SUPPLEMENTAL MATERIAL

The following supplemental material is available online:

SUPPLEMENTAL TEXT

GOHTAM and BLAST analyses.

SUPPLEMENTAL TABLES

Table S1 General features of reported esterases/lipases isolated from metagenomic resources. Data are based on bibliographic records that are specifically cited.

Table S2 List of primers used in the study.

Table S3 Percentage of identity between Arreo enzymes, determined by Matcher (EMBOSS package). Matches/alignment lengths (% identity) are specifically shown.

Table S4 Half-saturation (Michaelis) coefficient (K_m), the catalytic rate constant (k_{cat}) and the catalytic efficiency (k_{cat}/K_m) values for the wild-type α/β hydrolases from Lake Arreo. Kinetic parameters were calculated at 30ºC as outlined in Table 1 and Materials and Method section in 96-well microtiter plate where each well contained 0.388-0.882 μM enzyme solution and 0-100 mM substrate. For kinetic parameters determinations (performed in triplicate) a conventional Lineweaver and Burk model was used. Standard deviations (SD) are given.

SUPPLEMENTAL FIGURES

FIG S1 Location map (a), vegetation and use of drainage basin (b) and bathymetric map (c) of Lake Arreo. Sampling point is indicated by an asterict. The map has been generated based on batimetric studies previously published (ref. 13 and 52 in main text). Location map in (a) was made with the Geographic data sets found on the ESRI Data&Maps Kit of the SIG ArcGis 9.2 software. The limit of the drainage basin in (b) was established based on 1:25.000 maps of the digital cartographic base from the National Geographic Institute (*Instituto Geográfico Nacional de España*). The information was analyzed using the ArcGis 9.2 software. The correct establishment of the limit was further validated based on in situ measurements using topographic-geographic instrumentations (the digital free access information is available at the following URL "http://centrodedescargas.cnig.es/CentroDescargas", as established in the *Orden FOM/956/2008*, dated on 31th March 2008 [BOE 8th April 2008]). The vegetation map was generated based on the photo-interpretation of the orthophotos "PNOA © *Instituto Geográfico Nacional de España – Basque Country* (*Gobierno Vasco)*". The correct assignation of the vegetation units was established based on in situ visit to the studied area. To perform the bathymetric map (c) of Lake Arreo, an aerial photo obtained by *Diputación Foral de Álava* (1992) was used. The field study was performed in 1994, using a boat with electric engine and an ecosonda Furuno FE-4300 or a surveying rod. The obtained values were referenced and digitally treated using specific software (ArcGis v9.2 and Surfer v7.00). Further modifications and validations were done based on up-dated ortho-images.

FIG S2 Lake Arreo proteins, as overexpressed in the active form in *E. coli* at 16ºC. A Coomassie-stained SDS-PAGE gel showing the expression level of the Lake Arreo proteins is shown. As shown, a high percentage of protein is produced in a soluble form, which resulted in

a purity higher than 98% after a single His_6-tag purification step. Abbreviation: MW, molecular weight marker.

FIG S3 Chemical structures of substrates applied for activity profiling of esterases/lipases preparations used in the present study. Note: ethyl 2-methylacetoacetate, ethyl 4-chloroacetoacetate, ethyl (R)-(−)-3-hydroxybutyrate, ethyl propionate, ethyl acetate, ethyl hexanoate, ethyl 2-chloropropionate, ethyl caprate, ethyl 4-hydroxy-3-methoxycinnamate, methyl propionate, methyl 2,2-dimethyl-3-hydroxypropionate, methyl (S)-(+)-3-hydroxybutyrate, methyl (R)-(+)-3-bromo-2-methylpropionate, (−)-methyl L-lactate, (+)-methyl D-lactate, methyl 4-(hydroxymethyl)benzoote, methyl pyruvate, methyl acetoacetate, methyl 2-hydroxyisobutyrate, methyl (S)-(+)-3-hydroxy-2-methylpropionate, tert-butyl 3-hydroxypropionate, isobutyl acetate, vinyl pivalate, vinyl methacrylate, vinyl crotonoate, vinyl acetate, vinyl propionate, vinyl laurate, vinyl butyrate, glycine ethyl ester, (1S)-(+)-menthyl acetate, (1R)-(−)-menthyl acetate, (1S)-(+)-neomenthyl acetate, (1R)-(−)-neomenthyl acetate, were not used as substrates by any of the enzymes.

FIG S4 Structural models of esterases/lipases from the α/β hydrolase family characterised in this work. Residues belonging to the catalytic core are explicitly shown as follows: LAE1 (A), LAE2 (B), LAE3 (C), LAE4 (D), LAE5 (E), LAE6 (F), and LAE7 (G).

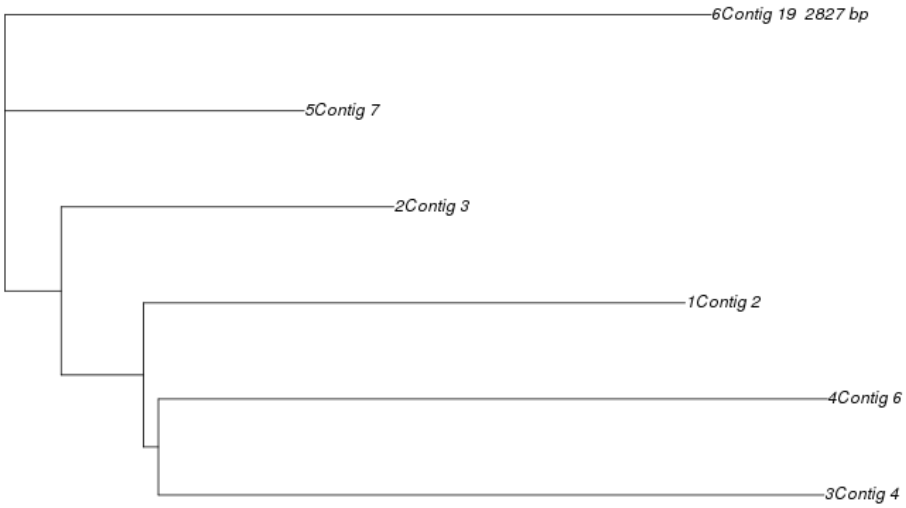
ANNEX

Nucleotide and amino acid sequences of esterases/lipases from Lake Arreo.

SUPPLEMENTAL TEXT

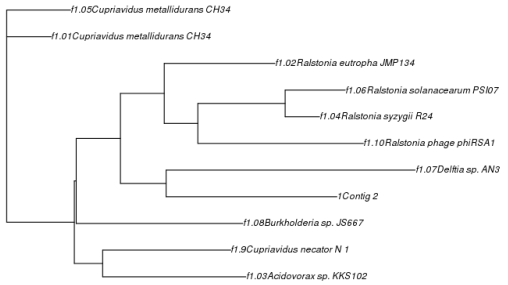
GOHTAM and BLAST analyses. Compositional similarity between the metagenomic fragments and the sequences of related bacterial chromosomes and plasmids was analyzed by the comparison of frequencies of tetranucleotides in DNA sequences using GOHTAM tool and protein blast of contig encoded proteins, the results of which are given below.

The tree of contigs by pattern similarity is shown below:



Analysis of DNA fragment (contig 2) containing LAE1 and LAE2 enzymes

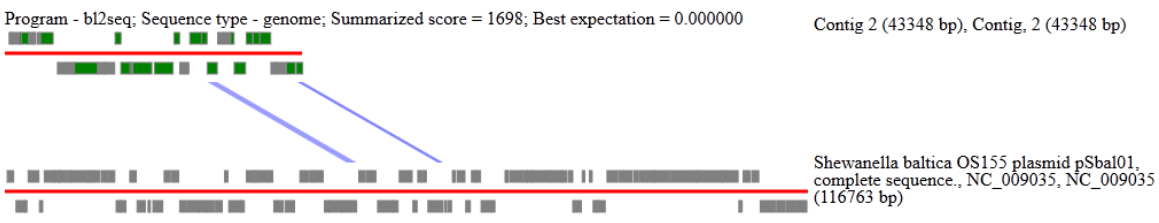
fasta 1 (length:43348) Contig 2 (43348 bp)								
Distance (A.U.Euclidean)	rRNA	Subject	Strain	Reference length (pb)	Origin	Taxonomy	similarity	confidence
102	no	Cupriavidus metallidurans CH34	GI	3928089	genomic	Bacteria	4/5	4.5/5
104	no	Ralstonia eutropha JMP134	GI	6532686	genomic	Bacteria	4/5	4.5/5
106	no	Acidovorax sp. KKS102	GI	68689	genomic	Bacteria	4/5	4.5/5
107	no	Ralstonia syzygii R24	GI	5420219	genomic	Bacteria	4/5	4.5/5
110	no	Cupriavidus metallidurans CH34	GI	2580084	plasmid	Bacteria	4/5	4.5/5
111	no	Ralstonia solanacearum PSI07	GI	2085000	plasmid	Bacteria	4/5	4.5/5
111	no	Delftia sp. AN3	GI	31303	genomic	Bacteria	4/5	4.5/5
113	no	Burkholderia sp. JS667	GI	30597	genomic	Bacteria	4/5	4.5/5
114	no	Cupriavidus necator N-1	GI	424140	plasmid	Bacteria	4/5	4.5/5
116	no	Ralstonia phage phiRSA1	GI	38760	genomic	Viruses	4/5	4.5/5



Protein blast hits against plasmid proteins

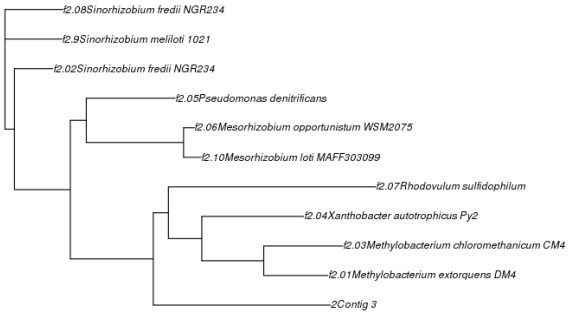
Number of hits	Subject plasmid
18	*Arthrobacter aurescens* TC1 plasmid TC2 [NC_008713]
14	*Shewanella baltica* OS155 plasmid pSbal01 [NC_009035]
11	*Synechococcus* sp. PCC 7002 plasmid pAQ7 [NC_010474]
10	*Erwinia billingiae* Eb661 plasmid pEB170 [NC_014305]
10	*Mesorhizobium* sp. BNC1 plasmid 2 [NC_008243]
10	*Agrobacterium radiobacter* K84 plasmid pAtK84c [NC_011987]

Significant protein blast hit:



Analysis of DNA fragment (contig 3) containing LAE3 enzyme

fasta 2 (length:39476) Contig 3 (39476 bp)								
Distance (A.U.Euclidean)	rRNA	Subject	Strain	Reference length (pb)	Origin	Taxonomy	similarity	confidence
121	no	Methylobacterium extorquens DM4	GI	141504	plasmid	Bacteria	3/5	4.5/5
124	no	Sinorhizobium fredii NGR234	GI	3925702	genomic	Bacteria	3/5	4.5/5
130	no	Methylobacterium chloromethanicum CM4	GI	380207	plasmid	Bacteria	3/5	4.5/5
131	no	Xanthobacter autotrophicus Py2	GI	316164	plasmid	Bacteria	3/5	4.5/5
133	no	Pseudomonas denitrificans	GI	39000	genomic	Bacteria	3/5	4.5/5
136	no	Mesorhizobium opportunistum WSM2075	GI	6884444	genomic	Bacteria	3/5	4.5/5
137	no	Rhodovulum sulfidophilum	GI	43978	genomic	Bacteria	3/5	4.5/5
137	no	Sinorhizobium fredii NGR234	GI	2430033	plasmid	Bacteria	3/5	4.5/5
138	no	Sinorhizobium meliloti 1021	GI	3654135	genomic	Bacteria	3/5	4.5/5
138	no	Mesorhizobium loti MAFF303099	GI	7036071	genomic	Bacteria	3/5	4.5/5

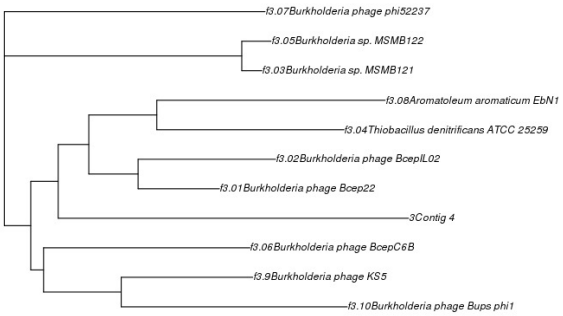


Protein blast hits against plasmid proteins

Number of hits	Subject plasmid
12	*Nitrobacter hamburgensis* X14 plasmid 1 [NC_007959]
7	*Azospirillum* sp. B510 plasmid pAB510f [NC_013860]

Analysis of DNA fragment (contig 4) containing LAE7

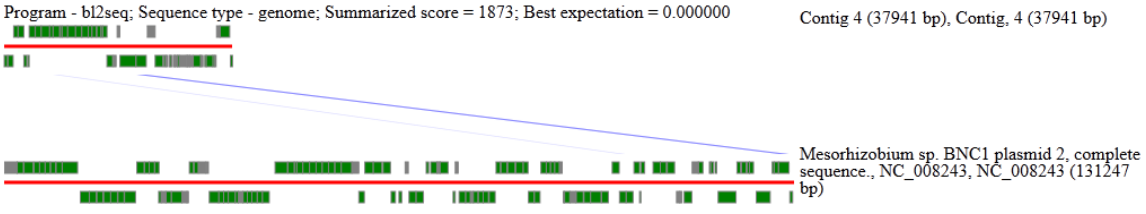
fasta 3 (length:37941) Contig 4 (37941 bp)								
Distance (A.U.Euclidean)	rRNA	Subject	Strain	Reference length (pb)	Origin	Taxonomy	similarity	confidence
116	no	Burkholderia phage Bcep22	GI	63879	genomic	Viruses	4/5	4.5/5
128	no	Burkholderia phage BcepIL02	GI	62715	genomic	Viruses	3/5	4.5/5
134	no	Burkholderia sp. MSMB121	GI	27120	genomic	Bacteria	3/5	4.5/5
136	no	Thiobacillus denitrificans ATCC 25259	GI	2909809	genomic	Bacteria	3/5	4.5/5
137	no	Burkholderia sp. MSMB122	GI	26280	genomic	Bacteria	3/5	4.5/5
138	no	Burkholderia phage BcepC6B	GI	42415	genomic	Viruses	3/5	4.5/5
144	no	Burkholderia phage phi52237	GI	37639	genomic	Viruses	3/5	4.5/5
145	no	Aromatoleum aromaticum EbN1	GI	4296230	genomic	Bacteria	3/5	4.5/5
145	no	Burkholderia phage KS5	GI	37236	genomic	Viruses	3/5	4.5/5
149	no	Burkholderia phage Bups phi1	GI	46216	genomic	Viruses	3/5	4.5/5



Protein blast hits against plasmid proteins

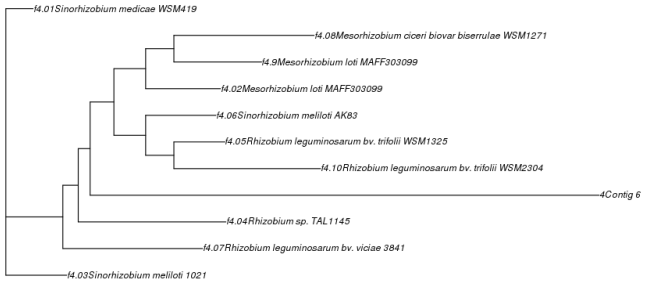
Number of hits	Subject plasmid
10	*Mesorhizobium* sp. BNC1 plasmid 2 [NC_008243]
8	*Polaromonas naphthalenivorans* CJ2 plasmid pPNAP04 [NC_008760]
8	*Agrobacterium radiobacter* K84 plasmid pAtK84c [NC_011987]

Significant protein blast hit:



Analysis of DNA fragment (contig 6) containing LAE4

fasta 4 (length:37591) Contig 6 (37591 bp)									
Distance (A.U.Euclidean)	rRNA	Subject	Strain	Reference length (pb)	Origin	Taxonomy	similarity	confidence
132	no	Sinorhizobium medicae WSM419	GI	1245408	plasmid	Bacteria	3/5	4.5/5
132	no	Mesorhizobium loti MAFF303099	GI	351911	plasmid	Bacteria	3/5	4.5/5
132	no	Sinorhizobium meliloti 1021	GI	1354226	plasmid	Bacteria	3/5	4.5/5
133	no	Rhizobium sp. TAL1145	GI	19998	genomic	Bacteria	3/5	4.5/5
134	no	Rhizobium leguminosarum bv. trifolii WSM1325	GI	516088	plasmid	Bacteria	3/5	4.5/5
134	no	Sinorhizobium meliloti AK83	GI	256269	plasmid	Bacteria	3/5	4.5/5
137	no	Rhizobium leguminosarum bv. viciae 3841	GI	488135	plasmid	Bacteria	3/5	4.5/5
138	no	Mesorhizobium ciceri biovar biserrulae WSM1271	GI	425539	plasmid	Bacteria	3/5	4.5/5
138	no	Mesorhizobium loti MAFF303099	GI	208315	plasmid	Bacteria	3/5	4.5/5
138	no	Rhizobium leguminosarum bv. trifolii WSM2304	GI	308747	plasmid	Bacteria	3/5	4.5/5



Protein blast hits against plasmid proteins

Number of hits Subject plasmid

13 *Azospirillum* sp. B510 plasmid pAB510f [NC_013860]

12 *Rhizobium leguminosarum* bv. trifolii WSM1325 plasmid pR132504 [NC_012852]

12 *Acaryochloris marina* MBIC11017 plasmid pREB1 [NC_009926]

11 *Rhizobium leguminosarum* bv. trifolii WSM1325 plasmid pR132505 [NC_012854]

11 *Rhizobium etli* CFN 42 plasmid p42c [NC_007764]

10 *Anabaena variabilis* ATCC 29413 plasmid C [NC_007412]

10 *Roseobacter denitrificans* plasmid pTB1 [NC_008386]

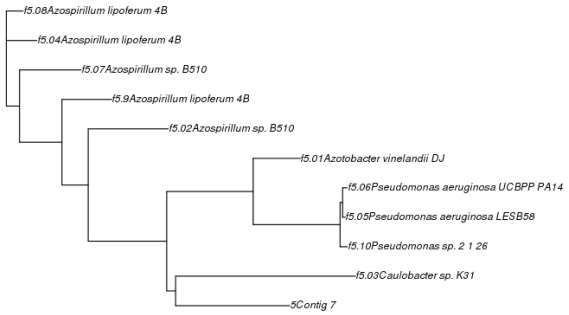
9 *Halomicrobium mukohataei* DSM 12286 plasmid pHmuk01 [NC_013201]

Significant protein blast hit:


Program - bl2seq; Sequence type - genome; Summarized score = 3065; Best expectation = 0.000000
Contig 6 (37591 bp), Contig, 6 (37591 bp)
Azospirillum sp. B510 plasmid pAB510f, complete sequence., NC_013860, NC_013860 (261596 bp)
Program - bl2seq; Sequence type - genome; Summarized score = 1699; Best expectation = 0.000000
Contig 6 (37591 bp), Contig, 6 (37591 bp)
Rhizobium leguminosarum bv. trifolii WSM1325 plasmid pR132504,, NC_012852, NC_012852 (350312 bp)

Analysis of DNA fragment (contig 7) containing LAE5

fasta 5 (length:36672) Contig 7 (36672 bp)								
Distance (A.U.Euclidean)	rRNA	Subject	Strain	Reference length (pb)	Origin	Taxonomy	similarity	confidence
120	no	Azotobacter vinelandii DJ	GI	5365318	genomic	Bacteria	4/5	4.5/5
128	no	Azospirillum sp. B510	GI	261596	plasmid	Bacteria	3/5	4.5/5
129	no	Caulobacter sp. K31	GI	177878	plasmid	Bacteria	3/5	4.5/5
130	no	Azospirillum lipoferum 4B	GI	2988332	genomic	Bacteria	3/5	4.5/5
131	no	Pseudomonas aeruginosa LESB58	GI	6601757	genomic	Bacteria	3/5	4.5/5
132	no	Pseudomonas aeruginosa UCBPP-PA14	GI	6537648	genomic	Bacteria	3/5	4.5/5
132	no	Azospirillum sp. B510	GI	723779	plasmid	Bacteria	3/5	4.5/5
133	no	Azospirillum lipoferum 4B	GI	1040425	plasmid	Bacteria	3/5	4.5/5
133	no	Azospirillum lipoferum 4B	GI	295744	plasmid	Bacteria	3/5	4.5/5
133	no	Pseudomonas sp. 2_1_26	GI	6447528	genomic	Bacteria	3/5	4.5/5



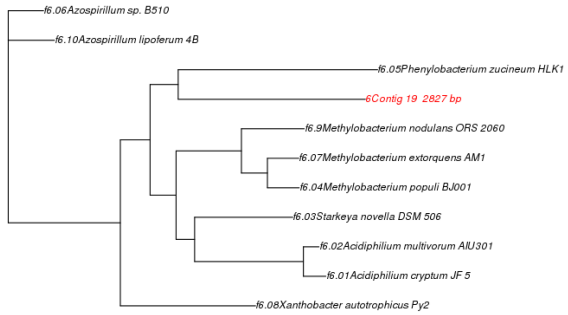
Protein blast hits against plasmid proteins

Number of hits	Subject plasmid
10	*Thermus thermophilus* HB8 plasmid pTT27 [NC_006462]
10	*Mesorhizobium* sp. BNC1 plasmid 2 [NC_008243]
10	*Rhodobacter sphaeroides* ATCC 17029 plasmid pRSPH01 [NC_009040]
9	*Agrobacterium radiobacter* K84 plasmid pAtK84c [NC_011987]

Significant protein blast hit:

Analysis of DNA fragment (contig 19) containing LAE6

fasta 6 (length:2827) Contig 19 (2827 bp)								
Distance (A.U.Euclidean)	rRNA	Subject	Strain	Reference length (pb)	Origin	Taxonomy	similarity	confidence
170	no	Acidiphilium cryptum JF-5	GI	3389227	genomic	Bacteria	2/5	3.5/5
173	no	Acidiphilium multivorum AIU301	GI	3749411	genomic	Bacteria	2/5	3.5/5
175	no	Starkeya novella DSM 506	GI	4765023	genomic	Bacteria	2/5	3.5/5
179	no	Methylobacterium populi BJ001	GI	5800441	genomic	Bacteria	2/5	3.5/5
186	no	Phenylobacterium zucineum HLK1	GI	3996255	genomic	Bacteria	2/5	3.5/5
186	no	Azospirillum sp. B510	GI	628837	plasmid	Bacteria	2/5	3.5/5
187	no	Methylobacterium extorquens AM1	GI	5511322	genomic	Bacteria	2/5	3.5/5
189	no	Xanthobacter autotrophicus Py2	GI	5308934	genomic	Bacteria	2/5	3.5/5
190	no	Methylobacterium nodulans ORS 2060	GI	7772460	genomic	Bacteria	2/5	3.5/5
191	no	Azospirillum lipoferum 4B	GI	645253	plasmid	Bacteria	2/5	3.5/5



Protein blast hits against plasmid proteins

Number of hits	Subject plasmid
3	*Sphingomonas* sp. KA1 plasmid pCAR3 [NC_008308]

Table S1 General features of reported esterases/lipases isolated from metagenomic resources. Data are based on bibliographic records that are specifically cited.

Source	Enzyme description	Substrates tested, kinetic parameters and pH and temperature optima[1]	Reference
Soil samples			
Fat contaminated soil collected from a wastewater treatment plant	1 Lipase	Opt. pH: 4.5-10.0 Opt Temp.: 60ºC Opt. NaCl: up to 3.7 M NaCl Spec. act.: 2187 $\mu molmin^{-1}mg^{-1}$ (tributyrin) Relevant properties: the pure enzyme has specific activities of 1722 U/mg and 1767 U/mg against olive oil and pig fat, respectively; it is highly stable in organic solvents at 15% and 30% (v/v).	**Glogauer A, Martini VP, Faoro H, Couto GH, Müller-Santos M, Monteiro RA, Mitchell DA, de Souza EM, Pedrosa FO, Krieger N.** 2011. Identification and characterization of a new true lipase isolated through metagenomic approach. Microb. Cell Fact. **10:**54.
Peat-swamp forest soil	1 Esterase/lipase	Opt. pH: 5.0 Opt Temp.: 50ºC K_m: 0.08 mM (pNPC$_4$) V_{max}: 88.75 $\mu molmin^{-1}$ (pNPC$_4$) k_{cat}/K_m: 583.88 $s^{-1}mM^{-1}$ (pNPC$_4$)	**Bunterngsook B, Kanokratana P, Thongaram T, Tanapongpipat S, Uengwetwanit T, Rachdawong S, Vichitsoonthonkul T, Eurwilaichitr L**. 2010. Identification and characterization of lipolytic enzymes from a peat-swamp forest soil metagenome. Biosci. Biotechnol. Biochem. 74:1848-1854
Oil contaminated soil	2 Lipases	Opt. pH: 10.0 Opt Temp.: 30ºC; the enzyme still displayed 28% residual activity at 0ºC and 16% at -5ºC. Spec. act.: up to 513.6 $\mu molmin^{-1}mg^{-1}$ (pNPC$_4$) Relevant properties: Stereo-selective for ibuprofen-pNP ester with a high preference for the (*R*) enantiomer of >91% ee and it demonstrated selectivity for esters of primary alcohols, whereas esters of secondary or tertiary alcohols were nearly not converted; one enzyme was highly enantioselective for (+)-menthylacetate	**Elend C, Schmeisser C, Hoebenreich H, Steele HL, Streit WR**. 2007. olation and characterization of a metagenome-derived and cold-active lipase with high stereospecificity for (R)-ibuprofen esters. J. Biotechnol 130:370-377
Antarctic Desert Soil	1 Esterase	Opt. pH: alkaline pH Opt Temp.: 7-54ºC K_m: 0.27 mM (pNPC$_3$) k_{cat}/K_m: 14.8 $s^{-1}mM^{-1}$ (pNPC$_3$) Relevant properties: -	**Heath C, Hu XP, Cary SC, Cowan D.** 2009. Identification of a novel alkaliphilic esterase active at low temperatures by screening a metagenomic library from antarctic desert soil. Appl. Environ. Microbiol. **75:**4657–4659
Alkaline-polluted soil samples were collected from the ground surface of a stream located in Guangxi Province, South China	1 Bifunctional β-glucosidase/lipase	Opt. pH: 8.0 Opt Temp.: 30ºC Spec. act.: 8.2 $\mu molmin^{-1}mg^{-1}$ (pNPC$_4$) K_m: 0.88 mM (pNPC$_4$) k_{cat}/K_m: 241 $min^{-1}mM^{-1}$ (pNPC$_3$) Relevant properties: β-glucosidase with lipolytic activity	**Jiang CJ, Chen G, Huang J, Huang Q, Jin K, Shen PH, Li JF, Wu B.** 2011. A novel β-glucosidase with lipolytic activity from a soil metagenome. Folia Microbiol. **56:**563–570
High Andean forest soil metagenome	1 Esterase	Opt. pH: 8.0 Opt Temp.: 10ºC Spec. act.: 0.142 $\mu molmin^{-1}mg^{-1}$ (pNPC$_4$)	**Jiménez DJ, Montaña JS, Alvarez D, Baena S.** 2012. A novel cold active esterase derived from Colombian high Andean forest soil metagenome. World J. Microbiol. Biotechnol. **28:**361–370
Top soil samples (5 to 10 cm) from vegetable soil	1 Pyrethroid-hydrolyzing esterase	Opt. pH: 5.5-9.0 (with maximum at 7.0) Opt Temp.: 40ºC	**Li G, Wang K, Liu YH.** 2008. Molecular cloning and characterization of a novel pyrethroid-

		Spec. act.: 429 µmolmin^{-1}mg^{-1} (pNPC8) Relevant properties: the enzyme shows a broader substrate specificities and high activity of the pyrethroid-hydrolyzing esterase (Pye3) make it an ideal candidate for in situ for detoxification of pyrethroids where they cause environmental contamination problems.	hydrolyzing esterase originating from the metagenome. Microb. Cell Fact. **7:**38
Soil samples of the German Biodiversity Exploratories: 10 German forest soils (A horizons) and six grassland soils (A and B horizons)	37 Lipolytic gene products	Opt. pH: - Opt Temp.: - Spec. act.: up to 0.835 µmolmin^{-1}ml^{-1} (pNPC$_4$) Relevant properties: 35 out of the 37 predicted proteins are members of known families of lipolytic enzymes	**Nacke H, Will C, Herzog S, Nowka B, Engelhaupt M, Daniel R.** 2011. Identification of novel lipolytic genes and gene families by screening of metagenomic libraries derived from soil samples of the German Biodiversity Exploratories. FEMS Microbiol. Ecol. **78:**188–201
Forest soil	6 Lipases	Opt. pH: - Opt Temp.: - Relevant properties: the identity values were far lower to a level of 20%-40%	**Hong KS, Lim HK, Chung EJ, Park EJ, Lee MH, Kim JC, Choi GJ, Cho KY, Lee SW.** 2007. Selection and characterization of forest soil metagenome genes encoding lipolytic enzymes. J. Microbiol. Biotechnol. **17:**1655-1660
Antarctic soil	1 Lipase	Opt. pH: 8.0 Opt Temp.: 35ºC Relevant properties: -	**Cieśliński H, Białkowskaa A, Tkaczuk K, Długołecka A, Kur J, Turkiewicz M.** 2009. Identification and molecular modeling of a novel lipase from an Antarctic soil metagenomic library. Pol. J. Microbiol. **58:**199-204
Forest topsoil	1 Lipases	Opt. pH: - Opt Temp.: - Spec. act.: up to >0.1 µmolmin^{-1}ml^{-1} (pNPC$_4$) Relevant properties: -	**Lee SW, Won K, Lim HK, Kim JC, Choi GJ, Cho KY.** 2004. Screening for novel lipolytic enzymes from uncultured soil microorganisms. Appl. Microbiol. Biotechnol. **65:**720–726
Soil samples	1 Lipase	Opt. pH: 7.0 Opt Temp.: 30ºC Spec. act.: 513 µmolmin^{-1}mg^{-1} (pNPC$_2$) Relevant properties: organic solvent stable lipase	**Khan M, Jithesh K.** 2012. Expression and purification of organic solvent stable lipase from soil metagenomic library. World J. Microbiol. Biotechnol. **28:**2417–2424
Soil	1 Esterase	Opt. pH: 5.0-10.0 Opt Temp.: 40ºC; retained about 50% maximal activity at 5–10ºC Spec. act.: 254 µmolmin^{-1}mg^{-1} (pNPC$_2$) Relevant properties: remarkable stability in up to 50% (v/v) benzene and alkanes; the purified enzyme also cleaved sterically hindered esters of tertiary alcohols and has potential for use in industrial processes	**Jin P, Pei X, Du P, Yin X, Xiong X, Wu H, Zhou X, Wang Q**. 2012. Overexpression and characterization of a new organic solvent-tolerant esterase derived from soil metagenomic DNA. Bioresour. Technol. **116:**234–240
Antarctic soil	14 Lipases/esterases	Opt. pH: - Opt Temp.: 35-55ºC Relevant properties: -	**Berlemont R, Pipers D, Delsaute M, Angiono F, Feller G, Galleni M, Power P.** 2011. Exploring the Antarctic soil metagenome as a source of novel cold-adapted enzymes and genetic mobile elements. Rev. Argent. Microbiol. **43:**94-103
Miscellaneous environmental samples	35 Esterases	Opt. pH: - Opt Temp.: - Relevant properties: high enantioselectivity (E > 100) in the kinetic resolution of arylaliphatic tertiary alcohols such as 1,1,1-trifluoro-2-phenylbut-3-yn-2-yl acetate.	Kourist R, Hari Krishna S, Patel JS, Bartnek F, Hitchman TS, Weiner DP, Bornscheuer UT. 2007. entification of a metagenome-derived esterase with high enantioselectivity in the kinetic resolution of arylaliphatic tertiary alcohols. Org. Biomol. Chem. 5:3310–3313

Pools of metagenomes from the Microbank of Microbial Genomics and Application Center (Taejon, South Korea)	1 Esterase	Opt. pH: 7.0-8.0 Opt Temp.: 65ºC Spec. act.: circa 22 $mmolmin^{-1}mg^{-1}$ (pNPC$_2$) Relevant properties: it shows specificity for (*S*)-ketoprofen	**Yoon S, Kim S, Ryu Y, Kim TD.** 2007. Identification and characterization of a novel (S)-ketoprofen-specific esterase. t J Biol Macromol. **41:**1–7
Alluvial soil metagenomic library	2 Esterases	Opt. pH: 7.0-9.0 Opt Temp.: 25-35ºC Spec. act.: up to circa 550 $mmolmin^{-1}mg^{-1}$ (pNPC$_2$) Relevant properties: reactivate chloramphenicol by counteracting chloramphenicol acetyltransferase	**Tao W, Lee MH, Yoon MY, Kim JC, Malhotra S, Wu J, Hwang EC, Lee SW.** 2011. Characterization of two metagenome-derived esterases that reactivate chloramphenicol by counteracting chloramphenicol acetyltransferase. J. Microbiol. Biotechnol. **21:**1203–1210
Environmental soils	1 Esterase	Opt. pH: 7.0 Opt Temp.: 25ºC K_m: 16.4 mM ((*R*,*S*)-ketoprofen ethyl ester) *V*max: 59.1U/mg ((*R*,*S*)-ketoprofen ethyl ester) Spec. act.: 103 $mmolmin^{-1}mg^{-1}$ (pNPC$_2$) K_m: 1.0 mM (pNPC$_2$) *V*max: 63.7U/mg (pNPC$_2$) Relevant properties: slight enantioselectivity toward (*R*)-ketoprofen ethyl ester: (eep» -4.6%)	**Kim YJ, Choi GS, Kim SB, Yoon GS, Kim YS, Ryu YW.** 2006. Screening and characterization of a novel esterase from a metagenomic library. Protein Expr Purif. **45:**315-323.
Glacier soil based	2 Lipases	Opt. pH: - Opt Temp.: ≤40ºC (had some low-temperature lipase characteristics, although they are not typical cold-active lipases) Relevant properties: -	**Yuhong Z, Shi P, Liu W, Meng K, Bai Y, Wang G, Zhan Z, Yao B.** 2009. Lipase diversity in glacier soil based on analysis of metagenomic DNA fragments and cell culture. J. Microbiol. Biotechnol. **19:**888–897
Plant rhizosphere soil	1 Esterase	Opt. pH: 8.0 Opt Temp.: 35ºC K_m: 0.0794 mM (pNPC$_4$) k_{cat}: 120.5 s^{-1} (pNPC$_4$) Relevant properties: the esterase exhibited an increase in enzymatic activity in the presence of 15% butanol and 15% methanol; phylogenetic analysis revealed that the lipolytic protein may be a member of a novel family of lipolytic enzymes	Lee MH, Hong KS, Malhotra S, Park JH, Hwang EC, Choi HK, Kim YS, Tao W, Lee SW. **2010. A new esterase EstD2 isolated from plant rhizosphere soil metagenome. Appl. Microbiol. Biotechnol.** 88:**1125-1134.**
Soil samples from Taishan (China) at three different altitudes (400, 800, 1,200 m)	2 Lipases	Opt. pH: 7.0-8.0 Opt Temp.: 20-40ºC; about 60% activity at 10ºC for one of the lipases Spec. act.: up to 166 $\mu molmin^{-1}mg^{-1}$ (pNPC$_{16}$) Relevant properties: thermostable lipase despite the low homology with some known lipases	Wei P, Bai L, Song W, Hao G. **2009. Characterization of two soil metagenome-derived lipases with high specificity for p-nitrophenyl palmitate. Arch. Microbiol.** 191:**233-240**
Antarctic desert soil	1 Esterase	Opt. pH: 11.0 Opt Temp.: 20ºC Spec. act.: - $mmolmin^{-1}mg^{-1}$ (pNPC$_4$) Relevant properties: esterases possessing such extreme alkaliphily are rare and so this enzyme represents an intriguing novel locus in protein sequence space	Hu, X.P., Heath, C., Taylor, M.P., Tuffin, M. & Cowan, D., **2012: A novel, extremely alkaliphilic and cold-active esterase from antarctic desert soil. Extremophiles** 16:**79-86**
Alluvial soil	1 Esterase	Opt. pH: 8.0 Opt Temp.: 40ºC K_m: 1.3 mM (pNPC$_4$) k_{cat}: 2293 s^{-1} (pNPC$_4$) k_{cat}/Km: 176.4 $s^{-1}mM^{-1}$ (pNPC$_4$) Relevant properties: tolerated well the presence of methanol and Tween 20; its activity is strongly	Tao, W., Lee, M.H., Wu, J., Kim, N.H. & Lee, S.W., **2011: Isolation and characterization of a family vii esterase derived from alluvial soil metagenomic library. J. Microbiol** 49:**178-185**

		inhibited by 1 mM Cu(2+) and Zn(2+), but stimulated by Fe(2+); it is high activity under alkaline conditions and stable in the presence of organic solvents	
Bioreactors and activated sludges			
Biomass enrichment in a fed-batch bioreactor	7 Lipases	Opt. pH: 8.5 Opt Temp.: 60ºC Spec. act.: 4284 $mmolmin^{-1}mg^{-1}$ ($pNPC_4$) Relevant properties: activity increases in the presence of 30% acetonitrile, ethanol, methanol and acetone	**Côté A, Shareck F.** 2010. Expression and characterization of a novel heterologous moderately thermostable lipase derived from metagenomics in Streptomyces lividans. J. Ind. Microbiol. Biotechnol. **37:**883–891
Activated sludge	1 Lipase	Opt. pH: 7.0 Opt Temp.: 10ºC K_m: 0.15 mM ($pNPC_4$) k_{cat}: 26.5 s^{-1} ($pNPC_4$) Spec. act.: 150.2 $\mu molmin^{-1}mg^{-1}$ (U/mg) ($pNPC_4$) k_{cat}/Km: 176.7 $s^{-1}mM^{-1}$ ($pNPC_4$)	**Roh C, Villatte F.** 2008. Isolation of a low-temperature adapted lipolytic enzyme from uncultivated micro-organism. J. Appl. Microbiol. **105:**116-123.
Biomass produced in an enriched sequencing fed-batch reactor	1 Lipase	Opt. pH: 10.5 Opt Temp.: 60ºC Relevant properties: exhibited maximal activity with $pNPC_{14}$ but not absolute value is given	**Meilleur C, Hupé JF, Juteau P, Shareck F.** 2009. Isolation and characterization of a new alkali-thermostable lipase cloned from a metagenomic library. J. Ind. Microbiol. Biotechnol. **36:**853–861
Activated sludge	1 Lipase	Opt. pH: 8.5 Opt Temp.: 35ºC Spec. act.: up to 4615 $\mu molmin^{-1}ml^{-1}$ ($pNPC_8$) Relevant properties: exhibited the same level of activity in the presence of metal ions or detergents and it showed high level of stability with unique substrate specificities makes it highly valuable for downstream biotechnological applications.	**JunGang L, KeGui Z, WenJun H.** 2010. Cloning and biochemical characterization of a novel lipolytic gene from activated sludge metagenome, and its gene product. Microb. Cell Fact. **9:**83
Activated sludge metagenome	1 Esterase	Opt. pH: 9.0 Opt Temp.: 35ºC Spec. act.: up to 5370 $\mu molmin^{-1}ml^{-1}$ ($pNPC_6$) Relevant properties: hydrolyze *p*NP esters of fatty acids with short chain lengths (≤ C8); it also exhibited the same level of stability in the presence of metal ions or detergents.	**Zhang T, Han WJ.** 2009. Gene cloning and characterization of a novel esterase from activated sludge metagenome. Microb. Cell Fact. **8:**67
Compost samples			
Compost	1 Esterase	Opt. pH: 9.0 Opt Temp.: 55ºC Relevant properties: Remarkable stability in up to 50% (v/v) dimethyl sulfoxide (DMSO) or dimethylformamide (DMF). The enzyme has the ability to cleave sterically hindered esters of tertiary alcohol, as well as to degrade polyurethanes, which are widely used in various industries	**Kang CH, Oh KH, Lee MH, Oh TK, Kim BH, Yoon J.** 2011. A novel family VII esterase with industrial potential from compost metagenomic library. Microb. Cell Fact. **10:**41
Compost containing poly(lactic acid) (PLA)-degrading microorganisms	3 Poly (DL-lactic acid) depolymerases	Opt. pH: - Opt Temp.: 70ºC Spec. act.: up to 234.8 $\mu molmin^{-1}mg^{-1}$ (U/mg) ($pNPC_6$) Relevant properties: The enzyme can effectively degrade PLA	**Mayumi D, Akutsu-Shigeno Y, Uchiyama H, Nomura N, Nakajima-Kambe T.** 2008. Identification and characterization of novel poly(DL-lactic acid) depolymerases from metagenome. Appl. Microbiol. Biotechnol. **79:**743–750
Leaf-Branch Compost	1 Cutinase with polyethylene terephthalate-degrading activity	Opt. pH: 8.5 Opt Temp.: 50ºC Spec. act.: 12 $\mu molh^{-1}mg^{-1}$ (U/mg) (polyethylene terephthalate) Spec. act.: 2.7 $\mu molmin^{-1}mg^{-1}$ (U/mg) ($pNPC_6$)	**Sulaiman S, Yamato S, Kanaya E, Kim JJ, Koga Y, Takano K, Kanaya S.** 2012. Isolation of a novel cutinase homolog with polyethylene terephthalate-degrading activity from leaf-branch compost by using

		Relevant properties: hydrolyzed various fatty acid monoesters with acyl chain lengths of 2 to 18, and had an ability to degrade poly(-caprolactone) and polyethylene terephthalate (PET). It is potentially applicable for surface modification and degradation of PET	a metagenomic approach. Appl. Environ. Microbiol. **78:**1556-1562.
Compost metagenomic library	1 Esterase	Opt. pH: 10.0 Opt Temp.: 50ºC Spec. act.: 17.1 µmolmin^{-1}mg^{-1} (U/mg) (pNPC$_6$) Relevant properties: stable in the presence of 30% methanol and exhibited a 2.4-fold higher activity in the presence of 5% methanol than in the presence of 1% isopropanol	**Kim YH, Kwon EJ, Kim SK, Jeong YS, Kim J, Yun HD, Kim H.** 2010. cular cloning and characterization of a novel family VIII alkaline esterase from a compost metagenomic library. Biochem. Biophys. Res. Commun. **393:**45-49.
Marine sediments			
Deep-sea metagenomic library	1 Esterase	Opt. pH: 9.0 Opt Temp.: 0-60ºC; best activity at 50°C, but was unstable at 60°C K_m: 0.46 mM (pNPC4) k_{cat}: 1200 s^{-1} (pNPC4) k_{cat}/K_m: 2600 s^{-1}mM^{-1} (pNPC4) Relevant properties: stable in the presence of 10% DMSO and 20% methanol	**Fu C, Hu Y, Xie F, Guo H, Ashforth EJ, Polyak SW, Zhu B, Zhang L.** 2011. Molecular cloning and characterization of a new cold-active esterase from a deep-sea metagenomic library. Appl. Microbiol. Biotechnol. **90:**961–970
Marine sediment	1 Lipase	Opt. pH: - Opt Temp.: 35ºC Spec. act.: circa 160 µmolmin^{-1}mg^{-1} (pNPC4)	**Hårdeman F, Sjöling S.** 2007. Metagenomic approach for the isolation of a novel low-temperature-active lipase from uncultured bacteria of marine sediment. FEMS Microbiol. Ecol. **59:**524–534
Deep-sea sediment	1 Esterase	Opt. pH: 10.0-11.0 Opt Temp.: 10-40ºC Spec. act.: up to 156 µmolmin^{-1}mg^{-1} (pNPC4) Relevant properties: Anionic detergents (SDS and Sarkosyl) showed a very weak inhibitory effect, and most neutral detergent had little activating effect	**Park HJ, Jeon JH, Kang SG, Lee JH, Lee SA, Kim HK.** 2007. Functional expression and refolding of new alkaline esterase, EM2L8 from deep-sea sediment metagenome. Protein Expr. Purif. **52:**340-347.
Arctic Sediment	2 Esterases	Opt. pH: 8.0 Opt Temp.: 20-40ºC Spec. act.: up to 59.8 µmolmin^{-1}mg^{-1} (pNPC4 and pNPC6) Relevant properties: the esterases could hydrolyze racemic ofloxacin esters, and hydrolyzed preferentially (S)-racemic ofloxacin butyl ester with an enantiomeric excess (eep) value of 70.3%.	**Jeon JH, Kim JT, Kang SG, Lee JH, Kim SJ.** 2009. Characterization and its potential application of two esterases derived from the arctic sediment metagenome. Mar. Biotechnol. **11:**307–316
Deep-sea sediment	1 Lipase	Opt. pH: 8.0 Opt Temp.: 25ºC (5-35ºC) Spec. act.: 203 µmolmin^{-1}mg^{-1} (trilaurin) Relevant properties: enzyme resistant to various detergents such as Triton X-100 and Tween 80. This study represents an example which developed a new cold-active lipase from a deep-sea sediment metagenome	**Jeon JH, Kim JT, Kim YJ, Kim HK, Lee HS, Kang SG, Kim SJ, Lee JH.** 2009. Cloning and characterization of a new cold-active lipase from a deep-sea sediment metagenome. Appl. Microbiol. Biotechnol. **81:**865–874
Deep-Sea Sediment	5 Esterases	Opt. pH: 8.0 Opt Temp.: 15-50ºC (depending on the enzyme) Spec. act.: up to 558.2 µmolmin^{-1}mg^{-1} (pNPC6) Relevant properties: high salt resistance with up to 4M NaCl	**Jeon JH, Kim JT, Lee HS, Kim SJ, Kang SG, Choi SH, Lee JH.** 2011. Novvel lipolytic enzymes identified from metagenomic library of deep-sea sediment. Evid. Based Complement. Alternat. Med. **2011:**271419.
Neritic sediments of the South China Sea	1 Esterase	Opt. pH: 8.5 Opt Temp.: 40ºC	**Peng Q, Zhang X, Shang M, Wang X, Wang G, Li B, Guan G, Li Y, Wang Y.** 2011. A novel esterase

		K_m: 0.858 mM (pNPC$_4$) V_{max}: 2260 µmolmin^{-1}mg^{-1} (pNPC$_4$) k_{cat}: 9850 s^{-1} (pNPC$_4$) Relevant properties: compared to other metagenomic esterases, its played a notable role in specificity for substrate pNPC$_4$ (k_{cat}/K_m value 11,500 s^{-1}mM^{-1}) and showed no inhibited by phenylmethylsulfonyl fluoride, suggested that the substrate binding pocket was suitable for substrate C$_4$ and the serine active-site residue was buried at the bottom of substrate binding pocket which sheltered by a lid structure	gene cloned from a metagenomic library from neritic sediments of the South China Sea. Microb. Cell Fact. **10:**95.
Deep-sea sediment	9 Lipases	Opt. pH: 7.5 Opt Temp.: 20ºC K_m: 0.89 mM (pNPC$_4$) V_{max}: 66.7 µmolmin^{-1}mg^{-1} (pNPC$_4$) Spec. act.: 104.41 µmolmin^{-1}mg^{-1} (pNPC4) Relevant properties: -	Jiang X, Xu X, Huo Y, Wu Y, Zhu X, Zhang X, Wu M. 2012. Identification and characterization of novel esterases from a deep-sea sediment metagenome. Arch. Microbiol. **194:**207–214
South China sea marine sediment	2 Lipases	Opt. pH: 8.0 Opt Temp.: 40-50ºC Spec. act.: up to 345.9 µmolmin^{-1}mg^{-1} (U/mg) (pNPC$_6$) Relevant properties: could not be assigned to any known family, thus probably representing a novel family of the bacterial lipolytic enzyme. 20% concentration of DMSO increased the activity to about 127%	**Hu Y, Fu C, Huang Y, Yin Y, Cheng G, Lei F, Lu N, Li J, Ashforth EJ, Zhang L, Zhu B.** 2010. Novel lipolytic genes from the microbial metagenomic library of the South China Sea marine sediment. FEMS Microbiol. Ecol. **72:**228–237
Tidal flat sediment	3 Esterase	Opt. pH: 9.0 Opt Temp.: 35ºC Spec. act.: up to 5370 µmolmin^{-1}ml^{-1} (U/mg) (pNPC$_6$) Relevant properties: the enzymes displayed salt tolerance with over 50% of the maximum activity remained in the presence of 3 M NaCl (or KCl)	**Jeon, J.H., Lee, H.S., Kim, J.T., Kim, S.J., Choi, S.H., Kang, S.G. & Lee, J.H.,** 2012: Identification of a new subfamily of salt-tolerant esterases from a metagenomic library of tidal flat sediment. Appl. Microbiol. Biotechnol **93:**623-631
Tial flat sediments on the Korean west coast	1 Phospholipase	Relevant properties: is the first experimentally characterized phospholipase A(1) with lipase activity obtained from a metagenomic library; the study provides insight into the evolution of lipases and phospholipases.	**Lee MH, Oh KH, Kang CH, Kim JH, Oh TK, Ryu CM, Yoon JH.** 2012. Novel metagenome-derived, cold-adapted alkaline phospholipase with superior lipase activity as an intermediate between phospholipase and lipase. Appl.Environ.Microbiol. **78:**4959-49.
Intertidal flat sediments	1 Lipase	Opt. pH: 8.0-9.0 Opt Temp.: 30ºC (retained 47% of the activity at 5°C) Relevant properties: pNPC12 is the best substrate	Kim EY, Oh KH, Lee MH, Kang CH, Oh TK, Yoon JH. 2009. **Novel cold-adapted alkaline lipase from an intertidal flat metagenome and proposal for a new family of bacterial lipases. Appl.Environ.Microbiol.** 75:**257-220.**
Freshwater samples			
Drinking water	2 Esterases	Opt. pH: 9.0-10.0 Opt Temp.: 47ºC Spec. act.: up to 513.6 µmolmin^{-1}mg^{-1} (pNPC$_4$) Relevant properties: Highly enantioselective for (-)-menthyl acetate; able to hydrolyze (+/–)-1-octin-3-ol, R-(+)-3-chlor-1-phenyl-1-propanol, trimethylsilylbutinol, **cis/trans**-1,2-cyclohexanediol and isopropylidenglycerol acetate	**Elend C, Schmeisser C, Leggewie C, Babiak P, Carballeira JD, Steele HL, Reymond JL, Jaeger KE, Streit WR.** 2006. Isolation and biochemical characterization of two novel metagenome-derived esterases. Appl. Environ. Microbiol. **72:**3637-3645.
Uncultured bacteria of pond	12 Lipases	Opt. pH: -	**Ranjan R, Grover A, Kapardar RK, Sharma R.**

water		Opt Temp.: - Relevant properties: one of the proteins has high similarity to yet uncharacterized α/β hydrolase protein family.	2005. Isolation of novel lipolytic genes from uncultured bacteria of pond water. Biochem. Biophys. Res. Commun. **335:**57-65
Metagenomic library of Yangtze River in China	2 Esterases	Opt. pH: 9.0 Opt Temp.: 50ºC K_m: 0.018 mM (*p*NPC_8) Relevant properties: Mg^{2+} was required for maximal activity; activity remained in the presence of 10% alcohol, acetone, isopropanol, and dimethyl sulfoxide	**Wu C, Sun B.** 2009. Identification of novel esterase from metagenomic library of Yangtze river. J. Microbiol. Biotechnol. **19:**187–193
Thailand hot spring	1 Patatin-like phospholipase and 1 esterase	Opt. pH: 7.0-10.0 Opt Temp.: 50-75ºC K_m: 0.14 mM (*p*NPC_4) k_{cat}: 574.17 s^{-1} (*p*NPC_4) V_{max}: 1065 µmol$min^{-1}mg^{-1}$ (*p*NPC_4) k_{cat}/K_m: 4101.2 $s^{-1}mM^{-1}$ (pNPC4) Relevant properties: -	**Tirawongsaroj P, Sriprang R, Harnpicharnchai P, Thongaram T, Champreda V, Tanapongpipat S, Pootanakit K, Eurwilaichitr L.** 2008. Novel thermophilic and thermostable lipolytic enzymes from a Thailand hot spring metagenomic library. J. Biotechnol. **133:**42-49.
Thermal environmental samples	1 Esterase	Opt. pH: - Opt Temp.: 30-90ºC Relevant properties: hyperthermostable enzyme	**Byun JS, Rhee JK, Kim ND, Yoon J, Kim DU, Koh E, Oh JW, Cho HS.** 2007. Crystal structure of hyperthermophilic esterase EstE1 and the relationship between its dimerization and thermostability properties. BMC Struct. Biol. **7:**47.
Yangtze River	1 Esterase	Opt. pH: 9.0 Opt Temp.: 50ºC Spec. act.: up to circa 400 µmol$min^{-1}mg^{-1}$ (pNPC_8) Relevant properties: Activity remained in the presence of 10% alcohol, acetone, isopropanol, and dimethyl sulfoxide	Wu C, Sun B. **2009. Identification of novel esterase from metagenomic library of Yangtze river. J. Microbiol. Biotechnol.**19:**187-193**
Marine water samples			
Intertidal Flat	1 Lipase	Opt. pH: 7.0-9.0 Opt Temp.: 30ºC (denatured above 55ºC) Relevant properties: No amino acid similarity to any known lipolytic enzyme except in the consensus region; it's a cold-adapted alkaline lipase; best substrate pNPC_{12}.	**Kim EY, Oh KH, Lee MH, Kang CH, Oh TK, Yoon JH.** 2009. Novel cold-adapted alkaline lipase from an intertidal flat metagenome and proposal for a new family of bacterial lipases. Appl. Environ. Microbiol. **75:**257-260.
Arctic intertidal sample	1 Esterase	Opt. pH: 7.5 Opt Temp.: 35ºC K_m: 0.039 mM (*p*NPC_6) k_{cat}: 25.8 s^{-1} (*p*NPC_6) Relevant properties: The cold adaptation features seem primarily related to a high number of methionine and glycine residues and flexible loops in the high-resolution structures	**Fu J, Leiros HK, de Pascale D, Johnson KA, Blencke HM, Landfald B.** 2012. Functional and structural studies of a novel cold-adapted esterase from an Arctic intertidal metagenomic library. Appl. Microbiol. Biotechnol. DOI 10.1007/s00253-012-4276-9
Urania deep-sea hypersaline anoxic basin	5 Esterases	Opt. pH: 8.0-9.0 Opt Temp.: 40-60ºC Spec. act.: up to 4500 µmol$min^{-1}mg^{-1}$ (pNPC2) k_{cat}/K_m: up to 3500–3900 $s^{-1}mM^{-1}$ Effect of NaCl: up top 180-fold activation by NaCl (2-4 M) Relevant properties: two had no significant sequence homology to known esterases, hydrolyzed both carboxylesters and thioesters, and exhibited unusual, habitat-specific characteristics (preference for high hydrostatic pressure and salinity). One has an unusual structural signature	**Ferrer M, Golyshina OV, Chernikova TN, Khachane AN, Martins Dos Santos VA, Yakimov MM, Timmis KN, Golyshin PN.** 2005. Microbial enzymes mined from the Urania deep-sea hypersaline anoxic basin. Chem. Biol. **12:**895-904.

		incorporating three catalytic active centers mediating distinct hydrolytic activities and an adaptive structures depending physico-chemical conditions. Some of the esterases have high activities, specificities, enantioselectivities, and exceptional stability in polar solvents, and they are therefore potentially useful for industrial biotransformations. One possesses the highest enantioselectivity toward an ester of the important chiral synthon solketal (*E*: 126[S]; 98%*ee*); the k_{cat}/K_m ratios of three of the five esterases greatly exceed published values for the hydrolysis of *p*NP-esters, paraben analogs, and α amino acid esters (600–3900 versus 400 $s^{-1}mM^{-1}$).	
Korean tidal flat metagenomic library	1 Lipase	Opt. pH: - Opt Temp.: - K_m: 0.8 mM ($pNPC_{16}$) k_{cat}: 1210 s^{-1} ($pNPC_{16}$) k_{cat}/K_m: 1090 $s^{-1}mM^{-1}$ (pNPC16) Spec. act.: 458.8 $\mu molmin^{-1}mg^{-1}$ (pNPC16) Relevant properties: -	**Lee MH, Lee CH, Oh TK, Song JK, Yoon JH.** 2006. Isolation and characterization of a novel lipase from a metagenomic library of tidal flat sediments: evidence for a new family of bacterial lipases. Appl. Environ. Microbiol. **72:**7406-7409.
Turban Basin metagenomic library	1 Pyrethroid-hydrolyzing enzyme	Opt. pH: 6.5 Opt Temp.: 55ºC Spec. act.: 772.9 $\mu molmin^{-1}mg^{-1}$ (pNPC2) Relevant properties: the enzyme efficiently degraded cyhalothrin, cypermethrin, sumicidin, and deltamethrin under assay conditions of 37°C for 15 min, with exceeding 95% hydrolysis rate	**Fan X, Liu X, Huang R, Liu Y.** 2012. Identification and characterization of a novel thermostable pyrethroid-hydrolyzing enzyme isolated through metagenomic approach. Microb. Cell Fact. **11:**33.
Marine metagenomic library derived from South China Sea	2 Esterases	Opt. pH: 6.5-7.5 Opt Temp.: 45-50ºC Spec. act.: up to 981 $\mu molmin^{-1}mg^{-1}$ (pNPC4) Relevant properties: habitat-specific characteristics such as its high level of stability in the presence of various divalent cations and at high concentrations of NaCl and highly stable in 30% methanol, ethanol, dimethylformamide, and dimethyl sulfoxide.	**Chu X, He H, Guo C, Sun B.** 2008. Identification of two novel esterases from a marine metagenomic library derived from South China Sea. Appl. Microbiol. Biotechnol. **80:**615–625
Landfill leachate			
Leachate metagenome library	1 Esterase	Opt. pH: - Opt Temp.: 30-40ºC K_m: 0.0342 mM ($pNPC_8$) k_{cat}: 278.9 s^{-1} (pNPC8) k_{cat}/K_m: 8100 $s^{-1}mM^{-1}$ (pNPC8) Relevant properties: exhibits promiscuous *β*-lactamase activity on nitrocefin; the organic solvent stability and the specificity towards esters of tertiary alcohols linalyl acetate (3,7-dimethyl-1,6-octadien-3-yl acetate) make EstC potentially useful in biocatalysis	**Rashamuse K, Magomani V, Ronneburg T, Brady D.** 2009. A novel family VIII carboxylesterase derived from a leachate metagenome library exhibits promiscuous beta-lactamase activity on nitrocefin. Appl. Microbiol. Biotechnol. **83:**491–500
Leachate metagenome library	1 Feruloyl esterase	Opt. pH: 6.5-7.0 Opt Temp.: 35ºC; thermolabile with a half life < 30 min at 50ºC K_m: 0.1 mM ($pNPC_5$) k_{cat}: 46710 s^{-1} (pNPC5) k_{cat}/K_m: 592580 $s^{-1}mM^{-1}$ (pNPC5) K_m: 0.7 mM (methyl sinapinate) k_{cat}: 12159 s^{-1} (methyl sinapinate) k_{cat}/K_m: 18851 $s^{-1}mM^{-1}$ (methyl sinapinate) Relevant properties: high affinity for methyl sinapate, methyl ferulate and ethyl ferulate suggest that the enzyme can be useful in hydrolyzing ferulated poly-saccharides in a biorefinery process	**Rashamuse K, Sanyika W, Ronneburg T, Brady D.** 2012. A feruloyl esterase derived from a leachate metagenome library. BMB Rep. **45:**14-19

Other samples			
China Holstein Cow Rumen	1 Feruloyl Esterase	Opt. pH: 8.0 Opt Temp.: 40ºC K_m: 1.63 mM (methyl ferulate) k_{cat}: 66.46 s^{-1} (methyl ferulate) Spec. act.: 259.52 µmol$min^{-1}mg^{-1}$ (methyl ferulate) k_{cat}/K_m: 40.84 $s^{-1}mM^{-1}$ (methyl ferulate) Relevant properties: the enzyme is as a good candidate to enhance biomass degradation and improve the health effects of food and forage	**Cheng F, Sheng J, Cai T, Jin J, Liu W, Lin Y, Du Y, Zhang M, Shen L.** 2012. A protease-insensitive feruloyl esterase from China Holstein cow rumen metagenomic library: expression, characterization, and utilization in ferulic acid release from wheat straw. J. Agric. Food Chem. **60:**2546–2553
China Holstein cow rumen	2 Lipases	Opt. pH: 6.5-8.5 Opt Temp.: 0-70ºC with maximal at 30ºC Spec. act.: up to 428 µmol$min^{-1}mg^{-1}$ (pNPC8) Relevant properties: the high specificity of both enzymes for long-chain fatty acid make them interesting targets for manipulation of rumen lipid metabolism	**Liu K, Wang J, Bu D, Zhao S, McSweeney C, Yu P, Li D.** 2009. Isolation and biochemical characterization of two lipases from a metagenomic library of China Holstein cow rumen. Biochem. Biophys. Res. Commun. **385:**605-611.
Cow rumen	12 Esterases	Opt. pH: - Opt Temp.: - Spec. act.: up to 7600 µmol$min^{-1}mg^{-1}$ (pNPC2) Spec. act.: up to 9400 µmol$min^{-1}mg^{-1}$ (tri-O-acetyl-glucal) Relevant properties: eight were entirely new and formed deep branched phylogenetic lineages with no close relatives among known ester hydrolases	**Ferrer M, Golyshina OV, Chernikova TN, Khachane AN, Reyes-Duarte D, Santos VA, Strompl C, Elborough K, Jarvis G, Neef A, Yakimov MM, Timmis KN, Golyshin PN.** 2005. Novel hydrolase diversity retrieved from a metagenome library of bovine rumen microflora. Environ. Microbiol. **7:**1996-2010.
Noncultured Rumen Bacterium	1 Esterase	Opt. pH: 7.0 Opt Temp.: 40ºC Relevant properties: homology to hypothetical proteins and it had no homology to previous known lipases and esterases.	**Kim MK, Kang TH, Kim J, Kim H, Yun HD.** 2012. Cloning and identification of a new group esterase (Est5S) from noncultured rumen bacterium. J. Microbiol. Biotechnol. **22:**1044–1053
Cow rumen	1 CE6 carbohydrate esterase	Opt. pH: 7.0 Opt Temp.: 40ºC K_m: 0.48 mM (pNPC$_3$) k_{cat}: 1210 s^{-1} (pNPC$_3$) k_{cat}/K_m: 2520 $s^{-1}mM^{-1}$ (pNPC3) K_m: 0.91 mM (glucose pentaacetate) k_{cat}: 5470 s^{-1} (glucose pentaacetate) k_{cat}/K_m: 6010 $s^{-1}mM^{-1}$ (glucose pentaacetate) K_m: 0.85 mM (methyl p-coumarate) k_{cat}: 950 s^{-1} (methyl p-coumarate) k_{cat}/K_m: 1117 $s^{-1}mM^{-1}$ (methyl p-coumarate) Relevant properties: the catalytic Ser and His have been identified in highly conserved sequences GQSX and DXXH in the CE6 family, respectively, and the active-site glutamate was part of a highly conserved sequence HQGE. This motif is situated near to the so-called Block III in the CE6 family and its role in catalysis has not been identified so far	**López-Cortés N, Reyes-Duarte D, Beloqui A, Polaina J, Ghazi I, Golyshina OV, Ballesteros A, Golyshin PN, Ferrer M.** 2007. Catalytic role of conserved HQGE motif in the CE6 carbohydrate esterase family. FEBS Lett. **581:**4657–4662
Symbionts of termite (*Coptotermes formosanus*) gut	1 Feruloyl esterase	Opt. pH: 7.0 Opt Temp.: 37ºC Spec. act.: 24.6 µmol$min^{-1}mg^{-1}$ (ethyl ferulate) Relevant properties: -	**Chandrasekharaiah M, Thulasi A, Bagath M, Kumar DP, Santosh SS, Palanivel C, Jose VL, Sampath KT.** 2011. Molecular cloning, expression and characterization of a novel feruloyl esterase enzyme from the symbionts of termite (*Coptotermes*

			formosanus) gut. BMB Rep. **44:** 52-57
Fecal samples of *Rusa unicolor* and *Equus burchelli*	2 Feruloyl esterases	Opt. pH: 5.0-8.0 Opt Temp.: 37ºC Spec. act.: up to 16.6 $\mu molmin^{-1}mg^{-1}$ (methyl ferulate) Relevant properties: -	**Chandrasekharaiah M, Thulasi A, Vijayarani K, Kumar DP, Santosh SS, Palanivel C, Jose VL, Sampath KT.** 2012. Expression and biochemical characterization of two novel feruloyl esterases derived from fecal samples of *Rusa unicolor* and *Equus burchelli*. Gene **500:**134–139
Marine sponge Haliclona simulans	1 Lipase	Opt. pH: 3.0-12.0 Opt Temp.: 4-60ºC Km: .093 mM (pNPC16) *Vmax:* 50 $\mu molmin^{-1}$ ($pNPC_{16}$) Relevant properties: high levels of stability in the presence of various solvents at NaCl concentrations as high as 5 M	**Selvin J, Kennedy J, Lejon DP, Kiran S, Dobson AD**. 2012. Isolation identification and biochemical characterization of a novel halo-tolerant lipase from the metagenome of the marine sponge *Haliclona simulans*. Microb. Cell Fact. **11:**72.
Microbial community in the pitcher fluid of the carnivorous plant *Nepenthes hybrida*	2 Lipases	Opt. pH: acidic conditions (5.0-6.0) Opt Temp.: - Relevant properties: capable of enzymatic activity in the acidic pitcher fluid.	**Morohoshi T, Oikawa M, Sato S, Kikuchi N, Kato N, Ikeda T.** 2011. Isolation and characterization of novel lipases from a metagenomic library of the microbial community in the pitcher fluid of the carnivorous plant *Nepenthes hybrida*. J. Biosci. Bioeng. **112:**315-320.
Marine sponge-associated bacteria	1 Esterase	Opt. pH: alkaline pH Opt Temp.: 30-50ºC Spec. act.: 5.6 $\mu molmin^{-1}mg^{-1}$ (pNPC2) Relevant properties: maintained activity in high concentrations of NaCl (up to 4M), indicating salt-tolerance; however, it showed maximal activity in the absence of salt	**Okamura Y, Kimura T, Yokouchi H, Meneses-Osorio M, Katoh M, Matsunaga T, Takeyama H.** 2010. Isolation and characterization of a GDSL esterase from the metagenome of a marine sponge-associated bacteria. Mar. Biotechnol. **12:**395–402
Salted shrimp	1 Esterase	Opt. pH: - Opt Temp.: - Spec. act.: 1344 $\mu molmin^{-1}mg^{-1}$ (pNPC4) Relevant properties: Comparison of the sequences revealed a very low sequence similarity (<5-11%) to those of established esterase family enzymes	**Park SY, Shin HJ, Kim GJ.** 2011. Screening and identification of a novel esterase EstPE from a metagenomic DNA library. J. Microbiol. **49:**7-14.

Table S2 List of primers used in the study.

Name	Sequence
LAE1Fwd	5´-GACGACGACAAGATG AACAGGATTGCC-3´
LAE1Rev	5´-GAGGAGAAGCCCGGTCAGTGGTGATTCTTCG-3´
LAE2Fwd	5´-GACGACGACAAGATGAGCAATTCTCTTGAACTG-3´
LAE2Rev	5´-GAGGAGAAGCCCGGTTAGCGGCCGGCCGGCGC-3´
LAE3Fwd	5´-GACGACGACAAGATGGCTAGCGCCGAGGC-3´
LAE3Rev	5´-GAGGAGAAGCCCGGTCATGGGGCGCTGGGGC-3´
LAE4Fwd	5´-GACGACGACAAGATGCGACATTCCATATCTC-3´
LAE4Rev	5´-GAGGAGAAGCCCGGTTACTTGGTTTGTTGGG-3´
LAE5Fwd	5´-GACGACGACAAGATGTATTTCGCAGCTGGC-3´
LAE5Rev	5´-GAGGAGAAGCCCGGTTAGCTATTCAGGCAGC-3´
LAE6Fwd	5´-GACGACGACAAGATG CTGCTGCCCGAGACC-3´
LAE6Rev	5´-GAGGAGAAGCCCGGTTACGTCCCGAAGTGCGC-3´
LAE7Fwd	5´-GACGACGACAAGATGCATTTCTTCGATCACG-3´
LAE7Rev	5´-GAGGAGAAGCCCGGTTAAGGACGCGCGCCC-3´

Table S3 Percentage of identity between Arreo enzymes, determined by Matcher (EMBOSS package). Matches/alignment lengths (% identity) are specifically shown.

	LAE1	**LAE2**	**LAE3**	**LAE4**	**LAE5**	**LAE6**	**LAE7**
LAE1		9/31 (29.0%)	3/31 (41.9%)	2/39 (30.8%)	5/10 (50.0%)	2/27 (44.4%)	1/18 (61.1%)
LAE2	9/31 (29.0%)		3/51 (25.5%)	9/19 (47.4%)	6/43 (37.2%)	8/18 (44.4%)	4/292 (59.6%)
LAE3	3/31 (41.9%)	3/51 (25.5%)		8/22 (36.4%)	4/42 (33.3%)	3/207 (30.4%)	3/48 (27.1%)
LAE4	2/39 (30.8%)	9/19 (47.4%)	8/22 (36.4%)		0/38 (26.3%)	9/48 (39.6%)	5/7 (71.4%)
LAE5	5/10 (50.0%)	6/43 (37.2%)	4/42 (33.3%)	0/38 (26.3%)		4/22 (18.2%)	5/43 (34.9%)
LAE6	2/27 (44.4%)	8/18 (44.4%)	3/207 (30.4%)	9/48 (39.6%)	4/22 (18.2%)		1/33 (33.3%)
LAE7	1/18 (61.1%)	4/292 (59.6%)	3/48 (27.1%)	5/7 (71.4%)	5/43 (34.9%)	9/37 (24.3%)	

Table S4 Half-saturation (Michaelis) coefficient (K_m), the catalytic rate constant (k_{cat}) and the catalytic efficiency (k_{cat}/K_m) values for the wild-type α/β hydrolases from Lake Arreo. Kinetic parameters were calculated at 30ºC as outlined in Table 1 and Materials and Method section in 96-well microtiter plate where each well contained 0.388-0.882 μM enzyme solution and 0-100 mM substrate. For kinetic parameters determinations (performed in triplicate) a conventional Lineweaver and Burk model was used. Standard deviations (SD) are given.

	K_m (mM)	SD K_m (mM)	k_{cat} (min^{-1})	SD k_{cat} (min^{-1})	k_{cat}/K_m ($min^{-1}mM^{-1}$)
LAE1					
*p*NP acetate	0.66	0.05	2.7	0.3	4.1
*p*NP propioante	0.29	0.02	6.2	0.7	21.4
*p*NP butyrate	0.13	0.02	32.5	0.9	250
*p*NP octanoate	0.08	0.05	3.4	0.3	42.5
*p*NP decanoate	0.02	0.02	0.6	0	30.0
Phenyl acetate	0.63	0.01	3.6	0.4	5.7
Triacetin	16.9	0.4	2	1.5	0.1
Tripropionin	0.32	0.18	10.7	26.3	33.4
Tributyrin	0.29	0.01	6.2	0.6	21.4
Methyl-(R)-(-)mandelate	3.58	0.15	9.0	3.3	2.5
Methyl-(S)-(+)-mandelate	37.4	2.8	10.8	4.2	0.3
Methyl α-bromoisobutyrate	0.65	0.01	4.3	0.5	6.6
Methyl 2-bromopropionate	8.58	0.69	25.2	2	2.9
Methyl bromoacetate	3.33	0.44	14.8	0.7	4.4
α-D-Glucose pentaacetate	7	0.28	13.9	1.4	2.0
Tri-O-acetyl-D-glucal	0.21	0.03	29.5	0.7	140
LAE2					
*p*NP acetate	0.12	0.04	166	15.6	1383
*p*NP propioante	0.04	0.01	11.7	0.9	293
*p*NP butyrate	0.1	0.03	2.7	0.3	27.0
Phenyl acetate	0.17	0.04	89.3	0.9	525
Triacetin	0.06	0.02	4.3	0.1	71.7
Methyl 4-bromobenzoate	21.5	0.5	22.4	1.9	1.0
α-D-Glucose pentaacetate	0.42	0.09	3.5	0.3	8.3
Tri-O-acetyl-D-glucal	0.41	0.09	3.5	0.4	8.5
LAE3					
*p*NP acetate	0.55	0.03	346	9.1	629
*p*NP propioante	1.07	0.16	10.2	0.3	9.5
*p*NP butyrate	0.46	0.11	25.8	0.5	56.1
*p*NP octanoate	0.2	0.26	1.1	0.1	5.5
Triacetin	11.2	3.7	1265	39.6	113
Tripropionin	6.7	0.3	1631	39	

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Tributyrin	2.94	0.28	36.5	0.3	12.4
Methyl-(R)-(-)mandelate	0.82	0.03	1004	25.2	1224
Methyl-(S)-(+)-mandelate	21	1.1	32.5	5	1.5
Methyl 4-bromobenzoate	0.28	0.12	7.1	0.7	25.4
Methyl 2-bromopropionate	1.88	0.18	80.3	5.6	42.7
α-D-Glucose pentaacetate	0.57	0.02	52.4	0.9	91.9
Tri-O-acetyl-D-glucal	3.4	0.01	132	19.2	38.8
Methyl *p*-coumarate	0.6	0.02	10.3	3.1	17.2
LAE4					
*p*NP acetate	0.03	0.01	1.9	0.1	63.3
*p*NP propioante	0.004	0.002	4.2	0.3	1050
*p*NP butyrate	0.02	0.01	4	0.1	200
Phenyl acetate	2.59	0.83	215	12.5	83.0
Triacetin	0.79	0.07	1005	75	1272
Tripropionin	0.78	0.08	2333	41.5	2991
Methyl-(S)-(-)mandelate	3.82	0.37	2.2	0.2	0.6
Methyl-(R)-(+)-mandelate	11.4	0.4	3.6	0.2	0.3
Methyl 4-bromobenzoate	1.21	0.07	114	22.9	94.2
Methyl *p*-coumarate	17	0.5	986	64.1	58.0
LAE5					
*p*NP acetate	0.09	0.03	35.9	0.9	399
*p*NP propioante	0.03	0.01	45.7	5	1523
*p*NP butyrate	0.21	0.07	0.3	0	1.4
Phenyl acetate	0.25	0.11	896	126	3584
Propyl acetate	11.4	3.3	32.3	0.8	2.8
Geranyl acetate	20.6	9	2.9	0.4	0.1
Triacetin	0.08	0.03	88.8	5.9	1110
Methyl 4-bromobenzoate	2.51	0.2	74.9	0.2	29.8
Methyl bromoacetate	0.57	0.02	5.7	0.8	10.0
α-D-Glucose pentaacetate	0.53	0.03	1418	35.4	2676
Tri-O-acetyl-D-glucal	0.38	0.03	1108	25.8	2916
Methyl sinapinate	1.65	0.06	3.3	4.7	2.0
LAE6					
*p*NP acetate	0.58	0.04	1229	154.4	2119
*p*NP propioante	0.26	0.01	4544	605.2	17500
*p*NP butyrate	0.83	0.03	2999	165.2	3613
*p*NP octanoate	0.33	0.03	122	6.8	370
*p*NP decanoate	0.09	0.01	34.8	1.4	387
*p*NP laurate	0.19	0.97	6.7	0.2	35.3
Phenyl acetate	0.08	0.02	266	9.5	3325
Propyl acetate	3.21	0.47	2.8	0.3	0.9
Ethyl benzoate	0.5	0.03	0.7	0.2	1.4
Ethyl butyrate	8.84	0.22	8.7	20.3	1.0
Triacetin	0.25	0.05	60.1	3.8	240
Tripropionin	0.24	0.04	600	31.8	2500
Tributyrin	0.49	0.15	77.5	10.7	158
Methyl (+/-)-α-bromophenylacetate	0.35	0.01	21.5	2.6	61.4
Methyl α-bromoisobutyrate	2.39	0.16	18.1	0.9	7.6
Methyl 4-bromobenzoate	0.31	0.08	32.6	3.2	

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Methyl bromoacetate	0.2	0.02	558	13.9	2790
Tri-O-acetyl-D-glucal	0.36	0.09	33.7	3.4	93.6
Methyl cinnamate	0.21	0.03	57	5.4	271
Methyl *p*-coumarate	0.57	0.06	7.1	0.3	12.5
(R)-(−)-Glycidyl butyrate	0.04	0.01	64.8	1	1620
(S)-(+)-Glycidyl butyrate	0.52	0.03	58.0	1.2	112
γ-Butyrolacton	0.38	0.02	57.6	0.8	152
LAE7					
*p*NP acetate	0.05	0.02	29.1	3.3	582
*p*NP propioante	0.03	0.02	5.1	0.1	170
*p*NP butyrate	0.05	0.01	0.8	0	16.0
Phenyl acetate	0.41	0.02	3.8	1.4	9.3
Triacetin	0.28	0.04	34.9	1.7	125
Tributyrin	1.17	0.27	4	0.2	3.4
Methyl 4-bromobenzoate	0.1	0.06	0.8	0.1	8.0
Methyl α-bromoisobutyrate	1.62	0.21	33.1	1.9	20.4
α-D-Glucose pentaacetate	2.53	0.11	6.7	2.3	2.6
Tri-O-acetyl-D-glucal	1.53	0.13	44.8	2	29.3

FIG S1 Location map (a), vegetation and use of drainage basin (b) and bathymetric map (c) of Lake Arreo. Sampling point is indicated by an asterict. The map has been generated based on batimetric studies previously published (ref. 13 and 52 in main text). Location map in (a) was made with the Geographic data sets found on the ESRI Data&Maps Kit of the SIG ArcGis 9.2 software. The limit of the drainage basin in (b) was established based on 1:25.000 maps of the digital cartographic base from the National Geographic Institute (*Instituto Geográfico Nacional de España*). The information was analyzed using the ArcGis 9.2 software. The correct establishment of the limit was further validated based on in situ measurements using topographic-geographic instrumentations (the digital free access information is available at the following URL "http://centrodedescargas.cnig.es/CentroDescargas", as established in the *Orden FOM/956/2008*, dated on 31th March 2008 [BOE 8th April 2008]). The vegetation map was generated based on the photo-interpretation of the orthophotos "PNOA © *Instituto Geográfico Nacional de España – Basque Country* (*Gobierno Vasco)*". The correct assignation of the vegetation units was established based on in situ visit to the studied area. To perform the bathymetric map (c) of Lake Arreo, an aerial photo obtained by *Diputación Foral de Álava* (1992) was used. The field study was performed in 1994, using a boat with electric engine and an ecosonda Furuno FE-4300 or a surveying rod. The obtained values were referenced and digitally treated using specific software (ArcGis v9.2 and Surfer v7.00). Further modifications and validations were done based on up-dated ortho-images.

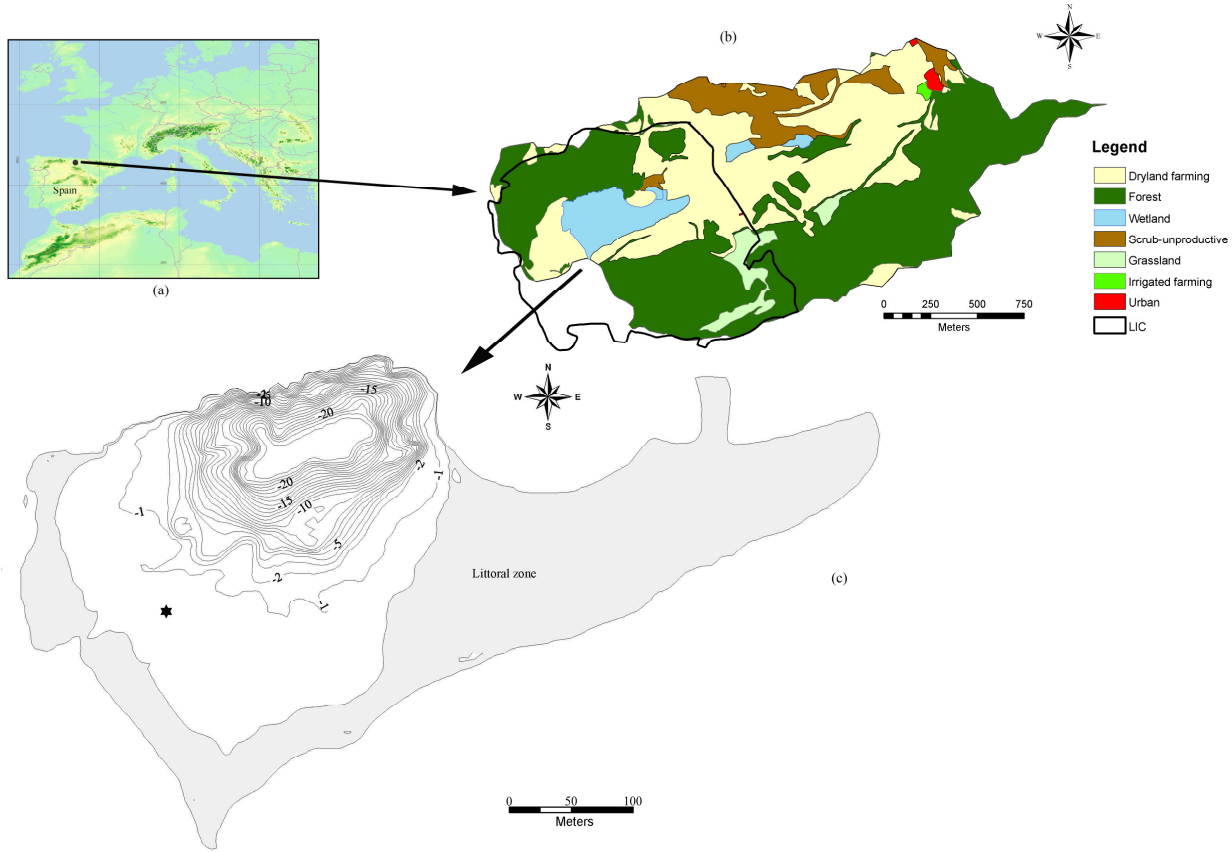


FIG S2 Lake Arreo proteins, as overexpressed in the active form in *E. coli* at 16ºC. A Coomassie-stained SDS-PAGE gel showing the expression level of the Lake Arreo proteins is shown. As shown, a high percentage of protein is produced in a soluble form, which resulted in a purity higher than 98% after a single His_6-tag purification step. Abbreviation: MW, molecular weight marker.

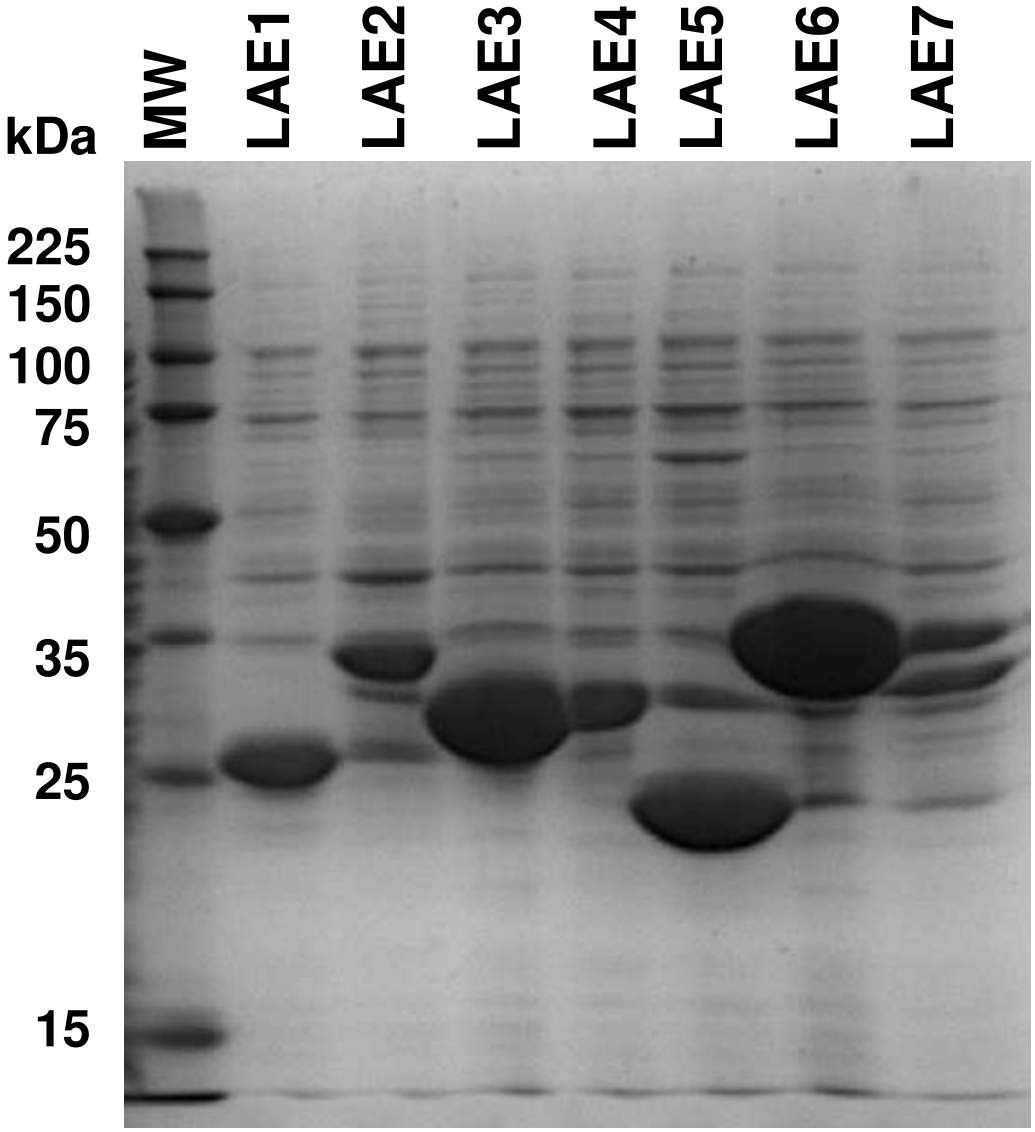


FIG S3 Chemical structures of substrates applied for activity profiling of esterases/lipases preparations used in the present study.Note: ethyl 2-methylacetoacetate, ethyl 4-chloroacetoacetate, ethyl (R)-(−)-3-hydroxybutyrate, ethyl propionate, ethyl acetate, ethyl hexanoate, ethyl 2-chloropropionate, ethyl caprate, ethyl 4-hydroxy-3-methoxycinnamate, methyl propionate, methyl 2,2-dimethyl-3-hydroxypropionate, methyl (S)-(+)-3-hydroxybutyrate, methyl (R)-(+)-3-bromo-2-methylpropionate, (−)-methyl L-lactate, (+)-methyl D-lactate, methyl 4-(hydroxymethyl)benzoote, methyl pyruvate, methyl acetoacetate, methyl 2-hydroxyisobutyrate, methyl (S)-(+)-3-hydroxy-2-methylpropionate, tert-butyl 3-hydroxypropionate, isobutyl acetate, vinyl pivalate, vinyl methacrylate, vinyl crotonoate, vinyl acetate, vinyl propionate, vinyl laurate, vinyl butyrate, glycine ethyl ester, (1S)-(+)-menthyl acetate, (1R)-(−)-menthyl acetate, (1S)-(+)-neomenthyl acetate, (1R)-(−)-neomenthyl acetate, were not used as substrates by any of the enzymes.

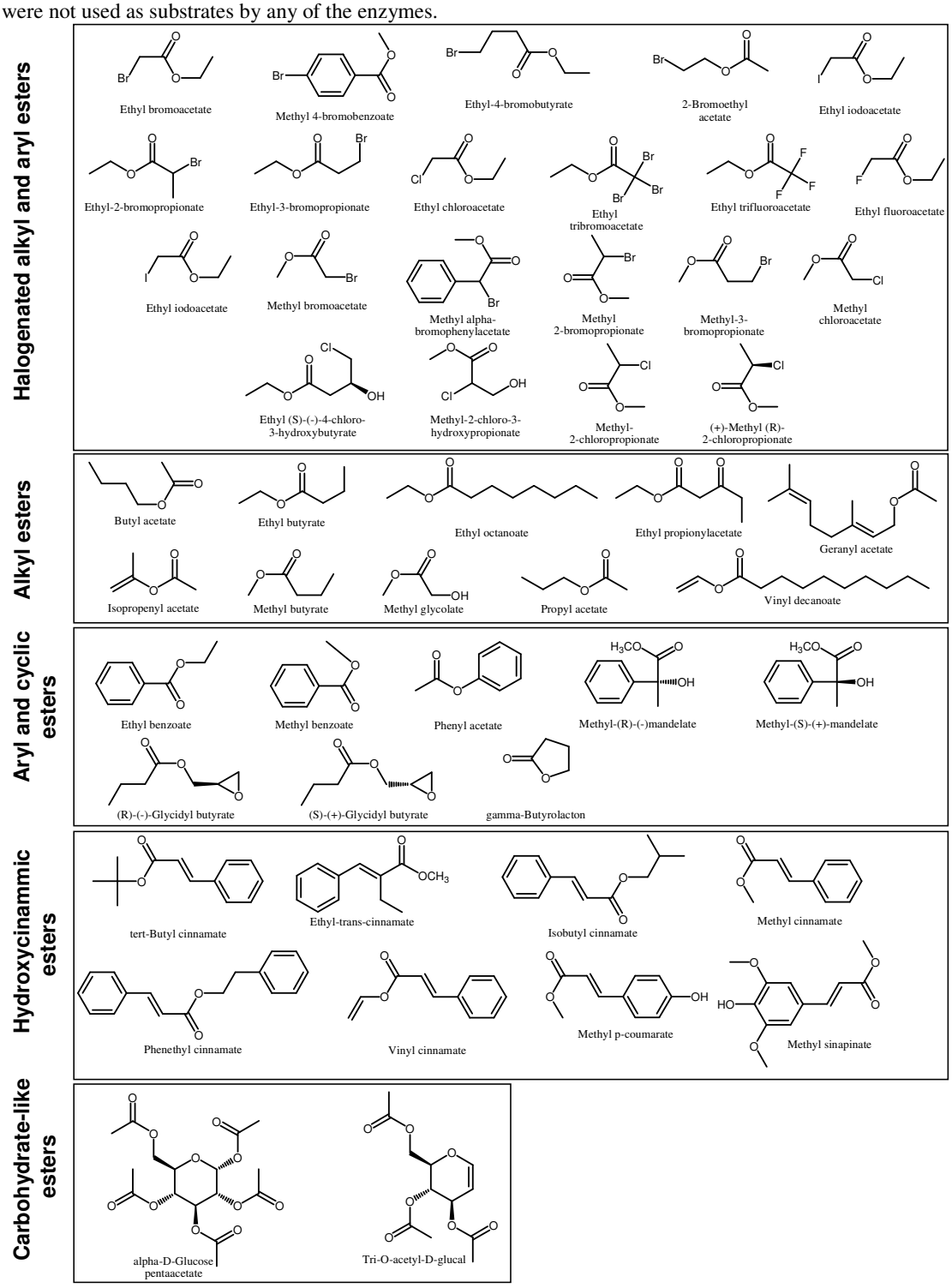
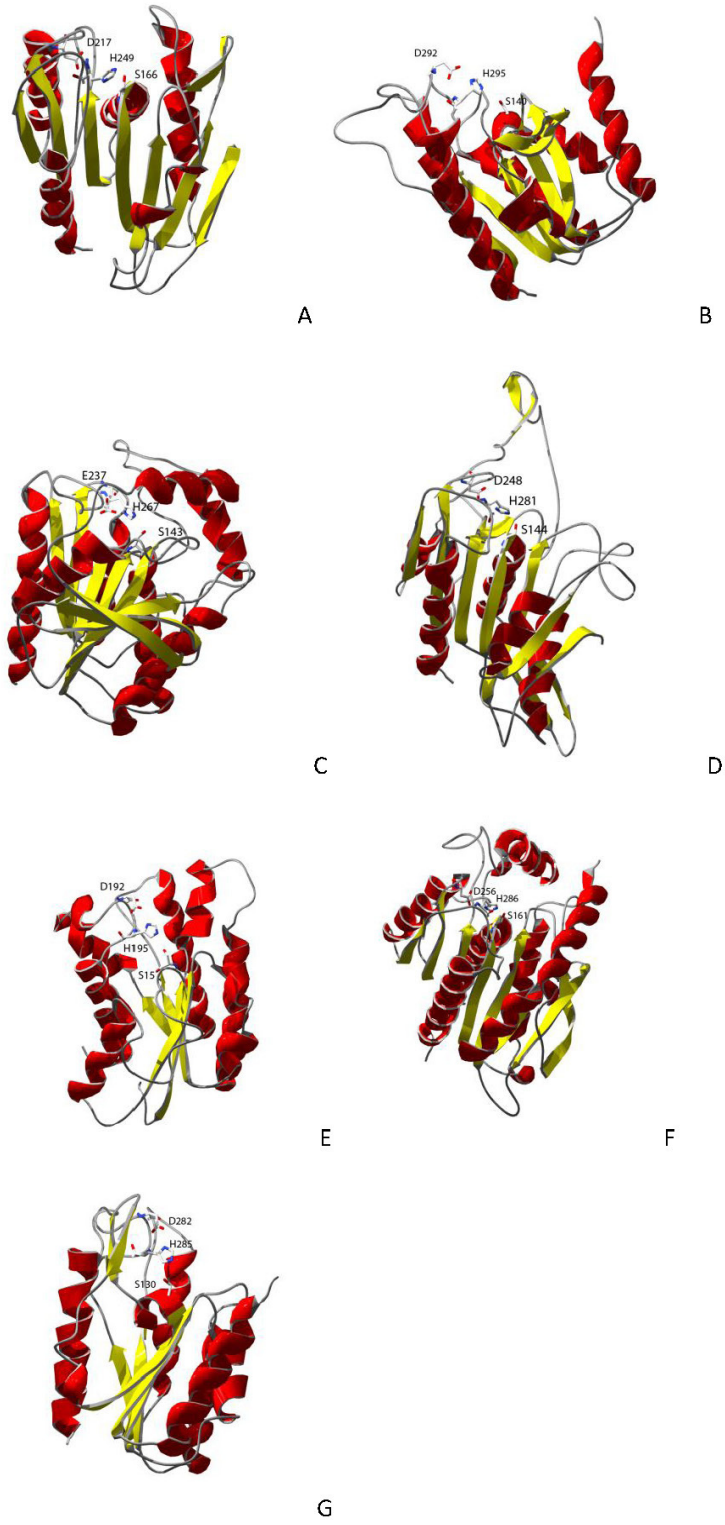


FIG S4 Structural models of esterases/lipases from the α/β hydrolase family characterised in this work. Residues belonging to the catalytic core are explicitly shown as follows: LAE1 (A), LAE2 (B), LAE3 (C), LAE4 (D), LAE5 (E), LAE6 (F), and LAE7 (G).



ANNEX:

Fosmid DNA isolated from each clone was sequenced as a pool using a Roche GS FLX DNA sequencer (1/16 plate), which produced 77,841 reads with an average length per read of 454.69 bp. Accordingly, a total of 38.5 Mbp of raw DNA sequences were obtained, which were assembled into 735,686 bp (19 contigs with length ranging from 1,854 to 43,416 bp. **Sequences are available at NCBI under accession number SRA059294.** A total number of 378 open reading frames were identified. Their amino acid and nucleotide sequences are described below. Seven genes encoding experimentally characterized esterases/lipases in this study, herein named LAE1 to LAE7, were identified and their sequences are given below:

LAE1 gene sequence

GTGAACAGGATTGCCGGGCTGTGCGTGCTGGCAGCCACGATGCAAGGCATCGGCTCCTCG
CCGGCGCATGCACAGCAAGCCGCACCCGGCGAGCAAGCCGCCAGCTTCGGGGCGCAGTTC
GGGCCGGTCGACCCGCGCGTCGAGATGCGGACCTACGTGTTTGCAGACACCGGTGAAAGG
ATTCCCTACGGCGTGTTCGTCTCTTCCAGCGTCACCATGGAGCGCAAGGCACCGCTGGTC
GTGGCGCTGCACGGGCTGGGCGCATCACAGGAATCCATGGTCCGCACCAGCTTCCGCGCT
GTCGAGCTGGCGGAAAAAGGCGGGTACATCCTGGTGGTACCGCTGGGGTACAACAGCAGC
GGCTGGTACGGCGTCCAGGCTGGCGGCAGCGTGCAGCAGCAGCGCGAGTACAGCGAAAAG
GACGTCATGAACGTGCTGGCGATGGTGCGCGATGAGTTCAACATAGATGACGATCGCATC
TTCCTGATGGGTCATTCGATGGGCGGTGCGGGCACATTGCACCTGGGCATGAAATACCCC
TCCATCTGGGCCGCCCTGGCGCCCATCGCGCCCGCCACTGCAGCTCTGGATCCGGACTCG
CTGGCCTCGATCCGCCACATGCCGGTGATCATCGTGCAGGGCGACGCGGACACGGCCGTT
CCGGTTGCCGTCACGCGGCGCTGGGCTTCCAAACTCCGGGAACTGCAGATGACCTTCCGC
TACATCGAAATTCCGGGCGGCGATCACATGGGCGTGATCGGCACGGGCATGCCGGACATC
TTCGCGTTCTTCGAGGCACACCCGAAGAATCACCACTGA

LAE1 protein sequence

VNRIAGLCVLAATMQGIGSSPAHAQQAAPGEQAASFGAQFGPVDPRVEMRTYVFADTGER
IPYGVFVSSSVTMERKAPLVVALHGLGASQESMVRTSFRAVELAEKGGYILVVPLGYNSS
GWYGVQAGGSVQQQREYSEKDVMNVLAMVRDEFNIDDDRIFLMGHSMGGAGTLHLGMKYP
SIWAALAPIAPATAALDPDSLASIRHMPVIIVQGDADTAVPVAVTRRWASKLRELQMTFR
YIEIPGGDHMGVIGTGMPDIFAFFEAHPKNHH

LAE2 gene sequence

ATGAGCAATTCTCTTGAACTGCGCAGCAAGCGCATGCCTGCCGCCCGCAACCTGCTGACC
GCCCTGCTGCTGGCCACAACTTGCGGCGCGCTCGCGCAGGTAGGCCCTGGCGGCCCGGGT
GGTCCGCGTGGCGGTGGTTTTGGCGCTCCGCCCACTCCGCCGGGACCGGCTGCCGAGGTA
CCCGCCGCGGTGCGCATGGCACGCCCCACCGCTGCCGAAGTGGCCTCGGTCCGCGCCAGC
GTCGACAAGCTCGACCTGGCGAAGAAGTATCCGGGCCTGGTGCAGATCAATGTGCCTGCG
CCCATGAGCGAGAACACGGCCATCCGGCCGTCGCTGTCCGGCGGGTTCCAGCAGAAGCAC
CAGGACAACCTGGCAGTGGCGAAGCAGGGCGACGCCGAACTGCTGTTCATGGGTGATTCC
ATCACCGACTGGTGGCGCAGCGAGACCGGCACCAACGCCGGCAAGGCCGTGCAGGACAAG
TACTTCGGCCAGTGGAAGGTGGCGAACTTCGGTATCGCCGGCGACACCACGCAGGGCGTG
CTCTACCGCCTGCAGAACGGCGAAGGCAAGGGCTTCAAGCCCAAGGCCGTCATGCTGATG
ATCGGCACCAACAATACGGGCCGGAATTCCGCCGGCGAGATCGCCGAGGGTGTCGGTGCC
GTGGTCCTGCAGCTGCAGAAGGATTTCCCCGACGCGCGTATCCTGCTGCTGGCCATCTTC

CCGCGTGGCAATCCGGGCGACGCGGCGCGCGGCACCATTGCCCAGATCAACGACACCATC
AAGAAGCTGGATGACGGCAAGAAGGTGTTCTACATGGACATCGGCAGGAACTTCCTCGAC
GCCAGCGGCGCGATACCGCGCGAGATCATGGGCGATGGACTGCATCCCACCACGGCGGGC
TACGAGATCTGGGCCAAGGCGGTCATCGATCCCATCACGGCGATGATGAACGGCCGCGCG
CCGGCCGGCCGCTAA

LAE2 protein sequence

MSNSLELRSKRMPAARNLLTALLLATTCGALAQVGPGGPGGPRGGGFGAPPTPPGPAAEV
PAAVRMARPTAAEVASVRASVDKLDLAKKYPGLVQINVPAPMSENTAIRPSLSGGFQQKH
QDNLAVAKQGDAELLFMGDSITDWWRSETGTNAGKAVQDKYFGQWKVANFGIAGDTTQGV
LYRLQNGEGKGFKPKAVMLMIGTNNTGRNSAGEIAEGVGAVVLQLQKDFPDARILLLAIF
PRGNPGDAARGTIAQINDTIKKLDDGKKVFYMDIGRNFLDASGAIPREIMGDGLHPTTAG
YEIWAKAVIDPITAMMNGRAPAGR

LAE3 gene sequence

ATGGCTAGCGCCGAGGCTTTGAAGATGATGGCGGAGCTGCGGGCGAACCGAAAGCCGGAT
CGGCCGCTTGCACAGGCTCGCGCCGAATGGGACATCGAAGCGGCTGCCCAGGCGCTGCCG
GCGGAGACCGTGGTCGAACCCGGCCAGATGGGCGGGGTCGCCTCGGAGTGGATCGTGTGC
GGTCCGGTGATCGAGCGTGGCGTCGTGCTGTTGCTGCATGGTGGCGGTTACCACGCCGGC
GGGCTGGTGACCCATCGCGCCTTCGCAGCCCGCGTCTCGCGCGCGCTCGGGCGGCGGGTA
TTGCAGATTGCCTATGCTCTTGCCCCGGAGCATCCCTTTCCGGCCGGGCTGCATGATGCT
CTGGCGGCCTACAAGGCGCTGATCGCCGAAGGCGTCGTCCCCGGCGAAATCGTCCTGCTC
GGCGACAGCGCCGGGGGCGGACTGGCCATTTCGTTGATGCTGGCCCTCAAACGCGACGGC
GACCCGCAGCCGGCCGGTGCGGTGCTCCTGTCGCCGTGGACCGATCTCGAATGCTCGGGC
ACCAGCTATCGGACGAACCTTTCGCGCGATCCCAACATGTCGCGCGAAGATCTGCTGGCG
GCAGCGGACGACTATCGCGGCAGCCTGCCGGCAAGCGAGCCGATGCTGTCCCCCATCCAT
GCCGACCTGTCCGGCCTGCCGCCCATGCTCGTCCAGGCGGGTGGCGGCGAGATCATGCTC
GACGATTCGATCTTTTTTGCCGAGCAGGCTCGCACCACCGGCTGCCGTGTGACGCTCGAC
GTGACGCCCGATCTCTGGCACGTCTTCCAGAACGGACCCGACGACATTCCCGAGGTGAGG
GCGGCCTTCGACCGCATGGGGACCTTCGTTGCCGGGGTGATGGCCCGCCCCAGCGCCCCA
TGA

LAE3 protein sequence

MASAEALKMMAELRANRKPDRPLAQARAEWDIEAAAQALPAETVVEPGQMGGVASEWIVC
GPVIERGVVLLLHGGGYHAGGLVTHRAFAARVSRALGRRVLQIAYALAPEHPFPAGLHDA
LAAYKALIAEGVVPGEIVLLGDSAGGGLAISLMLALKRDGDPQPAGAVLLSPWTDLECSG
TSYRTNLSRDPNMSREDLLAAADDYRGSLPASEPMLSPIHADLSGLPPMLVQAGGGEIML
DDSIFFAEQARTTGCRVTLDVTPDLWHVFQNGPDDIPEVRAAFDRMGTFVAGVMARPSAP

LAE4 gene sequence

ATGCGACATTCCATATCTCTCGTATTCCTGGTTGCCCTGGCCGCGCTGCCGTGGGCTTGT
CTTGCCGCCGAGGATGACAAAGCGCCGGACGTCCGCCGCACGGAAGACGTGATCTACGGC
CGAAAATCGGGCATGGCTTTGACGATGGACGTCTTCCAGCCCGCCAAACCGAACAAGTGC
GGCGTCGTCTTCCTCGTCAATGGCGGCTGGTTTTCCAGCAAGGCGACGCCGATGATGGAG
ACCGTTCAACCGGATCGCTACCAGCCGTTTCTCGATCGCGGCTACACGGTCTTTGCCGTG
GTCACCAGTTCCCAGCCTGTATTCACGATTCCCGACCTCATGCAAGACGTGCAGCGCGCC
GTCCGTTTCATACGGCACAATGCCGCGAGGTTCGGAGTTGATCCGAATCGACTCGGAGTG
ACCGGCGCAAGTTCCGGCGGCCAACTTTCCTTGACGATCGCAACGCAAGGCGGCCCAGGC
AAAGCCGACTCGCCCGATCCGGTCGAGCGTGAAAGCAGCGCCGTCCAGGCCGTCGCCTGC
TTCTTCCCGCCGACCGATTTTCTGAACTATGGCGGCCCCGGCGTCGATGGCGTGGGCCGC
GGCCCGTTGGAGCCCATCAAGGCGGCATTCGGTTCCCGCGCCCAGACGCTCGAAGGGCGC
CATGCCCTCGGCAAGGAAATATCGCCGATCTATTACGTCTCCGCGAAGCTGCCGCCGACG
CTGATCGTTCACGGCGATGCCGACAACGTCGTGCCTTTCCAGCAAGCCGAGAGTTTTATG
AAACGGGCCAAAGAGGCCGGCGCAAAGGACCTTCAACTCATCGTGCGTCCCGGCAAGGGA
CACGGCTGGGGCGATTTCTGGAAATCCGCCGAAGACGTCAACGCATTCGCCGACTGGTTC
GACAAGTTCCTACCCCAACAAACCAAGTAG

LAE4 protein sequence

MRHSISLVFLVALAALPWACLAAEDDKAPDVRRTEDVIYGRKSGMALTMDVFQPAKPNKC
GVVFLVNGGWFSSKATPMMETVQPDRYQPFLDRGYTVFAVVTSSQPVFTIPDLMQDVQRA
VRFIRHNAARFGVDPNRLGVTGASSGGQLSLTIATQGGPGKADSPDPVERESSAVQAVAC

FFPPTDFLNYGGPGVDGVGRGPLEPIKAAFGSRAQTLEGRHALGKEISPIYYVSAKLPPT
LIVHGDADNVVPFQQAESFMKRAKEAGAKDLQLIVRPGKGHGWGDFWKSAEDVNAFADWF
DKFLPQQTK

LAE5 gene sequence

ATGTATTTCGCAGCTGGCAGCAAGTTGGTCATCATCGGCGACTCGATCACGGACGCGGGA
CGGGACAAGGGGATCGGCGGCGAGGGGCTCTTCAACGCCCATGGCAGCGGCTATGTGGCA
TTGCTCAACGCCCACCTCTTCGCCCGCTTCCCCGAACGGCGGCTGCGGCTCGTCAACCAG
GGCAACAGCGGCAACACCGTGCGCGATCTGGCCGCCCGCTGGCAAAACGATGTCTTCGGC
CTGAAGCCCGACTATGTGGCGATGATGATCGGCATCAACGACGTCTGGCGCCAGTTCGAC
CTGCCGCTGATGACCGACCGCCATGTCTGCCCGGAAGAGTACGAGAAGACCCTCGACGAG
TTGGTGGCGCGCACCGCCCCGACGGTCAAGGGGATGATTCTGCTGACCCCCTACTTCATC
GAGCCTAACCGCGAGGACGCCATGCGCGCCCGCATGGACGTCTACGGCGACCTGATGCGC
CGCGTCGCCGAGCGTCACGGCTGCCTGCTGGTCGATGTCCAGGGAGCCTTCGACCGCTAT
CTGCAACACTACCATCCGGCGCAGCTCGCCTGGGACCGCATCCACCCCAACCTCGCGGGC
CACCAGGTCATCGCCAACGCCTTCCTCGCGGCCACCGGCTGCCTGAATAGCTGA

LAE5 protein sequence

MYFAAGSKLVIIGDSITDAGRDKGIGGEGLFNAHGSGYVALLNAHLFARFPERRLRLVNQ
GNSGNTVRDLAARWQNDVFGLKPDYVAMMIGINDVWRQFDLPLMTDRHVCPEEYEKTLDE
LVARTAPTVKGMILLTPYFIEPNREDAMRARMDVYGDLMRRVAERHGCLLVDVQGAFDRY
LQHYHPAQLAWDRIHPNLAGHQVIANAFLAATGCLNS

LAE6 gene sequence

ATGCTGCTGCCCGAGACCCGCAACCTGCTCGACCTGATGGACGCCGCCACCAGGGGCGGC
CGGCCCCGGCTGGAGACCCTGCCGCATGCCGTGGGGCGCAAGGCAGTCGACAAAATGTCG
GAGGATGGCGAGGCCGATCCGCCGGAAGTGGCCGAGGTGGCGAACGGCGGCTTCGCGGGA
CCGGCGAGCGAGATCCGGTTCCGGCGCTACCGTCCGCTAGGTGAGGCGGCCGGGCTGCTG
CCGACGCTGATCTACTATCACGGCGGCGGCTTCGTGATCGGCAACATCGAGACGCATGAT
TCGACCTGCCGGAGGCTCGCCAACAAAAGCCGCTGCCAGGTGATCTCGATCGACTACCGC
CTGGCGCCGGAGCATCCGTTCCCGGCGCCGATCGACGACGGGATCGCGGCGTTCCGGCAT
ATCCGCGACAACGCGGAGTCGTTCGGCGCCGATGCCGCGCGCCTCGCGGTGGGCGGCGAT
TCGGCGGGCGGCGCGATGGCGGCCGTGGTGTGCCAGGCCTGCCGCGACGCCGGCGAGACG
GGGCCCGCGTTCCAGATGCTGATCTATCCCGCGACGGACTCGAGCAGGGAGAGCGCCTCG
CGCGTGGCCTTCGCCGAAGGCTACTTCCTCAGCAAGGCGCTGATGGACTGGTTCTGGGAG
GCCTATGTGCCGGAGGACACCGACCTCACCGACCTCCGCCTCTCACCGCTCCTCGCCACG
GACTTCACCGGACTGCCGCCGGCCTTCGTGCTCACCGCCGGCTACGACCCGCTGCGCGAC
GAGGGCCGCGCCTATGCCGACAGGCTGATCGAGGCCGGCATCAAGACGACCTATGTGAAC
TATCCCGGCACCATCCACGGCTTCTTCTCGCTGACGCGATTCCTGAGCCAGGGACTGAAA
GCCAACGACGAGGCGGCGGCGGTGATGGGGGCGCACTTCGGGACGTAG

LAE6 protein sequence

MLLPETRNLLDLMDAATRGGRPRLETLPHAVGRKAVDKMSEDGEADPPEVAEVANGGFAG
PASEIRFRRYRPLGEAAGLLPTLIYYHGGGFVIGNIETHDSTCRRLANKSRCQVISIDYR
LAPEHPFPAPIDDGIAAFRHIRDNAESFGADAARLAVGGDSAGGAMAAVVCQACRDAGET
GPAFQMLIYPATDSSRESASRVAFAEGYFLSKALMDWFWEAYVPEDTDLTDLRLSPLLAT
DFTGLPPAFVLTAGYDPLRDEGRAYADRLIEAGIKTTYVNYPGTIHGFFSLTRFLSQGLK
ANDEAAAVMGAHFGT

LAE7 gene sequence

ATGCATTTCTTCGATCACGGTCGCGTGACTCGCAACTTCATGCTCGTGGCGCTGCTCGCC
GGCAGTGCCGGCGCGCATGCCCAAGCCCTGCGCGCTCCTGCGGCACAAGCGGCCCCTGTG
CCCGCCGCCGTGCCGGCCGCAGTGGCGATTCCGCGTCCCACGGCTGCCGAGGTCGAGCTC
GCGCGCGGCAGTCTCGCGAAGTTCCTTGCCTCGGCCGATGCTGCCACGCGCAGCGTGCTC
GAGAAATATCCCGGGCTGCTCGAAGTGCGGCCACCGGGGCCGAACAGCGCGATCCTGCCG
GGCTTGGCACCGCAGTTCCAGGCGAAACACCAGGCGAACCTCGAAGTCGCTCGCCAAGGC
GACGCCGAAGTGTTGTTCATGGGCGACTCGATCACCGATTTCTGGCGCAACGCCGAAGGT
CCGTTCGCAGGCAAGCCCGTGCTCGACCGGCATTTCGGGCACTGGAAGGTGGCGAACTTC
GGTATCGCCGGCGACACGACGCAGGGCGTTCTCTATCGGCTGCAGAACGGCGAGGGGCGC

GGTTTCAACCCGCGCGCCGTGATGTTGATGATCGGCACGAACAACACGGCCAGGAACACG
GCGGCCGAGATCGCCGAAGGCATCGGCGCCGTCGTGCTCGAGCTGCAGCGCGATTTTCCG
CAAGCGAAGATTCTGCTGCTCGGCGTATTTCCGCGCGGCCGGCCGAACGATCCGGTCCGC
GGCACGATTGCCGAGATCAACCGCGCGATCGCGAAGCTTCACGACGGCGACCGCGTCCAC
TATCTCGACATCGGCGCGCAATTCCTCGCTGCCGACGGCAGCATTCCGGCCGACGTCATG
AGCGATCTGCTGCACCCCGGGCCGAAGGGCTACGAGATCTGGGCGCAGGCCGTCAAGGAG
CCGTTGACGAAGCTGATGGGCGCGCGTCCTTAG

LAE7 protein sequence

MHFFDHGRVTRNFMLVALLAGSAGAHAQALRAPAAQAAPVPAAVPAAVAIPRPTAAEVEL
ARGSLAKFLASADAATRSVLEKYPGLLEVRPPGPNSAILPGLAPQFQAKHQANLEVARQG
DAEVLFMGDSITDFWRNAEGPFAGKPVLDRHFGHWKVANFGIAGDTTQGVLYRLQNGEGR
GFNPRAVMLMIGTNNTARNTAAEIAEGIGAVVLELQRDFPQAKILLLGVFPRGRPNDPVR
GTIAEINRAIAKLHDGDRVHYLDIGAQFLAADGSIPADVMSDLLHPGPKGYEIWAQAVKE
PLTKLMGARP