

Supporting Information Appendix for

# Mimicking the Fungal Decay Strategy for Promoting the Bacterial Production of Polyhydroxyalkanoate from Kraft Lignin

Xiao Fu <sup>†</sup>, Qing Gong <sup>†</sup>, Xuan Liu, Ze Zheng, Xiaoyu Zhang, Fuying Ma, Hongbo Yu <sup>\*</sup> and Shangxian Xie <sup>\*</sup>

Key Laboratory of Molecular Biophysics of MOE, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China; fuxiao@hust.edu.cn (X.F.)

<sup>\*</sup> Correspondence: yuhongbo@hust.edu.cn (H.Y.); shangxian\_xie@hust.edu.cn (S.X.)

<sup>†</sup> These authors contributed equally to this work.

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## SI Appendix, SI Experimental section

### *Formular of mediums*

10× M9 minimal medium: 30 g/L  $\text{KH}_2\text{PO}_4$ , 60 g/L  $\text{Na}_2\text{HPO}_4$ , 5 g/L NaCl, 0.65 g/L  $\text{NH}_4\text{Cl}$ .

Stock salt solution (1L): 22.94 g/L  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 2.0 g/L  $\text{CaCO}_3$ , 4.5 g/L  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.44 g/L  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.85 g/L  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 0.25 g/L  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.24 g/L  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.06 g/L  $\text{H}_3\text{BO}_3$ , and 51.3 mL HCl

Concentrated Goodies (100 mL): 3.009 g  $\text{MgSO}_4$ , 25 mL 1%  $\text{FeSO}_4$  (use concentrated HCl to dissolve, until clear), 50 mL stock salt solution.

100× Mg/Ca/B1/Goodies mix (300 mL): 159 mL ddH<sub>2</sub>O, 60 mL 1M  $\text{MgSO}_4$ , 3 mL 1M  $\text{CaCl}_2$ , 3 mL 10 mM thiamine, 75 mL concentrated Goodies.

### *Detection of glucose*

The medium after fermentation was collected and diluted with DI water. The glucose was determined by the DNS method. Briefly, we added 1 mL of DNS reagent to 1 mL of diluted medium, and heated the mixture at 100 °C for 10 minutes. Then, we immediately put the mixture into ice water to stop the reaction. The absorbance was measured at 540 nm under a UV-Vis spectrophotometer (Shimadzu Corporation, Tokyo, Japan). A glucose solution (0.1-1.0 mg/mL) was used to obtain the calibration curve.

### *Detection of mono-aromatic compounds*

The mono-aromatic compounds (*p*-coumaric acid, ferulic acid, *p*-hydroxybenzoic acid, vanillic acid) were detected by the Folin phenol method. A volume of 0.5 mL of diluted medium was added to 2.5 mL Folin phenol reagent solution (0.2 M) and 2 mL of 7.5% p/v sodium carbonate (Sinopharm Chemical Reagent Co. Ltd., Shanghai, China). The mixture was vortexed and reacted in the dark for 30 minutes at 25 °C. The absorbance was measured at 765 nm under a UV-Vis spectrophotometer. To obtain the phenolic compounds' concentration data, a calibration curve was constructed using different concentrations of gallic acid (10-70 mg/L).

### *Detection of short-chain organic products*

The succinic acid and lactic acid were analyzed with HPLC (LC-10AT, Shimadzu, Japan) with a HyperSep C18 column (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA). The injection volume was 10 µL, and the samples were detected by a SPD-10A detector. For succinic acid, the column was maintained at 35 °C at a flow rate of 1.0 mL/min. The mobile phase was made of eluent A (acetonitrile): eluent B (0.1% phosphoric acid in ultrapure water) at 50:50 with an isocratic elution program, and the sample was detected at 205 nm. For lactic acid, the column was maintained at 40 °C and the sample flow rate mentioned above; the mobile phase was made of eluent A (methanol): eluent B (0.05% phosphoric acid in ultrapure water) at 10:90 with an isocratic elution program. The sample was detected at 210 nm. The glycerol was detected by using an E1002 Glycerol test kit (Applygen Technologies Inc., Beijing, China).

## SI Appendix, Tables

**Table S1.** The concentration of reagent under different reaction conditions.

Kraft lignin	Fe <sup>2+</sup> (mM)	H <sub>2</sub> O <sub>2</sub> (mM)
5%	1	2
	2	
	4	
0	1	4
	2	
	4	
5%	1	4
	2	
	4	
0	1	4
	2	
	4	

**Table S2.** Relative abundance of the aromatic-compounds-derived peaks identified in the Py-GC/MS of Kraft lignin and Fenton-like-reaction-treated lignin.

Peak No. <sup>a</sup>	Retention time (min)	Compounds	Sidechain length <sup>b</sup>	Kraft lignin	Treated lignin
1 <sup>H</sup>	9.13	Phenol	0	2.27	9.03
2 <sup>H</sup>	10.93	2-Methylphenol	C <sub>α</sub>	1.60	7.43
3 <sup>H</sup>	11.50	4-Methylphenol	C <sub>α</sub>	1.24	6.87
4 <sup>G</sup>	11.77	Guaiacol	0	28.32	37.72
5 <sup>H</sup>	12.35	2,6-Dimethylphenol	C <sub>α</sub>	0.62	1.51
6 <sup>H</sup>	13.05	2-Ethylphenol	C <sub>β</sub>	0.00	2.45
7 <sup>G</sup>	13.22	1,2-Dimethoxybenzene	0	1.85	1.41
8 <sup>H</sup>	13.34	3,5-Dimethylphenol	C <sub>α</sub>	0.46	5.27
9 <sup>H</sup>	13.38	2,4-Dimethylphenol	C <sub>α</sub>	0.31	2.73
10 <sup>H</sup>	13.59	(1-Methyl-2-cyclopropen-1-yl)-benzene	C <sub>β</sub>	0.00	1.22
11 <sup>H</sup>	13.78	4-Ethylphenol	C <sub>α</sub>	0.00	1.41
12 <sup>G</sup>	14.04	2-Methoxy-5-methylphenol	C <sub>α</sub>	4.63	0.00
13 <sup>G</sup>	14.35	2-Methoxy-4-methylphenol,	C <sub>α</sub>	5.20	5.93
14 <sup>H</sup>	14.44	3,4-Dimethylphenol	C <sub>α</sub>	0.00	1.03
15 <sup>Mis.</sup>	14.48	1,2-Benzenediol	0	0.51	0.00
16 <sup>H</sup>	14.75	2,4,6-Trimethylphenol	C <sub>α</sub>	0.00	2.07
17 <sup>H</sup>	15.24	3-(1-Methylethyl)-phenol	C <sub>β</sub>	0.00	1.13
18 <sup>G</sup>	15.46	3,4-Dimethoxytoluene	C <sub>α</sub>	1.13	0.00
19 <sup>H</sup>	16.14	1-Ethenyl-4-methylbenzene	C <sub>β</sub>	0.00	0.94
20 <sup>G</sup>	16.37	4-Ethyl-2-methoxyphenol	C <sub>β</sub>	4.58	6.11
21 <sup>H</sup>	17.12	1,4-Bis(1-methylethenyl)-benzene		0.00	0.47
22 <sup>G</sup>	17.22	2-Methoxy-4-vinylphenol	C <sub>β</sub>	7.11	2.92
23 <sup>G</sup>	17.4	4-Ethyl-1,2-dimethoxybenzene	C <sub>β</sub>	0.72	0.00
24 <sup>G</sup>	18.17	2-Methoxy-3-(2-propenyl)-phenol	C <sub>γ</sub>	1.18	0.00

25 <sup>G</sup>	18.16	3-Allyl-6-methoxyphenol	C <sub>γ</sub>	0.00	1.03
26 <sup>G</sup>	19.13	Vanillin	C <sub>α</sub>	10.25	0.00
27 <sup>G</sup>	20.23	2-Methoxy-4-(1-propenyl)-phenol	C <sub>γ</sub>	4.17	1.32
28 <sup>G</sup>	20.85	3,4-Dimethoxybenzaldehyde	C <sub>α</sub>	1.75	0.00
29 <sup>G</sup>	20.96	1-(4-hydroxy-3-methoxyphenyl)-ethanone	C <sub>β</sub>	7.05	0.00
30 <sup>G</sup>	22.54	1-(3,4-Dimethoxyphenyl)-ethanone	C <sub>β</sub>	1.34	0.00
31 <sup>G</sup>	25.79	4-Hydroxy-2-methoxycinnamaldehyde	C <sub>γ</sub>	0.93	0.00
32 <sup>Mis.</sup>	28.00	Phthalic acid diisobutyl ester	0	4.79	0.00
33 <sup>Mis.</sup>	29.58	Dibutyl phthalate	0	5.56	0.00
34 <sup>Mis.</sup>	37.89	Diisooctyl phthalate	0	2.42	0.00

The compositional data were calculated based on H+G+Mis.=100%. <sup>a</sup>H: H-type lignin units, G: G-type lignin units, Mis: miscellaneous, <sup>b</sup>lignin-derived phenols with 0, 1, 2, and 3 carbons in the side chain.

**Table S3.** The assignments of <sup>1</sup>H-<sup>13</sup>C peaks in HSQC spectra.

Label	δC/δH (ppm)	Assignments
b <sub>α</sub>	86.8/5.43	C <sub>α</sub> -H <sub>α</sub> in phenylcoumaran substructures (PB)
c <sub>α</sub>	84.8/4.65	C <sub>α</sub> -H <sub>α</sub> in β-β' resinol substructures (c)
aβ(G)	83.4/4.27	C <sub>β</sub> -H <sub>β</sub> in β-O-4' substructures (a) linked to a G unit
aβ(H)	82.9/4.48	C <sub>β</sub> -H <sub>β</sub> in β-O-4' substructures (a) linked to a H-unit
aα(G)	70.9/4.71	C <sub>α</sub> -H <sub>α</sub> in β-O-4' substructures (a) linked to a G-unit
c <sub>γ</sub> and c <sub>γ</sub>	71.0/4.17 and 3.81	C <sub>γ</sub> -H <sub>γ</sub> in β-β' resinol substructures (c)
d <sub>γ</sub>	62.09/4.21	C <sub>γ</sub> -H <sub>γ</sub> in cinnamyl alcohol end-groups (d)
-OMe	55.6/3.73	C-H in methoxyls
c <sub>β</sub>	53.5/3.05	C <sub>β</sub> -H <sub>β</sub> in β-β' resinol substructures (c)
b <sub>β</sub>	53.1/3.43	C <sub>β</sub> -H <sub>β</sub> in phenylcoumaran substructures (b)

**Table S4.** GC/MS analysis of soluble compounds in liquid phase of black liquor before and after the *P. putida* KT2440 fermentation.

Peak No. <sup>a</sup>	Retention time (min)	Compounds	Peak area (%)	
			Soluble compounds	After fermentation <sup>b</sup>
1 <sup>Oc</sup>	9.93	Lactic acid	34.27	36.74
2 <sup>Oc</sup>	10.18	Glycolic acid	12.31	14.85
3 <sup>Oc</sup>	11.12	2-Hydroxybutanoic acid	18.72	16.54
4 <sup>Oc</sup>	11.18	2-Hydroxy-2-methylbutanoic acid	1.03	1.10
5 <sup>Oc</sup>	12.28	2-Hydroxypentanoic acid	0.73	0.98
6 <sup>Oc</sup>	12.40	Propanedioic acid	0.16	0.39
7 <sup>G</sup>	12.67	Guaiacol	1.17	1.20
8 <sup>Oc</sup>	12.83	4-Hydroxybutanoic acid	1.21	1.24
9 <sup>Oc</sup>	13.04	Propanedioic acid	0.11	ND
10 <sup>Oc</sup>	13.22	3-Methyl-4-hydroxybutanoic acid	0.17	0.14
11 <sup>Al</sup>	13.46	Glycerol	0.03	ND
12 <sup>Oc</sup>	13.96	Succinic acid	3.08	0.22

13 <sup>Oc</sup>	14.13	Methylsuccinic acid	1.88	3.75
14 <sup>Oc</sup>	14.27	Glyceric acid	0.31	0.45
15 <sup>Oc</sup>	14.42	Fumaric acid	0.45	ND
16 <sup>P</sup>	15.17	2,6-Dimethoxyphenol	0.89	0.39
17 <sup>Oc</sup>	15.37	2,4-Dihydroxybutanoic acid	0.76	0.40
18 <sup>Oc</sup>	15.62	3,4-Dihydroxybutanoic acid	0.62	0.79
19 <sup>Oc</sup>	16.34	Malic acid	1.48	0.70
20 <sup>G</sup>	16.96/17.08	Vanillin	1.63	0.00
21 <sup>Oc</sup>	17.38	2-Hydroxypentanedioic acid	2.66	3.42
22 <sup>Oc</sup>	17.75	2-Hydroxy-2-pentenedioic acid	0.44	0.17
23 <sup>H</sup>	17.92	<i>p</i> -Hydroxybenzoic acid	0.48	ND
24 <sup>G</sup>	17.96/17.99	Acetovanillone	1.14	0.92
25 <sup>Oc</sup>	18.49	2-Hydroxyhexanedioic acid	1.76	1.96
26 <sup>Oc</sup>	18.83	3-Deoxy-2-hydroxymethyl-D-erythro-Pentonic acid-1,4-lactone	0.51	1.49
27 <sup>G</sup>	19.45	Vanillic acid	1.68	1.20
28 <sup>G</sup>	19.56	Homovanillic acid	0.50	0.51
29 <sup>Oc</sup>	19.87	3-Hydroxy-3-methylpentanedioic acid	0.17	0.26
30 <sup>G</sup>	20.03	3-Guaiacyl-1-propanol	2.04	2.07
31 <sup>P</sup>	20.17	Homoprotocatechuic acid	0.20	0.12
32 <sup>P</sup>	21.49	Gallic acid	0.08	0.00
33 <sup>G</sup>	21.57	3-Guaiacyl-1,2-propanediol	0.16	0.17
34 <sup>G</sup>	22.1	Vanillylactic acid	3.86	4.65
35 <sup>P</sup>	22.53	Salvianic acid	0.19	0.00
36 <sup>P</sup>	23.22/23.27	3,4-Dimethoxymandelic acid	1.55	1.82
37 <sup>Hc</sup>	26.41	Tetracosanoic acid	0.68	0.72
38 <sup>Hc</sup>	28.25	13-Docosenamide	0.93	0.70

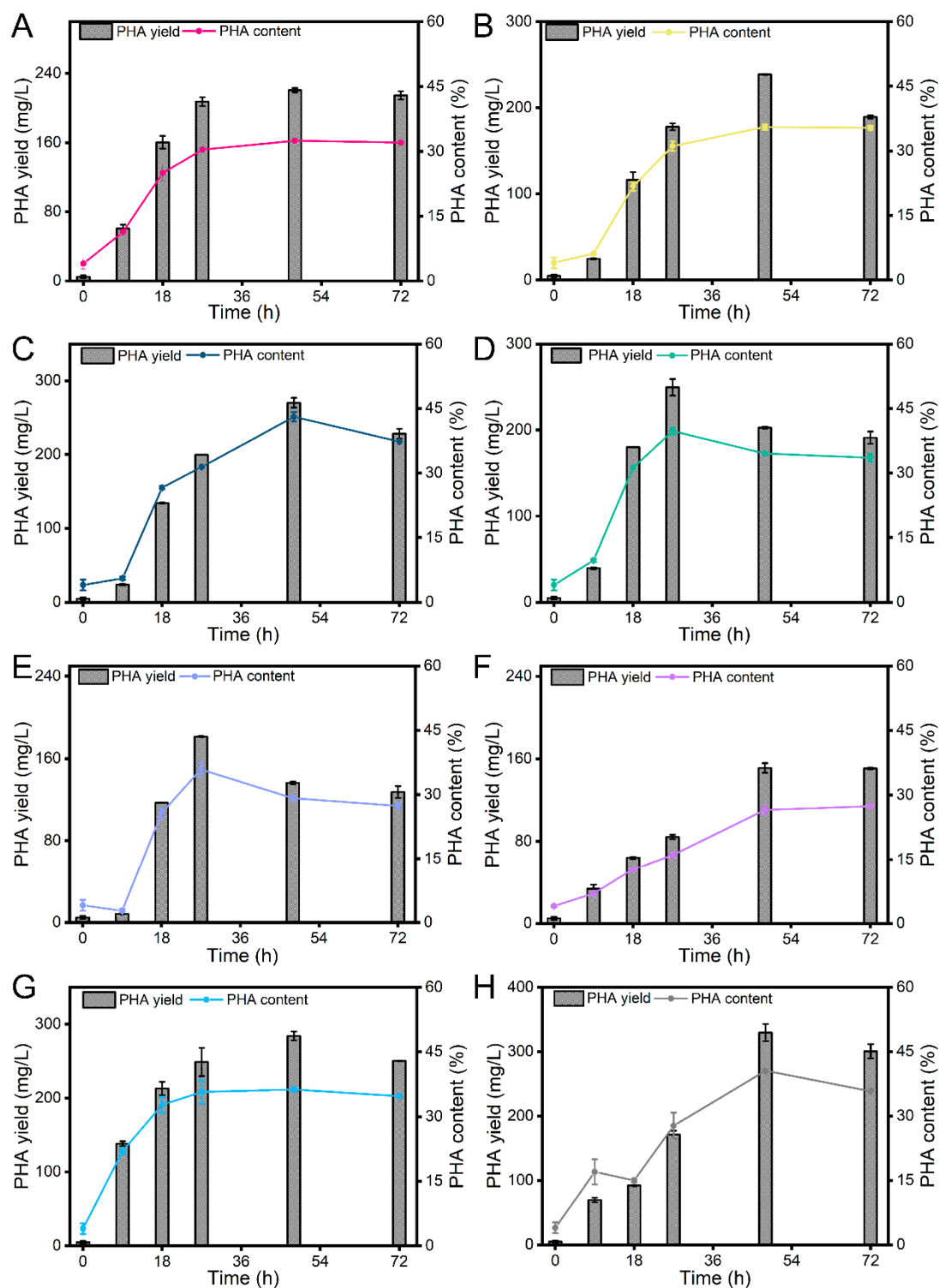
The compositional data were calculated based on Al+Oc+P+G+H+Hc=100%. <sup>a</sup>Al: alcohols, Oc: organic acids, P: phenols, G: sinapylic alcohol, H: *p*-coumarylic alcohol, Hc: hydrocarbons and their derivatives, <sup>b</sup>ND: not detected.

**Table S5.** Structural characterization in HSQC spectra and methoxy content of soluble lignin in the black liquor.

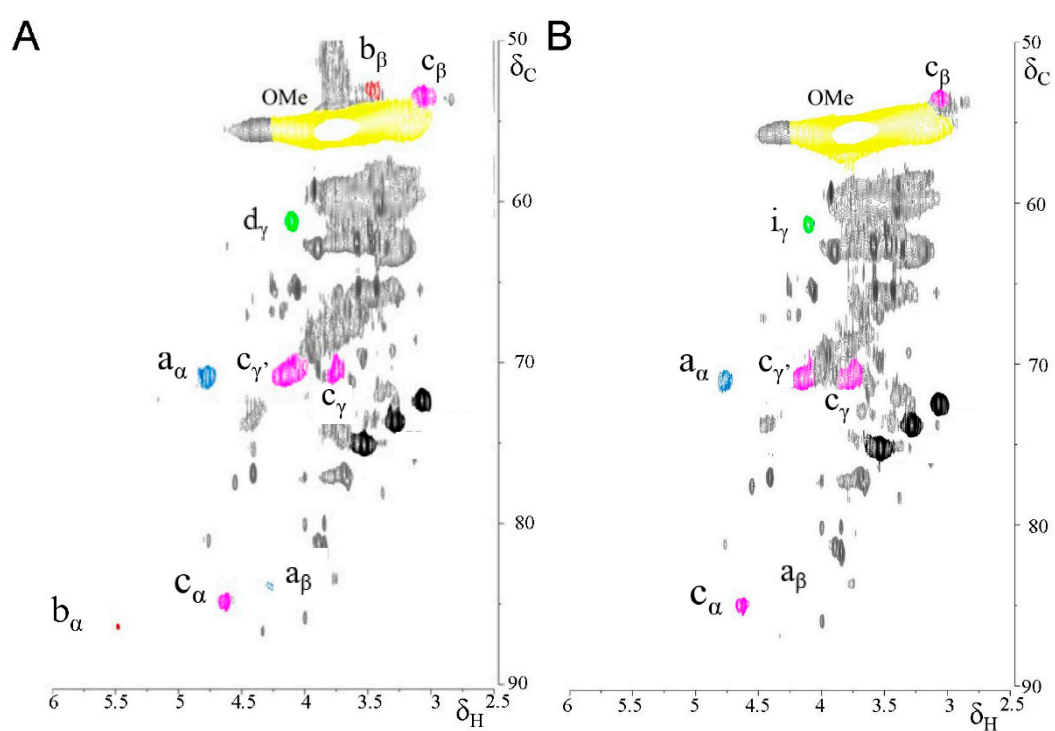
	Soluble lignin	After fermentation
Lignin inter-unit linkages <sup>a</sup>		
β-O-4'	0.30	0.22
β-5'	0.01	ND
β-β'	0.21	0.18
Lignin end-group <sup>b</sup>	0.29	0.15
Condensation degree <sup>c</sup>	0.68	0.80
OMe (%)	10.30	9.89

<sup>a</sup>Linkages are relative to the methoxy. <sup>b</sup>Cinnamyl alcohol. <sup>c</sup>Ratio of (β-β')/β-O-4'.

## SI Appendix, Figures



**Figure S1.** PHA yield and PHA content of glucose and lignin-depolymerized products: (A) glucose, (B) *p*-coumaric acid, (C) ferulic acid, (D) *p*-Hydroxybenzoic acid, (E) vanillic acid, (F) succinic acid, (G) lactic acid, and (H) glycerol.



**Figure S2.** Aliphatic ( $\delta_C/\delta_H$  50–90/2.5–6.0) regions on 2D HSQC NMR spectra of (A) soluble lignin in the black liquor and (B) lignin residue after fermentation.