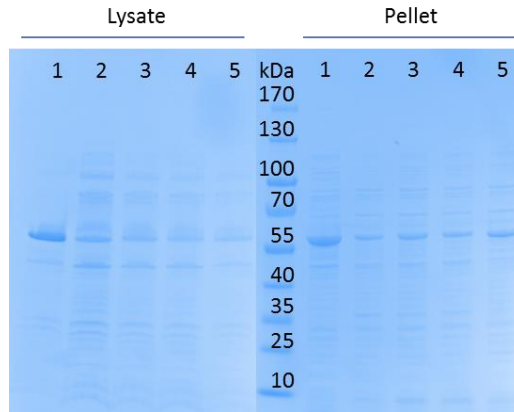
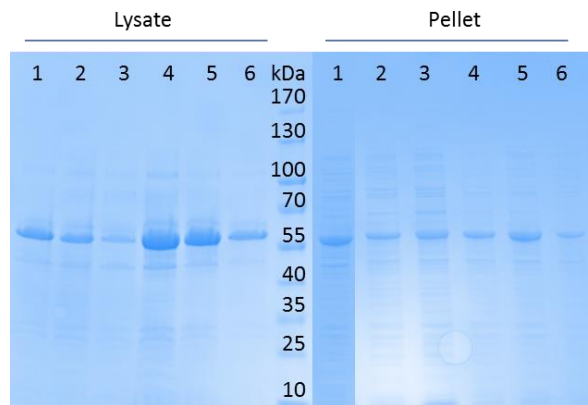


**Table S1.** Primers used for site-directed mutagenesis of Cbotu\_EstA gene. The inserted mutations are underlined.

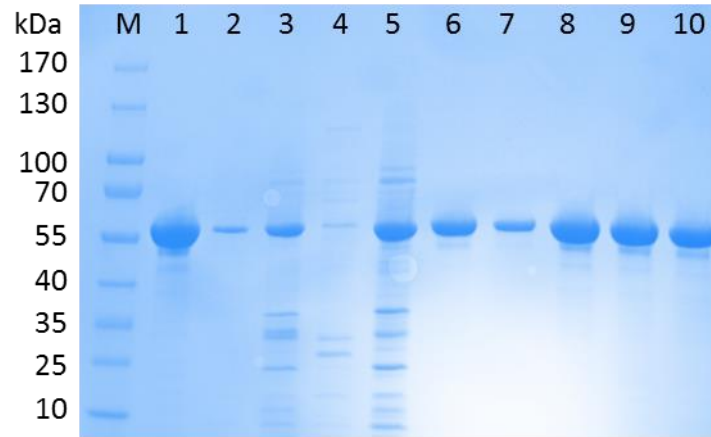
<b>Zn-coordination</b>	
Cbotu_EstA_D130L_F	5'-ACC GTT GGT CCG GTT AGC AGC AAT TGG <u>CTG</u> CGT GCA TGT GAA CTG ATG CAT ATA TTG T-3'
Cbotu_EstA_D130L_R	5'-ACA ATA TAT GCA TAC AGT TCA CAT GCA CGC <u>AGC</u> CAA TTG CTG CTA ACC GGA CCA ACG GT-3'
Cbotu_EstA_H150F_F	5'-GAT TAT GGT GAA GCA <u>TTT</u> GCC AAA AAA TTC-3'
Cbotu_EstA_H150F_R	5'-GAA TTT TTT GGC <u>AAA</u> TGC TTC ACC ATA ATC-3'
Cbotu_EstA_H156F_F	5'-CCA AAA AAT TCA AAT <u>TTA</u> ATC GTT ACG GTC G-3'
Cbotu_EstA_H156F_R	5'-CGA CCG TAA CGA TTA <u>AAT</u> TTG AAT TTT TTG G-3'
Cbotu_EstA_D302L_F	5'-ACA GCA CCA AAC TGA TTG CAA CCT ATG-3'
Cbotu_EstA_D302L_R	5'-CAT AGG TTG CAA <u>TCA</u> <u>GTT</u> TGG TGC TGT-3'
<b>Zn-cavity</b>	
Cbotu_EstA_S127A_F	5'-CCT ATA CCG CAA CCG TTG GTC CGG TTA <u>GCG</u> CCA ATT GGG ATC GTG CAT GTG AAC TGT ATG CA-3'
Cbotu_EstA_S127A_R	5'-TGC ATA CAG TTC ACA TGC ACG ATC CCA ATT <u>GGC</u> GCT AAC CGG ACC AAC GGT TGC GGT ATA GG -3'
Cbotu_EstA_F154Y_F	5'-CAC CGT TGA TTA TGG TGA AGC ACA TGC CAA AAA <u>ATA</u> <u>CAA</u> ACA TAA TCG TTA CGG TCG TAC CTA TCC-3'
Cbotu_EstA_F154Y_R	5'-GGA TAG GTA CGA CCG TAA CGA TTA TGT TTG <u>TAT</u> TTT TTG GCA TGT GCT TCA CCA TAA TCA ACG GTG-3'
Cbotu_EstA_W274H_F	5'-AGC AGC ATC TAT GAT CTG AAA CTG GAT CAG <u>CAC</u> GGT CTG AAA AAA CAG AAT GGT GAA AGC-3'
Cbotu_EstA_W274H_R	5'-GCT TTC ACC ATT CTG TTT TTT CAG ACC <u>GTG</u> CTG ATC CAG TTT CAG ATC ATA GAT GCT GCT-3'
<b>Cavity entrance</b>	
Cbotu_EstA_W129A_F	5'- CGC AAC CGT TGG TCC GGT TAG CAG CAA <u>TGC</u> GGA TCG TGC ATG TGA ACT GTA TGC ATA TAT TGT TGG T -3'
Cbotu_EstA_W129A_R	5'- ACC AAC AAT ATA TGC ATA CAG TTC ACA TGC ACG ATC <u>CGC</u> ATT GCT GCT AAC CGG ACC AAC GGT TGC G -3'
Cbotu_EstA_S199A_F	5'-ATT CGT ACC CTG ACC CAG CTG CTG AGC GAA GGT <u>GCC</u> GAA GAA GAA ATT AAC TGC GGT CAA GAA AAC AT-3'
Cbotu_EstA_S199A_R	5'- ATG TTT TCT TGA CCG CAG TTA ATT TCT TCT TCG <u>GCA</u> CCT TCG CTC AGC AGC TGG GTC AGG GTA CGA AT -3'



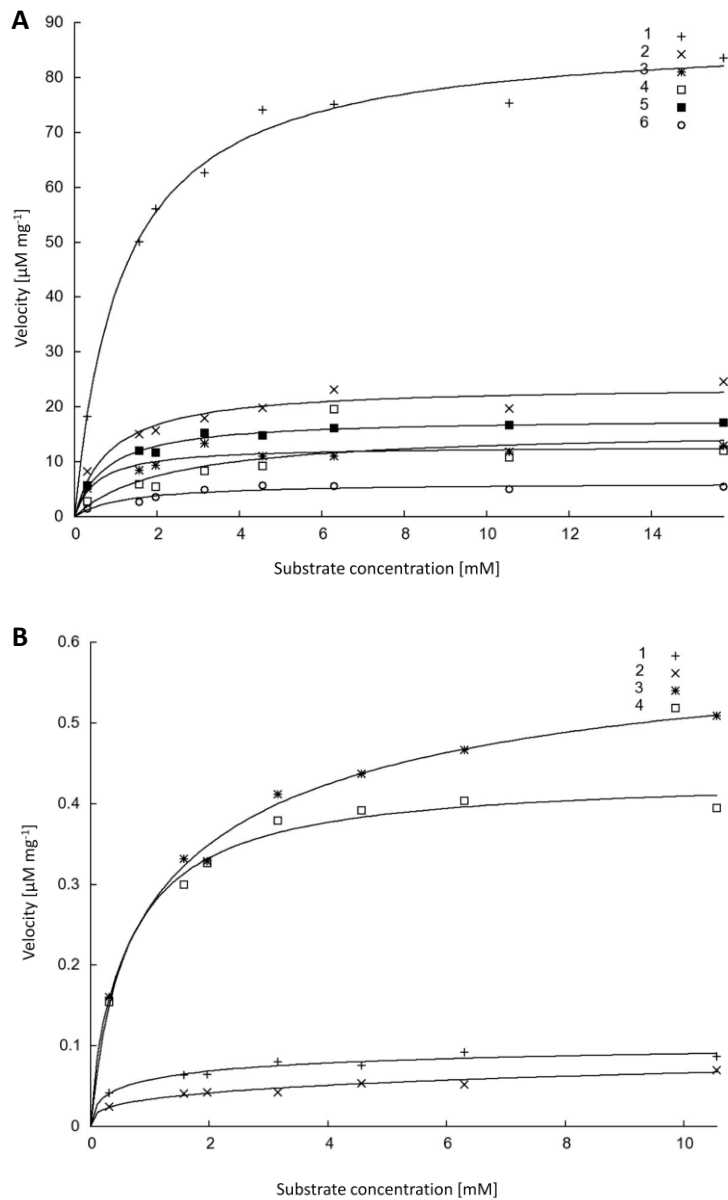
**Figure S1.** SDS-PAGE (4-15%) of Cbotu\_EstA wild-type and zinc-binding variants expressed in *E. coli* BL21-Gold(DE3). Samples were withdrawn after 20 h of induction, lysed and centrifuged to test the lysate and the pellet for soluble proteins and inclusion bodies, respectively. Lane 1: Cbotu\_EstA wild-type; lane 2-5: D130L, H150F, H156F, and D302L variant.



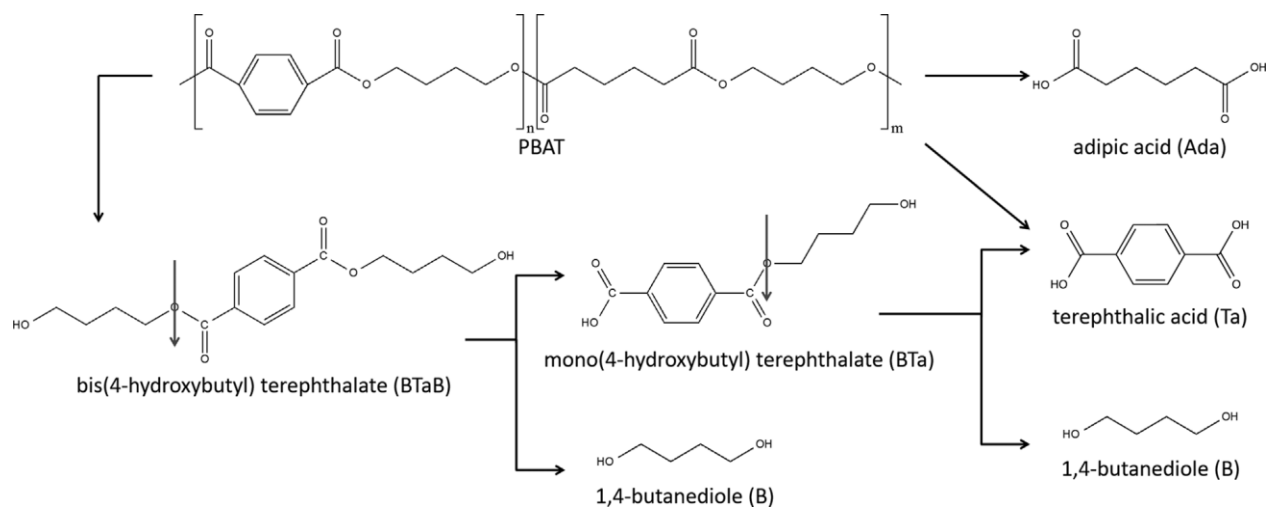
**Figure S2.** SDS-PAGE (4-15%) of Cbotu\_EstA wild-type and zinc cavity variants expressed in *E. coli* BL21-Gold(DE3). Samples were withdrawn after 20 h of induction, lysed and centrifuged to test the lysate and the pellet for soluble proteins and inclusion bodies, respectively. Lane 1: Cbotu\_EstA wild-type; lane 2-6: S127A, W129A, F154Y, S199A, and W274H variant.



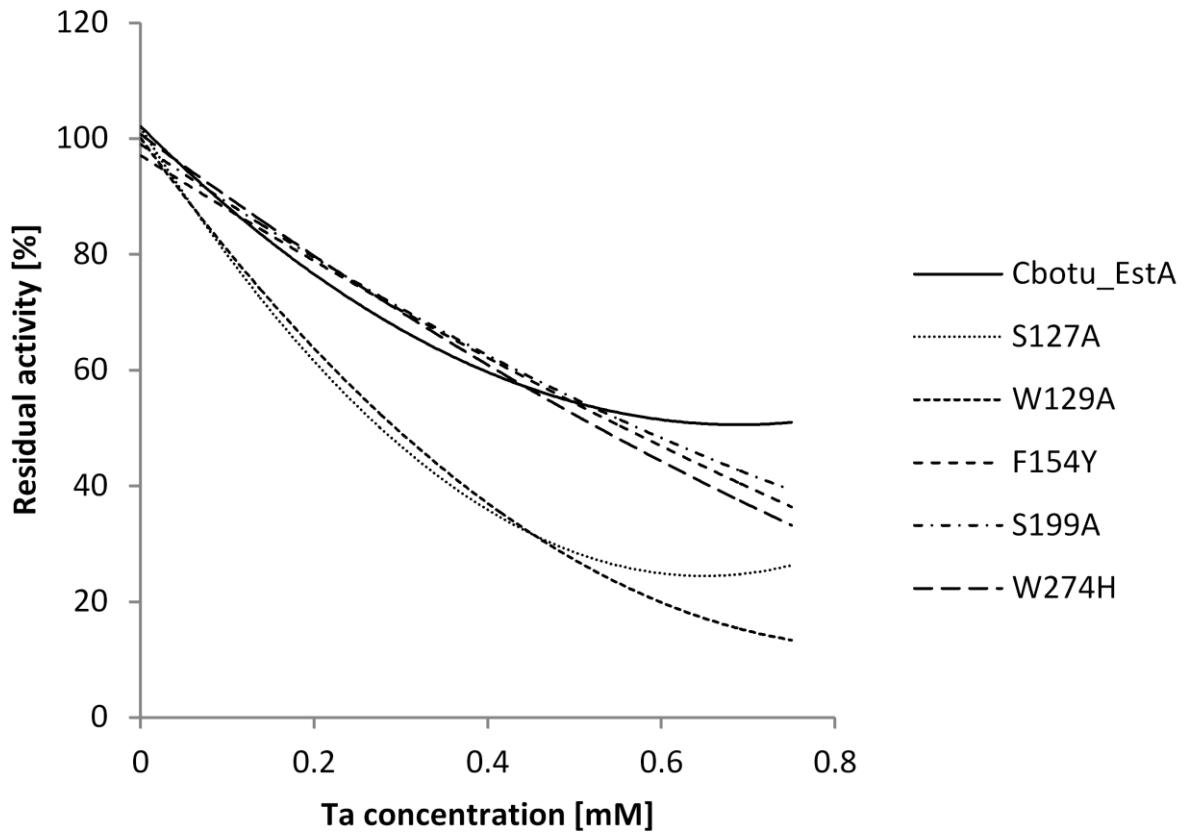
**Figure S3.** SDS-PAGE analysis (4-15%) of Cbotu\_EstA wild-type and variants expressed in *E. coli* BL21-Gold(DE3) with 0.05 mM IPTG and purified by IMAC. M: protein marker IV; lane 1: Cbotu\_EstA Wild-type; lane 2: D130L; lane 3: H150F; lane 4: H156F; lane 5: D302L; lane 6: S127A; lane 7: W129A; lane 8: F154Y; lane 9: S199A; lane 10: W274H.



**Figure S4.** Michaelis-Menten plot of Cbotu\_EstA wild-type and variants kinetics with the substrate *para*-nitrophenyl butyrate (*p*NPB) in a concentration range 0.3 - 15 mM. **(A)** Cbotu\_EstA wild-type and variants from the zinc cavity and the entrance to the cavity: Cbotu\_EstA (+); S127A (x); W129A (\*); F154Y (□); S199A (■); W274H (○). **(B)** Cbotu\_EstA variants from the zinc-coordination site: D130L (+); H150F (x); H156F (\*); D302L (□).



**Figure S5.** Possible PBAT hydrolysis patterns. Products terephthalic acid (Ta) and mono(4-hydroxybutyl) terephthalate (BTa) were detected by HPLC<sup>43</sup>.



**Figure S6.** Enzyme inhibition of Cbotu\_EstA wild-type and variants by terephthalic acid in a range of concentration of 0 - 0.75 mM.