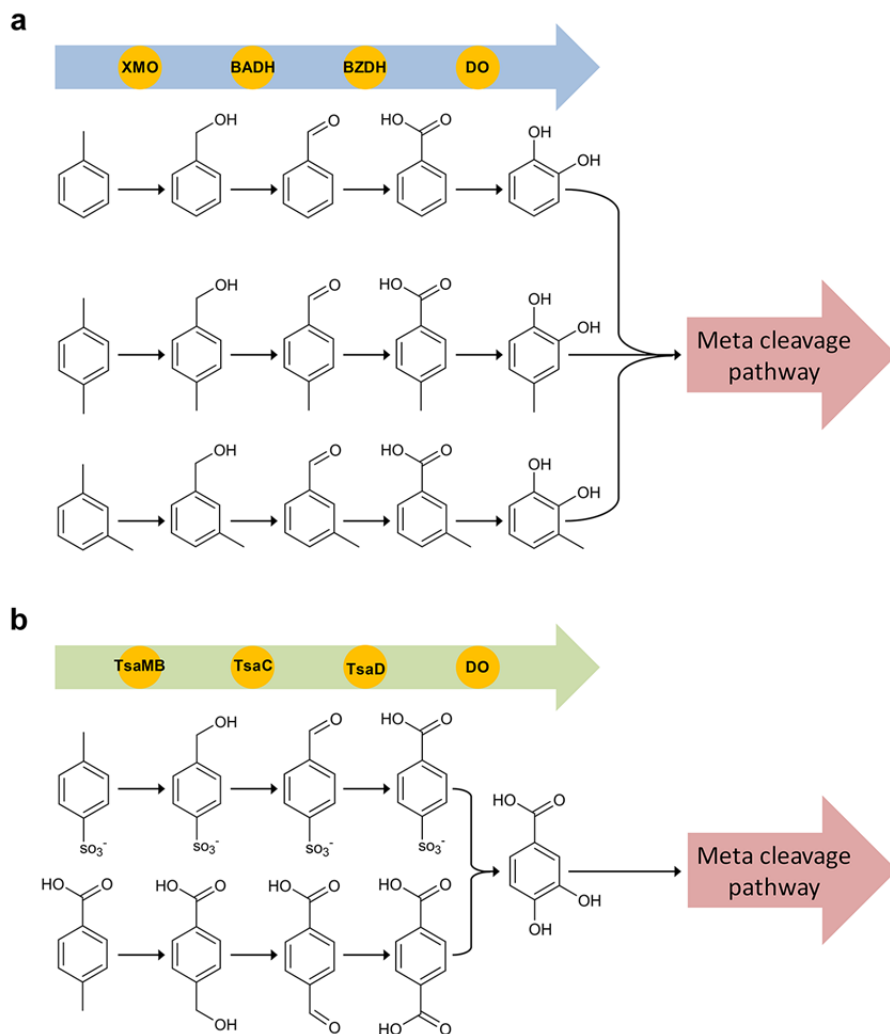


1 SUPPLEMENTARY FIGURES



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3 **Supplementary Figure 1. Natural degradation pathways of toluene and xylenes including**

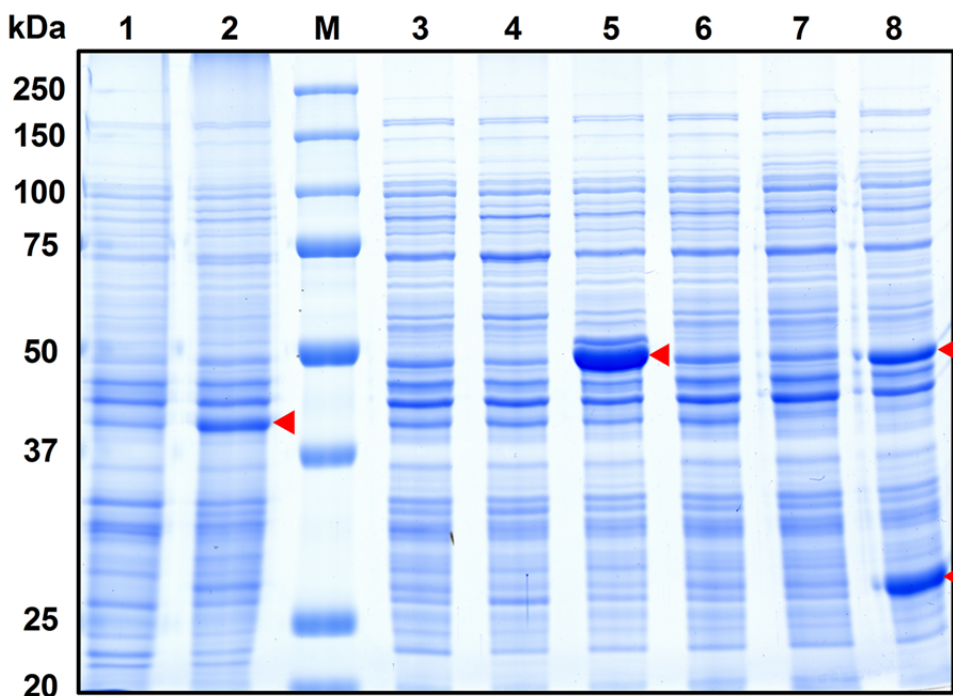
4 ***pX* and *mX* (a), *pTS* and *pTA* (b) in *P. putida* and *C. testosteroni* T-2, respectively. Enzymes:**

5 XMO, xylene monooxygenase; BADH, benzyl alcohol dehydrogenase; BZDH, benzaldehyde

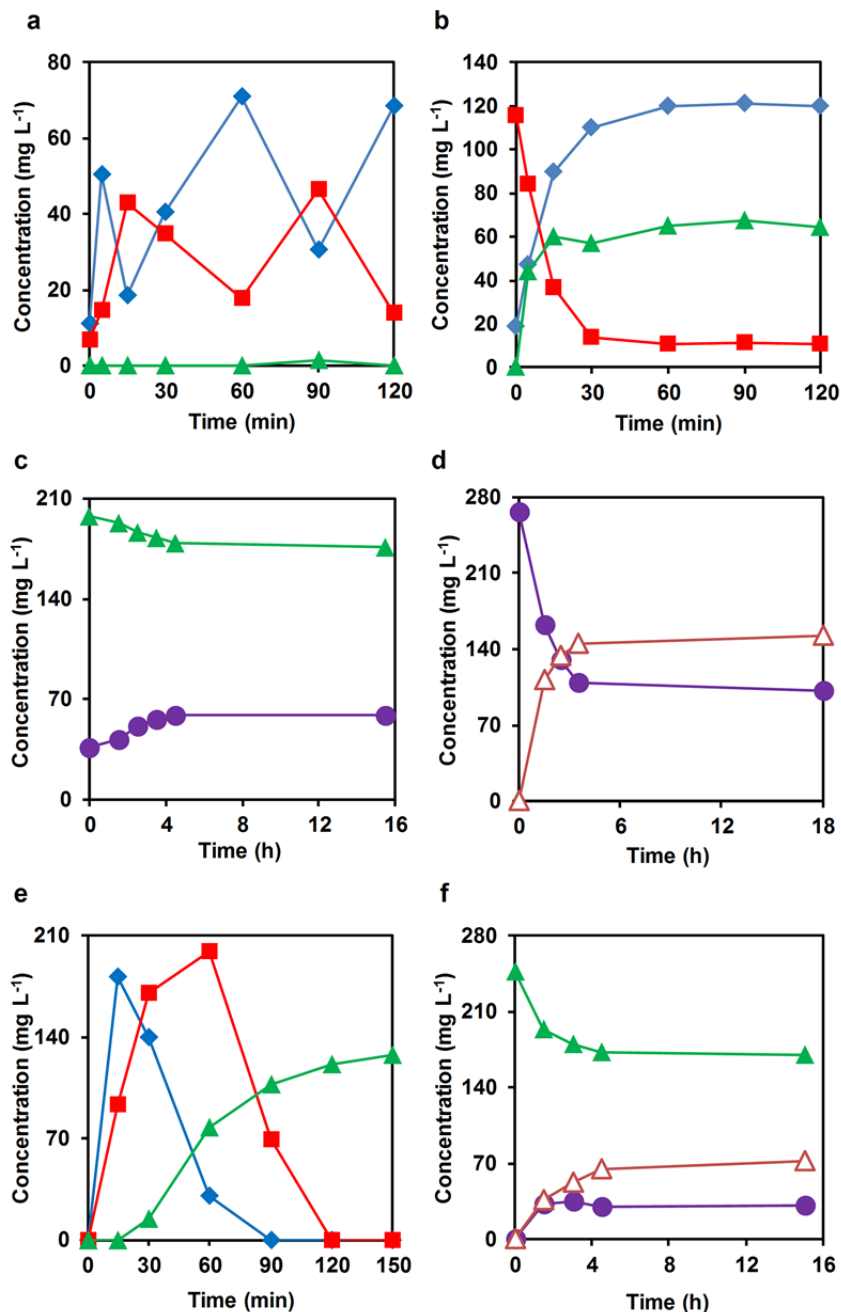
6 dehydrogenase; DO, dioxygenase; *pTS*, *p*-toluene sulfonate; TsaMB, *p*-toluene sulfonate

7 monooxygenase; TsaC, 4-carboxybenzyl alcohol dehydrogenase; TsaD, 4-carboxybenzaldehyde

8 dehydrogenase.



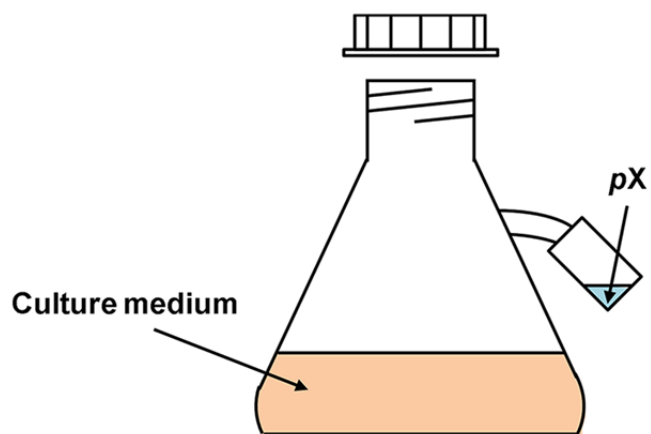
Supplementary Figure 2. SDS-PAGE analysis of the overexpression of heterologous pathway enzymes. Lane M, protein marker; lane 1, membrane fraction of DH5 α harboring pTrc99A; lane 2, membrane fraction of DH5 α harboring pTrcMA (XylM arrow-pointed); lane 3, soluble fraction of DH5 α harboring pTrc99A; lane 4, soluble fraction of DH5 α harboring pTrcMA; lane 5, soluble fraction of DH5 α harboring pTrcC (XylC arrow-pointed); lane 6, soluble fraction of DH5 α harboring pTrcMB; lane 7, soluble fraction of DH5 α harboring pTac15K; lane 8, soluble fraction of DH5 α harboring pTacCD (TsaC lower arrow-pointed, TsaD upper arrow-pointed). The expected molecular masses of XylM (membrane-bound), XylA, XylC, TsaM, TsaA, TsaC and TsaD are 43.2, 38.4, 52.9, 39.7, 34.3, 26.6 and 51.1 kDa, respectively.



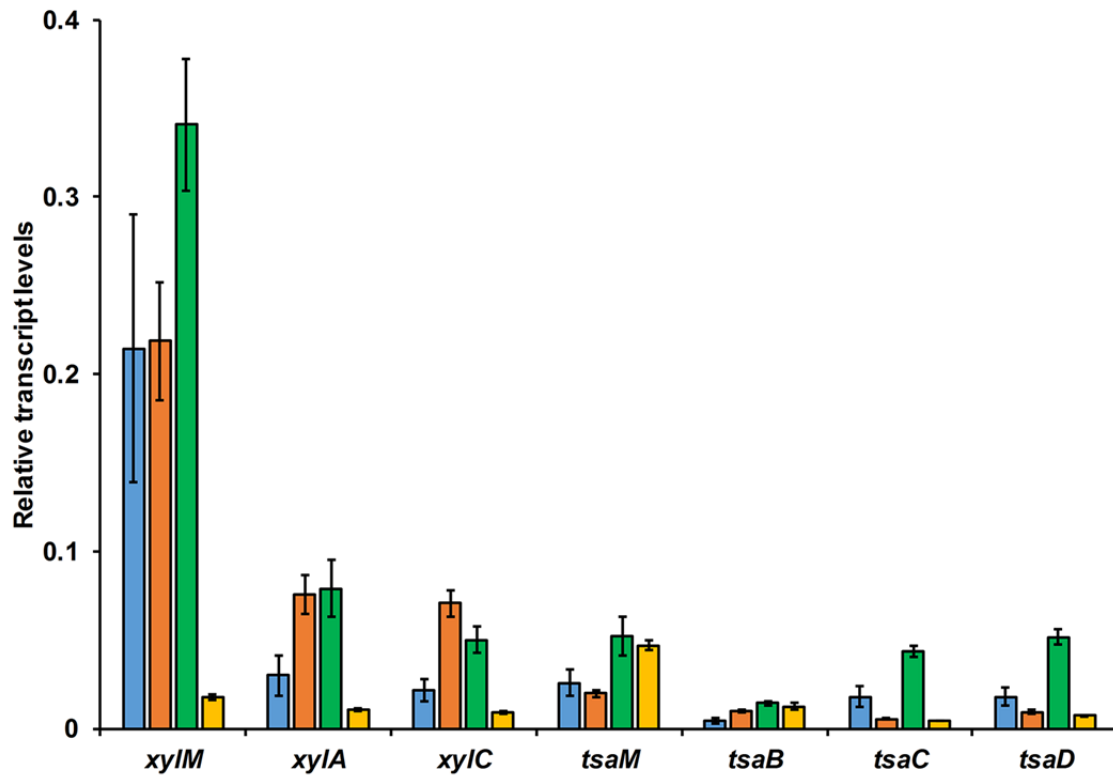
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23 **Supplementary Figure 3. Characterization of each steps of conversion by whole-cell activity**
 24 **assay against recombinant *E. coli* cells.** Recombinant cells employed are *E. coli* DH5 α
 25 harboring (a) pTrcMA, (b) pTrcC, (c) pTrcMB, (d) pTacCD, (e) pTrcMAC, and (f) pTacMBCD
 26 in the reaction mixtures containing substrates *pX*, *pTALD*, *pTA*, 4-CBAL, *pX* and *pTA*,
 27 respectively. The concentrations of substrates added in the reaction mixture are specified in

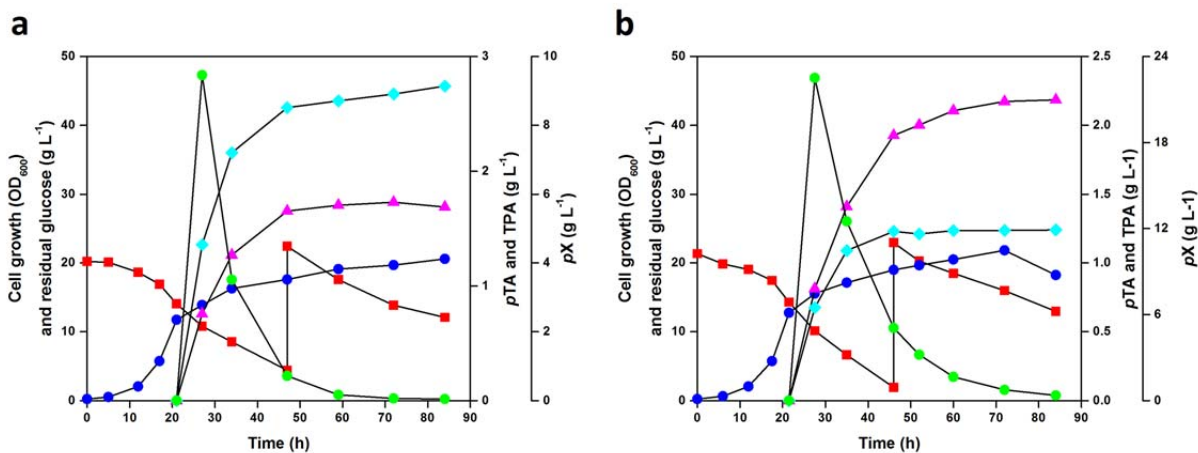
Methods section, while the substrate concentrations shown in above figures are actually measured values. It should be noted in **(b)** that the initial substrate (*p*TALD) concentration (118 mg L⁻¹) is much lower than that (200 mg L⁻¹) added in the reaction mixture. This is due to the time delay in measuring the concentrations in carrying out multiple reactions, while the conversion reaction of *p*TALD to *p*TALC is quite fast. The *p*X consumption profile is not shown owing to its insolubility in aqueous solution. Data for the wild-type strain as a control are not shown. Symbols are: blue diamond, *p*TALC; red rectangle, *p*TALD; solid green triangle, *p*TA; purple circle, 4-CBAL; open brown triangle, TPA.



Supplementary Figure 4. Schematic for the designed flask cultivation setup for *pX* to TPA conversion. The screw-cap flask (250 mL) containing 50 mL of culture medium is connected with a small attached vial into which *pX* is loaded and comes into contact with culture medium by evaporation.

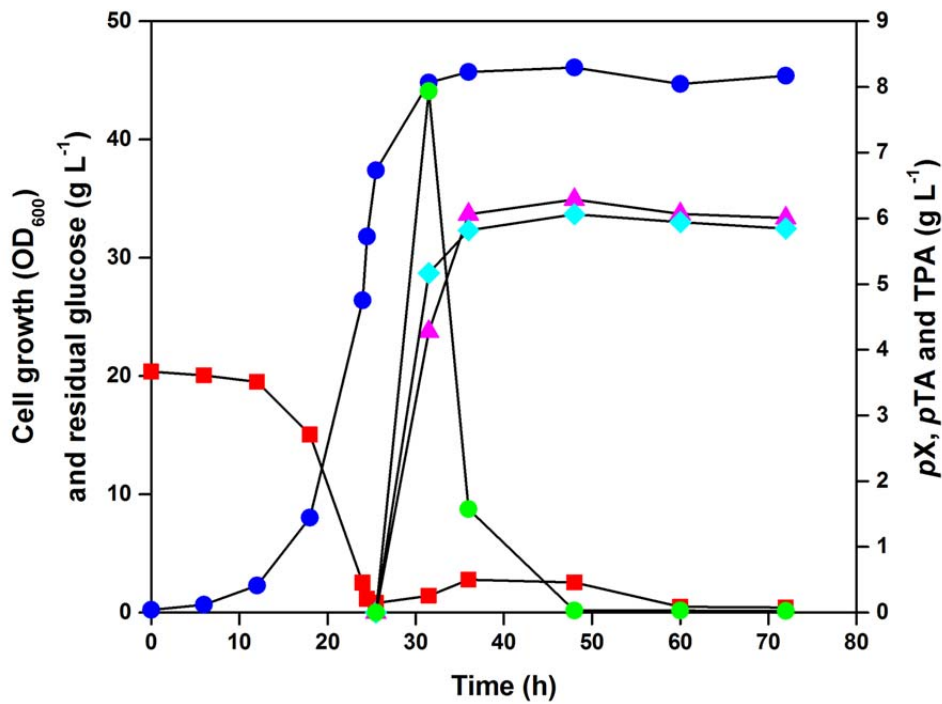


Supplementary Figure 5. Relative transcript levels of *xylM*, *xylA*, *xylC*, *tsaM*, *tsaB*, *tsaC* and *tsaD* in *E. coli* DH5 α cells harboring plasmids corresponding to Pi (blue box), Pii (orange box), Piii (green box) and Piv (yellow box), respectively, in the exponential phase. Values and error bars represent the mean and s.d. of triplicate experiments.

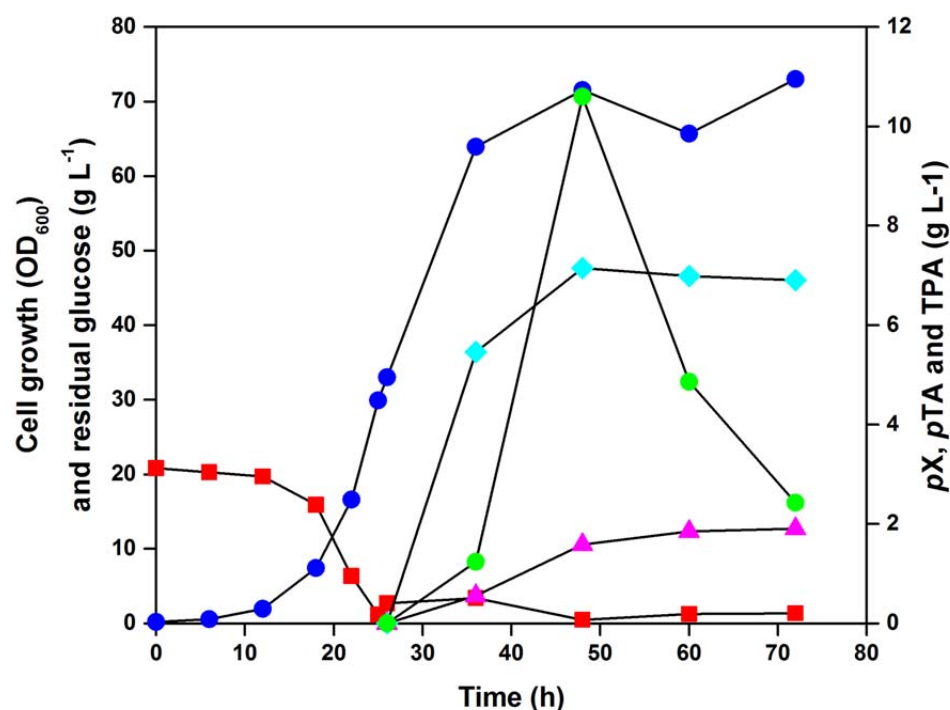


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73 **Supplementary Figure 6. Preliminary two-phase partitioning fermentation profiles of *E.***
 74 ***coli* DH5a harboring Piii for TPA production from 10 g (a) and 20 g (b) *pX*, respectively.**
 75 Symbols are: blue circle, cell growth (OD_{600}); red rectangle, residual glucose concentration ($g L^{-1}$);
 76 pink triangle, pTA concentration ($g L^{-1}$); light blue diamond, TPA concentration ($g L^{-1}$); green
 77 circle, residual pX concentration in oleyl alcohol ($g L^{-1}$).



Supplementary Figure 7. Two-phase partitioning DO-stat fed-batch fermentation profile of *E. coli* DH5α harboring Piii for TPA production from 20 g pX. Symbols are: blue circle, cell growth (OD₆₀₀); red rectangle, residual glucose concentration (g L⁻¹); pink triangle, pTA concentration (g L⁻¹); light blue diamond, TPA concentration (g L⁻¹); green circle, residual pX concentration in oleyl alcohol (g L⁻¹).



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88 **Supplementary Figure 8. Two-phase partitioning fermentation profile of *E. coli* DH5α**

89 **harboring Piii for TPA production from 20 g pX under DO-stat fed-batch conditions with**

90 **reduced pX feeding rate.** Symbols are: blue circle, cell growth (OD₆₀₀); red rectangle, residual

91 glucose concentration (g L⁻¹); pink triangle, pTA concentration (g L⁻¹); light blue diamond, TPA

92 concentration (g L⁻¹); green circle, residual pX concentration in oleyl alcohol (g L⁻¹).

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101 **Supplementary Table 1. Various chemical processes for TPA manufacturing from *p*X.**

Process	Commercialization	Reaction conditions	Characteristics	Reference
Amoco Process	Commercialized by BP	T: 175~225 °C; P: 15~30 bar; C: Co/Mn/Br; S: acetic acid O: air	Major TPA technology; Improved water recycling and liquid-solid separation methods	¹
INVISTA Process	Commercialized by DuPont	Same as Amoco Process conditions	Upgraded overall plant arrangement and solid-liquid separation techniques; Advanced residue recovery	²
Dow-Inca Process	Commercialized by Dow	Same as Amoco Process conditions	Novel high-pressure filtration and agitation system employed for enhanced mass transfer	³
Eastman Kodak Process	Commercialized by Eastman Kodak	T: 120~175 °C; P: 7.5~15 bar; C: Co/acetaldehyde; S: acetic acid O: air	High purity up to 99%; With acetic acid co-produced; No requirement of special materials for reactor coating due to replacement of bromide	⁴
Toray Process	Commercialized by Toray	T: 110~140 °C; P: 30 bar C: Co/paraldehyde; S: acetic acid O: air	High purity up to 99%; No requirement of special materials for reactor coating due to replacement of bromide	⁵
Teijin Process	Commercialized by Teijin	T: 100~130 °C; P: 10 bar C: Co S: acetic acid O: air	High purity up to 99%; No requirement of special materials for reactor coating due to omitting of bromide	⁶
Garcia-Verdugo's process	Not commercialized	T: 380 °C; P: 220 bar; C: MnBr ₂ ; S: water O: H ₂ O ₂	Simultaneously oxidizing xylene isomers in a continuous mode in supercritical water; Considerable reduction in overall energy input; Simplified downstream purification	⁷

Supplementary Table 1 (continued). Various chemical processes for TPA manufacturing from *p*X.

Process	Commercialization	Reaction conditions	Characteristics	Reference
Tashiro's process	Not commercialized	T: 100~150 °C; P: 20~30 bar; C: NHPI/Co/Mn; S: acetic acid O: air	Combined catalytic system with NHPI; Promoted oxidation leading to high product yield without any halogen ion	⁸
Li's process	Not commercialized	T: 200 °C; P: 15 bar; C: Co/Mn/Br; S: acetic acid O: O ₂ /CO ₂	Spray reactor system employed; Dramatic reduction in byproduct formation compared to conventional stirred reactor system	⁹

122 **Supplementary Table 2. Technologies for producing *p*X from bio-based resources.**

Company	Process description	Commercialization status	Reference
Gevo	Biomass-derived isobutanol by yeast fermentation, followed by chemical conversion to <i>p</i> X	Pilot facility in operation	¹⁰
Anellotech	Catalytic fast pyrolysis of lignocellulosic biomass to BTX, tradenamed Bio-TCAT TM	Pilot facility in operation	¹¹
Virent	Aqueous phase catalytic reforming of biomass to hydrocarbons including BTX, tradenamed BioForming TM	Pilot facility in operation	¹²
Ensyn	Fast pyrolysis of biomass to BTX, tradenamed Rapid Thermal Process TM	Pilot scale in operation	¹³
Micromidas	Dehydration of C6 carbohydrates to CMF, hydrogenolized to DMF, followed by [4+2] Diels-Alder cycloaddition with ethylene to produce <i>p</i> X	Pilot facility in operation	¹⁴
University of North Carolina at Chapel Hill	Bio-based ethylene conversion to <i>p</i> X	Not available	¹⁵

123 BTX, benzene, toluene and xylene; CMF, 5-chloromethylfurfural; DMF, 2,5-dimethylfuran;
 124 FDCA, 2,5-furandicarboxylic acid.

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Supplementary Table 3. Earlier studies on microbial conversion of *pX* into TPA.

Strains	Origin of isolation	Bioavailability of <i>pX</i>	TPA titer	Reference
Isolate IR10 that likely belongs to <i>Burkholderia</i>	Industrial wastewater bioreactor that is used to treat a waste stream containing <i>pX</i> , <i>pTA</i> and TPA	Bioavailable	89.0 mg L ⁻¹	16, 17
A mixture of <i>Stenotrophomonas maltophilia</i> and <i>Comamonas testosterone</i>	Soil	Bioavailable	5 mg L ⁻¹	18, 19

Supplementary Table 4. Candidates for xylene monooxygenase (XMO), benzyl alcohol dehydrogenase (BADH) and benzaldehyde dehydrogenase (BZDH), respectively.

Name	Size (aa)	Organism	Identity (%)	Accession No.
XMO				
XylM	376	<i>P. putida</i> F1	100	ABQ79033
	369	<i>P. putida</i> mt-2, pWW0	37.2	CAC86827
	375	<i>P. chlororaphis</i>	81.6	AAC27438
	367	<i>Novosphingobium aromaticivorans</i> , pNL1	34.2	ABP64090
XylA	349	<i>P. putida</i> F1	100	ABQ79032
	350	<i>P. putida</i> mt-2	41.1	CAC86826
	346	<i>N. aromaticivorans</i> , pNL1	42.7	ABP64091
BADH				
XylB	377	<i>P. putida</i> DOT-T1E	100	AFO51333
	366	<i>P. putida</i> mt-2, pWW0	53.6	CAC86825
	371	<i>Acinetobacter pittii</i> PHEA-2	52.5	ADY81899
	365	<i>N. aromaticivorans</i> , pNL1	52.0	ABP64142
	371	<i>Acinetobacter calcoaceticus</i>	50.4	AAC32671
BZDH				
XylC	492	<i>P. putida</i> KT2440	100	AAN67564
	487	<i>P. putida</i> mt-2	31.0	CAC86828
	501	<i>N. aromaticivorans</i> , pNL1	40.6	ABP64093
	484	<i>A. calcoaceticus</i>	32.6	AAC32670

169 **Supplementary Table 5. Strains and plasmids used in this study.**

Name	Relevant genotype	Reference
<i>E. coli</i> Strain		
DH5 α	F ⁻ <i>endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20 ϕ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17(<i>r</i>_K⁻ <i>m</i>_K⁺), λ⁻</i>	Invitrogen
Plasmids		
pTrc99A	Ap ^R , <i>trc</i> promoter, pBR322 origin, <i>lacI</i> ^q , 4.2-kb	Lab stock
pTac15K	Km ^R , <i>tac</i> promoter, p15A origin, 4.0-kb	Lab stock
pTrcMA	pTrc99A derivative containing <i>xylM</i> , <i>xylA</i> from <i>P. putida</i> F1	This study
pTrcC	pTrc99A derivative containing <i>xylC</i> from <i>P. putida</i> KT2440	This study
pTrcMAC	pTrcMA derivative containing <i>xylC</i> from <i>P. putida</i> KT2440	This study
pTrcMB	pTrc99A derivative containing <i>tsaM</i> , <i>tsaB</i> from <i>C. testosteroni</i> T-2	This study
pTacCD	pTac15K derivative containing <i>tsaC</i> , <i>tsaD</i> from <i>C. testosteroni</i> T-2	This study
pTacMBCD	pTacCD derivative containing <i>trc</i> - <i>tsaMB</i> -terminator cassette	This study
pTrcMAC-MBCD	pTrcMAC derivative containing RBS- <i>tsaMB</i> and RBS- <i>tsaCD</i> cassettes	This study
pTacMAC	pTac15K derivative containing <i>xylMA</i> - <i>xylC</i> from pTrcMAC	This study
pTrcMBCD	pTrcMB derivative containing RBS- <i>tsaCD</i>	This study

Abbreviations: Ap, ampicillin; Km, kanamycin; R, resistance.

Primer name	Sequence 5'-3'
pTrc(xylMA)-f	CGGACGGCCGGAACCTCCTAAGGAATTCGAGCTCGGTACCC
pTrc(xylMA)-r	TACTTGATGTATTCCACATATGGTCTGTTTCCTGTGTGA
xylMA(pTrc)-f	TCACACAGGAAACAGACCATATGTGGGAATACATCAAGTACT AC
xylMA(pTrc)-r	GGGTACCGAGCTCGAATTCCTTAGGAGTTCCGGCCGTCCG
pTrc(xylC)-f	CGACCATCATCCGCATTTGAGGAATTCGAGCTCGGTACCC
pTrc(xylC)-r	TACTGGCAAATACCGCCATATGGTCTGTTTCCTGTGTGA
xylC(pTrc)-f	TCACACAGGAAACAGACCATATGGCGGTATTTGCCAGTGA
xylC(pTrc)-r	GGGTACCGAGCTCGAATTCCTCAAATGCGGATGATGGTCG
xylC(pTrcMA)-f	GAACCTCCTAAGGAATTCGAGCTCGGTACCCGGGATGGCGGTA TTTGCCAG
xylC(pTrcMA)-r	CAGCCAAGCTTGCATGCCTGCAGGTCGACTCTAGAGTCAAAT GCGGATGATGGTC
tsaMB-f	AGACAGGAATTCATGTTTCATCCGCAATTGCTGG
tsaMB-r	AGACAGGGATCCTCAGATGTCCAGGACCAGC
tsaCD-f	AGACAGGAATTCATGAACCTGAACAAACAAGTGG
tsaCD-r	AGACAGCTGCAGTCAGGCCACGTAGTGCATGAAC
trcstaMB-f	GGTGATGCCGGCCACGATGCGTCCGGCGTAGAGTTGACAATT AATCATCC
trcstaMB-r	GAAGCATTGGTGCACCGTGCAGTCGATAAGCTCCGAAGAGT TTGTAGAAACG
RBStsaMB-f	CATCATCCGCATTTGACTCTAGAGTCGACCTGCATCACACAG GAAACAGAC
RBStsaMB-r	GAATTCTGTTTCCTGTGTGATCAGATGTCCAGGACCAGCC
RBStsaCD-f	GGCTGGTCCTGGACATCTGATCACACAGGAAACAGAATTC
RBStsaCD-r	GCCAAAACAGCCAAGCTTGCATGCCTGCATCAGGCCACGTA GTGCA
xylMAC(pTac)-f	TGAGCGGATAACAATTTACACACAGGAAACAGCCATATGTGGG AATAC
xylMAC(pTac)-r	ACTCTAGAGGATCCCCGGGTACCGAGCTCGTCAAATGCGGAT GATG
RBStsaCD(pTrcMB)-f	GCGGCGGCGGGCTGGTCCTGGACATCTGAGTCACACAGGAA ACAGAATTC
RBStsaCD(pTrcMB)-r	AGCTTGCATGCCTGCAGGTCGACTCTAGAGTCAGGCCACGTA GTGCATG
xylM(RT)-f	CCTGGTACAAGTCTTTGC
xylM(RT)-r	CAGCATGTCAAGGATGG
xylA(RT)-f	TTGCTGCTGATTTCTGCGAG
xylA(RT)-r	ACTCTTTCCAACGGTATGCC
xylC(RT)-f	ACCGTCTACACCAACGTCAAC
xylC(RT)-r	ACCGCCTCGTTTTCTGTCCTTG
tsaM(RT)-f	CCAAGACCTGCATCAAGAGC
tsaM(RT)-r	CAGAAGTAATCGACGATGTC
tsaB(RT)-f	GGTGTGGATTACGAAAAGC

188 **Supplementary Table 6 (continued). Oligonucleotides used in this study.**

Primer name	Sequence 5'-3'
tsaB(RT)-r	ATGGCGTAGATGGGCGTGAC
tsaC(RT)-f	ATGGCGACCGAACTCAAC
tsaC(RT)-r	CGTTGTTACACGATGTG
tsaD(RT)-f	CATGGAGGTGTTCAAGTTC
tsaD(RT)-r	TTGAGCACGATGGAACAACC

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