Table S1 Plasmids and bacterial strains used in this study. Summarized details of plasmids and recombinant A514 strains, including replicon,

antibiotic resistance, functional elements, and source.

Strain or Plasmid	Relevant characteristic(s)[a]	Source or Reference
Plasmids		
pPROBE-GT	pVS1/p15a Gm^r gfp	(Miller et al., 2000)
pUC57-Pmin-tat-lac	Amp^r; P$_{min}$::tat-Laccase	Personalbio Company
pUCP18-Gm	Amp^r; Gm^r, shuttle vector; lacZ with MCS	(23)
pGlac	pPROBE-GT derivative, P$_{xylA}$::Laccase	This study
pUlac	pUCP18-Gm derivative, P$_{xylA}$::Laccase	This study
pUI	pUCP18-Gm derivative, P$_{min}$::ABC transporters	This study
pUI-2539	pUI derivative, P$_{xylA}$::His·tag-Laccase-2539 C-terminal signal sequence	This study
pUI-2549	pUI derivative, P$_{xylA}$:: His·tag-Laccase-2549 C-terminal signal sequence	This study
pUI-rrnb	pUI derivative, His·tag-laccase-C-terminal signal sequence of 2549-T1/T2 terminators	This study
pGoprI	pPROBE-GT derivative, P$_{xylA}$::oprI-Laccase-His·tag	This study
pGpbP	pPROBE-GT derivative, P$_{xylA}$::pbP-Laccase-His·tag	This study
pGpelB	pPROBE-GT derivative, P$_{xylA}$::pelB-Laccase-His·tag	This study
pGtat	pPROBE-GT derivative, P$_{xylA}$::tat-Laccase-His·tag	This study
pG0383	pPROBE-GT derivative, P$_{xylA}$::0380-Laccase-His·tag	This study
pG0620	pPROBE-GT derivative, P$_{xylA}$::0620-Laccase-His·tag	This study
pG1543	pPROBE-GT derivative, P$_{xylA}$::1543-Laccase-His·tag	This study
pG1921	pPROBE-GT derivative, P$_{xylA}$::1921-Laccase-His·tag	This study

pG2816	pPROBE-GT derivative, P_{xylA}::2816-Laccase-His·tag	This study
pG4529	pPROBE-GT derivative, P_{xylA}::4529-Laccase-His·tag	This study
pG4580	pPROBE-GT derivative, P_{xylA}::4580-Laccase-His·tag	This study
pU2816	pUCP18-Gm derivative, P_{xylA}::2816-His·tag-Laccase	This study
pUV-ABC-2816	pU2816 derivative, P_{van}::tatABC	This study
pUM-ABC-2816	pU2816 derivative, P_{min}::tatABC	This study
pUV-1761	pU2816 derivative, P_{xylA}::2816-His·tag-Laccase-1761 β domain	This study
pUV-2541A	pU2816 derivative, P_{xylA}::2816-His·tag-Laccase-2541A β domain	This study
pUV-3548	pU2816 derivative, P_{xylA}::2816-His·tag-Laccase-3548 β domain	This study
pUV-4947	pU2816 derivative, P_{xylA}::2816-His·tag-Laccase-4947 β domain	This study
pUV2541B	pU2816 derivative, P_{xylA}::2816-His·tag-Laccase-2541B β domain	This study
pUV-2541C	pU2816 derivative, P_{xylA}::2816-His·tag-Laccase-2541C β domain	This study
pUV-2541D	pU2816 derivative, P_{xylA}::2816-His·tag-Laccase-2541D β domain	This study
pUV-rnb	pU2816 derivative, P_{xylA}::2816-His·tag-2541D β domain	This study
pUVV-ABC-2541D	pUV-2541D derivative, P_{van}::tatABC	This study
pUVM-ABC-2541D	pUV-2541D derivative, P_{min}::tatABC	This study
pUV-thromobin-2541D	pUV-2541D derivative, P_{xylA}::2816-His·tag-Laccase-thrombin cleavage site-2541D β domain	This study
pUV-MHT[a]- 2541A	pUV-2541A derivative, P_{xylA}::2816-MHT-2541A β domain	This study
pUV-MHT- 2541B	pUV-2541B derivative, P_{xylA}::2816-MHT-2541B β domain	This study
pUV-MHT- 2541C	pUV-2541C derivative, P_{xylA}::2816-MHT-2541C β domain	This study
pUV-MHT- 2541D	pUV-2541D derivative, P_{xylA}::2816-MHT-2541D β domain	This study
pUV-MHT-Lac-2541A	pUV-2541A derivative, P_{xylA}::2816-MHT-Laccase-2541A β domain	This study

pUV-MHT-Lac-2541B	pUV-2541B derivative, P_{xylA}::2816-MHT-Laccase-2541B β domain	This study
pUV-MHT-Lac-2541C	pUV-2541C derivative, P_{xylA}::2816-MHT-Laccase-2541C β domain	This study
pUV-MHT-Lac-2541D	pUV-2541D derivative, P_{xylA}::2816-MHT-Laccase-2541D β domain	This study
Strains		
E. coli Mach I	F- *delta lacX74 hsdR(rk^- mk^+) delta rec1398 endA1 tonA*	Takara
P. putida A514	Wild type	This study
A_{pGT}	A514 carrying pPROBE-GT	This study
A_{pGlac}	A514 carrying pGT-lac	This study
A_{pGoprI}	A514 carrying pGoprI	This study
A_{pGpbp}	A514 carrying pGpbp	This study
A_{pGpelb}	A514 carrying pGpelb	This study
A_{pGtat}	A514 carrying pGtat	This study
A_{pG383}	A514 carrying pG383	This study
A_{pG620}	A514 carrying pG620	This study
A_{pG1543}	A514 carrying pG1543	This study
A_{pG1921}	A514 carrying pG1921	This study
A_{pG2816}	A514 carrying pG2816	This study
A_{pG4529}	A514 carrying pG4529	This study
A_{pG4580}	A514 carrying pG4580	This study
A_{pU}	A514 carrying pUCP18-Gm	This study
A_{pUlac}	A514 carrying pUlac	This study
$A_{pUI-2539}$	A514 carrying pUI-2539	This study
$A_{pUI-2549}$	A514 carrying pUI-2549	This study

A$_{pUI\text{-}rrnb}$	A514 carrying pUI-rrnb	This study
A$_{pU2816}$	A514 carrying pU2816	This study
A$_{pUV\text{-}ABC\text{-}2816}$	A514 carrying pUV-ABC-2816	This study
A$_{pUM\text{-}ABC\text{-}2816}$	A514 carrying pUM-ABC-2816	This study
A$_{pUV\text{-}1761}$	A514 carrying pUV-1761	This study
A$_{pUV\text{-}2541A}$	A514 carrying pUV-2541A	This study
A$_{pUV\text{-}3548}$	A514 carrying pUV-3548	This study
A$_{pUV\text{-}4947}$	A514 carrying pUV-4947	This study
A$_{pUV\text{-}2541B}$	A514 carrying pUV-2541B	This study
A$_{pUV\text{-}2541C}$	A514 carrying pUV-2541C	This study
A$_{pUV\text{-}2541D}$	A514 carrying pUV-2541D	This study
A$_{pUV\text{-}rrnb}$	A514 carrying pUV-rrnb	This study
A$_{pUVV\text{-}ABC\text{-}2541D}$	A514 carrying pUVV-ABC-2541D	This study
A$_{pUVM\text{-}ABC\text{-}2541D}$	A514 carrying pUVM-ABC-2541D	This study
A$_{pUV\text{-}thromobin\text{-}2541D}$	A514 carrying pUV-thromobin-2541D	This study
A$_{pUV\text{-}MHT\text{-}2541A}$	A514 carrying pUV-MHT-2541A	This study
A$_{pUV\text{-}MHT\text{-}2541B}$	A514 carrying pUV-MHT-2541B	This study
A$_{pUV\text{-}MHT\text{-}2541C}$	A514 carrying pUV-MHT-2541C	This study
A$_{pUV\text{-}MHT\text{-}2541D}$	A514 carrying pUV-MHT-2541D	This study
A$_{pUV\text{-}MHT\text{-}Lac\text{-}2541A}$	A514 carrying pUV-MHT-Lac-2541A	This study
A$_{pUV\text{-}MHT\text{-}Lac\text{-}2541B}$	A514 carrying pUV-MHT-Lac-2541B	This study
A$_{pUV\text{-}MHT\text{-}Lac\text{-}2541C}$	A514 carrying pUV-MHT-Lac-2541C	This study
A$_{pUV\text{-}MHT\text{-}Lac\text{-}2541D}$	A514 carrying pUV-MHT-Lac-2541D	This study

[a]MHT, epitope containing the Myc epitope, His epitope, and thrombin protease recognition sequence

Table S2 Optimization of intracellular laccase production in the recombinant *P. putida* A514 strains.

Enzyme	Strain	Parameter		Range	Optimum range	Optimum Activity U/mL[b]
Laccase [a]	A$_{pGLac}$	Origin		\	\	0.73 ± 0.03
		pH		5.0-8.0	6.0	0.79 ± 0.20
		Metal ion	Mn^{2+}	0-2 mM	0	0.66 ± 0.04
			Cu^{2+}	0-0.2 mM	0.2 mM	0.88 ± 0.12
			Fe^{2+}	0 mM, 0.5mM and 1 mM	1 mM	1.01 ± 0.11
			Cu^{2+} with 1 mM Fe^{2+}	0.5-2 mM	1 mM	1.83 ± 0.12
		Temperature		20-30 ℃	20 ℃	2.28 ± 0.09
		Shaker speed		50-200 rpm	100 rpm	3.44 ± 0.17
		Incubation time		24-72 h	60 h	3.36 ± 0.06
	A$_{pULac}$	Vectors		pPROBE-GT pUCP18-Gm	pUCP18-Gm	99.73 ± 4.48

[a] For laccase activity, the absorption at 420 nm of intracellular extracts from A$_{pUCP18}$ or A$_{pGT}$ was employed as the blank control to eliminate the

interference of background enzyme activity.

[b] U/mL: the enzyme activity per one milliliter batch culture broth.

Table S3 Optimization of extracellular laccase production in the recombinant *P. putida* A514 strains via the TISS strategy.

Enzyme	Strain	Parameter	Range	Optimum range	Optimum U/mL	Activity
Laccase	$A_{pUI\text{-}2549}$	OD600	0-7.5	7.5	2.85 ± 0.13	
		Time point of adding 2 mM xylose	0 h, 8 h and 14 h	8 h	5.72 ± 0.29	
		Shaker speed	100-150 rpm	150 rpm	6.98 ± 0.31	
		Temperature	20-30 ℃	25 ℃	8.42 ± 0.41	
		Time point of adding 1 mM $CuSO_4$	0 h, 22 h and 44 h	22 h	15.47 ± 1.37	

A_{pUlac} was employed as the blank control to eliminate the interference of background enzyme activity.

Table S4 Optimization of extracellular laccase production from the recombinant *P. putida* A514 strains by the TVSS strategy.

Enzyme	Strain	Parameter	Range	Optimum range	Optimum Activity U/mL
Laccase (Peri.)[a]	A_{pG1543} A_{pG2816} A_{pG4529}	TAT signal peptides	1543 2816 4529	2816	16.65 ± 0.86
	A_{pG2816} A_{pU2816}	Vectors	pPROBE-GT pUCP18-Gm	pUCP18-Gm	49.16 ± 1.63
	A_{pU2816} $A_{pUV\text{-}ABC\text{-}2816}$ $A_{pUM\text{-}ABC\text{-}2816}$	TatABC transporters	Without TatABC P_{van}::*tatA tatB tatC* P_{min}::*tatA tatB tatC*	without TatABC	50.08 ± 1.65
Laccase (Extra. attached)[b]	$A_{pUV1761}$ $A_{pUV2541A}$ $A_{pUV3548}$ $A_{pUV4947}$	β domain	1761 2541A 3548 4947	2541A	15.99 ± 2.02
	$A_{pUV2541A}$ $A_{pUV2541B}$ $A_{pUV2541C}$ $A_{pUV2541D}$	Linker domain	2541A 2541B 2541C 2541D	2541D	23.42 ± 2.41
	$A_{pUV\text{-}2541D}$	Time point of adding 2 mM xylose	0 h 9 h 23 h	9 h	84.26 ± 1.81

	Temperature	20-28 °C	25 °C	109.29 ± 11.31
	Agitation	50-150 rpm	150 rpm	109.29 ± 11.31
	Time point of adding 1 mM $CuSO_4$	0 h 22 h 45 h	22 h	298.64 ± 13.71
$A_{pUV-2541D}$ $A_{pUVV-ABC-2541D}$ $A_{pUVM-ABC-2541D}$	TatABC transporters	Without TatABC P_{van}::*tatA tatB tatC* P_{min}::*tatA tatB tatC*	without TatABC	298.64 ± 13.71

[a] Laccase activity in the periplasmic space.

[b] Laccase was displayed on the cell surface. Its activity was detected by the whole-cell laccase activity assay, where A_{pUlac} was employed as the blank control to eliminate the interference of background enzyme activity.

Table S5 Cultivation optimization of the recombinant *P. putida* A514 strains in M9-lignin rich medium.

Strain	Parameter	Range	Optimum range	CFU growth folds	Lignin utilization
A_{pU}				450 ± 49	$2.65\% \pm 3.32\%$
$A_{pUI\text{-}2549}$	Origin	\	\	1141 ± 92	$4.29\% \pm 0.16\%$
$A_{pUV\text{-}2541D}$				937 ± 75	$4.78\% \pm 0.64\%$
A_{pU}		$10\ \mu M\ Cu^{2+}$ at 36 h plus $10\ \mu M$		2533 ± 250	$4.70\% \pm 0.03\%$
$A_{pUI\text{-}2549}$	Cu^{2+}	Cu^{2+} at 48 h	$20\ \mu M\ Cu^{2+}$ at 36 h	2050 ± 263	$7.27\% \pm 0.71\%$
$A_{pUV\text{-}2541D}$		$20\ \mu M\ Cu^{2+}$ at 36 h		1378 ± 125	$7.64\% \pm 0.31\%$
A_{pU}				301328 ± 1286	$4.56\% \pm 2.40\%$
$A_{pUI\text{-}2549}$	NH_4Cl	0.25, 0.5 and 1 g/L	0.5 g/L	2159289 ± 11593	$10.42\% \pm 1.80\%$
$A_{pUV\text{-}2541D}$				315436 ± 9012	$8.74\% \pm 0.46\%$
A_{pU}				411656 ± 1466	$4.97\% \pm 1.13\%$
$A_{pUI\text{-}2549}$	Yeast Extract	0.05% and 0.1%	0.1%	19875974 ± 290525	$13.72\% \pm 0.99\%$
$A_{pUV\text{-}2541D}$				21559069 ± 906720	$9.89\% \pm 0.47\%$
A_{pU}				14852708 ± 445258	$6.98\% \pm 0.96\%$
$A_{pUI\text{-}2549}$	xylose	3, 5, 7 and 12 mM	5 mM	31480027 ± 802956	$12.01\% \pm 0.99\%$
$A_{pUV\text{-}2541D}$				29228019 ± 308002	$9.09\% \pm 3.48\%$
A_{pU}				19607520 ± 84439	$4.62\% \pm 1.16\%$
$A_{pUI\text{-}2549}$	Inoculum size	10^3, 10^4 and 10^5 CFUs/mL	10^4 CFUs/mL	52682480 ± 132249	$12.58\% \pm 0.37\%$
$A_{pUV\text{-}2541D}$				49584584 ± 322426	$12.13\% \pm 0.72\%$

Table S6 Primers used in this study.

Primer	Sequence
Lac-Fwd(int)	5'-TGCCCAGCCGGAGATGGCCCCAGCTGCAAAGGGGATCA-3'
Lac-Rev	5'-TTATCGGTACCTCAGTGGTGGTGGT-3'
oprI-F	5'-AAAACTGCAGATGAACAACGTTCTGAAATTCTCTGC-3'
oprI-R	5'-GTGATCCCCTTTGCAGCTGGACCCATGCTGCTGCAACC-3'
pbp-F	5'-TTCCCTGCAGATGAAACTGAAACGTTTGATGGCGGCAATGACTTTTGTCGCTGCTGGCGT-3'
pbp-F(-lac)	5'-TTTTGTCGCTGCTGGCGTTGCGACCGTCAACGCGGTGGCCCCAGCTGCAAAGGGG-3'
pelb-F	5'-GCCGATCTGCAGATGAAATACCTGCTGCCGACC-3'
pelB-R	5'-TGATCCCCTTTGCAGCTGGGGCCATCGCCGGCTGGGCA-3'
383-F	5'-GTCCCTGCAGATGAACCCTAGCCTGACTGAT-3'
383-R	5'-GTGATCCCCTTTGCAGCTGGGGCAAAGCCGCTGGCC-3'
620-F	5'-GTCCCTGCAGATGACTACGCGCAACCCA-3'
620-R	5'-GTGATCCCCTTTGCAGCTGGGCCGCCCGTTGCGG-3'
1543-F	5'-AAAACTGCAGATGCACCGCAGGAATTTGC-3'
1543-R	5'-GTGATCCCCTTTGCAGCTGGACGGTTTGCGGCCCAG-3'
1921-F	5'-TCACACAAGGAGCTGCAGATGAGCAATGACGGCGTG-3'

1921-R	5'-GTGATCCCCTTTGCAGCTGGTGCACCTGCGGCTTTCG-3'
2816-F	5'-GTCCCTGCAGATGAGTGATTCAGAACAATTTTCAGC-3'
2816-R	5'-GTGATCCCCTTTGCAGCTGGGGCGGCGGCGGCCGCCATGGCCGGGCAGGT-3'
4529-F	5'-TTCCCTGCAGATGTCTGATCAAGATCAAGACAACC-3'
4529-R	5'-GTGATCCCCTTTGCAGCTGGCTTGGCCGGAGCGGC-3'
4580-F	5'-AAAACTGCAGATGTTAATCAAGATCCCCAAAGCG-3'
4580-R	5'-GTGATCCCCTTTGCAGCTGGAGCATCGTCCGCACTGG-3'
Gm F	5'-GCCGCTCGAGTTGACATAAGCCTGTTCGGTTC-3'
Gm R	5'-ACGGGGTACCTTAGGTGGCGGTACTTGGGT-3'
2544-F(XbaI)	5'-CTAGTCTAGAATATGACCGCCGGGATGATGA-3'
2542-R(Sac I)	5'-TTGCGAGCTCCGGTGAATCGGTGATCATCTAA-3'
Pmin-F(HindIII)	5'-ACGCAAGCTTATTGAAATAGCTGAGACACAAGCG-3'
Pmin-R(XbaI)	5'-ATGCTCTAGACTCCTAGTGTGAAATTGTTATCCGC-3'
rrnb-F(Sac I)	5'-AGACGAGCTCCAAATAAAACGAAAGGCTCAGTCG-3'
rrnb-R(-xylR)	5'-GCTAAGATCGTTCCCACGTGCGAAAAGGCCATCCGTCAGGAT-3'
xylR-F(BamHI)	5'-TCGCGGATCCGCACGTGGGAACGATCTTA-3'
xylA-R(SmaI)	5'-TTCCCCCGGGCTCCTTATTGTTCTTGGCGGTG-3'
LAC-F	5'-ATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCATGCCAGCTGCAAAGGGG-3'

LAC-F 2	5'-CACCGCCCGGGATGCATCATCATCATCATCACAGCAGCGGCC-3'
2539-F(for lac)	5'-CCTGGAAGTTCTGTTCCAGGGGCCCAATGCCGAACCGCACAAG-3'
2539-R(-rrnb)	5'-CTGAGCCTTTCGTTTTATTTGGGTGATCAGCCGATGCTGA-3'
2549-F(for lac)	5'-CCTGGAAGTTCTGTTCCAGGGGCCCAAGGGCGTTACCGTCGAGA-3'
2549-R(-rrnb)	5'-CTGAGCCTTTCGTTTTATTTGCCTAAGTCACGCTACGATGTCGCTG-3'
rrnb-R(XhoI)	5'-CCGCTCTAGAAAAAGGCCATCCGTCAGGAT-3'
2816-F(SmaI)	5'-GTCCCCCGGGATGAGTGATTCAGAACAATTTTCAGC-3'
Pvan-F(Hind III)	5'-GCCTAAGCTTCCTTGATGGGTTGGGTGTCC-3'
Pvan-R(XbaI)	5'-TCGCTCTAGACTCCTTGACATGGGAGGCTCTCCG-3'
tatA-F(XbaI)	5'-GGGCTCTAGAATGGGCATTTTTGACTGGAAACA-3'
tatC-R(-rrnb SacI)	5'-GACTGAGCCTTTCGTTTTATTTGGAGCTCAAGTAGCAGCAGGTTCACGG-3'
1761-F(KpnI)	5'-ACGCGGTACCCGTAACCAGACGAGTTTTGCC-3'
1761-R(XhoI)	5'-TCCGCTCGAGTTAGAAGCGGAATGTAAACCCG-3'
2541-F(KpnI)	5'-ACGGGGTACCCAAGCACCGAATACTATCGTGG-3'
2541-R(XhoI)	5'-TGCCCTCGAGCTAGAACTGCCAGTTCAGGCT-3'
3548-F(KpnI)	5'-ACTGGGTACCGCCGGTGCAGGTGCCAG-3'
3548-R(XhoI)	5'-ACCGCTCGAGTCAGAACGCGTAACGCGCTTTGA-3'
4947-F(KpnI)	5'-ACGGGGTACCCAACAACTGATCGCCGACTAC-3'

4947-R(XhoI)	5'-TACCCTCGAGTCAGAAATCCAGACTCACCCC-3'
2541-F K2	5'-ATGGGGTACCGGGCAGGGCGATGTGTG-3'
2541-F K3	5'-ATGGGGTACCGGCAGCCTGATCAAATCCG-3'
2541-F K4	5'-ACGGGGTACCAACAACGCCACATTCGCCAG-3'
xylR-F(BamHI)	5'-TCGCGGATCCGCACGTGGGAACGATCTTA-3'
2816-Myc R	5'-TCTGAGATGAGTTTTTGTTCAGCTGCGGCGGCGGCGGCGGCCGCCATGGCCGGGCAGGT-3'
myc-his R	5'-GATGATGATGGTCGACGGCGCTATTCAGATCCTCTTCTGAGATGAGTTTTTGTTCAGCT-3'
his-thrombin R	5'-CCTCCGCTGCCGCGCGGCACCAGGCCGCTGCTGTGATGATGATGATGATGGTCGACGGC-3'
thrombin-lac F	5'-ATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCGGAGGTTCCATGCCAGCTGCAAAGGGG-3'
lac-thrombin R(KpnI)	5'-TTGTGGTACCTGCGCTGCCGCGCGGCACCAGGCCGCTGCTGTGTTCGTGCTCATGGGC-3'
his-lac F	5'-CCGCCGCCGCAGCTCATCATCATCATCATCATAGCAGCGGCATGCCAGCTGCAAAGGGG-3'
2816-his R	5'-CTATGATGATGATGATGATGAGCTGCGGCGGCGGCGGCGGCCGCCATGGCCGGGCAGGT-3'
thrombin R(KpnI)	5'-TAAGGGTACCAGCTGGCATGGAACCTCC-3'