Supporting Information

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SI Materials and Methods

Construction of Plasmids. The genes (*CouP*, *ligV*, *ligM*, and *aroY*) used in this study are listed in Table 1. The gene of CouP was amplified from the genomic DNA of *R. palustris* (CGA009; ATCC) using primers 1 and 2. The amplicon was digested with *Dpn*I (NEB) and purified using QIAquick PCR purification kit (Qiagen). The DNA fragments of LigV, LigVM, and aroY with optimized ribosome binding sites and two DNA fragments of vector backbone were amplified from the vector pBbE1a-LigVM-aroY from a previous study (1) using primers 3–8. The amplicons of LigVM and aroY fragments of vector backbone were digested with *Dpn*I (NEB) and gel-purified using QIAquick Gel Extraction Kit (Qiagen). The purified DNA fragments of LigVM, CouP, and aroY were assembled into the plasmid pBbE1a using Gibson assembly kit (NEB) following the manufacturer's protocol to construct the plasmid pBbE1a-CouP-LigVM-aroY (Fig. S1). The fragment of ligV with cutting-site *EcoR*I and *Bgl*II was subcloned into plasmid pBbE1a to obtain pBbE1a-LigV (Fig. S1). The strain *S. cerevisiae* BY4742 was obtained from Joint BioEnergy Institute (accession no. JBx_042441). The genomic DNA of the strain was extracted using YeaStar Genomic DNA Kit (Zymo Research) following the manufacturer's protocol. The DNA sequence of promoter adh7 was amplified from the extracted genomic DNA of *S. cerevisiae* BY4742 using primers 11 and 12 and cleaned up as described above. The purified fragment was used to replace the pTrc promoter in the plasmid pBbE1a. To construct pBbE1a-adh7-LigVM-aroY (Fig. S1) the backbone of pBbE1a was amplified with primers 9 and 10 and the gel-purified PCR product was assembled with the adh7 PCR fragment and *EcoR*I- and *Xho*I-digested pBbE1a-LigVM-aroY using Gibson Assembly Kit (NEB). To construct pBbE1a-adh7-LigV (Fig. S1) the adh7-LigV fragment was PCR-amplified using primers 13 and 14 from pBbE1a-adh7-LigVM-aroY and assembled with *EcoR*I- and *Xho*I-digested pBbE1a-adh7-LigVM-aroY using the In-Fusion HD Cloning Kit (Clontech) following the manufacturer's instructions. The DNA fragment of CouP with restriction cutting site *EcoR*I and *Bsa*I was amplified using primers 15 and 16 and using pBbE1a-CouP-LigVM-aroY as PCR template. The amplicon was assembled with *EcoR*I- and *Bsa*I-digested pBbE1a-adh7-LigVM-aroY to achieve the vector pBbE1a-adh7-CouP-LigVM-aroY (Fig. S1) using the In-Fusion HD Cloning Kit (Clontech) following the manufacturer's protocol. The correctness of all of the gene sequences in the constructs was confirmed by DNA sequencing. All of the primers and strains used in this study are listed in Table 2 and Table S1.

Strain, Medium, and Cultivation Conditions. The *E. coli* strain DH1 was obtained from the Joint BioEnergy Institute. The plasmids pBbE1a-adh7-LigV, pBbE1a-CouP-LigVM-aroY, pBbE1a-adh7-LigVM-aroY, and pBbE1a-adh7-CouP-LigVM-aroY were transformed into strain DH1, respectively. The positive transformants of strain DH1 were cultivated in 5 mL of LB medium containing corresponding antibiotics (100 μg/mL ampicillin), overnight. One milliliter of overnight culture was transferred into 20 mL of fresh LB medium with the same antibiotics and cultured at 220 rpm at 37 °C until the OD reached 0.6. Then, the strains expressing vector pBbE1a-LigVM-aroY or pBbE1a-CouP-LigVM-aroY were induced by the addition of IPTG at 1 mM. Next, the strains expressing pBbE1a-adh7-LigV, pBbE1a-adh7-LigVM-aroY, or pBbE1a-adh7-CouP-LigVM-aroY were induced by the addition of vanillin at 0.5 g/L. The samples of the culture were taken at different time intervals for further analysis. All of the experiments were performed in duplicate. All of the strains used in this research are listed in Table 2.

To investigate the inhibitory effects of the vanillin, vanillic acid, and pyrocatechol on *E. coli* strain DH1, a single colony of DH1 was cultured in 5 mL of LB medium overnight. Then, 0.2 mL of the overnight culture was transferred into 20 mL of fresh LB medium with the addition of vanillin and pyrocatechol at concentrations of 0, 0.5, 1, 2, and 5 g/L or vanillic acid at concentrations of 0, 0.5, 0.75, 1, and 1.5 g/L, respectively. The ODs of the cultures were monitored at 600 nm at different time intervals.

Analytical Methods. The samples of the fermentation broth were centrifuged at 16,000 × *g*, 4 °C for 15 min and the supernatant was filtrated through a 0.2-μm polytetrafluoroethylene membrane before analysis. The concentrations of vanillin, vanillic acid, and catechol in the samples were analyzed by HPLC (Agilent 1100) using a Rezex ROA column (Phenomenex) at 65 °C under a UV detector (200 nm, 220 nm) for 65 min. The mobile phase was 0.005 N sulfuric acid at a flow rate of 0.6 mL/min.

1. Wu W, et al. (2017) Lignin valorization: Two hybrid biochemical routes for the conversion of polymeric lignin into value-added chemicals. *Sci Rep* 7:8420.

Fig. S1. Plasmid maps of the constructs in this study: (*A*) pBbEla-LigV, (*B*) pBbE1a-adh7-LigV, (*C*) pBbE1a-CouP-LigVM-aroY, (*D*) pBbEla-adh7-LigVM-aroY, and (*E*) pBbEla-adh7-CouP-LigVM-aroY.

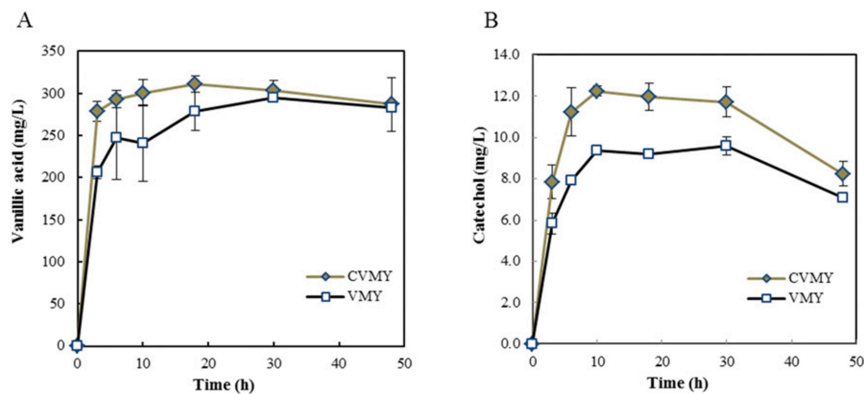

Fig. S2. Time-dependent production of vanillic acid and catechol with the expression of an aromatic transporter CouP under pTrc promoter. (*A*) Vanillic acid and (*B*) catechol production from the strains expressing the catechol biosyntheis pathway with (CVMY) and without (VMY) the aromatic transporter CouP under promoter pTrc.

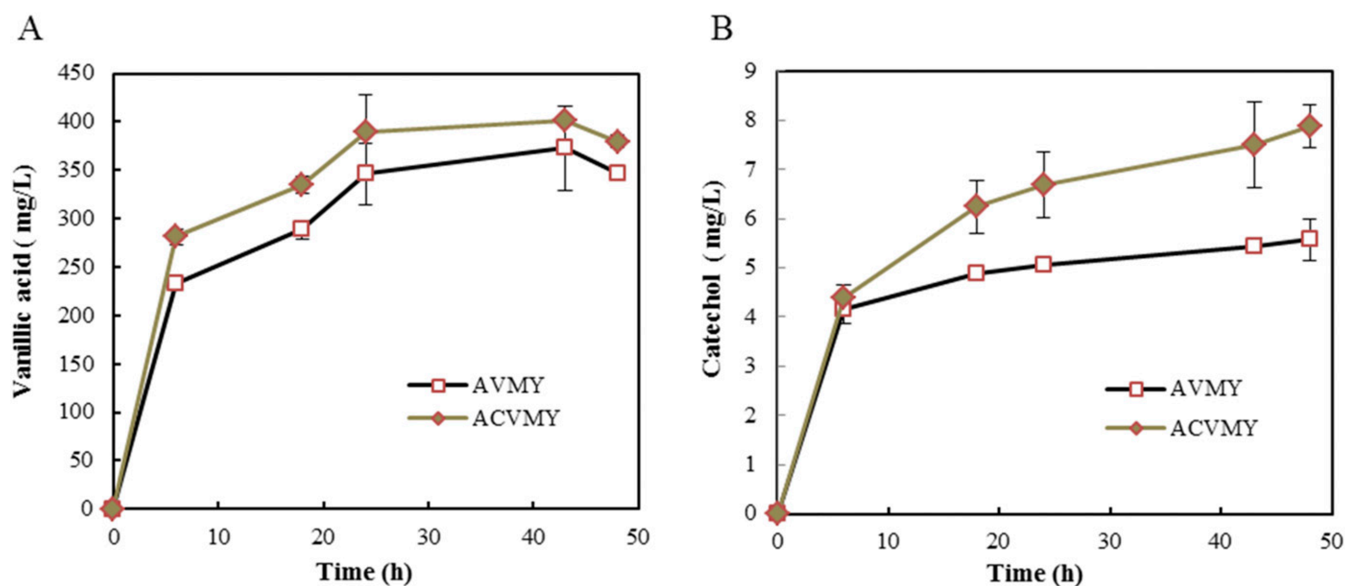

Fig. S3. Time-dependent production of vanillic acid and catechol with the expression of an aromatic transporter CouP under the ADH7 promter. (*A*) Vanillic acid and (*B*) catechol production from the strains expressing the catechol biosyntheis pathway with (ACVMY) and without (AVMY) the aromatic transporter CouP under promoter ADH7.

Table S1. Primers used for the design of constructs

No.	Name	Sequence	Description
1	pBbE1a-CouP-Gib-F	5-GTG TGG AAT TGT GAG CGG ATA ACA ATT TCA GAA TTC TGA CAG AAG TAA GGA GGT ATT CAG GTG ACC AAG TTC AAG CTA TCC-3	Amplify transporter CouP for Gibson Assembly to construct pBbE1a-CouP-LigVM-aroY
2	pBbE1a-CouP-Gib-R1	5-CGG TGT ACC TTA TTC TAT GTT GGG CTA TGG GAATTC TTA CTT CAC CAT CAC GTA TTT GC-3	Amplify transporter CouP for Gibson Assembly to construct pBbE1a-CouP-LigVM-aroY
3	LigV-pBbE1a-F	5-ATTTCAGAATTCCCATAGCCCAACATAGAATAAGGTACACCGAAATA ATG GAC AGC GCA CGT ATT GCT C-3	Amplify LigV to construct pBbE1a-LigV
4	LigV-pBbE1a-R	5-GGATCCAGATCT TTA AAT CGG AAA ATG GCCCGG TTG G-3	Amplify LigV to construct pBbE1a- LigV
5	LigV-LigM-Gib-F1	5-TAA GAATTC CCATAGCCCAACATAGAATAAGGTACACCGAAATA ATGGACAGCGCACG-3	Amplify LigV-LigM for Gibson Assembly to construct pBbE1a-CouP-LigVM-aroY
6	ligV-LigM-pBbE1a-GibR1	5-GTC CTT AAA TGA AAT GGT GGA TCC TTA CGC GGT CAC CGC CGC TTT AC-3	Amplify LigV-LigM for Gibson Assembly to construct pBbE1a-CouP-LigVM-aroY
7	AroY-pBbE1a-GibF1	5-CGGTGACCGCGTAA GGATCC ACCATTTCATTTAAGGACTACCACCGCAAC ATG ACA GCC CCT ATT CAA GAC TTA CG-3	Amplify aroY for Gibson Assembly to construct pBbE1a-CouP-LigVM-aroY
8	AroY-pBbE1a-GibR	5-TTT CGT TTT ATT TGA TGC CTG GAG ATC CTT A CTC GAG TT ATT TAG CGG AGC CTT GAT TTT TTT CCA-3	Amplify aroY for Gibson Assembly to construct pBbE1a-CouP-LigVM-aroY
9	pBbE1a-ColE1-Amp_G-F1	5-GGA TCC AAA CTC GAG TAA GGA TCT CCA GGC-3	Amplify backbone of pBbE1a for Gibson Assembly to construct pBbE1a-adh7-LigVM-aroY
10	pBbE1a-ColE1-Amp_G-R1	5-CAT TCG ATG GTG TCG ACG TCA GGT GGC ACT-3	Amplify backbone of pBbE1a for Gibson Assembly to construct pBbE1a-adh7-LigVM-aroY
11	adh7-pBbE1a -Gib-F	5-GCGAATTGATCTGGTTTGACAGCTTATCAT GAGACATGCCTTTACTAATGAAC-3	Amplify adh7 promoter for Gibson Assembly to construct pBbE1a-adh7-LigVM-aroY
12	adh7-pBbE1a-Gib-R	5-TAT TTC GGT GTA CCT TAT TCT ATG TTG GGC TAT GG GAA TTC T TTT TTT TTT GTA TTT TTC AGT GGT TCT ATG-3	Amplify adh7 promoter for Gibson Assembly to construct pBbE1a-adh7-LigVM-aroY
13	pBbE1a-adh7-LigV-F	5-CCA TTA CCG AGT CCG GGC-3	Amplify adh7 promoter for In-Fusion cloning to construct pBbE1a-adh7-LigV
14	pBbE1a-LigV-R	5-TTATTTGATGCCTGGAGATCCTTACTCGAG TTA AAT CGG AAA ATG GCC CG-3	Amplify adh7 promoter for In-Fusion cloning to construct pBbE1a-adh7-LigV
15	adh7-CouP-f	5-TAGAACCACTGAAAAATACAAAAAAAAAAGAATTC TGA CAG AAG TAA GGA GGT ATT CAG G-3	Amplify adh7 promoter for In-Fusion cloning to construct pBbE1a-adh7-CouP-LigVM-aroY
16	adh7-CouP-r	5-GGTCGCGTTCTTGATCCGGAGCAATACGTGCGCTGTCCATTATTTCGGTGTACCTTA TTCTATGTTGGGCTATGG TTA CTT CAC CAT CAC GTA TTT GCC-3	Amplify adh7 promoter for In-Fusion cloning to construct pBbE1a-adh7-CouP-LigVM-aroY