

Supplementary file

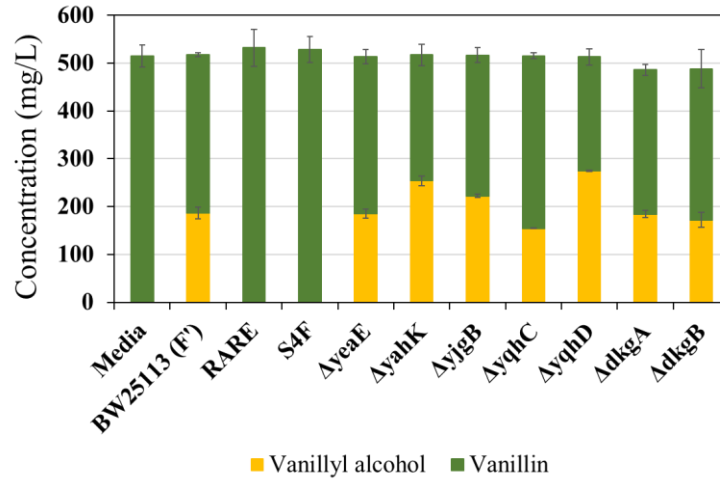
Towards Synthetic Ligninolysis via Microbial Utilization of Lignin Degraded Aromatics

Ruihua Zhang et. al.

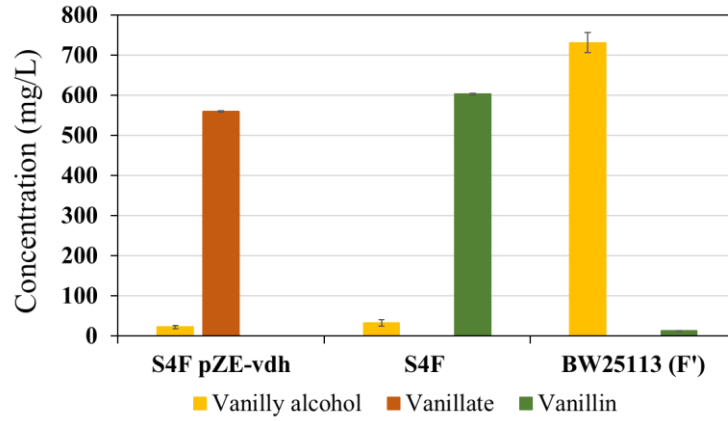
Supplementary Table 1. Plasmids and strains used in this study.

Plasmid	Description	Source
pETDuet-1	pT7lac; <i>colE</i> ori; Amp ^R	Promega
pZE12-luc	PLlacO1, <i>colE</i> ori, Amp ^R	1
pCS27	PLlacO1, <i>P15A</i> ori, Kan ^R	2
pCP20	Flippase, AmpR, and temperature-sensitive replicon	3
pET-xylE	pETDuet-1 harboring <i>xylE</i> from <i>P. putida</i> mt-2	This study
pET-xylG	pETDuet-1 harboring <i>xylG</i> from <i>P. putida</i> mt-2	This study
pET-xylF	pETDuet-1 harboring <i>xylF</i> from <i>P. putida</i> mt-2	This study
pZE-aroY	pZE12-luc harboring <i>aroY</i> from <i>K. pneumoniae</i> (ATCC 25955)	This study
pZE-aroY-hp1	pZE12-luc harboring <i>aroY-hp1</i> from <i>K. pneumoniae</i> (ATCC 25955)	This study
pZE-aroY-hp1-hp2	pZE12-luc harboring <i>aroY-hp1-hp2</i> from <i>K. pneumoniae</i> (ATCC 25955)	This study
pZE-aroY-kpdB	pZE12-luc harboring <i>aroY-kpdB</i> from <i>K. pneumoniae</i> (ATCC 25955)	This study
pZE-kpdBCD	pZE12-luc harboring <i>kpdBCD</i> from <i>K. pneumoniae</i> (ATCC 25955)	This study
pCS-xylE-aroY-hp1	pCS27 harboring <i>xylE</i> and <i>aroY-hp1</i>	This study
pZE-xylF-mhpD	pZE12-luc harboring <i>xylF</i> from <i>P. putida</i> mt-2 and <i>mhpD</i> from <i>E. coli</i>	This study
pZE-xylF-xylJ	pZE12-luc harboring <i>xylF</i> and <i>xylJ</i> from <i>P. putida</i> mt-2	This study
pZE-xylGHI-mhpD	pZE12-luc harboring <i>xylGHI</i> from <i>P. putida</i> mt-2 and <i>mhpD</i> from <i>E. coli</i>	This study
pZE-xylGHI-xylJ	pZE12-luc harboring <i>xylGHI</i> and <i>xylJ</i> from <i>P. putida</i> mt-2	This study
pCS-kivD-adh6	pCS27 harboring <i>kivD-adh6</i> operon	This study
pZE-FJKA	pZE12-luc harboring dual operons of <i>xylF-xylJ</i> and <i>kivD-adh6</i>	This study
pZE-FDKA	pZE12-luc harboring dual operons of <i>xylF-mhpD</i> and <i>kivD-adh6</i>	This study
pZE-GJKA	pZE12-luc harboring dual operons of <i>xylGHI-xylJ</i> and <i>kivD-adh6</i>	This study
pZE-GDKA	pZE12-luc harboring dual operons of <i>xylGHI-mhpD</i> and <i>kivD-adh6</i>	This study
pZE-vanAB	pZE12-luc harboring <i>vanAB</i> operon	This study
pZE-vdh	pZE12-luc harboring <i>vdh</i>	This study
pCS-VIV	pCS27 harboring <i>vanAB-iem-vdh</i> operon	This study
pZE-xylF-cimA*	pZE12-luc <i>xylF-cimA*</i> operon	This study
pZE-XCH	pZE12-luc harboring dual operons of <i>xylF-cimA*</i> and <i>aroY-hp1</i>	This study

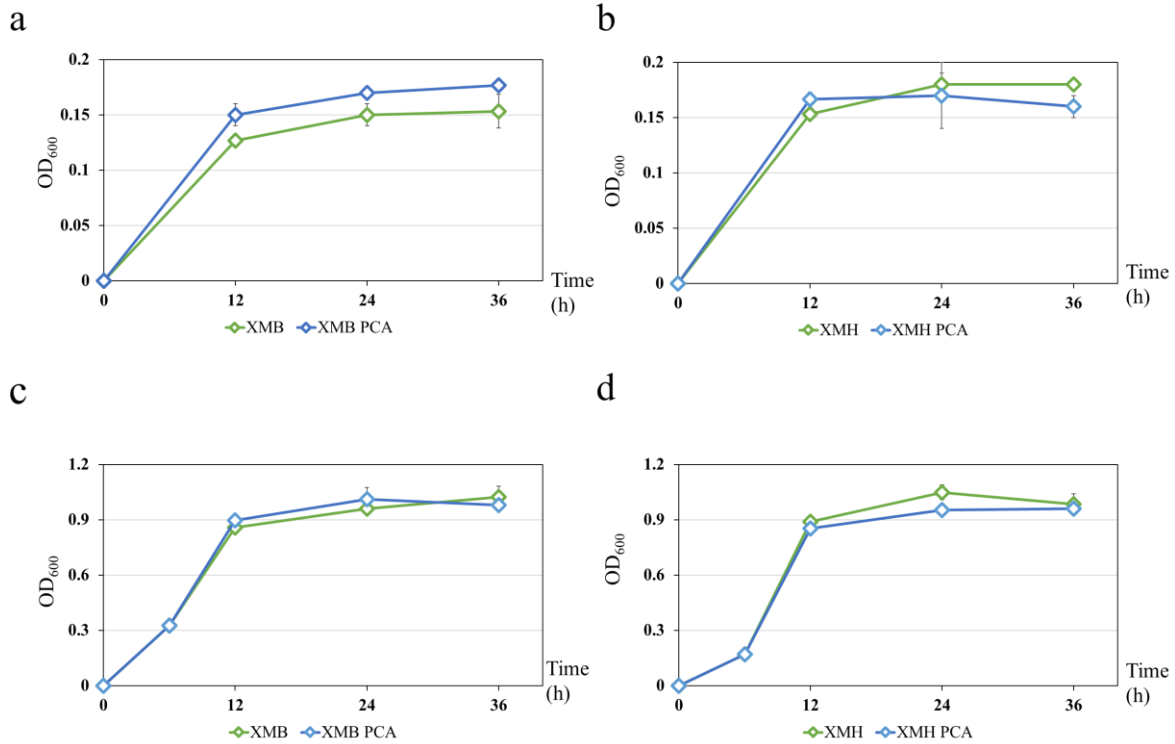
pZE-XCB	pZE12-luc harboring dual operons of <i>xylF-cimA*</i> and <i>aroY-kpdB</i>	This study
pZE-XCBCD	pZE12-luc harboring dual operons of <i>xylF-cimA*</i> and <i>kpdBCD</i>	This study
pCS-xylE	pCS27 harboring <i>xylE</i>	This study
pCS-xylE-mhpDFE	pCS27 harboring <i>xylE</i> and <i>mhpDFE</i> operons from <i>E. coli</i>	This study
pZE-xylE-mhpDFE	pZE12-luc harboring <i>xylE-mhpDFE</i> operon	This study
pCS-VIV-XEM	pCS27 harboring dual operons of <i>vanAB-iem-vdh</i> and <i>xylE-mhpDFE</i>	This study
pZE-vdh-xylF-cimA*	pZE12-luc harboring <i>vdh-xylF-cimA*</i> operon	This study
pZE-VXCH	pZE12-luc harboring dual operons of <i>vdh-xylF-cimA*</i> and <i>aroY-hp1</i>	This study
pZE-VXCB	pZE12-luc harboring dual operons of <i>vdh-xylF-cimA*</i> and <i>aroY-kpdB</i>	This study
Strains	Description	Source
<i>E. coli</i> XL1-Blue	<i>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac</i> [F' <i>proAB lacIqZ</i> Δ M15 Tn10 (TetR)]	Stratagene
<i>E. coli</i> BW25113 (F')	<i>rrnBT14 Δlac ZWJ16 hsdR514 ΔaraBADAH33 ΔrhaBADLD78 F'</i> [<i>traD36 proAB lacIqZ</i> Δ M15 Tn10 (TetR)] F'	This study
<i>E. coli</i> BL21 Star (DE3)	F-ompT hsdSB (rB ⁻ mB ⁻) gal dcm (DE3)	Invitrogen
BW25113 F' Δ <i>mhpE</i>	BW25113 (F') with Δ <i>mhpE</i> and Kanamycin resistance marker removed	This study
RARE	<i>E. coli</i> MG1655 (DE3) Δ <i>dkgB</i> Δ <i>yeaE</i> Δ (<i>yqhC-dkgA</i>) Δ <i>yahK</i> Δ <i>yjgB</i>	4
S4	<i>E. coli</i> MG1655 (DE3) Δ (<i>yqhC-dkgA</i>)::Kan Δ <i>yahK</i> Δ <i>yjgB</i>	4
S4F	S4 strain with F' plasmid and Kanamycin resistance marker removed	This study
FJKA	<i>E. coli</i> BW25113 (F') transformed with pZE-FJKA and pCS-xylE-aroY-hp1	This study
FDKA	<i>E. coli</i> BW25113 (F') transformed with pZE-FDKA and pCS-xylE-aroY-hp1	This study
GJKA	<i>E. coli</i> BW25113 (F') transformed with pZE-GJKA and pCS-xylE-aroY-hp1	This study
GDKA	<i>E. coli</i> BW25113 (F') transformed with pZE-GDKA and pCS-xylE-aroY-hp1	This study
XH	<i>E. coli</i> S4F transformed with pZE-XCH and pCS-xylE	This study
XB	<i>E. coli</i> S4F transformed with pZE-XCB and pCS-xylE	This study
XBCD	<i>E. coli</i> S4F transformed with pZE-XCBCD and pCS-xylE	This study
XMH	<i>E. coli</i> S4F transformed with pZE-XCH and pCS-xylE-mhpDFE	This study
XMB	<i>E. coli</i> S4F transformed with pZE-XCB and pCS-xylE-mhpDFE	This study
XMBCD	<i>E. coli</i> S4F transformed with pZE-XCBCD and pCS-xylE-mhpDFE	This study
VXMB	<i>E. coli</i> S4F transformed with pZE-VXCH and pCS-xylE-mhpDFE	This study
VXMH	<i>E. coli</i> S4F transformed with pZE-VXCB and pCS-xylE-mhpDFE	This study



Supplementary Figure 1. Screening of reductase deficient strains for vanillyl alcohol removal. Media: uninoculated media supplemented with vanillin. The error bars represent the standard deviations of two biological replications.



Supplementary Figure 2. Improvement of aromatic degradation efficiency by S4F and high-copy number *vdh*. All test groups were supplemented with 600 mg/L vanillin. The error bars represent the standard deviations of two biological replications.



Supplementary Figure 3. Investigation of growth promotion effect of lignin aromatics as the sole carbon source for the synthetic ligninolysis. (a) and (b) Growth profiles of strains grew in M9-0.5Y media. (c) and (d) Growth profiles of strains grew in M9-5Y media. PCA: strains were fed with 0.5 g/L PCA. The error bars represent the standard deviations of three biological replications.

References

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