Supporting Information

An esterase from anaerobic *Clostridium hathewayi* can hydrolyze aliphatic-aromatic polyesters

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Table S1. Crystallographic parameters of Chath_Est1 from *Clostridium hathewayi*

X-ray source	PETRA III, DESY Hamburg
wavelength (Å)	1.033
Temperature	100 K
Space group	$P2_1$
Cell dimensions	
a, b, c (Å)	62.05, 241.83, 83.58
β (°)	90.0
Resolution (Å)	
high resolution shell	60-1.90 (2.00-1.90)
Total no. reflections	471113
Unique no. reflections	163181
Multiplicity	2.9 (2.7)
Completeness (%)	84.1 (74.3)
R_{merge} (%)	8.8 (59.2)
$\langle I/\sigma I \rangle$	7.8 (2.1)
$R_{\text{work}} / R_{\text{free}}$	18.86/23.74
No. atoms	
Protein	17373
Water	1592
Mean B factor	24.9
R.m.s. deviations	
Bond lengths (Å)	0.005
Bond angles (°)	0.914
Ramachandran outliers (%)	0.2
Most favored residues (%)	95.6
PDB	5A2G

					
	360	370	380	390	400
EFC94627.1	GHGKELKTVF	AEAYPGKSPV	DLLTLDTIFR	GPTKEFVRSL	AAAGGSVYSY
WP_022032810	GHGKELKAVF	AEAYPGKSPV	DLLTLDTIFR	GPTKEFVRSL	AAAGGSVYSY
WP_025529448	GRGKELKAVF	AEAYPGKNPA	DLLTLDTIFR	GPTREFVRAL	AAAGGSVYSY
WP_002603106	GRGKELKAVF	AEAYPGKNPA	DLLTLDTIFR	GPTREFVRAL	AAAGGSVYSY
					
	410	420	430	440	450
EFC94627.1	LFALEFPYQN	QKTA-----	-----	-----	-----
WP_022032810	LFALEFPYQN	QKTAWHCSDI	PFIFHNTELV	PVANIPEVSD	RLEEQMFQAV
WP_025529448	LFALEFPYQN	QKTAWHCSDI	PFVFNHTELV	PVANIPGVSD	RLEEQIFQAV
WP_002603106	LFALEFPYQN	QKTAWHCSDI	PFVFNHTELV	PAANIPEVSD	RLQEQIFQAV
					
	460	470	480	490	500
EFC94627.1	-----	-----	-----	-----	-----
WP_022032810	MAFARSGKPE	YGGLPQWPAS	RENDEATMIF	DRVCEVRHNH	DNRLCLKLHAE
WP_025529448	MAFARTGKPE	YEGLPQWPAS	REDDEATMIF	DRVCEVRHNH	DDRLLKLHAE
WP_002603106	MAFARTGKPE	YEGLPQWPAS	REDDEATMIF	DRVCEVRHNH	DDRLLKLHAE
					
	510	520			
EFC94627.1	-----	-----			
WP_022032810	LSPKFDLAAV	MAEMGDEIQH			
WP_025529448	ASPKFDLAAM	MAKMGDEIQH			
WP_002603106	ASPKFDLAAM	MTKMGDEIQH			

Figure S1. Alignment of homologous sequences starting with amino acid 351. EFC94627.1 partial para-nitrobenzyl esterase from *Clostridium hathewayi* DSM-13479; WP_022032810 para-nitrobenzyl esterase from *Clostridium hathewayi* CAG:224; WP_025529448 carboxylesterase from *Hungatella hathewayi*; WP_002603106 hypothetical protein from *Hungatella hathewayi*.

Figure S2. SEM images of oPBAT pellets. A: untreated oPBAT sample. B: oPBAT sample after batch-wise incubation in abiotic medium. C: oPBAT sample with biofilm after batch-wise incubation in biogas sludge. The incubation was performed under the conditions described in the experimental section.

Figure S3. RP-HPLC profile of the oPBAT batch after 28 days of incubation. Liberated Ta: terephthalic acid was quantified.

Figure S4. RP-HPLC profiles of *C. hathewayi* cell supernatant samples "PYX + Glucose" (above) and "PYX + PBAT + oPBAT" (below). Liberated hydrolysis products Ta: terephthalic acid; BTa: mono(4-hydroxybutyl) terephthalate and BTaB: bis(4-hydroxybutyl) terephthalate were quantified.

Figure S5. Expression profile of the heterologous gene Chath_Est1 in *E. coli* BL21(DE3) at 20 °C and 160 rpm for 20 h after induction with 0.05 M IPTG. Lysates and pellets were withdrawn and analyzed after 2 , 4 h, and 20 h after induction with IPTG.

Figure S6. Released molecules after incubation of BaETaEBa with 6 μM Chath_Est1 for 23 h and 37 $^{\circ}\text{C}$ at different pHs. The release molecules are shown in μM , ETaE: bis(2-hydroxyethyl) terephthalate, ETa: mono(2-hydroxyethyl) terephthalate, Ta: terephthalic acid, BaE: 2-hydroxyethyl benzoate and Ba: benzoic acid were quantified by means of HPLC analysis. Each bar represents the average of three independent samples; error bars indicate the standard deviation.

Figure S7. Hydrolysis of PBAT, BTaBTaB and BTaB by 0.6 μ M Chath_Est1 at pH 7.0 and 37 °C. Released products (in μ M) were quantified by means of RP-HPLC analysis. Ta: terephthalic acid; BTa: mono(4-hydroxybutyl) terephthalate; BTaB: bis(4-hydroxybutyl) terephthalate. Each bar represents the average of three independent samples; error bars indicate the standard deviation.

Figure S8. α/β hydrolase fold represented on chain A: alpha helices (cyan), beta sheets (magenta) and loops (salmon).

Figure S9. Alignment of Chath_Est1 (green) with *Geobacillus stearothermophilus* carboxylesterase Est55 (cyan, left) and pNB esterase (blue, right)

Figure S10. Cut through the dimer structure to the active site pocket of Chath_Est1.