconate [60], muconic acid [60-68], 2-pyrone-4,6-dicarboxylic acid (PDC) [69], picolinic acid [70], 2-hydroxymuconic semialdehyde [70], pyridine 2,5-dicarboxylic acid (2,5-PDCA) and 2,5-dicarboxylic acid (2,4-PDCA) [71]. All these chemicals share common structures (two or more carboxylic acid groups), making these compounds, or their derivatives, useful raw materials for the synthesis of bio-based polymers.

The cleavage of aromatic ring has two modes: *ortho* ring-cleavage and *meta* ring-cleavage, which was carried out by intradiol dioxygenases or extraiol dioxygenases, respectively [72]. Ring cleavage can be conducted by two enzymes, a 1,2-catechol dioxygenase or a 2,3-catechol dioxygenase in catechol, and when in protocatechuate can be cleaved by three enzymes in the 2,3 (*meta*), 4,5 (*meta*), and 3,4 (*ortho*) positions [73].

Muconic acid, the recent focus of dicarboxylic acids, can be converted into the dicarboxylic acid, adipic acid, through hydrogenation. Adipic acid is an important precursor of nylon, plasticizers, lubricants and polyester polyols. The production of muconic acid through the aromatic catabolic pathway results in higher atom efficiency than its production through the sugar pathway. When benzoate and glucose were fermented in a DO stat fed-batch culture of KT2440-CJ102 and the pH value was maintained at 7.0 for 124 h, muconic acid was produced at a titer of 34.5 g. L^{-1} [64]. By deleting *Crc* gene, encoding catabolite repression control protein, from *P. pudita* KT 2440, muconate production is enhanced and the yield of muconate produced from *p*CA after 36 h was increased nearly 70% and the yield from FA after 72 h was more than doubled [66]. Moreover, *p*CA and FA from alkaline pretreated lignin were converted by engineered *P. putida* KT2440 to 0.70 g L^{-1} of muconic acid in 24 h and the molar yield was 67% [64]. Barton [67] and Sonoki [68] developed an engineered strain that could produce muconic acid with lignin or lignin hydrolysate. Recent research indicates that insufficient protocatechuate decarboxylase activity is considered to be the bottleneck in muconic acid production. Studies on the increase of protocatechuate decarboxylase activity have been performed for the improvement of muconic acid production. The results indicate that the muconic acid production could be increased by 50% with *p*CA as substrate after the improvement of protocatechuate decarboxylase activity [65].

The "upper pathways" in aromatic-compounds degrading organisms are utilized to integrate or funnel the heterogeneous lignin-depolymerizing slurry into a few common intermediates, such as CA (1,2-dihydroxybenzene) or *p*CA (3,4-dihydroxybenzoate).

Cleaving the aromatic rings gave rise to different products through the *ortho* (intradiol) or *meta* (extradiol) patterns, which are funneled into TCA cycle. The metabolic pathways of various ring-opened chemicals enter the central metabolism with different carbon efficiency and redox balance [73]. The replacement of the protocatechuate (PCA) *ortho* pathway in *P. pudita* Kt2440 with a *meta*-cleavage pathway from *Sphingobium* sp. SYK-6 results in a nearly five-fold increase in the yield of pyruvate, which indicates that the catabolic pathway from ring-cleavage to TCA cycle could be selected to optimize the yield of a desired product [74]. With synthetic lignin, *Phanerochaete chrysosporium* (white rot fungus) was demonstrated to accumulate succinic acid by the short-cut TCA cycles, providing a potential strain for the future lignin biorefinery [75].

The recent and rapid progress on prokaryotic lignin depolymerizing enzymes suggests that lignin valorization, involving fatty acid metabolism, might result in potential applications in the preparation of biofuels and biodegradable materials. Utilizing lignin through the fatty acid and lipid synthesis pathway began with the study on lignin model compounds (4-hydroxybenzoic acid, resorcinol, vanillic acid), and then has led to use of natural lignin in different microorganisms, such as *Aspergillus fumigatus* [76], *Cunninghamella echinulata* FR3 [28], *Rhodococcus opacus* [77, 78], *Rhodococcus jostii* [79], and *Trichosporon oleaginosus* [80]. The synergy between laccase and microbial lignin conversion and co-fermentation of two *R. jostii* strains have been examined in lipid production [79, 81]. Lipid accumulation by *Rhodococcus opacus* DSM 1069 reached 26.99 ± 2.88% of its cellular dry weight by utilizing pine organosolv pretreatment effluent [82]. Linger [53] reported that the alkaline depolymerized lignin could be converted by *Pseudomonas putida* KT2440 into medium chain length (mcl)-PHAs through integrated biological funneling pathway, and another *Pseudomonas putida* strain A514 was engineered to improve the yield of PHA on the basis of genomic and proteomic analysis, in which PHA content reached 73% per cell dry weight [83]. Shi [84] and Kumar [85] reported that *Cupriavidus basilensis* B-8 and *Pandoraea* sp. ISTKB could also bioconvert kraft lignin into PHA. All these metabolic conversions provide a roadmap for the further research on lignin biotransformation and suggest that more value-added products will one day be produced from lignin.

Combining Enzymatic Catalysis and Metabolic Engineering with a Multi- Disciplinary Approach for Lignin Valorization

Given the environmentally friendly and substrate-specific characterization, lignin valorization with enzymatic catalysis and metabolic engineering for the production of high value-added chemicals represent an attractive target. However, the economic feasibility

and efficiency of such processes remains a major challenge. Consequently, a multi-disciplinary approach relying on biology, chemistry and physics will be needed to develop practical process for lignin biological valorization (Figure 3).

The "funneling pathway" in microorganisms represents an practical approach for integrating different aromatic substances, obtained through lignin depolymerization, into some central intermediates for high-value chemical generation [52, 53]. The tools of synthetic biology and system biology, such as biosensors [86-89], "-omic" technologies [90], and tolerance engineering [91], can be applied to reconstruct the metabolic pathways to further improve the yield of target products. Next generation industrial biotechnologies (NGIB) represent a promising method for improving the economic feasibility of fermentation processes. Costs can be reduced by continuous bioprocessing under non-sterile (open) conditions using ceramic, cement or plastic bioreactors. Most lignin-utilizing microorganisms are aromatic-toxicity tolerant matching the NIGB requirements [92].

It is necessary to generate as many different lignin monomers and oligomers as possible using suitable pretreatment methods to improve the biotransformation efficiency of lignin. Unfortunately, the degradation of lignin using radical lignolytic enzymes requires weeks [93-94] and, thus, is unsuitable for the industrial applications. Chemical depolymerization usually results in the ring opening or can cause the competing re-polymerization of lignin-depolymerized products, thus, it is inefficient for lignin monomer production. More recently, the formic acid [95] and formaldehyde [96] processes, developed to produce lignin monomer in high yield, have recently become available. These pretreatments or depolymerization technologies may afford promising processes for lignin valorization through the combination of chemical depolymerization and biotransformation.

In addition to combining biotransformation and chemical catalysis in lignin depolymerization, industrial demands for saving energy and resources will also require the combination of the biotransformation and chemical or physical methods in other process steps. These combinations leverage the specificity of microorganisms and advantages of continuous reactions associated with chemical catalysis, thus, overcoming the drawbacks of either a purely chemical or biological approach for the industrial production of high-value chemicals from lignin. Studies by Linger [53] and Vardon [97] provide successful examples for the application of combination technologies in lignin bio-valorization, in which *mcl*-PHA and *cis, cis*-muconic acid from lignin bioconversion were used to produce alkenoic acids and adipic acid by chemical catalysis. Furthermore, the synthesis of picolinic acid, 2,5-PDCA and 2,4-PDCA [71] using *in vivo* metabolic conversion have also involved in such combinational technologies. Furthermore, besides the application in microorganism, the "funneling pathway" strategy can also be used to pool the high-value alkylphenols from pyrolysis bio-oil, generated from lignocellulosic biomass, which is referred to as the chemical "funneling pathway" [98]. The biotransformation of cellulose sugar could also provide many valuable clues to new approaches for lignin biological volarization, such as one pot conversion or platform molecules [99-100].

CONCLUSION

The insolubility and structural heterogeneity inherent to lignin and the complexity of its depolymerization provides a major challenge for lignin valorization. Recently, the enzymatic catalysis and metabolic engineering of lignin volarization provide some approaches for lignin bioconversion into high-value-added chemicals. These new approaches promise the generation of more varieties of lignin-derived high-value chemicals in the near future. However, enzymatic conversion *in vitro* shows its lowest efficiency towards natural lignin. This calls for studies on the mechanism of enzymatic catalysis and the identification of more β-etherases through protein engineering and gene mining. In addition, the chemical yields from natural lignin using metabolic conversion cannot meet the demands of industry. Thus, natural lignin will need to be depolymerized into more aromatic monomers or oligomers for the efficient bioconversion. Ultimately, the economic feasibility and effectiveness of industrial production will undoubtedly require the combination of biological, physical and chemical technologies for lignin biological valorization.

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REFERENCES

[1] Reiter, J., Strittmatter, H., Wiemann, L., Schieder, D. & Sieber, V. (2013). "Enzymatic cleavage of lignin β-O-4 aryl ether bonds via net internal hydrogen transfer." *Green Chem, 15*, 1373–1381.

[2] Picart, P., Dominguez de Maria, P. & Schallmey, A. (2015). "From gene to biorefinery: microbial β-etherases as promising biocatalysts for lignin valorization." *Front Microbiol, 6*, 916-923.

[3] Wong, D. W. S. (2009). "Structure and action mechanism of ligninolytic enzymes." *Appl Biochem Biotech, 157*, 174–209.

[4] Pollegioni, L., Tonin, F. & Rosini, E. (2015). "Lignin-degrading enzymes." *FEBS J, 282*, 1190–1213.

[5] Jeffries, T. W., Choi, S. & Kirk, T. K. (1981). "Nutritional regulation of lignin degradation by *Phanerochaete chrysosporium*." *Appl Environ Microbiol, 42*(2), 290–296.

[6] Kirk, T. K., Croan, S., Tien, M., Murtagh, K. E. & Farrell, R. L. (1986). "Production of multiple ligninases by *Phanerochaete chrysosporium*: effect of selected growth conditions and use of a mutant strain." *Enzyme Microb Technol, 8*(1), 27–32.

[7] Asina, F., Brzonova, I., Kozliak, E., Kubátová, A. & Ji, Y. (2017). "Microbial treatment of industrial lignin: successes, problems and challenges." *Renew Sust Energ Rev, 77*, 1179-1205.

[8] Wang, W., Zhang, C., Sun, X., Su, S., Li, Q. & Linhardt, R. J. (2017). "Efficient, environmentally-friendly and specific valorization of lignin: promising role of non-radical lignolytic enzymes." *World J Microb Biot, 33*(6), 125-139.

[9] Kamimura, N., Takahashi, K., Mori, K., Araki, T., Fujita, M., Higuchi, Y., et al. (2017). "Bacterial catabolism of lignin-derived aromatics: new findings in a recent decade: update on bacterial lignin catabolism." *Environ Microbiol Rep, 9*(6), 679-705.

[10] Bugg, T. D., Ahmad, M., Hardiman, E. M. & Rahmanpour, R. (2011). "Pathways for degradation of lignin in bacteria and fungi." *Nat Prod Rep, 28*, 1883-1896.

[11] Gall, D. L., Ralph, J., Donohue, T. J. & Noguera, D. R. (2014). "A group of sequence-related sphingomonad enzymes catalyzes cleavage of β-aryl ether linkages in lignin β-guaiacyl and β-syringyl ether dimers." *Environ Sci Technol, 48*(20), 12454–12463.

[12] Picart, P., Mueller, C., Mottweiler, J., Wiermans, L., Bolm, C., Dominguez de Maria, P. & Schallmey, A. (2014). "From gene towards selective biomass valorization: bacterial beta-etherases with catalytic activity on lignin-like polymers." *Chemsuschem, 7*, 3164–3171.

[13] Meux, E., Prosper, P., Masai, E., Mulliert, G., Dumarcay, S., Morel, M., Didierjean, C., Gelhaye, E. & Favier, F. (2012). "*Sphingobium sp.* SYK-6 Lig G involved in lignin degradation is structurally and biochemically related to the glutathione transferase omega class." *FEBS Lett, 586*, 3944–3950.

[14] Helmich, K. E., Pereira, J. H., Gall, D. L., Heins, R. A., McAndrew, R. P., Bingman, C., Deng, K., Holland, K. C., Noguera, D. R., Simmons, B. A., Sale, K. L., Ralph, J., Donohue, T. J., Adams, P. D. & Phillips, G. N. Jr. (2016). "Structural basis of stereospecificity in the bacterial enzymatic cleavage of β-aryl ether bonds in lignin." *J Biol Chem, 291*, 5234–5246.

[15] Pereira, J. H., Heins, R. A., Gall, D. L., McAndrew, R. P., Deng, K., Holland, K. C., Donohue, T. J., Noguera, D. R., Simmons, B. A., Sale, K. L., Ralph, J. & Adams, P. D. (2016). "Structural and biochemical characterization of the early and late enzymes in the lignin β-aryl ether cleavage pathway from *Sphingobium sp.* SYK-6." *J Biol Chem, 291*, 10228–10238.

[16] Jung, D. H., Kim, E. J., Jung, E., Kazlauskas, R. J., Choi, K. Y. & Kim, B. G. (2016). "Production of p-hydroxybenzoic acid from *p*-coumaric acid by *Burkholderia glumae* BGR1." *Biotechnol Bioeng, 113*(7), 1493–1503.

[17] Priefert, H., Rabenhorst, J. & Steinbüchel, A. (2001). "Biotechnological production of vanillin." *Appl Microbiol Biotechnol, 56*, 296–314.

[18] Pan, C., Oda, Y., Lankford, P. K., Zhang, B., Samatova, F. N., Pelletier, A. D., Harwood, S. C. & Hettich, L. R. (2008). "Characterization of anaerobic catabolism of *p*-coumarate in *Rhodopseudomonas palustris* by integrating transcriptomics and quantitative proteomics." *Mol Cell Proteom, 7*, 938–948.

[19] Gallage, N. J., Hansen, E. H., Kannangara, R., Olsen, C. E., Motawia, M. S., Jørgensen, K., Holme, I., Hebelstrup, K., Grisoni, M. & Møller, B. L. (2014). "Vanillin formation from ferulic acid in *Vanilla planifolia* is catalyzed by a single enzyme." *Nat Commun, 5*, 4037-4050.

[20] Gupta, J. K., Hamp, S. G., Buswell, J. A. & Eriksson, K. E. (1981). "Metabolism of trans-ferulic acid by white-rot fungus *Sporotrichum pulverulentum*." *Arch Microbiol, 128*, 349-354.

[21] Falconnier, B., Lapierre, C., Lesage-Meessen, L., Yonnet, G., Brunerie, P., Ceccaldi, B. C., Corrieu, G. & Asther, M. (1994). "Vanillin as a product of ferulic acid biotransformation by the white rot fungus *Pycnoporus cinnabarinus* I-37: identification of metabolic pathways." *J Biotechnol, 37*, 123–132.

[22] Rosazza, J. P., Huang, Z., Dostal, L., Volm, T. & Rousseau, B. (1995). "Biocatalytic transformations of ferulic acid: an abundant aromatic natural product." *J Ind Microbiol, 15*, 457–471.

[23] Peng, X., Misawa, N. & Harayama, S. (2003). "Isolation and characterization of *Thermophilic Bacilli* degrading cinnamic, 4-coumaric, and ferulic acids." *Appl Environ Microb, 69*, 1417–1427.

[24] Nazareth, S. & Mavinkurve, S. (1986). "Degradation of ferulic acid via 4-vinylguaiacol by *Fusarium solani* (Mart) Sacc." *Can J Microbiol, 32*, 494–497.

[25] Karmakar, B., Vohra, R. M., Nandanwar, H., Sharma, P., Gupta, K. G. & Sobti, R. C. (2000). "Rapid degradation of ferulic acid via 4-vinylguaiacol and vanillin by a newly isolated strain of *Bacillus coagulans*." *J Biotechnol, 80*, 195–202.

[26] Kadakol, C. & Kamanavalli, C. M. (2010). "Biodegradation of eugenol by *Bacillus cereus strain* PN24." *J Chem, 7*, 474–480.

[27] Xie, X. G., Huang, C. Y., Fu, W. Q. & Dai, C. C. (2016). "Potential of endophytic fungus *Phomopsis liquidambari* for transformation and degradation of recalcitrant pollutant sinapic acid." *Fungal Biol, 120*, 402-413.

[28] Xie, T., Liu, Z. C., Liu, Q. & Wang, G. G. (2015). "Structural insight into the oxidation of sinapic acid by CotA laccase." *J Struc Biol, 190*, 155-161.

[29] Jurkovfid, M. & Wurst, M. (1993). "Biodegradation of aromatic carboxylic acids by *Pseudomonas mira*," *FEMS Microbiol Lett, 111*, 245-250.

[30] Mukherjee, G., Sachan, A., Ghosh, S. & Mitra, A. (2006). "Conversion of sinapic acid to syringic acid by a filamentous fungus *Paecilomyces variotii*." *J Gen Appl Microbiol*, *52*, 131-135.

[31] Masai, E., Yamamoto, Y., Inoue, T., Takamura, K., Hara, H., Kasai, D., Katayama, Y. & Fukuda, M. (2007). "Characterization of ligV essential for catabolism of vanillin by *Sphingomonas paucimobilis* SYK-6.". *Biosci Biotechnol Biochem*, *71*, 2487-2892.

[32] Kasai, D., Masai, E., Miyauchi, K., Katayama, Y. & Fukuda, M. (2004). "Characterization of the 3-O-Methylgallate dioxygenase gene and evidence of multiple 3-O-methylgallate catabolic pathways in *Sphingomonas paucimobilis* SYK-6." *J Bacteriol*, *186*, 4951–4959.

[33] Abe, T., Masai, E., Miyauchi, K., Katayama, Y. & Fukuda, M. (2005). "A Tetrahydrofolate-dependent O-demethylase, LigM, is crucial for catabolism of vanillate and syringate in *Sphingomonas paucimobilis* SYK-6." *J Bacteriol*, *187*, 2030–2037.

[34] Zhu, D., Zhang, P., Xie, C., Zhang, W., Sun, J., Qian, W. J., et al. (2017). "Bidegradation of alkaline lignin by *Bacillus ligniniphilus* L1." *Biotechnol Biofuels*, *10*(1), 44-57.

[35] Deangelis, K. M., Sharma, D., Varney, R., Simmons, B., Isern, N. G., Markilllie, L. M., et al. (2013). "Evidence supporting dissimilatory and assimilatory lignin degradation in *Enterobacter lignolyticus* SCF1." *Front Microbiol*, *4*(4), 280-293.

[36] Michalska, K., Chang, C., Mack, J. C., Zerbs, S., Joachimiak, A. & Collart, F. R. (2012). "Characterization of transport proteins for aromatic compounds derived from lignin: benzoate derivative binding proteins." *J Mol Biol*, *423*(4), 555-575.

[37] Salmon, R. C., Cliff, M. J., Rafferty, J. B. & Kelly, D. J. (2013). "The CouPSTU and TarPQM transporters in *Rhodopseudomonas palustris*: redundant, promiscuous uptake systems for lignin-derived aromatic substrates." *Plos One*, *8*(3), e59844.

[38] Otani, H., Lee, Y. E., Casabon, I. & Eltis, L. D. (2014). "Characterization of phydroxycinnamate catabolism in a soil *Actinobacterium*." *J Bacteriol*, *196*(24), 4293-303.

[39] Otani, H., Stogios, P. J., Xu, X., Nocek, B., Li, S. N., Savchenko, A., et al. (2016). "The activity of couR, a marR family transcriptional regulator, is modulated through a novel molecular mechanism." *Nucleic Acids Res*, *44*(2), 595-607.

[40] Parke, D. & Ornston, L. N. (2003). "Hydroxycinnamate (*hca*) catabolic genes from *Acinetobacter* sp. strain ADP1 are repressed by HcaR and are induced by hydroxycinnamoyl-coenzyme A thioesters." *Appl Environ Microbiol*, *69*(9), 5398-5409.

[41] Calisti, C., Ficca, A. G., Barghini, P. & Ruzzi, M. (2008). "Regulation of ferulic catabolic genes in *Pseudomonas fluorescens* bf13: involvement of a marr family regulator." *Appl Microbiol Biotechnol*, *80*(3), 475-483.

[42] Hirakawa, H., Schaefer, A. L., Greenberg, E. P. & Harwood, C. S. (2012). "Anaerobic *p*-coumarate degradation by *Rhodopseudomonas palustris* and identification of CouR, a MarR repressor protein that binds *p*-coumaroyl coenzyme A." *J Bacteriol*, *194*(8), 1960.

[43] Valderrama, J. A., Duranterodríguez, G., Blázquez, B., García, J. L., Carmona, M. & Díaz, E. (2012). "Bacterial degradation of benzoate: cross-regulation between aerobic and anaerobic pathways." *J Biol Chem*, *287*(13), 10494-10508.

[44] Jiménez, J. I., Pérezpantoja, D., Chavarría, M., Díaz, E. & De, L. V. (2014). "A second chromosomal copy of the cata gene endows pseudomonas putida mt-2 with an enzymatic safety valve for excess of catechol." *Environ Microbiol*, *16*(6), 1767-78.

[45] Hernándezarranz, S., Moreno, R. & Rojo, F. (2013). "The translational repressor Crc controls the *Pseudomonas putida* benzoate and alkane catabolic pathways using a multi-tier regulation strategy." *Environ Microbiol*, *15*(1), 227-241.

[46] Luu, R. A., Kootstra, J. D., Nesteryuk, V., Brunton, C. N., Parales, J. V., Ditty, J. L., et al. (2015). "Integration of chemotaxis, transport and catabolism in *Pseudomonas putida* and identification of the aromatic acid chemoreceptor PcaY." *Mol Micicrobiol*, *96*(1), 134-147.

[47] Garcı´a, L. L., Rivas-Marı´n, E., Floriano, B., Bernhardt, R., Ewen, K. M., Reyes-Ramı´rez, F., Santero, E., et al. (2011). "ThnY is a ferredoxin reductase-like iron-sulfur flavoprotein that has evolved to function as a regulator of tetralin biodegradation gene expression." *J Biol Chem*, *286*(3), 1709-1718.

[48] Ohta, Y., Hasegawa, R., Kurosawa, K., Koizumi, T., Maeda, Allyn H., Nishimura, H., et al. (2017). "Enzymatic specific production and chemical functionalization of phenylpropanone platform monomers from lignin." *Chemsuschem*, *10*(2), 425-433.

[49] Picart, P., Liu, H., Grande, P. M., Anders, N., Zhu, L., Klankermayer, J., et al. (2017). "Multi-step biocatalytic depolymerization of lignin." *Appl Microbiol Biotec-hnol*, *101*(15), 6277-6287.

[50] Gall, D. L., Kontur, W. S., Lan, W., Kim, H., Li, Y., Ralph, J., et al. (2018). "*In vitro* enzymatic depolymerization of lignin with release of syringyl, guaiacyl, and tricin units." *Appl Environ Microbiol*, *84*(3), AEM.02076-17.

[51] Wang, C., Ouyang, X. H., Su, S. S., Liang, X., Zhang, C., Wang, W. Y., Yuan, Q. P. & Li, Q. (2016). "Effect of sulfonated lignin on enzymatic activity of the lignolytic enzymes Cα-dehydrogenase LigD and β-etherase LigF." *Enzyme Microb Technol*, *93*, 59–69.

[52] Fuchs, G., Boll, M. & Heider, J. (2011). "Microbial degradation of aromatic compounds—from one strategy to four." *Nature Rev Microbiol*, *9*, 803-816.

[53] Linger, J. G., Vardon, D. R., Guarnieri, M. T., Karp, E. M., Hunsinger, G. B., Franden, M. A., Johnson, C. W., Chupka, G., Strathmann, T. J., Pienkos, P. T. &

Beckham, G. T. (2014). "Lignin valorization through integrated biological funneling and chemical catalysis." *PNAS (USA), 111*, 12013-12018.

[54] Kaur, B. & Chakraborty, D. (2013). "Biotechnological and molecular approaches for vanillin production: a review." *Appl Biochem Biotechnol, 169*, 1353–1372.

[55] Davis, K. M., Rover, M., Brown, R. C., Bai, X. L., Wen, Z. Y. & Jarboe, L. R. (2016). "Recovery and utilization of lignin monomers as part of the biorefinery approach." *Energies, 9*, 808.

[56] Sainsbury, P. D., Hardiman, E. M., Ahmad, M., Otani, H., Seghezzi, N., Eltis, L. D. & Bugg, T. D. H. (2013). "Breaking down lignin to high-value chemicals: the conversion of lignocellulose to vanillin in a gene deletion mutant of *Rhodococcus jostii* RHA1." *ACS Chem Biol, 8*, 2151–2156.

[57] Austin, S., Kontur, W. S., Ulbrich, A., Oshlag, J. Z., Zhang, W. P., Higbee, A., et al. (2015). "Metabolism of multiple aromatic compounds in corn stover hydrolysate by *Rhodopseudomonas palustris*." *Environ Sci Technol, 49*(14), 8914-8922.

[58] Okamura-Abe, Y., Abe, T., Nishimura, K., Kawata, Y., Sato-Izawa, K., Otsuka, Y., Nakamura, M., Kajita, S., Masai, E., Sonoki, T. & Katayama, Y. (2016). "Beta-ketoadipic acid and muconolactone production from a lignin-related aromatic compound through the protocatechuate 3,4-metabolic pathway." *J Biosci Bioeng, 121*, 652-658.

[59] Kondo, S., Sugimura, K., Okamura, Y., Mase, K. & Sato-Izawa, K. (2016). "Stable Chiral Carboxymuconolactone Production from a Lignin-Related Aromatic Compound, Protocatechuic Acid." *Ferment Technol, 5*(135), 2-5.

[60] Gosling, A., Fowler, S. J., O'Shea, M. S., Straffon, M., Dumsday, G. & Zachariou, M. (2011). "Metabolic production of a novel polymer feedstock, 3-carboxy muconate, from vanillin." *Appl Microbiol Biotechnol, 90*, 107–116.

[61] van Duuren, J. B., Wijte, D., Karge, B., dos Santos, V. A., Yang, Y., Mars, A. E. & Eggink, G. (2012). "pH-stat fed-batch process to enhance the production of cis, cis-muconate from benzoate by *Pseudomonas putida* KT2440-JD1." *Biotechnol Prog, 28*, 85-92.

[62] Sonoki, T., Morooka, M., Sakamoto, K., Otsuka, Y., Nakamura, M., Jellison, J. & Goodell, B. (2014). "Enhancement of protocatechuate decarboxylase activity for the effective production of muconate from lignin-related aromatic compounds." *J Biotechnol, 192*, 71–77.

[63] Han, L., Liu, P., Sun, J., Wu, Y., Zhang, Y., Chen, W., Lin, J., Wang, Q. & Ma, Y. (2015). "Engineering catechol 1,2-dioxygenase by design for improving the performance of the *cis*, *cis*-muconic acid synthetic pathway in *Escherichia coli*." *Sci Rep-UK, 5*, 13435.

[64] Vardon, D. R., Franden, M. A., Johnson, C. W., Karp, E. M., Guarnieri, M. T., Linger, J. G., Salm, M. J., Strathmann, T. J. & Beckham, G. T. (2015). "Adipic acid production from lignin." *Energy Environ Sci, 8*, 617-628.

[65] Johnson, C. W., Salvachúa, D., Khanna, P., Smith, H., Peterson, D. J. & Beckham, G. T. (2016). "Enhancing muconic acid production from glucose and lignin-derived aromatic compounds via increased protocatechuate decarboxylase activity." *Metab Eng Commun, 3*,111–119.

[66] Johnson, C. W., Abraham, P. E., Linger, J. G., Khanna, P., Hettich, R. L. & Beckham, G. T. (2017). "Eliminating a global regulator of carbon catabolite repression enhances the conversion of aromatic lignin monomers to muconate in *Pseudomonas putidakt* 2440." *Metab Eng Commun, 5*, 19-25.

[67] Barton, N., Horbal, L., Starck, S., Kohlstedt, M., Luzhetskyy, A. & Wittmann, C. (2017). "Enabling the valorization of guaiacol-based lignin: integrated chemical and biochemical production of cis, cis-muconic acid using metabolically engineered amycolatopsis sp ATCC 39116." *Metab Eng, 45*, 200-210.

[68] Sonoki, T., Takahashi, K., Sugita, H., Hatamura, M., Azuma, Y., Sato, T., et al. (2017). "Glucose-free *cis*, *cis*-muconic acid production via new metabolic designs corresponding to the heterogeneity of lignin." *ACS Sustain Chem Eng, 6*(1), 1256-1264.

[69] Otsuka, Y., Nakamura, M., Shigehara, K., Sugimura, K., Masai, E., Ohara, S. & Katayama, Y. (2006). "Efficient production of 2-pyrone 4,6-dicarboxylic acid as a novel polymer-based material from protocatechuate by microbial function." *Appl Microbiol Biotechnol, 71*, 608–614.

[70] Asano, Y., Yamamoto, Y. & Yamada, H. (1994). "Catechol 2,3-dioxygenase-catalyzed synthesis of picolinic acids from catechols." *Biosci Biotechnol Biochem, 58*, 2054-2056.

[71] Mycroft, Z., Gomis, M., Mines, P., Law, P. & Bugg, T. D. (2015). "Biocatalytic conversion of lignin to aromatic dicarboxylic acids in *Rhodococcus jostii* RHA1 by re-routing aromatic degradation pathways." *Green Chem, 17*, 4974-4979.

[72] Vaillancourt, F. H., Bolin, J. T. & Eltis, L. D. (2006). "The ins and outs of ring-cleaving dioxygenases."*Crit Rev Biochem Mol Biol, 41*(4), 241-267.

[73] Beckham, G. T., Johnson, C. W., Karp, E. M., Salvachúa, D. & Vardon, D. R. (2016). "Opportunities and challenges in biological lignin valorization." *Curr Opin Biotech, 42*, 40-53.

[74] Johnson, C. W. & Beckham, G. T. (2015). "Aromatic catabolic pathway selection for optimal production of pyruvate and lactate from lignin." *Metab Eng, 28*, 240-247.

[75] Hong, C. Y., Ryu, S. H., Jeong, H., Lee, S. S., Kim, M. & Choi, I. G. (2017). "*Phanerochaete chrysosporium* multi-enzyme catabolic system for *in vivo* modification of synthetic lignin to succinic acid." *ACS Chemical Biology, 12*(7), 1749-1759.

[76] Eduardo, B. T., Juan Manuel, S. Y., Otoniel, B. D. & Liliana, M. B. (2015). "Production of short-chain fatty acids from the biodegradation of wheat straw lignin by aspergillus fumigatus." *Bioresource Technol*, *196*(2), 418-425.

[77] Kosa, M. & Ragauskas, A. J. (2012). "Bioconversion of lignin model compounds with oleaginous Rhodococci." *Appl Microbiol Biotechnol*, *93*, 891–900.

[78] Kosa, M. & Ragauskas, A. J. (2013). "Lignin to lipid bioconversion by oleaginous *Rhodococci*." *Green Chem*, *15*, 2070-2074.

[79] He, Y., Li, X., Ben, H., Xue, X. & Yang, B. (2017). "Lipid production from dilute alkali corn stover lignin by *Rhodococcus* strains." *ACS Sustain Chem Eng*, *5*(3), 2302-2311.

[80] Yaguchi, A., Robinson, A., Mihealsick, E. & Blenner, M. (2017). "Metabolism of aromatics by *Trichosporon oleaginosus*, while remaining oleaginous." *Microb Cell Fact*, *16*(1), 206.

[81] Zhao, C., Xie, S., Pu, Y., Zhang, R., Huang, F., Ragauskas, A. J. & Yuan, J. S. (2015). "Synergistic enzymatic and microbial lignin conversion." *Green Chem*, *18*, 1306-1312.

[82] Wells, T. J., Wei, Z. & Ragauskas, A. (2015). "Bioconversion of lignocellulosic pretreatment effluent via oleaginous *Rhodococcus opacus* DSM 1069."*Biomass Bioenerg*, *72*(4), 200-205.

[83] Lin, L., Cheng, Y., Pu, Y., Sun, S., Li, X. & Jin, M., et al. (2016). "Systems biology-guided biodesign of consolidated lignin conversion." *Green Chem*, *18*(20), 5536-5547.

[84] Shi, Y., Yan, X., Li, Q., Wang, X., Liu, M., Xie, S., et al. (2016). "Directed bioconversion of kraft lignin to polyhydroxyalkanoate by *Cupriavidus basilensis*, B-8 without any pretreatment." *Process Biochem*, *52*, 238-242.

[85] Kumar, M., Singhal, A., Verma, P. K. & Thakur, I. S. (2017). "Production and characterization of Polyhydroxyalkanoate from lignin derivatives by *Pandoraea* sp. ISTKB." *ACS Omega*, *2*(12), 9156-9163.

[86] Ho, J., Pawar, S. V., Hallam, S. J. & Yadav, V. G. (2018). "An improved whole-cell biosensor for the discovery of lignin-transforming enzymes in functional metagenomic screens." *ACS Synth Biol*, *2*(7), 392-398.

[87] Ni, J., Gao, Y. Y., Tao, F., Liu, H. Y. & Xu, P. (2017). "Temperature-directed biocatalysis for the sustainable production of aromatic aldehydes or alcohols." *Angew Chem Int Edit*, *57*(5), 1214-1217.

[88] Delorenzo, D. M., Henson, W. R. & Moon, T. S. (2017). "Development of chemical and metabolite sensors for *Rhodococcus opacus* PD630." *ACS Synth Biol*, *6*(10), 1973-1978.

[89] Nogales, J., García, J. L. & Díaz, E. (2017). "Degradation of Aromatic Compounds in Pseudomonas: A Systems Biology View." In *Aerobic Utilization of Hydrocarbons, Oils and Lipids*. Springer International Publishing., 1-49.

[90] Xie, S. X., Syrenne, R., Sun, S. & Yuan, J. S. (2014). "Exploration of natural biomass utilization systems (NBUS) for advanced biofuel - from systems biology to synthetic design." *Curr Opin Biotech, 27*(6), 195-203.

[91] Mukhopadhyay, A. (2015). "Tolerance engineering in bacteria for the production of advanced biofuels and chemicals." *Trends Microbiol, 23*(8), 498-508.

[92] Chen, G. Q. & Jiang, X. R. (2018). "Next generation industrial biotechnology based on extremophilic bacteria." *Curr Opin Biotech, 50*, 94-100.

[93] Chi, Y., Hatakka, A. & Maijala, P. (2007). "Can co-culturing of two white-rot fungi increase lignin degradation and the production of lignin-degrading enzymes." *Int Biodeter Biodegrad, 59*(1), 32-39.

[94] Huang, D. L., Zeng, G. M., Feng, C. L., Hu, S., Lai, C., Zhao, M. H., Su, F. F., Tang, L. & Liu, H. L. (2010). "Changes of microbial population structure related to lignin degradation during lignocellulosic waste composting." *Bioresour Technol, 101*(11), 4062-4067.

[95] Rahimi, A., Ulbrich, A., Coon, J. J. & Stahl, S. S. (2014). "Formic-acid-induced depolymerization of oxidized lignin to aromatics." *Nature, 515*, 249-252.

[96] Li, S., Masoud, T. A., Ydna, M. Questell-Santiago., Florent, H., Li, Y. D., Kim, H., Richard, M., Clint, C., John, R. & Jeremy, S. L. (2016). "Formaldehyde stabilization facilitates lignin monomer production during biomass depolymerization." *Science, 354*, 329-333.

[97] Vardon, D. R., Rorrer, N. A., Salvachúa, D., Settle, A. E., Johnson, C. W., Menart, M. J., Cleveland, N. S., Ciesielski, P. N., Steirer, K. X., Dorgan, J. R. & Beckham, G. T. (2016). "*cis*, *cis*-Muconic acid: separation and catalysis to bio-adipic acid for nylon-6, 6 polymerization." *Green Chem, 18*, 3397-3413.

[98] Cao, Z., Engelhardt, J., Dierks, M., Clough, M. T., Wang, G. H., Heracleous, E., et al. (2017). "Catalysis meets nonthermal separation for the production of (Alkyl)-phenols and hydrocarbons from Pyrolysis oil." *Angew Chem Int Edit, 129*(9), 2334-2339.

[99] Schwartz, T. J., O'Neill, B. J., Shanks, B. H. & Dumesic, J. A. (2014). "Bridging the chemical and biological catalysis gap: challenges and outlooks for producing sustainable chemicals." *ACS Catal, 4*, 2060-2069.

[100] Huo, Y., Zeng, H. & Zhang, Y. (2016). "Integrating metabolic engineering and heterogeneous chemocatalysis: New opportunities for biomass to chemicals." *ChemSusChem, 9*, 1078–1080.

[101] Plaggenborg, R., Overhage, J., Loos, A., Archer, J. A. C., Lessard, P., Sinskey, A. J., Steinbuchel, A. & Priefert, H. (2006). "Potential of *Rhodococcus* strains for biotechnological vanillin production from ferulic acid and eugenol." *Appl Microbiol Biotechnol, 72*, 745–755.

[102] Barghini, P., Di Gioia, D., Fava, F. & Ruzzi, M. (2007). "Vanillin production using metabolically engineered *Escherichia coli* under non-growing conditions." *Microb Cell Fact*, *6*(1), 13-23.

[103] Lee, E. G., Yoon, S. H., Das, A., Lee, S. H., Li, C., Kim, J. Y., Choi, M. S., Oh, D, K. & Kim, S. W. (2009). "Directing vanillin production from ferulic acid by increased acetyl-CoA consumption in recombinant *Escherichia coli*." *Biotechnol Bioeng*, *102*, 200–208.

[104] Tilay, A., Bule, M. & Annapure, U. (2010). "Production of biovanillin by one-step biotransformation using fungus *Pycnoporous cinnabarinus*." *J Agric Food Chem*, *58*, 4401–4405.