

**Structure-based discovery of a non-canonical
prototype long-chain monoacylglycerol lipase
through an endogenous catalysis intermediate
complex**

SUPPLEMENT

RESULTS

Structural comparison of Tth MGL with cinnamoyl and feruloyl esterases

The relations of cinnamoyl esterase from *Lactobacillus johnsonii* (UNIPROT code D3YEX6, PDB code 3S2Z, plus additional codes of the same sequence) and feruloyl esterase from *Butyrivibrio proteoclasticus* (UNIPROT code D2YW37, PDB code 2WTM, plus additional codes of the same sequence) to the sequence and structure of *Tth* MGL are at a comparable level, as illustrated by rmsd values of 1.2–1.3 Å and structure-based sequence identities of 30–32 % (**Figure 6, Supplementary Figure S2, Supplementary Table S8**). The lid domain in these three structures comprises a virtually identical two-stranded antiparallel β -hairpin (residues 163–165 and 170–172 in the *Tth* MGL), which is connected by a short hairpin motif of five or less residues (**Figure 1C, Figure 6A**). This hairpin shields a highly conserved histidine-glycine-phenylalanine (HGF) motif within the catalytic domain loop connecting β -strand 3 and α -helix B (residues 35–37 in the *Tth* MGL structure) and the small α -helix B' (residues 77–79 in the *Tth* MGL structure) next to the active site (**Figure 6B, Supplementary Figure S2**). Helix B' comprises a phenylalanine-serine-glutamate/aspartate (FSE/D) motif that is conserved in the sequences of the related esterases from *L. johnsonii*, *B. proteoclasticus*, and *T. siderophilus* (*Tth*) but not in other related esterase/lipases (**Supplementary Figure S2**). Phe37, Phe77 and Phe/Tyr175 from the C-terminal lid domain helix α -LC form a highly conserved aromatic residue cluster in these structures and play a crucial role in shaping the substrate-binding sites of the respective enzymes (**Figure 3A, Supplementary Figures S2 & S10**).

Based on these relations it is surprising that *Tth* MGL did not show measurable activity against phenylpropanoid FA ester substrates of the esterases from *L. johnsonii* and *B. proteoclasticus* (**Figure 6A**)¹³⁻¹⁴. Inspection of their structures revealed a plausible explanation of the distinct substrate specificities: First, in these esterases, there is an

additional second small β -hairpin interaction following the first lid helix α -LA, which is not found in any other HBH esterase/lipase lid domains including the structure of *Tth* MGL (**Figure 6A, Supplementary Figure S10**). This hairpin hovers over the central part of the active site of these two esterases to shield bound substrates and thereby further restricts the size of the substrate binding pocket. In the *Tth* MGL this hairpin structure is replaced by a second lid helix α -LB, which is more distal to the enzyme's active site (**Supplementary Figure S10, left panel**). Secondly, there are two residues in the *Tth* MGL lid structure, Leu145 from the N-terminal lid helix α -LA and Phe175 from the C-terminal lid helix α -LC that are replaced by residues with functional polar groups, aspartate (Asp138, only in the sequence of the *L. johnsonii* esterase sequence) and tyrosine (Tyr169 in the *L. johnsonii* and *B. proteoclasticus* esterase sequences) respectively (**Supplementary Figure S10**). Structures of the esterase from *L. johnsonii* in the presence of several ferulate derivatives (3PFB, 3PFC, 3S2Z) demonstrate the distinct roles of these residues in substrate binding, and removal of the polar side chain of Asp138 strongly impairs substrate turnover ¹⁴. In conclusion, the absence of these functional residue groups in the *Tth* MGL well explains the lack of any measurable activity towards hydroxycinnamic esters.

Supplementary Table S1: Thermostability of *Tth* variants

<i>Tth</i> lipase variant	T _m (°C)	ΔT _m (°C)
wt	77.2 ± 0.16	
wt-PMS	77.1 ± 0.04	- 0.1
ΔLid	82.8 ± 0.01	+ 5.6
E43A	76.1 ± 0.02	- 1.1
E43K	77.9 ± 0.21	+ 0.7
E72R	75.7 ± 0.05	- 1.5
S113A	81.8 ± 0.07	+ 4.6
Y154A	78.3 ± 0.14	+ 1.1
Y154R	78.3 ± 0.10	+ 1.1

Legend Supplementary Table S1: We did not detect significant changes in thermostability (< 2 degrees changes), except for a variant without the lid domain (Δ140-183) that revealed an increased melting temperature of 83 °C, which is 6 °C higher than one found for the wt *Tth* MGL (**Figure 1B**). This was within the melting temperature of the wt *Tth* MGL (**Figure 1B**). This was within expectation as the lid domain is highly exposed and presents the most dynamic part of the overall *Tth* MGL structure (**Figures 1 & 3**). Interestingly, we have also observed a similar level of thermal stabilization in the *Tth* MGL S113A active site mutant. The most plausible explanation is that a mutation of this active site residue could lead to a relieve of the high-energy ε-conformation of Ser113 found in both *wt* enzymes in the presence and absence of PMSF (**Supplementary Figure S3**).

Supplementary Table S2: X-ray structure determination and refinement

	Se-Met peak	SeMet inflection	PMSF derivative
PDB identifier	7Q4J		7Q4H
Crystal data			
Space group	P4 ₃ 2 ₁ 2	P4 ₃ 2 ₁ 2	P4 ₃ 22
Unit cell parameters (Å)	a=b= 137.5, c=67.8		a=b= 85.8, c=157.5
Mosaicity (deg.)	0.25	0.25	0.90
Data collection			
Beam line	BM14, ESRF	BM14, ESRF	BM14, ESRF
Wavelength (Å)	0.97873	0.97903	0.95372
Resolution range	50.0-2.56 (2.60-2.56)	20.0-1.91 (1.94-1.91)	30.0-2.00 (2.05-2.00)
R _{sym} (%)	0.059 (13.1)	5.1 (64.2)	8.2 (51.4)
I/σ (I)	35.3 (19.3)	21.4 (2.03)	24.0 (1.65)
Reflections measured/unique	482,920/21,648	543,030/49,383	728,975/38930
Redundancy	10.4 (10.0)	3.3 (2.9)	5.1 (2.7)
Completeness (%)	99.7 (100.0)	97.1 (82.2)	98.1 (82.2)
Refinement			
Program	Phenix, TLS		Phenix, TLS
R / R _{free} (%)	17.09/19.93		18.72/24.72
Reflections (work/test sets)	46,554/1,457		38,285/1,200
Protein atoms	4,190		4,148
Ligands*	1 x STE, 1 x D10, 7 x		1x OLC, 1 x Cl ⁻ , 5 x
	GOL, 4 x SO ₄ ²⁻		GOL, 3 x SO ₄ ²⁻
Solvent molecules	303		157
r.m.s.d. bonds (Å)	0.007		0.017
r.m.s.d. angles (deg)	1.446		1.894
Average B values for main chain atoms (Å ²)	39.45		46.46
Average B values for side chain and solvent molecules (Å ²)	45.37		49.84
Model quality (Ramachandran plot)			
Most favored (%)	90.8		89.5
Additionally allowed (%)	8.3		9.2
Generously allowed (%)	0.9		0.9
Disallowed (%)	0.0		0.4

*STE: stearic acid; D10: decan; GOL: glycerol; OLC: (2R)-2,3-dihydroxypropyl (9Z)-octadec-9-enoate

Supplementary Table S3: *In vitro* activity data

Figure 6A, left panel

***Tth* MGL (wt) activity towards *p*NP substrate [U mg⁻¹]**

C2	C4	C5	C6	C8	C10
1.23 ± 0.06	3.46 ± 0.15	3.56 ± 0.24	2.77 ± 0.18	2.49 ± 0.16	1.67 ± 0.13
C12	C14	C16	C18	ferulate	
0.98 ± 0.05	0.55 ± 0.04	0.04 ± 0.01	0.01 ± 0.01	0.03 ± 0.03	

Figure 6A, central panel

***Tth* MGL variant activity towards *p*NP substrate [U mg⁻¹]**

	C5	C6	C8	C10	C14
wt	3.53 ± 0.13	2.64 ± 0.02	2.14 ± 0.05	1.50 ± 0.04	0.46 ± 0.02
E43A	13.94 ± 0.51	11.04 ± 0.47	8.13 ± 0.14	6.90 ± 0.10	0.62 ± 0.06
S113A	0.01 ± 0.02	0.01 ± 0.01	-0.01 ± 0.02	0.00 ± 0.01	-0.01 ± 0.02
E72R	4.47 ± 0.31	3.84 ± 0.30	1.26 ± 0.08	1.28 ± 0.04	0.01 ± 0.01
ΔLid	0.01 ± 0.02	0.01 ± 0.02	-0.02 ± 0.00	0.00 ± 0.00	0.00 ± 0.01
Y154A	4.67 ± 0.11	3.58 ± 0.08	2.88 ± 0.13	2.11 ± 0.11	0.59 ± 0.07

Supplementary Table S4: Water solubility of *p*-nitrophenyl FA esters used for *Tth*

MGL activity measurements

Name	Corresponding acid	Water solubility (mg ml ⁻¹) ¹
4-Nitrophenyl acetate (<i>p</i> NP-C2)	Acetic/ethanoic acid	1160
4-Nitrophenyl butyrate (<i>p</i> NP-C4)	Butyric/Butanoic acid	24
4-Nitrophenyl valerate (<i>p</i> NP-C5)	Valeric/Pentanoic acid	9
4-Nitrophenyl hexanoate (<i>p</i> NP-C6)	Caproic/Hexanoic acid	15
4-Nitrophenyl octanoate (<i>p</i> NP-C8)	Caprylic/Octanoic acid	1.513
4-Nitrophenyl decanoate (<i>p</i> NP-C10)	Capric/Decanoic acid	0.1515
4-Nitrophenyl laurate (<i>p</i> NP-C12)	Lauric/Dodecanoic acid	0.01504
4-Nitrophenyl myristate (<i>p</i> NP-C14)	Myristic/Tetradecanoic acid	0.001481
4-Nitrophenyl palmitate (<i>p</i> NP-C16)	Palmitic/Hexadecanoic acid	0.0001449
4-Nitrophenyl stearate (<i>p</i> NP-C18)	Stearic/Octadecanoic acid	0.00001409

¹ Source: ChemSpider (<http://www.chemspider.com/>)

Supplementary Table S5: Steady-state kinetics of Ttl MGL variants with *p*-nitrophenyl substrates of different chain lengths

<i>p</i>NP-C2	K_M [mM]	k_{cat} [s ⁻¹]	k_{cat}/K_M [M ⁻¹ s ⁻¹]	$(k_{cat}/K_M)_{variant}/wt$
wt	2.3 ± 0.3	6.1 ± 0.7	2.7 ± 0.5 × 10 ³	1.0
E43A	1.9 ± 0.3	1.7 ± 0.2 × 10 ¹	8.7 ± 1.5 × 10 ³	3.3
E43K	6.3 ± 0.6 × 10 ⁻¹	1.0 ± 0.1	1.6 ± 0.2 × 10 ³	0.6
E72R	7.1 ± 0.7 × 10 ⁻¹	4.2 ± 0.2	6.0 ± 0.6 × 10 ³	2.2
Y154A	1.4 ± 1.1 × 10 ⁻¹	2.5 ± 1.8 × 10 ¹	1.8 ± 1.9 × 10 ³	0.7
Y154R	2.0 ± 0.3	1.5 ± 0.2 × 10 ¹	7.5 ± 1.4 × 10 ³	2.8
<i>p</i>NP-C4	K_M [mM]	k_{cat} [s ⁻¹]	k_{cat}/K_M [M ⁻¹ s ⁻¹]	$(k_{cat}/K_M)_{variant}/wt$
wt	7.1 ± 0.7 × 10 ⁻¹	1.0 ± 0.1 × 10 ¹	1.4 ± 0.2 × 10 ⁴	1.0
E43A	6.7 ± 1.9 × 10 ⁻¹	1.7 ± 0.3 × 10 ¹	2.5 ± 0.8 × 10 ⁴	1.7
E43K	8.2 ± 0.8 × 10 ⁻¹	4.6 ± 0.2	5.6 ± 0.6 × 10 ³	0.4
E72R	1.4 ± 2.0	5.8 ± 5.4	4.0 ± 6.8 × 10 ³	0.3
Y154A	3.4 ± 0.6	1.5 ± 0.2 × 10 ¹	4.5 ± 1.0 × 10 ³	0.3
Y154R	7.2 ± 1.9 × 10 ⁻¹	1.4 ± 0.2 × 10 ¹	1.9 ± 0.6 × 10 ⁴	1.3
<i>p</i>NP-C5	K_M [mM]	k_{cat} [s ⁻¹]	k_{cat}/K_M [M ⁻¹ s ⁻¹]	$(k_{cat}/K_M)_{variant}/wt$
wt	8.3 ± 1.3 × 10 ⁻¹	9.9 ± 0.8	1.2 ± 0.2 × 10 ⁴	1.0
E43A	6.5 ± 1.6 × 10 ⁻¹	1.5 ± 0.2 × 10 ¹	2.3 ± 0.6 × 10 ⁴	1.9
E43K	4.5 ± 0.8 × 10 ⁻¹	3.4 ± 0.3	7.6 ± 1.5 × 10 ³	0.6
E72R	9.4 ± 3.3 × 10 ⁻¹	1.2 ± 0.2 × 10 ¹	1.2 ± 0.5 × 10 ⁴	1.0
Y154A	1.4 ± 0.2	1.3 ± 0.1 × 10 ¹	9.6 ± 1.9 × 10 ³	0.8
Y154R	8.8 ± 2.2 × 10 ⁻¹	1.6 ± 0.2 × 10 ¹	1.8 ± 0.5 × 10 ⁴	1.5
<i>p</i>NP-C6	K_M [mM]	k_{cat} [s ⁻¹]	k_{cat}/K_M [M ⁻¹ s ⁻¹]	$(k_{cat}/K_M)_{variant}/wt$
wt	3.6 ± 0.8 × 10 ⁻¹	5.8 ± 0.5	1.6 ± 0.4 × 10 ⁴	1.0
E43A	7.1 ± 3.0 × 10 ⁻¹	1.3 ± 0.3 × 10 ¹	1.8 ± 0.9 × 10 ⁴	1.1
E43K	3.2 ± 0.9 × 10 ⁻¹	2.9 ± 0.3	9.1 ± 2.8 × 10 ³	0.6
E72R	6.3 ± 3.4 × 10 ⁻¹	8.7 ± 2.3	1.4 ± 0.8 × 10 ⁴	0.8
Y154A	4.8 ± 0.8 × 10 ⁻¹	6.7 ± 0.5	1.4 ± 0.3 × 10 ⁴	0.9
Y154R	7.5 ± 3.6 × 10 ⁻¹	1.4 ± 0.3 × 10 ¹	1.8 ± 1.0 × 10 ⁴	1.1

pNP-C8	K_M [mM]	k_{cat} [s ⁻¹]	k_{cat}/K_M [M ⁻¹ s ⁻¹]	$(k_{cat}/K_M)_{variant}/wt$
wt	$4.9 \pm 1.5 \times 10^{-1}$	6.5 ± 0.9	$1.3 \pm 0.4 \times 10^4$	1.0
E43A	$5.2 \pm 3.4 \times 10^{-1}$	$1.2 \pm 0.3 \times 10^1$	$2.3 \pm 1.6 \times 10^4$	1.7
E43K	$4.6 \pm 1.3 \times 10^{-1}$	3.7 ± 0.5	$8.0 \pm 2.5 \times 10^3$	0.6
E72R	$5.1 \pm 2.2 \times 10^{-1}$	6.7 ± 1.3	$1.3 \pm 0.6 \times 10^4$	1.0
Y154A	$6.2 \pm 2.3 \times 10^{-1}$	7.6 ± 1.4	$1.2 \pm 0.5 \times 10^4$	0.9
Y154R	$8.2 \pm 4.4 \times 10^{-1}$	$1.3 \pm 0.4 \times 10^1$	$1.6 \pm 1.0 \times 10^4$	1.2
pNP-C10	K_M [mM]	k_{cat} [s ⁻¹]	k_{cat}/K_M [M ⁻¹ s ⁻¹]	$(k_{cat}/K_M)_{variant}/wt$
wt	$3.0 \pm 0.7 \times 10^{-1}$	5.2 ± 0.5	$1.7 \pm 0.5 \times 10^4$	1.0
E43A	$2.7 \pm 1.0 \times 10^{-1}$	7.5 ± 1.0	$2.7 \pm 1.1 \times 10^4$	1.6
E43K	$3.3 \pm 1.1 \times 10^{-1}$	3.0 ± 0.4	$8.9 \pm 3.1 \times 10^3$	0.5
E72R	$1.3 \pm 0.7 \times 10^{-1}$	2.5 ± 0.4	$1.9 \pm 1.1 \times 10^4$	1.1
Y154A	$2.2 \pm 0.6 \times 10^{-1}$	3.4 ± 0.3	$1.6 \pm 0.5 \times 10^4$	0.9
Y154R	$3.9 \pm 1.1 \times 10^{-1}$	9.1 ± 1.1	$2.3 \pm 0.7 \times 10^4$	1.3
pNP-C12	K_M [mM]	k_{cat} [s ⁻¹]	k_{cat}/K_M [M ⁻¹ s ⁻¹]	$(k_{cat}/K_M)_{variant}/wt$
wt	$1.1 \pm 0.1 \times 10^{-1}$	2.3 ± 0.1	$2.1 \pm 0.3 \times 10^4$	1.0
E43A	$1.0 \pm 0.1 \times 10^{-1}$	3.4 ± 0.1	$3.4 \pm 0.5 \times 10^4$	1.6
E43K	$5.3 \pm 1.3 \times 10^{-2}$	1.0 ± 0.1	$1.9 \pm 0.5 \times 10^4$	0.9
E72R	$1.7 \pm 0.6 \times 10^{-2}$	$4.1 \pm 0.3 \times 10^{-1}$	$2.5 \pm 0.9 \times 10^4$	1.2
Y154A	$6.4 \pm 0.9 \times 10^{-2}$	1.3 ± 0.0	$2.0 \pm 0.3 \times 10^4$	0.9
Y154R	$1.2 \pm 0.2 \times 10^{-1}$	3.3 ± 0.1	$2.8 \pm 0.4 \times 10^4$	1.3
pNP-C14	K_M [mM]	k_{cat} [s ⁻¹]	k_{cat}/K_M [M ⁻¹ s ⁻¹]	$(k_{cat}/K_M)_{variant}/wt$
wt	$1.2 \pm 0.1 \times 10^{-1}$	$5.9 \pm 0.2 \times 10^{-1}$	$5.0 \pm 0.5 \times 10^3$	1.0
E43A	$1.0 \pm 0.1 \times 10^{-1}$	$8.6 \pm 0.2 \times 10^{-1}$	$8.5 \pm 0.8 \times 10^3$	1.7
E43K	$4.3 \pm 1.2 \times 10^{-2}$	$1.8 \pm 0.1 \times 10^{-1}$	$4.1 \pm 1.2 \times 10^3$	0.8
E72R	$4.4 \pm 1.4 \times 10^{-2}$	$3.5 \pm 0.3 \times 10^{-1}$	$8.0 \pm 2.7 \times 10^3$	1.6
Y154A	$9.0 \pm 1.3 \times 10^{-2}$	$4.5 \pm 0.2 \times 10^{-1}$	$5.0 \pm 0.8 \times 10^3$	1.0
Y154R	$1.7 \pm 0.7 \times 10^{-1}$	1.2 ± 0.2	$7.3 \pm 3.1 \times 10^3$	1.5

Supplementary Table S6: FAME data

Activity (mg FAMES/g bacteria)							
WT	Lid	E43A	E43K	S113A	Y154R	WT2	Y154A
0,4499	0,2568	0,8950	0,4008	0,2835	0,6647	2,6032	2,7885
0,4132	0,3726	0,8710	0,3757	0,2933	0,7022	2,4927	2,5948
0,4515	0,3500	0,7370	0,2559	0,1822	0,3148	3,4162	3,0043
0,4360	0,2076		0,2575				2,5359
0,3826	0,3964						
0,3663	0,2682						
	0,1863						
Normalized activity (Ratio mutant/WT)							
WT	Lid	E43A	E43K	S113A	Y154R	WT2	Y154A
1,080	0,616	2,148	0,962	0,680	1,595	0,917	0,983
0,992	0,894	2,091	0,902	0,704	1,686	0,879	0,915
1,084	0,840	1,769	0,614	0,437	0,756	1,204	1,059
1,046	0,498		0,618				0,894
0,918	0,952						
0,879	0,644						
	0,447						
Normalized activity (Ratio mutant/WT)							
WT	Lid	E43A	E43K	S113A	Y154A	Y154R	
1,080	0,616	2,148	0,962	0,680	0,983	1,595	
0,992	0,894	2,091	0,902	0,704	0,915	1,686	
1,084	0,840	1,769	0,614	0,437	1,059	0,756	
1,046	0,498		0,618		0,894		
0,918	0,952						
0,879	0,644						
	0,447						
Normalized activity (Ratio mutant/WT) REPORTED ON the GRAPH							
1,000	0,699	2,003	0,774	0,607	0,963	1,346	MEAN
0,086	0,198	0,204	0,184	0,148	0,075	0,513	SD
	0,0055	< 0,0001	0,0288	0,0013	0,4985	0,1285	p value vs WT
	**	****	*	**	ns	ns	p value summary

Supplementary Table S7: Sequences producing significant alignments with *Tth* (UNIPROT code: I9KU82)

	Score	E Value	Phylum
UR50:UniRef50_G2MWQ3 Alpha/beta hydrolase fold containing protein ...	513	0.0	Clostridia
UR50:UniRef50_A0A1I5TFW2 Hydrolase_4 domain-containing protein n=1...	266	5,00E-87	Clostridia
UR50:UniRef50_A0A1M6CI71 Peptidase_S9 domain-containing protein n=...	263	2,00E-85	Firmicutes
UR50:UniRef50_A0A1W1X6Q3 Hydrolase_4 domain-containing protein n=5...	245	2,00E-78	Clostridia
UR50:UniRef50_UPI000B980ED8alpha/beta fold hydrolase n=1 Tax=Petr...	229	2,00E-72	Clostridia
UR50:UniRef50_A0A177ZKP7Alpha/beta hydrolase n=17 Tax=Firmicutes ...	228	7,00E-72	Firmicutes
UR50:UniRef50_A0A355RZC9Alpha/beta hydrolase n=1 Tax=Clostridiace...	223	4,00E-70	Clostridia
UR50:UniRef50_A0A0C2UJT1 Peptidase_S9 domain-containing protein n=...	221	6,00E-69	Cohnella
UR50:UniRef50_T0BRH7 Hydrolase_4 domain-containing protein n=40 Ta...	216	5,00E-67	Firmicutes
UR50:UniRef50_A0A353N5E8Alpha/beta hydrolase n=6 Tax=Firmicutes T...	216	6,00E-67	Firmicutes
UR50:UniRef50_A0A0A8X650Alpha/beta superfamily hydrolase n=3 Tax=...	214	2,00E-66	Firmicutes
UR50:UniRef50_A0A660RIT6Alpha/beta hydrolase n=4 Tax=Thermotogae ...	209	2,00E-64	Thermotogae
UR50:UniRef50_A0A660TFB6Alpha/beta hydrolase n=2 Tax=Spirochaetes...	209	3,00E-64	Spirochaetes
UR50:UniRef50_A0A285CSW9 Hydrolase_4 domain-containing protein n=5...	208	5,00E-64	Firmicutes
UR50:UniRef50_UPI000A26B42Dalpha/beta hydrolase n=1 Tax=Clostridi...	205	1,00E-62	Clostridia
UR50:UniRef50_A0A6N7M3G8Alpha/beta fold hydrolase n=3 Tax=root Ta...	201	3,00E-61	
UR50:UniRef50_D1CF13 Dienelactone hydrolase n=1 Tax=Thermobaculum ...	200	6,00E-61	Chloroflexi
UR50:UniRef50_A0A1M6M0X5 Hydrolase_4 domain-containing protein n=7...	200	6,00E-61	
UR50:UniRef50_A0A1G6GNU3 Hydrolase_4 domain-containing protein n=3...	200	8,00E-61	Firmicutes
UR50:UniRef50_A0A1I2SBI9 Hydrolase_4 domain-containing protein n=4...	197	7,00E-60	Firmicutes
UR50:UniRef50_A0A173U362 Acetoin dehydrogenase E2 subunit dihydrol...	194	2,00E-58	Firmicutes
UR50:UniRef50_A0A101IKX3 Hydrolase of the alpha/beta superfamily-l...	193	3,00E-58	Parcubacteria
UR50:UniRef50_H8XW45 Carboxylesterase n=8 Tax=Bacteria TaxID=2 Rep...	192	5,00E-58	

UR50:UniRef50_C7N773 Peptidase_S9 domain-containing protein n=29 T...	187	1,00E-55	Actinobacteria
UR50:UniRef50_A0A7C2XQZ5Alpha/beta fold hydrolase n=5 Tax=root Ta...	185	4,00E-55	
UR50:UniRef50_UPI0004788E77alpha/beta hydrolase n=1 Tax=Alicyclob...	183	4,00E-54	Firmicutes
UR50:UniRef50_A0A0S7X983 Peptidase_S9 domain-containing protein n=...	182	9,00E-54	Planctomycetes
UR50:UniRef50_A0A7C3MWS3Alpha/beta fold hydrolase n=1 Tax=Firmicu...	181	1,00E-53	Firmicutes
UR50:UniRef50_A0A2V9K2M2Alpha/beta hydrolase n=8 Tax=Acidobacteri...	180	4,00E-53	Acidobacteria
UR50:UniRef50_A0A350X132Alpha/beta hydrolase n=1 Tax=Firmicutes b...	179	7,00E-53	Firmicutes
UR50:UniRef50_A0A1F7FLL2 Hydrolase_4 domain-containing protein n=1...	178	3,00E-52	
UR50:UniRef50_A0A7C6UT48Alpha/beta fold hydrolase n=1 Tax=Firmicu...	177	5,00E-52	Firmicutes
UR50:UniRef50_A0A0L8F3E0 Hydrolase_4 domain-containing protein n=2...	176	8,00E-52	Clostridia
UR50:UniRef50_B8E2K8 Dienelactone hydrolase n=8 Tax=Dictyoglomus T...	177	8,00E-52	Firmicutes
UR50:UniRef50_X1B567 Peptidase_S9 domain-containing protein n=1 Ta...	173	7,00E-51	
UR50:UniRef50_A0A3Q9HNM1 Peptidase_S9 domain-containing protein n=...	174	1,00E-50	Firmicutes
UR50:UniRef50_A0A1W9SG40 Peptidase_S9 domain-containing protein n=...	174	2,00E-50	
UR50:UniRef50_A0A1F9X5K1 Peptidase_S9 domain-containing protein n=...	173	3,00E-50	Elusimicrobia
UR50:UniRef50_A0A1G9X0F9 Hydrolase_4 domain-containing protein n=3...	172	7,00E-50	Firmicutes
UR50:UniRef50_A0A660R9K6Alpha/beta hydrolase n=1 Tax=Thermotoga s...	172	7,00E-50	Thermotoga
UR50:UniRef50_B9E0E1 AB hydrolase-1 domain-containing protein n=6 ...	172	7,00E-50	Clostridia
UR50:UniRef50_A0A1T4P9G4 Peptidase_S9 domain-containing protein n=...	171	1,00E-49	Firmicutes
UR50:UniRef50_A0A1H8CGU1 AB hydrolase-1 domain-containing protein ...	171	2,00E-49	Firmicutes
UR50:UniRef50_A0A386H4I4Alpha/beta hydrolase n=2 Tax=unclassified...	171	2,00E-49	Clostridia
UR50:UniRef50_D7RU28 Ferulic acid esterase n=2 Tax=Lactobacillus b...	170	3,00E-49	Firmicutes
UR50:UniRef50_A0A1V6BNQ8 Non-heme bromoperoxidase BPO-A1 n=1 Tax=T...	169	9,00E-49	Tenericutes
UR50:UniRef50_A0A0G2ZFB4 Peptidase_S9 domain-containing protein n=...	165	2,00E-47	Thermotogae
UR50:UniRef50_A0A143WVC9 Putative aminoacrylate hydrolase RutD n=2...	162	2,00E-46	Clostridia

UR50:UniRef50_A0A7C6N4Z9Alpha/beta fold hydrolase n=1 Tax=Acholep...	162	2,00E-46	Firmicutes
UR50:UniRef50_A0A7C5K5D5Alpha/beta fold hydrolase n=2 Tax=Verruco...	163	3,00E-46	Verrucomicrobia
UR50:UniRef50_B1BZ56 Hydrolase_4 domain-containing protein n=54 Ta...	162	3,00E-46	Firmicutes
UR50:UniRef50_A0A3D6D8K7Alpha/beta hydrolase n=14 Tax=Thermotogae...	162	4,00E-46	Thermotogae
UR50:UniRef50_A0A7C5IFR0Alpha/beta hydrolase n=1 Tax=bacterium Ta...	162	6,00E-46	
UR50:UniRef50_A0A3M2F2U0Alpha/beta fold hydrolase n=1 Tax=Candida...	163	1,00E-45	
UR50:UniRef50_A0A0S8HFA0 Peptidase_S9 domain-containing protein n=...	161	1,00E-45	Planctomycetes
UR50:UniRef50_A0A2H5WXV4 2-succinyl-6-hydroxy-2, 4-cyclohexadiene-...	160	2,00E-45	
UR50:UniRef50_A0A6J4MJQ0 Peptidase_S9 domain-containing protein n=...	159	7,00E-45	Chloroflexi
UR50:UniRef50_A0A1F5UGK4 Uncharacterized protein n=1 Tax=Candidatu...	157	2,00E-44	
UR50:UniRef50_A0A1V4QWG9 Peptidase_S9 domain-containing protein n=...	157	2,00E-44	
UR50:UniRef50_A0A7C1E965Alpha/beta fold hydrolase (Fragment) n=1 ...	159	4,00E-44	Firmicutes
UR50:UniRef50_A0A1Y4GAT0 Peptidase_S9 domain-containing protein n=...	156	9,00E-44	Synergistetes
UR50:UniRef50_A0A399FFE0 Esterase EstD n=1 Tax=Meiothermus granati...	156	1,00E-43	Deinococcus
UR50:UniRef50_A0A1G9G1K1 Hydrolase_4 domain-containing protein n=1...	155	2,00E-43	Actinobacteria
UR50:UniRef50_A0A496ABF8Alpha/beta hydrolase n=7 Tax=Candidatus P...	155	4,00E-43	
UR50:UniRef50_C9Z377 AB hydrolase-1 domain-containing protein n=30...	153	1,00E-42	Actinobacteria
UR50:UniRef50_A0A2H5V4E2 2-succinyl-6-hydroxy-2, 4-cyclohexadiene-...	151	4,00E-42	
UR50:UniRef50_UPI000C1BB749alpha/beta fold hydrolase n=1 Tax=Mini...	150	2,00E-41	Miniphocibacter
UR50:UniRef50_A0A1H6TBQ8 Hydrolase_4 domain-containing protein n=5...	149	4,00E-41	Deinococcus
UR50:UniRef50_A0A1F9XBG6 Peptidase_S9 domain-containing protein n=...	149	4,00E-41	Elusimicrobia
UR50:UniRef50_C0CTE6 Hydrolase_4 domain-containing protein n=5 Tax...	147	3,00E-40	Actinobacteria
UR50:UniRef50_A0A1V5Z8D4 Phosphoribosylformylglycinamide cyclo-l...	154	4,00E-40	
UR50:UniRef50_A0A4R2RPK9 Hydrolase_4 domain-containing protein n=5...	146	1,00E-39	Firmicutes
UR50:UniRef50_A0A3R7ASF0Alpha/beta fold hydrolase n=9 Tax=Candida...	145	2,00E-39	

UR50:UniRef50_A0A1F3XTK4 Peptidase_S9 domain-containing protein n=...	145	2,00E-39	Proteobacteria
UR50:UniRef50_S9UDC0 Hydrolase_4 domain-containing protein n=13 Ta...	145	2,00E-39	Firmicutes
UR50:UniRef50_A0A0Q3VY12 Hydrolase_4 domain-containing protein n=4...	144	6,00E-39	Firmicutes
UR50:UniRef50_A0A559IWC1Alpha/beta fold hydrolase n=3 Tax=Paeniba...	143	1,00E-38	Firmicutes
UR50:UniRef50_A0A410QE23Alpha/beta fold hydrolase n=2 Tax=Tissier...	142	2,00E-38	Firmicutes
UR50:UniRef50_A0A3D5RJW5 Hydrolase_4 domain-containing protein n=4...	142	3,00E-38	Actinobacteria
UR50:UniRef50_W4Q095 Peptidase S15 n=1 Tax=Bacillus wakoensis JCM ...	141	7,00E-38	Firmicutes
UR50:UniRef50_UPI000C2348F2alpha/beta fold hydrolase n=1 Tax=Baci...	141	8,00E-38	Firmicutes
UR50:UniRef50_S2ZQT5 Hydrolase_4 domain-containing protein n=65 Ta...	140	2,00E-37	
UR50:UniRef50_A0A511N521Alpha/beta hydrolase n=4 Tax=Deinococcus ...	139	2,00E-37	Deinococcus
UR50:UniRef50_E0SNK2 Dienelactone hydrolase n=3 Tax=Ignisphaera ag...	139	2,00E-37	
UR50:UniRef50_A0A6L3VAP3Alpha/beta hydrolase n=3 Tax=Bacillales T...	140	2,00E-37	Firmicutes
UR50:UniRef50_F7JM98 Hydrolase_4 domain-containing protein n=3 Tax...	139	3,00E-37	Firmicutes
UR50:UniRef50_A0A3R7AJM1Alpha/beta fold hydrolase n=1 Tax=Candida...	142	4,00E-37	
UR50:UniRef50_A0A6N7INW5Alpha/beta fold hydrolase n=2 Tax=Desulfo...	139	6,00E-37	Thermodesulfobacteria
UR50:UniRef50_UPI0009EC19CCalpha/beta fold hydrolase n=1 Tax=Limn...	139	6,00E-37	
UR50:UniRef50_A0A562QML1 Peptidase_S9 domain-containing protein n=...	138	1,00E-36	
UR50:UniRef50_A0A7C4YG41Alpha/beta fold hydrolase n=5 Tax=Bacteri...	137	2,00E-36	
UR50:UniRef50_A0A2D5YTE8 Peptidase_S9 domain-containing protein n=...	137	3,00E-36	Planctomycetes
UR50:UniRef50_A0A7C2Z7G5Alpha/beta fold hydrolase n=1 Tax=Armatim...	139	7,00E-36	Armatimonadetes
UR50:UniRef50_U6SV57 Peptidase_S9 domain-containing protein n=4 Ta...	135	9,00E-36	Firmicutes
UR50:UniRef50_A0A7C5W765Alpha/beta fold hydrolase n=1 Tax=Plancto...	135	9,00E-36	Planctomycetes
UR50:UniRef50_A0A1V5Y0E8Fermentation/respiration switch protein n...	134	3,00E-35	
UR50:UniRef50_A0A1W1VKA4 Hydrolase_4 domain-containing protein n=2...	133	5,00E-35	Deinococcus
UR50:UniRef50_X1UDA6 Peptidase_S9 domain-containing protein (Fragm...	133	5,00E-35	

UR50:UniRef50_UPI0009EB71D7alpha/beta fold hydrolase n=2 Tax=Lagi...	133	9,00E-35	Firmicutes
UR50:UniRef50_A0A7C6Y8R1Alpha/beta hydrolase n=1 Tax=Acholeplasma...	132	1,00E-34	Firmicutes
UR50:UniRef50_A0A517YSM1Fermentation/respiration switch protein n...	132	1,00E-34	Planctomycetes
UR50:UniRef50_L1PMY6 Hydrolase_4 domain-containing protein n=37 Ta...	132	2,00E-34	Bacteroidetes
UR50:UniRef50_A0A4S8QDR3Alpha/beta fold hydrolase n=1 Tax=Glycomy...	134	2,00E-34	Glycomyces
UR50:UniRef50_A0A2V9JLX7Alpha/beta hydrolase (Fragment) n=1 Tax=A...	128	4,00E-34	Acidobacteria
UR50:UniRef50_A0A090CYN4 Peptidase_S9 domain-containing protein n=...	132	4,00E-34	Chlamydiae
UR50:UniRef50_C2BEE5 Hydrolase, alpha/beta domain protein n=20 Tax...	130	1,00E-33	Clostridia
UR50:UniRef50_A0A0E2H8K7 Hydrolase_4 domain-containing protein n=2...	129	1,00E-33	
UR50:UniRef50_A0A7C6HYB2Alpha/beta hydrolase n=2 Tax=Acholeplasma...	129	2,00E-33	Firmicutes
UR50:UniRef50_A0A094YYJ9 Hydrolase_4 domain-containing protein n=4...	129	2,00E-33	Firmicutes
UR50:UniRef50_UPI000DC1D062alpha/beta fold hydrolase n=1 Tax=Meso...	129	3,00E-33	Thermotogae
UR50:UniRef50_A0A2S0UCN1 Hydrolase_4 domain-containing protein n=5...	130	3,00E-33	Firmicutes
UR50:UniRef50_UPI000854E465alpha/beta fold hydrolase n=1 Tax=Cand...	129	3,00E-33	
UR50:UniRef50_A0A6M2A136 Esterase FrsA n=1 Tax=Chlamydiae bacteriu...	129	4,00E-33	Chlamydiae
UR50:UniRef50_A0A2M7QX46 Peptidase_S9 domain-containing protein n=...	128	4,00E-33	
UR50:UniRef50_UPI0002F3A25Dalpha/beta fold hydrolase n=2 Tax=Anaer...	128	8,00E-33	Clostridia
UR50:UniRef50_A0A2Z4Y4K2Alpha/beta superfamily hydrolase n=2 Tax=...	127	2,00E-32	
UR50:UniRef50_A0A5N7IN55Alpha/beta hydrolase n=1 Tax=Clostridium ...	124	2,00E-32	Clostridia
UR50:UniRef50_A0A0A0C164Alpha/beta hydrolase n=2 Tax=Cellulomonas...	127	2,00E-32	Actinobacteria
UR50:UniRef50_F0RJ91 Hydrolase, putative n=4 Tax=Deinococcus TaxID...	126	3,00E-32	Deinococcus
UR50:UniRef50_A0A2N2PNJ1 AB hydrolase-1 domain-containing protein ...	127	5,00E-32	
UR50:UniRef50_D6YWC6 Peptidase_S9 domain-containing protein n=2 Ta...	125	7,00E-32	Chlamydiae
UR50:UniRef50_UPI00145F20CAalpha/beta fold hydrolase n=1 Tax=Bifi...	125	7,00E-32	Actinobacteria
UR50:UniRef50_A0A644TNS7 2-succinyl-6-hydroxy-2, 4-cyclohexadiene-...	125	2,00E-31	

UR50:UniRef50_A0A072MVI6 Hydrolase_4 domain-containing protein n=2...	124	2,00E-31	Actinobacteria
UR50:UniRef50_A0A256XMB4 Hydrolase_4 domain-containing protein n=4...	124	2,00E-31	
UR50:UniRef50_E6K0Z8 AB hydrolase-1 domain-containing protein n=6 ...	124	2,00E-31	Bacteroidetes
UR50:UniRef50_A0A2X2RLV4 Dihydrolipoyllysine-residue acetyltransfe...	124	3,00E-31	Bacteroidetes
UR50:UniRef50_A0A3G1KRL6 AB hydrolase-1 domain-containing protein ...	124	4,00E-31	Firmicutes
UR50:UniRef50_A3XN04 Hydrolase of alpha-beta family protein n=2 Ta...	124	7,00E-31	Bacteroidetes
UR50:UniRef50_Q6MCU6 Hydrolase_4 domain-containing protein n=6 Tax...	123	7,00E-31	Chlamydiae
UR50:UniRef50_A0A564TCI22-succinyl-6-hydroxy-2,4-cyclohexadiene-1...	122	8,00E-31	Actinobacteria
UR50:UniRef50_A0A0A6V9N7Alpha/beta hydrolase n=3 Tax=Bacillus Tax...	123	8,00E-31	
UR50:UniRef50_A0A7C1ISM4Alpha/beta hydrolase (Fragment) n=1 Tax=F...	120	1,00E-30	Firmicutes
UR50:UniRef50_S7SRT6Alpha/beta hydrolase family protein n=7 Tax=B...	122	1,00E-30	Firmicutes
UR50:UniRef50_A0A410QE14Alpha/beta fold hydrolase n=2 Tax=Tissier...	122	1,00E-30	Firmicutes
UR50:UniRef50_UPI0011F069F2alpha/beta hydrolase n=2 Tax=Cellulomo...	122	1,00E-30	
UR50:UniRef50_A0A7C6Z9V6Alpha/beta hydrolase n=3 Tax=Bacteria Tax...	124	1,00E-30	
UR50:UniRef50_A0A1M3CT29 Peptidase_S9 domain-containing protein n=...	122	1,00E-30	Chlamydiae
UR50:UniRef50_S5ZG56 Hydrolase_4 domain-containing protein n=8 Tax...	121	2,00E-30	
UR50:UniRef50_A0A7C2V0M0Alpha/beta fold hydrolase n=2 Tax=TACK gr...	121	2,00E-30	
UR50:UniRef50_UPI00047F6E0Dalpha/beta fold hydrolase n=1 Tax=Dein...	117	2,00E-30	Deinococcus
UR50:UniRef50_A0A0F0LDC52-hydroxy-6-oxononadienedioate/2-hydroxy-...	121	3,00E-30	Actinobacteria
UR50:UniRef50_A0A0X8JGL0 Hydrolase_4 domain-containing protein n=3...	120	5,00E-30	Actinobacteria
UR50:UniRef50_C2CIF9 Hydrolase_4 domain-containing protein n=6 Tax...	120	7,00E-30	Clostridia
UR50:UniRef50_A0A1J4WQ76 Hydrolase_4 domain-containing protein n=3...	120	9,00E-30	
UR50:UniRef50_A0A318S4U1 Hydrolase_4 domain-containing protein n=3...	119	1,00E-29	Deinococcus
UR50:UniRef50_A0A7C2LD26Alpha/beta fold hydrolase n=1 Tax=Thermof...	124	2,00E-29	
UR50:UniRef50_A0A256XSA7 AB hydrolase-1 domain-containing protein ...	119	2,00E-29	

UR50:UniRef50_A0A2G9N4W7 Peptidase_S9 domain-containing protein n=...	119	3,00E-29	
UR50:UniRef50_F8KZH9 Hydrolase_4 domain-containing protein n=3 Tax=...	119	3,00E-29	Chlamydiae
UR50:UniRef50_A0A4V4HPV7Alpha/beta fold hydrolase n=1 Tax=Glycomy...	120	5,00E-29	Actinobacteria
UR50:UniRef50_A0A517YUI7Alpha/beta hydrolase family protein n=1 T...	119	5,00E-29	Planctomycetes
UR50:UniRef50_J2I3F4 Hydrolase_4 domain-containing protein n=20 Ta...	118	7,00E-29	Firmicutes
UR50:UniRef50_A0A7C5DQC2Alpha/beta fold hydrolase (Fragment) n=1 ...	120	7,00E-29	
UR50:UniRef50_A0A2M8TDW9Alpha/beta hydrolase n=1 Tax=Clostridium ...	117	9,00E-29	Clostridia
UR50:UniRef50_A0A4P6M108 Aminoacrylate hydrolase RutD n=10 Tax=Ter...	117	9,00E-29	Actinobacteria
UR50:UniRef50_A0A2J0KW78 Peptidase_S9 domain-containing protein n=...	117	1,00E-28	Parcubacteria
UR50:UniRef50_A0A0G0A911 Peptidase_S9 domain-containing protein n=...	117	1,00E-28	
UR50:UniRef50_UPI00096DB779alpha/beta hydrolase n=1 Tax=Paenibaci...	117	1,00E-28	Firmicutes
UR50:UniRef50_UPI000D19F70Dalpha/beta fold hydrolase n=1 Tax=Stre...	115	1,00E-28	Actinobacteria
UR50:UniRef50_A0A3A0CXX4 Peptidase_S9 domain-containing protein n=...	118	1,00E-28	Planctomycetes
UR50:UniRef50_A0A662G586 Hydrolase_4 domain-containing protein n=2...	116	3,00E-28	
UR50:UniRef50_UPI0006931F19alpha/beta fold hydrolase n=1 Tax=Clos...	117	3,00E-28	Clostridia
UR50:UniRef50_A0A7C4VHZ4Alpha/beta fold hydrolase (Fragment) n=1 ...	112	4,00E-28	
UR50:UniRef50_I3E8P1 PGAP1 family protein n=3 Tax=Bacillus TaxID=1...	116	4,00E-28	
UR50:UniRef50_A0A1V5HKM8 2-hydroxy-6-oxo-6-(2'-aminophenyl)hexa-2,...	115	4,00E-28	Actinobacteria
UR50:UniRef50_A0A396KRW0Alpha/beta fold hydrolase n=3 Tax=Terraba...	115	5,00E-28	Actinobacteria
UR50:UniRef50_A0A1G9L876 AB hydrolase-1 domain-containing protein ...	115	5,00E-28	Actinobacteria
UR50:UniRef50_A0A1U7NRK1 Dienelactone hydrolase n=5 Tax=Deinococcu...	115	5,00E-28	Deinococcus
UR50:UniRef50_I9NRE5 Hydrolase_4 domain-containing protein n=1 Tax...	115	7,00E-28	Firmicutes
UR50:UniRef50_C0Z4X2 Hydrolase_4 domain-containing protein n=63 Ta...	115	8,00E-28	
UR50:UniRef50_A0A1I7IGP7 Peptidase_S9 domain-containing protein n=...	114	1,00E-27	Clostridia
UR50:UniRef50_A0A497FIW7 Peptidase_S9 domain-containing protein n=...	114	1,00E-27	

UR50:UniRef50_A0A662Q0B5 Peptidase_S15 domain-containing protein n...	114	2,00E-27	
UR50:UniRef50_C2ER94 Hydrolase_4 domain-containing protein n=1 Tax...	114	2,00E-27	Firmicutes
UR50:UniRef50_A0A380NMF6 Acetoin dehydrogenase E2 subunit dihydrol...	115	2,00E-27	Firmicutes
UR50:UniRef50_R5VL94 Uncharacterized protein n=1 Tax=Clostridium s...	114	2,00E-27	Clostridia
UR50:UniRef50_G9QH89 AB hydrolase-1 domain-containing protein n=15...	114	2,00E-27	Firmicutes
UR50:UniRef50_A0A1M3BBP6 Hydrolase_4 domain-containing protein n=1...	114	2,00E-27	Chlamydiae
UR50:UniRef50_A0A4V2I6X8Alpha/beta hydrolase n=1 Tax=Alteromonas ...	113	5,00E-27	Proteobacteria
UR50:UniRef50_A0A0Q3WN81 Hydrolase_4 domain-containing protein n=5...	112	8,00E-27	Firmicutes
UR50:UniRef50_A0A6N6L3B6Alpha/beta fold hydrolase n=1 Tax=Chlorof...	112	9,00E-27	Chloroflexi
UR50:UniRef50_A0A1H8RZ11 Hydrolase_4 domain-containing protein n=4...	112	1,00E-26	
UR50:UniRef50_A0A6M8SQ21Alpha/beta fold hydrolase n=3 Tax=Deefgea...	111	1,00E-26	Proteobacteria
UR50:UniRef50_A0A1H6I7Y9 Hydrolase_4 domain-containing protein n=3...	112	1,00E-26	
UR50:UniRef50_A0A0J1JD40 Peptidase_S15 domain-containing protein n...	112	2,00E-26	Proteobacteria
UR50:UniRef50_A0A5J6YNP1Alpha/beta fold hydrolase n=2 Tax=Deinoco...	114	2,00E-26	Deinococcus
UR50:UniRef50_A0A1I1JJ50 Hydrolase_4 domain-containing protein n=1...	111	2,00E-26	
UR50:UniRef50_D1QPE8 Antioxidant, AhpC/TSA family n=20 Tax=root Ta...	114	2,00E-26	
UR50:UniRef50_UPI0005C4D4D5alpha/beta hydrolase n=1 Tax=Prevotell...	111	3,00E-26	Bacteroidetes
UR50:UniRef50_A0A089LPB3 DLH domain-containing protein n=14 Tax=Fi...	113	3,00E-26	Firmicutes
UR50:UniRef50_A0A150LL17 Hydrolase_4 domain-containing protein n=2...	110	3,00E-26	Firmicutes
UR50:UniRef50_C3MSB7 Hydrolase of the alpha/beta superfamily n=6 T...	110	5,00E-26	
UR50:UniRef50_A0A1F5Z3K4 Hydrolase_4 domain-containing protein n=1...	110	5,00E-26	
UR50:UniRef50_UPI001177A7F4alpha/beta fold hydrolase n=2 Tax=uncl...	111	6,00E-26	Clostridia
UR50:UniRef50_A0A1H7GTL4 Hydrolase_4 domain-containing protein n=1...	110	6,00E-26	Firmicutes
UR50:UniRef50_UPI00101D386Balpha/beta fold hydrolase n=1 Tax=Bact...	113	6,00E-26	Bacteroidetes
UR50:UniRef50_A0A0E3H183 Feruloyl esterase n=1 Tax=uncultured micr...	110	7,00E-26	

UR50:UniRef50_U2BHA2 Hydrolase, alpha/beta domain protein n=9 Tax=...	109	7,00E-26	Firmicutes
UR50:UniRef50_A0A2D5Z891 Peptidase_S9 domain-containing protein n=...	109	8,00E-26	Bacteroidetes
UR50:UniRef50_A0A101X5F5 Peptidase_S9 domain-containing protein n=...	110	8,00E-26	
UR50:UniRef50_A0A497G9W0 Hydrolase_4 domain-containing protein (Fr...	107	1,00E-25	
UR50:UniRef50_A0A3N5SCD3Alpha/beta fold hydrolase (Fragment) n=1 ...	105	1,00E-25	Chloroflexi
UR50:UniRef50_A0A0M9GU80 Hydrolase_4 domain-containing protein n=3...	109	1,00E-25	Firmicutes
UR50:UniRef50_A0A2D5V1B3 Hydrolase_4 domain-containing protein n=1...	109	1,00E-25	
UR50:UniRef50_A0A133Z6N3 X-Pro dipeptidyl-peptidase n=1 Tax=Bacter...	109	1,00E-25	Bacteroidetes
UR50:UniRef50_UPI00166C434Dalpha/beta fold hydrolase n=2 Tax=Paen...	109	1,00E-25	Firmicutes
UR50:UniRef50_UPI00105DDEB9alpha/beta fold hydrolase n=4 Tax=Cand...	111	1,00E-25	
UR50:UniRef50_U1QU38 Hydrolase_4 domain-containing protein n=11 Ta...	110	2,00E-25	Actinobacteria
UR50:UniRef50_X0V080 Hydrolase_4 domain-containing protein (Fragme...	107	2,00E-25	
UR50:UniRef50_E6K5Q2 Hydrolase_4 domain-containing protein n=15 Ta...	110	2,00E-25	Bacteroidetes
UR50:UniRef50_A0A3D0N7L6 Uncharacterized protein n=1 Tax=Armatimon...	105	3,00E-25	Armatimonadetes
UR50:UniRef50_G6AH16 Hydrolase_4 domain-containing protein n=3 Tax...	109	3,00E-25	Bacteroidetes
UR50:UniRef50_A0A1F7Y214 Hydrolase_4 domain-containing protein n=3...	108	3,00E-25	
UR50:UniRef50_A0A4R1NQQ4 Peptidase_S15 domain-containing protein n...	108	4,00E-25	
UR50:UniRef50_A0A2H6EVT0Alpha/beta hydrolase family protein n=2 T...	107	4,00E-25	
UR50:UniRef50_A0A1M6HZI0 Hydrolase_4 domain-containing protein n=5...	108	4,00E-25	Clostridia
UR50:UniRef50_A0A0V8J792 Peptidase_S9 domain-containing protein n=...	108	4,00E-25	Firmicutes
UR50:UniRef50_A0A3S4VC53 Non-haem bromoperoxidase BPO-A2 n=86 Tax=...	107	5,00E-25	Actinobacteria
UR50:UniRef50_A0A1X7LL25 Hydrolase_4 domain-containing protein n=1...	108	5,00E-25	Proteobacteria
UR50:UniRef50_A0A4P6JQU8Alpha/beta fold hydrolase n=1 Tax=Ktedono...	107	6,00E-25	Chloroflexi
UR50:UniRef50_A0A1H6SYR9 Hydrolase_4 domain-containing protein n=2...	108	6,00E-25	Firmicutes
UR50:UniRef50_UPI00068FCB42alpha/beta fold hydrolase n=1 Tax=Haem...	108	7,00E-25	Proteobacteria

UR50:UniRef50_A0A0R1T798 Alpha beta fold family hydrolase family p...	107	8,00E-25	Firmicutes
UR50:UniRef50_A0A380JFQ6 Cinnamoyl ester hydrolase n=22 Tax=Strept...	107	8,00E-25	Firmicutes
UR50:UniRef50_A0A1H5RRD6 Hydrolase_4 domain-containing protein n=2...	107	9,00E-25	Firmicutes
UR50:UniRef50_A0A0G0K0B6Alpha/beta hydrolase fold protein n=6 Tax...	107	9,00E-25	Bacteroidetes
UR50:UniRef50_A0A255SZ99 Hydrolase_4 domain-containing protein n=3...	108	9,00E-25	Bacteroidetes
UR50:UniRef50_A0A223HZC4Alpha/beta superfamily hydrolase n=1 Tax=...	102	1,00E-24	Firmicutes
UR50:UniRef50_A0A165WGN5 Hydrolase_4 domain-containing protein n=7...	107	1,00E-24	Firmicutes
UR50:UniRef50_A0A3A9C967Alpha/beta fold hydrolase n=1 Tax=bacteri...	107	2,00E-24	
UR50:UniRef50_A0A271JE86 Hydrolase_4 domain-containing protein n=1...	106	2,00E-24	
UR50:UniRef50_A0A1H6F7Y1 Esterase n=1 Tax=Thiotrichales bacterium ...	106	2,00E-24	Proteobacteria
UR50:UniRef50_A0A081C1G4 Peptidase S15 n=4 Tax=Bacteria TaxID=2 Re...	106	2,00E-24	
UR50:UniRef50_A0A4Q5NDK7Alpha/beta hydrolase n=1 Tax=bacterium Ta...	105	2,00E-24	
UR50:UniRef50_A0A2G8DDL3 Peptidase_S15 domain-containing protein n...	107	2,00E-24	Mesotoga
UR50:UniRef50_A0A292YFF2 Permease n=3 Tax=Bacillales TaxID=1385 Re...	106	3,00E-24	Firmicutes
UR50:UniRef50_A0A0G1NYN3 Dienelactone hydrolase n=2 Tax=Microgenom...	105	3,00E-24	
UR50:UniRef50_A0A1I1U6X8 Peptidase_S9 domain-containing protein n=...	105	3,00E-24	Firmicutes
UR50:UniRef50_A0A6M1ZSY0 2-succinyl-6-hydroxy-2, 4-cyclohexadiene-...	105	4,00E-24	Chlamydiae
UR50:UniRef50_R5PAK8 Hydrolase_4 domain-containing protein n=3 Tax...	105	4,00E-24	Bacteroidales
UR50:UniRef50_A0A2X0RBE42-succinyl-6-hydroxy-2,4-cyclohexadiene-1...	105	4,00E-24	
UR50:UniRef50_A0A3N2MZV7Alpha/beta fold hydrolase n=4 Tax=Muribac...	108	6,00E-24	Bacteroidetes
UR50:UniRef50_A0A076IS58Alpha/beta fold hydrolase n=6 Tax=Bactero...	107	6,00E-24	Bacteroidales
UR50:UniRef50_A0A5J5HT44Alpha/beta hydrolase n=2 Tax=Bacillus Tax...	105	6,00E-24	
UR50:UniRef50_A0A353BU96 Peptidase_S9 domain-containing protein n=...	101	7,00E-24	Firmicutes
UR50:UniRef50_A0A6N7XJW8Alpha/beta fold hydrolase n=1 Tax=Clostri...	104	8,00E-24	Clostridia

UR50:UniRef50_A0A370AVS2 Peptidase_S9 domain-containing protein n=...	105	8,00E-24	
UR50:UniRef50_A0A2M8KL77 Hydrolase_4 domain-containing protein n=3...	104	1,00E-23	
UR50:UniRef50_A0A137SUL4 Putative esterase n=1 Tax=Clostridiales b...	107	1,00E-23	Clostridia
UR50:UniRef50_A0A7C5LB81Alpha/beta hydrolase n=1 Tax=Deltaproteob...	103	1,00E-23	Proteobacteria
UR50:UniRef50_A0A0D0SH11 TWA4_scaffold00001, whole genome shotgun			
...	104	1,00E-23	Firmicutes
UR50:UniRef50_A0A1M5C420 Peptidase_S9 domain-containing protein n=...	104	1,00E-23	Proteobacteria
UR50:UniRef50_A0A4Y9FM63Alpha/beta hydrolase n=5 Tax=Streptococcu...	104	1,00E-23	Firmicutes
UR50:UniRef50_UPI00168B8E03alpha/beta fold hydrolase n=1 Tax=Baci...	103	2,00E-23	Firmicutes
UR50:UniRef50_A0A0U2W8A5Alpha/beta hydrolase n=2 Tax=Paenibacillu...	103	2,00E-23	Firmicutes
UR50:UniRef50_D9ZEN7 Peptidase_S9 domain-containing protein n=4 Ta...	103	2,00E-23	
UR50:UniRef50_A0A1F9I336 Peptidase_S9 domain-containing protein n=...	103	2,00E-23	
UR50:UniRef50_I2F4G1 LysM domain-containing protein,prolyl oligope...	104	3,00E-23	
UR50:UniRef50_F3P8S0 Carboxymuconolactone decarboxylase family pro...	107	3,00E-23	Actinomyces
UR50:UniRef50_A0A318KX93 Uncharacterized protein YhfF n=2 Tax=Diel...	107	3,00E-23	Firmicutes
UR50:UniRef50_A0A3P3XQ87 Peptidoglycan-binding lysin domain protei...	103	3,00E-23	Firmicutes
UR50:UniRef50_A0A2M7LW84 Hydrolase_4 domain-containing protein n=6...	103	4,00E-23	
UR50:UniRef50_UPI000783B427alpha/beta fold hydrolase n=1 Tax=Mass...	102	4,00E-23	Firmicutes
UR50:UniRef50_A0A1G7LLD5 Hydrolase_4 domain-containing protein n=6...	102	5,00E-23	
UR50:UniRef50_A0A564T726Fermentation/respiration switch protein n...	103	5,00E-23	Actinobacteria
UR50:UniRef50_L1P102 Hydrolase_4 domain-containing protein n=2 Tax...	99.8	5,00E-23	Bacteroidetes
UR50:UniRef50_A0A1H0BRC0 Peptidase_S9 domain-containing protein n=...	102	6,00E-23	Firmicutes
UR50:UniRef50_A0A661IDL4 Hydrolase_4 domain-containing protein n=7...	101	6,00E-23	
UR50:UniRef50_H3NGP5 Hydrolase_4 domain-containing protein n=5 Tax...	102	6,00E-23	Firmicutes
UR50:UniRef50_A0A6M1UEP4Alpha/beta hydrolase n=2 Tax=unclassified...	102	7,00E-23	Firmicutes
UR50:UniRef50_A0A1H9SN66 Peptidase_S9 domain-containing protein n=...	102	7,00E-23	Firmicutes

UR50:UniRef50_A0A6P1AI72 Prolyl oligopeptidase family serine pepti...	102	7,00E-23	
UR50:UniRef50_UPI0006CF9B7Dalpha/beta fold hydrolase n=1 Tax=Thal...	99.8	7,00E-23	Firmicutes
UR50:UniRef50_A0A3E0JMI5Alpha/beta hydrolase (Fragment) n=1 Tax=P...	99.0	7,00E-23	Firmicutes
UR50:UniRef50_A0A2E3QXW6Alpha/beta hydrolase n=1 Tax=Rhodothermac...	103	8,00E-23	Bacteroidetes
UR50:UniRef50_A0A6L3BG93 Hydrolase_4 domain-containing protein n=3...	102	9,00E-23	Firmicutes
UR50:UniRef50_A0A6N9I3X8Alpha/beta fold hydrolase n=2 Tax=Lactoba...	102	9,00E-23	Firmicutes
UR50:UniRef50_A0A5A7RTX7Alpha/beta fold hydrolase n=9 Tax=Thermop...	100	1,00E-22	
UR50:UniRef50_A0A2Z4L6Y5 Peptidase_S9 domain-containing protein n=...	101	1,00E-22	
UR50:UniRef50_A0A7C3Z5F7Alpha/beta hydrolase n=1 Tax=Deltaproteob...	100	1,00E-22	Proteobacteria
UR50:UniRef50_A0A1F6GWG6 Hydrolase_4 domain-containing protein n=8...	101	1,00E-22	Firmicutes
UR50:UniRef50_A0A357APR6 Peptidase_S9 domain-containing protein n=...	102	1,00E-22	Firmicutes
UR50:UniRef50_A0A024KFU3Alpha/beta superfamily hydrolase n=17 Tax...	101	2,00E-22	
UR50:UniRef50_F9ED99 Feruloyl esterase n=10 Tax=Actinomyces TaxID=...	102	2,00E-22	Actinomyces
UR50:UniRef50_UPI0012D8CA25alpha/beta fold hydrolase n=1 Tax=Paen...	100	2,00E-22	Firmicutes
UR50:UniRef50_A0A1G9S4D7 Hydrolase_4 domain-containing protein n=8...	100	2,00E-22	
UR50:UniRef50_A0A0G1H0A8 Carboxylesterase n=3 Tax=Parcubacteria gr...	100	2,00E-22	Parcubacteria
UR50:UniRef50_D9SNU3Alpha/beta superfamily hydrolase n=2 Tax=Clos...	101	2,00E-22	Clostridia
UR50:UniRef50_J4VLU0 Peptidase, S9A/B/C family, catalytic domain p...	101	2,00E-22	Bacteroidetes
UR50:UniRef50_A0A6M2ADF3 Multifunctional-autoprocessing repeats-in...	100	3,00E-22	Chlamydiae
UR50:UniRef50_A0A345Z1H0 Est1E (Fragment) n=52 Tax=Bacteria TaxID=...	100	3,00E-22	
UR50:UniRef50_A0A0U1P777 Hydrolase_4 domain-containing protein n=2...	100	3,00E-22	Proteobacteria
UR50:UniRef50_R5EN90 Hydrolase_4 domain-containing protein n=2 Tax...	100	3,00E-22	Proteobacteria
UR50:UniRef50_W0FQD8 Feruloyl esterase n=1 Tax=uncultured bacteriu...	101	3,00E-22	
UR50:UniRef50_UPI0014029154alpha/beta fold hydrolase n=1 Tax=Subd...	100	3,00E-22	Firmicutes
UR50:UniRef50_UPI00174C1BADalpha/beta fold hydrolase n=1 Tax=Cand...	103	4,00E-22	

UR50:UniRef50_S5XZS6 AB hydrolase-1 domain-containing protein n=16...	100	4,00E-22	Proteobacteria
UR50:UniRef50_UPI000C7C2368alpha/beta fold hydrolase n=1 Tax=Pygm...	100	4,00E-22	
UR50:UniRef50_A0A2G9N5X2 Peptidase_S9 domain-containing protein n=...	100	4,00E-22	
UR50:UniRef50_A0A2D6LJQ3 Peptidase_S9 domain-containing protein n=...	99.8	4,00E-22	
UR50:UniRef50_A0A2H0TBI0 Peptidase_S9 domain-containing protein n=...	100	4,00E-22	
UR50:UniRef50_A0A7C5TGY3Alpha/beta fold hydrolase (Fragment) n=2 ...	96.7	4,00E-22	
UR50:UniRef50_A0A7C4BFQ0Alpha/beta fold hydrolase (Fragment) n=1 ...	99.4	4,00E-22	
UR50:UniRef50_A0A7C3HHR4 Hydrolase_4 domain-containing protein (Fr...	97.8	5,00E-22	Bacteroidetes
UR50:UniRef50_A0A7D5Z3X2Alpha/beta fold hydrolase n=5 Tax=Chromob...	99.8	5,00E-22	Proteobacteria
UR50:UniRef50_UPI001370834Balpha/beta hydrolase n=1 Tax=Clostridi...	100	5,00E-22	Clostridia
UR50:UniRef50_A0A1M5JF38 Peptidase_S9 domain-containing protein n=...	100	5,00E-22	Proteobacteria
UR50:UniRef50_C6LBC1 Acetyl xylan esterase (AXE1) n=11 Tax=Bacteri...	100	5,00E-22	
UR50:UniRef50_U2LY33 Acetyl xylan esterase n=2 Tax=Treponema lecit...	101	5,00E-22	Spirochaetes
UR50:UniRef50_A0A223HZ34Alpha/beta superfamily hydrolase n=1 Tax=...	96.7	6,00E-22	Firmicutes
UR50:UniRef50_UPI0006B67ADEalpha/beta fold hydrolase n=1 Tax=Niam...	100	6,00E-22	Actinobacteria
UR50:UniRef50_A0A3N9NNQ0 Peptidase_S9 domain-containing protein n=...	99.4	7,00E-22	
UR50:UniRef50_A0A1C5ZT52 Prolyl tripeptidyl peptidase n=12 Tax=Clo...	100	7,00E-22	Clostridia
UR50:UniRef50_A0A3S4WJW1 Acetoin dehydrogenase E2 subunit dihydrol...	99.4	7,00E-22	Actinomyces
UR50:UniRef50_A0A0P9DTF9Alpha/beta hydrolase family protein n=1 T...	98.6	8,00E-22	
UR50:UniRef50_M2NDT4 Peptidase_S9 domain-containing protein n=3 Ta...	99.0	8,00E-22	Firmicutes
UR50:UniRef50_I8AKB0 PGAP1 family protein n=1 Tax=Fictibacillus ma...	99.4	9,00E-22	Firmicutes
UR50:UniRef50_E0E1U4 Hydrolase_4 domain-containing protein n=3 Tax...	99.0	1,00E-21	Firmicutes
UR50:UniRef50_Q822R5 Hydrolase_4 domain-containing protein n=71 Ta...	100	1,00E-21	Chlamydiae
UR50:UniRef50_A0A555ZF10Alpha/beta fold hydrolase n=5 Tax=Pantoea...	99.8	1,00E-21	Proteobacteria
UR50:UniRef50_A0A3R8MIT0Alpha/beta fold hydrolase n=1 Tax=Biberst...	99.4	1,00E-21	Proteobacteria

UR50:UniRef50_A0A4R4PJ68Alpha/beta fold hydrolase n=2 Tax=Strepto...	98.2	1,00E-21	Actinobacteria
UR50:UniRef50_A0A329NCT8Alpha/beta fold hydrolase n=19 Tax=Aeroco...	100	1,00E-21	Firmicutes
UR50:UniRef50_A0A1V6GYM0Alpha/beta hydrolase family protein n=1 T...	99.4	2,00E-21	Thermotogae
UR50:UniRef50_A0A1I4HQ83 Peptidase_S9 domain-containing protein n=...	98.6	2,00E-21	Firmicutes
UR50:UniRef50_A0A380K4G1 Cinnamoyl ester hydrolase n=2 Tax=Strepto...	98.6	2,00E-21	Firmicutes
UR50:UniRef50_A0A135YPI0 3-dehydroquinase dehydratase n=5 Tax=Pept...	101	2,00E-21	Firmicutes
UR50:UniRef50_J6HBL6 Peptidase, S9A/B/C family, catalytic domain p...	98.2	2,00E-21	Firmicutes
UR50:UniRef50_A0A0G0PZX8 Hydrolase of the alpha/beta superfamily n...	98.2	2,00E-21	
UR50:UniRef50_UPI0009340A05alpha/beta fold hydrolase n=1 Tax=Lact...	98.2	2,00E-21	Firmicutes
UR50:UniRef50_A0A437SCN9 Hydrolase_4 domain-containing protein n=2...	100	2,00E-21	Firmicutes
UR50:UniRef50_UPI000F7782A8alpha/beta fold hydrolase n=1 Tax=Lact...	99.4	2,00E-21	Firmicutes
UR50:UniRef50_A0A2E0L7X2Alpha/beta hydrolase n=1 Tax=Anaerolineac...	98.6	2,00E-21	Chloroflexi
UR50:UniRef50_UPI0009E6DB92alpha/beta hydrolase n=4 Tax=Leptotric...	98.6	2,00E-21	Fusobacteria
UR50:UniRef50_Q9Z923 Acyltransferase family n=7 Tax=Chlamydia Taxl...	98.2	3,00E-21	Chlamydiae
UR50:UniRef50_A0A0G1I5A7 Hydrolase n=2 Tax=Parcubacteria group Tax...	97.8	3,00E-21	Parcubacteria
UR50:UniRef50_A0A554MFT6 Hydrolase_4 domain-containing protein n=2...	97.4	4,00E-21	Parcubacteria
UR50:UniRef50_UPI0003F95ED9alpha/beta fold hydrolase n=1 Tax=Alic...	98.6	4,00E-21	Alicyclobacillus
UR50:UniRef50_S3XCK4 Hydrolase_4 domain-containing protein n=13 Ta...	98.6	4,00E-21	Actinobacteria
UR50:UniRef50_D3R4M0 Cinnamoyl ester hydrolase n=42 Tax=Bifidobact...	97.8	4,00E-21	Bifidobacterium
UR50:UniRef50_UPI0009DBBE97alpha/beta hydrolase n=1 Tax=Butyrivib...	97.8	4,00E-21	Firmicutes
UR50:UniRef50_A0A259UH65 Putative aminoacylate hydrolase RutD n=8...	97.4	4,00E-21	Firmicutes
UR50:UniRef50_R5XBI4 Uncharacterized protein n=1 Tax=Anaerotruncus...	98.6	5,00E-21	Firmicutes
UR50:UniRef50_A0A0G1UCW1 Hydrolase_4 domain-containing protein n=3...	97.1	5,00E-21	
UR50:UniRef50_UPI0012B7D3BEalpha/beta hydrolase n=1 Tax=Paenibaci...	97.1	5,00E-21	Firmicutes
UR50:UniRef50_R5QHM6 Feruloyl esterase n=1 Tax=Ruminococcus sp. CA...	98.2	5,00E-21	Firmicutes

UR50:UniRef50_A0A7C4SRC4Alpha/beta fold hydrolase n=1 Tax=Candida...	95.9	5,00E-21	
UR50:UniRef50_A0A6L3TJK6Alpha/beta hydrolase n=2 Tax=Vibrionaceae...	97.4	6,00E-21	Proteobacteria
UR50:UniRef50_A0A3C0IYR8Alpha/beta hydrolase n=3 Tax=Firmicutes b...	97.8	6,00E-21	Firmicutes
UR50:UniRef50_A0A1Y0FS27 Hydrolase_4 domain-containing protein n=1...	97.8	6,00E-21	Proteobacteria
UR50:UniRef50_M8DB53 Hydrolase_4 domain-containing protein n=11 Ta...	97.1	7,00E-21	Firmicutes
UR50:UniRef50_A0A4S3B3M6Alpha/beta fold hydrolase n=2 Tax=Vagococ...	97.8	8,00E-21	Firmicutes
UR50:UniRef50_UPI0009FE83ABalpha/beta fold hydrolase n=1 Tax=Deme...	96.3	9,00E-21	Actinobacteria
UR50:UniRef50_A0A0J1IQ41 Hydrolase_4 domain-containing protein n=6...	96.3	1,00E-20	
UR50:UniRef50_UPI0009F92FE1alpha/beta fold hydrolase n=1 Tax=Alic...	96.7	1,00E-20	Alicyclobacillus
UR50:UniRef50_A6VR29 Cinnamoyl ester hydrolase n=1 Tax=Actinobacil...	96.7	1,00E-20	Proteobacteria
UR50:UniRef50_A0A260EDK6Alpha/beta hydrolase n=1 Tax=Rhodococcus ...	96.3	1,00E-20	Actinobacteria
UR50:UniRef50_A0A1T5IU94 Hydrolase_4 domain-containing protein n=1...	95.5	2,00E-20	Bacteroidetes
UR50:UniRef50_A0A3D2N8D8 Thioredoxin domain-containing protein (Fr...	97.1	2,00E-20	Bacteroidetes
UR50:UniRef50_A0A496Q2A4 Hydrolase_4 domain-containing protein n=3...	95.5	2,00E-20	Verrucomicrobia
UR50:UniRef50_A0A353SAF3 Cinnamoyl ester hydrolase n=2 Tax=Bacteri...	96.3	2,00E-20	
UR50:UniRef50_UPI00160DE3FAalpha/beta fold hydrolase n=1 Tax=Cate...	96.3	3,00E-20	Firmicutes
UR50:UniRef50_I3PVE7 Ferulic acid esterase 3 n=1 Tax=uncultured ba...	94.7	3,00E-20	
UR50:UniRef50_A0A1M4MW73 Putative alpha/beta hydrolase n=4 Tax=Don...	95.1	3,00E-20	Proteobacteria
UR50:UniRef50_C2EMB3 Feruloyl esterase n=53 Tax=Lactobacillus Taxl...	95.9	3,00E-20	Firmicutes
UR50:UniRef50_A0A1D2X1C7 Hydrolase_4 domain-containing protein n=1...	94.7	4,00E-20	Alicyclobacillus
UR50:UniRef50_UPI0004145E31alpha/beta hydrolase n=1 Tax=Ruminococ...	94.7	4,00E-20	Firmicutes
UR50:UniRef50_D1NU52 Hydrolase_4 domain-containing protein n=4 Tax...	95.1	4,00E-20	Bifidobacterium
UR50:UniRef50_UPI00040108C7alpha/beta fold hydrolase n=1 Tax=Alic...	95.1	4,00E-20	Alicyclobacillus
UR50:UniRef50_A0A3B9FYI3Alpha/beta hydrolase n=1 Tax=Erysipelotri...	94.7	4,00E-20	Firmicutes
UR50:UniRef50_A0A0Q5GFR9 AB hydrolase-1 domain-containing protein ...	94.4	4,00E-20	Proteobacteria

UR50:UniRef50_C5ESK4 Hydrolase_4 domain-containing protein n=11 Ta...	94.4	5,00E-20	Clostridiales
UR50:UniRef50_A0A0N0GPE5 Putative aminoacrylate hydrolase RutD n=4...	94.0	6,00E-20	Proteobacteria
UR50:UniRef50_A0A520KX16Alpha/beta fold hydrolase n=4 Tax=Candida...	93.2	6,00E-20	
UR50:UniRef50_UPI0015EF4CE9 hypothetical protein n=1 Tax=Paenactin...	94.0	6,00E-20	Actinomyces
UR50:UniRef50_A0A1F7UVW0 Hydrolase_4 domain-containing protein n=2...	94.0	7,00E-20	Parcubacteria
UR50:UniRef50_A0A1Y4KAL8 Hydrolase_4 domain-containing protein n=3...	95.1	8,00E-20	Actinobacteria
UR50:UniRef50_A0A6B1E4X9Alpha/beta hydrolase n=1 Tax=Chloroflexi ...	95.1	8,00E-20	Chloroflexi
UR50:UniRef50_C5NUM3 Feruloyl esterase n=5 Tax=Terrabacteria group...	94.7	9,00E-20	Actinobacteria
UR50:UniRef50_A0A0L0BGU9 Hydrolase_4 domain-containing protein n=1...	94.0	9,00E-20	Actinobacteria
UR50:UniRef50_A0A1G2U5D4 Hydrolase_4 domain-containing protein n=3...	93.6	9,00E-20	Proteobacteria
UR50:UniRef50_A0A561N4N3 Peptidase_S15 domain-containing protein n...	94.4	1,00E-19	Proteobacteria
UR50:UniRef50_A0A136LZ38Alpha/beta hydrolase family protein n=1 T...	93.2	1,00E-19	
UR50:UniRef50_A0A0V8S178 Hydrolase_4 domain-containing protein n=2...	93.2	1,00E-19	Actinobacteria
UR50:UniRef50_F9VK43 Hydrolase_4 domain-containing protein n=18 Ta...	93.6	1,00E-19	Firmicutes
UR50:UniRef50_A0A3G9JW11 Cinnamoyl ester hydrolase n=1 Tax=Intesti...	94.0	1,00E-19	
UR50:UniRef50_A0A329LBX6Alpha/beta hydrolase n=1 Tax=Paenibacillu...	93.6	1,00E-19	Firmicutes
UR50:UniRef50_F6LQK6 Feruloyl esterase III n=1 Tax=Cellulosilyticu...	95.1	1,00E-19	Firmicutes
UR50:UniRef50_UPI0003F61EF9alpha/beta fold hydrolase n=2 Tax=Fuso...	93.6	2,00E-19	Fusobacteria
UR50:UniRef50_UPI0008F96E33alpha/beta fold hydrolase n=1 Tax=Trao...	93.2	2,00E-19	
UR50:UniRef50_A0A2N5M6E1Alpha/beta hydrolase n=2 Tax=Peribacillus...	93.2	2,00E-19	Firmicutes
UR50:UniRef50_A0A1H1LNM3 Hydrolase_4 domain-containing protein n=2...	93.2	2,00E-19	Verrucomicrobia
UR50:UniRef50_A0A559NLZ7 Hydrolase_4 domain-containing protein n=3...	92.8	2,00E-19	Proteobacteria
UR50:UniRef50_A0A177XJV8 Hydrolase_4 domain-containing protein n=1...	93.2	2,00E-19	Actinobacteria
UR50:UniRef50_A0A1D7QRA3Alpha/beta superfamily hydrolase n=1 Tax=...	92.8	2,00E-19	Firmicutes
UR50:UniRef50_A0A1F9LWW8 Hydrolase_4 domain-containing protein (Fr...	91.3	2,00E-19	Proteobacteria

UR50:UniRef50_R7CWQ7 Hydrolase_4 domain-containing protein n=17 Ta...	94.0	2,00E-19	Bacteroidetes
UR50:UniRef50_UPI000F794EF8alpha/beta fold hydrolase n=1 Tax=Agri...	93.2	2,00E-19	Firmicutes
UR50:UniRef50_S3JS07 Peptidase_S9 domain-containing protein n=9 Ta...	93.2	2,00E-19	
UR50:UniRef50_A0A1I2U708 Hydrolase_4 domain-containing protein n=1...	92.4	3,00E-19	Firmicutes
UR50:UniRef50_A0A497N1W1 Hydrolase_4 domain-containing protein (Fr...	93.2	3,00E-19	
UR50:UniRef50_UPI00166AE99Falpha/beta hydrolase n=1 Tax=Paenibaci...	92.8	3,00E-19	Firmicutes
UR50:UniRef50_D7CN41 Putative hydrolase n=1 Tax=Syntrophothermus l...	92.4	3,00E-19	Firmicutes
UR50:UniRef50_A0A662FA48 Hydrolase_4 domain-containing protein n=1...	92.4	3,00E-19	
UR50:UniRef50_UPI0015FC81A4alpha/beta fold hydrolase n=1 Tax=Limo...	92.8	3,00E-19	Firmicutes
UR50:UniRef50_A0A202DGJ1 Uncharacterized protein n=1 Tax=archaeon ...	92.0	3,00E-19	
UR50:UniRef50_A0A1H7FMX4 Hydrolase_4 domain-containing protein n=2...	92.0	3,00E-19	
UR50:UniRef50_UPI0011E59630alpha/beta fold hydrolase n=2 Tax=Neis...	92.4	3,00E-19	Proteobacteria
UR50:UniRef50_A0A0R1W5M7 Alpha-beta superfamily hydrolase n=2 Tax=...	92.4	3,00E-19	Firmicutes
UR50:UniRef50_A0A1G9NTZ2 Peptidase_S9 domain-containing protein n=...	92.4	3,00E-19	Actinobacteria
UR50:UniRef50_A0A0M2PHI3 Hydrolase_4 domain-containing protein n=5...	91.7	3,00E-19	
UR50:UniRef50_UPI0013694A57alpha/beta fold hydrolase n=1 Tax=Lact...	91.7	4,00E-19	Firmicutes
UR50:UniRef50_A0A1F9C1G2 Hydrolase_4 domain-containing protein n=6...	91.7	4,00E-19	Proteobacteria
UR50:UniRef50_UPI0004149386alpha/beta hydrolase n=3 Tax=unclassif...	90.9	5,00E-19	Firmicutes
UR50:UniRef50_A0A1F6H6E8 Hydrolase_4 domain-containing protein n=9...	91.3	5,00E-19	
UR50:UniRef50_A0A1E5XPH1 Peptidase_S15 domain-containing protein n...	92.4	5,00E-19	Proteobacteria
UR50:UniRef50_A0A2H0SXA8 Peptidase_S9 domain-containing protein n=...	91.7	5,00E-19	
UR50:UniRef50_A0A4P8L0E3Alpha/beta fold hydrolase n=2 Tax=Desulfo...	92.0	5,00E-19	Proteobacteria
UR50:UniRef50_A0A1Q5PUB6 Hydrolase_4 domain-containing protein n=3...	93.2	6,00E-19	Actinobacteria
UR50:UniRef50_A0A2N0Z197Alpha/beta hydrolase n=2 Tax=Bacillus nea...	91.7	6,00E-19	Firmicutes
UR50:UniRef50_Q74J82 Hydrolase_4 domain-containing protein n=32 Ta...	91.3	6,00E-19	Actinobacteria

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UR50:UniRef50_A0A3D0XXE5 Hydrolase_4 domain-containing protein n=1...	91.3	7,00E-19	Clostridiales
UR50:UniRef50_A0A2T6DB01 Hydrolase_4 domain-containing protein n=1...	93.2	8,00E-19	Verrucomicrobia
UR50:UniRef50_A0A430JHL9Alpha/beta fold hydrolase n=1 Tax=Paeniba...	94.4	8,00E-19	Firmicutes
UR50:UniRef50_W0FJ07 Cinnamoyl ester hydrolase n=1 Tax=uncultured ...	90.9	8,00E-19	
UR50:UniRef50_A0A2K1P362 Peptidase_S9 domain-containing protein n=...	92.0	9,00E-19	Thermotogae
UR50:UniRef50_A0A074LQC2 Peptidase_S9 domain-containing protein n=...	91.3	9,00E-19	Actinobacteria
UR50:UniRef50_A0A2H0V175 Hydrolase_4 domain-containing protein n=1...	90.5	1,00E-18	
UR50:UniRef50_P94315 CinII n=3 Tax=Lachnospiraceae TaxID=186803 Re...	91.3	1,00E-18	Firmicutes
UR50:UniRef50_A0A2N1SD64 Peptidase_S9 domain-containing protein n=...	91.3	1,00E-18	Spirochaetes
UR50:UniRef50_A0A3D0Y834 Hydrolase_4 domain-containing protein n=1...	91.3	1,00E-18	Clostridiales
UR50:UniRef50_A0A0G0YHJ5 PGAP1 family protein n=1 Tax=candidate di...	90.5	1,00E-18	
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UR50:UniRef50_A0A350J963Alpha/beta hydrolase n=1 Tax=Clostridiale...	90.9	2,00E-18	Clostridiales
UR50:UniRef50_A0A397S100 Hydrolase_4 domain-containing protein n=1...	89.7	2,00E-18	Tenericutes
UR50:UniRef50_F7V472 Hydrolase_4 domain-containing protein n=2 Tax...	90.9	2,00E-18	Clostridiales
UR50:UniRef50_A0A357FXM6 Uncharacterized protein n=1 Tax=Rhodospir...	90.5	2,00E-18	Proteobacteria
UR50:UniRef50_A0A0M0G2K1 AB hydrolase-1 domain-containing protein ...	90.1	2,00E-18	Actinobacteria

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UR50:UniRef50_A0A1F6GXQ6 Hydrolase_4 domain-containing protein n=2...	89.4	3,00E-18	
UR50:UniRef50_A0A1I6KBM2 Uncharacterized protein n=2 Tax=Clostridi...	90.1	3,00E-18	Clostridiales
UR50:UniRef50_A0A1F6JW03 Peptidase_S9 domain-containing protein n=...	89.4	3,00E-18	
UR50:UniRef50_UPI001302B074alpha/beta fold hydrolase n=1 Tax=Neie...	90.5	3,00E-18	
UR50:UniRef50_B9JMY9 Peptidase_S15 domain-containing protein n=34 ...	90.1	4,00E-18	Proteobacteria
UR50:UniRef50_A0A0R1M4F8 Hydrolase_4 domain-containing protein n=4...	89.4	4,00E-18	Firmicutes
UR50:UniRef50_A0A2D6NUQ7 Hydrolase_4 domain-containing protein n=1...	89.4	4,00E-18	
UR50:UniRef50_A0A1G1WQF5 Peptidase_S9 domain-containing protein n=...	89.4	4,00E-18	
UR50:UniRef50_A0A7C3E629Alpha/beta fold hydrolase n=2 Tax=Proteob...	89.0	5,00E-18	Proteobacteria
UR50:UniRef50_J8ZQX3 DLH domain-containing protein n=7 Tax=Bacilla...	89.4	5,00E-18	Firmicutes
UR50:UniRef50_A0A661KJ40 Hydrolase_4 domain-containing protein n=5...	88.6	5,00E-18	Proteobacteria
UR50:UniRef50_B9Y2M8 Hydrolase_4 domain-containing protein n=4 Tax...	90.1	5,00E-18	Firmicutes
UR50:UniRef50_A0A3A4QXA2Alpha/beta hydrolase n=1 Tax=Candidatus A...	89.0	5,00E-18	
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UR50:UniRef50_A0A3N2D7Y1 Hydrolase_4 domain-containing protein n=3...	90.1	5,00E-18	Actinobacteria
UR50:UniRef50_A0A2H0RIP5 Hydrolase_4 domain-containing protein n=1...	89.0	6,00E-18	
UR50:UniRef50_A0A3D9TET4 Hydrolase_4 domain-containing protein n=1...	89.0	6,00E-18	Actinobacteria
UR50:UniRef50_A0A367GEL2 AB hydrolase-1 domain-containing protein ...	90.1	6,00E-18	
UR50:UniRef50_A0A2J0KVP3 Hydrolase_4 domain-containing protein n=1...	87.0	6,00E-18	Parcubacteria
UR50:UniRef50_A0A2M7V3Q0 Hydrolase_4 domain-containing protein n=1...	88.6	6,00E-18	
UR50:UniRef50_UPI0017489C35alpha/beta fold hydrolase n=2 Tax=Cand...	88.6	8,00E-18	
UR50:UniRef50_A0A259T3W1 Hydrolase_4 domain-containing protein n=7...	88.6	8,00E-18	Firmicutes
UR50:UniRef50_A0A559Q1I7 Hydrolase_4 domain-containing protein n=6...	89.0	9,00E-18	Proteobacteria
UR50:UniRef50_A0A358M881 Uncharacterized protein n=1 Tax=Clostridi...	90.5	9,00E-18	Clostridiales

UR50:UniRef50_A0A1F6ZG60 Uncharacterized protein n=1 Tax=Candidatu...	88.2	9,00E-18	
UR50:UniRef50_A0A3A4KEE1Alpha/beta hydrolase n=1 Tax=Myxococcales...	88.2	1,00E-17	Proteobacteria
UR50:UniRef50_A0A173XYS6 Trifunctional nucleotide phosphoesterase ...	91.3	1,00E-17	
UR50:UniRef50_A0A256ZTN4 Peptidase_S9 domain-containing protein n=...	88.2	1,00E-17	
UR50:UniRef50_A0A4V1ALQ1Alpha/beta fold hydrolase n=1 Tax=Acetila...	87.8	1,00E-17	Firmicutes
UR50:UniRef50_A0A1Y4RKX6 Uncharacterized protein n=1 Tax=Lachnoclo...	90.5	1,00E-17	Clostridia
UR50:UniRef50_R2XTC0 Hydrolase_4 domain-containing protein n=2 Tax...	89.0	1,00E-17	Firmicutes
UR50:UniRef50_R7KXY0 Uncharacterized protein n=2 Tax=environmental...	90.9	1,00E-17	
UR50:UniRef50_A0A6L9HLT7Alpha/beta fold hydrolase n=1 Tax=Lachnos...	86.3	1,00E-17	Firmicutes
UR50:UniRef50_A0A1Q4AZQ5 Hydrolase_4 domain-containing protein n=1...	87.8	1,00E-17	Firmicutes
UR50:UniRef50_A0A1A5I0W9 Hydrolase_4 domain-containing protein n=6...	88.6	1,00E-17	Proteobacteria
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UR50:UniRef50_A0A0R2LSN0 Hydrolase_4 domain-containing protein n=3...	87.8	1,00E-17	Firmicutes
UR50:UniRef50_A0A1I4W7R2 Peptidase_S9 domain-containing protein n=...	88.2	1,00E-17	Bacteroidetes
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UR50:UniRef50_A0A0G0JDF6 Peptidase_S9 domain-containing protein n=...	87.4	2,00E-17	
UR50:UniRef50_A0A387BUD8Alpha/beta fold hydrolase n=2 Tax=Microba...	88.6	2,00E-17	Actinobacteria
UR50:UniRef50_A0A646JSY2Alpha/beta hydrolase n=3 Tax=Lactobacilla...	87.4	2,00E-17	Firmicutes
UR50:UniRef50_A0A151BXR6 AB hydrolase-1 domain-containing protein ...	87.4	2,00E-17	Actinobacteria
UR50:UniRef50_A0A6N9Q4P4Alpha/beta hydrolase n=2 Tax=Chengkuiheng...	87.8	2,00E-17	
UR50:UniRef50_A0A3G8ZMP4 Hydrolase_4 domain-containing protein n=2...	89.0	2,00E-17	Actinobacteria
UR50:UniRef50_A0A351U0D7 Peptidase_S9 domain-containing protein n=...	87.4	2,00E-17	Proteobacteria
UR50:UniRef50_A0A0K9R3E3 Hydrolase_4 domain-containing protein n=1...	87.4	2,00E-17	

UR50:UniRef50_UPI00067B2E5B	alpha/beta fold hydrolase n=1 Tax=Olse...	87.8	2,00E-17	Actinobacteria
UR50:UniRef50_A0A0S2W8M7	5'-nucleotidase n=8 Tax=Clostridiales Tax...	90.1	3,00E-17	Clostridiales

Legend Supplementary Table S7: A search against the UniProtKB50 protein sequence data base revealed the top scoring 1000 sequence families with a minimal threshold score of 70 and an E value of 7×10^{-4} and archaeal phyla, to the extent organisms of origin were unambiguously determined.

Supplementary Table S8: *Tth* MGL sequence/structure relations to other α/β hydrolases

Sequence ID	Protein name	Organism	Phylum	Reference	Score ^a	E value ^a	PDB ID ^{b,c}	Q score ^b	Rmsd ^b	Seq. identity ^b	Lid ^d type	Lid length ^d	Assembly
I9KU82	Monoacylglycerol lipase	<i>T. siderophilus</i>	Firmicutes				7Q4J				HBH	43	Dimer
D3YEX6	Cinnamoyl esterase	<i>L. johnsonii</i>	Firmicutes	¹⁴	117,00	1,00E-31	3S2Z*	0,72	1,18	0,32	HBH	46	Dimer
D2YW37	Feruloyl esterase	<i>B. proteoclasticus</i>	Firmicutes	¹³	102,00	1,00E-25	2WTM*	0,68	1,31	0,30	HBH	46	Dimer
D0MHY8	Osmotically inducible protein C	<i>R. marinus</i>	Bacteroidetes	¹⁵	82,00	2,00E-17	5CML	0,44	2,22	0,23	HBH**	48	Dimer
B9JYM4	Hydroxymuconic semialdehyde hydrolase	<i>A. vitis</i>	Proteobacteria	unpublished	55,80	9,00E-09	3LLC	0,56	1,88	0,22	HBH	50	Monomer
Q6PE15	Palmitoyl-protein thioesterase A	<i>M. musculus</i>	(Vertebrate)	¹⁶	51,60	3,00E-07	6NY9	0,52	1,99	0,20	HBH	51	Monomer
Q83AV9	Alpha/beta hydrolase	<i>C. burnetii</i>	Proteobacteria	¹⁷	47,40	4,00E-06	3TRD				no		Monomer
O30361	Xylan esterase	<i>T. saccharolyticum</i>	Firmicutes	unpublished	39,30	0,003	3FCY	0,32	2,11	0,20	HB	43	Trimer
Q65EQ1	Carboxylesterase	<i>B. licheniformis</i>	Firmicutes	unpublished	38,50	0,005	6NKG				HB	54	Monomer
Q1QEU6	Triacylglycerol lipase	<i>P. cryohalolentis</i>	Proteobacteria	unpublished	37,40	0,014	4NS4	0,36	2,03	0,22	HB	54	Monomer

A0A6I8WFT7	Monoacylglycerol lipase	<i>P. ferrophilus</i>	Euryarchaeota	unpublished	34,70	0,1	6QE2					HBH	66	Dimer
B0V9K7	Dienelactone hydrolase	<i>A. baumannii</i>	Proteobacteria	unpublished	34,70	0,089	4OPM					HB	70	Dimer
A0A6L8NUA5	Bromoperoxidase	<i>B. anthracis</i>	Firmicutes	unpublished	33,50	0,23	3FOB					HB	80	Trimer
P82597	Monoacylglycerol lipase	<i>Bacillus sp.</i>	Firmicutes	¹⁸	32,70	0,41	4KE8*	0,45	2,16	0,21		HBH	51	Dimer
O07427	Monoacylglycerol lipase	<i>M. tuberculosis</i>	Actinobacteria	¹⁹	30,40	2,30	6EIC	0,45	1,86	0,18		HBH	70	Trimer
A0A0B5WSQ6	Lysophospho-lipase	<i>B. coagulans</i>	Firmicutes	²⁰	28,90	7,7	5O7G*	0,39	2,13	0,15		HB	88	Monomer
J4WAT9	AB hydrolase-1	<i>B. bassiana</i>	Ascomycota	²¹			7D79*	0,49	2,00	0,19		HBH	84	Dimer

A BLAST search using the *Tth* MGL sequence (A0A1I1X7Z7) against UniProtKB restricted to sequences with known 3D structures revealed 106 hits. From this pool of sequences, 15 non-redundant validated targets were selected (**Figure 7A**). These sequences were further analyzed by structural superposition using the PDBeFold server ¹⁰. For ten out of fifteen selected hits, PDBeFold produced a reliable structure-based alignment for both catalytic domain segments (**Supplementary wS2**). In addition, an α/β hydrolase from *Beauveria bassiana* (J4WAT9) was included, which was detected in the PDBeFold search but not in the BLAST search (**Supplementary Table S8**).

^a Taken from **Supplementary Table S7**.

^b Taken from PDBeFold output using the structure of *Tth* MGL as template against the PDB.

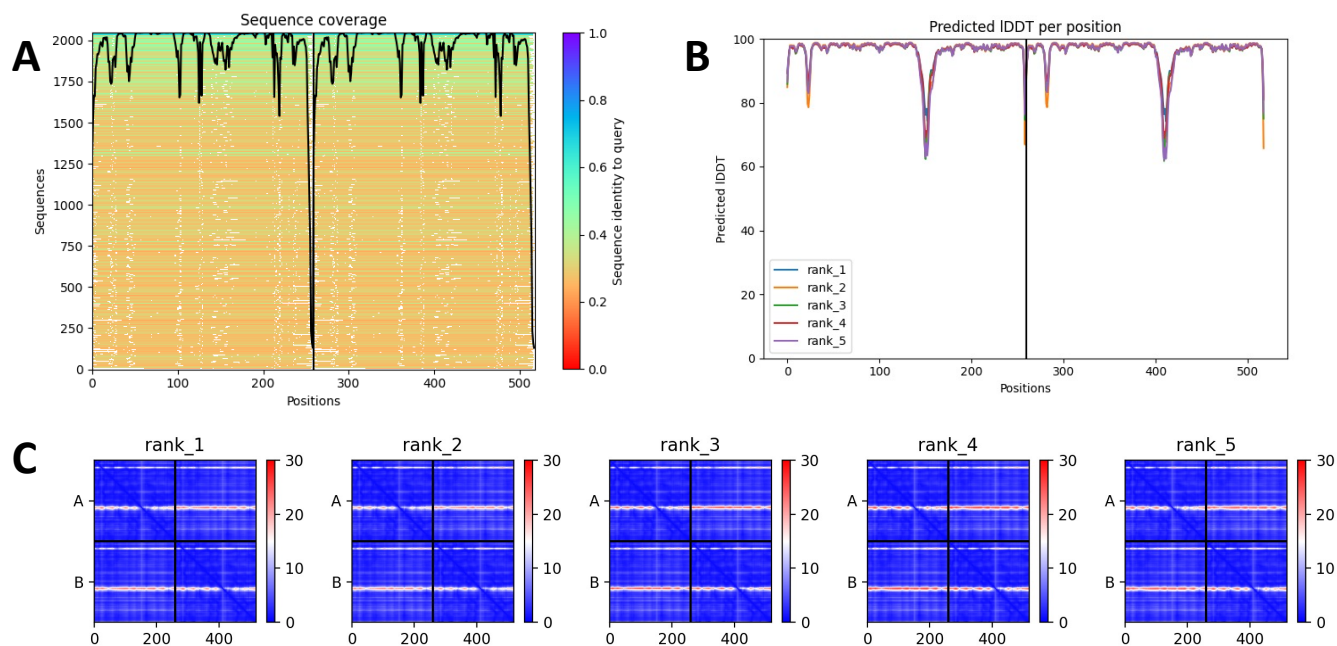
^c, *, indicates PDB code cited represents multiple structures deposited in PDB of the same sequence.

^d, for further details see **Figure 7A**; **, indicates a non-canonical HBH lid domain (for further details, see text)

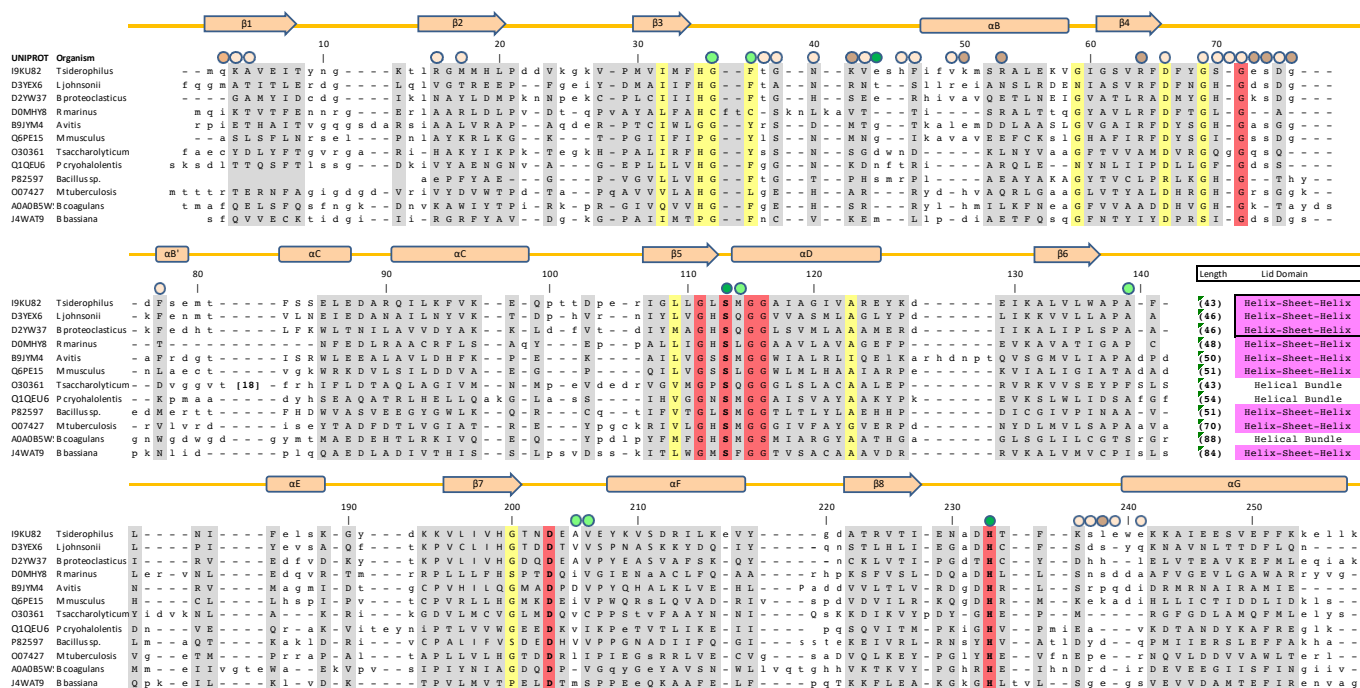
References:

1. Royter, M.; Schmidt, M.; Elend, C.; Hobenreich, H.; Schafer, T.; Bornscheuer, U. T.; Antranikian, G., Thermostable lipases from the extreme thermophilic anaerobic bacteria *Thermoanaerobacter thermohydrosulfuricus* SOL1 and *Caldanaerobacter subterraneus* subsp. *tengcongensis*. *Extremophiles* **2009**, 13 (5), 769-83.
2. Rypniewski, W. R.; Holden, H. M.; Rayment, I., Structural consequences of reductive methylation of lysine residues in hen egg white lysozyme: an X-ray analysis at 1.8-Å resolution. *Biochemistry* **1993**, 32 (37), 9851-8.
3. Otwinowski, Z.; Minor, W., Processing of X-ray diffraction data collected in oscillation mode. *Method Enzymol* **1997**, 276, 307-326.
4. Sheldrick, G. M., A short history of SHELX. *Acta Crystallogr A* **2008**, 64 (Pt 1), 112-22.
5. Terwilliger, T. C.; Grosse-Kunstleve, R. W.; Afonine, P. V.; Moriarty, N. W.; Zwart, P. H.; Hung, L.-W.; Read, R. J.; Adams, P. D., Iterative model building, structure refinement and density modification with the PHENIX AutoBuild wizard. *Acta Crystallographica Section D* **2008**, 64 (1), 61-69.
6. Langer, G.; Cohen, S. X.; Lamzin, V. S.; Perrakis, A., Automated macromolecular model building for X-ray crystallography using ARP/wARP version 7. *Nat Protoc* **2008**, 3 (7), 1171-9.
7. Winn, M. D.; Ballard, C. C.; Cowtan, K. D.; Dodson, E. J.; Emsley, P.; Evans, P. R.; Keegan, R. M.; Krissinel, E. B.; Leslie, A. G.; McCoy, A.; McNicholas, S. J.; Murshudov, G. N.; Pannu, N. S.; Potterton, E. A.; Powell, H. R.; Read, R. J.; Vagin, A.; Wilson, K. S., Overview of the CCP4 suite and current developments. *Acta Crystallogr D Biol Crystallogr* **2011**, 67 (Pt 4), 235-42.
8. Emsley, P.; Cowtan, K., Coot: model-building tools for molecular graphics. *Acta Crystallogr D Biol Crystallogr* **2004**, 60 (Pt 12 Pt 1), 2126-32.
9. Afonine, P. V.; Grosse-Kunstleve, R. W.; Echols, N.; Headd, J. J.; Moriarty, N. W.; Mustyakimov, M.; Terwilliger, T. C.; Urzhumtsev, A.; Zwart, P. H.; Adams, P. D., Towards automated crystallographic structure refinement with phenix.refine. *Acta Crystallogr D Biol Crystallogr* **2012**, 68 (Pt 4), 352-67.
10. Krissinel, E.; Henrick, K., Secondary-structure matching (SSM), a new tool for fast protein structure alignment in three dimensions. *Acta Crystallogr D Biol Crystallogr* **2004**, 60 (Pt 12 Pt 1), 2256-68.
11. Lan  elle, M. A.; Spina, L.; Nigou, J.; Lemassu, A.; Daff  , M., Lipid and Lipoarabinomannan Isolation and Characterization. *Methods Mol Biol* **2021**, 2314, 109-150.

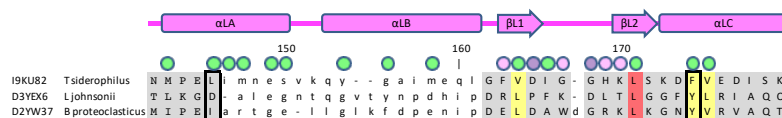
12. Kotov, V.; Mlynek, G.; Vesper, O.; Pletzer, M.; Wald, J.; Teixeira-Duarte, C. M.; Celia, H.; Garcia-Alai, M.; Nussberger, S.; Buchanan, S. K.; Morais-Cabral, J. H.; Loew, C.; Djinojic-Carugo, K.; Marlovits, T. C., In-depth interrogation of protein thermal unfolding data with MoltenProt. *Protein Sci* **2021**, 30 (1), 201-217.
13. Goldstone, D. C.; Villas-Bôas, S. G.; Till, M.; Kelly, W. J.; Attwood, G. T.; Arcus, V. L., Structural and functional characterization of a promiscuous feruloyl esterase (Est1E) from the rumen bacterium *Butyrivibrio proteoclasticus*. *Proteins* **2010**, 78 (6), 1457-69.
14. Lai, K. K.; Stogios, P. J.; Vu, C.; Xu, X.; Cui, H.; Molloy, S.; Savchenko, A.; Yakunin, A.; Gonzalez, C. F., An inserted α/β subdomain shapes the catalytic pocket of *Lactobacillus johnsonii* cinnamoyl esterase. *PLoS One* **2011**, 6 (8), e23269.
15. Jensen, M. V.; Horsfall, L. E.; Wardrope, C.; Togneri, P. D.; Marles-Wright, J.; Rosser, S. J., Characterisation of a New Family of Carboxyl Esterases with an OsmC Domain. *PLoS One* **2016**, 11 (11), e0166128.
16. Cao, Y.; Qiu, T.; Kathayat, R. S.; Azizi, S. A.; Thorne, A. K.; Ahn, D.; Fukata, Y.; Fukata, M.; Rice, P. A.; Dickinson, B. C., ABHD10 is an S-depalmitoylase affecting redox homeostasis through peroxiredoxin-5. *Nat Chem Biol* **2019**, 15 (12), 1232-1240.
17. Franklin, M. C.; Cheung, J.; Rudolph, M. J.; Burshteyn, F.; Cassidy, M.; Gary, E.; Hillerich, B.; Yao, Z. K.; Carlier, P. R.; Totrov, M.; Love, J. D., Structural genomics for drug design against the pathogen *Coxiella burnetii*. *Proteins* **2015**, 83 (12), 2124-36.
18. Rengachari, S.; Aschauer, P.; Schittmayer, M.; Mayer, N.; Gruber, K.; Breinbauer, R.; Birner-Gruenberger, R.; Dreveny, I.; Oberer, M., Conformational plasticity and ligand binding of bacterial monoacylglycerol lipase. *J Biol Chem* **2013**, 288 (43), 31093-104.
19. Aschauer, P.; Zimmermann, R.; Breinbauer, R.; Pavkov-Keller, T.; Oberer, M., The crystal structure of monoacylglycerol lipase from *M. tuberculosis* reveals the basis for specific inhibition. *Sci Rep* **2018**, 8 (1), 8948.
20. De Vitis, V.; Nakhnoukh, C.; Pinto, A.; Contente, M. L.; Barbiroli, A.; Milani, M.; Bolognesi, M.; Molinari, F.; Gourlay, L. J.; Romano, D., A stereospecific carboxyl esterase from *Bacillus coagulans* hosting nonlipase activity within a lipase-like fold. *Febs j* **2018**, 285 (5), 903-914.
21. Gao, D. W.; Jamieson, C. S.; Wang, G.; Yan, Y.; Zhou, J.; Houk, K. N.; Tang, Y., A Polyketide Cyclase That Forms Medium-Ring Lactones. *J Am Chem Soc* **2021**, 143 (1), 80-84.



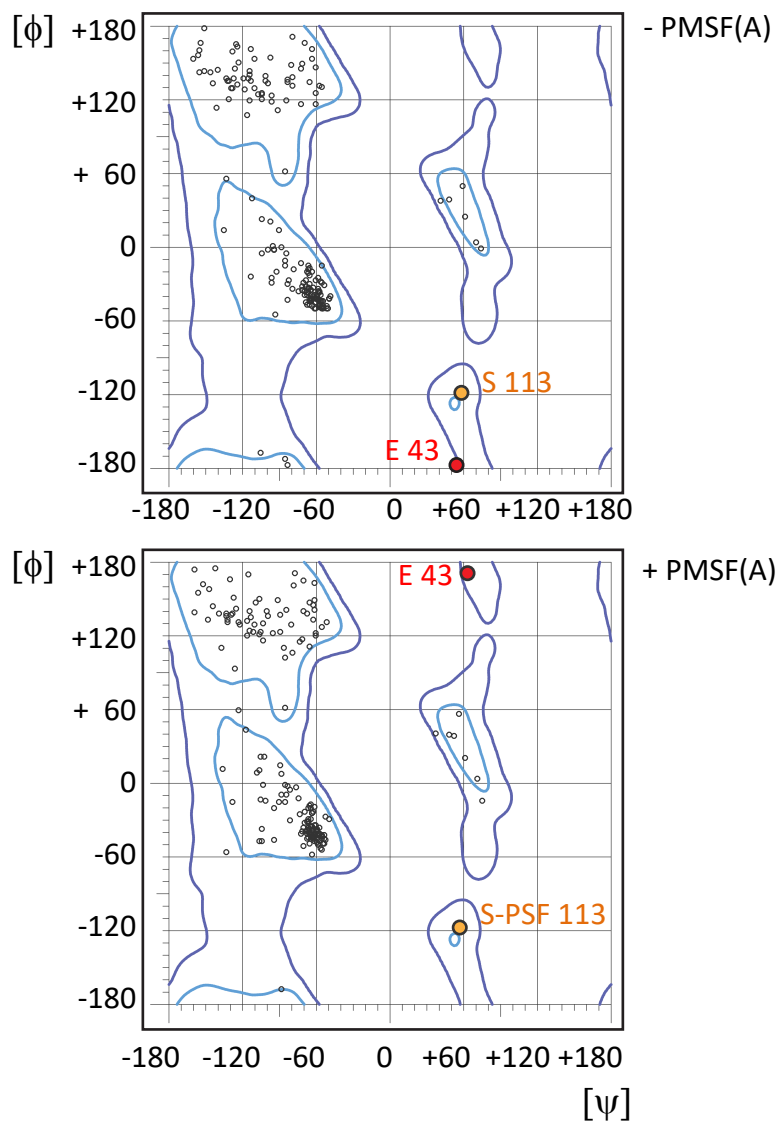
Alpha Fold 2 predicted structures of dimeric *Tth* MGL. The overall root mean square deviations of the five top predicted structures with the experimental *Tth* MGL structures are in the range of 0.4-0.5 Å. The majority of the *Tth* dimer interface specific interactions (**Figure 1F**) are not recovered in the predicted structures. In addition, any information on protein-ligand interactions (**Figures 2 & 3**) is missing in the predicted models. The vertical and horizontal lines shown in the plots indicate the border between the first and second sequence chains (A, B) of the predicted models. **A**, Sequence coverage of the predicted models; **B**, Local Distance Difference Test, indicating a major peak within the sequence segment of the *Tth* MGL lid domain; **C**, Distance matrices of the top five ranking models, indicating the highest level of uncertainty at the sequence segment of the *Tth* MGL lid domain.



Alignment of 12 CD sequences, for which an overall structure-based alignment was possible. UNIPROT codes and organisms of aligned sequences are indicated to the left. Structurally aligned residues are shown in capital letters and respective aligned positions are shaded in grey; other residues are in small letters. As a structure-based alignment of the lid domain segment, inserted into the N-terminal and C-terminal part of each CD sequence, was not possible due to high level of sequence and structural diversity, instead the length and type of lid domains of all chosen esterase/lipases are indicated. HBH-type lid domains with structural relations to the *Tth* MGL lid domain are colored in magenta. Sequence numbers indicated in 10-residues intervals on top of the alignment refer to the *Tth* MGL sequence. Helices and β -strands, indicated by cylinders and arrows, respectively, are taken from the *Tth* MGL coordinates. The coloring codes of these secondary structural elements are as in **Figures 1 & 2**. Long insertions of single sequences are shown by numbers, indicating the length of the inserted sequence. Conserved residue positions are highlighted in red (invariant) or yellow (highly conserved). Residues involved in ligand interactions (glycerol, C18 mono-acyl) in the *Tth* MGL structure without PMSF are indicated by green circles on top of the alignment: hydrogen bond interactions, dark green; other interactions, light green. Residues involved in *Tth* MGL dimer interactions are indicated by orange circles: hydrogen bond interactions, dark orange; other interactions, light orange.

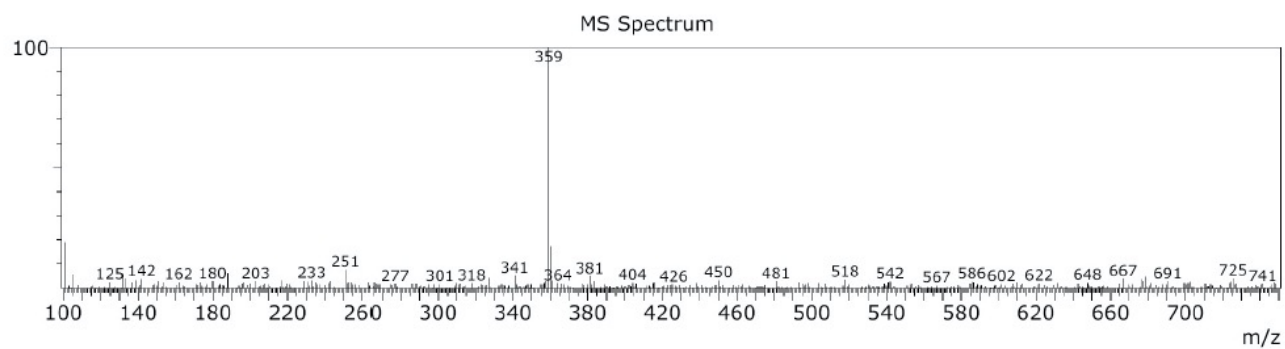


Alignment of the HBH lid domains of three sequences closely related to *Tth* MGL, for which a structure-based alignment was possible. The color codes are as for panel A, except residues involved into lid domain dimer interface interactions are highlighted with magenta circles according to the color codes from **Figure 2** on top of the alignment. Residues with changed properties for active site ligand binding are boxed.

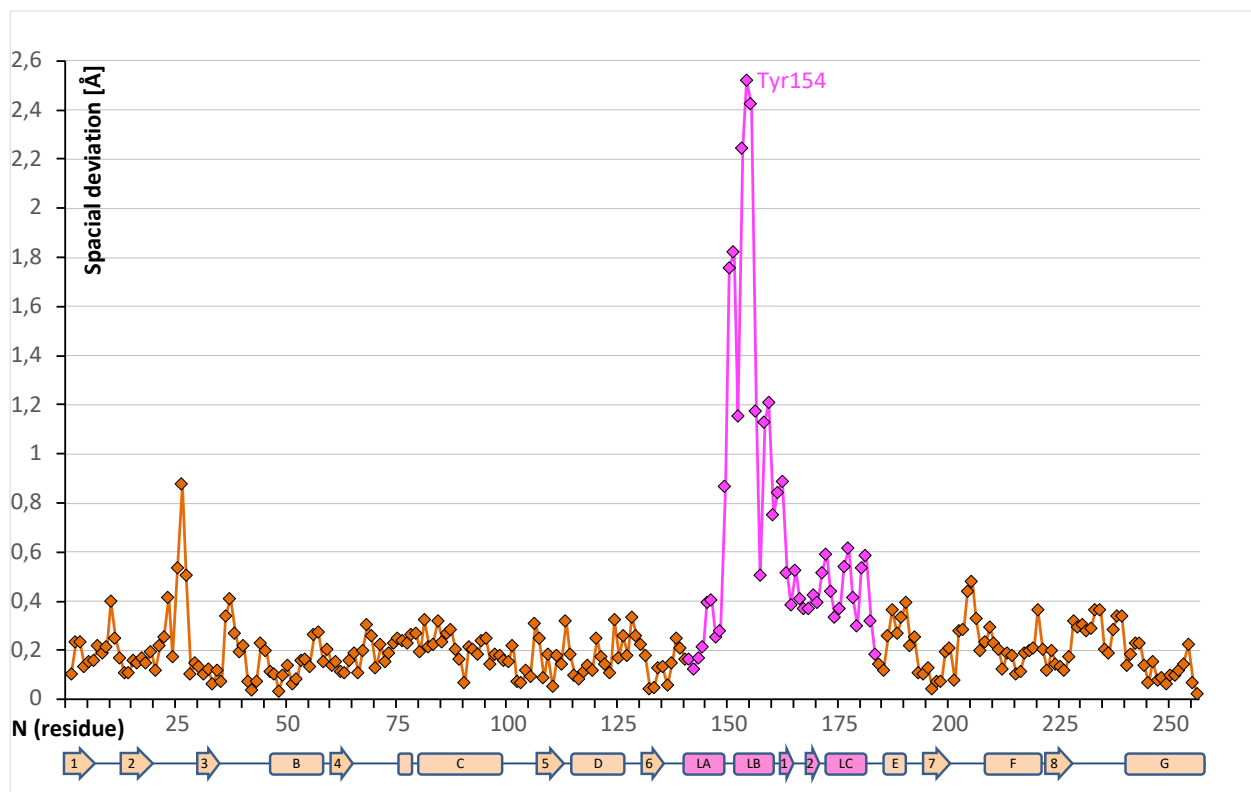


Ramachandran plot of *Tth* MGL structures, in the absence and presence of PMSF. In both structures, Glu43 and Ser113 have unusual dihedral angles, due to functional reasons (for details, see text).

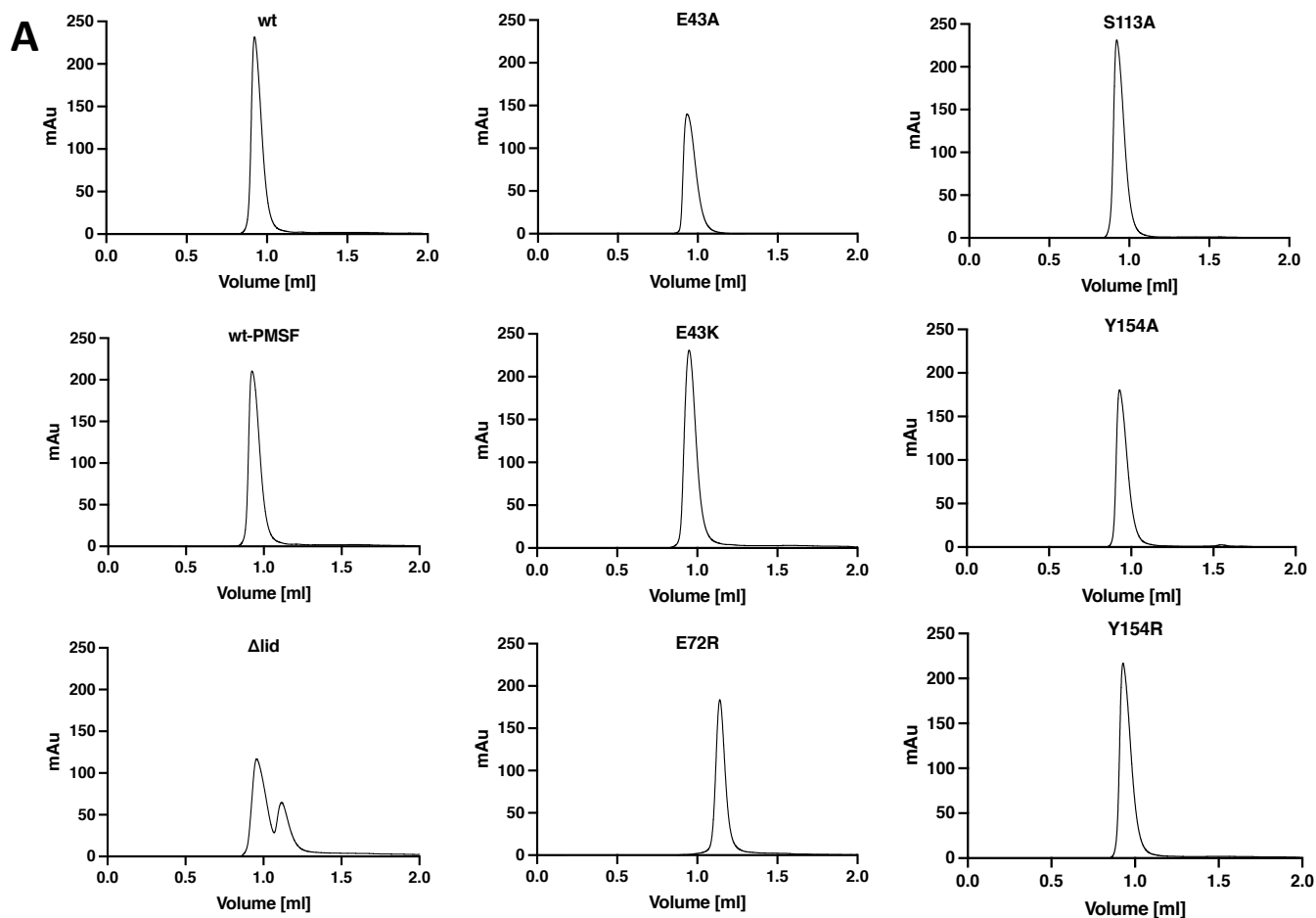
Supplementary Figure S3



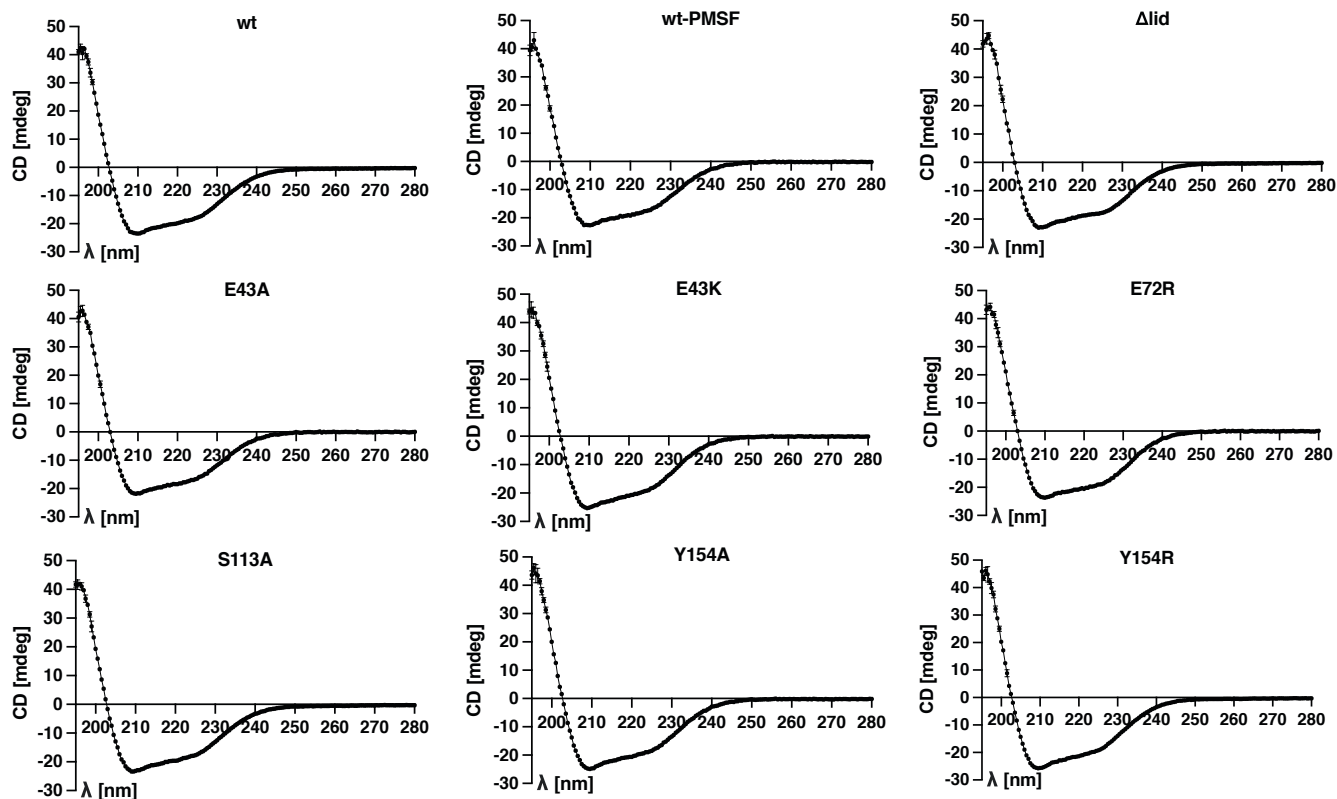
Reverse phase chromatography analysis of bound ligands in *Tth* MGL crystals in the absence of PMSF.



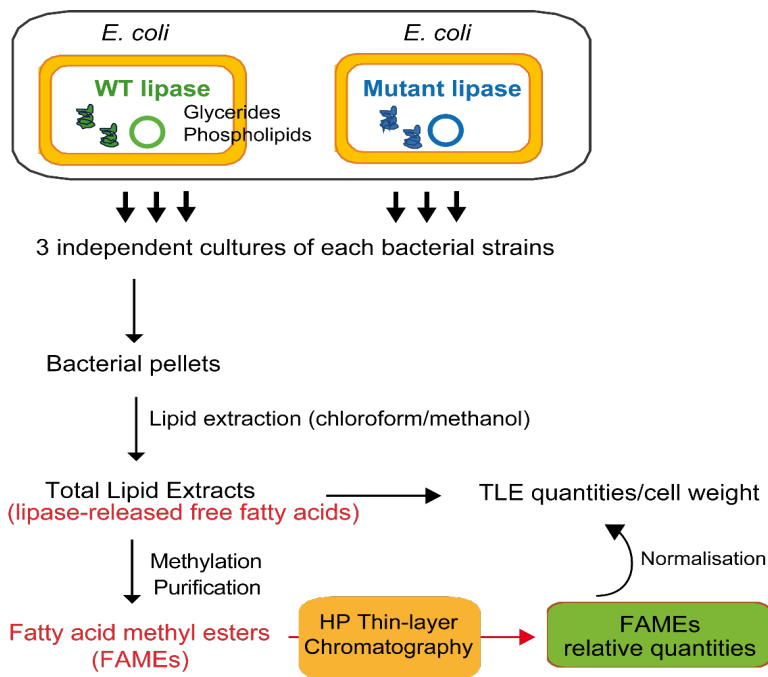
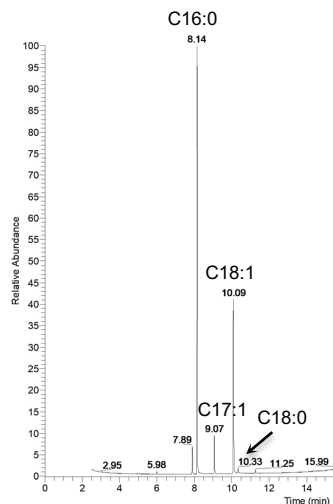
Dynamic substrate tunnel opening in the *Tth* MGL lid domain (cf. Figure 3D-E). Spatial differences of the C α positions of the *Tth* MGL structures, with and without PMSF. Color codes are as in Figures 1-3. The approximate positions of secondary structural elements are indicated (Figure 1C, Supplementary Figure S2). The comparison indicates peak movements of lid domain residues next to the tunnel opener Tyr154.



Analytic SEC profiles of all *Tth* MGL variants investigated. The respective elution profiles – except the Δ Lid and E72R mutants – were interpreted as showing a dimeric state of the enzyme.

A

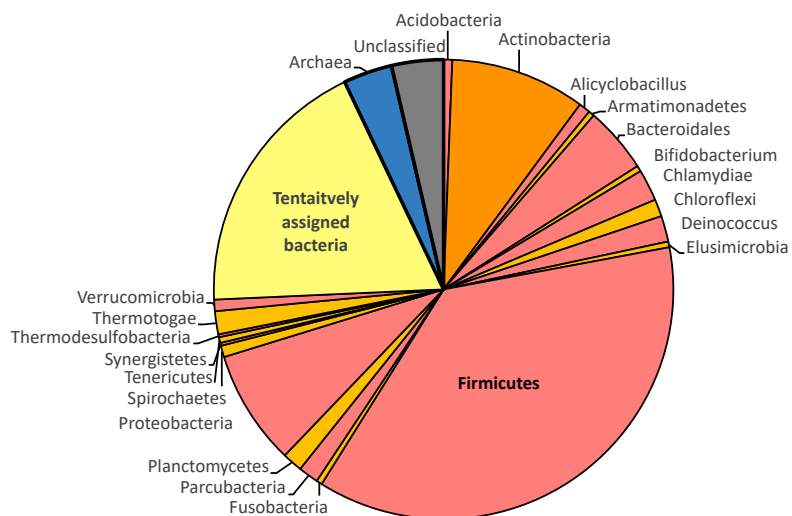
CD spectra for all MGL *Tth* variants investigated, showing no significant deviations from wt *Tth* MGL.

A**B**

A, Schematic workflow of sample preparation and lipid cell-based data analysis. *E. coli* cultures were transformed with *Tth* MGL variants, induced with IPTG for 60 min. Bacterial pellets were used to prepare total lipid extracts (TLE) following CHCl₃/CH₃OH extraction. Lipid extracts were methylated to yield Fatty Acid Methyl Esters (FAMES), further purified through florisil columns and the resulting enriched FAMES were analyzed by high-pressure thin-layer chromatography (HPTLC) and quantified. Plate visualization was realized using primuline dye and scanning. Fluorescence associated with FAME bands was quantified, and normalization to TLE and bacterial mass was performed to correct for variations across samples. **B**, representative gas chromatogram used for FAME analysis.

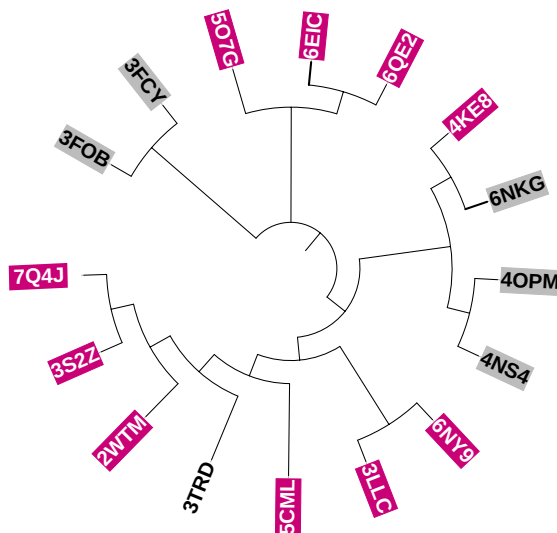
Supplementary Figure S8

A



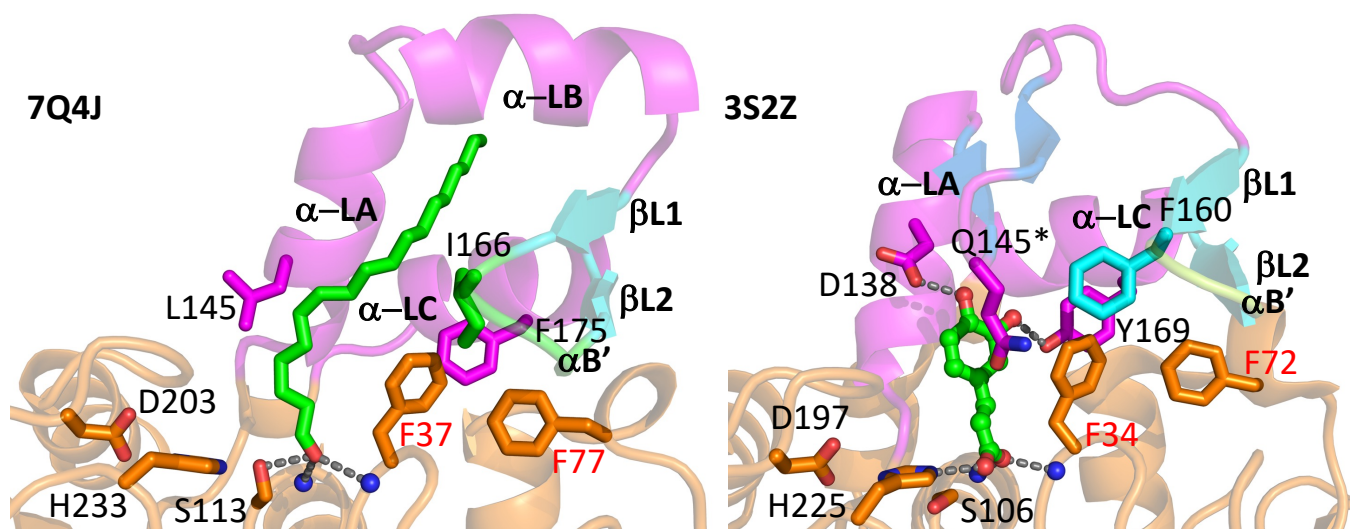
Taxonomic analysis of most closely related 496 sequence groups as defined in UniprotKB50, indicating that all related sequences (to the extent unambiguously assigned) are either of bacterial or archaeal origin. The statistics for different bacterial phyla are shown in alphabetic order and in alternating colors (red, orange). Statistics of unclassified or tentatively assigned bacterial sequences are shown in yellow, archaeal sequences in blue, and unclassified sequences are in grey. The complete list of sequence groups is in **Supplementary Table S7**.

B



Phylogenetic tree presentation of sequences with known 3D structures and distant relations to *Tth* MGL, indicating the respective PDB ID codes (cf. **Supplementary Table S8**). Sequences containing a HBH lid domain are highlighted in pink (cf. **Figure 7**), other sequences with helical lid domains are in grey. The precise phylogenetic distances are not reliable because of only distant relations and hence have no meaning in this presentation. Conditions for target selection: a) presence of a complete α/β hydrolase catalytic triad (Ser113, Asp203, His233 in the *Tth* MGL sequence) to discard any false-positive hits with unrelated function, b) coverage of both the N-terminal and C-terminal segments of the *Tth* MGL domain with the lid domain inserted in the resulting pairwise sequence alignments.

Supplementary Figure S9



Structural comparison of the ligand bound HBH lid domains and active site area for *Tth* MGL in the presence of C18 monoacyl (left) and the esterase from *L. johnsonii* (PDB code: 3S2Z) in the presence of ferulate (right), indicating different substrate specificities. Two residues critical for specific hydrogen bond interactions with ferulate in structure of the esterase from *L. johnsonii* (Asp138, Tyr169) are substituted by hydrophobic residues in *Tth* MGL (equivalent residues Leu145, Phe175). Other key residues with critical roles for substrate binding are also shown. In the structure of the esterase from *L. johnsonii*, an additional N-terminal lid β -sheet is formed (colored in blue) that hovers over the CD active site. At the end of the short turn connecting the two β -strands, there is a highly exposed residue, which is Gln145 in this structure. Residues that are part of the conserved HGF motif (residues 35-37 in *Tth* MGL, residues 32-34 in the *L. johnsonii* esterase) and less conserved FSE/D motif (residues 77-79 in *Tth* MGL, residues 70-72 in the *L. johnsonii* esterase) are labeled in red (**Supplementary Figure S2**).

Supplementary Figure S10