FIGURE 1:

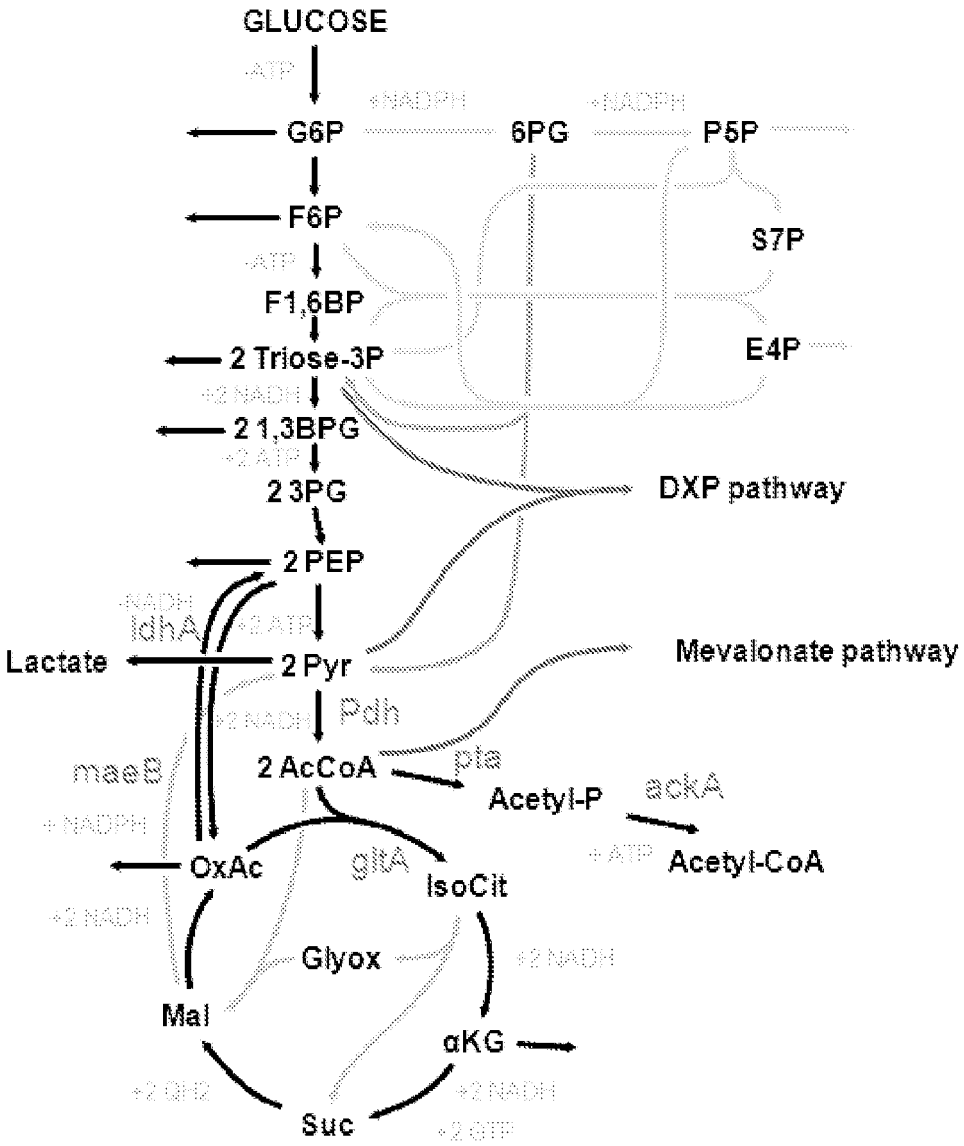

GLUCOSE
ATP
G6P
+NADPH
6PG
+NADPH
P5P
F6P
S7P
ATP
F1,6BP
2 Triose-3P
E4P
+2 NADH
2 1,3BPG
+2 ATP
2 3PG
DXP pathway
2 PEP
+NADH
IdhA
+2 ATP
Lactate
2 Pyr
Mevalonate pathway
+2 NADH
Pdh
maeB
2 AcCoA
pta
Acetyl-P
ackA
+ NADPH
gltA
+ ATP
Acetyl-CoA
OxAc
IsoCit
+2 NADH
Glyox
+2 NADH
Mal
αKG
+2 QH2
+2 NADH
Suc
+2 GTP

FIGURE 2:

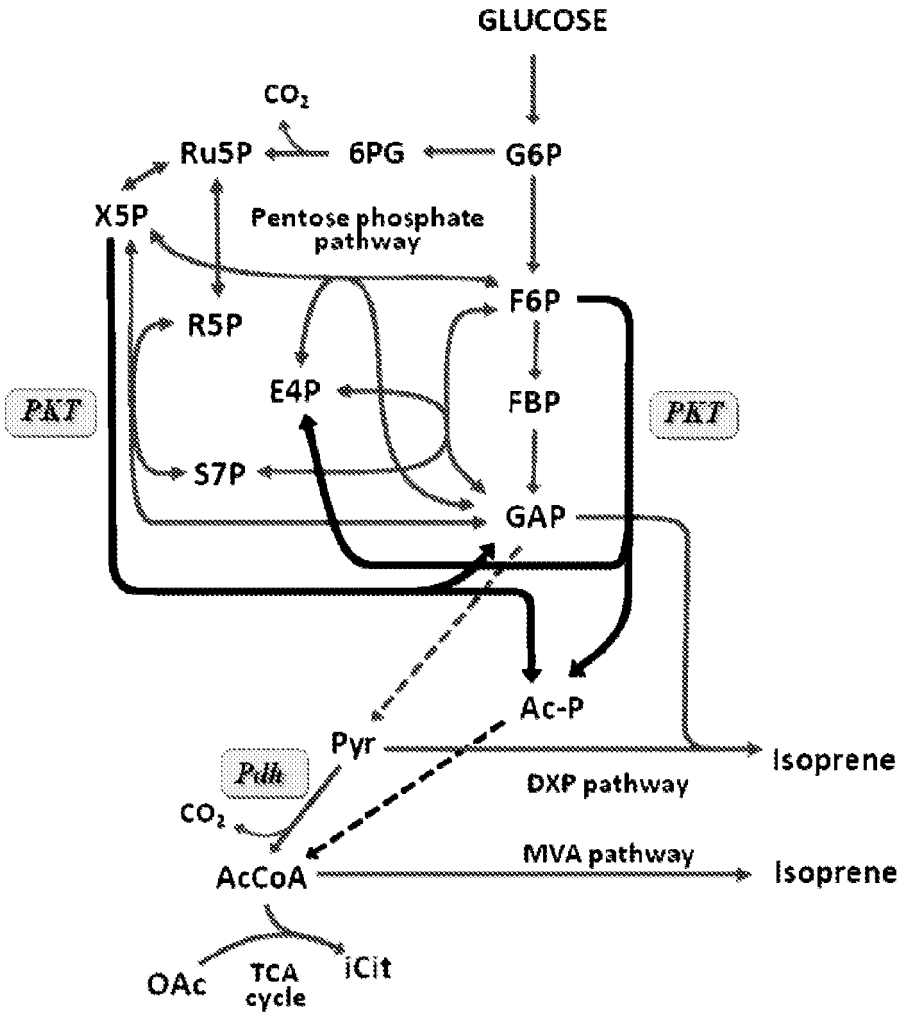

GLUCOSE
CO_2
Ru5P
6PG
G6P
X5P
Pentose phosphate pathway
F6P
R5P
E4P
FBP
PKT
PKT
S7P
GAP
Ac-P
Pyr
Isoprene
Pdh
DXP pathway
CO_2
MVA pathway
AcCoA
Isoprene
OAc
TCA cycle
iCit

FIGURE 3:

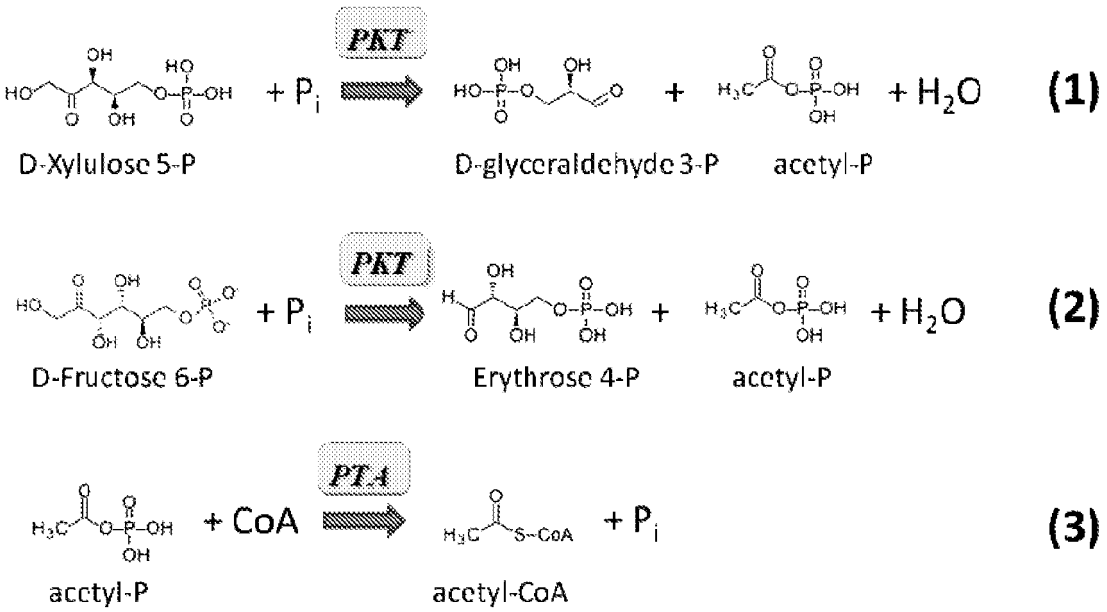

PKT
+ P_i
+ H_2O
(1)
D-Xylulose 5-P
D-glyceraldehyde 3-P
acetyl-P
PKT
+ P_i
+ H_2O
(2)
D-Fructose 6-P
Erythrose 4-P
acetyl-P
PTA
+ CoA
+ P_i
(3)
acetyl-P
acetyl-CoA

FIGURE 4:

Organsim	Accession		Organsim	Accession
Clostridium carboxidivorans P7	ZP_05390294.1		Ferrimonas balearica DSM 9799	ADN77608.1
Roseburia intestinalis L1-82	ZP_04743029.2		Methylobacter tundripaludum SV96	ZP_08779585.1
Subdoligranulum variabile DSM 15176	ZP_05979027.2		Acidithiobacillus ferrivorans SS3	AEM46566.1
Ruminococcaceae bacterium D16	ZP_08418749.1		Anaeromyxobacter dehalogenans 2CP-1	YP_002493710.1
Bacteroides capillosus ATCC 29799	ZP_02038271.1		Stigmatella aurantiaca DW4/3-1	ZP_01464191.1
Oscillibacter valericigenes Sjm18-20	BAK98480		Chlorobaculum parvum NCIB 8327	ACF12009.1
Listeria grayi DSM 20601	ZP_07053045.1		Chlorobium ferrooxidans DSM 13031	EAT58960.1
Bacillus coagulans 36D1	AEP00547.1		Aspergillus fumigatus Af293	XP_754543.1
Paenibacillus polymyxa E681	ADM68092.1		Paracoccidioides brasiliensis Pb18	EEH44652.1
Mesorhizobium opportunistum WSM2075	AEH87451.1		Ajellomyces dermatitidis SLH14081	EEQ76387.1
Lactobacillus reuteri DSM 20016	YP_001272262.1		Phaeodactylum tricornutum CCAP 1055/1	EEC47950.1
Bifidobacterium longum subsp. Infantis ATCC 15697	YP_002323176.1		Acidobacterium sp. MP5ACTX9	YP_004218333.1
Allochromatium vinosum DSM 180	ADC61973.1		Terriglobus saanensis SP1PR4	YP_004181316.1

FIGURE 5.

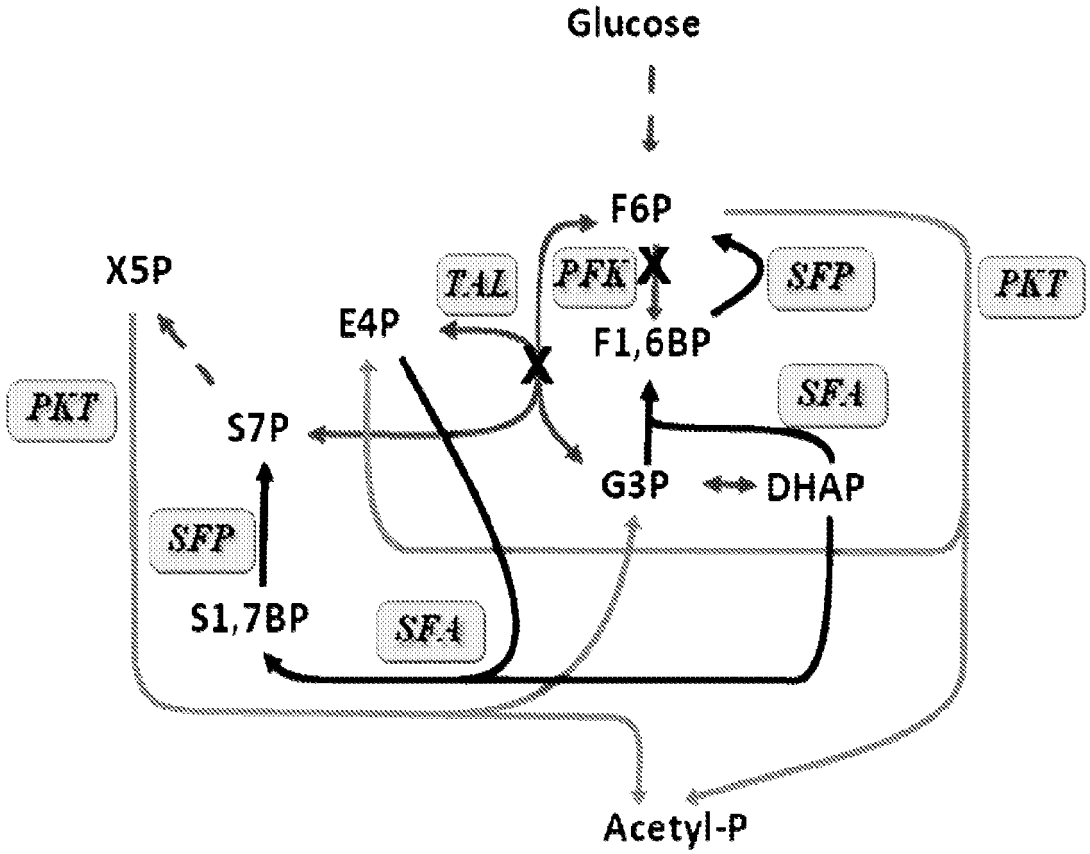

Glucose
F6P
X5P
TAL
PFK
SFP
PKT
E4P
F1,6BP
PKT
SFA
S7P
G3P
DHAP
SFP
S1,7BP
SFA
Acetyl-P

FIGURE 6.

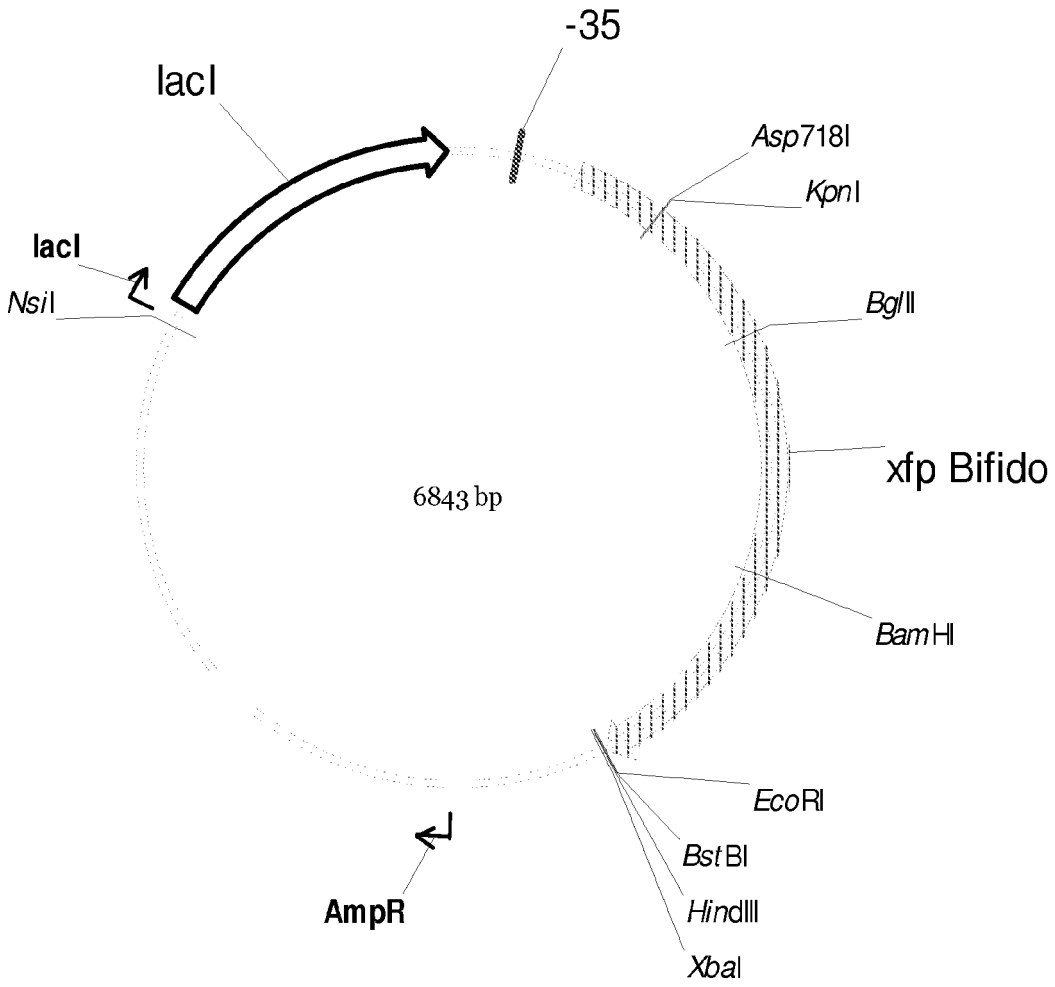

-35
lacI
Asp718I
KpnI
lacI
NsiI
BglII
xfp Bifido
6843 bp
BamHI
EcoRI
BstBI
HindIII
XbaI
AmpR

FIGURE 7.

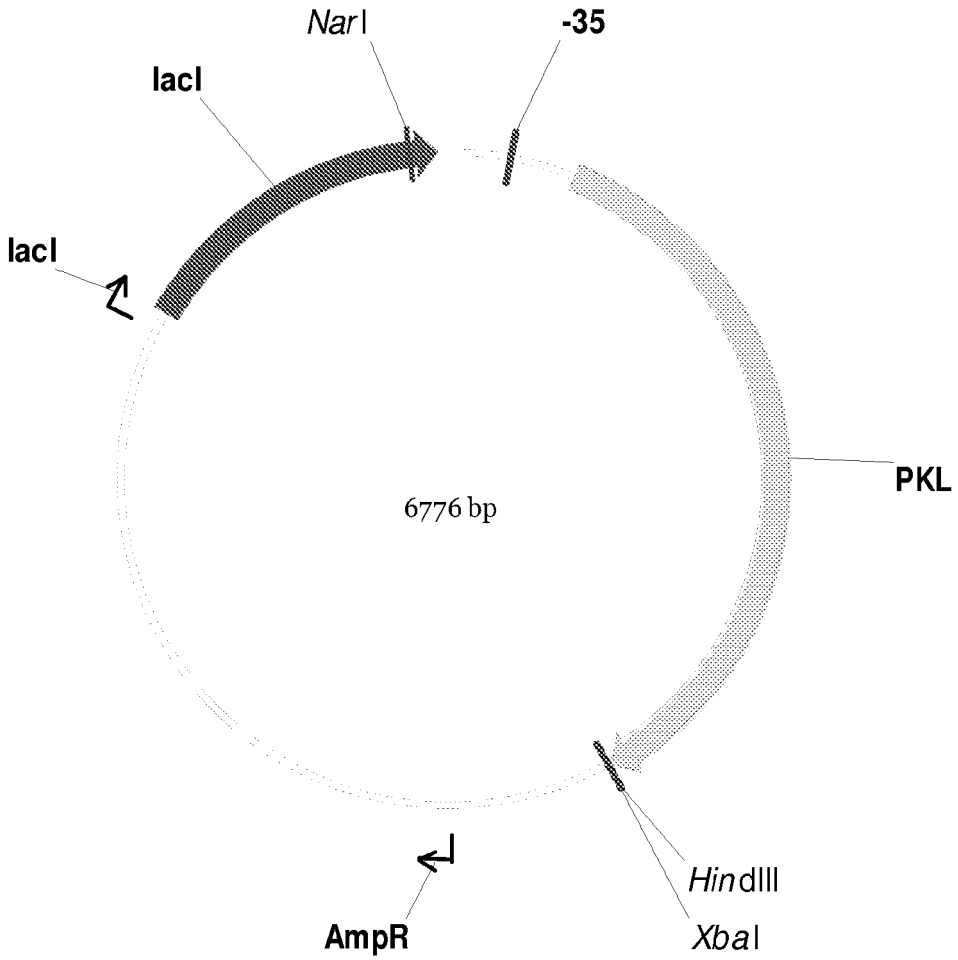


FIGURE 8.

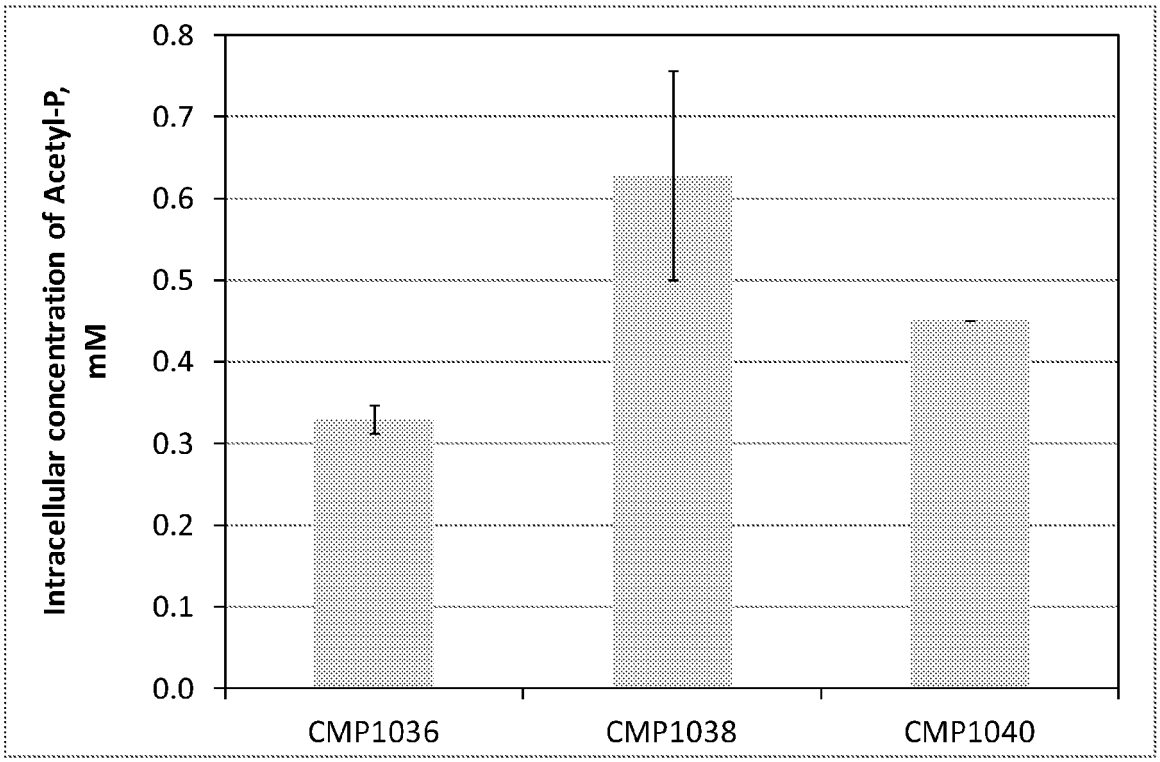

Intracellular concentration of Acetyl-P, mM
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
CMP1036
CMP1038
CMP1040

FIGURE 9.

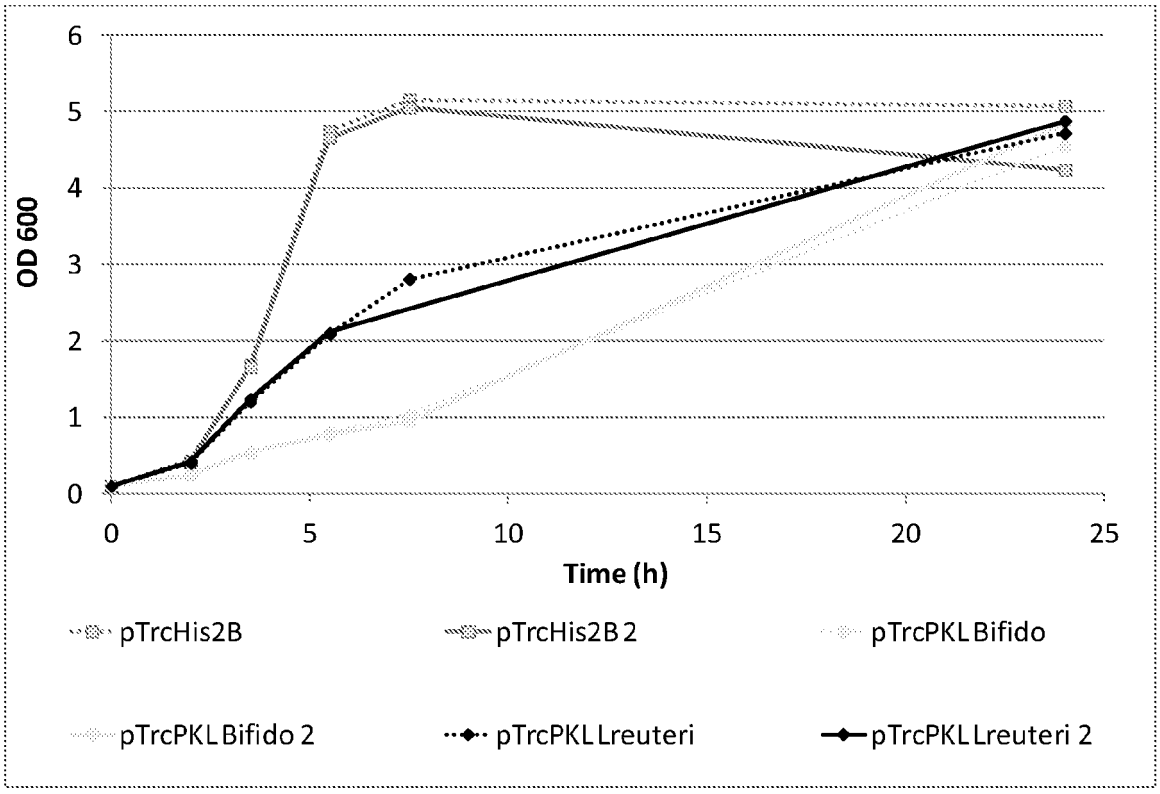

OD 600
6
5
4
3
2
1
0
0
5
10
15
20
25
Time (h)
pTrcHis2B
pTrcHis2B 2
pTrcPKL Bifido
pTrcPKL Bifido 2
pTrcPKL Lreuteri
pTrcPKL Lreuteri 2

FIGURE 10.

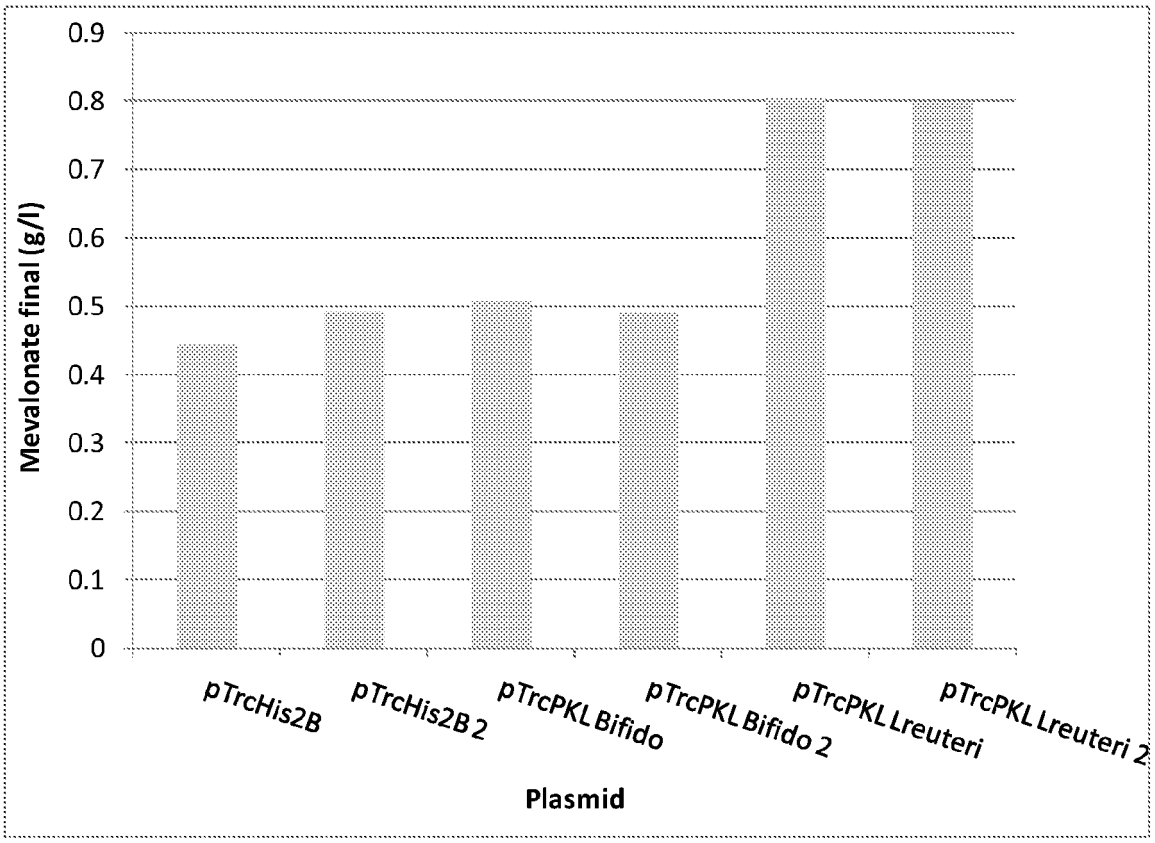
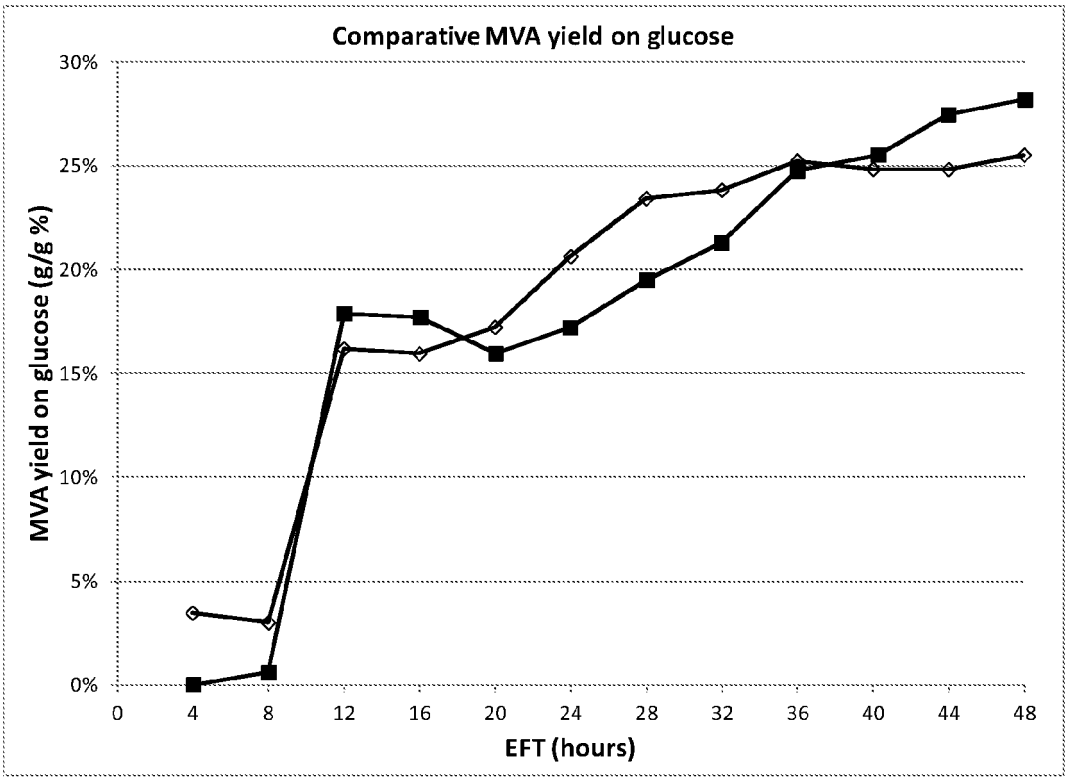

0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Mevalonate final (g/l)
pTrcHis2B
pTrcHis2B 2
pTrcPKL Bifido
pTrcPKL Bifido 2
pTrcPKL Lreuteri
pTrcPKL Lreuteri 2
Plasmid

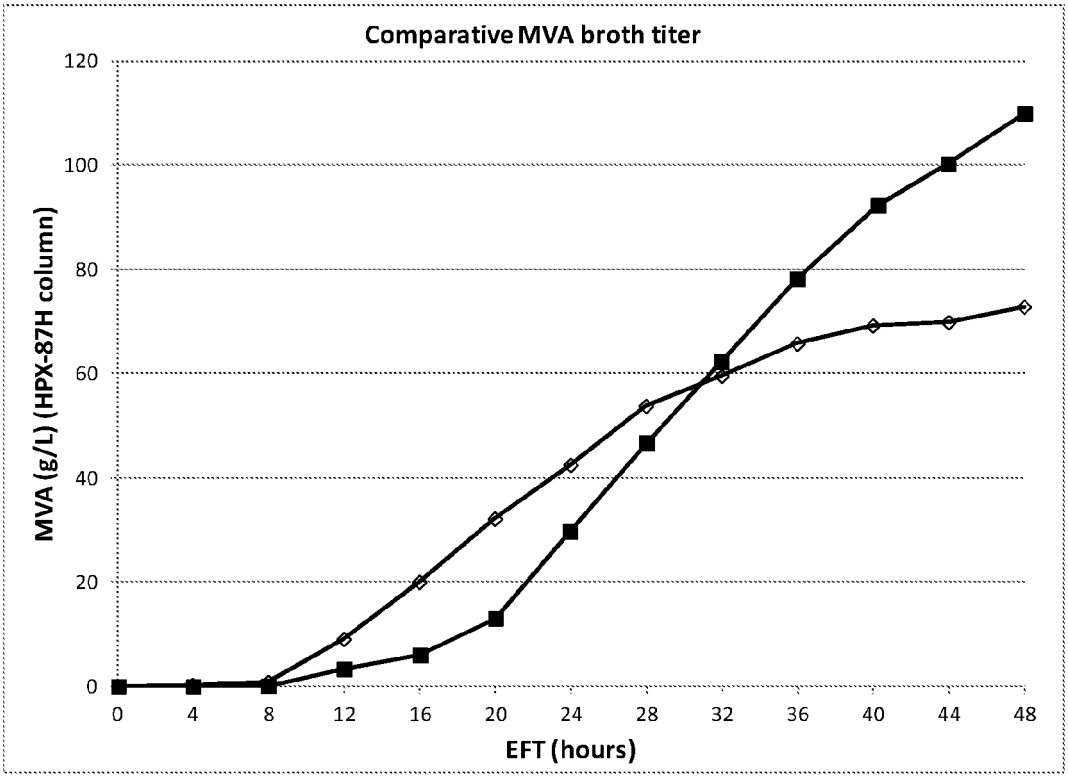
FIGURE 11.



a) SHG0863 CMP1053 (open diamonds) control strain contain empty pTrcHis2B plasmid

b) SHG0864 CMP1057 (closed black squares) strain expressing phosphoketolase off of pTrcPhosphoketolase Lreuteri plasmid

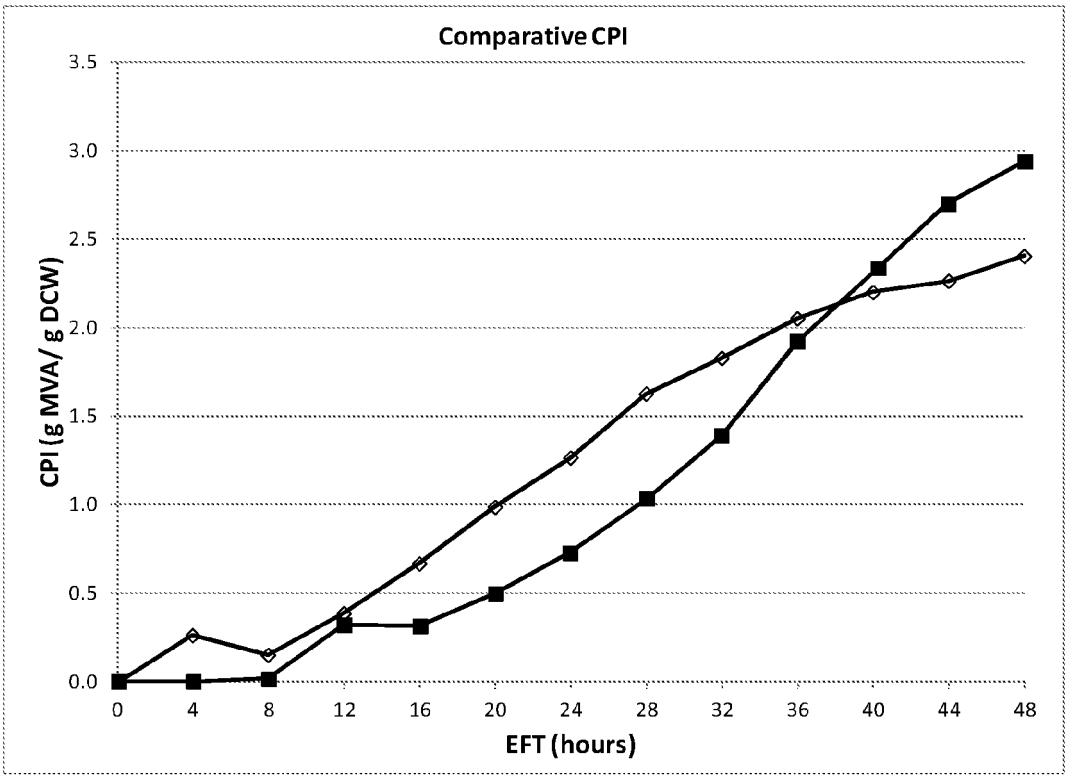
FIGURE 12.



a) SHG0863 CMP1053 (open diamonds) control strain contain empty pTrcHis2B plasmid

b) SHG0864 CMP1057 (closed black squares) strain expressing phophoketolase off of pTrcPhosphoketolase Lreuteri plasmid

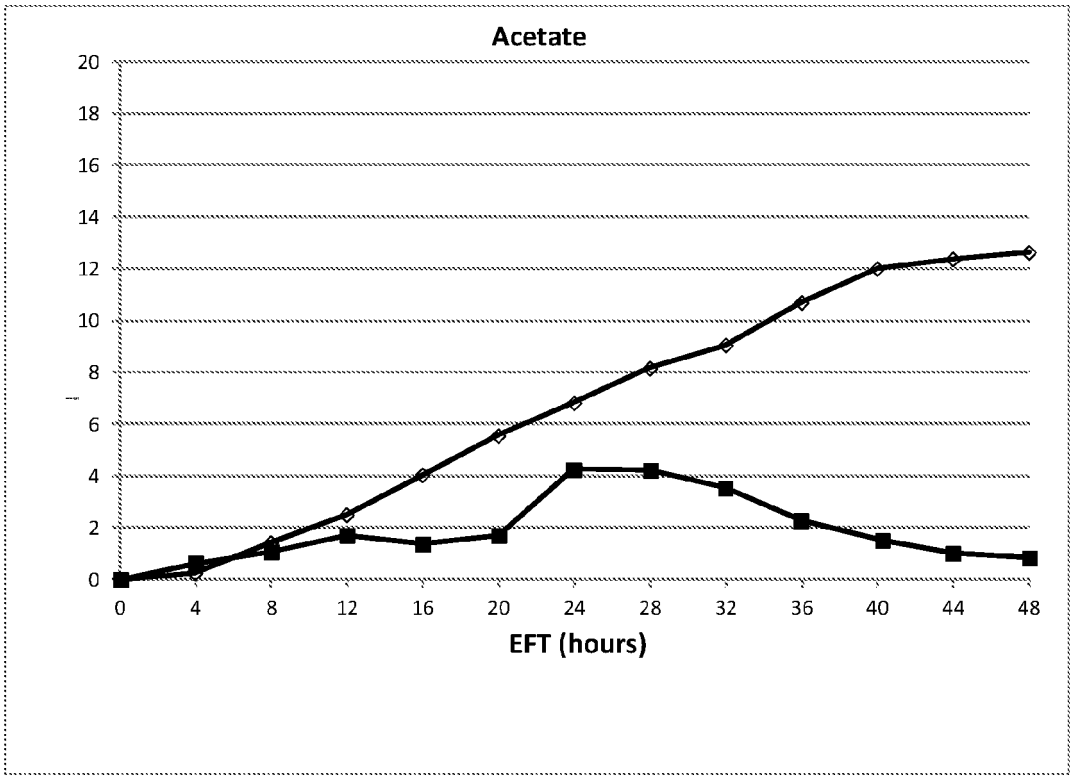
FIGURE 13.



a) SHG0863 CMP1053 (open diamonds) control strain contain empty pTrcHis2B plasmid

b) SHG0864 CMP1057 (closed black squares) strain expressing phophoketolase off of pTrcPhosphoketolase Lreuteri plasmid

FIGURE 14.



a) SHG0863 CMP1053 (open diamonds) control strain contain empty pTrcHis2B plasmid

b) SHG0864 CMP1057 (closed black squares) strain expressing phophoketolase off of pTrcPhosphoketolase Lreuteri plasmid

FIGURE 15.

atggcagtagattacgattccaagaaatacttggaaagtgttgatgcttactggcgtgcagctaactacctttcagttggtacattgtacttaatgggt
gatccattacttcgccaaccattaaaggcagaagatgttaagcctaagccaattggtcactggggtactattgttcctcaaaacttcatttacgcaca
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acatccgttcagaaggtaaggacttaccagaagttactaactggacttggaagggtcttaagtaa **(SEQ ID NO:1)**

FIGURE 16.

MAVDYDSKKYLESVDAYWRAANYLSVGTLYLMGDPLLRQPLKAEDVKPKPIGHWGTIVP
QNFIYAHLNRVIKKYDLDMFYIEGSGHGGQVMVNNSYLDGSYTEIYPEYTQDTKGMAKLF
KHFSFPGGTASHAAPETPGSIHEGGELGYSLSHGVGAILDNPEVIAAVEIGDGEAETGPLMA
SWFSDKFINPIKDGAVLPIIQVNGFKISNPTILSWMSDEELTKYFEGMGWKPYFVSAYKEAD
RDGEFKGYKPHMEVHEEMAKTLDKVVEEIKAIQKNARENNDNSLPQWPMIIFRAPKGWTG
PKTDLDGNPIENSFRAHQIPVPVSQDDMEHKDILVDWLKSYKPEELFDEDGHPVALVEENT
PEGNRRMAMNPITNGGIDPKPLVLPNYRDFAIDVQNPGSVVKQDMLEWGKYLNKMAELN
PTNFRGFGPDESKSNRLYAFLDGQKRQWMESVHEPNDEDVAPQGRMIDSQLSEHQAEGFL
EGYTLTGRHGFFATYEAFGRVVDSMLTQHMKWLRKAKDLYWRHQYPALNFVDTSTVFQ
QDHNGYTHQDPGLLTHLFEKERPDLVKEYLPADTNSLMAVSNKAFRNQECINLFVTSKHP
RAQWFSIDEATQLADNGLGYIDWASTDQGTEPDVVFASSGTEPTEEALAAIDILHDNFPELK
IRYINIIEIMRLMNTDKNPEGLTDAEFNSYFTTDKPVIFAWHGFRDMIQALFFDRANRNVHIH
SYEENGDITTPFDMRVLNELDRFHLAKDAIQSVPGYEQKSAAFVAKMDNMINKHNHYIRSE
GKDLPEVTNWTWKGLK (**SEQ ID NO: 2**)

FIGURE 17.

atgacgagtcctgttattggcacccctggaagaagctcagcgctccggtttccgaggaagccctcgaaggcgttgacaagtactggcgcgttg
ccaactacctttccatcggccagatttatctgcgttccaacccgctgatgaaggagcccttcacccgcgaagatgtgaagcatcgtctggtgggcc
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gaccgacaacgtgcaccgtccgttctacccgatgctgatcttccgcaccccgaagggctggacctgcccgaagtacatcgacggcaagaaga
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aacaccggcaagaagggtgccgtcaccgctaccgccgctaccgctggcgacaacgagtga **(SEQ ID NO: 3)**

FIGURE 18.

MTSPVIGTPWKKLSAPVSEEALEGVDKYWRVANYLSIGQIYLRSNPLMKEPFTREDVKHRL
VGHWGTTPGLNFLIGHINRFIADHGQNTVIIMGPGHGGPAGTSQSYLDGTYTETFPKITKDE
AGLQKFFRQFSYPGGIPSHFAPETPGSIHEGGELGYALSHAYGAIMDNPSLFVPAIVGDGEA
ETGPLATGWQSNKLVNPRTDGIVLPILHLNGYKIANPTILSRISDEELHEFFHGMGYEPYEFV
AGFDDEDHMSIHRRFAELWETIWDEICDIKATAQTDNVHRPFYPMLIFRTPKGWTCPKYID
GKKTEGSWRSHQVPLASARDTEAHFEVLKNWLESYKPEELFDANGAVKDDVLAFMPKGE
LRIGANPNANGGVIRDDLKLPNLEDYEVKEVAEFGHGWGQLEATRSLGAYTRDIIKNNPRD
FRIFGPDETASNRLQASYEVTNKQWDAGYISDEVDEHMRVSGQVVEQLSEHQMEGFLEAY
LLTGRHGIWSSYESFVHVIDSMLNQHAKWLEATVREIPWRKPIASMNLLVSSHVWRQDHN
GFSHQDPGVTSVLLNKCFHNDHVIGIYFATDANMLLAIAEKCYKSTNKINAIIAGKQPAAT
WLTLDEARAELEKGAAAWDWASTAKTNDEAEIVLAAAGDVPTQEIMAASDKLKELGIKF
KVVNVVDLLSLQSAKENDEALSNEEFADIFTADKPVLFAYHSYAHDVRGLIYDRPNHDNF
NVHGYEEEGSTTTPYDMVRVNRIDRYELTAEALRMIDADKYADKIDELEKFRDEAFQFAV
DKGYDHPDYTDWVYSGVNTGKKGAVTATAATAGDNE (**SEQ ID NO: 4**)

FIGURE 19.

MTDVRFRIIGTGAYVPERIVSNDEVGAPAGVDDDWITRKTGIRQ
RRWAADDQATSDLATAAGRAALKAAGITPEQLTVIAVATSTPDRPQPPTAAYVQHHLG
ATGTAAFDVNAVCSGTVFALSSVAGTLVYRGGYALVIGADLYSRILNPADRKTVVLFG
DGAGAMVLGPTSTGTGPIVRRVALHTFGGLTDLIRVPAGGSRQPLDTDGLDAGLQYFA
MDGREVRRFVTEHLPQLIKGFLHEAGVDAADISHFVPHQANGVMLDEVFGELHLPRAT
MHRTVETYGNTGAASIPITMDAAVRAGSFRPGELVLLAGFGGGMAASFALIEW (**SEQ ID NO: 5**)

FIGURE 20:

L.grayi mvaE:

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ctcttggtgtggctttgaatctgagggtcaatgataagagttataccatcccactagcaactgaggaaccgagtgtaatcgctgcctgtaataatggt
gcaaaaatggcaaaccacctgggcggttttcagtcagaattaaaagatggtttcctgcgtgggcaaattgtacttatgaacgtcaaagaacccgca
actatcgagcatacgatcacggcagagaaagcggcaatttttcgtgccgcagcgcagtcacatccatcgattgtgaaacgaggtgggggtctaa
aagagatagtagtgcgtacgttcgatgatgatccgacgttcctgtctattgatctgatagttgatactaaagacgcaatgggcgctaacatcattaac
accattctcgagggtgtagccggctttctgagggaaatccttaccgaagaaattctgttctctatttttatctaattacgcaaccgaatcaattgtgacc
gccagctgtcgcataccttacgaagcactgagtaaaaaaggtgatggtaaacgaatcgctgaaaaagtggctgctgcatctaaatttgcccagtta
gatccttatcgagctgcaacccacaacaaaggtattatgaatggtattgaggccgtcgttttggcctcaggaaatgacacacgggcggtcgcggc
agccgcacatgcgtatgcttcacgcgatcagcactatcggggcttaagccagtggcaggttgcagaaggcgcgttacacggggagatcagtct
accacttgcactcggcagcgttggcggtgcaattgaggtcttgcctaaagcgaaggcggcattcgaaatcatggggatcacagaggcgaagga
gctggcagaagtcacagctgcggtagggctggcgcaaaacctggcggcgttaagagcgcttgttagtgaaggaatacagcaaggtcacatgtc
gctccaggctcgctctcttgcattatcggtaggtgctacaggcaaggaagttgaaatcctggccgaaaaattacagggctctcgtatgaatcaggc
gaacgctcagaccatactcgcagagatcagatcgcaaaaagttgaattgtga **(SEQ ID NO: 6)**

FIGURE 21:

E. faecium mvaE:

atgaaagaagtggttatgattgatgcggctcgcacacccattgggaaatacagaggtagtcttagtccttttacagcggtggagctggggacact
ggtcacgaaagggctgctggataaaacaaagcttaagaaagacaagatagaccaagtgatattcggcaatgtgcttcaggcaggaaacggaca
aaacgttgcaagacaaatagccctgaacagtggcttaccagttgacgtgccggcgatgactattaacgaagtttgcgggtccggaatgaaagcg
gtgattttagcccgccagttaatacagttaggggaggcagagttggtcattgcagggggtacggagtcaatgtcacaagcacccatgctgaaac
cttaccagtcagagaccaacgaatacggagagccgatatcatcaatggttaatgacgggctgacggatgcgttttccaatgctcacatgggtctta
ctgccgaaaaggtggcgacccagttttcagtgtcgcgcgaggaacaagaccggtacgcattgtccagccaattgaaagcagcgcacgcggttg
aagccggggtgttctcagaagagattattccggttaagattagcgacgaggatgtcttgagtgaagacgaggcagtaagaggcaacagcacttt
ggaaaaactgggcaccttgcggacggtgttttctgaagagggcacggttaccgctggcaatgcttcaccgctgaatgacggcgctagtgtcgtg
attcttgcatcaaaagaatacgcggaaaacaataatctgccttacctggcgacgataaaggaggttgcggaagttggtatcgatccttctatcatgg
gtattgccccaataaaggccattcaaaagttaacagatcggtcgggcatgaacctgtccacgattgatctgttcgaaattaatgaagcattcgcgg
catctagcattgttgtttctcaagagctgcaattggacgaagaaaaagtgaatatctatggcggggcgatagctttaggccatccaatcggcgcaa
gcggagcccggatactgacaaccttagcatacggcctcctgcgtgagcaaaagcgttatggtattgcgtcattatgtatcggcggtggtcttggtc
tggccgtgctgttagaagctaatatggagcagacccacaaagacgttcagaagaaaaagttttaccagcttacccccctccgagcggagatcgca
gcttatcgagaagaacgttctgactcaagaaacggcacttattttccaggagcagacgttgtccgaagaactgtccgatcacatgattgagaatca
ggtctccgaagtggaaattccaatgggaattgcacaaaattttcagattaatggcaagaaaaaatggattcctatggcgactgaagaaccttcagt
aatagcggcagcatcgaacggcgccaaaatctgcgggaacatttgcgcggaaacgcctcagcggcttatgcgcgggcagattgtcctgtctgg
caaatcagaatatcaagccgtgataaatgccgtgaatcatcgcaaagaagaactgattctttgcgcaaacgagtcgtacccgagtattgttaaacg
cgggggaggtgttcaggatatttctacgcgggagtttatgggttcttttcacgcgtatttatcaatcgactttctggtggacgtcaaggacgcaatgg
gggcaaacatgatcaactctattctcgaaagcgttgcaaataaactgcgtgaatggttcccggaagaggaaatactgttctccatcctgtcaaactt
cgctacggagtccctggcatctgcatgttgcgagattccttttgaaagacttggtcgtaacaaagaaattggtgaacagatcgccaagaaaattca
acaggcaggggaatatgctaagcttgacccttaccgcgcggcaacccataacaaggggattatgaacggtatcgaagccgtcgttgccgcaac
gggaaacgacacacgggctgtttccgcttctattcacgcatacgccgcccgtaatggcttgtaccaaggtttaacggattggcagatcaagggcg
ataaactggttggtaaattaacagtcccactggctgtggcgactgtcggtggcgcgtcgaacatattaccaaaagccaaagcttccctcgccatgc
tggatattgattccgcaaaagaactggcccaagtgatcgccgcggtaggtttagcacagaatctggcggcgttacgtgcattagtgacagaagg
cattcagaaaggacacatgggcttgcaagcacgttctttagcgatttcgataggtgccatcggtgaggagatagagcaagtcgcgaaaaaactg
cgtgaagctgaaaaaatgaatcagcaaacggcaatacagattttagaaaaaattcgcgagaaatga **(SEQ ID NO: 7)**

FIGURE 22:

E. gallinarum mvaE:

atggaagaagtggtaattatagatgcacgtcggactccgattggtaaatatcacgggtcgttgaagaagttttcagcggtggcgctggggacggc
cgtggctaaagacatgttcgaacgcaaccagaaaatcaaagaggagatcgcgcaggtcataattggtaatgtcttgcaggcaggaaatggcca
gaaccccgcgcggcaagttgctcttcaatcagggttgtccgttgacattcccgcttctacaattaacgaggtttgtgggtctggtttgaaagctatctt
gatgggcatggaacaaatccaactcggcaaagcgcaagtagtgctggcaggcggcattgaatcaatgacaaatgcgccaagcctgtcccacta
taacaaggcggaggatacgtatagtgtcccagtgtcgagcatgacactggatggtctgacagacgcattttctagtaaacctatgggattaacagc
ggaaaacgtcgcacagcgctacggtatctcccgtgaggcgcaagatcaattcgcatatcaatctcagatgaaagcagcaaaagcgcaggcag
aaaacaaattcgctaaggaaattgtgccactggcgggtgaaactaaaaccatcacagctgacgaagggatcagatcccaaacaacgatggaga
aactggcaagtctcaaacctgtttttaaaaccgatggcactgtaaccgcagggaatgctagcaccattaatgacggggccgcccttgtgctgcttg
ctagcaaaacttactgcgaaactaatgacataccgtaccttgcgacaatcaaagaaattgttgaagttggaatcgatccggagattatgggcatctc
tccgataaaagcgatacaaacattgttacaaaatcaaaaagttagcctcgaagatattggagttttgaaataaatgaagcctttgccgcaagtagc
atagtggttgaatctgagttgggattagatccggctaaagttaaccgttatgggggtggtatatccttaggtcatgcaattggggcaaccggcgctc
gcctggccacttcactggtgtatcaaatgcaggagatacaagcacgttatggtattgcgagcctgtgcgttggtggtggacttggactggcaatgc
ttttagaacgtccaactattgagaaggctaaaccgacagacaaaaagttctatgaattgtcaccagctgaacggttgcaagagctggaaaatcaac
agaaaatcagttctgaaactaaacagcagttatctcagatgatgcttgccgaggacactgcaaaccatttgatagaaaatcaaatatcagagattga
actcccaatgggcgtcgggatgaacctgaaggttgatgggaaagcctatgttgtgccaatggcgacggaagagccgtccgtcatcgcggccat
gtctaatggtgccaaaatggccggcgaaattcacactcagtcgaaagaacggctgctcagaggtcagattgttttcagcgcgaagaatccgaat
gaaatcgaacagagaatagctgagaaccaagctttgattttcgaacgtgccgaacagtcctatccttccattgtgaaaagagagggaggtctccg
ccgcattgcacttcgtcattttcctgccgattctcagcaggagtctgcggaccagtccacatttttatcagtggacctttttgtagatgtgaaagacgc
gatgggggcaaatatcataaatgcaatacttgagggcgtcgcagccctgtttcgcgaatggttccccaatgaggaaattctttttttctattctctcgaa
cttggctacggagagcttagtcacggctgtttgtgaagtcccatttagtgcacttagcaagagaggtggtgcaacggtggcccagaaaattgtgc
aggcgtcgctcttcgcaaagacagacccataccgcgcagtgacccacaacaaagggattatgaacggtgtagaggctgttatgcttgccacag
gcaacgacacgcgcgcagtctcagccgcttgtcatggatacgcagcgcgcaccggtagctatcagggtctgactaactggacgattgagtcgg
atcgcctggtaggcgagataacactgccgctggccatcgctacagttggaggcgctaccaaagtgttgcccaaagctcaagcggcactggaga
ttagtgatgttcactcttctcaagagcttgcagccttagcggcgtcagtaggtttagtacaaaatctcgcggccctgcgcgcactggtttccgaagg
tatacaaaaagggcacatgtccatgcaagcccggtctctcgcaatcgcggtcggtgctgaaaaagccgagatcgagcaggtcgccgaaaagtt
gcggcagaacccgccaatgaatcagcagcaggcgctccgttttcttggcgagatccgcgaacaatga (**SEQ ID NO: 8**)

FIGURE 23:

E. casseliflavus mvaE:

atggaagaagttgtcatcattgacgcactgcgtactccaataggaaagtaccacggttcgctgaaagattacacagctgttgaactggggacagt
agcagcaaaggcgttgctggcacgaaatcagcaagcaaaagaacacatagcgcaagttattattggcaacgtcctgcaagccggaagtgggc
agaatccaggccgacaagtcagtttacagtcaggattgtcttctgatatccccgctagcacgatcaatgaagtgtggctcgggtatgaaagcga
ttctgatgggtatggagcaaattcagctgaacaaagcctctgtggtcttaacaggcggaattgaaagcatgaccaacgcgccgctgtttagttatta
caacaaggctgaggatcaatattcggcgccggttagcacaatgatgcacgatggtctaacagatgctttcagttccaaaccaatgggcttaaccg
cagagaccgtcgctgagagatatggaattacgcgtaaggaacaagatgaatttgcttatcactctcaaatgaaggcggccaaagcccaggcgg
cgaaaaagtttgatcaggaaattgtacccctgacggaaaaatccggaacggttctccaggacgaaggcatcagagccgcgacaacagtcgag
aagctagctgagcttaaaacggtgttcaaaaaagacggaacagttacagcgggtaacgcctctacgataaatgatggcgctgctatggtattaat
agcatcaaaatcttattgcgaagaacaccagattccttatctggccgttataaaggagatcgttgaggtgggttttgcccccgaaataatgggtattt
cccccattaaggctatagacaccctgctgaaaaatcaagcactgaccatagaggatataggaatatttgagattaatgaagcctttgctgcgagttc
gattgtggtagaacgcgagttgggcctggaccccaaaaaagttaatcgctatggcggtggtatatcactcggccacgcaattggggcgacggg
agctcgcattgcgacgaccgttgcttatcagctgaaagatacccaggagcgctacggtatagcttccttatgcgttggtgggggtcttggattggc
gatgcttctggaaaacccatcggccactgcctcacaaactaattttgatgaggaatctgcttccgaaaaaactgagaagaagaagttttatgcgcta
gctcctaacgaacgcttagcgtttttggaagcccaaggcgctattaccgctgctgaaaccctggtcttccaggagatgaccttaaacaaagagac
agccaatcacttaatcgaaaaccaaatcagcgaagttgaaattcctttaggcgtgggcctgaacttacaggtgaatgggaaagcgtataatgttcc
tctggccacggaggaaccgtccgttatcgctgcgatgtcgaatggcgccaaaatggctggtcctattacaacaacaagtcaggagaggctgtta
cggggtcagattgtcttcatggacgtacaggacccagaagcaatattagcgaaagttgaatccgagcaagctaccattttcgcggtggcaaatga
aacataccgtctatcgtgaaaagaggaggaggtctgcgtagagtcattggcaggaatttcagtccggccgaaagtgacttagccacggcgtat
gtatcaattgacctgatggtagatgttaaggatgcaatgggtgctaatatcatcaatagtatcctagaaggtgttgcggaattgtttagaaaatggttc
ccagaagaagaaatcctgttctcaattctctccaatctcgcgacagaaagtctggtaacggcgacgtgctcagttccgtttgataaattgtccaaaa
ctgggaatggtcgacaagtagctggtaaaatagtgcacgcggcggactttgctaagatagatccatacagagctgccacacacaataaaggtatt
atgaatggcgttgaagcgttaatcttagccaccggtaatgacacccgtgcggtgtcggctgcatgccacggttacgcggcacgcaatgggcgaa
tgcaagggcttacctcttggacgattatcgaagatcggctgataggctctatcacattacctttggctattgcgacagtgggggtgccacaaaaat
cttgccaaaagcacaggccgccctggcgctaactggcgttgagacggcgtcggaactggccagcctggcggcgagtgtgggattagttcaaa
atttggccgctttacgagcactagtgagcgagggcattcagcaagggcacatgagtatgcaagctagatccctggccattagcgtaggtgcgaa
aggtactgaaatagagcaactagctgcgaagctgagggcagcgacgcaaatgaatcaggagcaggctcgtaaatttctgaccgaaataagaaa
ttaa **(SEQ ID NO: 9)**

FIGURE 24:

L.grayi mvaS:

atgaccatgaacgttggaatcgataaaatgtcattctttgttccaccttactttgtggacatgactgatctggcagtagcacgggatgtcgatcccaat
aagtttctgattggtattggccaggaccagatggcagttaatccgaaaacgcaggatattgtgacatttgccacaaatgctgccaaaaacatactgt
cagctgaggaccttgataaaattgatatggtcatagtcggcaccgagagtggaatcgatgaatccaaagcgagtgccgtagtgcttcacaggttg
ctcggtatccagaagtttgctcgctcctttgaaatcaaagaagcctgttatgggggtaccgcggctttacagttcgctgtaaaccacattaggaatc
atcctgaatcaaaggttcttgtagttgcatcagatatcgcgaaatacggcctggcttctggaggtgaaccaacgcaaggtgcaggcgctgtggct
atgctcgtctcaactgaccctaagatcattgctttcaacgacgatagcctcgcgcttacacaagatatctatgacttctggcgaccagttggacatga
ctatcctatggtcgacgggcctcttagtacagagacctacatccagtcatttcagaccgtatggcaggaatacacaaaacggtcgcagcatgcac
tggcagactttgctgcccttagctttcatatcccgtatactaaaatgggcaaaaaggcgctgcttgcaatccttgaaggcgaatcagaggaggctc
agaaccgtatactagcaaaatatgaaaagagtatagcctactccagaaaggcgggtaacctgtataccggtagcctgtatctaggacttatttcact
tctggaaaatgcagaagaccttaaagctggtgatttaataggcctcttttcttacggttccggtgctgttgcggagtttttctcaggaaggctggttga
ggactatcaggaacagctacttaaaacaaaacatgccgaacagctggcccatagaaagcaactgacaatcgaggagtacgaaacgatgttctc
cgatcgcttggacgtggacaaagacgccgaatacgaagacacattagcttatagcatttcgtcagtccgaaacaccgtacgtgagtacaggagtt
ga (**SEQ ID NO:10)**

FIGURE 25:

E. faecium mvaS:

atgaaaatcggtattgaccgtctgtccttcttcatcccgaatttgtatttggacatgactgagctggcagaatcacgcggggatgatccagctaaata
tcatattggaatcggacaagatcagatggcagtgaatcgcgcaaacgaggacatcataacactgggtgcaaacgctgcgagtaagatcgtgac
agagaaagaccgcgagttgattgatatggtaatcgttggcacggaatcaggaattgaccactccaaagcaagcgccgtgattattcaccatctcct
taaaattcagtcgttcgcccgttctttcgaggtaaaagaagcttgctatggcggaactgctgccctgcacatggcgaaggagtatgtcaaaaatcat
ccggagcgtaaggtcttggtaattgcgtcagacatcgcgcgttatggtttggccagcggaggagaagttactcaaggcgtgggggccgtagcc
atgatgattacacaaaaccccggattctttcgattgaagacgatagtgtttttctcacagaggatatctatgatttctggcggcctgattactccgagt
tccctgtagtggacgggccccttcaaactcaacgtatatagagagttttcagaaagtttggaaccggcacaaggaattgtccggaagagggctg
gaagattatcaagctattgcttttcacataccctatacgaagatgggtaagaaagcgctccagagtgttttagaccaaaccgatgaagataaccag
gagcgcttaatggctagatatgaggagtctattcgctatagccggagaattggtaacctgtacacaggcagcttgtaccttggtcttacaagcttgtt
ggaaaactctaaaagtttacaaccgggagatcggatcggcctcttttcctatggcagtggtgcggtgtccgagttctttaccgggtatttagaagaa
aattaccaagagtacctgttcgctcaaagccatcaagaaatgctggatagccggactcggattacggtcgatgaatacgagaccatcttttcagag
actctgccagaacatggtgaatgcgccgaatatacgagcgacgtccccttttctataaccaagattgagaacgacattcgttattataaaatctga

(SEQ ID NO: 11)

FIGURE 26:

E. gallinarum mvaS:

atgaacgtcggcattgacaaaattaatttttcgttccaccgtattatctggatatggtcgacctggcccacgcacgcgaagtggacccgaacaaat
ttacaattggaattggacaggatcagatggctgtgagcaaaaagacgcacgatatcgtaacattcgcggctagtgccgcgaaggaaattttagaa
cctgaggacttgcaagctatagacatggttatagttggtaccgaatcgggcattgacgagagcaaagcatccgcggtcgttttacatcgtttgttgg
gcgtacaacctttcgctcgcagttttgaaattaaagaagcctgttacggggcaaccgcaggcattcagtttgccaagactcatatacaagcgaacc
cggagagcaaggtcctggtaattgcaagcgatatagctcggtatggtcttcggtcaggtggagagcccacacaaggcgcaggggcagttgcta
tgcttctcacggcaaatcccagaatcctgaccttcgaaaacgacaatctgatgttaacgcaggatatttatgacttctggagaccacttggtcacgct
taccctatggtagatggccacctttccaatcaagtctatattgacagttttaagaaggtctggcaagcacattgcgaacgcaatcaagcttctatatc
cgactatgccgcgattagttttcatattccgtatacaaaaatgggtaagaaagccctgctcgctgtttttgcagatgaagtggaaactgaacaggaa
cgcgttatggcacggtatgaagagtctatcgtatattcacgccggatcggcaacttgtatacgggatcattgtacctggggctgatatccttattgga
aaacagttctcacctgtcggcgggcgaccggataggattgtttagttatgggagtggcgctgtcagcgaatttttctccggtcgtttagtggcaggc
tatgaaaatcaattgaacaaagaggcgcatacccagctcctggatcagcgtcagaagctttccatcgaagagtatgaggcgatttttacagattcct
tagaaattgatcaggatgcagcgttctcggatgacctgccatattccatccgcgagataaaaaacacgattcggtactataaggagagctga

(SEQ ID NO:12)

FIGURE 27:

E. casseliflavus mvaS:

atgaacgttggaattgataaaatcaatttttcgttccgccctatttcattgatatggtggatctcgctcatgcaagagaagttgaccccaacaagttcactataggaa
taggccaagatcagatggcagtaaacaagaaaacgcaagatatcgtaacgttcgcgatgcacgccgcgaaggatattctgactaaggaagatttacaggcca
tagatatggtaatagtggggactgagtctgggatcgacgagagcaaggcaagtgctgtcgtattgcatcggcttttaggtattcagccttttgcgcgctcctttga
aattaaggaggcatgctatggggccactgccggccttcagtttgcaaaagctcatgtgcaggctaatccccagagcaaggtcctggtggtagcttccgatatag
cacgctacggactggcatccggaggagaaccgactcaaggtgtaggtgctgtggcaatgttgatttccgctgatccagctatcttgcagttagaaaatgataatc
tcatgttgacccaagatatatacgatttttggcgcccggtcgggcatcaatatcctatggtagacggccatctgtctaatgccgtctatatagacagctttaaacaa
gtctggcaagcacattgcgagaaaaaccaacggactgctaaagattatgctgcattgtcgttccatattccgtacacgaaaatgggtaagaaagctctgttagcg
gtttttgcggaggaagatgagacagaacaaaagcggttaatggcacgttatgaagaatcaattgtatacagtcgtcggactggaaatctgtatactggctcactc
tatctgggcctgatttccttactggagaatagtagcagtttacaggcgaacgatcgcataggtctgtttagctatggttcaggggccgttgcggaatttttcagtgg
cctcttggtaccgggttacgagaaacaattagcgcaagctgcccatcaagctcttctggacgaccggcaaaaactgactatcgcagagtacgaagccatgttta
atgaaaccattgatattgatcaggaccagtcatttgaggatgacttactgtactccatcagagagatcaaaaacactattcgctactataacgaggagaatgaata
a **(SEQ ID NO:13)**

FIGURE 28.

pCMP1090 (PKL from *Bifidobacterium infantis*)

gtttgacagcttatcatcgactgcacggtgcaccaatgcttctggcgtcaggcagccatcggaagctgtggtatggctgtgcaggtcgtaaatcac
tgcataattcgtgtcgctcaaggcgcactcccgttctggataatgttttttgcgccgacatcataacggttctggcaaatattctgaaatgagctgttg
acaattaatcatccggctcgtataatgtgtggaattgtgagcggataacaatttcacacaggaaacagcgccgctgagaaaaagcgaagcggca
ctgctctttaacaatttatcagacaatctgtgtgggcactcgaccggaattatcgattaactttattattaaaaattaaagaggtatatattaatgtatcga
ttaaataaggaggaataaaccatgacgagtcctgttattggcaccccttggaagaagctcagcgctccggtttccgaggaagccctcgaaggcg
ttgacaagtactggcgcgttgccaactacctttccatcggccagatttatctgcgttccaacccgctgatgaaggagcccttcacccgcgaagatg
tgaagcatcgtctggtgggccactggggcactacccctggcctgaacttcctcatcggccacatcaaccgtttcattgctgaccacggccagaac
accgtgatcatcatgggcccgggccacggtggcccggccggtacctcccagtcctacctggacggcacctacaccgagaccttcccgaagat
caccaaggacgaagctggtctgcagaagttcttccgtcagttctcttacccgggcggtattccgtcccacttcgctccggagaccccgggctcca
tccacgagggtggtgagctgggttacgctctgtcccacgcttacggcgccatcatggacaacccgagcctgttcgtcccggccatcgtcggcga
cggcgaggctgagaccggcccgctggctaccggttggcagtccaacaagctcgtgaacccgcgcaccgacggtatcgtgctgccgatcctgc
acctcaacggctacaagatcgccaacccgaccatcctgtcccgcatctccgacgaagagctccacgagttcttccacggcatgggttacgagcc
ctacgagttcgtcgctggcttcgatgatgaggaccacatgtccatccaccgccgcttcgccgagctgtgggagaccatctgggacgagatctgc
gacatcaaggccaccgctcagaccgacaacgtgcaccgtccgttctaccgatgctgatcttccgcaccccgaagggctggacctgcccgaag
tacatcgacggcaagaagaccgaaggctcctggcgttcccaccaggtgccgctggcctccgcccgcgacaccgaggcccacttcgaggtcct
caagaactggctcgagtcctacaagccggaagagctgttcgacgccaacggcgccgtcaaggacgacgtcctcgccttcatgccgaagggcg
agctgcgtatcggtgccaacccgaacgccaacggcggtgtgatccgcgacgacctgaagctgccgaacctcgaggactacgaggtcaagga
agtggccgagttcggccacggctggggccagctcgaggccacccgctccctgggcgcctacacccgcgacatcatcaagaacaacccgcgt
gacttccgcatcttcggaccggatgagaccgcttccaaccgtctgcaggcttcctacgaagtcaccaacaagcagtgggatgccggctacatct
ccgacgaggtcgacgagcacatgcgcgtctccggccaggtcgtcgagcagctgtccgagcaccagatggaaggcttcctcgaggcctacctg
ctgaccggccgtcacggcatctggagctcctacgagtccttcgtccacgtgatcgactccatgctgaaccagcacgccaagtggcttgaggcta
ccgtccgcgagattccgtggcgcaagccgatcgcctccatgaacctgctggtctcctcccacgtctggcgtcaggaccacaacggcttctccca
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FIGURE 28 (continued).

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gagaattatgcagtgctgccataaccatgagtgataacactgcggccaacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttt
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acgcaaaccgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccgactggaaagcgggcagtgagcgcaacg
caattaatgtgagttagcgcgaattgatctg **(SEQ ID NO:15)**

FIGURE 29.

pCMP1029 (PKL from *Lactobacillus reuteri*)

gtttgacagcttatcatcgactgcacggtgcaccaatgcttctggcgtcaggcagccatcggaagctgtggtatggctgtgcaggtcgtaaatcac
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Figure 29 (continued).

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caattaatgtgagttagcgcgaattgatctg **(SEQ ID NO:16)**

Figure 30

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gataaaggtctggatcatccggattataccgattgggtttggcctggtgttaaaaccgataaacctggtgcggttaccgcaaccg
cagccaccgcaggcgataatgaataa **(SEQ ID NO:17)**

Figure 31

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Figure 32

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Figure 33

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(SEQ ID NO:20)

Figure 34

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Figure 35

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Figure 36

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Figure 37

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Figure 38

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ataa **(SEQ ID NO:25)**

Figure 39

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gtttacccgtgaagatgttaaacatcgtctggttggtcattggggcaccacaccgggtctgaattttctgattggtcatattaatcgc
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Figure 40

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Figure 41

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Figure 42

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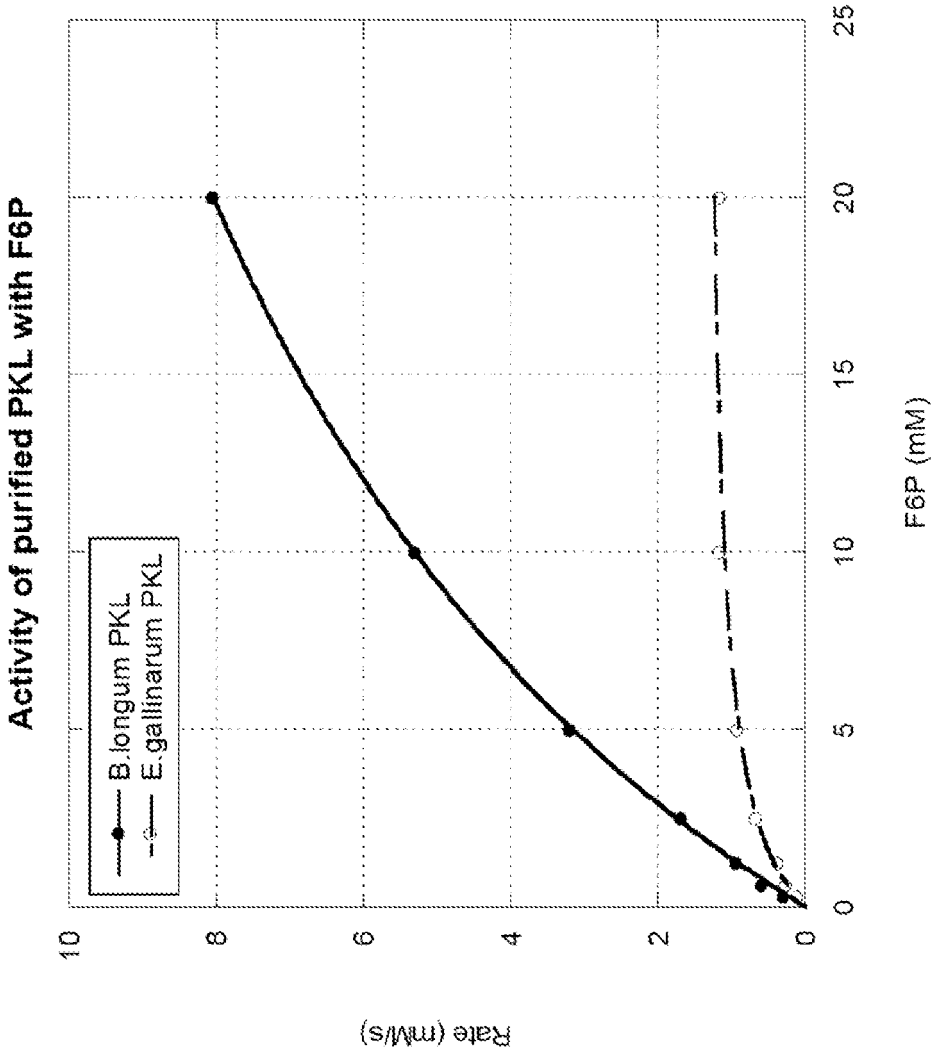

Activity of purified PKL with F6P
B.longum PKL
E.gallinarum PKL
Rate (mM/s)
F6P (mM)
0
2
4
6
8
10
0
5
10
15
20
25

Figure 43

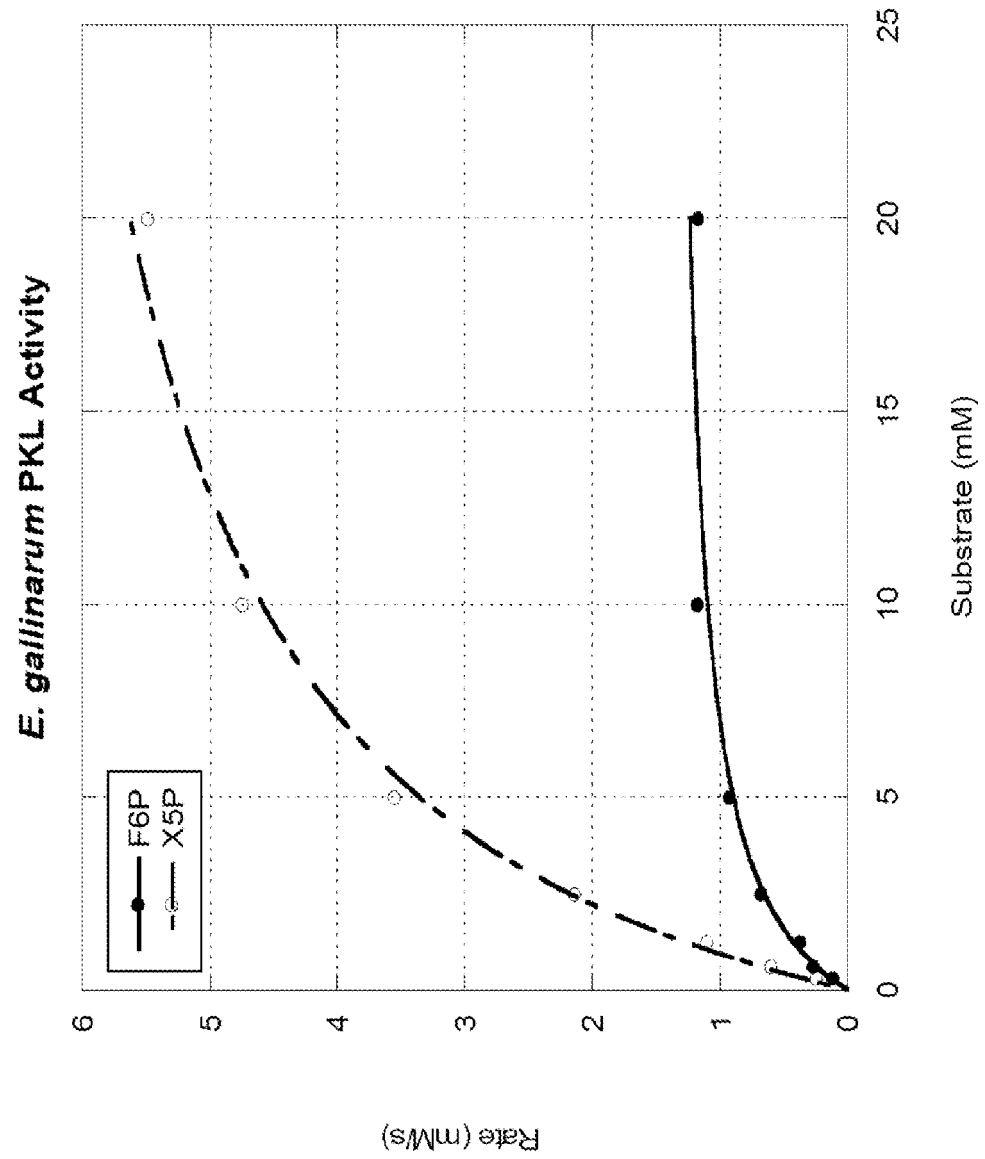

E. gallinarum PKL Activity
F6P
X5P
Rate (mM/s)
Substrate (mM)
0
1
2
3
4
5
6
0
5
10
15
20
25

Figure 44

Figure 45

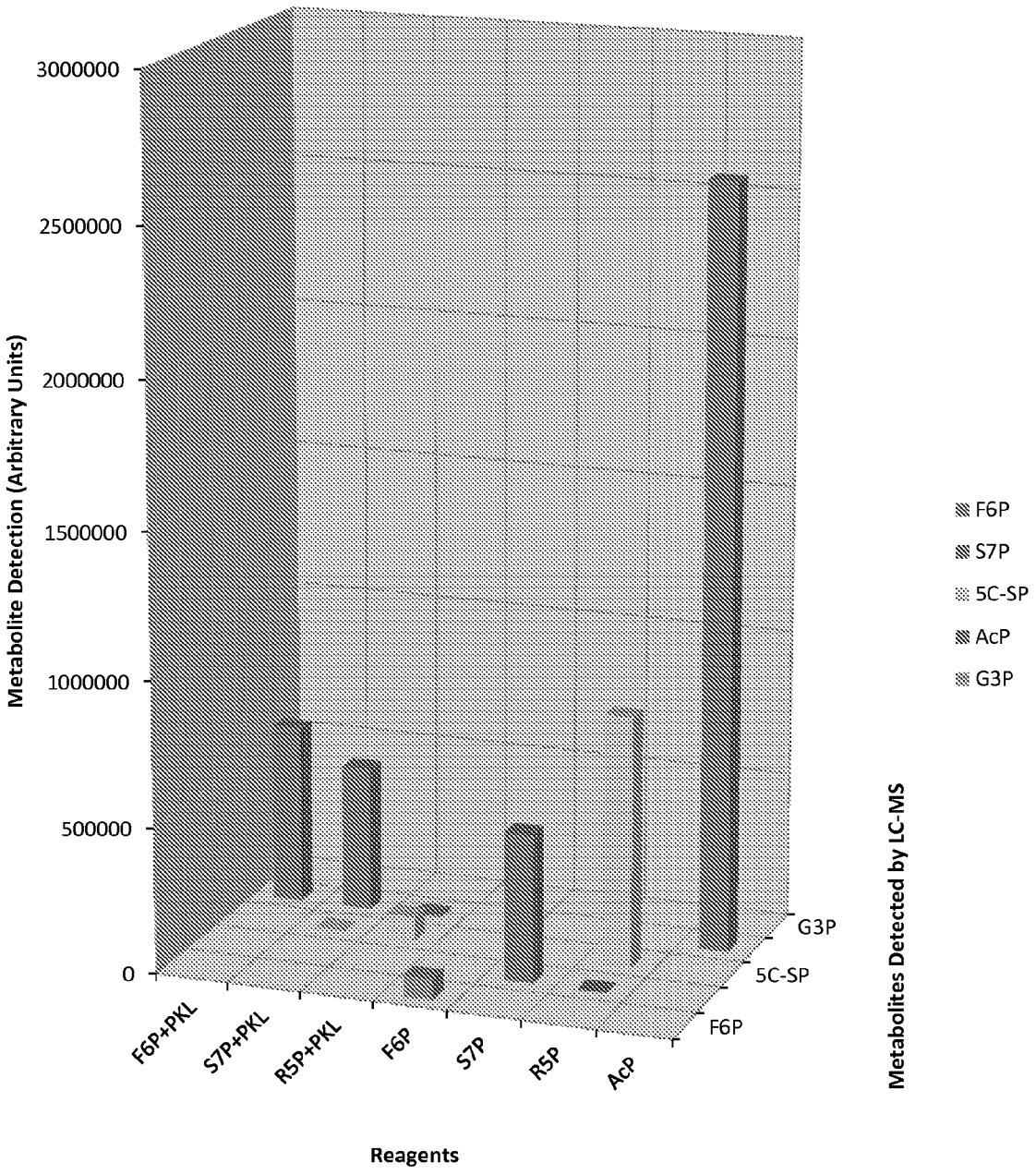
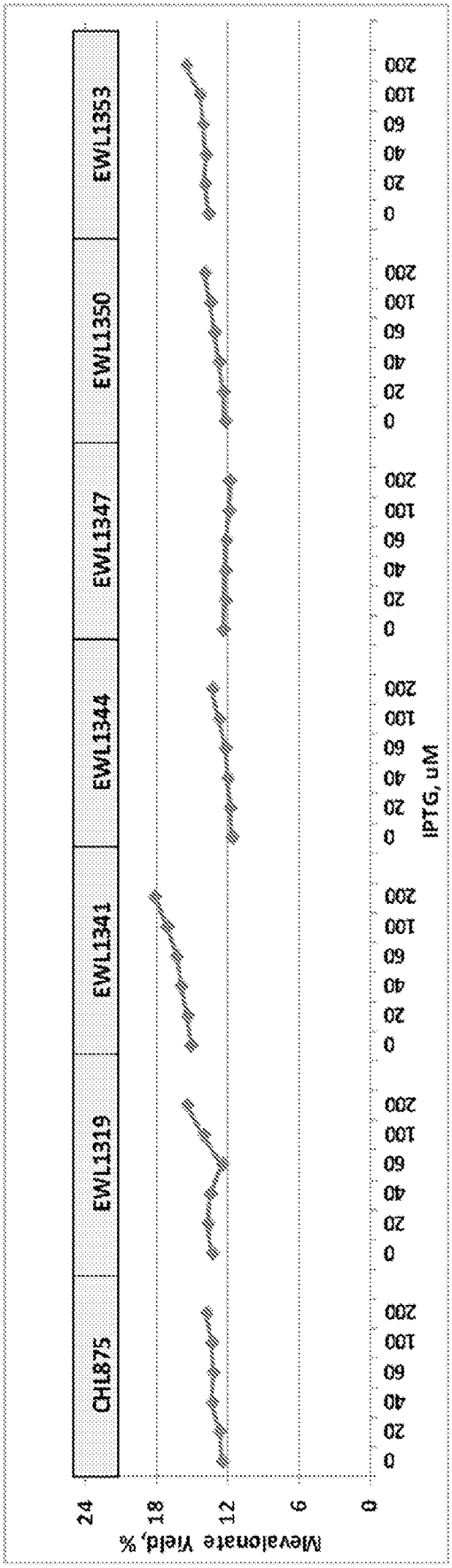

3000000
2500000
2000000
1500000
1000000
500000
0
Metabolite Detection (Arbitrary Units)
F6P+PKL
S7P+PKL
R5P+PKL
F6P
S7P
R5P
AcP
Reagents
G3P
5C-SP
F6P
Metabolites Detected by LC-MS
F6P
S7P
5C-SP
AcP
G3P

Figure 46


CH1875
EWL1319
EWL1341
EWL1344
EWL1347
EWL1350
EWL1353
Mevalonate Yield, %
0
6
12
18
24
IPTG, uM
0
20
40
60
100
200

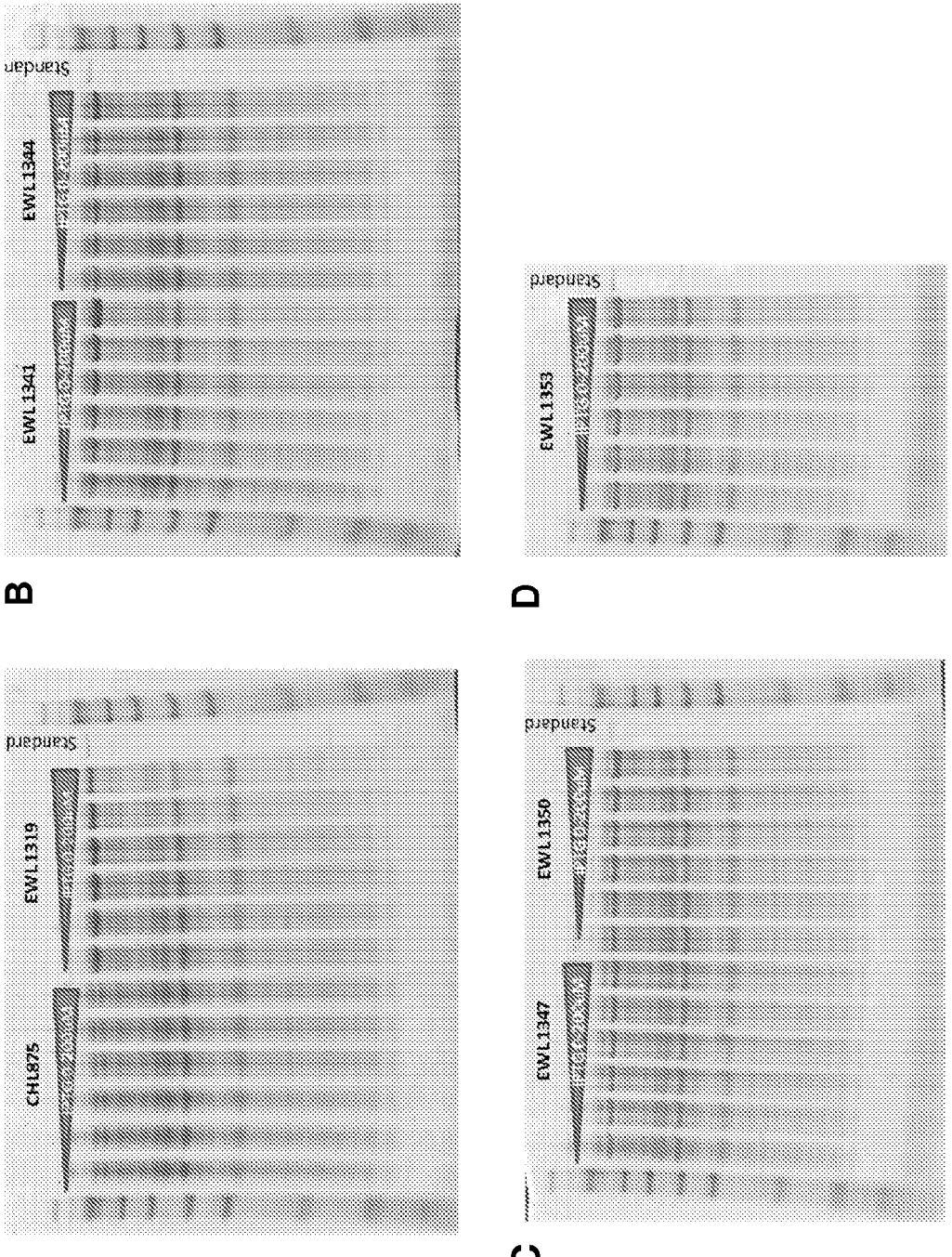

A
CHL875
EWL1319
Standard
B
EWL1341
EWL1344
Standard
C
EWL1347
EWL1350
Standard
D
EWL1353
Standard

Figure 47

Figure 48

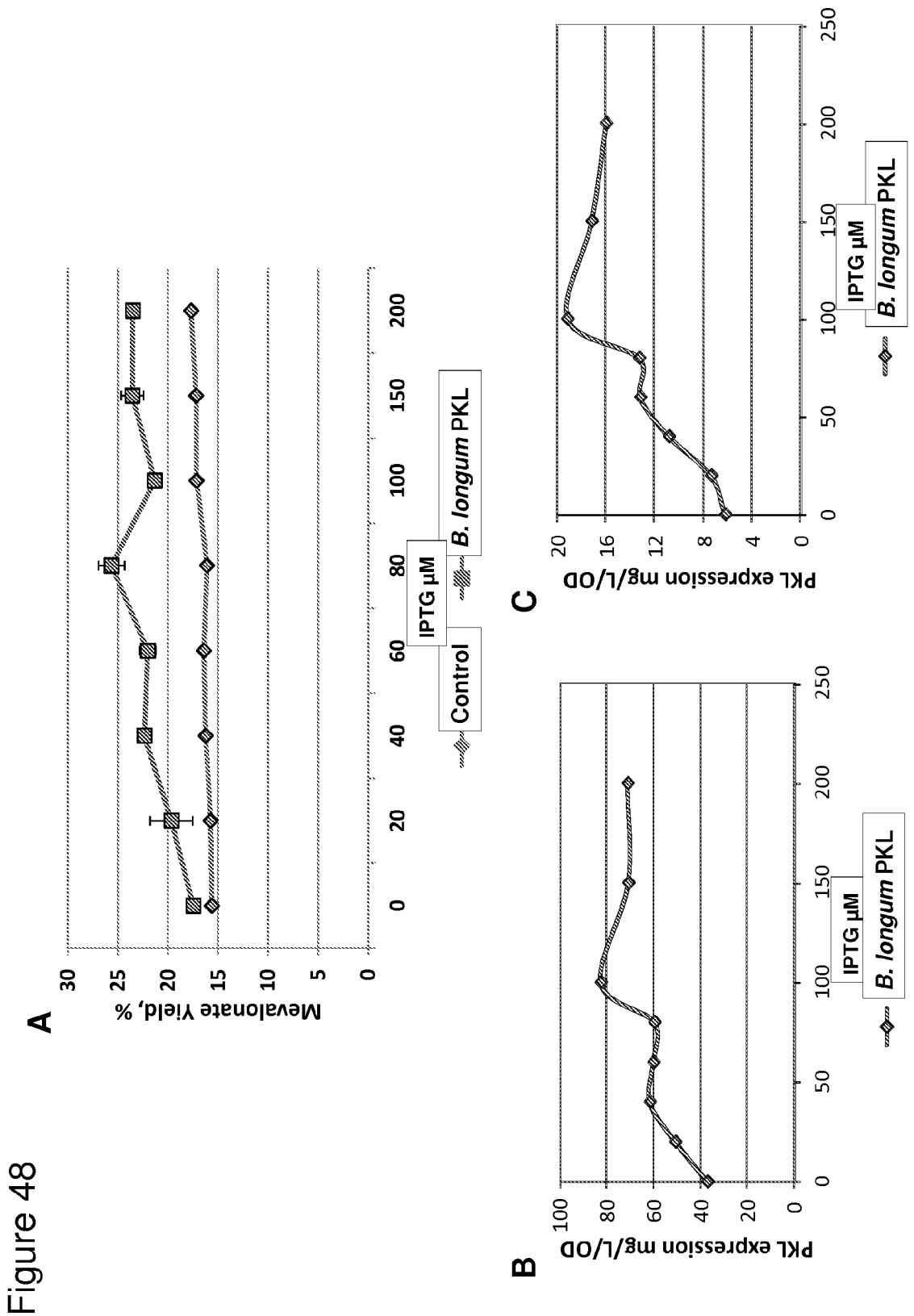
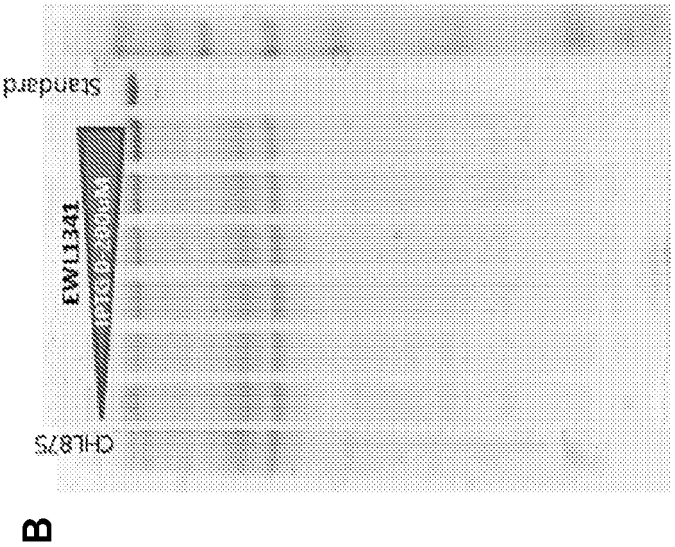

A
Mevalonate Yield, %
30
25
20
15
10
5
0
0
20
40
60
80
100
150
200
IPTG µM
Control
B. longum PKL
B
PKL expression mg/L/OD
100
80
60
40
20
0
0
50
100
150
200
250
IPTG µM
B. longum PKL
C
PKL expression mg/L/OD
20
16
12
8
4
0
0
50
100
150
200
250
IPTG µM
B. longum PKL

Figure 49


B
CHL875
EW11341
Standard

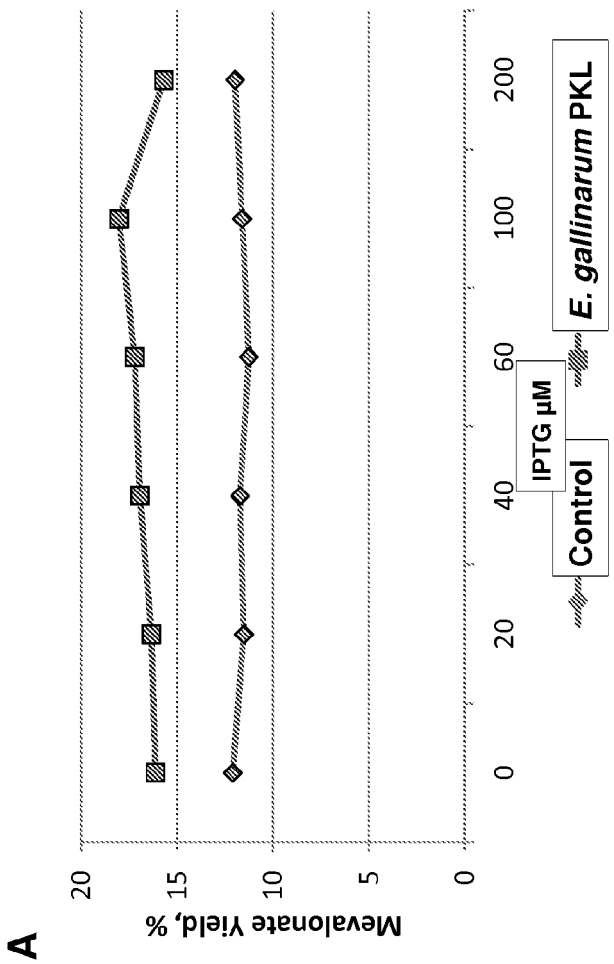
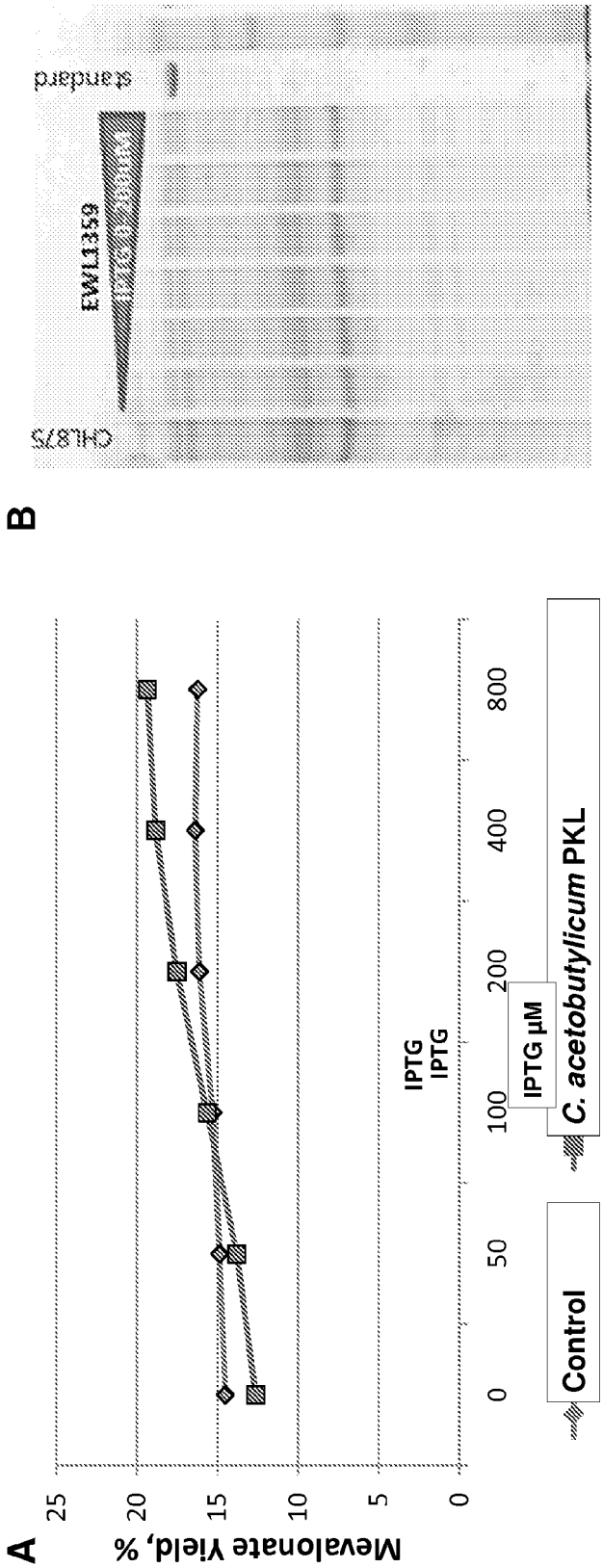
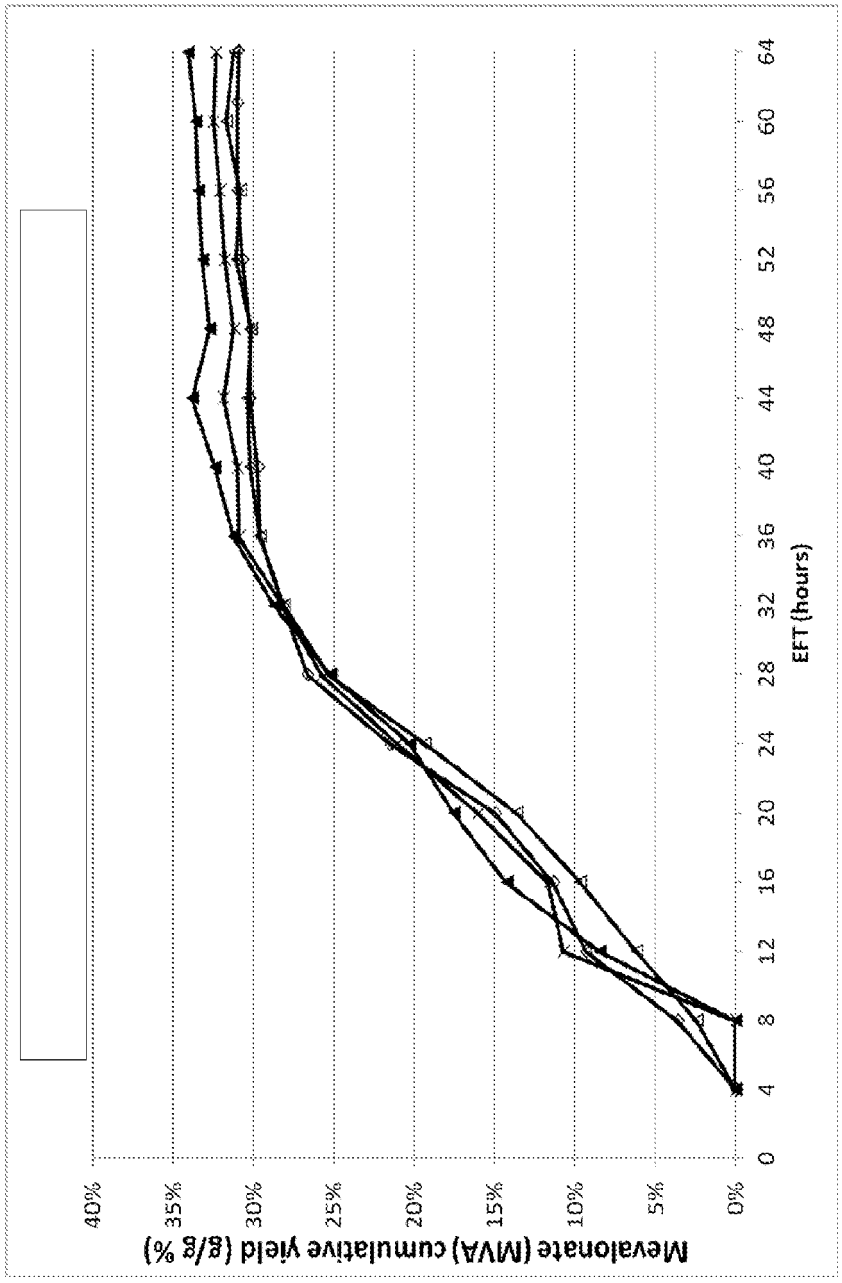

A
Mevalonate Yield, %
20
15
10
5
0
0
20
40
60
100
200
Control
IPTG µM
E. gallinarum PKL

Figure 50

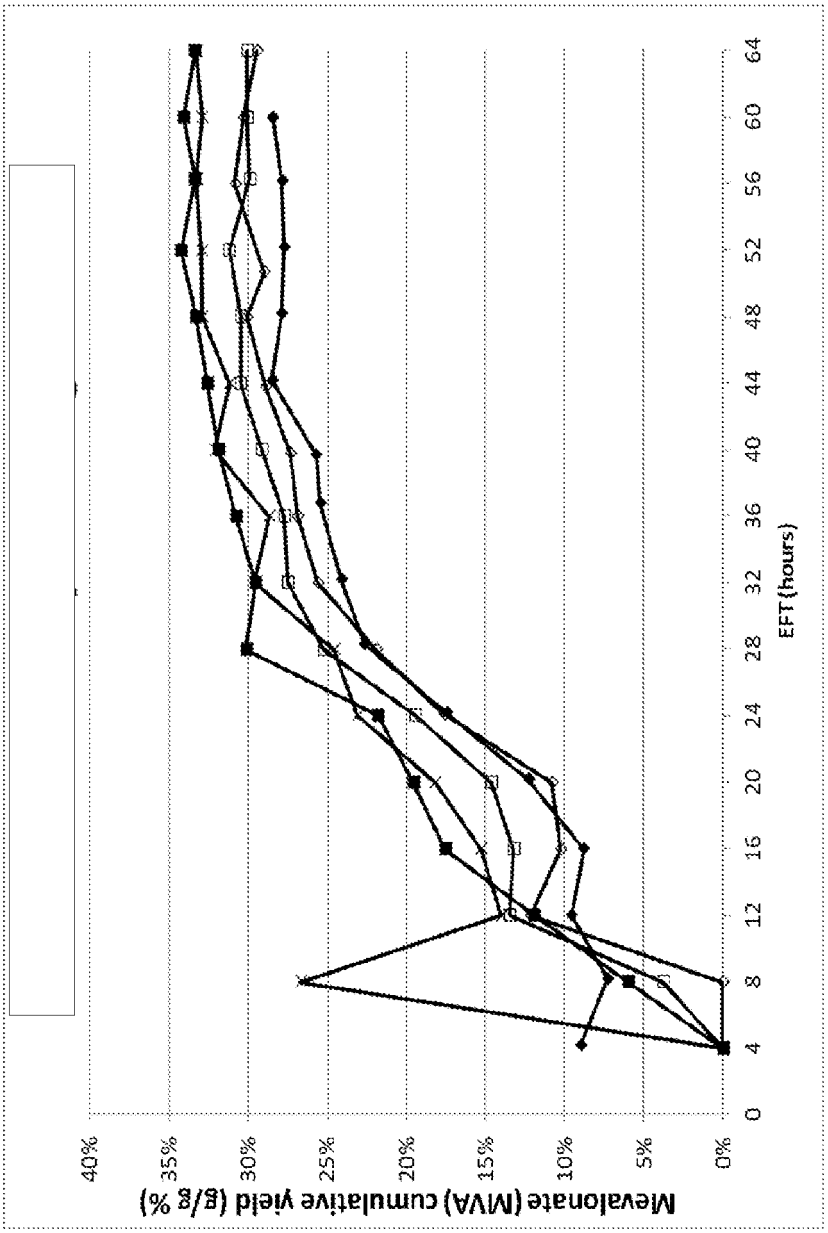

B
CHL875
EWL1359
standard
A
Mevalonate Yield, %
25
20
15
10
5
0
0
50
100
200
400
800
IPTG µM
IPTG
IPTG
Control
C. acetobutylicum PKL



20121058: EWL1359 induced at 100uM IPTG (closed triangles)
20121057: EWL1359 induced at 50uM IPTG (x symbol)
20121056: EWL1359 with no added IPTG (open triangles)
20121059: CHL875 induced at 100uM IPTG (open diamonds)

Figure 51

Figure 52



20120979: EWL1341 induced at 400uM IPTG (closed squares)
20120978: EWL1341 induced at 100uM IPTG (x symbols)
20120977: EWL1341 with no added IPTG (open squares)
20120976: CHL875 with no added IPTG (open diamonds)
20120821: CHL875 with no added IPTG (closed diamonds)

Figure 53

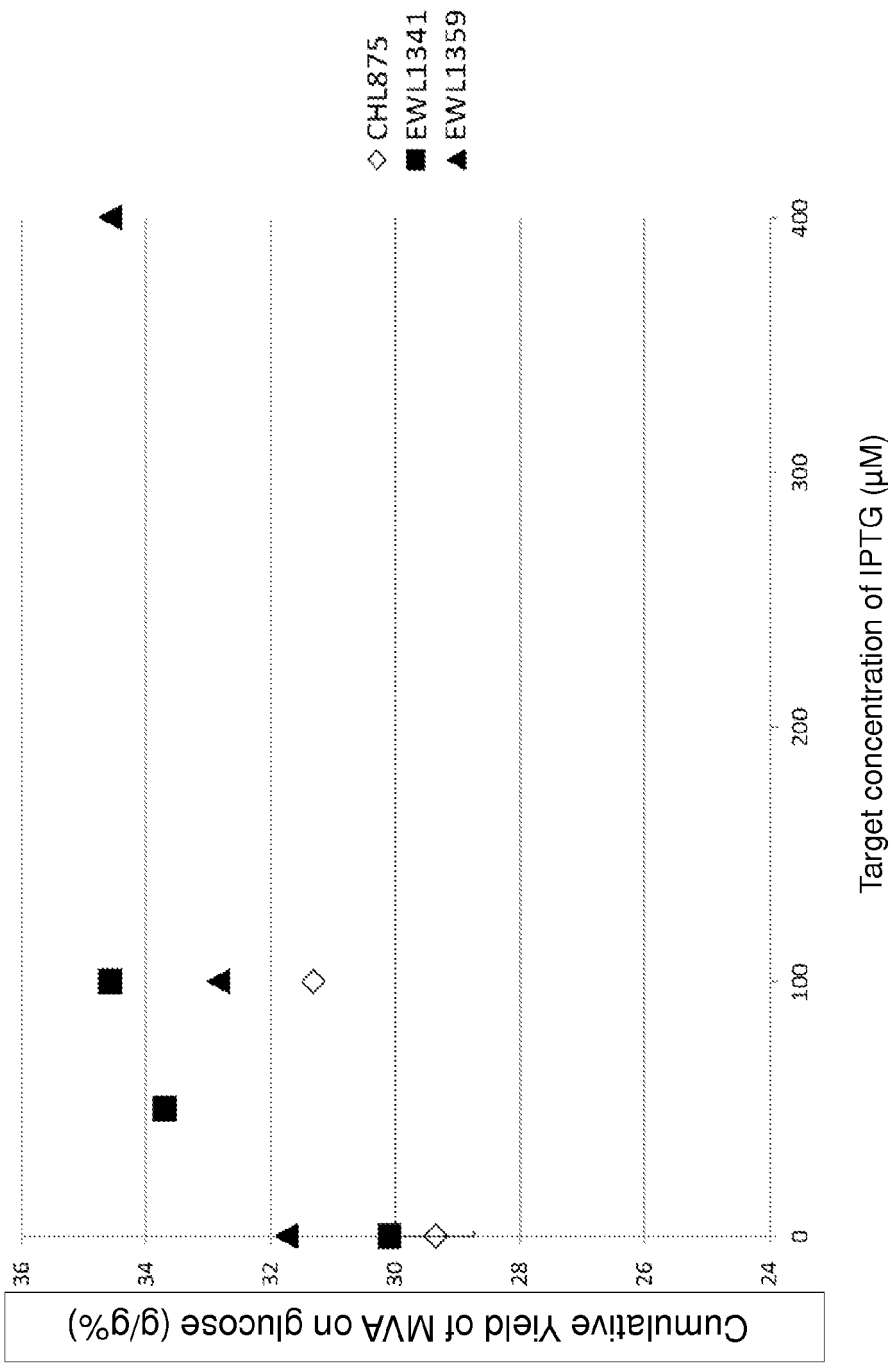

Cumulative Yield of MVA on glucose (g/g%)
36
34
32
30
28
26
24
0
100
200
300
400
Target concentration of IPTG (μM)
CHL875
EWL1341
EWL1359

Figure 54

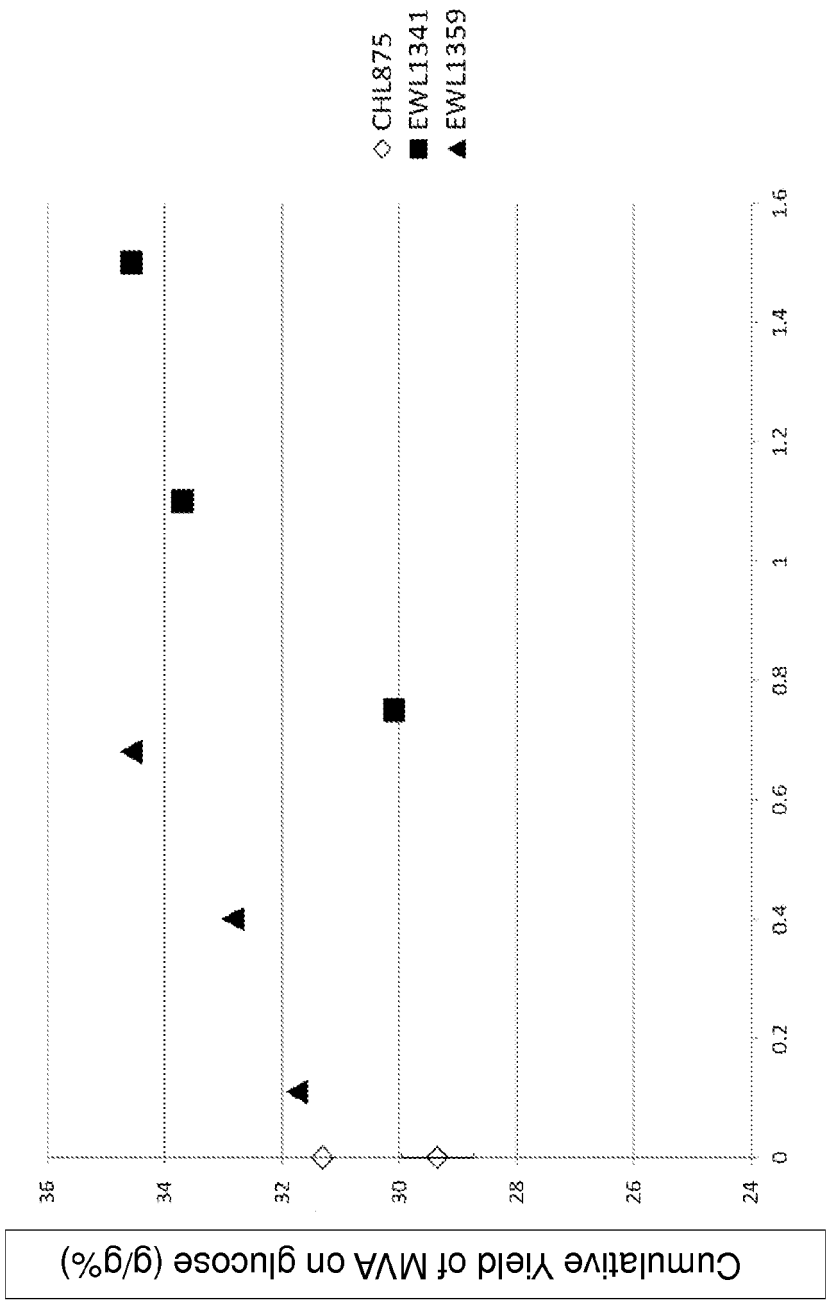
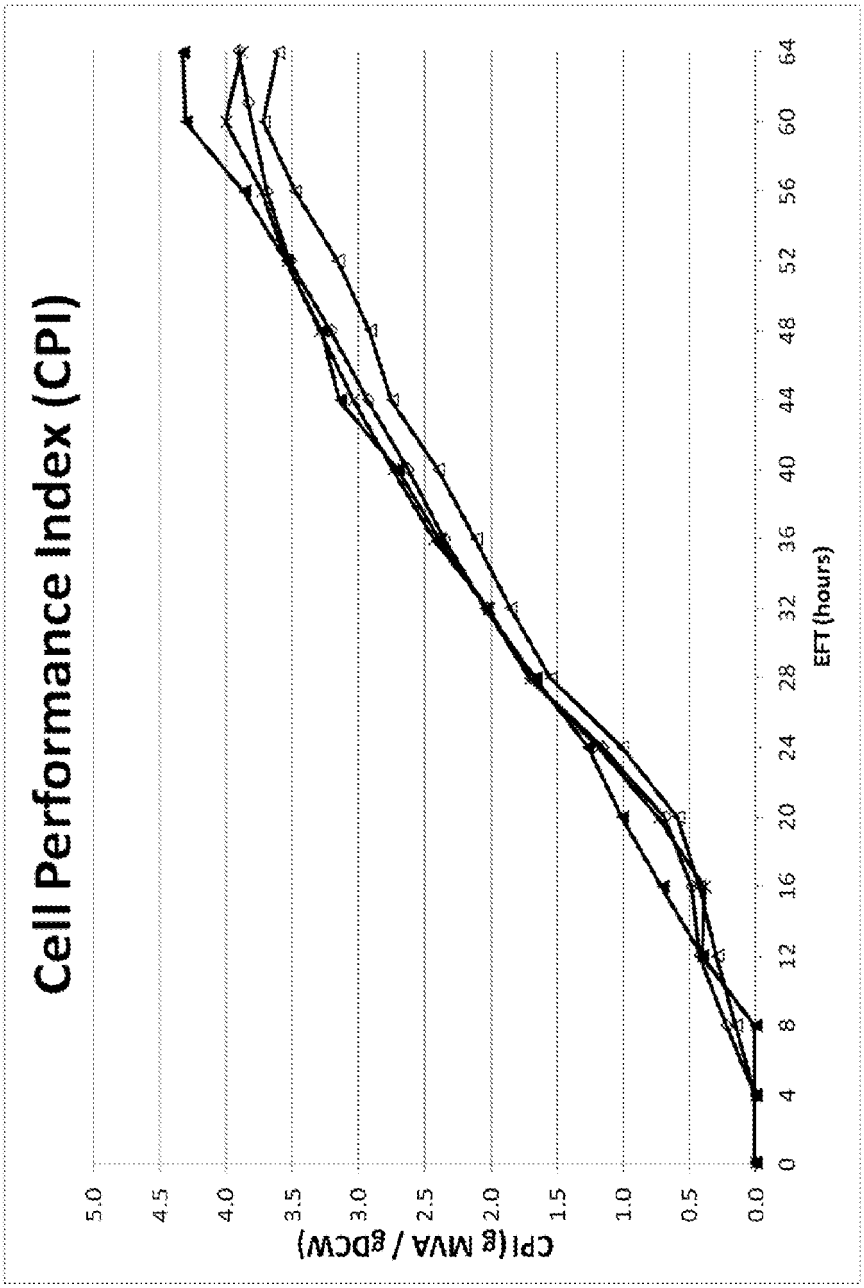
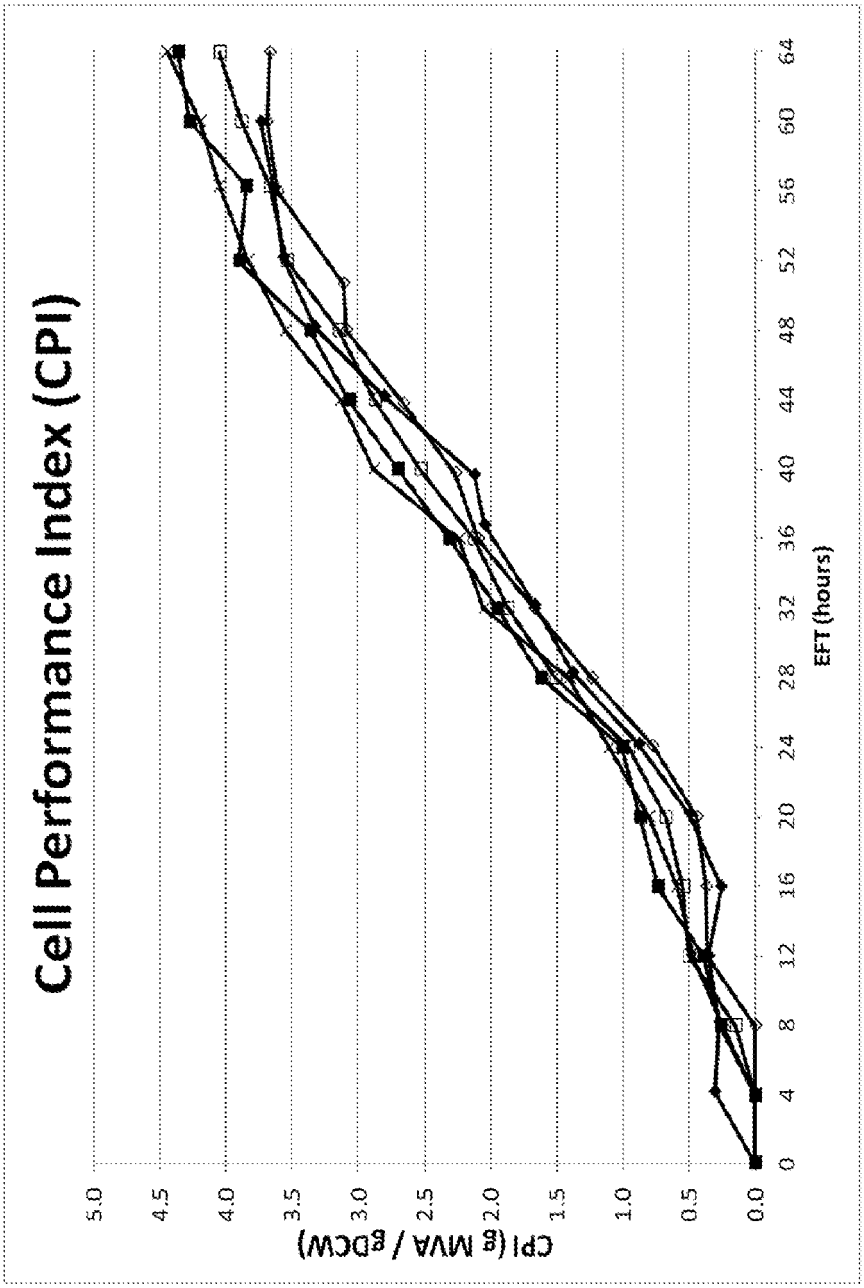

Cumulative Yield of MVA on glucose (g/g%)
36
34
32
30
28
26
24
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
PKL Specific Activity (mmol AcP/L/hr/OD) at 36hrs EFT
CHL875
EWL1341
EWL1359

Figure 55



20121058: EWL1359 induced at 400uM IPTG (closed triangles)
20121057: EWL1359 induced at 100uM IPTG (x symbol)
20121056: EWL1359 with no added IPTG (open triangles)
20121059: CHL875 induced at 100uM IPTG (open diamonds)

Figure 56



20120979: EWL1341 induced at 100uM IPTG (closed squares in Chart 6 above)
20120978: EWL1341 induced at 50uM IPTG (x figures in Chart 6 above)
20120977: EWL1341 with no added IPTG (open squares in Chart 6 above)
20120976: CHL875 with no added IPTG (open diamonds in Chart 6 above)
20120821: CHL875 with no added IPTG (closed diamonds in Chart 6 above)

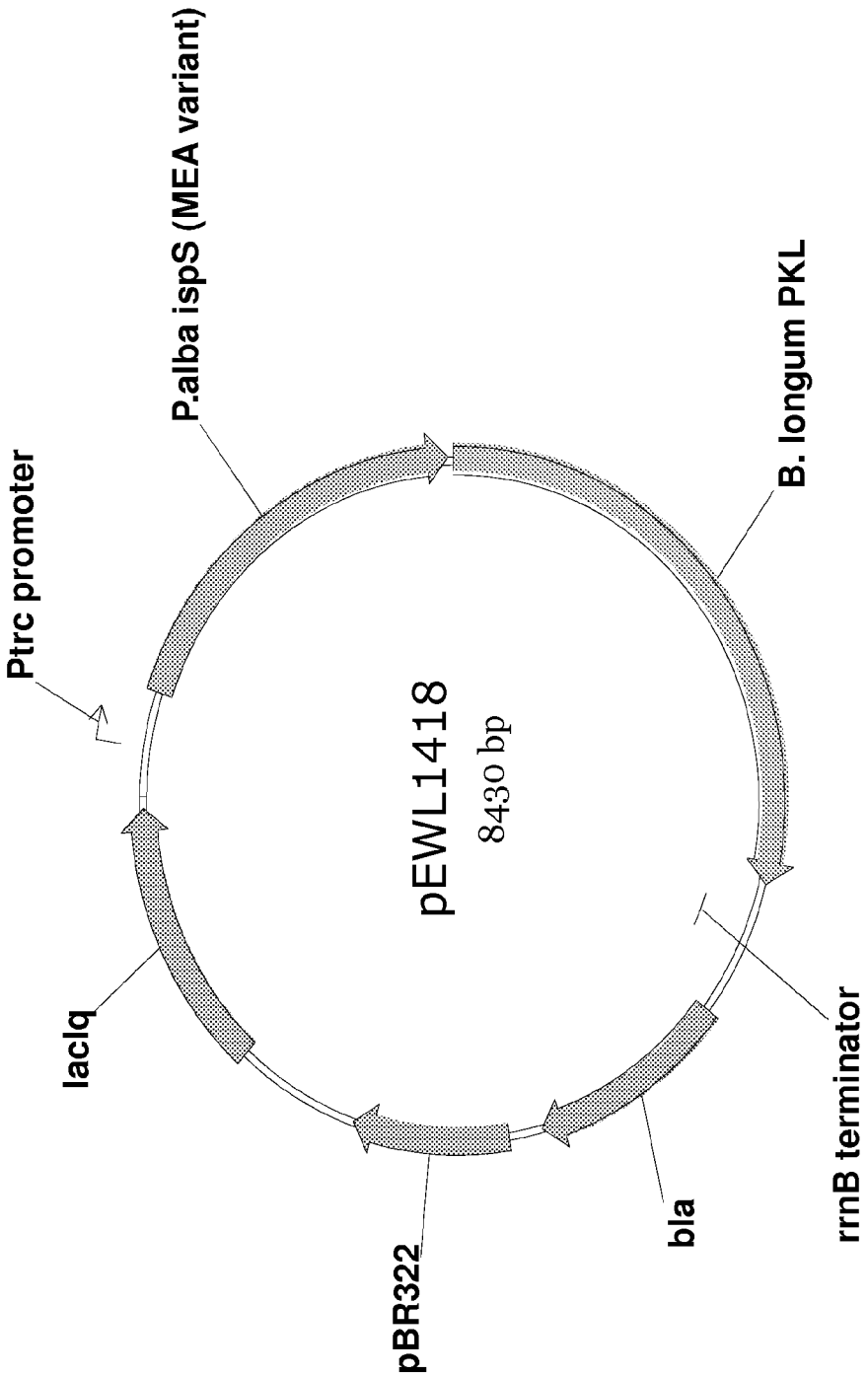

P.alba ispS (MEA variant)
B. longum PKL
Ptrc promoter
pEWL1418
8430 bp
lacIq
pBR322
bla
rrnB terminator

Figure 57

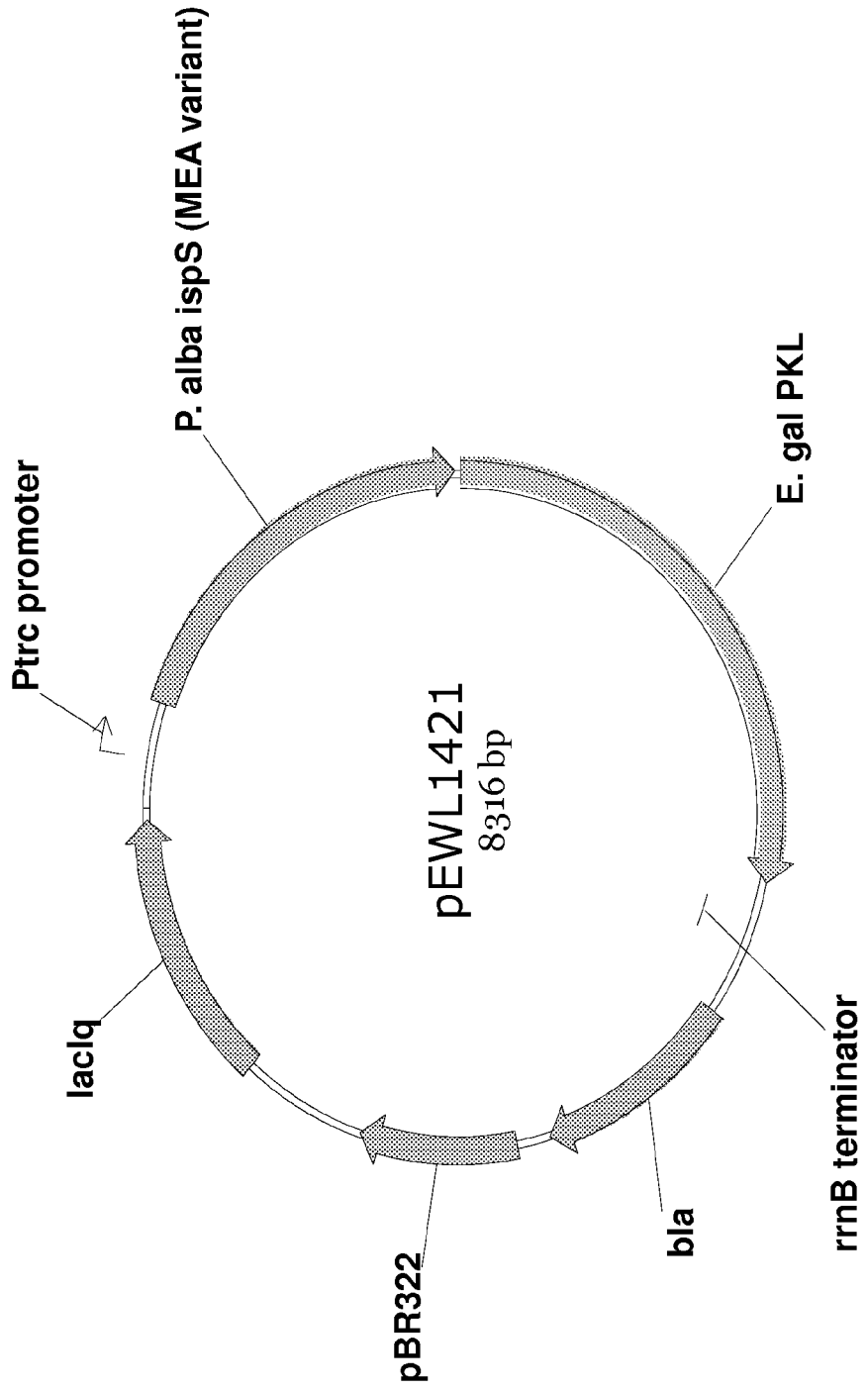

P. alba ispS (MEA variant)
E. gal PKL
Ptrc promoter
pEWL1421
8316 bp
lacIq
pBR322
bla
rrnB terminator

Figure 58

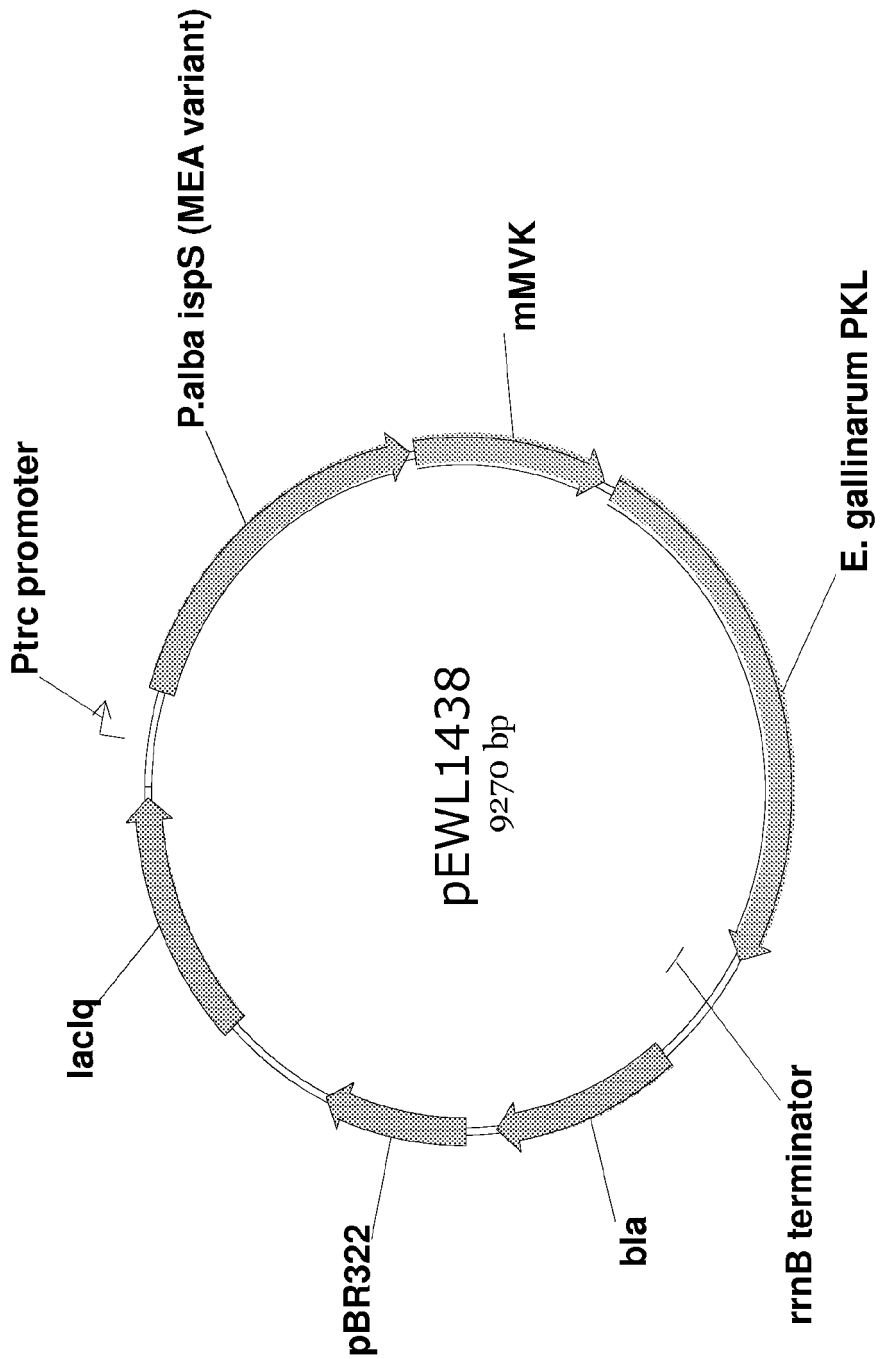

P.alba ispS (MEA variant)
mMVK
E. gallinarum PKL
Ptrc promoter
pEWL1438
9270 bp
lacIq
pBR322
bla
rrnB terminator

Figure 59

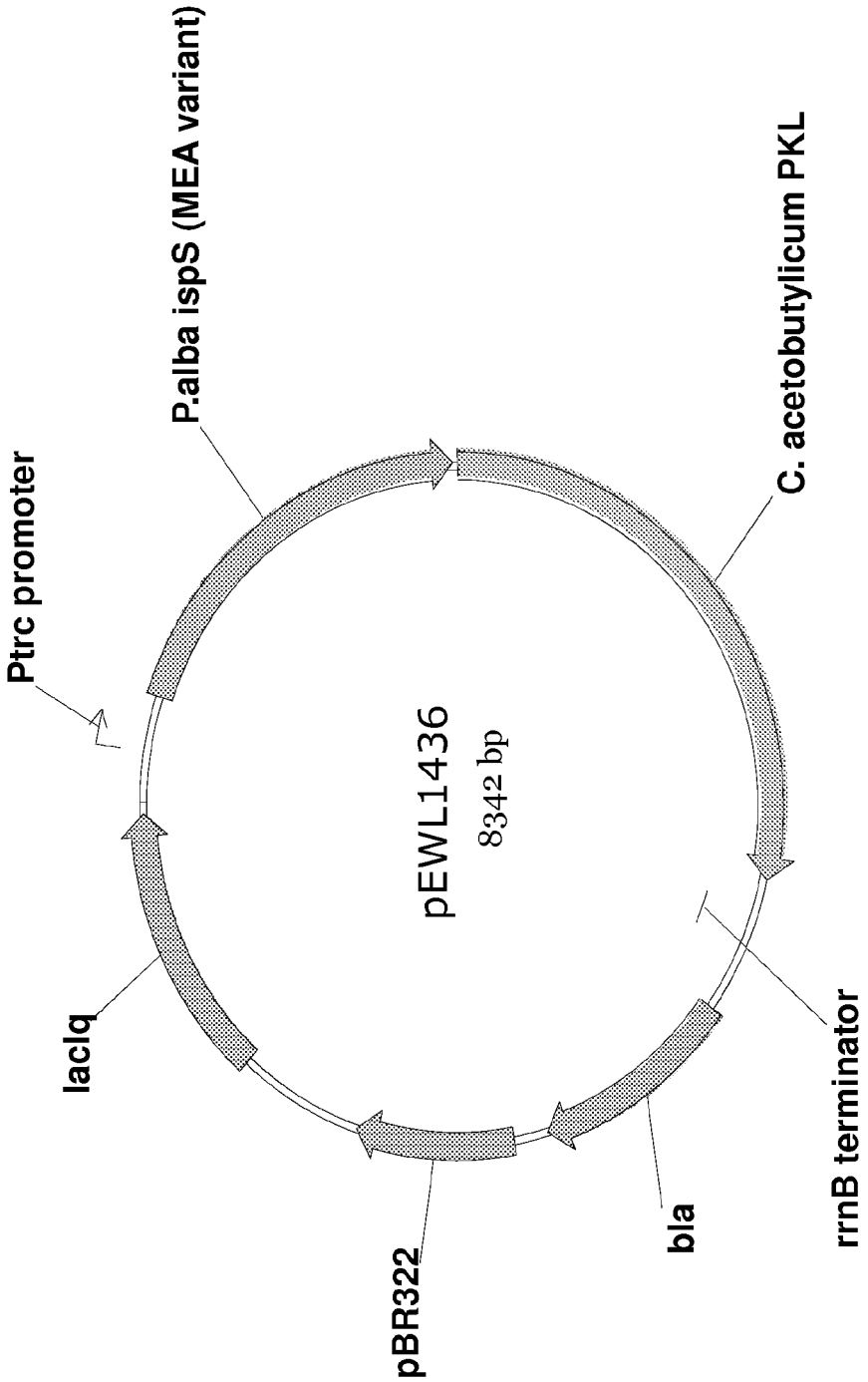

P.alba ispS (MEA variant)
C. acetobutylicum PKL
Ptrc promoter
pEWL1436
8342 bp
lacIq
pBR322
bla
rrnB terminator

Figure 60

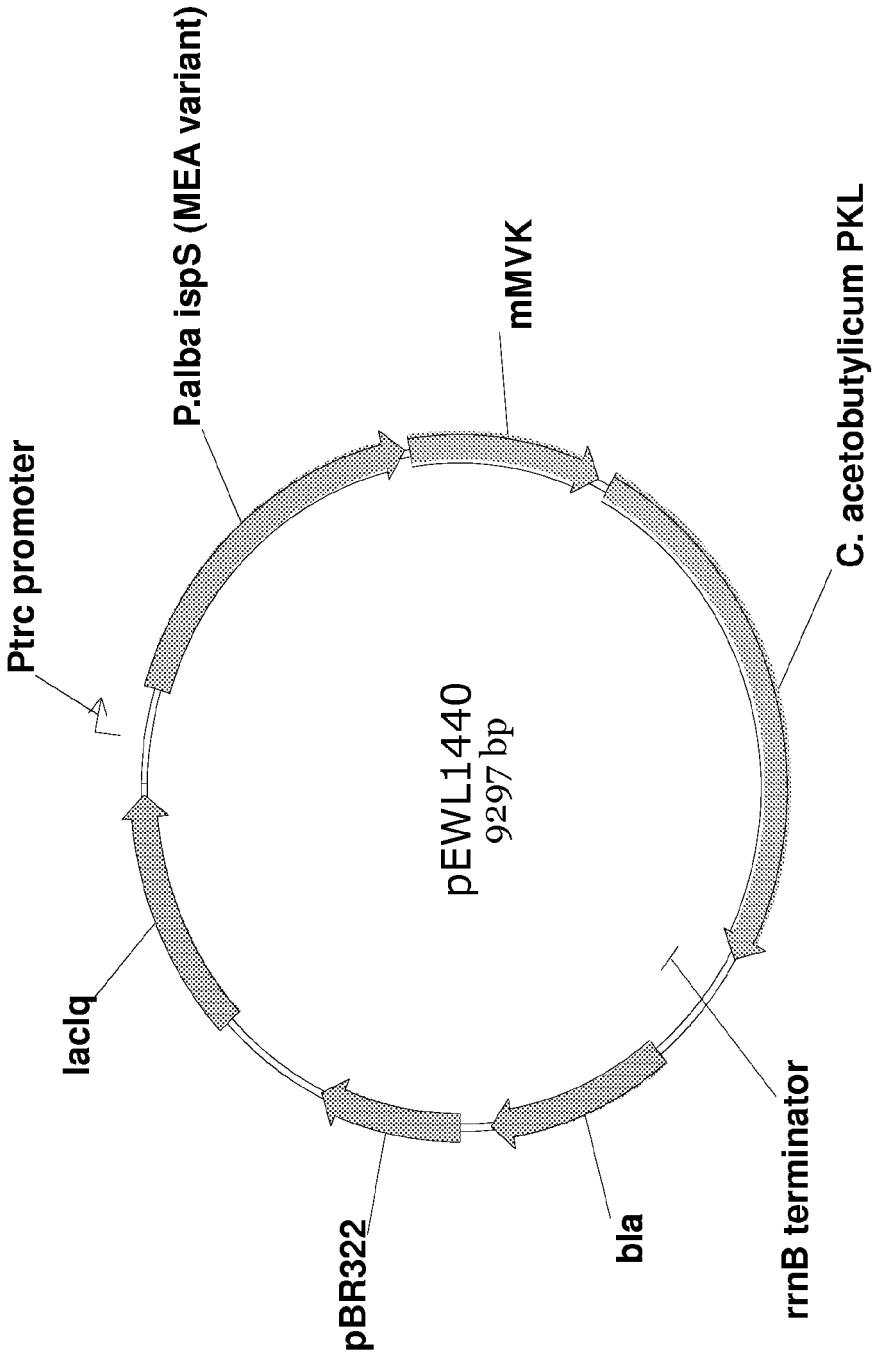
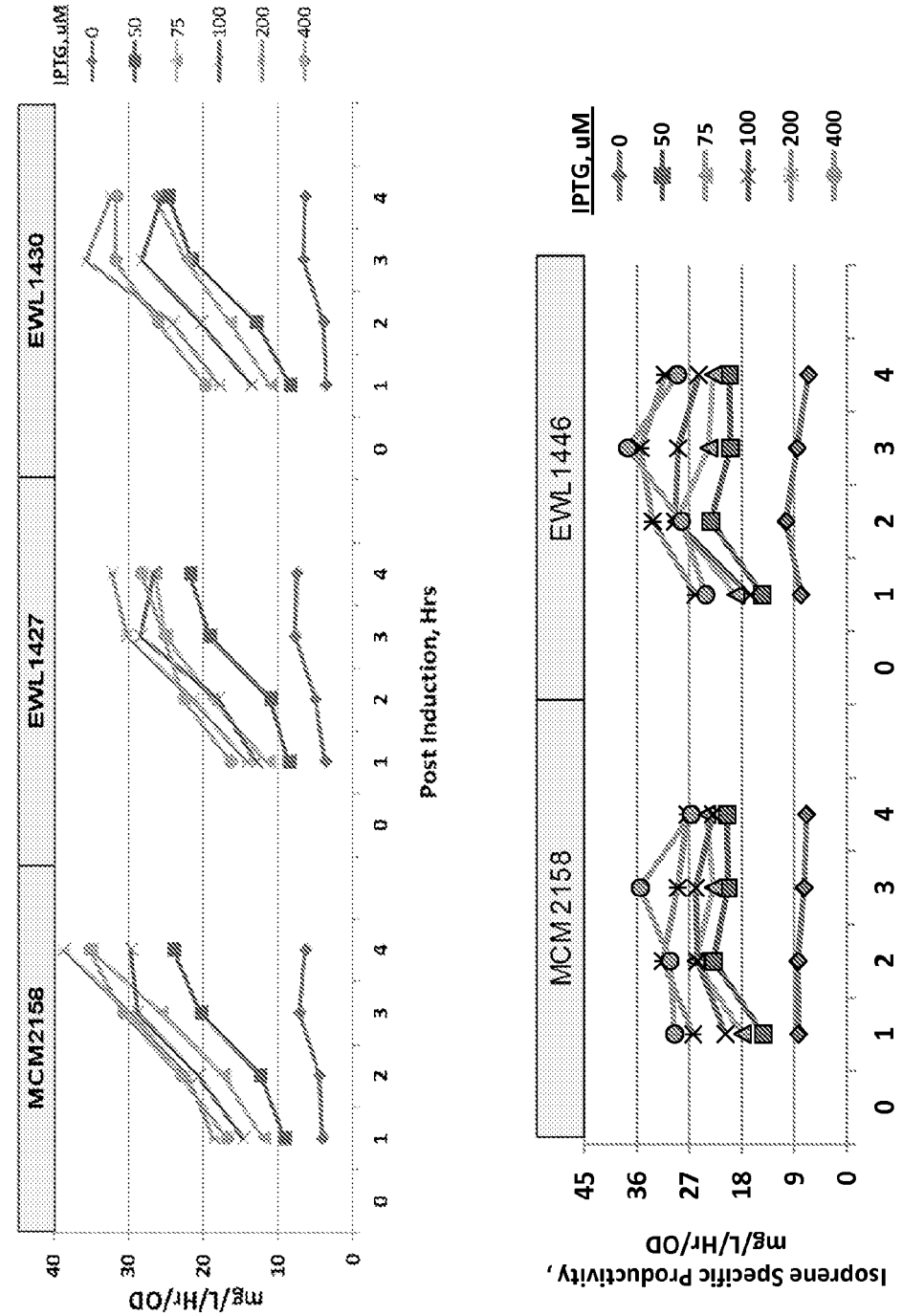
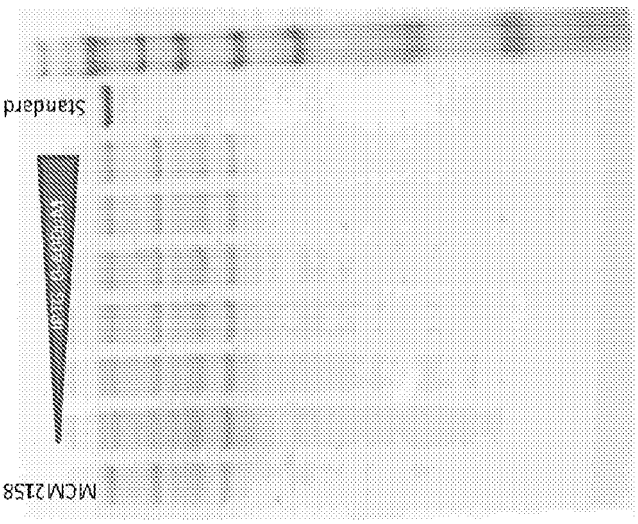

P.alba ispS (MEA variant)
mMVK
C. acetobutylicum PKL
rrnB terminator
bla
pBR322
laclq
Ptrc promoter
pEWL1440
9297 bp

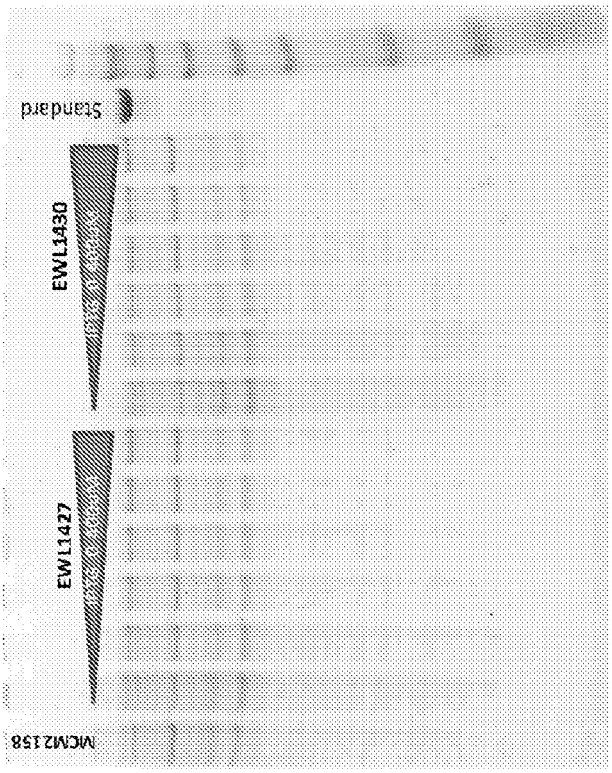
Figure 61

Figure 62


A
MCM2158
EWL1427
EWL1430
IPTG, uM
0
50
75
100
200
400
Isoprene Specific Productivity, mg/L/Hr/OD
Post Induction, Hrs
B
MCM 2158
EWL1446
IPTG, uM
0
50
75
100
200
400
Isoprene Specific Productivity, mg/L/Hr/OD
Post Induction, Hrs



B



A

Figure 63

Figure 64

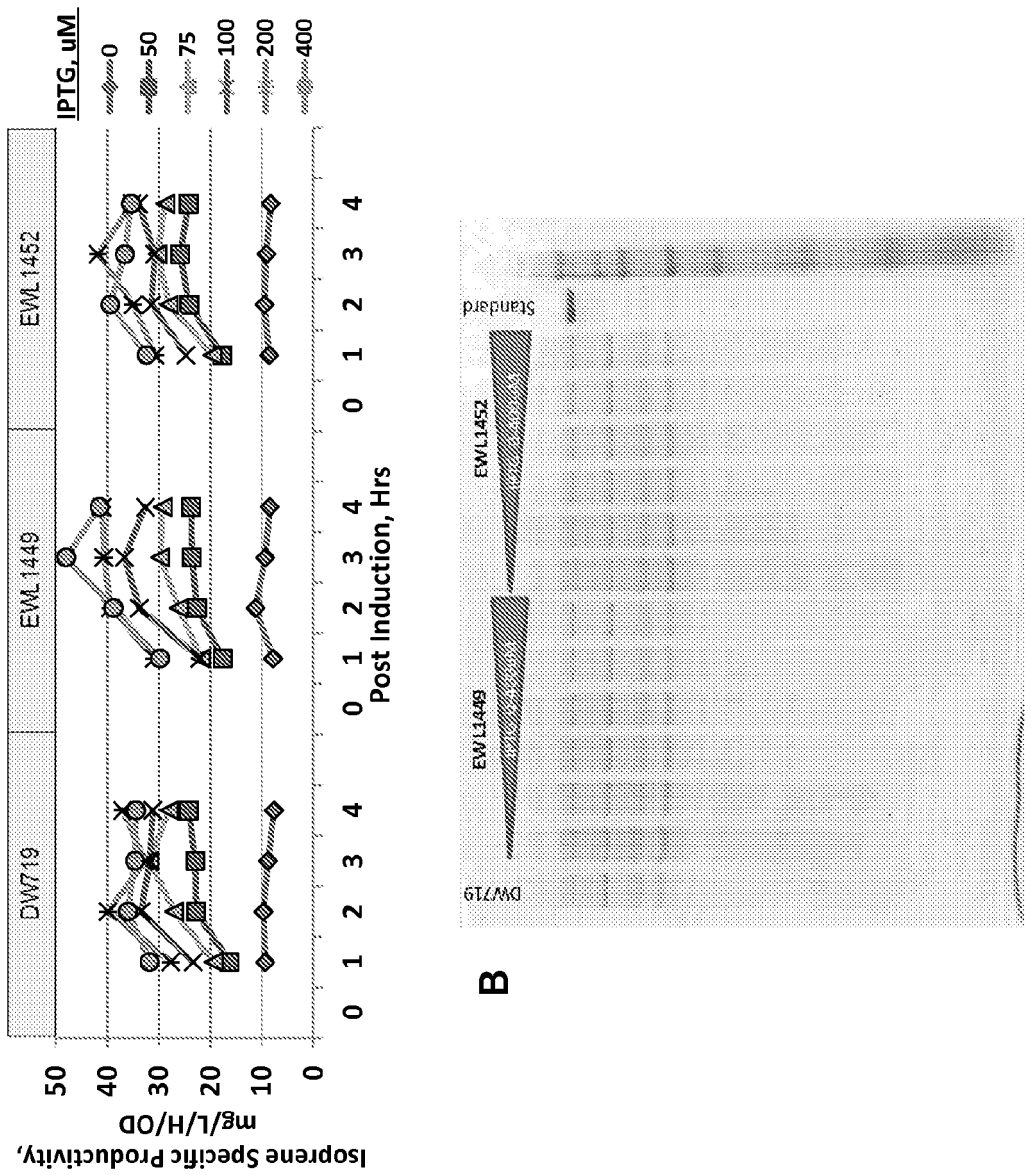
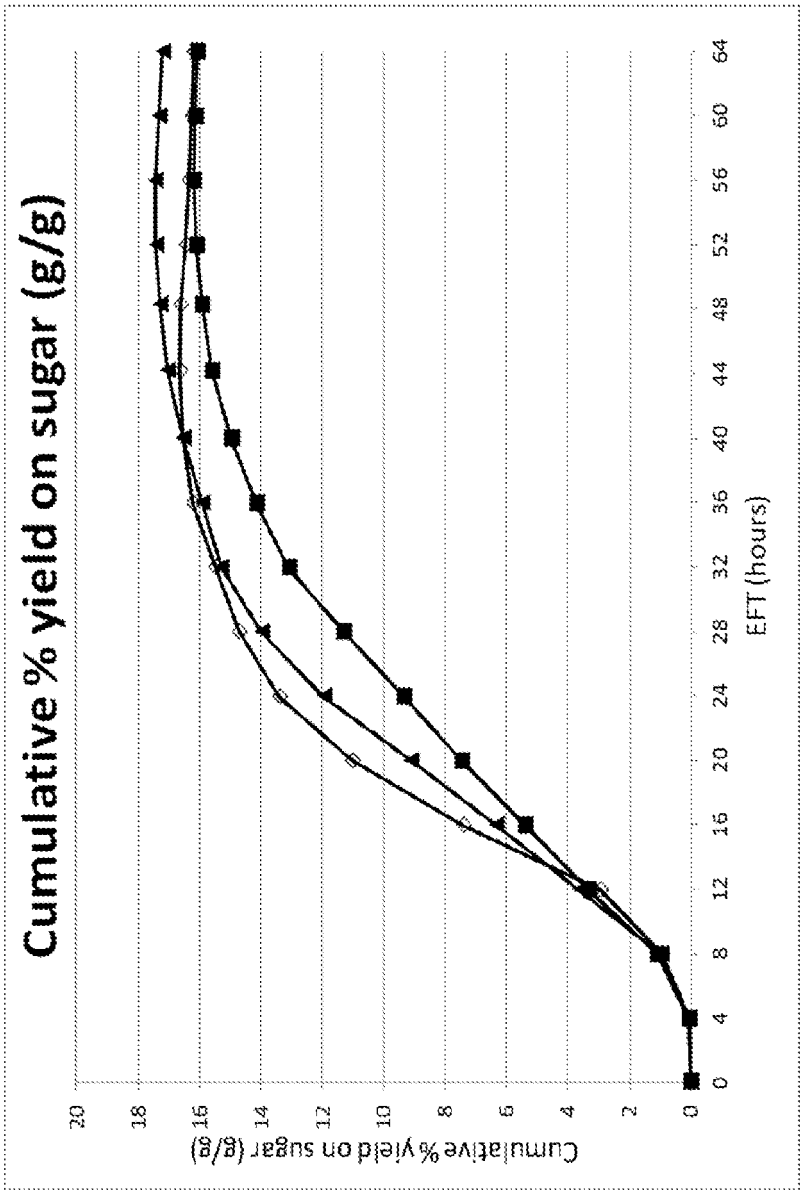

A
Isoprene Specific Productivity, mg/L/H/OD
50
40
30
20
10
0
DW719
EWL1449
EWL1452
0 1 2 3 4 0 1 2 3 4 0 1 2 3 4
Post Induction, Hrs
IPTG, uM
0
50
75
100
200
400
B
DW719
EWL1449
EWL1452
Standard

Figure 65



20121136: EWL1430 induced at 100uM IPTG (closed triangles)
20121135: EWL1427 induced at 100uM IPTG (closed squares)
20121134: MCM2158 induced at 100uM IPTG (open diamonds)

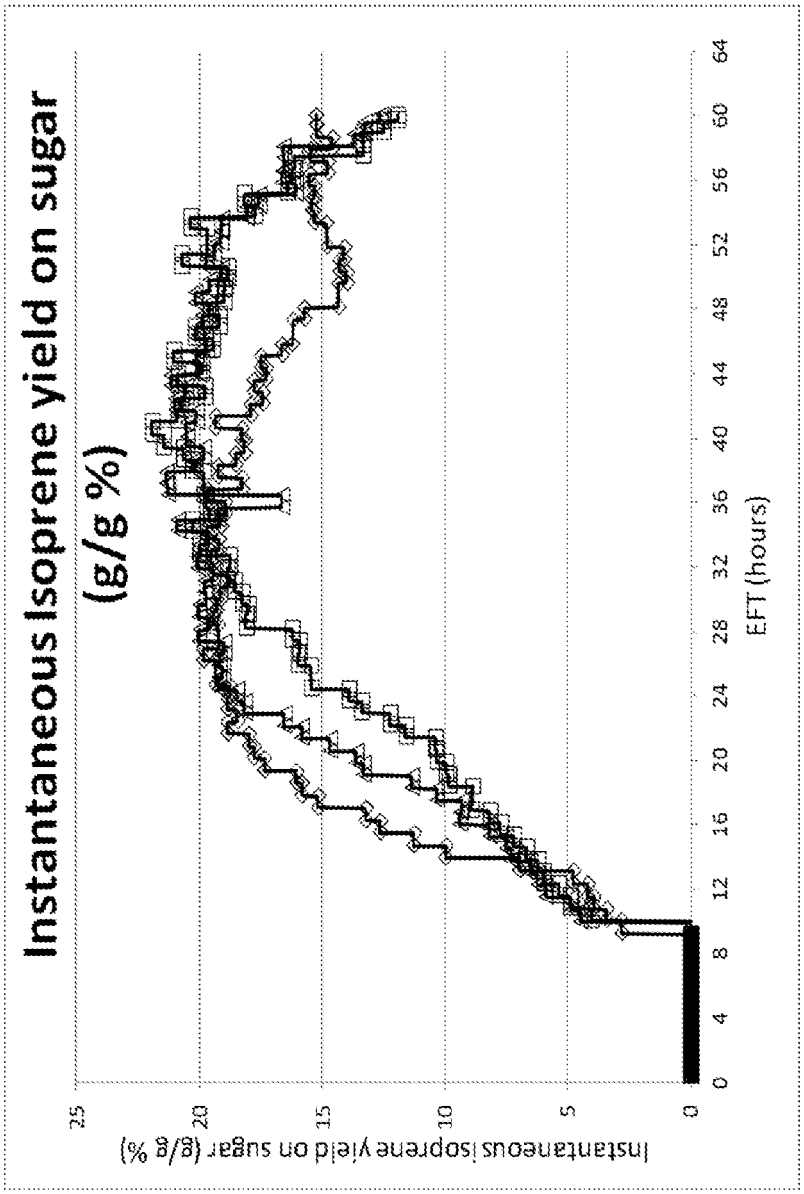

Instantaneous Isoprene yield on sugar (g/g %)
Instantaneous isoprene yield on sugar (g/g %)
25
20
15
10
5
0
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64
EFT (hours)
20121136: EWL1430 induced at 100uM IPTG (open triangles)
20121135: EWL1427 induced at 100uM IPTG (open squares)
20121134: MCM2158 induced at 100uM IPTG (open diamonds)

Figure 66

Figure 67

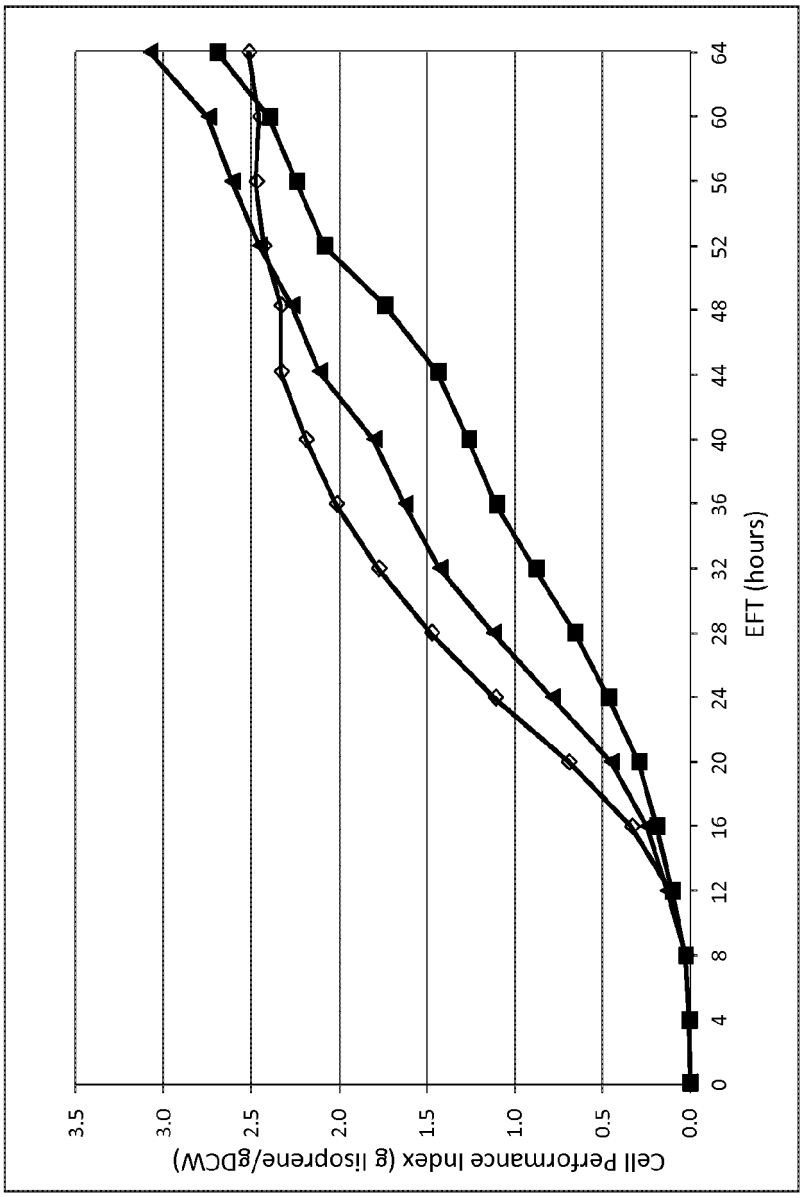

Cell Performance Index (g Isoprene/gDCW)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
EFT (hours)
20121136: EWL1430 induced at 100uM IPTG (closed triangles)
20121135: EWL1427 induced at 100uM IPTG (closed squares)
20121134: MCM2158 induced at 100uM IPTG (open diamonds)

Figure 68

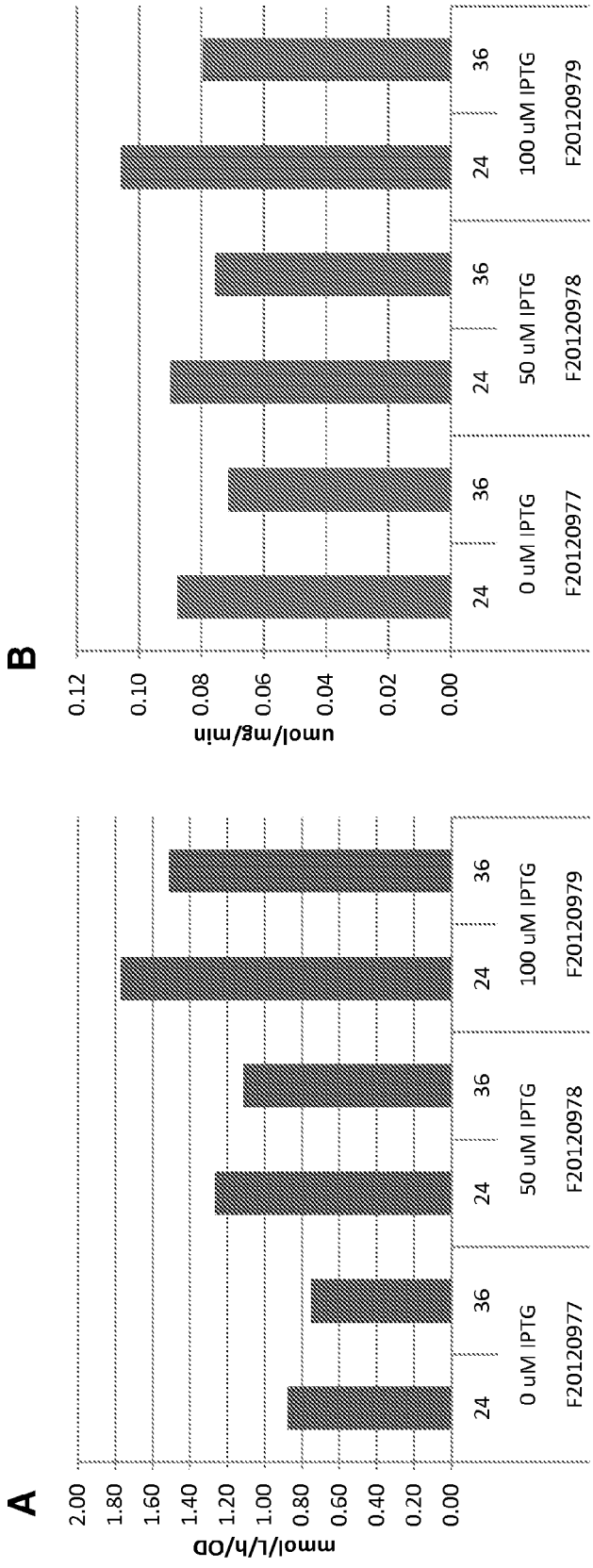
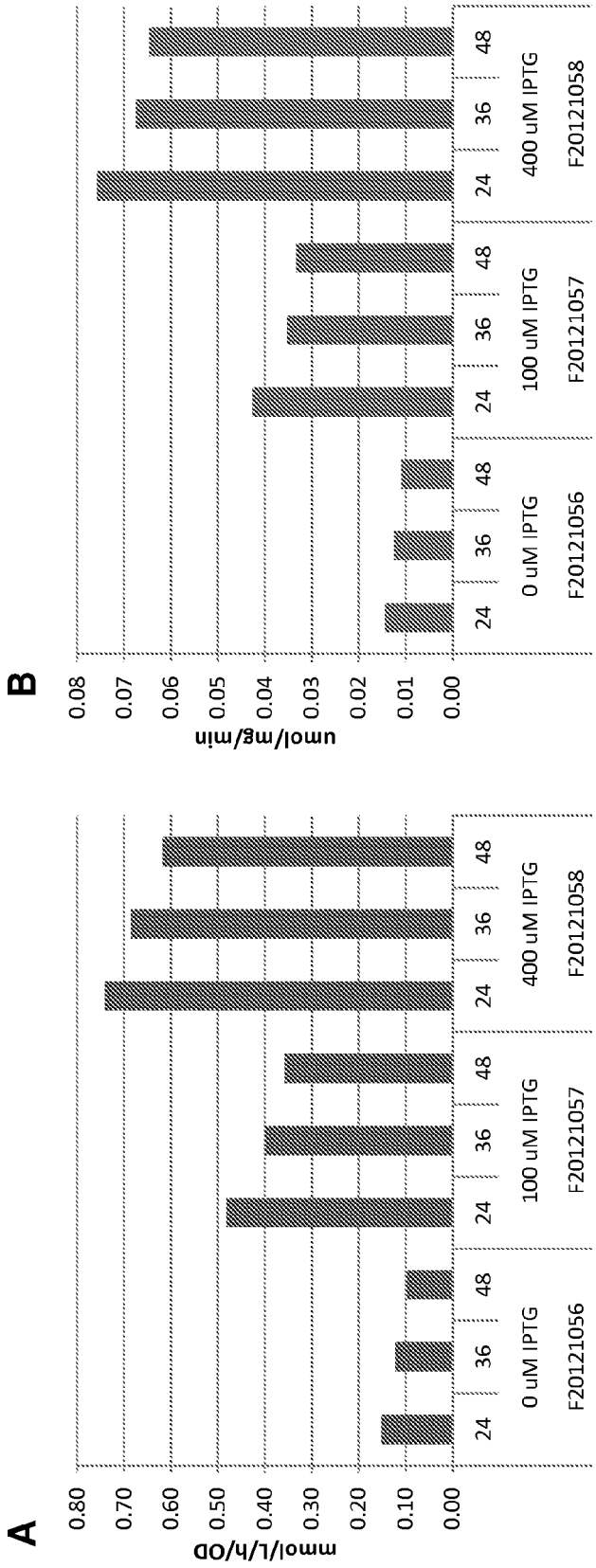

A
mmol/L/h/OD
2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
24
36
0 uM IPTG
F20120977
24
36
50 uM IPTG
F20120978
24
36
100 uM IPTG
F20120979
B
umol/mg/min
0.12
0.10
0.08
0.06
0.04
0.02
0.00
24
36
0 uM IPTG
F20120977
24
36
50 uM IPTG
F20120978
24
36
100 uM IPTG
F20120979

Figure 69


A
mmol/L/h/OD
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
24
36
48
0 uM IPTG
F20121056
100 uM IPTG
F20121057
400 uM IPTG
F20121058
B
umol/mg/min
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00

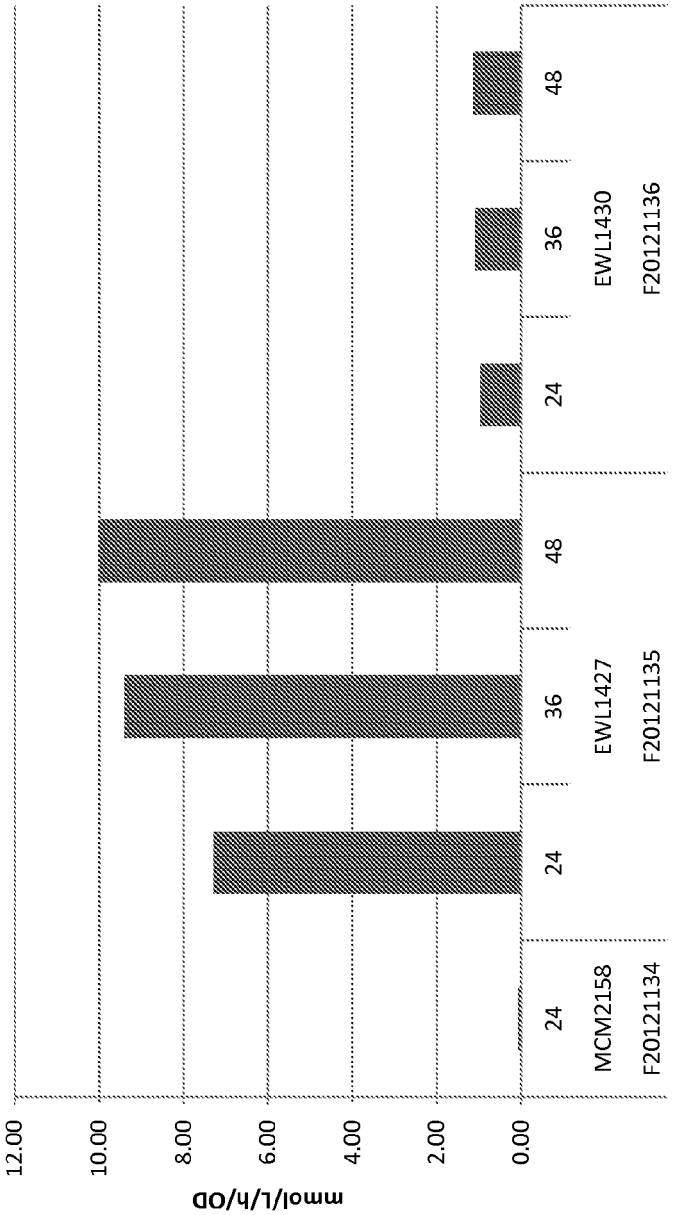

mmol/L/h/OD
12.00
10.00
8.00
6.00
4.00
2.00
0.00
MCM2158
F20121134
24
24
EWL1427
F20121135
36
48
EWL1430
F20121136
24
36
48

Figure 70

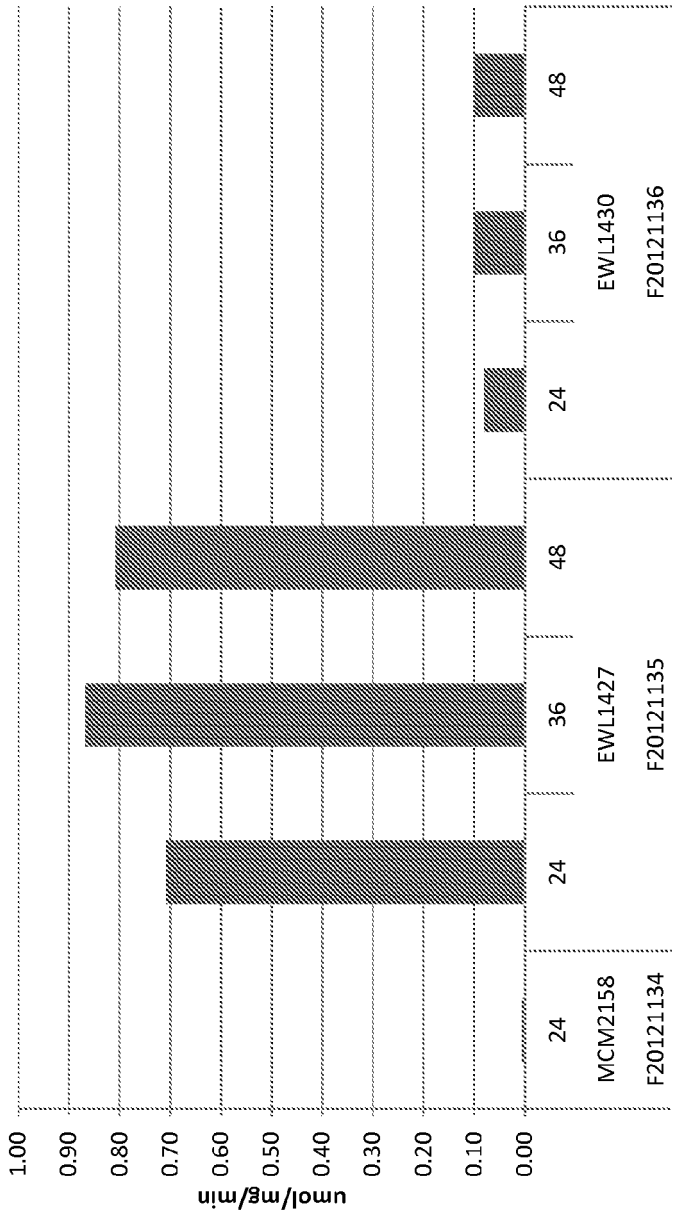

umol/mg/min
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
MCM2158
F20121134
24
24
EWL1427
F20121135
36
48
EWL1430
F20121136
24
36
48

Figure 71

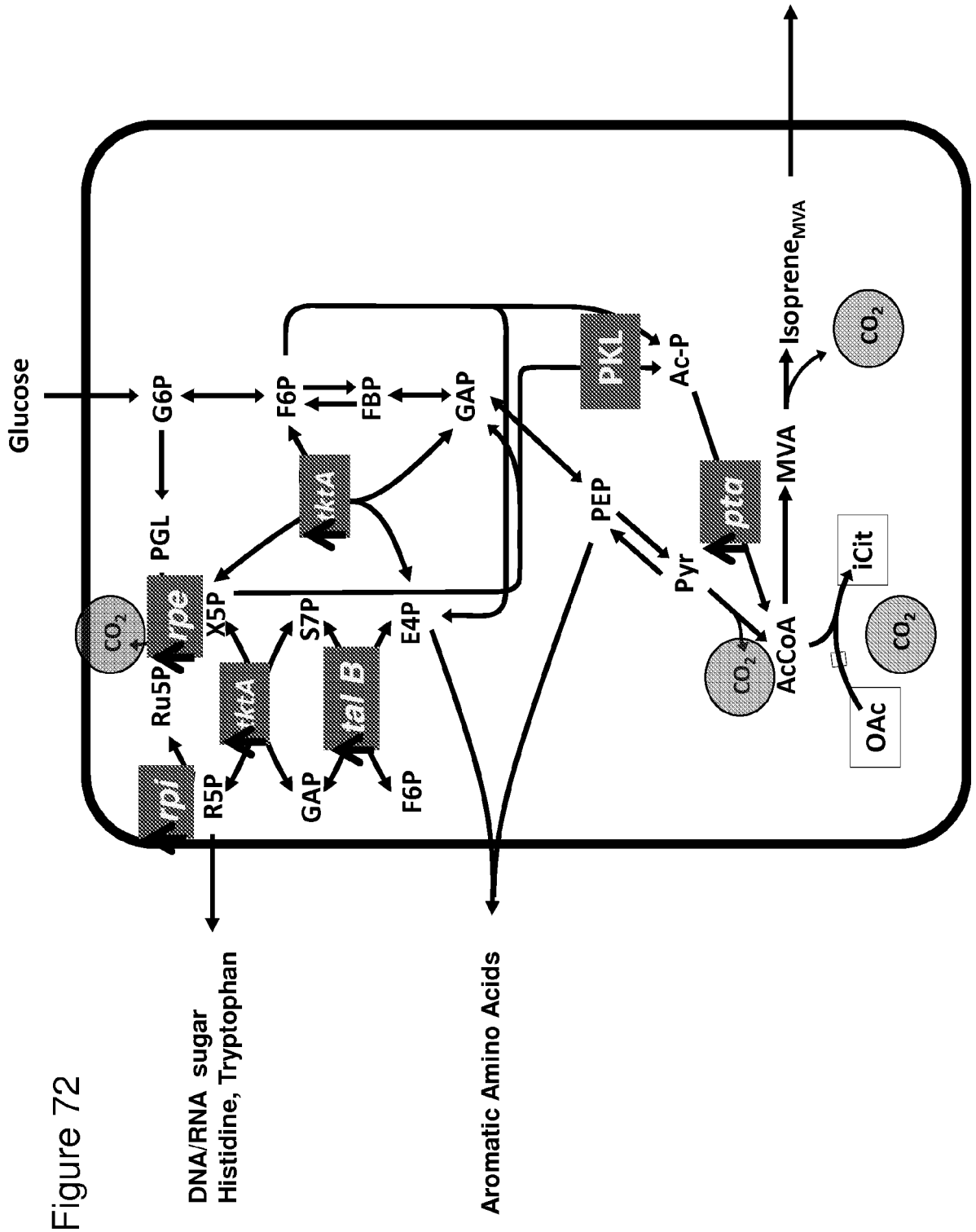

Glucose
G6P
PGL
F6P
FBP
GAP
PKL
Ac-P
Isoprene$_{MVA}$
CO_2
PEP
Pyr
MVA
AcCoA
iCit
OAc
CO_2
CO_2
X5P
Ru5P
R5P
S7P
E4P
GAP
F6P
DNA/RNA sugar
Histidine, Tryptophan
Aromatic Amino Acids

Figure 72

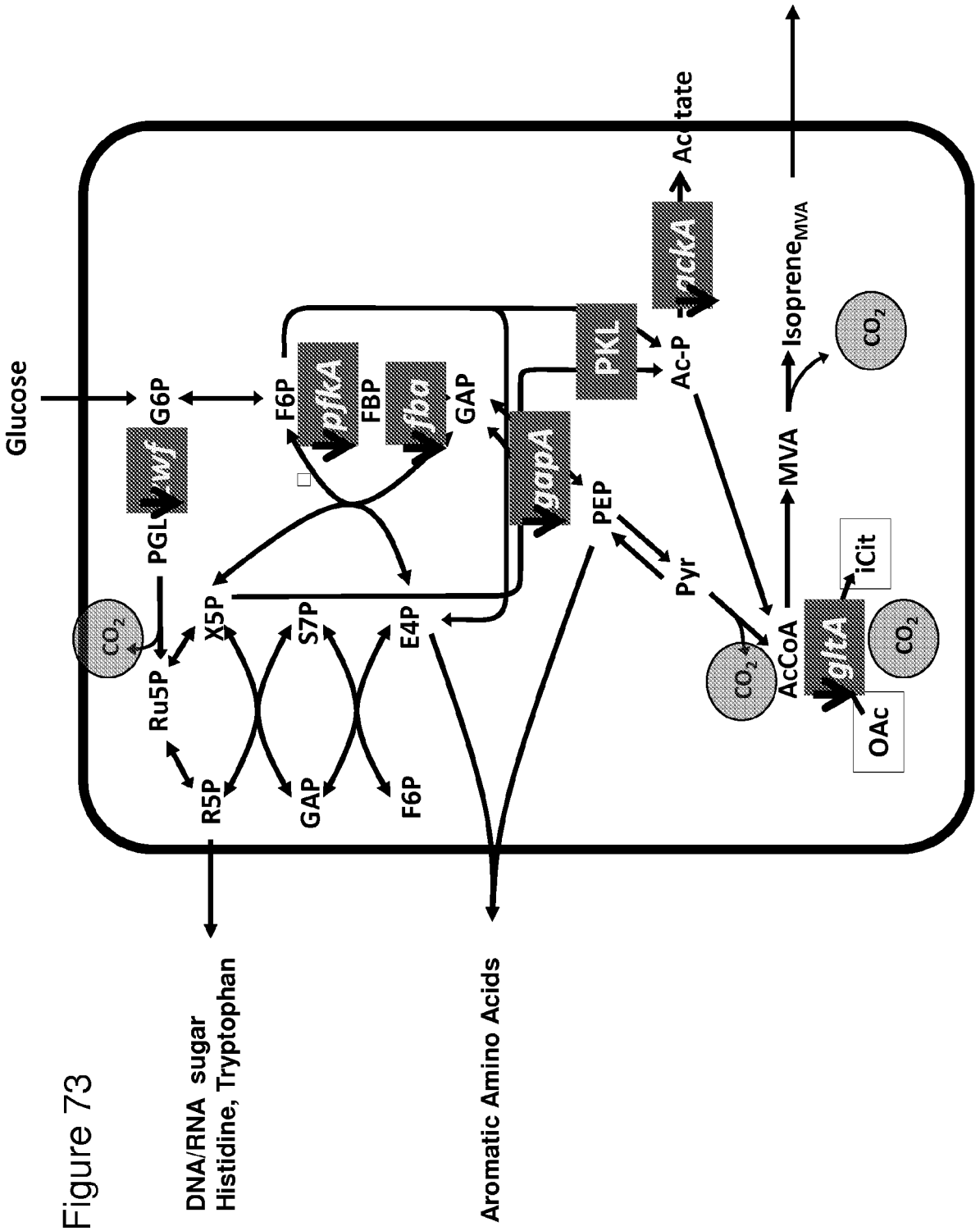

Glucose
G6P
PGL
F6P
FBP
GAP
PEP
Pyr
Ac-P
Acetate
Isoprene$_{MVA}$
MVA
AcCoA
iCit
OAc
X5P
Ru5P
R5P
S7P
GAP
E4P
F6P
CO_2
pfkA
fbp
gapA
PKL
pta
gltA
DNA/RNA sugar
Histidine, Tryptophan
Aromatic Amino Acids

Figure 73