

1 **Biochemical and Structural Insights Concerning Triclosan Resistance in a Novel YX7K**  
2 **Type Enoyl-Acyl Carrier Protein Reductase from Soil Metagenome**

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4 Running title: Triclosan resistant enoyl-ACP reductase in eukaryotes and prokaryotes

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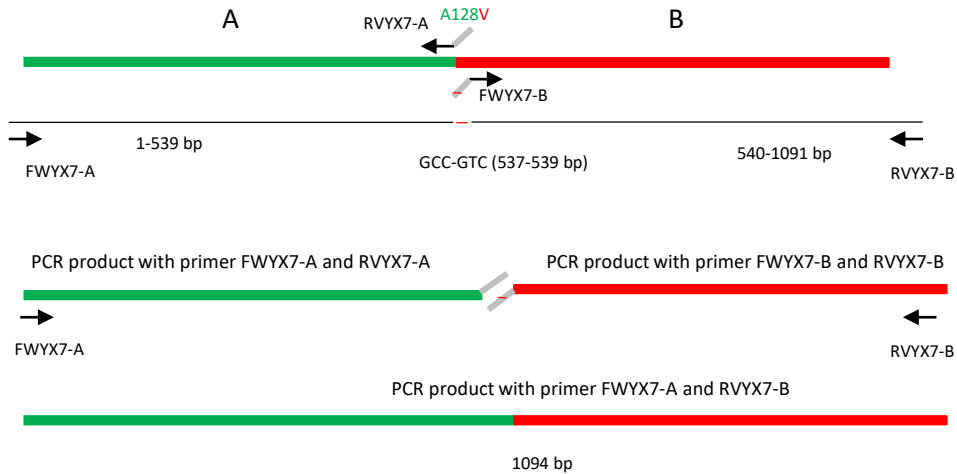
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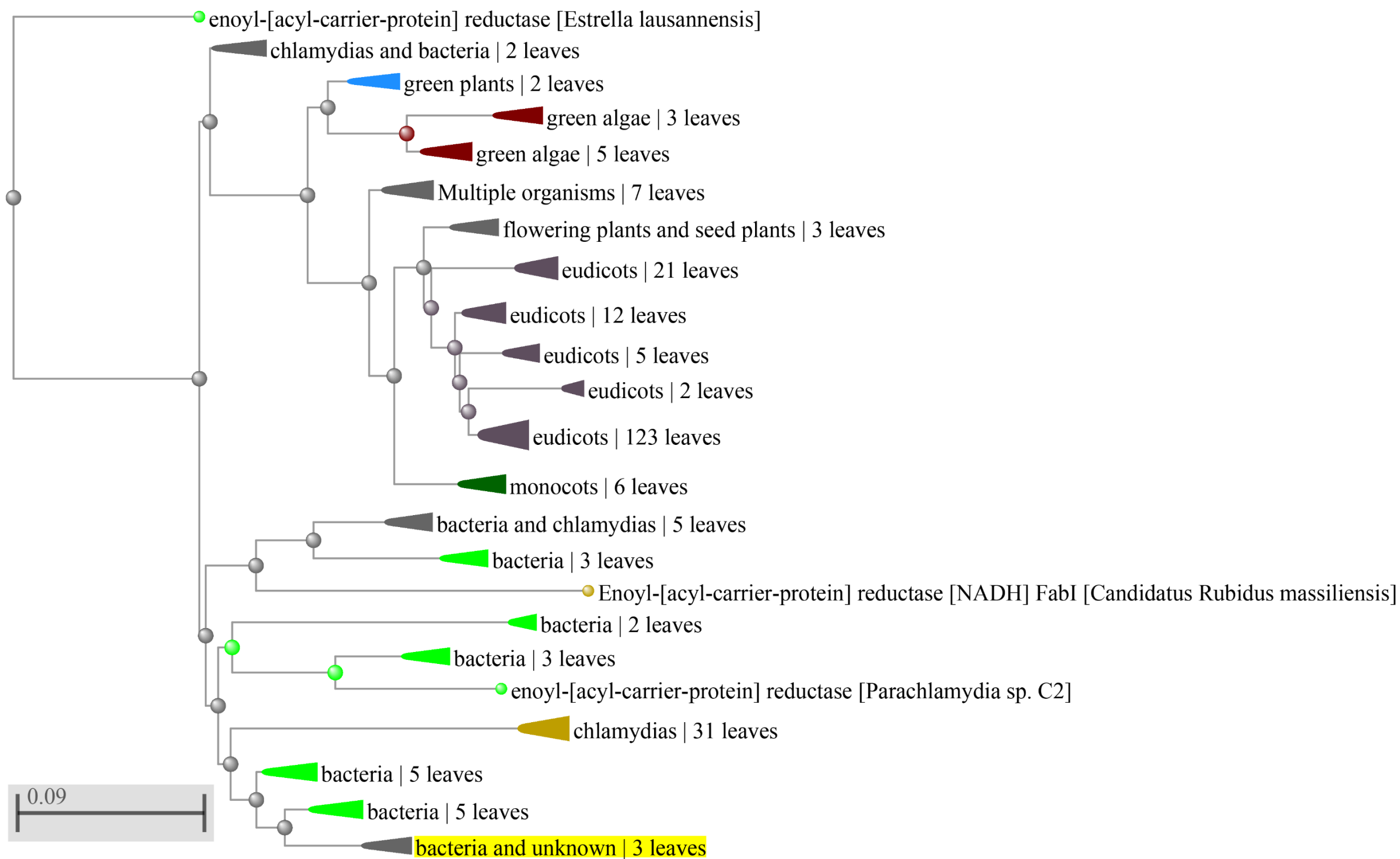
25 <sup>†</sup>These authors contributed equally to this work.

## [Fig. S1, Raees Khan et al.]

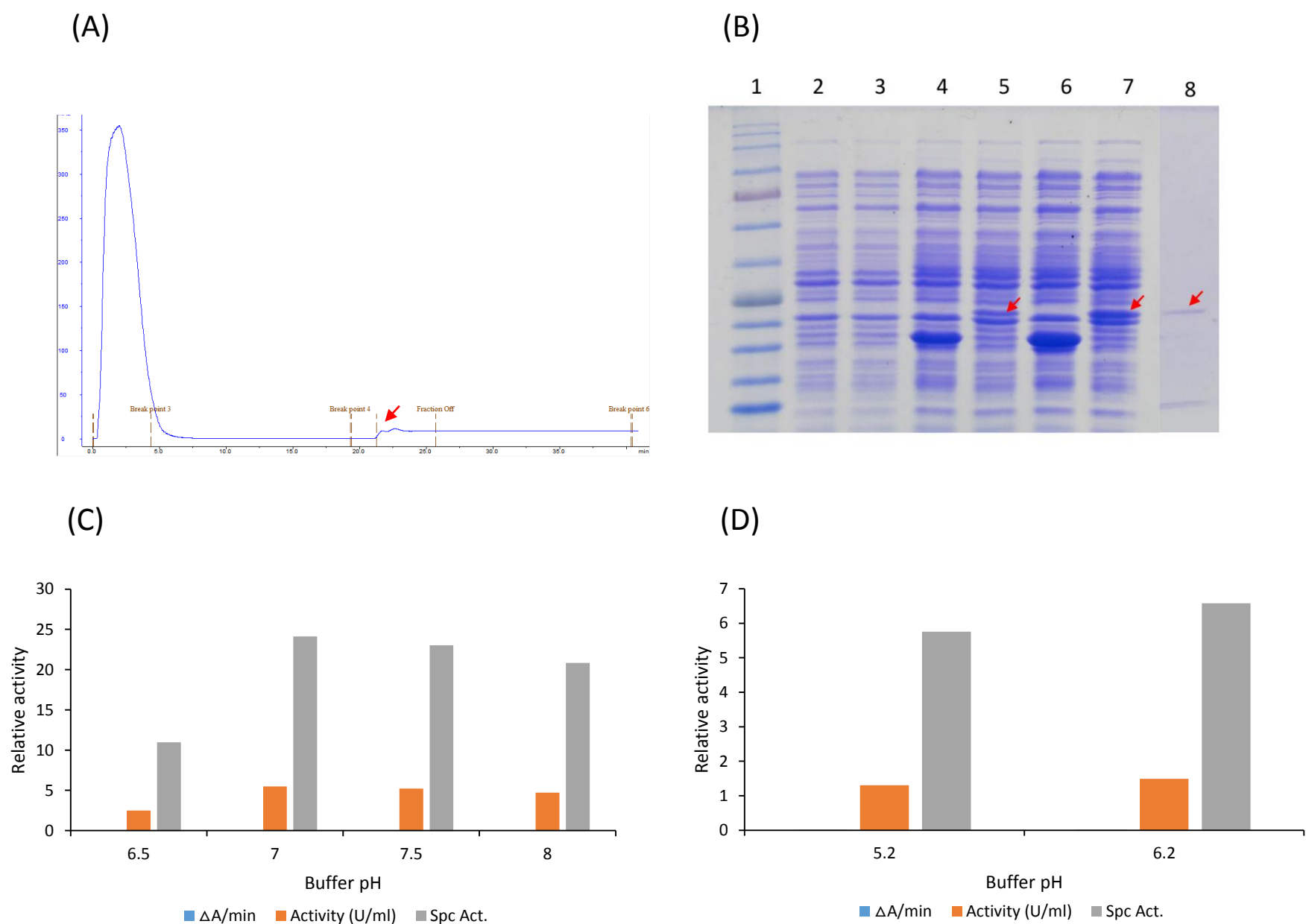


**Fig. S1. Site-directed mutagenesis using overlap extension PCR to substitute A128V.** The *FabI2* gene sequence along with additional nucleotides on the 5' and 3' ends (for primer designing and promoter regions) were divided into two regions; A and B. Region "A" contain the A128 encoding region of *FabI2* which will be substituted with V128, already incorporated in the RVYX7-A primer. Fragments "A" and "B" were amplified using mentioned primers which carries complementary overlapping nucleotide sequences (presented as gray overhangs) for the purpose of fusion PCR. Both fragments were finally fused using primers FWYX7-A and RVYX7-B to create mFabI2 with the A128V substitution.

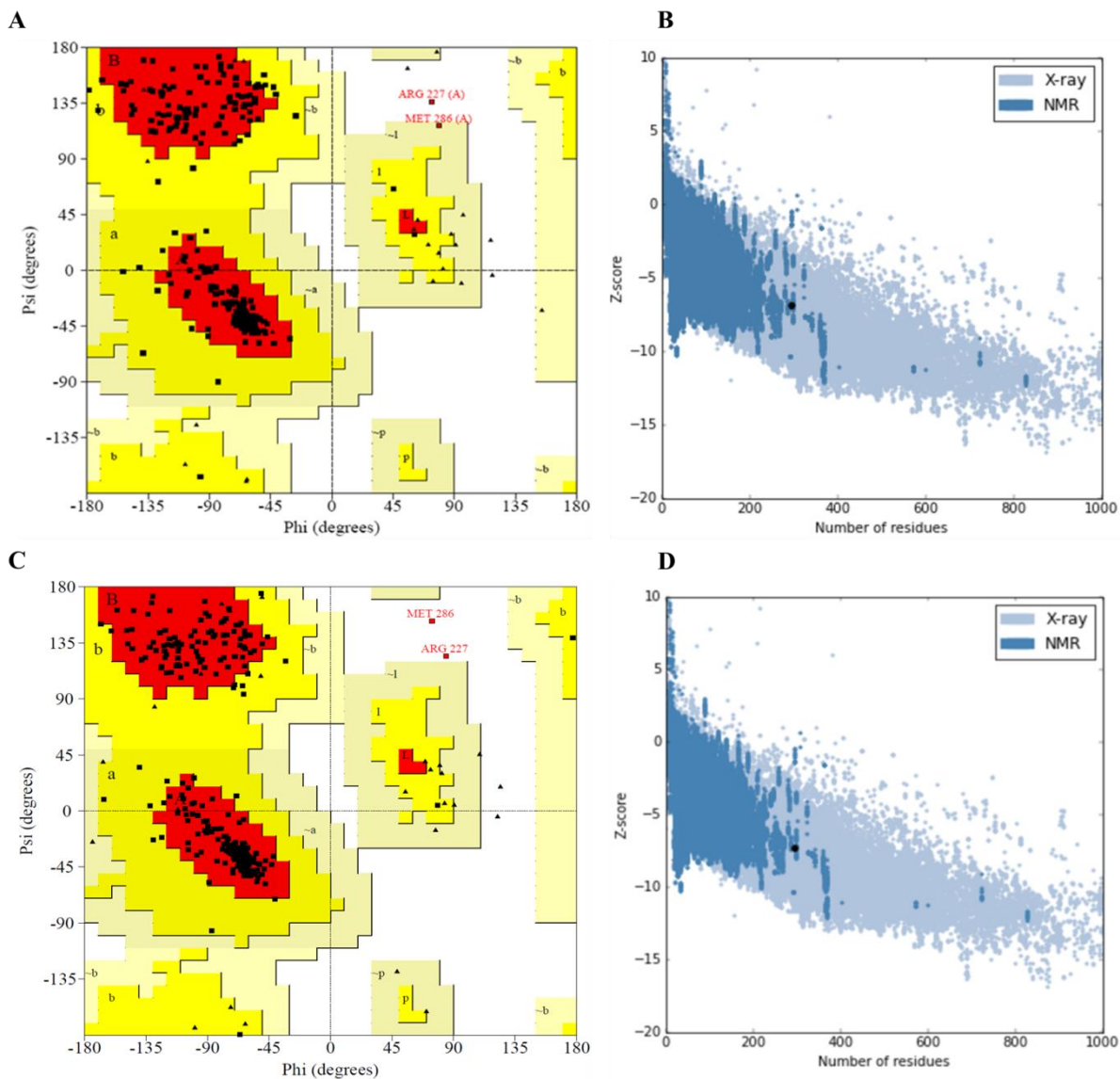
[Fig. S2, Raees Khan et al.]



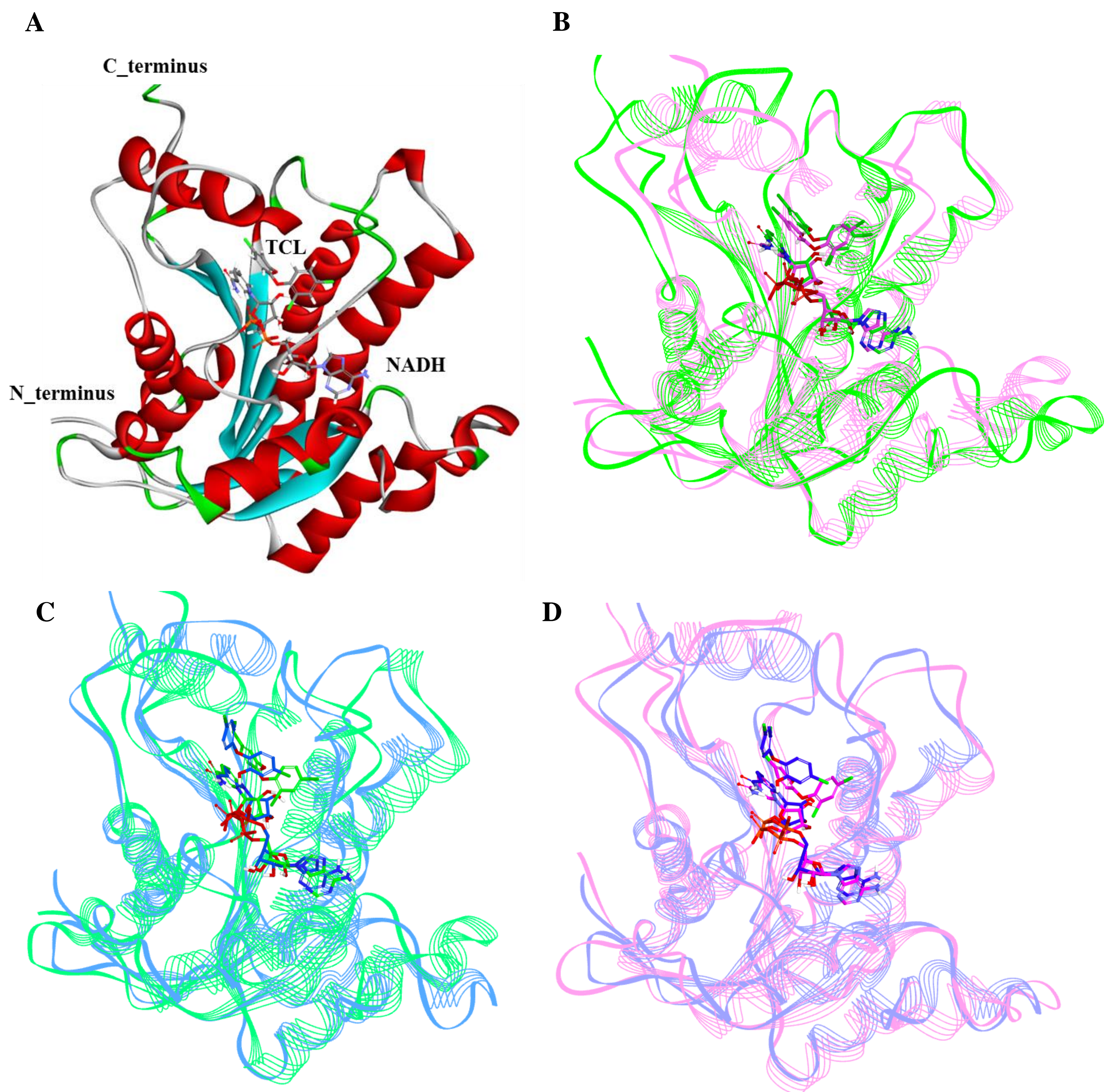
**Fig. S2. Distance tree of FabI2 ENR.** Fast minimum evolution method based phylogenetic tree was constructed using the “Distance tree of results” option of the NCBI blastp results. The closest 250 hits were selected, sharing 66-84% identity to FabI2. The maximum sequence difference was set at 0.85 and the scale bar represents 0.09 estimated amino acid substitutions per residue. Label color for the query is yellow, whereas for other closest hits various label colors have been assigned. The size of the leaf represents the number of hits corresponding to it.



**Fig. S3. Purification of FabI2 protein.** (A) FPLC chromatogram of FabI2 protein. Red arrow indicates the chromatogram peak corresponding to desired fusion protein (B) FabI2 protein was purified by affinity chromatography. Gel electrophoresis indicate the purity of FabI2 protein. Lane 1, standards; lane 2, uninduced *E. coli* DE3 cultures (+ve control) carrying FabL2 in pET-30b(+); lane 3, Uninduced *E. coli* DE3 cultures carrying FabI2 in pET-30b(+); Lane 4, *E. coli* DE3 cultures carrying FabL2 in pET-30b(+) 2 hours post IPTG (1 mM) induction; Lane 5, *E. coli* DE3 cultures carrying FabI2 in pET-30b(+) 2 hours post IPTG (1 mM) induction; lane 6, *E. coli* DE3 cultures carrying FabL2 in pET-30b(+) 4 hours post IPTG (1 mM) induction; Lane 7, *E. coli* DE3 cultures carrying FabI2 in pET-30b(+) 4 hours post IPTG (1 mM) induction; Lane 8 purified FabI2 (37.74 kDa) protein (cropped from a separate SDS gel picture and merged here). (C) Enzyme activity of the purified FabI2 protein in 100 mM sodium phosphate buffer (pH; 6.5-8). (D) Enzyme activity of the purified FabI2 protein in 100 mM sodium citrate buffer (pH; 3.2-6.2). Highest activity was observed at pH 7.0 in case of sodium phosphate buffer and at pH 6.2 for sodium citrate buffer. Orange bar represents enzyme unit (Unit/ml) of FabI2 and gray bar represents specific activity, whereas the blue bars represents change in absorbance per minute.



**Fig. S4. Validation of FabI2 and mFabI2 models.** Ramachandran plots of phi/psi ( $\Phi/\Psi$ ) distribution of homology models, calculated by PROCHECK, of (A) FabI2 and (C) mFabI2. The Z-score plot for modeled structures of (B) FabI2 and (D) mFabI2. Both the analyses suggested that modeled structures of FabI2 and mFabI2 were reasonably accurate and were within the range typically observed for native proteins of similar size.



**Fig. S5. Structure of BnENR (template) and superimposition of BnENR, FabI2 and mFabI2.** (A) Crystal structure of *Brassica napus* Enoyl-acyl carrier protein reductase (BnENR, PDB ID: 1D7O) has been used as template for homology modeling of FabI2. NADH and TCL are depicted as sticks representations. (B) Superimposition of BnENR and FabI2 in complex with NADH and TCL. Structure of BnENR is shown as green wire representation, while FabI2 is depicted as magenta wire representation. Color scheme for NADH and TCL is tailing their respective protein partner and are shown as sticks representation. (C) Superimposition of BnENR and mFabI2 in complex with NADH and TCL. Structure of BnENR and its associated NADH and TCL is shown as green wire and sticks model, respectively. mFabI2 and its associated NADH and TCL are colored blue. (D) Superimposition of FabI2 and mFabI2 in complex with NADH and TCL. FabI2 and mFabI2 are depicted as wire representations, while NADH and TCL are shown as sticks. Magenta and blue colors reflect FabI2 and mFabI2, respectively.

# [Fig. S6, Raees Khan et al.]

FabI2	HTISLTYMASEKAVPGYGG-GMSSAKAALESDNRVLAWEAGRK	Metagenomic FabI2	
Q9SLA8	ASISLTYIASERIIPGYGG-GMSSAKAALESDTRVLAYEAGRK	FabI2 homologues having YX <sub>7</sub> K type catalytic domain	
A0A061GSG8	SSISLTYIASERIIPGYGG-GMSSAKAALESDTRVLAFEAGRK		
A0A0A0LXD8	SSISLTYIASERIIPGYGG-GMSSAKAALESDTRVLAFEAGRK		
B8BXA1	NMLSLTYIASSEKVIIPGYGG-GMSSAKAAQLESDTRTLAFEAGRK		
B9T5D7	SSISLTYIASERIIPGYGG-GMSSAKAALESDTRVLAFEAGRK		
U6GHH8	AALALSYIASERVIIPGYGG-GMSSAKAALESDCRTLAFEAGRQ		
Q6X5U8	SILSLTFIASERVVPGYGG-GMSSAKAAQLESDTKVLAWEAGRK		
Q6TEI5	SVVSLTYQASQKVVPGYGG-GMSSAKAALESDTRVLAYYLGRK		
FabI	ALLTLSYLGAERAI PNYN--VMGLAKASLEANVRYMANAMGPE		Prototypic FabI
O67505	AIVTLSYYGAEKVVPHYN--VMGIAKAALESTVRYLAYDIAKH		FabI homologues having YX <sub>6</sub> K type catalytic domain
C6J746	SIMTMTYMGSERVMRNYN--VMGVAKAGLEASVRYLANDLGPK		
Q3A2X2	SVLTMTYLGAQRAVPNYN--VMGVAKAALEASVRYLAAEVGSQ		
Q820V5	GIVTTLTYLGSERAI PNYN--MMGIAKASLETAVKYLAFAELAAD		
L0RE87	SVMAMTYLGASKVITNYN--VMGVAKAALEASVRYLSSDMGSS		
Q81GI3	NILTLTYLGCERVVKNYN--VMGVAKASLEASVKYLANDLGQH		
O24990	SVLTLSYLGSTKYMAYN--VMGLAKAALESAVRYLAVDLGKH		
WP_037465251	TIVTLTHMGSEKRVLPNYH--VMGVAKAGLESSIRYLANELGPA		
B9M6K7	SILALTYYGQKVFPNYN--VMGVAKAALEMSIRYLAFAVGP		
P73016	SIITLTYFGGVKVI PNYN--LMGVAKAGLEMTVRYLAAELGPQ		
FABL bas sub	HIVSISSLGSI RYLENYT--TVGVSKAALEALTRYLAVELSPK	Prototypic FabL	
B3QVF0	KIVALSSIGSLRCLPAYA--AVGTAKAGLEAMVKYLAVELGPK	FabL homologues having YX <sub>6</sub> K type catalytic domain	
A0A098G835	RVVALSSLGASRAI PNYYA--FIGASKAALESLVRSLSLELAIY		
WP_038326851	RIVAMSSLGAQRAMPHYG--FIGASKAALESLVRTLAQELGPR		
A0A098GBD7	RVIALSSLGASRAI PNYYG--FIGASKAALEALVRSLSLELAEY		
WP_044743084	KIVSISSLGSI RYLENYT--AVGVSKAALEALTRYLAVELAPK		
A0A0E0T904	KIVSISSLGSI RYLENYT--AVGVSKAALEALTRYLAVELAPK		
WP_035487157	AMLALSSFSGSERVFPYYT--SVGASKAALEALVRYLAVELAPR		
J9HD84	AMLALSSFSGADRVPYYV--SVGSSKAALESLVRYFAIELAPR		
WP_028582575	RIITISSLGSVRALPDYT--AVGASKAALESLVRHLAVELGPK		
W7B1Y2	KIIGVSSIGAIRYLENYT--TVGVSKAAVEALTRYLAVELAPL		
FabL2	AVVTMSSTGNLIYIENYA--GHGINKAAVEAMSRYAAREL GEM	Prototypic FabL2	
CJ-lab7alpha	SIISISSTGNLVYIENYS--GHGTAKAAVEAMARYAATELG EK	FabL2 homologues having YX <sub>6</sub> K type catalytic domain	
HPLAB-7ALP	AIVSLSSTGNLVYMPNYA--GHGNSKNAVETMVKYAAVDLGEF		
Q17WU6	AIVSLSSTGNLVYMPNYA--GHGNSKNAIETMVKYAAVDLGEF		
I0ETE1	AIVSLSSTGNLVYMPNYA--GHGNSKNAVETMVKYAATDLGEF		
T2SLB2	AIVSLSSTGNLVYMPNYA--GHGNSKNAVE-----		
WP_029520276	KIIAISSTGTRDYMPNYA--IHGAASKALEALVRYAAVEFGPK		
WP_029523144	KIIAISSTGTRDYMPNYA--IHGAASKALEALVRYAAVEFGPK		
FabV Vib Albensis	KTVAYSYIGTELTWPIYWDGALGRAKMDLDRAATALNEKLAAK		Prototypic FabV
A0A0H6Q9B8	KTVAYSYIGTELTWPIYWDGALGRAKMDLDRAATALNEKLAAK		FabV homologues having YX <sub>8</sub> K type catalytic domain
Q2P9J6	RSVAFSYIGTEITWPIYWHGALGKAKVDLDRTAQRLNARLAKH		
A0A160SZK9	KTISYSYIGTISITWPIYRHGTLGKAKEHLENTAKNINKKLFEN		
Q62L02	KTTAFTYLGQVTHDIYWNGSIGEAKKDLDRTVLALRGKLAAR		
H9UIZ3	VTLALS YIGPEITTA VYRDGTIGQAKKHLEKTAHSLSESLKDI		
A0A098G1G8	ITVAYSYIGPPVSHAIYKDGTIGKAKEHLQQTAKHLNQLKLEKI		
Q73Q47	ITLAYS YIGPEATQALYRKGTIGKAKEHLEATAHRLNKENPSI		
Q6LP67	KTVAYSYVGP KATYSIYHQGTIGRAK AHLHATADQLNDKMSEM		
A0A143XGL5	RTVAYSYIGPEMTPYIYTEGTIGQAKQHLYHTALQLN--AEGI		

**Fig. S6. The YX<sub>7</sub>K catalytic domain was unique to metagenomic FabI2 ENR and its homologs from UniRef50**

**database.** Multiple alignment (partial) of metagenomic FabI2 ENR and its homologs with prototypic well characterized ENRs and their homologs from UniRef50 database. The YX<sub>7</sub>K catalytic domain (boxed in red) was unique to FabI2 ENR and was strictly conserved among its homologs. On the other hand, other prototypic ENRs has different catalytic domains; FabI (YX<sub>6</sub>K type, boxed in green ), FabL (YX<sub>6</sub>K type, boxed in dark yellow ), FabL2 (YX<sub>6</sub>K type, boxed in dark brown ), and FabV, (YX<sub>8</sub>K type, boxed in cyan).

	310	320	330	340	350
FabI2	MNAGGSTISLTYMASEKAVPGYGGGMSSAKAALES	DNRVLAW	EAGRK	WGW	
WP 032125249.1	LNAGGSALSILTYLASEKIIPGYGGGMSSAKAALES	DTRTLAW	EAGRK	WWS	
WP 059060327.1	LNAGGSALSILTYLASEKIIPGYGGGMSSAKAALES	DTRTLAW	EAGRK	WWS	
AUX13448.1 eno	MSDGGSAINLTYLASERAVPGYGGGMSSAKAALES	DTRTLAW	EAGRK	WVN	
OGN58662.1 eno	MPALGASLSLSYIASERAIPGYGGGMSSAKAALES	DTRTLAF	EAGRK	WVG	
OGN53367.1 eno	MPALGASLSLSYIASERAIPGYGGGMSSAKAALES	DTRTLAF	EAGRK	WVG	
PIS02783.1 eno	MNQGGSVLSLTYIASERVIPGYGGGMSSAKAALES	DTRTLAW	EAGRK	WVN	
OJV07209.1 eno	MNPGGATLSLTYIASERKAIPGYGGGMSSAKAALES	DTRSLAW	EAGRK	WVN	
WP 041418840.1	MPPGSSALNLTLYLASERAVPGYGGGMSSAKAALES	DTRTLAW	EVGRK	WVG	
CCB88976.1 eno	MPPGSSALNLTLYLASERAVPGYGGGMSSAKAALES	DTRTLAW	EVGRK	WVG	
WP 042239046.1	INAGGATLSLTYIASERKAIPGYGGGMSSAKAALES	DTRTLAW	EAGRK	WVN	
WP 044882332.1	INAGGATLSLTYIASERKAIPGYGGGMSSAKAALES	DTRTLAW	EAGRK	WVN	
KIC73781.1 Eno	INAGGATLSLTYIASERKAIPGYGGGMSSAKAALES	DTRTLAW	EAGRK	WVN	
WP 039384665.1	INAGGATLSLTYIASERKAIPGYGGGMSSAKAALES	DTRTLAW	EAGRK	WVN	
OGN63603.1 eno	MNEGGSVLSLTYIASVRAIPGYGGGMSSAKAALES	DTRTLAW	EAGRK	WVK	
WP 042103672.1	MPPGSSCISLTYIAAEKVIPGYGGGMSSAKAALES	DTQVLA	FEAGR	KWN	
WP 088245030.1	MREGGSVLSLTYLASERVVPGYGGGMSSAKAALES	DTRTLAW	EAGRK	WVG	
PCI94168.1 eno	MSHGGSCLTLYIASERAVPGYGGGMSSAKAALES	DTRTLAF	EAGRK	WVG	
WP 013925330.1	MNAGGSTLSLTYIASERKAIPGYGGGMSSAKAALES	DTRTLAW	EAGRK	WVN	
WP 006339971.1	MNAGGSTLSLTYIASERKAIPGYGGGMSSAKAALES	DTRTLAW	EAGRK	WVN	
OGN56192.1 eno	MNEGGAAALCLTYIASERAIPSYGGGMSSAKAALES	DTRTLAW	EAGRK	WVN	
CCB91849.1 Eno	MNAGGSAISLTYFASERAVPGYGGGMSSAKAALES	DTRILSW	EAGRK	WVK	
WP 013181731.1	MNAGGSAISLTYFASERAVPGYGGGMSSAKAALES	DTRILSW	EAGRK	WVK	
GAQ86187.1 Pla	MTQGGATVSLTYFASEKVIPGYGGGMSSAKAALES	DTQVLA	W	EAGRK	YVG
ABK25822.1 unk	MNPGGSTISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	RRYN	
CDZ80974.1 Eno	MNEGAATISLTYAASEKAIPGYGGGMSSAKAALES	DTRVLA	YEAPR	KFG	
PNR39341.1 hyp	MNPGGSSLSLTYVASEEQIIPGYGGGMSSAKAALES	DTRVLA	FEVGR	KYV	
CDP10132.1 unn	MNPGGATISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHR	
XP 015898944.1	INPGGATISLTYIASERTIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHK	
AMY26635.1 red	MNPGGATISLTYLASEKIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHN	
XP 001768068.1	MNPGGSSLSLTYVASEEQIIPGYGGGMSSAKAALES	DTRVLA	FEVGR	KYV	
XP 022020754.1	MNRRGGATISLTYIASERIIIPGYGGGMSSAKAALES	DTQVLA	FEAGR	KHG	
XP 023914015.1	MNPGGASISLTYIASERIIIPGYGGGMSSAKAALES	DTQVLA	FEAGR	KHK	
WP 013712757.1	MNSGGCTISLTYLASMRAVPGYGGGMSSAKAALES	DTKTLAW	EAGR	RWN	
PON52209.1 Glu	MNPGGASISLTYIASERKIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHK	
WP 021756559.1	MNSGGCTISLTYLASMRAVPGYGGGMSSAKAALES	DTKTLAW	EAGR	RWN	
POF25828.1 eno	MNPGGASISLTYIASERIIIPGYGGGMSSAKAALES	DTQVLA	FEAGR	KHK	
ACZ06071.1 eno	MNPGGSSISLTYIASERKIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KRK	
XP 004142140.1	INPGGSSLSLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHK	
WP 063329268.1	MNSGGCTISLTYLASMRAVPGYGGGMSSAKAALES	DTKTLAW	EAGR	RWN	
PON81019.1 Glu	MNPGGASISLTYIASERKIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHK	
XP 017623717.1	MNPGGSSISLTYIASERVIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHK	
AJQ20621.1 Eno	INPGGSSISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHK	
XP 008449761.1	INPGGSSLSLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHK	
XP 022944685.1	INPGGSSLSLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHK	
XP 017430721.1	LNPGGSSISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KRK	
ACJ07149.1 eno	MNPGGSSISLTYIASERKIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KRK	
XP 012472974.1	MNPGGSSISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHK	
XP 014502579.1	LNPGGSSISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KRK	
KTF28607.1 eno	MNSGGCTISLTYLASMRAVPGYGGGMSSAKAALES	DTKTLAW	EAGR	RWN	
XP 006660484.1	MNPGGATISLTYIASERTIPGYGGGMSSAKAALES	DTRVLA	YEAGR	KGK	
WP 098038283.1	MPPGSSLTLTYLAAQRAIPGYGGGMSSAKAALES	DTKTLAW	EAGR	KWG	
XP 011078829.1	MNPGGATISLTYIASERKIIPGYGGGMSSAKAALES	DTKVLAF	FEAGR	KHN	
XP 017433127.1	LNPGGSSISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KRK	
ACJ07148.1 eno	MNPGGSSISLTYIASERKIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KRK	
WP 100934847.1	MNRRGGSTISLTYLASMRAVPGYGGGMSSAKAALES	DTKTLAW	EAGR	RWG	
XP 019450932.1	LNPGGASISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KRK	
WP 068470377.1	MNPHGSALSILTYLASERVIPGYGGGMSSAKAALES	DTQTLAW	EAGR	KWN	
XP 022985712.1	INPGGSSLSLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHK	
KZN26630.1 eno	MNSGGCTISLTYLASMRAVPGYGGGMSSAKAALES	DTKTLAW	EAGR	RWN	
XP 023513365.1	INPGGSSLSLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHK	
XP 010265894.1	MNPGGSAISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHK	
WP 011175702.1	LNSQGSALTLYLASEKIIPGYGGGMSSAKAALES	DTRTLAW	EAGR	KWN	
XP 022154116.1	MNPGGSSISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KNK	
WP 010883049.1	MNRRGGSTISLTYLASMRAVPGYGGGMSSAKAALES	DTKTLAW	EAGR	RWG	
XP 009348968.1	MNPGGASLSLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHN	
WP 080126519.1	MNEGASTISLTYLASTRAVPGYGGGMSSAKAALES	DTKVLAW	EAGR	RWG	
XP 009348967.1	MNPGGASLSLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHN	
XP 014493530.1	LNPGGSSISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KRK	
BAT81583.1 hyp	LNPGGSSISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KRK	
WP 035405642.1	MNEGASTISLTYLASMRAVPGYGGGMSSAKAALES	DTKVLAW	EAGR	RWG	
WP 080124232.1	MNEGASTISLTYLASMRAVPGYGGGMSSAKAALES	DTKVLAW	EAGR	RWG	
XP 009369620.1	MNPGGASLSLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHN	
ADV16377.1 eno	MNPGGASISLTYIASERIIIPGYGGGMSSAKAALES	DTQVLA	FEAGR	KHG	
KZV36401.1 NAD	MNPGGSTISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHN	
XP 008387259.1	MNPGGASLSLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHN	
XP 011025228.1	MNRRGGSSISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KNR	
XP 008367361.1	MNPGGASLSLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHN	
WP 010230294.1	MNEGASTISLTYLASMRAVPGYGGGMSSAKAALES	DTKVLAW	EAGR	RWG	
WP 009873559.1	MNAGASTISLTYLASMRAVPGYGGGMSSAKAALES	DTKVLAW	EAGR	RWG	
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XP 022018336.1	MNPGGASISLTYIASERIIIPGYGGGMSSAKAALES	DTQVLA	FEAGR	KHG	
AMY26631.1 red	MNPGGATISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHK	
PNT46771.1 hyp	MNPGGSSISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KNR	
XP 002297662.2	MNPGGSSISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KNR	
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NP 001280885.1	MNPGGASLSLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHN	
XP 011037915.1	MNPGGSSISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KNR	
WP 080122016.1	MNEGASTISLTYLASTRAVPGYGGGMSSAKAALES	DTKVLAW	EAGR	RWG	
WP 011006351.1	MHPGGSSISLTYLASSRAVPGYGGGMSSAKAALES	DTKMLAW	EAGR	KWG	
XP 002304026.1	MNPGGSSISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KNR	
WP 080127032.1	MNEGASTISLTYLASMRAVPGYGGGMSSAKAALES	DTKVLAW	EAGR	RWG	
XP 009376124.1	MKPGGASLSLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHN	
WP 080128631.1	MNEGASTISLTYLASMRAVPGYGGGMSSAKAALES	DTKVLAW	EAGR	RWG	
XP 004304370.1	MNPGGASISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHR	
XP 021830626.1	INPGGASISLTYIASERIIIPGYGGGMSSAKAALES	DTRVLA	FEAGR	KHN	

**Fig. S7. The YX<sub>7</sub>K catalytic domain was unique to metagenomic FabI2 ENR and its top 100 homologs from NCBI database. Multiple alignment (partial) of metagenomic FabI2 ENR and its homologs (see supplementary data 1 for the details) with best hits from NCBI database. The YX<sub>7</sub>K catalytic domain (boxed in red) was unique to FabI2 ENR and was strictly conserved among its homologs.**

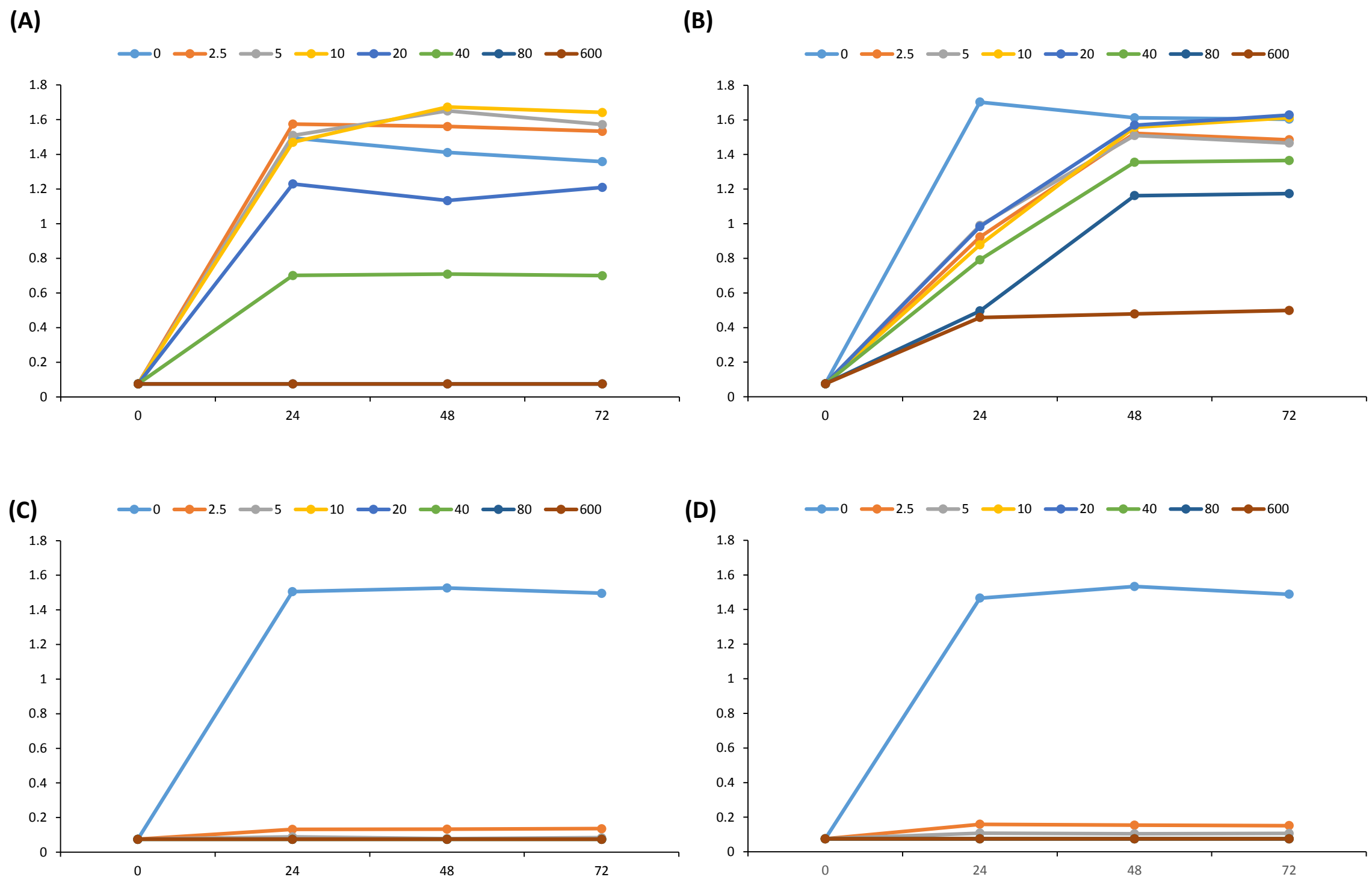


[Fig. S8, Raees Khan et al.]

1. pAF1 5 yx7k	YMASEKAVPG - YGGGMSSAKAALES	SDNRVLA	Metagenomic FabI2	
2. UniRef50 A0A061GSG8	YIASERIIIPG - YGGGMSSAKAALES	SDTRVLA		
3. UniRef50 A0A0A0LXD8	YIASERIIIPG - YGGGMSSAKAALES	SDTRVLA	Uniref 50 FabI2 homologues having YX <sub>7</sub> K type catalytic domain	
4. UniRef50 A0A0S3G5D1	YLASERVIIPG - YGGGMSSAKAALES	SDTKVLA		
5. UniRef50 A0A142XNM6	YVAGERVVPH - YGGGMGTCKAALQMDAKQLS			
6. UniRef50 B8BXA1	YIASEKVIIPG - YGGGMSSAKAAQLES	SDTRTLA		
7. UniRef50 Q9SLA8	YIASERIIIPG - YGGGMSSAKAALES	SDTRVLA		
8. UniRef50 B9T5D7	YIASERIIIPG - YGGGMSSAKAALES	SDTRVLA		
9. UniRef50 FOVRC4	EYWSSALIPVG - YGGGMSSAKAALES	SDTRTLA		
10. UniRef50 Q6TEI5	YQASQKVVPG - YGGGMSSAKAALES	SDTRVLA		
11. UniRef50 Q6X5U8	FIASERVVPG - YGGGMSSAKAAQLES	SDTKVLA		
12. UniRef50 U6GHH8	YIASERVIIPG - YGGGMSSAKAALES	SDCRTLA		
13. UniRef50 Q6Z0I4	YIASERAIPG - YGGGMSSAKAALES	SDTKVLA		
14. UniRef50 A0A067CC20	YLGAVKAI PN - YN - IMGPAKAALE	EATCRALA		Uniref 50 FabI2 homologues YX <sub>6</sub> K type catalytic domain
15. UniRef50 A0A068NVM5	YLGSTRAMKN - YN - VMGVCKAALE	EASVRYLA		
16. UniRef50 A0A098G8M8	YYGSQKVILN - YN - LMGVVKAALE	EASVRYLA		
17. UniRef50 A0A0F6A7R7	YLGSERAVQN - YN - VMGPVK	SALDSSVRYLA		
18. UniRef50 A0A0S4PDV7	YLGSERVVPG - YN - VMGVAKAALE	EASVRYLA		
19. UniRef50 A0A0U5BK22	YYGSEKVV TN - YN - VMGVAKAALE	ESSIRYLA		
20. UniRef50 A0A0W0WIB4	YYGAEKVIKN - YN - LMGPVKAALE	EASVRYLA		
21. UniRef50 A0A142WRH8	YLGGEKVI PG - YN - LMGLCK	SALESATEYCA		
22. UniRef50 A0A167IRJ7	YLGSQRAIQN - YN - VMGPVK	SALDSSVRYLA		
23. UniRef50 A0A177W1C0	YLGAQKVMQN - YN - MMGPIKAALE	EASIKYLA		
24. UniRef50 A0A1C7PE22	YLGAEKVV PN - YN - LMGVSKAALE	EATTRYLA		
25. UniRef50 A0A1E5GRH2	YLGSERAIPN - YN - MMGIKAA	SLETAVKYLA		
26. UniRef50 A0A1F3SNZ3	YLGGRVLP G - YG - IMGPAKAALE	SCVRYLS		
27. UniRef50 A0A1F3VNS9	YHGSQKVLKG - YN - VMGSAKAALE	SAIRYLA		
28. UniRef50 A0A1F8SC12	YYGAEKVV TH - YN - VMGVAKAALE	EASVRYLA		
29. UniRef50 A0A1F9KZF7	HEGSRRAVPG - YC - TMGVAKAALE	ESTVRYLA		
30. UniRef50 A0A1G3LCV0	YIGSIKALPS - YN - AMGVAKAALE	SAVRYLA		
31. UniRef50 A0A1J5F0K6	YLGATAVVQN - YN - VMGVAKAALE	SCVRYLA		
32. UniRef50 A0A1Q3SQ15	FHGSQKIVEH - YN - LMGPVKAALE	SAVRYMA		
33. UniRef50 A1K7E2	YLGSEKVI PH - YG - VMGLCKAALE	EATRYMA		
34. UniRef50 A4Z1H2	YYGADKVV NN - YN - IMGLAKAALE	EASVRYLA		
35. UniRef50 A6DIN9	YLGSERICTN - YN - VMGVAKAALE	GLECSTRYLA		
36. UniRef50 B3DWM4	YYGSEKVISN - YK - IMGPAKAALE	ESTVRYLA		
37. UniRef50 C1CY09	YHASQQVVPK - YN - VMGVAKAALE	EATRYLA		
38. UniRef50 C6XEY3	YGGSMRVV PN - YN - AMAPAK	SALESSTKYLA		
39. UniRef50 D8IVG6	YIGAEEVV VN - YG - VMGPVKAALE	SAVRYLA		
40. UniRef50 E0I8M3	YLGSNRVCAG - YN - VLGIAK	SALETTCKYLA		
41. UniRef50 E0XQH6	YAASNRFVPS - YG - IMSMAKAALE	ECWTRELA		
42. UniRef50 G7Q4C7	YYGSGRVV AN - YN - AMGVAK	SALEASVRYLA		
43. UniRef50 G8NCG2	YYASEKVV PK - YN - VMATAKAALE	EASVRYLA		
44. UniRef50 I0IG62	YYGGEKVA PG - YN - VMGVAK	ACLEHTTRYLA		
45. UniRef50 I0IPP1	YLGSERVV PH - YN - VMGVAKAALE	SAVRYLA		
46. UniRef50 I5BTJ8	YYGAEKVM PH - YN - VMGVAKAALE	EASVRYLA		
47. UniRef50 K0ABY2	YLGGEKMPV PN - YN - VMGVAK	AALDASVRYLA		
48. UniRef50 M1X3I0	YLGSI RAVPN - YN - IMGVAKAALE	EASVRYLA		
49. UniRef50 N9WGL0	YLGSERVIPN - YN - VMGVAKAALE	EASVRYLA		
50. UniRef50 O67505	YYGAEKVV PH - YN - VMGIKAALE	ESTVRYLA		
51. UniRef50 P54616	YLGGE LVPN - YN - VMGVAK	ASLDASVKYLA		
52. UniRef50 P73016	YFGGVKVI PN - YN - LMGVAKAALE	GLEMTVRYLA		
53. UniRef50 Q05069	YLCGVRAV PN - YN - VMGVAKAALE	EASVRYLA		
54. UniRef50 Q1LQX5	YDGANRVV PN - YD - LMGPVKAALE	ASCRYLA		
55. UniRef50 Q1RH28	YYGAEKVI PN - YN - VMGVAKAALE	EASVRYLA		
56. UniRef50 Q2BKW9	YLGSA LATGD - YN - IMGCAKAALE	EATRYLA		
57. UniRef50 Q2FZQ3	YLGGEFAVQN - YN - VMGVAKAALE	ANVKYLA		
58. UniRef50 Q3A2X2	YLGAQRAV PN - YN - VMGVAKAALE	EASVRYLA		
59. UniRef50 Q7UK85	YYGGEKCV PG - YN - VMGVCK	AALDASMRVLA		
60. UniRef50 Q81GI3	YLGGERVVKN - YN - VMGVAKAALE	ASVVKYLA		

Fig. S8. The YX<sub>7</sub>K catalytic domain was unique to metagenomic FabI2 ENR and its closely related homologs from UniRef50 database. Multiple alignment (partially shown) of metagenomic FabI2 ENR and its homologs with best hits from UniRef50 database. The YX<sub>7</sub>K catalytic domain (boxed in red) was unique to FabI2 ENR and was strictly conserved among few of its homologs. On the other hand, most of the closely related homologues of FabI2 from UniRef50 database shared YX<sub>6</sub>K type catalytic domain (boxed in black).

# [Fig. S9, Raees Khan et al.]



**Fig. S9. Comparative growth assay for FabI2 and mFabI2 under various concentrations of TCL.** All strains were grown in LB broth supplemented with various TCL concentrations (0–600 µg/ml). (A) Growth assay of *Escherichia coli* carrying FabI2 in pUC119. (B) Growth assay of *E. coli* carrying mFabI2 in pUC119. Two negative controls included were; (C) *E. coli* carrying prototypic FabI in pUC119 from *E. coli* K12 MG1655, and (D) *E. coli* carrying pUC119 vector only. Both of the negative controls were unable to grow at higher TCS concentrations (2.5–600 µg/ml). Legends are overlapped for higher TCL concentrations indicating no growth.

**Table S1.** Comparative identity of the metagenome derived novel FabI2 ENRs to known prototypic ENRs.

Identity of FabI2 to known ENRs						
<i>EcFabI</i>	<i>VcFabV</i>	<i>BsFabL</i>	<i>FabL2</i>	<i>S.pneFabK</i>	<i>CtFabI</i>	<i>TgFabI</i>
30%	Partial	27%	24%	Partial	67%	64 %

**Symbols and abbreviations;** Partial, these ENRs showed very low levels of similarity, only to a small region of the target prototypic ENR; *EcFabI*, FabI ENR from *Escherichia. coli*; *VcFabV*, FabV ENR from *Vibrio Cholera*; *BsFabL*, FabL from *Bacillus Subtillis*; *FabL2*, previously identified novel FabL like metagenomic ENR, *S.pneFabK*, FabK from *Streptococcus pneumonia*; *CtFabI*, FabI from *Chlamydia trachomatis*; *TgFabI*, FabI from *Toxoplasma gondii*.

**Table S2.** Kinetics of the metagenomic FabI2 ENR.

<b>Substrate/cofactor</b>	<b>K<sub>m</sub> (μM)</b>	<b>K<sub>cat</sub> (min<sup>-1</sup>)</b>	<b>K<sub>cat</sub>/K<sub>m</sub> (μM<sup>-1</sup>min<sup>-1</sup>)</b>	<b>V<sub>max</sub> (μM/min)</b>
Crotonyl-CoA	13.85	0.19396	0.014	4.441
NADH	18.21	0.9546	0.052	14.67

**Table S3:** Comparison of the type of YX<sub>n</sub>K catalytic domain of various enoyl-ACP reductases.

<b>ENR</b>	<b>Catalytic domain type</b>	<b>Reference</b>
Most SDR members	YX <sub>3</sub> K	[1, 2]
FabI ( <i>Eschericia coli</i> )	YX <sub>6</sub> K	[3]
FabL ( <i>Bacills subtilis</i> )	YX <sub>6</sub> K	[4]
FabL2 (Metagenome/ Epsilonprtoeobacteria)	YX <sub>6</sub> K	[5]
FabV ( <i>Vibrio cholerae</i> )	YX <sub>8</sub> K	[6]
FabK ( <i>Streptococcus pneumoniae</i> )	FMN type	[7]
<i>Brassica napus</i> ENR		[3]
<i>Arabidopsis thaliana</i> ENR	YX <sub>7</sub> K	
<i>Nicotinia tabacum</i> ENR		
<b>FabI2</b>	YX <sub>7</sub> K	[This study]

Symbols and abbreviations; X in YX<sub>n</sub>K is any amino acid

**Table S4:** MIC of TCL for wild type FabI2 and its mutant mFabI2 (A128V).

Clone	TCL concentration in LB agar growth media ( $\mu\text{g/ml}$ )						
	5	20	50	100	200	400	600
mFabI2	+	+	+	+	+	+	+
FabI2	+	+	-	-	-	-	-

Symbols and Abbreviations: +; Positive growth, -; No growth

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