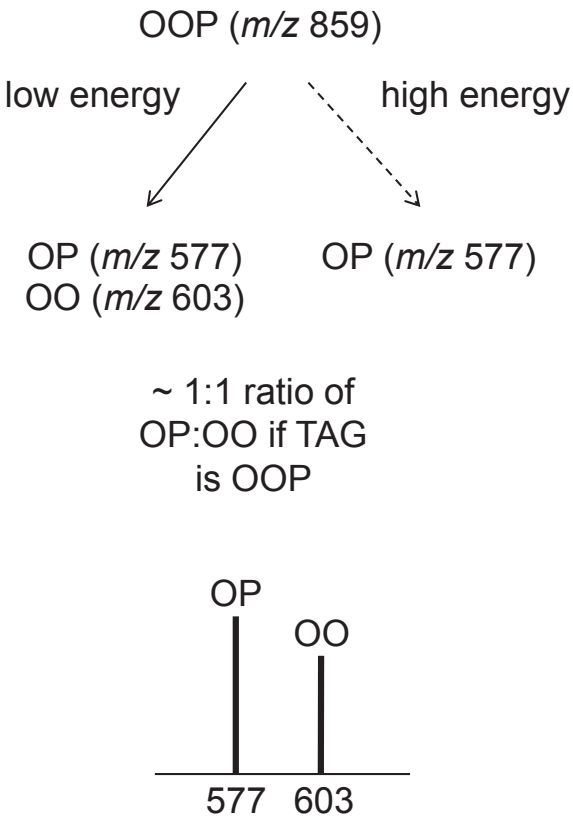
OOP (*m/z* 859)
low energy
high energy
OP (*m/z* 577)
OO (*m/z* 603)
OP (*m/z* 577)
~ 1:1 ratio of
OP:OO if TAG
is OOP
OP
OO
577
603

FIG. 1A

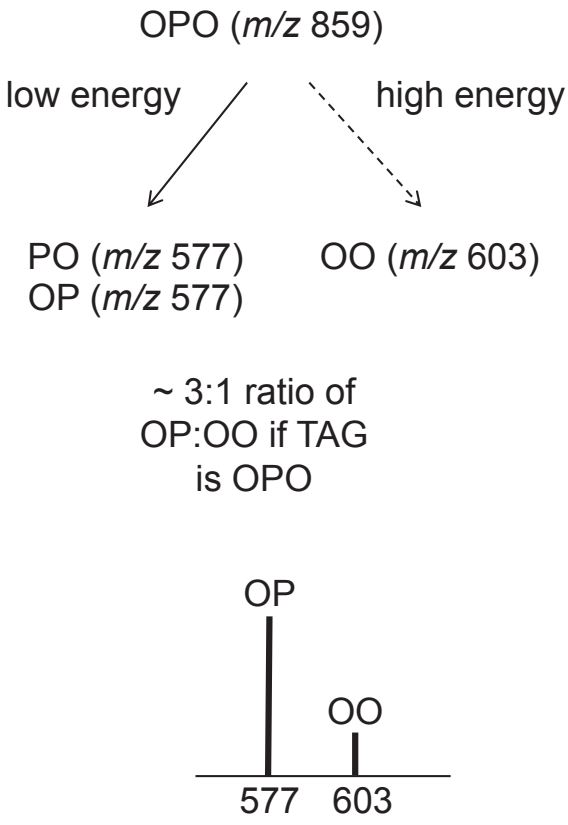
OPO (*m/z* 859)
low energy
high energy
PO (*m/z* 577)
OP (*m/z* 577)
OO (*m/z* 603)
~ 3:1 ratio of
OP:OO if TAG
is OPO
OP
OO
577
603

FIG. 1B

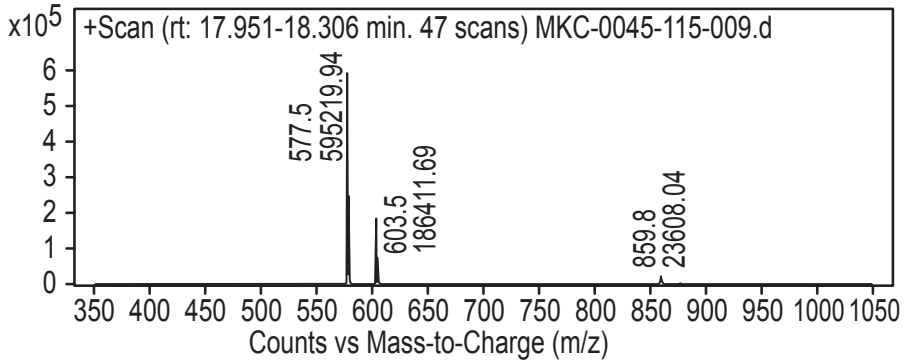

FIG. 2A

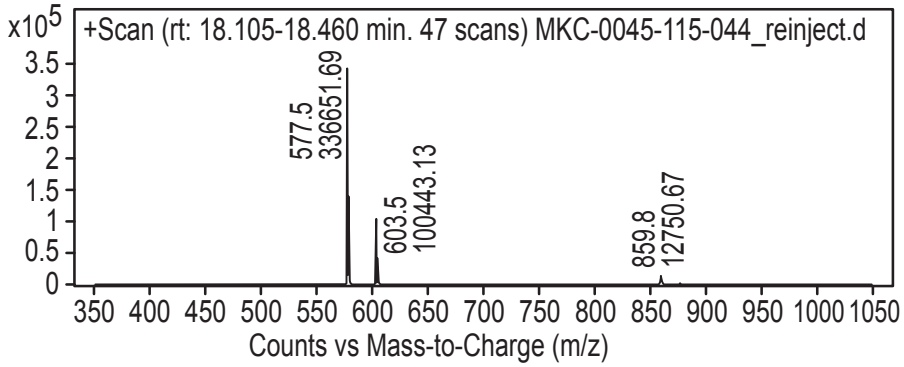

FIG. 2B

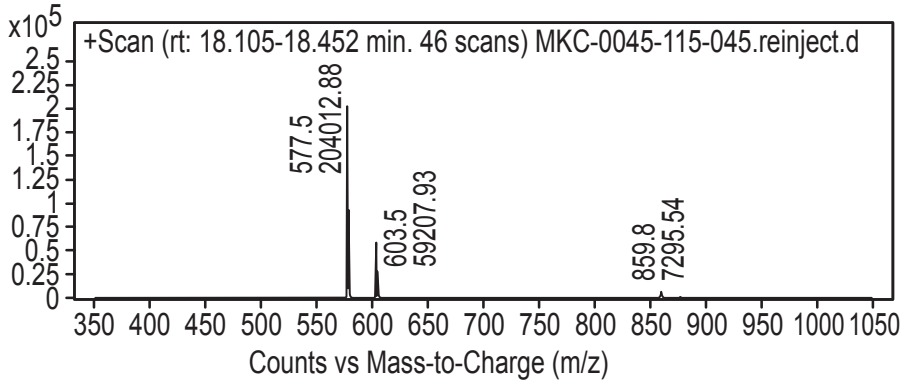

FIG. 2C

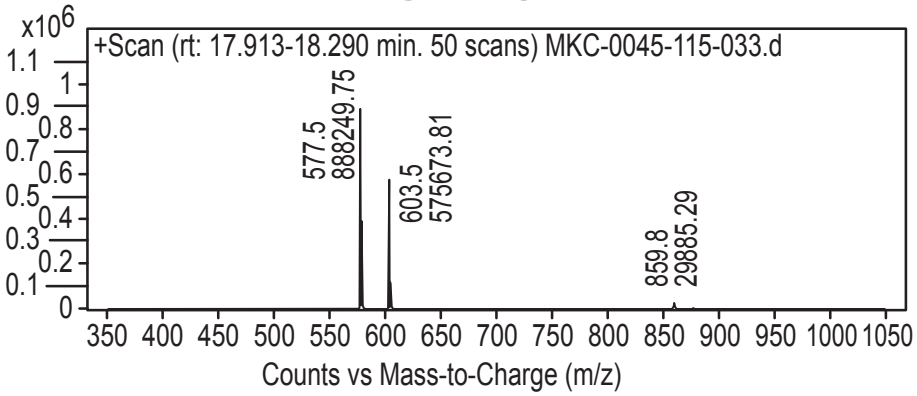

FIG. 2D

```
                +         +         +         +         +         +         +         +         +
Consensus       MAREGLDSVETADGGXXXXXXXAXXVXEXXPXXSXPXXXXQXXLXRXXXXXAXXXXRXXXXXAXXXXAXXXXXXRXXXVXXC
NoLPAT3         ------------------------------------------------------------------------------------
NoLPAT4         ------------------------------------------------------------------------------------
OlLPAAT2        ------------------------------------------------------------------------------------
PmLPAAT2        ------------------------------------------------------------------------------------
CosLPAAT2       ------------------------------------------------------------------------------------
VcLPAAT2        ------------------------------------------------------------------------------------
VaLPAAT2        ------------------------------------------------------------------------------------
CrLPAAT2        ------------------------------------------------------------------------------------
ChsLPAAT2       ------------------------------------------------------------------------------------
CiLPAAT2        ------------------------------------------------------------------------------------
PmLPAAT1        MAREGLDSVETADGGAKPENGAGEDVREGRSSPSDEDEALPAELEGLYPPEAPIHGPVDAGFAERVMTLQAKHDLAGKGDAL
EcPlsC          ------------------------------------------------------------------------------------
Sll1848         ------------------------------------------------------------------------------------
CrLPAAT1        ---------------MARKSSLAQAAIERKPVLLRP----QLNVPRMSGITALPMERRPLPVAPSPSAKPELPARSALV--C
BnBAT2          -----------------MDVASARGVSSHPPYYSKPICSSQSSLIRIPISKGCCFARSSNLITSLHAASRGVTRRTSGVQWC

                      +         +         +         +         +         +         +         +
Consensus       XXXXXXXPXXXXDXXXXXXSXXXDAXGXAXXEXTXPMSVLTKXLGLPSFLFSVFVFY--WSLP-LFAIXYRIRFXSLG--KR
NoLPAT3          ----------------------------------MWGLLWKHMTLRLLFVSVCLVTSSIGAN-MMALALFLVIRPFSRSLY
NoLPAT4          -----------------MSGPADARGPARVSDTIIGAPDRHHVGLTILVALLLSAF--CFCE-FFMLPVAMALLPVPGTTS
OlLPAAT2        ---------------------------------------------MAPVFALS-------FQLS-IFASTDGARVDRRA--RR
PmLPAAT2        ---------------------------------MTLAASIKLALRWPLFLLSMALLY--WSVV-VGSLGGYIKFLDLKSEQR
CosLPAAT2       ----------------------------------MGVLEKIQTLPAFFLGVFFLY--WSLP-LAALMNKVKFLKLAG-KR
VcLPAAT2        ----------------------------------MSVPSKALSLPSFLFSVFVFY--WSLP-LFAIIYRIRFANVG--KR
VaLPAAT2        ----------------------------------MSVIRKALSLPSFLFSVFVFY--WSLP-FFAIIYRIRFINVG--KR
CrLPAAT2        ----------------------------------MSVLTKWLGLPSFLFSVFVFY--WSLP-IFAILYRIRFASLG--KR
ChsLPAAT2       ----------------------------------MSALTKLLGLPSFLFSVFVFY--WSLP-IFAIFYRIRFASLG--KR
CiLPAAT2        ----------------------------------MSALTKLLGLPSFLFSVFVFY--WSLP-IFAILYRIRFASLG--KR
PmLPAAT1        LKGTLEETTQLRLRKGEAGSEAGPAGSGSLYELARSSPFIDLQHPIDAYELAKMVFL--APLF-LFRALILLVLVASAYLSV
EcPlsC          ---------------------------------------------MLYIFRLIITVIY--SILVCVFGSIYCL------FSPR
Sll1848         ---------------------------MDSEINHRGGLSAPRPRETSLNLALYRGL--K--------------------
CrLPAAT1        HAAAASVPLPNSD-------------------SAPQPNV-LLAKIRAIMFFAWSFL--LSLP-LFVTMMVMAPLVLAFDKY
BnBAT2          YRSIRFDPFKVNDKNSRTVTVRSDLSGAATPESTYPEPEIKLSSRLRGICFCLVAGI--SAIV-LIVLMIIGHPFVLLFDRY
```

FIG. 3A

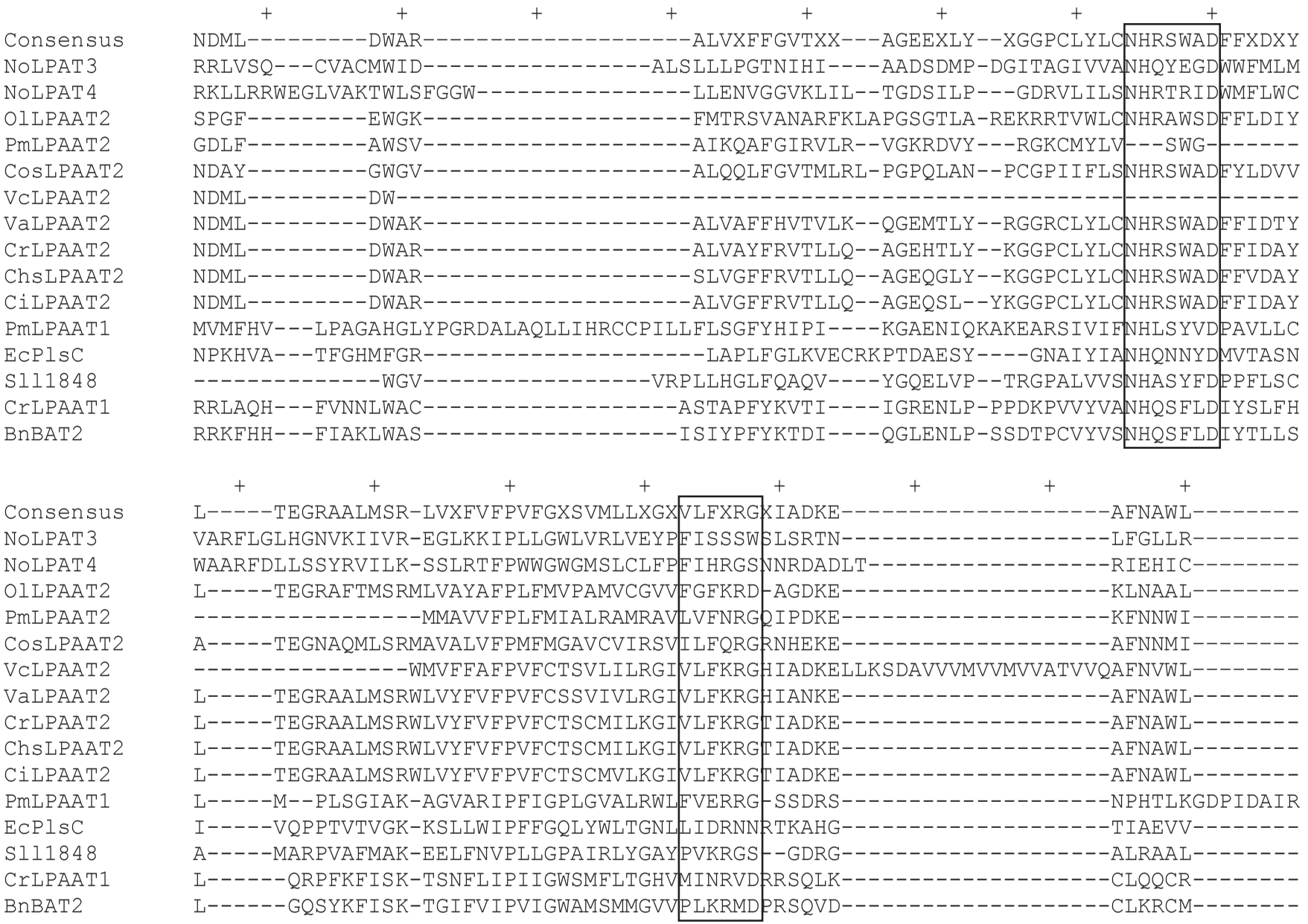
Consensus NDML---------DWAR--------------------ALVXFFGVTXX---AGEEXLY--XGGPCLYLCNHRSWADFFXDXY
NoLPAT3 RRLVSQ---CVACMWID----------------ALSLLLPGTNIHI----AADSDMP-DGITAGIVVANHQYEGDWWFMLM
NoLPAT4 RKLLRRWEGLVAKTWLSFGGW----------------LLENVGGVKLIL--TGDSILP---GDRVLILSNHRTRIDWMFLWC
OlLPAAT2 SPGF---------EWGK--------------------FMTRSVANARFKLAPGSGTLA-REKRRTVWLCNHRAWSDFFLDIY
PmLPAAT2 GDLF---------AWSV--------------------AIKQAFGIRVLR--VGKRDVY---RGKCMYLV---SWG-------
CosLPAAT2 NDAY---------GWGV--------------------ALQQLFGVTMLRL-PGPQLAN--PCGPIIFLSNHRSWADFYLDVV
VcLPAAT2 NDML---------DW----------
VaLPAAT2 NDML---------DWAK--------------------ALVAFFHVTVLK--QGEMTLY--RGGRCLYLCNHRSWADFFIDTY
CrLPAAT2 NDML---------DWAR--------------------ALVAYFRVTLLQ--AGEHTLY--KGGPCLYLCNHRSWADFFIDAY
ChsLPAAT2 NDML---------DWAR--------------------SLVGFFRVTLLQ--AGEQGLY--KGGPCLYLCNHRSWADFFVDAY
CiLPAAT2 NDML---------DWAR--------------------ALVGFFRVTLLQ--AGEQSL--YKGGPCLYLCNHRSWADFFIDAY
PmLPAAT1 MVMFHV---LPAGAHGLYPGRDALAQLLIHRCCPILLFLSGFYHIPI----KGAENIQKAKEARSIVIFNHLSYVDPAVLLC
EcPlsC NPKHVA---TFGHMFGR--------------------LAPLFGLKVECRKPTDAESY----GNAIYIANHQNNYDMVTASN
Sll1848 --------------WGV-----------------VRPLLHGLFQAQV----YGQELVP--TRGPALVVSNHASYFDPPFLSC
CrLPAAT1 RRLAQH---FVNNLWAC--------------------ASTAPFYKVTI----IGRENLP-PPDKPVVYVANHQSFLDIYSLFH
BnBAT2 RRKFHH---FIAKLWAS--------------------ISIYPFYKTDI----QGLENLP-SSDTPCVYVSNHQSFLDIYTLLS
Consensus L-----TEGRAALMSR-LVXFVFPVFGXSVMLLXGXVLFXRGXIADKE----------AFNAWL--------
NoLPAT3 VARFLGLHGNVKIIVR-EGLKKIPLLGWLVRLVEYPFISSSWSLSRTN----------LFGLLR--------
NoLPAT4 WAARFDLLSSYRVILK-SSLRTFPWWGWGMSLCLFPFIHRGSNNRDADLT----------RIEHIC--------
OlLPAAT2 L-----TEGRAFTMSRMLVAYAFPLFMVPAMVCGVVFGFKRD-AGDKE----------KLNAAL--------
PmLPAAT2 ----------MMAVVFPLFMIALRAMRAVLVFNRGQIPDKE----------KFNNWI--------
CosLPAAT2 A-----TEGNAQMLSRMAVALVFPMFMGAVCVIRSVILFQRGRNHEKE----------AFNNMI--------
VcLPAAT2 ----------WMVFFAFPVFCTSVLILRGIVLFKRGHIADKELLKSDAVVVMVVMVVATVVQAFNVWL--------
VaLPAAT2 L-----TEGRAALMSRWLVYFVFPVFCSSVIVLRGIVLFKRGHIANKE----------AFNAWL--------
CrLPAAT2 L-----TEGRAALMSRWLVYFVFPVFCTSCMILKGIVLFKRGTIADKE----------AFNAWL--------
ChsLPAAT2 L-----TEGRAALMSRWLVYFVFPVFCTSCMILKGIVLFKRGTIADKE----------AFNAWL--------
CiLPAAT2 L-----TEGRAALMSRWLVYFVFPVFCTSCMVLKGIVLFKRGTIADKE----------AFNAWL--------
PmLPAAT1 L-----M--PLSGIAK-AGVARIPFIGPLGVALRWLFVERRG-SSDRS----------NPHTLKGDPIDAIR
EcPlsC I-----VQPPTVTVGK-KSLLWIPFFGQLYWLTGNLLIDRNNRTKAHG----------TIAEVV--------
Sll1848 A-----MARPVAFMAK-EELFNVPLLGPAIRLYGAYPVKRGS--GDRG----------ALRAAL--------
CrLPAAT1 L------QRPFKFISK-TSNFLIPIIGWSMFLTGHVMINRVDRRSQLK----------CLQQCR--------
BnBAT2 L------GQSYKFISK-TGIFVIPVIGWAMSMMGVVPLKRMDPRSQVD----------CLKRCM--------

FIG. 3B


Consensus DRTLG--XSHVPGLLVYPEGXRSTKP----------RSLPLKRGMLHYAHSR----KLPVQPITTR--GKEEVX
NoLPAT3 SFNAD---DFPVLLFQFPEGDRIDAKVRQQSLAFAAKEQRPHLLHVLLPRTTGFNTCIEALRTSHPPVYDMTIAIPGTTGQP
NoLPAT4 QYLSE--LGVPNSLILFPEGTDLSQSNQKRDRDYALAKNLPIYHNVLHPRSGAFIASLTAMRPHLDAVVDLTIGYVDYTPGE
OlLPAAT2 DAHFDAFGGEFAGMVCYPEGTRNVRE----------NSMPLKRGMLRYAHSR----KMAVQCVCTA--RKERVF
PmLPAAT2 DKELA--NTPQTGLAVYPEGHRSVLS----------DSLPLKRGMLHYAYSR----KMTVQCIICA--NKEAVL
CosLPAAT2 DRKMA--ASPVDGLIVYPEGHRSTLR----------HSLPLKRGMLNYAYIR----KIPVQVIFCN-------
VcLPAAT2 DATLR--KSTVPGLLVYPEGHRSLKP----------RSLPLKRGMLHFAFSR----HLAVQLVITR--GKEEVM
VaLPAAT2 DVTLG--KSTVPGLLVYPEGHRSLKP----------TSLPLKRGMLHFAYSR----KLPVQIIVTR--GKEEVL
CrLPAAT2 DQTLG--SSHVPGLLVYPEGHRSTKP----------ASLPLKRGMLHYAHSR----KLPVQIVVTR--GKDEVL
ChsLPAAT2 DRTLG--DSHVPGLLVYPEGHRSTKP----------TSLPLKRGMLHYAHSR----KLPVQVIVTR--GKDEVM
CiLPAAT2 DRTLG--DSHVPGLLVYPEGHRSTKP----------ASLPLKRGMLHYAHSR----KLPVQVIITR--GKDEVL
PmLPAAT1 ARRAD---HSAPLLVMAPEATTKAQQ----------CLLKFRRGAFTS--------GCPVTPVLIR--------
EcPlsC NHFKK----RRISIWMFPEGTRSRGR----------GLLPFKTGAFHAAIAA----GVPIIPVCVS--------
Sll1848 -TALG--DGWLVGVF--LEGTRTKDG----------RIHQPKLGAAMIAAKA----QVPIIPVSLG--GVEQIF
CrLPAAT1 DLLAE--G---APVLFFPEGTRSLDC----------KMAGFKKGAFSVAAKA----GVEVVPITLL--GTGSLM
BnBAT2 ELVKK--G---ASVFFFPEGTRSKDG----------RLGPFKKGAFTIAAKT----GVPVVPITLM--GTGKIM
Consensus SEKXXS---GXX-----X----------VXXX-D---FXAFF-X-QXTWDACW-AXY---------G-X
NoLPAT3 SSSSSSSSPSAAAAAASPSAGAAAAAAAAASNTSTNPSSNPPSSSSTDTSPAAAAAAAADAAAAESHDASLFHTFLRFCNGEG
NoLPAT4 RPSELSLLKGRLPHEVHINMKRWDIKTT----------PLLRENPSDRAEQFLRESFDRKEALLTAFYSNNTDTSGGGLK
OlLPAAT2 SSHLTCAERGLALPVYFAE----------VIHAEDYPDFEDFYNEIRSRWDNAWSAAN-------GVSAA
PmLPAAT2 SEKYQVARWGQTVWAGYSD----------PINAADFDSFEAFMTTVQSTWDTLWKEVY---------G-A
CosLPAAT2 ----------
VcLPAAT2 SEKTCSARWGRTLVTNYSKVCAGGRWGG----------VIKPTDFDSFDAFFNEIQTTWDACWQLSY---------H-Q
VaLPAAT2 SEKTLSARWGRTLVVTFSH----------VLKPSDYDSFDAFFDDIQHKWDACWKSSY----------DK
CrLPAAT2 SEKSQSVHFGRTCVTTFSK----------VLKSADYPNFEAFFTDLQATWDSCWAATY---------GLE
ChsLPAAT2 SEKGQSVHFGRTCVTTFSK----------VLKPADYPNFEAFFADLQATWDACWAATY---------GLK
CiLPAAT2 SEKSQSVHFGRTCVTTFSK----------VIKPSDFPNFEAFFADLQATWDAGWTATY---------GLK
PmLPAAT1 ----------YRFRHFNPAWGLCRSTPL---------HVY
EcPlsC ----------TTSNKINLNRLHN----------
Sll1848 QP----------GSPWPHPV----------
CrLPAAT1 PSGKES----------QLRPG----------
BnBAT2 PTGSEGIL----------NHG

FIG. 3C

```
                +         +         +         +         +         +         +         +
Consensus   DLXGVPR----LSMPPPQAYD----------------------YXEX MWAQQLAX----TLXSIVVFAAVCYGS-----
NoLPAT3     PRDVHIR-LKRYSLNDVLADP----------------------HWLDTKWAEKDRVLTY-FSRHACFPAPLPPHMANGGG
NoLPAT4     QTSSLLV----SSSSSPFSNSSAPLGFPSFSSPSTSVASSYNEPVYVHNAHWTYGKGLLGTGIAMALVLMLACCLPA-----
OlLPAAT2    EAESLPD-------YTPREHV----------------------VRCTPRHDAVIVS----SALLTVLGVALT--------
PmLPAAT2    DPTGLPD----LEMDDPVLDT----------------------PAHL-RYAVSASV----VLVDVVFFLVLWFGARFAIW
CosLPAAT2   ------------------------------------------------------AVL----CLL
VcLPAAT2    PIAGVPR----LSLKDAHEYD----------------------YPPS-MRWLQLGV----TLLSIVILAAVLYGS-----
VaLPAAT2    PTAGVPE----LSMKGAHEYD----------------------YPRS-MWWLQLGM----TLMSIVILIGVMWGS-----
CrLPAAT2    DLKNVPR----FSMPGPQAYS----------------------YSSS-MWVQQLAI----TLVSILVFAGVCYGS-----
ChsLPAAT2   DLKGVPR----FSMPSPQAYT----------------------YSAK-TWAQQLAI----TLVSIVVFAAVCYGS-----
CiLPAAT2    DLKGVPR----FSMPSPQAYT----------------------YSSK-MWAQQLAI----TLFSIPVFAGVCYGS-----
PmLPAAT1    RLLVQPVNHVSLEVLPPYFPD----------------------EQETKDWTRFASN----VRVRMAQALQAPMVE-----
EcPlsC      ---GLVI----VEMLPPIDVS----------------------QYGK-DQVRELAA----HCRSIMEQKIAELD------
Sll1848     PLTIRIG----KAIAPPVKNR----------------------KPEL-EAVTKACQ----AQIHEMLDLGRD
CrLPAAT1    QVTIVVH----KALPPNKNAD----------------------QLCD-AARQAVAS----SLPPELVGSATEMAPDEQ
BnBAT2      DVRVIIH----KPIYGSKADV----------------------LCEEARNKIAESM----NLLS

                     +         +         +         +         +         +         +         +
Consensus   ----------WRGLAAXLAVTGAA----------------------------------------------------QQVVX
NoLPAT3     STQTHGGRGGPRGPAGSHAVVGAAGNAQYLRSFNSRKFKAETSFLALARLLLTPLCLPLLLLMAC--------PLMTLYVCV
NoLPAT4     -----VAFCGYSIVVFLLFVTVNSVWEG----------------------------------------------------FDTLE
OlLPAAT2    ----------WRACSKLL
PmLPAAT2    ATSPLGPLRGYLLFAAILTVVASFVVYSEPFNSVAYNARKHQRRAAADPNRVRATGASISDKPAG--------VAPPTDLVA
CosLPAAT2
VcLPAAT2    ----------WVVLRAGVLSLGSSVTQQ----------------------------------------------------AVVVL
VaLPAAT2    ----------WVALRTGLAVLGAA----------------------------------------------------QQPVV
CrLPAAT2    ----------WRGLAAALAATGAA----------------------------------------------------QQVVA
ChsLPAAT2   ----------WRGLAAALAATGAAQQVRGQQQQQQQQQRRRRLSSSGGWAEGRGAQAGGRLQWDGGDWGRWVALPLSLEVVT
CiLPAAT2    ----------WRVLAAALAATGAA----------------------------------------------------QQVVT
PmLPAAT1    ----QGIAEERRLLKAGVTTDLAGS----------------------------------------------------RIIFR
EcPlsC      ------------------------------------------------------------------------------KEVAE
Sll1848
CrLPAAT1
BnBAT2
```

FIG. 3D

```
                +         +         +         +
Consensus       LXLAGWVGGSVLRSFLXXXXXXXGDAVYESVNWSV
NoLPAT3         STVRRWLGEEIVFSPGGREGGREGDAVYESVNWSV
NoLPAT4         LNLAVWWGGQAGQTKQE
OlLPAAT2
PmLPAAT2        HSVKDVTGDSLLVDGELDSKKDA
CosLPAAT2
VcLPAAT2        GGAGGWLGLSLLRSFL
VaLPAAT2        LCAAVWIGASMLRAFF
CrLPAAT2        LVLAAWVGSSVLRSFL
ChsLPAAT2       VLLAAWVGGSVLRSFL
CiLPAAT2        LALAGWVGGSVLRSFL
PmLPAAT1        RKVAEQATSPRKRD
EcPlsC          REAAGKV
Sll1848
CrLPAAT1
BnBAT2
```

FIG. 3E

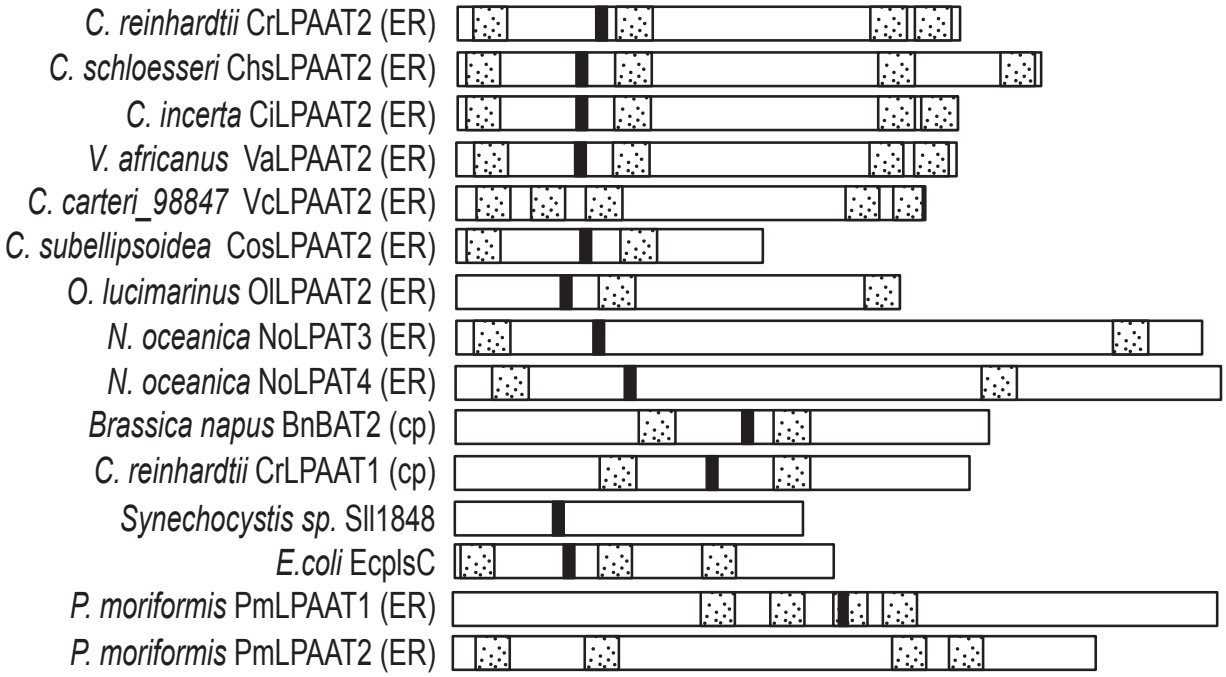
C. reinhardtii CrLPAAT2 (ER)
C. schloesseri ChsLPAAT2 (ER)
C. incerta CiLPAAT2 (ER)
V. africanus VaLPAAT2 (ER)
C. carteri_98847 VcLPAAT2 (ER)
C. subellipsoidea CosLPAAT2 (ER)
O. lucimarinus OlLPAAT2 (ER)
N. oceanica NoLPAT3 (ER)
N. oceanica NoLPAT4 (ER)
Brassica napus BnBAT2 (cp)
C. reinhardtii CrLPAAT1 (cp)
Synechocystis sp. Sll1848
E.coli EcplsC
P. moriformis PmLPAAT1 (ER)
P. moriformis PmLPAAT2 (ER)

FIG. 4

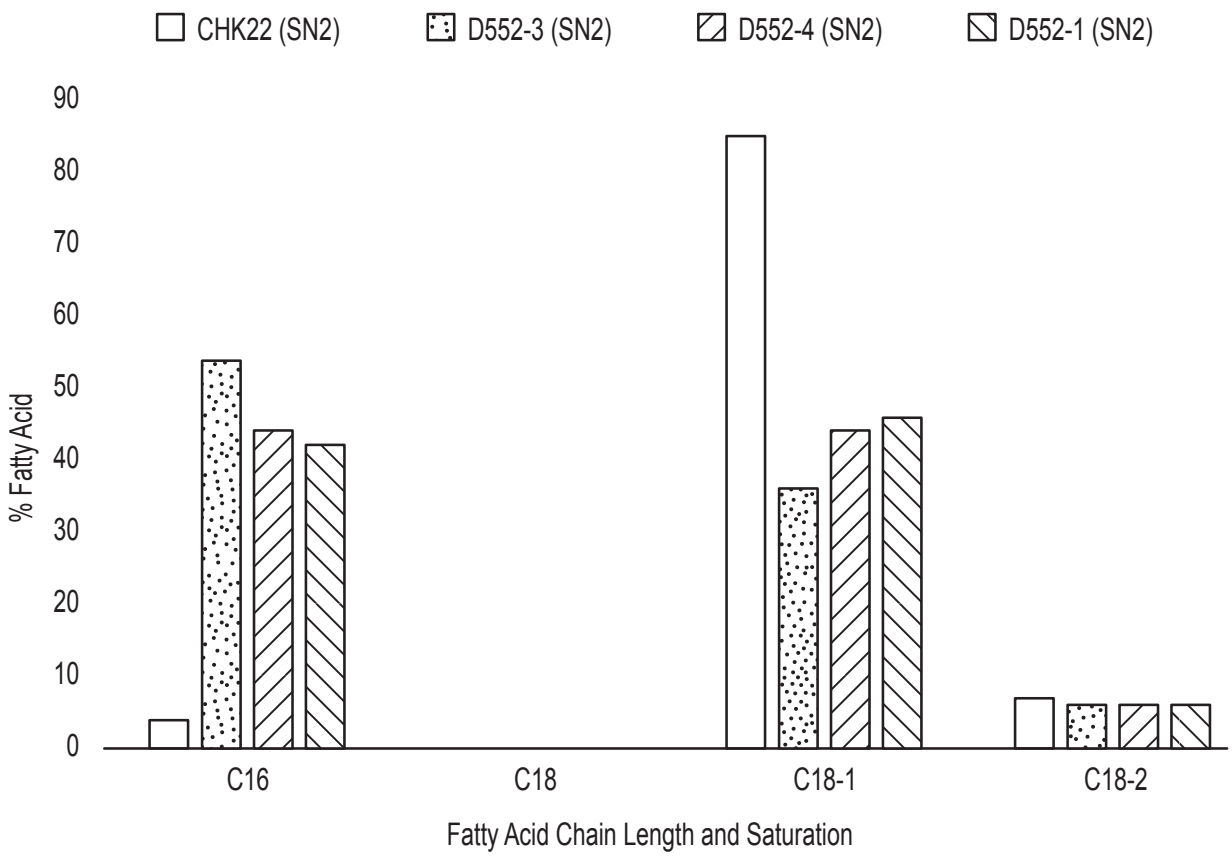
CHK22 (SN2)
D552-3 (SN2)
D552-4 (SN2)
D552-1 (SN2)
90
80
70
60
50
40
30
20
10
0
% Fatty Acid
C16
C18
C18-1
C18-2
Fatty Acid Chain Length and Saturation

FIG. 5

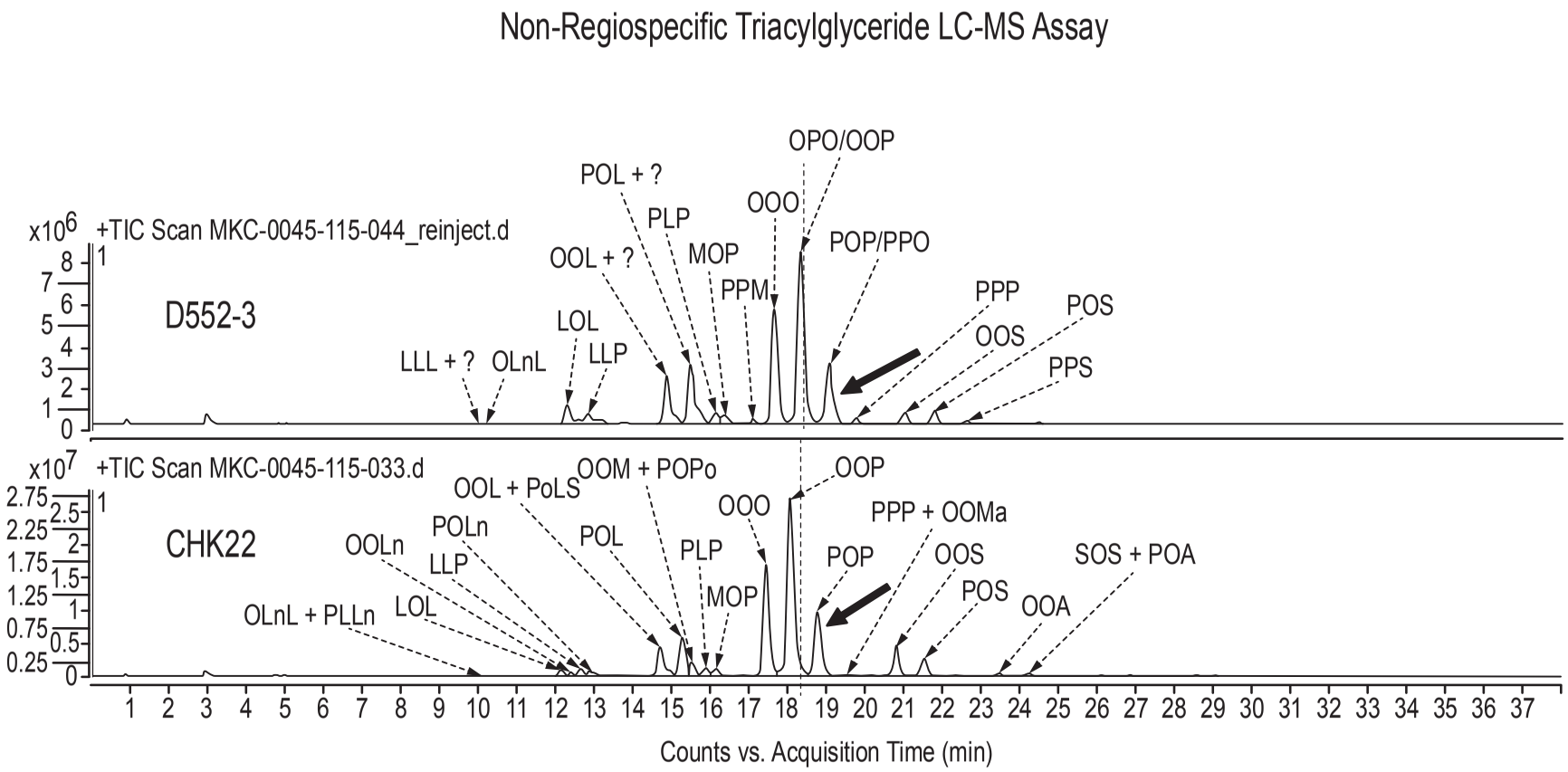
Non-Regiospecific Triacylglyceride LC-MS Assay
x10^6 +TIC Scan MKC-0045-115-044_reinject.d
D552-3
LLL + ?
OLnL
LOL
LLP
OOL + ?
POL + ?
PLP
MOP
PPM
OOO
OPO/OOP
POP/PPO
PPP
OOS
POS
PPS
x10^7 +TIC Scan MKC-0045-115-033.d
CHK22
OLnL + PLLn
LOL
OOLn
LLP
POLn
OOL + PoLS
POL
OOM + POPo
PLP
MOP
OOO
OOP
POP
PPP + OOMa
OOS
POS
OOA
SOS + POA
Counts vs. Acquisition Time (min)

FIG. 6

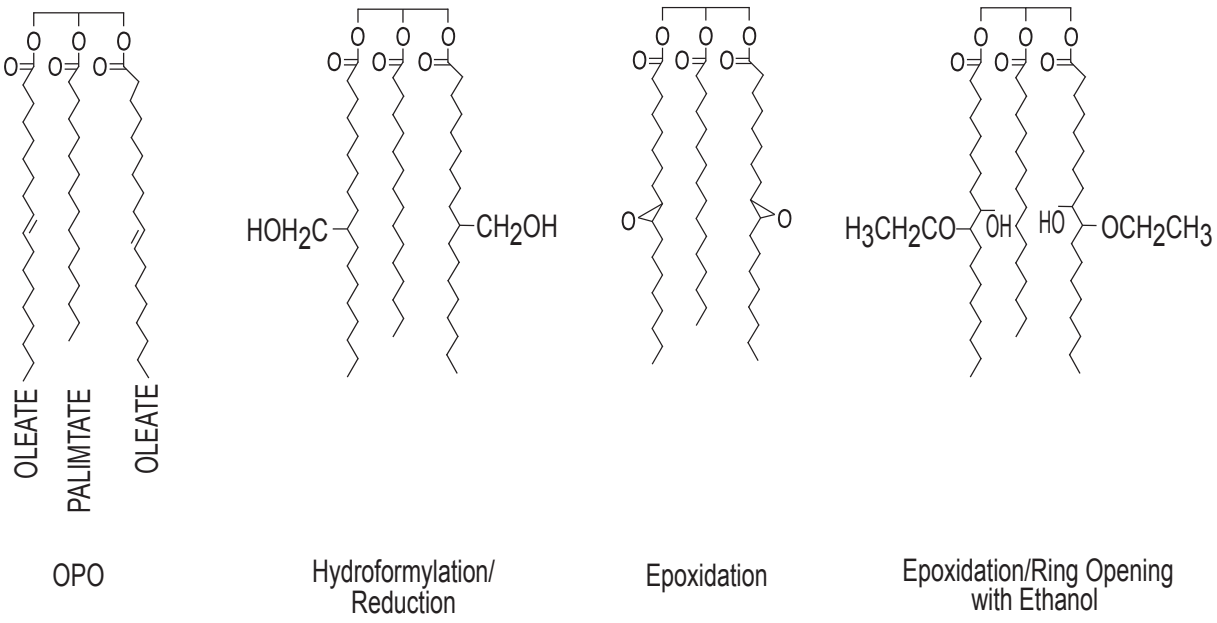
OLEATE
PALIMTATE
OLEATE
HOH2C
CH2OH
O
H3CH2CO
OH
HO
OCH2CH3
OPO
Hydroformylation/
Reduction
Epoxidation
Epoxidation/Ring Opening
with Ethanol

FIG. 7

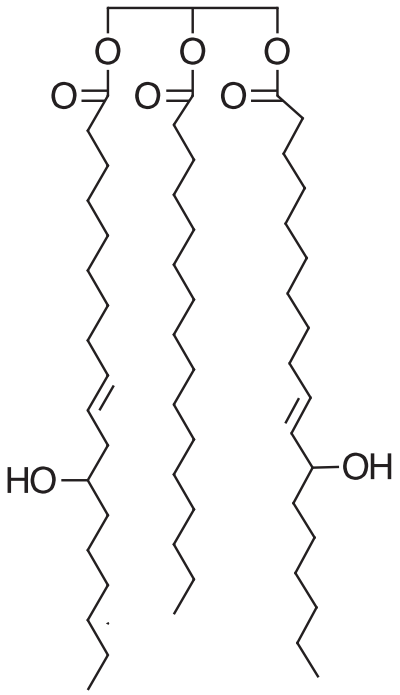
HO
OH

FIG. 8

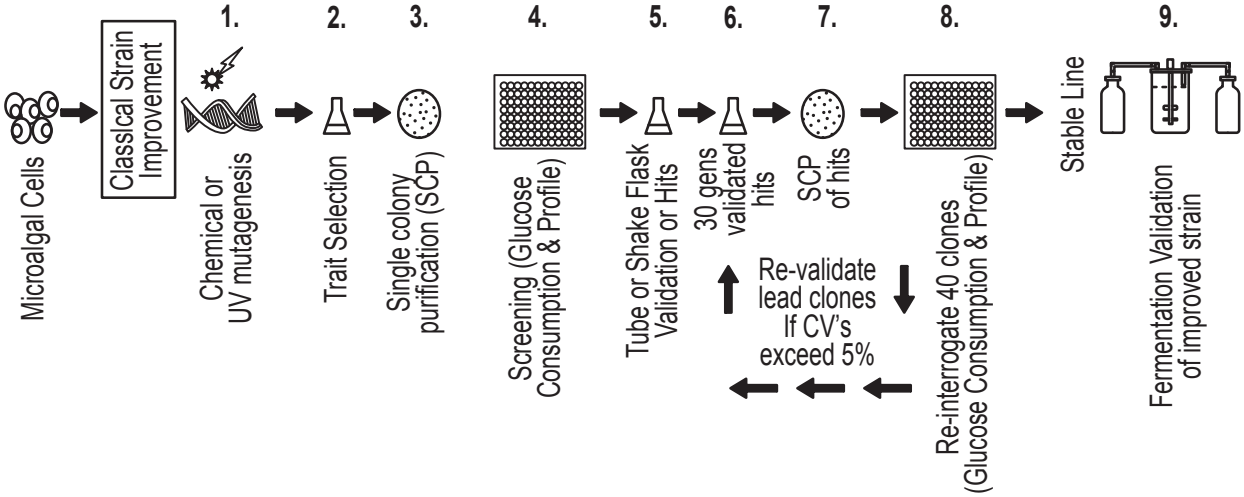
Microalgal Cells
Classical Strain Improvement
1.
Chemical or UV mutagenesis
2.
Trait Selection
3.
Single colony purification (SCP)
4.
Screening (Glucose Consumption & Profile)
5.
Tube or Shake Flask Validation or Hits
6.
30 gens validated hits
7.
SCP of hits
8.
Re-interrogate 40 clones (Glucose Consumption & Profile)
Re-validate lead clones If CV's exceed 5%
9.
Stable Line
Fermentation Validation of improved strain

FIG. 9

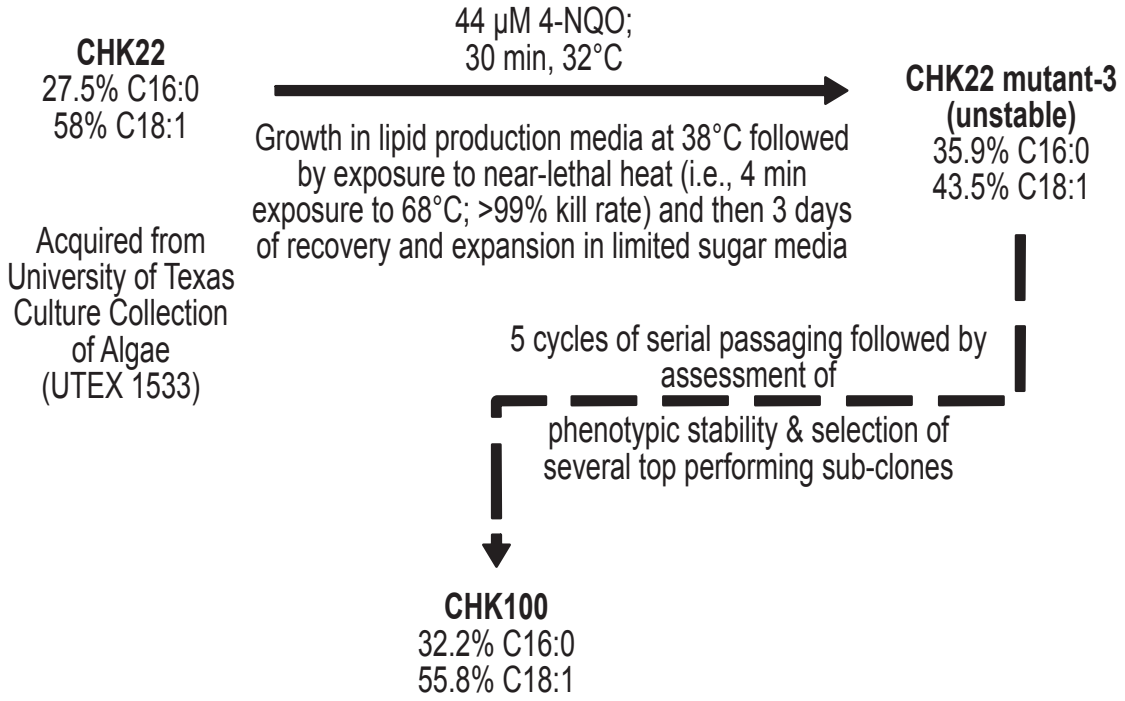
CHK22
27.5% C16:0
58% C18:1
Acquired from University of Texas Culture Collection of Algae (UTEX 1533)
44 µM 4-NQO;
30 min, 32°C
Growth in lipid production media at 38°C followed by exposure to near-lethal heat (i.e., 4 min exposure to 68°C; >99% kill rate) and then 3 days of recovery and expansion in limited sugar media
CHK22 mutant-3 (unstable)
35.9% C16:0
43.5% C18:1
5 cycles of serial passaging followed by assessment of
phenotypic stability & selection of several top performing sub-clones
CHK100
32.2% C16:0
55.8% C18:1

FIG. 10

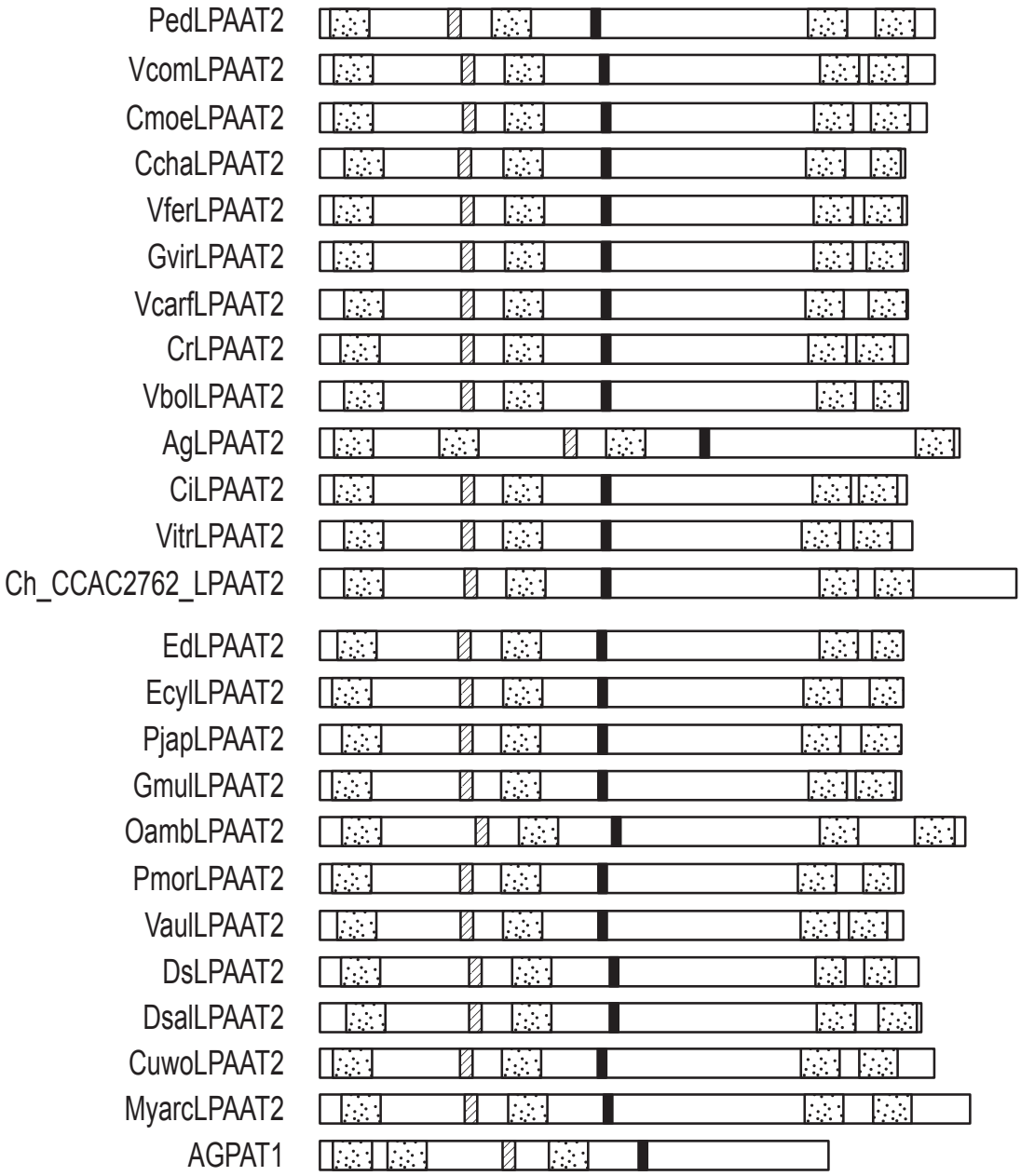
PedLPAAT2
VcomLPAAT2
CmoeLPAAT2
CchaLPAAT2
VferLPAAT2
GvirLPAAT2
VcarfLPAAT2
CrLPAAT2
VbolLPAAT2
AgLPAAT2
CiLPAAT2
VitrLPAAT2
Ch_CCAC2762_LPAAT2
EdLPAAT2
EcylLPAAT2
PjapLPAAT2
GmulLPAAT2
OambLPAAT2
PmorLPAAT2
VaulLPAAT2
DsLPAAT2
DsalLPAAT2
CuwoLPAAT2
MyarcLPAAT2
AGPAT1

FIG. 11

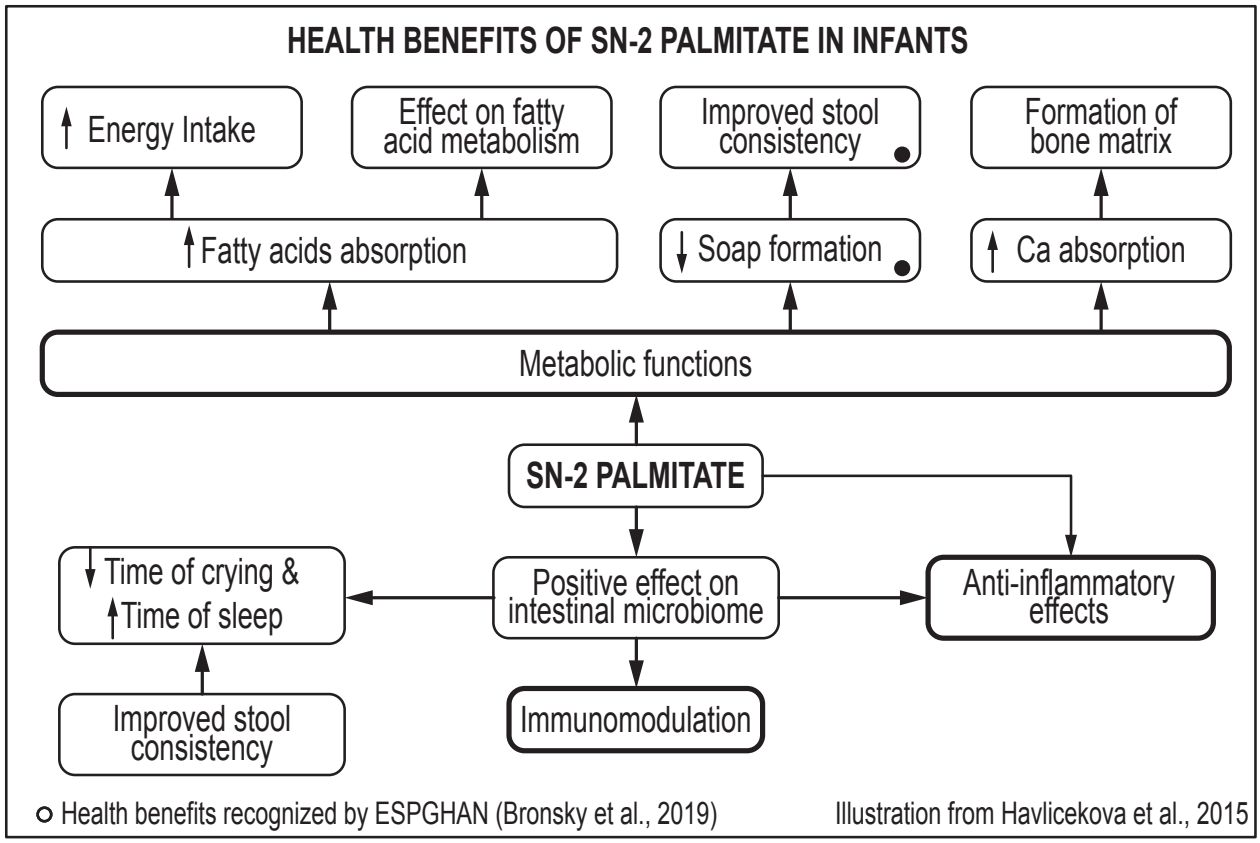
HEALTH BENEFITS OF SN-2 PALMITATE IN INFANTS
Energy Intake
Effect on fatty acid metabolism
Improved stool consistency
Formation of bone matrix
Fatty acids absorption
Soap formation
Ca absorption
Metabolic functions
SN-2 PALMITATE
Time of crying & Time of sleep
Positive effect on intestinal microbiome
Anti-inflammatory effects
Improved stool consistency
Immunomodulation
Health benefits recognized by ESPGHAN (Bronsky et al., 2019)
Illustration from Havlicekova et al., 2015

FIG. 12

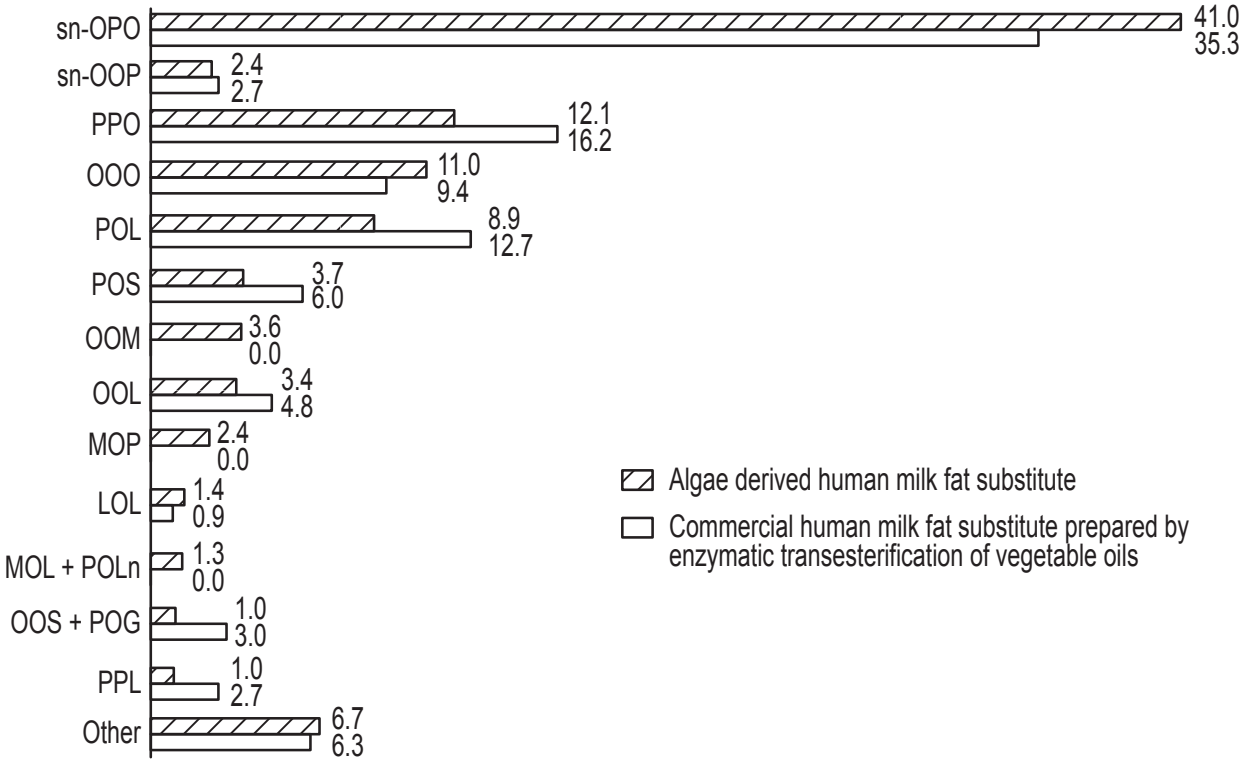
sn-OPO
sn-OOP
PPO
OOO
POL
POS
OOM
OOL
MOP
LOL
MOL + POLn
OOS + POG
PPL
Other
41.0
35.3
2.4
2.7
12.1
16.2
11.0
9.4
8.9
12.7
3.7
6.0
3.6
0.0
3.4
4.8
2.4
0.0
1.4
0.9
1.3
0.0
1.0
3.0
1.0
2.7
6.7
6.3
Algae derived human milk fat substitute
Commercial human milk fat substitute prepared by enzymatic transesterification of vegetable oils

FIG. 13