

Supplementary Information

For the manuscript: **Cold-adapted carboxylesterases from *Alcanivoracaceae* active with a wide range of synthetic polyesters**

Co-authored by Hairong Ma et al.

Content

Supplementary tables

	Page:
SI Table 1. α/β -fold hydrolases from <i>Alcanivoracaceae</i> characterised in this study	2
SI Table 2. Amino acid sequence identities of studied proteins	3
SI Table 3. HPLC product analysis and plate assay results.	4
SI Table 4. Amino acid content in previously characterised enzymes and enzymes from this study	5

Supplementary figures

SI Figure 1. Multiple sequence alignments of polyesterases	6-8
SI Figure 2. SDS-PAGE analysis of purified enzymes	9
SI Figure 3. Agarose-based screening of purified proteins for polyesterase activity	10
SI Figure 4. DSF analysis for the purified enzymes	11

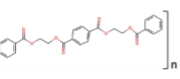
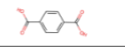
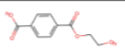
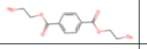
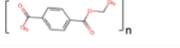
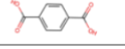
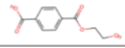
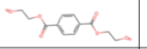
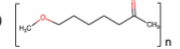
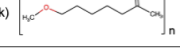
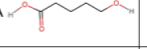
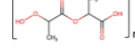
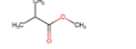
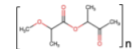
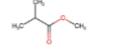
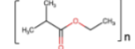
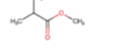
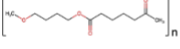
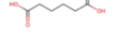
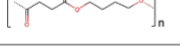
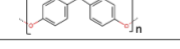
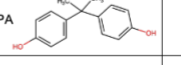
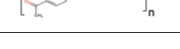
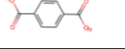
SI Table 1. α/β -fold hydrolases from *Alcanivoracaceae* characterised in this study.

Protein name (this work)	Accession	Organism/species	Signal peptide	Clade location
1. AGE_3	WP_138772051	<i>Alloalcanivorax (Alcanivorax) gelatiniphagus</i> MEBiC08158 ^T	-	IV
2. AGE_8	WP_138772014	<i>Alloalcanivorax (Alcanivorax) gelatiniphagus</i> MEBiC08158 ^T	-	I
3. AGE_12	WP_138772717	<i>Alloalcanivorax (Alcanivorax) gelatiniphagus</i> MEBiC08158 ^T	-	III
4. APA_3	WP_035204471	<i>Isoalcanivorax (Alcanivorax) pacificus</i> W11-5 ^T	-	IV
5. APA_4	WP_008736028	<i>Isoalcanivorax (Alcanivorax) pacificus</i> W11-5 ^T	-	IV
6. APA_5	WP_008737557	<i>Isoalcanivorax (Alcanivorax) pacificus</i> W11-5 ^T	+	IV
7. ALC24_1162	WP_133490829	<i>Alloalcanivorax (Alcanivorax)</i> sp. 24	-	III
8. AGE_2	WP_138771864	<i>Alloalcanivorax (Alcanivorax) gelatiniphagus</i> MEBiC08158 ^T	-	I
9. APA_2	WP_008737455	<i>Isoalcanivorax (Alcanivorax) pacificus</i> W11-5 ^T	-	I
10. APA_8	WP_008737253	<i>Isoalcanivorax (Alcanivorax) pacificus</i> W11-5 ^T	-	I
11. ALC24_3989	WP_133493397	<i>Alloalcanivorax (Alcanivorax)</i> sp. 24	-	IV
12. ALC24_1328	WP_133490973	<i>Alloalcanivorax (Alcanivorax)</i> sp. 24	-	IV
13. ALC24_2069	WP_133491672	<i>Alloalcanivorax (Alcanivorax)</i> sp. 24	-	III
14. ALC24_4107	WP_133493510	<i>Alloalcanivorax (Alcanivorax)</i> sp. 24	-	I
15. APA_6	WP_008739160	<i>Isoalcanivorax (Alcanivorax) pacificus</i> W11-5	+	IV
16.ABO_0116	Q0VTH9	<i>Alcanivorax borkumensis</i> SK2 ^T	-	I
17.ABO_1197	Q0VQA3	<i>Alcanivorax borkumensis</i> SK2 ^T	+	III
18.ABO_1251	Q0VQ49	<i>Alcanivorax borkumensis</i> SK2 ^T	-	IV
19.ABO_1483	Q0VPG7	<i>Alcanivorax borkumensis</i> SK2 ^T	-	I
20.ABO_1895	Q0VNA5	<i>Alcanivorax borkumensis</i> SK2 ^T	+	I
21.ABO_2249	Q0VLQ1	<i>Alcanivorax borkumensis</i> SK2 ^T	-	III

SI Table 2. Amino acid sequence identities of studied proteins.

	ABO_0116	ABO_1197	ABO_1251	ABO_1483	ABO_1895	ABO_2449	AGE_2	AGE_3	AGE_8	AGE_12	APA_2	APA_3	APA_4	APA_5	APA_6	APA_8	ALC24_3989	ALC24_1328	ALC24_1162	ALC24_2069	ALC24_4107
ABO_0116		8.4	5.6	10.1	9	12.2	5.6	7.9	6.9	11.9	8.7	6.8	8.7	5.2	10.4	6.8	7.3	5.6	9.2	10.9	6.7
ABO_1197	8.4		7.4	11.5	8.8	15.5	7.2	6.8	8.8	16	10.4	8.7	7.2	7.9	10.8	7	9.5	7.1	52.6	14.9	7.1
ABO_1251	5.6	7.4		9.6	6.1	8.3	4.5	8.1	7	8.1	9.1	8.4	7.4	58.3	6.6	6.4	7.9	63.7	7.7	8.4	9.4
ABO_1483	10.1	11.5	9.6		6.6	11.5	6.5	6.7	12.3	13.1	11.7	8.2	6.4	8.6	8.4	11.9	7.8	9.5	12.5	14.6	7.2
ABO_1895	9	8.8	6.1	6.6		8.9	5.9	5.3	7.2	10	8.3	6.6	6.8	6.2	10.6	7.4	7.8	5.9	11.1	11.7	7.3
ABO_2449	12.2	15.5	8.3	11.5	8.9		11.9	4.9	12.1	67.2	10.1	8	6.7	8.1	9.5	10.7	9.1	8	16.6	62.9	7
AGE_2	5.6	7.2	4.5	6.5	5.9	11.9		5.4	5.7	12.5	9.8	5.2	5	5.6	6	5	4.7	4.7	9.8	11.5	5.4
AGE_3	7.9	6.8	8.1	6.7	5.3	4.9	5.4		7.7	7.2	7.2	11.1	34	7.3	7.5	6.5	10.8	8.5	6.9	6.7	7.5
AGE_8	6.9	8.8	7	12.3	7.2	12.1	5.7	7.7		13.2	7.8	7	7.7	6.2	7.7	67.7	8	7.3	8.8	11.3	9
AGE_12	11.9	16	8.1	13.1	10	67.2	12.5	7.2	13.2		10.3	9	8.2	7.7	9.2	11.5	10.5	9	16	66.5	7.6
APA_2	8.7	10.4	9.1	11.7	8.3	10.1	9.8	7.2	7.8	10.3		9.4	7.4	8.2	12.9	8	10.1	9.6	12.4	10.7	4.6
APA_3	6.8	8.7	8.4	8.2	6.6	8	5.2	11.1	7	9	9.4		10.1	7.7	8.2	6.8	67.1	8.4	9.1	8.2	9.5
APA_4	8.7	7.2	7.4	6.4	6.8	6.7	5	34	7.7	8.2	7.4	10.1		7.8	7.7	6.3	9.3	7.4	7.8	8.5	7.8
APA_5	5.2	7.9	58.3	8.6	6.2	8.1	5.6	7.3	6.2	7.7	8.2	7.7	7.8		6	5.5	7.9	57.5	7.8	7.4	9
APA_6	10.4	10.8	6.6	8.4	10.6	9.5	6	7.5	7.7	9.2	12.9	8.2	7.7	6		6.1	8.1	6.1	11.6	9.4	6.2
APA_8	6.8	7	6.4	11.9	7.4	10.7	5	6.5	67.7	11.5	8	6.8	6.3	5.5	6.1		6.6	6.2	8.1	10.2	9.3
ALC24_3989	7.3	9.5	7.9	7.8	7.8	9.1	4.7	10.8	8	10.5	10.1	67.1	9.3	7.9	8.1	6.6		8.5	9.8	9.8	10.2
ALC24_1328	5.6	7.1	63.7	9.5	5.9	8	4.7	8.5	7.3	9	9.6	8.4	7.4	57.5	6.1	6.2	8.5		6.6	9.1	8.9
ALC24_1162	9.2	52.6	7.7	12.5	11.1	16.6	9.8	6.9	8.8	16	12.4	9.1	7.8	7.8	11.6	8.1	9.8	6.6		15.1	8.2
ALC24_2069	10.9	14.9	8.4	14.6	11.7	62.9	11.5	6.7	11.3	66.5	10.7	8.2	8.5	7.4	9.4	10.2	9.8	9.1	15.1		7.8
ALC24_4107	6.7	7.1	9.4	7.2	7.3	7	5.4	7.5	9	7.6	4.6	9.5	7.8	9	6.2	9.3	10.2	8.9	8.2	7.8	

SI Table 3. HPLC product analysis and plate assay results. +/- indicate presence/absence of activity on agar plates with polymers' suspensions. Numbers in brackets indicate average concentration (mM) of monomers quantified by HPLC after overnight incubation at 30 °C in reaction mixtures of the total volume of 200 µl, 50 mM CHES pH 9.0, 50 µg enzyme/reaction, 50 µl polyester nanoparticles)

Substrate	Product	ABO_0116	ABO_1197	ABO_1251	ABO_1483	ABO_1895	ABO_2249	ALC24_1328	AGE_12	APA_2	APA_3	APA_5
3PET 	TA 	-	++(0.45)	-(0.22)	-	-	+(0.18)	+(0.03)	-(0.12)	-(0.01)	-	-(0.11)
	MHET 	-(0.11)	++(0.46)	-(0.78)	-	-	+(0.13)	+(0.41)	-(0.06)	-(0.11)	-(0.02)	-(0.49)
	BHET 	-	++	-	-	-	+	+	-	-	-	-
aPET 	TA 	-	-	-	-	-	-	-	-	-	-	-
	MHET 	-	-	-	-	-	-	-	-	-	-	-
	BHET 	-	-	-	-	-	-	-	-	-	-	-
PCL (2k) 	6-HHA 	-(0.17)	++(0.50)	-(0.44)	-(0.06)	-(0.29)	+(0.26)	-(0.48)	-(0.39)	-(0.38)	-(0.24)	-(0.46)
PCL (14k) 	6-HHA 	-	++(1.7)	-(1.10)	-	-	+(0.90)	-(0.20)	-	-	-	-(0.40)
PLLA 	LA 	-	-	-	-	-	-	-	-	-	-	-
PDLA 	LA 	-	-	-	-	-	-	-	-	-	-	-
PDLLA 	LA 	-	-(0.4)	-(0.30)	-	-	-(0.20)	-(0.10)	-	-	-	-(0.6)
PBA 	AA 	-	+(0.14)	-(0.06)	-	-(0.11)	+	-(0.10)	-(0.06)	-	-	-
PBS 	SA 	-	-	-	-	-	-	-	-	-	-	-
PC 	BPA 	-	-	-	-	-	-	-	-	-	-	-
PBT 	TA 	-	-	-	-	-	-(0.007)	-	-	-	-	-

SI Table 4. Amino acid content in previously characterised enzymes and enzymes from this study

Enzyme name	Amino acids % in polypeptide					Reference
	Gly	Ser	Met	Pro	Arg	
HotPETase	7.8	10.0	2.8	6.0	4.1	Wang et al., 2024
CBA10055	8.8	5.0	2.2	8.0	6.6	Ma et al., 2025
EstB (<i>A. dieselolei</i>)	7.6	5.9	1.5	6.2	6.5	Zhang et al., 2014
Est97	13.1	5.8	3.9	4.6	5.8	Fu et al., 2013
ABO_1197	6.9	5.7	3.1	5.0	4.1	Tchigvintsev et al., 2015
ABO_1251	9.3	5.1	2.4	5.9	5.7	Tchigvintsev et al., 2015
ABO_2249	9.8	7.2	4.3	7.5	6.6	Martínez-Martínez et al., 2018
APA_5	11.8	4.1	1.9	6.8	7.8	this study
ALC24_1328	10.6	4.9	2.8	7.5	8.7	this study

A)

ABO1197	1	MQLKHLFLFIVCSFFLSGCEDITNW	AYEKLAMEKN	RAGLKDQILTTADG	ITWHILTSETSAPIAEKEAV	LLIHGFSADSSNW	84
ABO1483	1	MDAIER KFSVYQQTISA		LQWPG	EVDRNDAPPI	LALHGWLDNAASF	45
APA_12	1	MES LRYSPADLTCR	IERTGGY	YQGTWCAATLYRPA	ATGDAALPAI	LMVHGWGGTQDTLT	59
ABO1895	1	MKTASV FTLTALLGLSAI			STFAEARPPW		28
AGE_8	1	MKIVIH ALLGALLLLG		LSGPAT	AGYTETRHPI	VIVHGLPGFDITAAG	46
APA_8	1	MCKRLP VLLCACLCLE		LSLPASAFFGWLKDTYTETRYPI		VIVHGLPGFDSLIG	53
AGE_2	1	MWGRLL			IVL		9
ALC24_4107	1	MTSRPM RSLVIAFLTLVAAAAALAGAGAWQNNLSLGGFNKVHLYTP			DGDSPVGNKGALLIVLHGC	TQSIDAYK TANLEVAEEYGMV	88
ABO0116	1	MSDIE LVSATKSPGGW	LKRYKHHSQA	LNCEMIFA LYLPP	AAEGNDVPLL	WWLSGLTCTDENFMQKAGAHRTAAELGLAI	79
ABO1197	85	VRFANELEGDFPI VPDLP	GHG	ETT RNLDLTYTMSAQATRLTLMDALQIQ	HPHVAGNSHGG	AISSALAAQAPQ	169
ABO1483	46	APLSRFIQR	LLAMDFS	GHG	HSDHRPCGVVTHLVHVRDVLAVVDQLGWK	RFTLMGHS	129
APA_12	60	VFFYEETFRAGFVVMSEFYT		GWG	DSAGLPRH	TISARGVRREADAALFLKSR	148
ABO1895	29			ENG QRIDTR		THVQLVDNRHDSYR	52
AGE_8	47	VDYW	YRIPEELRRS	GAQVYVAQVS	AA	EDT	130
APA_8	54	VDYF	YGVPGELQRS	GARVEVAQVA	AA	NST	137
AGE_2	10	CLIVPAAVRAEGVIVLGDSTSA	AYG	IDFS	EGWVQLLRQRIETQCSGLPVI	NASVSGST	93
ALC24_4107	89	VAVPDAMNKAGFSCWSYWGTKSR	SAG	DY	KNLINLANTLSGDAAR	GIDPNQVYIAG	172
ABO0116	80	VCPTSPRGTDLPGEHD	SYDFGS	GAGFYVNATQSPWSQHYRMYDYVTEELPALVQEHFP		LNGKQAISSGSHGG	175
ABO1197	170	G LTRQTEGFKT		ILADSNS NPLIPHTA	EQFOA TLQ	WAMEEPFPLPSFV	233
ABO1483	130	G PPSYDG	KDVASNLRKALDDSSASL	AGRRKPIYAHVE	DAIDARTKG	PGGLNHKASALL	217
APA_12	149	PMLEGLAAVR	AVPLARI	LRFGLYCIVDLIKPG	QPYYIQV	SAPGA	216
ABO1895	53					YSSREREVLQR	89
AGE_8	131	N	WGARLADVLCQV	ADNVVPFSGNLIAYLGNSLAGLIDL	FSSGGNEQDILAALK	ALTTAETVA	219
APA_8	138	N	WGAPMADVLQGV	SDHIFPGDDVINGLGNTLAGHINLISGGGAQDISAAMK	SITTAETLR	FNALYPEGMP	226
AGE_2	94	N	DGLRGDP	PPELTDNLATMIRISREAGA	EPVLLGMRIPP	NYGRAYTER	157
ALC24_4107	173	S	VGTSSSGATGTC	QADVESRCDRL	GGGYQSAFDTQVAS	IAHGDAITTVDT	258
ABO0116	176	CNPSCQPCWGEKALGGYLG		EDKERWKAWDSCELLKRS	G SPLPLMV	DQGDADNFYR	249
ABO1197	234	VWR				DLLEDPGMMLEDKNVLP	273
ABO1483	198	WWR				ADSRILITSLRLITEEQVE	240
APA_12	217					YDNRVAARSIMTMGPYRPF	253
ABO1895	90	GWQ	KKLQKGGY		LP	DTLYYNSERLPSYSGIRHIDGVSVDVIDNE	138
AGE_8	220	SWSGAKP			LTNVLDPS	DLVLLGATSI	275
APA_8	227	SWSGGRT			LTNLLDVTDP	EMAAISIVFLGEKND	282
AGE_2	158				LP=LL	DGVVEAGQLQDD	188
ALC24_4107	259	LWQDGRVSMLEWFEGLDHSWGGQ	GASGSYVSGASINYARYLG	GGFAEHNARI	DRNRVPTLA	QVDVTASADRIQVSGQAS	365
ABO0116	250	TLR					252
ABO1197	274	NVGAF	LEELP	QSRRAIVLDGIGHVPM	AEAPGK	SADA	308
ABO1483	241	GNGMF	DHRLGWLS	GATIVRLPGRH	HLHM	EEPQS	277
APA_12	254	SVAPF	IEGKIRRVN	NNPHLTVD	VEANHFEPYEPV		288
ABO1895	139	ATQTI					143
AGE_8	276	EVNQV	LGLHDLWS		TDPV		292
APA_8	283	EVNQA	FGLRDIFE		TSPV		299
AGE_2	189	NAWV			IEPAL	PASC	202
ALC24_4107	366	NNGYF	QATSDPLADGLYT	VAVTAADDGGESEPA	IRTVRVGPEPPASAPV	LSDIASVSDGQC	473
ABO0116	253				IQPG		256
ABO1197	309	FRAFWRE	VR				317
ABO1483	278	INTFLGETDDL	LSRA			GD	294
APA_12	289	FSSVISKQLAF	LKSLVA				305
ABO1895	144				IDVFGL		149
AGE_8	293	TLVRQQANRL	KNYNF				307
APA_8	300	TVFRQQANRL	KNA				312
AGE_2	203						202
ALC24_4107	474	QNATVSAE	DEGGLSSGQ	IDFV	DAGQTATLDQHIS	AGRLDYTNAYDCYLEYGADAFKIL	582
ABO0116	257	YDHSYFFIASFIDH	RLRYF			YHLSQ	281
ABO1197	318				P		
ABO1483	295				D		
APA_12	306						
ABO1895	150				R		
AGE_8	308						
APA_8	313						
AGE_2	203				R		
ALC24_4107	583	YHKVAGRAYSTGN	YAPDYFAQGS	DEPMAGSTWGLT	ALYSEDG	GGNVRLGEC	
ABO0116	282						

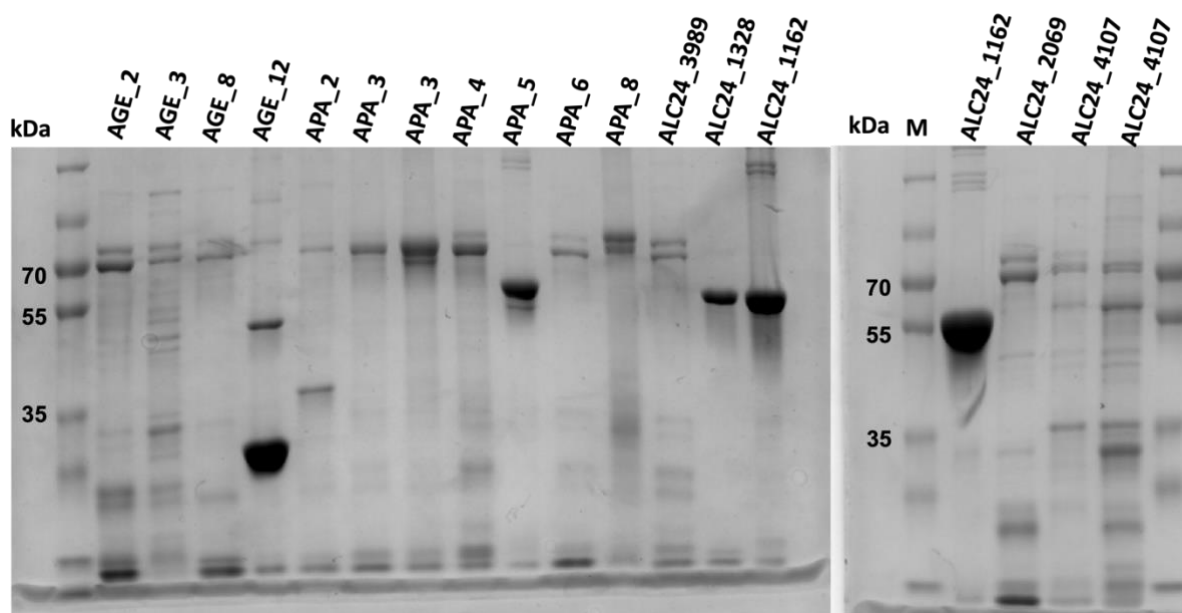
B)

ABO1197	1	MQ	LKHLFLFTVCSFFFLSGCEDITNWAYEKLAMEKNRAGLKDQILTTADGITWEILTSETSAEPIAEKEAVLILH GFSA ADSSNW VRFANEL	92
ABO2249	1	MQ	SGTVASNGIELFYESRGPEN GE PMVFVM GLSA QMVFWPDTLLDALA	48
ALC24_2069	1	MS	SGIISKNGMELYDARGPED GE PIIIFIM GLSA QWVFWEALLNLALA	48
ALC24_1162	1	MTWRRVGLILAVAVLGAAI	LGQCCHTRRWAYHQGIAPFQWRAGLESIRVSSDSGLWHEILRSKIG AHVPAVILILH GFCA DARNW VRFANQ L	91
AGE_12	1	MD	YILPCVELDP RQPADSSVIW LRGIGAGGHDF EPVVF LG	40
ABO1197	92	EGDFFFIIVPDLPGHGETT	RNLDLT YTMSAQATRLIT LMDA LQIQHFHVAG NSMGG AI SLAL AQQ	155
ABO2249	49	AKGYRVIRFDNRDVGKSTQIRKPIKQGPVSAILRRIIIGLPVES	PYTLHDMVADTV GLDA LNIEKAHFV GASMGG MI SQLMAGT	132
ALC24_2069	49	RRGYRVIAFDNRDAGMSTAIRAPIRQGPVGAMARYMIGMPVAA	PYTLVDMVADTV GLDA IGIARAHV GASMGG HAQLTAAT	132
ALC24_1162	92	EGEYRFIIIPDLPGHGAT	LEPLTTSM EYGIIDKQATRIFF LIDH IGVERFHVAG NSMGG AIATFMA RC	156
AGE_12	41	RKSTRFVFPHAP	QIPFTVNGGMVMPAWYDIVSLGGNGAVDEEGIRRSAAQLD LAL IEREIERGVPAERILVAG FSQGG AVAYEA ALR	126
ABO1197	156	APQ R VLISIGLIDSAGLTRQTEGFKTI	LADSNENPLI PH TAEQFQATL QWAMEEPPYLPSPVVDIMGKKKAAN AAVAQKVWRDLLEDPGM MLE	247
ABO2249	133	HPE R VLISITS	IMSSNNSSIM PP KPSALRVLLAPRVKVFETERQFVTFGLEMMSKLAGTLPQGEFLAAMYRAAWARGINPRGIRNQ	218
ALC24_2069	133	RSD R VLISITS	IMSSNNSPFI PP KPAAIKTIVAPKVVFETLDQYLAFGRDMMSRIGGTLPQDQDLERAPRQSWERGINPRGIRNQ	218
ALC24_1162	157	HPD R LISIGLVDAAGVTILQTQEFERDV	LAQSPGNPLI PR RAKDFETTL DWAAERSVGMPPDFAITLMGEEKKAAN AEVAEKVWADINSDPAM RLG	248
AGE_12	127	HPK R LLAGLMALSTYFATADSVAFSEANAGLPVFIAGHTFDP PMVPE	QGGRRAA DTLSAKGY PTE	189
ABO1197	248	D KNVLPISIQIPTILVLWGR ED RLLGVDNVGAPLEELPQSRATVLGX GH VP	MAHAPGKSADAPRAFWRFV RP	318
ABO2249	219	FLAITATGSLSKTLKQIQCPPTTVIHGG AD PLIRPAGGKASARATRGAKIVIIIPGM GH DEPPPSVIDRIGELIAETAGRANSV	VPPAVG	305
ALC24_2069	219	FMAILATGSLINRYLRRVQCPAQIIHGGS DP LIRPAGGKASAKATPGARLATLEGM GH DLPPAVLPRVGELIAATARRA	GASRPLGGEGEHLADV	312
ALC24_1162	249	Q AGVLASINTPTILVLWGR ED RLLGVDNVVEFTQTLPNARAVILDGV GH VP	MAEAAGQSADAPRVFWNRIP ARP	321
AGE_12	190		YKTYPMEHAVCLEELQD I AGFLAKVFD	216
ABO1197	319			
ABO2249	306			
ALC24_2069	313	AVSVDPGHQ		
ALC24_1162	322	R		
AGE_12	217			

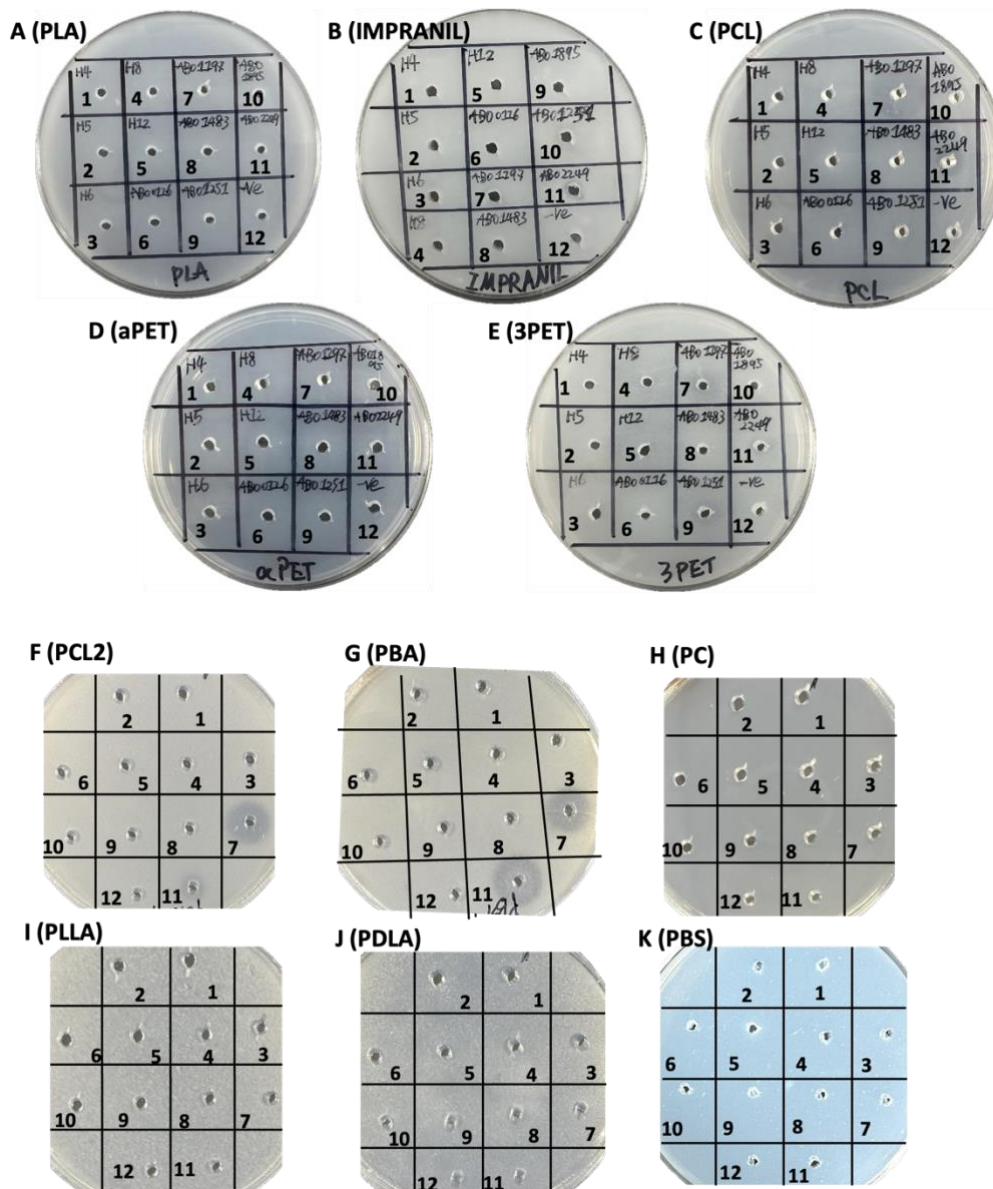
C)

ABO1197	1	MQLK	HLFLFVCSF	FLSGCEDITNWAY		EKGLAMEKNRAGL	KDQILTAD	GCTW	HILTSETS	61	
ABO1251	1	M	TATIRQGRYQGL	SSKGVTEYRGIPFAKAPLGEWRFKAPQLPDSEDCVNADRYPLASLQPRNFMIGQE					SGEDCLYLNINWAFEGE	86	
ALC24_1328	1	MANE	QCTVLSQGRYQGV	PGEVHIEFGVPYALPLGERFRKAPQLPDSDQMQRADRYPLPSLQRRNPLMGVEE					SGEDCLYLNINWAFEGE	90	
APA_5	1	M	TAVQLDSGRITGLRRDGVTEYRGIPFAAPFVGALRFRAPOQVPVGGGERACDRTALPAWQKKNFVMGVSA						IGDDCLALNVVWFEGD	88	
APA_3	1	MD				SSSNRGGLAPKTTTPDAVYSGVAMDPALMGPY		TVAEHEYDPGRMEV		47	
ALC24_3989	1	MS	INTPPVSAGKAGAFYLFISL	LLSGC		GGGNSSDAPPVAKTEPDPTTNTVAADAAATGDY		GVSENEYQPRMTV		75	
APA_6	1	MR	QIATAMVTSL				ALLSTGGPAMA	DVVVKVAYDID		35	
AGE_3	1	MRH	STVLIVLLIL			NCYAGAAATDAKPRITGGED	APSAPS	WMVEVRRSL		50	
APA_4	1	MR	ELGLFGLVMAL			CLTSQGVAGGEVEARVIGGTE	LEQGNKP	WMAAVNVYFG		52	
ABO1197	62		AEPI	AEKEAVLLIHGF		SADSSNWVWFANELEG		DEFFIVPOLPHGH		106	
ABO1251	87			GPFPMVMWFHG	GGYM	AGSTSQALYNGAELARSQVWVVVNAAYRIGAM		GFADFSAVAPRLDAOTNIGL		153	
ALC24_1328	91			GPFPMVLWYHG	GGYT	AGSVSQMLYNGAELARHGKVVVNAAYRIGAH		GFADFSAVLP	GADSNLGL	155	
APA_5	89			GPFPMVMWLHG	GGYV	AGSPSQMLYHGAELARSQVWVVVNAAYRIGVM		GYGWFRTIAP	PSLFSOSNIGL	155	
APA_3	48	YDKRDRHYETTLHGVIQYFEDLDGSAPVVLHLHGRHGTCYD	RGYT	PUTCPTPIPSYRGYEYLSLENASHGYAVISVDANYINAN				DSQPSNG	DSGALQ	145	
ALC24_3989	76	VDNKNNGDSYETDIHGVIQYFENFAGFPFVFLHGRHGTCYDGRGID	DDDCNP	PIPSYRGYQYISENLASHGYAVISVDANDINCH				DSQPSNG	DTGALA	174	
APA_6	36	GTFPSGLMIVYDOSTGARPLGLMLPNW		MGPTDDAARKAARVAGNRYVVEADMYGTDR				PQNPPQQAQAAGAV		108	
AGE_3	51	GNPNYAGTRCGGAIAPRVWLTAACHV	RDDNGTFD	PGLTFAALGHVNRFPATPRETIIV		RAVHVHDGYRSDIYRNDLALLELS	PSQAQAADIAK			144	
APA_4	53	PESTRKSLICGGTLIAPRVWLTAACHV	INSQQQRTLP	ONLLVVVSGAHRDGSGGGHLRV		AAVRTHENYNRDGLHNDIALLQLAS	PATVAFVNVAG			147	
ABO1197	107		ETTRN	LDTYMS	AQATRLTLMDALQIQHF			HVAGNSMG	AISLALQAQAPQR	VLS	162
ABO1251	154	RDQL		AALQWQENIAAFAG	DDKQV			TLFGESAGSGFSVCSLLACQADE		LPQ	203
ALC24_1328	156	RDQI		AALWVNRHIEAFGG	RADQV			TLFGESAGSGFSVASIMAAPRAPR		LPQ	205
APA_5	156	RDQV		AALWVQRNAGAFGG	DPSQV			TVFGESAGSGFSVATLLATPAADG		LPQ	205
APA_3	146	RAQL	VVRH	LDEFREINTLAGGYGMDLVLGKLDMSAT				GLMGHSRGSGGVNKTIVTYNTRPEPHNIVAVFS		213	
ALC24_3989	175	RAML	VVRH	LDEFHAINQAGGNGLDULMGKLLMSRI				GLMGHSRGSGGVNQTIVLYNRAQPEPHNITAVFA		242	
APA_6	109	RSNR	PIMRHRAAALLWTRQQT	PDSKILSTIDTRL				GAVGFCFGSGV	DEIARSGE	163	
AGE_3	145	SAEMQRLEBGNADALRALWGGQTES	GS	MSLLQQVLDYVPLSSCSGYWNNLGSGQLCAGEMNPVAGVDDTCH	GLSGGP			LLYRTADG	LWL	236	
APA_4	148	AAQIDNYTGAVGRNALPTALWGRTRANDPAS	GARILHEVSLGYVFGKQCRSTWRSVTGSQT	CAGGAGAV		DDCTGSGGP		LVLRHGT	QWT	237	
ABO1197	163	IGLIDSAGL		TRQTEGFKTILA	DNSNPLIP	HTAFQFQA	TLQWAMEFPYLPSE	VVDIMGKKKAANAAYA	EKVVRO	237	
ABO1251	204	AALVQSGGADFVL	APDQVKKVITNAFVAALPG	DGSAAEKLLSADNKGWIKAQNAAVKVLVDRLRTTT	PQAMNPLPMVDGJVLPLPVDALA	AGAAANKRVMA				306	
ALC24_1328	206	RATVQSGGADFVL	SPREFSRRTAFIAALPG	EGIVADRLIQADGEAWLAAQGFALRTSVARGLRDST	PQGMNPLPMVDGJVLPRVPVQAMA	EGACADIRILA				308	
APA_5	206	RAIVQSGAADMVL	MPALAAVITALMCALPG	DGMAERLLAAEPRAIVRAQAALKTAVQRLRDTT	PQGMVEMPVVDGJLLPRSPLEALA	AGCAORALLA				308	
APA_3	214	LAPTQYNTL		TVANVAPTALAGYCDGJVEDIMG	NFAFDTSRYAVSNQPY	PKF		QLTPMGANHNFY	NTYWADETL	285	
ALC24_3989	243	LAPTQYNTQ		TVSNVNFSLAGYCDGJVEDIMG	NFAFDTSRYAVSNQPY	AKF		QLTPMGANHNFY	NTCVW	311	
APA_6	164									163	
AGE_3	237	AGITSYGARQCAS	GVPA	VYTRVSAYLDWIERTSAGAMVDLESTTEGEA	HYASPGQTLTLSTRISNKS	V		VNEARAVGLRIYHQGLAVWA	DDL	328	
APA_4	238	VGITSYGARQCASGVPGV	VYTGAAASFGWMEQTATQQLVILASSRSBN	TSGSRPTTVVTSATSNESA				LTQASAVGWRLRSDAPVSVRLDGL		331	
ABO1197	238	LLDPGMMLEDK			NVLPS				IQIPTLV	261	
ABO1251	307	GVCRDEPNFFQ		YAGVLAGTTTMDALREISDE	EIVSRFE	RALPGNGRRAP	DYYQTAVEPDARRSLD	WLAAMESDRLEFRVPTVR		389	
ALC24_1328	309	GVCRDEPNFFQ		YSGPLAGFPTEQMRAMDDA	EILRRFE	RAAPGNGQRLP	DYYRAHITPHPRSSNMD	WFSAMESDRLEFRVPTLR		391	
APA_5	309	GVCRDEYHLFQ		YAAPFNGGQTIDALRALDD	AILSRFE	RALPQHGERAP	EYREAVTPDPARSHLD	WFSAMESDRLEFRVPTQR		391	
APA_3	286	ESRFPDWSIWDNRQDDPHCGTAAAGNGHDTAEVQKAQGLE			PMASFF	RYIVG	GETEF	AGYWNQATVPGNICPDGVGPODDORYLLSVLAPACRLVDD		383	
ALC24_3989	312	DTRFPDWTILDSGDGDPWCGENAAAGNGRDTPEMQAHGLE			FTASFF	RYFIG	GRDEF	GAYWNGRTVPVETILCPAGETSCDDRFLSVHAPAAQRLVIDD		409	
APA_6	164	LEGAD				PLNGVVSF	HGNLDTFNP	EDARAQTPIVL		192	
AGE_3	329	QQQDQGEYTL		CQQWNSLGTGATGDMHQVRLSSRSGETVAGIA	RIVPVSDRHDYTTANERYSLVFSIQP	NCLTD		ATAVRSNGTVRVATV		418	
APA_4	332	TCTQRGGITD		CRSQTVLSVGGQANRRKFEISY	SGQTDADVELEVTPIASQHNRYQRANDVIGLRFSEK	DESLH		LLREVLADRRARVTVQL		422	
ABO1197	262	LWGRE			DRLLGVNVGAFLEELPQS	RAIVLDGIGHVPM		EAPGKSADA		308	
ABO1251	390	LILDAQ	SQFAQCW	GFQPTWFSPPFVPIGACHVVDVVF	VFGVTDT	PAGM	YFTGGTSFARALSHQVQAAGWTFARGDAPGNWAWQSDRQVQC			479	
ALC24_1328	392	LLDAQASRQPRTW		GFQPTWFSQPGIIMGACHVIDVVF	VFGITDT	PVGQ	YFTGGGEPPKQLARHVQQVWSDFEAGRGADWPAWGESRRVHQ			482	
APA_5	392	LILDAHHAAGGRAW		GYEFTWEVSI PGVPLGACHVVDVVF	VFGITDT	PVGE	YFTGGGEFAAALSQRVQQCWGNFARGGSPGWADWAGLAGRAHH			482	
APA_3	384	TLDPASLFLVNNLGGNVTFDGSFSGVQTNGRFEGGCAVAEPTENTAEQLYMWVDT	TATY				RTCELLGLNVVYQVLSMRIGVSHGDLONVDGQPTLVLEOVAGNR			488	
ALC24_3989	410	TLDPASLTLTNLISGAVRFDFGSNFGICQTNGRFEGGCAVAEPTENTAEQLYMWVDT	AATY				RSELLSTDATGYQVLSVRVGTISHGDTDNIDGQDQGVVLEMDGNR			514	
APA_6	193	LEGAD				DYVPAGQVAAFESEEMRAADVMDQLVSGGAVH		SFSDPTA	DSPPQAYNAX	248	
AGE_3	419	RNLADHRDARNAWVEPOLPGGWDETL	PEGC	VASVPV	RCALGDL	PRGGSAAARTLVLTSGSGGRVPMN	AGNADGDFPSGDTNAPVMPSPRAGTESA			512	
APA_4	423	ANDAPHVAAGTQLRVFLPGGTRIANLE	AAGC	VNGVPV	VCDLGLTRPEEARDLPLDIEVSGSGEGLRFE		LAANGSYPLSDAER	SMPASGLLASA		517	
ABO1197	309		FRAFW		REVRP						
ABO1251	480	LG	PGETMASLLESSEGLW		RDIIIPV						
ALC24_1328	483	FG	PGDSAAALDEQGEALW		EVVTPRP						
APA_5	483	FG	REAGSGPLLDPAARAALW		ADIIPTATGG		DGA				
APA_3	489	VSVLASDYSGAIFYPPGRAF	AESGSQKTVINAVDV	PLTAFEGVDYVQTLNATELVFDQTAAGSVQMTDVLPRV							
ALC24_3989	515	AAVVASDYGNGLYFPFGHTFYDDANRGSQKTLNADV1	PLTAFEGVDYVQTLNATELVFDQTAAGTVQITDLAMQRVDFQP								
APA_6	249	VA	GRAF				DMNITFFGEQ	FGR			
AGE_3	513	RS	EASSSGGGGGGGGAAGLW	LAL			VLAGLGLMRHR				
APA_4	518	RS	GGGGGGSTIGW	LAL	P		ALALILRLRQRR	H			

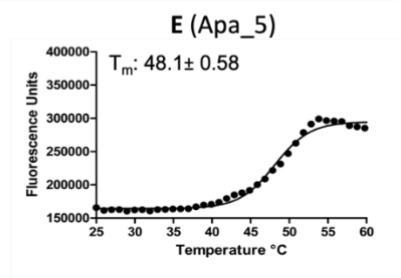
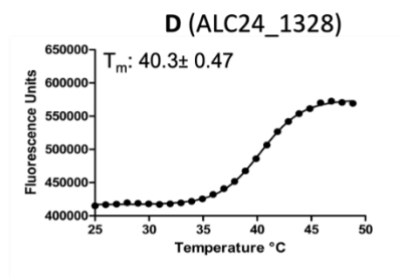
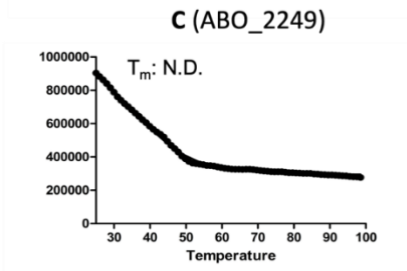
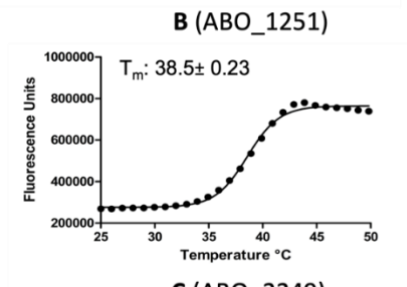
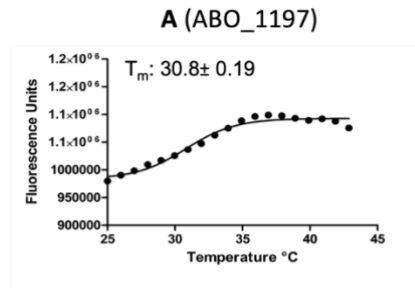
SI Figure 1. Multiple sequence alignments of polyesterses from this study from three phylogenetic clusters (see Fig. 1 for reference): Cluster I (A); Cluster III (B) and Cluster IV (C). Catalytic triad residues (Ser, Asp, and His) are also highlighted in bold among the conserved positions.



SI Figure 2. SDS-PAGE analysis of purified enzymes. 6His-tagged proteins were recombinantly expressed in *E. coli* LOBSTR cells and affinity-purified using nickel-chelate chromatography followed by SDS-PAGE analysis of purified protein (2 µg/well) and Coomassie staining. M, protein molecular weight maker (PageRuler™Plus Prestained Protein Ladder, Thermo Scientific).



SI Figure 3. Agarose-based screening of purified proteins for polyesterase activity against various polyesters. Plates/polyesters: (A), PLA; (B), IMPRANIL; (C), PCL14; (D), aPET; (E), 3PET; (F), PCL2; (G), PBA; (H), PC; (I), PLLA; (J), PDLA; (K), PBS. The presence of polyesterase activity is indicated by the formation of a clear zone around the wells loaded with indicated proteins (50 µg/well, 72 h at 30°C). Proteins: (1), AGE_12; (2), APA_5; (3), APA_3; (4), APA_5; (5), ALC24_1328; (6), ABO_0116; (7), ABO_1197; (8), ABO_1483; (9), ABO_1251; (10), ABO_1895; (11), ABO_2249; (12), -VE. The wells labelled “-VE” represent negative controls (no protein).



SI Figure 4. Thermostability of the purified enzymes: DSF analysis. Reaction conditions: 10 μg of protein in 50 mM CHES buffer (pH 9.0 CHES), 25X SYPRO Orange, emission 300 nm, excitation 472 nm. “N.D.”, not detectable, low quality of the curve (for ABO_2249).