World J Microbiol Biotechnol (2017) 33:125
DOI 10.1007/s11274-017-2286-6

REVIEW

Efficient, environmentally-friendly and specific valorization of lignin: promising role of non-radical lignolytic enzymes

Wenya Wang[1,2] · Chao Zhang[1] · Xinxiao Sun[1] · Sisi Su[1] · Qiang Li[3] · Robert J. Linhardt[4,5,6]

Received: 23 February 2017 / Accepted: 16 May 2017

Abstract Lignin is the second most abundant bio-resource in nature. It is increasingly important to convert lignin into high value-added chemicals to accelerate the development of the lignocellulose biorefinery. Over the past several decades, physical and chemical methods have been widely explored to degrade lignin and convert it into valuable chemicals. Unfortunately, these developments have lagged because of several difficulties, of which high energy consumption and non-specific cleavage of chemical bonds in lignin remain the greatest challenges. A large number of enzymes have been discovered for lignin degradation and these are classified as radical lignolytic enzymes and non-radical lignolytic enzymes. Radical lignolytic enzymes, including laccases, lignin peroxidases, manganese peroxidases and versatile peroxidases, are radical-based bio-catalysts, which degrade lignins through non-specific cleavage of chemical bonds but can also catalyze the radical-based re-polymerization of lignin fragments. In contrast, non-radical lignolytic enzymes selectively cleave chemical bonds in lignin and lignin model compounds and, thus, show promise for use in the preparation of high value-added chemicals. In this mini-review, recent developments on non-radical lignolytic enzymes are discussed. These include recently discovered non-radical lignolytic enzymes, their metabolic pathways for lignin conversion, their recent application in the lignin biorefinery, and the combination of bio-catalysts with physical/chemical methods for industrial development of the lignin refinery.

Keywords Non-radical lignolytic enzymes · Lignin · Valorization · Metabolic engineering · High value added chemicals

Wenya Wang and Chao Zhang have contributed equally to this work.

✉ Wenya Wang
wangwy@mail.buct.edu.cn

[1] College of Life Science and Technology, Beijing University of Chemical Technology, Beijing 100029, China

[2] State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China

[3] Department of Chemical Engineering, Tsinghua University, Beijing 100084, China

[4] Departments of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, USA

[5] Chemical and Biological Engineering, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, USA

[6] Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, 110 8th Street, Troy, NY 12180, USA

Introduction

Lignin is a major component of lignocellulose and is also the most abundant aromatic polymers on earth. Lignin has a highly branched three-dimensional poly-phenolic structure that includes three phenylpropane units, *p*-coumaryl, coniferyl and sinapyl. Although 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, beginning with the extracellular oxidative degradation of natural lignin into low-molecular weight aromatics. Extracellular oxidative degradation is followed by intracellular metabolic degradation and

Published online: 24 May 2017

bioconversion of aromatic substances 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 (Reiter et al. 2013; Picart et al. 2015). Over the past few decades, the radical-dependent lignolytic enzymes have been studied extensively and thoroughly reviewed (Wong 2009; Pollegioni et al. 2015). The non-radical lignolytic enzymes, involving intracellular metabolic degradation and bioconversion of lignin with a low-molecular weight and lignin model compounds, have been the subject of recent intensive studies. Usually, radical lignolytic enzymes are first secreted extracellularly to depolymerize the natural lignin by microorganisms. The resulting aromatic molecules are then transported into microbial cells for further catabolism. The "funneling pathway", including upper pathways and lower pathways, is present in many microorganisms for the metabolism of aromatic compounds (Fuchs et al. 2011). In the upper pathways, aromatic molecules are catabolized into some conserved intermediates, including catechol (CA) in bacteria and protocatechuate (PCA) in most fungi and some bacteria (Fuchs et al. 2011). 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 (Harwood and Parales 1996; Fuchs et al. 2011; Linger et al. 2014).

Since the biodegradation of lignin is a time-consuming process, in practice natural lignin is usually pretreated with chemicals to obtain low-molecular weight compounds before its conversion by microorganisms to higher value chemicals. Over the past several decades, significant progress had been made in lignin depolymerization by different chemical methods, relying on alkali (Linger et al. 2014), formic acid (Rahimi et al. 2014), and formaldehyde (Shuai et al. 2016). A high yield of aromatics, especially the lignin monomers such as *p*-coumaric acid, vanillic acid, ferulic acid, vanillin, benzoic acid, syringaldehyde, and syringic acid can often be obtained from lignin depolymerization. These lignin deploymerized monomers can be classified as hydroxy-phenyl lignin unit (H-type), guajacyl lignin unit (G-type), syringyl lignin unit (S-type) and their derivatives. The G-/S-/H- type lignin units can be metabolized into various valuable chemicals by "funneling pathway" (Linger et al. 2014).

In this review, we focus on recent progress on non-radical enzymes that catalyze the reactions of G-/S-/H- type lignin aromatic monomers to PCA or CA, as these enzymes constitute the upper part of "funneling pathway" (Linger et al. 2014; Vardon et al. 2015), and plays a critical role in the biological valorization of lignin depolymerization. The enzymes involved in the aromatic catabolism of PCA and CA for entry into the TCA cycle, used for lignin valorization, are not included in this review since they have already been extensively reviewed (Harwood et al. 1996; Fuchs et al. 2011). In the case of non-radical lignolytic enzymes degrading lignin oligomers (dimer and beyond), only β-etherase is reviewed, for information on other enzymes readers can refer to papers by Bugg et al. (2011a, b) and Masai et al. (2007b). Taken together, the recent progress of non-radical lignolytic enzymes, their application in lignin biological valorization and combinational technologies for lignin biological valorization will be summarized and discussed in this review.

The progress in non-radical lignolytic enzymes

β-ether degrading enzymes

Lignin contains a variety of different chemical bonds that link the three types of its monolignol units. The *β–O–*4 bond is the most abundant one of these accounting for 50–70% of total linkages. Efficient enzymatic cleavage of *β–O–*4 bond would provide a specific, environmentally-friendly and effective method for depolymerizing lignin to afford high-value added chemicals.

The model lignin dimer, containing a *β–O–*4 bond can be split by β-ether enzyme systems, of which the β-etherase system of *Sphingobium* sp. SYK-6 is the most well-known. The degradation of *β–O–*4 bond by *Sphingobium* sp. SYK-6 includes dehydrogenase (LigD), etherase enzyme (LigF) and glutathione-lyase (LigG). The LigDFG system catalyzes the following reaction steps: (1) LigD oxidizes the hydroxyl group of C_α in the substrate to form the ketone group in the presence of NAD^+; (2) LigF breaks the ether bonds by attacking the C_β with reduced glutathione; (3) LigG, a glutathione transferase, releases an oxidized dimer glutathione and an aromatic monomer from the substrate with the aid of another reduced glutathione (Fig. 1).

The genes encoding these three enzymes have a number of family members, which are summarized in Tables 1 and 2. The different family members show a variety of pH optima, thermal stabilities and substrate specificities. Different lignin model compounds have been prepared to test the novel family members of LigD, LigF and LigG. In addition to their enantioselective differences, other groups can also significantly impact the activity of these enzymes including, the groups on the aromatic rings, the linkage between two aromatic rings, the group at C_α, the side chain of C_β, and the positions of methoxy groups on aromatic ring. 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 the C_α position is replaced by carbonyl, the β-etherase

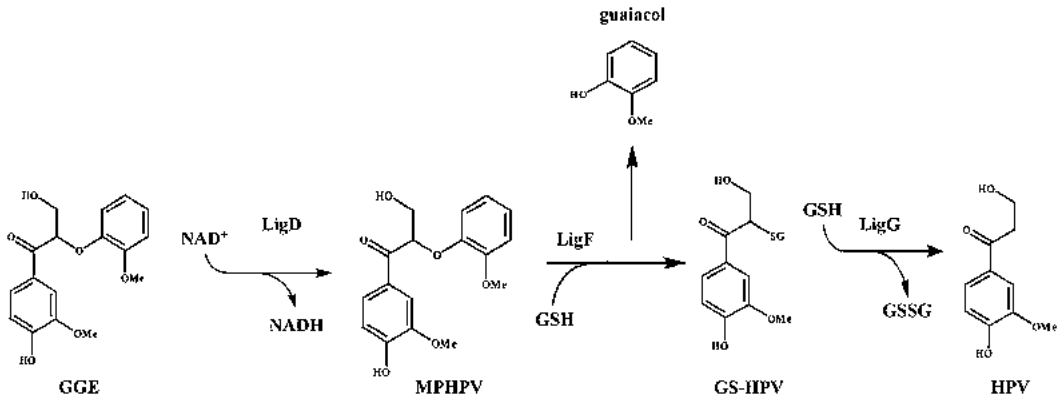

Fig. 1 Reaction procedure of the LigDFG enzyme system. *GGE* guaiacylglycerol-*β*-guaniacyl ether; *MPHPV* *α*-(2-methoxyphenoxy)-*β*-hydroxypropiovanillone; *GS-HPV* *α*-glutathionyl-*β*-hydroxypropiovanillone; *HPV* *β*-hydroxypropiovanillone

Table 1 The summary of reported *β*-etherases in LigDFG reaction system

Name	Strain	Substrate	Stereospecificity	References
Lig F (SsLig F1)	*Sphingobium* sp. SYK-6	MPHPV, GVG, GVE, 2,6-MP-VG, 3,5-MP-VG, MUAV, DHP-MUAV, GS, SS, GG, SG	*S*	Gall et al. (2014) Picart et al. (2014)
LigF-NS (NsLigF)	*Novosphingobium* sp. PP1Y	GVG, GVE, 2,6-MP-VG, MUAV, DHP-MUAV, GS, SS, GG, SG	*S*	Picart et al. (2014) Gall et al. (2014)
LigF-NA (NaLigF1)	*N.aromaticovorans*DSM 12444	GVG, GVE, 2,6-MP-VG, 3,5-MP-VG, MUAV, DHP-MUAV, GS, SS, GG, SG	*S*	Picart et al. (2014) Gall et al. (2014)
NaLigF2	*N.aromaticovorans*DSM 12444	GVG, GVE, 2,6-MP-VG, 3,5-MP-VG, MUAV, DHP-MUAV, GSvSS, GGvSG	*S*	Picart et al. (2014) Gall et al. (2014)
LigE (SsLigE)	*Sphingobium* sp. SYK-6	MPHPV, GVG, GVE, 2,6-MP-VGvMUAV, DHP-MUAV, GS, SS, GG, SG	*R*	Picart et al. (2014) Gall et al. (2014)
LigE-NS (NsLigE)	*Novosphingobium* sp. PP1Y	GVG, GVE, 2,6-MP-VG, MUAV, DHP-MUAV, GS, SS, GG, SG	*R*	Picart et al. (2014) Gall et al. (2014)
LigE-NA (NaLigE)	*N. Aromaticovorans* DSM 12444	GVG, GVE, 2,6-MP-VG, MUAV, DHP-MUAV, GS, SS, GG, SG	*R*	Picart et al. (2014) Gall et al. (2014)
LigP (SsLigP)	*Sphingobium* sp. SYK-6	MPHPV, GVG, GVE, 2,6-MP-VG, MUAV, DHP-MUAV, GS, SS, GG, SG	*R*	Picart et al. (2014) Gall et al. (2014)
GST4	*Novosphingobium* sp. MBES04	MPHPV	*S*	Ohta et al. (2015)
GST5	*Novosphingobium* sp. MBES04	MPHPV	*R*	Ohta et al. (2015)

MPHPV *α*-(2-methoxyphenoxy)-*β*-hydroxypropiovanillone, *GVG* *β*-guaiacyl-*α*-veratrylethanone, *GVE* *β*-guaiacyl-α-veratrylethanone, *2,6-MP-VG* *β*-(2,6-methoxyphenoxy)-*α*-veratrylglycerone, *3,5-MP-VG* *β*-(3,5-methoxyphenoxy)-*α*-veratrylglycerone, *MUAV* *α-O-*(*β*-methoxylumbelliferyl)acetovanillone, *DHP-MUAV* the multiple polymer of coniferyl alcohol and α-*O*-(β-methoxylumbelliferyl)acetovanillone, *GS* *α*-(4-*O*-Me)-guaiacylglycerone-*β*-(1′-formyl)-syringyl ether, *SS* *α*-(4-*O*-Me)-syringylglycerone-*β*-(1′-formyl)-syringyl ether, *GG* *α*-(4-*O*-Me)-guaiacylglycerone-β-(1′-formyl)-guaiacyl ether, *SG* *α*-(4-*O*-Me)-syringylglycerone-β-(1′-formyl)-guaiacyl ether

can effectively cleave the *β*-ether bond linking the aromatic groups in dimer compounds (Gall et al. 2014; Picart et al. 2014).

The large number of gene family members might have evolved to adapt to the environment and the intrinsic heterogeneity of lignin in order to effectively utilize lignin as carbon and energy source. However, from the point of industrial application, the presence of too many enzymes makes a process unduly complex. Thus, it is essential to study the catalytic mechanism of enzymatic reaction and 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 (Meux et al. 2012; Helmich et al. 2016; Pereira et al. 2016) and these will undoubtedly be used in combination with gene mining and protein engineering to promote studies on *β*-ether degrading enzymes.

Table 2 The summary of reported dehydrogenases and glutathione-lyase in LigDEF reaction system

Name	Strain	Substrate	Stereospecificity	References
Lig D	*Sphingobium* sp. SYK-6	GGE	*R*	Masai et al. (1993) Pereira et al. (2016)
LigO	*Sphingobium* sp. SYK-6	GGE	*R*	Sato et al. (2009) Pereira et al. (2016)
LigL	*Sphingobium* sp. SYK-6	GGE	*S*	Sato et al. (2009) Pereira et al. (2016)
LigN	*Sphingobium* sp SYK-6	GGE	*S*	Sato et al. (2009)
LigG	*Sphingobium* sp. SYK-6	GS-VG	*R*	Meux et al. (2012) Picart et al. (2015)
LigG-NS	*Novosphingobium* sp. PP1Y	GS-VG	*R*	Picart et al. (2015)
LigG-TD	*Thiobacillus denitrificans* ATCC 25259	GS-VG	*R*	Picart et al. (2015)
GST3	*Novosphingobium sp. MBES04*	GS-GHP	*S,R*	Ohta et al. (2015)
GST6	*Novosphingobium sp. MBES04*	GS-GHP	*S*	Ohta et al. (2015)

GGE guaiacylglycerol-*β*-guaniacyl ether, *GS-VG* S-glutathionyl-*α*-veratryglycerone, *GS-GHP* glutathione adduct of guaiacylhydroxylpropanone

The enzymes in hydroxy-phenyl lignin catabolism (H-type lignin unit)

As one of three ligninol units, *p*-coumaric acid (pCA) is abundant in biomass with low lignin content, such as straw, wheat and barley (Jung et al. 2016). pCA can be catabolized to *p*-hydroxybenzoic acid (pHBA), and then further converted into PCA or CA. There are both CoA-dependent *β*-oxidation and CoA-dependent non-*β*-oxidation pathways in microorganisms that can transform pCA to pHBA (Fig. 2A, B) (Pan et al. 2008; Trautwein et al. 2012; Jung et al. 2016). In the CoA-dependent *β*-oxidation pathway, the catabolism of pCA resembles fatty acid *β*-oxidation. pCA is initially catalytically converted to *p*-hydroxycinnamoyl-CoA by *p*-hydroxycinnamoyl-CoA synthetase (pHCS), and subsequently hydrated, oxidized and thiolyzed

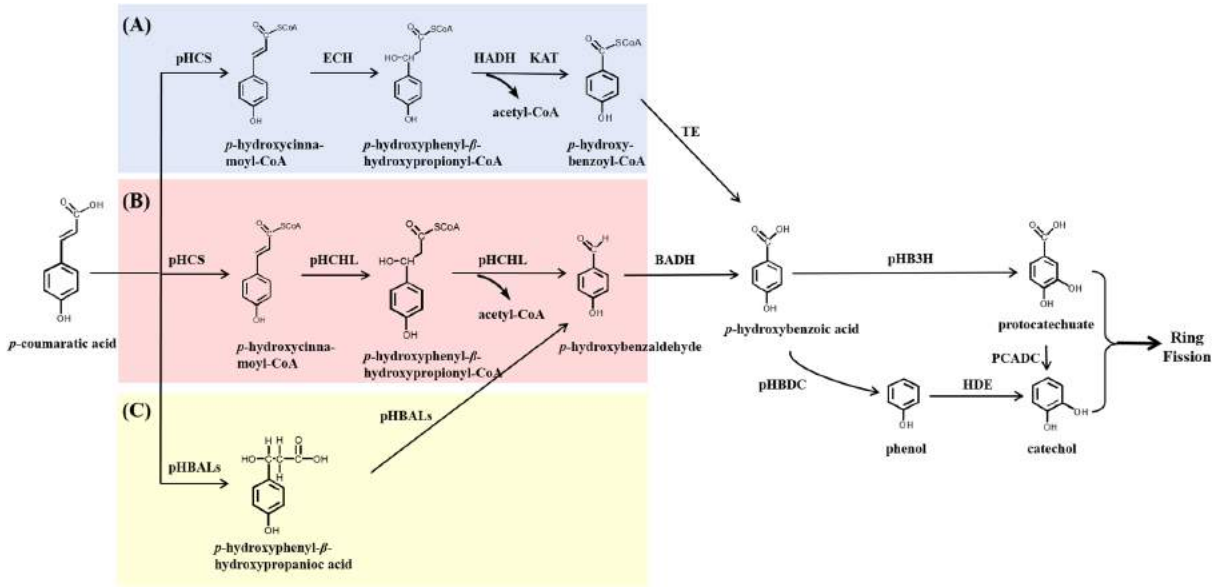

Fig. 2 The catabolism of *p*-coumaric acid. **A** CoA-dependent *β*-oxidation pathway; **B** CoA-dependent non *β*-oxidation pathway; **C** CoA-independent pathway. *BADH* benzaldehdye dehydrogenase, *ECH* enoyl-CoA hydratase, *HADH* 3-hydroxyacyl-CoA dehydrogenase, *HDE* hydroxylase, *pHBALS* *p*-hydroxybenzaldehydesynthase, *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

by enoyl-CoA hydratase (ECH), 3-hydroxyacyl-CoA dehydrogenase (HADH) and 3-ketoacyl-CoA thiolase (KAT) to produce *p*-hydroxybenzoyl-CoA. Finally, the hydrolysis of *p*-hydroxybenzoyl-CoA by thiolase (TE) generates pHBA (Jung et al. 2016). In the phototrophic bacterium *Rhodopseudomonas palustris* CGA010, a non-*β*-oxidation pathway is involved in the anaerobic degradation of *p*-coumarate, in which pHCS and *p*-hydroxycinnamoyl-CoA hydratase/lyase (pHCHL) are employed to generate *p*-hydroxyphenyl-*β*-hydroxypropionyl-CoA (pHPβHPC), the same intermediate formed in the *β*-oxidation pathway. Further thiolysis of pHPβHPC by pHCHL and oxidation of *p*-hydroxybenzaldehyde by *p*-hydroxybenzaldehyde dehydrogenase (BADH) produces *p*-hydroxybenzoic acid (pHBA), not observed in the *β*-oxidation pathway (Pan et al. 2008; Jung et al. 2016). In the plants *Glechoma* sp. and *Vanilla* sp., a CoA-independent pathway produces pHBA from pCA, and is proposed to involve one reductase (pHBALS) and one decarboxylase (pHBALS) (Fig. 2C) (Gallage et al. 2014; Jung et al. 2016), however, no genes corresponding to these enzymes have been reported yet in microorganisms.

pHBA can be hydroxylated by *p*-hydroxybenzoate-3-hydroxylase (pHB3H) to form protocatechuate (PCA) in *Pseudomonas* sp., (Meijnen et al. 2011) in some *Bacillus* sp., *Enterobacter* sp., and *Klebsiella* sp. Decarboxylation and hydroxylation of pHBA through the successive action of *p*-hydroxybenzoate decarboxylase (pHBDC) and hydroxylase (HDE) generates catechol (CA) (Fig. 2) (Bains and Boulanger 2007; Lupa et al. 2008; Pérez-Pantoja et al. 2012). The protocatechuat decarboxylase (pCADC) catalyzes the formation of CA from PCA. Finally, the aromatic ring of PCA or CA can be cleaved by dioxygenase to enter the TCA cycle (Fig. 2).

The enzymes in guajacyl lignin catabolism (G-type lignin unit)

Ferulic acid is commonly considered the typical model of a G-type lignin monomer. The ferulic acid monomer can be found under different deploymerzing conditions, including alkaline hydrolysis, acid hydrolysis and hydrogenolysis. Over the past several decades, the degradation of ferulic acid in microorganisms has been classified into four categories: non-oxidative decarboxylation, non-*β*-oxidation, *β*-oxidation, and side chain reduction (Rosazza et al. 1995; Priefert el al. 2001; Peng et al. 2003).

It was first reported in *Rhodococcus* sp. I24 in 2006 that ferulic acid was believed to be degraded through the *β*-oxidation pathway (Fig. 3C) (Plaggenbor et al. 2006). In *S. paucimobilis* SYK-6, the degradation of ferulic acid involves a CoA-dependent, non-*β*-oxidation pathway, in which feruloyl CoA synthetase (FerA) and feruloyl CoA hydratase/lyase (FerB) convert ferulic acid to vanillin, which is then successively transformed into vanillic acid and PCA by vanillin dehydrogenase (ligV), vanillate/3-*O*-methylgallate *O*-demethylase (ligM) (Fig. 3B) (Kasai et al. 2012). The same catabolic pathway was also discovered in *Pseudomonas* sp. strain HR199, *Pseudomonas fluorescens* AN103, and *Delftia acidovorans*, (Priefert el al. 2001; Peng et al. 2003). There is also a CoA-independent non-*β*-oxidation pathway that initiates the hydration of the *trans* double bond of ferulic acid to give 4-hydroxy-3-methoxyphenyl-*β*-hydroxypropionic acid as a transient intermediate, followed by aldolase cleavage to vanillin and acetate (not shown in Fig. 3) (Priefert el al. 2001).

In the non-oxidative decarboxylation pathway, ferulic acid is catalytically decarboxylated by ferulic acid decarboxylase (FDC) to form 4-vinylguaiacol. The step in which ferulic acid is converted into 4-vinylguaiacol was discovered in fungi, yeast and bacteria (Priefert el al. 2001), and the genes encoding this decarboxylase have been cloned and characterized from different microorganisms (Priefert el al. 2001). A 4-vinyl guaiacol dehydrogenase (VGDH) transforms the 4-vinylguaiacol into vanillin or vanillic acid (Fig. 3A), and this has been established in *Fusarium solani* (Mart) Sacc. (Nazareth and Mavinkurve 1986), *Bacillus coagulans* (Karmakar et al. 2000), and *Bacillus cereus* strain PN24 (Kadakol and Kamanavalli 2010).

In *Corynebacterium glutamicum*, dihydroferulic acid was identified as a metabolite (Labuda et al. 1992) indicative of the reducing pathway of ferulic acid degradation. Ferulic acid 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 (Priefert et al. 2001). In addition, ferulic acid can also be reduced to coniferyl alcohol, which is further degraded to vanillic acid (Gupta et al. 1981; Falconnier et al. 1994) (Fig. 3D).

Among the four pathways of ferulic acid degradation, the enzymes present in CoA-dependent non-*β*-oxidation pathway are well-developed from a variety of different microorganisms. The enzymes present in the other three pathways still require further investigation.

The enzymes in syringyl lignin catabolism (S-type lignin unit)

Sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) is believed to participate in the formation of syringyl-lignin and is often found in the depolymerized lignin. There are two methoxy groups on the aromatic ring of sinapic acid. There are fewer degrading microorganisms of sinapic acid in comparison with that of *p*-coumaric acid and ferulic acid. Most catabolic microorganisms for *p*-coumaric acid and ferulic acid cannot degrade sinapic acid, which

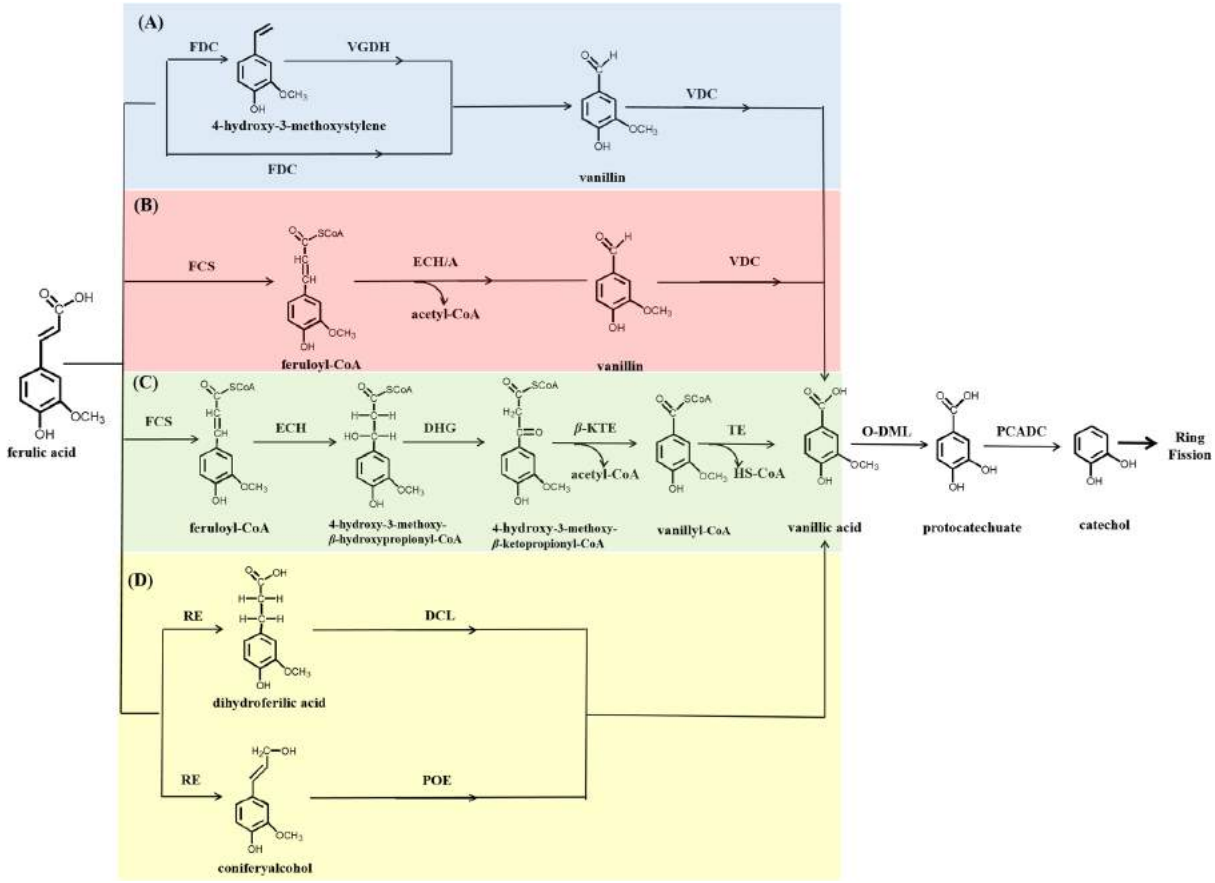

Fig. 3 The catabolism of ferulic acid. **A** Non-oxidative decarboxylation pathway; **B** non *β*-oxidation pathway; **C** *β*-oxidation pathway; **D** side chain reduction pathway. *DCL* decarboxylase, *DHG* dehydrogenase, *ECH* enoyl-CoA-hydratase, *ECH/A* enoyl-CoA-hydratase/aldolase, *FCS* feruloyl-CoA synthelase, *FDC* ferulic acid decarboxylase, *β-KTE* *β*-keto thiolase, *O-DML* *O*-demethylase, *PCADC* protocatechuate deccarboxylase, *POE* phenol oxidase, *RE* reductase, *TE* thiolase, *VD* vanillin decarboxylase, *VGDH* 4-vinylguaiaol dehydrogenase

indicates the adverse impact of aromatic methoxyl groups on sinapic acid catabolism (Xie et al. 2016). The proposed catabolic pathway of sinapic acid is presented in Fig. 4. The initial step from sinapic acid to syringic acid is proposed to be catalyzed by radical lignolytic enzymes (laccases) (Xie et al. 2015, 2016) or non-radical lignolytic enzymes (decarboxylases) (Jurkovfid and Wurst 1993; Mukherjee et al. 2006). In the case of the non-radical catalysis, no genes corresponding to these decarboxylases have yet been reported. Sinapic acid 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 (Masai et al. 2007a; Kasai et al. 2004; Abe et al. 2005). There is currently no evidence to support the transformation of syringic acid to PCA or CA prior to ring-opening.

Progress in biotransforming production of chemicals from depolymerized lignin and lignin model compounds

There are two approaches to biotransforming depolymerized lignin or lignin model compounds into chemicals. The first is in vitro enzymatic conversion and the second is in vivo metabolic conversion. Due to lignin`s heterogeneous structure and its inhibitory effect on lignolytic enzymes, the efficiency of its in vitro enzymatic conversion is low and most recent publications on lignin biological

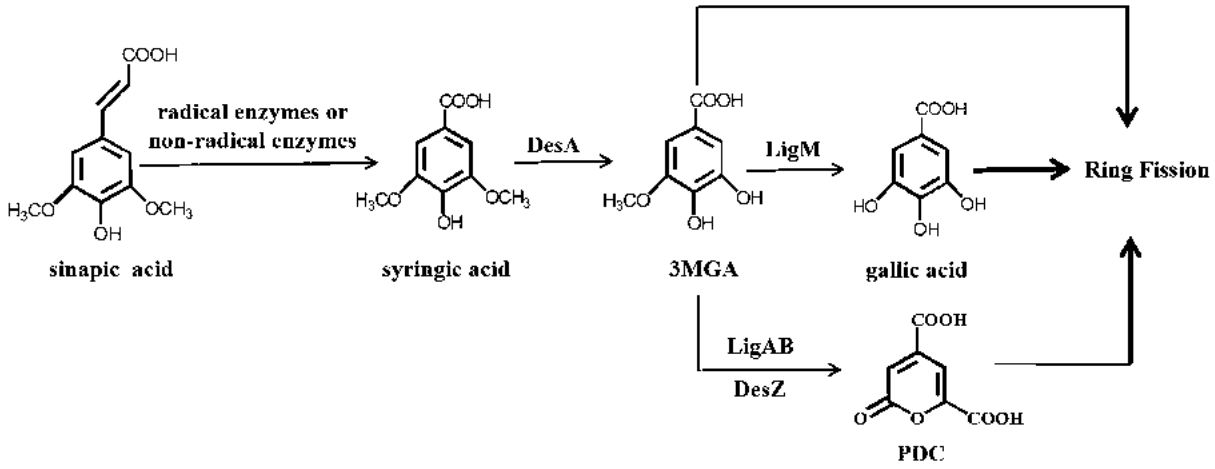

Fig. 4 The catabolism of sinapic acid. *3MGA* 3-*O*-methylgallate, *PDC* pyrone-4,6-dicarboxylic acid

valorization focus on in vivo metabolic conversion. Moreover, although some value-added chemicals have been produced from lignin using microorganisms, most reports rely on lignin model compounds to study the metabolic conversion in vivo.

Enzymatic conversion in vitro

Given the complexity of lignin structures, it is difficult to depolymerize lignin into value-added functional aromatic compounds at large scale using chemical or physical treatment (Picart et al. 2015). Enzymatic methods, particularly using non-radical lignolytic enzymes, represent an alternative approach for lignin valorization using environmentally-friendly and selective conditions (Picart et al. 2015). Since *β-O*-4 aryl ether is the most prevalent type of linkages between lignin monomers, accounting for 50–70% of linkages within lignin, recent in vitro enzymatic conversion of lignin has focused on *β*-etherase systems.

Picart et al. (2014) 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 indicated 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. The cleavage of natural lignin by the LigDFG *β*-etherase system had also been carried out by Reiter et al. (2013), in which softwood/hardwood alkali-lignin and bagasse organosolv-lignin were selected as substrates. GPC analysis showed that, softwood and hardwood alkali-lignin were degraded only slightly, but this did not occur in bagasse-organosolv lignin even after 7 days of treatment.

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 (Reiter et al. 2013; Picart et al. 2014). This might result from the problems of enzymatic enantioselectivity, substrate availability, or the inhibiting effect of lignin on enzyme activity (Wang et al. 2016). Consequently, more research is still required to understand the mechanism of *β*-etherase catalysis and the interaction between the enzymes and the lignin.

Metabolic conversion in vivo

Recently, researchers have explored the biotransformation of lignin and lignin-derived monomers using metabolic engineering for the purpose of lignin valorization into different chemicals (Fig. 5; Table 3).

Vanillin

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is one of the most widely used flavoring agents and is also a precursor to pharmaceuticals, household products, deodorants, air fresheners, floor polishes and herbicides (Priefert et al. 2001; Davis et al. 2016). Originally, vanillin was extracted from the orchid *Vanilla planifolia, Vanilla tahitiensis* and *Vanilla pompona* (Kaur and Chakraborty 2013; Gallage et al. 2014). 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, ferulic acid,

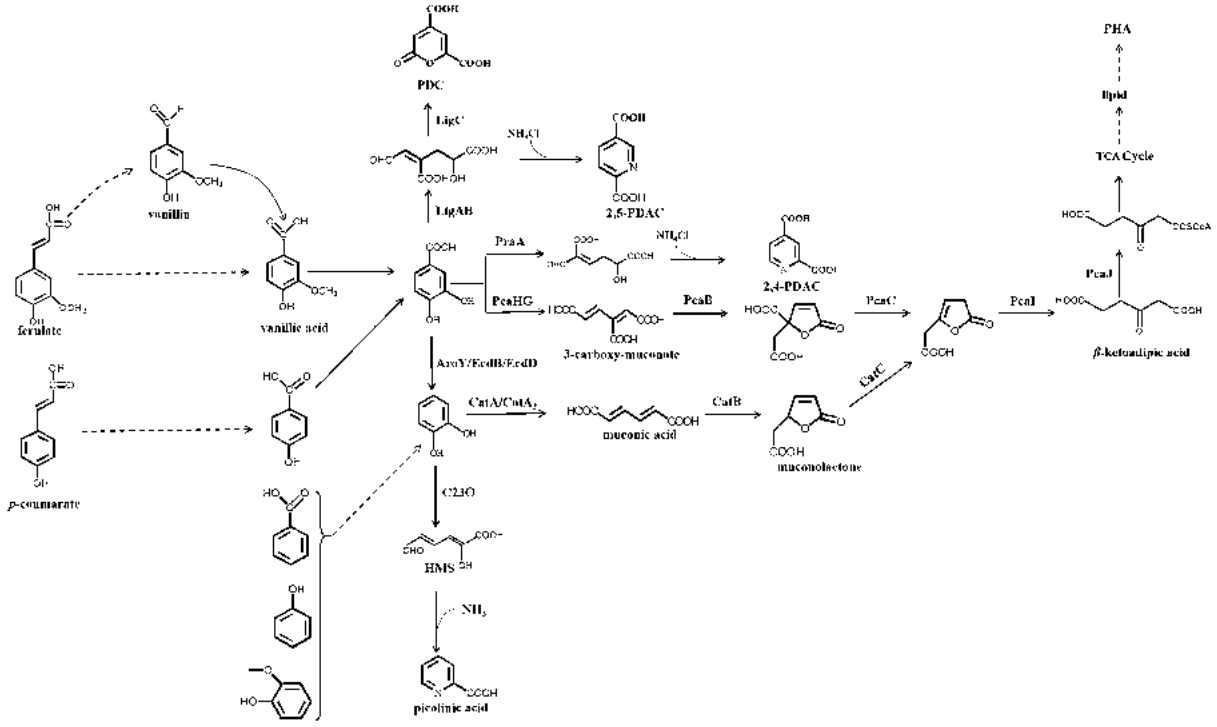

Fig. 5 Lignin valorization by in vivo metabolic conversion. *Dashed arrows* indicate multiple-step of enzymatic catalysis

Table 3 The reported chemicals biotransforming from lignin or lignin model compounds

Molecule	Substrate	Host	Reference
Pyruvate, lactate	Benzoate and *p*-coumarate	*P. pudita* KT2440	Johnson and Beckham (2015)
β-Ketoadipic acid, muconolactone	Protocatechuic acid	*P. pudita* KT2440	Okamura-Abe et al. (2016)
Muconic acid	Catechol, *p*-coumarate, benzoate, vanillin, lignin	*E. coli* *E. coli* XL-1 Blue *P. pudita* KT2440	van Duuren et al. (2012); Sonoki et al. (2014); Han et al. (2015); Vardon et al. (2015); Johnson et al. (2016)
Picolinic acid, HMS[a]	Catechol	*Pseudomonas putida* (arvilla) mt-2 ATCC 23973	Asano et al. (1994)
3-Carboxy-muconate	Vanillin	*Escherichia coli* BL21-Gold (DE3)	Gosling et al. (2011)
Fluorescent substance	Syringaldehyde	*Pseudomonas* sp. ITH-SA1	Iwabuchi et al. (2015)
2,5-PDCA 2,4-PDCA[b]	Wheat straw lignocellulose	*Rhodococcus jostii* RHA1	Mycroft et al. (2015)
Fatty acid, PHA	Alkaline pretreated lignin	*P. pudita* KT2440	Linger et al. (2014)
Lipid	4-hydroxybenzoic acid, vanillic acid, lignin	*Rhodococcus opacus* DSM 1069 and PD630	Kosa and Ragauskas (2012, 2013)
Vanillin	Eugenol, isoeugenol, ferulic acid, vanillic acid, lignin	*E. coli, Pseudomonas putida, Rhodococcus, Pycnoporous*	Plaggenborg et al. (2006); Barghini et al. (2007); Lee et al. (2009); Tilay et al. (2010); Sainsbury et al. (2013); Davis et al. (2016)
PDC[c]	Protocatechuate	*P. putida* PpY1100	Otsuka et al. (2006)

[a]2-Hydroxymuconic semialdehyde (HMS)

[b]Pyridine 2,5-dicarboxylic acid (2,5-PDCA) and pyridine 2,4-dicarboxylic acid (2,4-PDCA)

[c]2-Pyrone-4,6-dicarboxylic acid (PDC)

vanillic acid (Priefert et al. 2001; Kaur and Chakraborty 2013; Davis et al. 2016). 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 (Sainsbury et al. 2013). In addition, ferulate catabolic pathways and *β*-aryl ether cleavage produce vanillin as one intermediate metabolite within different lignin degradation pathways, which provides valuable approaches for the bio-production of vanillin from lignin. Consequently, the application of metabolic engineering for the enhancement of upstream gene expression and the knock out of the downstream genes could help to further improve the biotransformation of lignin into vanillin.

Dicarboxylic acid and its derivatives

Currently dicarboxylic acid and its derivatives account for the major microbial bio-transformation products coming from lignin or lignin monomer. These derivatives include *β*-ketoadipic acid (Okamura-Abe et al. 2016), muconotactone (Okamura-Abe et al. 2016), 3-carboxy-muconate (Gosling et al. 2011), muconic acid (van Duuren et al. 2012; Sonoki et al. 2014; Han et al. 2015; Vardon et al. 2015; Johnson et al. 2016), 2-pyrone-4,6-dicarboxylic acid (PDC) (Otsuka et al. 2006), picolinic acid (Asano et al. 1994), 2-hydroxymuconic semialdehyde (Asano et al. 1994), pyridine 2,5-dicarboxylic acid (2,5-PDCA) and 2,5-dicarboxylic acid (2,4-PDCA) (Mycroft et al. 2015).

When PCA is cleaved at its *ortho* position by PcaHG, 3-carboxy-muconate is obtained, subsequent enzymatic conversion of 3-carboxy-muconate by PcaB/C/I forms *β*-ketoadipic acid (Gosling et al. 2011; Okamura-Abe et al. 2016). CA is cleaved at its *ortho* position by CatA or CatA2, producing muconic acid (Johnson et al. 2016; Okamura-Abe et al. 2016). When PCA is cleaved at 2,3(*meta*) or 4,5(*meta*) positions by PraA or LigAB, following ammonia cyclization, 2,5-PDCA or 2,4-PDCA are afforded (Mycroft et al. 2015). Moreover, PCA is catalyzed successively by ligAB and ligC forming PDC (Otsuka et al. 2006). All these chemicals have shared structures (two or more carboxylic acid groups), making these compounds, or their derivatives, useful raw materials for the synthesis of bio-based polymers.

Muconic acid, the recent focus of these dicarboxylic acids, can be converted into the important 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. Attempts have also been made to produce muconic acid from *p*-coumarate, 4-hydroxybenzoate, benzoate, and vanillate using engineered strains (Sonoki et al. 2014; Vardon et al. 2015; Johnson et al. 2016). 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} (Vardon et al. 2016). Moreover, *p*-coumarate and ferulate 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% (Vardon et al. 2015). 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*-coumaric acid as substrate after the improvement of protocatechuate decarboxylase activity (Johnson et al. 2016).

Chemicals from TCA cycle

The "upper pathways" in aromatic-compounds degrading organisms are utilized to integrate or funnel the heterogeneous lignin depolymerization into a few common intermediates, such as CA (1,2-dihydroxybenzene) or PCA (3,4-dihydroxybenzoate). The "lower pathways" then cleave the aromatic rings through the action of dioxygenase enzymes through *ortho* (intradiol) or *meta* (extradiol) pattern. The split products are catabolized through different metabolic pathways to produce different molecules in the TCA metabolism.

The *ortho*-cleavage pattern of CA and PCA produce succinate and acetyl-CoA (Jiménez et al. 2002). The 2,3 *meta*-cleavage of CA and PCA yields pyruvate and acetyl-CoA, while 4,5 meta-cleavage of PCA affords two molecules of pyruvate (Johnson and Beckham 2015). Since the pyruvate is a key intermediate for bio-based production of amino acids, alcohols, terpenoids and lactate, Johnson and Beckham (2015) compared the yield of pyruvate from CA and PCA through *ortho* (intradiol) or *meta* (extradiol) cleavage. The replacement of the *ortho* catechol degradation pathway in *Pseudomonas putida* KT2440 with a *meta*-cleavage pathway from *P. putida* mt-2 increases yields of pyruvate. Furthermore, replacing the protocatechuate (PCA) *ortho* pathway with a *meta*-cleavage pathway from *Sphingobium sp*. SYK-6 results in a nearly five-fold increase in pyruvate yield. This metabolic strategy can also be applied for the production of succinic acid or succinic acid-based products from lignin.

Lipid, fatty acids and its polymer

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 synthetic pathway to produce lipids began with the study on lignin model compounds, hydroxybenzoic and vanillic acid, (Kosa and Ragauskas 2012) and has recently led to use of natural lignin (Kosa and Ragauskas 2013). The synergism between laccase and microbial lignin conversion has also been examined for lipid production (Zhao et al. 2015). Lipid accumulation by *Rhodococcus opacus* DSM 1069 in nitrogen limited medium reached nearly 20% of its weight (Kosa and Ragauskas 2012). Linger et al. (2014) reported that the alkaline depolymerized lignin could be converted by *Pseudomonas putida* KT2440 into medium chain length (mcl)-PHAs through integrated biological funneling pathway. The isolated mcl-PHAs showed similar physicochemical properties to conventional carbohydrate-derived mcl-PHAs.

In addition to the above chemicals, Iwabuchi et al. (2015) recently isolated *Pseudomonas* sp. ITH-SA-1 from seawater, which produced water soluble fluorescent substances without aromatic rings from the lignin-derived aromatic, syringaldehyde (SYAL). All these metabolic conversions provide a road-map for the further research on lignin biotransformation, and suggests that more value-added products will one day be produced from lignin.

Combinational technologies for biological valorization of lignin with non-radical lignolytic enzymes

Biological valorization of lignin 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 mutlidisciplinary approach relying on biology, chemistry and physics will be needed to develop practical process for lignin biological valorization.

The "funneling pathway" in microorganism represents an excellent approach to integrating the different aromatic substances obtained through lignin depolymerization into some central intermediates for high-value chemical generation. Thus, it is necessary to generate as many different lignin monomers and oligomers as possible using suitable pretreatment methods. Unfortunately, the degradation of lignin using radical lignolytic enzymes requires weeks (Chi et al. 2007; Huang et al. 2010) and, thus, is unsuitable for the industrial applications. Chemical depolymerization usually results in the ring-opening or can cause the competing repolymerizaiton of lignin depolymerized products, thus, it is inefficient for lignin monomer production. Fortunately, the formic acid and formaldehyde processes, developed to produce lignin monomer in high yield, have recently become available. In Rahimi's et al. (2014) report, treatment of aspen under the aerobic conditions, the formic acid/sodium formate at 110 °C depolymerizes 62.1% wt of oxidized lignin into soluble lignin products and more than 52% of the original lignin is converted to G-/S-/H-type aromatic compounds. Subsequently, Shuai et al. (2016) reports that adding formaldehyde during biomass pre-treatment produced a soluble lignin fraction that could be converted to guaiacyl and syringyl monomers after hydrogenolysis in near theoretical yields (47 mol% of Klason lignin for beech and 78 mol% for a high-syringyl transgenic poplar). These pretreatments or depolymerization technologies may afford promising processes for lignin valorization through the combination of chemical depolymerization and bio-transformation.

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. Linger et al. (2014) and Vardon's et al. (2016) work give successful examples for the application of combination technologies in lignin biovalorization, in which *mcl*-PHA and *cis,cis*-muconic acid from lignin bioconverion 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 (Mycroft et al. 2015) using in vivo metabolic conversion have also involved in such combinational technologies (Fig. 6). Moreover, the biotransformation of cellulose sugar could provide many valuable suggestions of approaches for lignin biological volarization, such as one pot conversion or platform molecules (Schwartz et al. 2014; Huo et al. 2016).

Conclusion

The insolubility and structural heterogeneity inherent to lignin and the complexity of its depolymerization provides a major challenge for lignin valorization. Recently, the rapid progress of non-radical lignolytic enzymes, such as the funneling pathway and enzymatic/metabolic conversion, suggest a new approach for lignin bio-conversion into high-value-added chemicals. These new approaches

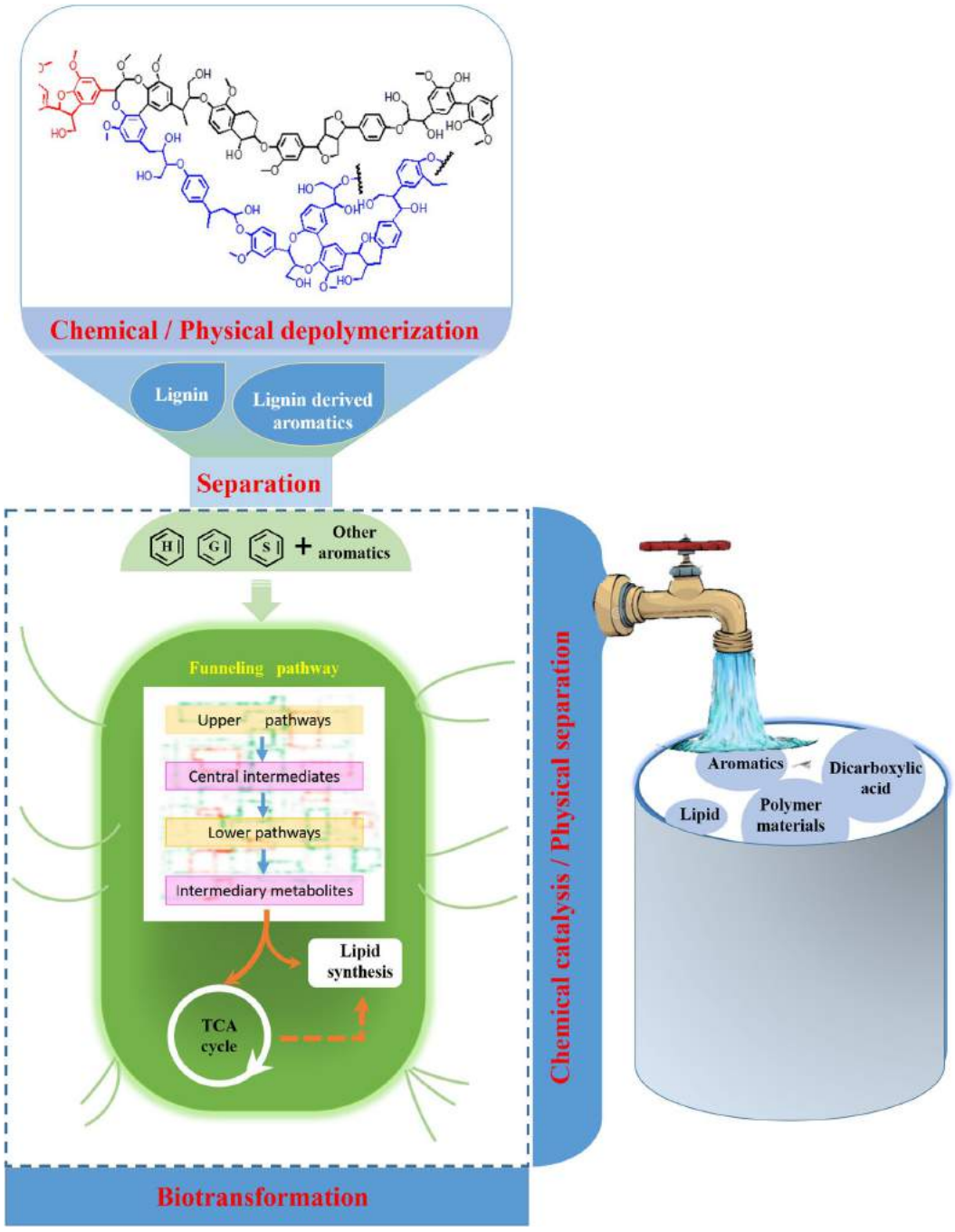

Fig. 6 Lignin valorization with a combination of technologies

promise the generation of more varieties of lignin-derived high-value chemicals in the near future. However, enzymatic conversion 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 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 many different 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.

Acknowledgements We thank the National Key Research Programme (2016YFD0400601), State Key Laboratory of Pulp and Paper Engineering (201760), Natural Science Foundation of China (NSFC 21576153), Beijing Natural Science Foundation (5162019), National High-tech Research and Development Program (2015AA021001) and Chinese 111 Project (B13005) for the generous financial support.

References

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

Asano Y, Yamamoto Y, Yamada H (1994) Catechol 2,3-dioxygenase-catalyzed synthesis of picolinic acids from catechols. Biosci Biotechnol Biochem 58:2054–2056

Bains J, Boulanger MJ (2007) Biochemical and structural characterization of the paralogous benzoate CoA ligases from *Burkholderia xenovorans* LB400: Defining the entry point into the novel benzoate oxidation (box) pathway. J Mol Biol 373:965–977

Barghini P, Di Gioia D, Fava F, Ruzzi M (2007) Vanillin production using metabolically engineered *Escherichia coli* under non-growing conditions. Microb Cell Factories 6:13. doi:10.1186/1475-2859-6-13

Bugg TD, Ahmad M, Hardiman EM, Rahmanpour R (2011a) Pathways for degradation of lignin in bacteria and fungi. Nat Product Rep 28:1883–1896

Bugg TD, Ahmad M, Hardiman EM, Singh R (2011b) The emerging role for bacteria in lignin degradation and bio-product formation. Curr Opin Biotechnol 22:394–400

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 Biodeterior Biodegrad 59(1):32–39

Davis KM, Rover M, Brown RC, Bai XL, Wen ZY, Jarboe LR (2016) Recovery and utilization of lignin monomers as part of the biorefinery approach. Energies 9:808. doi:10.3390/en9100808

Falconnier B, Lapierre C, Lesage-Meessen L, Yonnet G, Brunerie P, Ceccaldi BC, 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

Fuchs G, Boll M, Heider J (2011) Microbial degradation of aromatic compounds—from one strategy to four. Nat Rev Microbiol 9:803–816. doi:10.1038/nrmicro2652

Gall DL, Ralph J, Donohue TJ, Noguera DR (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

Gallage NJ, Hansen EH, Kannangara R, Olsen CE, Motawia MS, Jørgensen K, Holme I, Hebelstrup K, Grisoni M, Møller BL (2014) Vanillin formation from ferulic acid in *Vanilla planifolia* is catalyzed by a single enzyme. Nat Commun 5:4037

Gosling A, Fowler SJ, O'Shea MS, 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

Gupta JK, Hamp SG, Buswell JA, Eriksson KE (1981) Metabolism of trans-ferulic acid by white-rot fungus *Sporotrichum pulverulentum*. Arch Microbiol 128:349–354

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 5:13435. doi:10.1038/srep13435

Harwood CS, Parales RE (1996) The β-ketoadipate pathway and the biology of self-identity. Annu Rev Microbiol 50(1):553–590. doi:10.1146/annurev.micro.50.1.553

Helmich KE, Pereira JH, Gall DL, Heins RA, McAndrew RP, Bingman C, Deng K, Holland KC, Noguera DR, Simmons BA, Sale KennethL, Ralph J, Donohue TJ, Adams PD, Phillips GN Jr (2016) Structural basis of stereospecificity in the bacterial enzymatic cleavage of β-aryl ether bonds in lignin. J Biol Chem 291:5234–5246. doi:10.1074/jbc.M115.694307

Huang DL, Zeng GM, Feng CL, Hu S, Lai C, Zhao MH, Su FF, Tang L, Liu HL (2010) Changes of microbial population structure related to lignin degradation during lignocellulosic waste composting. Bioresour Technol 101(11):4062–4067

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

Iwabuchi N, Takiguchi H, Hamaguchi T, Takihara H, Sunairi M, Matsufuji H (2015) Transformation of lignin-derived aromatics into nonaromatic polymeric substances with fluorescent activities (NAPSFA) by *Pseudomonas* sp. ITH-SA-1. ACS Sustain Chem Eng 3:2678–2685

Jiménez JI, Miñambres B, García JL, Díaz E (2002) Genomic analysis of the aromatic catabolic pathways from *Pseudomonas putida* KT2440. Environ Microbiol 4:824–841

Johnson CW, Beckham GT (2015) Aromatic catabolic pathway selection for optimal production of pyruvate and lactate from lignin. Metab Eng 28:240–247. doi:10.1016/j.ymben.2015.01.005

Johnson CW, Salvachúa D, Khanna P, Smith H, Peterson DJ, Beckham GT (2016) Enhancing muconic acid production from glucose and lignin-derived aromatic compounds via increased protocatechuate decarboxylase activity. Metab Eng Commun 3:111–119

Jung DH, Kim EJ, Jung E, Kazlauskas RJ, Choi KY, Kim BG (2016) Production of *p*-hydroxybenzoic acid from *p*-coumaric acid by *Burkholderia glumae* BGR1. Biotechnol Bioeng 113(7):1493–1503

Jurkovfid M, Wurst M (1993) Biodegradation of aromatic carboxylic acids by *Pseudomonas mira*. FEMS Microbiol Lett 111:245–250

Kadakol C, Kamanavalli CM (2010) Biodegradation of eugenol by *Bacillus cereus* strain PN24. J Chem 7:474–480

Karmakar B, Vohra RM, Nandanwar H, Sharma P, Gupta KG, Sobti RC (2000) Rapid degradation of ferulic acid via 4-vinylguaiacol and vanillin by a newly isolated strain of *Bacillus coagulans*. J Biotechnol 80:195–202

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

Kasai D, Kamimura N, Tani K, Umeda S, Abe T, Fukuda M, Masai E (2012) Characterization of FerC, a MarR-type transcriptional regulator, involved in transcriptional regulation of the ferulate catabolic operon in *Sphingobium* sp. strain SYK-6. FEMS Microbiol Lett 332:68–75

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

Kosa M, Ragauskas AJ (2012) Bioconversion of lignin model compounds with oleaginous *Rhodococci*. Appl Microbiol Biotechnol 93:891–900

Kosa M, Ragauskas AJ (2013) Lignin to lipid bioconversion by oleaginous *Rhodococci*. Green Chem 15:2070–2074

Labuda IM, Goers SK, Keon KA (1992) Bioconversion process for the production of vanillin. Patent application US 5128253

Lee EG, Yoon SH, Das A, Lee SH, Li C, Kim JY, Choi MS, Oh DK, Kim SW (2009) Directing vanillin production from ferulic acid by increased acetyl-CoA consumption in recombinant *Escherichia coli*. Biotechnol Bioeng 102:200–208

Linger JG, Vardon, DR, Guarnieri MT, Karp EM, Hunsinger GB, Franden MA, Johnson CW, Chupka G, Strathmann TJ, Pienkos PT, Beckham GT (2014). Lignin valorization through integrated biological funneling and chemical catalysis. PNAS 111:12013–12018.doi:10.1073/pnas.1410657111

Lupa B, Lyon D, Shaw LN, Sieprawska-Lupa M, Wiegel J (2008) Properties of the reversible nonoxidative vanillate/4-hydroxybenzoate decarboxylase from *Bacillus subtilis*. Can J Microbiol 54:75–81

Masai E, Kubota S, Katayama Y, Kawai S, Yamasaki M, Morohoshi N (1993) Characterization of the Cα-dehydrogenase gene involved in the cleavage of *β*-aryl ether by *Pseudomonas paucimohilis*. Biosci Biotechnol Biochem 57:1655–1659

Masai E, Yamamoto Y, Inoue T, Takamura K, Hara H, Kasai D, Katayama Y, Fukuda M (2007a) Characterization of ligV essential for catabolism of vanillin by *Sphingomonas paucimobilis* SYK-6. Biosci Biotechnol Biochem 71:2487–2892. doi:10.1271/bbb.70267

Masai E, Katayama Y, Fukuda M (2007b) Genetic and biochemical investigations on bacterial catabolic pathwaysfor lignin-derived aromatic compounds. Biosci Biotechnol Biochem 71:1–15

Meijnen JP, Verhoef S, Briedjlal AA, de Winde JH, Ruijssenaars HJ (2011) Improved *p*-hydroxybenzoic acid production by engineered *Pseudomonas putida* S12 by using a mixed-substrate feeding strategy. Appl Microbiol Biotechnol 90:885–893

Meux E, Prosper P, Masai E, Mulliert G, Dumarcay S, Morel M, Didierjean C, Gelhaye E, Favier F (2012) *Sphingobium* sp. SYK-6 LigG involved in lignin degradation is structurally and biochemically related to the glutathione transferase omega class. FEBS Lett 586:3944–3950. doi:10.1016/j.febslet.2012.09.036

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

Mycroft Z, Gomis M, Mines P, Law P, Bugg TD (2015) Biocatalytic conversion of lignin to aromatic dicarboxylic acids in *Rhodococcus jostii* RHA1 by re-routing aromatic degradation pathways. Green Chem 17:4974–4979

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

Ohta Y, Nishi S, Hasegawa R, Hatada Y (2015) Combination of six enzymes of a marine *Novosphingobium* converts the stereoisomers of beta-O-4 lignin model dimers into the respective monomers. Sci Rep 5:15105. doi:10.1038/srep15105

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

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

Pan C, Oda Y, Lankford PK, Zhang B, Samatova FN, Pelletier AD, Harwood SC, Hettich LR (2008) Characterization of anaerobic catabolism of *p*-coumarate in *Rhodopseudomonas palustris* by integrating transcriptomics and quantitative proteomics. Mol Cell Proteom 7:938–948

Peng X, Misawa N, Harayama S (2003) Isolation and characterization of *Thermophilic Bacilli* degrading cinnamic, 4-coumaric, and ferulic acids. Appl Environ Microbiol 69:1417–1427

Pereira JH, Heins RA, Gall DL, Mcandrew RP, Deng K, Holland KC, Donohue TJ, Noguera DR, Simmons BA, Sale KL, Ralph J, Adams PD (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. doi:10.1074/jbc.M115.700427

Pérez -Pantoja D, Donoso R, Agulló L, Córdova M, Seeger M, Pieper DH, González B (2012) Genomic analysis of the potential for aromatic compounds biodegradation in *Burkholderiales*. Environ Microbiol 14:1091–1117

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

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. doi:10.3389/fmicb.2015.00916

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

Pollegioni L, Tonin F, Rosini E (2015) Lignin-degrading enzymes. FEBS J 282:1190–1213. doi:10.1111/febs.13224

Priefert H, Rabenhorst J, Steinbüchel A (2001) Biotechnological production of vanillin. Appl Microbiol Biotechnol 56:296–314

Rahimi A, Ulbrich A, Coon JJ, Stahl SS (2014) Formic-acid-induced depolymerization of oxidized lignin to aromatics. Nature 515:249–252. doi:10.1038/nature13867

Reiter J, Strittmatter H, Wiemann LO, 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

Rosazza JP, 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

Sainsbury PD, Hardiman EM, Ahmad M, Otani H, Seghezzi N, Eltis LD, Bugg TDH (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

Sato Y, Moriuchi H, Hishiyama S, Otsuka Y, Oshima K, Kasai D, Nakamura M, Ohara S, Katayama Y, Fukuda M, Masai E (2009) Identification of three alcohol dehydrogenase genes involved in the stereospecific catabolism of arylglycerol-β-aryl ether by *sphingobium* sp. strain SYK-6. Appl Environ Microbiol 75(16):5195–5201

Schwartz TJ, O'Neill BJ, Shanks BH, Dumesic JA (2014) Bridging the chemical and biological catalysis gap: challenges and outlooks for producing sustainable chemicals. ACS Catal 4:2060–2069

Shuai L, Amiri MT, Questell-Santiago YM, Héroguel F, Li Y, Kim H, Meilan R, Chapple C, Ralph J, Luterbacher JS (2016) Formaldehyde stabilization facilitates lignin monomer production during biomass depolymerization. Science 354:329–333. doi:10.1126/science.aaf7810

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

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

Trautwein K, Wilkes H, Rabus R (2012) Proteogenomic evidence for *β*-oxidation of plant-derived 3-phenylpropanoids in "*Aromatoleum aromaticum*" EbN1. Proteomics 12:1402–1413

van Duuren JB, Wijte D, Karge B, dos Santos VA, Yang Y, Mars AE, 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

Vardon DR, Franden MA, Johnson CW, Karp EM, Guarnieri MT, Linger JG, Salm MJ, Strathmann TJ, Beckham GT (2015) Adipic acid production from lignin. Energy Environ Sci 8:617–628. doi:10.1039/c4ee03230f

Vardon DR, Rorrer NA, Salvachúa D, Settle AE, Johnson CW, Menart MJ, Cleveland NS, Ciesielski PN, Steirer KX, Dorgan JR, Beckham GT (2016) *cis, cis*-Muconic acid: separation and catalysis to bio-adipic acid for nylon-6, 6 polymerization. Green Chem 18:3397–3413

Wang C, Ouyang XH, Su SS, Liang X, Zhang C, Wang WY, Yuan QP, Li Q (2016) Effect of sulfonated lignin on enzymatic activity of the lignolytic enzymes C_{α}-dehydrogenase LigD and *β*-etherase LigF. Enzyme Microb Technol 93:59–69

Wong DWS (2009) Structure and action mechanism of ligninolytic enzymes. Appl Biochem Biotech 157:174–209. doi:10.1007/s12010-008-8279-z

Xie T, Liu ZC, Liu Q, Wang GG (2015) Structural insight into the oxidation of sinapic acid by CotA laccase. J Struc Biol 190:155–161. doi:10.1016/j.jsb.2015.03.005

Xie XG, Huang CY, Fu WQ, Dai CC (2016) Potential of endophytic fungus *Phomopsis liquidambari* for transformation and degradation of recalcitrant pollutant sinapic acid. Fungal Biol 120:402–413

Yamada M, Okada Y, Yoshida T, Nagasawa T (2007) Biotransformation of isoeugenol to vanillin by *Pseudomonas putida* IE27 cells. Appl Microbiol Biotechnol 73:1025–1030

Zhao C, Xie S, Pu Y, Zhang R, Huang F, Ragauskas AJ, Yuan JS (2015) Synergistic enzymatic and microbial lignin conversion. Green Chem 18:1306–1312