

Table S1 High-TPKM contigs containing full or partial open reading frames for putative *L. bostrychophila* esterases

Contig ID	Contig length	TPKM (gene) ^{*1}	Blastn TopHit (percent identity) ^{*2}	Blastn TopHit ID	ORF status ^{*3}	Amino acids seq length	UniProt Blast TopHit Accession	UniProt Blast TopHit (percent identity)	UniProt E-value	Molecular Function (GO ID)	Molecular Function (GO Term)
comp59220_c1_seq1	1885	438.8	No hits		complete	554	E0VEL6	<i>Pediculus humanus corporis</i> Carboxylic ester hydrolase (46.7%)	6.80E-172	GO:0052689//GO:0016787	carboxylic ester hydrolase activity/hydrolase activity
comp62262_c0_seq1	1989	349.44	No hits		complete	577	A0A8K0DIJ4	<i>Ignelater luminosus</i> Carboxylic ester hydrolase (47.8%)	1.50E-154	GO:0052689//GO:0016787	carboxylic ester hydrolase activity/hydrolase activity
comp62654_c0_seq7	1559	261.07	No hits		internal	519	E0VEL6	<i>Pediculus humanus corporis</i> Carboxylic ester hydrolase (44.1%)	3.40E-152	GO:0052689//GO:0016787	carboxylic ester hydrolase activity/hydrolase activity
comp61411_c0_seq3	1961	171.8	No hits		complete	590	E0W271	<i>Pediculus humanus corporis</i> Carboxylic ester hydrolase (28.4%)	6.30E-48	GO:0052689//GO:0016787	carboxylic ester hydrolase activity/hydrolase activity
comp62642_c0_seq2	2127	170.37	<i>Liposcelis bostrychophila</i> esterase 4 mRNA (64.78%)	KR905662.1	complete	565	A0A067QFP9	<i>Zootermopsis nevadensis</i> Carboxylic ester hydrolase (42.5%)	2.30E-147	GO:0052689//GO:0016787	carboxylic ester hydrolase activity/hydrolase activity
comp62769_c0_seq2	1792	153.15	<i>Pediculus humanus corporis</i> Esterase FE4 precursor, putative mRNA (65.99%)	XM_002424515.1	5'-prime_partial	537	E0VEL6	<i>Pediculus humanus corporis</i> Carboxylic ester hydrolase (49.8%)	0.00E+00	GO:0052689//GO:0016787	carboxylic ester hydrolase activity/hydrolase activity
comp60632_c1_seq4	1514	135.05	No hits		complete	392	A0A2J7Q6N7	<i>Cryptotermes secundus</i> Carboxylic ester hydrolase (41.6%)	5.70E-102	GO:0052689//GO:0016787	carboxylic ester hydrolase activity/hydrolase activity
comp61080_c0_seq5	1140	123.22	<i>Liposcelis bostrychophila</i> esterase 4 mRNA (94.95%)	KR905662.1	3'-prime_partial	349	A0A2J7QJE6	<i>Cryptotermes secundus</i> Carboxylic ester hydrolase (54.4%)	3.90E-124	GO:0052689//GO:0016787	carboxylic ester hydrolase activity/hydrolase activity
comp63171_c1_seq4	1423	85.86	No hits		5'-prime_partial	415	A0A1W4WNW7	<i>Agrilus planipennis</i> Carboxylic ester hydrolase (43.8%)	4.50E-106	GO:0052689//GO:0016787	carboxylic ester hydrolase activity/hydrolase activity
comp61270_c1_seq2	1807	70.51	No hits		5'-prime_partial	540	A0A2J7RMN7	<i>Cryptotermes secundus</i> Carboxylic ester hydrolase (41.6%)	2.30E-126	GO:0052689//GO:0016787	carboxylic ester hydrolase activity/hydrolase activity
comp51226_c0_seq1	2142	63.88	No hits		complete	570	Q7PWX6	<i>Anopheles gambiae</i> Carboxylic ester hydrolase (31.2%)	4.10E-52	GO:0052689//GO:0016787	carboxylic ester hydrolase activity/hydrolase activity
comp61175_c0_seq1	2518	47.09	<i>Liposcelis bostrychophila</i> esterase 1 mRNA (96.48%)	EU854151.1	complete	610	A0A2J7RMN7	<i>Cryptotermes secundus</i> Carboxylic ester hydrolase (48.3%)	3.00E-164	GO:0052689//GO:0016787	carboxylic ester hydrolase activity/hydrolase activity
comp62403_c1_seq1	3095	30.76	<i>Liposcelis bostrychophila</i> esterase 2 mRNA (97.11%)	EU854152.1	complete	617	E0W271	<i>Pediculus humanus corporis</i> Carboxylic ester hydrolase (44.8%)	2.30E-165	GO:0052689//GO:0016787	carboxylic ester hydrolase activity/hydrolase activity

^{*1} Filtered with the criterion of a FPKM value > 30.

^{*2} Filtered with the criterion of a query coverage rate > 30%.

^{*3} Filtered with the criterion of ORF length > 1,000 nt.

Table S2 Carboxylesterases reported to be associated with insecticide resistance

Description	Organism	Signal sequence	Accession	Reference (DOI)
estalpha2 esterase	<i>Culex quinquefasciatus</i>	No	CAA88030.1	10.1042/bj3250359
serine esterase	<i>Culex quinquefasciatus</i>	No	CAA83643.1	10.1042/bj3050651
serine esterase	<i>Culex quinquefasciatus</i>	No	CAA83644.1	10.1042/bj3050651
E3	<i>Lucilia cuprina</i>	No	AAB67728.1	10.1016/s0965-1748(96)00065-3
carboxylesterase E3	<i>Cochliomyia hominivorax</i>	No	ACR56068.1	10.1111/j.1365-2915.2008.00788.x
esterase-1	<i>Culex nigripalpus</i>	No	AIT56591.1	10.1111/jvec.12195
esterase	<i>Diabrotica virgifera virgifera</i>	No	ALP46202.1	10.1111/imb.12194
esterase B1	<i>Musca domestica</i>	No	NP_001295905.1	10.1016/s0965-1748(99)00035-1
carboxylesterase MdaE7	<i>Musca domestica</i>	No	AAD29685.1	10.1016/s0965-1748(99)00035-1
carboxylesterase (Esterase FE4)	<i>Myzus persicae</i>	Yes	CAA52649.1	10.1042/bj2940569
carboxylesterase (Esterase E4)	<i>Myzus persicae</i>	Yes	CAA52648.1	10.1042/bj2940569

Table S3 BLASTP-hits for LBCE1-homologous proteins against the PDB database

Description	Organism	Query Cover (%)	E value	Per. Ident (%)	Accession
alpha-esterase-7 in complex with [3-bromo-5-(pyrrolidin-1-yl)phenyl]borinic acid	<i>Lucilia cuprina</i>	95	4E-101	36.28	5TYM_A
E3 alpha-esterase-7 carboxylesterase	<i>Lucilia cuprina</i>	95	3E-100	35.99	5CH3_A
The alpha-esterase-7 carboxylesterase, E3, from the blowfly <i>Lucilia cuprina</i>	<i>Lucilia cuprina</i>	95	4E-100	35.99	4FNG_A
The alpha-esterase-7 carboxylesterase, E3, from the blowfly <i>Lucilia cuprina</i>	<i>Lucilia cuprina</i>	95	4E-100	35.99	4FNM_A
The alpha-esterase-7 carboxylesterase, E3, from the blowfly <i>Lucilia cuprina</i> : phosphorylated-enzyme ensemble refinement	<i>Lucilia cuprina</i>	95	4E-100	35.99	5IVK_A
<i>Lucilia cuprina</i> alpha esterase 7: Gly137Asp	<i>Lucilia cuprina</i>	95	2E-99	35.8	5C8V_A
alpha-esterase-7 in complex with (3-bromo-5-phenoxyphenyl)boronic acid	<i>Lucilia cuprina</i>	95	2E-99	35.8	5TYJ_A
<i>Culex quinquefasciatus</i> carboxylesterase B2	<i>Culex quinquefasciatus</i>	85	2E-72	35.58	5W1U_A
Crystal Structure of Juvenile Hormone Esterase from <i>Manduca sexta</i> , with OTFP covalently attached	<i>Manduca sexta</i>	90	5E-82	34.36	2FJ0_A
Chain A, Acetylcholinesterase	<i>Anopheles gambiae</i>	90	1E-66	33.52	5X61_A
Chain A, Acetylcholinesterase	<i>Anopheles gambiae</i>	90	3E-66	33.52	5YDH_A
Chain A, Carboxylesterase-24	<i>Epiphyas postvittana</i>	94	2E-83	33.52	7MP4_A
Crystal structure of an insecticide-resistant acetylcholinesterase mutant from the malaria vector <i>Anopheles gambiae</i> in complex with a difluoromethyl ketone inhibitor	<i>Anopheles gambiae</i>	91	7E-66	33.33	6ARY_A
Crystal structure of an insecticide-resistant acetylcholinesterase mutant from the malaria vector <i>Anopheles gambiae</i> in the ligand-free state	<i>Anopheles gambiae</i>	90	1E-65	33.33	6ARX_A
Crystal structure of the Fab410-BfAChE complex	<i>Bungarus fasciatus</i>	94	3E-73	32.36	4QWW_A
<i>Mus musculus</i> Acetylcholinesterase in complex with N-(2-Diethylamino- ethyl)-3-trifluoromethyl-benzenesulfonamide	<i>Mus musculus</i>	94	1E-81	32.31	4B84_A
Crystal structure of the Y337A mutant of mouse acetylcholinesterase	<i>Mus musculus</i>	94	4E-81	32.31	2XUD_A
Crystal Structure Of Mache-Y337a-Tz2pa6 Anti Complex (1 Mth)	<i>Mus musculus</i>	94	4E-81	32.31	2XUF_A
Crystal structure of the mouse acetylcholinesterase-gallamine complex	<i>Mus musculus</i>	94	4E-81	32.31	1N5M_A
Chain A, ACETYLCHOLINESTERASE	<i>Electrophorus electricus</i>	94	4E-81	32.31	1C2B_A

Table S4 Docking result of ligand binding to LBCE1

Ligand	Mode	Affinity (kcal/mol)	Distance from RMSD lower bound	Distance from RMSD upper bound	Interacting residues
Malathion	1	-4.7	0	0	R351, M90, L447, A462, I142, F446
	2	-4.6	1.68	2.485	M90, L447, A462, F446, I142
	3	-4.2	1.563	4.35	R351, I347, M90, A462, I142, F446
	4	-4.2	2.537	4.714	R351, I347, M90, L447, A462, I142
	5	-4.1	2.283	6.391	M90, F446, A462, I142
	6	-4.1	1.729	4.384	R351, M90, A462, F446, I142
	7	-4.1	1.471	4.674	R351, I347, M90, A462, I142, F446
	8	-3.7	2.143	5.915	R351, M90, A462, F446, I142
	9	-3.7	2.023	5.542	R351, I347, M90, L447, A462, I142, F446
Diazinon	1	-5.5	0	0	M90, A462, I142, F446
	2	-5.2	1.562	3.641	M90, R351, A462, L447, F446, I142
	3	-4.9	1.553	2.79	M90, R351, A462, F446, I142
	4	-4.7	3.293	5.947	M90, R351, A462, I142
	5	-4.5	1.795	3.218	M90, R351, L447, A462, F446, I142
	6	-4.4	1.834	2.255	R351, M90, A462, I142, F446
	7	-4.1	1.887	3.386	M90, I142, A462, F446, L447
	8	-4	3.334	5.248	R351, I347, M90, A462, I142
	9	-3.7	3.632	6.132	R351, I347, M90, A462, I142

Table S5 Docking result of ligand binding to LC α E7

Ligand	Mode	Affinity (kcal/mol)	Distance from RMSD lower bound	Distance from RMSD upper bound	Interacting residues
Malathion	1	-2.5	0	0	F309, M308, G136, S218, F354, M460
	2	-1.7	1.994	5.724	M308, S218, G136, F354, M460
	3	-1.2	2.091	5.557	F309, M308, G136, S218, F354, M460
	4	-0.5	2.36	5.946	F309, M308, G136, S218, F354, M460
	5	-0.2	1.614	4.715	F309, M308, G136, S218, F354, M460
Diazinon	1	-1.8	0	0	M308, G136, S218, F354, M460
	2	-1.2	0.832	2.718	F309, M308, G136, S218, F354, M460
	3	-0.5	3.062	6.123	F309, M308, G136, S218, F354, M460
	4	-0.5	1.35	2.773	M308, G136, S218, F354, M460
	5	0.4	2.923	5.877	F309, M308, G136, S218, F354, M460
	6	1.1	1.95	3.833	F309, M308, G136, S218, F354, M460

ATG CAG TTC GGC TCC GAC CTT CGA ACG AGT TAC AAT CGC GAA AAA AAA AAA ATG ACT GAA
 M Q F G S D L R T S Y N R E K K K M T E
 AGC CAA CCG ATT ATC CGC ATC GCG GAT GGC TCG ATC CGA GGG GAA AAA TCG GAT TCA ATT
 S Q P I I R I A D G S I R G E K S D S I
 CGC GGT GGT TCT TAT TAC AGC TTT AAG GGG ATC CCT TAT GCC AAA CCT CCT GTT GGG GAT
 R G G S Y Y S F K G I P Y A K P P V G D
 TTG AGG TTT AAG GCC CCG GTA CCG GTG GAA CCT TGG ACA GGT GTA AGA GAT GCC TTA AAA
 L R F K A P V P V E P W T G V R D A L K
 CAT GGA AGC GAA GCC CCG GCA AAG GAC ATG TTG AAA CAT GAA TAT ATG GAA AAT ACG AGT
 H G S E A P A K D M L K H E Y M E N T S
 GAG GAT TGC CTG TTC ATC AAC GTC TAC ACG CCA GAA CTT CCG AAA AGC AAA AAT GAC AAA
 E D C L F I N V Y T P E L P K S K N D K
 TTG AAA TCA GTC CTC GTC TGG GTG CAC GGA GGA GGA TTC TCC ATG GGA TCT GGA AAC TCT
 L K S V L V W H G G F S M G S G N S
 GAA ATC TAC GGC CCC GAC TAC CTC ATC ACG GAA GAC GTC GTC CTG ACT TTC AAC TAT
 E I Y G P D Y L I T E D V V L V T F N Y
 CGG TTG GGA GTT TTG GGA TTC CTC AGT CTC GGA ACA GTC GAA TGC CCC GGG AAC TTC GGT
 R L G V L G F L S L G T V E C P G N F G
 TTG AAG GAT ATG GTC CTT GCC TTA AAA TGG GTT CAA AAG AAC ATT GCC GCT TTC GGC GGA
 L K D M V L A L K W V Q K N I A A F G G
 GAT CCG AAC AAC GTC ACG ATT TTC GGT GAA AGC GCG GGA GGA GCC GCC GTT CAG TAC CTT
 D P N N V T I F G E S A G G A A V Q Y L
 TTG ATT TCG AAA GCG ACC AGA GGA TTG TTC CAT AAG GCC ATT TCC CAA TCG GGA ACC ACT
 L I S K A T R G L F H K A I S Q S G T T
 TTG GAC CCG TGG GCG CAT AGA GAT CCG AGA GAT TTC CCG TTT GCT TTG GCG GAA GAG
 L D P W A H R L N P R D F A F A L G E E
 TTG GGA TGC AAA ACA ACC GAC GAC AAA GTG CTT CTC GAC TTC TTG AAA AAA GCA TCG CAA
 L G C K T T D D K V L L D F L K K A S Q
 AAA GAT TTC GTA GAA AAA GAA GGG GAC TTG CCG AAG AAG CTG TAC CCC GAC AGG ATT TTT
 K D F V E K E G D L P K K L Y P D R I F
 CTC CAG TTA TCG TTT GTT CCC GTA GTC GAA CCC GAA CAC GAA GGG GCC TTT TTA ACC AAA
 L Q L S F P V V E P E H E G A V L T K
 AGC CCA AGG GAA ATT ATT CAA AGC GGG GAT TTC AAT GAT GTC CCG TAT ATT ATC GGA GGA
 S P R E I I Q S G D F N D V P Y I I G G
 GTT AGC TTG GAA GGC CTT ATT ATT ATC TAC AGA AAT TTC GAA TAT AAA GAA TCG ACG GCG
 V S L E G L I I I Y R N F E Y K E S T A
 GAT GAG GAT TTG GAA CAA GTC CTC CCT CTG GGA ACA TTA AAC ATT CAA AAG GGA TCG AAA
 D E D L E Q V L P L G T L N I Q K G S K
 GAA TCC AAG GAA ATA ACG AAA ATT CGG GAC TTT TAC TTC CCC AAC GGA TAT GAG AAG
 E S K E I T K K I R D F Y F P N G Y E K
 GAG AAA CTA GTA GCT GTT CTC TCC GCC ATT TAT TTT CTG AAC GGA ATC GGC AAA ACC TGC
 E K L V A V L S A I Y F L N G I G K T C
 GAT TGG ATC GGC AGA TTA AAG AAC AGA AAT TCT CCC ACT TAT CTG TAC CAT TTC CTG TTC
 D W I G R L K N R N S P T Y L Y H F L F
 GAC GGA ACC AAG GCC TTC CTT AAG CAT CTT ATA GGC TAC GGG GAT TGG AAA GGA ACT TGC
 D G T K A F L K H L I G Y G D W K G T C
 CAT GCT GAC GAG CTC GGC TAT CTC TTC CAC ATG CCC ATG CTC CAA GCT AAA CTC GAG CCG
 H A D E L G Y L F H M P M L Q A K L E P
 AAC ACC CCT GAA TAT ACG ACA GTT CAA CGC ATG ACC AAA TTA TGG ACC GAT TTT GCG AAA
 N T P E Y T T V Q R M T K L W T D F A K
 ACC GGA AAC CCG ACG CCG AAG GAT AAC TCC TGG AAA CCG ATA TCT GAG AAT GAC AAC ACG
 T G N P T P K D N S W K P I S E N D N T
 TAT CTG GAA ATC GAA AAA GAA TTA ACT CTC AAG AAG AAT TTC AAC GAG AAA GAG GCG AAA
 Y L E I E K E L T L K K N F N E K E A K
 TTG TGG AAT GAA ATT TAC AAA TCC GTT TGC ACA AGA CAC AAG TAA
 L W N E I Y K S V C T R H K *

Figure S1 Nucleotide sequences of the longest ORF of Comp59220 and the deduced amino acid sequence



Figure S2 Next-generation sequencing reads sharing homologous nucleotide sequences with the CDS of LBCE1. The LBCE1-homologous reads were retrieved from the *L. bostrychophila* RNA (ERR073018) and genomic DNA (SRR17191995 and SRR17191998) sequencing datasets using the blastn suite-SRA tool (shown as red lines). The reads from the dataset ERR073018, SRR17191995 and SRR17191998 show at minimum 92.5-100%, 100%, and 99.33-100% identities with the nucleotide sequence of LBCE1, respectively.

Score	Expect	Method	Identities	Positives	
1097 bits(2838)	0.0	Compositional matrix adjust.	532/537(99%)	535/537(99%)	
LBCE1		MTESQPIIRIADGSIRGEKLD	IRGGSYYSFKGIPYAKPPV	DLRFKAPVPVEPWTGVRD	60
GAYV02033066.1		MTESQPIIRIADGSIRGEK	DSIRGGSYYSFKGIPYAKPPV	DLRFKAPVPVEPWTGVRD	60
LBCE1		ALKHGSEAPAKDMLKHEYMENT	SEDCLFINVYTPPELPKSKNDK	LKSVLVVWHGGGFSMGS	120
GAYV02033066.1		ALKHGSEAPAKDMLKHEYMENT	SEDCLFINVYTPPELPKSKNDK	LKSVLVVWHGGGFSMGS	120
LBCE1		GNSEIYGPDYLI TEDVVL VTFN	YRLGVLGFLSLGTVECPGNFGL	KDMVLALKWVQKNIAA	180
GAYV02033066.1		GNSEIYGPDYLI TEDVVL VTFN	YRLGVLGFLSLGTVECPGNFGL	KDMVLALKWVQKNIAA	180
LBCE1		FGGDPNNVTIFGESAGGAAVQYLL	ISKATRGLFHKAISQSGTTLP	WAHRLNPRDFAFAL	240
GAYV02033066.1		FGGDPNNVTIFGESAGGAAVQYLL	ISKATRGLFHKAISQSGTTLP	WAHRLNPRDFAFAL	240
LBCE1		GEELGCKTTDDKVL DFLKKA	SQKDFVEKEGELPKKLYPDR	IFLQLSFVPVVEPEHEGAF	300
GAYV02033066.1		GEELGCKTTDDKVL DFLKKA	SQKDFVEKEGDLPKKLYPDR	IFLQLSFVPVVEPEHEGAF	300
LBCE1		LTKSPREI IQSGDFNDVPYI	IGGVSLEGLII IYRNFEYKE	STADEDLEQVPLGTLN	IKK 360
GAYV02033066.1		LTKSPREI IQSGDFNDVPYI	IGGVSLEGLII IYRNFEYKE	STADEDLEQVPLGTLN	+K 360
LBCE1		GSKESKEITKKIRDFYFPNGYE	KEKLVAVLSAIYFLNIGKTC	DWIGRLKNRNSPTYLYH	420
GAYV02033066.1		GSKESKEITKKIRDFYFPNGYE	KEKLVAVLSAIYFLNIGKTC	DWIGRLKNRNSPTYLYH	420
LBCE1		FLFDGTKAFLKHLIGYGDWKG	TCHADELGYLFHMPMLQAKLE	PNTPEYTTVQRM	TKLWTD 480
GAYV02033066.1		FLFDGTKAFLKHLIGYGDWKG	TCHADELGYLFHMPMLQAKLE	PNTPEYTTVQRM	TKLWTD 480
LBCE1		FAKTGNPTPKDNSWKP ISEN	DNTYLEIEKEL TLKKNFN	EAKLWNEIYKSVCTRHK	537
GAYV02033066.1		FAKTGNPTPKDNSWKP ISEN	DNTYLEIEKEL TLKKNFN	+EAKLWNEIYKSVCTRHK	537

Figure S3 Sequence alignment between LBCE1 and the deduced amino acid sequence retrieved from the Shotgun Assembly Database entry GAYV02033066.1

SignalP-5.0

Summary of 1 predicted sequences from Eukarya

Predictions list. Use the help page for more detailed description of the output page.

Predicted proteins

Sequence

Prediction: Other

Protein type	Signal Peptide (Sec/SPI)	Other
Likelihood	0.0013	0.9987

Download: PNG (/services/SignalP-5.0/tmp/5F086556000069D6FDC500E9/output_Sequence_plot.png) / EPS (/services/SignalP-5.0/tmp/5F086556000069D6FDC500E9/output_Sequence_plot.eps) / Tabular (/services/SignalP-5.0/tmp/5F086556000069D6FDC500E9/output_Sequence_pred.txt)

SignalP-5.0 prediction (Eukarya): Sequence

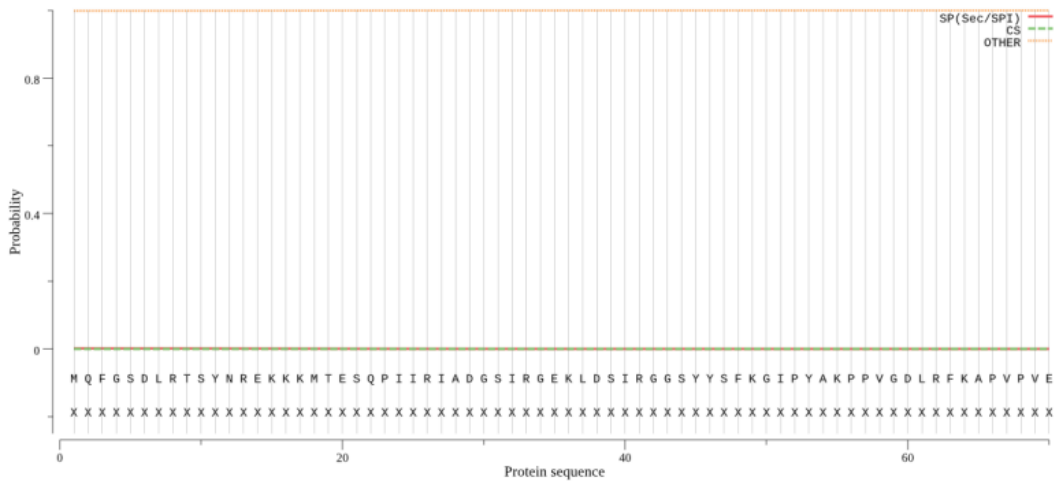


Figure S4 Prediction of the presence/absence of signal sequences in LBCE1 using the SignalP tool. The probability of the presence of signal sequences was calculated to be 0.0013.

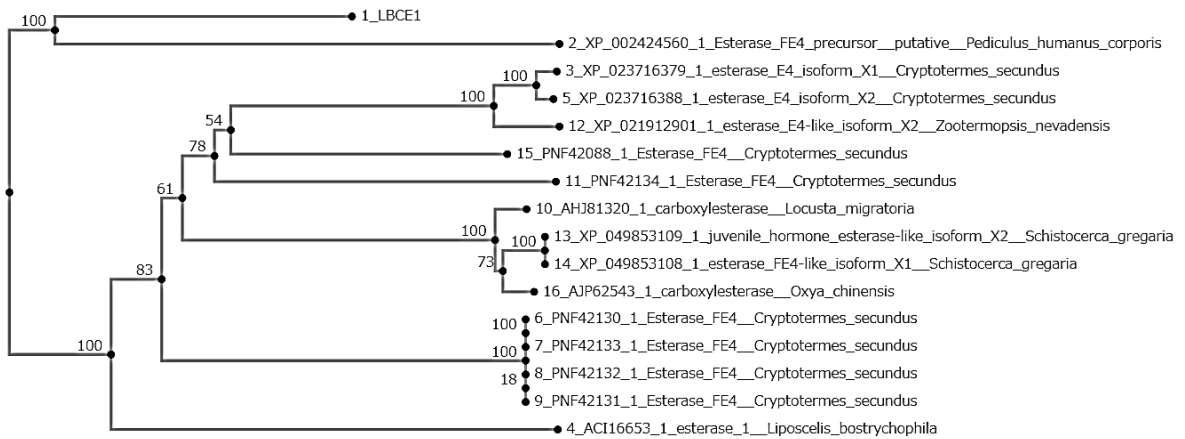


Figure S5 Phylogenetic tree showing the relationship among LBCE1 and homologous carboxylesterases. Numbers shown besides the nodes indicate bootstrap values.

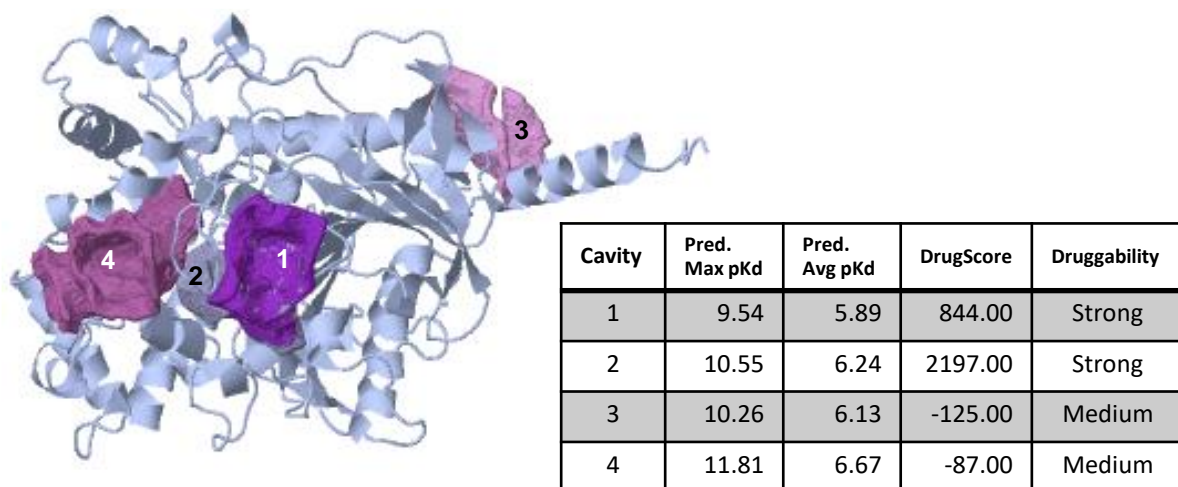


Figure S6 Prediction of the druggable surface cavities of LBCE1 using the CavityPlus tool.