Electronic Supplementary Material (ESI) for Green Chemistry.

Supplementary Information

Microbial synthesis of vanillin from waste poly(ethylene terephthalate)

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Contents

1 General materials and methods 3

2 Cloning 5

2.1 pVan1 and pVan2 plasmid construction 5

2.2 Plasmid maps of pVan1 and pVan2 10

3 Experimental methods 10

3.1 Co-expression of pVan1, pVan2 and pSfp in *E. coli* RARE 10

3.2 Whole cell biotransformation of TA to vanillin 10

3.3 Preparation of semi-purified LCC WCCG 11

3.4 One-pot preparation of vanillin from post-consumer poly(ethylene) terephthalate 11

3.5 HPLC sample preparation from biotransformation reactions 12

4 Supplementary data 13

4.1 Exemplar HPLC chromatograms 13

4.2 UV absorbance spectrum of vanillin 14

4.3 Mass spectrometry of biotransformation sample 14

4.4 Optimisation of vanillin pathway expression conditions 15

4.5 Additives screen 16

4.6 SDS-PAGE analysis of protein expression 17

4.7 Reaction vessel headspace 18

4.8 L-Met 18

4.9 Biotransformation buffer pH 19

4.10 Biotransformation OD 20

4.11 Toxicity of TA and vanillin to *E. coli* RARE_pVan 21

4.12 Substrate concentration 22

4.13 NMR Spectroscopy 22

4.14 LCC catalysed PET degradation 24

4.15 HPLC standard curves 25

5 References 25

1 General materials and methods

Unless otherwise stated, starting materials and reagents were obtained from commercial suppliers and were used without further purification. All water used experimentally was purified with a Suez Select purification system (18 MΩ.cm, 0.2 µM filter). The following analyte abbreviations are used throughout: protocatechuate (PC), 2, 4-dihydroxybenzaldehyde (DHBAl), vanillic acid (VA), terephthalate (TA), benzyl alcohol (BnOH), ferrous sulfate (FS), ferric citrate (FC), ferric ammonium citrate (FAC). Disodium terephthalate was used for all experiments using TA.

NMR: Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded using a AVA500 NMR spectrometer at the specified frequency at 298 K. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent. NMR solvents were used as purchased from commercial suppliers.

HPLC: High performance liquid chromatography (HPLC) analysis was carried out using a Thermo Scientific Dionex UltiMate 3000 Series UHPLC instrument and a HyperSil Gold C18 column (150x3 mm x 3 µm). Analytes were detected at 206 nm and quantified by comparison to a caffeine internal standard, added to 50 µM final concentration. All HPLC solvents were purchased from commercial suppliers. Samples were analyzed using the following method:

Solvent A: Water+0.1% v/v trifluoroacetic acid (TFA)
Solvent B: Acetonitrile+0.1% v/v TFA

Time (min)	Flow rate (mL/min)	%B
0 to 4	0.4	5
4 to 20	0.4	5 to 30
21 to 30	0.4	5

Molecular Biology: All synthetic genes were codon-optimised for *E. coli* BL21(DE3) and synthesised using GeneArtTM (Thermo Scientific). Oligonucleotide primers were synthesised by Integrated DNA Technologies. Gene products from PCR were gel purified with a Zymoclean Gel DNA Recovery Kit (Zymo Research), eluting in 10-20 µL sterile Milli-Q H_2O. Recombinant plasmid DNA was purified with a Miniprep Kit (Qiagen) from *E. coli* DH5α. Plasmid pET22b(+) was kindly provided by Prof. Chris French (University of Edinburgh). Plasmids pET21a-NiCAR and pCDF1b-sfp were kindly provided by the laboratory of Prof. Nicholas Turner (University of Manchester). pRSFDuet-1 was purchased from Novagen. All restriction enzymes were purchased from Thermo Fisher as FastDigest™ enzymes. All restriction enzyme digests were carried out at 37 °C using FastDigest™ Green buffer. All plasmids were sequenced by Sanger sequencing at Edinburgh Genomics (Edinburgh, UK). OneTaq 2X premix (New England Biolabs) was used for all colony PCR reactions. Phusion High-Fidelity DNA

Polymerase (New England Biolabs) was used for all other PCR reactions, which contained 0.5 μM forward and reverse primers, 200 μM each dNTP, 10-20 ng template DNA and 1X HF buffer. T4 DNA ligase (Thermo Scientific) was used for all ligation reactions.

Colony PCR reactions were performed using the following conditions: initial denaturation (95 °C, 10 minutes), 35 thermocycles (20 seconds denaturation at 95 °C, annealing at 50-65 °C for 30 seconds, and extension at 68 °C for 60 s/kb), and final extension (68 °C for 10 minutes). All standard Phusion PCR reactions were performed using the following conditions: initial denaturation (98 °C, 30 seconds), 30 thermocycles (15 seconds denaturation at 98 °C, annealing at 50-72 °C for 20 seconds, and extension at 72 °C for 30 s/kb), and final extension (72 °C for 7 minutes). For agarose gel electrophoresis, agarose (1% w/v) TAE gels containing a 1 kB GeneRuler ladder (Thermo Scientific) were run at 100 V for 40 minutes and visualised using SYBR SafeTM. For SDS-PAGE, 12-well 12% acrylamide Bis-Tris NuPAGE gels (Thermo Scientific) containing an unstained Precision plus standard ladder (BioRad) were used to analyse samples. Gels were run in 1X MES buffer (Novagen) at 50 V for 30 minutes followed by 150 V for 2 hours. Optical densities of *E. coli* cultures were determined using a DeNovix DS-11 UV/Vis spectrophotometer by measuring absorbance at 600 nm. Unless stated otherwise, *E. coli* cells were cultured at 37 ˚C with shaking at 220 rpm in an incubator shaker with a 5.1 cm orbit throw.

All chemically competent cells were prepared via treatment with calcium chloride[1]. For use in protein expression and cloning, chemically competent *E. coli* BL21(DE3), *E. coli* RARE (RARE genotype: MG1655(DE3) Δ*dkgB* Δ*yeaE* Δ*yqhC* Δ*yqhD* Δ*dkgA* Δ*yahK* Δ*yjgB* Δ*endA* Δ*recA*[2]) and *E. coli* DH5α cells were prepared and transformed with an appropriate plasmid via heat-shock at 42 ˚C for 45 seconds. Cells were recovered in 1 mL of SOC media for 1 hour at 37 ˚C. Transformants were selected by plating on LB agar containing appropriate antibiotics and incubating at 37 ˚C (*E. coli* BL21(DE3) and *E. coli* DH5α) or 30 ˚C (*E. coli* RARE) overnight.

Media recipes

Lysogeny Broth (LB) Medium: Bacto-tryptone (10 g/L), yeast extract (5 g/L) and NaCl (10 g/L) were dissolved in Milli-Q H_2O. LB was autoclaved at 121 ˚C for 20 min, cooled and stored at room temperature. LB agar was made using the same recipe but with the addition of agar (15 g/L).

Terrific Broth (TB) Medium: Yeast extract (24 g/L), tryptone (20 g/L), glycerol (4 mL/L), potassium phosphate buffer (72 mM K_2HPO_4; 12.5 g/L) were dissolved in in 1 L Milli-Q H_2O. TB was autoclaved at 121 ˚C for 20 min, cooled and stored at room temperature.

2XTY Autoinduction Medium (AIM): 2XYT AIM including trace elements (Formedium™) (45.85 g/L) was dissolved in 1 L Milli-Q H_2O and was autoclaved at 121 ˚C for 20 min, cooled and stored at room temperature.

M9 Minimal Media (M9-Glucose, M9-Glycerol and M9-CA) stock solutions: A 5X stock solution of M9 salts containing $Na_2HPO_4 \cdot 12H_2O$ (85.5 g/L), KH_2PO_4 (15 g/L), NH_4Cl (5 g/L) and NaCl (2.5 g/L) was prepared and autoclaved at 121 ˚C for 20 minutes, cooled and stored at room temperature. Stock solutions of 20% w/v glucose and 20% w/v glycerol were prepared and autoclaved at 121 ˚C for 20 minutes, cooled and stored at room temperature. Stock solutions of $MgSO_4$ (1 M), $CaCl_2$ (50 mM), thiamine hydrochloride (50 mg/mL) and casamino acids (10% w/v) were prepared and filter sterilized.
M9-Glucose: For 1 L media: 5X M9 salts (200 mL, 1X final concentration), $MgSO_4$ (2 mL, 2 mM final concentration), $CaCl_2$ (2 mL, 0.1 mM final concentration), thiamine chloride (0.8 mL, 40 µL/mL final concentration) and glucose (25 mL, 0.5% w/v final concentration) were combined and sterile water was added to a final volume of 1 L.
M9-Glycerol: For 1 L media: 5X M9 salts (200 mL, 1X final concentration), $MgSO_4$ (2 mL, 2 mM final concentration), $CaCl_2$ (2 mL, 0.1 mM final concentration), thiamine chloride (0.8 mL, 40 µL/mL final concentration) and glycerol (25 mL, 0.5% w/v final concentration) were combined and sterile water was added to a final volume of 1 L.
M9-CA: For 1 L media: 5X M9 salts (200 mL, 1X final concentration), $MgSO_4$ (2 mL, 2 mM final concentration), $CaCl_2$ (2 mL, 0.1 mM final concentration), thiamine chloride (0.8 mL, 40 µL/mL final concentration), glycerol (25 mL, 0.5% w/v final concentration) and casamino acids (20 mL, 0.2% w/v) were combined and sterile water was added to a final volume of 1 L.
Biotransformation buffer: For 1 L media: M9-Glucose was prepared as described above, making up to 800 mL with sterile water. L-Methionine was added to a final concentration of 10 mM, *n*-butanol was added to a final concentration 1% v/v and the buffer was adjusted to pH 5.5 with aq. HCl. Sterile water was added to a final volume of 1 L and the buffer was stored at 4 °C and used within 1 week.

2 Cloning

2.1 pVan1 and pVan2 plasmid construction

Synthetic gene strings for TPADO (UniProt IDs Q3C1E0, Q3C1D5 and Q3C1D4), DCDDH (UniProt ID Q3C1E1) and S-COMT (UniProt ID: P22734-2) were synthesized using GeneArt™ (Thermo Scientific) (Table S1) and were cloned into pUC19 vectors using Seamless Ligation Cloning Extract (SLiCE) cloning and sequenced prior to use for construction of pVan1 and pVan2. SLiCE cloning mix was prepared from unmodified *E. coli* JM109 and used according to the published protocol[3]. All SLiCE cloning was designed with 20-25 bp overlaps using a 1:2 vector:insert or 1:2:2 vector:insert-1:insert-2 ratio.

pVan1

An operon encoding TphA1, TphA2, TphA3 and DCDDH was designed using the Salis lab operon calculator tool, with a target protein translation level of 15 000 (au)[4,5]. The operon was manually split

into 4 DNA strings which were ordered as gene strings, each encoding an RBS, pathway gene and overlap sequences for cloning. Pathway genes were amplified by PCR using the primers shown in Table S2 (entries 3-10) and purified by gel electrophoresis and extraction. Adjacent pairs of genes (i.e. (1) TphA1+TphA2 and (2) TphA3 and DCDDH) were concatenated by overlap extension PCR (OE PCR) using primer pairs (1) TphA1 fwd + TphA2 rev and (2) TphA3 fwd + DCDDH rev. OE PCR products were purified by gel electrophoresis and extraction and ligated into a linearized pET21a(+) vector using SLiCE cloning. Clones harboring the desired insert were identified by colony PCR using T7 forward and reverse primers and verified by Sanger sequencing using pVan1 sequencing primers (Table S2, entries 15 to 20).

pVan2

pVan2 was constructed using the commercially available pRSFDuet-1 vector. S-COMT was amplified from the gene string using S-COMT fwd and rev (Table S2, entries 11-12) and the PCR product was purified by gel electrophoresis and extraction. pVan2 was assembled by the sequential ligation of (1) NiCAR (restriction digest with NcoI and NotI) and (2) S-COMT (restriction digest with NdeI and XhoI) into the two multiple cloning sites of pRSFDuet-1. The final construct was confirmed by Sanger sequencing using pVan2 sequencing primers (Table S2, entries 20-25). pCFD1b-Sfp was used as received without further modification.

Table S1. Nucleotide sequences of synthesized gene strings used in this study. Blue: 5' and 3' cloning sequences for ligation into linearized pUC19. Red: RBS sequence. Yellow highlight: start codon.

Name	Nucleotide sequence (5' to 3')
TphA1	GGCCAGTGAATTCGAGCTCGGTACTTCGCGAGATACAGGGGAGCGCTAAA TGAATCATCAGATACATATTCACGATTCGGATATAGCATTTCCTTGCGCACC CGGGCAATCTGTTTTGGATGCGGCTCTGCAAGCAGGCATAGAATTGCCATA CAGCTGCCGTAAAGGTTCCTGTGGCAACTGCGCATCAGCCCTATTGGATGG GAATATTACGTCTTTTAATGGCATGGCTGTCCGATCGGAGCTGTGCACATC AGAGCAAGTACTCTTGTGTGGCTGCACTGCTGCATCAGATATCAGAATTCA ACCGAGCAGCTTTCGGAGGCTGGACCCGGAAGCTCGCAAGAGGTTTACTGC AAAGGTGTACTCCAATACTCTGGCGGCACCGGATGTGTCTCTCCTCCGTCTC AGGTTACCAGTTGGCAAGCGAGCGAAATTCGAAGCGGGCCAATATTTACTT ATTCACCTTGATGATGGCGAATCACGGTCATACAGTATGGCCAATCCGCCC CACGAGAGCGATGGGATAACGCTTCACGTACGCCACGTTCCTGGCGGACG ATTCTCCACAATAGTGCAACAACTGAAAAGCGGCGATACATTGGAAATTGA GCTGCCCTTTGGCTCGATTGCGCTCAAACCGGATGATACGAGGCCACTGAT ATGCGTTGCTGGGGGTACGGGTTTTGCGCCAATTAAATCAGTTCTCGACGA TTTAGCTAAGCGAAAAGTTCAGCGCGACATCACCTTGATCTGGGGCGCCAG GAATCCTAGCGGCTTATACTTGCCTAGCGCGATCGACAAGTGGAGGAAAAC ATGGCCACAGTTCCGATACATTGCGGCAATCACAGACTTGGGCAACGTTCC GGCAGACGCGCACGCTGGACGTGTTGACGATGCCTTGCGCACCCACTTTGG AAACCTTCACGATCATGTTGTTCATTGCTGCGGGTCGCCTAGCCTGGTTCAA TCGGTCCGGACCGCTGCCAGCGACATGGGACTTTTGGCCCAGAATTTTCAC GCAGACGTGTTTGCAACCAGCCCGACGGGTTCCCATTAAGATCCTCTAGAG TCGACCTGCAGG
TphA2	GGCCAGTGAATTCGAGCTCGGTACTCCCGCAATCCGTTAAGGAGGGTAGAT GCAAGAGTCAATTATCCAGTGGCATGGTGCAACAAACACTCGGGTACCATT TGGCATCTATACTGACACTGCCAATGCAGATCAAGAGCAACAGAGGATCTA TCGGGGGGAGGTCTGGAACTATTTGTGTCTGGAGTCAGAAATCCCCGGAGC GGGAGATTTCCGAACAACCTTCGCGGGAGAAACGCCAATTGTAGTTGTTCG CGACGCTGATCAAGAAATCTACGCCTTTGAAAACCGGTGTGCCCACCGCGG AGCACTGATCGCGTTGGAGAAGAGTGGAAGGACGGACTCTTTCCAATGCGT GTATCATGCCTGGTCGTACAACCGTCAAGGCGATCTGACCGGCGTTGCGTT TGAAAAAGGGGTCAAGGGCCAAGGTGGTATGCCGGCCTCCTTTTGCAAGG AAGAGCACGGGCCGCGAAAGCTCAGGGTCGCTGTGTTCTGCGGACTTGTCT TTGGTTCATTTAGCGAGGACGTACCCTCGATCGAGGATTATCTGGGTCCTG AAATCTGCGAACGCATAGAGCGCGTTCTGCACAAACCCGTAGAGGTCATA GGCCGGTTCACGCAGAAGCTGCCTAACAATTGGAAACTTTACTTTGAGAAC GTAAAAGATTCCTACCACGCATCTCTCCTACATATGTTTTTTACGACATTTG AGCTCAACCGGTTATCACAAAAGGGGGGAGTAATTGTGGATGAGTCAGGC GGGCATCATGTATCATACTCTATGATCGATCGCGGTGCCAAAGACGATTCT TACAAAGATCAAGCGATCAGGAGCGATAACGAACGGTACCGGCTCAAAGA CCCTTCATTACTCGAAGGTTTCGAAGAATTCGAGGACGGCGTAACTCTTCA GATCTTGTCCGTTTTCCCAGGCTTTGTACTTCAACAGATTCAGAACAGCATA GCAGTTAGACAACTGCTGCCTAAATCGATCAGTAGTAGCGAATTGAACTGG ACATACCTAGGCTATGCTGACGATTCGGCTGAGCAGAGAAAAGTCCGGCTG AAACAGGCCAACCTCATAGGTCCAGCAGGGTTTATCTCTATGGAAGATGGT GCAGTTGGCGGCTTCGTTCAGCGCGGGATTGCTGGGGCCGCCAATCTCGAT GCCGTTATCGAGATGGGCGGTGACCACGAAGGCTCCTCAGAGGGACGAGC GACAGAAACAAGCGTAAGAGGGTTTTGGAAAGCCTATCGTAAACATATGG GCCAAGAAATGCAAGCATAATCTAGAGTCGACCTGCAGG
TphA3	GGCCAGTGAATTCGAGCTCGGTACTACGAATACAATACCGGGGTCTTCAAT GATAAACGAAATACAGATAGCCGCCTTTAATGCGGCCTATGCGAAAACGA TTGATAGTGATGCCATGGAACAGTGGCCCACGTTCTTCACCAAGGATTGCC

	ATTACTGCGTGACGAACGTGGATAACCACGATGAGGGCCTGGCTGCCGGA ATCGTGTGGGCTGACTCACAAGATATGTTGACTGACAGAATTTCCGCATTA CGCGAAGCAAACATTTACGAGCGGCATCGTTATCGGCATATCCTCGGATTA CCATCGATTCAATCTGGAGACGCGACGCAAGCGTCCGCGTCTACTCCGTTC ATGGTCCTTCGAATTATGCACACGGGTGAGACAGAAGTCTTTGCCAGCGGG GAATACTTAGACAAATTTACCACAATTGATGGCAAGCTCCGCTTACAAGAA CGAATTGCGGTCTGTGATTCAACGGTCACCGACACTCTGATGGCACTGCCT CTGTAATCTAGAGTCGACCTGCAGG
DCDD H	GGCCAGTGAATTCGAGCTCGGTACGTTACCTCAGTCAGGAGGATCTTTTATG ACAATTGTGCACCGGCGCTTAGCACTCGCGATCGGTGATCCACATGGCAT AGGCCCTGAAATCGCCTTGAAGGCTCTGCGACAACTCAGCGCGAATGAGC GATCCTTAATCAAAGTGTATGGTCCATGGAGCGCACTTGAACAGGCCGCTC AAATTTGCCAGATGGAATCTCTCCTCCAGGATCTGATACACGAAGAAGCTG GATCATTAGCGCAACCCGCGCAGTGGGGAGAAATCACCCCCCAGGCGGGA CTTAGCACTGTCCAGAGTGCGACTGCTGCCATTCGGGCGTGCGAGAATGGA GAAGTGGATGCTGTCATCGCATGCCCGCACCACGAAACTGCGATCCATCGC GCTGGTATAGCATTCAGCGGCTACCCGTCTCTGCTTGCCAACGTTCTTGGTA TGAACGAAGATCAGGTCTTTCTTATGCTTGTAGGGGCCGGCTTGCGCATCG TACACGTGACCCTCCATGAATCAGTAAGGAGCGCCTTGGAACGGTTATCGC CGCAGCTGGTTGTAAATGCAGTTCAGGCAGCAGTGCAGACGTGCACACTAT TGGGGGTTCCCAAACCACAAGTGGCCGTGTTCGGTATAAACCCACATGCGT CCGAAGGGCAACTGTTTGGGCTGGAAGACAGTCAAATCACCGCGCCGGCC GTTGAAACATTACGTAAGTGCGGACTCGCGGTCGACGGTCCTATGGGAGCC GACATGGTTTTGGCCCAACGCAAACATGATCTCTATGTGGCTATGCTGCAT GATCAAGGACACATACCGATTAAACTTTTGGCGCCGAATGGGGCCTCAGCT CTGTCTATAGGTGGGCGTGTGGTCTTGAGTTCCGTAGGCCACGGAAGCGCT ATGGACATAGCAGGTCGTGGTGTCGCCGATTCAACCGCGCTGTTGAGGACT ATAGCCCTATTGGGTGCGCAGCCCGGTTGATCTAGAGTCGACCTGCAGG
S- COMT	GGCCAGTGAATTCGAGCTCGGTACTTAAGTCCTGGAAAATAGTAGGCGGA AATTATGGGTGATACCAAAGAGCAGCGTATCCTGCGTTACGTTCAACAGAA CGCAAAGCCGGGTGACCCGCAGTCTGTACTGGAAGCTATCGACACCTACTG TACCCAGAAAGAATGGGCCATGAATGTTGGTGACGCGAAAGGTCAGATCA TGGACGCGGTGATCCGCGAGTACTCTCCATCCCTGGTTCTGGAACTGGGCG CTTATTGCGGTTATTCTGCAGTGCGTATGGCTCGTCTGCTGCAACCGGGTGC ACGTCTGCTGACTATGGAGATGAACCCGGATTACGCCGCGATTACCCAGCA GATGCTGAATTTCGCTGGCCTGCAGGACAAAGTAACTATCCTGAACGGTGC TAGCCAAGACCTGATCCCGCAACTGAAAAAAAAATATGATGTTGACACCCT GGATATGGTCTTCCTGGACCACTGGAAAGACCGCTATCTGCCGGACACCCT GCTGCTGGAAAAATGTGGTCTGCTGCGTAAAGGCACTGTGCTGCTGGCTGA CAACGTTATCGTCCCAGGCACGCCGGATTTCCTGGCATACGTTCGCGGCTC TTCCTCCTTCGAGTGTACGCACTACAGCTCTTATCTGGAACTGATGAAGGTG GTGGACGGCCTGGAAAAGGCAATTTATCAGGGTCCATCTTCCCCGGATAAA TCTTAAGATCCTCTAGAGTCGACCTGCAGG

Table S2. Primers used for the construction of pVan1 and pVan2.

Entry	Name	Sequence
1	pUC19 op. fwd	GATCCTCTAGAGTCGACCTGCAG
2	pUC19 op. rev	GTACCGAGCTCGAATTCACTGG
3	TphA1 fwd	TTCGCGAGATACAGGGGAGC
4	TphA1 rev	TACCCTCCTTAACGGATTGCGGGATTAATGGGAACCCGTCGGG
5	TphA2 fwd	TCCCGCAATCCGTTAAGGAGG
6	TphA2 rev	GAAGACCCCGGTATTGTATTCGTATTATGCTTGCATTTCTTGGCCC
7	TphB2 fwd	TACGAATACAATACCGGGGTCTTC
8	TphB2 rev	AAAGATCCTCCTGACTGAGGTAACTTACAGAGGCAGTGCCATCAGAG
9	DCDDH fwd	GTTACCTCAGTCAGGAGGATCTTTTATG
10	DCDDH rev	TCAACCGGGCTGCGCAC
11	S-COMT fwd	GTATAAGAAGGAGATATACATATGGGTGATACCAAAGAGCAG
12	S-COMT rev	ACCAGACTCGAGGGTACCGACGTTTAAGATTTATCCGGGGAAGATGG
13	pET21a fwd	CTATTGGGTGCGCAGCCCGGTTGACAAAGCCCGAAAGGAAGCTG
14	pET21a rev	TAGCGCTCCCCTGTATCTCGCGAAATTATTTCTAGAGGGGAATTGTTATCCGC
15	pVan1 seq1	GAATCCTAGCGGCTTATACTTGCCTAG
16	pVan1 seq2	GCGAACGCATAGAGCGC
17	pVan1 seq3	GTGATGCCATGGAACAGTGGC
18	pVan1 seq4	CTGCTTGCCAACGTTCTTGG
19	T7 fwd	TAATACGACTCACTATAGGG
20	T7 term. rev	GCTAGTTATTGCTCAGCGG
21	pVan2 seq 1	GGTGATTGTGGAAACCCTGGATG
22	pVan2 seq 2	CGATATTGTGGCGGAACTGGAAC
23	pVan2 seq 3	CCTGGAATGGCTGGAACGTC
24	pVan2 seq 4	CGGAACAGGATATTCCGCATCTG
25	DuetUP2 fwd	TTGTADACGGCCGCATAATC

2.2 Plasmid maps of pVan1 and pVan2

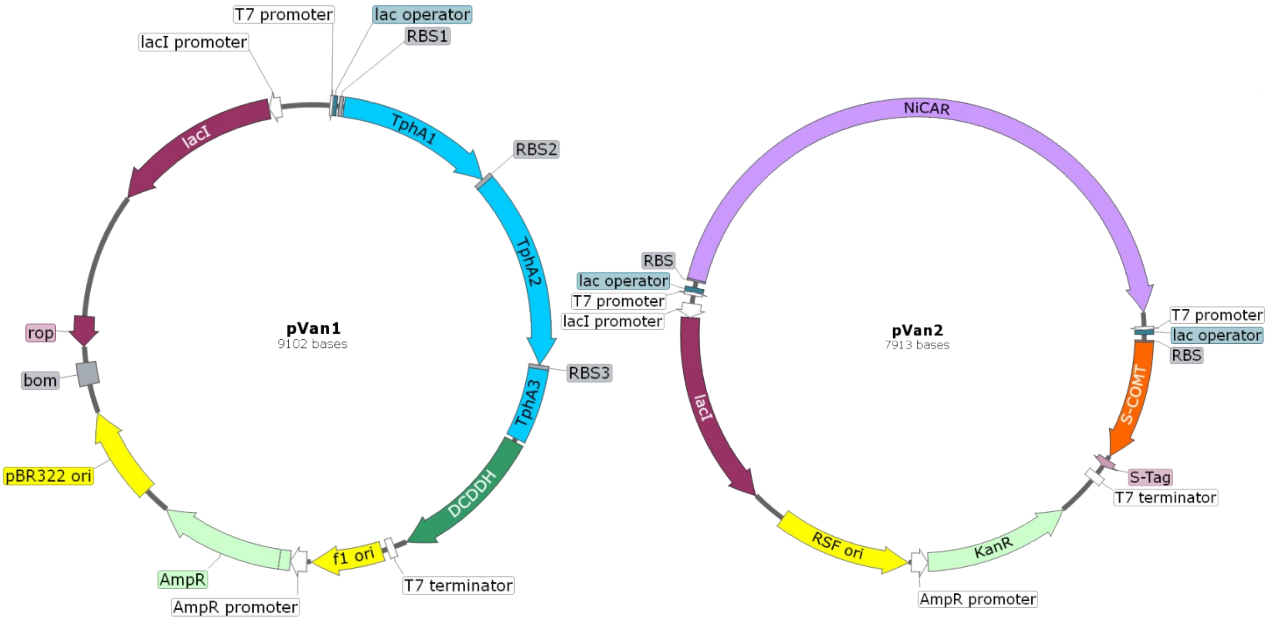


Figure S1. Plasmid maps of pVan1 and pVan2.

3 Experimental methods

3.1 Co-expression of pVan1, pVan2 and pSfp in *E. coli* RARE

Chemically competent *E. coli* RARE were co-transformed with pVan1, pVan2 and pSfp using the heat shock method. Positive transformants were selected for on Lysogeny Broth Agar (LBA) plates containing kanamycin (50 µg/mL), ampicillin (100 µg/mL) and streptomycin (100 µg/mL), which were incubated at 30 °C for 16-20 hours. The next day, a single colony was used to inoculate 10 mL Lysogeny Broth (LB) containing kanamycin (50 µg/mL), ampicillin (100 µg/mL) and streptomycin (100 µg/mL) and cultures were incubated with orbital shaking at 30 °C for 16 hours. Expression cultures were prepared by inoculating the desired culture volume (typically 300 mL or 1 L in a 1 L or 2.8 L baffled conical flask, respectively) of M9-CA media (see Section 1) containing kanamycin (40 µg/mL), ampicillin (80 µg/mL) and streptomycin (80 µg/mL) with 1.5% v/v overnight culture. The cultures were incubated with orbital shaking at 30 °C until OD_{600} = 0.2-0.3 and benzyl alcohol (BnOH) was added to a final concentration of 10 mM. The cultures were incubated at 30 °C for a further 30 minutes before adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 1 mM. Cultures were incubated at 30 °C for 24 hours before harvesting cells for biotransformation reactions. Typical harvest OD_{600} = 1.8-2.

3.2 Whole cell biotransformation of TA to vanillin

Freshly prepared cells from expression cultures were harvested by centrifugation (6118xg, 4 °C, 20 minutes) and the supernatant carefully removed. Cell pellets were resuspended in biotransformation

buffer (M9-Glucose containing 10 mM L-Met, 1% v/v *n*-BuOH, pH 5.5) to OD_{600}=80. A 500 µL aliquot of resuspended cells was added to a 50 mL Falcon™ tube per screening reaction. TA (added as the disodium salt) was added to a final concentration of 1 mM (from a 100 mM stock solution in biotransformation buffer) and, if using ISPR, oleyl alcohol (125 µL, 20% v/v) was added. Reactions were incubated at 22 °C for 24 hours and then analysed by HPLC using sample preparation Method 1 or Method 2 as specified for each experiment. All reactions were performed in triplicate, with the mean and standard deviation reported in each case.

3.3 Preparation of semi-purified LCC WCCG

A gene encoding LCC WGGC[6] was ordered in a pMA-RQ vector (GeneArt, ThermoFisher). The gene was cloned into pET22b(+) using XhoI and NdeI restriction sites and positive clones were identified by colony PCR with T7 forward and T7 term. reverse primers. The resulting plasmid, named pET22b(+)-LCC, was used to transform an aliquot of chemically competent *E. coli* BL21 (DE3) using the heat-shock method. Positive transformants were selected for on LBA plates containing ampicillin (100 µg/mL), incubated at 37 °C overnight. A single colony from the transformation was used to inoculate 100 mL LB containing ampicillin (100 µg/mL) and the cultures were incubated at 37 °C for 16 hours. 1 L 2XYT autoinduction medium (Formedium™) containing ampicillin (100 µg/mL) was inoculated with 1% v/v overnight culture and incubated with orbital shaking at 22 °C for 24 hours. Cells were harvested by centrifugation (3260xg, 4 °C, 20 minutes) and the supernatant was discarded. The cell pellet was resuspended in 20 mL lysis buffer (100 mM potassium phosphate, 300 mM NaCl, pH 8) and lysed by sonication (10 seconds on, 10 seconds off, on ice, 5 minutes total, 10 µm amplitude). The cell lysate was clarified by centrifugation (3260xg, 4 °C, 20 minutes) and the supernatant transferred to a fresh tube and heated to 60 °C for 30 minutes. The soluble (thermostable) fraction was separated by centrifugation (3260xg, 4 °C, 20 minutes) and the supernatant concentrated to ~5 mL final volume (~3 mg/mL total protein concentration) by centrifugal filtration using a 10 kDA molecular weight cut off filter. The resulting protein was stored at -20 °C until required.

3.4 One-pot preparation of vanillin from post-consumer poly(ethylene) terephthalate

Post-consumer PET from a plastic water bottle (Sainsbury's Caldonian Clear™ Sparkling Spring Water, 2 L bottle found littering a pavement) was prepared by cleaning with ethanol and cutting in to ~4 cm^2 squares. The PET squares were heated to 250 °C for 30 minutes then cooled to room temperature. The amorphous material was ground to 1-2 mm size pellets using a pestle and mortar, which were used directly as a substrate for LCC catalyzed hydrolysis. To the PET granules (20 mg) was added 450 µL potassium phosphate buffer (100 mM, pH 10) and 50 µL semi-purified LCC (Section 3.3) and the reactions were incubated at 72 °C, 400 rpm in a Thermoshaker (3 mm mixing orbit) for 48 hours. The pH was adjusted to 6 with aq. 2 M HCl and 125 µL of a 5X concentrate of biotransformation buffer was added. The mixture was added to a 50 mL Falcon Tube and freshly prepared *E. coli* RARE_pVan were

added to final OD_{600}=80. The reactions were incubated at 22 °C for 24 hours before analyzing by HPLC using sample preparation Method 1 and sample preparation Method 2 for the sample containing an oleyl alcohol overlay.

3.5 HPLC sample preparation from biotransformation reactions

Method 1: A 250 µL sample was removed from the biotransformation reaction and quenched with 250 µL acetonitrile containing 0.2% v/v trifluoroacetic acid (TFA). Samples were vortexed and incubated at 4 °C for 30 minutes before clarifying by centrifugation (1378xg, 4 °C, 10 minutes). A 300 µL aliquot of the supernatant was transferred to a fresh tube and the solvent allowed to evaporate in a fume cupboard for 16-24 hours. MilliQ-H_2O containing 0.2% v/v TFA was added to a final volume of 350 µL and the samples were clarified by centrifugation (1378xg, 4 °C, 10 minutes). 145 µL of the resulting supernatant was added to 145 µL caffeine solution (100 µM in 0.1% v/v TFA in water) to give a final internal standard concentration of 50 µM and samples were analysed by HPLC according to the method described above.

Method 2: 500 µL biotransformation reactions were extracted with MTBE (3 x 400 µL) and the combined organic fractions were concentrated by evaporation in a fume cupboard for 16-24 hours. Aqueous NaOH (200 µL of a 0.25 M stock) was added and the samples were vortexed then phases separated by centrifugation (1378xg, 22 °C, 2 minutes). 120 µL of the aqueous phase was transferred to a fresh tube and the extraction was repeated with a further 150 µL aq. NaOH (0.25 M), transferring 120 µL to the second tube. The combined aqueous fractions were acidified to pH 2 with aq. HCl (50 µL of a 2 M stock) and clarified by centrifugation (1378xg, 4 °C, 10 minutes). 145 µL of the resulting supernatant was added to 145 µL caffeine solution (100 µM in 0.1% v/v TFA in water) to give a final internal standard concentration of 50 µM and samples were analysed by HPLC according to the method described above.

4 Supplementary data

4.1 Exemplar HPLC chromatograms

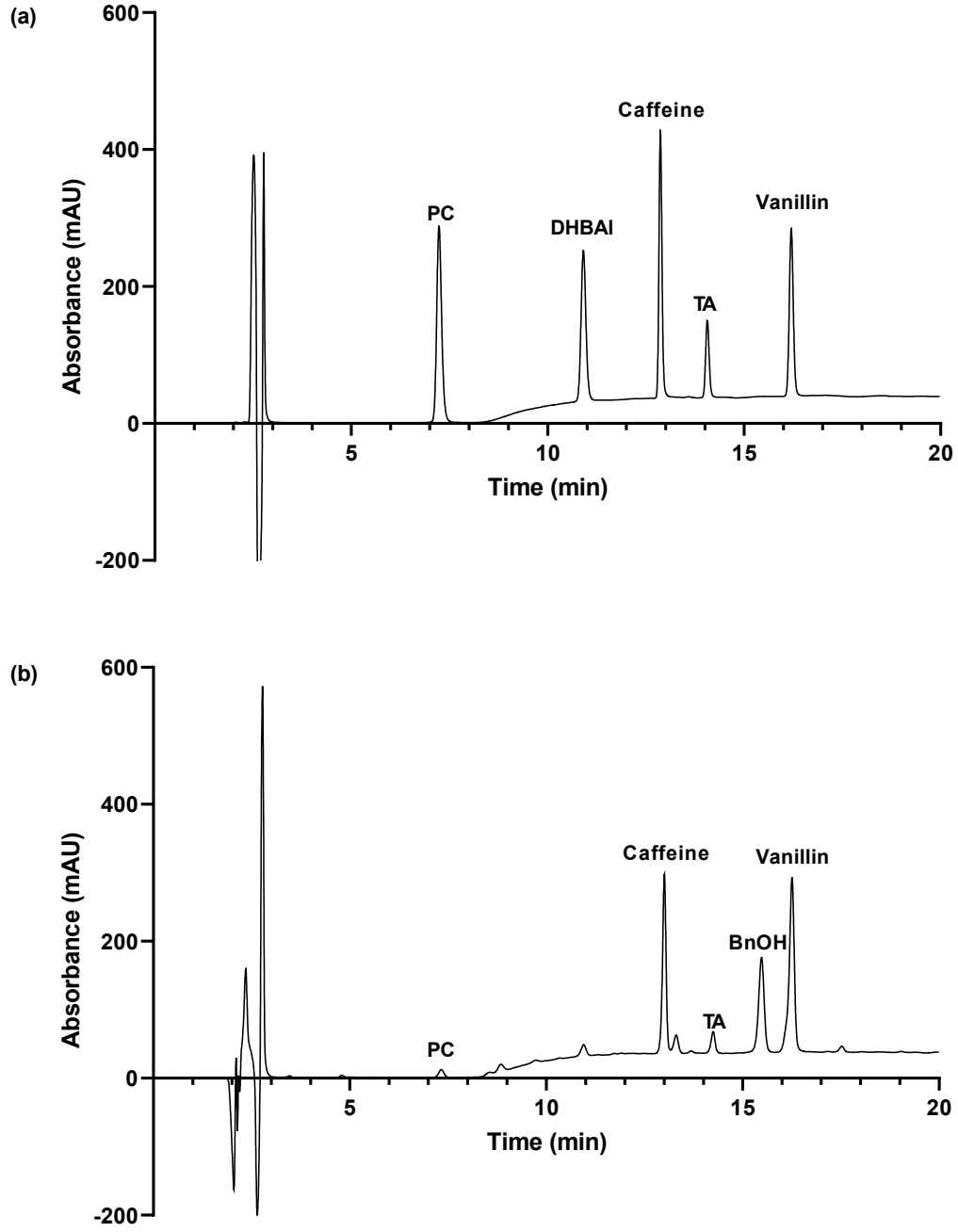


Figure S2. (a) HPLC chromatograph of a mixture of commercial standards of PC, DHBAl, caffeine (internal standard), TA and vanillin. (b) Exemplar chromatogram of 24-hour reaction sample from whole cell catalysed TA to vanillin biotransformation under optimized conditions.

4.2 UV absorbance spectrum of vanillin

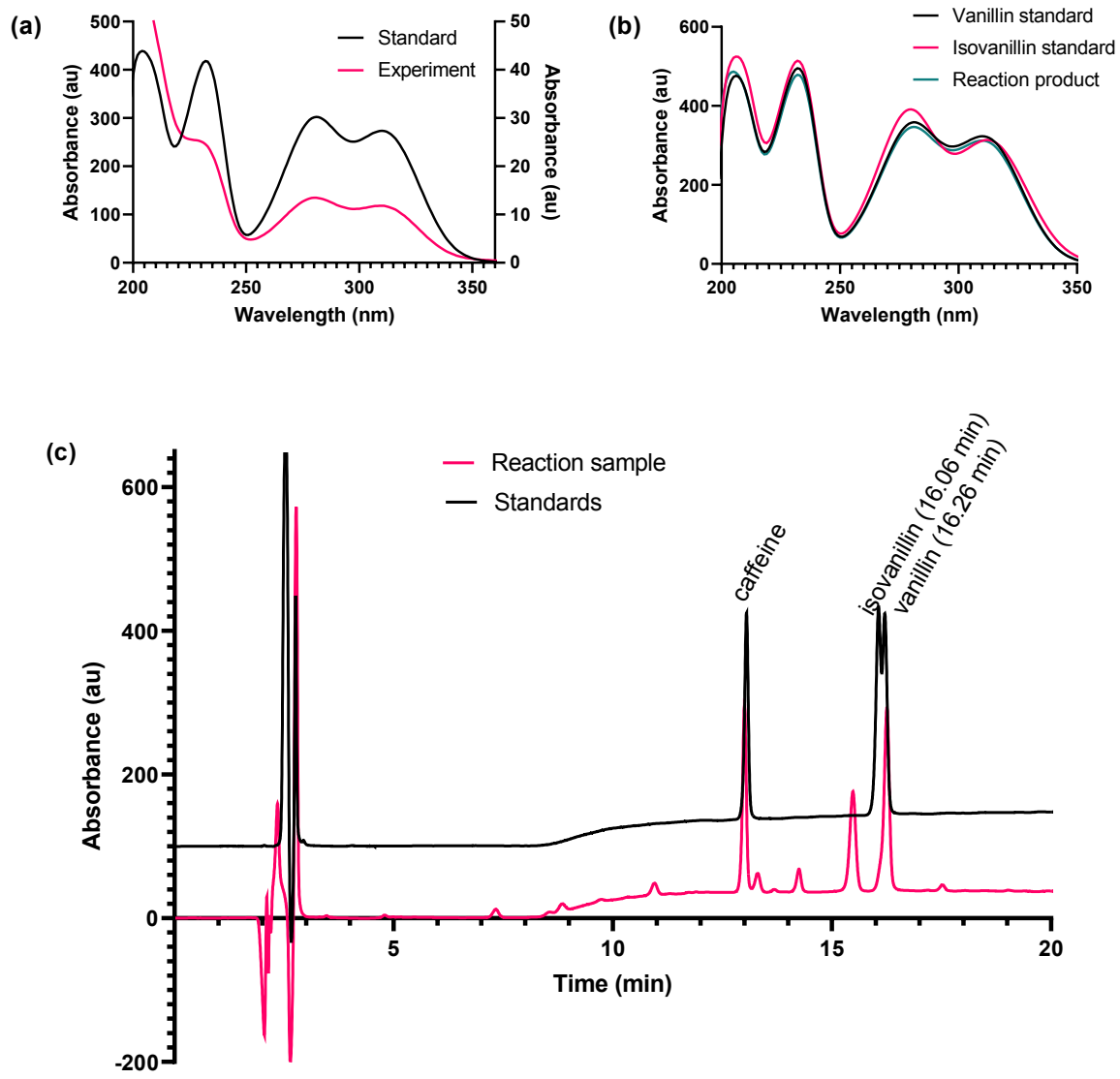


Figure S3. (a) UV absorbance spectra of a vanillin standard (left hand axis) and the putative vanillin peak at 16.2 minutes (right hand axis) from pre-optimisation reaction sample (b) UV absorbance spectra of vanillin and isovanillin standards overlayed with UV spectrum from the post-optimisation reaction sample peak at 16.26 minutes. (c) HPLC alignment of post-optimisation reaction sample to commercial standards of vanillin and isovanillin.

4.3 Mass spectrometry of biotransformation sample

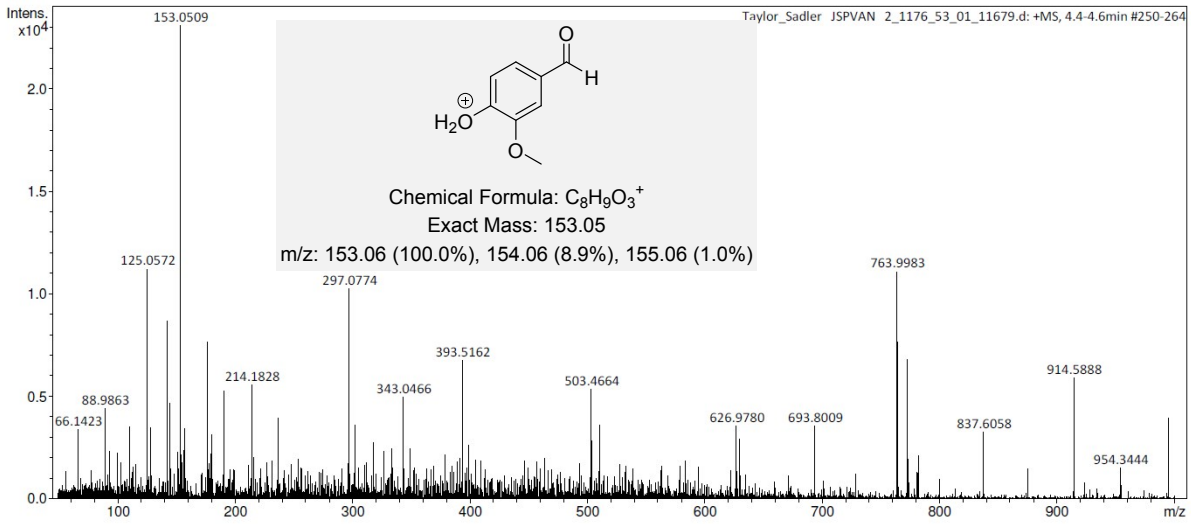


Figure S4. LC-MS spectrum of 24-hour reaction sample from whole cell catalysed TA to vanillin biotransformation under optimized conditions.

4.4 Optimisation of vanillin pathway expression conditions

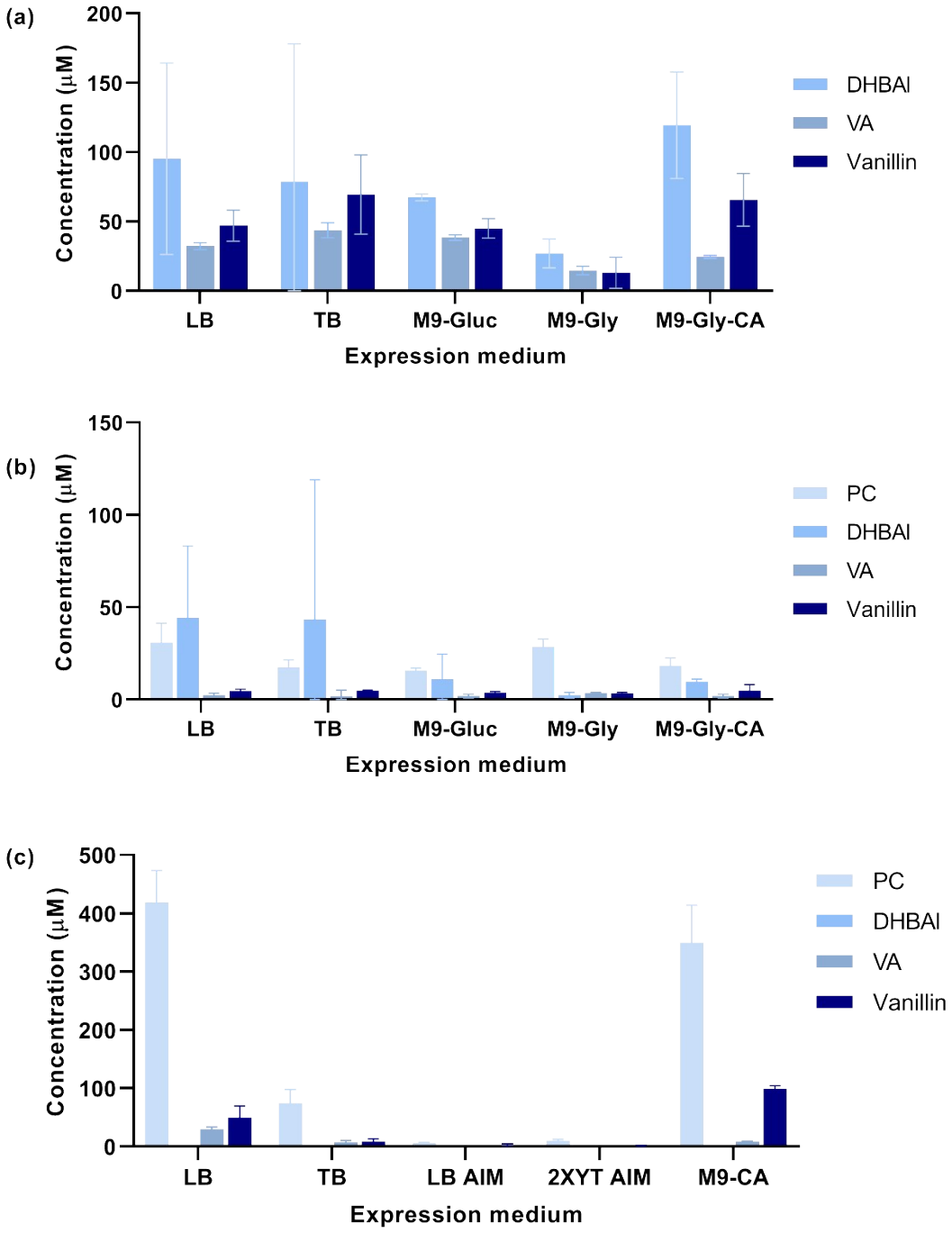


Figure S5. Screen of media for expression of vanillin pathway enzymes using (a) PC and (b) TA as substrate. *Biotransformation conditions:* (a) and (b) 200 µL scale in 96 well plate, 30 °C, 5 mM TA substrate. (c) 30 °C, 250 µL in 50 mL Falcon Tube, 1 mM TA substrate.

4.5 Additives screen

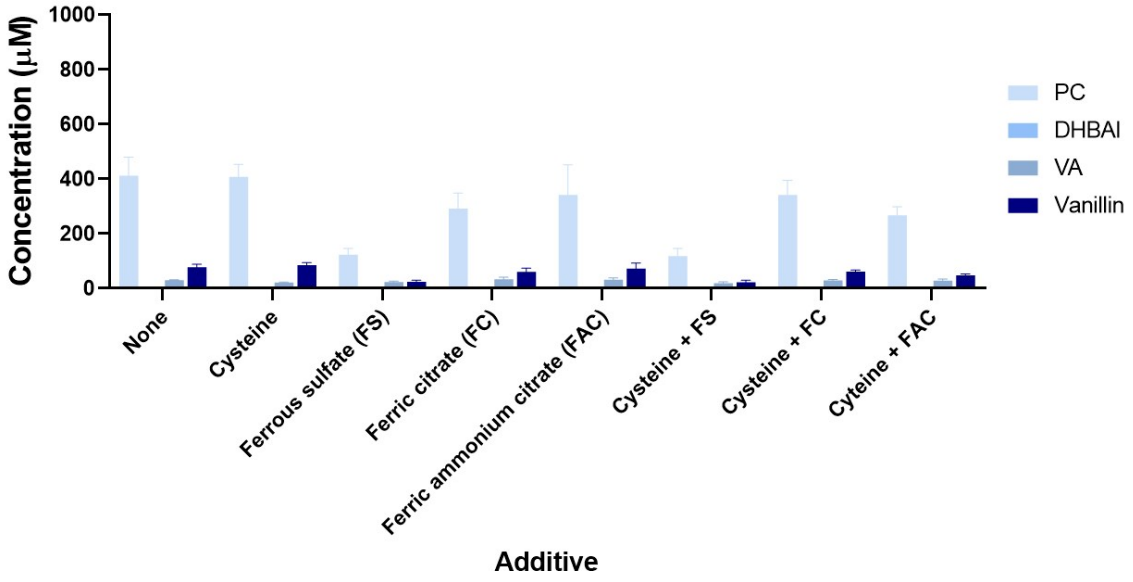


Figure S6. Effect of addition of Fe and S containing additives to expression medium on conversion of 5 mM TA to vanillin and pathway intermediates. Biotransformation reactions were incubated at 30 °C for 24 hours and analysed sample preparation Method 1. FS: ferrous sulfate; FC: ferric citrate; FAC: ferric ammonium citrate.

4.6 SDS-PAGE analysis of protein expression

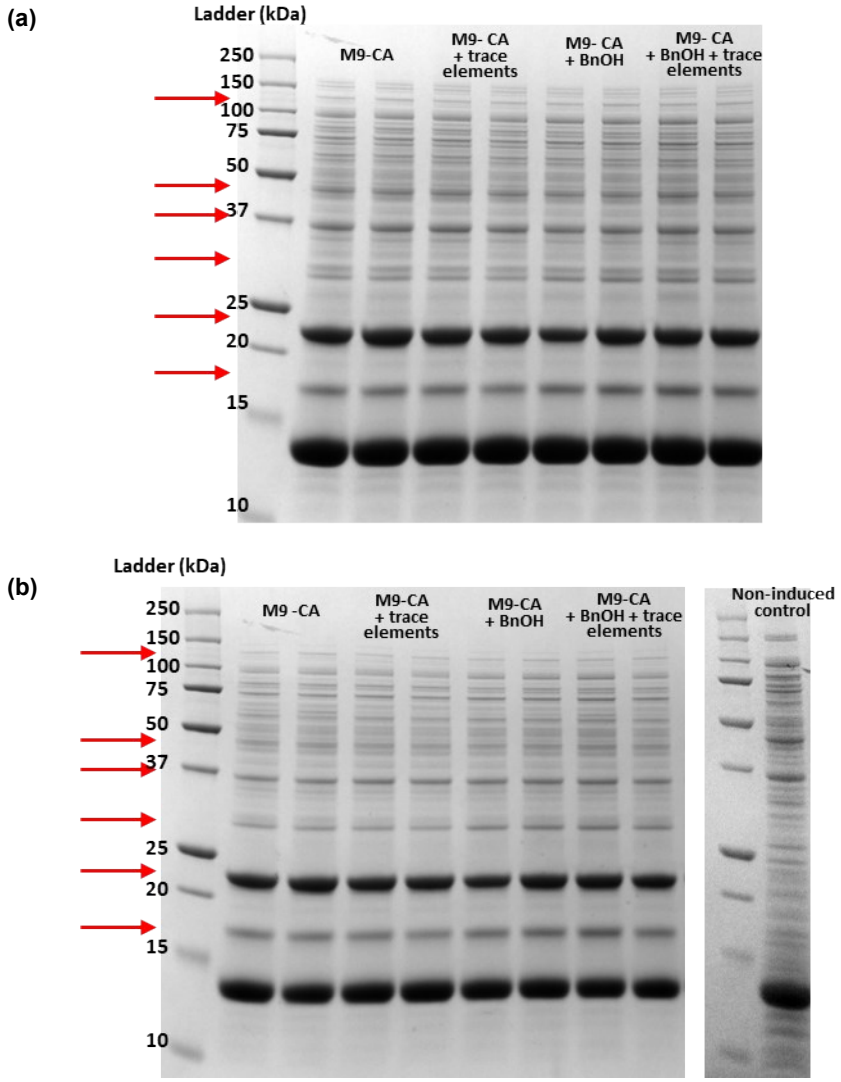


Figure S7. SDS-PAGE analysis of expression of pVan1+pVan2+pSfp by *E. coli* RARE in M9-CA medium with and without trace elements and/or benzyl alcohol (BnOH). (a) Total protein expression; (b) soluble protein expression. Trace elements were added to expression culture from a 100X stock solution containing $FeCl_3$-$6H_2O$ (3.1 mM) $ZnCl_2$ (0.62 mM), $CuCl_2$-$2H_2O$ (76 µM), $CoCl_2$-$2H_2O$ (42 µM), H_3BO_3 (162 µM) and $MnCl_2$-$4H_2O$ (8.1 µM). Theoretical masses of target protein monomers (kDa): TphA1: 36.3; TphA1: 46.2; TphA3: 17.2; DCDDH: 32.9; NiCAR: 128.1; S-COMT: 24.7; Sfp: 26.2.

4.7 Reaction vessel headspace

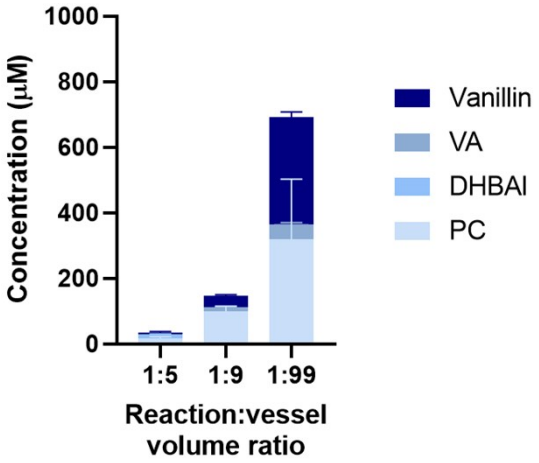


Figure S8. Effect of biotransformation vessel headspace on conversion of 1 mM TA to vanillin and pathway intermediates. Biotransformation reactions incubated at 30 °C for 24 hours without ISPR and analysed by sample preparation Method 1.

4.8 L-Met

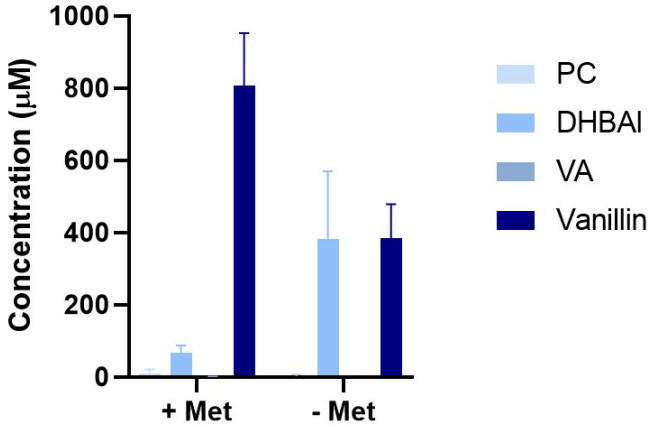


Figure S9. Effect of addition of 10 mM L-Met to biotransformation buffer on conversion of 1 mM TA to vanillin and pathway intermediates. Biotransformation reactions incubated at 22 °C with oleyl alcohol overlay and analysed by sample preparation Method 2.

4.9 Biotransformation buffer pH

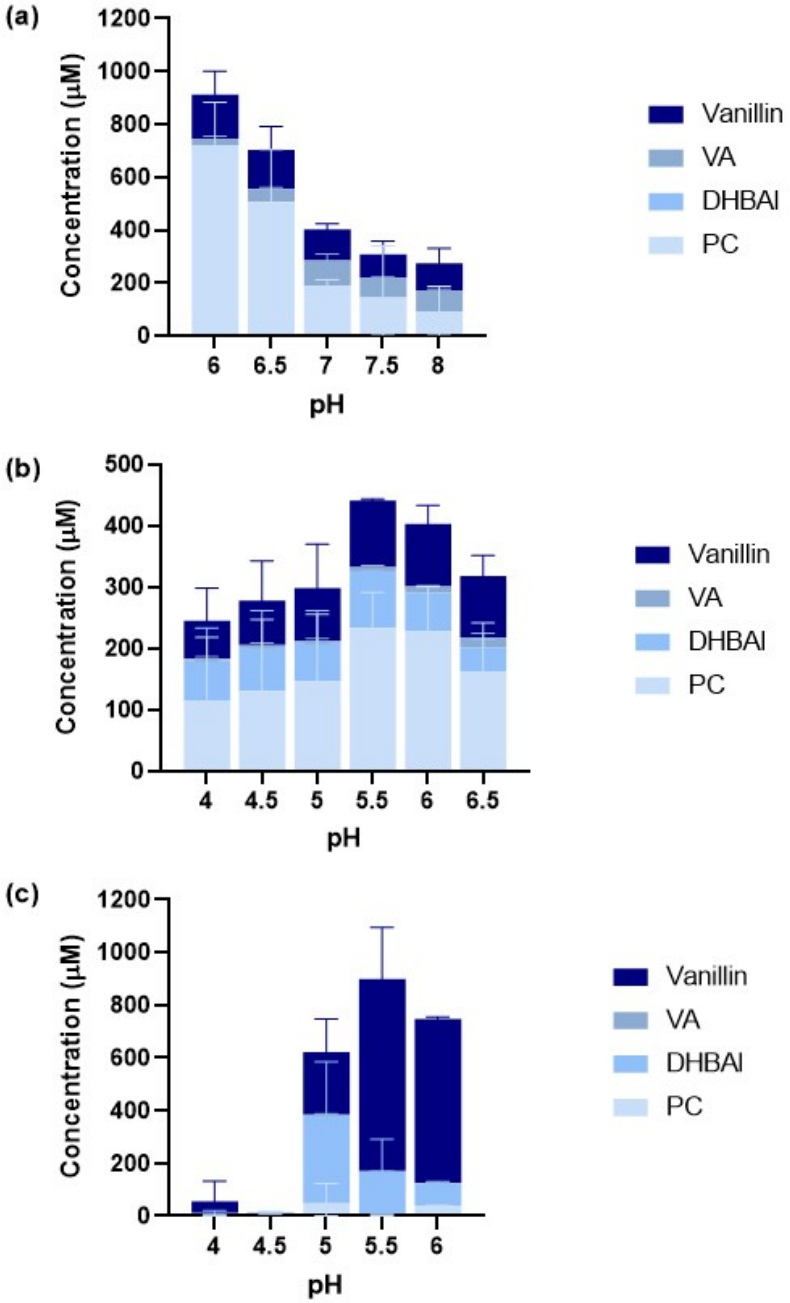


Figure S10. Effect of biotransformation buffer pH on conversion of 1 mM TA into vanillin and pathway intermediates. (a) Biotransformation reactions incubated at 30 °C for 24 hours without IPSR and analysed by sample preparation Method 1. (b) Biotransformation reactions incubated at 22 °C for 24 hours without IPSR and analysed by sample preparation Method 1. (c) Biotransformation reactions incubated at 22 °C for 24 hours with oleyl alcohol overlay and analysed by sample preparation Method 2.

4.10 Biotransformation OD

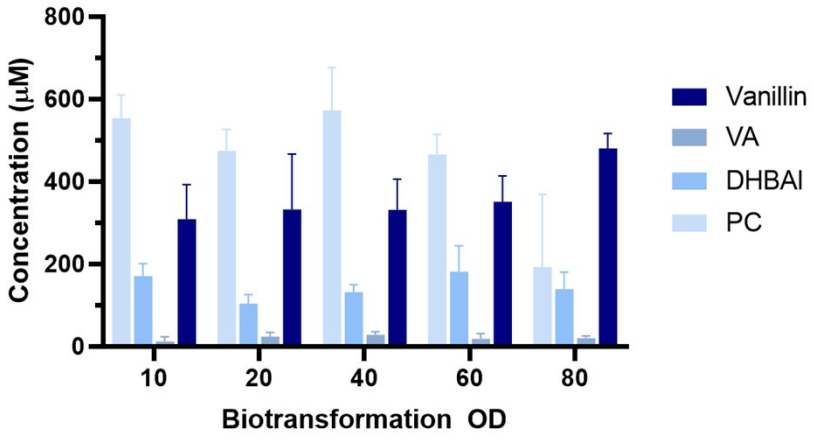


Figure S11. Effect of biotransformation OD on conversion of 1 mM TA to vanillin and pathway intermediates. Biotransformation reactions incubated at 22 °C for 24 hours with an oleyl alcohol overlay and analysed by sample preparation Method 2.

4.11 Toxicity of TA and vanillin to *E. coli* RARE_pVan

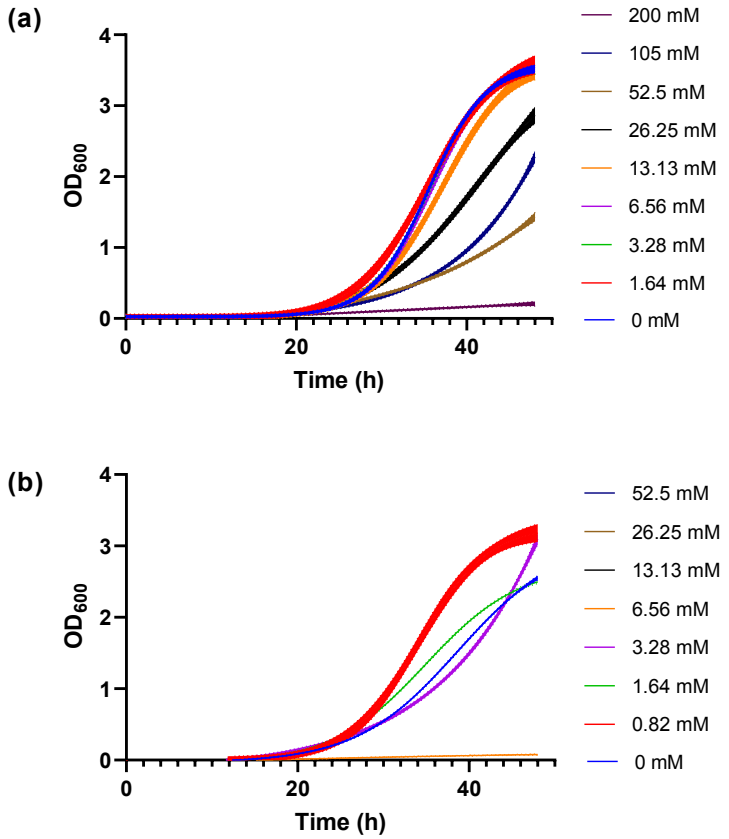


Figure S12. *E. coli* RARE_pVan growth curves in the presence of (a) TA and (b) vanillin. Cultures were grown in triplicate in a 96-well plate in M9-CA at 22 °C for 48 hours. Growth curves were fitted to Sigmoidal curves using Prism GraphPad software.

4.12 Substrate concentration

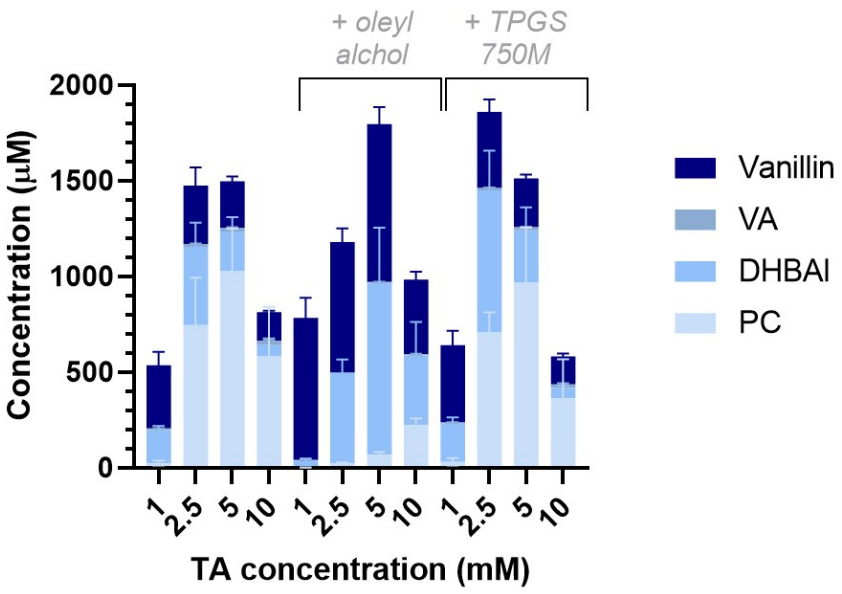
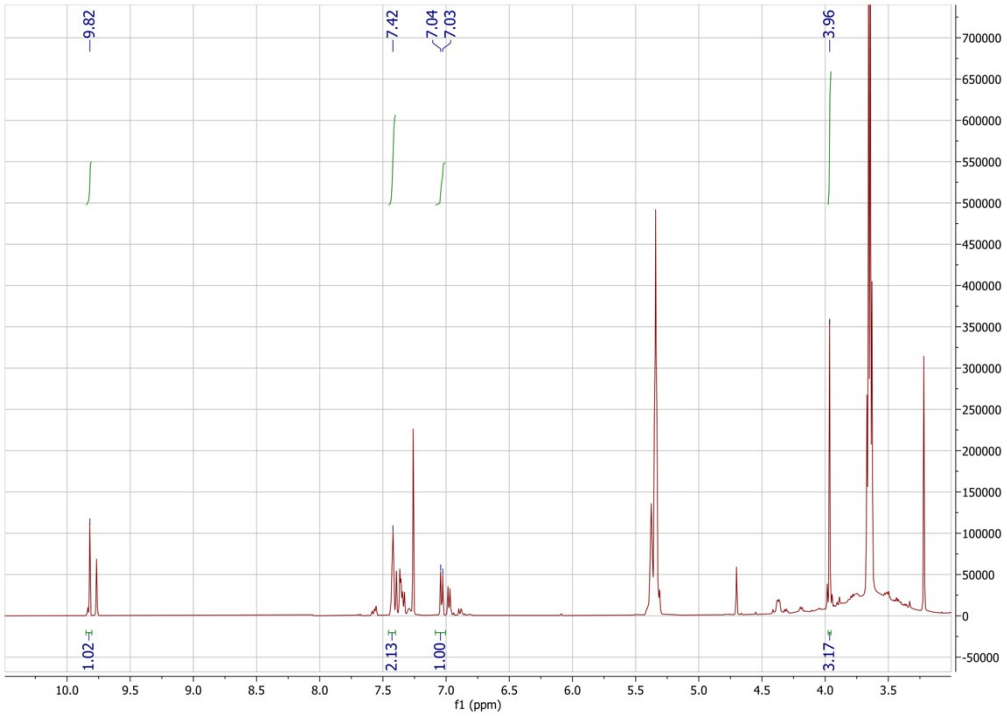


Figure S13. Effect of substrate concentration on conversion of TA to vanillin and pathway intermediates with and without ISPR. Biotransformation reactions incubated at 22 °C for 24 hours and analysed by sample preparation Method 2.

4.13 NMR Spectroscopy



(a)

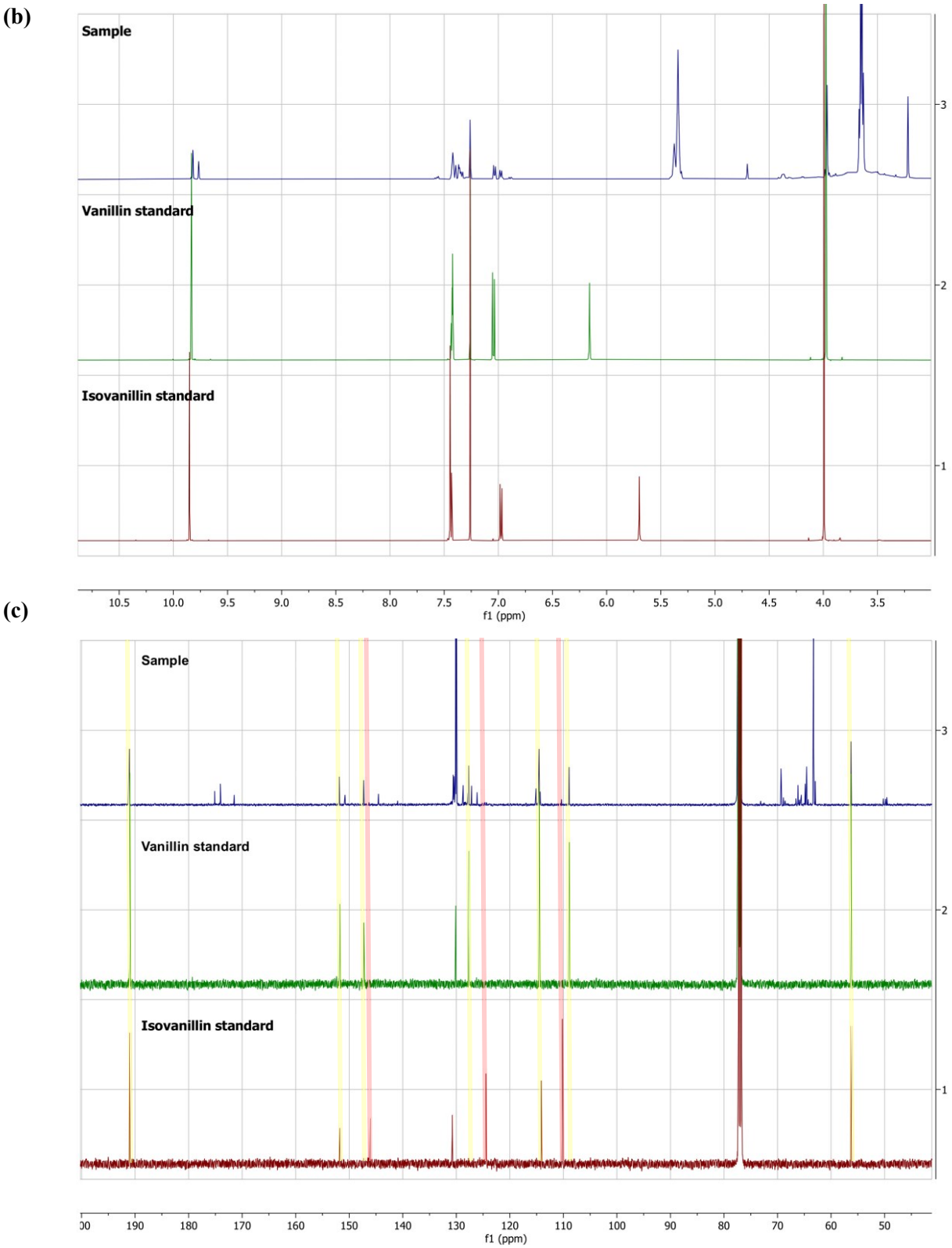


Figure S14. (a) ^{1}H NMR spectrum of crude reaction product from a 40 mL scale biotransformation of TA to vanillin. (b) Alignment of crude product ^{1}H NMR with commercial standards of vanillin and isovanillin. (c) Alignment of crude product ^{13}C NMR with commercial standards of vanillin and isovanillin showing vanillin to be the major product. Alignment of vanillin peaks highlighted in yellow; position of isovanillin peaks highlighted in red.

4.14 LCC catalysed PET degradation

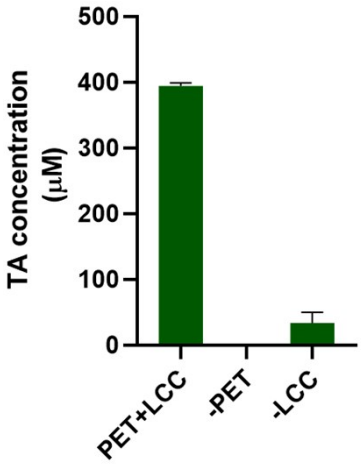


Figure S15. LCC WCCG catalysed degradation of post-consumer PET. Reation conditions: 100 mM potassium phosphate buffer, pH10 at 72 °C for 48 hours in a Thermoshaker at 300 rpm (3 mm shaking orbit).

4.15 HPLC standard curves

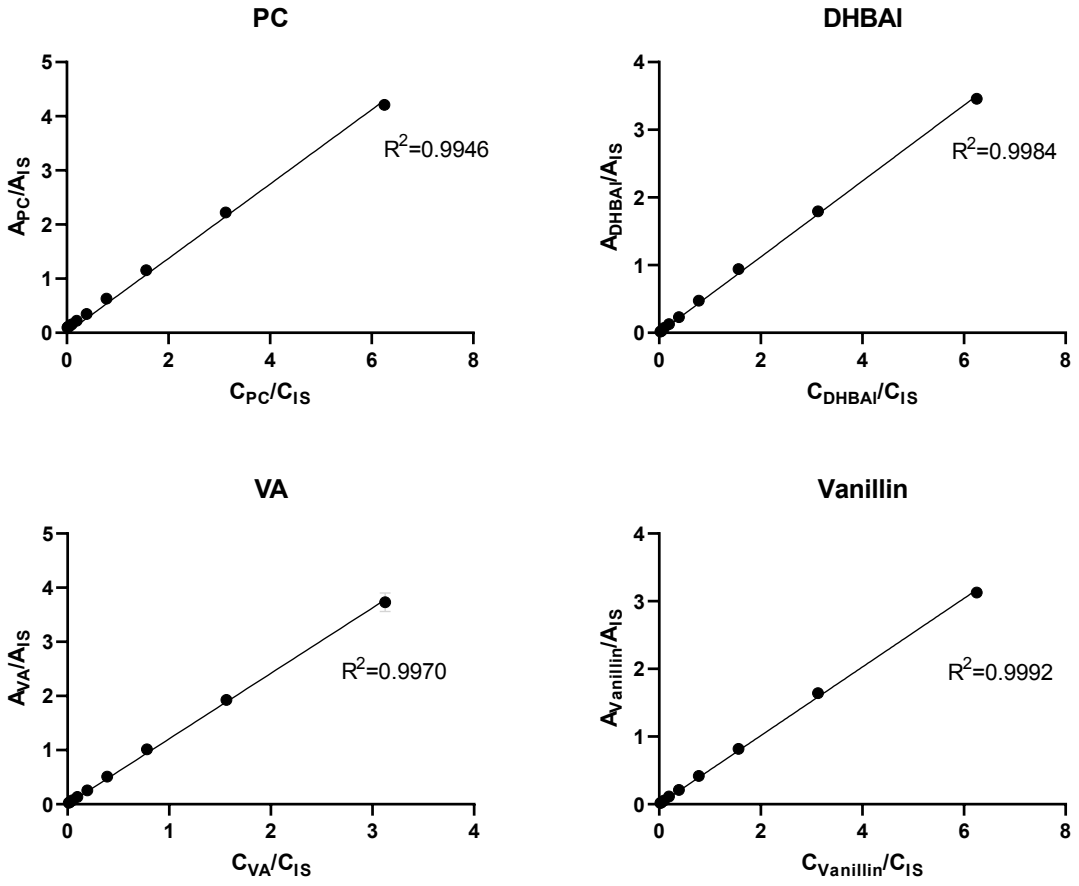


Figure S16. Response curves for vanillin pathway intermediates and vanillin in comparison to a caffeine internal standard (IS). Reaction samples were diluted to within the linear range prior to analysis by HPLC. Response curves generated using the mean values of triplicate runs for each analyte concentration.

5 References

1. Cohen, S. N., Chang, A. C. & Hsu, L. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of Escherichia coli by R-factor DNA. *Proc. Natl. Acad. Sci. U. S. A.* **69**, 2110–2114 (1972).
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diverse bacteria. *Mol. Syst. Biol.* **10**, 731 (2014).

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