

Supplementary Materials

Characterization of a Novel Family IV Esterase Containing a Predicted CzcO Domain and a Family V Esterase with Broad Substrate-Specificity from the Oil-Polluted Mud Flat Metagenomic Library

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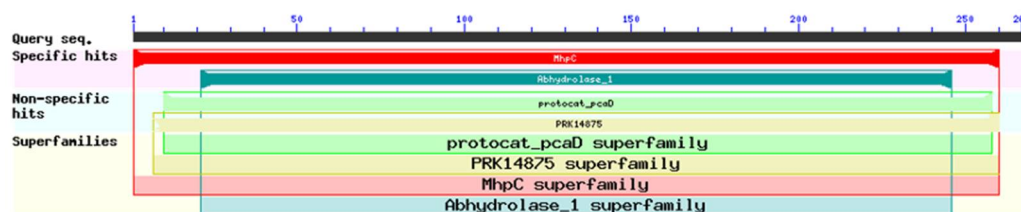
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(a)



(b)

Figure S1. BLASTp of Est2L (a) and Est4L (b). Est2L was matched to two domains of a CzcO superfamily domain (10-419 amino acid residues) and an acylesterase superfamily (573-811 amino acid residues).

| | | | | | |
|-------------------|--|-----|-------------------|---|-----|
| Est2L Czco domain | THFTIDSATFLPAIIICGCGCGMAIIRHRAQVHR...F | 44 | Est2L Czco domain | EEKRLIRCFWPMKIIFPAHYLHFESFALGFTRLGLTHW | 269 |
| O07085 |MYCTIVICAGACISIGYMRKSECK...F | 27 | O07085 |LVYFFIMIG.....KFSIFFW | 208 |
| NP_244544 |MESIKWVINGGAGIAGMGYIVKKEVE...F | 29 | NP_244544 |FFFLFLKIL.....NKSMFSW | 211 |
| NP_355926 | EAAEIGYAEQFYVINGGCGGIALGAFIRKLGVE...T | 196 | NP_355926 | GMTTRKALLIRASLYRIMHEEFVFYIRIRREQLAEFYPA | 433 |
| NP_390541 |MYCTIVICAGACISIGYMRKSECK...F | 27 | NP_390541 |LVYFFIMIG.....KFSIFFW | 208 |
| NP_601977 |MSTINYEAIICAGACIAPAAFIISRFGEFGKLE | 34 | NP_601977 |ETCFEEIAGWGIAVEFAVRENTENGAFAS | 236 |
| Consensus | y iigagcagla gyl q gv f | | Consensus | f pl ig s f w | |
| Est2L Czco domain | IIIEFGGCVGGVWRINSYF(PACLVSESHLYSESEFNFW | 84 | Est2L Czco domain | VVGGPFKKLIHTSV...FEIRRCMTFYIFGCHRIIS | 306 |
| O07085 | IIIEKSHVEGESMKIR.YLSIVIFTSFMYSSIECMHLEGE | 66 | O07085 | FKRLGVLIHASTSIVG...RFICRKGFEVFGHEIRFAIRQ | 245 |
| NP_244544 | MIILANECVGSWRNR.YLSIVIFTSFYSCIEGFMIDGA | 68 | NP_244544 | LEWGGIYAGIITTRG...RWFRRKCFIFGKELKSLIK | 248 |
| NP_355926 | IIIEFNREFGDSMRK.YKSLCIHFEVWYHLEFYIFFEN | 235 | NP_355926 | LERAGFMILFGIESGLMKRYLRGSGYIIVGACILVID | 473 |
| NP_390541 | IIIEKSHVEGESMKIR.YLSIVIFTSFMYSSIECMHLEGE | 66 | NP_390541 | FKRLGVLIHASTSIVG...RFICRKGFEVFGHEIRFAIRQ | 245 |
| NP_601977 | IVILSNLGHGCAFRHR.WLSITIGFAEGIALIGLIMNRP | 73 | NP_601977 | VVRTTGIFCIHFIYLAG.VIRGLIVSRGMFEIITFTGVVFG | 275 |
| Consensus | iildkn vg swdr ydvlvlftpr ysslpq p g | | Consensus | k g l a hds g k l k dp fg elk ik | |
| Est2L Czco domain | SRVFAFQAEIHGYIHCACALVELKHFIFEGAEVFAEFET | 124 | Est2L Czco domain | NCYKTFQCFHVHLLTRIRKTIHETGIIETLGEFFAVIAI | 346 |
| O07085 | KHGEISKNEIVAYKKYVKHFEIF..ICLPTEVISVLKIK | 104 | O07085 | KEIILRK.....RVIPAKNEIIFKESSTIEVNNI | 275 |
| NP_244544 | FNGEITIKENASYICCYANFNLE..MRHHTKIVIRVIRKQ | 106 | NP_244544 | GCIIHLKF.....RVMNVCGKEVEFAIHSRISFIRI | 278 |
| NP_355926 | WEVETIKKVKGLKEMVIRAMEIN..YWGSTTCFSAQYCE | 273 | NP_355926 | GSIKLRSG.....VLVSHITENAVVIKGTETIAIV | 505 |
| NP_390541 | KHGEISKNEIVAYKKYVKHFEIF..ICLPTEVISVLKIK | 104 | NP_390541 | KEIILRK.....RVIPAKNEIIFKESSTIEVNNI | 275 |
| NP_601977 | LEKTIASTLVAGIYCAENEFSER..VVFVYVSRVEFTS | 111 | NP_601977 | EFFAERSEG..IGFSAGTILAVFSWKFYAGTHILVVI | 313 |
| Consensus | gfp k ei yl y felp i tev sv | | Consensus | i lk rv a enei fkdgs l vd i | |
| Est2L Czco domain | AN...ACWHTICADGCFRFAPIIISATGCLSRPAHLEIFG | 161 | Est2L Czco domain | IYATGEPANTEFISF.MRITGFDGLINCAWCCGAPAYIGI | 385 |
| O07085 | N.....YFLIKTNREYQIKNLVIAATGFFHTENISISK | 138 | O07085 | IWATGERNFICWIN...IKGVLICGRIIHHRGVSFVEGL | 312 |
| NP_244544 | NG.....REHLKTINGWIEPKVVIATGAFCKEYILFVID | 141 | NP_244544 | IWATGGSFSYEWIL...IDGVIAINGWFIHNRGITNIPGL | 315 |
| NP_355926 | TIGETVVVVEFAGKEVVLRFKQLVIAIGMSGFANVKKFG | 313 | NP_355926 | VYATGYSGMNGAPAILISREVAIKVKGWGLSITTLKFG | 545 |
| NP_390541 | N.....YFLIKTNREYQIKNLVIAATGFFHTENISISK | 138 | NP_390541 | IWATGERNFICWIN...IKGVLICGRIIHHRGVSFVEGL | 312 |
| NP_601977 | ELFS.SFLRVSSIDGREWITFMVLNATGWTNIFYVYIFG | 150 | NP_601977 | FWATGEPALRHAEMKIRGCGRIELICEVSEASLAR.V | 352 |
| Consensus | n f kt e tk lviatg f pn p ipg | | Consensus | iwatgfr l wi i gv d g iih rg s ql | |
| Est2L Czco domain | MASEFGRIHSAFMDHEYFIAGKFAVHTETAPICQEVFE | 201 | Est2L Czco domain | SVHGFNFNFMLYGFNTINIGHNSIIHMLSCISHVMARQA | 425 |
| O07085 | LISEININLSSCYKNSKCIAYGNVWVGGSNSACIAYE | 178 | O07085 | YFICGLF...WCHKRGSAILCGVG.....NLAEYIVKMN | 343 |
| NP_244544 | SANNMSQVSSAYRNACIFGKSVWVGGSNSACIAYE | 181 | NP_244544 | YFICGLF...WQYCRGSAILCGVG.....RLAEYIVFEIK | 346 |
| NP_355926 | QIVFEGECSSCHFGFLAYAGKRVWVGGSNSAHLICPA | 353 | NP_355926 | FWEGECRNMRKFTICAIWFHGGNLHCSRHSYCYLSICIK | 585 |
| NP_390541 | LISEININLSSCYKNSKCIAYGNVWVGGSNSACIAYE | 178 | NP_390541 | YFICGLF...WCHKRGSAILCGVG.....NLAEYIVKMN | 343 |
| NP_601977 | IKKFKKGLITVNYRFAELFKGRKRVWVGGSNSAVCEILE | 190 | NP_601977 | ILVYVG..STASTVQATFAGRVAG....RVAAKRLIVVS | 386 |
| Consensus | sf g qlhsscy n qlagk vlvvggnsaaaiave | | Consensus | yf glp wq rgsal gvg daeylv q | |
| Est2L Czco domain | VARQVAELKVFQSFAYIME.....FPIRFYSA | 229 | Est2L Czco domain | IVESLASHVEVEICR... | 440 |
| O07085 | ISKEFVTYLACSNK..... | 192 | O07085 | .GE..... | 345 |
| NP_244544 | IAKEFNVMIAISEF..... | 195 | NP_244544 | HGCK..... | 350 |
| NP_355926 | IWEACALVIMLCFSSTHIVFSISIMEIGLGLYSEFAYAN | 393 | NP_355926 | ARCAGLKTFVYGLCKEHHL | 604 |
| NP_390541 | ISKEFVTYLACSNK..... | 192 | NP_390541 | .GE..... | 345 |
| NP_601977 | LEGIAETIWAIFEFN..... | 206 | NP_601977 | FHVN..... | 390 |
| Consensus | l ker t a sr | | Consensus | g | |

(a)

| | | |
|--------------|---|-----|
| Est2L | HCRFQLGICRRRLATSVWSGCKSWYVDERGHNSTNWPFTW | 480 |
| PHS53692 | LLTFSSAFFILVVSMTFSASTSGASTHNEDTASASKCKAD | 42 |
| WP_082823965 | TLRFIFSFILVVSMTFSSTSEASAHNTDTAKT..... | 38 |
| AVF72281 | | 0 |
| AKG92633 | | 0 |
| Consensus | | |
| Est2L | SYRWLARYAGLAAYRLSSPLDTCSPACEGCRIAAFRDWLE | 520 |
| PHS53692 | TVKISNESPLTFPAKTLPLPSASSDELKSAISCYPMPSVD | 82 |
| WP_082823965 | ...TTEPLLTVPTKMVPLPSAASDELKSAISEYPMPSVD | 74 |
| AVF72281 |MASFELD | 7 |
| AKG92633 |MCRFN | 5 |
| Consensus | | |
| Est2L | RANAFLRVFLRVSRALIGFPFALASCRKIVDLLSWLMP | 560 |
| PHS53692 | EVINNTFQSIQCWRELICIRNADCKKKIKEMRKCFDVEDS | 122 |
| WP_082823965 | EIINNTFQSVQCWRELICRNGDCCKKKIKNMKCFDVEDS | 114 |
| AVF72281 | TVF...CMIKWGENFGTIEDNRLAYEKLVEPLPWVDD | 43 |
| AKG92633 | CKLA..WMPFRIRNRVTFRVIQALLRSSCMVAGNKLLKH | 43 |
| Consensus | | |
| Est2L | GCLGVNRRSMCLDGLSLQIVFGTRAPKGVILYLHGGAFG | 600 |
| PHS53692 | ...LEKINGVPVRRLLTPKTIAREFN..KVFIDVHGGAYV | 157 |
| WP_082823965 | ...LEKINGVTVRRLLTPKTIAREFN..KVFIDVHGGAYV | 149 |
| AVF72281 | ...VKTERVAGSAPAEWIIASGAEDG..PILLYLHGGGYV | 79 |
| AKG92633 | ...GLCAESRRVGSVFVRIRIRKGAAG..GVVLDIHGGGWV | 79 |
| Consensus | p hgg | |
| Est2L | LGSFRSHYSVTSRLARDSGCANWVFDYSLATEHYFAALE | 640 |
| PHS53692 | FFAGLPSIEESLLIAHRVGITVISIDYRMPPHAFPAALN | 197 |
| WP_082823965 | FFAGLPSIEESLLIAHRVGITVISIDYRMPPHAFPAALN | 189 |
| AVF72281 | MGSMTTRHVMIAHISRAAGARVLGLDYRLATEFVFPAQVE | 119 |
| AKG92633 | IGNACMNDLNVAMVNACEVAVSVVDYRLAVNTVEGILE | 119 |
| Consensus | v dy | |
| Est2L | GCLACDAIRACLC..PADKLLRAGISAGGNVLAALALM | 678 |
| PHS53692 | DVVSIVYSSVAEH...GACNLFEGGHSAGAGVLSAVCQL | 234 |
| WP_082823965 | DVVSIVYSSVAEH...DAHNLFEGGHSAGAGVLSAVCQL | 226 |
| AVF72281 | DAVAAIRWLLIANGS..DPKFIIVGGHSAGGGMVATLVAM | 157 |
| AKG92633 | DCLATARWLLDCEEFAGLPVIVVGSAGGHAATLLAM | 159 |
| Consensus | d a g sag l l | |
| Est2L | KEPENGIAAGIMLLSPVTEPDLGGASMCSPCEATPMIR.. | 716 |
| PHS53692 | IADKQFLPAAYVAGTFWADLTKEGDTLYTNEGVRILVTY | 274 |
| WP_082823965 | IETKQSLPLAVYAGTFWADLTKEGDTLYTNEGIRILLTY | 266 |
| AVF72281 | RYLGEPMPAAGVGLSVWVMEGIGETFITNAEVTIMVC.. | 195 |
| AKG92633 | KCSPELLARVSGAVLYYGVYDLTGCTFSVTRTAGRTLLDG | 199 |
| Consensus | g | |
| Est2L | RDWLECALRAYA...APVCALSHRPLEADLRGIPPMIIQ | 752 |
| PHS53692 | CGFLEAAANLYAGS..ESLTHSSISPLYGNFEGFPPTFLI | 312 |
| WP_082823965 | CGFLAASAKLYAGS..ESLTHPSISSLYGNFEGFPPTLLV | 304 |
| AVF72281 | KDLILCIAGVYLG...KDPRAPIASPIHADLRGIPPELLQ | 233 |
| AKG92633 | PGMVEALRLITPLSDECCRCPFLISPLYGLDLGIPFALMF | 239 |
| Consensus | g PP | |
| Est2L | VGDCELLLPSSTRLAERARQSCACGLEIHCGRWVFCLO | 792 |
| PHS53692 | SGTREMFLDITVRVNRKLRDAGVTCLEVFEGLSHADYVV | 352 |
| WP_082823965 | SGTREMFLDITVRVNRKLRNANVTCLEVFEGLSHADYVV | 344 |
| AVF72281 | VGSIEITLLPSNCLARLAKADQEVKVEVWDEMHWVFCDF | 273 |
| AKG92633 | VSELDFIPDITLCMAERNWAGSDVFLLPAAHGFIHFPTAM | 279 |
| Consensus | g l d v h | |
| Est2L | AFYLRARNALLGLARFARQCLETAGCTFCAADSFRTTCP | 832 |
| PHS53692 | AYETPESHVYQELKCFLLSVCTKSSD..... | 379 |
| WP_082823965 | AYETPESLSVYQELKCFLLSVSTNSFD..... | 371 |
| AVF72281 | APILPEALCAIDGIGEFIKKHTG..... | 296 |
| AKG92633 | S.....GRVLAISREWITGRLSVG..... | 299 |
| Consensus | | |
| Est2L | ATDFQP | 838 |
| PHS53692 | | 379 |
| WP_082823965 | | 371 |
| AVF72281 | | 296 |
| AKG92633 | | 299 |
| Consensus | | |

(b)

| | | | |
|-----------|---|-------------|-----|
| ABL95965 | ..MENNTFVSFLKVLLLGTIVFYISTHLS | TSRIVEYVS.. | 36 |
| AAC21862 |MIFIFISLFAKIFFNNDFFTN | SHVKIMAK.. | 30 |
| CAA37863 | MLLKRLGLAALFSLSMVGCTTAPNTLAVNTTCKRIICYERS | | 40 |
| CAA47949 | MLLKRLCFAALFSLSMVGCTNAPNALAVNTTCKRIICYERN | | 40 |
| AAC67392 | .MNRDAIKILGAGAIAGALATRIPLSISCTPPCINYS.. | | 37 |
| Est4L | | | 0 |
| Consensus | | | |
| ABL95965 |QDAKKLTIEGICVAYREYKGNFTETIVFLMGFAG | | 70 |
| AAC21862 |SLLNYCFHQVKQTIN.TPVLIFIHGLFG | | 57 |
| CAA37863 | KSDLEVKSLTLASGDKMVYAENDNVTG..EPILLINGFGG | | 78 |
| CAA47949 | KSDLEIKSLTLASGDKMVYAENGNAV..EPILLINGFGG | | 78 |
| AAC67392 | ..NLVPKEFGWAFVNNIHIYYEYIGSG..EPLIMINGVLG | | 73 |
| Est4L |MAYFEMEGOTLHYEYVHG..APLILIHGLGS | | 30 |
| Consensus | | g | |
| ABL95965 | SSYDNKVLIDV.LSENVHCIAFGIPFFELSEKKNDFDYSD | | 109 |
| AAC21862 | DMDNLGVIAARA.FSEHNSILRIIDLNNHCHSFHS..EKMN | | 94 |
| CAA37863 | NKDNFTRIADK..LEGYHLIIBCLLGFENSSKPMADYRA | | 116 |
| CAA47949 | NKDNFTRIARQ..LEGYHLIIBCLLGFENSSKPMADYRS | | 116 |
| AAC67392 | NLESWGPPIIINGLASCHVEIINDNRGTGSGTVGQDFLMD | | 113 |
| Est4L | SCLEWELCVPV.LSGHYRLVVVVRGHERSDKP.RERYSI | | 68 |
| Consensus | | y d g s | |
| ABL95965 | ESIVRLILKSLDSLGIE.....CFTLVGSHMGGYLSLAI | | 143 |
| AAC21862 | CLMAEDVIAVIRHNLIS.....KVILGHSMGGKTAMKI | | 128 |
| CAA37863 | DACATRLHELMCAKGLA.....SNTHVCGNSMGGCAISVAY | | 151 |
| CAA47949 | EAQTRLHELMCAKGLA.....SNTHVCGNSMGGCAISVAY | | 151 |
| AAC67392 | ALTYTIPLYASDTIGLLNYLGYSNLNVIGWSMGGFVACCI | | 153 |
| Est4L | AGFTADLVALIEHLCIP.....PAHVVGLSMGGMIAFCL | | 102 |
| Consensus | | g smgg | |
| ABL95965 | *SIISRRVERPILFPAAYDVNSEDLQNPFPFLKDEHLL | | 183 |
| AAC21862 | TALCHELVERPILVIEMSE.....MPYEGFGHKDVFNGLF | | 162 |
| CAA37863 | AAKSEKEIKSNWLVTAG.....FWSAGVFKSLEGATL | | 184 |
| CAA47949 | AAKSEKEIKSNWLVTAG.....FWSAGVFKSLEGATL | | 184 |
| AAC67392 | AIDNPSYVNRVLLCTAFNI...YLPPFKVSPCSIITGFT | | 190 |
| Est4L | AVDBEQMLKSNLIVNSAF.....QVKVRSASDYWCWAK | | 135 |
| Consensus | | p l | |
| ABL95965 | KFYCIFLDVGLKTYPLFKFVYRNSLAEGEILNAEHFDYLF | | 223 |
| AAC21862 | AVRNAKFPENRQCAKFIKQEIN.DEDVVCFMLRSFDVNSA | | 201 |
| CAA37863 | ENNPLLINSKEDFYMYDFVMYKPPYIPKSVKAVFACERI | | 224 |
| CAA47949 | ENNPLLINSKEDFYMYDFVMYKPPYIPKSVKAVFACERI | | 224 |
| AAC67392 | ASDFTVTVETIIPYLVPDNLQAHFDVAKYVLTLEKYFI | | 230 |
| Est4L | RWSLARILSLNTIGKALGSLFPKPECAELRRKMAERWAK | | 175 |
| Consensus | | | |
| ABL95965 | SCNYFLPAEILIKFTKDKAAKFLKIDLEGITARTLIYIG | | 263 |
| AAC21862 | DCFRFNLTALENNYANIMDEK.....VRVFTETIFIKG | | 235 |
| CAA37863 | NN.KALDTFILEQIVTENVEER..AKIIAKYNIITLVVWG | | 261 |
| CAA47949 | NN.KALDAKILEQIVTENVEER..AKIIAQYKISTLVVWG | | 261 |
| AAC67392 | S....YTSVLKCTNALATFNS..VGQLCNITARTIVVGG | | 263 |
| Est4L | N....DKRAYLASFEAIVGWGV..CECLSKISCTIVVISA | | 209 |
| Consensus | | t1 | |
| ABL95965 | *EKQCITPFSIGEYLSKSIKSKFMLIP.NEGEMPLSN..R | | 300 |
| AAC21862 | GNSSYIKIENSEKILECFENATAFTIN.GSGHWVHAKEPD | | 274 |
| CAA37863 | DRDQVIKPETTTELKELIPQACQVIMN.DVGEVPMVEAVK | | 300 |
| CAA47949 | DRDQIKPEETVNLIKKIIPQACQVIMME.DVGEVPMVEALD | | 300 |
| AAC67392 | DSDLLPFCNSQYLAENINAGLYIFSPDAGEGLIYCYFT | | 303 |
| Est4L | DHDYTPVACK.EIIVKLLDARLVVIE.DSPKATPLDQFE | | 247 |
| Consensus | | h | |
| ABL95965 | LVIELVRNFLIDILE.... | | 315 |
| AAC21862 | FVIRAIKRFNLNKN..... | | 287 |
| CAA37863 | DTANDYKAFRDGLKK.... | | 315 |
| CAA47949 | ETADNYKAFRSILEACR.. | | 317 |
| AAC67392 | CFINLVTSFLG..... | | 314 |
| Est4L | RFNNZLLDELKTVEETTQD | | 266 |
| Consensus | | f | |

(c)

Figure S2. Multiple sequence alignments of a CzCO domain of Est2L (a), an acetyltransferase domain of Est2L (b), and Est4L (c) using the Clustal W method in DNA/MAN. Completely and highly conserved sequence(s) in the alignments are shown in black and pink boxes, respectively. Marks with star represent catalytic residues.

References for MSA sequences(Figure S2)

(Figure S2.a)

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(Figure S2.b)

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(Figure S2.c)

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