

Article

Cloning, expression, characterization and immobilization of a recombinant carboxylesterase from the halophilic archaeon, *Halobacterium salinarum* NCR-1

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Supplementary Materials:

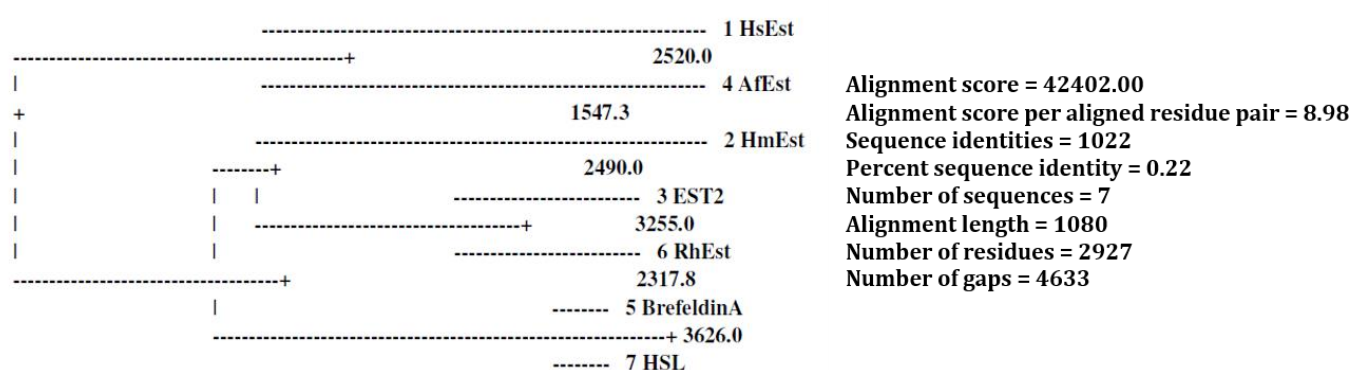


Figure S1. PRALINE tree representation of alignment of the amino acid sequence of *H. salinarum* esterase (HsEst, GenBank: AAG19778.1) with other characterized proteins of the HSL proteins family. HmEst (NSSF): *H. marismortui* esterase Lip C (GenBank: AAV45166.1); EST2 (NSSF): *Alicyclobacillus acidocaldarius* esterase (NCBI Reference Sequence: WP_012812080.1; PDB: 1U4N); BrefeldinA: *Bacillus subtilis* Brefeldin A esterase (GenBank: AAC12774.1; PDB ID:1JKM), RhEST (NSSF): *Rhodococcus* sp. heroin esterase (NCBI Reference Sequence: WP_259118130.1; PDB: 4RNC); AfEst (31.35% Per. Ident.): *Archaeoglobus fulgidus* esterase (NCBI Reference Sequence: PDB: 5FRD); and HSL (NSSF): hormone sensitive lipase (NCBI Reference Sequence: NP_005348.2). The characterized proteins are marked with (*). Per. Ident.: Percentage identity with HsEst determined with NCBI protein-protein blast. NSSF: No significant similarity found determined with NCBI protein-protein blast.

		Pentapeptide		
(a)				
HsEst	90	ASVLV GNSL GGAVVLTA	106	
HmEst	121	RVVGV GHSAG ANLIVLA	137	
EST2	148	RIAVG GDSAG GNLAAVT	164	
BrefeldinA	195	-VVVG GESGG GNLAIAT	211	
RhEst	153	RIAVG GESAG GGLAAGT	169	
rHSL	716	RICLA GDSAG GNLCITV	632	
(b)		Oxyanion hole loop		
HsEst	23	TVLFV HGSG GTHAVWKA	39	
HmEst	50	VAVLV RGGAF TSGDKGE	66	
EST2	76	ALVYY HGGG WVVDLET	93	
BrefeldinA	120	GLVYT HGGG MTILTTDN	136	
RhEst	81	VLLWI HGGG FAIGTAES	97	
rHSL	644	LVVHI HGGG FVAQTSKS	660	

Figure S2. Alignment of the amino acid sequence of HsEST with other proteins of the HSL family showing the pentapeptide containing the active site serine (a) and the oxyanion-hole loop (b). *H. salinarum* esterase (HsEst), *H. marismortui* esterase Lip C (HmEst), esterase from *Alicyclobacillus acidocaldarius* (EST2), *B. subtilis* Brefeldin A esterase (BrefeldinA), *Rhodococcus* sp. heroin esterase (RhEST) and hormone sensitive lipase (rHSL). The residues marked in bold indicate the most conserved residues within the amino acid sequences. The catalytic serine is marked (*).

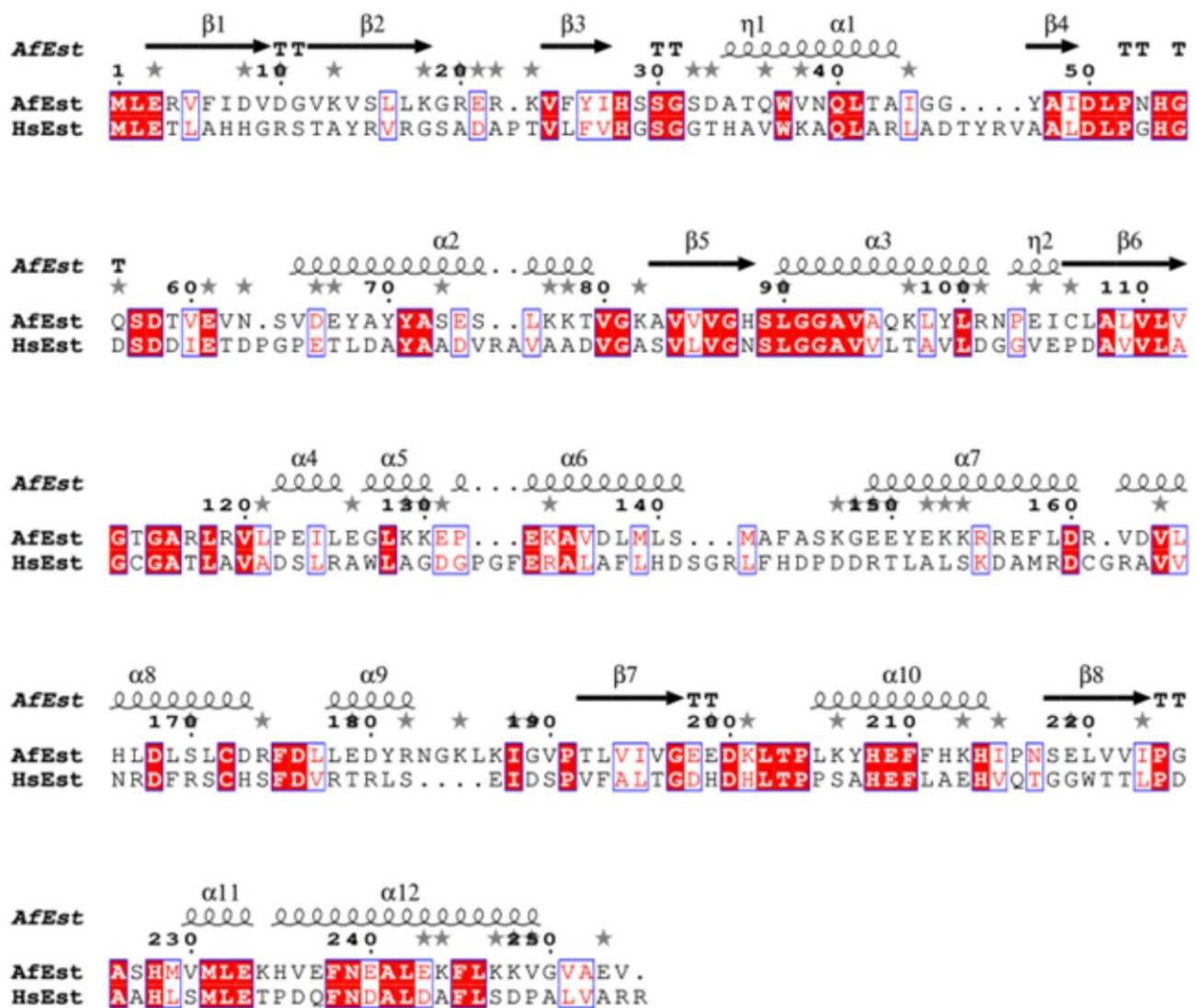


Figure S3. Pairwise sequence alignment with ESPrict 3.0 between HsEst and AfEst (PDB: 5FRD) that share 31.25 % identity. The dots in the alignment and in the structural elements indicate gaps. The numbers at the top of sequence for each line correspond to the amino acid position of AfEst. The secondary structure elements of both enzymes are shown above the alignment: alpha-helix (α), beta-strand (β). The conserved amino acids are highlighted in red while the conserved regions are contained within blue lines. Amino acid residues of HsEst (S97, D211 and H239) and AfEst (S89, D200 and H228) involved in the active site are signalled with *.

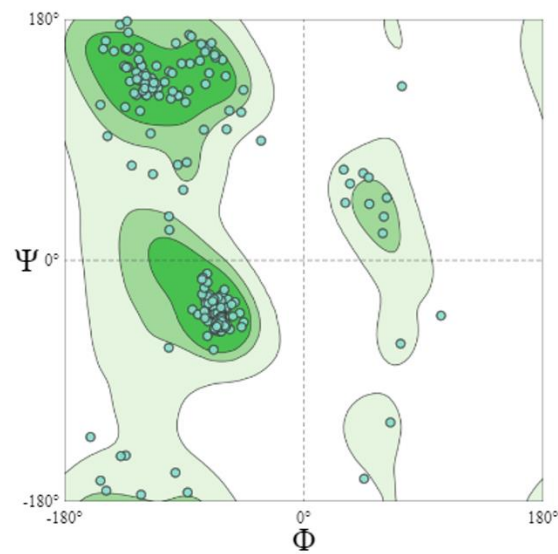


Figure S4. Structure assessment of HsEst model analyzed Molprobit in Swiss model. Ramachandran score 89.77% for HsEst model obtained with Phyre2.

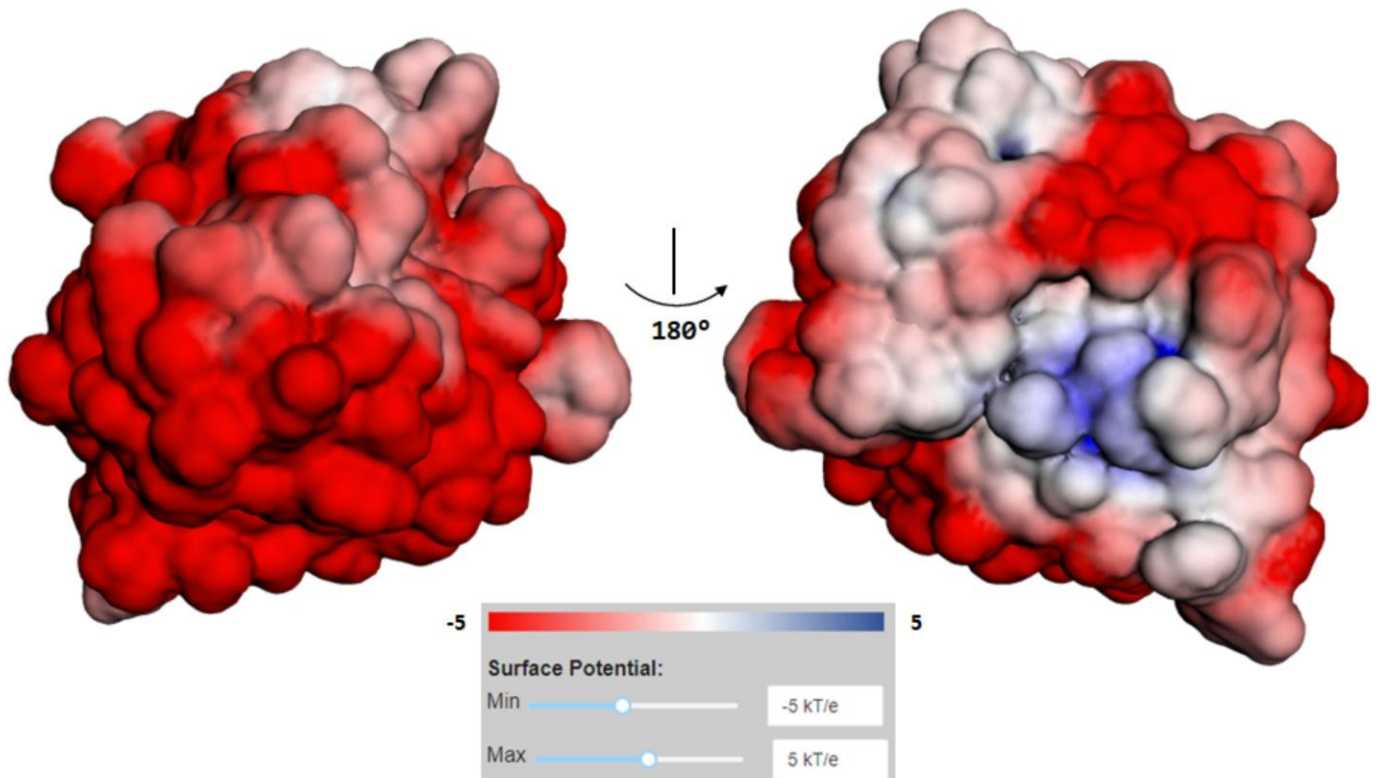


Figure S5. Relative surface electrostatic potentials of HsEst as obtained using PDB2PQR with PARSE forcefield online program. Online visualization with 3Dmol: the red surface corresponding to negatively charged residues (Asp, Glu) and the blue surface corresponding to positively charged residues (Arg, Lys).

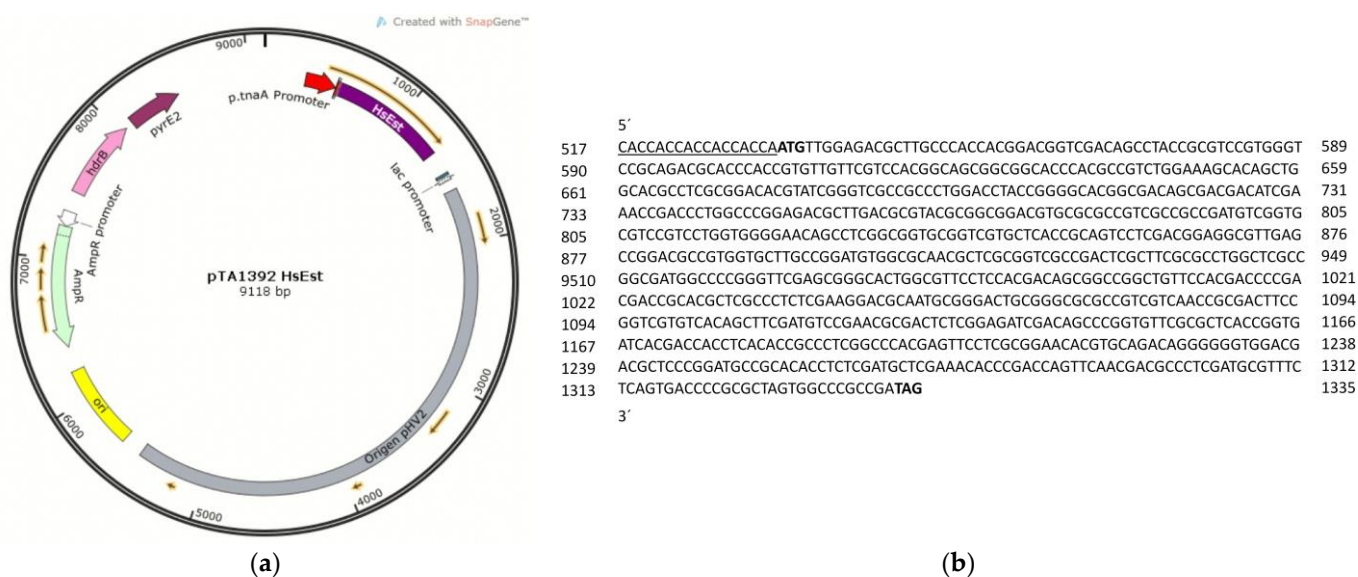


Figure S6. Schematic representation of the plasmid (pTA1392-HsEst), showing the insertion site of AAG19778.1 gene from *H. salinarum* NRC-1, which encodes a putative carboxylesterase generated with SnapGene™ (a). Nucleotide sequence of HsEst in frame contained in plasmid pTA1392, where the start (ATG) and stop (TAG) codons for protein synthesis are indicated in bold, the 6xHis-tag in N-terminus is underlined, in bold and italics the codon for the initial methionine (ATG) of HsEst (b). In (a) the numbers close to the circle indicate the nucleotide position for each feature, in (b) the numbers on the sides of the sequence indicate the position of the first and last base in each row.

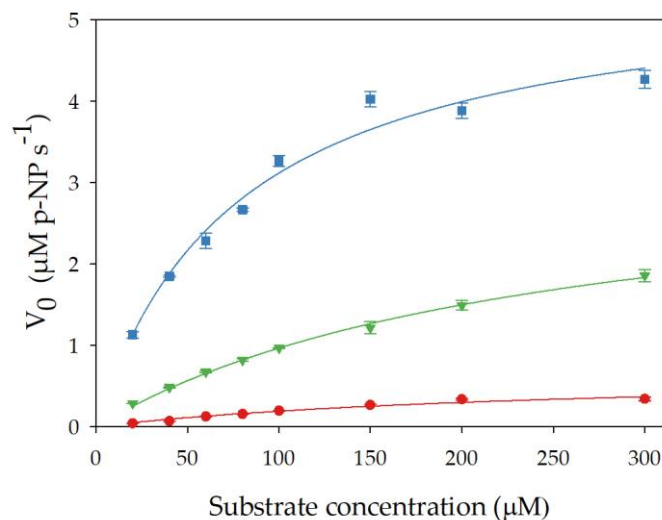


Figure S7. Enzymatic kinetics of rHsEst of the hydrolysis of *p*-nitrophenyl acetate (●), *p*-nitrophenyl valerate (■) and *p*-nitrophenyl octanoate (▼). Each point represents the average value of three independent replicates and the bars indicate the standard error. Solid lines represent the fit of Michaelis-Menten model.