Supplementary Information

PET hydrolysing enzymes catalyse bioplastics precursor synthesis under aqueous conditions

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Contents

1. Production and Purification of PETase and TfH

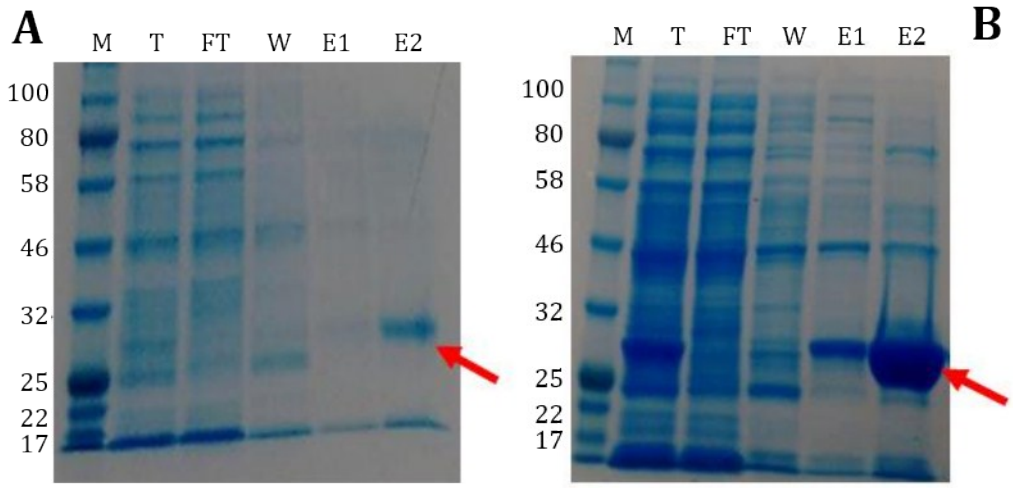


Figure S1: SDS-PAGE analysis of PETase (A) and TfH (B) expression in *E. coli*. Legend: **M** cell total lysate; **F.T.** unbound sample eluted from his-trap column; **W** first resin wash; **E1** column elution with 50 mM imidazole; **E2** column elution with 250 mM imidazole. Final product is indicated by the red arrow.

PETase and tfH genes were ordered from GeneMill - University of Liverpool in codon optimized form for expression in *E. coli*. Genes were subcloned into the pET21a vector using NheI and XhoI restriction sites for expression of the C-terminal His tagged protein. *E. coli* BL21 RIPL cells were transformed and grown in 1L 2YT media containing Ampicillin at 37°C until an O.D. 0.8 was reached. After induction by the addition of 1mM isopropyl β-D-1-thiogalactopyranoside (IPTG), the culture was further incubated for 18 h., 18°C. The cells were harvested by centrifugation at 4000 *x g* for 15 min at 4°C. The cell pellets were resuspended in Buffer A (50mM Tris-HCl pH 7.5 and 300mM NaCl) supplemented with ethylenediaminetetraacetic acid (EDTA)-free protease inhibitor cocktail (Roche), 2.5 µg/mL DNase I (2000 units/mL), and disrupted by ultrasonication (7 cycles of 20 s 14% amplitude, intervaled by 40 s rest). The soluble fraction was obtained by centrifugation at 15,000 *x g*, and applied to a 1 mL HisTrap ™ High Performance (GE Healthcare) column. The column was washed with 10 column volumes (CV) of BufferA + 0 mM imidazole), and the bound protein was finally eluted with Buffer A + 250 mM imidazole. All fractions were checked on 10% SDS-PAGE gel (Figure S1). Eluted protein volume was reduced to 2.5 mL using Amicon ultra concentrators (Merck-Millipore) and loaded onto a PD-10 column for removal of imidazole. The purified protein was concentrated to 25 µM and stored at -70°C. CAL-A and CAL-B enzymes were purchased from Sigma-Aldrich®.

2. Transesterification of FDME **3** with PETase: effect of Temperature and pH.

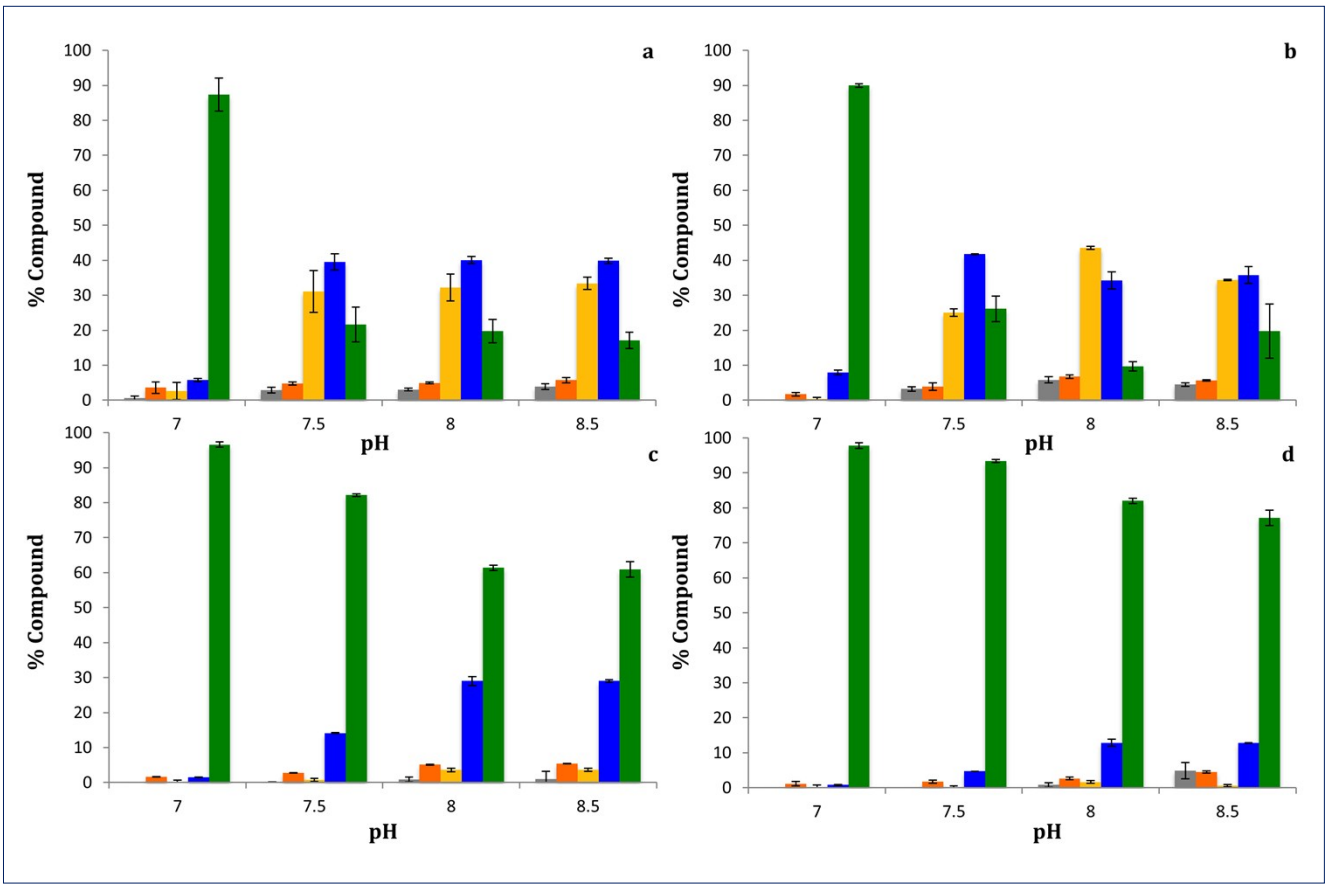


Figure S2: Bioconversion of FDME with PETase in Tris-HCl buffer (50mM)/BDO (1:1) at (A) 18°C, (B) 25°C, (C) 37°C, (D) 42°C. Product ratios as percentage of total after 24hr: Orange - mono methylester **7** (B)**;** grey - mono BDO ester **8**; yellow – Bis-BDO ester **4**; blue - methyl BDO ester **6**; green FDME **3.**

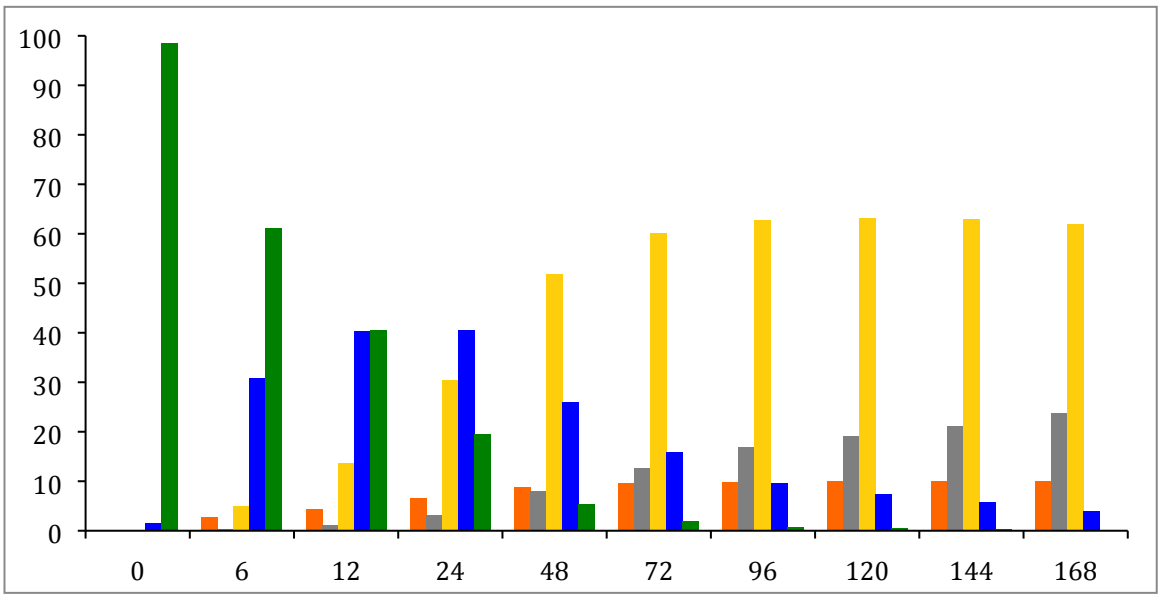


Figure S2B: Bioconversion of FDME with PETase in Tris-HCl buffer (50mM)/BDO (1:1) over 7 days.

3. Transesterification of FDME **3** with TfH: effect of temperature and pH.

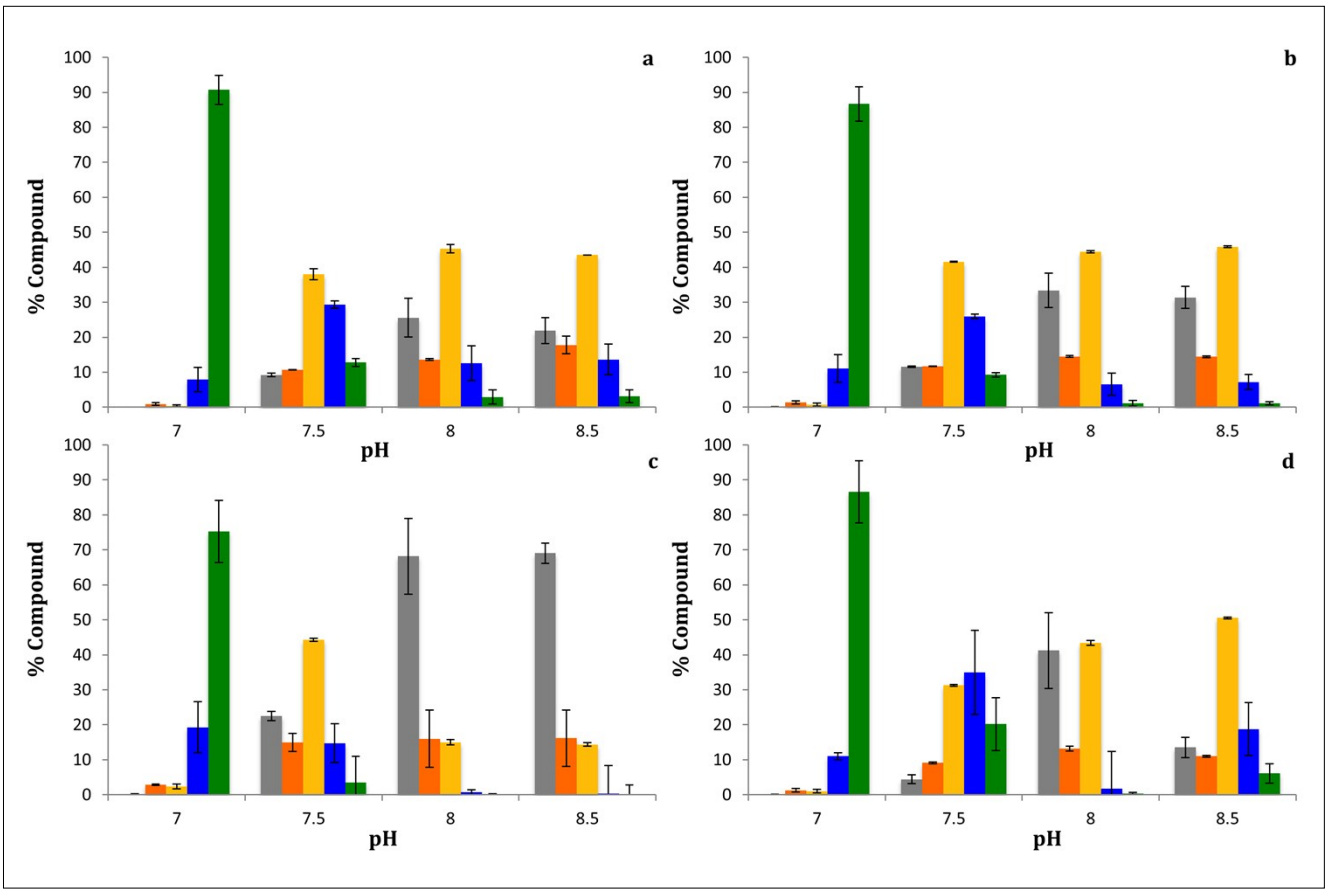


Figure S3: Bioconversion of FDME with TfH in Tris-HCl buffer (50mM)/BDO (1:1) at (A) 18°C, (B) 25°C, (C) 37°C, (D) 42°C. Product ratios as percentage of total after 24hr: Orange - mono methylester **7** (B)**;** grey - mono BDO ester **8**; yellow – Bis-BDO ester **4**; blue - methyl BDO ester **6**; green FDME **3.**

4. Transesterification of FDME **3** with CAL-A: effect of temperature and pH.

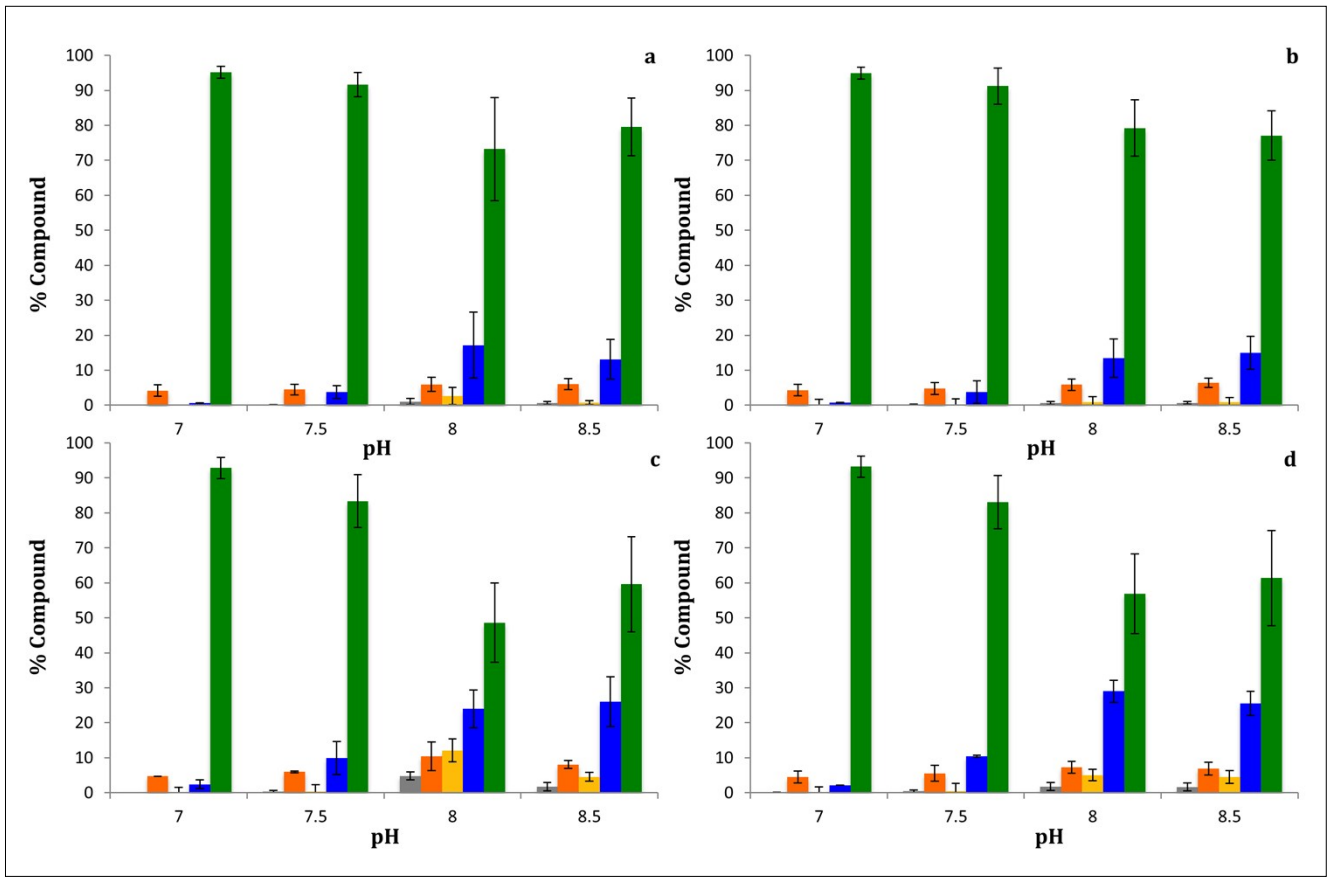


Figure S4: Bioconversion of FDME with CAL-A in Tris-HCl buffer (50mM)/BDO (1:1) at (A) 18°C, (B) 25°C, (C) 37°C, (D) 42°C. Product ratios as percentage of total after 24hr: Orange - mono methylester **7** (B)**;** grey - mono BDO ester **8**; yellow - Bis-BDO ester **4**; blue - methyl BDO ester **6**; green FDME **3.**

5. Transesterification of FDME **3** with CAL-B: effect of Temperature and pH.

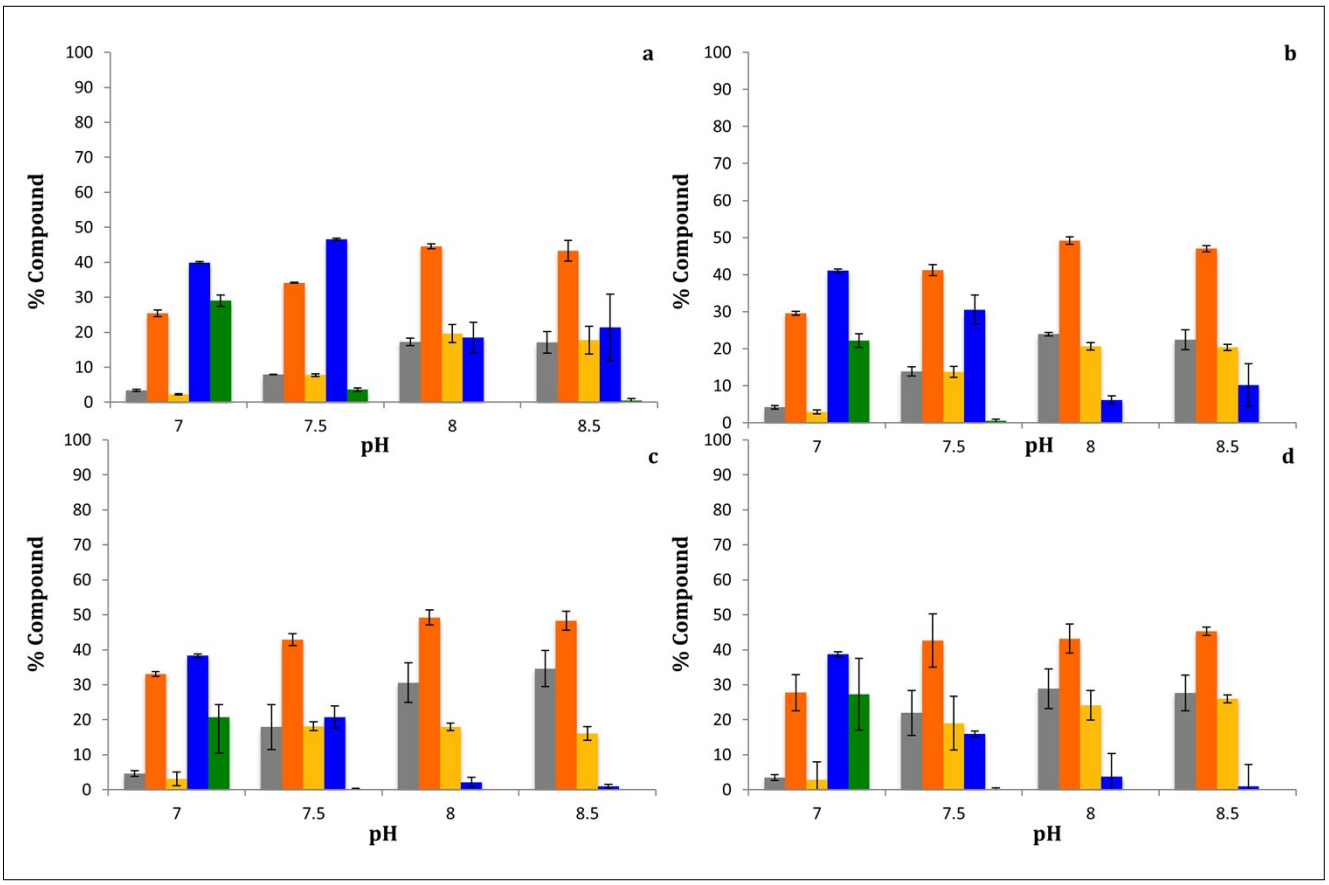


Figure S5: Bioconversion of FDME with CAL-B in Tris-HCl buffer (50mM)/BDO (1:1) at (A) 18°C, (B) 25°C, (C) 37°C, (D) 42°C. Product ratios as percentage of total after 24hr: Orange - mono methylester **7** (B)**;** grey - mono BDO ester **8**; yellow - Bis-BDO ester **4**; blue - methyl BDO ester **6**; green FDME **3.**

6. Control transesterification of FDME **3** with no enzyme: effect of Temperature and pH.

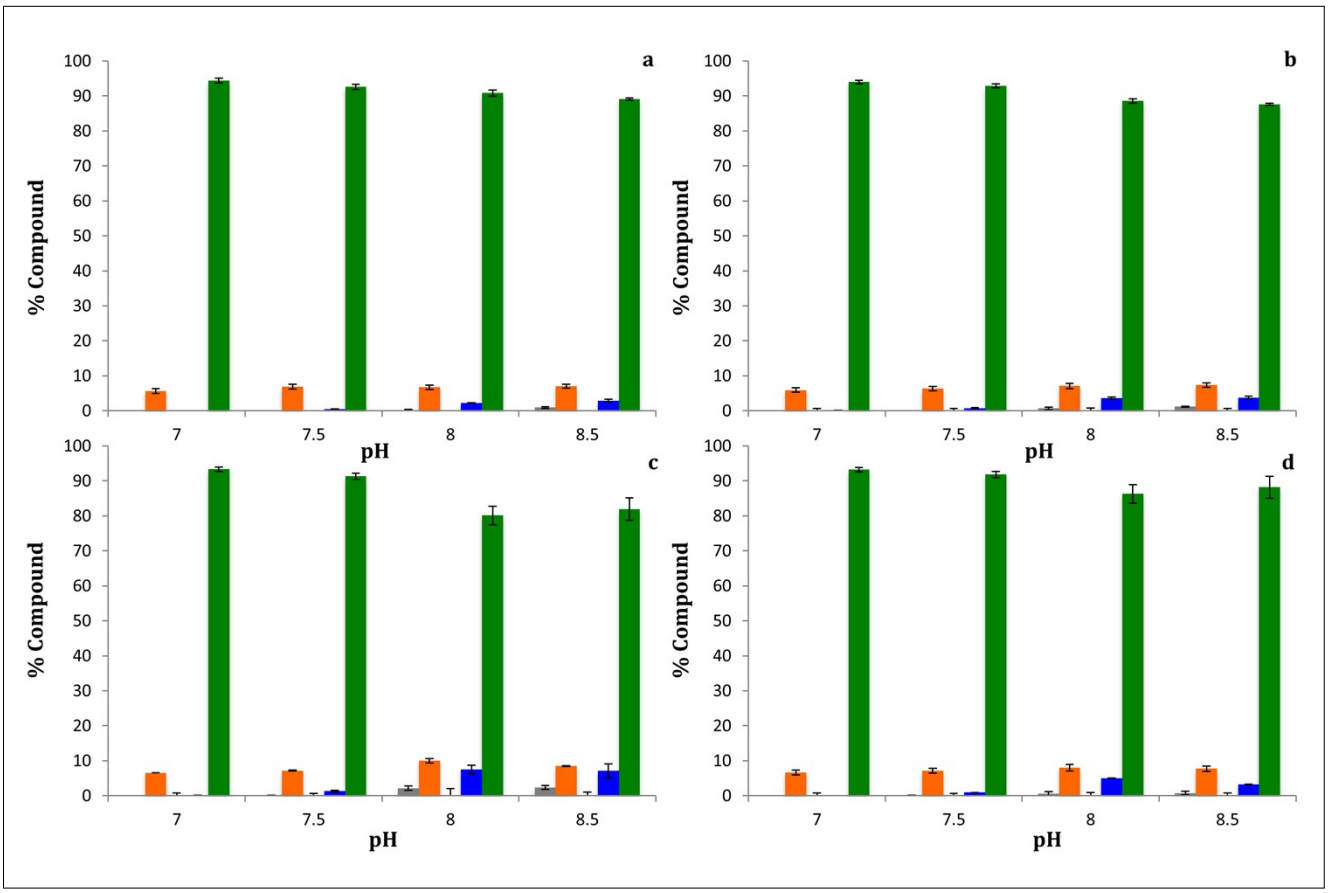


Figure S6: Bioconversion of FDME with no enzyme in Tris-HCl buffer (50mM)/BDO (1:1) at (A) 18°C, (B) 25°C, (C) 37°C, (D) 42°C. Product ratios as percentage of total after 24hr: Orange - mono methylester **7** (B)**;** grey - mono BDO ester **8**; yellow - Bis-BDO ester **4**; blue - methyl BDO ester **6**; green FDME **3.**

7. Analysis of transesterification vs hydrolysis at different water:BDO ratios

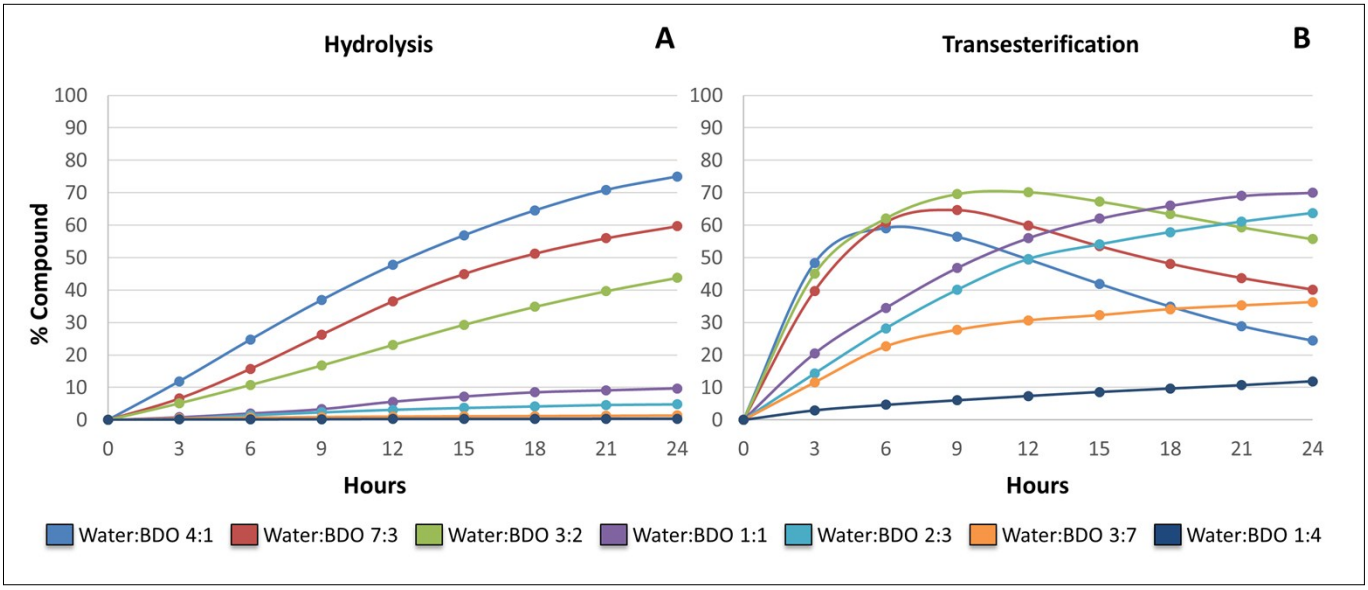


Figure S7: Bioconversion of FDME with PETase at 25°C with different ratio of Tris-HCl buffer (50mM)/BDO. Product ratios as percentage of total after 24hr.

8. Circular dichroism of PETase and TfH

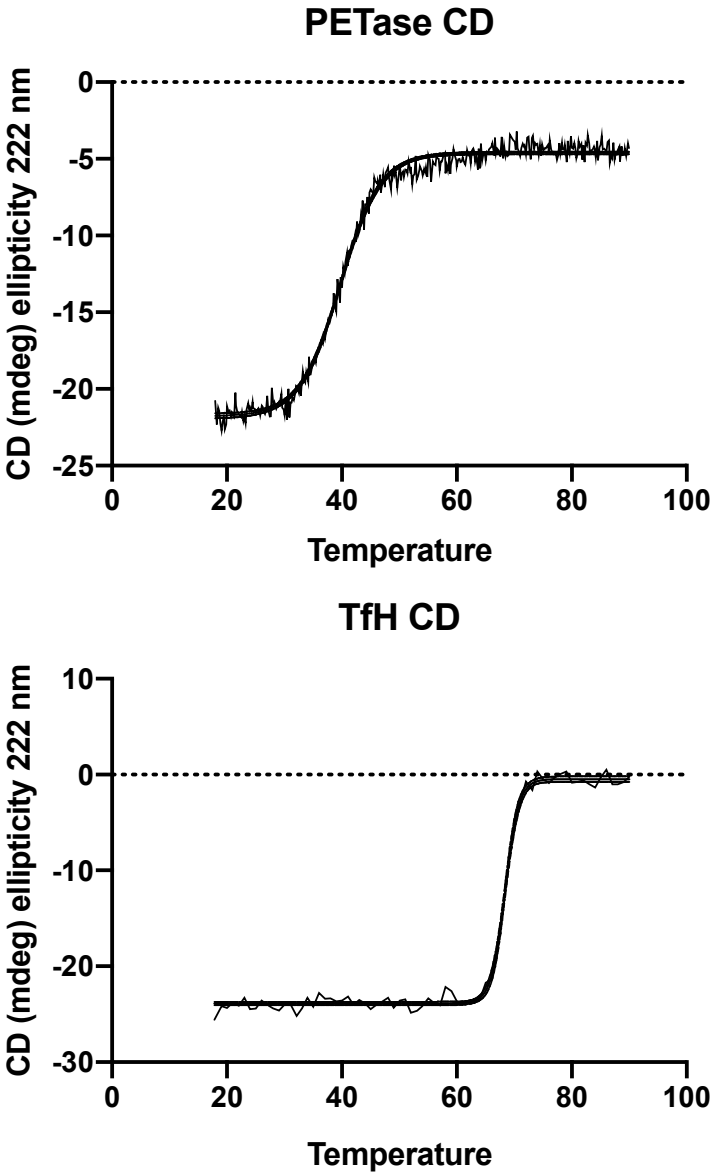


Figure S8: thermal denaturation curve of PETase and TfH. Temperature range from 20°C to 95°C, wavelength 222nm.

9. Materials and Analysis

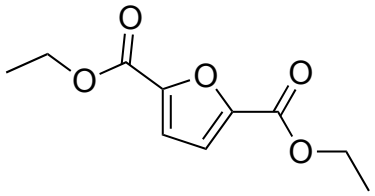
Unless stated otherwise, all reagents were commercially available. Solvents for extraction and FCC were technical grade. Reported solvent mixtures for both TLC and FCC were volume/volume mixtures. Analytical thin layer chromatography (TLC) was performed on Whatman F254 precoated silica gel plates (250 μm thickness). Visualization was accomplished with a UV light and/or a $KMnO_4$ solution. Flash column chromatography (FCC) was performed using Whatman Silica Gel 60Å (230-400 mesh).

Normal and reverse phase HPLC was performed on an Agilent system (Santa Clara, CA, USA) equipped with a G1379A degasser, G1312A binary pump, a G1329 well plate autosampler unit, a G1315B diode array detector and a G1316A temperature controlled column compartment. The University of Liverpool analytical services department provided Mass spectrometry. ^{1}H NMR and ^{13}C NMR were recorded on Bruker AV 500 MHz & Bruker DPX 400 MHz NMR spectrometers in the indicated deuterated solvents.

All values for ^{1}H NMR and ^{13}C NMR chemical shifts for deuterated solvents were obtained from Cambridge Isotope Labs. Data are reported in the following order: chemical shift in ppm (δ), integration, (multiplicity, which are indicated by s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet)) and coupling constants (*J*, Hz).

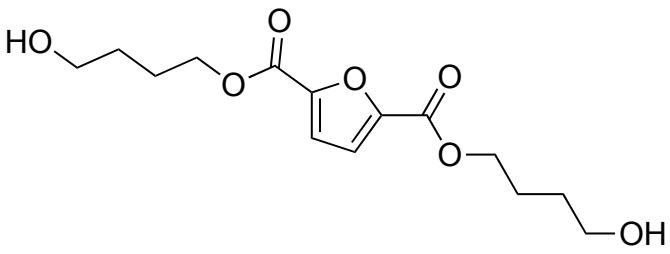
FDEE and furan bis-BDO ester were kindly provided by the University of York.

<u>FDEE (**3**) characterisation data</u>



NMR ^{1}H (400 MHz, DMSO) δ 7.40 (2 H, s, Ar-*H*), 4.36-4.31 (4 H, q, *J* = 7 Hz, CH_2), 1.33-1.29 (6 H, t, *J* = 7 Hz, CH_3). ^{13}C (100 MHz, DMSO) δ 157.9, 146.7, 119.4, 61.8, 14.5. *m/z* 213 ([M+H]$^{+}$, 100).

<u>FDCA bis-BDO ester (**5**)</u>



NMR ^{1}H (400 MHz, DMSO) δ 7.41 (2 H, s, Ar-*H*), 4.32-4.29 (4 H, t, *J* = 7 Hz, CH_2), 3.46-3.43 (4 H, t, *J* = 7 Hz, CH_2), 1.77-1.70 (4 H, p, *J* = 7 Hz CH_2) 1.55-1.48 (4 H, p, *J* = 7 Hz CH_2). ^{13}C (100 MHz, DMSO) δ 157.9, 146.7, 119.4, 65.7, 60.7, 29.2, 25.4. *m/z* 301 ([M+H]$^+$, 100).

Synthesis

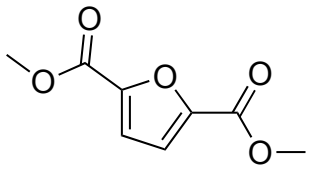
General method for esterification of furan mono/diacids (method **A**)

Potassium carbonate was added to a stirring solution of the acid in DMF/acetone (10 mL) at 0 °C. Alkyl iodide was added slowly over 10 min and the reaction was stirred at 0 °C for an additional 30 min. The reaction was warmed to room temperature and left to stir for 1h. Distilled water (30 mL) was added to the reaction and the whole was extracted with ethyl acetate (2 x 25 mL). The organic extracts were washed with brine and dried over sodium sulfate before purification by FCC (1:1 hexane:ethyl acetate)

General method for mono hydrolysis of furan di esters (method **B**)

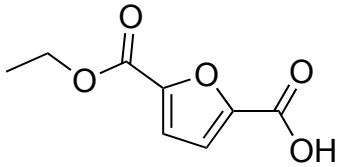
The starting ester was dissolved in a mixture of THF (2 mL) and water (20 mL) and cooled to 0 °C. NaOH (0.25 M, 24 mL) was added in 5 mL portions and the reaction was followed by TLC, where upon completion the reaction was left to stir another 30 min. The reaction was then acidified with HCL (1 M, 2 mL), solid NaCl was added and the whole was extracted with ethyl acetate (4 x 15 mL). The organic extracts were dried over sodium sulfate and the crude material was purified by FCC (30 % methanol, 70 % DCM).

Synthesis of FDCA dimethyl ester FDME (**4**)



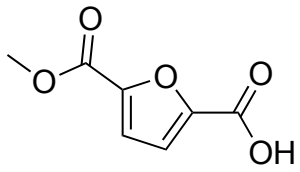
General method A was followed using potassium carbonate (0.65 g, 4.8 mM), FDCA (0.5 g, 3.2 mM) and methyl iodide (100 µL, 1.6 mM) in DMF to furnish the diester (250 mg, 42 %). NMR ^{1}H (400 MHz, $CDCl_3$) δ 7.24 (2 H, s, Ar-*H*), 3.95 (2 H, s, CH_3). ^{13}C (100 MHz, $CDCl_3$) δ 158.4, 146.7, 118.5, 52.4, 29.7. *m/z* 185 ([M+H]$^+$, 100).

Synthesis of FDCA monoethyl ester (**10**)



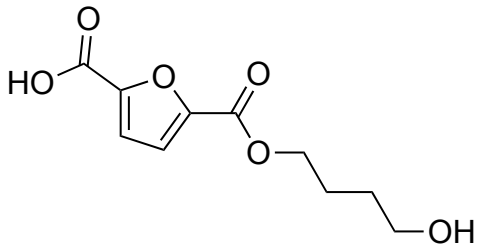
General method B was followed using FDEE (180 mg, 0.8 mM) to furnish the monoester (24 mg, 15 %). NMR ^{1}H (400 MHz, MeOD) δ 7.26-7.25 (1 H, d, *J* = 4 Hz, Ar-*H*), 7.14-7.13 (1 H, d, *J* = 4 Hz, Ar-*H*), 4.40-4.35 (2 H, q, *J* = 7 Hz, CH_2), 1.41-1.37 (3 H, t, *J* = 7 Hz, CH_3). ^{13}C (100 MHz, MeOD) δ 156.9, 144.7, 118.2, 117.4, 61.8, 14.5. *m/z* 185 ([M+H]$^+$, 100).

Synthesis of FDCA monomethyl ester (**11**)



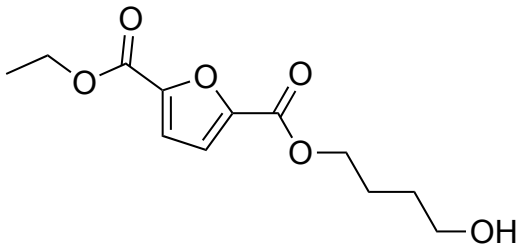
General method B was followed using FDME (500 mg, 2.7 mM) to furnish the monoester (202 mg, 43 %). NMR ^{1}H (400 MHz, MeOD) δ 7.31-7.30 (1 H, d, *J* = 4 Hz, Ar-*H*), 7.28-7.27 (1 H, d, *J* = 4 Hz, Ar-*H*), 3.93 (3 H, s, CH_3). ^{13}C (100 MHz, MeOD) δ 158.5, 146.4, 118.2, 117.9, 51.3. *m/z* 171 ([M+H]$^+$, 100).

Synthesis of FDCA monoBDO ester (**9**)



General method B was followed using bis-BDO ester (300 mg, 1 mM) to furnish the monoester (105 mg, 46 %). NMR ^{1}H (400 MHz, MeOD) δ 7.27-7.26 (1 H, d, *J* = 4 Hz, Ar-*H*), 7.13-7.12 (1 H, d, *J* = 4 Hz, Ar-*H*), 4.37-4.35 (2 H, t, *J* = 7 Hz, CH_2), 3.65-3.62 (2 H, t, *J* = 7 Hz, CH_2), 1.88-1.82 (2 H, p, *J* = 7 Hz, CH_2), 1.71-1.65 (2 H, p, *J* = 7 Hz, CH_2). ^{13}C (100 MHz, MeOD) δ 158.6, 145.2, 118.3, 115.7, 64.8, 61.0, 28.6, 24.9. *m/z* 229 ([M+H]$^+$, 100).

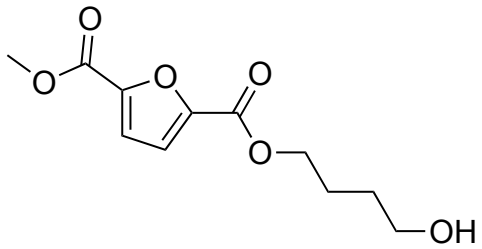
Synthesis of FDCA ethyl BDO diester (**7**)



General method A was followed using potassium carbonate (70 mg, 0.5 mM), FDCA monoBDO ester (30 mg, 0.13 mM) and ethyl iodide (10 µL, 0.2 mM) in acetone to furnish the diester (10 mg, 30 %). NMR ^{1}H (400 MHz, DMSO) δ 7.17-7.16 (1 H, d, *J* = 4 Hz, Ar-*H*), 6.67-6.66 (1 H, d, *J* = 4 Hz, Ar-*H*), 4.49-4.46 (1 H, t, *J* = 7 Hz, CH_2), 4.40-4.37 (1 H, t, *J* = 7 Hz, CH_2), 4.26-4.22 (2 H, t, *J* = 7 Hz, CH_2), 3.46-3.41 (2 H, q, *J* = 7 Hz, CH_2), 1.75-1.68 (2 H, p, *J* = 7 Hz, CH_2), 1.55-1.48 (2 H, p, *J* = 7

Hz, CH_2), 1.45-1.42 (3 H, t, *J* = 7 Hz, CH_3). ^{13}C (100 MHz, DMSO) δ 159.0, 134.8, 119.6, 112.5, 64.9, 61.3, 60.8, 29.8, 29.4, 25.5. *m/z* 257 ([M+H]$^+$, 100).

Synthesis of FDCA methyl BDO diester (**8**)



General method A was followed using potassium carbonate (70 mg, 0.5 mM), FDCA monoBDO ester (30 mg, 0.13 mM) and methyl iodide (10 µL, 0.2 mM) in acetone to furnish the diester (15 mg, 47 %). NMR ^{1}H (400 MHz, DMSO) δ 7.52-7.51 (1 H, d, *J* = 4 Hz, Ar-*H*), 7.06-7.05 (1 H, d, *J* = 4 Hz, Ar-*H*), 4.6 (3 H, s, CH_3), 4.48-4.45 (1 H, t, *J* = 7 Hz, CH_2), 4.38-4.35 (1 H, t, *J* = 7 Hz, CH_2), 4.26-4.22 (2 H, t, *J* = 7 Hz, CH_2), 1.73-1.68 (2 H, m, CH_2), 1.55-1.49 (2 H, m, CH_2). ^{13}C (100 MHz, DMSO) δ 159.6, 147.7, 119.0, 112.1, 65.5, 61.2, 52.5, 42.7, 29.4 25.1. *m/z* 243 ([M+H]$^+$, 100).

10. HPLC analysis and Statistical tests

Calibration curves were established for all intermediates. Samples were prepared in water containing 2% TFA and diluted to have a maximum peak height near 1 absorbance unit (AU), minimising errors due to detector and solution absorbance nonlinearity. All sample concentrations were analysed using serial dilution from 10 to 0.05mM of each analyte. All measurements were conducted at room temperature (25 °C) applying a 0.6 mL/min flow rate, with an injection volume of 5 µL. Absorbance was monitored at 254 nm, 230 nm, 210 nm and 280nm.

No significant differences in absorbance have been observed for all the compounds analysed in the concentration range between 1-2 mM (Figure S8a). Integration of two tailed peaks was done using drop integration method, which proved to be suitable for resolved peaks as shown in Figure S9. As stated in Table 1, the different spectrophotometric contribution of the compound conversion doesn't alter the total absorbance, suggesting how any spectrophotometric contribution is mainly determined by the furan ring, rather than the alcohol/ester/acid groups.

All bioconversions reported in this paper were diluted within a range of 1-2mM for HPLC analysis so that all measurements would appear in the linear range according to the calibration curves.

All measurements were repeated in triplicate.

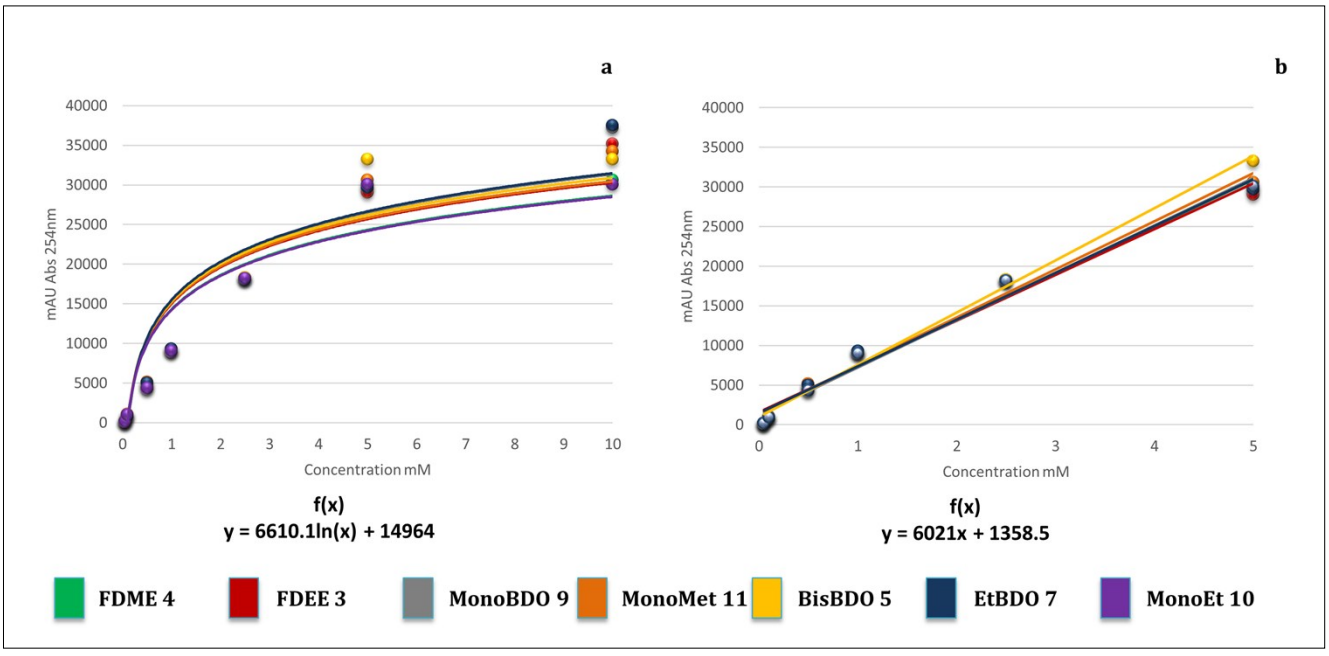
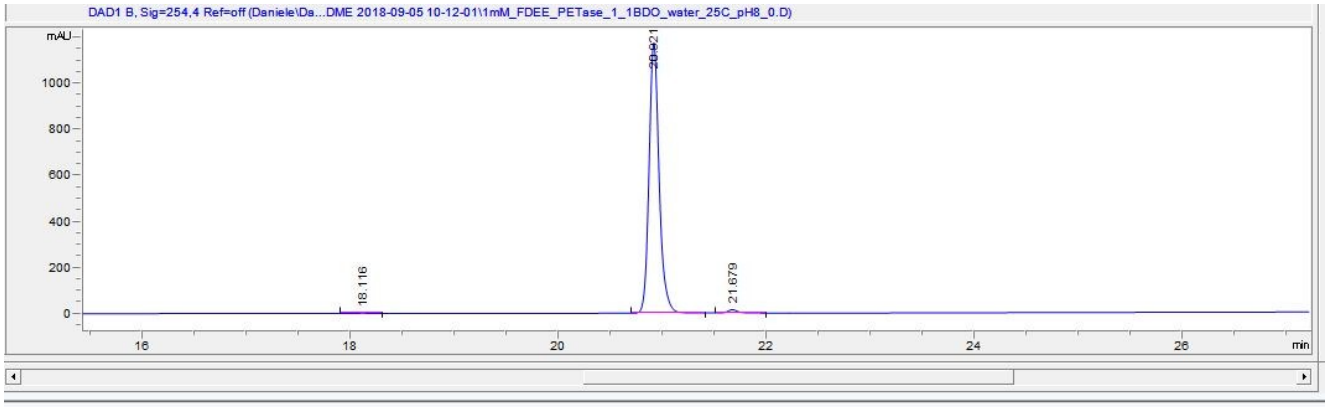


Figure S9: Calibration reference with nonlinear **(a)** and linear **(b)** interpolation.

Sample	1 MonoBDO	2 Monomethyl ester	3 Bis-BDO	4 MeBDO ester	5 FDME	Total integration	Time
0	0	7	0	92	7901	8000	1 min
1	0	67	50	1036	7049	8202	36 min
2	9	123	170	1785	6274	8361	72 min
3	20	175	338	2346	5515	8394	108 min
4	35	231	558	2838	4948	8610	144 min
5	58	305	855	3397	4712	9327	180 min
6	67	292	966	3071	3445	7841	216 min
7	88	328	1204	3180	2995	7795	252 min
8	113	364	1462	3259	2625	7823	288 min
9	142	396	1709	3269	2289	7805	324 min
10	171	427	1972	3244	1984	7798	360 min
11	204	457	2217	3194	1734	7806	396 min
12	237	482	2416	3089	1504	7728	432 min
13	273	509	2650	3006	1317	7755	468 min
14	311	533	2866	2901	1153	7764	504 min
15	351	558	3088	2805	1015	7817	540 min
16	386	572	3242	2655	879	7734	576 min
17	426	591	3420	2534	773	7744	612 min
18	466	608	3582	2413	679	7748	648 min
19	508	625	3746	2298	599	7776	684 min
20	550	640	3882	2176	526	7774	720 min
21	588	652	3989	2047	460	7736	756 min
22	628	663	4095	1927	405	7718	792 min
23	673	677	4218	1826	359	7753	828 min
24	717	692	4336	1727	317	7789	864 min
25	756	699	4405	1617	279	7756	900 min
26	801	712	4506	1527	247	7793	936 min
27	840	718	4551	1427	218	7754	972 min
28	877	722	4593	1332	191	7715	1008 min
29	923	734	4670	1255	170	7752	1044 min
30	965	741	4716	1176	150	7748	1080 min
31	1011	754	4791	1109	134	7799	1116 min
32	1050	757	4806	1034	118	7765	1152 min
33	1085	759	4811	962	104	7721	1188 min
34	1129	766	4851	903	93	7742	1224 min
35	1169	773	4893	848	83	7766	1260 min
36	1209	778	4892	790	73	7742	1296 min
37	1253	785	4924	740	65	7767	1332 min
38	1300	794	4960	696	58	7808	1368 min
39	1327	791	4922	694	51	7785	1404

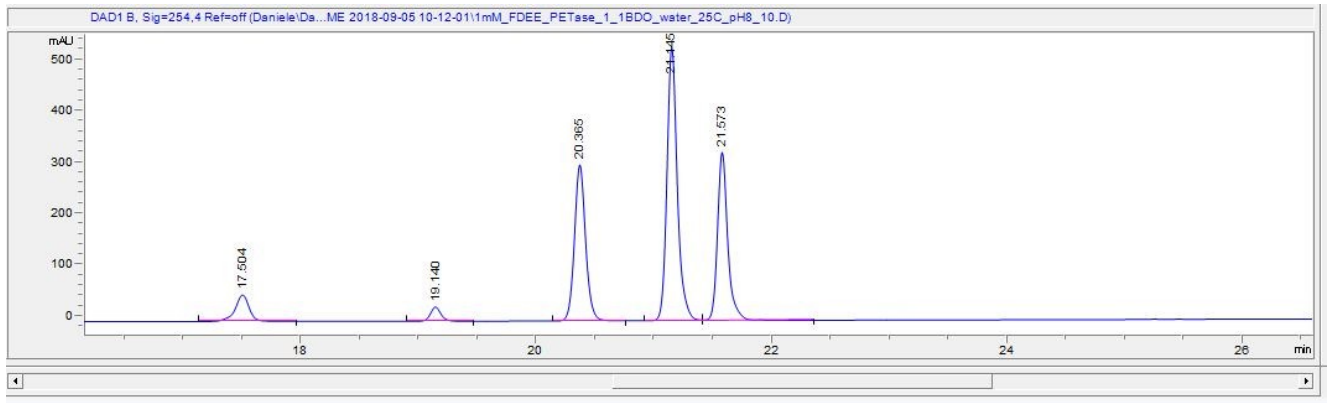
Table S1: FDEE (1mM) conversion plot with total integration conversion values

a



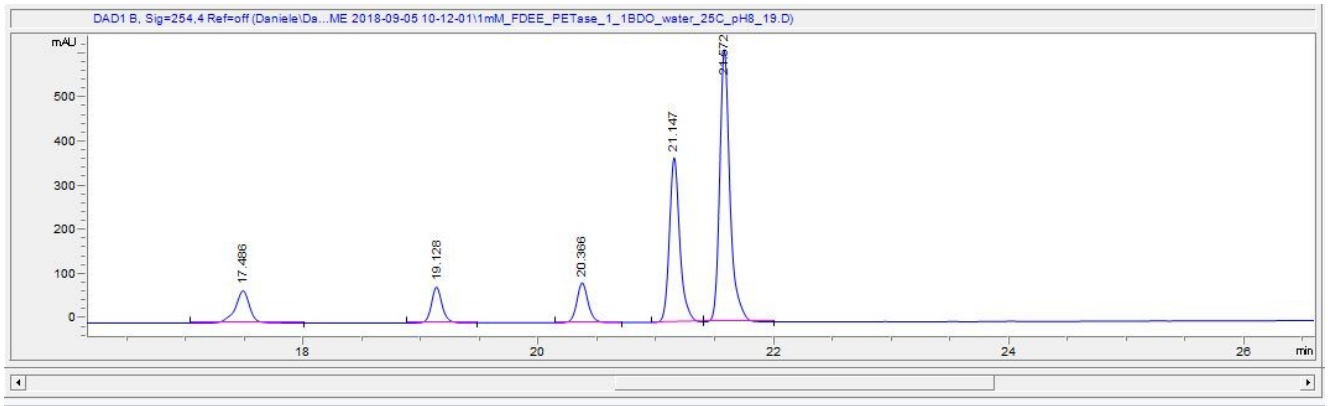
#	Time	Area	Height	Width	Area%	Symmetry
1	1.604	63.1	10.9	0.0895	0.757	0.43
2	1.802	58.5	6.3	0.124	0.702	0.209
3	1.995	43.5	8.2	0.0782	0.521	1.427
4	2.086	153.7	11.8	0.1666	1.845	0.26
5	18.116	7.4	1	0.1114	0.089	0.946
6	20.921	7901.2	1173.8	0.1025	94.829	0.821
7	21.679	92.2	14.5	0.0959	1.106	0.789
8	29.961	12.5	1.7	0.1061	0.150	0.759

b



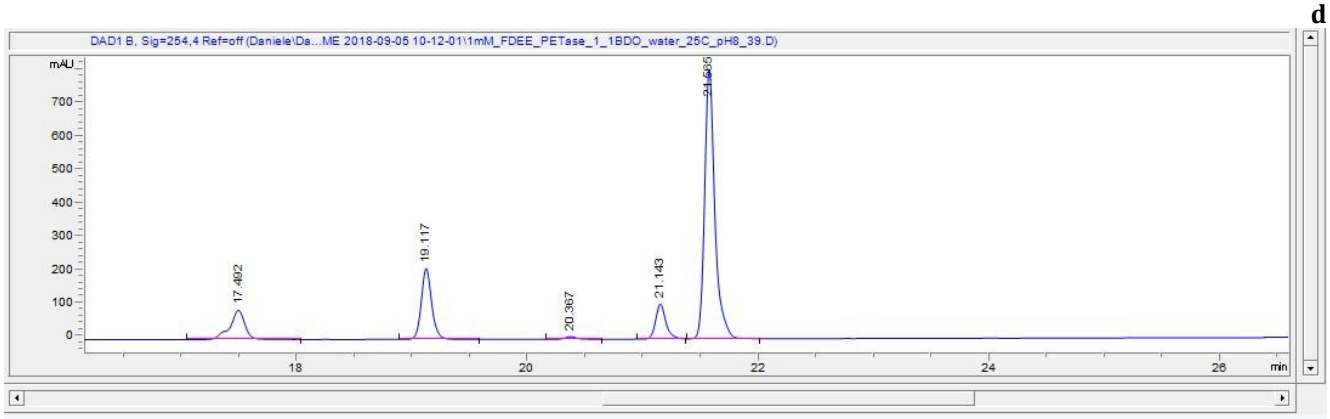
#	Time	Area	Height	Width	Area%	Symmetry
2	0.697	121.3	15.2	0.1052	1.107	0.435
3	0.84	111.4	13.5	0.1246	1.017	0.427
4	1.531	943.4	43	0.2698	8.611	3.373
5	1.788	211.5	25.1	0.1443	1.930	1.05
6	1.966	1452.2	23.9	0.736	13.256	5.49E-2
7	15.657	39.2	5.2	0.1139	0.358	1.079
8	17.504	427	51.5	0.1228	3.898	1.131
9	19.14	171.3	27.7	0.0943	1.564	0.882
10	20.365	1984.4	303.1	0.0984	18.114	0.887
11	21.145	3243.6	531.4	0.0932	29.608	0.813
12	21.573	1972.1	326.8	0.0924	18.002	0.772

c



#	Time	Area	Height	Width	Area%	Symmetry
2	0.699	122.6	14.7	0.1091	1.116	0.416
3	0.839	113.4	12.9	0.1148	1.033	0.299
4	1.533	918.4	42.6	0.2656	8.366	3.336
5	1.792	213.2	24.6	0.1491	1.942	1.055
6	1.975	1515.9	23.6	0.7808	13.807	5.28E-2
7	15.646	48.5	6.6	0.1117	0.442	1.104
8	17.486	625.3	72.9	0.1281	5.696	1.181
9	19.128	507.6	81.1	0.095	4.623	0.864
10	20.366	599.2	90.2	0.0995	5.458	0.876
11	21.147	2298.5	373.5	0.0938	20.936	0.803
12	21.572	3745.6	617.2	0.0928	34.117	0.752

d



#	Time	Area	Height	Width	Area%	Symmetry
1	0.951	615.1	20	0.3701	5.017	33.291
2	0.992	363.7	20.6	0.2141	2.966	8.1E-2
3	1.777	1005.8	52.8	0.236	8.204	2.411
4	2.081	310.3	28.6	0.1445	2.531	1.116
5	2.285	2183.7	29.2	0.9022	17.812	4.63E-2
6	15.663	45.1	6.4	0.1086	0.368	1.129
7	17.492	791.3	87.2	0.1339	6.454	1.348
8	19.117	1327.5	211.9	0.0951	10.828	0.852
9	20.367	51.3	7.7	0.1019	0.418	0.87
10	21.143	644.1	104.4	0.094	5.254	0.803
11	21.565	4922	805.4	0.0913	40.147	0.743

Figure S10: Peak integration examples of samples 0 **(a)**, 10 **(b)**, 19 **(c)** and 39 **(d)** from Table S1. Integration from 0 to 5 min represents background absorbance.

11. Gene sequences

PETase - from *Ideonella sakaiensis* 201-F6 (Genbank GAP38373.1), codon optimized for expression in *E. coli* RiplBL21

atggctagccagaccaatccgtatgcacgtggtccgaatccgaccgcagcaagcctggaagcaagcgcaggtccgtttaccgttcgtagctt
taccgttagccgtccgagcggttatggtgcaggcaccgtttattatccgaccaatgccggtggcaccgttggtgcaattgccattgttccgggt
tataccgcacgtcagagcagcattaaatggtggggtccgcgtctggcaagtcatggttttgttgttattaccattgataccaacagcaccctgg
atcagccgagcagccgtagcagtcagcagatggcagcactgcgtcaggttgccagcctgaatggcaccagcagcagcccgatttatggtaa
agttgatacagcacgtatgggtgttatgggttggagcatgggtggtggtggtagcctgattagcgcagcaaataatccgagcctgaaagcag
cagcaccgcaggctccgtgggatagcagcaccaattttagcagcgttaccgttccgacactgatttttgcatgtgaaaatgatagcattgcac
cggttaatagcagcgcactgccgatctatgatagtatgagccgtaatgcaaaacagtttctggaaattaatggtggcagccatagctgtgcaa
atagcggtaatagcaatcaggcactgatcggtaaaaaaggtgttgcatggatgaaacgcttcatggataatgatacccgttatagcacctttg
cctgcgaaaatccgaatagcacccgtgttagcgattttcgtaccgcaaattgtagcctcgagcaccaccaccaccaccac

TfH - from *Thermobifida fusca* YXT(WSH 03-11), codon optimized for expression in *E. coli* RiplBL21

atggctagcgccaatccgtatgaacgtggtccgaatccgaccgatgcactgctggaagcaagcagcggtccgtttagcgttagcgaagaaa
atgttagccgtctgagcgcaagcggttttggtggtggcaccatctattatccgcgtgaaaataacacctatggtgcagttgcaattagtccggg
ttataccggcaccgaagccagcattgcatggctgggtgaacgtattgcaagccatggttttgttgtgattaccattgataccattaccacactg
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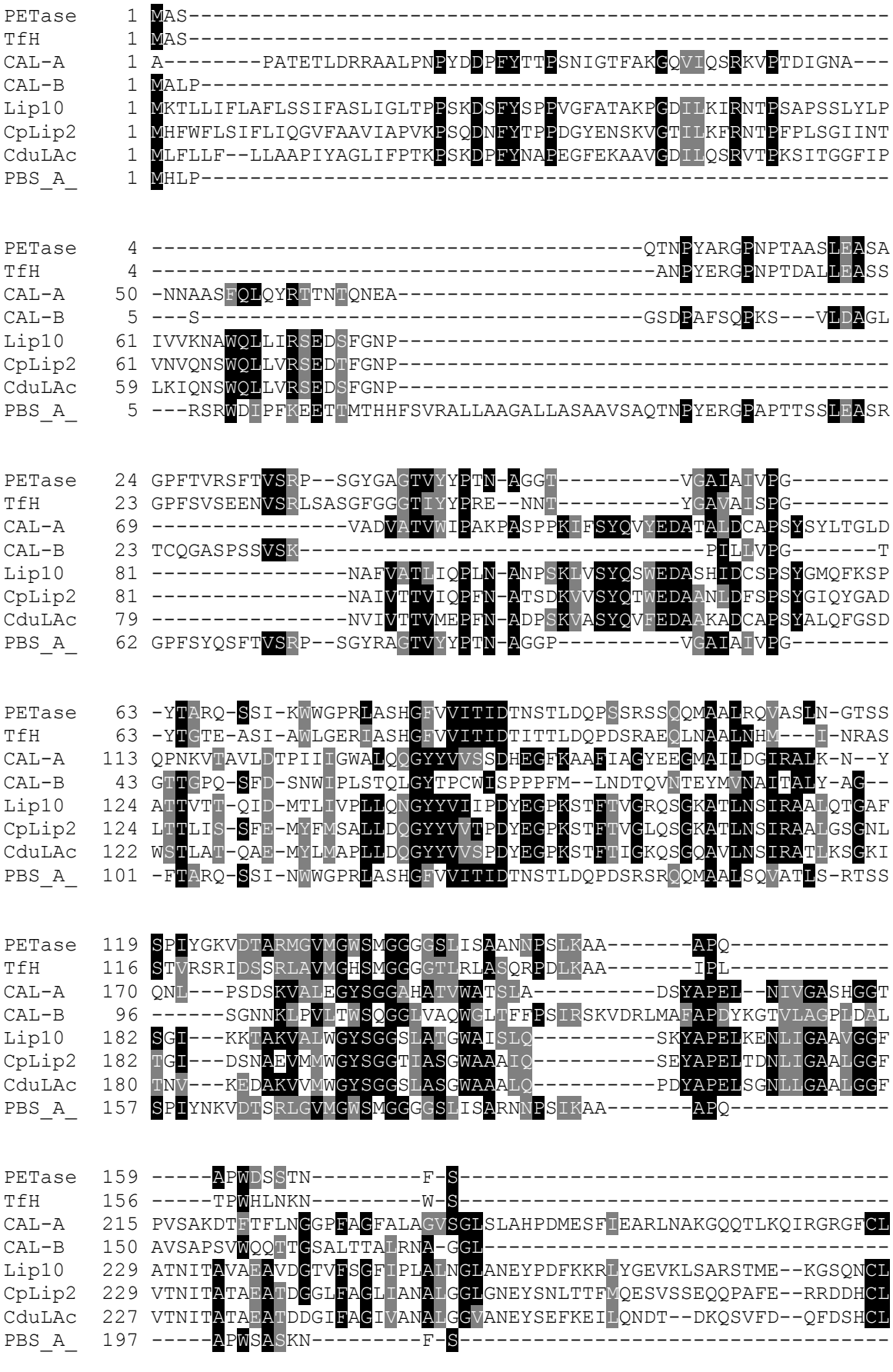
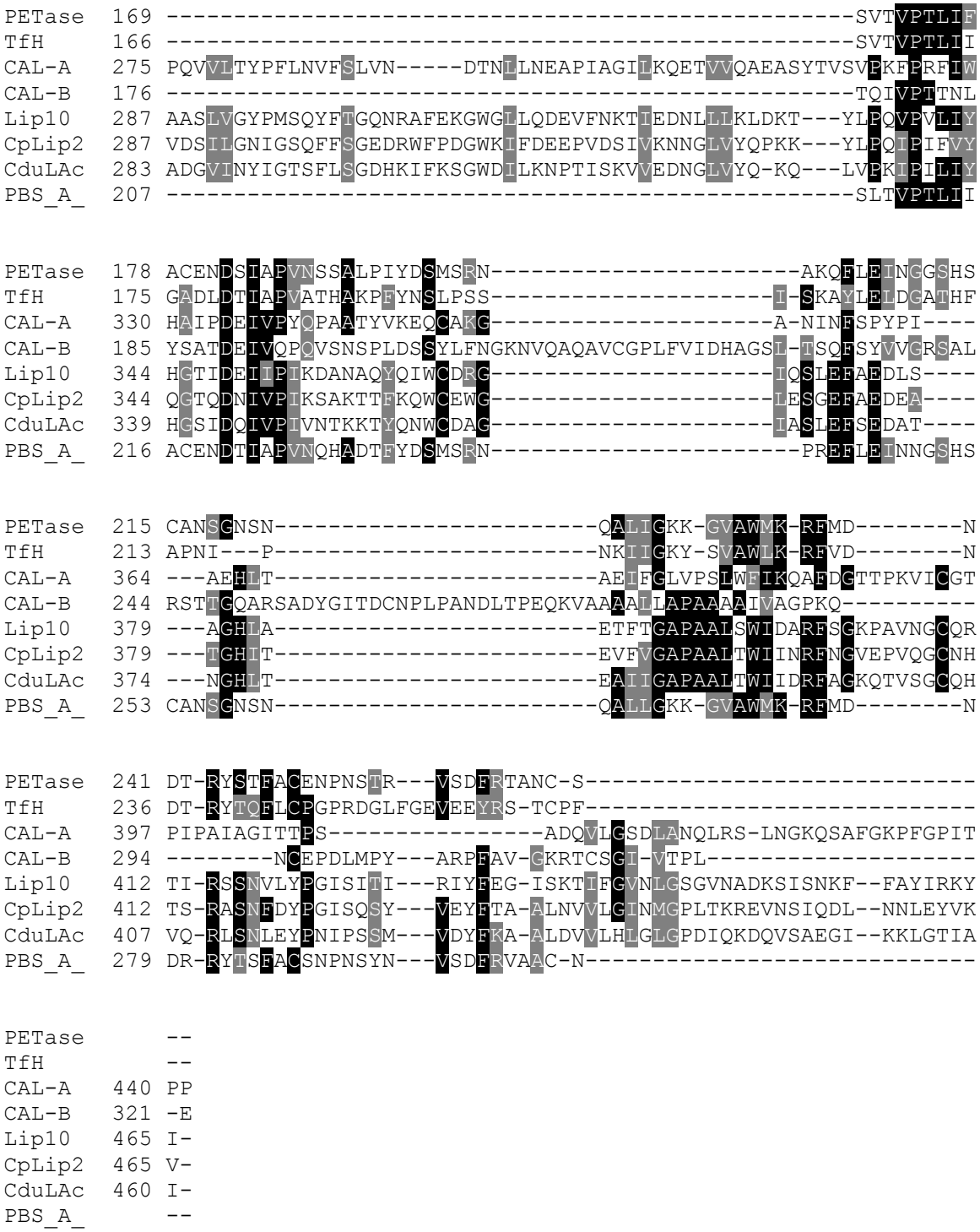
tgatggtctgtttggtgaagttgaagaatatcgtagcacctgtccgtttctcgagcaccaccaccaccaccactg

12. Protein alignments

Amino-acid sequence alignment of enzymes using ClustalW.




PETase 169 ----------SVTVPTLIF
TfH 166 ----------SVTVPTLII
CAL-A 275 PQVVLTYPFLNVFSLVN-----DTNLLNEAPIAGILKQETVVQAEASYTVSVPKFPRFIW
CAL-B 176 ----------TQIVPTTNL
Lip10 287 AASLVGYPMSQYFTGQNRAFEKGWGLLQDEVFNKTIEDNLLLKLDKT---YLPQVPVLIY
CpLip2 287 VDSILGNIGSQFFSGEDRWFPDGWKIFDEEPVDSIVKNNGLVYQPKK---YLPQIPIFVY
CduLAc 283 ADGVINYIGTSFLSGDHKIFKSGWDILKNPTISKVVEDNGLVYQ-KQ---LVPKIPILIY
PBS_A_ 207 ----------SLTVPTLII
PETase 178 ACENDSIAPVNSSALPIYDSMSRN----------AKQFLEINGGSHS
TfH 175 GADLDTIAPVATHAKPFYNSLPSS----------I-SKAYLELDGATHF
CAL-A 330 HAIPDEIVPYQPAATYVKEQCAKG----------A-NINFSPYPI----
CAL-B 185 YSATDEIVQPQVSNSPLDSSYLFNGKNVQAQAVCGPLFVIDHAGSL-TSQFSYVVGRSAL
Lip10 344 HGTIDEIIPIKDANAQYQIWCDRG----------IQSLEFAEDLS----
CpLip2 344 QGTQDNIVPIKSAKTTFKQWCEWG----------IESGEFAEDEA----
CduLAc 339 HGSIDQIVPIVNTKKTYQNWCDAG----------IASLEFSEDAT----
PBS_A_ 216 ACENDTIAPVNQHADTFYDSMSRN----------PREFLEINNGSHS
PETase 215 CANSGNSN----------QALIGKK-GVAWMK-RFMD--------N
TfH 213 APNI---P----------NKIIGKY-SVAWLK-RFVD--------N
CAL-A 364 ---AEHLT----------AEIFGLVPSLWFIKQAFDGTTPKVICGT
CAL-B 244 RSTTGQARSADYGITDCNPLPANDLTPEQKVAAAALLAPAAAAIVAGPKQ----------
Lip10 379 ---AGHLA----------ETFTGAPAALSWIDARFSGKPAVNGCQR
CpLip2 379 ---TGHIT----------EVFVGAPAALTWIINRFNGVEPVQGCNH
CduLAc 374 ---NGHLT----------EAIIGAPAALTWIIDRFAGKQTVSGCQH
PBS_A_ 253 CANSGNSN----------QALLGKK-GVAWMK-RFMD--------N
PETase 241 DT-RYSTFACENPNSTR---VSDFRTANC-S----------
TfH 236 DT-RYTQFLCPGPRDGLFGEVEEYRS-TCPF----------
CAL-A 397 PIPAIAGITTPS----------ADQVLGSDLANQLRS-LNGKQSAFGKPFGPIT
CAL-B 294 --------NCEPDLMPY---ARPFAV-GKRTCSGI-VTPL----------
Lip10 412 TI-RSSNVLYPGISITI---RIYFEG-ISKTIFGVNLGSGVNADKSISNKF--FAYIRKY
CpLip2 412 TS-RASNFDYPGISQSY---VEYFTA-ALNVVLGINMGPLTKREVNSIQDL--NNLEYVK
CduLAc 407 VQ-RLSNLEYPNIPSSM---VDYFKA-ALDVVLHLGLGPDIQKDQVSAEGI--KKLGTIA
PBS_A_ 279 DR-RYTSFACSNPNSYN---VSDFRVAAC-N----------
PETase --
TfH --
CAL-A 440 PP
CAL-B 321 -E
Lip10 465 I-
CpLip2 465 V-
CduLAc 460 I-
PBS_A_ --

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