

Microbial upcycling of plastic waste to levodopa

In the format provided by the
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S1 General materials and methods

Unless otherwise stated, starting materials and reagents were obtained from commercial suppliers and were used without further purification. Water was purified using a Suez Select purification system (18 M Ω .cm, 0.2 μ M filter). Terephthalic acid (TPA) was employed as the disodium salt (disodium terephthalate), except for waste PET-derived TPA that was used as the free acid. Hot stamping foil was donated by API Foilmakers Ltd. (Livingston, UK). PET bottle samples were collected from domestic rubbish (Edinburgh, UK). PET food packaging samples were received from the Uttamapinant lab (Vidyasirimedhi Institute of Science and Technology, Thailand). Bread waste glucose was obtained from C-Source Renewables Ltd.

NMR: Proton nuclear magnetic resonance spectra (^1H NMR) were recorded using an AVA500 – (DS) Bruker AVIII 500 (500 MHz) or an AVA600 – (DS) Bruker AVIII 600 (500 MHz) at 298 K. Carbon nuclear magnetic resonance spectra (^{13}C NMR) were recorded using an AVA500 – (DS) Bruker AVIII 500 (125 MHz) or an AVA600 – (DS) Bruker AVIII 600 (125 MHz) at 298 K. Fluorine nuclear magnetic resonance spectra (^{19}F NMR) were recorded using a PRO500 – (DS) Bruker AVIII 500 (470 MHz) at 298 K.. Chemical shift is reported in parts per million (δ , ppm) and are relative to the deuterated solvent or residual TMS peak. Data is reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), and coupling constants (J , Hz). For all quantitative NMR, 1,3,5-trimethoxybenzene (TMB) was used as an internal standard.

HPLC method: Reverse phase high performance liquid chromatography (HPLC) was performed using a Vanquish Core HPLC (Thermo Fisher Scientific) fitted with a HyperSil Gold C18 column (150 mm $\times$ 3 mm $\times$ 3 μ m, Thermo Fisher Scientific), HPLC grade mobile phases with trifluoroacetic acid (TFA) as modifier. A gradient program was used for elution (flow rate = 0.4 mL/min, 30 $^{\circ}\text{C}$, 15 μ L sample injection, **Table S1** details the gradient for PCA, CAT, and L-DOPA and **Table S2** for TPA. Analytes were quantified by measuring absorbance at 206 nm (PCA, CAT, and L-DOPA), and 240 nm (TPA) by comparing analyte, known authentic standard and internal standard (caffeine) peak areas.

Table S1 HPLC gradient for PCA, CAT, and L-DOPA analysis

Time (min)	H ₂ O + 0.1% v/v TFA (%)	Acetonitrile + 0.1% v/v TFA (%)
0	95	5
5	95	5
10	85	15
20	50	50
20.2	95	5
25 (end of method)	95	5

Table S2 HPLC gradient for TPA analysis

Time (min)	H ₂ O + 0.1% v/v TFA (%)	Acetonitrile + 0.1% v/v TFA (%)
0	80	20
1	80	20
6	60	40
7	10	90
10	10	90
11	80	20
16 (end of method)	80	20

HPLC sample preparation: For PCA, CAT, and L-DOPA, 100 µL of the resulting biotransformation was added to 300 µL HCl (100 mM) containing caffeine (0.01 g/L), vortexed and clarified by centrifugation at 13,000 rpm before the supernatant was analysed by HPLC. For TPA quantification, 100 µL of the supernatant from the biotransformation was added to 300 µL of Tris-HCl-NaCl (25 mM Tris, 150 mM NaCl, pH 7.5), vortexed and clarified by centrifugation at 13,000 rpm before the supernatant was analysed by HPLC.

Gas chromatography method: Gases within samples were identified by headspace gas chromatography with thermal conductivity detection (150 °C) using a Shimadzu GC 2030 equipped with a headspace injector and a ShinCarbon packed column (column length: 2 metres; internal diameter: 0.53 mm; Restek) held at 45 °C. To improve detection of CO₂, samples were heated to 150 °C for 30 seconds then 1 mL equilibrated headspace gas was injected. Total flow rate was 80 mL/min using N₂ as the carrier gas.

S2 Media and culture condition

Luria–Bertani (LB) Medium: Bacto-tryptone (10 g/L), yeast extract (5 g/L) and NaCl (10 g/L) were dissolved in Milli-Q H₂O, autoclaved at 121 °C for 20 min, cooled and stored at room temperature. Solid media was made using the same recipe but with the addition of agar (15 g/L).

M9 Minimal Media: Na₂HPO₄ (6 g/L), KH₂PO₄ (3 g/L), NH₄Cl (1 g/L) and NaCl (0.5 g/L) were dissolved in Milli-Q H₂O, autoclaved at 121 °C for 20 min and the media was stored at room temperature.

Tris-acetate-phosphate (TAP) medium: prepared following the recipe outlined by Gorman and Levine¹ as well as Kropat *et al*². This was dissolved in Milli-Q H₂O, autoclaved at 121 °C for 20 min, cooled and stored at room temperature. Solid media was made using the same recipe but with the addition of agar (15 g/L).

Unless stated otherwise, *Escherichia coli* (*E. coli*) cells were cultured at 37 °C with shaking at 220 rpm in an incubator shaker. Optical densities (OD) of *E. coli* cultures were monitored using a DeNovix DS-11 UV/Vis spectrophotometer by measuring absorbance at 600 nm. For bacterial growth and protein induction, appropriate antibiotics for selective growth of each strain were added to media at the following concentrations: 100 µg/mL ampicillin, 30 µg/mL chloramphenicol, 50 µg/mL kanamycin, 25 µg/mL spectinomycin.

Chlamydomonas reinhardtii CC1690 (*C. reinhardtii*) starter cultures were produced by inoculating 20 mL TAP medium from a single colony grown on TAP agar and incubated under continuous light (27 °C, 130 rpm, 7 days, 90 µmol photons/m²/s). For experimental cultures, 150 mL TAP was inoculated with 1.5 mL starter culture (1% v/v) and incubated (27 °C, 130 rpm, 2 days) to acquire an actively growing culture.

S3 Molecular biology

SapphireAmp Fast PCR (Takara Bio) was used for colony PCR and Phusion High-Fidelity DNA Polymerase (New England Biolabs, NEB) was used for homology-mediated DNA assembly, following manufacturer recommendation. PCR products were gel purified using a Zymoclean Gel DNA Recovery Kit (Zymo Research). Restriction enzymes and T4 DNA ligase (Thermo Fisher)

were used following manufacturer recommendations. Plasmid DNA was purified with a Miniprep Kit (Qiagen) from *E. coli* DH5a.

Table S3 Custom synthesised DNA sequences used in this study

Gene code	Nucleotide sequence (5' to 3')
<i>tphA1</i>	ATGAATCATCAGATACATATTCACGATTCGGATATAGCATTTCTTGCGCACC CGGGCAATCTGTTTTGGATGCGGCTCTGCAAGCAGGCATAGAATTGCCATAC AGCTGCCGTAAAGGTTCTGTGGCAACTGCGCATCAGCCCTATTGGATGGGA ATATTACGTCTTTTAATGGCATGGCTGTCCGATCGGAGCTGTGCACATCAGAG CAAGTACTCTTGTGTGGCTGCACTGCTGCATCAGATATCAGAATTCAACCGAG CAGCTTTCGGAGGCTGGACCCGGAAGCTCGCAAGAGGTTTACTGCAAAGGTG TACTCCAATACTCTGGCGGCACCGGATGTGTCTCTCTCCGTCTCAGGTTACC AGTTGGCAAGCGAGCGAAATTCGAAGCGGGCCAATATTTACTTATTACCTTG ATGATGGCGAATCACGGTCATACAGTATGGCCAATCCGCCCCACGAGAGCGA TGGGATAACGCTTCACGTACGCCACGTTCTGCGGACGATTCTCCACAATAG TGCAACAACCTGAAAAGCGGCGATACATTGGAAATTGAGCTGCCCTTTGGCTC GATTGCGCTCAAACCGGATGATACGAGGCCACTGATATGCGTTGCTGGGGGT ACGGGTTTTGCGCCAATTAATCAGTTCTCGACGATTTAGCTAAGCGAAAAGT TCAGCGCGACATCACCTTGATCTGGGGCGCCAGGAATCCTAGCGGCTTATACT TGCCTAGCGCGATCGACAAGTGGAGGAAAAACATGGCCACAGTTCCGATACAT TGCGGCAATCACAGACTTGGGCAACGTTCCGGCAGACGCGCACGCTGGACGT GTTGACGATGCCTTGCGCACCCACTTTGGAAACCTTCACGATCATGTTGTTCA TTGCTGCGGGTCGCCTAGCCTGGTTCAATCGGTCCGGACCGCTGCCAGCGACA TGGGACTTTTGGCCCAGAATTTTACGCAGACGTGTTTGCAACCAGCCCGACG GGTTCCCATTA
<i>tphA2</i>	ATGCAAGAGTCAATTATCCAGTGGCATGGTGCAACAAACACTCGGGTACCAT TTGGCATCTATACTGACACTGCCAATGCAGATCAAGAGCAACAGAGGATCTA TCGGGGGGAGGTCTGGAACATTTTGTGTCTGGAGTCAGAAATCCCCGGAGCG GGAGATTTCCGAACAACCTTCGCGGGAGAAACGCCAATTGTAGTTGTTTCGCG ACGCTGATCAAGAAATCTACGCCTTTGAAAACCGGTGTGCCACCGCGGAGC ACTGATCGCGTTGGAGAAGAGTGGAAGGACGGAATCTTTCCAATGCGTGTAT CATGCCTGGTTCGTACAACCGTCAAGGCGATCTGACCGGCGTTGCGTTTGA AAGGGGTCAAGGGCCAAGGTGGTATGCCGGCCTCCTTTTGAAGGAAGAGCA CGGGCCGCGAAAGCTCAGGGTCGCTGTGTTCTGCGGACTTGTCTTTGGTTTCA TTAGCGAGGACGTACCCTCGATCGAGGATTATCTGGGTCTGAAATCTGCGA ACGCATAGAGCGCGTTCTGCACAAACCCGTAGAGGTCATAGGCCGGTTTACG CAGAAGCTGCCTAACAATTGGAACCTTTACTTTGAGAACGTAAAAGATTCTCT ACCACGCATCTCTCTACATATGTTTTTTACGACATTTGAGCTCAACCGGTTAT CACAAAAGGGGGGAGTAATTGTGGATGAGTCAGGCGGGCATCATGTATCATA CTCTATGATCGATCGCGGTGCCAAAGACGATTCTTACAAAGATCAAGCGATC AGGAGCGATAACGAACGGTACCGGCTCAAAGACCTTCATTACTCGAAGGTT TCGAAGAATTGAGGACGGCGTAACCTCTCAGATCTTGTCCGTTTTCCAGGC TTTGTACTTCAACAGATTTCAGAACAGCATAGCAGTTAGACAACTGCTGCCTAA ATCGATCAGTAGTAGCGAATTGAACTGGACATACCTAGGCTATGCTGACGAT TCGGCTGAGCAGAGAAAAAGTCCGGCTGAAACAGGCCAACCTCATAGGTCCAG CAGGGTTTATCTCTATGGAAGATGGTGCAGTTGGCGGCTTCGTTACGCGCGGG ATTGCTGGGGCCGCAATCTCGATGCCGTTATCGAGATGGGCGGTGACCACG AAGGCTCCTCAGAGGGACGAGCGACAGAAACAAGCGTAAGAGGGTTTTGGA AAGCCTATCGTAAACATATGGGCCAAGAAATGCAAGCATAA
<i>tphA3</i>	ATGATAAACGAAATACAGATAGCCGCCTTTAATGCGGCCTATGCGAAAACGA TTGATAGTGATGCCATGGAACAGTGGCCACGTTCTTACCAAGGATTGCCAT TACTGCGTGACGAACGTGGATAACCACGATGAGGGCCTGGCTGCCGGAATCG TGTGGGCTGACTCACAAGATATGTTGACTGACAGAATTTCCGCATTACGCGAA

	GCAAACATTTACGAGCGGCATCGTTATCGGCATATCCTCGGATTACCATCGAT TCAATCTGGAGACGCGACGCAAGCGTCCGCGTCTACTCCGTTTCATGGTCCCTTC GAATTATGCACACGGGTGAGACAGAAGTCTTTGCCAGCGGGGAATACTTAGA CAAATTTACCACAATTGATGGCAAGCTCCGCTTACAAGAACGAATTGCGGTCT GTGATTCAACGGTCACCGACACTCTGATGGCACTGCCTCTGTAA
<i>dcddh</i>	ATGACAATTGTGCACCGGCGCTTAGCACTCGCGATCGGTGATCCACATGGCAT AGGCCCTGAAATCGCCTTGAAGGCTCTGCGACAACCTCAGCGCGAATGAGCGA TCCTTAATCAAAGTGTATGGTCCATGGAGCGCACTTGAACAGGCCGCTCAAAT TTGCCAGATGGAATCTCTCCTCCAGGATCTGATACACGAAGAAGCTGGATCAT TAGCGCAACCCGCGCAGTGGGGAGAAATCACCCCCAGGCGGGACTTAGCAC TGTCCAGAGTGCAGTCTGCTGCCATTCTGGGCGTGCAGAGAATGGAGAAGTGGAT GCTGTCATCGCATGCCCCGACCACGAAACTGCGATCCATCGCGCTGGTATAGC ATTACAGCGGCTACCCGTCTCTGCTTGCCAACGTTCTTGGTATGAACGAAGATC AGGTCTTTCTTATGCTTGTAGGGGCGGCTTGCGCATCGTACACGTGACCCTC CATGAATCAGTAAGGAGCGCCTTGGAACGGTTATCGCCGCAGCTGGTTGTAA ATGCAGTTCAGGCAGCAGTGCAGACGTGCACACTATTGGGGGTTCCCAAACC ACAAGTGGCCGTGTTCCGTATAAACCACATGCGTCCGAAGGGCAACTGTTT GGGCTGGAAGACAGTCAAATCACCGCGCCGGCCGTTGAAACATTACGTAAGT GCGGACTCGCGGTCGACGGTCTATGGGAGCCGACATGGTTTTGGCCCAACG CAAACATGATCTCTATGTGGCTATGCTGCATGATCAAGGACACATACCGATTA AACTTTTGGCGCCGAATGGGGCCTCAGCTCTGTCTATAGGTGGGCGTGTGGTC TTGAGTTCCGTAGGCCACGGAAGCGCTATGGACATAGCAGGTCTGTGGTGTG CCGATTCAACCGCGCTGTTGAGGACTATAGCCCTATTGGGTGCGCAGCCCGGT TAA
<i>tpak</i>	ATGTCCTTAGCACCATCGCGCGTGACACTGCCGGATTCATCGATTGCGCGCC AGTGAGTCGATACCAGTACATCGTGATCGCCCTGTGTGGGGTGGTCATGTTCA TCGACGGCTTCGACACCCAGAGCATCAGCTACATGGCGCCCCACATCGCCGA GGAGTGGGGCCTGTCGAAACAGGTGCTCGGGCCCATCTTCTCCGCCGCTCTCG CCGGCCTCATGGTCGGCTATCTGGCGCTCTCGCCGCTGTCCGAGCGGTTCCGGC CACCGCCGATGATCCTCACGAGCACGGTCATCTTCGCGCTCGGCACGTTGGC GGCGGCCTGGTCACAGAACGTACCCGAACCTGATGGCGTTGCGCTTCATCACC GGAATGGGGCTCGGTGCCGCCGCGCCGAGTGCCATCGCACTGACGGGCGAAT TCAGCCCCAAGCGCCTTCGAGCAACCTTCGTCCTGGTGATCTATTGCGGCTTC TCCCTCGGATTCGTGCGGGCAGGGCTGGTTTCCGGTTGGCTGATCCCGATCCT CGGCTGGCGGTGGTACTCGTCGTGCGCGCAGTAGCACCGCTGCTCCTGCTCC CGGCGTTGCTGCGCTACCTGCCGGACTCACTACCTCAATGATCAACCGAGGC GCGGAGCCCCAACAGAATCCAGGCGATCTTCCGCAAAATGGATCCCGCCCTCG CCGTGCGCCCCGACATCACTACGAGGCCGAGAAGCGGACCGACGACAACG CACTGCACTGAGGAGCCTGTTACCCGTGACCGGGTCTTGGAACCCCTGCTGC TGTGGCTGGTCTTTGTCATCAACCTCGGCGAGTTTTACGCGCTGCAGAGCTGG CTACCGTCGATCATGACGAGCCTGGACTACGACATGGGTACGGTGGTCACCG CCACCACCCTCACGACTGTGCGCGGCATCGCCGCTGCAATTTGTACCGGGCCC TGTATGGACCGACTCGGCGCGTACGTCACCCTCGGAACCGTTTATGTCGTCGG ATTGCGATTCTGTTGCGCTGACCGGTGTGCGCTTTACGGCGCCGTTGTGGGTCC TATTGACGGCCAATTTTTTTGCGGGGGTCTGCATCAGCGGTGGACAGAAGAG CCTCATCGCGCTGTCCGCGGTGTTCTATCCGACACCGATGCGGTCCACCGGGG TTGGATGGGCGTTGGGTGTTGGCCGCTCGGCGGCATCGTGGGCGCGATCGC GGTCGGAGCGGCACTCGGCATGGGCTGGTCCGCCAGTGCAGTCTTTTACGCA ATGTCAGTCCCCATGCTCGTCGCCGAGCTGCGGTCTTTCTCCTCGGCCGCTG GGTCCGAAGCGACAATCACCCGATCGCAAGTCGGCAGAAAGTCATTGCTC GCCCCGAAGTAA
<i>aroY</i>	ATGACTGCGCCAATTCAGGATCTGCGCGACGCGATCGCGTTGTTACAGCAGC ATGACAACCAGTATTTAGAGACCGATCACCCAGTTGACCCGAACGCGGAAC GGCAGGGGTATATCGTCATATTGGCGCGGGAGGCACGGTGAAACGGCCGACC CGTATTGGACCGGCAATGATGTTTAAACAATATTAAGGGGTACCCGCATTACG

	TATTCTTGTGGGCATGCACGCCTCTCGGCAACGTGCGGCACTGCTGCTGGGCT GCGAGGCATCGCAGTTGGCTTTAGAAGTGGGGAAGGCCGTAAAGAAACCGGT CGCACCAGTTGTGGTCCCGGCAAGTTCCGCACCTTGTGAGGAACAGATCTTTC TTGCAGATGACCCGGACTTCGACCTGCGCACCTTGCTTCCCGCCCCGACGAAT ACGCCGATTGACGCGGGTCCGTTTTTTTTGTCTGGGACTCGCTTTAGCGTCCGA TCCTGTCGATGCCTCCCTGACGGATGTGACGATTACCCGCCTGTGCGTGCAGG GCCGTGATGAATTAAGCATGTTTTTGGCGGCGGGGCGTCACATCGAAGTCTTC CGCCAGAAGGCTGAAGCCGCGGGTAAGCCCCTTCCGATCACGATTAACATGG GCTTAGATCCAGCTATTTATATCGGGGCATGTTTTGAAGCGCCAACCACTCCC TTTGGCTACAACGAAC GGGGGTGGCGGGTGCCCTGCGGCAACGCCCCGTGGAACCTGGTCCAAGGAGTT TCTGTACCTGAAAAAGCGATTGCGCGCGCAGAAATCGTCATTGAAGGAGAAT TGTTGCCAGGGGTACGTGTCCGCGAAGATCAGCACACGAATTCAGGTCATGC GATGCCCTGAGTTTCCAGGCTACTGCGGTGGGGCAAATCCTTCTCTGCCCCGTTA TCAAAGTTAAGGCGGTGACTATGCGTAACAACGCCATCCTGCAAACGCTTGT GGGACCGGGTGAAGAACATACGACCTTGCCCGGTCTGCCGACCGAAGCATCT ATTTGGAACGCGGTGGAAGCCGCGATCCCCGGTTTCCTCCAAAACGTTTATGC CCACACGGCAGGAGGGGGGAAATTCCTTAGGTATCCTGCAGGTTAAAAAGCGT CAGCCGGCTGACGAGGGTCGTCAAGGACAAGCCGCATTGCTGGCATTAGCGA CCTACTCCGAGCTGAAAAACATCATCTGGTTGATGAAGATGTCGACATTTTT GATTCTGACGATATCCTGTGGGCCATGACAACCCGGATGCAGGGTGACGTTA GCATACCACGATCCCCGGCATTCGCGGTATCAGTTAGACCCGAGCCAAAC TCCTGAGTATTCACCCAGTATCCGCGGTAACGGTATTAGCTGCAAGACCATCT TTGACTGCACCGTGCCGTGGGCCCTTAAAAGTCATTTTGAGCGTGCGCCATTC GCTGACGTTGACCCGCGCCCTTTTGCCCCGGAATATTCGCCCGTCTCGAAAA AAACCAAGGCAGCGCGAAATCATCA
<i>kpdB</i>	ATGAAACTCATTATCGGCATGACCGGCGCGACCGGCGCACCTTTAGGCGTTG CCTTGTTGCAAGCTCTGCGCGATATGCCGGAGGTTGAAACTCACCTGGTAATG TCGAAATGGGCTAAAACCACGATCGAGCTGGAAGCTCCTTGGACGGCGCGTG AAGTAGCAGCCTTGGCAGATTTTAGCCATTACCCGGCTGACCAGGCTGCGAC GATCAGTTCAGGTAGCTTTCGCACTGATGGGATGATTGTTATTCCTGTTCAA TGAAAACCCTTGCGGGTATCCGTGCCGGCTATGCCGAAGGCTTGGTCGGACG CGCAGCGGACGTTGTTCTGAAAGAGGGGCGTAAACTGGTCCTGGTTCCGCGG GAAATGCCGCTGTCGACAATTCATCTGGAACACATGCTGGCTCTGTCCCGAT GGGTGTGGCAATGGTTCCCCCATGCCGCGTATTACAATACCCGGAGACC GTTGACGACATTACCAATCACATCGTTACCCGTGTATTAGATCAGTTTGGCCT GGACTATCACAAGCGCGCCGCTGGAACGGCCTTCGTACAGCCGAACAGTTT GCCCAAGAAATTGAATCATCA
<i>fnrpl</i>	ATGCGTTTCGAGGACTACCCGGCAGAGCCGTTTCGCATTAAAGTCAGTCGAAA CCGTGAAGATGATCGACAAGGCGGCACGTGAGGAAGTAATAAAGAAAGCTG GTTACAATACATTCTTATCAATAGTGAAGATGTATACATAGACCTTCTTACT GACAGCGGCACTAATGCTATGTCTGACAAGCAATGGGGCGGCTTAATGCAGG GCGACGAGGCGTACGCTGGTTCTCGCAATTTCTTCCATCTTGAAGAAACGGTT AAAGAAATTTTCGGATTCAAGCACATTGTACCTACACACCAGGGCCGAGGGG CGGAAAATATCTTATCGCAAATTGCCATTAAACCGGGCCAATACGTACCAGG TAATATGTATTTACGACGACACGTTATCACCAGGAGCGCAATGGTGGTATTT TCAAGGACATTATACGCGACGAGGCGCATGACGCCACCCTGAATGTGCCCTT CAAAGGAGACATAGATTTGAACAAGTTACAGAAGCTTATAGCAGGAGGTGGGT GCTGAGAACATAGCGTACGTATGTCTTGCAAGTTACAGTGAATTTGGCAGGCG GACAACCAGTTAGTATGAAGAACATGAAAGCTGTGCGCGAGCTCACTAAGAA ACACGGGATTAAGGTTTTCTATGACGCGACTCGCTGCGTTGAGAACGCATACT TCATAAAGGAACAGGAAGAGGGTTACCAGGATAAAAACGATTAAGGAAATAG TTCACGAGATGTTTTCATATGCGGACGGATGTACTATGTCAGGCAAGAAAGA TTGCTTAGTAAATATTGGCGGGTTCTTGTGCATGAATGACGAGGACCTTTTCT TAGCCGCGAAGGAAATCGTCGTTGTATATGAGGGCATGCCTAGCTACGGTGG

	GCTTGCTGGTCGAGACATGGAAGCTATGGCCATTGGGTTGAGAGAGAGTCTG CAATACGAGTATATCCGTCACCGCATATTACAGGTACGTTACTTAGGGGAGA AGCTCAAGGAAGCGGGTGTACCAATCTTAGAGCCGGTGGGTGGACACGCAGT ATTTCTCGACGCCCGGCGCTTTTGCCCGCACATCCCACAAGAGGAATTCCTG CCCAAGCGTTAGCAGCGGCAATCTATGTCGAGTGCGGTGTGCGGACTATGGA GCGTGGGATAATCTCCGCCGGGCGCGATGTTAAGACGGGAGAGAACCACAAG CCGAAGCTTGAAACCGTTCGGGTACAATTCCGCGTCGCGTGTATACTTACAA GCACATGGACGTCGTTGCCGAAGGCATTATCAAGCTCTACAAACACAAAGAG GACATTAAGCCTCTGGAGTTCGTATACGAGCCCAAACAGTTAAGATTCTTCAC GGCAAGATTTCGGTATTAAGAAGTAA
<i>hisfntpl</i>	CATCACCATCATCATCACATGCGTTTCGAGGACTACCCGGCAGAGCCGTTTCG CATTAAAGTCAGTCGAAACCGTGAAGATGATCGACAAGGCGGCACGTGAGGAA GTAATAAAGAAAGCTGGTTACAATACATTTCCTTATCAATAGTGAAGATGTAT ACATAGACCTTCTTACTGACAGCGGCACTAATGCTATGTCTGACAAGCAATGG GGCGGCTTAATGCAGGGCGACGAGGCGTACGCTGGTTCTCGCAATTTCTTCCA TCTTGAAGAAACGGTTAAAGAAATTTTCGGATTCAAGCACATTGTACCTACAC ACCAGGGCCGAGGGGCGGAAAATATCTTATCGCAAATTGCCATTAAACCGGG CCAATACGTACCAGGTAATATGTATTTACGACGACACGTTATCACCAGGAG CGCAATGGTGGTATTTTCAAGGACATTATACGCGACGAGGCGCATGACGCCA CCCTGAATGTGCCCTTCAAAGGAGACATAGATTTGAACAAGTTACAGAAGCT TATAGACGAGGTGGGTGCTGAGAACATAGCGTACGTATGTCTTGCAGTTACA GTGAATTTGGCAGGCGGACAACCAGTTAGTATGAAGAACATGAAAGCTGTGC GCGAGCTCACTAAGAAACACGGGATTAAGGTTTTCTATGACGCGACTCGCTG CGTTGAGAACGCATACTTCATAAAGGAACAGGAAGAGGGTTACCAGGATAAA ACGATTAAGGAAATAGTTCACGAGATGTTTTCATATGCGGACGGATGTACTAT GTCAGGCAAGAAAGATTGCTTAGTAAATATTGGCGGGTCTTGTGCATGAAT GACGAGGACCTTTTCTTAGCCGCGAAGGAAATCGTCGTTGTATATGAGGGCA TGCCTAGCTACGGTGGGCTTGCTGGTCGAGACATGGAAGCTATGGCCATTGG GTTGAGAGAGAGTCTGCAATACGAGTATATCCGTCACCGCATATTACAGGTA CGTTACTTAGGGGAGAAGCTCAAGGAAGCGGGTGTACCAATCTTAGAGCCGG TGGGTGGACACGCAGTATTTCTCGACGCCCGGCGCTTTTGCCCGCACATCCCA CAAGAGGAATTCCCTGCCCAAGCGTTAGCAGCGGCAATCTATGTCGAGTGCG GTGTGCGGACTATGGAGCGTGGGATAATCTCCGCCGGGCGCGATGTTAAGAC GGGAGAGAACCACAAGCCGAAGCTTGAAACCGTTCGGGTACAATTCCGCGT CGCGTGTATACTTACAAGCACATGGACGTCGTTGCCGAAGGCATTATCAAGCT CTACAAACACAAAGAGGACATTAAGCCTCTGGAGTTCGTATACGAGCCCAA CAGTTAAGATTCTTCACGGCAAGATTTCGGTATTAAGAAGTAA

Table S4 List of plasmids used in this study

Plasmid Name	Description	Reference
pEV	pJUMP29-1A backbone (pBR322/ROP oriV, KanR) with empty cloning site	This work
pPCA1	pGro7-derived backbone (p15a oriV, CamR, T7 IPTG induction) with genes <i>tphA1A2A3</i> and <i>dcddh</i> .	⁴
pPCA3	pGro7-derived backbone (p15a oriV, CamR, T7 IPTG induction) genes <i>tphA1A2A3</i> , <i>dcddh</i> , and <i>tpaK</i>	This work
pPCA4	pJUMP49-2B backbone (pBR322/ROP oriV, SpecR, T7 IPTG induction) with genes <i>tphA1A2A3</i> , <i>dcddh</i> , and <i>tpaK</i>	This work

pTpaK	pJUMP29-1D' backbone (pBR322/ROP oriV, KanR, T7 IPTG induction) with gene <i>tpaK</i>	This Work
pCAT1	pQlinkN backbone (pUC oriV, AmpR, T5-LacI IPTG induction) with genes <i>aroY</i> and <i>kpdB</i>	⁴
pFnTPL	pJUMP29-1A backbone (pBR322/ROP oriV, KanR, T7 IPTG induction) with gene <i>Fusobacteriu. nucleatum tpl</i>	This work
pCAT-FnTPL	pJUMP49-2A backbone (pBR322/ROP oriV, SpecR, T7 IPTG induction) with genes <i>aroY</i> , <i>kpdB</i> , and <i>Fusobacterium nucleatum tpl</i>	This work
pHisFnTPL	pJUMP29-1A backbone (pBR322/ROP oriV, KanR, T7 IPTG induction) with N-terminal his tagged gene <i>Fusobacterium nucleatum tpl</i>	This work
pHisLCC	pET-21a backbone (colE1 oriV, AmpR, T7 IPTG induction) with C-terminal his tagged leaf branch compost cutinase variant ICCM.	Uttamapinant lab

Details on plasmid assemblies

pPCA3

pPCA1 was first linearized by *HindIII*. The cassette encoding for *tpak* was acquired through PCR of plasmid pTpaK with primers (pTpaK-F and pTpaK-R) to add *HindIII* cut sites and homologous ends. These were then assembled by NEBuilder HiFi DNA Assembly Cloning.

pPCA4

Expression cassette assembled in a pJUMP49-2B backbone, includes a T7 promoter, pET RBS, coding sequence for *tpaA1* (1A backbone). Includes pET RBS for coding sequences *tpaA2* (1B) and *tpaA3* (1C). Includes pET RBS and terminator b0015 for coding sequence *dcddh* (1D'). Finally, there is J23107 constitutive promoter, BBa_B0032_RBS, coding sequence for *tpak* (1E), and terminator L1U1H09.

pTpaK

Expression cassette assembled in a pJUMP29-1D' backbone, includes a J23107 constitutive promoter, BBa_B0032_RBS, coding sequence for *tpak*, and terminator L1U1H09.

pFnTPL

Expression cassette assembled in a pJUMP29-1A backbone, includes a T7 promoter, pET RBS, coding sequence for *fntrl*, and terminator L3S1P32.

pCAT-*FnTPL*

Genes were assembled in a pJUMP49-2A backbone. Expression cassette is formed of *aroY* with a T7 promoter, pET RBS, and terminator L2UH09; *kpdB* with promoter J23107, BBA_B0034 RBS, and terminator BBA_B0015; and *fntpl* with T7 promoter, PET RBS, and terminator L3S1P32.

Transformation: Chemically competent *E. coli* DH5 α cells were prepared via the calcium chloride treatment⁶ and transformed with assembled plasmids. Competent cells were transformed by heat-shock at 42 °C for 30 s, then recovered in 300 μ L of SOC media for 1 h and incubated on LB agar plates containing appropriate antibiotics overnight at 37 °C. Purified plasmids from *E. coli* DH5 α were transferred into *E. coli* BL21(DE3) for protein expression using the same protocol.

Protein gel: For SDS-PAGE, 15-well 4-12% Bis-TRIS NuPAGE Bolt gels (Thermo Scientific) and 4 μ L of PageRuler unstained protein ladder (Thermo Scientific) or Precision Plus Protein prestained ladder (Bio-Rad) were used. Gels were run in 1 $\times$ MES buffer (Novagen) at 200 V for 25 min.

S4 Protein purification and biotransformations

Protein expression and reaction conditions are described in the main text, alternative reaction conditions are shown in the supplementary figures with varied carbon source, temperature and cell density (**Figure S18** and **S19**).

Purification of *FnTPL*

A post expression *E. coli*_His*FnTPL* cell pellet was resuspended in 50 mL of buffer A (50 mM sodium phosphate buffer pH 7.5, containing 150 mM NaCl, 0.5 mM PLP, and 20 mM imidazole) and kept on ice. 50 mL of the resulting cell suspension was lysed by sonication (Fisherbrand™ Model 120 Sonic Dismembrator equipped with 6 mm diameter tip, 40% amplitude with 140 cycles of 3 s on/off). The resulting solution was clarified by centrifugation at 10,000 $\times$ g for 30 min at 4 °C before the supernatant was filtered (0.4 μ M) and kept on ice. Protein purification was carried out using an ÄKTA go (Cytiva) chromatography system fitted with a 1 mL HisTrap HP column (Cytiva). Protein was eluted using a gradient of buffer A to buffer B (50 mM sodium phosphate buffer pH 7.5, containing 150 mM NaCl, 0.5 mM PLP, and 300 mM imidazole). Fractions

containing the enzyme were verified by SDS-PAGE, pooled, and protein concentrations measured by Bradford assay. Purified His*Fn*TPL was used in biotransformations at (final concentration = 100 ug/mL) on the same day.

Purification of LCC

A post expression *E. coli*_HisLCC cell pellet was resuspended in 20 mL of buffer C (20 mM Tris-HCl, pH 8.0 containing 200 mM NaCl, and 20 mM imidazole) and kept on ice. 20 mL of the resulting cell suspension was lysed by sonication (Fisherbrand™ Model 120 Sonic Dismembrator equipped with 6 mm diameter tip, 40% amplitude with 140 cycles of 3 s on/off). The resulting solution was clarified by centrifugation at 10,000 x g for 30 min and 4 °C before the supernatant was filtered (0.4 µM) and kept on ice. Purification was carried out by incubating the supernatant with 2 mL of pre-equilibrated Nickel beads for 1 h, 10,000 rpm at 4 °C. The supernatant was discarded and beads were washed with 5 × 2 mL buffer C and 2 mL buffer D (20 mM Tris-HCl, pH 8.0 containing 200 mM NaCl, and 50 mM imidazole). Elution was then carried out with 2 mL buffer E (20 mM Tris-HCl, pH 8.0 containing 200 mM NaCl, and 300 mM imidazole). Fractions containing the enzyme were verified by SDS-PAGE, pooled, and protein concentrations verified by Bradford assay.

Two-step biotransformation for the conversion of TPA to L-DOPA

Harvested *E. coli*_pPCA3_pCAT1 post expression cells were washed with PBS buffer, resuspended at OD₆₀₀ = 30 in M9 medium and 3 mL of the resulting cell suspension was used for each biotransformation. Disodium terephthalate (final concentration = 5 mM) and glucose (final concentration = 5% w/v) were added to the resulting cell suspension and incubated in a 15 mL Falcon tube at 21 °C, 220 rpm. After 24 h, 600 µL of *E. coli*_p*Fn*TPL post expression cells (OD₆₀₀ = 150 in M9 medium) and M9 medium (containing sodium pyruvate (480 mM), ammonium chloride (1.2 M), potassium chloride (8 mM), disodium EDTA (24 mM), sodium sulfite (56 mM) and pyridoxal phosphate (4 mM) were added and the pH was adjusted to 8.0 with ammonium hydroxide before incubation at 21 °C, 220 rpm for 3 h.

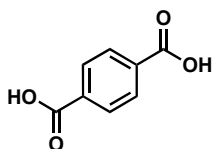
Scale up of PET waste to L-DOPA biotransformation

Pre-grown *E. coli*_pPCA3_pCat1 was inoculated at OD₆₀₀ = 0.1 and incubated in LB medium at 37 °C until OD₆₀₀ reaches 0.6-0.8. At this time, IPTG (0.5 mM) was added and protein expression was carried out at 30 °C, 220 rpm overnight. The *E. coli* cells were washed with PBS buffer and resuspended to OD₆₀₀ = 30 in 500 mL M9 medium containing waste derived TPA (10.7 mM) and

glucose (5% w/v) and the reaction was incubated in a 2 L Erlenmeyer flask at 21 °C, 220 rpm for 24 h. After 24 h, 50 mL *E. coli_pFnTPL* (post enzyme expression cells in M9 medium at OD600 = 360) and fresh M9 medium (50 mL, containing sodium pyruvate (720 mM), ammonium chloride (3 M), potassium chloride (12 mM), disodium EDTA (36 mM), sodium sulfite (84 mM) and pyridoxal phosphate (6 mM) were added and pH was adjusted with ammonium hydroxide if needed. The reaction was incubated under the same condition for 3 h.

S5 Analysis of PET hydrolysis and isolated L-DOPA

NMR and purity PET waste hydrolysis to TPA

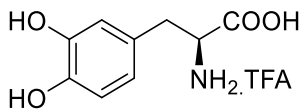


^1H NMR (600 MHz, DMSO- d_6) δ : 8.04 (s, 4H). ^{13}C NMR (150 MHz, DMSO- d_6) δ : 166.66, 134.46, 129.46. Spectroscopic data is in agreement with reported literature⁴. For quantification of waste derived terephthalic acid, 10 mg of terephthalic acid powder isolated from the hydrolysed PET was dissolved in 1 mL DMSO- d_6 containing 10 mM 1,3,5-trimethoxybenzene as an internal standard (see **Figure S20**).

Table S5 Terephthalic acid powder obtained from the plastic hydrolysis

	PET loading (g)	Resulting TPA (g)	TPA purity in the powder (% w/w)	TPA yield from the hydrolysis (%)
PET foil (#1)	10	5.53	83.07	43.3
PET foil (#2)	20	11.91	95.00	52.3
PET bottle	10	0.50	51.35	2.6

NMR of the TFA salt of L-DOPA



^1H NMR (500 MHz, D $_2$ O) δ 6.89 (d, J = 8.1 Hz, 1H, CHCHCOH), 6.81 (d, J = 2.2 Hz, 1H, CCHCO), 6.72 (dd, J = 8.1, 2.2 Hz, 1H, CCHCH), 4.25 (dd, J = 7.7, 5.4 Hz, 1H, CHN), 3.21 (dd,

$J = 14.7, 5.4$ Hz, 1H, CH_AH_BCN), 3.08 (dd, $J = 14.7, 7.7$ Hz, 1H, CH_AH_BCN). ^{13}C NMR (125 MHz, D_2O) δ 171.6, 162.9 (q, $J = 35.5$ Hz), 144.3, 143.6, 126.4, 121.8, 117.0, 116.6, 116.5 (q, $J = 290.9$ Hz), 54.2, 34.9. ^{19}F NMR (470 MHz, D_2O) δ -75.58. **HRMS** $\text{C}_9\text{H}_{12}\text{NO}_4^+$ calcd. = 198.07608, found = 198.07610. ^1H NMR corresponds to an authentic commercial L-DOPA at pH 7.4 in PBS buffered- D_2O (see **Figure S21**).

S6 *In silico* docking

Structural modeling of *Fn*TPL

The three-dimensional structure of the *Fn*TPL dimer was obtained using AlphaFold3⁷. Active site residues were identified by the alignment of the *Fn*TPL-dimer with a TPL homolog from *Citrobacter freundii* (PDB ID 2TPL)⁸.

Molecular docking

Docking simulations were performed using the molecular docking package GNINA⁹. Initially, the cofactor pyridoxal phosphate (PLP) was covalently docked to the *Fn*TPL-dimer. To achieve this the oxygen atom of the aldehyde in PLP was removed manually. This resulted in a new covalent bond between the carbon of the aldehyde in PLP and the NZ atom of Lys260 on chain A. Another round of docking was performed to bind the ligands CAT and PCA to the *Fn*TPL-dimer-PLP covalent complex. The residues Arg384 on chain A and Tyr74 on chain B were treated as flexible during ligand docking simulations. Binding poses were selected using the lowest binding energy score from each docking simulation, and residue ligand distances are shown in **Table S6**.

Table S6. Reside-ligand distances (Å) between docked catechol and PCA molecules in *Fn*TPL.

Residue	Residue-catechol distance	Residue-PCA distance
D53	3.9	4.1
F126	4.8	4.8
F452	6.4	3.6
PLP (K260)	3.3	4.9
M382	4.9	4
R220	3.3	2.1
R384	2.8	4.6
R407	2.8	3.9
T127	5.8	2.8
T52	4.2	2.8

S7 Supplementary figures

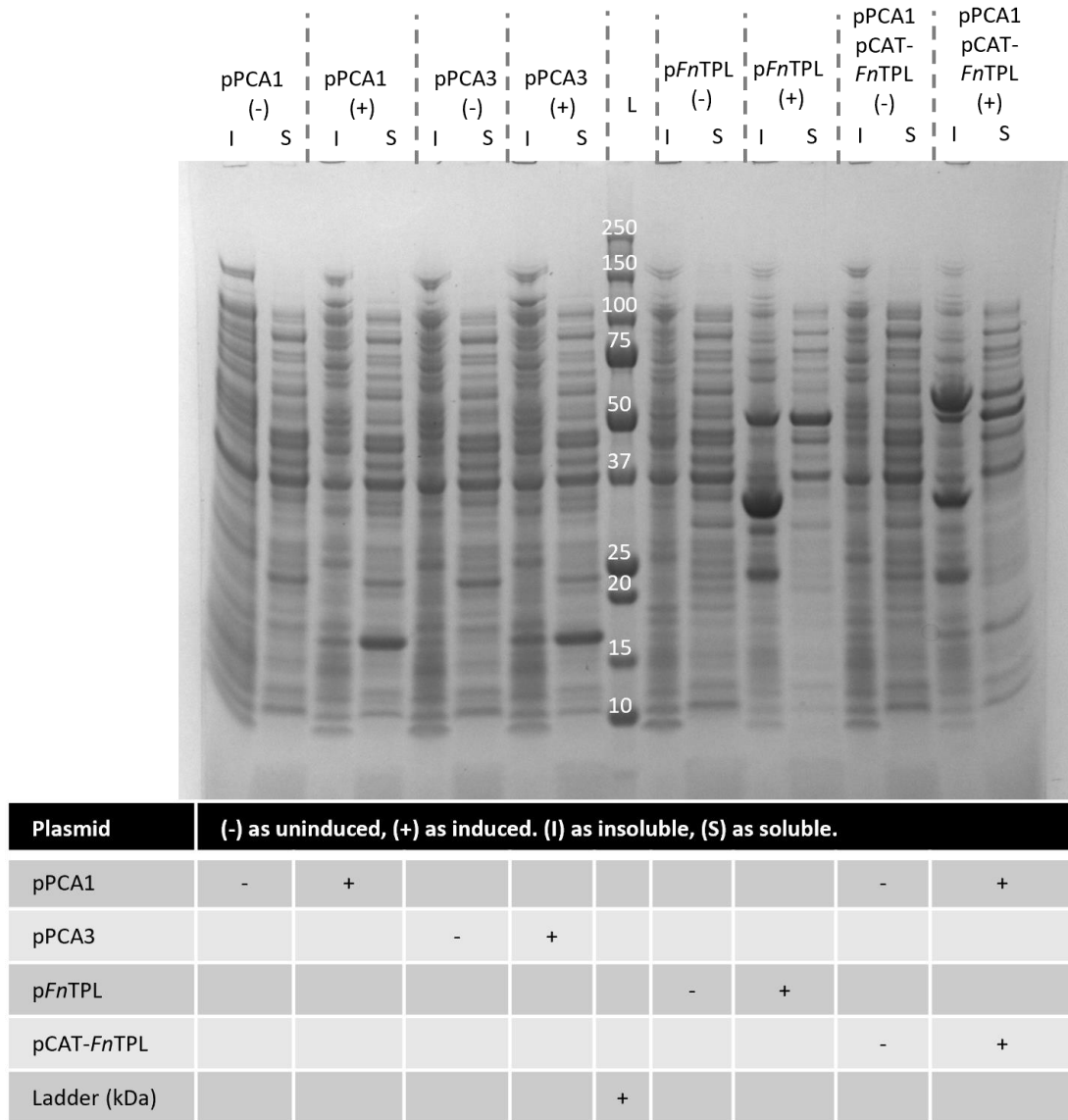


Figure S1. SDS-PAGE analysis of proteins expressed by *E. coli* BL21(DE3) containing different pathway plasmids (Part 1). “S” and “I” indicate soluble and insoluble fractions respectively. Plasmids pPCA encode for *tph* complex and *DCDDH*, pCAT for *aroY* and *kpdB*, pFnTPL for *fnTPl*, and pCAT-FnTPL for *fnTPl*, *aroY*, and *kpdB*. Expected band sizes (kDa) are TphA1: 36.3, TphA2: 46.2, TphA3: 17.2, DCDDH: 32.9, TpaK: 48.9, AroY: 54, KpdB: 21.6, *KiTPL*: 51.5, *FnTPL*: 52.2

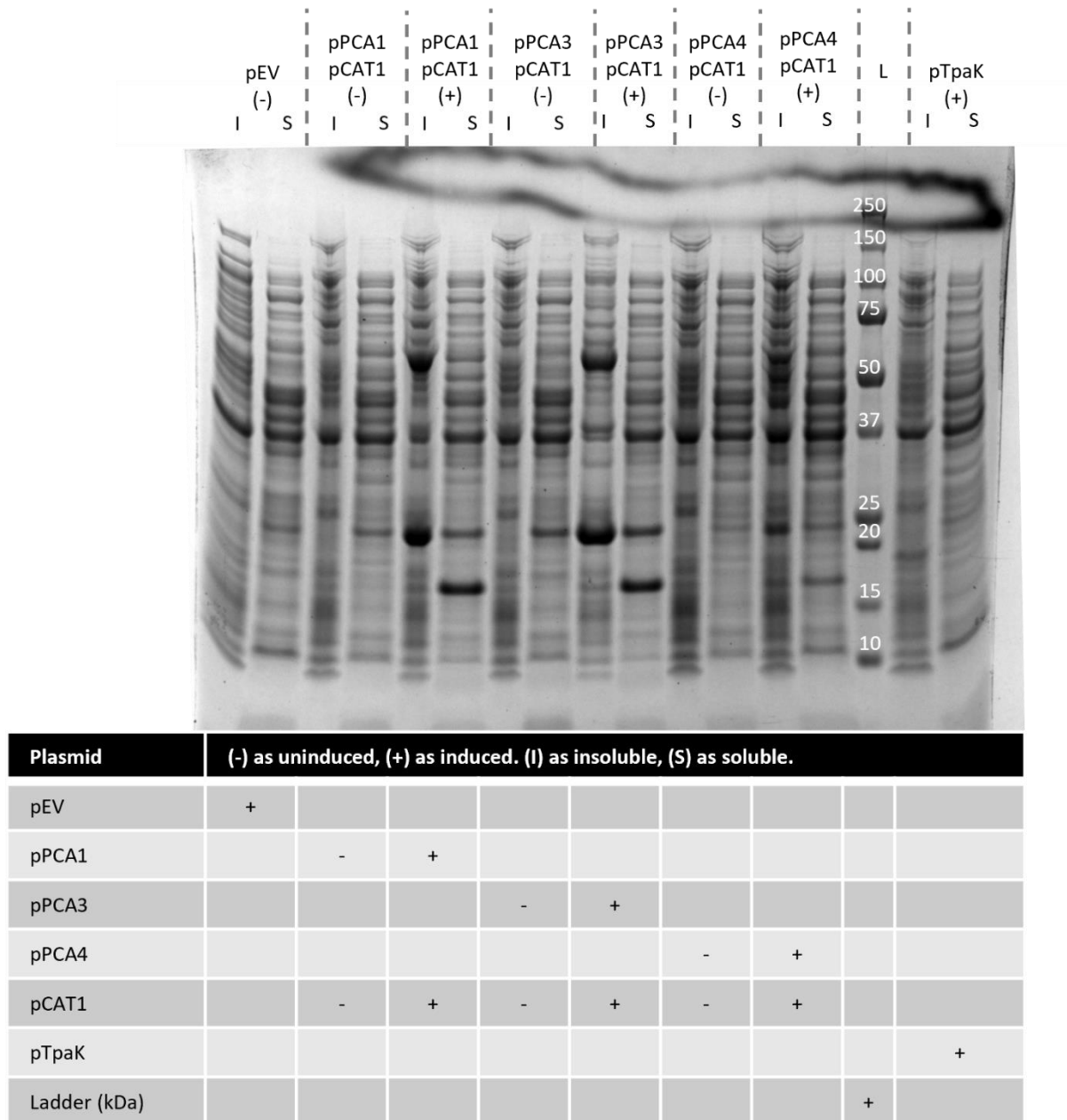


Figure S2. SDS-PAGE analysis of proteins expressed by *E. coli* BL21(DE3) containing different pathway plasmids (Part 2). “S” and “I” indicate soluble and insoluble fractions respectively. Plasmids pPCA encode for *tph* complex and *DCDDH*, pCAT for *aroY* and *kpdB*, p*FnTPL* for *fnTpl*, and pTpaK for *tpaK*. Expected band sizes (kDa) are TphA1: 36.3, TphA2: 46.2, TphA3: 17.2, DCDDH: 32.9, TpaK: 48.9, AroY: 54, KpdB: 21.6, *KiTPL*: 51.5, *FnTPL*: 52.2

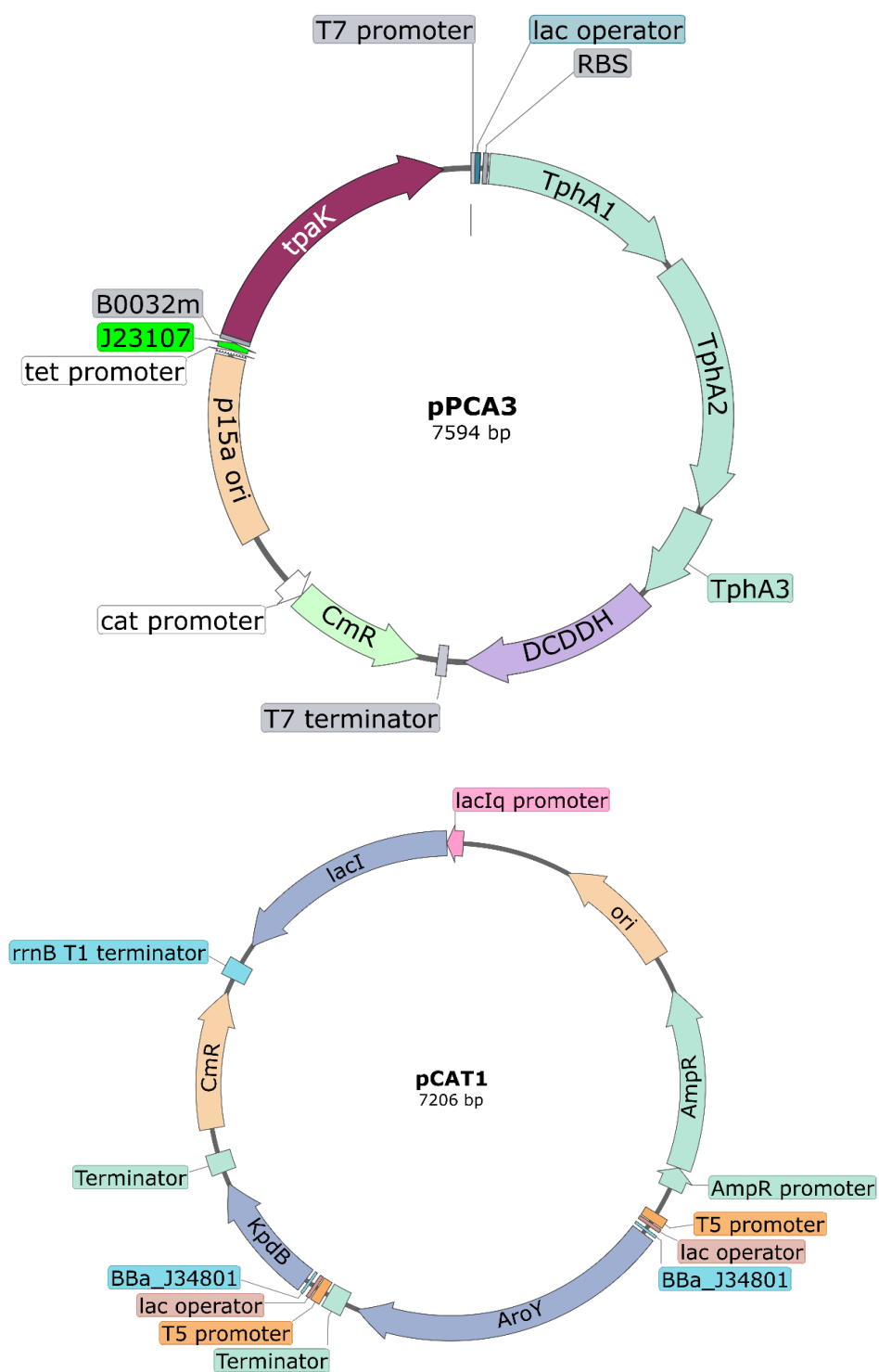


Figure S3. Plasmid maps of pPCA3 and pCAT1

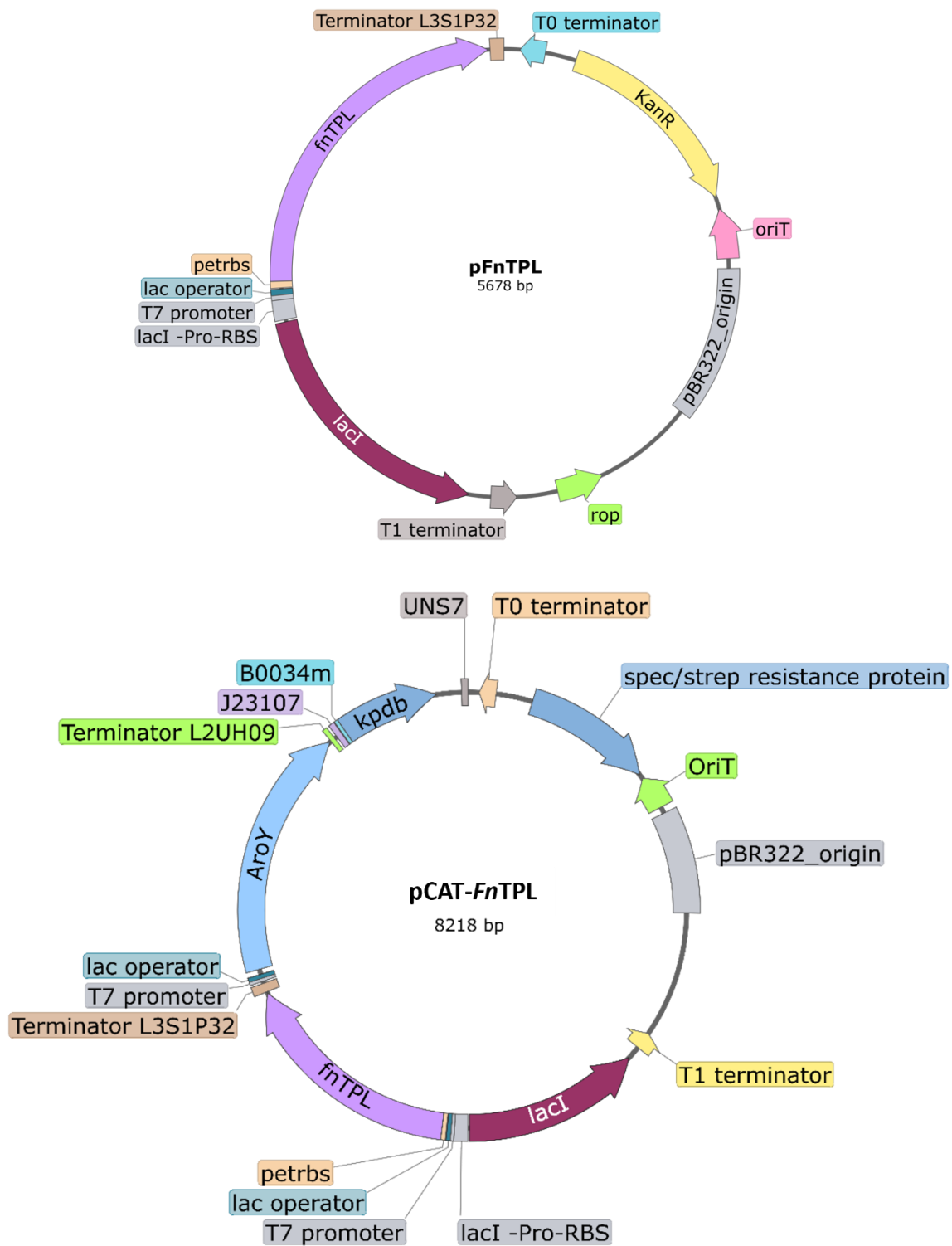


Figure S4. Plasmid maps of p*FnTPL* and pCAT-*FnTPL*.

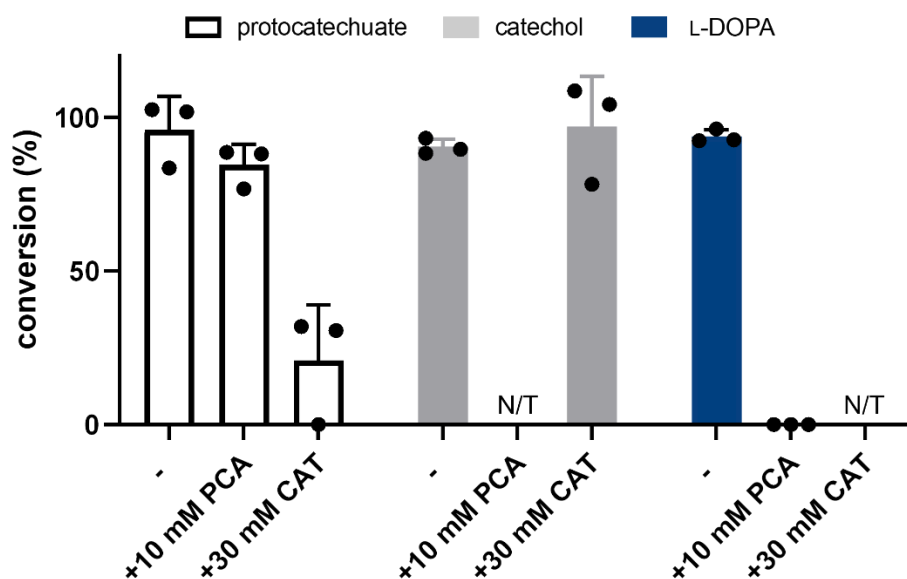


Figure S5. Effect of intermediates on biotransformation conversion. Reactions were performed using *E. coli_pPCA3* (white, 5-25 mM TPA as substrate), *E. coli_pCAT1* (grey, 5 mM PCA as substrate) for 24 h or *E. coli_pFnTPL* (blue, 5 mM CAT as substrate) for 3 h at 21 °C. N/T = not tested. All data as n = 3. Error bars shown as standard deviation.

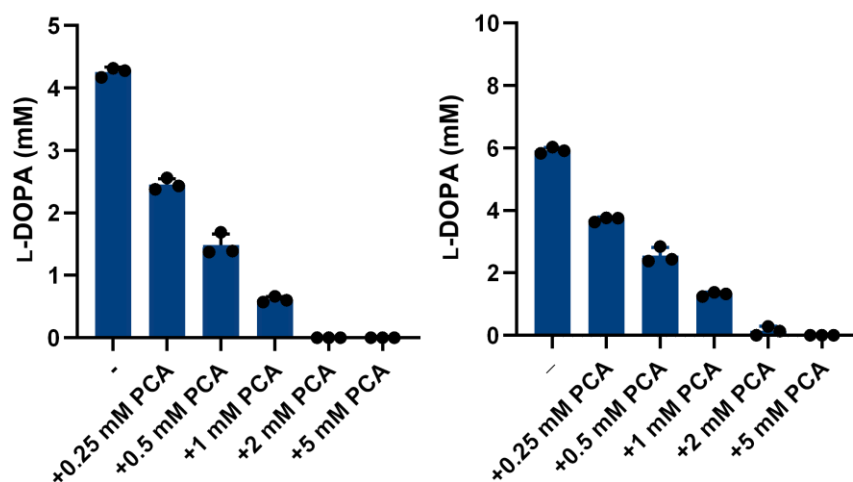


Figure S6. *FnTPL* inhibition by PCA. Reactions were performed using *E. coli_pFnTPL* (5 mM CAT as substrate) at 21 °C for 3 h (left) or purified *FnTPL* enzyme (10 mM catechol used as substrate) 21 °C for 3 h (right). All data as n = 3. Error bars shown as standard deviation.

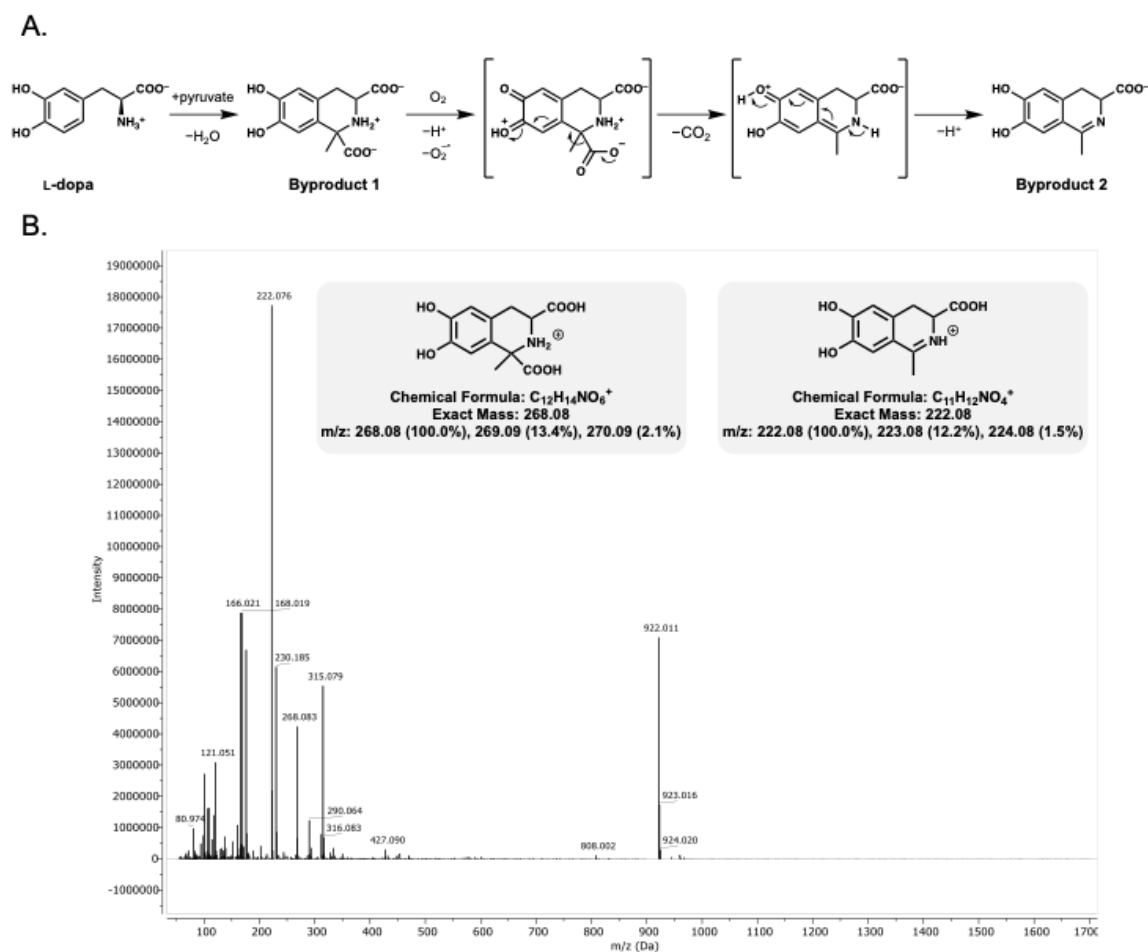


Figure S7. Biotransformation by-product formation. A: Proposed mechanism for the by-product formation in the biotransformation by Pictet-Spengler reaction with pyruvate and oxidative decarboxylation¹⁰. B: LC-MS spectrum of a reaction sample from whole cell catalysed CAT to L-DOPA biotransformation run for extended period. Reactions were performed using *E. coli* *pFnTPL* (5 mM CAT as substrate) at 21 °C for 48 h.

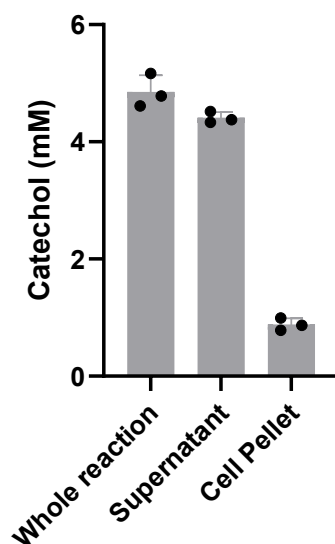


Figure S8. CAT concentration in supernatant and cell pellet following conversion of TPA to CAT. Reactions were performed using *E. coli*_pPCA3_pCAT1 and 5 mM TPA at 21 °C for 24 h (n = 3). Error bars shown as standard deviation.

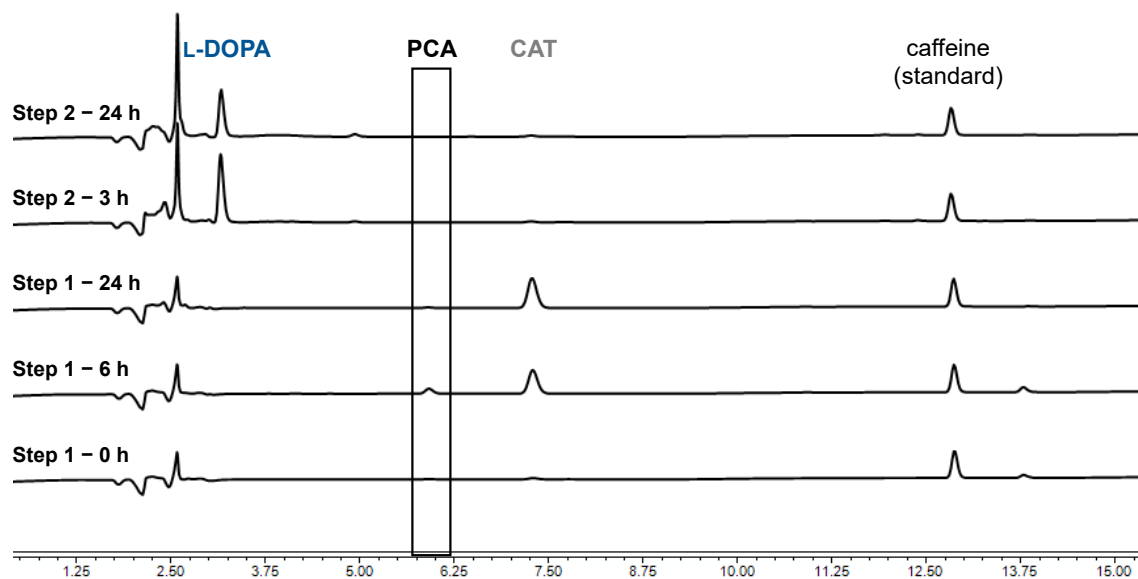


Figure S9. HPLC chromatograms depicting the two-step whole cell biotransformation of TPA to L-DOPA at different stages and time points. Biotransformations were performed using *E. coli*_pPCA3_pCAT1 (5 mM TPA as substrate) at 21 °C for 24 h followed by the incubation with *E. coli*_pFnTPL at 21 °C for further 24 h.

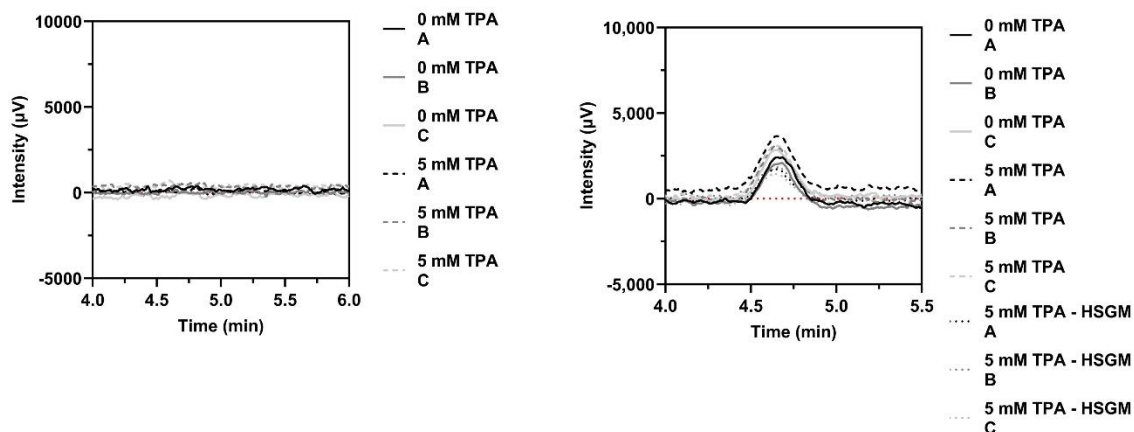


Figure S10. Gas chromatography traces of CO₂ produced before (left) and after (right) biotransformations of *E. coli_pCA4_pCAT1* with either 0 or 5 mM TPA at 21 °C for 24 h. Baseline shown in red dotted line. A, B, C denote individual test runs within the triplicate set. HSGM: Headspace gas mixture.

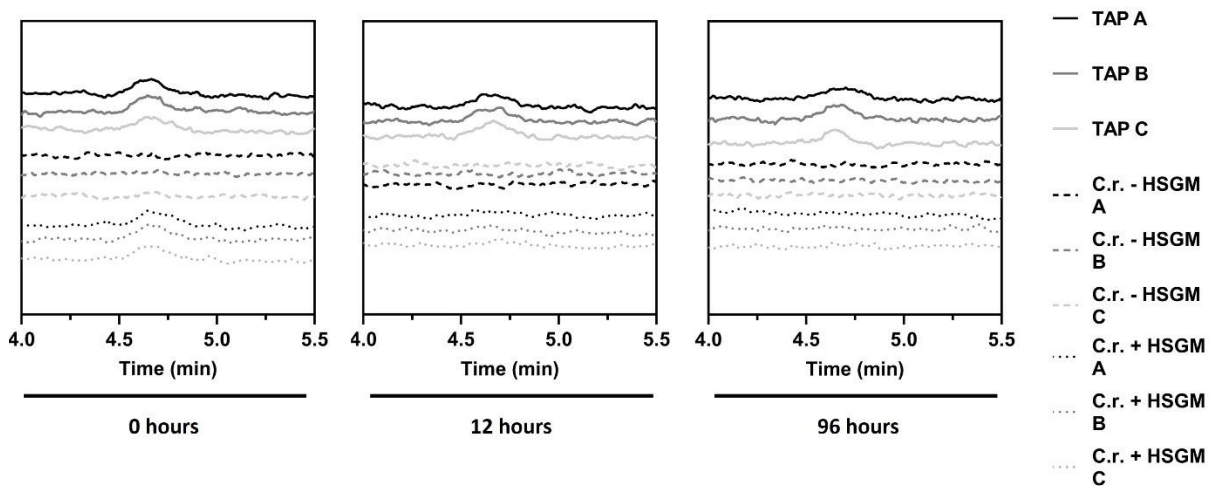


Figure S11. Gas chromatography traces of CO₂ produced by *C. reinhardtii* (C.r.) and TAP medium at timepoints of 0, 12, and 96 hours. A, B, C denote individual test runs within the triplicate set. HSGM: Headspace gas mixture.



HGSM	Replicate	Culture OD ₇₅₀	Chlorophyll a+b (µg/mL)
Y	1	2.84	45.99
Y	2	2.71	44.04
Y	3	2.73	40.77
N	1	1.62	30.19
N	2	1.61	29.86
N	3	1.62	29.24

Figure S12. Effect of headspace gas mixture (HSGM) from *E. coli*_pPCA4_pCAT1 on the photomixotrophic growth of *C. reinhardtii* cultures. (Top image) The three samples on the left (dark green) are with HSGM, whereas the three samples on the right (light green) are without. (Bottom table) Recorded culture optical density at OD₇₅₀ and estimated chlorophyll content (µg/mL) via absorbance measurements at wavelengths 647, 664, and 750 nm after 3 days incubation with or without HSGM.

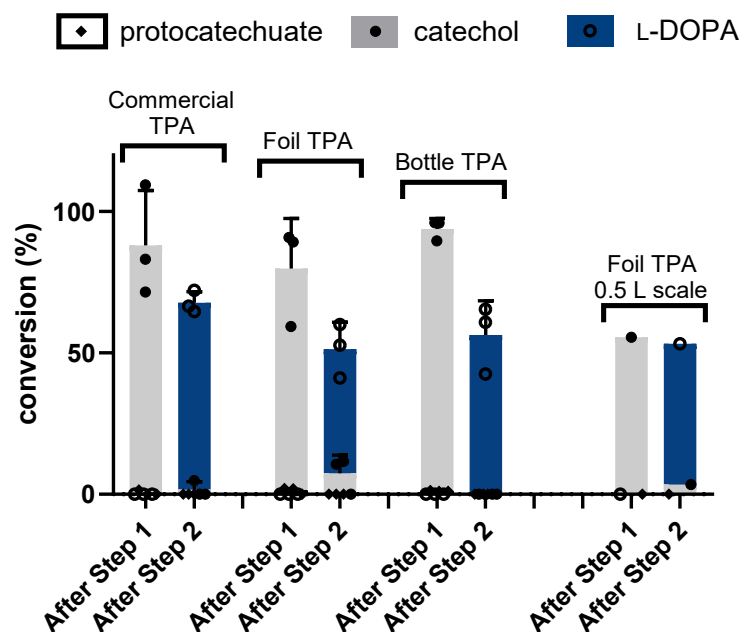


Figure S13. Two-step biotransformation of different PET waste-derived TPA to L-DOPA. Reactions were performed using *E. coli*_pPCA3_pCAT1 (5 mM TPA disodium terephthalate or TPA prepared by base hydrolysis of PET waste) at 21 °C for 24 h followed by the incubation with at 21 °C for further 3 h (n = 3). Foil TPA at 500 mL scale was performed at 10.7 mM (n=1). A scaling factor of 0.83 was applied to account for dilution effects from the addition of two strains. Error bars shown as standard deviation.

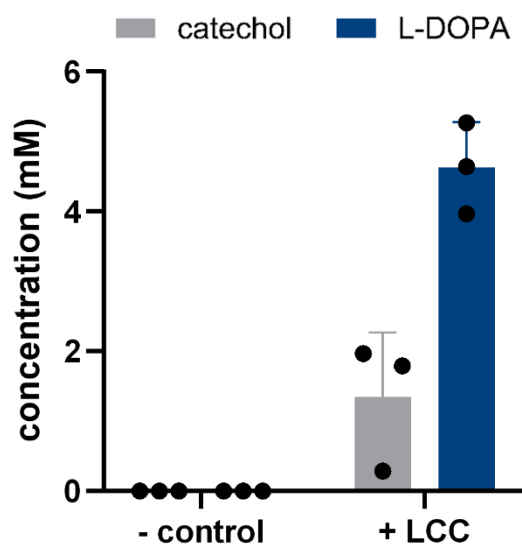


Figure S14. L-DOPA production from post-consumer PET following LCC depolymerization. Biotransformations were performed using *E. coli*_pPCA3_pCAT1 at 21 °C for 24 h followed by the incubation with *E. coli*_pFnTPL at 21 °C for further 3 h (n = 3). Error bars shown as standard deviation.

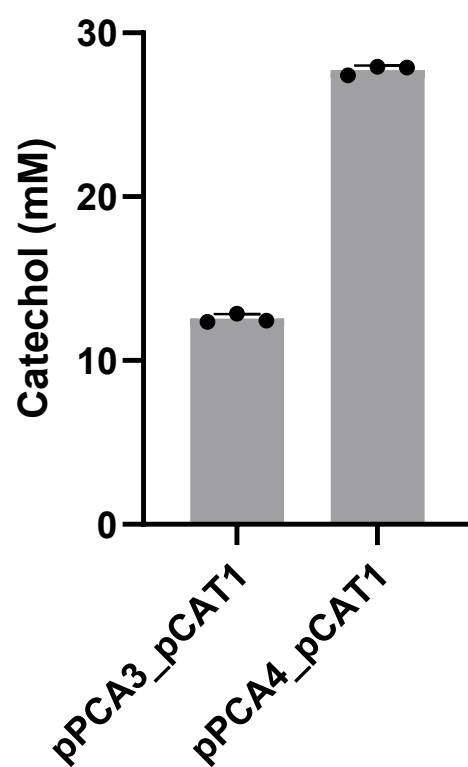


Figure S15. Comparison of catechol generated from 30 mM TPA between *E. coli* strains with the pPCA3 plasmid (left) and the pPCA4 plasmid (right). Biotransformations were performed using *E. coli* strains at 21 °C for 24 h (n = 3). Error bars shown as standard deviation.

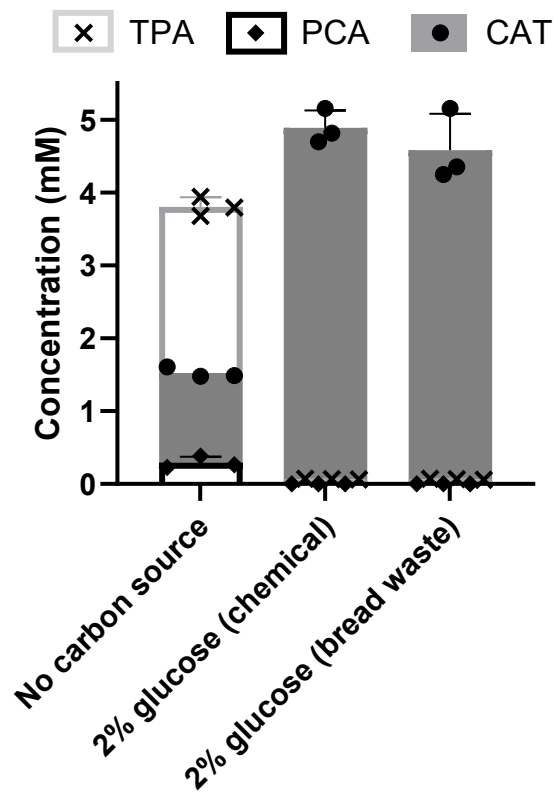


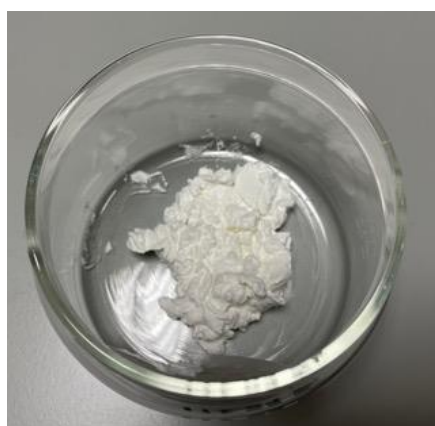
Figure S16. Effect of bread waste carbon source on the conversion of TPA to CAT. Biotransformations were performed using *E. coli*_pPCA3_pCAT1 and 5 mM TPA at 21 °C for 24 h (n = 3). Error bars shown as standard deviation.



Mixed with base/ethanol
and heated



Precipitate collected
from acidified supernatant



Used for biotransformation
without further purification

Figure S17. Plastic waste hydrolysis.

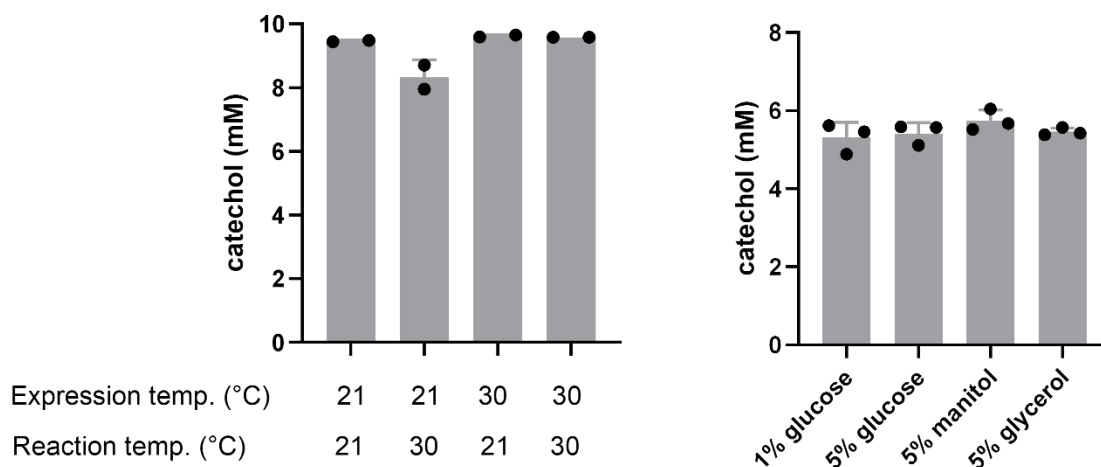


Figure S18. Effect of protein expression and reaction temperature (left) as well as carbon source (right) on the conversion of TPA to CAT. Biotransformations were performed using *E. coli*_pPCA3_pCAT1 and 5 (right) or 12 (left) mM TPA at 21 °C for 24 h. Data on the left as duplicates and on the right as triplicates. Error bars shown as standard deviation.

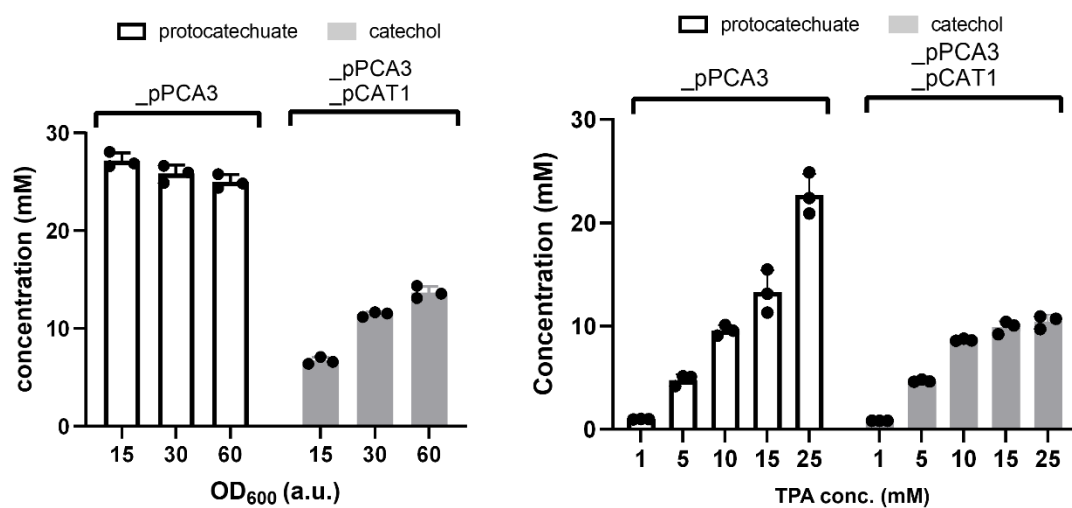


Figure S19. Effect of cell density (left) and initial substrate concentration on TPA conversion to PCA or CAT. Biotransformations were performed using *E. coli*_pPCA3 or *E. coli*_pPCA3_pCAT1 at 21 °C for 24 h. For cell density, 25 or 15 mM TPA was used for *E. coli*_pPCA3 and *E. coli*_pPCA3_pCAT1 respectively. All data as n = 3, error bars shown as standard deviation.

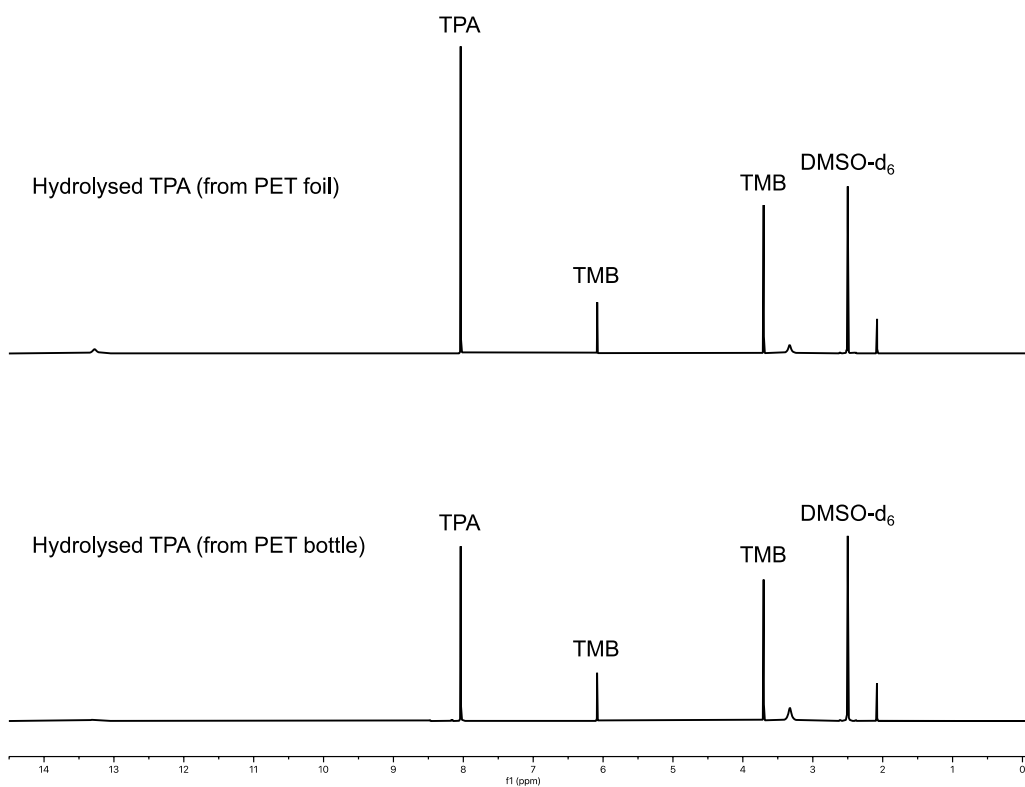


Figure S20. ^1H NMR spectra of TPA obtained from the hydrolysis of PET foil and bottle waste.

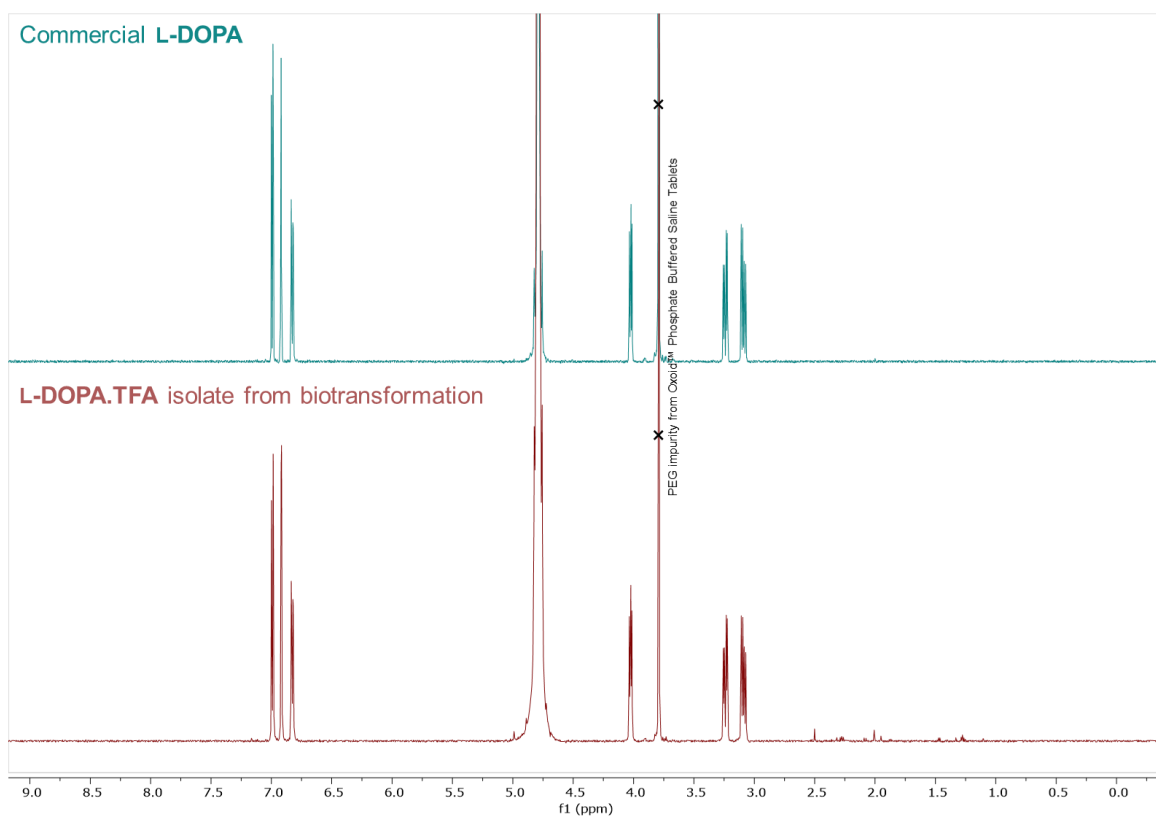


Figure S21. Comparison of waste PET-derived L-DOPA.TFA isolated from biotransformation with an authentic commercial standard by ¹H NMR in PBS buffered-D₂O (pH 7.4).

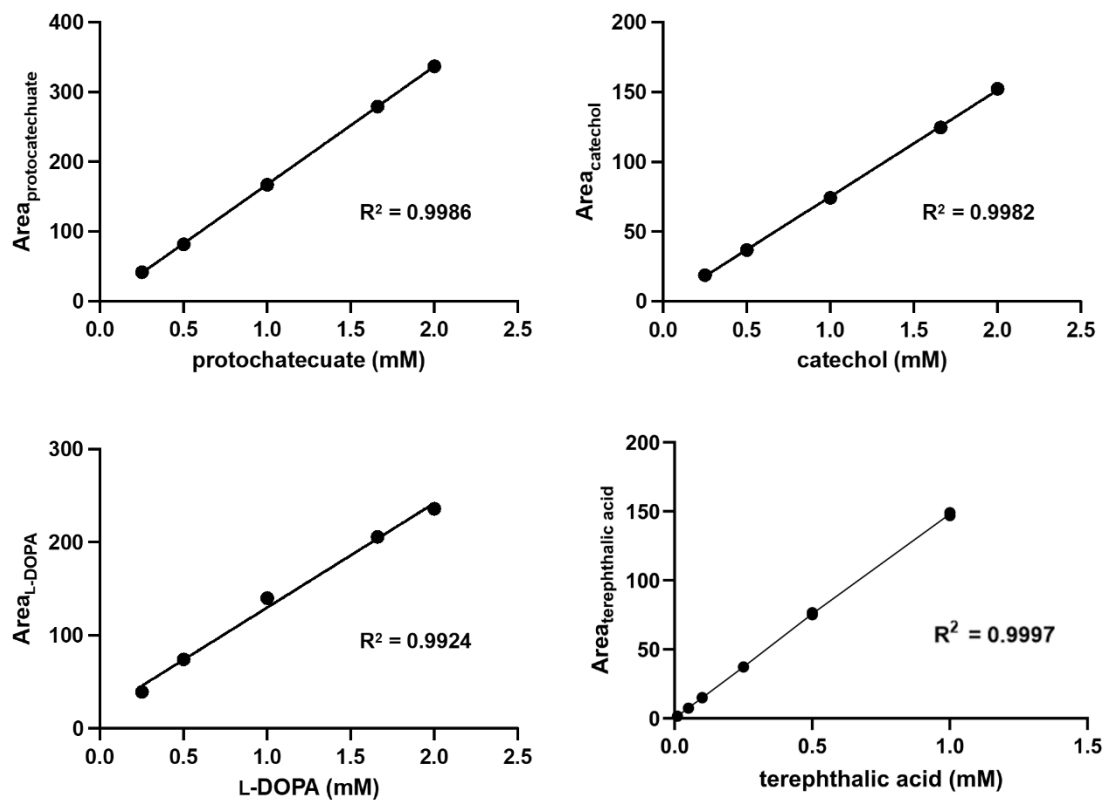
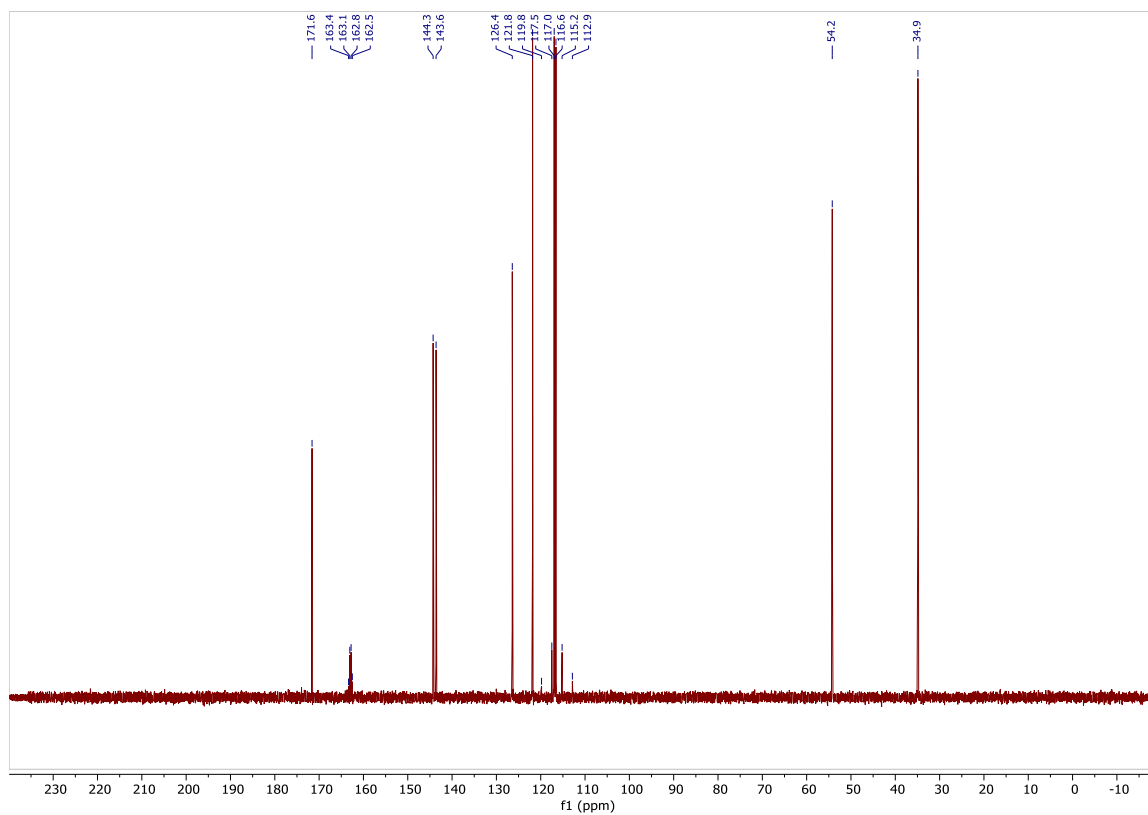
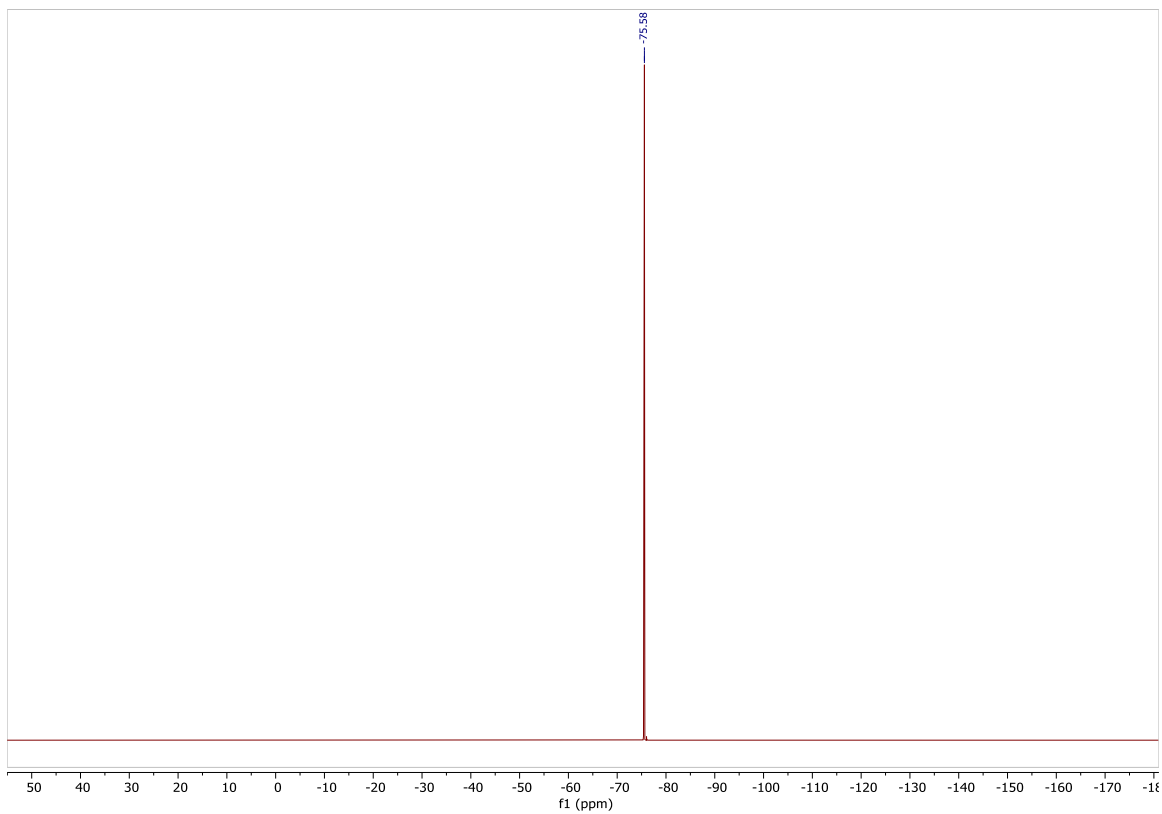


Figure S22. HPLC calibration curves for pathway analytes. For analysis samples were diluted to within the linear range. Calibration curves were generated using the mean values of triplicate injections at each analyte concentration.

^{13}C NMR



^{19}F NMR



S9 References

1. Gormant, D. S. & Levine, R. P. Cytochrome f and plastocyanin: their sequence in the photosynthetic electron transport chain of *Chlamydomonas reinhardtii*. *PNAS* **54**, 1665-1669 (1965).
2. Kropat, J. *et al.* A revised mineral nutrient supplement increases biomass and growth rate in *Chlamydomonas reinhardtii*. *Plant Journal* **66**, 770–780 (2011).
3. Sadler, J. C. & Wallace, S. Microbial synthesis of vanillin from waste poly(ethylene terephthalate). *Green Chemistry* **23**, 4665–4672 (2021).
4. Valenzuela-Ortega, M., Sutor, J. T., White, M. F. M., Hinchcliffe, T. & Wallace, S. Microbial Upcycling of Waste PET to Adipic Acid. *ACS Cent Sci* **9**, 2057–2063 (2023).
5. Valenzuela-Ortega, M. & French, C. Joint universal modular plasmids (JUMP): A flexible vector platform for synthetic biology. *Synth Biol* **6**, (2021).
6. Cohen, S. N., Chang, A. C. Y. & Hsu, L. Nonchromosomal Antibiotic Resistance in Bacteria: Genetic Transformation of *Escherichia coli* by R-Factor DNA*. *PNAS* **69**, 2110–2114 (1972).
7. Abramson, J. *et al.* Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* **630**, 493–500 (2024).
8. Sundararaju, B. *et al.* The Crystal Structure of *Citrobacter freundii* Tyrosine Phenol-lyase Complexed with 3-(4'-Hydroxyphenyl)propionic Acid, Together with Site-Directed Mutagenesis and Kinetic Analysis, Demonstrates That Arginine 381 Is Required for Substrate Specificity,. *Biochemistry* **36**, 6502–6510 (1997).
9. McNutt, A. T. *et al.* GNINA 1.0: molecular docking with deep learning. *J Cheminform* **13**, 43 (2021).
10. Kumagai, H., Katayama, T., Koyanagi, T. & Suzuki, H. Research overview of L-DOPA production using a bacterial enzyme, tyrosine phenol-lyase. *Proceedings of the Japan Academy, Series B* **99**, 75–101 (2023).