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Yoshikuni et al.

(54) **BIOFUEL PRODUCTION**

(75) Inventors: **Yasuo Yoshikuni**, Seattle, WA (US); **Yuki Kashiyama**, Seattle, WA (US)

Correspondence Address:
SEED INTELLECTUAL PROPERTY LAW GROUP PLLC
701 FIFTH AVE, SUITE 5400
SEATTLE, WA 98104 (US)

(73) Assignee: **BIO ARCHITECTURE LAB, INC.**, Seattle, WA (US)

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Publication Classification

(51) **Int. Cl.**

C10L 1/18	(2006.01)
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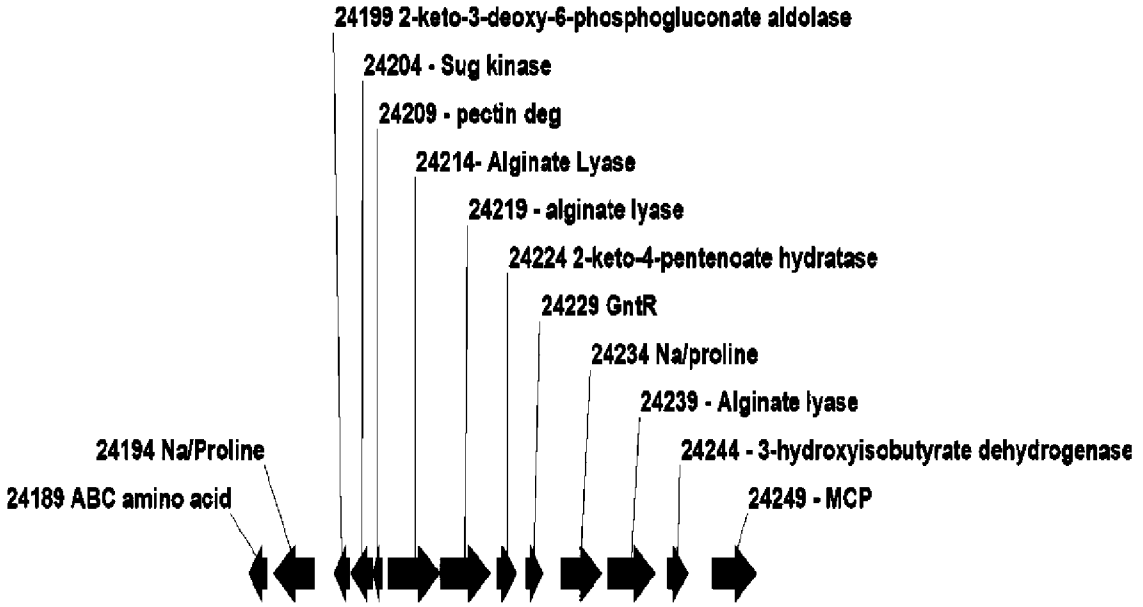
C12P 7/04	(2006.01)
C12N 1/21	(2006.01)
C07C 31/08	(2006.01)

(52) **U.S. Cl.** **44/307**; 435/161; 435/157; 435/252.3; 568/840

(57) **ABSTRACT**

Methods, enzymes, recombinant microorganism, and microbial systems are provided for converting polysaccharides, such as those derived from biomass, into suitable monosaccharides or oligosaccharides, as well as for converting suitable monosaccharides or oligosaccharides into commodity chemicals, such as biofuels. Commodity chemicals produced by the methods described herein are also provided. Commodity chemical enriched, refinery-produced petroleum products are also provided, as well as methods for producing the same.

Figure 1


24199 2-keto-3-deoxy-6-phosphogluconate aldolase
24204 - Sug kinase
24209 - pectin deg
24214- Alginate Lyase
24219 - alginate lyase
24224 2-keto-4-pentenoate hydratase
24229 GntR
24234 Na/proline
24239 - Alginate lyase
24244 - 3-hydroxyisobutyrate dehydrogenase
24249 - MCP
24194 Na/Proline
24189 ABC amino acid

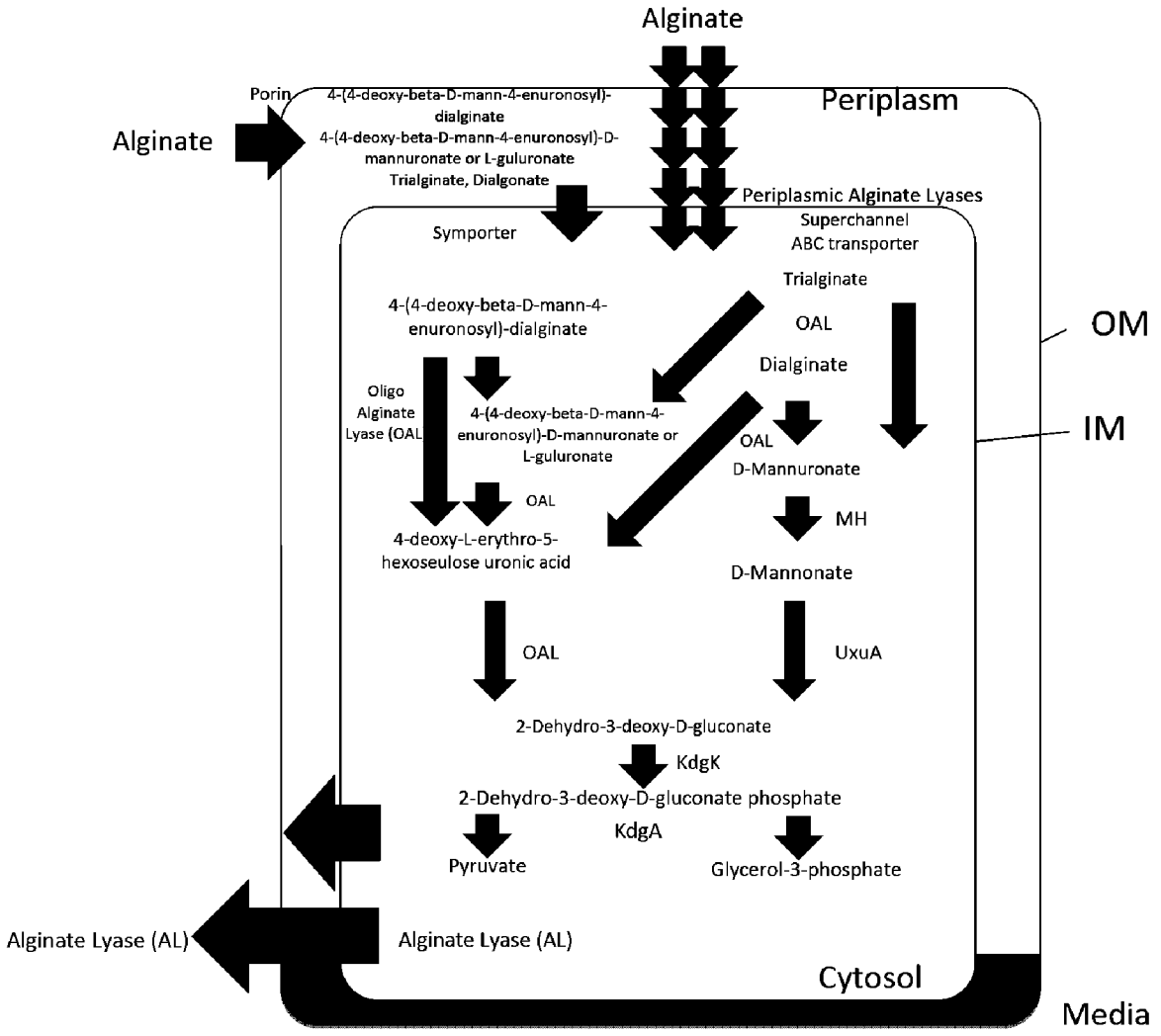
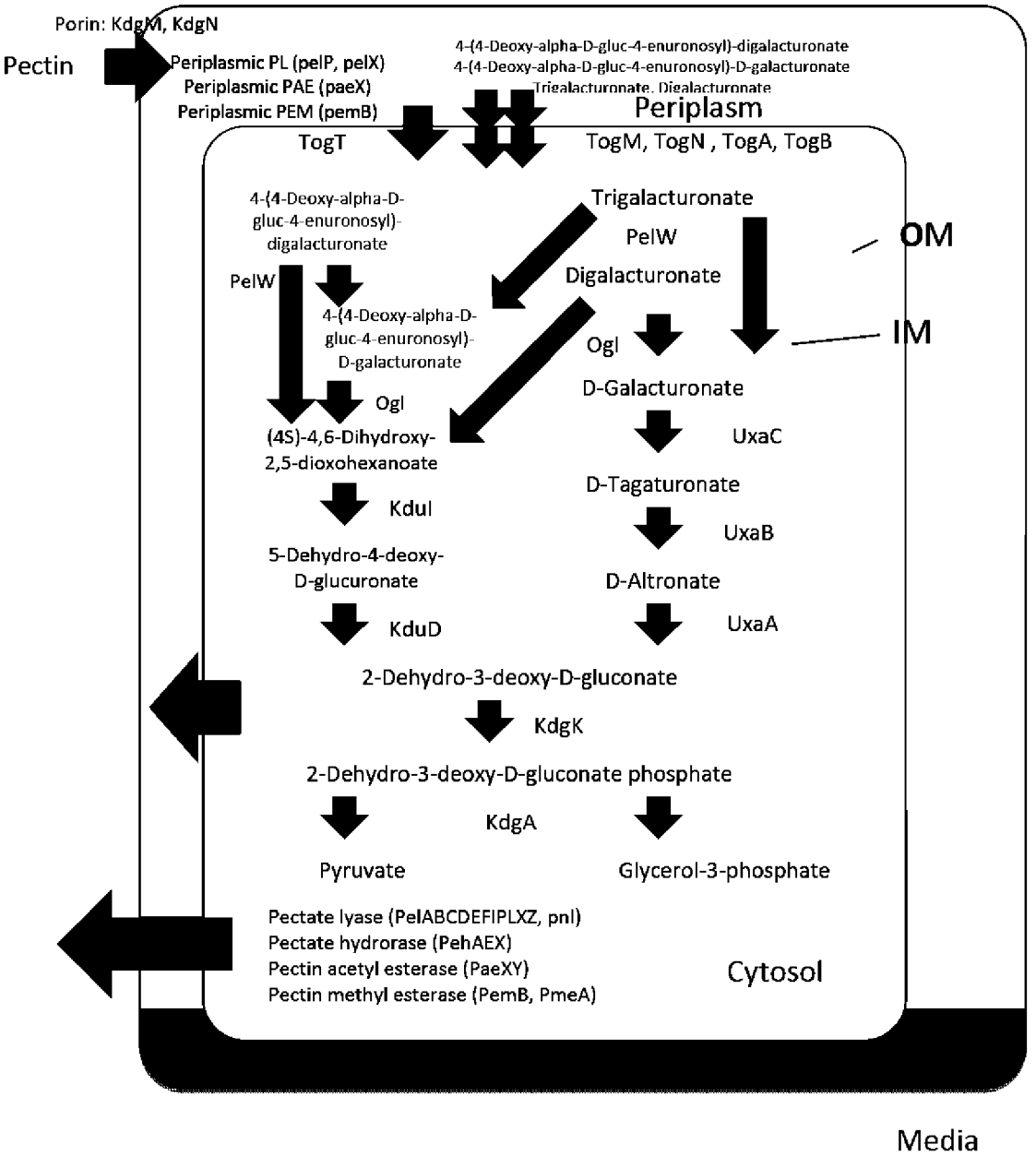

Alginate
Porin
Alginate
4-(4-deoxy-beta-D-mann-4-enuronosyl)-dialginate
4-(4-deoxy-beta-D-mann-4-enuronosyl)-D-mannuronate or L-guluronate
Trialginate, Dialgonate
Periplasm
Periplasmic Alginate Lyases
Superchannel
ABC transporter
Symporter
Trialginate
OAL
Dialginate
OM
IM
4-(4-deoxy-beta-D-mann-4-enuronosyl)-dialginate
Oligo Alginate Lyase (OAL
4-(4-deoxy-beta-D-mann-4-enuronosyl)-D-mannuronate or L-guluronate
OAL
D-Mannuronate
OAL
MH
4-deoxy-L-erythro-5-hexoseulose uronic acid
D-Mannonate
OAL
UxuA
2-Dehydro-3-deoxy-D-gluconate
KdgK
2-Dehydro-3-deoxy-D-gluconate phosphate
KdgA
Pyruvate
Glycerol-3-phosphate
Alginate Lyase (AL)
Alginate Lyase (AL)
Cytosol
Media

Figure 2

Figure 3


Porin: KdgM, KdgN
Pectin
Periplasmic PL (pelP, pelX)
Periplasmic PAE (paeX)
Periplasmic PEM (pemB)
4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-digalacturonate
4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-galacturonate
Trigalacturonate, Digalacturonate
Periplasm
TogT
TogM, TogN , TogA, TogB
4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-digalacturonate
Trigalacturonate
PelW
OM
PelW
Digalacturonate
4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-galacturonate
IM
Ogl
D-Galacturonate
Ogl
(4S)-4,6-Dihydroxy-2,5-dioxohexanoate
UxaC
D-Tagaturonate
KduI
UxaB
5-Dehydro-4-deoxy-D-glucuronate
D-Altronate
KduD
UxaA
2-Dehydro-3-deoxy-D-gluconate
KdgK
2-Dehydro-3-deoxy-D-gluconate phosphate
KdgA
Pyruvate
Glycerol-3-phosphate
Pectate lyase (PelABCDEFIPLXZ, pnl)
Pectate hydrorase (PehAEX)
Pectin acetyl esterase (PaeXY)
Pectin methyl esterase (PemB, PmeA)
Cytosol
Media

E. coli Growing on Alginate

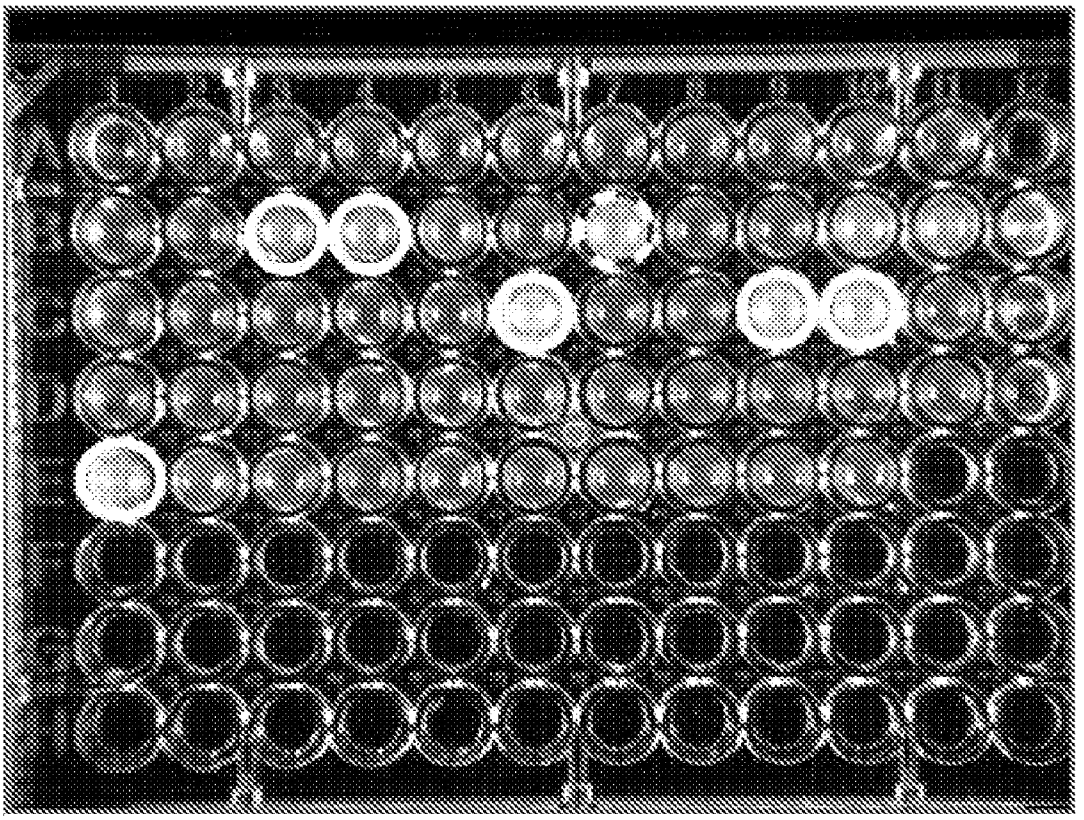


FIG. 4

Figure 5A

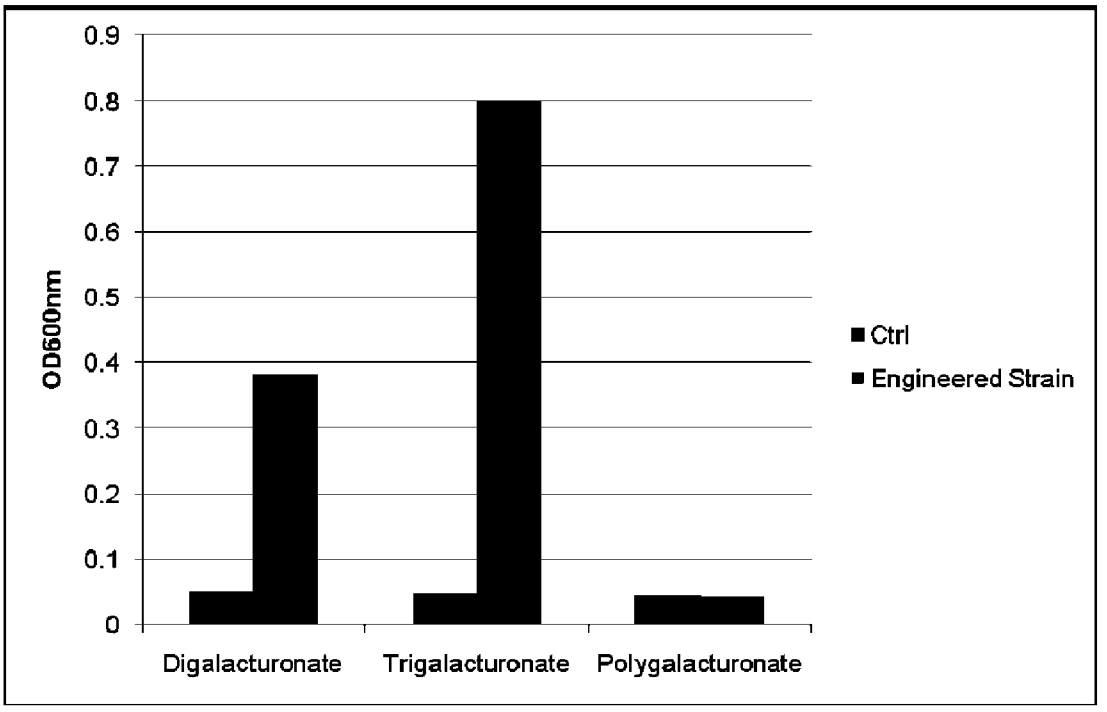
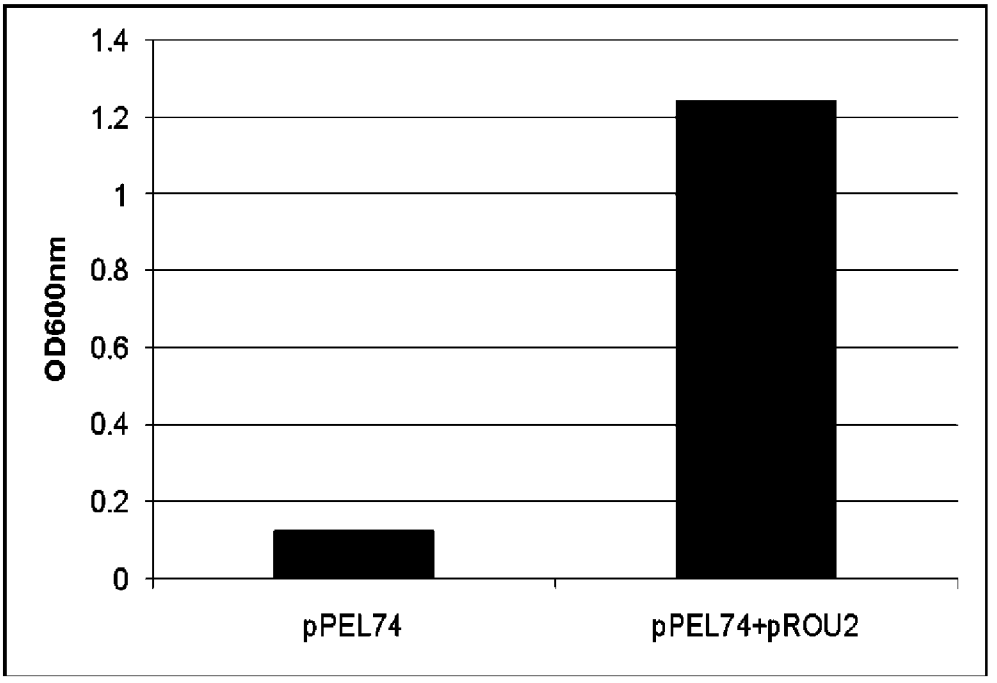


Figure 5B



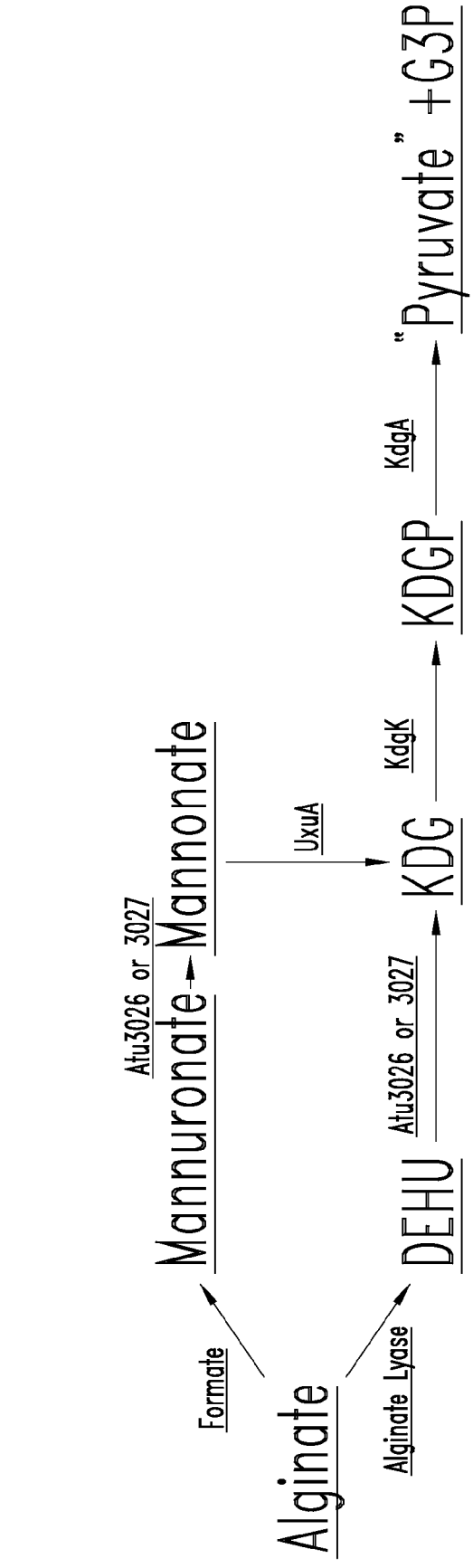

Alginate
Formate
Alginate Lyase
Mannuronate-Mannonate
Atu3026 or 3027
DEHU
Atu3026 or 3027
UxuA
KDG
KdgK
KDGP
KdgA
"Pyruvate" +G3P

FIG. 6A

Figure 6B

Pyruvate formation from alginate (enzymatic degradation route)

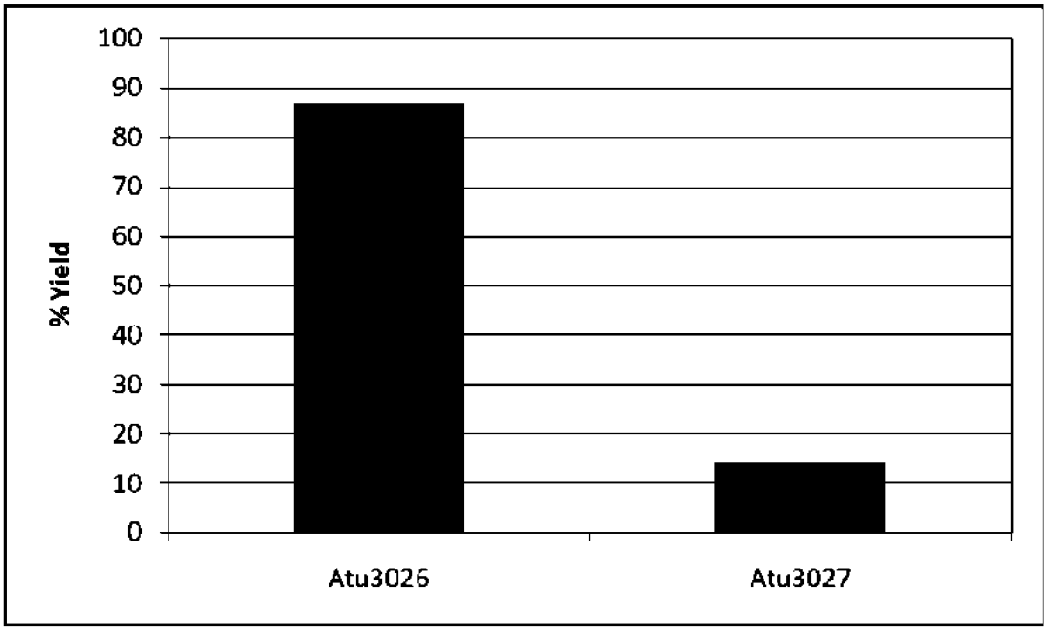


Figure 6C

Pyruvate formation from alginate (chemical degradation route).

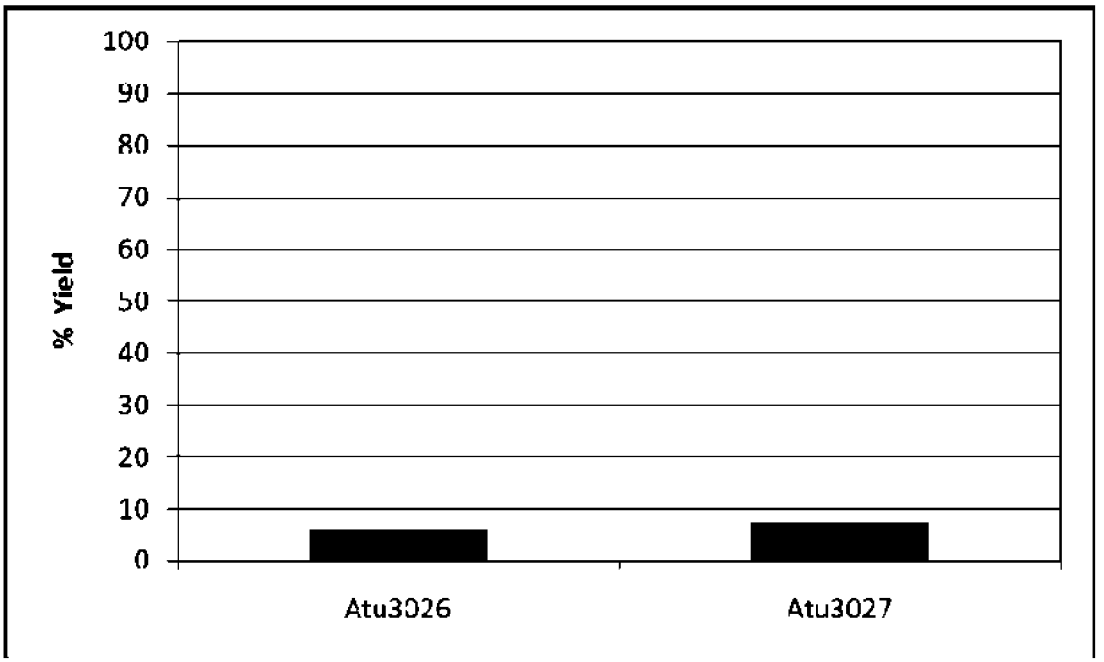


Figure 7A

DEHU hydrogenase activity monitored by NADPH consumption

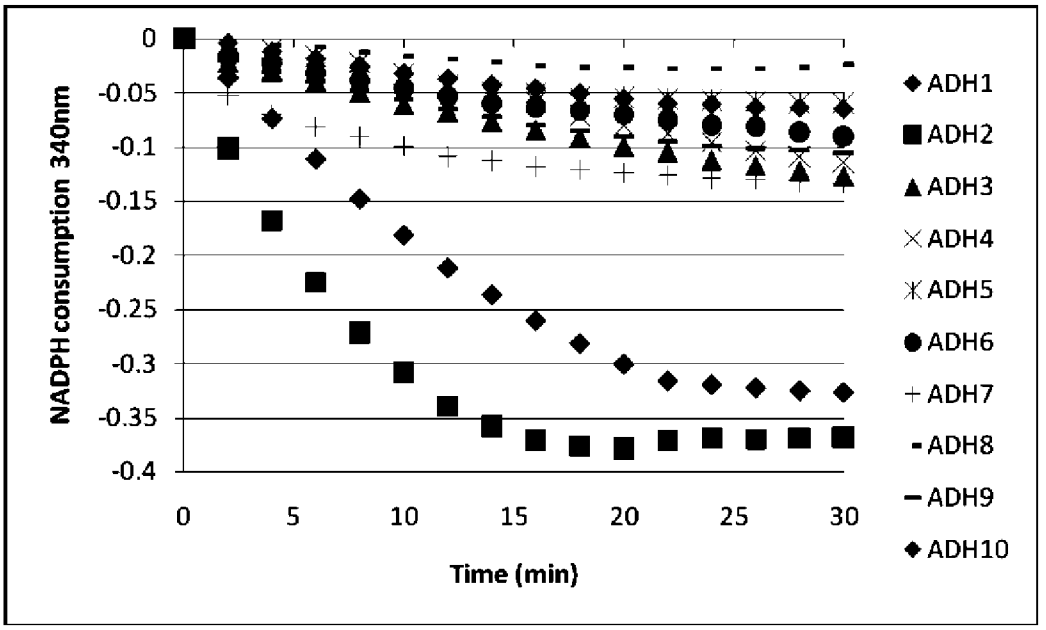
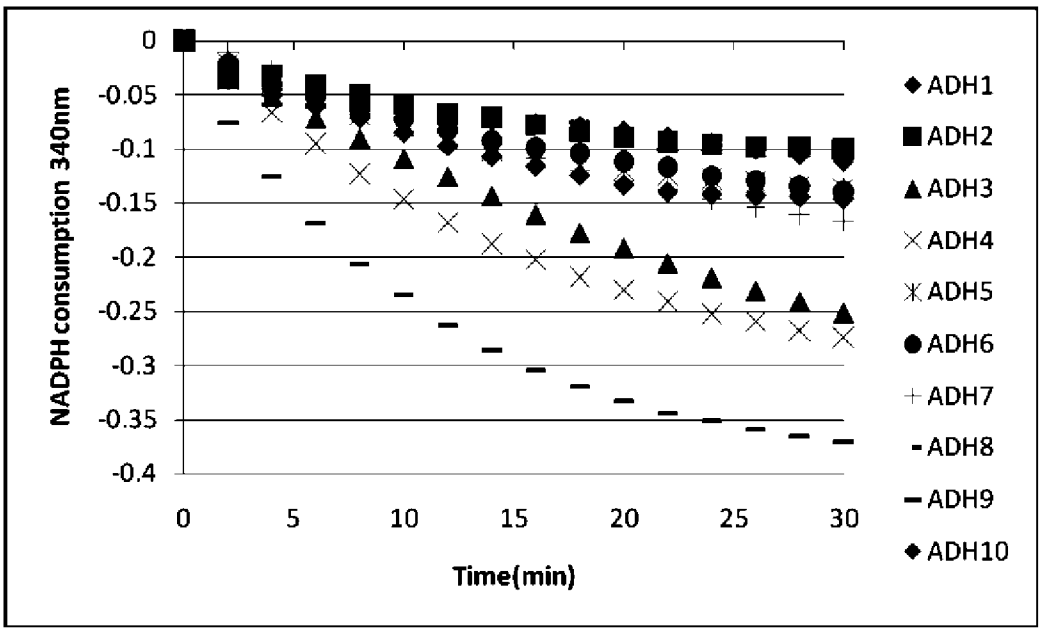


Figure 7B

Mannuronate hydrogenase activity monitored by NADPH consumption



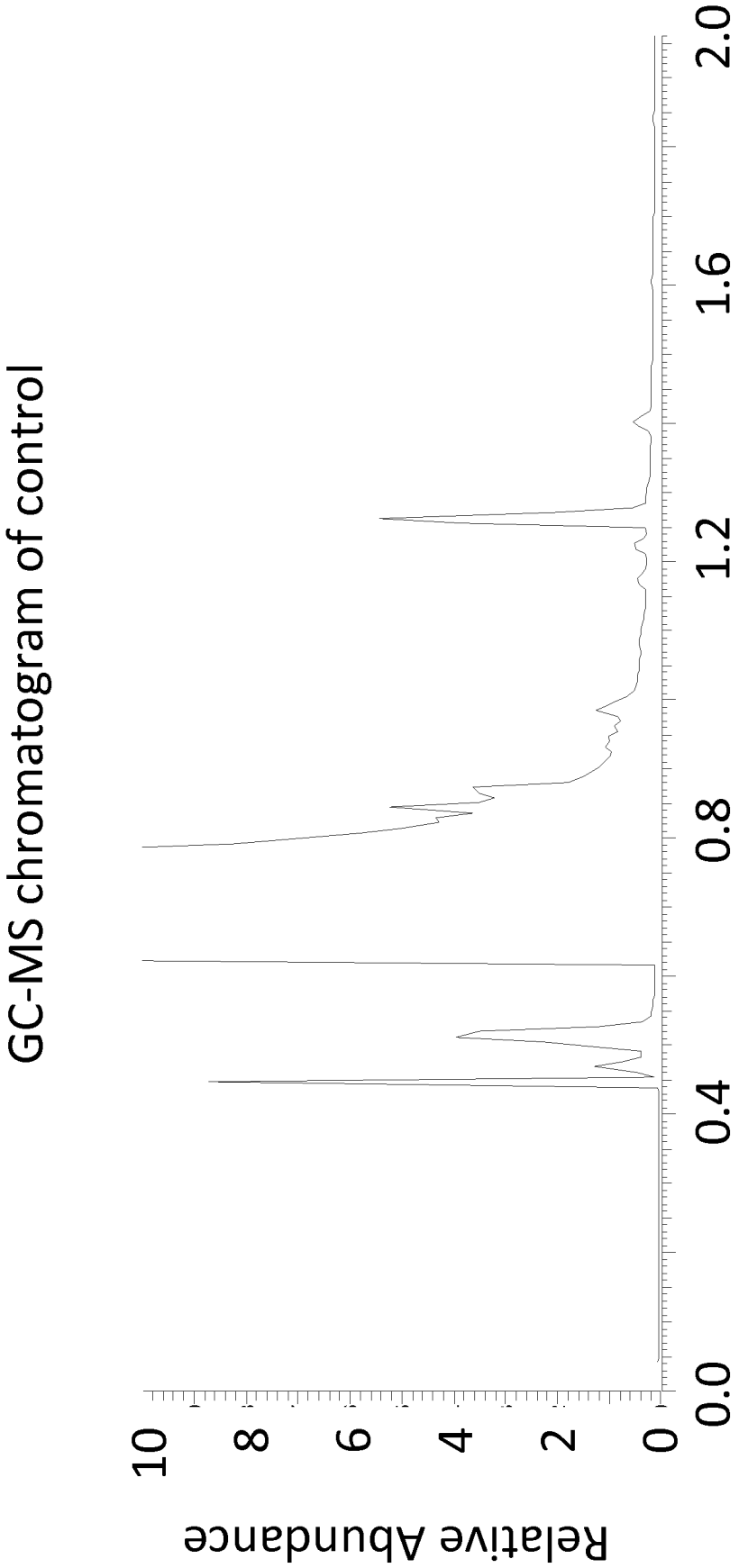

GC-MS chromatogram of control
Relative Abundance
Time (min)
0
2
4
6
8
10
0.0
0.4
0.8
1.2
1.6
2.0

FIG. 8A

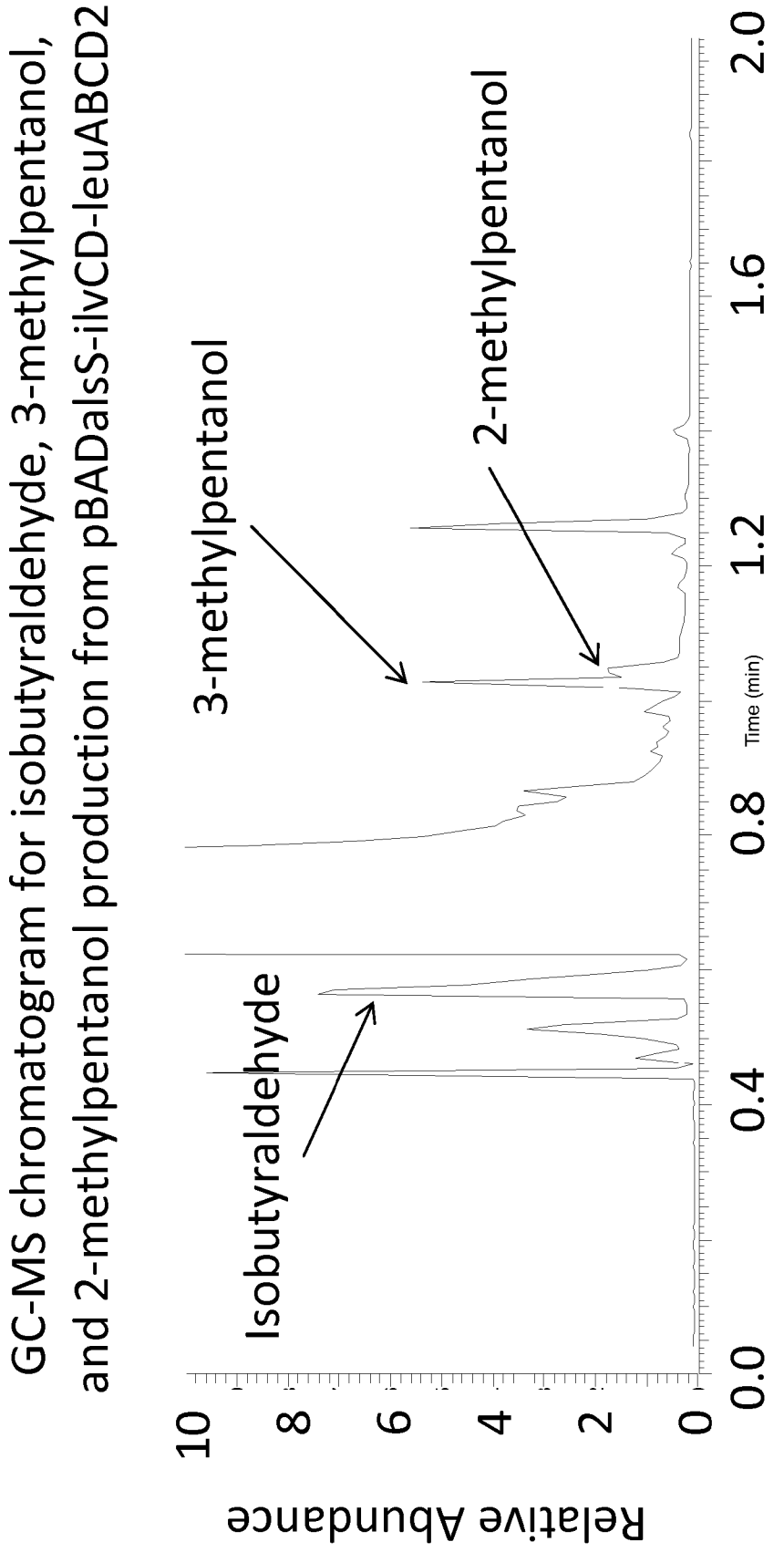

GC-MS chromatogram for isobutyraldehyde, 3-methylpentanol, and 2-methylpentanol production from pBADalsS-ilvCD-leuABCD2
Isobutyraldehyde
3-methylpentanol
2-methylpentanol
Relative Abundance
Time (min)
0
2
4
6
8
10
0.0
0.4
0.8
1.2
1.6
2.0

FIG. 8B

Figure 9A
GC-MS chromatogram of Control

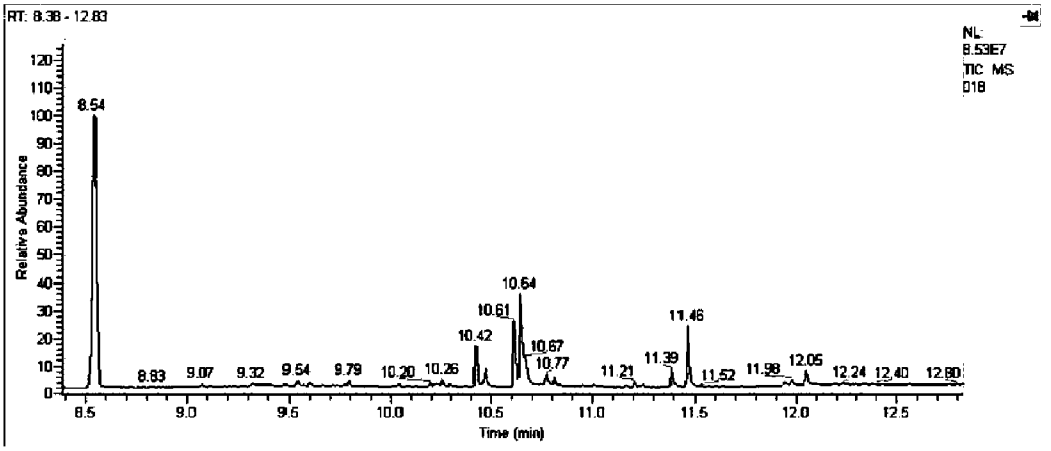


Figure 9B
GC-MS chromatogram for 4-hydroxyphenylethanol and indole-3-ethanol production from pBADtyrA-aroLAC-aroG-tktA-aroBDE and pTrcBALK

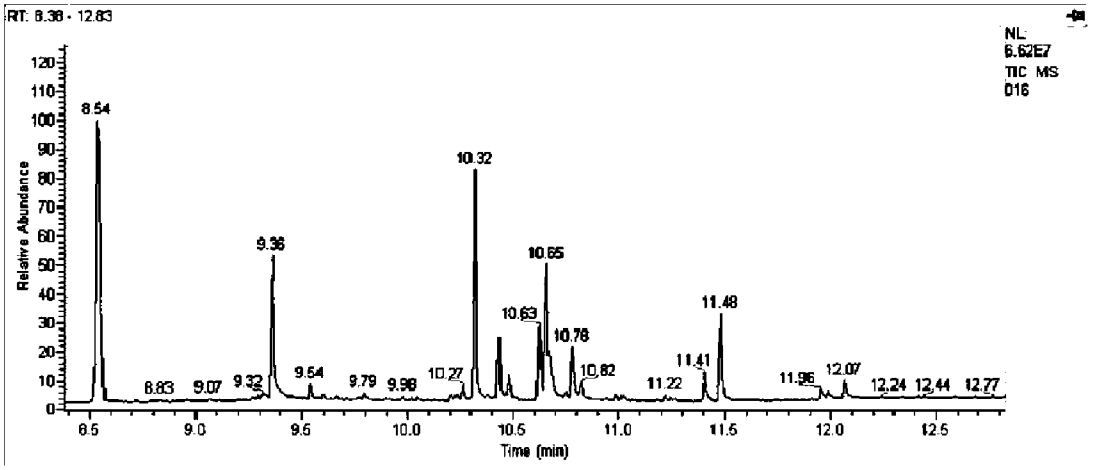


Figure 10A

Mass spectrometry of isobutanal

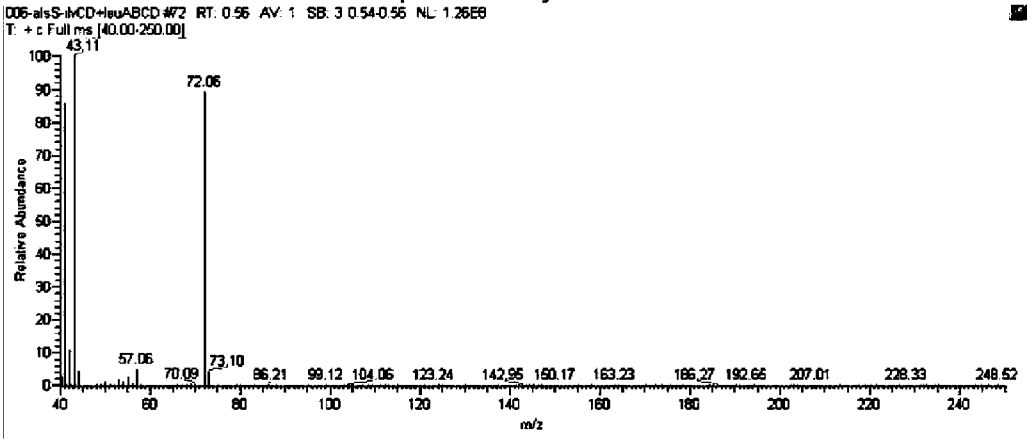


Figure 10B

Mass spectrometry of 3-methylpentanol

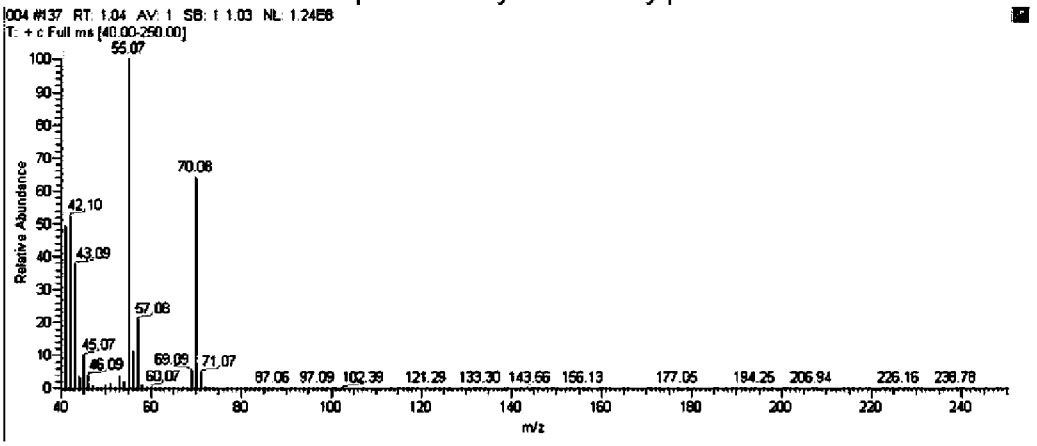


Figure 10C

Mass spectrometry of 2-methylpentanol

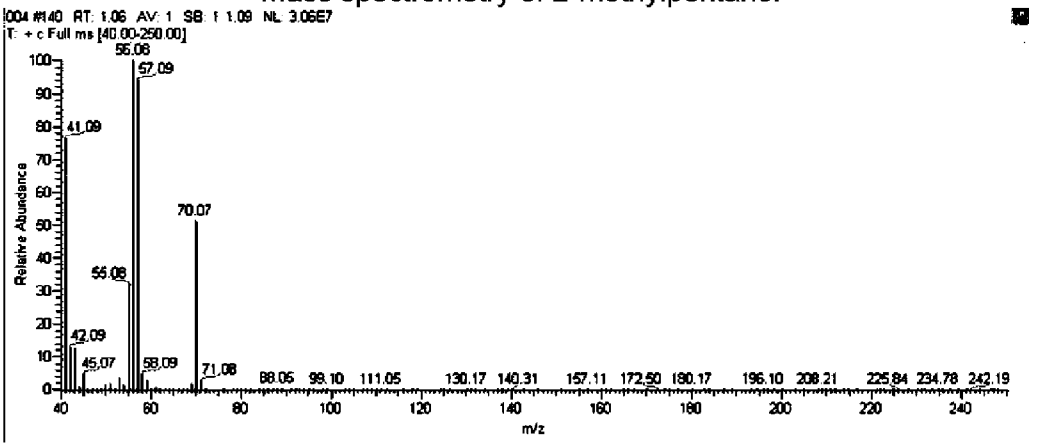


Figure 11A

Mass spectrometry of 2-phenylethanol

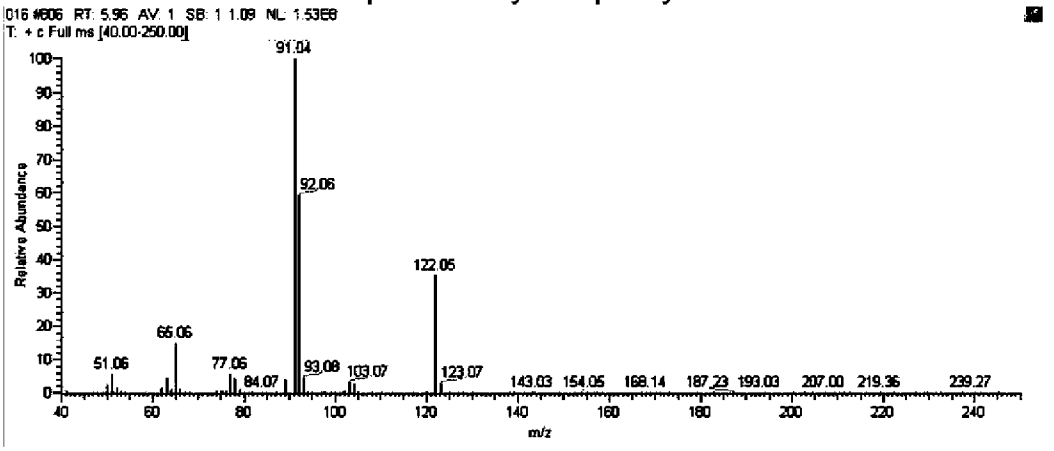


Figure 11B

Mass spectrometry of 4-hydroxyphenylethanol

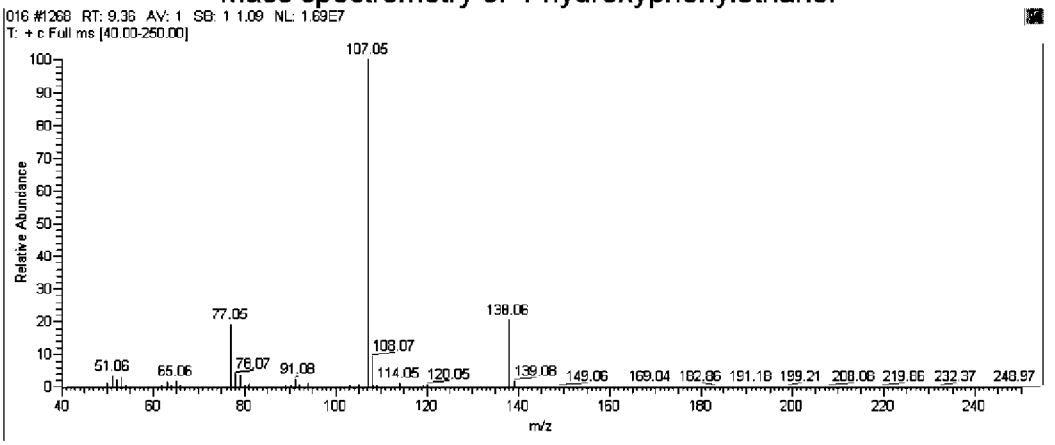


Figure 11C

Mass spectrometry of indole-3-ethanol

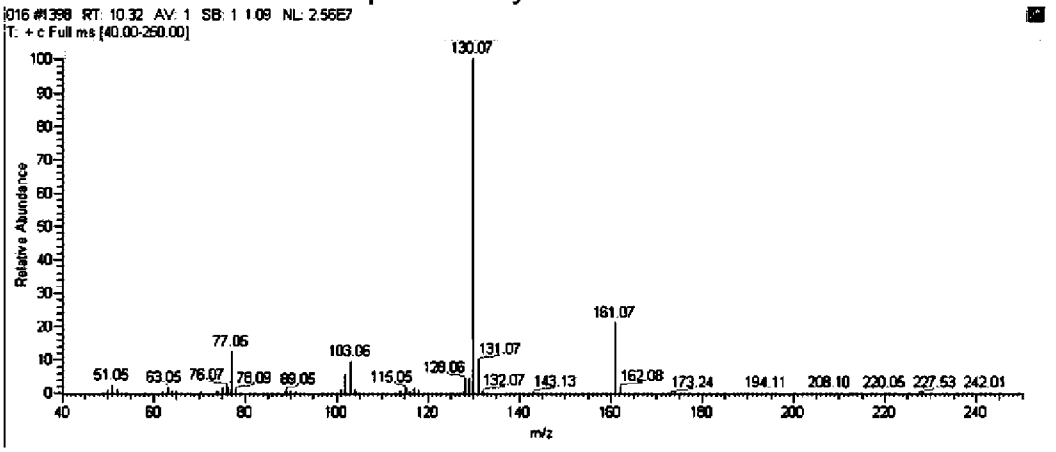


Figure 12A

Reduction of butyroin by *ddh*1, *ddh*2, and *ddh*3 monitored by NADH consumption.

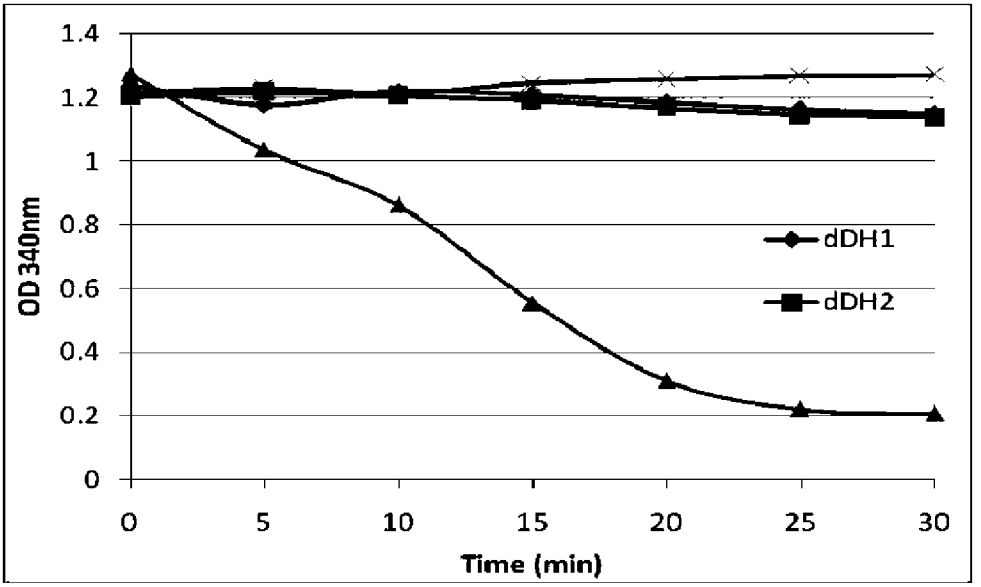


Figure 12B

Oxidation activity of *ddh*3 towards 1,2-cyclopentanediol and 1,2-cyclohexanediol as measured by NADH production.

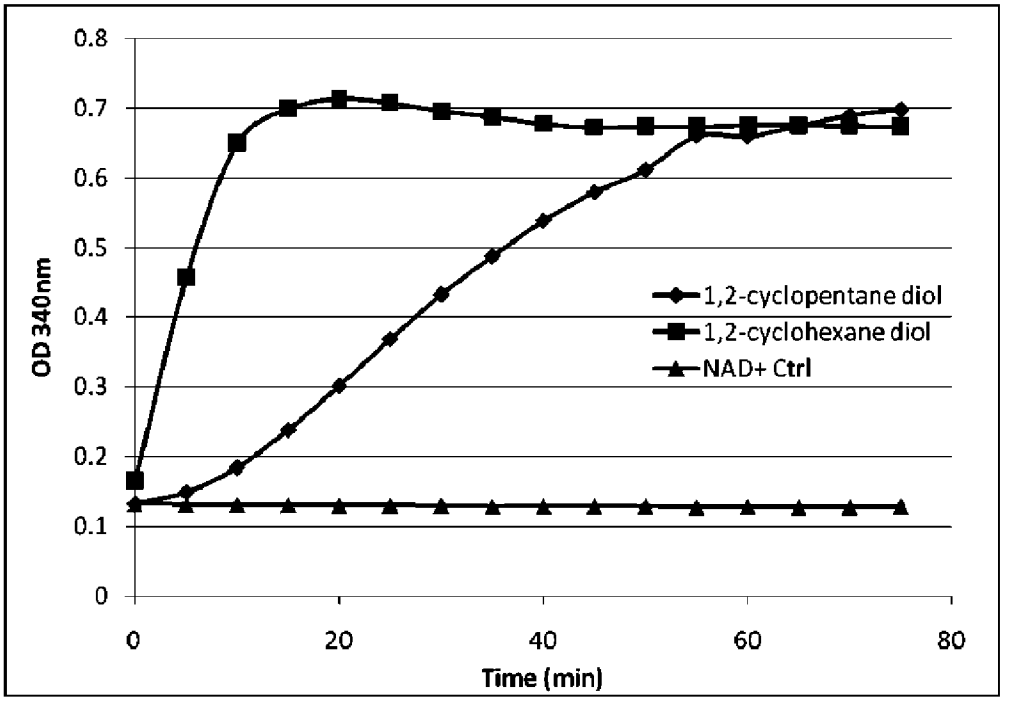
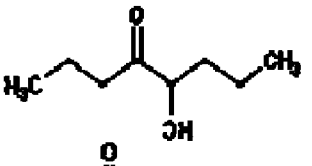
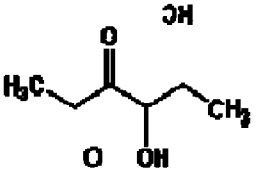


Figure 13

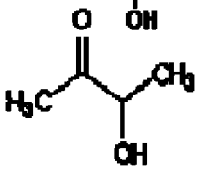
- Butyroin: k_{cat}/K_M = 139 $sec^{-1}M^{-1}$ +/- 9



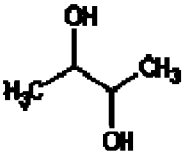
- Propioin: k_{cat}/K_M – 709 $sec^{-1}M^{-1}$ +/- 40



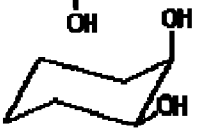
- Acetoin: k_{cat}/K_M = 1990 $sec^{-1}M^{-1}$ +/- 210



- meso-2,3-butanediol: k_{cat}/K_M – 1450 $sec^{-1}M^{-1}$ +/- 150



- cis-1,2-cyclohexanediol-: k_{cat}/K_M = 108 $sec^{-1}M^{-1}$ +/- 15



- trans-1,2-cyclopentandiol: k_{cat}/K_M – 19.1 $sec^{-1}M^{-1}$ +/- 4.1

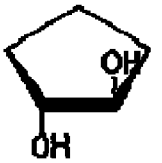


Figure 14A

Nucleotide sequence of diol dehydrogenase DDH1 isolated from *Lactobaccilus brevis* ATCC 367

ATGGCATCAAATGGAAAAGTAGCAATGGTTACCGGTGGCGGACAAGGAATTGGTGAAGC
CATCTCGAAACGGTTAGCTAACGACGGCTTTGCTGTGGCAATTGCTGATTTGAACTTGG
ACAATGCCAACAAGGTCGTTTCTGATATTGAAGCTGCTGGTGGCAAGGCCATTGCGGTC
AAGACCGATGTCTCTGATCGTGATAGCGTGTTTGCTGCGGTTAATGAAGCGGCCGACAA
GCTGGGCGGCTTTGACGTTATCGTTAATAACGCCGGCCTTGGCCCAACCACGCCAATTG
ACACCATCACCCAAGAACAGTTTGATACGGTTTATCACGTTAACGTGGGTGGGGTTCTT
TGGGGCATTCAAGCAGCCCATGCGAAGTTCAAGGAATTGGGTCATGGTGGGAAGATCAT
TTCCGCGACGTCTCAAGCCGGGGTTGTTGGTAACCCGAACTTAGCTCTGTACAGTGGAA
CTAAGTTTGCCATTCGTGGTGTGACCCAAGTTGCGGCGCGTGACTTAGCCGCTGAAGGT
ATCACGGTCAATGCTTATGCACCCGGGATTGTTAAGACACCAATGATGTTTGACATCGC
TCACAAGGTTGGTCAAAATGCTGGTAAAGACGACGAATGGGGGATGCAAACCTTCTCAA
AGGACATCGCTTTATGTCGATTGTCAGAACCAGAAGATGTGGCTAACGGGGTGGCTTTC
TTAGCCGGTCCCGATTCTAACTACATTACGGGTCAAACACTTGAAGTTGATGGTGGGAT
GCAGTTCCACTAA (SEQ ID NO:97)

Figure 14B

Polypeptide primary sequence of diol dehydrogenase DDH1 isolated from *Lactobaccilus brevis* ATCC 367

MASNGKVAMVTGGGQGIGEAISKRLANDGFAVAIADLNLDNANKVVSDIEAAGGKAIAV
KTDVSDRDSVFAAVNEAADKLGGFDVIVNNAGLGPTTPIDTITQEQFDTVYHVNVGGVL
WGIQAAHAKFKELGHGGKIISATSQAGVVGNPNLALYSGTKFAIRGVTQVAARDLAAEG
ITVNAYAPGIVKTPMMFDIAHKVGQNAGKDDEWGMQTFSKDIALCRLSEPEDVANGVAF
LAGPDSNYITGQTLEVDGGMQFH (SEQ ID NO:98)

Figure 15A

Nucleotide sequence of diol dehydrogenase DDH2 isolated from *Pseudomonas putida* KT2440

ATGAATGACCTGAGCCACACCCACATGCGCGCGGCCGTCTGGCATGGCCGCCACGATAT
TCGTGTCGAACAGGTACCTTTGCCGGCCGACCCTGCGCCGGGCTGGGTGCAGATCAAGG
TGGACTGGTGCGGCATCTGCGGCTCCGACCTGCACGAATATGTTGCCGGCCCGGTGTTC
ATCCCGGTAGAGGCCCCGCACCCGCTGACCGGCATTCAGGGCCAGTGCATCCTCGGCCA
CGAATTCTGCGGCCACATCGCCAAGCTTGGCGAAGGCGTGGAAGGCTATGCCGTAGGCG
ACCCGGTGGCGGCAGACGCGTGCCAGCATTGTGGTACCTGCTATTACTGCACCCATGGC
CTGTACAACATCTGCGAACGCCTGGCGTTCACCGGCCTGATGAACAACGGTGCCTTCGC
CGAGCTGGTCAACGTGCCCGCCAACCTGCTCTACCGGCTGCCGCAGGGCTTCCCTGCCG
AAGCCGGGGCACTGATCGAGCCGCTGGCGGTGGGTATGCACGCGGTGAAAAAGGCCGGC
AGCCTGCTTGGGCAAACCGTTGTAGTGGTTGGGGCCGGCACCATCGGCCTGTGCACCAT
CATGTGCGCCAAGGCTGCAGGTGCGGCACAGGTCATCGCCCTTGAGATGTCCTCTGCGC
GCAAAGCCAAGGCCAAGGAAGCGGGCGCCAACGTGGTGCTGGACCCCAGCCAGTGCGAT
GCCCTGGCGGAAATCCGCGCACTGACTGCTGGGCTGGGCGCCGATGTGAGTTTTGAGTG
CATCGGCAACAAACATACGGCCAAGCTGGCCATCGACACCATCCGCAAAGCAGGCAAGT
GCGTGCTGGTGGGTATTTTCGAAGAGCCCAGCGAGTTCAACTTCTTCGAGCTGGTGTCC
ACCGAGAAGCAAGTGCTGGGGGCGTTGGCGTACAACGGCGAGTTTGCTGACGTGATTGC
CTTCATTGCTGATGGTCGGCTGGATATTCGCCCGCTGGTAACCGGCCGGATCGGATTGG
AGCAGATTGTCGAGCTGGGCTTCGAGGAACTGGTGAACAACAAAGAGGAGAACGTGAAG
ATCATCGTTTCACCAGGTGTGCGCTGA (SEQ ID NO:99)

Figure 15B

Polypeptide sequence of diol dehydrogenase DDH2 isolated from *Pseudomonas putida* KT2440

MNDLSHTHMRAAVWHGRHDIRVEQVPLPADPAPGWVQIKVDWCGICGSDLHEYVAGPVF
IPVEAPHPLTGIQGQCILGHEFCGHIAKLGEGVEGYAVGDPVAADACQHCGTCYYCTHG
LYNICERLAFTGLMNNGAFAELVNVPANLLYRLPQGFPAEAGALIEPLAVGMHAVKKAG
SLLGQTVVVVGAGTIGLCTIMCAKAAGAAQVIALEMSSARKAKAKEAGANVVLDPSQCD
ALAEIRALTAGLGADVSFECIGNKHTAKLAIDTIRKAGKCVLVGIFEEPSEFNFFELVS
TEKQVLGALAYNGEFADVIAFIADGRLDIRPLVTGRIGLEQIVELGFEELVNNKEENVK
IIVSPGVR (SEQ ID NO:100)

Figure 16A

Nucleotide sequence of diol dehydrogenase DDH3 isolated from *Klebsiella pneumoniae* MGH78578

ATGAAAAAAGTCGCACTTGTTACCGGCGCCGGCCAGGGGATTGGTAAAGCTATCGCCCT
TCGTCTGGTGAAGGATGGATTTGCCGTGGCCATTGCCGATTATAACGACGCCACCGCCA
AAGCGGTCGCCTCGGAAATCAACCAGGCCGGCGGACACGCCGTGGCGGTGAAAGTGGAT
GTCTCCGACCGCGATCAGGTATTTGCCGCCGTTGAACAGGCGCGCAAAACGCTGGGCGG
CTTCGACGTCATCGTCAATAACGCCGGTGTGGCACCGTCTACGCCGATCGAGTCCATTA
CCCCGGAGATTGTCGACAAAGTCTACAACATCAACGTCAAAGGGGTGATCTGGGGTATT
CAGGCGGCGGTCGAGGCCTTTAAGAAAGAGGGGCACGGCGGGAAAATCATCAACGCCTG
TTCCCAGGCCGGCCACGTCGGCAACCCGGAGCTGGCGGTGTATAGCTCCAGTAAATTCG
CGGTACGCGGCTTAACCCAGACCGCCGCTCGCGACCTCGCGCCGCTGGGCATCACGGTC
AACGGCTACTGCCCGGGGATTGTCAAAACGCCAATGTGGGCCGAAATTGACCGCCAGGT
GTCCGAAGCCGCCGGTAAACCGCTGGGCTACGGTACCGCCGAGTTCGCCAAACGCATCA
CTCTCGGTCGTCTGTCCGAGCCGGAAGATGTCGCCGCCTGCGTCTCCTATCTTGCCAGC
CCGGATTCTGATTACATGACCGGTCAGTCGTTGCTGATCGACGGCGGGATGGTATTTAA
CTAA (SEQ ID NO:101)

Figure 16B

Polypeptide sequence of diol dehydrogenase DDH3 isolated from *Klebsiella pneumoniae* MGH78578

MKKVALVTGAGQGIGKAIALRLVKDGFAVAIADYNDATAKAVASEINQAGGHAVAVKVD
VSDRDQVFAAVEQARKTLGGFDVIVNNAGVAPSTPIESITPEIVDKVYNINVKGVIWGI
QAAVEAFKKEGHGGKIINACSQAGHVGNPELAVYSSSKFAVRGLTQTAARDLAPLGITV
NGYCPGIVKTPMWAEIDRQVSEAAGKPLGYGTAEFAKRITLGRLSEPEDVAACVSYLAS
PDSDYMTGQSLLIDGGMVFN (SEQ ID NO:102)

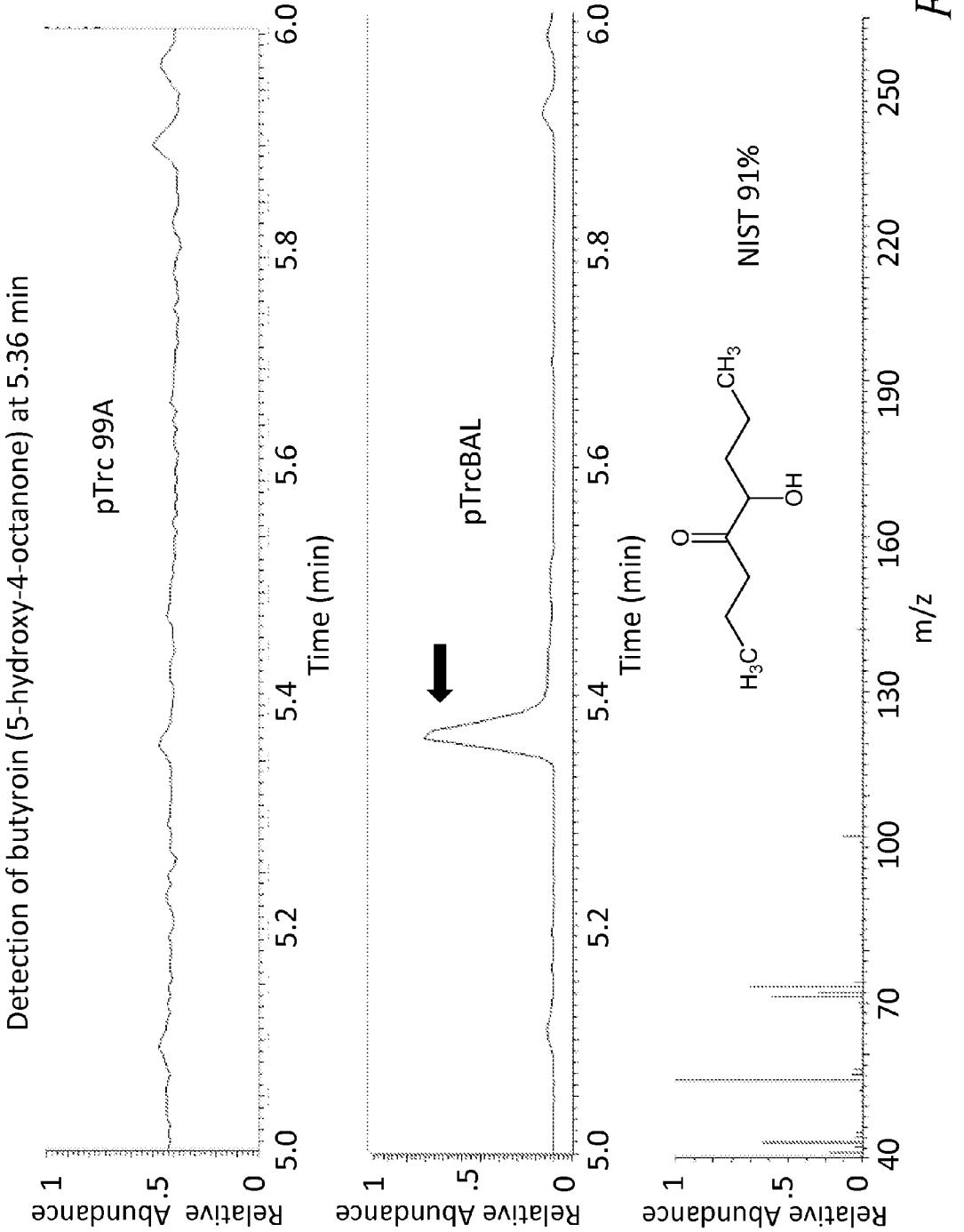

Detection of butyroin (5-hydroxy-4-octanone) at 5.36 min
pTrc 99A
pTrcBAL
Relative Abundance
Time (min)
m/z
NIST 91%
CH_3
OH
O
H_3C

FIG. 17A

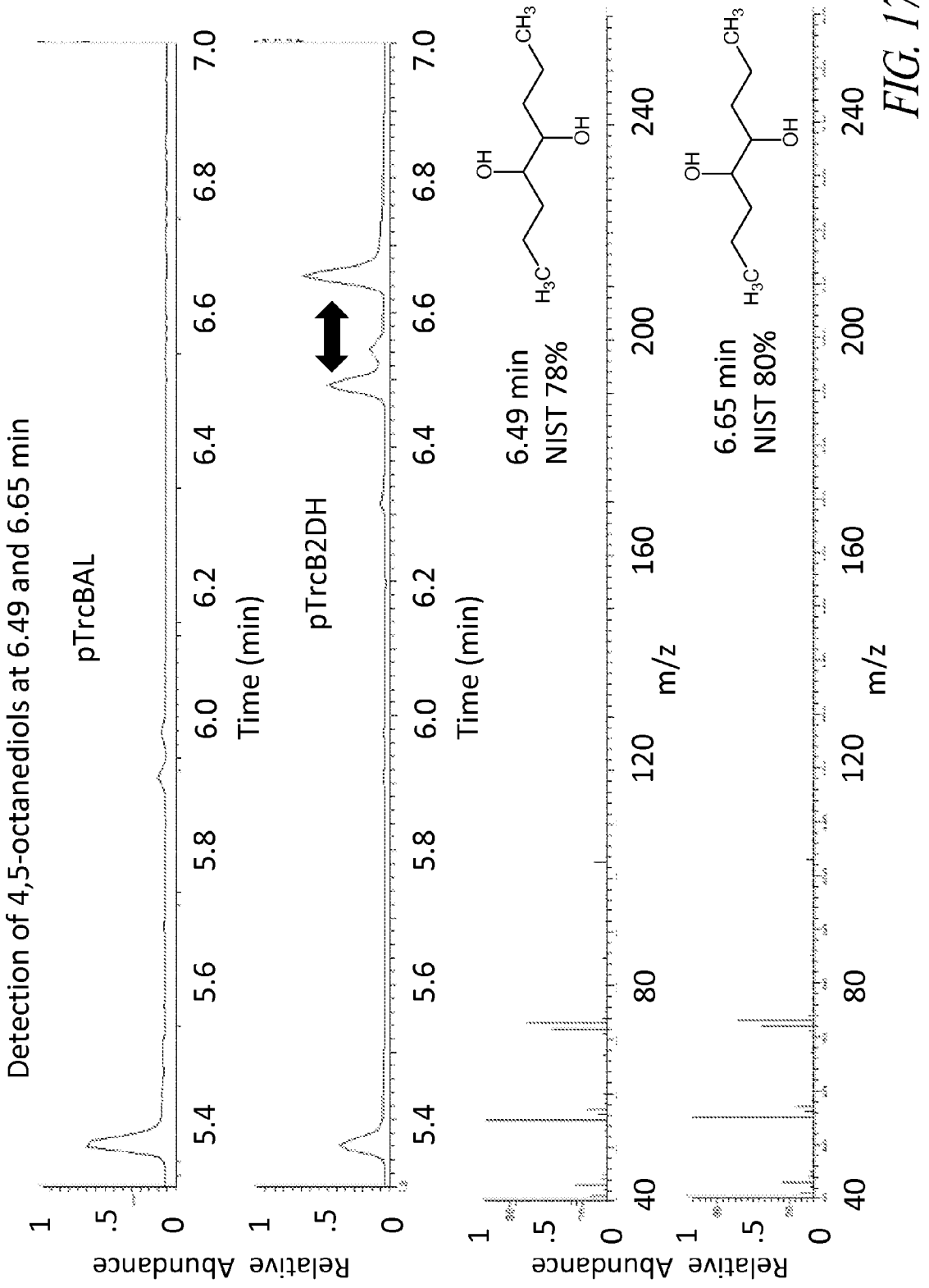

Detection of 4,5-octanediols at 6.49 and 6.65 min
pTrcBAL
pTrcB2DH
Relative Abundance
Time (min)
5.4
5.6
5.8
6.0
6.2
6.4
6.6
6.8
7.0
6.49 min
NIST 78%
6.65 min
NIST 80%
OH
OH
H3C
CH3
m/z
40
80
120
160
200
240

FIG. 17B

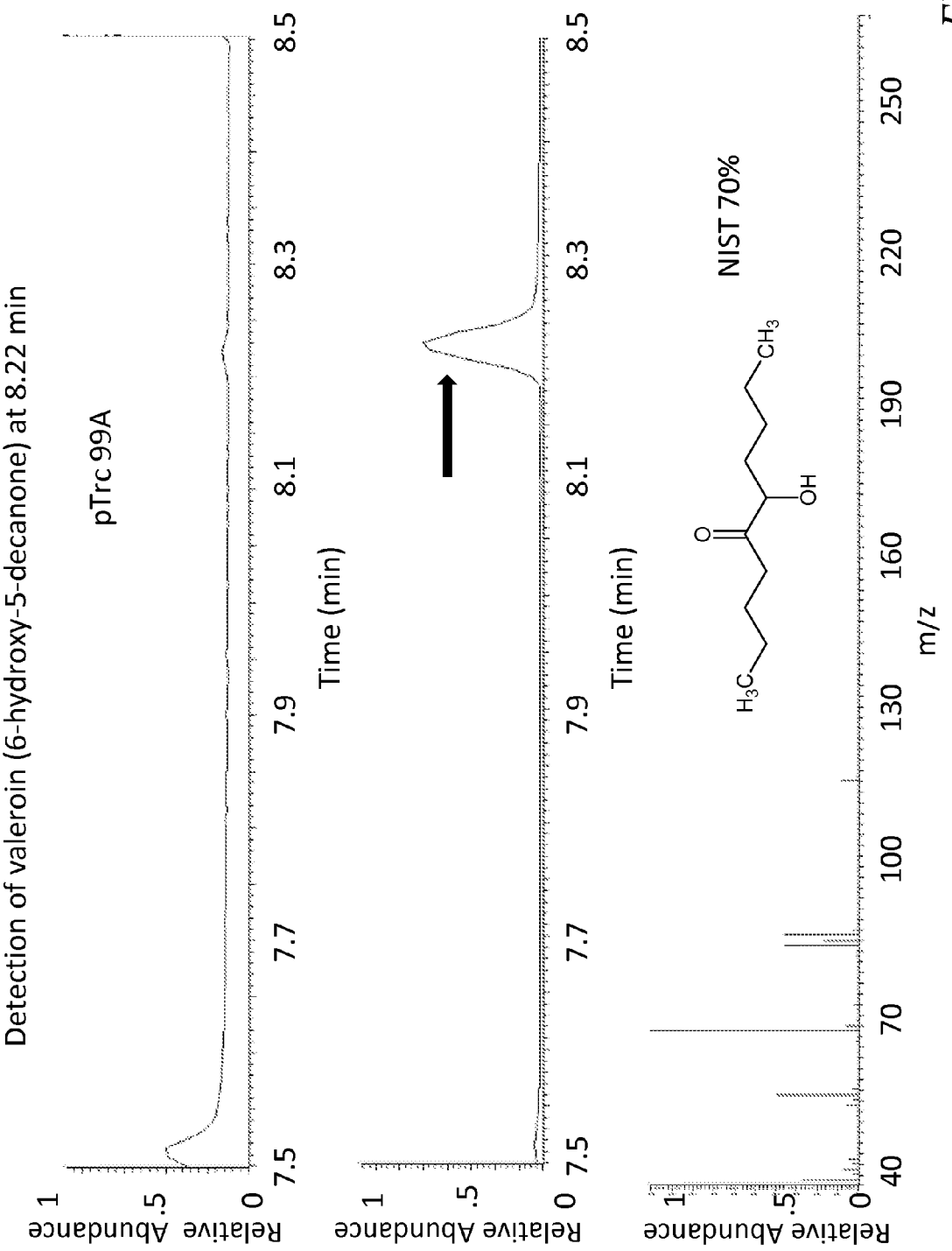

Detection of valeroin (6-hydroxy-5-decanone) at 8.22 min
pTrc 99A
Relative Abundance
Time (min)
NIST 70%
m/z
O
OH
CH3
H3C

FIG. 18A

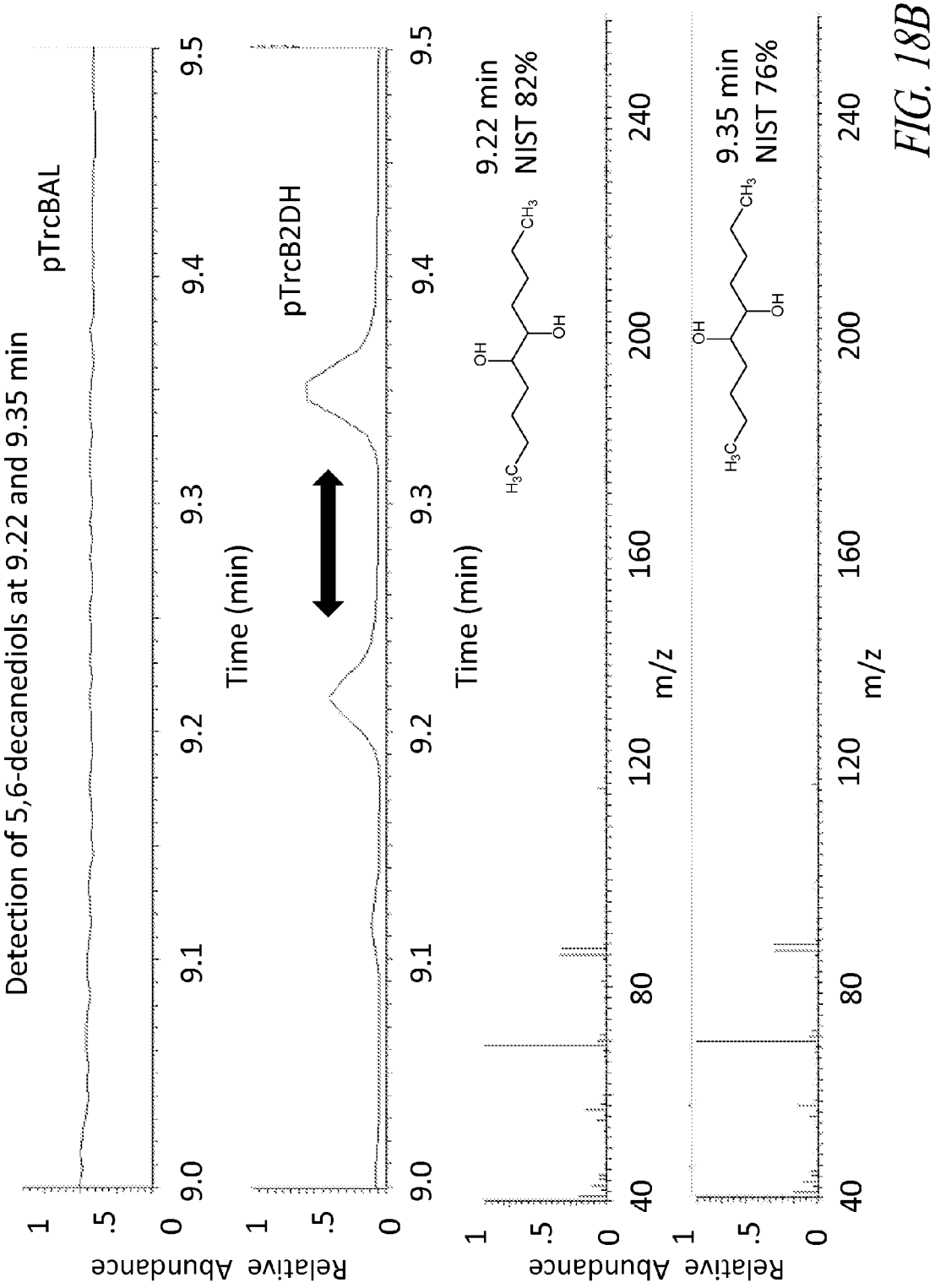

Detection of 5,6-decanediols at 9.22 and 9.35 min
pTrcBAL
pTrcB2DH
Relative Abundance
Time (min)
9.0
9.1
9.2
9.3
9.4
9.5
0
.5
1
9.22 min
NIST 82%
9.35 min
NIST 76%
m/z
40
80
120
160
200
240
OH
CH3
H3C

FIG. 18B

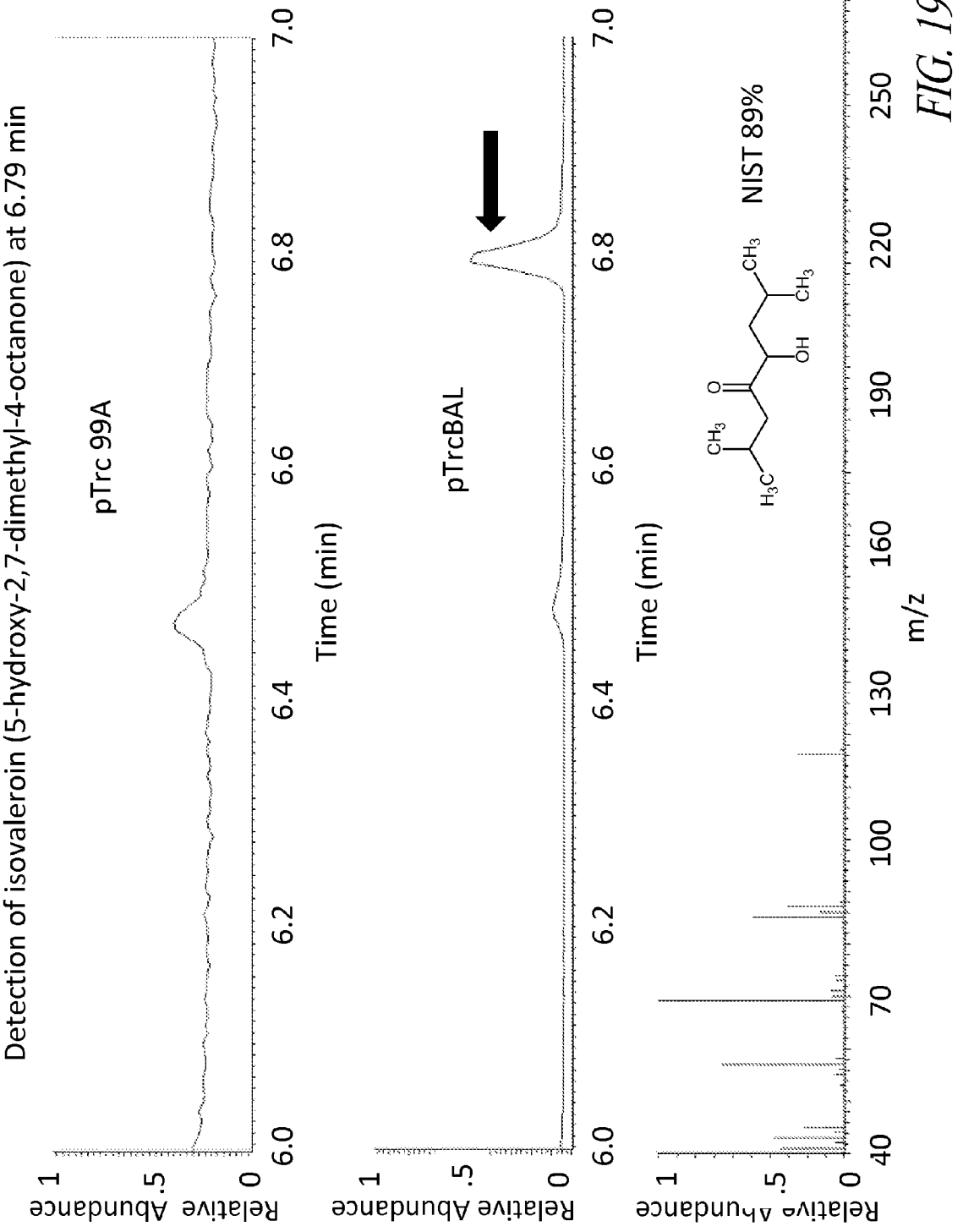

Detection of isovaleroin (5-hydroxy-2,7-dimethyl-4-octanone) at 6.79 min
pTrc 99A
Relative Abundance
Time (min)
pTrcBAL
NIST 89%
m/z

FIG. 19A

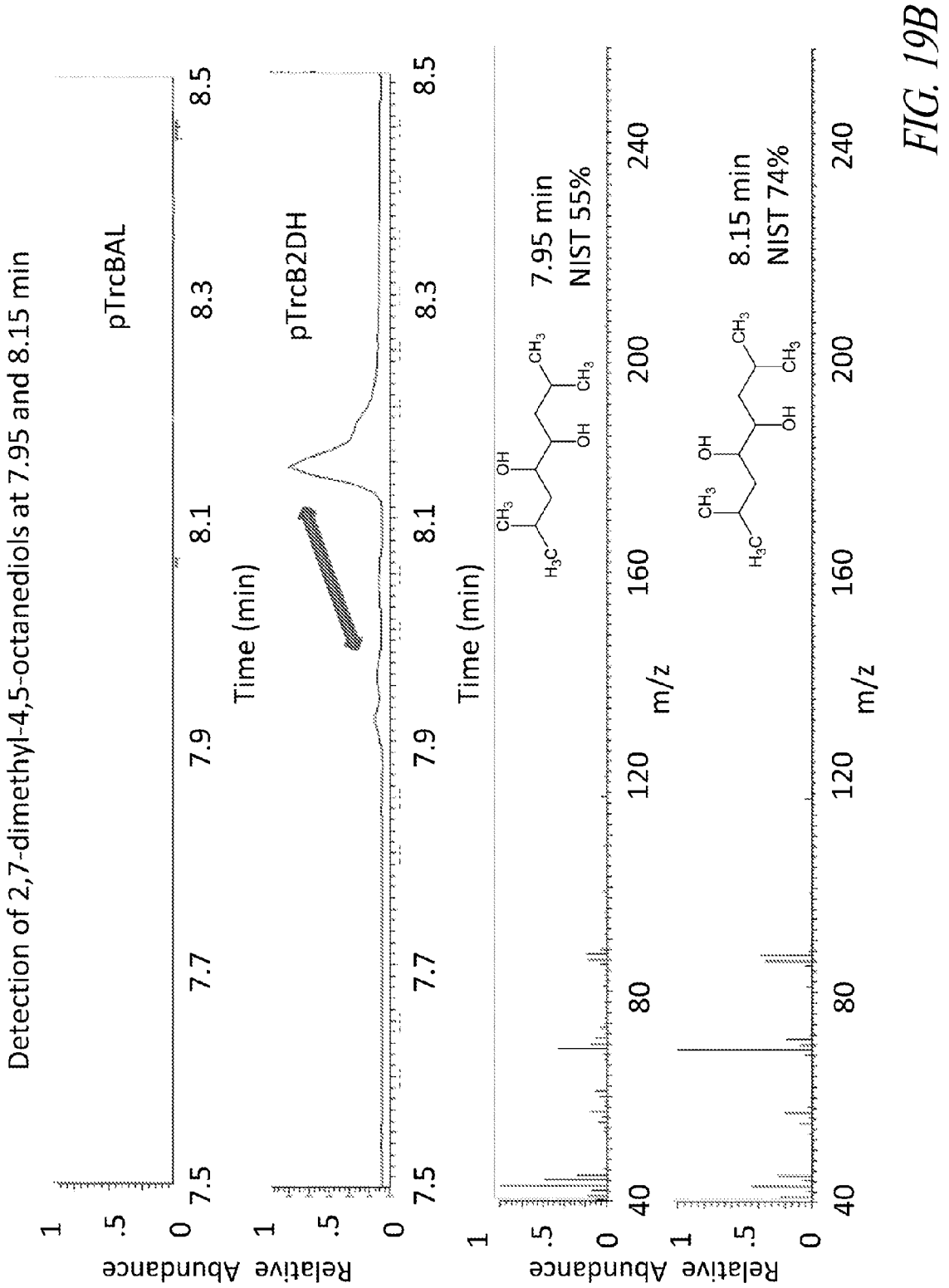

Detection of 2,7-dimethyl-4,5-octanediols at 7.95 and 8.15 min
pTrcBAL
pTrcB2DH
Relative Abundance
Time (min)
7.5
7.7
7.9
8.1
8.3
8.5
m/z
40
80
120
160
200
240
7.95 min
NIST 55%
8.15 min
NIST 74%

FIG. 19B

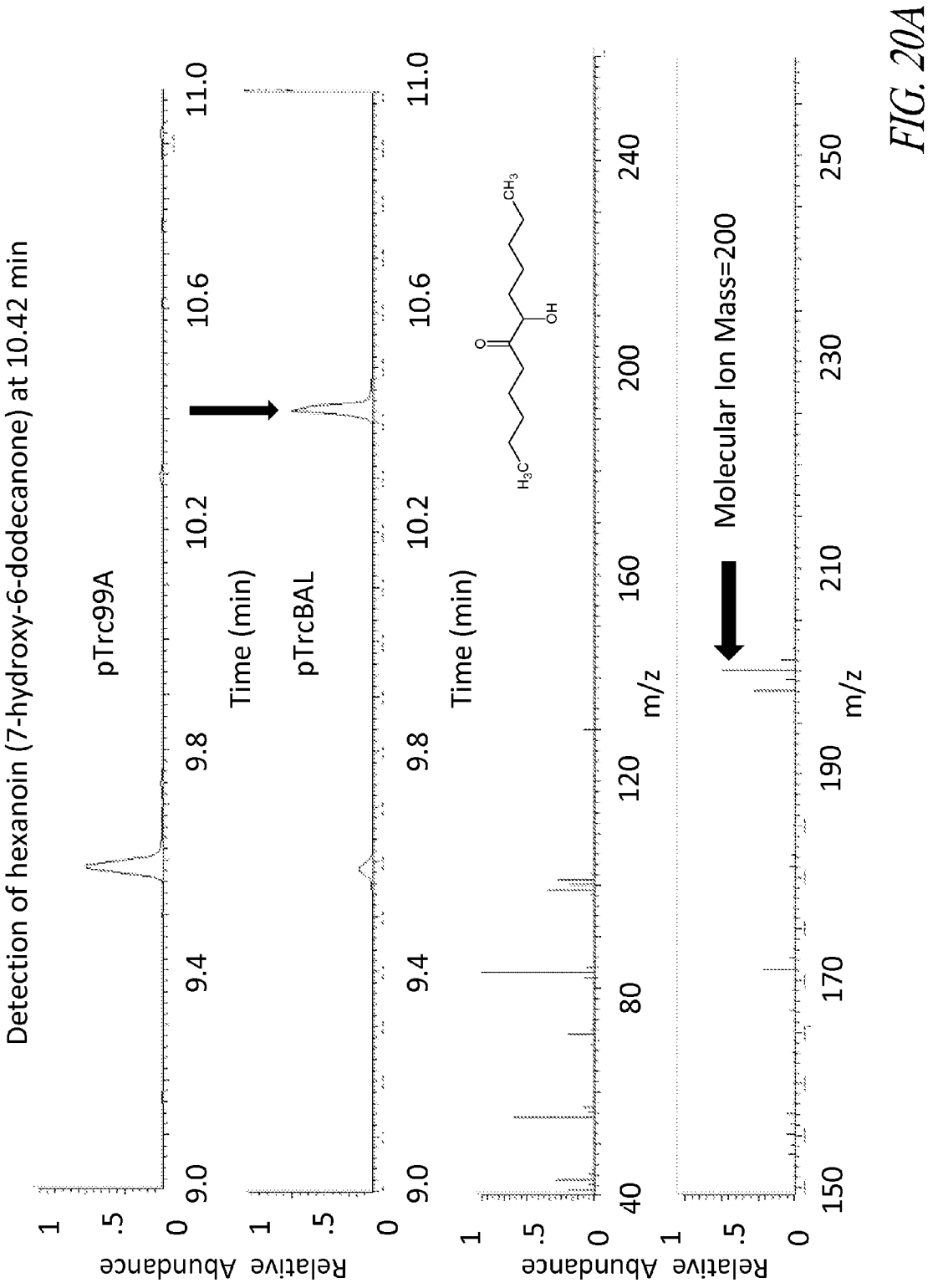

Detection of hexanoin (7-hydroxy-6-dodecanone) at 10.42 min
pTrc99A
pTrcBAL
Time (min)
Relative Abundance
m/z
Molecular Ion Mass=200
H3C
CH3
OH
O

FIG. 20A

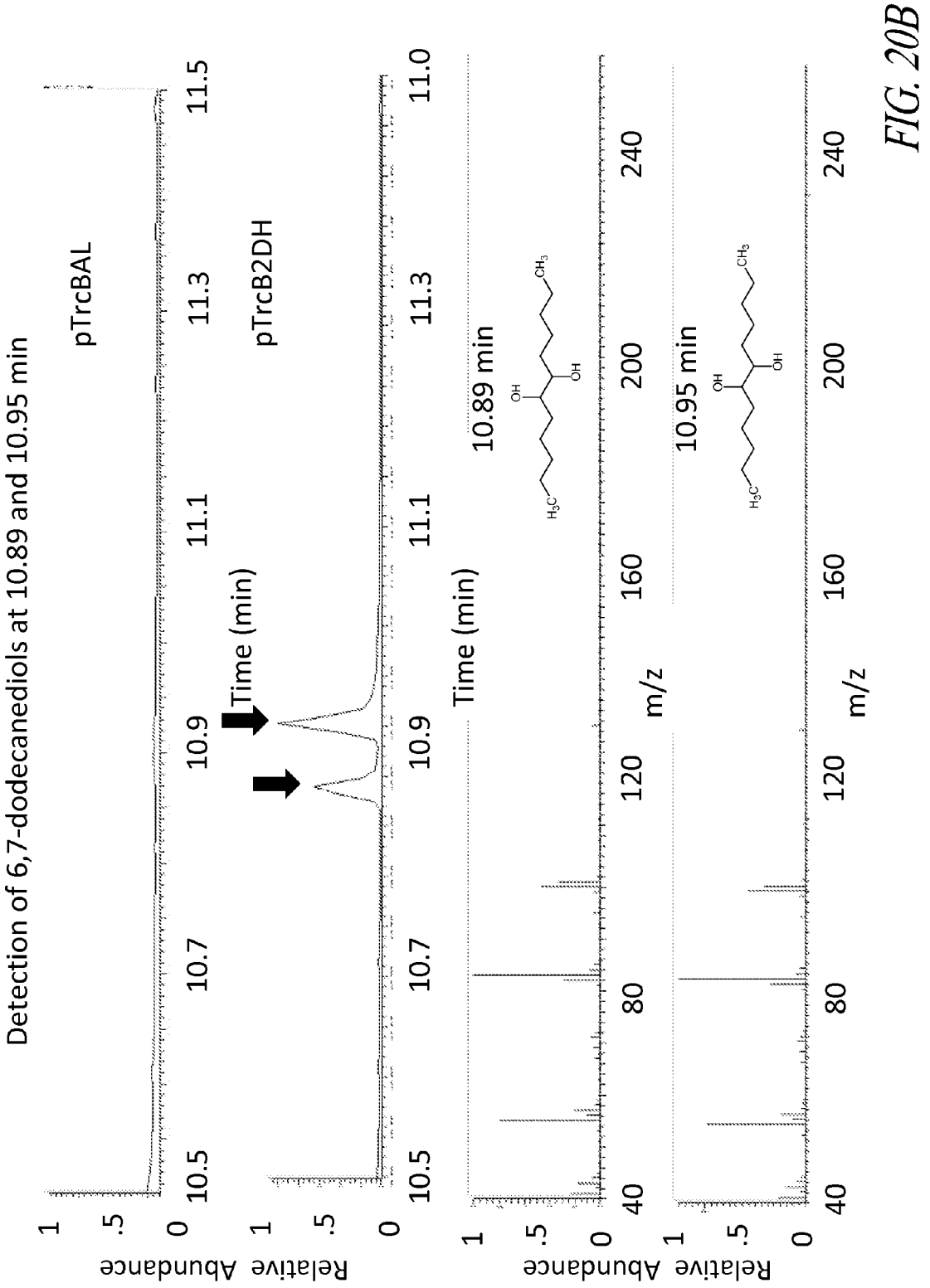

Detection of 6,7-dodecanediols at 10.89 and 10.95 min
pTrcBAL
pTrcB2DH
Relative Abundance
Time (min)
10.89 min
10.95 min
m/z

FIG. 20B

Figure 21A

Detection of isohexanoin (2,9-Dimethyl-6-hydroxy-5-decanone) at 9.45 min

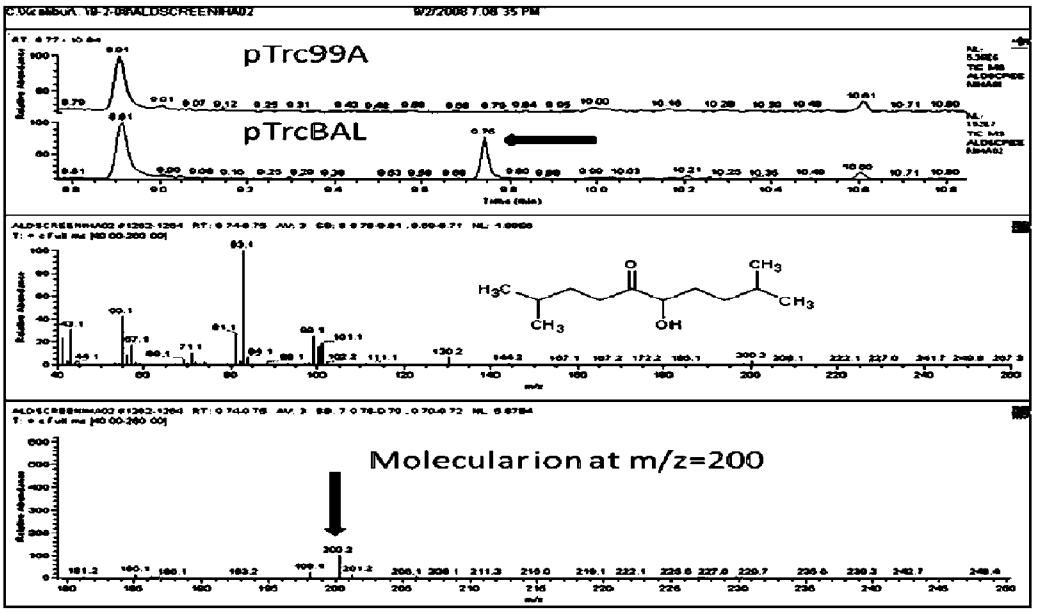


Figure 21B

Detection of 2,9-dimethyl-5,6-decanediol at 10.38 and 10.44 min

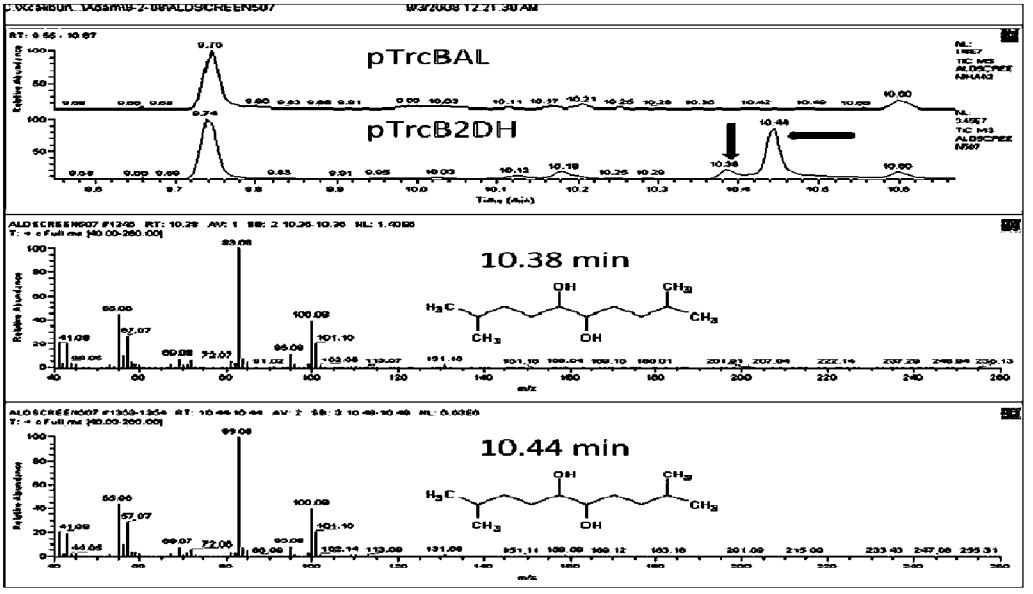


Figure 22

Detection of octanoin (9-Hydroxy-8- hexadecanone) at 12.35 min

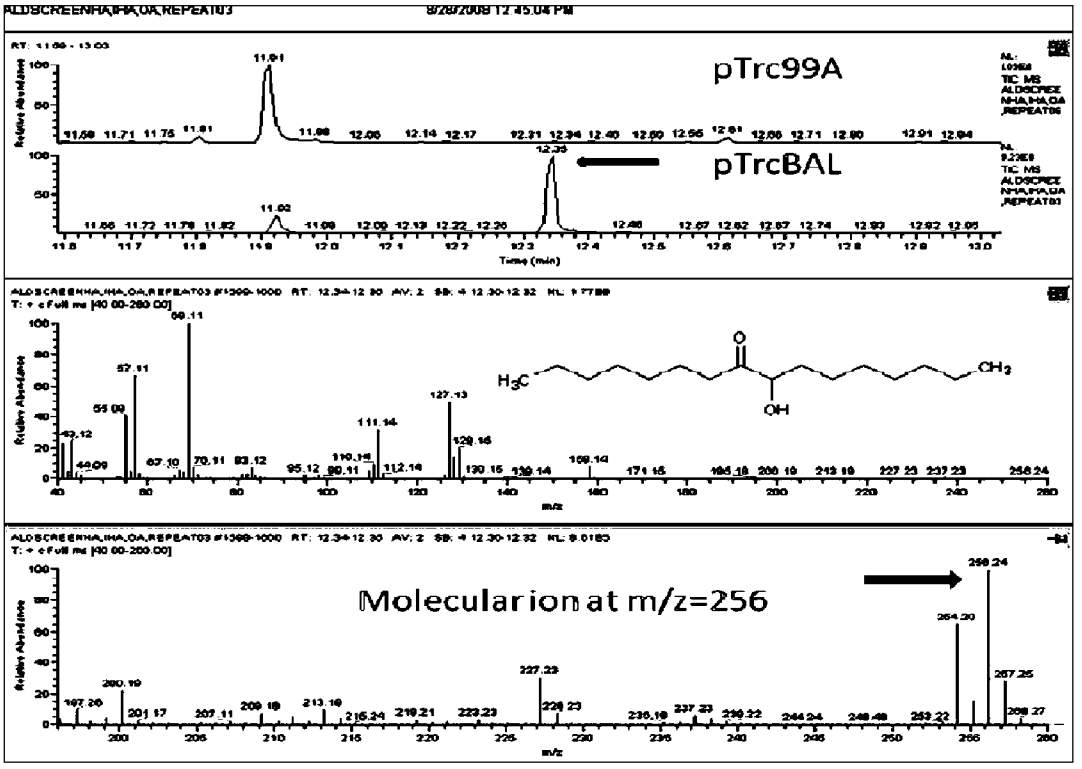


Figure 23

Detection of 3-hydroxy-2-butanone (acetoin) rt=0.91 min

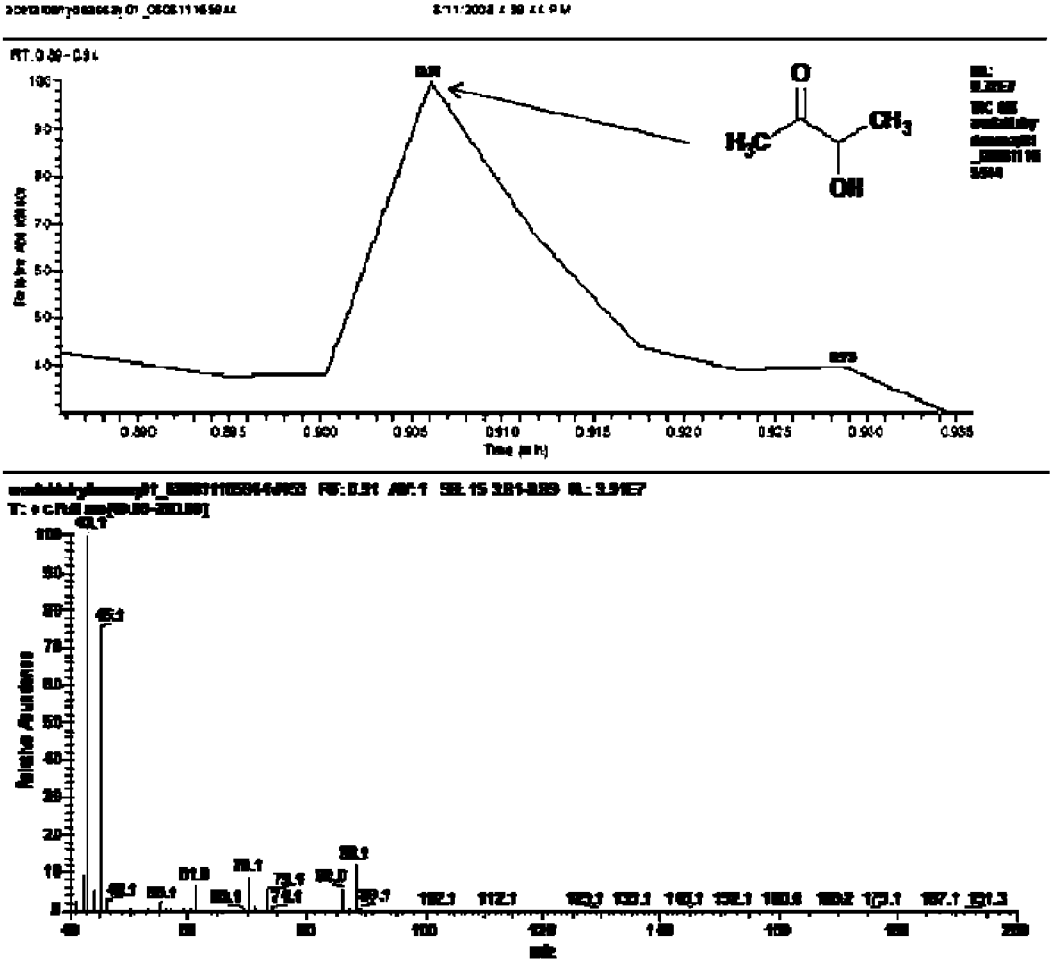


Figure 24A

Detection of 4-hydroxy-3-hexanone (propioin) rt=2.62 min

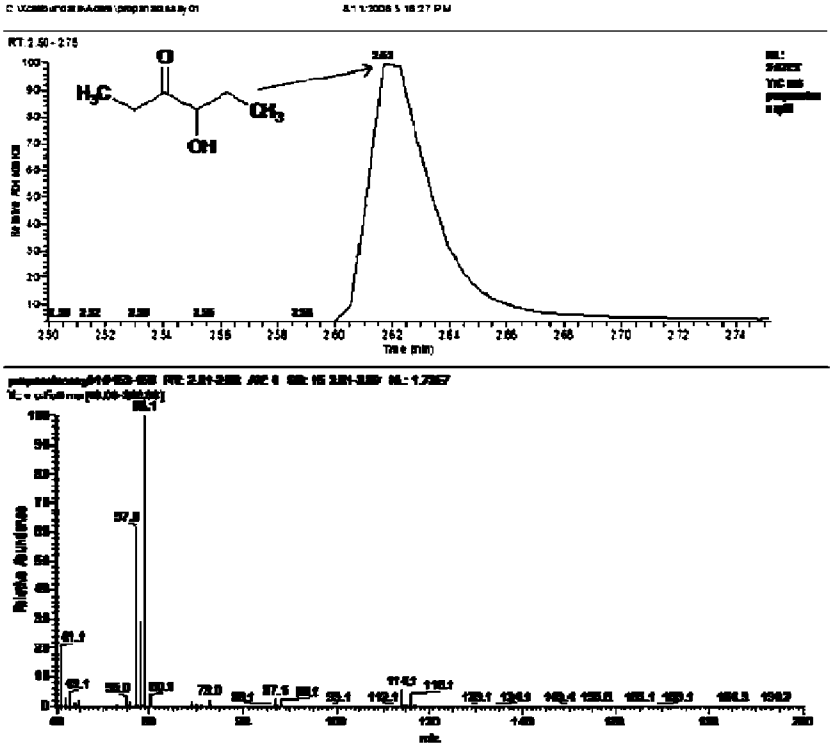


Figure 24B

Detection of 3,4-hexanediol rt=3.79 min

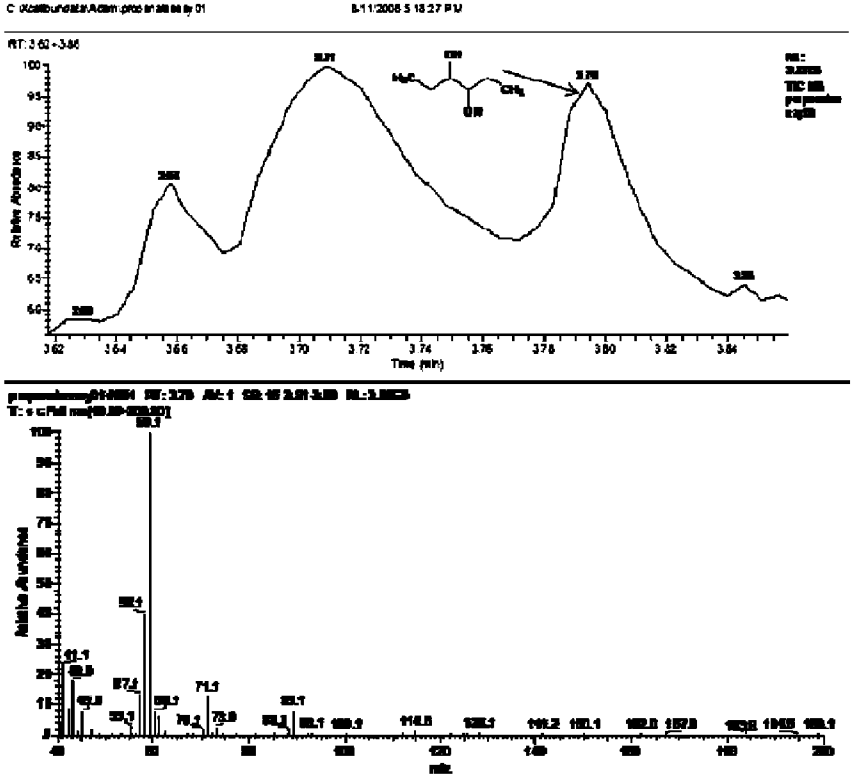


Figure 25

Detection of 1,4-diphenyl-3-hydroxy-2-butanone rt=13.66 min

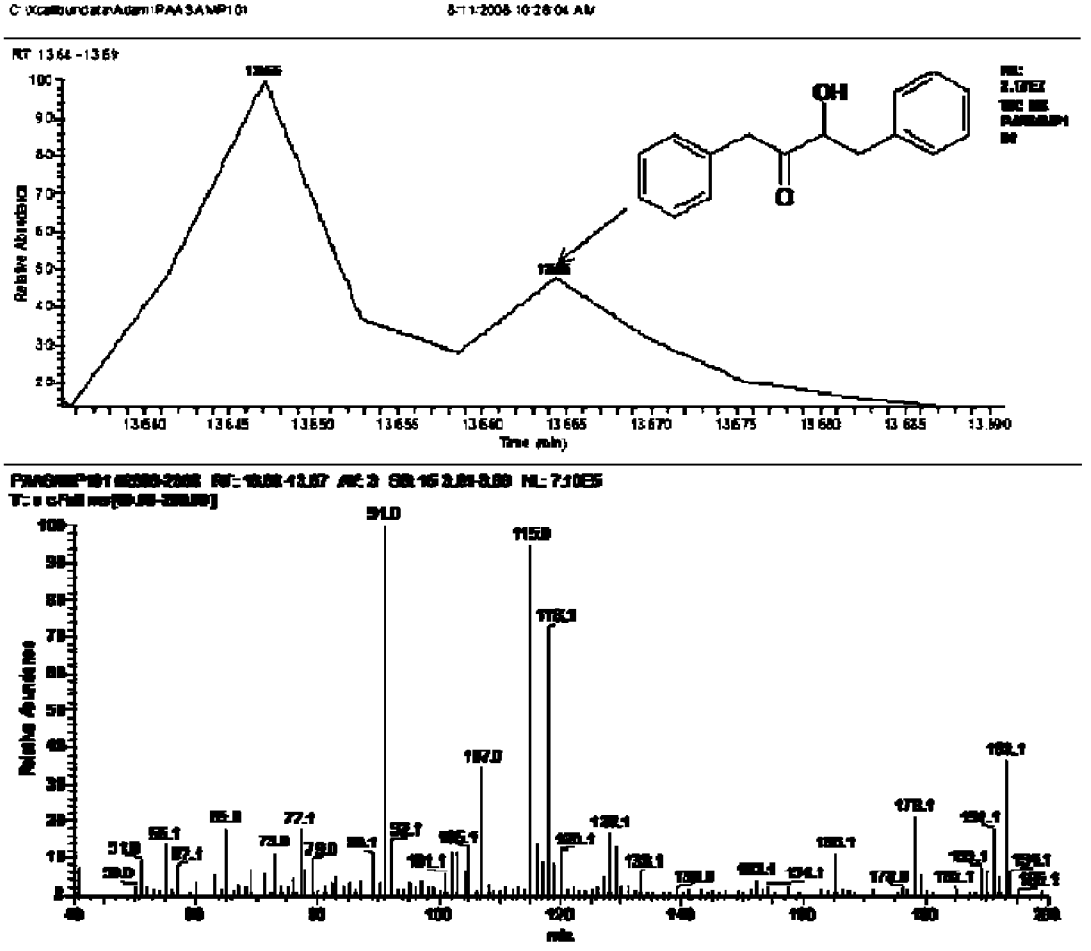


Figure 26A

GC-MS data confirming the presence of 4,5-octanediol in the sample extraction

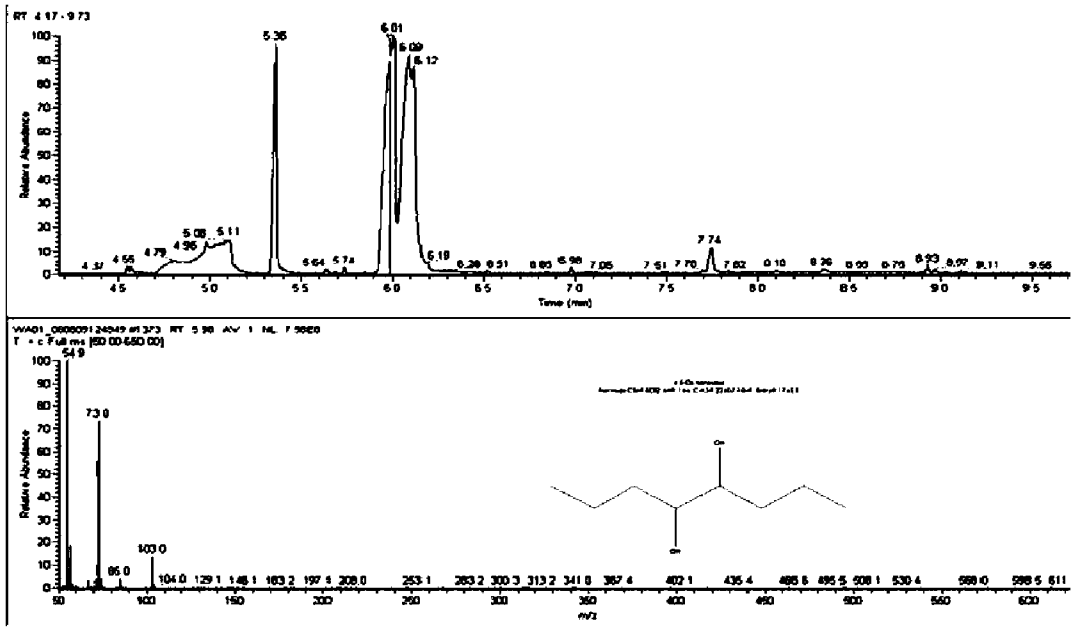
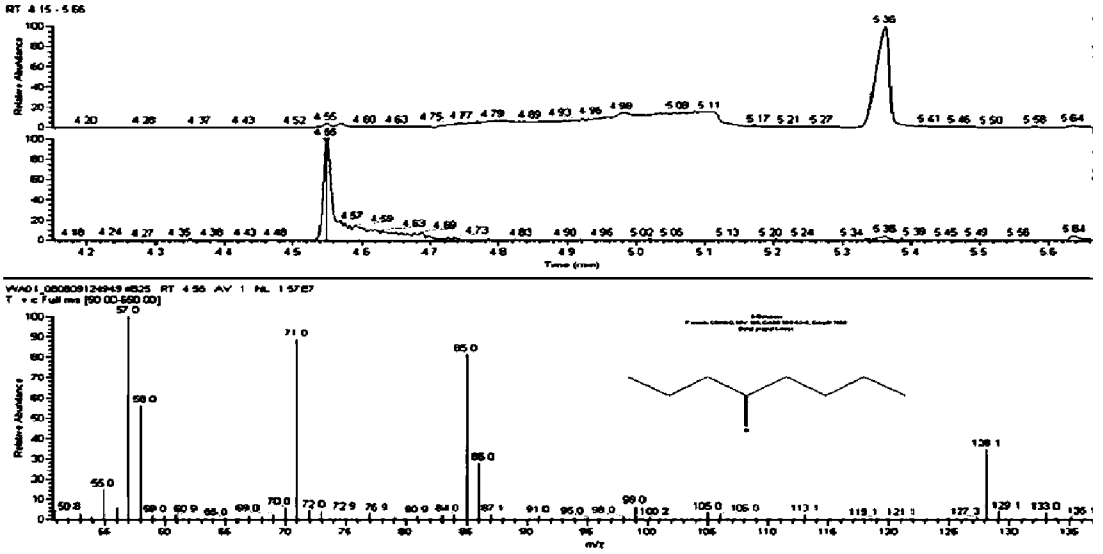


Figure 26B

GC-MS data confirming the presence of 4-octanone in the sample extraction

Sample Solution – Peak at 4.55 identified as 4-octanone



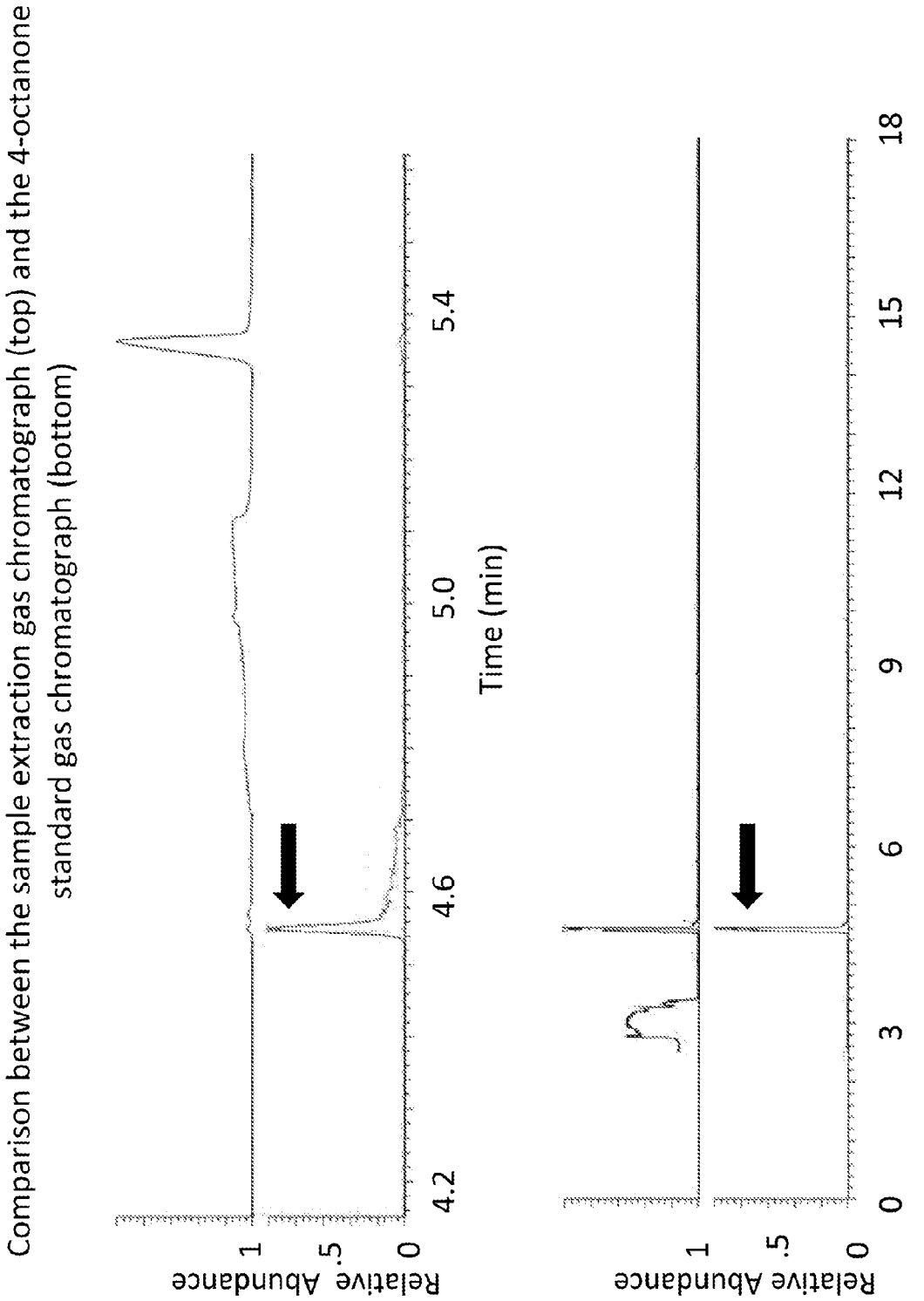

Comparison between the sample extraction gas chromatograph (top) and the 4-octanone standard gas chromatograph (bottom)
Relative Abundance
1
.5
0
4.2
4.6
5.0
5.4
Time (min)
Relative Abundance
1
.5
0
0
3
6
9
12
15
18
Time (min)

FIG. 27A

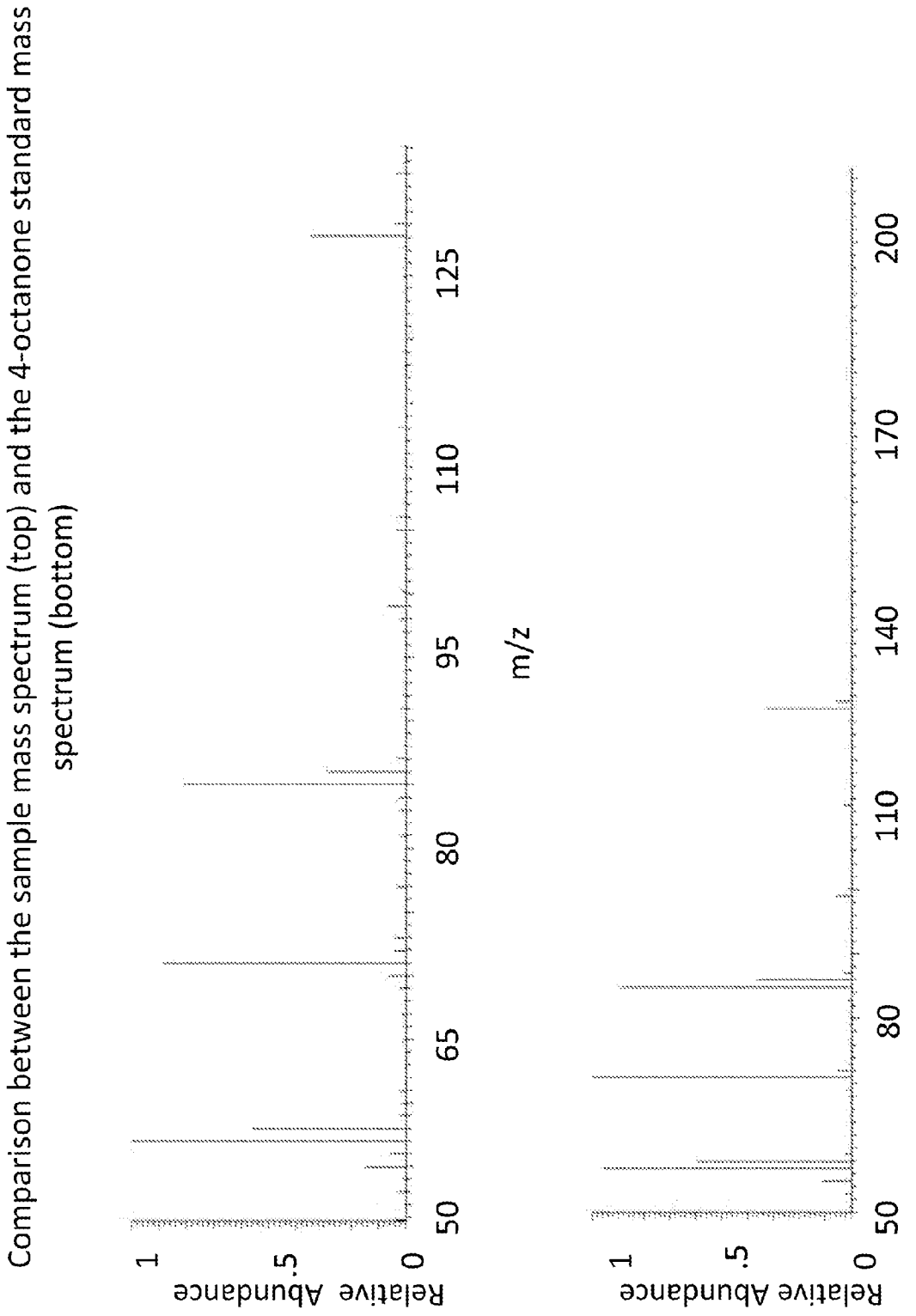

Comparison between the sample mass spectrum (top) and the 4-octanone standard mass spectrum (bottom)
Relative Abundance
1
.5
0
50
65
80
95
110
125
m/z
Relative Abundance
1
.5
0
50
80
110
140
170
200
m/z

FIG. 27B

Figure 28A

Nucleotide sequence of diol dehydratase large subunit (*pduC*) isolated from *Klebsiella pneumoniae* MGH78578

ATGAGATCGAAAAGATTTGAAGCACTGGCGAAACGCCCTGTGAATCAGGATGGTTTCGTTAAGGA
GTGGATTGAAGAGGGCTTTATCGCGATGGAAAGCCCTAACGATCCCAAACCTTCTATCCGCATCG
TCAACGGCGCGGTGACCGAACTCGACGATAAACCGGTTGAGCAGTTCGACCTGATTGACCACTTT
ATCGCGCGCTACGGCATTAATCTCGCCCGGGCCGAAGAAGTGATGGCCATGGATTCGGTTAAGCT
CGCCAACATGCTCTGCGACCCGAACGTTAAACGCAGCGACATCGTGCCGCTCACTACCGCGATGA
CCCCGGCGAAAATCGTGGAAGTGGTGTCGCATATGAACGTGGTCGAGATGATGATGGCGATGCAA
AAAATGCGCGCCCGCCGCACGCCGTCCCAGCAGGCGCATGTCACTAATATCAAAGATAATCCGGT
ACAGATTGCCGCCGACGCCGCTGAAGGCGCATGGCGCGGCTTTGACGAGCAGGAGACCACCGTCG
CCGTGGCGCGCTACGCGCCGTTCAACGCCATCGCCCTGCTGGTCGGTTCACAGGTTGGCCGCCCC
GGCGTCCTCACCCAGTGTTCGCTGGAAGAAGCCACCGAGCTGAAACTGGGCATGCTGGGCCACAC
CTGCTATGCCGAAACCATTTCGGTATACGGTACGGAACCGGTGTTTACCGATGGCGATGACACCC
CGTGGTCGAAAGGCTTCCTCGCCTCCTCCTACGCCTCGCGCGGCCTGAAAATGCGCTTTACCTCC
GGTTCCGGCTCGGAGGTGCAGATGGGCTATGCCGAAGGCAAATCGATGCTTTATCTCGAAGCGCG
CTGCATCTACATCACCAAAGCCGCCGGGGTGCAAGGCCTGCAGAATGGCTCCGTCAGCTGTATCG
GCGTGCCGTCCGCCGTGCCGTCCGGGATCCGCGCCGTACTGGCGGAAAACCTGATCTGCTCAGCG
CTGGATCTGGAGTGCGCCTCCAGCAACGATCAAACCTTTACCCACTCGGATATGCGGCGTACCGC
GCGTCTGCTGATGCAGTTCCTGCCAGGTACCGACTTTATCTCCTCCGGTTACTCGGCGGTGCCGA
ACTACGACAACATGTTCGCCGGTTCCAACGAAGATGCCGAAGACTTCGATGACTACAACGTGATC
CAGCGCGACCTGAAGGTCGATGGCGGCCTGCGGCCGGTGCGTGAAGAGGACGTGATCGCCATTCG
CAACAAAGCCGCCCGCGCGCTGCAGGCGGTATTTGCCGGCATGGGTTTGCCGCCTATTACGGATG
AAGAAGTAGAAGCCGCCACCTACGCCCACGGTTCAAAAGATATGCCTGAGCGCAATATCGTCGAG
GACATCAAGTTTGCTCAGGAGATCATCAACAAGAACCGCAACGGCCTGGAGGTGGTGAAAGCCCT
GGCGAAAGGCGGCTTCCCCGATGTCGCCCAGGACATGCTCAATATTCAGAAAGCCAAGCTCACCG
GCGACTACCTGCATACCTCCGCCATCATTGTTGGCGAGGGCCAGGTGCTCTCGGCCGTGAATGAC
GTGAACGATTATGCCGGTCCGGCAACAGGCTACCGCCTGCAAGGCGAGCGCTGGGAAGAGATTAA
AAATATCCCGGGCGCGCTCGATCCCAATGAACTTGGCTAA (SEQ ID NO:103)

Figure 28B

Polypeptide sequence of diol dehydratase large subunit was isolated from *Klebsiella pneumoniae* MGH78578 (*pduC*)

MRSKRFEALAKRPVNQDGFVKEWIEEGFIAMESPNDPKPSIRIVNGAVTELDDKPVEQFDLIDHF
IARYGINLARAEEVMAMDSVKLANMLCDPNVKRSDIVPLTTAMTPAKIVEVVSHMNVVEMMMAMQ
KMRARRTPSQQAHVTNIKDNPVQIAADAAEGAWRGFDEQETTVAVARYAPFNAIALLVGSQVGRP
GVLTQCSLEEATELKLGMLGHTCYAETISVYGTEPVFTDGDDTPWSKGFLASSYASRGLKMRFTS
GSGSEVQMGYAEGKSMLYLEARCIYITKAAGVQGLQNGSVSCIGVPSAVPSGIRAVLAENLICSA
LDLECASSNDQTFTHSDMRRTARLLMQFLPGTDFISSGYSAVPNYDNMFAGSNEDAEDFDDYNVI
QRDLKVDGGLRPVREEDVIAIRNKAARALQAVFAGMGLPPITDEEVEAATYAHGSKDMPERNIVE
DIKFAQEIINKNRNGLEVVKALAKGGFPDVAQDMLNIQKAKLTGDYLHTSAIIVGEGQVLSAVND
VNDYAGPATGYRLQGERWEEIKNIPGALDPNELG (SEQ ID NO:104)

Figure 29A

Nucleotide sequence of diol dehydratase medium subunit isolated from *Klebsiella pneumoniae* MGH78578 (*pduD*)

ATGGAAATTAACGAAACGCTGCTGCGCCAGATTATCGAAGAGGTGCTGTCGGAGATGAAATCAGG
CGCAGATAAGCCGGTCTCCTTTAGCGCGCCTGCGGCTTCTGTCGCCTCTGCCGCGCCGGTCGCCG
TTGCGCCTGTGTCCGGCGACAGCTTCCTGACGGAAATCGGCGAAGCCAAACCCGGCACGCAGCAG
GATGAAGTCATTATTGCCGTCGGGCCAGCGTTTGGTCTGGCGCAAACCGCCAATATCGTCGGCAT
TCCGCATAAAAATATTCTGCGCGAAGTGATCGCCGGCATTGAGGAAGAAGGCATCAAAGCCCGGG
TGATCCGCTGCTTTAAGTCTTCTGACGTCGCCTTCGTGGCAGTGGAAGGCAACCGCCTGAGCGGC
TCCGGCATCTCGATCGGTATTCAGTCGAAAGGCACCACCGTCATCCACCAGCGCGGCCTGCCGCC
GCTTTCCAATCTGGAACTCTTCCCGCAGGCGCCGCTGCTGACGCTGGAAACCTACCGTCAGATTG
GCAAAAACGCCGCGCGCTACGCCAAACGCGAGTCGCCGCAGCCGGTGCCGACGCTTAACGATCAG
ATGGCTCGTCCCAAATACCAGGCGAAGTCGGCCATTTTGCACATTAAAGAGACCAAATACGTGGT
GACGGGCAAAAACCCGCAGGAACTGCGCGTGGCGCTTTAA (SEQ ID NO:105)

Figure 29B

Polypeptide sequence of diol dehydratase medium subunit isolated from *Klebsiella pneumoniae* MGH78578 (*pduD*)

MEINETLLRQIIEEVLSEMKSGADKPVSFSAPAASVASAAPVAVAPVSGDSFLTEIGEAKPGTQQ
DEVIIAVGPAFGLAQTANIVGIPHKNILREVIAGIEEEGIKARVIRCFKSSDVAFVAVEGNRLSG
SGISIGIQSKGTTVIHQRGLPPLSNLELFPQAPLLTLETYRQIGKNAARYAKRESPQPVPTLNDQ
MARPKYQAKSAILHIKETKYVVTGKNPQELRVAL (SEQ ID NO:106)

Figure 29C

Nucleotide sequence of diol dehydratase small subunit isolated from *Klebsiella pneumoniae* MGH78578 (*pduE*)

ATGAATACCGACGCAATTGAATCCATGGTACGCGACGTGCTGAGCCGGATGAACAGCCTACAGGA
CGGGATAACGCCCGCGCCAGCCGCGCCGACAAACGACACCGTTCGCCAGCCAAAAGTTAGCGACT
ACCCGTTAGCGACCCGCCATCCGGAGTGGGTCAAAACCGCTACCAATAAAACGCTCGATGACCTG
ACGCTGGAGAACGTATTAAGCGATCGCGTTACGGCGCAGGACATGCGCATCACTCCGGAAACGCT
GCGTATGCAGGCGGCGATCGCCCAGGATGCCGGACGCGATCGGCTGGCGATGAACTTTGAGCGGG
CCGCAGAGCTCACCGCGGTTCCCGACGACCGAATCCTTGAGATCTACAACGCCCTGCGCCCATAC
CGTTCCACCCAGGCGGAGCTACTGGCGATCGCTGATGACCTCGAGCATCGCTACCAGGCACGACT
CTGTGCCGCCTTTGTTCGGGAAGCGGCCGGGCTGTACATCGAGCGTAAGAAGCTGAAAGGCGACG
ATTAA (SEQ ID NO:107)

Figure 29D

Polypeptide sequence of diol dehydratase small subunit isolated from *Klebsiella pneumoniae* MGH78578 (*pduE*)

MNTDAIESMVRDVLSRMNSLQDGITPAPAAPTNDTVRQPKVSDYPLATRHPEWVKTATNKTLDDL
TLENVLSDRVTAQDMRITPETLRMQAAIAQDAGRDRLAMNFERAAELTAVPDDRILEIYNALRPY
RSTQAELLAIADDLEHRYQARLCAAFVREAAGLYIERKKLKGDD (SEQ ID NO:108)

Figure 30A

Oxidation of 4-octanol monitored by NADH production (2ADH 1-10)

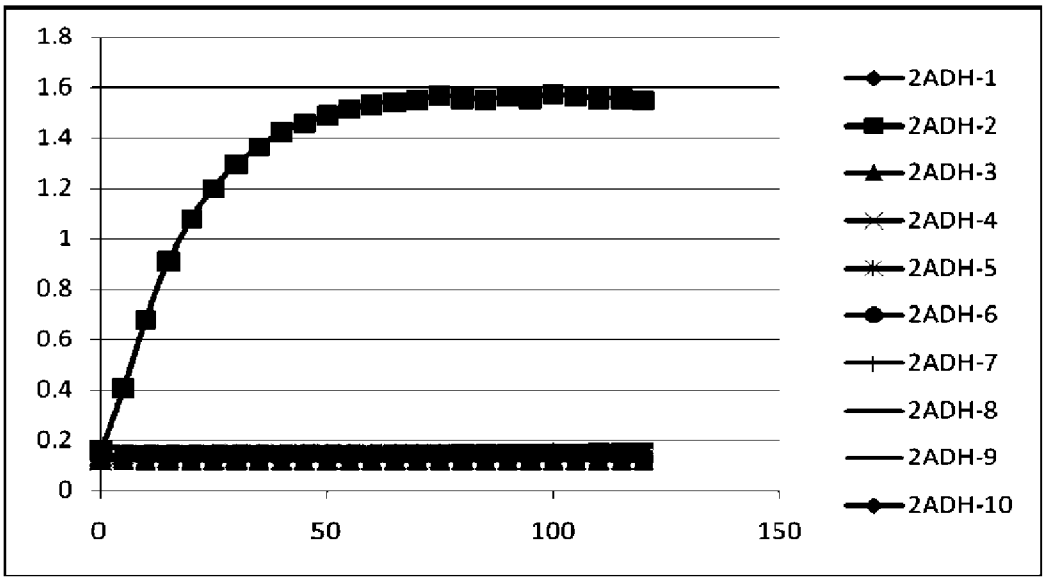


Figure 30B

Oxidation of 4-octanol monitored by NADPH production (2ADH 1-10)

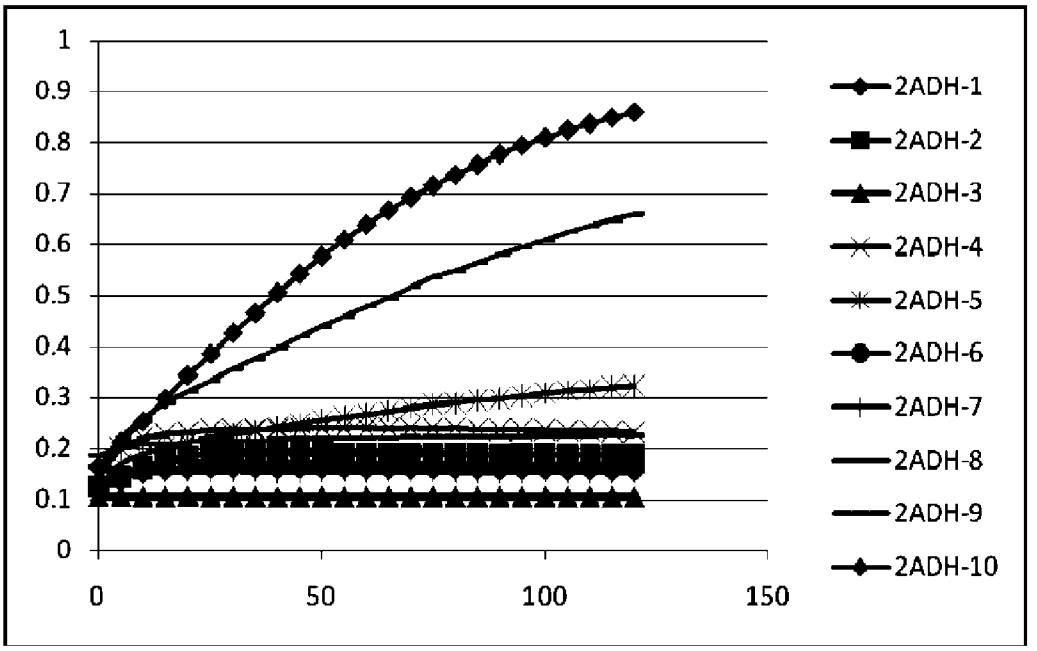


Figure 31A

Oxidation of 4-octanol monitored by NADH production (2ADH11-18)

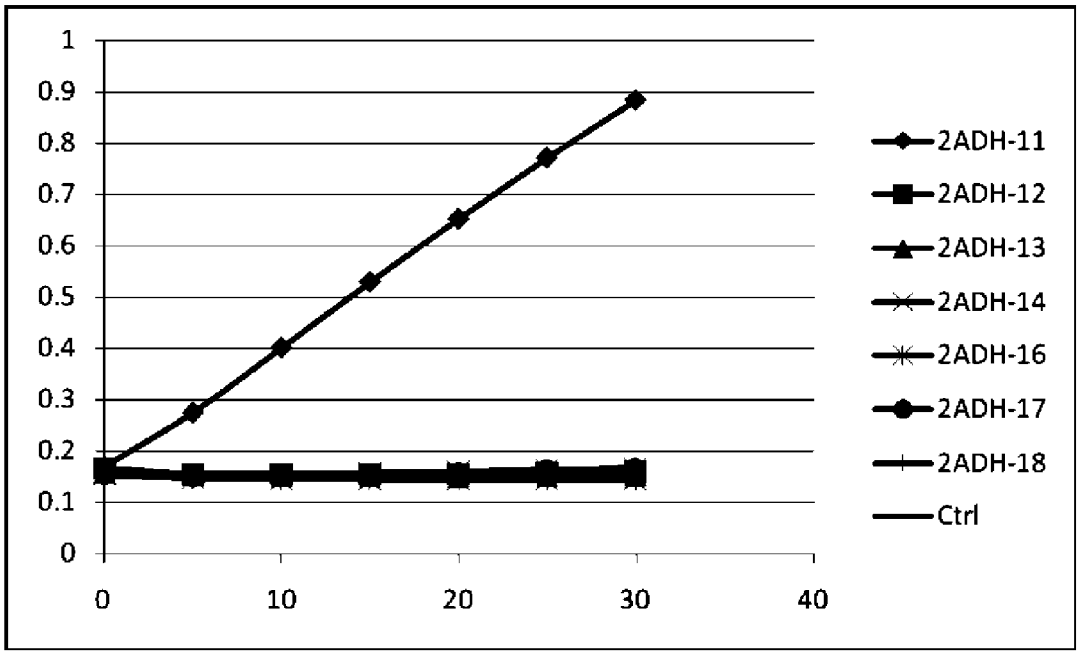


Figure 31B

Oxidation of 4-octanol monitored by NADPH production (2ADH 11-18)

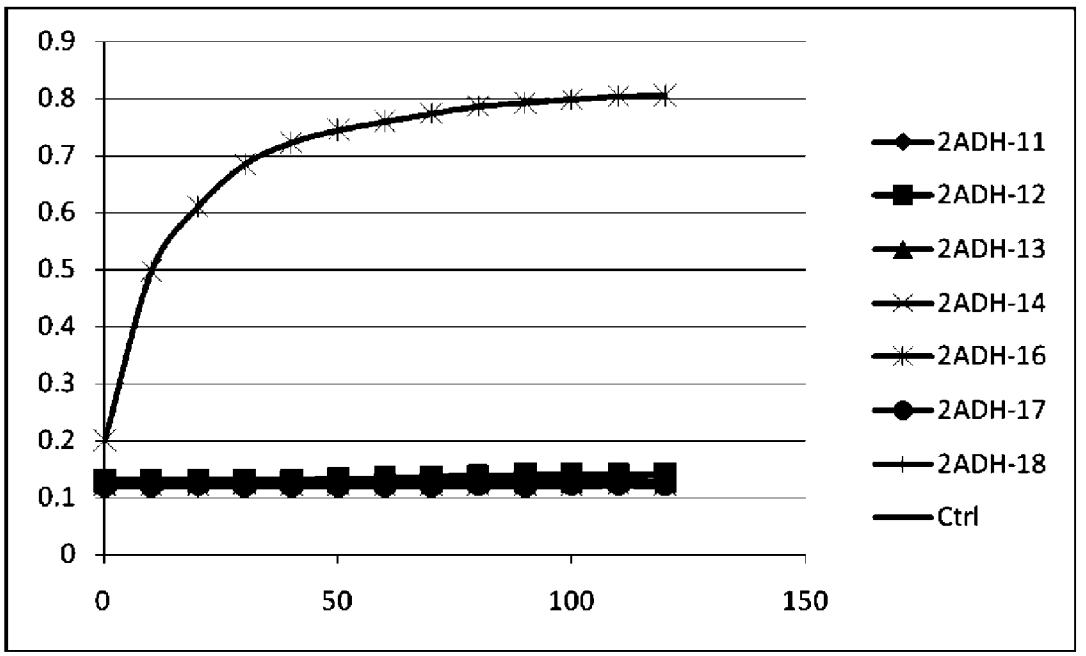


Figure 32A

Oxidation of 2,7-dimethyl octanol monitored by NADH production (2ADH 11-18)

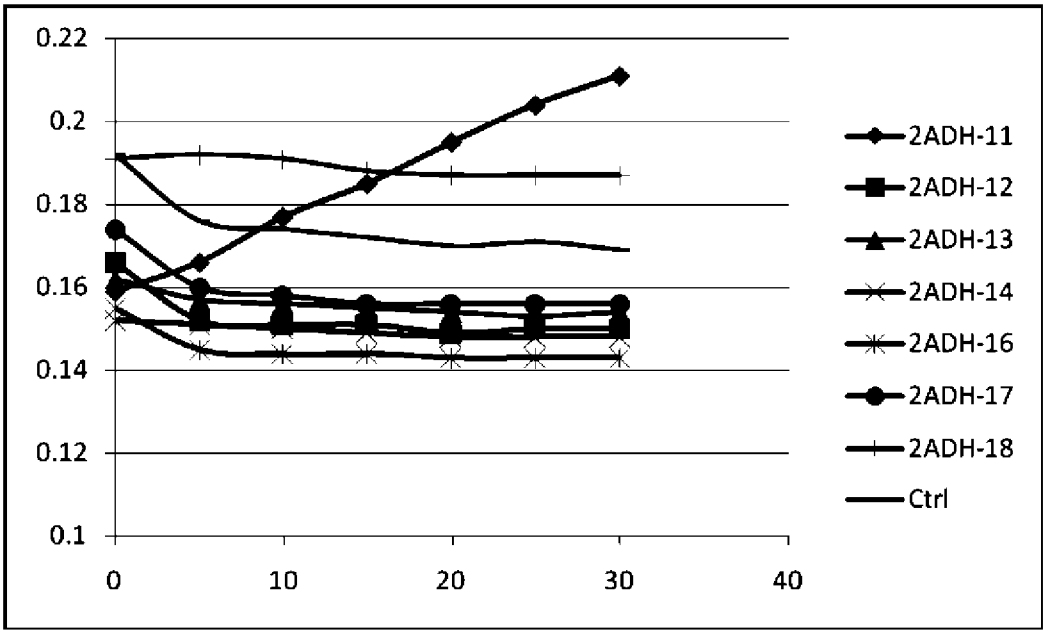


Figure 32B

Oxidation of 2,7-dimethyl octanol monitored by NADPH production (2ADH 11-18)

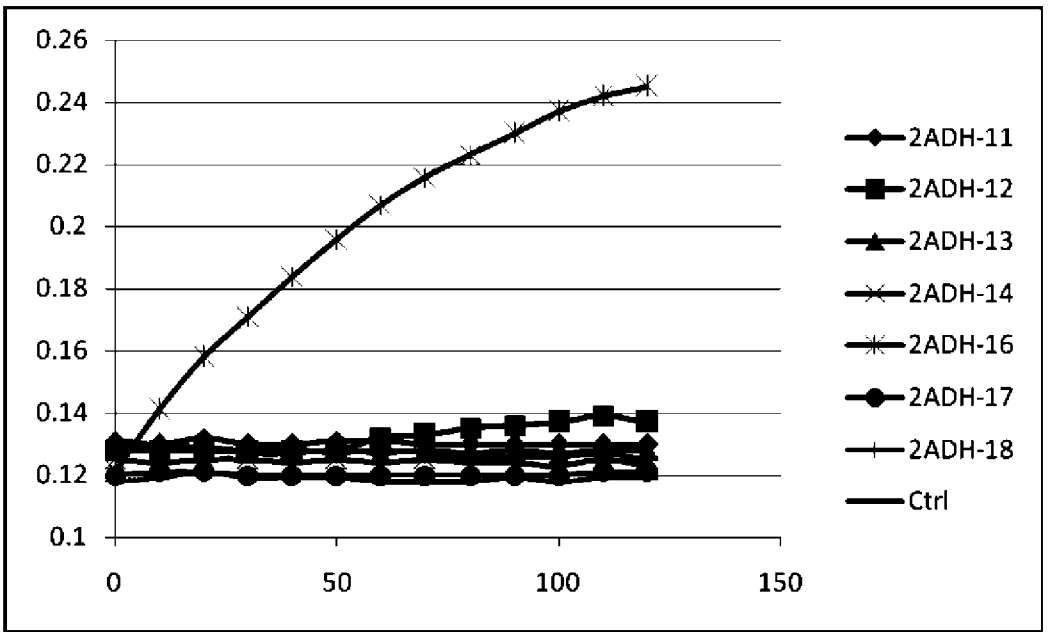


Figure 33A

Reduction of of 2,7-dimethyl octanol monitored by NADPH consumption

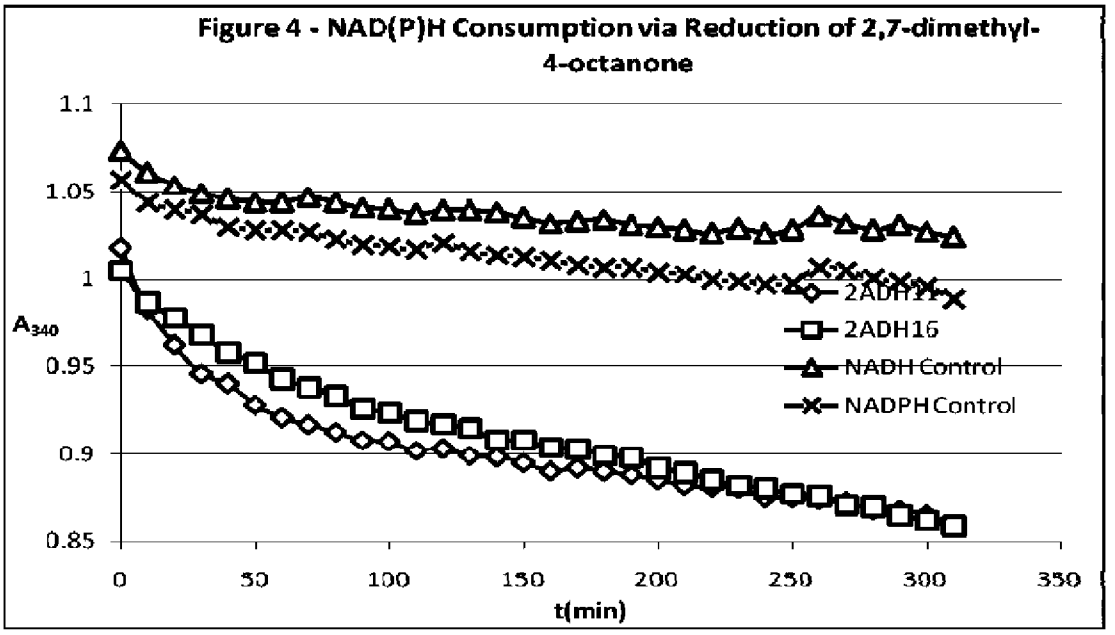


Figure 33B

Activity of 2ADH11 and 2ADH16 Towards Various Substrates

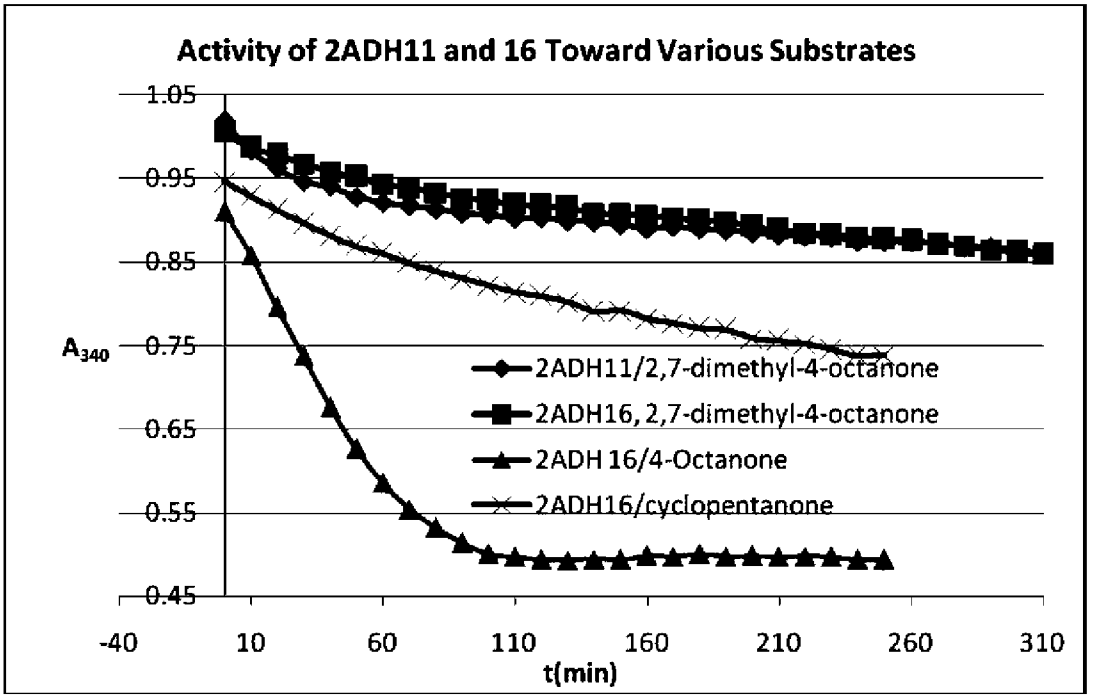


Figure 34A

Oxidation of cyclopentanol catalyzed by 2ADH as monitored by NADH or NADPH formation

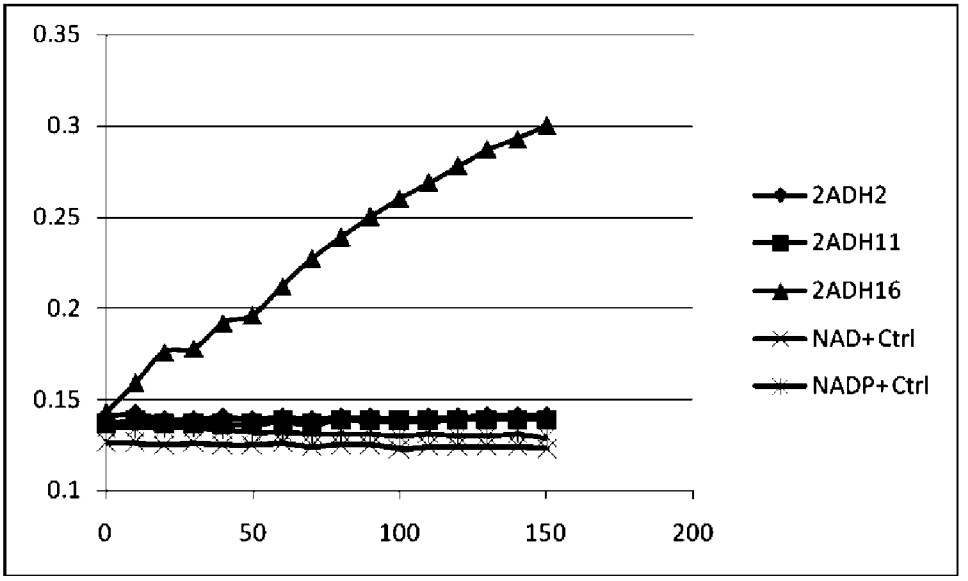


Figure 34B

Reduction of cyclopentanonone catalyzed by 2ADH as monitored by NADPH consumption

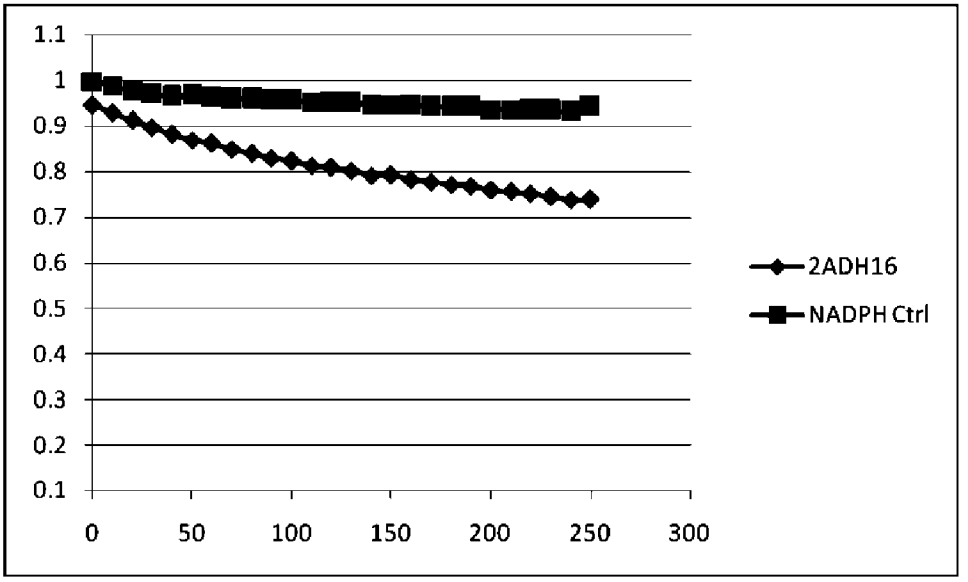
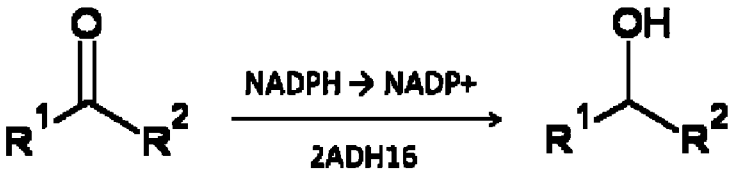
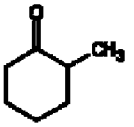


Figure 35
Calculated Rate Constants for Reduction Reactions of 2ADH16

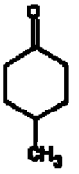


- Reduction Reaction

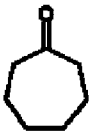
- 2-methylcyclohexanone: k_{cat}/K_M = 211 $sec^{-1}M^{-1}$ +/- 18



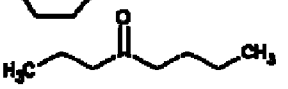
- 4-methylcyclohexanone: k_{cat}/K_M – 17.5 $sec^{-1}M^{-1}$ +/- 2.0



- cycloheptanone: k_{cat}/K_M = 4.08 – $sec^{-1}M^{-1}$ +/- 0.62



- 4-octanone: k_{cat}/K_M = 44.7 – $sec^{-1}M^{-1}$ +/- 3.7



-4-decanone: k_{cat}/K_M approx 150 $sec^{-1}M^{-1}$

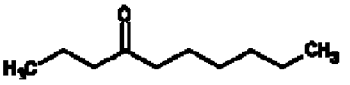
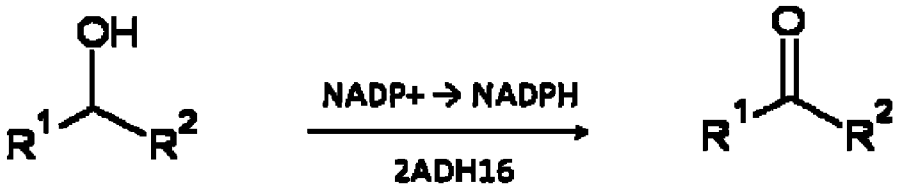


Figure 36

Calculated Rate Constants for Oxidation Reactions of 2ADH16



- **Oxidation Reaction**
- 4-octanol: k_{cat}/K_M = 1430 $sec^{-1}M^{-1}$ +/- 200
- 4-decanol: k_{cat}/K_M = 1260 $sec^{-1}M^{-1}$ +/- 290
- cycloheptanol: k_{cat}/K_M – 198 $sec^{-1}M^{-1}$ +/- 19
- 3-hexanol: k_{cat}/K_M = 148 $sec^{-1}M^{-1}$ +/- 16
- 2-methyl-3-octanol: k_{cat}/K_M = 123 $sec^{-1}M^{-1}$ +/- 16
- 5-methyl-3-hexanol: k_{cat}/K_M – 17.9 $sec^{-1}M^{-1}$ +/- 3.5
- 2,5-dimethyl -3-hexanol: k_{cat}/K_M = 1.55 $sec^{-1}M^{-1}$ +/- .24
- 3-methyl-2-pentanol: k_{cat}/K_M = 8.54 $sec^{-1}M^{-1}$ +/- 2.04
- 2-methylcyclohexanol: k_{cat}/K_M – 97.4 $sec^{-1}M^{-1}$ +/- 14.4
- 4-methylcyclohexanol: k_{cat}/K_M = 10.4 $sec^{-1}M^{-1}$ +/- 21.1

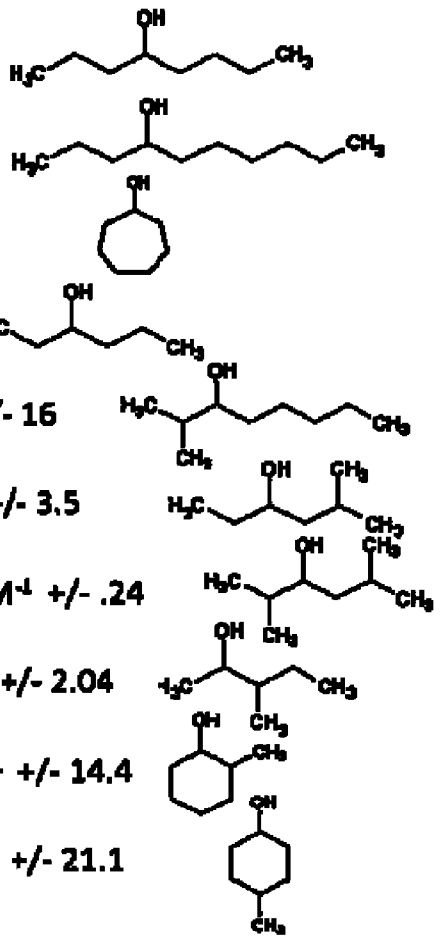


Figure 37A
Alginate Lyases

Protein	Organism	GenBank/ GenPept	
Family 5			
alginate lyase (AlgL)	Azotobacter chroococcum ATCC 4412	AJ223605	CAA11481.1
alginate lyase (AlgL)	Azotobacter vinelandii	AF027499	AAC04567.1
		AF037600	AAC32313.1
alginate lyase (Alg)	Cobetia marina N-1	AB018795	BAA33966.1
alginate lyase (AlgL)	Pseudomonas aeruginosa 8830	L14597	AAA71990.1
alginate lyase (AlgL)	Pseudomonas aeruginosa FRD1	U27829	AAA91127.1
alginate lyase (AlgL;PA3547)	Pseudomonas aeruginosa PAO1	AE004775	AAG06935.1
		NC_002516	NP_252237.1
alginate lyase (AlgL)	Pseudomonas sp. QD03	AY380832	AAR23929.1
alginate lyase (AlgL)	Pseudomonas sp. QDA	AY163384	AAN63147.1
alginate lyase (AlgL)	Pseudomonas syringae pv. syringae FF5	AF222020	AAF32371.1
alginate lyase (aly;A1-I/PolyG+PolyM;A1-II/PolyG;A1-III/PolyM)	Sphingomonas sp. A1	-	2009330A
		AB011415	BAB03312.1
Family 6			
alginate lyase (AlyP)	Pseudomonas sp. OS-ALG-9	D10336	BAA01182.1
Family 7			
guluronate lyase (alyPG)	Corynebacterium sp. ALY-1	AB030481	BAA83339.1
poly(-L-guluronate) lyase (AlyA)	Klebsiella pneumoniae subsp. aerogenes	L19657	AAA25049.1
alginate lyase / poly-mannuronate lyase (AlxM)	Photobacterium sp. ATCC 43367	X70036	CAA49630.1
alginate lyase (PA1167)	Pseudomonas aeruginosa PAO1	AE004547	AAG04556.1
		NC_002516	NP_249858.1
alginate lyase (A1-II')	Sphingomonas sp. A1	AB120939	BAD16656.1
alginate lyase (aly;A1-I/PolyG+PolyM;A1-II/PolyG;A1-III/PolyM)	Sphingomonas sp. A1	-	2009330A
		AB011415	BAB03312.1

Figure 37B
Alginate Lyases

Protein	Organism	GenBank	GenPept
Family 7			
poly(a-L-guluronate) lyase (AlyVGI;AlyVG1)	Vibrio halioticoli IAM14596T	AF114039	AAF22512.1
alginate lyase / poly-mannuronate lyase (AlyVOA)	Vibrio sp. O2	DQ235160	ABB36771.1
alginate lyase / poly-mannuronate lyase (AlyVOB)	Vibrio sp. O2	DQ235161	ABB36772.1
alginate lyase (AlyVI)	Vibrio sp. QY101	AY221030	AAP45155.1
exo-oligoalginate lyase (HdAlex;HdAlex-1)	Haliotis discus hannai	AB234872	BAE81787.1
alginate lyase (HdAly)	Haliotis discus hannai	AB110094	BAC87758.1
polysaccharide lyase acting on glucuronic acid (vAL-1)	Chlorella virus CVK2	AB044791	BAB19127.1
alginate lyase (AlyII)	Pseudomonas sp. OS-ALG-9	AB003330	BAA19848.1
Family18			
alginate lyase	Pseudoalteromonas sp. 272		
alginate lyase (Aly)	Pseudoalteromonas sp. IAM14594	AF082561	AAD16034.1
Family15			
exotype alginate lyase (Atu3025)	Agrobacterium tumefaciens str. C58	AE009232	AAL43841.1
		NC_003305	NP_533525.1
exotype alginate lyase (AGR_L_3558p)	Agrobacterium tumefaciens str. C58 (Cereon)	AE008381	AAK90358.1
		NC_003063	NP_357573.1
oligo alginate lyase (A1-IV)	Sphingomonas sp. A1	AB011415	BAB03319.1
alginate lyase (A1-IV')	Sphingomonas sp. A1	AB176667	BAD90006.1

Figure 38A
Pectate Lyases

Protein	Organism	GenBank/GenPept	
Family1			
pectate lyase	Bacillus agaradhaerens	-	AAE59745.1
		-	AAS29292.1
pectate lyase	Bacillus halodurans	-	AAE59748.1
		-	AAS29295.1
pectate lyase (BH3819)	Bacillus halodurans C-125	AP001520	BAB07538.1
		NC_002570	NP_244686.1
pectate lyase (BH0698)	Bacillus halodurans C-125	AP001509	BAB04417.1
		NC_002570	NP_241564.1
pectate lyase (PelA)	Bacillus licheniformis 14A	-	AAE59746.1
		-	AAN26179.1
		AJ517194	CAD56882.1
		-	AAS29293.1
BLi04129 or BL00947 (PelII)	Bacillus licheniformis DSM 13 ATCC 14580	CP000002	AAU25568.1
		AE017333	AAU42942.1
pectate lyase (Pel)	Bacillus licheniformis RN1	AB428424	BAG12908.1
pectate lyase (PelB)	Bacillus pumilus DKS1	EU652988	ACD11362.1
pectate lyase (fragment)	Bacillus sp. KSM-P7	AB015043	BAA76884.1
pectate lyase	Bacillus sp. AAI12	-	AAE59747.1
		-	AAS29294.1
pectate lyase	Bacillus sp. I534	-	AAE59749.1
		-	AAR65348.1
		-	AAS29296.1
pectate lyase (Pel-103)	Bacillus sp. KSM-P103	AB015044	BAA76885.1
pectate lyase (Pel-34K)	Bacillus sp. P-2850	AB080666	BAC11008.1
pectate lyase (Pel-4A)	Bacillus sp. P-4-N	AB041769	BAA96477.1
pectate lyase (Pel-4B)	Bacillus sp. P-4-N	AB042100	BAA96478.1
pectate lyase (Pl47)	Bacillus sp. TS-47	AB045986	BAB40336.1
pectate lyase (PelK)	Bacillus sp. YA-14	D26349	BAA05383.1
pectate lyase	Bacillus subtilis	AX601431	CAD67509.1
		AX601436	CAD67510.1
		AX601448	CAD67511.1
		AX951870	CAF05441.1
pectate lyase (Pel)	Bacillus subtilis AC327	D86417	BAA22313.1

Figure 38B
Pectate Lyases

Protein	Organism	GenBank	GenPept
pectin lyase (Ppr)	Bacillus subtilis IFO 3134	D83791	BAA12119.1
pectate lyase (Pel)	Bacillus subtilis SO113	X74880	CAA52866.1
pectate lyase (Pel;BSU07560)	Bacillus subtilis subsp. subtilis str. 168	-	AAR45489.1
		D86417	BAA22313.1
		X74880	CAA52866.1
		Z99108	CAB12585.1
		NC_000964	NP_388637.1
pectate lyase (Pel-1;Pel1)	Erwinia carotovora 71	L32171	AAA73933.1
Pel9.5 (fragment)	Erwinia carotovora EC14	X61088	CAA43402.1
pectin lyase (Pnl) (probable fragment)	Erwinia carotovora ER	M65057	AAA24857.1
pectate lyase 1 (Pel1;PelI)	Erwinia carotovora ER / IAM1068 / atroseptica EC / atroseptica C18	M18859	AAA24845.1
		S51490	AAC60423.1
		D00217	BAA00155.1
		X81847	CAA57439.1
pectate lyase 2 (Pel2;PelII)	Erwinia carotovora ER / IAM1068 / atroseptica EC / atroseptica C18	M17364	AAA24848.1
		S51475	AAC60422.1
		X81847	CAA57440.1
ECA4067 (PelA)	Erwinia carotovora subsp. atroseptica SCRI1043	BX950851	CAG76964.1
pectate lyase (PelZ)	Erwinia chrysanthemi 3937	X97119	CAA65785.1
pectate lyase A (PelA)	Erwinia chrysanthemi 3937	M77808	AAA24846.1
pectate lyase B (PelB)	Erwinia chrysanthemi 3937	X67475	CAA47821.1
			S25262
pectate lyase (PelD)	Erwinia chrysanthemi 3937	AJ132101	CAA10570.1
pectate lyase E (PelE)	Erwinia chrysanthemi 3937 / B374	M33584	AAA24854.1
		X17284	CAA35175.1
pectate lyase D (PelD)	Erwinia chrysanthemi B374	X17284	CAA35176.1
pectate lyase (PelA)	Erwinia chrysanthemi EC16	M14509	AAA24843.1
pectate lyase (PelC)	Erwinia chrysanthemi EC16	M19411	AAA24849.1
		-	AAR45490.1
		-	AAW11900.1
pectate lyase (PelB;PlB)	Erwinia chrysanthemi EC16	M14510	AAA24847.1
pectate lyase (PelE)	Erwinia chrysanthemi EC16	M14509	AAA24844.1

Figure 38C
Pectate Lyases

Protein	Organism	GenBank	GenPept
pectate lyase C (PelC)	Erwinia chrysanthemi strain 3937	AJ132325	CAA10642.1
pectin lyase (PnlA)	Pectobacterium carotovorum Ecc71	M59909	AAA24856.1
pectate lyase III (Pel3;PelC)	Pectobacterium carotovorum Er	D10064	BAA00953.1
pectate lyase B (PelB)	Pseudoalteromonas haloplanktis 505	AF278705	AAF86343.1
		AF278705	AAF86343.2
pectate lyase A	Pseudoalteromonas haloplanktis ANT/505	AF278706	AAF86344.2
pectate lyase (Pel)	Pseudomonas fluorescens CY091	L41673	AAA93535.1
		L38902	AAB46399.1
pectin lyase (PnL) (fragment)	Pseudomonas marginalis N6301	M84971	AAA92512.1
		D32121	BAA06847.1
pectate lyase (PeL)	Pseudomonas marginalis N6301	S65042	AAC60448.1
		D32122	BAA06848.1
pectate lyase P (PelP)	Pseudomonas syringae pv. lachrymans	U75414	AAB17879.1
pectate lyase (Pel;Pstru-4)	Pseudomonas viridiflava	L38901	AAB46398.1
		L38574	AAC41521.1
		DQ273695	ABB55454.1
		D44611	BAA08077.1
pectate lyase (Pel)	Pseudonocardia sp.	AF002241	AAC38059.1
pectate lyase (SCO2821;SCBAC17F8.12c)	Streptomyces coelicolor A3(2)	AL596030	CAC44284.1
		NC_003888	NP_627050.1
pectate lyase (SCO1880;SCI39.27c)	Streptomyces coelicolor A3(2)	AL591322	CAC38815.1
		NC_003888	NP_626147.1
α pectate lyase A (PelA;TM0433)	Thermotoga maritima MSB8	AE001722	AAD35518.1
		NC_000853	NP_228243.1
XC_1298	Xanthomonas campestris pv. campestris str. 8004	CP000050	AAY48367.1
XC_3590	Xanthomonas campestris pv. campestris str. 8004	CP000050	AAY50632.1
pectate lyase (Pel;XCC0645)	Xanthomonas campestris pv. campestris str. ATCC 33913	AE012162	AAM39961.1
		NC_003902	NP_636037.1
pectate lyase II (PelB;XCC2815)	Xanthomonas campestris pv. campestris str. ATCC 33913	AE012393	AAM42087.1
		NC_003902	NP_638163.1

Figure 38D
Pectate Lyases

Protein	Organism	GenBank	GenPept
pectate lyase (PelB;Pl;Pstru-3)	Xanthomonas campestris pv. malvacearum strain B414	L38573	AAC41522.1
pectin lyase (AN2331.2)	Aspergillus nidulans FGSC A4	DQ490478	ABF50854.1
		AACD01000038	EAA64442.1
pectin lyase (AN2569.2)	Aspergillus nidulans FGSC A4	AACD01000043	EAA64674.1
		DQ490480	ABF50856.1
pectate lyase (PelA;AN0741.2)	Aspergillus nidulans FGSC A4	U05592	AAA80568.1
		DQ490468	ABF50844.1
		EF452421	ABO38859.1
		AACD01000012	EAA65383.1
pectate lyase (AN7646.2)	Aspergillus nidulans FGSC A4	AACD01000130	EAA61832.1
		DQ490513	ABF50889.1
pectin lyase A (PelA) - **Pl1A**	Aspergillus niger CBS 120.49 / N400	X55784	CAA39305.1
		X60724	CAA43130.1
pectin lyase C (PelC)	Aspergillus niger CBS 120.49 / N400	AY839647	AAW03313.1
pectin lyase F (PelF)	Aspergillus niger CBS 120.49 / N400	AJ489943	CAD34589.1
pectate lyase A (PlyA)	Aspergillus niger CBS 120.49 / N400	AJ276331	CAC33162.1
pectin lyase B (PelB)	Aspergillus niger CBS 120.49 / N400	A12248	CAA01023.1
		X65552	CAA46521.1
An14g04370 (PelA)	Aspergillus niger CBS 513.88	AM270321	CAK48529.1
An03g00190 (PelB)	Aspergillus niger CBS 513.88	AM270043	CAK37997.1
An15g07160 (PelF)	Aspergillus niger CBS 513.88	AM270351	CAK48551.1
An19g00270 (PelD)	Aspergillus niger CBS 513.88	AM270415	CAK47350.1
pectate lyase I (PlyA;An10g00870)	Aspergillus niger CBS 513.88	AM270216	CAK40523.1
pectin lyase D (PelD)	Aspergillus niger N756	M55657	AAA32701.1
pectin lyase 2 (Pel2)	Aspergillus oryzae KBN616	AB029323	BAB82468.1
pectin lyase 1 (Pel1)	Aspergillus oryzae KBN616	AB029322	BAB82467.1
pectin lyase 1 (Pel1;AO090010000504)	Aspergillus oryzae RIB 40	EF452419	ABO38857.1
		AP007175	BAE66352.1
pectin lyase 2 (Pel2;AO090010000030)	Aspergillus oryzae RIB 40	AP007175	BAE65949.1

Figure 38E
Pectate Lyases

Protein	Organism	GenBank	GenPept
pectate lyase (PelB)	Colletotrichum gloeosporioides	AF052632	AAD09857.1
pectin lyase (PnlA)	Colletotrichum gloeosporioides	L22857	AAA21817.1
pectate lyase 2 (Pel-2)	Colletotrichum gloeosporioides f. sp. malvae	AF156985	AAD43566.1
pectin lyase (Pnl1;Pnl-1)	Colletotrichum gloeosporioides f. sp. malvae	AF158256	AAF22244.1
pectin lyase 2 (Pnl2;Pnl-2)	Colletotrichum gloeosporioides f. sp. malvae	AF156984	AAD43565.1
pectate lyase 1 (Pel-1)	Colletotrichum gloeosporioides f. sp. malvae	AF156983	AAD43564.1
pectate lyase (LLP-52)	Lilium longiflorum	L18911	AAA33398.1
		EF026017	ABM68553.1
		Z17328	CAA78976.1
pectate lyase (PelI;PI1;MwPI1;Ban17)	Musa acuminata Williams	AF206319	AAF19195.1
		DQ663594	ABG74583.1
		X92943	CAA63496.1
pectate lyase	Nicotiana tabacum	X61102	CAA43414.1
		X67158	CAA47630.1
		X67159	CAA47631.1
pectate lyase	Zinnia elegans	Y09541	CAA70735.1
		AX005936	CAC05181.1

Figure 39A
Rhamnogalacturonases

Protein	**Organism**	**GenBank/GenPept**	
rhamnogalacturonate lyase (RhiE)	Erwinia chrysanthemi 3937	AJ438339	CAD27359.1
rhamnogalacturonan lyase (RhgB)	Aspergillus aculeatus KSM 510	L35500	AAA64368.1
rhamnogalacturonan lyase (AN6395.2)	Aspergillus nidulans FGSC A4	AACD01000108	EAA58417.1
		DQ490501	ABF50877.1
rhamnogalacturonan lyase (AN7135.2)	Aspergillus nidulans FGSC A4	AACD01000122	EAA61387.1
		DQ490504	ABF50880.1
rhamnogalacturonan lyase (YesW;BSU07050)	Bacillus subtilis subsp. subtilis str. 168	Z99107	CAB12524.1
		NC_000964	NP_388586.1
exo-unsaturated rhamnogalacturonan lyase (YesX;BSU07060)	Bacillus subtilis subsp. subtilis str. 168	Z99107	CAB12525.1
		NC_000964	NP_388587.1
rhamnogalacturonan lyase - **Rgl11A**	Cellvibrio japonicus (formerly Pseudomonas cellulosa)	AY026755	AAK20911.1
CJA_3559 (rhamnogalacturonan lyase) - **Rgl11A**	Cellvibrio japonicus Ueda107	CP000934.1	ACE83155.1
rhamnogalacturonan lyase Y - **Rgl11Y**	Clostridium cellulolyticum ATCC 35319	AF316823	AAG45161.1

Figure 39B
Rhamnogalacturonate Hydrolases

Protein	Organism	GenBank/GenPept	
GH family 105			
unsaturated rhamnogalacturonyl hydrolase (BSU30120; YteR)	Bacillus subtilis subsp. subtilis str. 168	Z99119	CAB14990.1
unsaturated rhamnogalacturonyl hydrolase (BSU07000; YesR)	Bacillus subtilis subsp. subtilis str. 168	Z99107	CAB12519.1
		NC_000964	NP_388581.1

Figure 40A
Pectate Methyl Esterases

Protein	Organism	GenBank/GenPept	
Family 8			
ECA3253 (PemA)	Erwinia carotovora subsp. atroseptica SCRI1043	BX950851	CAG76151.1
ECA0107 (PmeB)	Erwinia carotovora subsp. atroseptica SCRI1043	BX950851	CAG73027.1
pectin methylesterase b	Erwinia chrysanthemi 3937	X84665	CAA59151.1
pectin methylesterase A (PemA;Pem)	Erwinia chrysanthemi 3937 / B374	L07644	AAA24852.1
		-	AAR64146.1
		Y00549	CAA68628.1
pectate lyase A	Pseudoalteromonas haloplanktis ANT/505	AF278706	AAF86344.2
pectin methylesterase (Pme5; Vgd1;At2g47040/F14M4.13)	Arabidopsis thaliana	AC004411	AAC34240.1
		AY091768	AAM10316.1
		BT001120	AAN64511.1
		AY830948	AAV91508.1
		AJ250430	CAB58974.1
		NM_130272	NP_182227.1
pectin methylesterase (Pme1)	Aspergillus aculeatus	U49378	AAB42153.1
pectin methyl esterase (AN3390.2)	Aspergillus nidulans FGSC A4	DQ490489	ABF50865.1
		AACD01000055	EAA63358.1
pectin methylesterase (Pme1)	Aspergillus niger RH 5344	A34997	CAA02198.1
		A35006	CAA02201.1
		A35008	CAA02202.1
		X52902	CAA37084.1
		X54145	CAA38084.1
pectin methylesterase (PmeA)	Aspergillus oryzae KBN616	AB011211	BAA75474.1
pectin methylesterase (PmeA;AO090012000749)	Aspergillus oryzae RIB 40	AP007161	BAE60873.1
pectin methylesterase (Bcpme2)	Botryotinia fuckeliana Bd90	AJ428403	CAD21438.1
pectin methyl esterase (Bcpme1)	Botryotinia fuckeliana T4	AJ309701	CAC29255.1
pectin methylesterase 1.1 (PECS-1.1)	Citrus sinensis	U82973	AAB57667.1
		U82976	AAB57670.1

Figure 40B
Pectate Methyl Esterases

Protein	Organism	GenBank	GenPept
pectin methylesterase (PME1)	Cochliobolus carbonum	AF159252	AAD43340.1
pectin methylesterase	Daucus carota		
pectinesterase FaPE1	Fragaria x ananassa	AY324809	AAQ21124.1
pectin methyl-esterase (Pef1)	Medicago truncatula	AJ249611	CAB65291.1
pectin methyl-esterase (Per)	Medicago truncatula	AJ249611	CAB65290.2
pectin methylesterase	Nicotiana benthamiana	AY238968	AAO85706.1
pectin methylesterase	Nicotiana plumbaginifolia	Z71752	CAA96434.1
pectin methylesterase (NtPPME1)	Nicotiana tabacum	AY772945	AAX13972.1
pectin methylesterase	Nicotiana tabacum	AJ401158	CAB95025.1
pectin methylesterase (PME1) (fragment)	Orobanche cumana	AY072720	AAL66865.1
pectin methylesterase (fragment)	Orobanche cumana	AF333068	AAG49395.1
pectin methylesterase	Petunia inflata	L27101	AAA33714.1
pectin methyl esterase (PttPME1)	Populus tremula x Populus tremuloides	AJ277547	CAC01624.1
pectin methylesterase PME1 (fragment)	Prunus armeniaca	AF184079	AAG12248.1
pectin methylesterase (SgPME1)	Salix gilgiana	AB029461	BAA89480.1
pectin methylesterase	Sitophilus oryzae	AY841894	AAW28928.1
pectin methylesterase 2	Solanum lycopersicum	U50985	AAB67739.1
		-	AAQ71552.1
		A15983	CAA01257.1
		A17011	CAA01315.1
		X07910	CAA30746.1
		X74639	CAA52704.1
pectin methylesterase (PmeU1)	Solanum lycopersicum	U49330	AAD09283.1
		AY046596	AAL02367.1
pectin methylesterase 1 (PME1.9)	Solanum lycopersicum	U50986	AAB67740.1
		A17010	CAA01314.1
		X74638	CAA52703.1
pectin methyl esterase (Pest1)	Solanum tuberosum	AF152171	AAF23891.1
pectin methyl esterase (Pest2)	Solanum tuberosum	AF152172	AAF23892.1
pectin methylesterase isoform alpha (PME2) (fragment)	Vigna radiata	AF229849	AAF35897.1
pectin methylesterase (PME)	Vitis riparia	AF178989	AAD51853.1

Figure 41
Pectate Acetyl Esterases

Protein	Organism	GenBank/GenPept	
Family12			
acetyl xylan esterase (Rgae;BH1115)	Bacillus halodurans C-125	AP001511	BAB04834.1
		NC_002570	NP_241981.1
cephalosporin C deacetylase	Bacillus sp. KCCM10143	AF184175	AAF25818.1
acetyl xylan esterase (YesT;BSU07020)	Bacillus subtilis subsp. subtilis str. 168	Z99107	CAB12521.1
		NC_000964	NP_388583.1
ECA3252 (PaeY)	Erwinia carotovora subsp. atroseptica SCRI1043	BX950851	CAG76150.1
pectin acetylesterase (PaeY)	Erwinia chrysanthemi 3937	Y09828	CAA70971.1
rhamnogalacturonan acetylesterase (Rha1)	Aspergillus aculeatus KSM 510	X89714	CAA61858.1
rhamnogalacturonan acetylesterase (AN2528.2)	Aspergillus nidulans FGSC A4	DQ490479	ABF50855.1
		AACD01000043	EAA64633.1
pectin acetylesterase	Vigna radiata Wilzeck	X99348	CAA67728.1

Figure 42A

Production of 2-phenyl ethanol (24 hrs)

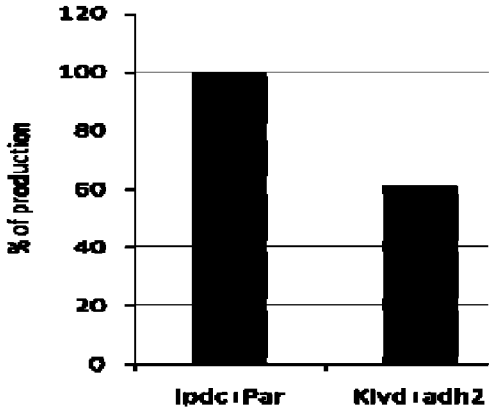


Figure 42B

Production of 2-(4-hydroxyphenyl) ethanol (24 hrs)

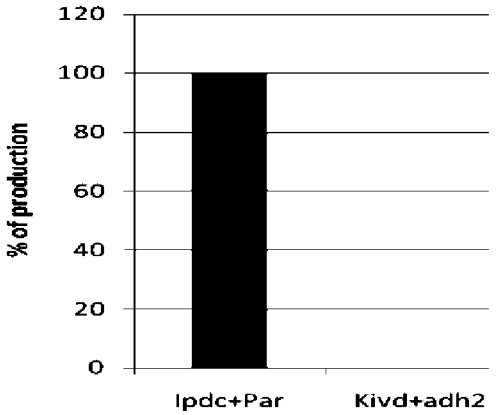


Figure 42C

Production of 2-(indole-3-)ethanol (24 hrs)

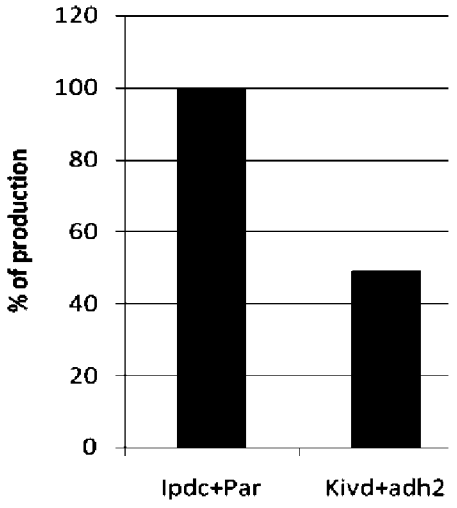


Figure 43A

GC-MS chromatogram for control (pBAD33 and pTrc99A) (one week)

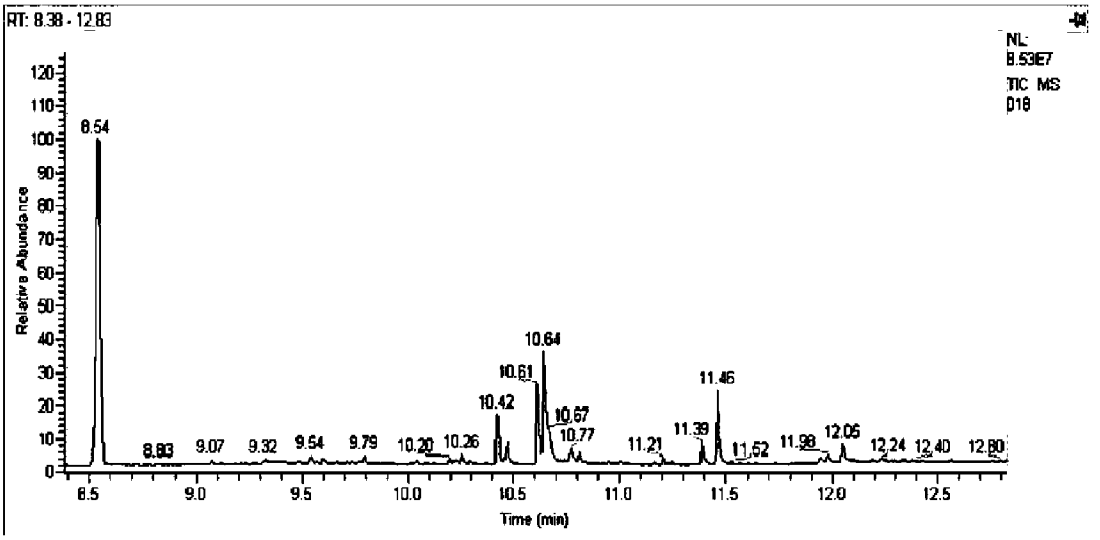


Figure 43B

GC-MS chromatogram for 2-phenylethanol (5.97 min) production from pBADpheA-aroLAC-aroG-tktA-aroBDE and pTrcBALK (one week)

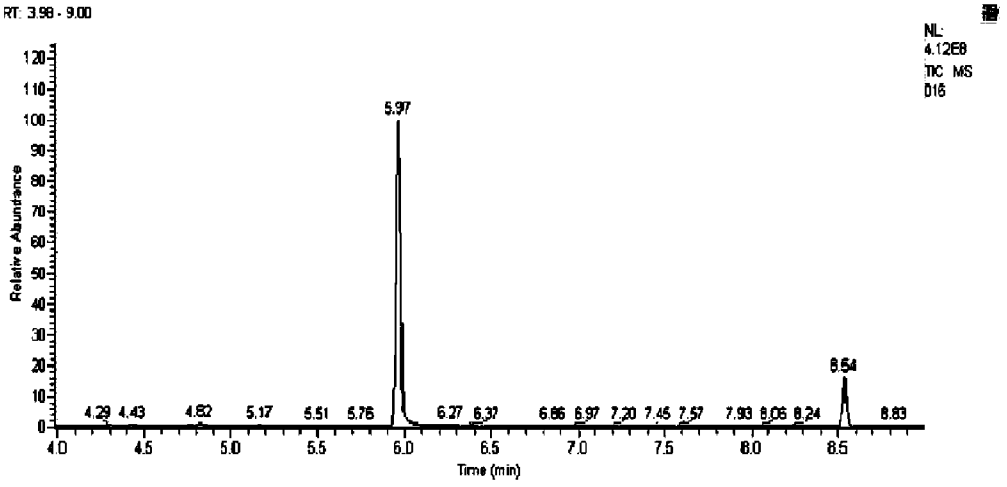
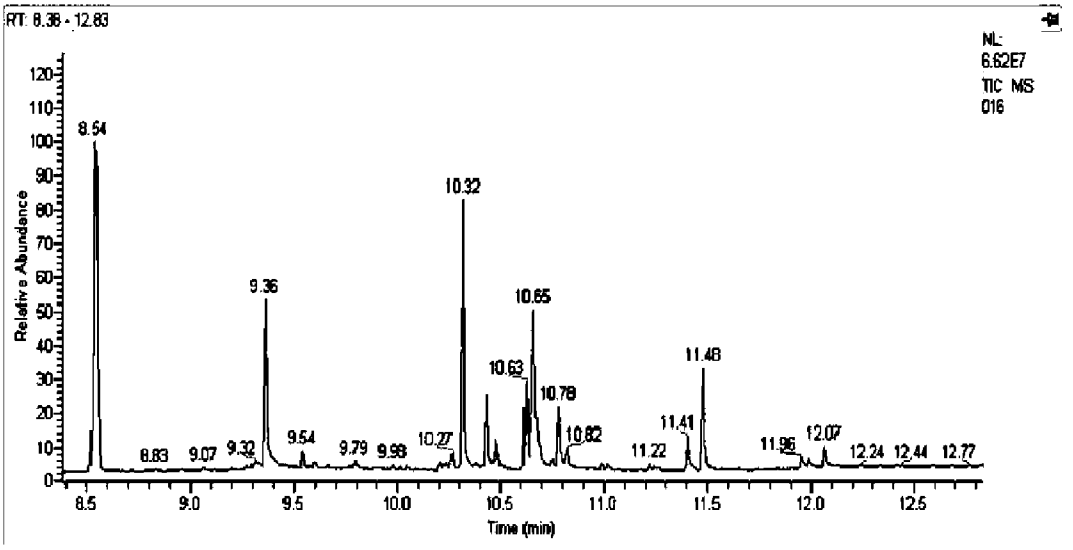


Figure 44

GC-MS chromatogram for 2-(4-hydroxyphenyl) ethanol (9.36 min) and 2-(indole-3) ethanol (10.32 min) production from pBADtyrA-aroLAC-aroG-tktA-aroBDE and pTrcBALK (one week)



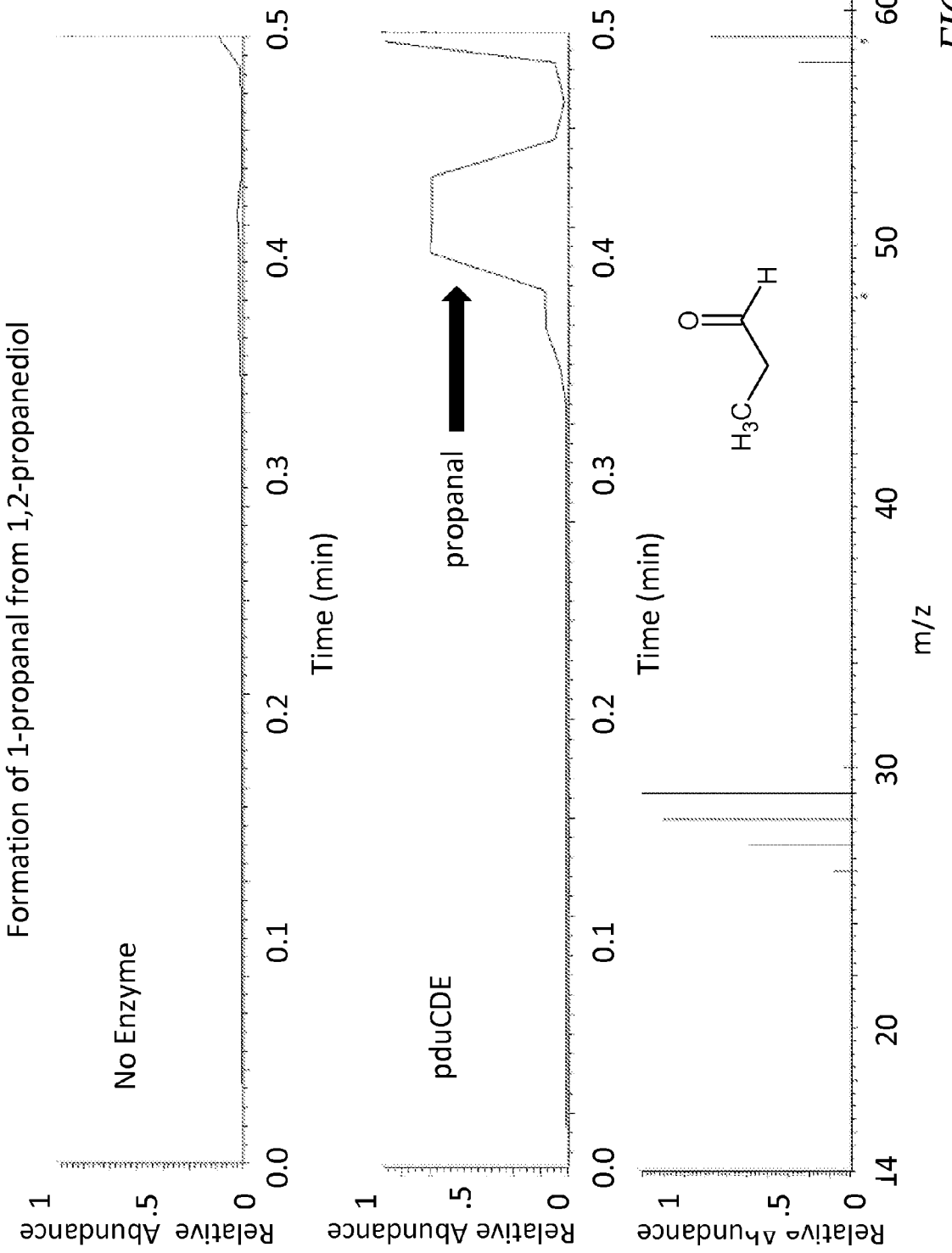

Formation of 1-propanal from 1,2-propanediol
No Enzyme
pduCDE
propanal
Relative Abundance
Time (min)
m/z
0.0
0.1
0.2
0.3
0.4
0.5
0
.5
1
20
30
40
50
60
O
H
H3C

FIG. 45

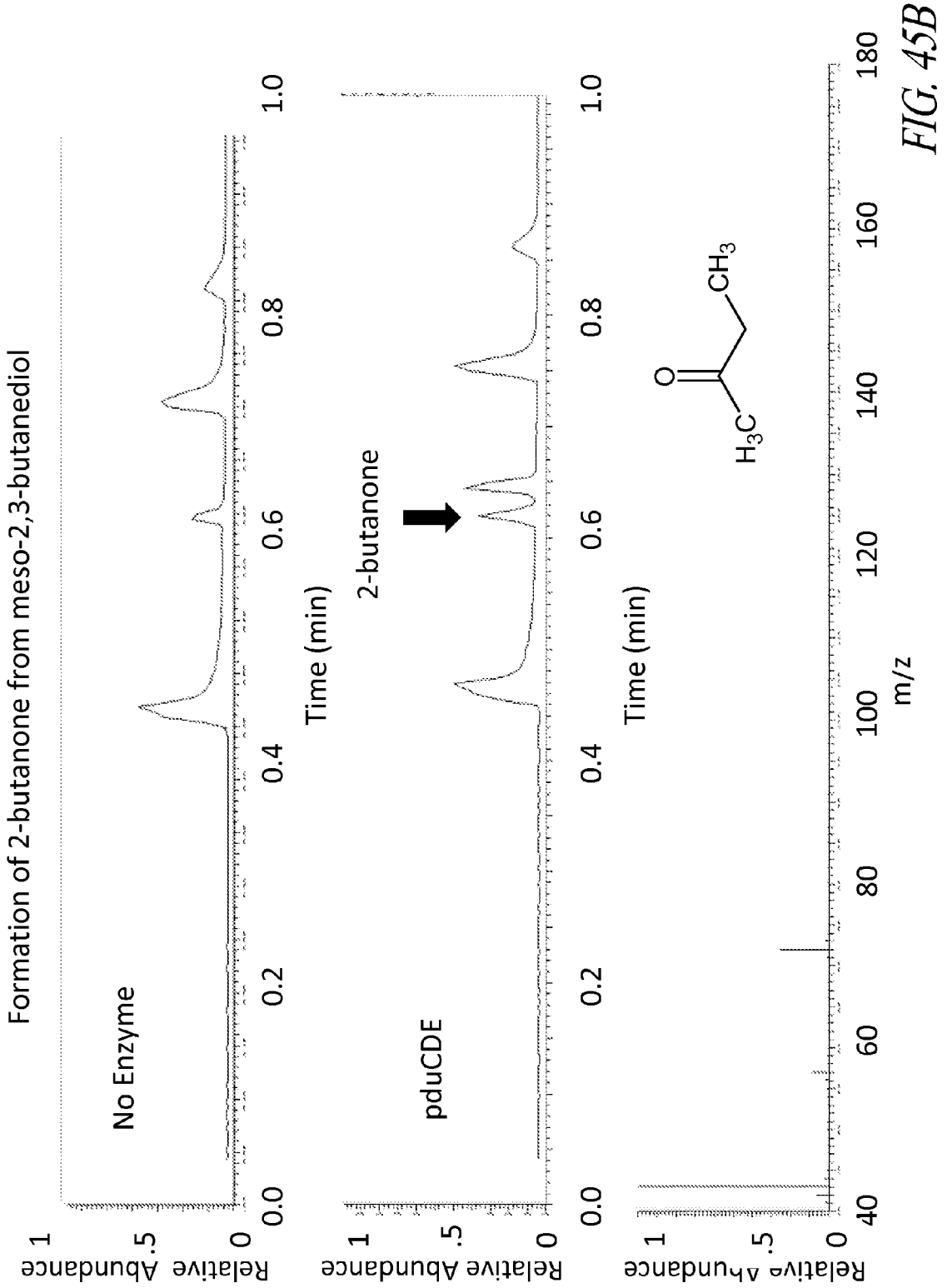

Formation of 2-butanone from meso-2,3-butanediol
No Enzyme
pduCDE
2-butanone
Relative Abundance
Time (min)
m/z
0.0
0.2
0.4
0.6
0.8
1.0
40
60
80
100
120
140
160
180
0
.5
1
CH_3
O
H_3C

FIG. 45B

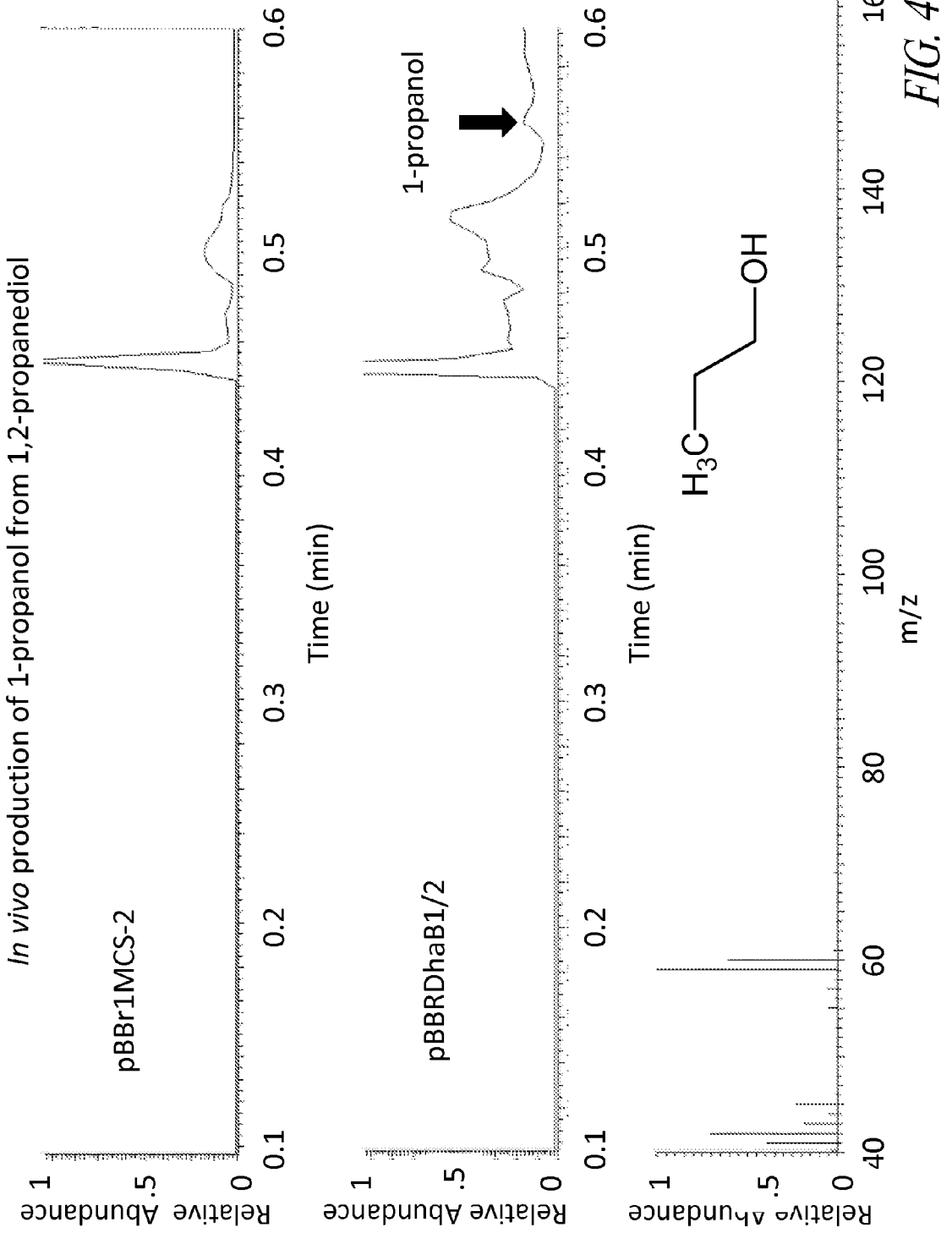

In vivo production of 1-propanol from 1,2-propanediol
pBBr1MCS-2
Relative Abundance
Time (min)
pBBRDhaB1/2
1-propanol
Relative Abundance
Time (min)
H3C
OH
Relative Abundance
m/z

FIG. 46A

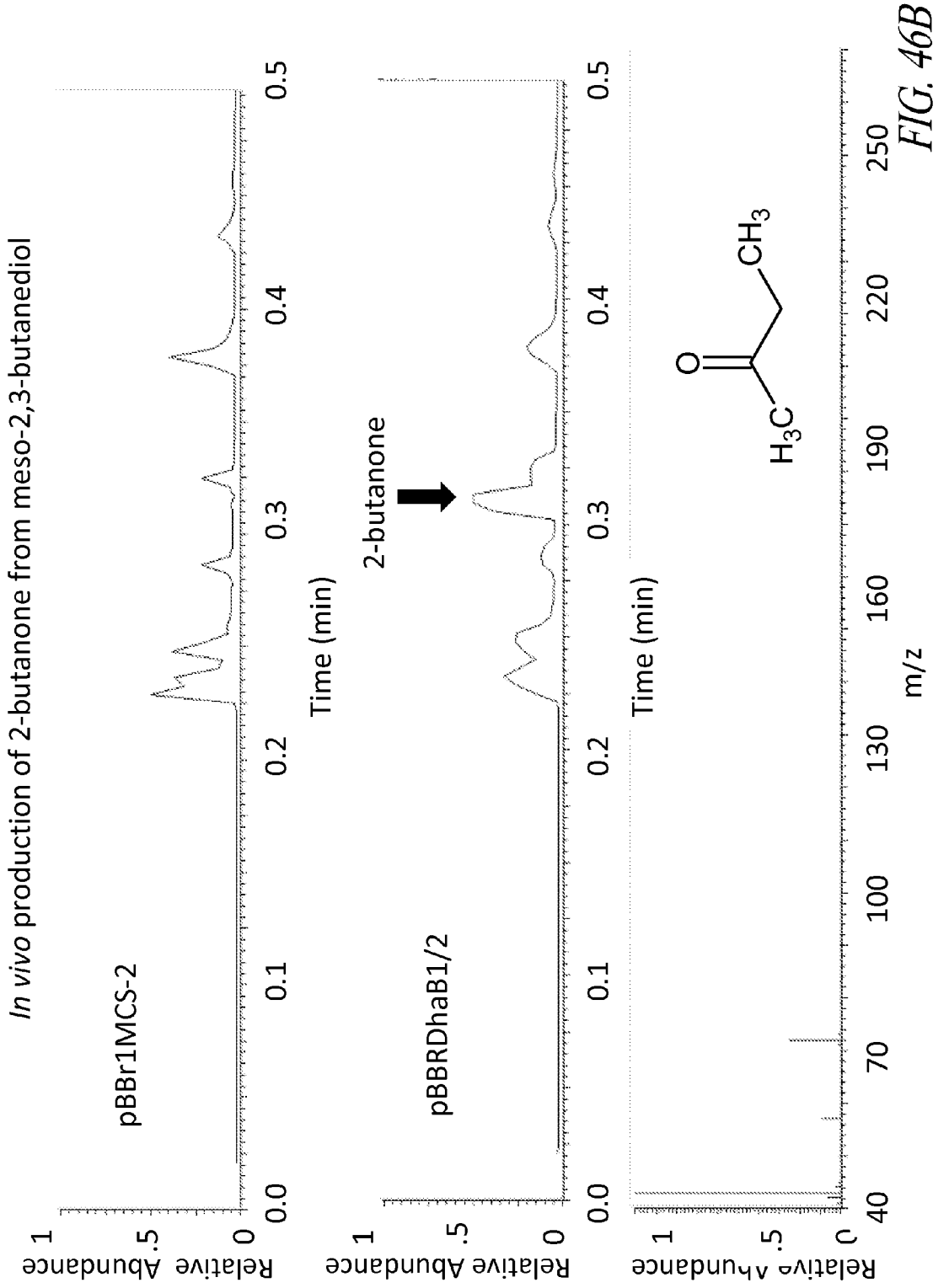

In vivo production of 2-butanone from meso-2,3-butanediol
pBBr1MCS-2
Relative Abundance
Time (min)
2-butanone
pBBRDhaB1/2
Relative Abundance
Time (min)
CH_3
O
H_3C
Relative Abundance
m/z

FIG. 46B

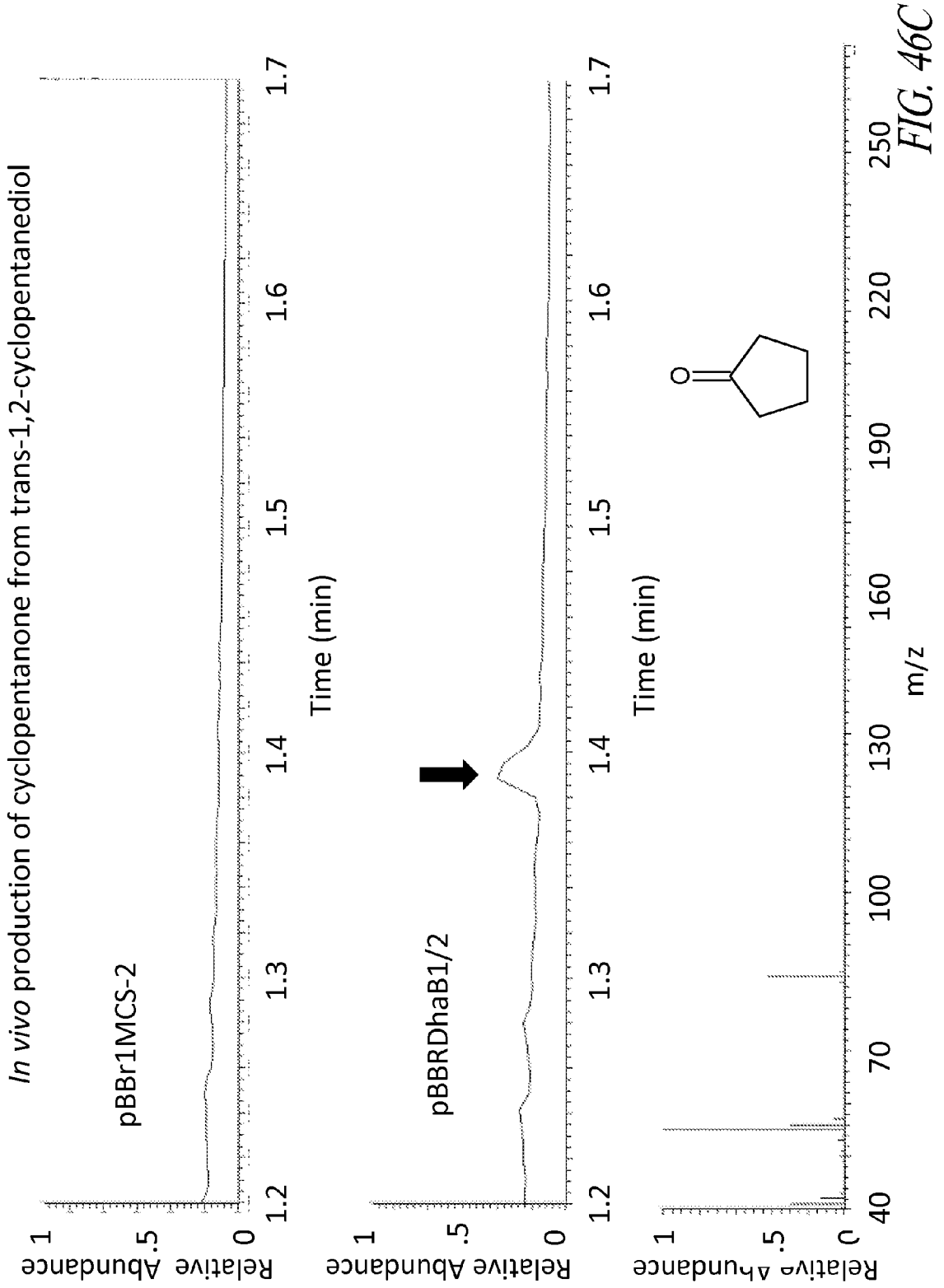

In vivo production of cyclopentanone from trans-1,2-cyclopentanediol
pBBr1MCS-2
pBBRDhaB1/2
Relative Abundance
Time (min)
m/z
O

FIG. 46C

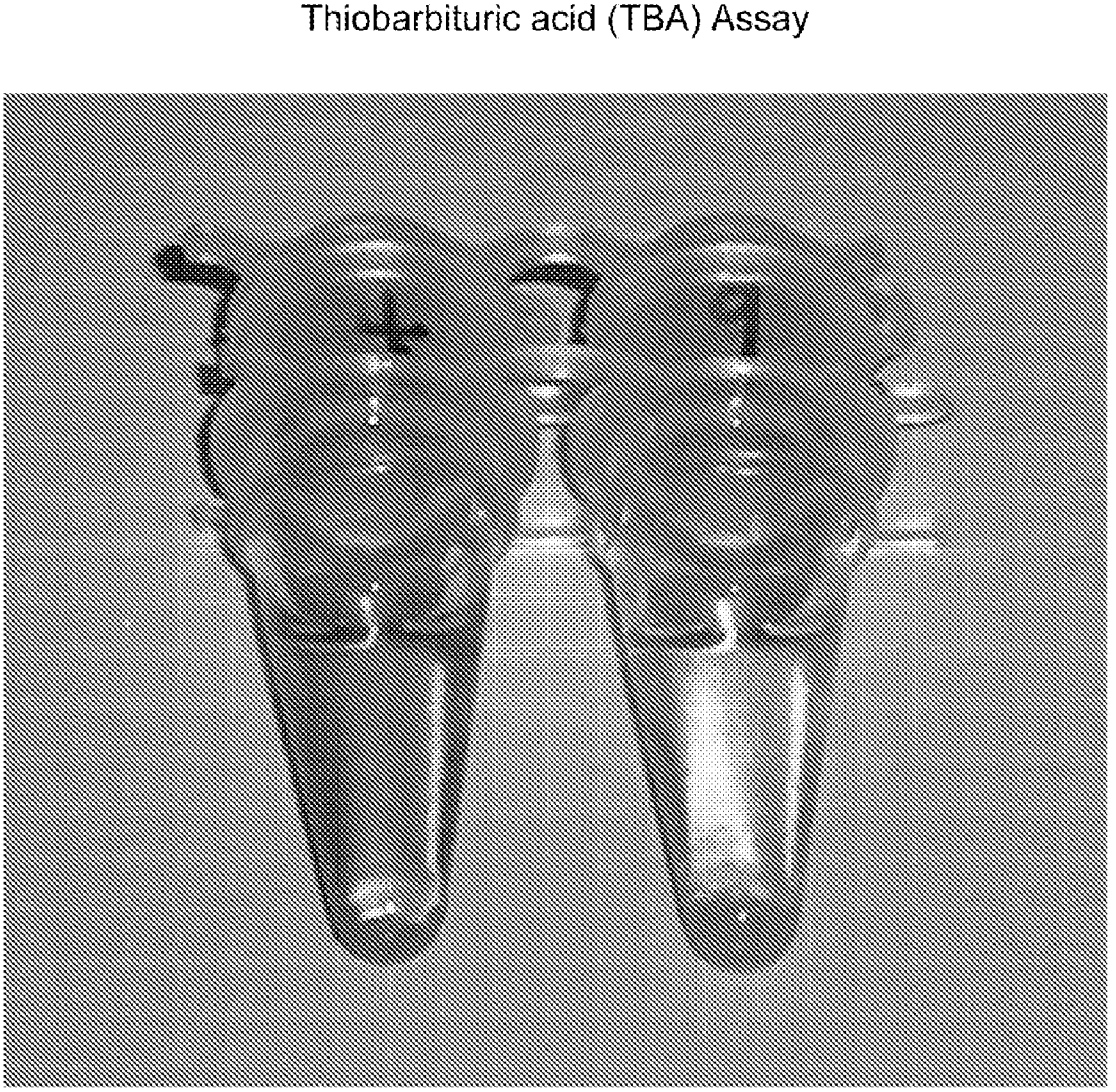


FIG. 47

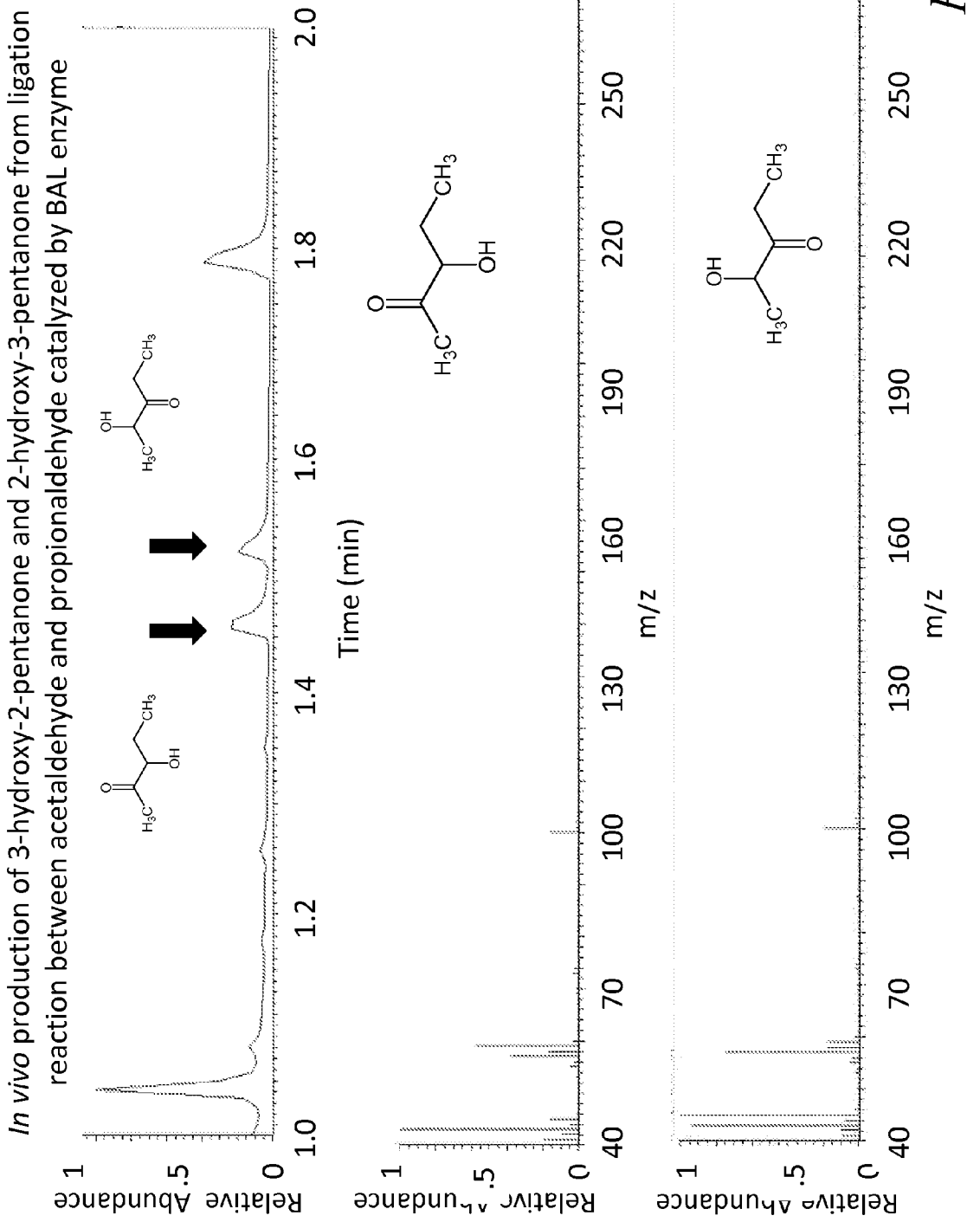

In vivo production of 3-hydroxy-2-pentanone and 2-hydroxy-3-pentanone from ligation reaction between acetaldehyde and propionaldehyde catalyzed by BAL enzyme
Relative Abundance
Time (min)
m/z
m/z

FIG. 48A

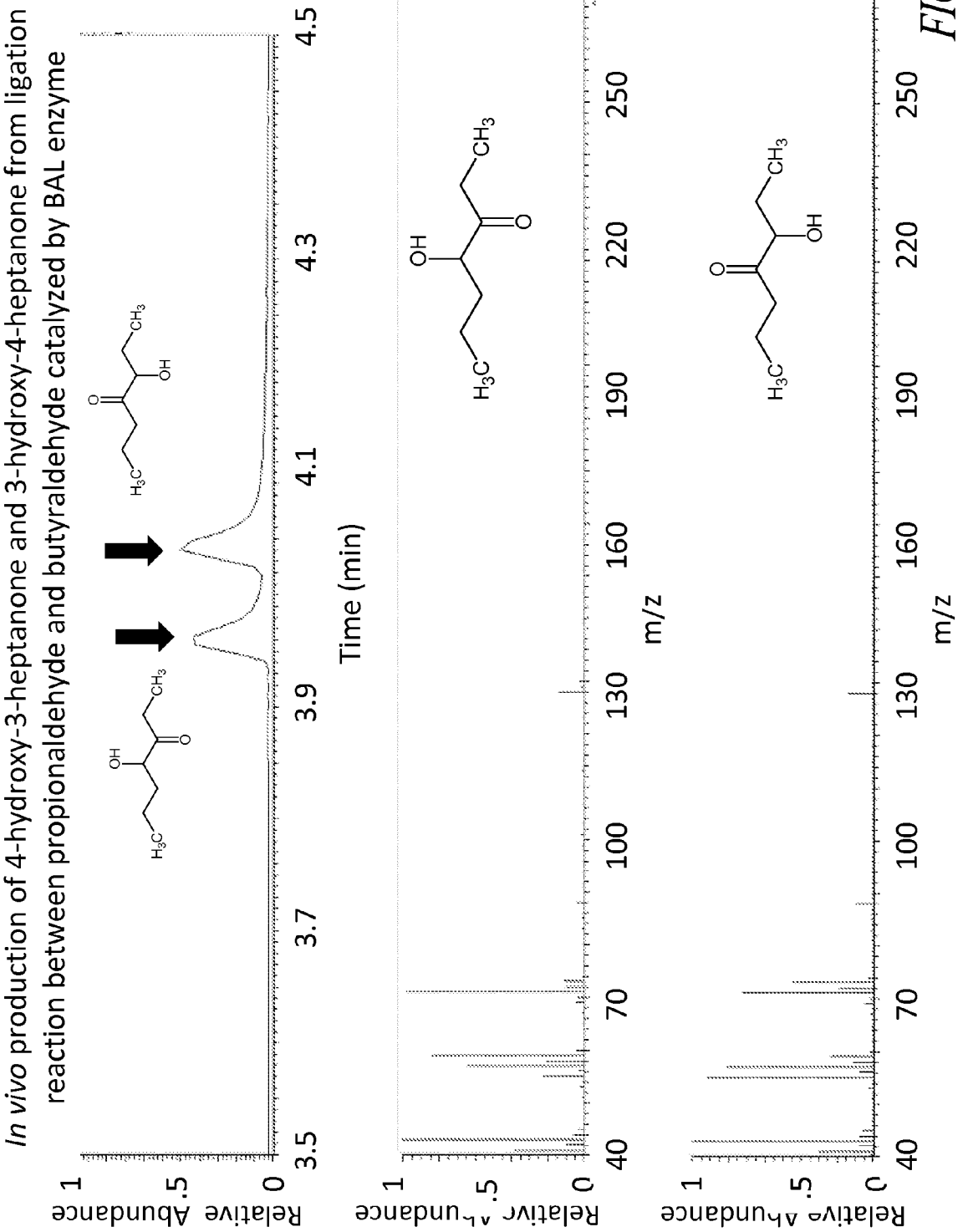

In vivo production of 4-hydroxy-3-heptanone and 3-hydroxy-4-heptanone from ligation reaction between propionaldehyde and butyraldehyde catalyzed by BAL enzyme
Relative Abundance
Time (min)
m/z
m/z

FIG. 48B

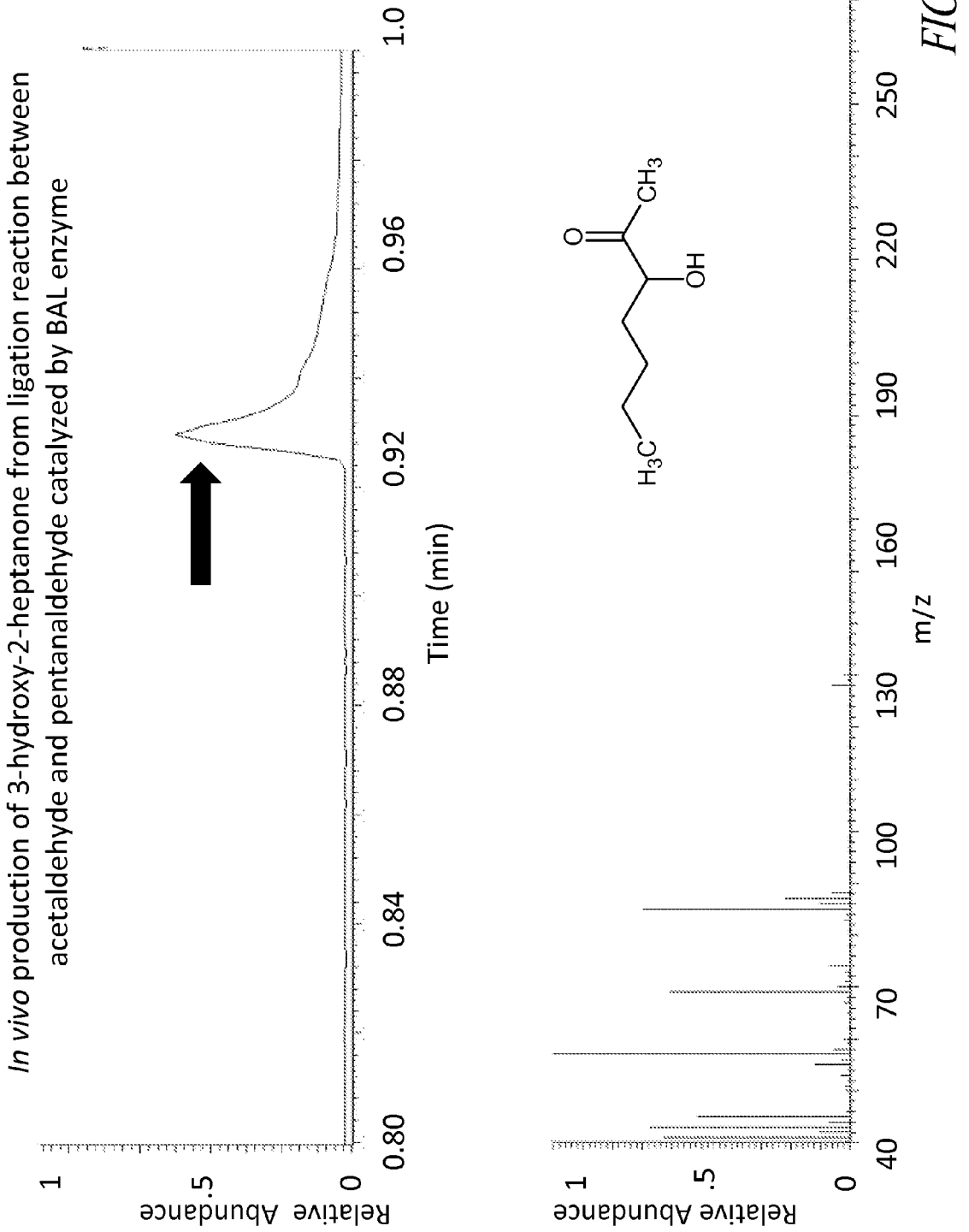

In vivo production of 3-hydroxy-2-heptanone from ligation reaction between acetaldehyde and pentanaldehyde catalyzed by BAL enzyme
Relative Abundance
1
.5
0
0.80
0.84
0.88
0.92
0.96
1.0
Time (min)
O
CH3
OH
H3C
Relative Abundance
1
.5
0
40
70
100
130
160
190
220
250
m/z

FIG. 49A

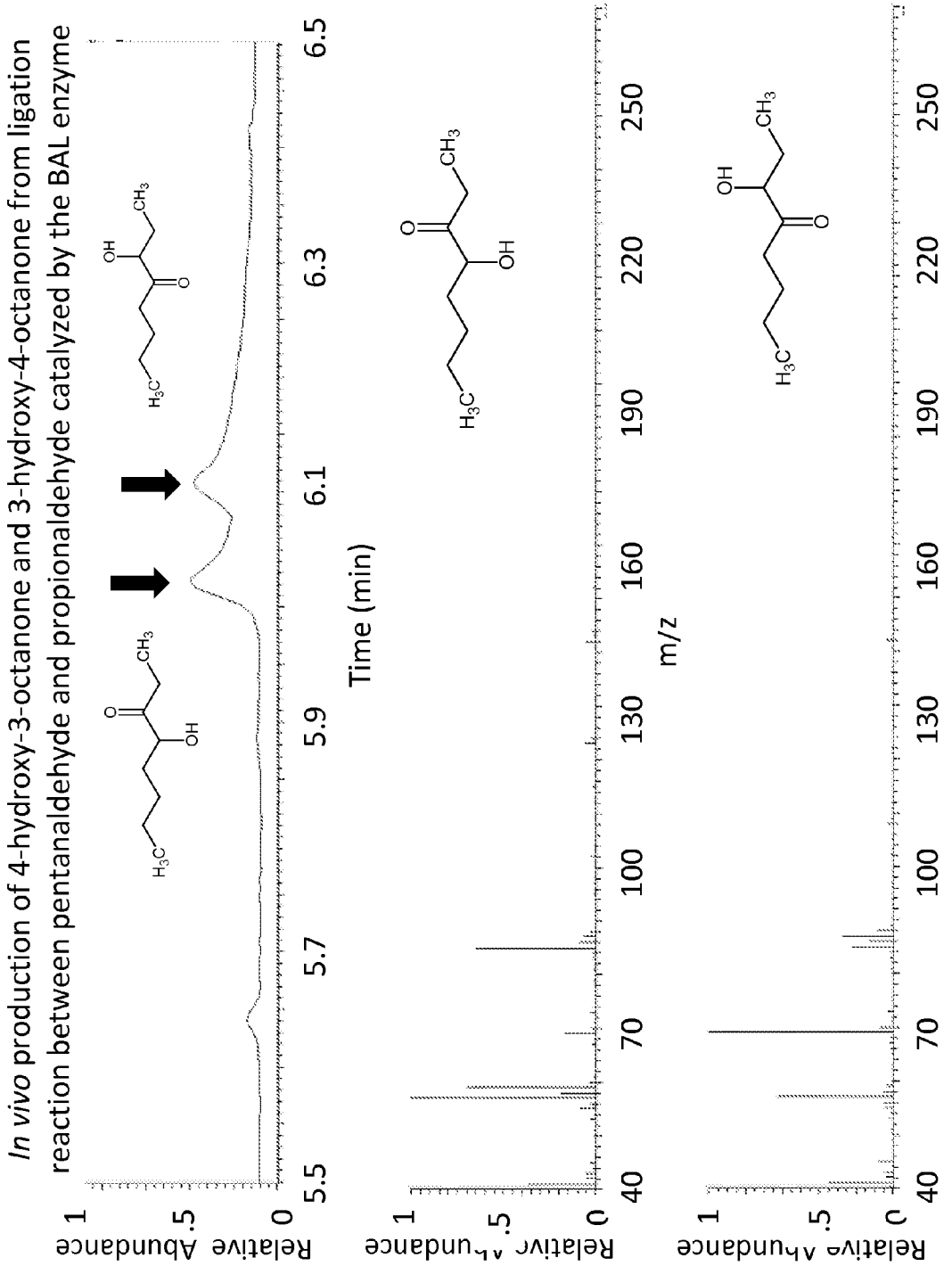

In vivo production of 4-hydroxy-3-octanone and 3-hydroxy-4-octanone from ligation reaction between pentanaldehyde and propionaldehyde catalyzed by the BAL enzyme
Relative Abundance
Time (min)
m/z
Relative Abundance
m/z
Relative Abundance

FIG. 49B

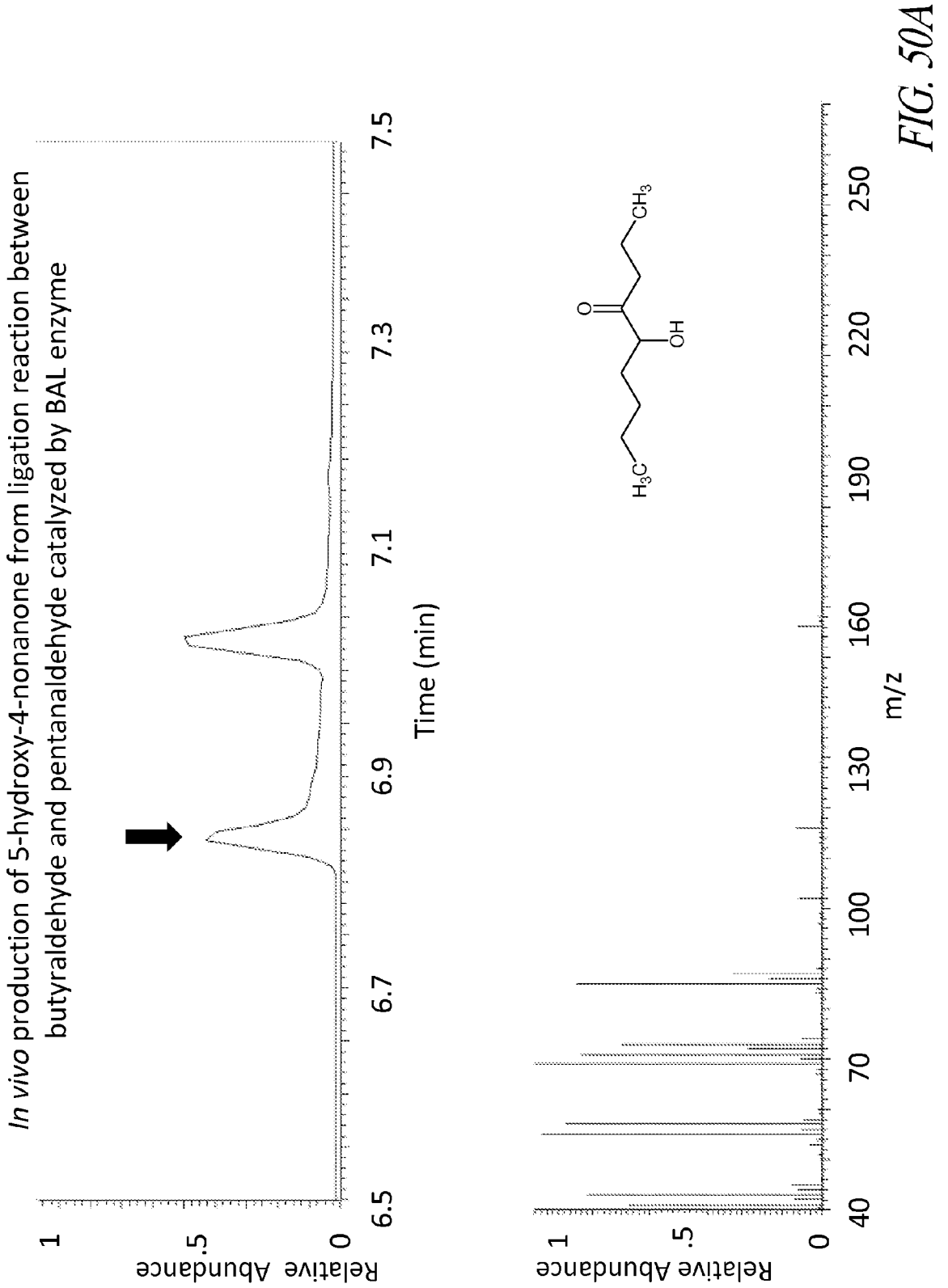

In vivo production of 5-hydroxy-4-nonanone from ligation reaction between butyraldehyde and pentanaldehyde catalyzed by BAL enzyme
Relative Abundance
Time (min)
6.5
6.7
6.9
7.1
7.3
7.5
0
.5
1
O
OH
CH3
H3C
m/z
40
70
100
130
160
190
220
250

FIG. 50A

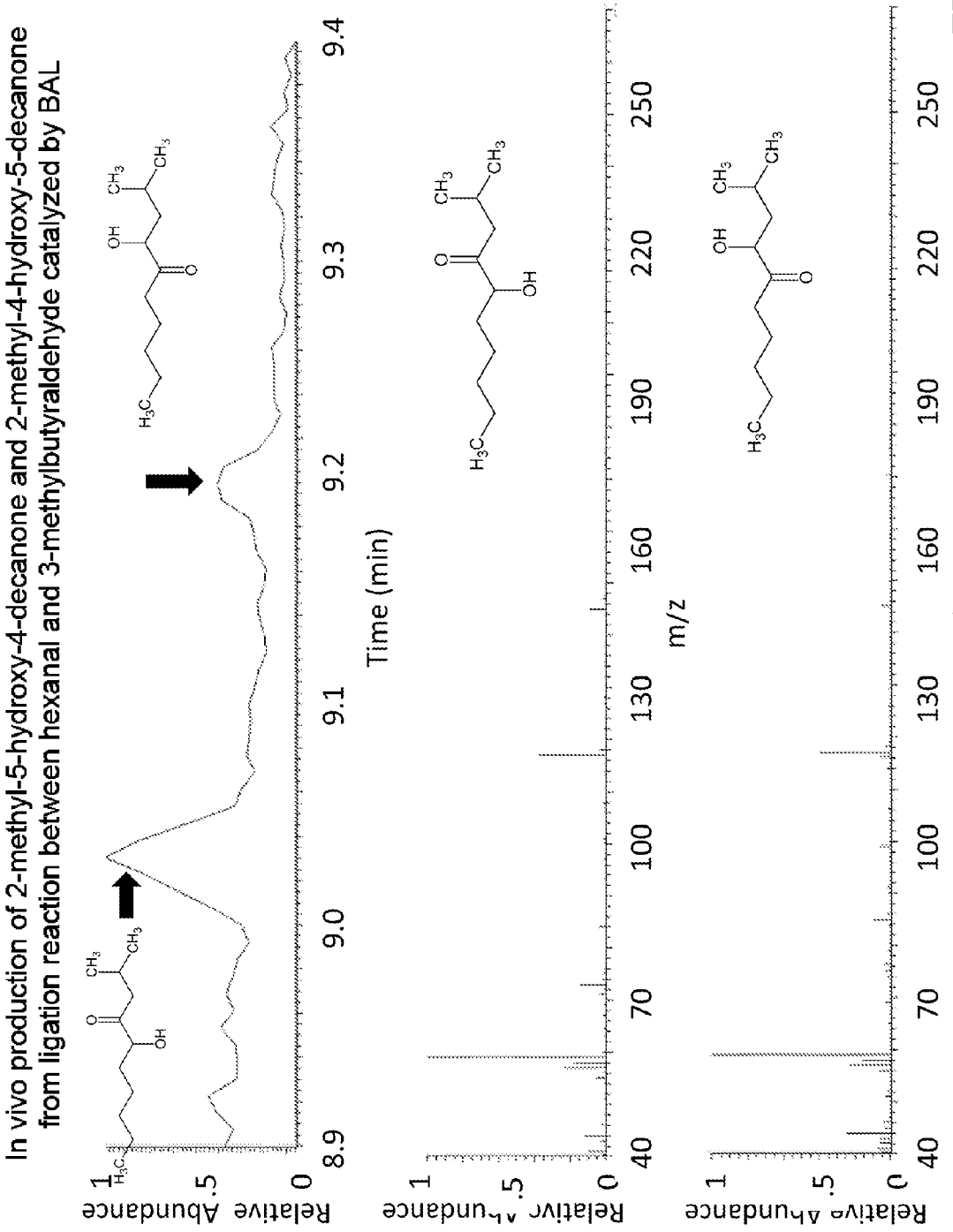

In vivo production of 2-methyl-5-hydroxy-4-decanone and 2-methyl-4-hydroxy-5-decanone from ligation reaction between hexanal and 3-methylbutyraldehyde catalyzed by BAL
Relative Abundance
Time (min)
m/z
m/z

FIG. 50B

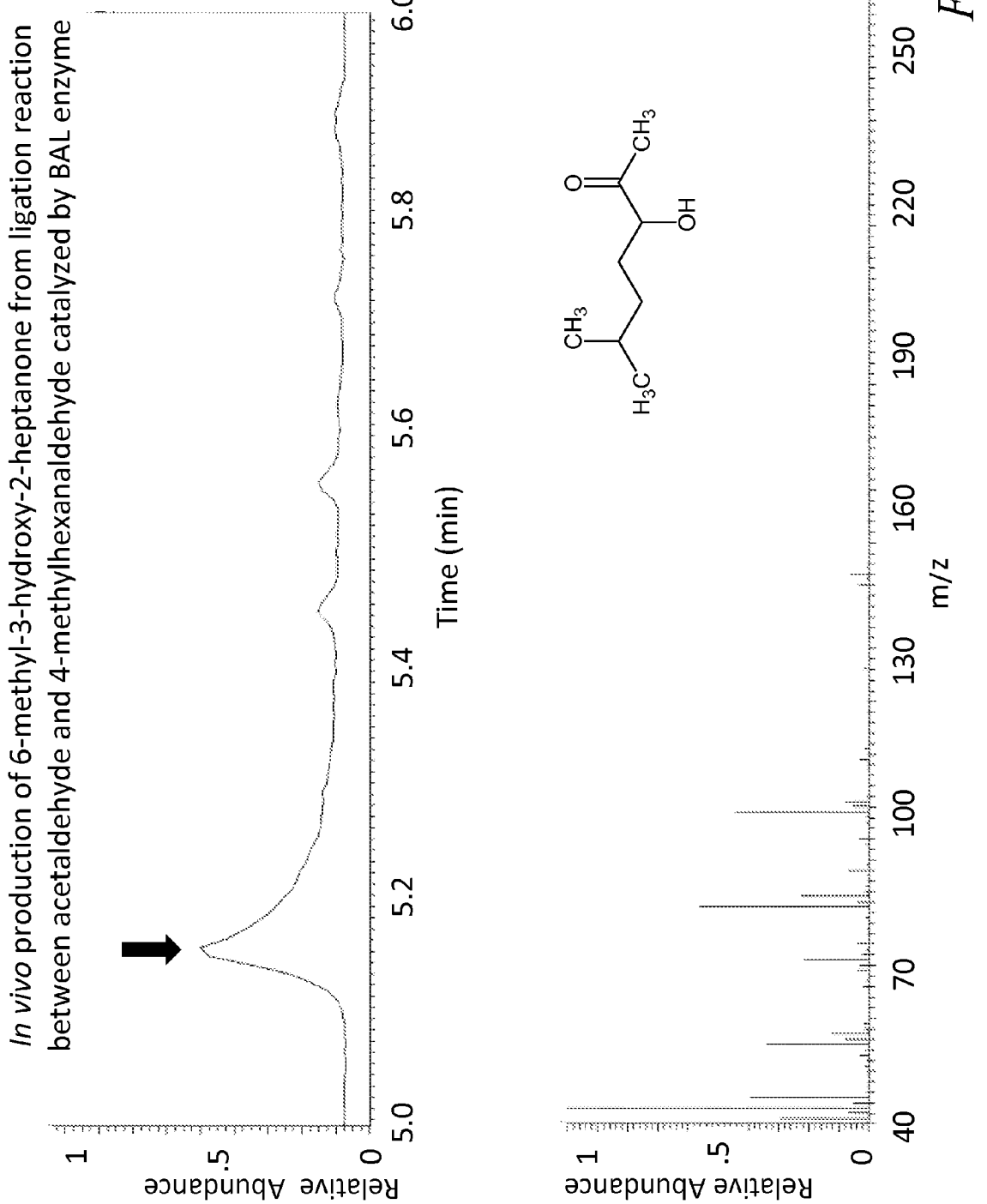
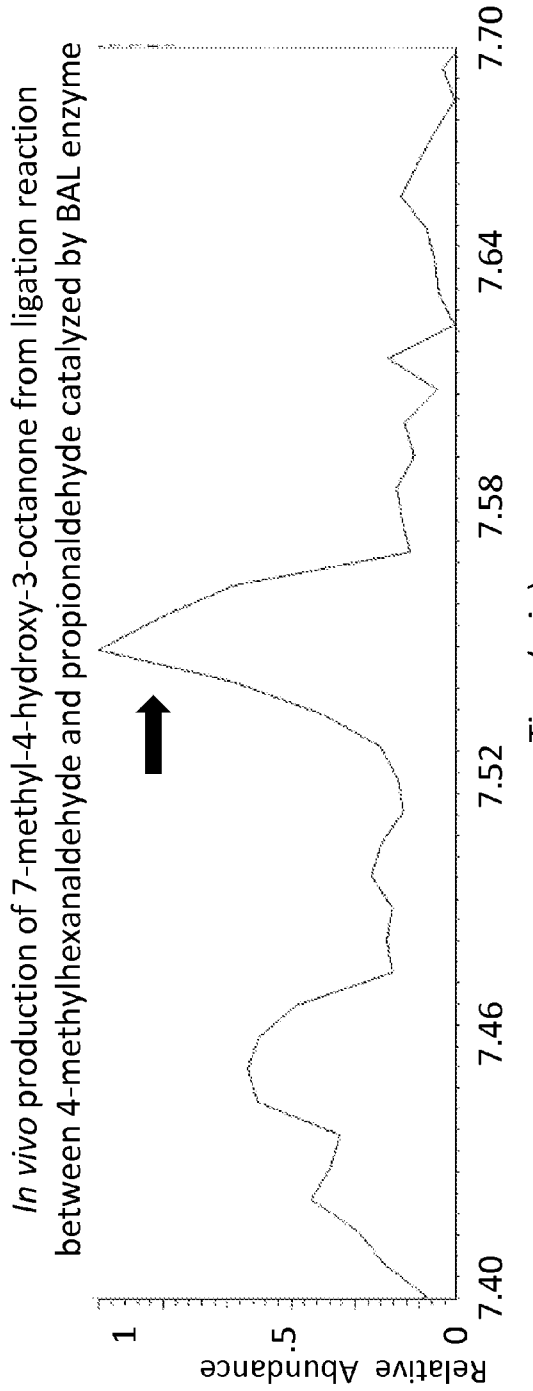
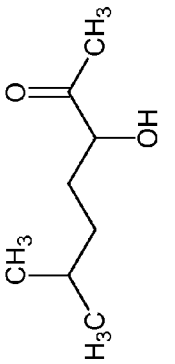

In vivo production of 6-methyl-3-hydroxy-2-heptanone from ligation reaction between acetaldehyde and 4-methylhexanaldehyde catalyzed by BAL enzyme
Relative Abundance
Time (min)
5.0
5.2
5.4
5.6
5.8
6.0
0
.5
1
O
CH3
OH
CH3
H3C
Relative Abundance
m/z
40
70
100
130
160
190
220
250

FIG. 51A


In vivo production of 7-methyl-4-hydroxy-3-octanone from ligation reaction between 4-methylhexanaldehyde and propionaldehyde catalyzed by BAL enzyme
Relative Abundance
1
.5
0
7.40
7.46
7.52
7.58
7.64
7.70
Time (min)


O
CH3
OH
CH3
H3C

Relative Abundance
1
.5
0
40
70
100
130
160
190
220
250
m/z

FIG. 51B

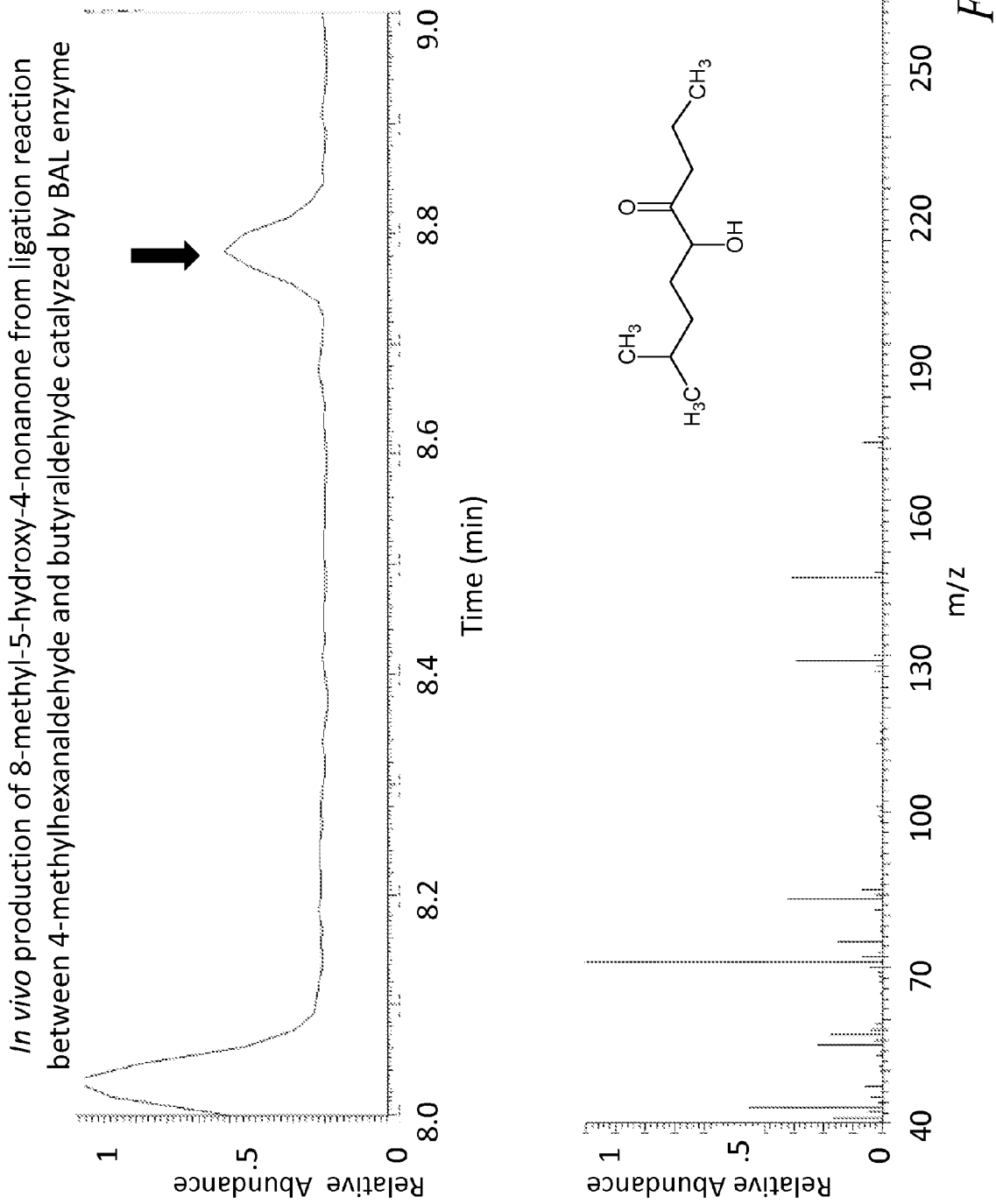

In vivo production of 8-methyl-5-hydroxy-4-nonanone from ligation reaction between 4-methylhexanaldehyde and butyraldehyde catalyzed by BAL enzyme
Relative Abundance
Time (min)
m/z
O
OH
CH3
H3C
CH3

FIG. 52A

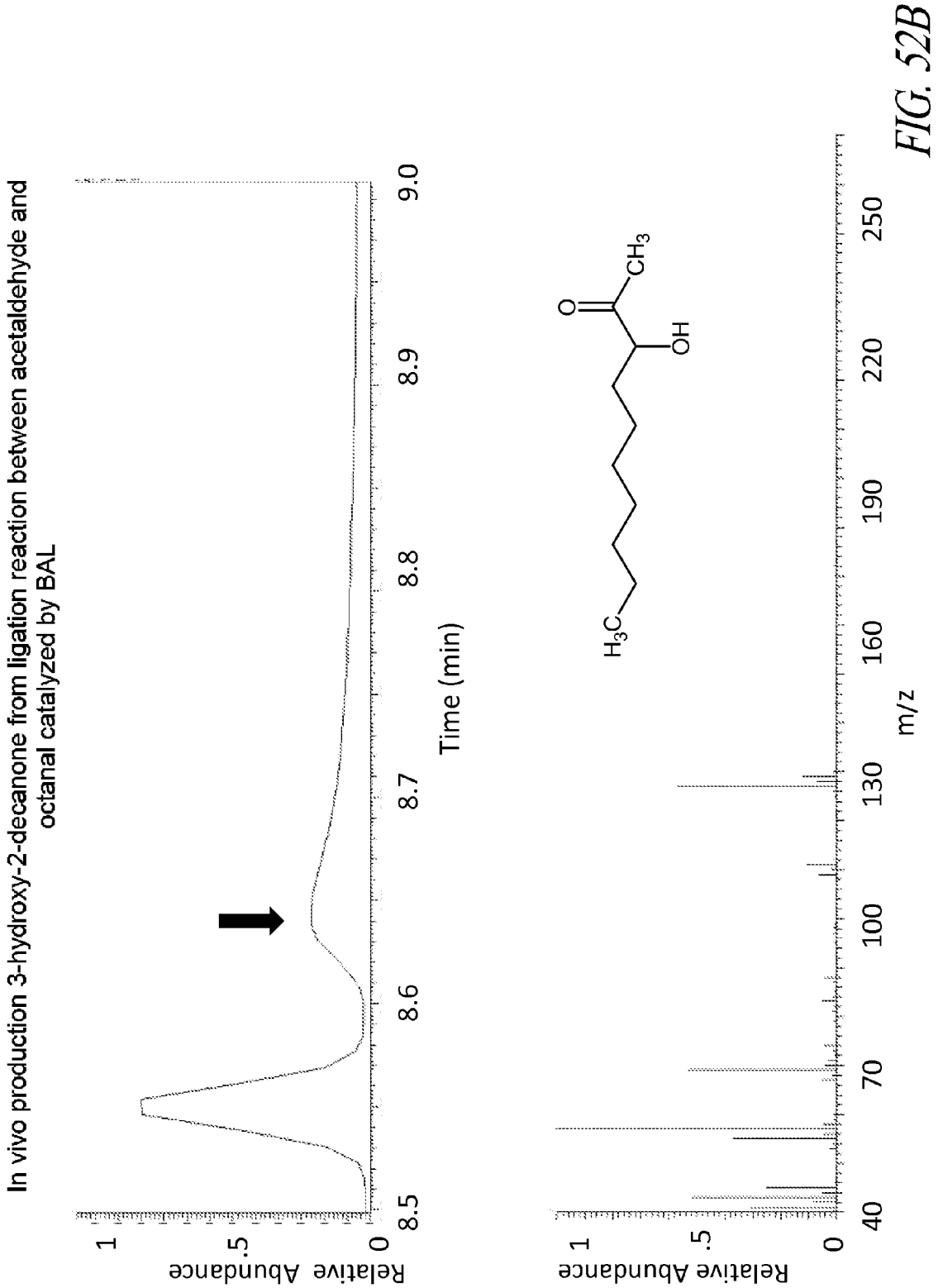

In vivo production 3-hydroxy-2-decanone from ligation reaction between acetaldehyde and octanal catalyzed by BAL
Relative Abundance
1
.5
0
8.5
8.6
8.7
8.8
8.9
9.0
Time (min)
O
CH3
OH
H3C
Relative Abundance
1
.5
0
40
70
100
130
160
190
220
250
m/z

FIG. 52B

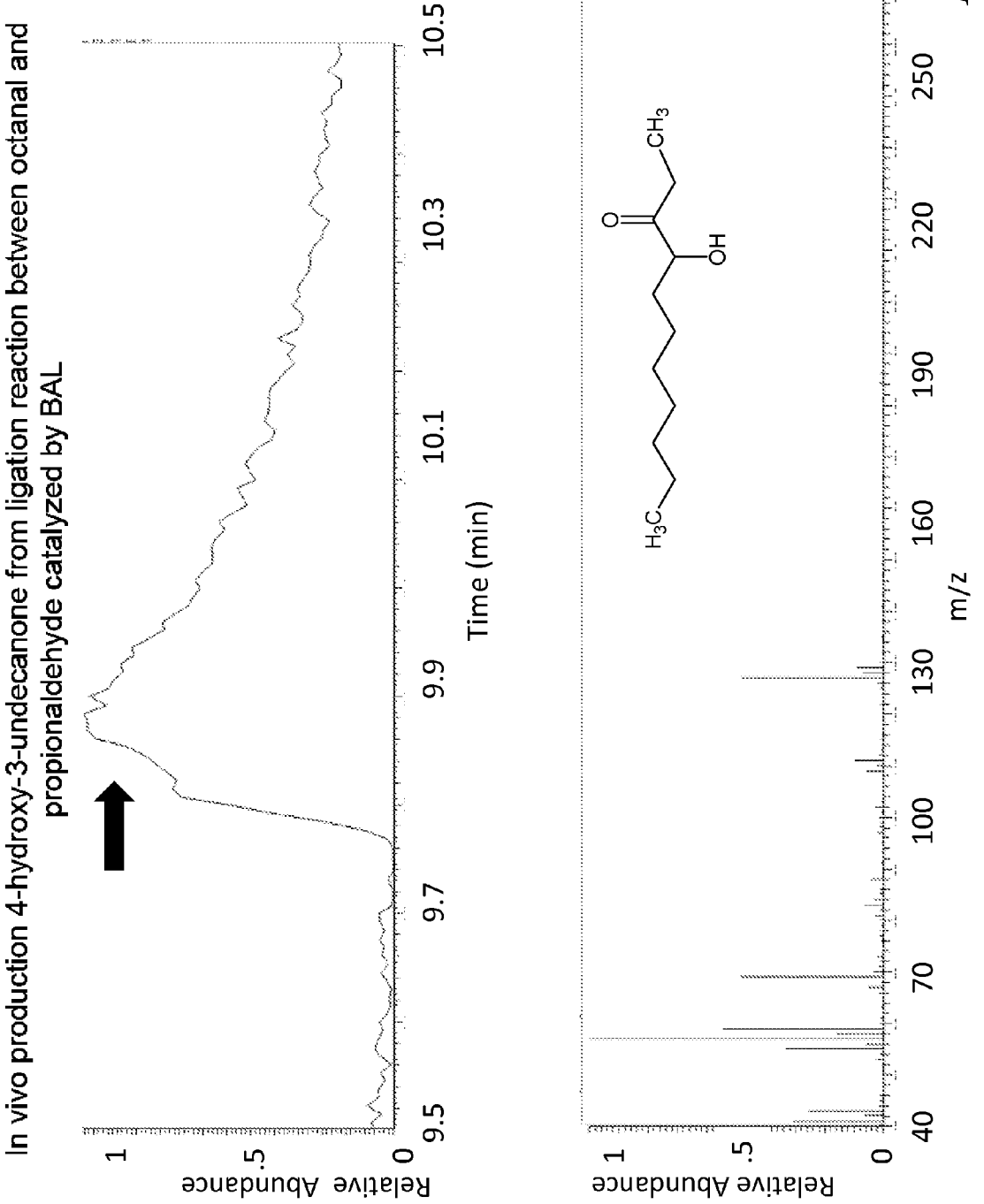

In vivo production 4-hydroxy-3-undecanone from ligation reaction between octanal and propionaldehyde catalyzed by BAL
Relative Abundance
Time (min)
9.5
9.7
9.9
10.1
10.3
10.5
0
.5
1
O
OH
CH3
H3C
m/z
40
70
100
130
160
190
220
250

FIG. 53A

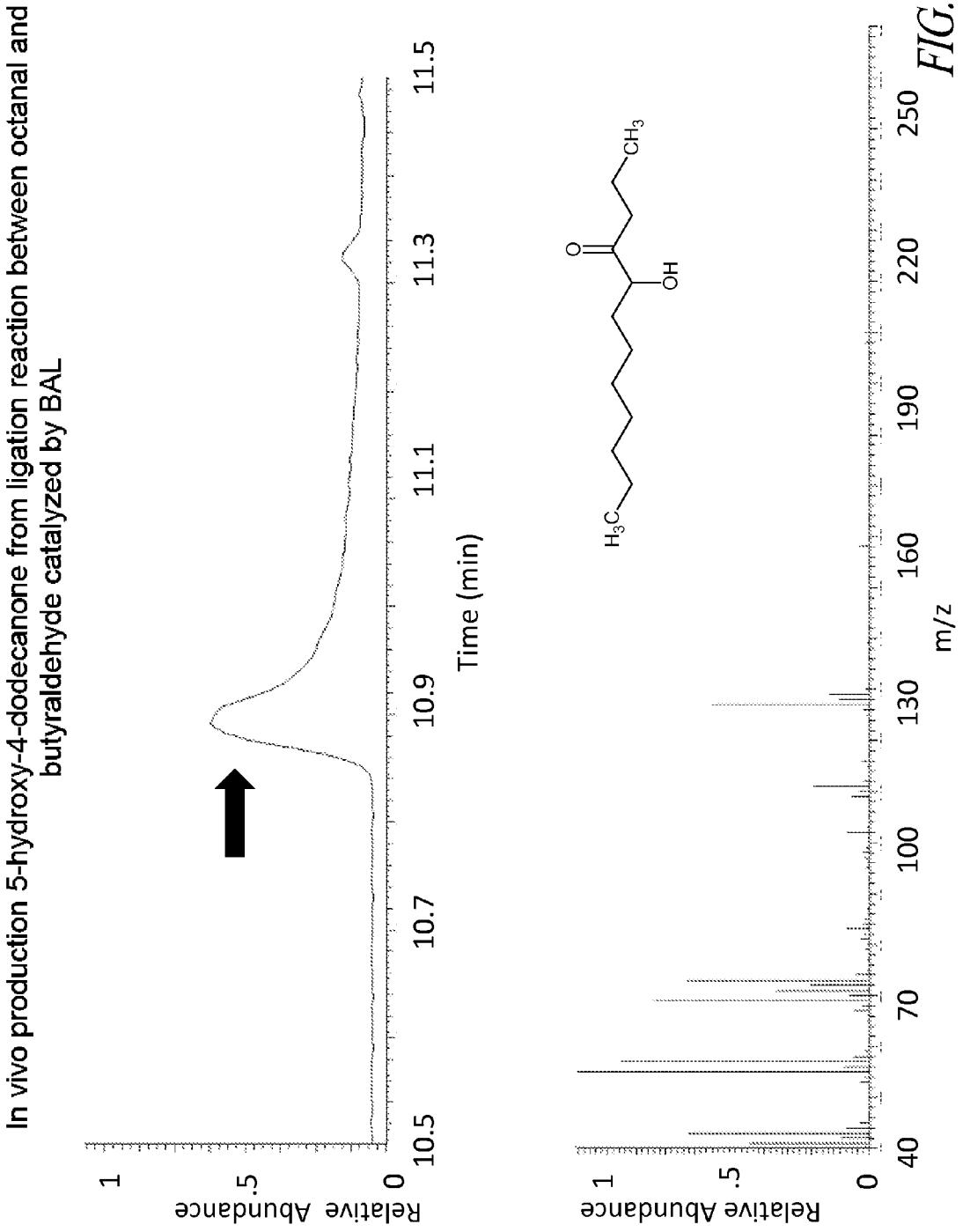

In vivo production 5-hydroxy-4-dodecanone from ligation reaction between octanal and butyraldehyde catalyzed by BAL
Relative Abundance
Time (min)
10.5
10.7
10.9
11.1
11.3
11.5
0
.5
1
O
OH
CH_3
H_3C
m/z
40
70
100
130
160
190
220
250

FIG. 53B

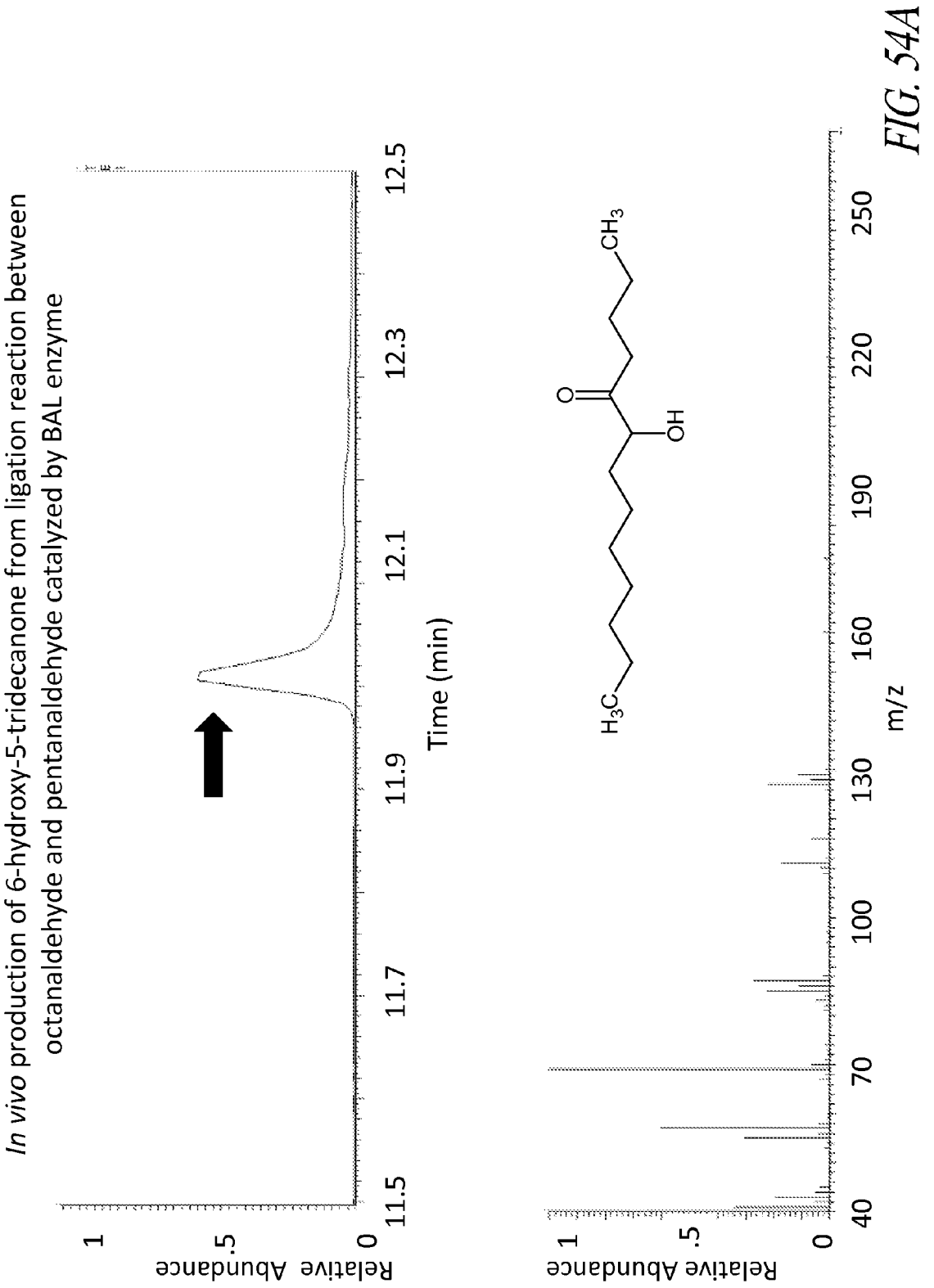

In vivo production of 6-hydroxy-5-tridecanone from ligation reaction between octanaldehyde and pentanaldehyde catalyzed by BAL enzyme
Relative Abundance
Time (min)
11.5
11.7
11.9
12.1
12.3
12.5
0
.5
1
O
OH
CH3
H3C
m/z
40
70
100
130
160
190
220
250

FIG. 54A

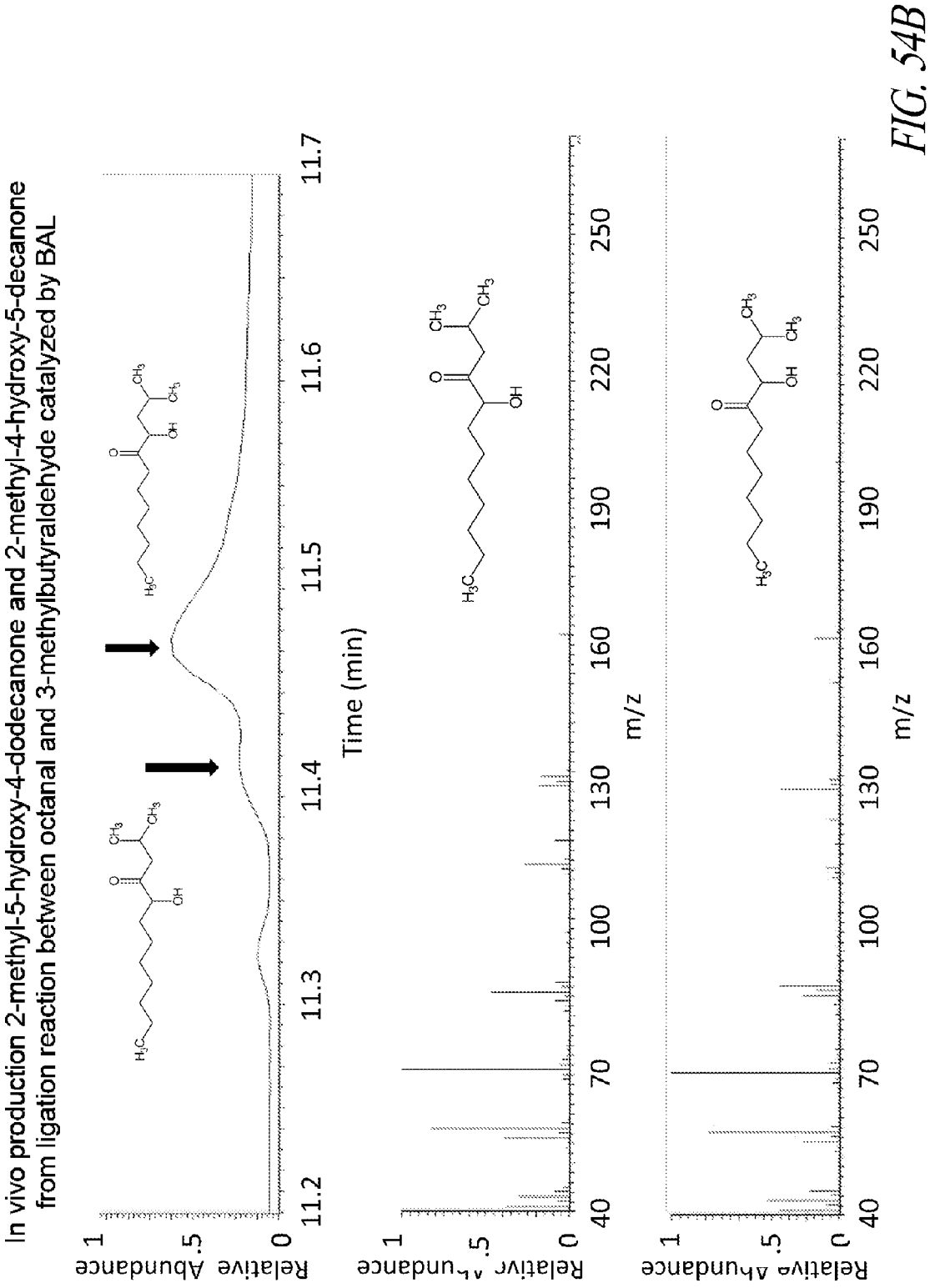

In vivo production 2-methyl-5-hydroxy-4-dodecanone and 2-methyl-4-hydroxy-5-decanone
from ligation reaction between octanal and 3-methylbutyraldehyde catalyzed by BAL
Relative Abundance
Time (min)
m/z
Relative Abundance
m/z
Relative Abundance

FIG. 54B

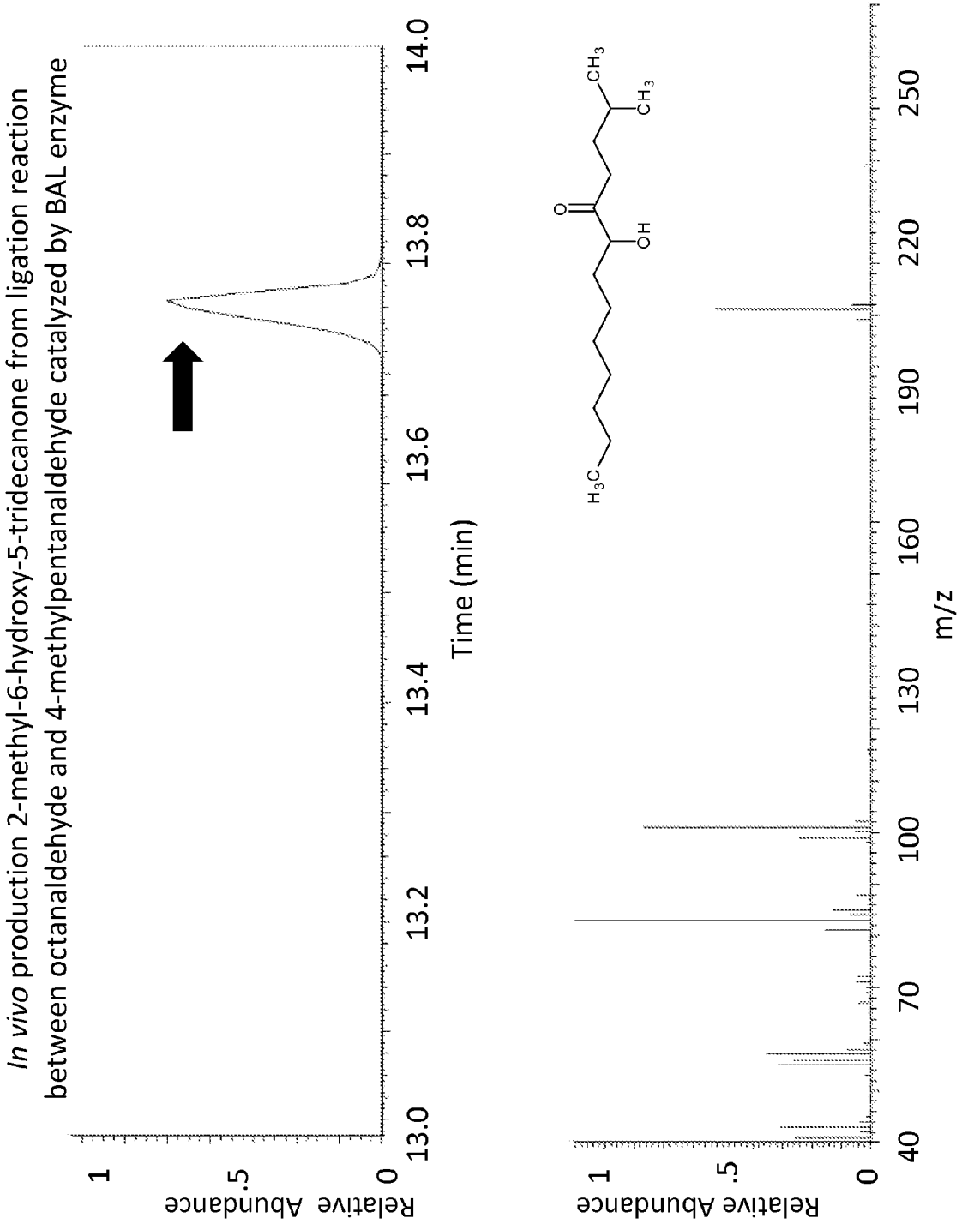

In vivo production 2-methyl-6-hydroxy-5-tridecanone from ligation reaction between octanaldehyde and 4-methylpentanaldehyde catalyzed by BAL enzyme
Relative Abundance
1
.5
0
13.0
13.2
13.4
13.6
13.8
14.0
Time (min)
O
OH
CH3
CH3
H3C
Relative Abundance
1
.5
0
40
70
100
130
160
190
220
250
m/z

FIG. 55

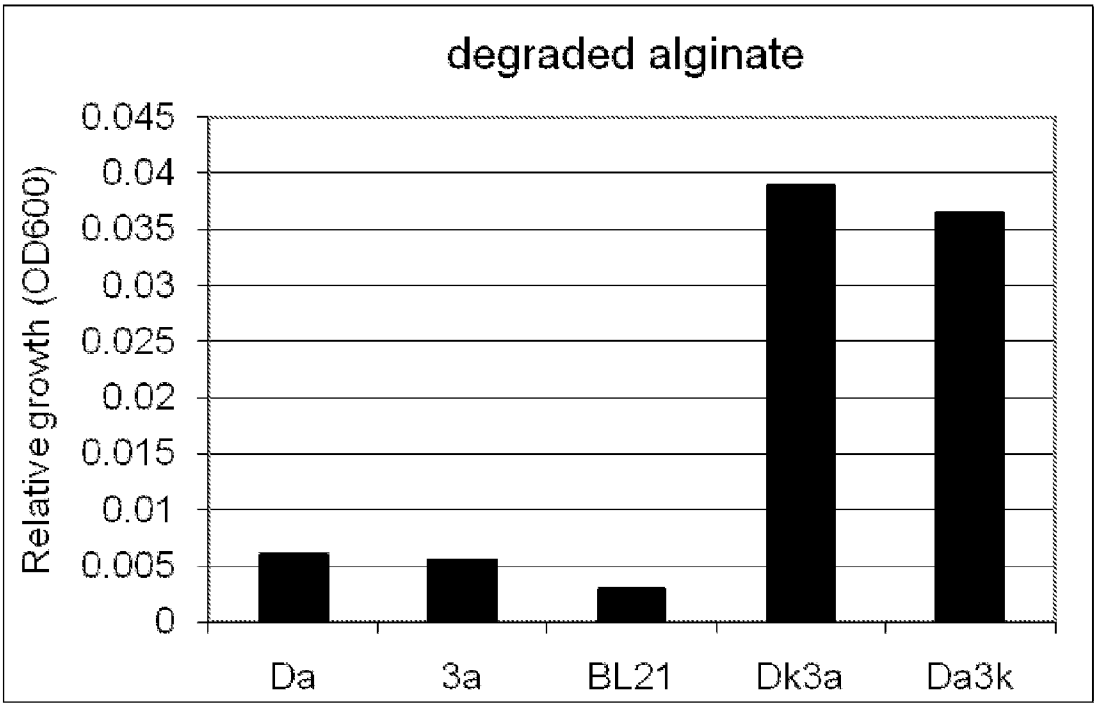


FIG. 56A

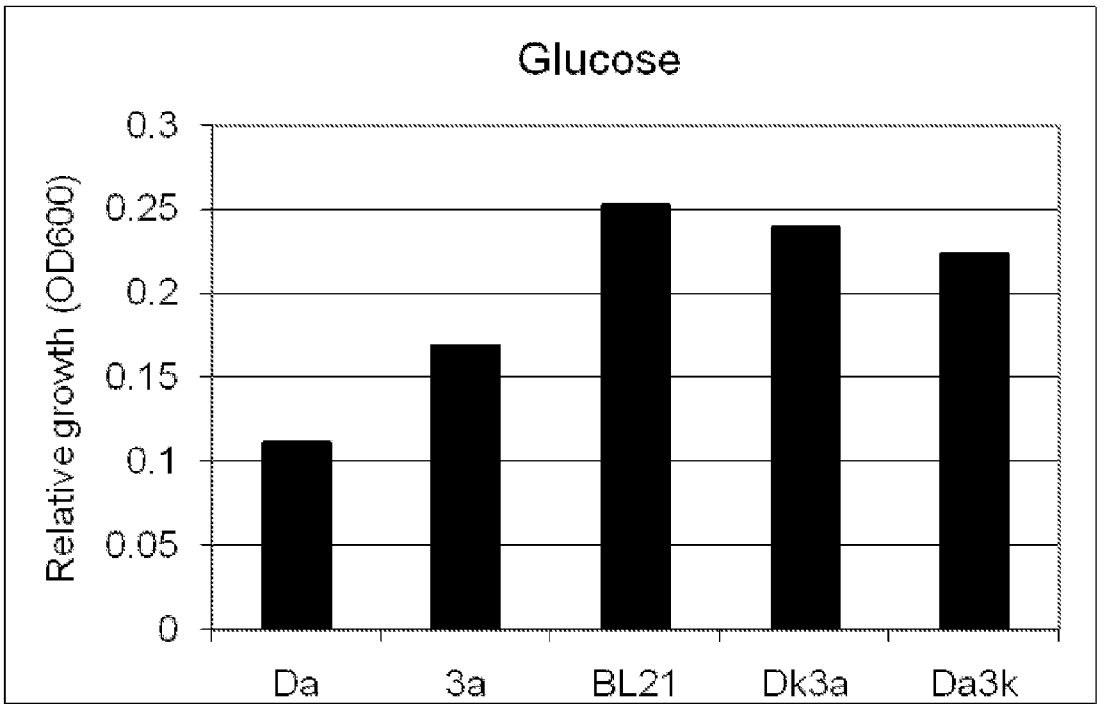
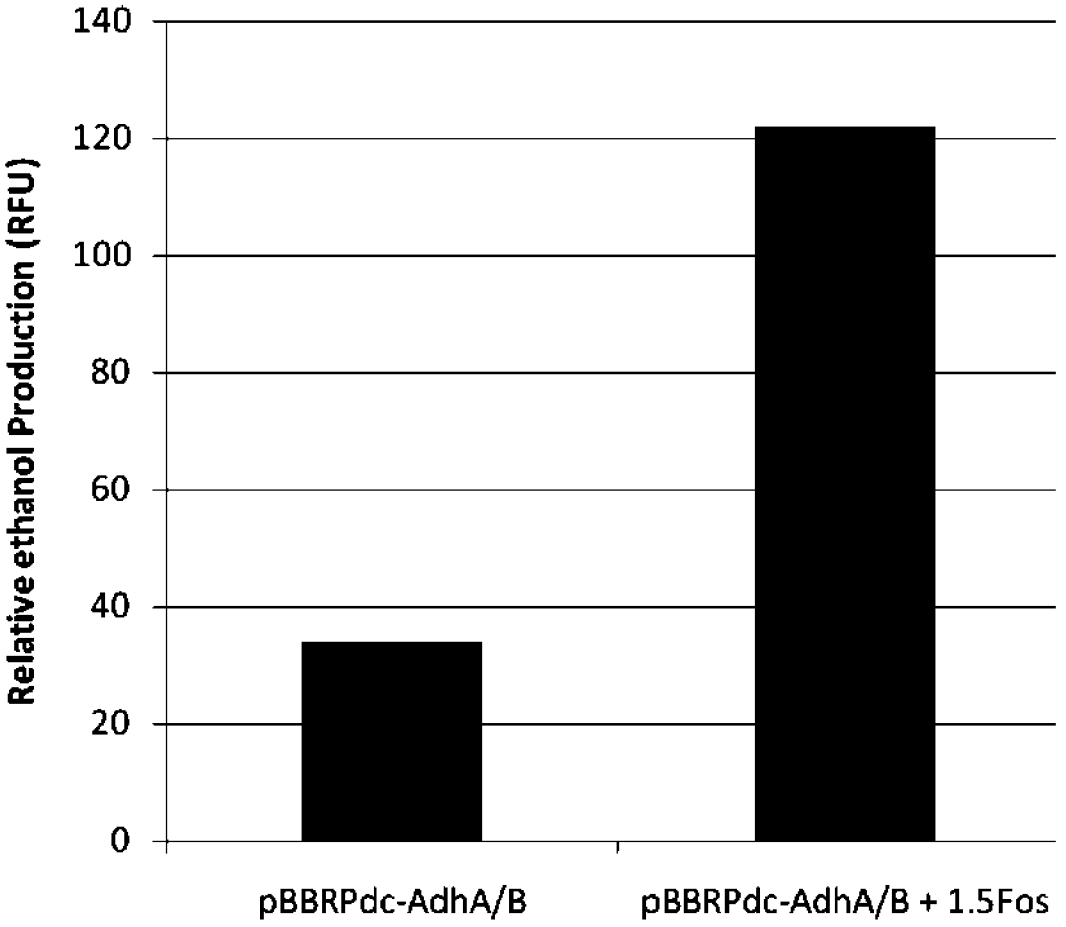


FIG. 56B

Figure 57
Production of Ethanol from Alginate



BIOFUEL PRODUCTION

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application No. 60/977,628 filed Oct. 4, 2007, which application is incorporated herein by reference in its entirety.

STATEMENT REGARDING SEQUENCE LISTING

[0002] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is 150097_40101_SEQUENCE_LISTING.txt. The text file is 519 KB, was created on Oct. 3, 2008, and is being submitted electronically via EFS-Web.

TECHNICAL FIELD

[0003] The present application relates generally to the use of microbial and chemical systems to convert biomass to commodity chemicals, such as biofuels/biopetrols.

BACKGROUND

[0004] Petroleum is facing declining global reserves and contributes to more than 30% of greenhouse gas emissions driving global warming. Annually 800 billion barrels of transportation fuel are consumed globally. Diesel and jet fuels account for greater than 50% of global transportation fuels.

[0005] Significant legislation has been passed, requiring fuel producers to cap or reduce the carbon emissions from the production and use of transportation fuels. Fuel producers are seeking substantially similar, low carbon fuels that can be blended and distributed through existing infrastructure (e.g., refineries, pipelines, tankers).

[0006] Due to increasing petroleum costs and reliance on petrochemical feedstocks, the chemicals industry is also looking for ways to improve margin and price stability, while reducing its environmental footprint. The chemicals industry is striving to develop greener products that are more energy, water, and CO_2 efficient than current products. Fuels produced from biological sources, such as biomass, represent one aspect of process.

[0007] Presents method for converting biomass into biofuels focus on the use of lignocellulolic biomass, and there are many problems associated with using this process. Large-scale cultivation of lignocellulolic biomass requires substantial amount of cultivated land, which can be only achieved by replacing food crop production with energy crop production, deforestation, and by recultivating currently uncultivated land. Other problems include a decrease in water availability and quality and an increase in the use of pesticides and fertilizers.

[0008] The degradation of lignocellulolic biomass using biological systems is a very difficult challenge due to its substantial mechanistic strength and the complex chemical components. Approximately thirty different enzymes are required to fully convert lignocellulose to monosaccharides. The only available alternate to this complex approach requires a substantial amount of heat, pressure, and strong acids. The art therefore needs an economic and technically simple process for converting biomass into hydrocarbons for use as biofuels or biopetrols.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] FIG. **1** shows the *Vibrio splendidus* genomic region of the fosmid clone described in Example 1. Genes are indicated with orange arrows. Labels show the numerical gene indices and the predicted function of the proteins.

[0010] FIG. **2** illustrates the pathways involved in certain embodiment in which *E. coli* may be engineered to grow on alginate as a sole source of carbon.

[0011] FIG. **3** illustrates the pathways involved in certain embodiment in which *E. coli* may be engineered to grow on pectin as a sole source of carbon.

[0012] FIG. **4** shows the results of engineered or recombinant *E. coli* growing on alginate as a sole source of carbon (see solid circles). *Agrobacterium tumefaciens* cells provide a positive control (see hatched circles). The well to the immediate left of the of the *A. tumefaciens* positive control contains DH10B *E. coli* cells, which provide a negative control.

[0013] FIG. **5** shows the growth of recombinant strain of *E. coli* on galacturonates and pectin. FIG. **5**A shows the growth of *E. coli* on various lengths of galacturonate after 24 hr. The recombinant strain in FIG. **5**A is the *E. coli* BL21(DE3) strain harboring pTrlogl-kdgR+pBBRGal3P, and the control strain is the BL21(DE3) strain harboring pTrc99A+pBBR1MCS-2, as described in Example 2. FIG. **5**B shows the growth of recombinant *E. coli* on pectin after 3-4 days. The recombinant strain in FIG. **5**B is *E. coli* DH5a strain containing pPEL74 (Ctrl) and pPEL74 and pROU2, as described in Example 2.

[0014] FIG. **6** shows the degradation of alginate to form pyruvate. FIG. **6**A illustrates a simplified metabolic pathway for alginate degradation and metabolism. FIG. **6**B shows the results of in vitro degradation of alginate to form pyruvate by an enzymatic degradation route. FIG. **6**C shows the results of in vitro degradation of alginate to form pyruvate by a chemical degradation route.

[0015] FIG. **7** shows the biological activity of various alcohol dehydrogenases isolated from *Agrobacterium tumefaciens* C58. FIG. **7**A shows DEHU hydrogenase activity as monitored by NADPH consumption, and FIG. **7**B shows mannuronate hydrogenase activity as monitored by NADPH consumption.

[0016] FIG. **8** shows the GC-MS chromatogram results for the control sample (FIG. **8**A) and for isobutyraldehyde, 3-methylpentanol, and 2-methylpentanal production from pBAD-alsS-ilvCD-leuABCD2 and pTrcBALK (FIG. **8**B).

[0017] FIG. **9** shows the GC-MS chromatogram results for the control sample (FIG. **9**A) and for 4-hydroxyphenylethanol and indole-3-ethanol production from pBADtyrA-aroLAC-aroG-tktA-aroBDE and pTrcBALK (FIG. **9**B).

[0018] FIG. **10** shows the mass spectrometry results for isobutanal (FIG. **10**A), 3-methylpentanol (FIG. **10**B), and 2-methylpentanol (FIG. **10**C).

[0019] FIG. **11** shows the mass spectrometry results for phenylethanol (FIG. **11**A), 4-hydroxyphenylethanol (FIG. **11**B), and indole-3-ethanol (FIG. **11**C).

[0020] FIG. **12** shows the biological activity of diol dehydratases. FIG. **12**A shows the reduction of butyroin by ddh1, ddh2, and ddh3 as monitored by NADH consumption. FIG. **12**B shows the oxidation activity of ddh3 towards 1,2-cyclopentanediol and 1,2-cyclohexanediol as measured by NADH production.

[0021] FIG. **13** summarizes the results of kinetic studies for various substrates in the oxidation reactions catalyzed by the DDH polypeptides. These reactions were NAD+dependent.

[0022] FIG. **14** shows the nucleotide sequence (FIG. **14**A) (SEQ ID NO:97) and polypeptide sequence (FIG. **14**B) (SEQ ID NO:98) of diol dehydrogenase DDH1 isolated from *Lactobaccilus brevis* ATCC 367.

[0023] FIG. **15** shows the nucleotide sequence (FIG. **15**A) (SEQ ID NO:99) and polypeptide sequence (FIG. **15**B) (SEQ ID NO:100) of diol dehydrogenase DDH2 isolated from *Pseudomonas putida* KT2440.

[0024] FIG. **16** shows the nucleotide sequence (FIG. **16**A) (SEQ ID NO:101) and polypeptide sequence (FIG. **16**B) (SEQ ID NO:102) of diol dehydrogenase DDH3 isolated from *Kiebsiella pneumoniae* MGH78578.

[0025] FIG. **17** shows the sequential in vivo biological activity of a benzaldehyde lyase (bal) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a ddh gene isolated from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (DDH3). This reaction illustrates the sequential conversion of butanal into 5-hydroxy-4-octanone and then 4,5-octanonediol. FIG. **17**A shows the detection of butyroin (5-hydroxy-4-octanone) at 5.36 minutes, and FIG. **17**B shows the detection of 4,5-octanediol at 6.49 and 6.65 minutes.

[0026] FIG. **18** shows the sequential in vivo biological activity of a benzaldehyde lyase (bal) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a ddh gene isolated from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (DDH3). This Figure illustrates the sequential conversion of n-pentanal into 6-hydroxy-5-decanone and then 5,6-decanediol. FIG. **18**A shows the detection of valeroin (6-hydroxy-5-decanone) at 8.22 minutes, and FIG. **18**B shows the detection of 5,6 decanediol at 9.22 and 9.35 minutes.

[0027] FIG. **19** shows the sequential in vivo biological activity of a benzaldehyde lyase (bal) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a ddh gene isolated from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (DDH3). This Figure illustrates the sequential conversion of 3-methylbutanal into 2,7-dimethyl-5-hydroxy-4-octanone and then 2,7-dimethyl-4,5-octanediol. FIG. **19**A shows the detection of isoveraloin (2,7-dimethyl-5-hydroxy-4-octanone) at 6.79 minutes, and FIG. **19**B shows the detection of 2,7-dimethyl-4,5-octanediol at 7.95 and 8.15 minutes.

[0028] FIG. **20** shows the sequential in vivo biological activity of a benzaldehyde lyase (bal) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a ddh gene isolated from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (DDH3). This Figure illustrates the sequential conversion of n-hexanal into 7-hydroxy-6-dodecanone and then 6,7-dodecanediol. FIG. **20**A shows the detection of hexanoin (7-hydroxy-6-decanone) at 10.42 minutes, and FIG. **20**B shows the detection of 6,7 dodecanediol at 10.89 and 10.95 minutes.

[0029] FIG. **21** shows the sequential in vivo biological activity of a benzaldehyde lyase (bal) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a ddh gene isolated from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (DDH3). This Figure illustrates the sequential conversion of 4-methylpentanal into 2,9-dimethyl-6-hydroxy-5-decanone and then 2,9-dimethyl-5,6-decanediol. FIG. **21**A shows the detection of isohexanoin (2,9-Dimethyl-6-hydroxy-5-decanone) at 9.45 minutes, and FIG. **21**B shows the detection of 2,9-dimethyl-5,6-decanediol at 10.38 and 10.44 minutes.

[0030] FIG. **22** shows the in vivo biological activity of a benzaldehyde lyase (bal) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a ddh gene isolated from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (DDH3). This Figure illustrates the conversion of n-octanal into 9-hydroxy-8-hexadecanone by showing the detection of detection of octanoin (9-hydroxy-8-hexadecanone) at 12.35 minutes.

[0031] FIG. **23** shows the in vivo biological activity of a benzaldehyde lyase (bal) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a ddh gene isolated from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (DDH3). This Figure illustrates the conversion of acetaldehyde into 3-hydroxy-2-butanone by showing the detection of acetoin (3-hydroxy-2-butanone) at rt=0.91 minutes.

[0032] FIG. **24** shows the sequential in vivo biological activity of a benzaldehyde lyase (bal) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a ddh gene isolated from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (DDH3). This Figure illustrates the sequential conversion of n-propanal into 4-hydroxy-3-hexanone and then 3,4-hexanediol. FIG. **24**A shows the detection of propioin (4-hydroxy-3-hexanone) at rt=2.62 minutes, and FIG. **24**B shows the detection of 3,4-hexanediol at rt=3.79 minutes.

[0033] FIG. **25** the in vivo biological activity of a benzaldehyde lyase (bal) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and a ddh gene isolated from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (DDH3). This Figure illustrates the conversion of phenylacetoaldehyde into 1,4-diphenyl-3-hydroxy-2-butanone by showing the detection of 1,4-diphenyl-3-hydroxy-2-butanone at rt=13.66 minutes.

[0034] FIG. **26** shows the sequential biological activity of a diol dehydrogenase ddh from *Kiebsiella pneumoniae* MGH 78578 (DDH3) and a diol dehydratase pduCDE from *Kiebsiella pneumoniae* MGH 78578. FIG. **26**A shows GC-MS data which confirms the presence of 4,5-octanediol in the sample extraction, which is the expected product resulting from the reduction of butyroin by ddh3. FIG. **26**B shows GC-MS data confirming the presence of 4-octanone in the sample extraction, which is the expected product resulting from the sequential dehydrogenation of butyroin and dehydration of 4,5-octanediol by ddh3 and pduCDE, respectively.

[0035] FIG. **27** shows the sequential biological activity of a diol dehydrogenase ddh from *Kiebsiella pneumoniae* MGH 78578 (DDH3) and a diol dehydratase pduCDE from *Kiebsiella pneumoniae* MGH 78578. FIGS. **27**A and **27**B show comparisons between the sample extraction gas chromatograph/mass spectrum and the 4-octanone standard gas chromatograph/mass spectrum, confirming that 4-octanone was produced from butyroin using the enzymes diol dehydrogenase (ddh3) and a diol dehydratase (pduCDE).

[0036] FIG. **28** shows the nucleotide sequence (FIG. **28**A) (SEQ ID NO:103) and polypeptide sequence (FIG. **28**B) (SEQ ID NO:104) of a diol dehydratase large subunit (pduC) isolated from *Klebsiella pneumoniae* MGH78578.

[0037] FIG. **29** shows the nucleotide sequence (FIG. **29**A) (SEQ ID NO:105) and polypeptide sequence (FIG. **29**B) (SEQ ID NO:106) of a diol dehydratase medium subunit

isolated from *Klebsiella pneumoniae* MGH78578 (pduD), in addition to the nucleotide sequence (FIG. **29**C) (SEQ ID NO:107) and polypeptide sequence (FIG. **29**D) (SEQ ID NO:108) of a diol dehydratase small subunit isolated from *Klebsiella pneumoniae* MGH78578 (pduE).

[0038] FIG. **30** shows the oxidation of 4-octanol by secondary alcohol dehydrogenases as monitored by NADH production (FIG. **30**A) and NADPH production (FIG. **30**B).

[0039] FIG. **31** shows the oxidation of 4-octanol by secondary alcohol dehydrogenases as monitored by NADH production (FIG. **31**A) and NADPH production (FIG. **31**B).

[0040] FIG. **32** shows the oxidation of 2,7-dimethyl octanol by secondary alcohol dehydrogenases as monitored by NADH production (FIG. **32**A) and NADPH production (FIG. **32**B).

[0041] FIG. **33** shows the oxidation and reduction activity of 2ADH11 and 2ADH16. FIG. **33**A shows the reduction of 2,7-dimethyl-4-octanone as measured by NADPH consumption. FIG. **33**B shows the reduction of 2,7-dimethyl-4-octanone, 4-octanone, and cyclolypentanone.

[0042] FIG. **34** shows the oxidation and reduction of cyclopentanol by secondary alcohol dehydrogenases. FIG. **34**A shows the oxidation of cyclopentanol as monitored by NADH or NADPH formation. FIG. **34**B shows the reduction of cyclopentanol as monitored by NADPH consumption.

[0043] FIG. **35** shows the calculated rate constants for the illustrated reduction reactions for each substrate catalyzed by secondary alcohol dehydrogenase ADH-16 (SEQ ID NO:138).

[0044] FIG. **36** shows the calculated rate constants for the illustrated oxidation reactions for each substrate catalyzed by secondary alcohol dehydrogenase ADH-16 (SEQ ID NO:138).

[0045] FIG. **37** shows a list of alginate lyases genes/proteins that may be utilized according to the methods and recombinant microorganisms described herein.

[0046] FIG. **38** shows a list of pectate lyase genes/proteins that may be utilized according to the methods and recombinant microorganisms described herein.

[0047] FIG. **39**A shows a list of rhamnogalacturonan lyase genes/proteins that may be utilized according to the methods and recombinant microorganisms described herein. FIG. **39**B shows a list of rhamnogalacturonate hydrolase genes/proteins that may be utilized according to the methods and recombinant microorganisms described herein.

[0048] FIG. **40** shows a list of pectin methyl esterase genes/proteins that may be utilized according to the methods and recombinant microorganisms described herein.

[0049] FIG. **41** shows a list of pectin acetyl esterase genes/proteins that may be utilized according to the methods and recombinant microorganisms described herein.

[0050] FIG. **42** shows the production of 2-phenyl ethanol (FIG. **42**A), 2-(4-hydroxyphenyl)ethanol (FIG. **42**B), and 2-(indole-3-)ethanol (FIG. **42**C) at 24 hours from the recombinant microorganisms described in Example 4, which comprise functional 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and 2-(indole-3-)ethanol biosynthesis pathways.

[0051] FIG. **43** shows the GC-MS chromatogram results that confirm the production of 2-phenyl ethanol (FIG. **43**B) at one week from the recombinant microorganisms described in Example 4 (pBADpheA-aroLAC-aroG-tktA-aroBDE and pTrcBALK). FIG. **43**A shows the negative control cells (pBAD33 and pTrc99A).

[0052] FIG. **44** shows the GC-MS chromatogram results that confirm the production of 2-(4-hydroxyphenyl)ethanol (9.36 min) and 2-(indole-3) ethanol (10.32 min) at one week from the recombinant microorganisms described in Example 4 (pBADtyrA-aroLAC-aroG-tktA-aroBDE and pTrcBALK).

[0053] FIG. **45** confirms both the formation of 1-propanal from 1,2-propanediol (FIG. **45**A), and the formation of 2-butanone from meso-2,3-butanediol (FIG. **45**B), both of which were catalyzed in vitro by an isolated B12 independent diol dehydratase, as described in Example 9.

[0054] FIG. **46**A shows the in vivo production of 1-propanol from 1,2-propanediol. FIG. **46**B shows the in vivo production of 2-butanol from meso-2,3 butanediol. FIG. **46**C shows the in vivo production of cyclopentanone from trans-1,2-cyclopentanediol. These experiments were performed as described in Example 9.

[0055] FIG. **47** shows the results of the TBA assay, as performed in Example 10. The left tube in FIG. **47** represents media taken from an overnight culture of cells expressing Vs24254, showing secretion of an alginate lyase, while the right hand tube shows the TBA reaction using media from cells expressing Vs24259 (negative control). The lack of pink coloration in the negative control indicates that little or no cleavage of the alginate polymer has occurred.

[0056] FIG. **48** shows the in vivo biological activity of a C—C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show that codon-optimized benzaldehyde lyase (BAL) catalyzed the in vivo production of 3-hydroxy-2-pentanone and 2-hydroxy-3-pentanone from a ligation reaction between acetaldehyde and propionaldehyde (FIG. **48**A), and catalyzed the in vivo production of 4-hydroxy-3-heptanone and 3-hydroxy-4-heptanone from a ligation reaction between propionaldehyde and butyraldehyde (FIG. **48**B).

[0057] FIG. **49** shows the in vivo biological activity of a C—C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show that codon-optimized BAL catalyzed the in vivo production of 3-hydroxy-2-heptanone from a ligation reaction between acetaldehyde and pentanal (FIG. **49**A), and catalyzed the in vivo production of 4-hydroxy-3-octanone and 3-hydroxy-4-octanone from a ligation reaction between pentanal and propionaldehyde (FIG. **49**B).

[0058] FIG. **50** shows the in vivo biological activity of a C—C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show that codon-optimized BAL catalyzed the in vivo production of 5-hydroxy-4-nonanone from ligation reaction between butyraldehyde and pentanal (FIG. **50**A), and catalyzed the in vivo production of 2-methyl-5-hydroxy-4-decanone and 2-methyl-4-hydroxy-5-decanone from ligation reaction between hexanal and 3-methylbutyraldehyde (FIG. **50**B).

[0059] FIG. **51** shows the in vivo biological activity of a C—C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show that codon-optimized BAL catalyzed the in vivo production of 6-methyl-3-hydroxy-2-heptanone from ligation reaction between acetaldehyde and 4-methylhexanal (FIG. **51**A), and catalyzed the in vivo production of 7-methyl-4-hydroxy-3-octanone from a ligation reaction between 4-methylhexanal and propionaldehyde (FIG. **51**B).

[0060] FIG. **52** shows the in vivo biological activity of a C—C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show

that codon-optimized BAL catalyzed the in vivo production of 8-methyl-5-hydroxy-4-nonanone from ligation reaction between 4-methylhexanal and butyraldehyde (FIG. **52**A), and catalyzed the in vivo production of 3-hydroxy-2-decanone from a ligation reaction between acetaldehyde and octanal (FIG. **52**B).

[0061] FIG. **53** shows the in vivo biological activity of a C—C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show that codon-optimized BAL catalyzed the in vivo production of 4-hydroxy-3-undecanone from ligation reaction between octanal and propionaldehyde (FIG. **53**A), and catalyzed the in vivo production of 5-hydroxy-4-dodecanone from a ligation reaction between octanal and butyraldehyde (FIG. **53**B).

[0062] FIG. **54** shows the in vivo biological activity of a C—C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show that codon-optimized BAL catalyzed the in vivo production of 6-hydroxy-5-tridecanone (FIG. **54**A) from ligation reaction between octanal and pentanal, and catalyzed the in vivo production of 2-methyl-5-hydroxy-4-dodecanone and 2-methyl-4-hydroxy-5-decanone from a ligation reaction between octanal and 3-methylbutyraldehyde (FIG. **54**B).

[0063] FIG. **55** shows the in vivo biological activity of a C—C ligase isolated from *Pseudomonas fluorescens* and cloned into *E. coli*. The GC-MS chromatogram results show that codon-optimized BAL catalyzed the in vivo production of 2-methyl-6-hydroxy-5-tridecanone from a ligation reaction between octanal and 4-methylpentanal.

[0064] FIG. **56** shows the growth of recombinant *E. coli* on alginate as a sole source of carbon (FIG. **56**A), as described in Example 10. Growth on glucose (FIG. **56**B) provides a positive control. The cells were transformed with either no plasmid (BL21—negative control), one plasmid (e.g., Da or 3a), or two plasmids (e.g., Dk3a and Da3k). The plasmids are indicated by the lower case letter: "a" refers to the pET-DEST42 plasmid backbone and "k" refers to the pENTR/D/TOPO backbone. "D" indicates that the plasmid contains the genomic region Vs24214-24249, while "3" indicates that the plasmid contains the genomic region Vs24189-24209. Thus, Da would be pET-DEST42-Vs24214-24249, Da3k would be pET-DEST42-Vs24214-24249 and pENTR/D/TOPO-Vs24189-24209 and so on. These results show that the combined genomic regions Vs24214-24249 and Vs24189-24209 are sufficient to confer on *E. coli* the ability to grow on alginate as a sole source of carbon.

[0065] FIG. **57** shows the production of ethanol by *E. coli* growing on alginate, as performed in Example 11. *E. coli* was transformed with either pBBRPdc-AdhA/B or pBBRPdc-AdhA/B+1.5 FOS and allowed to grow in m9 media containing alginate.

BRIEF SUMMARY

[0066] Embodiments of the present invention include methods for converting a polysaccharide to a commodity chemical, comprising (a) contacting the polysaccharide, wherein the polysaccharide is optionally derived from biomass, with a polysaccharide degrading or depolymerizing metabolic system, wherein the metabolic system is selected from; (i) enzymatic or chemical catalysis, and (ii) a microbial system, wherein the microbial system comprises a recombinant microorganism, wherein the recombinant microorganism comprises one or exogenous genes that allow it to grow on the polysaccharide as a sole source of carbon, thereby converting the polysaccharide to a suitable monosaccharide or oligosaccharide; and (b) contacting the suitable monosaccharide or oligosaccharide with commodity chemical biosynthesis pathway, wherein the commodity chemical biosynthesis pathway comprises an aldehyde or ketone biosynthesis pathway, thereby converting the polysaccharide to the commodity chemical.

[0067] In certain aspects, the biomass is selected from marine biomass and vegetable/fruit/plant biomass. In certain aspects, the marine biomass is selected from kelp, giant kelp, sargasso, seaweed, algae, marine microflora, microalgae, and sea grass. In certain aspects, the vegetable/fruit/plant biomass comprises plant peel or pomace. In certain aspects, the vegetable/fruit/plant biomass is selected from citrus, potato, tomato, grape, gooseberry, carrot, mango, sugar-beet, apple, switchgrass, wood, and stover.

[0068] In certain aspects, the polysaccharide is selected from alginate, agar, carrageenan, fucoidan, pectin, polygalacturonate, cellulose, hemicellulose, xylan, arabinan, and mannan. In certain aspects, the suitable monosaccharide or oligosaccharide is selected from 2-keto-3-deoxy D-gluconate (KDG), D-mannitol, guluronate, mannuronate, mannitol, lyxose, glycerol, xylitol, glucose, mannose, galactose, xylose, arabinose, glucuronate, galacturonates, and rhamnose.

[0069] In certain aspects, the commodity chemical is selected from methane, methanol, ethane, ethene, ethanol, n-propane, 1-propene, 1-propanol, propanal, acetone, propionate, n-butane, 1-butene, 1-butanol, butanal, butanoate, isobutanal, isobutanol, 2-methylbutanal, 2-methylbutanol, 3-methylbutanal, 3-methylbutanol, 2-butene, 2-butanol, 2-butanone, 2,3-butanediol, 3-hydroxy-2-butanone, 2,3-butanedione, ethylbenzene, ethenylbenzene, 2-phenylethanol, phenylacetaldehyde, 1-phenylbutane, 4-phenyl-1-butene, 4-phenyl-2-butene, 1-phenyl-2-butene, 1-phenyl-2-butanol, 4-phenyl-2-butanol, 1-phenyl-2-butanone, 4-phenyl-2-butanone, 1-phenyl-2,3-butandiol, 1-phenyl-3-hydroxy-2-butanone, 4-phenyl-3-hydroxy-2-butanone, 1-phenyl-2,3-butanedione, n-pentane, ethylphenol, ethenylphenol, 2-(4-hydroxyphenyl)ethanol, 4-hydroxyphenylacetaldehyde, 1-(4-hydroxyphenyl) butane, 4-(4-hydroxyphenyl)-1-butene, 4-(4-hydroxyphenyl)-2-butene, 1-(4-hydroxyphenyl)-1-butene, 1-(4-hydroxyphenyl)-2-butanol, 4-(4-hydroxyphenyl)-2-butanol, 1-(4-hydroxyphenyl)-2-butanone, 4-(4-hydroxyphenyl)-2-butanone, 1-(4-hydroxyphenyl)-2,3-butandiol, 1-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 4-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-2,3-butanonedione, indolylethane, indolylethene, 2-(indole-3-)ethanol, n-pentane, 1-pentene, 1-pentanol, pentanal, pentanoate, 2-pentene, 2-pentanol, 3-pentanol, 2-pentanone, 3-pentanone, 4-methylpentanal, 4-methylpentanol, 2,3-pentanediol, 2-hydroxy-3-pentanone, 3-hydroxy-2-pentanone, 2,3-pentanedione, 2-methylpentane, 4-methyl-1-pentene, 4-methyl-2-pentene, 4-methyl-3-pentene, 4-methyl-2-pentanol, 2-methyl-3-pentanol, 4-methyl-2-pentanone, 2-methyl-3-pentanone, 4-methyl-2,3-pentanediol, 4-methyl-2-hydroxy-3-pentanone, 4-methyl-3-hydroxy-2-pentanone, 4-methyl-2,3-pentanedione, 1-phenylpentane, 1-phenyl-1-pentene, 1-phenyl-2-pentene, 1-phenyl-3-pentene, 1-phenyl-2-pentanol, 1-phenyl-3-pentanol, 1-phenyl-2-pentanone, 1-phenyl-3-pentanone, 1-phenyl-2,3-pentanediol, 1-phenyl-2-hydroxy-3-pentanone, 1-phenyl-3-hydroxy-2-pentanone, 1-phenyl-2,3-pentanedione, 4-methyl-1-phenylpentane, 4-methyl-1-phenyl-1-pen-

tene, 4-methyl-1-phenyl-2-pentene, 4-methyl-1-phenyl-3-pentene, 4-methyl-1-phenyl-3-pentanol, 4-methyl-1-phenyl-2-pentanol, 4-methyl-1-phenyl-3-pentanone, 4-methyl-1-phenyl-2-pentanone, 4-methyl-1-phenyl-2,3-pentanediol, 4-methyl-1-phenyl-2,3-pentanedione, 4-methyl-1-phenyl-3-hydroxy-2-pentanone, 4-methyl-1-phenyl-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl) pentane, 1-(4-hydroxyphenyl)-1-pentene, 1-(4-hydroxyphenyl)-2-pentene, 1-(4-hydroxyphenyl)-3-pentene, 1-(4-hydroxyphenyl)-2-pentanol, 1-(4-hydroxyphenyl)-3-pentanol, 1-(4-hydroxyphenyl)-2-pentanone, 1-(4-hydroxyphenyl)-3-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanediol, 1-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-1-(4-hydroxyphenyl) pentane, 4-methyl-1-(4-hydroxyphenyl)-2-pentene, 4-methyl-1-(4-hydroxyphenyl)-3-pentene, 4-methyl-1-(4-hydroxyphenyl)-1-pentene, 4-methyl-1-(4-hydroxyphenyl)-3-pentanol, 4-methyl-1-(4-hydroxyphenyl)-2-pentanol, 4-methyl-1-(4-hydroxyphenyl)-3-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2,3-pentanediol, 4-methyl-1-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, 1-indole-3-pentane, 1-(indole-3)-1-pentene, 1-(indole-3)-2-pentene, 1-(indole-3)-3-pentene, 1-(indole-3)-2-pentanol, 1-(indole-3)-3-pentanol, 1-(indole-3)-2-pentanone, 1-(indole-3)-3-pentanone, 1-(indole-3)-2,3-pentanediol, 1-(indole-3)-2-hydroxy-3-pentanone, 1-(indole-3)-3-hydroxy-2-pentanone, 1-(indole-3)-2,3-pentanedione, 4-methyl-1-(indole-3-)pentane, 4-methyl-1-(indole-3)-2-pentene, 4-methyl-1-(indole-3)-3-pentene, 4-methyl-1-(indole-3)-1-pentene, 4-methyl-2-(indole-3)-3-pentanol, 4-methyl-1-(indole-3)-2-pentanol, 4-methyl-1-(indole-3)-3-pentanone, 4-methyl-1-(indole-3)-2-pentanone, 4-methyl-1-(indole-3)-2,3-pentanediol, 4-methyl-1-(indole-3)-2,3-pentanedione, 4-methyl-1-(indole-3)-3-hydroxy-2-pentanone, 4-methyl-1-(indole-3)-2-hydroxy-3-pentanone, n-hexane, 1-hexene, 1-hexanol, hexanal, hexanoate, 2-hexene, 3-hexene, 2-hexanol, 3-hexanol, 2-hexanone, 3-hexanone, 2,3-hexanediol, 2,3-hexanedione, 3,4-hexanediol, 3,4-hexanedione, 2-hydroxy-3-hexanone, 3-hydroxy-2-hexanone, 3-hydroxy-4-hexanone, 4-hydroxy-3-hexanone, 2-methylhexane, 3-methylhexane, 2-methyl-2-hexene, 2-methyl-3-hexene, 5-methyl-1-hexene, 5-methyl-2-hexene, 4-methyl-1-hexene, 4-methyl-2-hexene, 3-methyl-3-hexene, 3-methyl-2-hexene, 3-methyl-1-hexene, 2-methyl-3-hexanol, 5-methyl-2-hexanol, 5-methyl-3-hexanol, 2-methyl-3-hexanone, 5-methyl-2-hexanone, 5-methyl-3-hexanone, 2-methyl-3,4-hexanediol, 2-methyl-3,4-hexanedione, 5-methyl-2,3-hexanediol, 5-methyl-2,3-hexanedione, 4-methyl-2,3-hexanediol, 4-methyl-2,3-hexanedione, 2-methyl-3-hydroxy-4-hexanone, 2-methyl-4-hydroxy-3-hexanone, 5-methyl-2-hydroxy-3-hexanone, 5-methyl-3-hydroxy-2-hexanone, 4-methyl-2-hydroxy-3-hexanone, 4-methyl-3-hydroxy-2-hexanone, 2,5-dimethylhexane, 2,5-dimethyl-2-hexene, 2,5-dimethyl-3-hexene, 2,5-dimethyl-3-hexanol, 2,5-dimethyl-3-hexanone, 2,5-dimethyl-3,4-hexanediol, 2,5-dimethyl-3,4-hexanedione, 2,5-dimethyl-3-hydroxy-4-hexanone, 5-methyl-1-phenylhexane, 4-methyl-1-phenylhexane, 5-methyl-1-phenyl-1-hexene, 5-methyl-1-phenyl-2-hexene, 5-methyl-1-phenyl-3-hexene, 4-methyl-1-phenyl-1-hexene, 4-methyl-1-phenyl-2-hexene, 4-methyl-1-phenyl-3-hexene, 5-methyl-1-phenyl-2-hexanol, 5-methyl-1-phenyl-3-hexanol, 4-methyl-1-phenyl-2-hexanol, 4-methyl-1-phenyl-3-hexanol, 5-methyl-1-phenyl-2-hexanone, 5-methyl-1-phenyl-3-hexanone, 4-methyl-1-phenyl-2-hexanone, 4-methyl-1-phenyl-3-hexanone, 5-methyl-1-phenyl-2,3-hexanediol, 4-methyl-1-phenyl-2,3-hexanediol, 5-methyl-1-phenyl-3-hydroxy-2-hexanone, 5-methyl-1-phenyl-2-hydroxy-3-hexanone, 4-methyl-1-phenyl-3-hydroxy-2-hexanone, 4-methyl-1-phenyl-2-hydroxy-3-hexanone, 5-methyl-1-phenyl-2,3-hexanedione, 4-methyl-1-phenyl-2,3-hexanedione, 4-methyl-1-(4-hydroxyphenyl)hexane, 5-methyl-1-(4-hydroxyphenyl)-1-hexene, 5-methyl-1-(4-hydroxyphenyl)-2-hexene, 5-methyl-1-(4-hydroxyphenyl)-3-hexene, 4-methyl-1-(4-hydroxyphenyl)-1-hexene, 4-methyl-1-(4-hydroxyphenyl)-2-hexene, 4-methyl-1-(4-hydroxyphenyl)-3-hexene, 5-methyl-1-(4-hydroxyphenyl)-2-hexanol, 5-methyl-1-(4-hydroxyphenyl)-3-hexanol, 4-methyl-1-(4-hydroxyphenyl)-2-hexanol, 4-methyl-1-(4-hydroxyphenyl)-3-hexanol, 5-methyl-1-(4-hydroxyphenyl)-2-hexanone, 5-methyl-1-(4-hydroxyphenyl)-3-hexanone, 4-methyl-1-(4-hydroxyphenyl)-2-hexanone, 4-methyl-1-(4-hydroxyphenyl)-3-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2,3-hexanediol, 4-methyl-1-(4-hydroxyphenyl)-2,3-hexanediol, 5-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-hexanone, 4-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-hexanone, 4-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2,3-hexanedione, 4-methyl-1-(4-hydroxyphenyl)-2,3-hexanedione, 4-methyl-1-(indole-3-)hexane, 5-methyl-1-(indole-3)-1-hexene, 5-methyl-1-(indole-3)-2-hexene, 5-methyl-1-(indole-3)-3-hexene, 4-methyl-1-(indole-3)-1-hexene, 4-methyl-1-(indole-3)-2-hexene, 4-methyl-1-(indole-3)-3-hexene, 5-methyl-1-(indole-3)-2-hexanol, 5-methyl-1-(indole-3)-3-hexanol, 4-methyl-1-(indole-3)-2-hexanol, 4-methyl-1-(indole-3)-3-hexanol, 5-methyl-1-(indole-3)-2-hexanone, 5-methyl-1-(indole-3)-3-hexanone, 4-methyl-1-(indole-3)-2-hexanone, 4-methyl-1-(indole-3)-3-hexanone, 5-methyl-1-(indole-3)-2,3-hexanediol, 4-methyl-1-(indole-3)-2,3-hexanediol, 5-methyl-1-(indole-3)-3-hydroxy-2-hexanone, 5-methyl-1-(indole-3)-2-hydroxy-3-hexanone, 4-methyl-1-(indole-3)-3-hydroxy-2-hexanone, 4-methyl-1-(indole-3)-2-hydroxy-3-hexanone, 5-methyl-1-(indole-3)-2,3-hexanedione, 4-methyl-1-(indole-3)-2,3-hexanedione, n-heptane, 1-heptene, 1-heptanol, heptanal, heptanoate, 2-heptene, 3-heptene, 2-heptanol, 3-heptanol, 4-heptanol, 2-heptanone, 3-heptanone, 4-heptanone, 2,3-heptanediol, 2,3-heptanedione, 3,4-heptanediol, 3,4-heptanedione, 2-hydroxy-3-heptanone, 3-hydroxy-2-heptanone, 3-hydroxy-4-heptanone, 4-hydroxy-3-heptanone, 2-methylheptane, 3-methylheptane, 6-methyl-2-heptene, 6-methyl-3-heptene, 2-methyl-3-heptene, 2-methyl-2-heptene, 5-methyl-2-heptene, 5-methyl-3-heptene, 3-methyl-3-heptene, 2-methyl-3-heptanol, 2-methyl-4-heptanol, 6-methyl-3-heptanol, 5-methyl-3-heptanol, 3-methyl-4-heptanol, 2-methyl-3-heptanone, 2-methyl-4-heptanone, 6-methyl-3-heptanone, 5-methyl-3-heptanone, 3-methyl-4-heptanone, 2-methyl-3,4-heptanediol, 2-methyl-3,4-heptanedione, 6-methyl-3,4-heptanediol, 6-methyl-3,4-heptanedione, 5-methyl-3,4-heptanediol, 5-methyl-3,4-heptanedione, 2-methyl-3-hydroxy-4-heptanone, 2-methyl-4-hydroxy-3-heptanone, 6-methyl-3-hydroxy-4-heptanone, 6-methyl-4-hydroxy-3-heptanone, 5-methyl-3-hydroxy-4-heptanone, 5-methyl-4-hydroxy-3-heptanone, 2,6-dimethylheptane, 2,5-dimethylheptane, 2,6-dimethyl-2-heptene, 2,6-dimethyl-3-heptene, 2,5-dimethyl-2-heptene, 2,5-dimethyl-

3-heptene, 3,6-dimethyl-3-heptene, 2,6-dimethyl-3-heptanol, 2,6-dimethyl-4-heptanol, 2,5-dimethyl-3-heptanol, 2,5-dimethyl-4-heptanol, 2,6-dimethyl-3,4-heptanediol, 2,6-dimethyl-3,4-heptanedione, 2,5-dimethyl-3,4-heptanediol, 2,5-dimethyl-3,4-heptanedione, 2,6-dimethyl-3-hydroxy-4-heptanone, 2,6-dimethyl-4-hydroxy-3-heptanone, 2,5-dimethyl-3-hydroxy-4-heptanone, 2,5-dimethyl-4-hydroxy-3-heptanone, n-octane, 1-octene, 2-octene, 1-octanol, octanal, octanoate, 3-octene, 4-octene, 4-octanol, 4-octanone, 4,5-octanediol, 4,5-octanedione, 4-hydroxy-5-octanone, 2-methyloctane, 2-methyl-3-octene, 2-methyl-4-octene, 7-methyl-3-octene, 3-methyl-3-octene, 3-methyl-4-octene, 6-methyl-3-octene, 2-methyl-4-octanol, 7-methyl-4-octanol, 3-methyl-4-octanol, 6-methyl-4-octanol, 2-methyl-4-octanone, 7-methyl-4-octanone, 3-methyl-4-octanone, 6-methyl-4-octanone, 2-methyl-4,5-octanediol, 2-methyl-4,5-octanedione, 3-methyl-4,5-octanediol, 3-methyl-4,5-octanedione, 2-methyl-4-hydroxy-5-octanone, 2-methyl-5-hydroxy-4-octanone, 3-methyl-4-hydroxy-5-octanone, 3-methyl-5-hydroxy-4-octanone, 2,7-dimethyloctane, 2,7-dimethyl-3-octene, 2,7-dimethyl-4-octene, 2,7-dimethyl-4-octanol, 2,7-dimethyl-4-octanone, 2,7-dimethyl-4,5-octanediol, 2,7-dimethyl-4,5-octanedione, 2,7-dimethyl-4-hydroxy-5-octanone, 2,6-dimethyloctane, 2,6-dimethyl-3-octene, 2,6-dimethyl-4-octene, 3,7-dimethyl-3-octene, 2,6-dimethyl-4-octanol, 3,7-dimethyl-4-octanol, 2,6-dimethyl-4-octanone, 3,7-dimethyl-4-octanone, 2,6-dimethyl-4,5-octanediol, 2,6-dimethyl-4,5-octanedione, 2,6-dimethyl-4-hydroxy-5-octanone, 2,6-dimethyl-5-hydroxy-4-octanone, 3,6-dimethyloctane, 3,6-dimethyl-3-octene, 3,6-dimethyl-4-octene, 3,6-dimethyl-4-octanol, 3,6-dimethyl-4-octanone, 3,6-dimethyl-4,5-octanediol, 3,6-dimethyl-4,5-octanedione, 3,6-dimethyl-4-hydroxy-5-octanone, n-nonane, 1-nonene, 1-nonanol, nonanal, nonanoate, 2-methylnonane, 2-methyl-4-nonene, 2-methyl-5-nonene, 8-methyl-4-nonene, 2-methyl-5-nonanol, 8-methyl-4-nonanol, 2-methyl-5-nonanone, 8-methyl-4-nonanone, 8-methyl-4,5-nonanediol, 8-methyl-4,5-nonanedione, 8-methyl-4-hydroxy-5-nonanone, 8-methyl-5-hydroxy-4-nonanone, 2,8-dimethylnonane, 2,8-dimethyl-3-nonene, 2,8-dimethyl-4-nonene, 2,8-dimethyl-5-nonene, 2,8-dimethyl-4-nonanol, 2,8-dimethyl-5-nonanol, 2,8-dimethyl-4-nonanone, 2,8-dimethyl-5-nonanone, 2,8-dimethyl-4,5-nonanediol, 2,8-dimethyl-4,5-nonanedione, 2,8-dimethyl-4-hydroxy-5-nonanone, 2,8-dimethyl-5-hydroxy-4-nonanone, 2,7-dimethylnonane, 3,8-dimethyl-3-nonene, 3,8-dimethyl-4-nonene, 3,8-dimethyl-5-nonene, 3,8-dimethyl-4-nonanol, 3,8-dimethyl-5-nonanol, 3,8-dimethyl-4-nonanone, 3,8-dimethyl-5-nonanone, 3,8-dimethyl-4,5-nonanediol, 3,8-dimethyl-4,5-nonanedione, 3,8-dimethyl-4-hydroxy-5-nonanone, 3,8-dimethyl-5-hydroxy-4-nonanone, n-decane, 1-decene, 1-decanol, decanoate, 2,9-dimethyldecane, 2,9-dimethyl-3-decene, 2,9-dimethyl-4-decene, 2,9-dimethyl-5-decanol, 2,9-dimethyl-5-decanone, 2,9-dimethyl-5,6-decanediol, 2,9-dimethyl-6-hydroxy-5-decanone, 2,9-dimethyl-5,6-decanedionen-undecane, 1-undecene, 1-undecanol, undecanal. undecanoate, n-dodecane, 1-dodecene, 1-dodecanol, dodecanal, dodecanoate, n-dodecane, 1-decadecene, 1-dodecanol, ddodecanal, dodecanoate, n-tridecane, 1-tridecene, 1-tridecanol, tridecanal, tridecanoate, n-tetradecane, 1-tetradecene, 1-tetradecanol, tetradecanal, tetradecanoate, n-pentadecane, 1-pentadecene, 1-pentadecanol, pentadecanal, pentadecanoate, n-hexadecane, 1-hexadecene, 1-hexadecanol, hexadecanal, hexadecanoate, n-heptadecane, 1-heptadecene, 1-heptadecanol, heptadecanal, heptadecanoate, n-octadecane, 1-octadecene, 1-octadecanol, octadecanal, octadecanoate, n-nonadecane, 1-nonadecene, 1-nonadecanol, nonadecanal, nonadecanoate, eicosane, 1-eicosene, 1-eicosanol, eicosanal, eicosanoate, 3-hydroxy propanal, 1,3-propanediol, 4-hydroxybutanal, 1,4-butanediol, 3-hydrxy-2-butanone, 2,3-butandiol, 1,5-pentane diol, homocitrate, homoisocitorate, b-hydroxy adipate, glutarate, glutarsemialdehyde, glutaraldehyde, 2-hydroxy-1-cyclopentanone, 1,2-cyclopentanediol, cyclopentanone, cyclopentanol, (S)-2-acetolactate, (R)-2,3-Dihydroxy-isovalerate, 2-oxoisovalerate, isobutyryl-CoA, isobutyrate, isobutyraldehyde, 5-amino pentaldehyde, 1,10-diaminodecane, 1,10-diamino-5-decene, 1,10-diamino-5-hydroxydecane, 1,10-diamino-5-decanone, 1,10-diamino-5,6-decanediol, 1,10-diamino-6-hydroxy-5-decanone, phenylacetoaldehyde, 1,4-diphenylbutane, 1,4-diphenyl-1-butene, 1,4-diphenyl-2-butene, 1,4-diphenyl-2-butanol, 1,4-diphenyl-2-butanone, 1,4-diphenyl-2,3-butanediol, 1,4-diphenyl-3-hydroxy-2-butanone, 1-(4-hydeoxyphenyl)-4-phenylbutane, 1-(4-hydeoxyphenyl)-4-phenyl-1-butene, 1-(4-hydeoxyphenyl)-4-phenyl-2-butene, 1-(4-hydeoxyphenyl)-4-phenyl-2-butanol, 1-(4-hydeoxyphenyl)-4-phenyl-2-butanone, 1-(4-hydeoxyphenyl)-4-phenyl-2,3-butanediol, 1-(4-hydeoxyphenyl)-4-phenyl-3-hydroxy-2-butanone, 1-(indole-3)-4-phenylbutane, 1-(indole-3)-4-phenyl-1-butene, 1-(indole-3)-4-phenyl-2-butene, 1-(indole-3)-4-phenyl-2-butanol, 1-(indole-3)-4-phenyl-2-butanone, 1-(indole-3)-4-phenyl-2,3-butanediol, 1-(indole-3)-4-phenyl-3-hydroxy-2-butanone, 4-hydroxyphenylacetoaldehyde, 1,4-di(4-hydroxyphenyl)butane, 1,4-di(4-hydroxyphenyl)-1-butene, 1,4-di(4-hydroxyphenyl)-2-butene, 1,4-di(4-hydroxyphenyl)-2-butanol, 1,4-di(4-hydroxyphenyl)-2-butanone, 1,4-di(4-hydroxyphenyl)-2,3-butanediol, 1,4-di(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3-)butane, 1-(4-hydroxyphenyl)-4-(indole-3)-1-butene, 1-di(4-hydroxyphenyl)-4-(indole-3)-2-butene, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanol, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3)-2,3-butanediol, 1-(4-hydroxyphenyl-4-(indole-3)-3-hydroxy-2-butanone, indole-3-acetoaldehyde, 1,4-di(indole-3-)butane, 1,4-di(indole-3)-1-butene, 1,4-di(indole-3)-2-butene, 1,4-di(indole-3)-2-butanol, 1,4-di(indole-3)-2-butanone, 1,4-di(indole-3)-2,3-butanediol, 1,4-di(indole-3)-3-hydroxy-2-butanone, succinate semialdehyde, hexane-1,8-dicarboxylic acid, 3-hexene-1,8-dicarboxylic acid, 3-hydroxy-hexane-1,8-dicarboxylic acid, 3-hexanone-1,8-dicarboxylic acid, 3,4-hexanediol-1,8-dicarboxylic acid, 4-hydroxy-3-hexanone-1,8-dicarboxylic acid, fucoidan, iodine, chlorophyll, carotenoid, calcium, magnesium, iron, sodium, potassium, and phosphate.

[0070] Certain embodiments of the present invention include methods for converting a polysaccharide to a suitable monosaccharide or oligosaccharide, comprising: (a) contacting the polysaccharide, wherein the polysaccharide is optionally obtained from biomass, with a microbial system for a time sufficient to convert the polysaccharide to a suitable monosaccharide or oligosaccharide, wherein the microbial system comprises, (i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase, wherein the lyase and/or hydrolase optionally comprises at least one signal peptide or at least one autotransporter domain; (ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide trans-

porter, a trisaccharide transporter, an oligosaccharide transporter, a polysaccharide transporter, and a superchannel; and (iii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase, thereby converting the polysaccharide to a suitable monosaccharide or oligosaccharide.

[0071] Certain embodiments of the present invention include methods for converting a polysaccharide to a suitable monosaccharide or oligosaccharide, comprising: (a) contacting the polysaccharide, wherein the polysaccharide is optionally obtained from biomass, with a chemical or enzymatic catalysis pathway for a time sufficient to convert the polysaccharide to a first monosaccharide or oligosaccharide; and (b) contacting the first monosaccharide or oligosaccharide with a microbial system for a time sufficient to convert the first monosaccharide or oligosaccharide to the suitable monosaccharide or oligosaccharide, wherein the microbial system comprises, (i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase, (ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, a polysaccharide transporter, and a superchannel; and (ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase, thereby converting the polysaccharide to the suitable monosaccharide or oligosaccharide.

[0072] In certain aspects, the lyase is selected from an alginate lyase, a pectate lyase, a polymannuronate lyase, a polygluronate lyase, a polygalacturonate lyase and a rhamnogalacturonate lyase. In certain aspects, the hydrolase is selected from an alginate hydrolase, a rhamnogalacturonate hydrolase, a polymannuronate hydrolase, a pectin hydrolase, and a polygalacturonate hydrolase. In certain aspects, the transporter is selected from an ABC transporter, a symporter, and an outer membrane porin. In certain aspects, the ABC transporter is selected from Atu3021, Atu3022, Atu3023, Atu3024, algM1, algM2, AlgQ1, AlgQ2, AlgS, OG2516_05558, OG2516_05563, OG2516_05568, OG2516_05573, TogM, TogN, TogA, TogB, and functional variants thereof. In certain aspects, the symporter is selected from V12B01_24239 (SEQ ID NO:26), V12B01_24194 (SEQ ID NO:8), and TogT, and functional variants thereof. In certain aspects, the outermembrane porin comprises a porin selected from V12B01_24269, KdgM, and KdgN, and functional variants thereof.

[0073] Certain embodiments include a recombinant microorganism that is capable of growing on a polysaccharide as a sole source of carbon, wherein the polysaccharide is selected from alginate, pectin, tri-galacturonate, di-galacturonate, cellulose, and hemi-cellulose. In certain aspects, the polysaccharide is alginate. In certain aspects, the polysaccharide is pectin. In certain aspects, the polysaccharide is tri-galacturonate.

[0074] Certain embodiments include a recombinant microrganism, comprising (i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase, wherein the lyase or hydrolase optionally comprises at least one signal peptide or at least one autotransporter domain; (ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, a polysaccharide transporter, and a superchannel; and (iii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase. In certain aspects, the microorganism is capable of growing on a polysaccharide as a sole source of carbon. In certain aspects, the polysaccharide is selected from alginate, pectin, and tri-galacturonate.

[0075] Certain embodiments include methods for converting a suitable monosaccharide or oligosaccharide to a first commodity chemical comprising, (a) contacting the suitable monosaccharide or oligosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide or oligosaccharide to the commodity chemical, wherein the microbial system comprises a recombinant microorganism, wherein the microorganism comprises a commodity chemical biosynthesis pathway, thereby converting the suitable monosaccharide or oligosaccharide to the first commodity chemical. In certain aspects, the commodity chemical pathway comprises one or more genes encoding an aldehyde or ketone biosynthesis pathway.

[0076] In certain aspects, the aldehyde or ketone biosynthesis pathway is selected from one or more of an acetoaldehyde, a propionaldehyde, a butyraldehyde, an isobutyraldehyde, a 2-methyl-butyraldehyde, a 3-methyl-butyraldehyde, a 2-phenyl acetaldehyde, a 2-(4-hydroxyphenyl)acetaldehyde, a 2-Indole-3-acetoaldehyde, a glutaraldehyde, a 5-amino-pentaldehyde, a succinate semialdehyde, and a succinate 4-hydroxyphenyl acetaldehyde biosynthesis pathway. In certain aspects, the aldehyde or ketone biosynthesis pathway comprises an acetoaldehyde biosynthesis pathway and a biosynthesis pathway selected from a propionaldehyde, butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl)acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

[0077] In certain aspects, the aldehyde or ketone biosynthesis pathway comprises a propionaldehyde biosynthesis pathway and a biosynthesis pathway selected from a butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, and phenylacetoaldehyde biosynthesis pathway. In certain aspects, the aldehyde or ketone biosynthesis pathway comprises a butyraldehyde biosynthesis pathway and a biosynthesis pathway selected from an isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl)acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway. In certain aspects, the aldehyde or ketone biosynthesis pathway comprises an isobutyraldehyde biosynthesis pathway and a biosynthesis pathway selected from a 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl)acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

[0078] In certain aspects, the aldehyde or ketone biosynthesis pathway comprises a 2-methyl-butyraldehyde biosynthesis pathway and a biosynthesis pathway selected from a 3-methyl-butyraldehyde, a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl)acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway. In certain aspects, the aldehyde or ketone biosynthesis pathway comprises a 3-methyl-butyraldehyde biosynthesis pathway and a biosynthesis pathway selected from a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl)acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway. In certain aspects, the aldehyde or ketone biosynthesis pathway comprises a 2-phenyl acetoaldehyde

biosynthesis pathway and a biosynthesis pathway selected from a 2-(4-hydroxyphenyl)acetaldehyde and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

[0079] In certain aspects, the aldehyde or ketone biosynthesis pathway comprises a 2-(4-hydroxyphenyl)acetaldehyde biosynthesis pathway and a 2-Indole-3-acetoaldehyde biosynthesis pathway. In certain aspects, the first commodity chemical is further enzymatically and/or chemically reduced and dehydrated to a second commodity chemical.

[0080] Certain embodiments include methods for converting a suitable monosaccharide or oligosaccharide to a commodity chemical comprising, (a) contacting the suitable monosaccharide or oligosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide or oligosaccharide to the commodity chemical, wherein the microbial system comprises; (i) one or more genes encoding and expressing an aldehyde biosynthesis pathway, wherein the aldehyde biosynthesis pathway comprises one or more genes encoding and expressing a decarboxylase enzyme; and (ii)

[0081] one or more genes encoding and expressing an aldehyde reductase, thereby converting the suitable monosaccharide or oligosaccharide to the commodity chemical. In certain aspects, the decarboxylase enzyme is an indole-3-pyruvate decarboxylase (IPDC). In certain aspects, the IPDC comprises an amino acid sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 312. In certain aspects, the aldehyde reductase enzyme is a phenylacetaldehyde reductase (PAR). In certain aspects, the PAR comprises an amino acid sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 313. In certain aspects, the commodity chemical is selected from 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and indole-3-ethanol.

[0082] Certain embodiments include a recombinant microorganism, comprising (i) one or more genes encoding and expressing an aldehyde biosynthesis pathway, wherein the aldehyde biosynthesis pathway comprises one or more genes encoding and expressing a decarboxylase enzyme; and (ii) one or more genes encoding and expressing an aldehyde reductase. In certain aspects, the aldehyde biosynthesis pathway further comprises one or more genes encoding and expressing an enzyme selected from a CoA-linked aldehyde dehydrogenase, an aldehyde dehydrogenase, and an alcohol dehydrogenase. In certain aspects, the decarboxylase enzyme is an indole-3-pyruvate decarboxylase (IPDC). In certain aspects, the aldehyde reductase enzyme is a phenylacetoaldehyde reductase (PAR). In certain aspects, the microorganism is capable of converting a suitable monosaccharide or oligosaccharide to a commodity chemical. In certain aspects, the commodity chemical is selected from 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and indole-3-ethanol.

[0083] Certain embodiments include a recombinant microorganism, wherein the microorganism comprises reduced ethanol production capability compared to a wild-type microorganism. In certain aspects, the microorganism comprises a reduction or inhibition in the conversion of acetyl-coA to ethanol. In certain aspects, the recombinant microorganism comprises a reduction of an ethanol dehydrogenase, thereby providing a reduced ethanol production capability. In certain aspects, the ethanol dehydrogenase is an adhE, homolog or variant thereof. In certain aspects, the microorganism comprises a deletion or knockout of an adhE, homolog or variant thereof. In certain aspects, the recombinant microorganism comprises one or more deletions or knockouts in a gene encoding an enzyme selected from an enzyme that catalyzes the conversion of acetyl-coA to ethanol, an enzyme that catalyzes the conversion of pyruvate to lactate, an enzyme that catalyzes the conversion of fumarate to succinate, an enzyme that catalyzes the conversion of acetyl-coA and phosphate to coA and acetyl phosphate, an enzyme that catalyzes the conversion of acetyl-coA and formate to coA and pyruvate, and an enzyme that catalyzes the conversion of alpha-keto acid to branched chain amino acids.

[0084] Certain embodiments include wherein the microbial systems or recombinant microorganisms described herein comprise a microorganism selected from *Acetobacter aceti, Achromobacter, Acidiphilium, Acinetobacter, Actinomadura, Actinoplanes, Aeropyrum pernix, Agrobacterium, Alcaligenes, Ananas comosus* (M), *Arthrobacter, Aspargillus niger, Aspargillus oryze, Aspergillus melleus, Aspergillus pulverulentus, Aspergillus saitoi, Aspergillus sojea, Aspergillus usamii, Bacillus alcalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus lentus, Bacillus licheniformis, Bacillus macerans, Bacillus stearothermophilus, Bacillus subtilis, Bifidobacterium, Brevibacillus brevis, Burkholderia cepacia, Candida cylindracea, Candida rugosa, Carica papaya* (L), *Cellulosimicrobium, Cephalosporium, Chaetomium erraticum, Chaetomium gracile, Clostridium, Clostridium butyricum, Clostridium acetobutylicum, Clostridium thermocellum, Corynebacterium* (glutamicum), *Corynebacterium efficiens, Escherichia coli, Enterococcus, Erwina chrysanthemi, Gliconobacter, Gluconacetobacter, Haloarcula, Humicola insolens, Humicola nsolens, Kitasatospora setae, Klebsiella, Klebsiella oxytoca, Kluyveromyces, Kluyveromyces fragilis, Kluyveromyces lactis, Kocuria, Lactlactis, Lactobacillus, Lactobacillus fermentum, Lactobacillus sake, Lactococcus, Lactococcus lactis, Leuconostoc, Methylocystis, Methanolobus siciliae, Methanogenium organophilum, Methanobacterium bryantii, Microbacterium imperiale, Micrococcus lysodeikticus, Microlunatus, Mucor javanicus, Mycobacterium, Myrothecium, Nitrobacter, Nitrosomonas, Nocardia, Papaya carica, Pediococcus, Pediococcus halophilus, Penicillium, Penicillium camemberti, Penicillium citrinum, Penicillium emersonii, Penicillium roqueforti, Penicillum lilactinum, Penicillum multicolor, Paracoccus pantotrophus, Propionibacterium, Pseudomonas, Pseudomonas fluorescens, Pseudomonas denitrificans, Pyrococcus, Pyrococcus furiosus, Pyrococcus horikoshii, Rhizobium, Rhizomucor miehei, Rhizomucor pusillus Lindt, Rhizopus, Rhizopus delemar, Rhizopus japonicus, Rhizopus niveus, Rhizopus oryzae, Rhizopus oligosporus, Rhodococcus, Sccharomyces cerevisiae, Sclerotina libertina, Sphingobacterium multivorum, Sphingobium, Sphingomonas, Streptococcus, Streptococcus thermophilus* Y-1, *Streptomyces, Streptomyces griseus, Streptomyces lividans, Streptomyces murinus, Streptomyces rubiginosus, Streptomyces violaceoruber, Streptoverticillium mobaraense, Tetragenococcus, Thermus, Thiosphaera pantotropha, Trametes, Trichoderma, Trichoderma longibrachiatum, Trichoderma reesei, Trichoderma viride, Trichosporon penicillatum, Vibrio alginolyticus, Xanthomonas*, yeast, *Zygosaccharomyces rouxii, Zymomonas*, and *Zymomonus mobilis.*

[0085] Certain embodiments include a commodity chemical produced by the methods described herein. Certain aspects include a blended commodity chemical comprising a commodity chemical produced by the methods provided

herein and a refinery-produced petroleum product. In certain aspects, the commodity chemical is selected from a C10-C12 hydrocarbon, 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and indole-3-ethanol. In certain aspects, the C10-C12 hydrocarbon is selected from 2,7-dimethyloctane and 2,9-dimethyldecane. In certain aspects, the refinery-produced petroleum product is selected from jet fuel and diesel fuel.

[0086] Certain embodiments include methods of producing a commodity chemical enriched refinery-produced petroleum product, comprising (a) blending the refinery-produced petroleum product with the commodity chemical produced by the methods described herein, thereby producing the commodity chemical enriched refinery-produced petroleum product.

DETAILED DESCRIPTION

[0087] Embodiments of the present invention relate to the unexpected discovery that microorganisms which are otherwise incapable of growing on certain polysaccharides derived from biomass as a sole source of carbon, can be engineered to grow on these polysaccharides as a sole source of carbon. Such microorganisms can include both prokaryotic and eukaryotic microorganisms, such as bacteria and yeast. In some aspects, certain laboratory and/or wild-type strains of *E. coli* can be engineered to grow on biomass derived from either alginate or pectin as a sole source of carbon to produce suitable monosaccharides or other molecules. Among other uses apparent to a person skilled in the art, the monosaccharides and other molecules produced by the growth of these engineered or recombinant microorganisms on alginate or pectin may be utilized as feedstock in the production of various commodity chemicals, such as biofuels.

[0088] Alginate and pectin provide advantages over other biomass sources in the production of biofuel feedstocks. For example, large-scale aquatic-farming can generate a significant amount of biomass without replacing food crop production with energy crop production, deforestation, and recultivating currently uncultivated land, as most of hydrosphere including oceans, rivers, and lakes remains untapped. As one particular example, the Pacific coast of North America is abundant in minerals necessary for large-scale aqua-farming. Giant kelp, which lives in the area, grows as fast as 1 m/day, the fastest among plants on earth, and grows up to 50 m. Additionally, aqua-farming has other benefits including the prevention of a red tide outbreak and the creation of a fish-friendly environment.

[0089] As an additional advantage, and in contrast to lignocellulolic biomass, biomass derived from aquatic, fruit, plant and/or vegetable sources is easy to degrade. Such biomass typically lacks lignin and is significantly more fragile than lignocellulolic biomass and can thus be easily degraded using either enzymes or chemical catalysts (e.g., formate). As one example, aquatic biomass such as seaweed may be easily converted to monosaccharides using either enzymes or chemical catalysis, as seaweed has significantly simpler major sugar components (Alginate: 30%, Mannitol: 15%) as compared to lignocellulose (Glucose: 24.1-39%, Mannose: 0.2-4.6%, Galactose: 0.5-2.4%, Xylose: 0.4-22.1%, Arabinose 1.5-2.8%, and Uronic acids: 1.2-20.7%, and total sugar contents are corresponding to 36.5-70% of dried weight).

[0090] As an additional example, biomass from plants such as fruit and/or vegetable contains pectin, a heteropolysaccharide derived from the plant cell wall. The characteristic structure of pectin is a linear chain of α-(1-4)-linked D-galacturonic acid that forms the pectin-backbone, a homogalacturonan. Pectin can be easily converted to oligosaccharides or suitable monosaccharides using either enzymes, chemical catalysis, and/or microbial systems designed to utilize pectin as a source of carbon, as described herein. Saccharification and fermentation using aquatic, fruit, and/or vegetable biomass is much easier than using lignocellulose.

[0091] In this regard, embodiments of the present invention also relate to the surprising discovery that certain microorganisms can be engineered to produce various commodity chemicals, such as biofuels. In certain aspects, these biofuels may include alkanes, such as medium to long chain alkanes, which provide advantages over ethanol based biofuels. In certain aspects, the monosaccharides (e.g., 2-keto-3-deoxy D-gluconate; KDG) and other molecules produced by the growth of various engineered or recombinant microorganisms (e.g., recombinant microorganisms growing on pectin or alginate as a source of carbon) may be useful in the production of commodity chemicals, such as biofuels. As one example, suitable monosaccharides such as KDG may be utilized by recombinant microorganisms to produce alkanes, such as medium to long chain alkanes, among other chemicals. In certain aspects, such recombinant microorganisms may be utilized to produce such commodity chemical as 2,7 dimethyl octane and 2,9 dimethyl decane, among others provided herein and known in the art.

[0092] Such processes produce biofuels with significant advantages over other biofuels. In particular, medium to long chain alkanes provide a number of important advantages over the existing common biofuels such as ethanol and butanol, and are attractive long-term replacements of petroleum-based fuels such as gasoline, diesels, kerosene, and heavy oils in the future. As one example, medium to long chain alkanes and alcohols are major components in all petroleum products and jet fuel in particular, and hence alkanes we produce can be utilized directly by existing engines. By way of further example, medium to long chain alcohols are far better fuels than ethanol, and have a nearly comparable energy density to gasoline.

[0093] As another example, n-alkanes are major components of all oil products including gasoline, diesels, kerosene, and heavy oils. Microbial systems or recombinant microorganisms may be used to produce n-alkanes with different carbon lengths ranging, for example, from C7 to over C20: C7 for gasoline (e.g., motor vehicles), C10-C15 for diesels (e.g., motor vehicles, trains, and ships), and C8-C16 for kerosene (e.g., aviations and ships), and for all heavy oils.

[0094] As one aspect of the invention, the commodity chemicals produced by the methods and recombinant microorganisms described herein may be utilized by existing petroleum refineries for the purposes of blending with petroleum products produced by traditional refinery methods. To this end, as noted above, fuel producers are seeking substantially similar, low carbon fuels that can be blended and distributed through existing infrastructure (refineries, pipelines, tankers). As hydrocarbons, the commodity chemicals produced according to the methods herein are substantially similar to petroleum derived fuels, reduce green house gas emissions by more than 80% from petroleum derived fuels, and are compatible with existing infrastructure in the oil and gas industry. For instance, certain of the commodity chemicals produced herein, including, for example, various C10-C12 hydrocarbons such as 2,7 dimethyloctane, 2,7 dimethyldecanone,

among others, are blendable directly into refinery-produced petroleum products, such as jet and diesel fuels. By using such biologically produced commodity chemicals as a blendstock for jet and diesel fuels, refineries may reduce Green House Gas emissions by more than 80%.

[0095] Accordingly, certain embodiments of the present invention relate generally to methods for converting biomass to a commodity chemical, comprising obtaining a polysaccharide from biomass; contacting the polysaccharide with a polysaccharide degrading or depolymerizing pathway, thereby converting the polysaccharide to a suitable monosaccharide. The suitable monosaccharide obtained from such as process may be used for any desired purpose. For instance, in certain aspects, the suitable monosaccharide may then be converted to a commodity chemical (e.g., biofuel) by contacting the suitable monosaccharide with a biofuel biosynthesis pathway, whether as part of a recombinant microorganism, an in vitro enzymatic or chemical pathway, or a combination thereof, thereby converting the monosaccharide to a commodity chemical.

[0096] In other aspects, in producing a commodity chemical such as a biofuel, a suitable monosaccharide may be obtained directly from any available source and converted to a commodity chemical by contacting the suitable monosaccharide with a biofuel biosynthesis pathway, as described herein. Among other uses apparent to a person skilled in the art, such biofuels may then be blended directly with refinery produced petroleum products, such as jet and diesel fuels, to produce commodity chemical enriched, refinery-produced petroleum products.

DEFINITIONS

[0097] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below. All references referred to herein are incorporated by reference in their entirety.

[0098] The articles "a" and "an" are used herein to refer to one or to more than one (i.e. to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0099] By "about" is meant a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much as 30, 25, 20, 25, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1% to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

[0100] The term "biologically active fragment", as applied to fragments of a reference polynucleotide or polypeptide sequence, refers to a fragment that has at least about 0.1, 0.5, 1, 2, 5, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, 100, 110, 120, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1000% or more of the activity of a reference sequence.

[0101] The term "reference sequence" refers generally to a nucleic acid coding sequence, or amino acid sequence, of any enzyme having a biological activity described herein (e.g., saccharide dehydrogenase, alcohol dehydrogenase, dehydratase, lyase, transporter, decarboxylase, hydrolase, etc.), such as a "wild-type" sequence, including those reference sequences exemplified by SEQ ID NOS:1-144, and 308-313. A reference sequence may also include naturally-occurring, functional variants (i.e., orthologs or homologs) of the sequences described herein.

[0102] Included within the scope of the present invention are biologically active fragments of at least about 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 500, 600 or more contiguous nucleotides or amino acid residues in length, including all integers in between, which comprise or encode a polypeptide having an enzymatic activity of a reference polynucleotide or polypeptide. Representative biologically active fragments generally participate in an interaction, e.g., an intra-molecular or an inter-molecular interaction. An inter-molecular interaction can be a specific binding interaction or an enzymatic interaction. Examples of enzymatic interactions or activities include saccharide dehydrogenase activities, alcohol dehydrogenase activities, dehydratases activities, lyase activities, transporter activities, isomerase activities, kinase activities, among others described herein. Biologically active fragments typically comprise one or more active sites or enzymatic/binding motifs, as described herein and known in the art.

[0103] By "coding sequence" is meant any nucleic acid sequence that contributes to the code for the polypeptide product of a gene. By contrast, the term "non-coding sequence" refers to any nucleic acid sequence that does not contribute to the code for the polypeptide product of a gene.

[0104] Throughout this specification, unless the context requires otherwise, the words "comprise", "comprises" and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements.

[0105] By "consisting of," is meant including, and limited to, whatever follows the phrase "consisting of" Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present.

[0106] By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[0107] The terms "complementary" and "complementarity" refer to polynucleotides (i.e., a sequence of nucleotides) related by the base-pairing rules. For example, the sequence "A-G-T," is complementary to the sequence "T-C-A." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands.

[0108] By "corresponds to" or "corresponding to" is meant (a) a polynucleotide having a nucleotide sequence that is substantially identical or complementary to all or a portion of a reference polynucleotide sequence or encoding an amino

acid sequence identical to an amino acid sequence in a peptide or protein; or (b) a peptide or polypeptide having an amino acid sequence that is substantially identical to a sequence of amino acids in a reference peptide or protein.

[0109] By "derivative" is meant a polypeptide that has been derived from the basic sequence by modification, for example by conjugation or complexing with other chemical moieties (e.g., pegylation) or by post-translational modification techniques as would be understood in the art. The term "derivative" also includes within its scope alterations that have been made to a parent sequence including additions or deletions that provide for functionally equivalent molecules.

[0110] By "enzyme reactive conditions" it is meant that any necessary conditions are available in an environment (i.e., such factors as temperature, pH, lack of inhibiting substances) which will permit the enzyme to function. Enzyme reactive conditions can be either in vitro, such as in a test tube, or in vivo, such as within a cell.

[0111] As used herein, the terms "function" and "functional" and the like refer to a biological or enzymatic function.

[0112] By "gene" is meant a unit of inheritance that occupies a specific locus on a chromosome and consists of transcriptional and/or translational regulatory sequences and/or a coding region and/or non-translated sequences (i.e., introns, 5' and 3' untranslated sequences).

[0113] "Homology" refers to the percentage number of amino acids that are identical or constitute conservative substitutions. Homology may be determined using sequence comparison programs such as GAP (Deveraux et al., 1984, *Nucleic Acids Research* 12, 387-395) which is incorporated herein by reference. In this way sequences of a similar or substantially different length to those cited herein could be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

[0114] The term "host cell" includes an individual cell or cell culture which can be or has been a recipient of any recombinant vector(s) or isolated polynucleotide of the invention. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change. A host cell includes cells transfected, transformed, or infected in vivo or in vitro with a recombinant vector or a polynucleotide of the invention. A host cell which comprises a recombinant vector of the invention is a recombinant host cell, recombinant cell, or recombinant microrganism.

[0115] By "isolated" is meant material that is substantially or essentially free from components that normally accompany it in its native state. For example, an "isolated polynucleotide", as used herein, refers to a polynucleotide, which has been purified from the sequences which flank it in a naturally-occurring state, e.g., a DNA fragment which has been removed from the sequences that are normally adjacent to the fragment. Alternatively, an "isolated peptide" or an "isolated polypeptide" and the like, as used herein, refer to in vitro isolation and/or purification of a peptide or polypeptide molecule from its natural cellular environment, and from association with other components of the cell, i.e., it is not associated with in vivo substances.

[0116] By "increased" or "increasing" is meant the ability of one or more recombinant microorganisms to produce a greater amount of a given product or molecule (e.g., commodity chemical, biofuel, or intermediate product thereof) as compared to a control microorganism, such as an unmodified microorganism or a differently modified microorganism. An "increased" amount is typically a "statistically significant" amount, and may include an increase that is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30 or more times (including all integers and decimal points in between, e.g., 1.5, 1.6, 1.7. 1.8, etc.) the amount produced by an unmodified microorganism or a differently modified microorganism.

[0117] By "obtained from" is meant that a sample such as, for example, a polynucleotide extract or polypeptide extract is isolated from, or derived from, a particular source, such as a desired organism, typically a microorganism. "Obtained from" can also refer to the situation in which a polynucleotide or polypeptide sequence is isolated from, or derived from, a particular organism or microorganism. For example, a polynucleotide sequence encoding a benzaldehyde lyase enzyme may be isolated from a variety of prokaryotic or eukaryotic microorganisms, such as *Pseudomonas*.

[0118] The term "operably linked" as used herein means placing a gene under the regulatory control of a promoter, which then controls the transcription and optionally the translation of the gene. In the construction of heterologous promoter/structural gene combinations, it is generally preferred to position the genetic sequence or promoter at a distance from the gene transcription start site that is approximately the same as the distance between that genetic sequence or promoter and the gene it controls in its natural setting; i.e. the gene from which the genetic sequence or promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting; i.e., the genes from which it is derived. "Constitutive promoters" are typically active, i.e., promote transcription, under most conditions. "Inducible promoters" are typically active only under certain conditions, such as in the presence of a given molecule factor (e.g., IPTG) or a given environmental condition (e.g., CO_2 concentration, nutrient levels, light, heat). In the absence of that condition, inducible promoters typically do not allow significant or measurable levels of transcriptional activity.

[0119] The recitation "polynucleotide" or "nucleic acid" as used herein designates mRNA, RNA, cRNA, rRNA, cDNA or DNA. The term typically refers to polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

[0120] As will be understood by those skilled in the art, the polynucleotide sequences of this invention can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the hand of man.

[0121] Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. Additional coding or non-coding sequences may, but need not, be present within a

polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

[0122] Polynucleotides may comprise a native sequence (i.e., an endogenous sequence) or may comprise a variant, or a biological functional equivalent of such a sequence. Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions, as further described below, preferably such that the enzymatic activity of the encoded polypeptide is not substantially diminished relative to the unmodified polypeptide, and preferably such that the enzymatic activity of the encoded polypeptide is improved (e.g., optimized) relative to the unmodified polypeptide. The effect on the enzymatic activity of the encoded polypeptide may generally be assessed as described herein.

[0123] The polynucleotides of the present invention, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a polynucleotide fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol.

[0124] The terms "polynucleotide variant" and "variant" and the like refer to polynucleotides that display substantial sequence identity with any of the reference polynucleotide sequences or genes described herein, and to polynucleotides that hybridize with any polynucleotide reference sequence described herein, or any polynucleotide coding sequence of any gene or protein referred to herein, under low stringency, medium stringency, high stringency, or very high stringency conditions that are defined hereinafter and known in the art. These terms also encompass polynucleotides that are distinguished from a reference polynucleotide by the addition, deletion or substitution of at least one nucleotide. Accordingly, the terms "polynucleotide variant" and "variant" include polynucleotides in which one or more nucleotides have been added or deleted, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide, or has increased activity in relation to the reference polynucleotide (i.e., optimized). Polynucleotide variants include, for example, polynucleotides having at least 50% (and at least 51% to at least 99% and all integer percentages in between) sequence identity with a reference polynucleotide described herein.

[0125] The terms "polynucleotide variant" and "variant" also include naturally-occurring allelic variants that encode these enzymes. Examples of naturally-occurring variants include allelic variants (same locus), homologs (different locus), and orthologs (different organism). Naturally occurring variants such as these can be identified and isolated using well-known molecular biology techniques including, for example, various polymerase chain reaction (PCR) and hybridization-based techniques as known in the art. Naturally occurring variants can be isolated from any organism that encodes one or more genes having a suitable enzymatic activity described herein (e.g., C—C ligase, diol dehyodrogenase, pectate lyase, alginate lyase, diol dehydratase, transporter, etc.).

[0126] Non-naturally occurring variants can be made by mutagenesis techniques, including those applied to polynucleotides, cells, or organisms. The variants can contain nucleotide substitutions, deletions, inversions and insertions. Variation can occur in either or both the coding and non-coding regions. In certain aspects, non-naturally occurring variants may have been optimized for use in a given microorganism (e.g., *E. coli*), such as by engineering and screening the enzymes for increased activity, stability, or any other desirable feature. The variations can produce both conservative and non-conservative amino acid substitutions (as compared to the originally encoded product). For nucleotide sequences, conservative variants include those sequences that, because of the degeneracy of the genetic code, encode the amino acid sequence of a reference polypeptide. Variant nucleotide sequences also include synthetically derived nucleotide sequences, such as those generated, for example, by using site-directed mutagenesis but which still encode a biologically active polypeptide. Generally, variants of a particular reference nucleotide sequence will have at least about 30%, 40% 50%, 55%, 60%, 65%, 70%, generally at least about 75%, 80%, 85%, 90% to 95% or more, and even about 97% or 98% or more sequence identity to that particular nucleotide sequence as determined by sequence alignment programs described elsewhere herein using default parameters.

[0127] As used herein, the term "hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions" describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in Ausubel et al., "Current Protocols in Molecular Biology", John Wiley & Sons Inc, 1994-1998, Sections 6.3.1-6.3.6. Aqueous and non-aqueous methods are described in that reference and either can be used.

[0128] Reference herein to "low stringency" conditions include and encompass from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1 M to at least about 2 M salt for hybridization at 42° C., and at least about 1 M to at least about 2 M salt for washing at 42° C. Low stringency conditions also may include 1% Bovine Serum Albumin (BSA), 1 mM EDTA, 0.5 M $NaHPO_4$ (pH 7.2), 7% SDS for hybridization at 65° C., and (i) 2×SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM $NaHPO_4$ (pH 7.2), 5% SDS for washing at room temperature. One embodiment of low stringency conditions includes hybridization in 6× sodium chloride/sodium citrate (SSC) at about 45° C., followed by two washes in 0.2×SSC, 0.1% SDS at least at 50° C. (the temperature of the washes can be increased to 55° C. for low stringency conditions).

[0129] "Medium stringency" conditions include and encompass from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M salt for hybridization at 42° C., and at least about 0.1 M to at least about 0.2 M salt for washing at 55° C. Medium stringency conditions also may include 1% Bovine Serum Albumin (BSA), 1 mM EDTA, 0.5 M $NaHPO_4$ (pH 7.2), 7% SDS for hybridization at 65° C., and (i) 2×SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM $NaHPO_4$ (pH 7.2), 5% SDS for washing at 60-65° C. One embodiment of medium

stringency conditions includes hybridizing in 6×SSC at about 45° C., followed by one or more washes in 0.2×SSC, 0.1% SDS at 60° C.

[0130] "High stringency" conditions include and encompass from at least about 31% v/v to at least about 50% v/v formamide and from about 0.01 M to about 0.15 M salt for hybridization at 42° C., and about 0.01 M to about 0.02 M salt for washing at 55° C. High stringency conditions also may include 1% BSA, 1 mM EDTA, 0.5 M $NaHPO_4$ (pH 7.2), 7% SDS for hybridization at 65° C., and (i) 0.2×SSC, 0.1% SDS; or (ii) 0.5% BSA, 1 mM EDTA, 40 mM $NaHPO_4$ (pH 7.2), 1% SDS for washing at a temperature in excess of 65° C. One embodiment of high stringency conditions includes hybridizing in 6×SSC at about 45° C., followed by one or more washes in 0.2×SSC, 0.1% SDS at 65° C.

[0131] One embodiment of "very high stringency" conditions includes hybridizing in 0.5 M sodium phosphate, 7% SDS at 65° C., followed by one or more washes in 0.2×SSC, 1% SDS at 65° C.

[0132] Other stringency conditions are well known in the art and a skilled addressee will recognize that various factors can be manipulated to optimize the specificity of the hybridization. Optimization of the stringency of the final washes can serve to ensure a high degree of hybridization. For detailed examples, see Ausubel et al., supra at pages 2.10.1 to 2.10.16 and Sambrook et al., Current Protocols in Molecular Biology (1989), at sections 1.101 to 1.104.

[0133] While stringent washes are typically carried out at temperatures from about 42° C. to 68° C., one skilled in the art will appreciate that other temperatures may be suitable for stringent conditions. Maximum hybridization rate typically occurs at about 20° C. to 25° C. below the T_m for formation of a DNA-DNA hybrid. It is well known in the art that the T_m is the melting temperature, or temperature at which two complementary polynucleotide sequences dissociate. Methods for estimating T_m are well known in the art (see Ausubel et al., supra at page 2.10.8).

[0134] In general, the T_m of a perfectly matched duplex of DNA may be predicted as an approximation by the formula: T_m=81.5+16.6 ($\log_{10}$M)+0.41 (% G+C)−0.63 (% formamide)−(600/length) wherein: M is the concentration of Na^+, preferably in the range of 0.01 molar to 0.4 molar; % G+C is the sum of guano sine and cytosine bases as a percentage of the total number of bases, within the range between 30% and 75% G+C; % formamide is the percent formamide concentration by volume; length is the number of base pairs in the DNA duplex. The T_m of a duplex DNA decreases by approximately 1° C. with every increase of 1% in the number of randomly mismatched base pairs. Washing is generally carried out at T_m−15° C. for high stringency, or T_m−30° C. for moderate stringency.

[0135] In one example of a hybridization procedure, a membrane (e.g., a nitrocellulose membrane or a nylon membrane) containing immobilized DNA is hybridized overnight at 42° C. in a hybridization buffer (50% deionizer formamide, 5×SSC, 5× Reinhardt's solution (0.1% fecal, 0.1% polyvinylpyrollidone and 0.1% bovine serum albumin), 0.1% SDS and 200 mg/mL denatured salmon sperm DNA) containing a labeled probe. The membrane is then subjected to two sequential medium stringency washes (i.e., 2×SSC, 0.1% SDS for 15 min at 45° C., followed by 2×SSC, 0.1% SDS for 15 min at 50° C.), followed by two sequential higher stringency washes (i.e., 0.2×SSC, 0.1% SDS for 12 min at 55° C. followed by 0.2×SSC and 0.1% SDS solution for 12 min at 65-68° C.

[0136] Polynucleotides and fusions thereof may be prepared, manipulated and/or expressed using any of a variety of well established techniques known and available in the art. For example, polynucleotide sequences which encode polypeptides of the invention, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of a selected enzyme in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express a given polypeptide.

[0137] As will be understood by those of skill in the art, it may be advantageous in some instances to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence. Such nucleotides are typically referred to as "codon-optimized." Any of the nucleotide sequences described herein may be utilized in such a "codon-optimized" form. For example, the nucleotide coding sequence of the benzaldehyde lyase from *Pseudomonas fluorescens* may be codon-optimized for expression in *E. coli.*

[0138] Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, expression and/or activity of the gene product.

[0139] In order to express a desired polypeptide, a nucleotide sequence encoding the polypeptide, or a functional equivalent, may be inserted into appropriate expression vector, i.e., a vector that contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook et al., Molecular Cloning, A Laboratory Manual (1989), and Ausubel et al., Current Protocols in Molecular Biology (1989).

[0140] "Polypeptide," "polypeptide fragment," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues are synthetic non-naturally occurring amino acids, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers. In certain aspects, polypeptides may include enzymatic polypeptides, or "enzymes," which typically catalyze (i.e., increase the rate of) various chemical reactions.

[0141] The recitation polypeptide "variant" refers to polypeptides that are distinguished from a reference polypeptide sequence by the addition, deletion or substitution of at least one amino acid residue. In certain embodiments, a

polypeptide variant is distinguished from a reference polypeptide by one or more substitutions, which may be conservative or non-conservative. In certain embodiments, the polypeptide variant comprises conservative substitutions and, in this regard, it is well understood in the art that some amino acids may be changed to others with broadly similar properties without changing the nature of the activity of the polypeptide. Polypeptide variants also encompass polypeptides in which one or more amino acids have been added or deleted, or replaced with different amino acid residues.

[0142] The present invention contemplates the use in the methods described herein of variants of full-length polypeptides having any of the enzymatic activities described herein, truncated fragments of these full-length polypeptides, variants of truncated fragments, as well as their related biologically active fragments. Typically, biologically active fragments of a polypeptide may participate in an interaction, for example, an intra-molecular or an inter-molecular interaction. An inter-molecular interaction can be a specific binding interaction or an enzymatic interaction (e.g., the interaction can be transient and a covalent bond is formed or broken). Biologically active fragments of a polypeptide/enzyme an enzymatic activity described herein include peptides comprising amino acid sequences sufficiently similar to, or derived from, the amino acid sequences of a (putative) full-length reference polypeptide sequence. Typically, biologically active fragments comprise a domain or motif with at least one enzymatic activity, and may include one or more (and in some cases all) of the various active domains. A biologically active fragment of a an enzyme can be a polypeptide fragment which is, for example, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 450, 500, 600 or more contiguous amino acids, including all integers in between, of a reference polypeptide sequence. In certain embodiments, a biologically active fragment comprises a conserved enzymatic sequence, domain, or motif, as described elsewhere herein and known in the art. Suitably, the biologically-active fragment has no less than about 1%, 10%, 25%, 50% of an activity of the wild-type polypeptide from which it is derived.

[0143] The term "exogenous" refers generally to a polynucleotide sequence or polypeptide that does not naturally occur in a wild-type cell or organism, but is typically introduced into the cell by molecular biological techniques, i.e., engineering to produce a recombinant microorganism. Examples of "exogenous" polynucleotides include vectors, plasmids, and/or man-made nucleic acid constructs encoding a desired protein or enzyme. The term "endogenous" refers generally to naturally occurring polynucleotide sequences or polypeptides that may be found in a given wild-type cell or organism. For example, certain naturally-occurring bacterial or yeast species do not typically contain a benzaldehyde lyase gene, and, therefore, do not comprise an "endogenous" polynucleotide sequence that encodes a benzaldehyde lyase. In this regard, it is also noted that even though an organism may comprise an endogenous copy of a given polynucleotide sequence or gene, the introduction of a plasmid or vector encoding that sequence, such as to over-express or otherwise regulate the expression of the encoded protein, represents an "exogenous" copy of that gene or polynucleotide sequence. Any of the of pathways, genes, or enzymes described herein may utilize or rely on an "endogenous" sequence, or may be provided as one or more "exogenous" polynucleotide sequences, and/or may be utilized according to the endogenous sequences already contained within a given microorganism.

[0144] A "recombinant" microorganism typically comprises one or more exogenous nucleotide sequences, such as in a plasmid or vector.

[0145] The recitations "sequence identity" or, for example, comprising a "sequence 50% identical to," as used herein, refer to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" may be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, I) or the identical amino acid residue (e.g., Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

[0146] Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity" and "substantial identity". A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (i.e., only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of at least 6 contiguous positions, usually about 50 to about 100, more usually about 100 to about 150 in which a sequence is compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. The comparison window may comprise additions or deletions (i.e., gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, Wis., USA) or by inspection and the best alignment (i.e., resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul et al., 1997, *Nucl. Acids Res.* 25:3389. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel et al., "Current Protocols in Molecular Biology", John Wiley & Sons Inc, 1994-1998, Chapter 15.

[0147] "Transformation" refers generally to the permanent, heritable alteration in a cell resulting from the uptake and

incorporation of foreign DNA into the host-cell genome; also, the transfer of an exogenous gene from one organism into the genome of another organism.

[0148] By "vector" is meant a polynucleotide molecule, preferably a DNA molecule derived, for example, from a plasmid, bacteriophage, yeast or virus, into which a polynucleotide can be inserted or cloned. A vector preferably contains one or more unique restriction sites and can be capable of autonomous replication in a defined host cell including a target cell or tissue or a progenitor cell or tissue thereof, or be integrable with the genome of the defined host such that the cloned sequence is reproducible. Accordingly, the vector can be an autonomously replicating vector, i.e., a vector that exists as an extra-chromosomal entity, the replication of which is independent of chromosomal replication, e.g., a linear or closed circular plasmid, an extra-chromosomal element, a mini-chromosome, or an artificial chromosome. The vector can contain any means for assuring self-replication. Alternatively, the vector can be one which, when introduced into the host cell, is integrated into the genome and replicated together with the chromosome(s) into which it has been integrated. Such a vector may comprise specific sequences that allow recombination into a particular, desired site of the host chromosome. A vector system can comprise a single vector or plasmid, two or more vectors or plasmids, which together contain the total DNA to be introduced into the genome of the host cell, or a transposon. The choice of the vector will typically depend on the compatibility of the vector with the host cell into which the vector is to be introduced. In the present case, the vector is preferably one which is operably functional in a bacterial cell, such as a cyanobacterial cell. The vector can include a reporter gene, such as a green fluorescent protein (GFP), which can be either fused in frame to one or more of the encoded polypeptides, or expressed separately. The vector can also include a selection marker such as an antibiotic resistance gene that can be used for selection of suitable transformants.

[0149] The terms "wild-type" and "naturally occurring" are used interchangeably to refer to a gene or gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild type gene or gene product (e.g., a polypeptide) is that which is most frequently observed in a population and is thus arbitrarily designed the "normal" or "wild-type" form of the gene.

[0150] Examples of "biomass" include aquatic or marine biomass, fruit-based biomass such as fruit waste, and vegetable-based biomass such as vegetable waste, among others. Examples of aquatic or marine biomass include, but are not limited to, kelp, giant kelp, seaweed, algae, and marine microflora, microalgae, sea grass, and the like. In certain aspects, biomass does not include fossilized sources of carbon, such as hydrocarbons that are typically found within the top layer of the Earth's crust (e.g., natural gas, nonvolatile materials composed of almost pure carbon, like anthracite coal, etc).

[0151] Examples of fruit and/or vegetable biomass include, but are not limited to, any source of pectin such as plant peel and pomace including citrus, orange, grapefruit, potato, tomato, grape, mango, gooseberry, carrot, sugar-beet, and apple, among others.

[0152] Examples of polysaccharides, oligosaccharides, monosaccharides or other sugar components of biomass include, but are not limited to, alginate, agar, carrageenan, fucoidan, pectin, gluronate, mannuronate, mannitol, lyxose, cellulose, hemicellulose, glycerol, xylitol, glucose, mannose, galactose, xylose, xylan, mannan, arabinan, arabinose, glucuronate, galacturonate (including di- and tri-galacturonates), rhamnose, and the like.

[0153] Certain examples of alginate-derived polysaccharides include saturated polysaccharides, such as β-D-mannuronate, α-L-gluronate, dialginate, trialginate, pentalginate, hexylginate, heptalginate, octalginate, nonalginate, decalginate, undecalginate, dodecalginate and polyalginate, as well as unsaturated polysaccharides such as 4-deoxy-L-erythro-5-hexoseulose uronic acid, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-D-mannuronate or L-guluronate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-dialginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-trialginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-tetralginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-pentalginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-hexylginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-heptalginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-octalginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-nonalginate, 4-(4-deoxy-beta-D-mann-4-enuronosyl)-undecalginate, and 4-(4-deoxy-beta-D-mann-4-enuronosyl)-dodecalginate.

[0154] Certain examples of pectin-derived polysaccharides include saturated polysaccharides, such as galacturonate, digalacturonate, trigalacturonate, tetragalacturonate, pentagalacturonate, hexagalacturonate, heptagalacturonate, octagalacturonate, nonagalacturonate, decagalacturonate, dodecagalacturonate, polygalacturonate, and rhamnopolygalacturonate, as well as saturated polysaccharides such as 4-deoxy-L-threo-5-hexosulose uronate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-galacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-digalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-trigalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-tetragalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-pentagalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-hexagalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-heptagalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-octagalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-nonagalacturonate, 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-decagalacturonate, and 4-(4-Deoxy-alpha-D-gluc-4-enuronosyl)-D-dodecagalacturonate.

[0155] These polysaccharide or oligosaccharide components may be converted into "suitable monosaccharides" or other "suitable saccharides," such as "suitable oligosaccharides," by the microorganisms described herein which are capable of growing on such polysaccharides or other sugar components as a source of carbon (e.g., a sole source of carbon).

[0156] A "suitable monosaccharide" or "suitable saccharide" refers generally to any saccharide that may be produced by a recombinant microorganism growing on pectin, alginate, or other saccharide (e.g., galacturonate, cellulose, hemi-cellulose etc.) as a source or sole source of carbon, and also refers generally to any saccharide that may be utilized in a biofuel biosynthesis pathway of the present invention to produce hydrocarbons such as biofuels or biopetrols. Examples of suitable monosaccharides or oligosaccharides include, but are not limited to, 2-keto-3-deoxy D-gluconate (KDG), D-mannitol, gluronate, mannuronate, mannitol, lyxose, glycerol, xylitol, glucose, mannose, galactose, xylose, arabinose, glucuronate, galacturonates, and rhamnose, and the like. As noted herein, a "suitable monosaccharide" or "suitable saccharide" as used herein may be produced by an engineered or

recombinant microorganism of the present invention, or may be obtained from commercially available sources.

[0157] The recitation "commodity chemical" as used herein includes any saleable or marketable chemical that can be produced either directly or as a by-product of the methods provided herein, including biofuels and/or biopetrols. General examples of "commodity chemicals" include, but are not limited to, biofuels, minerals, polymer precursors, fatty alcohols, surfactants, plasticizers, and solvents. The recitation "biofuels" as used herein includes solid, liquid, or gas fuels derived, at least in part, from a biological source, such as a recombinant microorganism.

[0158] Examples of commodity chemicals include, but are not limited to, methane, methanol, ethane, ethene, ethanol, n-propane, 1-propene, 1-propanol, propanal, acetone, propionate, n-butane, 1-butene, 1-butanol, butanal, butanoate, isobutanal, isobutanol, 2-methylbutanal, 2-methylbutanol, 3-methylbutanal, 3-methylbutanol, 2-butene, 2-butanol, 2-butanone, 2,3-butanediol, 3-hydroxy-2-butanone, 2,3-butanedione, ethylbenzene, ethenylbenzene, 2-phenylethanol, phenylacetaldehyde, 1-phenylbutane, 4-phenyl-1-butene, 4-phenyl-2-butene, 1-phenyl-2-butene, 1-phenyl-2-butanol, 4-phenyl-2-butanol, 1-phenyl-2-butanone, 4-phenyl-2-butanone, 1-phenyl-2,3-butandiol, 1-phenyl-3-hydroxy-2-butanone, 4-phenyl-3-hydroxy-2-butanone, 1-phenyl-2,3-butanedione, n-pentane, ethylphenol, ethenylphenol, 2-(4-hydroxyphenyl)ethanol, 4-hydroxyphenylacetaldehyde, 1-(4-hydroxyphenyl) butane, 4-(4-hydroxyphenyl)-1-butene, 4-(4-hydroxyphenyl)-2-butene, 1-(4-hydroxyphenyl)-1-butene, 1-(4-hydroxyphenyl)-2-butanol, 4-(4-hydroxyphenyl)-2-butanol, 1-(4-hydroxyphenyl)-2-butanone, 4-(4-hydroxyphenyl)-2-butanone, 1-(4-hydroxyphenyl)-2,3-butandiol, 1-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 4-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-2,3-butanonedione, indolylethane, indolylethene, 2-(indole-3-)ethanol, n-pentane, 1-pentene, 1-pentanol, pentanal, pentanoate, 2-pentene, 2-pentanol, 3-pentanol, 2-pentanone, 3-pentanone, 4-methylpentanal, 4-methylpentanol, 2,3-pentanediol, 2-hydroxy-3-pentanone, 3-hydroxy-2-pentanone, 2,3-pentanedione, 2-methylpentane, 4-methyl-1-pentene, 4-methyl-2-pentene, 4-methyl-3-pentene, 4-methyl-2-pentanol, 2-methyl-3-pentanol, 4-methyl-2-pentanone, 2-methyl-3-pentanone, 4-methyl-2,3-pentanediol, 4-methyl-2-hydroxy-3-pentanone, 4-methyl-3-hydroxy-2-pentanone, 4-methyl-2,3-pentanedione, 1-phenylpentane, 1-phenyl-1-pentene, 1-phenyl-2-pentene, 1-phenyl-3-pentene, 1-phenyl-2-pentanol, 1-phenyl-3-pentanol, 1-phenyl-2-pentanone, 1-phenyl-3-pentanone, 1-phenyl-2,3-pentanediol, 1-phenyl-2-hydroxy-3-pentanone, 1-phenyl-3-hydroxy-2-pentanone, 1-phenyl-2,3-pentanedione, 4-methyl-1-phenylpentane, 4-methyl-1-phenyl-1-pentene, 4-methyl-1-phenyl-2-pentene, 4-methyl-1-phenyl-3-pentene, 4-methyl-1-phenyl-3-pentanol, 4-methyl-1-phenyl-2-pentanol, 4-methyl-1-phenyl-3-pentanone, 4-methyl-1-phenyl-2-pentanone, 4-methyl-1-phenyl-2,3-pentanediol, 4-methyl-1-phenyl-2,3-pentanedione, 4-methyl-1-phenyl-3-hydroxy-2-pentanone, 4-methyl-1-phenyl-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl) pentane, 1-(4-hydroxyphenyl)-1-pentene, 1-(4-hydroxyphenyl)-2-pentene, 1-(4-hydroxyphenyl)-3-pentene, 1-(4-hydroxyphenyl)-2-pentanol, 1-(4-hydroxyphenyl)-3-pentanol, 1-(4-hydroxyphenyl)-2-pentanone, 1-(4-hydroxyphenyl)-3-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanediol, 1-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-1-(4-hydroxyphenyl) pentane, 4-methyl-1-(4-hydroxyphenyl)-2-pentene, 4-methyl-1-(4-hydroxyphenyl)-3-pentene, 4-methyl-1-(4-hydroxyphenyl)-1-pentene, 4-methyl-1-(4-hydroxyphenyl)-3-pentanol, 4-methyl-1-(4-hydroxyphenyl)-2-pentanol, 4-methyl-1-(4-hydroxyphenyl)-3-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2,3-pentanediol, 4-methyl-1-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, 1-indole-3-pentane, 1-(indole-3)-1-pentene, 1-(indole-3)-2-pentene, 1-(indole-3)-3-pentene, 1-(indole-3)-2-pentanol, 1-(indole-3)-3-pentanol, 1-(indole-3)-2-pentanone, 1-(indole-3)-3-pentanone, 1-(indole-3)-2,3-pentanediol, 1-(indole-3)-2-hydroxy-3-pentanone, 1-(indole-3)-3-hydroxy-2-pentanone, 1-(indole-3)-2,3-pentanedione, 4-methyl-1-(indole-3-)pentane, 4-methyl-1-(indole-3)-2-pentene, 4-methyl-1-(indole-3)-3-pentene, 4-methyl-1-(indole-3)-1-pentene, 4-methyl-2-(indole-3)-3-pentanol, 4-methyl-1-(indole-3)-2-pentanol, 4-methyl-1-(indole-3)-3-pentanone, 4-methyl-1-(indole-3)-2-pentanone, 4-methyl-1-(indole-3)-2,3-pentanediol, 4-methyl-1-(indole-3)-2,3-pentanedione, 4-methyl-1-(indole-3)-3-hydroxy-2-pentanone, 4-methyl-1-(indole-3)-2-hydroxy-3-pentanone, n-hexane, 1-hexene, 1-hexanol, hexanal, hexanoate, 2-hexene, 3-hexene, 2-hexanol, 3-hexanol, 2-hexanone, 3-hexanone, 2,3-hexanediol, 2,3-hexanedione, 3,4-hexanediol, 3,4-hexanedione, 2-hydroxy-3-hexanone, 3-hydroxy-2-hexanone, 3-hydroxy-4-hexanone, 4-hydroxy-3-hexanone, 2-methylhexane, 3-methylhexane, 2-methyl-2-hexene, 2-methyl-3-hexene, 5-methyl-1-hexene, 5-methyl-2-hexene, 4-methyl-1-hexene, 4-methyl-2-hexene, 3-methyl-3-hexene, 3-methyl-2-hexene, 3-methyl-1-hexene, 2-methyl-3-hexanol, 5-methyl-2-hexanol, 5-methyl-3-hexanol, 2-methyl-3-hexanone, 5-methyl-2-hexanone, 5-methyl-3-hexanone, 2-methyl-3,4-hexanediol, 2-methyl-3,4-hexanedione, 5-methyl-2,3-hexanediol, 5-methyl-2,3-hexanedione, 4-methyl-2,3-hexanediol, 4-methyl-2,3-hexanedione, 2-methyl-3-hydroxy-4-hexanone, 2-methyl-4-hydroxy-3-hexanone, 5-methyl-2-hydroxy-3-hexanone, 5-methyl-3-hydroxy-2-hexanone, 4-methyl-2-hydroxy-3-hexanone, 4-methyl-3-hydroxy-2-hexanone, 2,5-dimethylhexane, 2,5-dimethyl-2-hexene, 2,5-dimethyl-3-hexene, 2,5-dimethyl-3-hexanol, 2,5-dimethyl-3-hexanone, 2,5-dimethyl-3,4-hexanediol, 2,5-dimethyl-3,4-hexanedione, 2,5-dimethyl-3-hydroxy-4-hexanone, 5-methyl-1-phenylhexane, 4-methyl-1-phenylhexane, 5-methyl-1-phenyl-1-hexene, 5-methyl-1-phenyl-2-hexene, 5-methyl-1-phenyl-3-hexene, 4-methyl-1-phenyl-1-hexene, 4-methyl-1-phenyl-2-hexene, 4-methyl-1-phenyl-3-hexene, 5-methyl-1-phenyl-2-hexanol, 5-methyl-1-phenyl-3-hexanol, 4-methyl-1-phenyl-2-hexanol, 4-methyl-1-phenyl-3-hexanol, 5-methyl-1-phenyl-2-hexanone, 5-methyl-1-phenyl-3-hexanone, 4-methyl-1-phenyl-2-hexanone, 4-methyl-1-phenyl-3-hexanone, 5-methyl-1-phenyl-2,3-hexanediol, 4-methyl-1-phenyl-2,3-hexanediol, 5-methyl-1-phenyl-3-hydroxy-2-hexanone, 5-methyl-1-phenyl-2-hydroxy-3-hexanone, 4-methyl-1-phenyl-3-hydroxy-2-hexanone, 4-methyl-1-phenyl-2-hydroxy-3-hexanone, 5-methyl-1-phenyl-2,3-hexanedione, 4-methyl-1-phenyl-2,3-hexanedione, 4-methyl-1-(4-hydroxyphenyl)hexane, 5-methyl-1-(4-hydroxyphenyl)-1-hexene, 5-methyl-1-(4-hydroxyphenyl)-2-hexene, 5-methyl-1-(4-hydroxyphenyl)-3-hexene, 4-methyl-1-(4-hydroxyphenyl)-1-hexene, 4-methyl-

1-(4-hydroxyphenyl)-2-hexene, 4-methyl-1-(4-hydroxyphenyl)-3-hexene, 5-methyl-1-(4-hydroxyphenyl)-2-hexanol, 5-methyl-1-(4-hydroxyphenyl)-3-hexanol, 4-methyl-1-(4-hydroxyphenyl)-2-hexanol, 4-methyl-1-(4-hydroxyphenyl)-3-hexanol, 5-methyl-1-(4-hydroxyphenyl)-2-hexanone, 5-methyl-1-(4-hydroxyphenyl)-3-hexanone, 4-methyl-1-(4-hydroxyphenyl)-2-hexanone, 4-methyl-1-(4-hydroxyphenyl)-3-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2,3-hexanediol, 4-methyl-1-(4-hydroxyphenyl)-2,3-hexanediol, 5-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-hexanone, 4-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-hexanone, 4-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2,3-hexanedione, 4-methyl-1-(4-hydroxyphenyl)-2,3-hexanedione, 4-methyl-1-(indole-3-)hexane, 5-methyl-1-(indole-3)-1-hexene, 5-methyl-1-(indole-3)-2-hexene, 5-methyl-1-(indole-3)-3-hexene, 4-methyl-1-(indole-3)-1-hexene, 4-methyl-1-(indole-3)-2-hexene, 4-methyl-1-(indole-3)-3-hexene, 5-methyl-1-(indole-3)-2-hexanol, 5-methyl-1-(indole-3)-3-hexanol, 4-methyl-1-(indole-3)-2-hexanol, 4-methyl-1-(indole-3)-3-hexanol, 5-methyl-1-(indole-3)-2-hexanone, 5-methyl-1-(indole-3)-3-hexanone, 4-methyl-1-(indole-3)-2-hexanone, 4-methyl-1-(indole-3)-3-hexanone, 5-methyl-1-(indole-3)-2,3-hexanediol, 4-methyl-1-(indole-3)-2,3-hexanediol, 5-methyl-1-(indole-3)-3-hydroxy-2-hexanone, 5-methyl-1-(indole-3)-2-hydroxy-3-hexanone, 4-methyl-1-(indole-3)-3-hydroxy-2-hexanone, 4-methyl-1-(indole-3)-2-hydroxy-3-hexanone, 5-methyl-1-(indole-3)-2,3-hexanedione, 4-methyl-1-(indole-3)-2,3-hexanedione, n-heptane, 1-heptene, 1-heptanol, heptanal, heptanoate, 2-heptene, 3-heptene, 2-heptanol, 3-heptanol, 4-heptanol, 2-heptanone, 3-heptanone, 4-heptanone, 2,3-heptanediol, 2,3-heptanedione, 3,4-heptanediol, 3,4-heptanedione, 2-hydroxy-3-heptanone, 3-hydroxy-2-heptanone, 3-hydroxy-4-heptanone, 4-hydroxy-3-heptanone, 2-methylheptane, 3-methylheptane, 6-methyl-2-heptene, 6-methyl-3-heptene, 2-methyl-3-heptene, 2-methyl-2-heptene, 5-methyl-2-heptene, 5-methyl-3-heptene, 3-methyl-3-heptene, 2-methyl-3-heptanol, 2-methyl-4-heptanol, 6-methyl-3-heptanol, 5-methyl-3-heptanol, 3-methyl-4-heptanol, 2-methyl-3-heptanone, 2-methyl-4-heptanone, 6-methyl-3-heptanone, 5-methyl-3-heptanone, 3-methyl-4-heptanone, 2-methyl-3,4-heptanediol, 2-methyl-3,4-heptanedione, 6-methyl-3,4-heptanediol, 6-methyl-3,4-heptanedione, 5-methyl-3,4-heptanediol, 5-methyl-3,4-heptanedione, 2-methyl-3-hydroxy-4-heptanone, 2-methyl-4-hydroxy-3-heptanone, 6-methyl-3-hydroxy-4-heptanone, 6-methyl-4-hydroxy-3-heptanone, 5-methyl-3-hydroxy-4-heptanone, 5-methyl-4-hydroxy-3-heptanone, 2,6-dimethylheptane, 2,5-dimethylheptane, 2,6-dimethyl-2-heptene, 2,6-dimethyl-3-heptene, 2,5-dimethyl-2-heptene, 2,5-dimethyl-3-heptene, 3,6-dimethyl-3-heptene, 2,6-dimethyl-3-heptanol, 2,6-dimethyl-4-heptanol, 2,5-dimethyl-3-heptanol, 2,5-dimethyl-4-heptanol, 2,6-dimethyl-3,4-heptanediol, 2,6-dimethyl-3,4-heptanedione, 2,5-dimethyl-3,4-heptanediol, 2,5-dimethyl-3,4-heptanedione, 2,6-dimethyl-3-hydroxy-4-heptanone, 2,6-dimethyl-4-hydroxy-3-heptanone, 2,5-dimethyl-3-hydroxy-4-heptanone, 2,5-dimethyl-4-hydroxy-3-heptanone, n-octane, 1-octene, 2-octene, 1-octanol, octanal, octanoate, 3-octene, 4-octene, 4-octanol, 4-octanone, 4,5-octanediol, 4,5-octanedione, 4-hydroxy-5-octanone, 2-methyloctane, 2-methyl-3-octene, 2-methyl-4-octene, 7-methyl-3-octene, 3-methyl-3-octene, 3-methyl-4-octene, 6-methyl-3-octene, 2-methyl-4-octanol, 7-methyl-4-octanol, 3-methyl-4-octanol, 6-methyl-4-octanol, 2-methyl-4-octanone, 7-methyl-4-octanone, 3-methyl-4-octanone, 6-methyl-4-octanone, 2-methyl-4,5-octanediol, 2-methyl-4,5-octanedione, 3-methyl-4,5-octanediol, 3-methyl-4,5-octanedione, 2-methyl-4-hydroxy-5-octanone, 2-methyl-5-hydroxy-4-octanone, 3-methyl-4-hydroxy-5-octanone, 3-methyl-5-hydroxy-4-octanone, 2,7-dimethyloctane, 2,7-dimethyl-3-octene, 2,7-dimethyl-4-octene, 2,7-dimethyl-4-octanol, 2,7-dimethyl-4-octanone, 2,7-dimethyl-4,5-octanediol, 2,7-dimethyl-4,5-octanedione, 2,7-dimethyl-4-hydroxy-5-octanone, 2,6-dimethyloctane, 2,6-dimethyl-3-octene, 2,6-dimethyl-4-octene, 3,7-dimethyl-3-octene, 2,6-dimethyl-4-octanol, 3,7-dimethyl-4-octanol, 2,6-dimethyl-4-octanone, 3,7-dimethyl-4-octanone, 2,6-dimethyl-4,5-octanediol, 2,6-dimethyl-4,5-octanedione, 2,6-dimethyl-4-hydroxy-5-octanone, 2,6-dimethyl-5-hydroxy-4-octanone, 3,6-dimethyloctane, 3,6-dimethyl-3-octene, 3,6-dimethyl-4-octene, 3,6-dimethyl-4-octanol, 3,6-dimethyl-4-octanone, 3,6-dimethyl-4,5-octanediol, 3,6-dimethyl-4,5-octanedione, 3,6-dimethyl-4-hydroxy-5-octanone, n-nonane, 1-nonene, 1-nonanol, nonanal, nonanoate, 2-methylnonane, 2-methyl-4-nonene, 2-methyl-5-nonene, 8-methyl-4-nonene, 2-methyl-5-nonanol, 8-methyl-4-nonanol, 2-methyl-5-nonanone, 8-methyl-4-nonanone, 8-methyl-4,5-nonanediol, 8-methyl-4,5-nonanedione, 8-methyl-4-hydroxy-5-nonanone, 8-methyl-5-hydroxy-4-nonanone, 2,8-dimethylnonane, 2,8-dimethyl-3-nonene, 2,8-dimethyl-4-nonene, 2,8-dimethyl-5-nonene, 2,8-dimethyl-4-nonanol, 2,8-dimethyl-5-nonanol, 2,8-dimethyl-4-nonanone, 2,8-dimethyl-5-nonanone, 2,8-dimethyl-4,5-nonanediol, 2,8-dimethyl-4,5-nonanedione, 2,8-dimethyl-4-hydroxy-5-nonanone, 2,8-dimethyl-5-hydroxy-4-nonanone, 2,7-dimethylnonane, 3,8-dimethyl-3-nonene, 3,8-dimethyl-4-nonene, 3,8-dimethyl-5-nonene, 3,8-dimethyl-4-nonanol, 3,8-dimethyl-5-nonanol, 3,8-dimethyl-4-nonanone, 3,8-dimethyl-5-nonanone, 3,8-dimethyl-4,5-nonanediol, 3,8-dimethyl-4,5-nonanedione, 3,8-dimethyl-4-hydroxy-5-nonanone, 3,8-dimethyl-5-hydroxy-4-nonanone, n-decane, 1-decene, 1-decanol, decanoate, 2,9-dimethyldecane, 2,9-dimethyl-3-decene, 2,9-dimethyl-4-decene, 2,9-dimethyl-5-decanol, 2,9-dimethyl-5-decanone, 2,9-dimethyl-5,6-decanediol, 2,9-dimethyl-6-hydroxy-5-decanone, 2,9-dimethyl-5,6-decanedionen-undecane, 1-undecene, 1-undecanol, undecanal. undecanoate, n-dodecane, 1-dodecene, 1-dodecanol, dodecanal, dodecanoate, n-dodecane, 1-decadecene, 1-dodecanol, ddodecanal, dodecanoate, n-tridecane, 1-tridecene, 1-tridecanol, tridecanal, tridecanoate, n-tetradecane, 1-tetradecene, 1-tetradecanol, tetradecanal, tetradecanoate, n-pentadecane, 1-pentadecene, 1-pentadecanol, pentadecanal, pentadecanoate, n-hexadecane, 1-hexadecene, 1-hexadecanol, hexadecanal, hexadecanoate, n-heptadecane, 1-heptadecene, 1-heptadecanol, heptadecanal, heptadecanoate, n-octadecane, 1-octadecene, 1-octadecanol, octadecanal, octadecanoate, n-nonadecane, 1-nonadecene, 1-nonadecanol, nonadecanal, nonadecanoate, eicosane, 1-eicosene, 1-eicosanol, eicosanal, eicosanoate, 3-hydroxy propanal, 1,3-propanediol, 4-hydroxybutanal, 1,4-butanediol, 3-hydrxy-2-butanone, 2,3-butandiol, 1,5-pentane diol, homocitrate, homoisocitorate, b-hydroxy adipate, glutarate, glutarsemialdehyde, glutaraldehyde, 2-hydroxy-1-cyclopentanone, 1,2-cyclopentanediol, cyclopentanone, cyclopentanol, (S)-2-acetolactate, (R)-2,3-Dihydroxy-isovalerate, 2-oxoisovalerate, isobutyryl-CoA, isobutyrate, isobutyraldehyde, 5-amino pentaldehyde, 1,10-diaminodecane, 1,10-diamino-5-decene, 1,10-diamino-5-

hydroxydecane, 1,10-diamino-5-decanone, 1,10-diamino-5,6-decanediol, 1,10-diamino-6-hydroxy-5-decanone, phenylacetoaldehyde, 1,4-diphenylbutane, 1,4-diphenyl-1-butene, 1,4-diphenyl-2-butene, 1,4-diphenyl-2-butanol, 1,4-diphenyl-2-butanone, 1,4-diphenyl-2,3-butanediol, 1,4-diphenyl-3-hydroxy-2-butanone, 1-(4-hydeoxyphenyl)-4-phenylbutane, 1-(4-hydeoxyphenyl)-4-phenyl-1-butene, 1-(4-hydeoxyphenyl)-4-phenyl-2-butene, 1-(4-hydeoxyphenyl)-4-phenyl-2-butanol, 1-(4-hydeoxyphenyl)-4-phenyl-2-butanone, 1-(4-hydeoxyphenyl)-4-phenyl-2,3-butanediol, 1-(4-hydeoxyphenyl)-4-phenyl-3-hydroxy-2-butanone, 1-(indole-3)-4-phenylbutane, 1-(indole-3)-4-phenyl-1-butene, 1-(indole-3)-4-phenyl-2-butene, 1-(indole-3)-4-phenyl-2-butanol, 1-(indole-3)-4-phenyl-2-butanone, 1-(indole-3)-4-phenyl-2,3-butanediol, 1-(indole-3)-4-phenyl-3-hydroxy-2-butanone, 4-hydroxyphenylacetoaldehyde, 1,4-di(4-hydroxyphenyl)butane, 1,4-di(4-hydroxyphenyl)-1-butene, 1,4-di(4-hydroxyphenyl)-2-butene, 1,4-di(4-hydroxyphenyl)-2-butanol, 1,4-di(4-hydroxyphenyl)-2-butanone, 1,4-di(4-hydroxyphenyl)-2,3-butanediol, 1,4-di(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3-)butane, 1-(4-hydroxyphenyl)-4-(indole-3)-1-butene, 1-di(4-hydroxyphenyl)-4-(indole-3)-2-butene, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanol, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3)-2,3-butanediol, 1-(4-hydroxyphenyl-4-(indole-3)-3-hydroxy-2-butanone, indole-3-acetoaldehyde, 1,4-di(indole-3-)butane, 1,4-di(indole-3)-1-butene, 1,4-di(indole-3)-2-butene, 1,4-di(indole-3)-2-butanol, 1,4-di(indole-3)-2-butanone, 1,4-di(indole-3)-2,3-butanediol, 1,4-di(indole-3)-3-hydroxy-2-butanone, succinate semialdehyde, hexane-1,8-dicarboxylic acid, 3-hexene-1,8-dicarboxylic acid, 3-hydroxy-hexane-1,8-dicarboxylic acid, 3-hexanone-1,8-dicarboxylic acid, 3,4-hexanediol-1,8-dicarboxylic acid, 4-hydroxy-3-hexanone-1,8-dicarboxylic acid, fucoidan, iodine, chlorophyll, carotenoid, calcium, magnesium, iron, sodium, potassium, phosphate, and the like.

[0159] The recitation "optimized" as used herein refers to a pathway, gene, polypeptide, enzyme, or other molecule having an altered biological activity, such as by the genetic alteration of a polypeptide's amino acid sequence or by the alteration/modification of the polypeptide's surrounding cellular environment, to improve its functional characteristics in relation to the original molecule or original cellular environment (e.g., a wild-type sequence of a given polypeptide or a wild-type microorganism). Any of the polypeptides or enzymes described herein may be optionally "optimized," and any of the genes or nucleotide sequences described herein may optionally encode an optimized polypeptide or enzyme. Any of the pathways described herein may optionally contain one or more "optimized" enzymes, or one or more nucleotide sequences encoding for an optimized enzyme or polypeptide.

[0160] Typically, the improved functional characteristics of the polypeptide, enzyme, or other molecule relate to the suitability of the polypeptide or other molecule for use in a biological pathway (e.g., a biosynthesis pathway, a C—C ligation pathway) to convert a monosaccharide or oligosaccharide into a biofuel. Certain embodiments, therefore, contemplate the use of "optimized" biological pathways. An exemplary "optimized" polypeptide may contain one or more alterations or mutations in its amino acid coding sequence (e.g., point mutations, deletions, addition of heterologous sequences) that facilitate improved expression and/or stability in a given microbial system or microorganism, allow regulation of polypeptide activity in relation to a desired substrate (e.g., inducible or repressible activity), modulate the localization of the polypeptide within a cell (e.g., intracellular localization, extracellular secretion), and/or effect the polypeptide's overall level of activity in relation to a desired substrate (e.g., reduce or increase enzymatic activity). A polypeptide or other molecule may also be "optimized" for use with a given microbial system or microorganism by altering one or more pathways within that system or organism, such as by altering a pathway that regulates the expression (e.g., up-regulation), localization, and/or activity of the "optimized" polypeptide or other molecule, or by altering a pathway that minimizes the production of undesirable by-products, among other alterations. In this manner, a polypeptide or other molecule may be "optimized" with or without altering its wild-type amino acid sequence or original chemical structure. Optimized polypeptides or biological pathways may be obtained, for example, by direct mutagenesis or by natural selection for a desired phenotype, according to techniques known in the art.

[0161] In certain aspects, "optimized" genes or polypeptides may comprise a nucleotide coding sequence or amino acid sequence that is 50% to 99% identical (including all integeres in between) to the nucleotide or amino acid sequence of a reference (e.g., wild-type) gene or polypeptide. In certain aspects, an "optimized" polypeptide or enzyme may have about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100 (including all integers and decimal points in between e.g., 1.2, 1.3, 1.4, 1.5, 5.5, 5.6, 5.7, 60, 70, etc.), or more times the biological activity of a reference polypeptide.

[0162] Certain aspects of the invention also include a commodity chemical, such as a biofuel, that is produced according to the methods and recombinant microorganisms described herein. Such a biofuel (e.g., medium to long chain alkane) may be distinguished from other fuels, such as those fuels produced by traditional refinery from crude carbon sources, by radio-carbon dating techniques. For instance, carbon has two stable, nonradioactive isotopes: carbon-12 (^{12}C), and carbon-13 (^{13}C). In addition, there are trace amounts of the unstable isotope carbon-14 (^{14}C) on Earth. Carbon-14 has a half-life of 5730 years, and would have long ago vanished from Earth were it not for the unremitting impact of cosmic rays on nitrogen in the Earth's atmosphere, which create more of this isotope. The neutrons resulting from the cosmic ray interactions participate in the following nuclear reaction on the atoms of nitrogen molecules (N_2) in the atmospheric air:

$$n + {}^{14}_{7}N \rightarrow {}^{14}_{6}C + p$$

[0163] Plants and other photosynthetic organisms take up atmospheric carbon dioxide by photosynthesis. Since many plants are ingested by animals, every living organism on Earth is constantly exchanging carbon-14 with its environment for the duration of its existence. Once an organism dies, however, this exchange stops, and the amount of carbon-14 gradually decreases over time through radioactive beta decay.

[0164] Most hydrocarbon-based fuels, such as crude oil and natural gas derived from mining operations, are the result of compression and heating of ancient organic materials (i.e., kerogen) over geological time. Formation of petroleum typically occurs from hydrocarbon pyrolysis, in a variety of

mostly endothermic reactions at high temperature and/or pressure. Today's oil formed from the preserved remains of prehistoric zooplankton and algae, which had settled to a sea or lake bottom in large quantities under anoxic conditions (the remains of prehistoric terrestrial plants, on the other hand, tended to form coal). Over geological time the organic matter mixed with mud, and was buried under heavy layers of sediment resulting in high levels of heat and pressure (known as diagenesis). This process caused the organic matter to chemically change, first into a waxy material known as kerogen which is found in various oil shales around the world, and then with more heat into liquid and gaseous hydrocarbons in a process known as catagenesis. Most hydrocarbon based fuels derived from crude oil have been undergoing a process of carbon-14 decay over geological time, and, thus, will have little to no detectable carbon-14. In contrast, certain biofuels produced by the living microorganisms of the present invention will comprise carbon-14 at a level comparable to all other presently living things (i.e., an equilibrium level). In this manner, by measuring the carbon-12 to carbon-14 ratio of a hydrocarbon-based biofuel of the present invention, and comparing that ratio to a hydrocarbon based fuel derived from crude oil, the biofuels produced by the methods provided herein can be structurally distinguished from typical sources of hydrocarbon based fuels.

[0165] Embodiments of the present invention include methods for converting a polysaccharide to a suitable monosaccharide comprising, (a) obtaining the polysaccharide; and (b) contacting the polysaccharide with a recombinant microorganism or microbial system comprising such a microorganism for a time sufficient to convert the polysaccharide to a suitable monosaccharide, wherein the microbial system comprises, (i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase, wherein the lyase and/or hydrolase optionally comprises at least one signal peptide or at least one autotransporter domain; (ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, and a polysaccharide transporter; and (iii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase, thereby converting the polysaccharide to a suitable monosaccharide.

[0166] Alternatively, certain aspects may include methods for converting a polysaccharide to a suitable monosaccharide comprising, (a) obtaining the polysaccharide; and (b) contacting the polysaccharide with a microbial system for a time sufficient to convert the polysaccharide to a suitable monosaccharide, wherein the microbial system comprises, (i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase; (ii) at least one gene encoding and expressing a superchannel; and (iii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase, thereby converting the polysaccharide to a suitable monosaccharide.

[0167] In certain embodiments, a microbial system or isolated microorganism is capable of growing using a polysaccharide (e.g., alginate, pectin, etc.) as a sole source of carbon and/or energy. A "sole source of carbon" refers generally to the ability to grow on a given carbon source as the only carbon source in a given growth medium.

[0168] With regard to alginate, approximately 50 percent of seaweed dry-weight comprises various sugar components, among which alginate and mannitol are major components corresponding to 30 and 15 percent of seaweed dry-weight, respectively. With regard to pectin, although microorganisms such as *E. coli* are generally considered as a host organisms in synthetic biology, and although such microorganism are able to metabolize mannitol, they completely lack the ability to degrade and metabolize alginate. In this regard, many laboratory or wild-type microorganisms, such as *E. coli*, are unable to grow on alginate as a sole source of carbon. Similarly, many organisms such as *E. coli* are unable to degrade and metabolize pectin, a polysaccharide found in many food waste products, and, thus are unable to grown on pectin as a sole source of carbon. Accordingly, embodiments of the present application include engineered microorganisms, such as *E. coli*, or microbial systems containing such engineered microorganisms, that are capable of using polysaccharides, such as alginate and pectin, as a sole source of carbon and/or energy.

[0169] Alginate is a block co-polymer of β-D-mannuronate (M) and α-D-gluronate (G) (M and G are epimeric about the C5-carboxyl group). Each alginate polymer comprises regions of all M (polyM), all G (polyG), and/or the mixture of M and G (polyMG). To utilize alginate to produce one or more suitable monosaccharides, certain aspects of the present invention provide an engineered or recombinant microorganism or microbial system that is able to degrade or de-polymerize alginate and to use it as a source of carbon and/or energy. As one means of accomplishing this purpose, such recombinant microorganisms may incorporate a set of polysaccharide degrading or depolymerizing enzymes such as alginate lyases (ALs) to the microbial system.

[0170] ALs are mainly classified into two distinctive subfamilies depending on their acts of catalysis: endo-(EC 4.2.2.3) and exo-acting (EC 4.2.2.-) ALs. Endo-acting ALs are further classified based on their catalytic specificity; M specific and G specific ALs. The endo-acting ALs randomly cleave alginate via a 1-elimination mechanism and mainly depolymerize alginate to di-, tri- and tetrasaccharides. The uronate at the non-reducing terminus of each oligosaccharide are converted to unsaturated sugar uronate, 4-deoxy-α-L-erythro-hex-4-ene pyranosyl uronates. The exo-acting ALs catalyze further depolymerization of these oligosaccharides and release unsaturated monosaccharides, which may be non-enzymatically converted to monosaccharides, including α-keto acid, 4-deoxy-α-L-erythro-hexoselulose uronate (DEHU). Certain embodiments of an engineered microbial system or isolated, engineered microorganism may include endoM-, endoG- and exo-acting ALs to degrade or depolymerize aquatic or marine-biomass polysaccharides such as alginate to a monosaccharide such as DEHU.

[0171] Embodiments of the present invention may also include lyases such as alginate lyases isolated from various sources, including, but not limited to, marine algae, mollusks, and wide varieties of microbes such as genus *Pseudomonas, Vibrio*, and *Sphingomonas*. Many alginate lyases are endo-acting M specific, several are G specific, and few are exo-acting. For example, ALs isolated from *Sphingomonas* sp. strain Al include five endo-acting ALs, Al-I, Al-II, Al-II', Al-III, and Al-IV' and an exo-acting AL, Al-IV.

[0172] Typically, Al-I, Al-II, and Al-III have molecular weights of 66 kDa, 25 kDa, and 40 kDa, respectively. AI-II and AI-III are self-splicing products of Al-1. AI-II may be

more specific to G and Al-III may be specific to M. Al-I may have high activity for both M and G. Al-IV has molecular weight of about 85 kDa and catalyzes exo-lytic depolymerization of oligoalginate. Although both Al-II' and Al-IV' are functional homologues of Al-II and Al-IV. Al-II' has endolytic activity and may have no preference to M or G. Al-IV has primarily endo-lytic activity. In addition to these ALs, exolytic AL Atu3025 derived from *Agrobacterium tumefaciens* has high activity for depolymerization of oligoalginate, and may be used in certain embodiments of the present invention. Certain embodiments may incorporate into the microbial system or isolated microorganism the genes encoding Al-I, Al-II', Al-IV, and Atu3025, and may include optimal codon usage for the suitable host organisms, such as *E. coli.*

[0173] Certain examples of alginate lyases or oligoalginate lyases that may be utilized herein include enzymes or polypeptides sharing at least 60%, 70%, 80%, 90%, 95%, 98%, or more sequence identity (including all integers in between) to SEQ ID NOS:67-68, which show the nucleotide (SEQ ID NO:67) and polyeptide (SEQ ID NO:68) sequences of oligoalginate lyase Atu3025 isolated from *Agrobacterium tumefaciens*. Certain examples of alginate lyases that may be utilized herein include enzymes or polypeptides sharing at least 60%, 70%, 80%, 90%, 95%, 98%, or more sequence identity (including all integers in between) to the alginate lyase enyzmes described in FIG. **37**, as well as the secreted alginate lyase encoded by Vs24254 from *Vibrio splendidus.*

[0174] In certain embodiments, a microbial system or recombinant microorganism may be engineered to secrete or display the lyases or alginate lyases (ALs) to the culture media, such as by incorporating a signal peptide or autotransporter domain into the lyase. In this regard, it is typically understood that bacteria have at least four different types of protein secretion machinery (type I, II, III and IV). For example, in *E. coli*, the type II secretion machinery is used for the secretion of recombinant proteins. The type II secretion machinery may comprise a two-step process: the translocation of premature proteins tagged with signal peptides to the periplasm fraction and processing to the mature proteins followed by secretion to media.

[0175] The first process may proceed by any of three different pathways: secB-dependent pathway, signal recognition particle (SRP) pathway, or twin-arginine translocation (TAT) pathway. Recombinant proteins may be secreted into periplasm fraction. The fates of the mature proteins vary dependent on the type of proteins. For example, some proteins are secreted spontaneously by diffusion or passively by a secretion apparatus named secretion that consists of 12-16 proteins, and others stay in periplasm fraction and are eventually degraded.

[0176] Some proteins may also be secreted by an autotransporter apparatus, such as by utilizing an autotransporter domain. The proteins secreted by autotransporter domains typically comprise an N-terminal signal peptide that plays a role in translocation to the periplasm, which may be mediated by secB or SRP pathways, passenger domain, and/or C-terminal translocation unit (UT) having a characteristic β-barrel structure. The β-barrel portion of the UT builds an aqueous pore channel across the outer membrane and helps the transportation of passenger domain to media. Autodisplayed passenger proteins are often cleaved by the autotransporter and set free to media.

[0177] The type I secretion machinery may also be used for the secretion of recombinant proteins in *E. coli*. The type I secretion machinery may be used for the secretion of high-molecular-weight toxins and exoenzymes. The type I secretion machinery consist of two inner membrane proteins (HlyB and HlyD) that are the member of the ATP binding cassette (ABC) transporter family, and an endogenous outer membrane protein (TolC). The secretion of recombinant proteins based on type I secretion machinery may utilize the C-terminal region of α-haemolysin (HlyA) as a signal sequence. The recombinant proteins may readily pass through the inner membrane, periplasm, and outer membrane through the type I secretion machinery.

[0178] Depending on the types of linker and signal peptides utilized by various embodiments of the present application, both autotransporter and type I secretion machinery can be altered to the cell surface display machinery. Alternatively, a system specific to cell surface display may be used. For example, in this system, target proteins may be fused to PgsA protein (a poly-γ-glutamate synthetase complex) that is natively displayed on the surface of *Bacillus subtilis.*

[0179] Certain embodiments may include lyases such as alginate lyases fused with various signal peptides and/or autotransporter domains found in proteins secreted by both type I and type II secretion machinery. Other embodiments may include lyases such as alginate lyases fused with any combination of signal peptides and or autotransporter domains found in proteins secreted transport machinery as described herein or known to a person skilled in the art. Embodiments may also include signal peptides or autotransporter domains that are experimentally redesigned to maximize the secretion of lyases such as alginate lyases to the culture media, and may also include the use of many different linker sequences that fuse signal peptides, lyases, and autotransporters that improve the efficiency of secretion or the cell surface presentation of lyases.

[0180] Certain embodiments may include a microbial system or isolated microorganism that comprise saccharide transporters, which are able to transport monosaccharides (e.g., DEHU) and oligosaccharides from the media to the cytosol to efficiently utilize these monosaccharides as a source of carbon and/or energy. For instance, genes encoding monosaccharide permeases (i.e., monosaccharide transporters) such as DEHU permeases may be isolated from bacteria that grow on polysaccharides such as alginate as a source of carbon and/or energy, and may be incorporated into embodiments of the present microbial system or isolated microorganism. As an additional example, embodiments may also include redesigned native permeases or transporters with altered specificity for monosaccharide (e.g., DEHU) transportation.

[0181] In this regard, *E. coli* contains several permeases able to transport monosaccharides, which include, but are not limited to, KdgT for 2-keto-3-deoxy-D-gluconate (KDG) transporter, ExuT for aldohexuronates such as D-galacturonate and D-glucuronate transporter, GntT, GntU, GntP, and GntT for gluconate transporter, and KgtP for proton-driven α-ketoglutarate transporter. Microbial systems or recombinant microorganisms described herein may comprise any of these permeases, in addition to those permeases known to a person of skill in the art and not mentioned herein, and may also include permease enzymes redesigned to transport other monosaccharides, such as DEHU.

[0182] A microbial system or recombinant microorganism according to the present invention may also comprise permeases/transporters/superchannels/porins that catalyze the

transport of monosaccharides (e.g., D-mannuronate and D-lyxose) from media to the periplasm or cytosol of a microorganism. For example, genes encoding the permeases of D-mannuronate in soil *Aeromonas* may be incorporated into a microbial system as described herein.

[0183] As one alternative example, a microbial system or microorganism may comprise native permeases/transporters that are redesigned to alter their specificity for efficient monosaccharide transportation, such as for D-mannuronate and D-lyxose transportation. For instance, E. coli contains several permeases that are able to transport monosaccharides or sugars such as D-mannonate and D-lyxose, including KdgT for 2-keto-3-deoxy-D-gluconate (KDG) transporter, ExuT for aldohexuronates such as D-galacturonate and D-glucuronate transporter, GntPTU for gluconate/fructuronate transporter, uidB for glucuronide transporter, fucP for L-fucose transporter, galP for galactose transporter, yghK for glycolate transporter, dgoT for D-galactonate transporter, uhpT for hexose phosphate transporter, dctA for orotate/citrate transporter, gntUT for gluconate transporter, malEGF for maltose transporter: alsABC for D-allose transporter, idnT for L-idonate/D-gluconate transporter, KgtP for proton-driven α-ketoglutarate transporter, lacY for lactose/galactose transporter, xylEFGH for D-xylose transporter, araEFGH for L-arabinose transporter, and rbsABC for D-ribose transporter. In certain embodiments, a microbial system or recombinant microorganism may comprise permeases or transporters as described above, including those that are re-designed or optimized for improvided transport of certain monosaccharides, such as D-mannuronate, DEHU, and D-lyxose.

[0184] Certain aspects may employ a recombinant microorganism that comprises a "superchannel," by which aquatic or marine-biomass polysaccharides such as alginate polymers, or fruit or vegetable biomass such as pectin polymers, may be directly incorporated into the cytosol and degraded inside the microbial system. For instance, a group of bacteria characterized as Sphingomonads have a wide range in capability of degrading environmentally hazardous compounds such as polychlorinated polycyclic aromatics (dioxin). These bacteria contain characteristic large pleat-like molecules on their cell surfaces. In this regard, certain Sphingomonads have structures characterized as "superchannels" that enable the bacteria to directly take up macromolecules.

[0185] As one particular example of a microorganism comprising a superchannel, *Sphingomonas* sp. strain Al directly incorporates polysaccharides such as alginate through a superchannel. Such superchannels may consist of a pit on the outer membrane (e.g., AlgR), alginate-binding proteins in the periplasm (e.g., AlgQ1 and Alg Q2), and an ATP-binding cassette (ABC) transporter (e.g., AlgM1, AlgM2, and AlgS). Incorporated polysaccharides such as alginate may be readily depolymerized by lyases such as alginate lyases produced in the cytosol. Thus, certain embodiments may incorporate genes encoding a superchannel (e.g., ccpA, algS, algM1, algM2, algQ1, algQ2) to introduce this ability to the microbial system or recombinant microorganism. Other embodiments may include microorganisms such as *Sphingomonas subarctica* IFO 16058^T, which harbor the plasmid containing genes that encode a superchannel, and which have significantly improved ability to utilize marine or aquatic biomass polysaccharides such as alginate as a source of carbon and/or energy. Certain recombinant microorganisms may employ these superchannel encoding plasmid sequences contained within *Sphingomonas subarctica* IFO 16058^T.

[0186] Certain examples of alginate ABC transporters that may be utilized herein, include ABC transporters Atu3021, Atu3022, Atu3023, Atu3024, algM1, algM2, AlgQ1, AlgQ2, AlgS, OG2516_05558, OG2516_05563, OG2516_05568, and OG2516_05573, including functional variants thereof. Certain examples of alginate symporters that may be utilized herein include symporters V12B01_24239 and V12B01_24194, among others, including functional variants thereof. One additional example of an alginate porin includes V12B01_24269, and variants thereof.

[0187] As noted above, certain embodiments may include recombinant microorganisms that comprise one or more monosaccharide dehydrogenases, isomerases, dehydratases, kinases, and aldolases. With regard to monosaccharide dehydrogenases, certain microbial systems or recombinant microorganism may incorporate enzymes that reduce various monosaccharides (e.g., DEHU, mannuronate) to a monosaccharide that is suitable for biofuel biosynthesis, such as 2-keto-3-deoxy-D-gluconate (KDG) or D-mannitol. Such exemplary enzymes, include, for example, DEHU hydrogenases and mannuronate hydrogenases, in addition to various alcohol dehydrogenases having DEHU hydrogenase and/or mannuronate dehydrogenase activity, such as the novel ADH1 through ADH12 enzymes isolated from *Agrobacterium tumefaciens* C58 (see, e.g., SEQ ID NOS:69-92).

[0188] For more detail on the ADH1 through ADH12 enzymes, SEQ ID NO:69 shows the nucleotide and SEQ ID NO:70 shows the polypeptide sequence of ADH1 Atu1557 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:71 shows the nucleotide and SEQ ID NO:72 shows the polypeptide sequence of ADH2 Atu2022 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:73 shows the nucleotide and SEQ ID NO:74 shows the polypeptide sequence of ADH3 Atu0626 isolated from *Agrobacterium tumefaciens* C58.

[0189] SEQ ID NO:75 shows the nucleotide and SEQ ID NO:76 shows the polypeptide sequence of ADH4 Atu5240 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:77 shows the nucleotide and SEQ ID NO:78 shows the polypeptide sequence of ADH5 Atu3163 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:79 shows the nucleotide and SEQ ID NO:80 shows the polypeptide sequence of ADH6 Atu2151 isolated from *Agrobacterium tumefaciens* C58.

[0190] SEQ ID NO:81 shows the nucleotide and SEQ ID NO:82 shows the polypeptide sequence of ADH7 Atu2814 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:83 shows the nucleotide and SEQ ID NO:84 shows the polypeptide sequence of ADH8 Atu5447 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:85 shows the nucleotide and SEQ ID NO:86 shows the polypeptide sequence of ADH9 Atu4087 isolated from *Agrobacterium tumefaciens* C58.

[0191] SEQ ID NO:87 shows the nucleotide and SEQ ID NO:88 shows the polypeptide sequence of ADH10 Atu4289 isolated from *Agrobacterium tumefaciens* C58.

[0192] SEQ ID NO:89 shows the nucleotide and SEQ ID NO:90 shows the polypeptide sequence of ADH11 Atu3027 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:91 shows the nucleotide and SEQ ID NO:92 shows the polypeptide sequence of ADH12 Atu3026 isolated from *Agrobacterium tumefaciens* C58.

[0193] Further examples of enzymes having dehydrogenase activity include Atu3026, Atu3027, OG2516_05543,

OG2516_05538 and V12B01_24244. The microorganisms and methods of the present invention may also utilize biologically active fragments and variants of these hydrogenase enzymes, including optimized variants thereof.

[0194] As a further example, *Pseudomonas* grown using alginate as a sole source of carbon and energy comprises a DEHU hydrogenase enzyme that uses NADPH as a co-factor, is more stable when $NADP^+$ is present in the solution, and is active at ambient pH. Thus, certain embodiments of a microbial system or a recombinant microorganism as described herein may incorporate genes encoding hydrogenases such as DEHU or mannuronate hydrogenase derived or obtained from various microbes, in which these microbes may be capable of growing on polysaccharides such as alginate or pectin as a source of carbon and/or energy.

[0195] Certain embodiments may incorporate components of a microbial system or isolated microorganism that is capable of efficiently growing on monosaccharides such as D-mannuronate or D-lyxose as a source of carbon and energy. For instance, both *Aeromonas* and *Aerobacter aerogenes* PRL-R3 comprise genes encoding monosaccharide dehydrogenases such as D-mannuronate hydrogenase and D-lyxose isomerase. Thus, certain microbial systems or recombinant microorganisms may comprise monosaccharide dehydrogenases such as D-mannuronate hydrogenase and D-lyxose isomerase from *Aeromonas, Aerobacter aerogenes* PRL-R3, or various other suitable microorganisms, including those microorganisms capable of growing on D-mannuronate or D-lyxose as a source of carbon and energy.

[0196] Certain embodiments may include a microbial system or isolated microorganism with enhanced efficiency for converting monosaccharides such as D-mannonate and D-xylulose into monosaccharides suitable for a biofuel biosynthesis pathway such as KDG. Merely by way of explanation, D-mannonate and D-xylulose are metabolites in microbes such as *E. coli*. D-mannonate is converted by a D-mannonate dehydratase to KDG. D-xylulose enters the pentose phosphate pathway. Thus, to increase conversion of D-mannonate to KDG, an exogenous or endogenous D-mannonate dehydratase (e.g., uxuA) gene may be over-expressed an a recombinant microorganism of the invention. Similarly, in other embodiments, suitable endogenous or exogenous genes such as kinases (e.g., kdgK), nad, as well as KDG aldolases (e.g., kdgA and eda) may be either incorporated or overexpressed in a given recombinant microorganism (see SEQ ID NOS:93-96), including biologically active variants or fragments thereof, such as optimized variants of these genes. SEQ ID NO:93 shows the nucleotide sequence and SEQ ID NO:94 shows the polypeptide sequence of a 2-keto-deoxy gluconate kinase (KdgK) from *Escherichia coli* DH10B. SEQ ID NO:95 shows the nucleotide sequence and SEQ ID NO:96 shows the polypeptide sequence of a 2-keto-deoxy gluconate-6-phosphate aldorase (KdgA) from *Escherichia coli* DH10B.

[0197] In certain aspects, as noted above, a recombinant microorganism that is capable of growing on alginate or pectin as a sole source of carbon may utilize a naturally-occurring or endogenous copy of a dehyradratase, kinase, and/or aldolase. For instance, *E. coli* contains endogenous dehydratases, kinases, and aldolases that are capable of catalyzing the appropriate steps in the conversion of polysaccharides to a suitable monosaccharide. In these and other related aspects, the naturally-occurring dehydratase or kinase may also be over-expressed, such as by providing an exogenous copy of the naturally-occurring dehydratase, kinase or aldolase operable linked to a highly constitutive or inducible promoter.

[0198] As one exemplary source of enzymes for engineering a recombinant microorganism to grow on alginate as a sole source of carbon, *Vibrio splendidus* is known to be able to metabolize alginate to support growth. For example, SEQ ID NO:1 shows a secretome region carrying certain *Vibrio splendidus* genes (V12B01_02425 to V12B01_02480), which encodes a type II secretion apparatus. SEQ ID NO:2 shows the nucleotide sequence of an entire genomic region between V12B01_24189 to V12B01_24249, which was derived from *Vibrio splendidus*, and which when transformed into *E. coli* as a fosmid clone was sufficient to confer the ability to grow on alginate as a sole source of carbon. SEQ ID NOS:3-64 show the individual putative genes contained within SEQ ID NO:2. Thus, in certain aspects, a recombinant microorganism (e.g., *E. coli*) that is able to grow on alginate as a sole source of carbon and/or energy may comprise one or more nucleotide or polypeptide reference sequences described in SEQ ID NOS:1-64, including biologically active fragments or variants thereof, such as optimized variants.

[0199] In certain aspects, a recombinant microorganism that is able to grow on alginate as a sole source of carbon may contain certain coding nucleotide or polypeptide sequences contained within SEQ ID NO:2, such as the sequences in SEQ ID NOS:3-64, or biologically active fragments or variants thereof, including optimized variants. These sequences are described in further detail below.

[0200] SEQ ID NO:3 shows the nucleotide coding sequence of the putative protein V12B01_24184. This putative coding sequence is contained within the polynucleotide sequence of SEQ ID NO:2, and encodes a polypeptide that is similar to an autotransporter adhesion or type I secretion target ggxgxdxxx (SEQ ID NO:145) repeat. SEQ ID NO:4 shows the polypeptide sequence of putative protein V12B01_24184, encoded by the polynucleotide of SEQ ID NO:3. This putative polypeptide is similar to autotransporter adhesion or type I secretion target ggxgxdxxx (SEQ ID NO:145) repeat.

[0201] SEQ ID NO:5 shows the nucleotide sequence that encodes the putative protein V12B01_24189. SEQ ID NO:6 shows the polypeptide sequence of the putative protein V12B01_24189, which is similar to cyclohexadienyl dehydratase.

[0202] SEQ ID NO:7 shows the nucleotide sequence that encodes the putative protein V12B01_24194. SEQ ID NO:8 shows the polypeptide sequence of the putative protein V12B01_24194, which is similar to a Na/proline transporter.

[0203] SEQ ID NO:9 shows the nucleotide sequence that encodes the putative protein V12B01_24199. SEQ ID NO:10 shows the polypeptide sequence of the putative protein V12B01_24199, which is similar to a keto-deoxy-phosphogluconate aldolase.

[0204] SEQ ID NO:11 shows the nucleotide sequence that encodes the putative protein V12B01_24204. SEQ ID NO:12 shows the polypeptide sequence of the putative protein V12B01_24204, which is similar to 2-dehydro-3-deoxy-gluconokinase.

[0205] SEQ ID NO:13 shows the nucleotide sequence that encodes the putative protein V12B01_241209. SEQ ID NO:14 shows the polypeptide sequence of the putative protein V12B01_241209.

[0206] SEQ ID NO:15 shows the nucleotide sequence that encodes the putative protein V12B01_24214. SEQ ID

NO:16 shows the polypeptide sequence of the putative protein V12B01_24214, which is similar to a chondroitin AC/alginate lyase.

[0207] SEQ ID NO:17 shows the nucleotide sequence that encodes the putative protein V12B01_24219. SEQ ID NO:18 shows the polypeptide sequence of the putative protein V12B01_24219, which is similar to a chondroitin AC/alginate lyase.

[0208] SEQ ID NO:19 shows the nucleotide sequence that encodes the putative protein V12B01_24224. SEQ ID NO:20 shows the polypeptide sequence of the putative protein V12B01_24224, which is similar to a 2-keto-4-pentenoate hydratase/2-oxohepta-3-ene-1,7-dioic acid hydratase.

[0209] SEQ ID NO:21 shows the nucleotide sequence that encodes the putative protein V12B01_24229. SEQ ID NO:22 shows the polypeptide sequence of the putative protein V12B01_24229, which is similar to a GntR-family transcriptional regulator.

[0210] SEQ ID NO:23 shows the nucleotide sequence that encodes the putative protein V12B01_24234. SEQ ID NO:24 shows the polypeptide sequence of the putative protein V12B01_24234, which is similar to a Na^+/proline symporter.

[0211] SEQ ID NO:25 shows the nucleotide sequence that encodes the putative protein V12B01_24239. SEQ ID NO:26 shows the polypeptide sequence of the putative protein V12B01_24239, which is similar to an oligoalginate lyase.

[0212] SEQ ID NO:27 shows the nucleotide sequence that encodes the putative protein V12B01_24244. SEQ ID NO:28 shows the polypeptide sequence of putative protein V12B01_24244, which is similar to a 3-hydroxyisobutyrate dehydrogenase.

[0213] SEQ ID NO:29 shows the nucleotide sequence that encodes the putative protein V12B01_24249. SEQ ID NO:30 shows the polypeptide sequence of the putative protein V12B01_24249, which is similar to a methyl-accepting chemotaxis protein.

[0214] SEQ ID NO:31 shows the nucleotide sequence that encodes the putative protein V12B01_24254. SEQ ID NO:32 shows the polypeptide sequence of putative protein V12B01_24254, which is similar to an alginate lyase.

[0215] SEQ ID NO:33 shows the nucleotide sequence that encodes the putative protein V12B01_24259. SEQ ID NO:34 shows the polypeptide sequence of putative protein V12B01_24259, which is similar to an alginate lyase.

[0216] SEQ ID NO:35 shows the nucleotide sequence that encodes the putative protein V12B01_24264. SEQ ID NO:36 shows the polypeptide sequence of putative protein V12B01_24264.

[0217] SEQ ID NO:37 shows the nucleotide sequence that encodes the putative protein V12B01_24269. SEQ ID NO:38 shows the polypeptide sequence of putative protein V12B01_24269, which is similar to a putative oligogalacturonate specific porin.

[0218] SEQ ID NO:39 shows the nucleotide sequence that encodes the putative protein V12B01_24274. SEQ ID NO:40 shows the polypeptide sequence of putative protein V12B01_24274, which is similar to an alginate lyase.

[0219] FIG. **32** shows the nucleotide coding sequence and polypeptide sequence of putative protein V12B01_02425. FIG. **32**A shows the nucleotide sequence that encodes the putative protein V12B01_02425 (SEQ ID NO:41). FIG. **32**B shows the polypeptide sequence of putative protein V12B01_02425 (SEQ ID NO:42), which is similar to a type II secretory pathway component EpsC.

[0220] SEQ ID NO:43 shows the nucleotide sequence that encodes the putative protein V12B01_02430. SEQ ID NO:44 shows the polypeptide sequence of putative protein V12B01_02430, which is similar to a type II secretory pathway component EpsD.

[0221] SEQ ID NO:45 shows the nucleotide sequence that encodes the putative protein V12B01_02435. SEQ ID NO:46 shows the polypeptide sequence of putative protein V12B01_02435, which is similar to a type II secretory pathway component EpsE.

[0222] SEQ ID NO:47 shows the nucleotide sequence that encodes the putative protein V12B01_02440. SEQ ID NO:48 shows the polypeptide sequence of putative protein V12B01_02440, which is similar to a type II secretory pathway component EpsF.

[0223] SEQ ID NO:49 shows the nucleotide sequence that encodes the putative protein V12B01_02445. SEQ ID NO:50 shows the polypeptide sequence of putative protein V12B01_02445, which is similar to a type II secretory pathway component EpsG.

[0224] SEQ ID NO:51 shows the nucleotide sequence that encodes the putative protein V12B01_02450. SEQ ID NO:52 shows the polypeptide sequence of putative protein V12B01_02450, which is similar to a type II secretory pathway component EpsH.

[0225] SEQ ID NO:53 shows the nucleotide sequence that encodes the putative protein V12B01_02455. SEQ ID NO:54 shows the polypeptide sequence of putative protein V12B01_02455, which is similar to a type II secretory pathway component EpsI.

[0226] SEQ ID NO:55 shows the nucleotide sequence that encodes the putative protein V12B01_02460. SEQ ID NO:56 shows the polypeptide sequence of putative protein V12B01_02460, which is similar to a type II secretory pathway component EpsJ.

[0227] SEQ ID NO:57 shows the nucleotide sequence that encodes the putative protein V12B01_02465. SEQ ID NO:58 shows the polypeptide sequence of putative protein V12B01_02465, which is similar to a type II secretory pathway component EpsK.

[0228] SEQ ID NO:59 shows the nucleotide sequence that encodes the putative protein V12B01_02470. SEQ ID NO:60 shows the polypeptide sequence of putative protein V12B01_02470, which is similar to a type II secretory pathway component EpsL.

[0229] SEQ ID NO:61 shows the nucleotide sequence that encodes the putative protein V12B01_02475. SEQ ID NO:62 shows the polypeptide sequence of putative protein V12B01_02475, which is similar to a type II secretory pathway component EpsM.

[0230] SEQ ID NO:63 shows the nucleotide sequence that encodes the putative protein V12B01_02480. SEQ ID NO:64 shows the nucleotide sequence that encodes the putative protein V12B01_02480, which is similar to a type II secretory pathway component EpsC.

[0231] As a further exemplary source of enzymes for engineering a microorganism to grow on alginate, *Agrobacterium tumefaciens* C58 is able to metabolize relatively small sizes of alginate molecules (1000 mers) as a sole source of carbon and energy. Since *A. tumefaciens* C58 has long been used for plant biotechnology, the genetics of this organism has been rela-

tively well studied, and many genetic tools are available and compatible with other gram-negative bacteria such as *E. coli*. Thus, certain aspects may employ this microbe, or the genes therein, for the production of suitable monosaccharides. For instance, as noted above, the present disclosure provides a series of novel ADH genes having both DEHU and mannuronate hydrogenase activity that were obtained from *Agrobacterium tumefaciens* C58 (see SEQ ID NOS: 67-92).

[0232] As noted above, certain aspects may include a recombinant microorganism or microbial system that is capable of growing on pectin as a sole source of carbon and/or energy. Pectin is a linear chain of α-(1-4)-linked D-galacturonic acid that forms the pectin-backbone, a homogalacturonan. Into this backbone, there are regions where galacturonic acid is replaced by (1-2)-linked L-rhamnose. From rhamnose, side chains of various neutral sugars typically branch off. This type of pectin is called rhamnogalacturonan I. Over all, about up to every 25th galacturonic acid in the main chain is exchanged with rhamnose. Some stretches consisting of alternating galacturonic acid and rhamnose—"hairy regions", others with lower density of rhamnose—"smooth regions." The neutral sugars mainly comprise D-galactose, L-arabinose and D-xylose; the types and proportions of neutral sugars vary with the origin of pectin. In nature, around 80% of carboxyl groups of galacturonic acid are esterified with methanol. Some plants, like sugar-beet, potatoes and pears, contain pectins with acetylated galacturonic acid in addition to methyl esters. Acetylation prevents gel-formation but increases the stabilising and emulsifying effects of pectin. Certain pectin degradation and metabolic pathways are exemplified in FIG. **3**.

[0233] In addition to the genes, enzymes, and biological pathways described above, certain recombinant microorganisms may incorporate features that are useful for growth on pectin as a sole source of carbon. For instance, to degrade and metabolize pectin as a sole source of carbon, pectin methyl and acetyl esterases first catalyze the hydrolysis of methyl and acetyl esters on pectin. Examples of pectin methyl esterases include, but are not limited to, pemA and pmeB. Examples of pectin acetyl esterases include, but are not limited to, PaeX and PaeY. Further examples of pectin methyl esterases that may be utilized herein include enzymes or polypeptides sharing at least 60%, 70%, 80%, 90%, 95%, 98%, or more sequence identity (including all integers in between) to the pectate methyl esterases in FIG. **40**. Further examples of pectate acetyl esterases that may be utilized herein include enzymes or polypeptides sharing at least 60%, 70%, 80%, 90%, 95%, 98%, or more sequence identity (including all integers in between) to the pectate acetyl esterases described in FIG. **41**.

[0234] Further to this end, pectate lyases and hydrolases may catalyze the endolytic cleavage of pectate via β-elimination and hydrolysis, respectively, to produce oligopectates. Other enzymes that may be utilized to metabolize pectin include Examples of pectate lyases include, but are not limited to, PelA, PelB, PelC, PelD, PelE, Pelf, PelI, PelL, and PelZ. Examples of pectate hydrolases include, but are not limited to, PehA, PehN, PehV, PehW, and PehX. Further examples of pectate lyases include polypeptides or enzymes sharing at least 60%, 70%, 80%, 90%, 95%, 98%, or more sequence identity (including all integers in between) to the pectate lyases described in FIG. **38**.

[0235] Polygalacturonases, rhamnogalacturonan lyases, and rhamnogalacturonan hydrolyases may also be utilized herein to degrade and metabolize pectin. Examples of rhamnogalacturonan lyases include polypeptides or enzymes sharing at least 60%, 70%, 80%, 90%, 95%, 98%, or more sequence identity (including all integers in between) to the rhamnoglacturonan lyases (i.e., rhamnogalacturonases) described in FIG. **39**A. Examples of rhamnogalacturonate hydrolyases include polypeptides or enzymes sharing at least 60%, 70%, 80%, 90%, 95%, 98%, or more sequence identity (including all integers in between) to the rhamnogalacturonate hydrolases described in FIG. **39**B.

[0236] Thus, to degrade and metabolize pectin, certain of the recombinant microorganisms and methods of the present invention may incorporate one or more of the above noted methy and acetyl esterases, lyases, and/or hydrolases, among others known in the art. These may enzymes may be encoded and expressed by endogenous or exogenous genes, and may also include biologically active fragments or variants thereof, such as homologs, orthologs, and/or optimized variants of these enzymes.

[0237] To further metabolize the degradation products of pectin, oligopectates may be transported into the periplasm fraction of gram-negative bacteria by outer membrane porins, where they are further degraded into such components as di- and tri-galactonurates. Examples of outer membrane porins include that can transport oligopectates into the periplasm include, but are not limited to, kdgN and kdgM. Certain recombinant microorganism may incorporate these or similar genes.

[0238] Di- and tri-galactonurates may then be transported into the cytosol for further degradation. Bacteria contain at least two different transporter systems responsible for di- and tri-galacturonate transportation, including symporter and ABC transporter (e.g., TogT and TogMNAB, respectively). Thus, certain of the recombinant microorganisms provided herein may comprise one or more a di- or tri-galacturonate transoporter systems, such as TogT and/or TogMNAB.

[0239] Once di- and trigalacturonate are incorporated into the cytosol, short pectate or galacturonate lyases, break them down to D-galacturonate and (4S)-4,6-dihydroxy-2,5-dioxohexuronate. Examples of short pectate or galacturonate lyases include, but are not limited to, PelW and Ogl, which genes may be either endogenously or exogenously incorporated into certain recombinant microorganisms provided herein. D-galacturonate and (4S)-4,6-dihydroxy-2,5-dioxohexuronate are then converted to 5-dehydro-4-deoxy-D-glucuronate and further to KDG, which steps may be catalyzed by KduI and KduD, respectively. The KduI enzyme has an isomerase activity, and the KduD enzyme has a dehydrogenase activity, such as a 2-deoxy-D-gluconate 3-dehydrogenase activity. Accordingly, certain recombinant microorganisms provided herein may comprise one or more short pectate or galacturonate lyases, such as PelW and/or Ogl, and may optionally comprise one or more isomerases, such as KduI, as well as one or more dehydrogenases, such as KduD, to convert di- and trigalacturonates into a suitable monosaccharide, such as KDG.

[0240] In certain aspects, a recombinant microorganism, such as *E. coli*, that is able to grown on pectin or tri-galacturonate as a sole source of carbon and/or energy may comprise one or more of the gene sequences contained within SEQ ID NOS:65 and 66, including biologically active fragments or variants thereof, such as optimized variants. SEQ ID NO:65 shows the nucleotide sequence of the kdgF-PaeX region from *Erwinia carotovora* subsp. *Atroseptica* SCRI1043. SEQ ID

NO:66 shows the nucleotide sequence of ogl-kdgR from *Erwinia carotovora* subsp. *Atroseptica* SCRI1043.

[0241] In certain aspects, a recombinant microorganism, such as *E. coli*, that is able to grown on pectin or tri-galacturonate as a sole source of carbon and/or energy may comprise one or more genomic regions of *Erwinia chrysanthemi*, comprising several genes (kdgF, kduI, kduD, pelW, togM, togN, togA, togB, kdgM, paeX, ogl, and kdgR) encoding enzymes (kduI, kduD, ogl, pelW, and paeX), transporters (togM, togN, togA, togB, and kdgM), and regulatory proteins (kdgR) responsible for degradation of di- and trigalacturonate, as well as several genes (pelA, pelE, paeY, and pem) encoding pectate lyases (pelA and pelE), pectin acetylesterases (paeY), and pectin methylesterase (pem) (see Example 2).

[0242] Additional examples of isomerases that may be utilized herein include glucoronate isomerases, such as those in the family uxaC, as well as 4-deoxy-L-threo-5-hexylose uronate isomerases, such as those in the family KduI. Additional examples of reductases that may be utilized herein include tagaturonate reductases, such as those in the family uxaB. Additional examples of dehyadratases that may be utilized herein include altronate dehydratases, such as those in the family uxaA. Additional examples of dehydrogenases that may be utilized herein include 2-deoxy-D-gluconate 3-dehydrogenases, such as those in the family kduD.

[0243] Certain aspects my also utilize recombinant microorganisms engineered to enhance the efficiency of the KDG degradation pathway. For instance, in bacteria, KDG is a common metabolic intermediate in the degradation of hexuronates such as D-glucuronate and D-galacturonate and enters into Entner Doudoroff pathway where it is converted to pyruvate and glyceraldehyde-3-phosphate (G3P). In this pathway, KDG is first phosphorylated by KDG kinase (KdgK) followed by its cleavage into pyruvate and glyceraldehyde-3-phosphate (G3P) using 2-keto-3-deoxy-D-6-posphate-gluconate (KDPG) aldolase (KdgA). The expression of these enzymes concurrently with KDG permease (e.g., KdgT) is negatively regulated by KdgR and is almost none at basal level. The expression is dramatically (3-5-fold) induced upon the addition of hexuronates, and a similar result has been reported in *Pseudomonas* grown on alginate. Hence, to increase the conversion of KDG to pyruvate and G3P, the negative regulator KdgR may be removed. To further improve the pathway efficiency, exogenous copies of KdgK and KdgA may also be incorporated into a given recombinant microorganism.

[0244] In certain aspects, a recombinant microorganism that is able to grow on a polysaccharide (e.g., alginate, pectin, etc) as a sole source of carbon may be capable of producing an increased amount of a given commodity chemical (e.g., ethanol) while growing on that polysaccharide. For example, *E. coli* engineered to grown on alginate may be engineered to produced an increased amount of ethanol from alginate as compared to *E. coli* that is not engineered to grown on alginate (see Example 11). Thus, certain aspects include a recombinant microorganism that is capable of growing on alginate or pectin as a sole source carbon, and that is capable of producing an increased amount of ethanol, such as by comprising one or more genes encoding and expressing a pyruvate decarboxylase (pdc) and/or an alcohol dehydrogenase, including functional variants thereof. In certain aspects, such a recombinant microorganism may comprise a pyruvate decarboxylase (pdc) and two alcohol dehydrogenases (adhA and adhB) obtained from *Zymomonas mobilis*.

[0245] Embodiments of the present invention also include methods for converting polysaccharide to a suitable monosaccharide comprising, (a) obtaining a polysaccharide; (b) contacting the polysaccharide with a chemical catalysis or enzymatic pathway, thereby converting the polysaccharide to a first monosaccharide or oligosaccharide; and (c) contacting the first monosaccharide with a microbial system for a time sufficient to convert the first monosaccharide or oligosaccharide to the suitable monosaccharide, wherein the microbial system comprises, (i) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, and a polysaccharide transporter; and (ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase, thereby converting the polysaccharide to a suitable monosaccharide.

[0246] In certain aspects of the present invention, aquatic or marine-biomass polysaccharides such as alginate may be chemically degraded using chemical catalysts such as acids. Similarly, biomass-derived pectin may be chemically degraded. For instance, the reaction catalyzed by chemical catalysts is typically through hydrolysis, as opposed to the β-elimination type of reactions catalyzed by enzymatic catalysts. Thus, certain embodiments may include boiling alginate or pectin with strong mineral acids to liberate carbon dioxide from D-mannuronate, thereby forming D-lyxose, a common sugar metabolite utilized by many microorganisms. Such embodiments may use, for example, formate, hydrochloric acid, sulfuric acid, in addition to other suitable acids known in the art as chemical catalysts.

[0247] An enzymatic pathway may utilized one or more enzymes described herein that are capable of catalyzing the degradation of polysaccharides, such as alginate or pectin.

[0248] Other embodiments may use variations of chemical catalysis similar to those described herein or known to a person skilled in the art, including improved or redesigned methods of chemical catalysis suitable for use with biomass related polysaccharides. Certain embodiments include those wherein the resulting monosaccharide uronate is D-mannuronate.

[0249] As noted above, the suitable monosaccharides or suitable oligosaccharides produced by the recombinant microorganisms and microbial systems of the present invention may be utilized as a feedstock in the production of commodity chemicals, such as biofuels, as well as commodity chemical intermediates. Thus, certain embodiments of the present invention relate generally to methods for converting a suitable monosaccharide or oligosaccharide to a commodity chemical, such as a biofuel, comprising, (a) obtaining a suitable monosaccharide or oligosaccharide; (b) contacting the suitable monosaccharide or oligosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide to the biofuel, thereby converting the suitable monosaccharide to the biofuel.

[0250] Certain aspects include methods for converting a suitable monosaccharide to a first commodity chemical such as a biofuel, comprising, (a) obtaining a suitable monosaccharide; (b) contacting the suitable monosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide to the first commodity chemical, wherein the microbial system comprises one or more genes

encoding a aldehyde or ketone biosynthesis pathway, thereby converting the suitable monosaccharide to the first commodity chemical.

[0251] In these and other related aspects, depending on the particular ketone or aldehyde biosynthesis pathway employed, the first commodity chemical may be further enzymatically and/or chemically reduced and dehydrated to a second commodity chemical. Examples of such second commodity chemicals include, but are not limited to, butene or butane; 1-phenylbutene or 1-phenylbutane; pentene or pentane; 2-methylpentene or 2-methylpentane; 1-phenylpentene or 1-phenylpentane; 1-phenyl-4-methylpentene or 1-phenyl-4-methylpentane; hexene or hexane; 2-methylhexene or 2-methylhexane; 3-methylhexene or 3-methylhexane; 2,5-dimethylhexene or 2,5-dimethylhexane; 1-phenylhexene or 1-phenylhexane; 1-phenyl-4-methylhexene or 1-phenyl-4-methylhexane; 1-phenyl-5-methylhexene or 1-phenyl-5-methylhexane; heptene or heptane; 2-methylheptene or 2-methylheptane; 3-methylheptene or 3-methylheptane; 2,6-dimethylheptene or 2,6-dimethylheptane; 3,6-dimethylheptene or 3,6-dimethylheptane; 3-methyloctene or 3-methyloctane; 2-methyloctene or 2-methyloctane; 2,6-dimethyloctene or 2,6-dimethyloctane; 2,7-dimethyloctene or 2,7-dimethyloctane; 3,6-dimethyloctene or 3,6-dimethyloctane; and cyclopentane or cyclopentene.

[0252] Certain embodiments of the present invention may also include methods for converting a suitable monosaccharide or oligosaccharide to a commodity chemical comprising (a) obtaining a suitable monosaccharide or oligosaccharide; (b) contacting the suitable monosaccharide or oligosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide or oligosaccharide to the commodity chemical, wherein the microbial system comprises; (i) one or more genes encoding a biosynthesis pathway; (ii) one or more genes encoding and expressing a C—C ligation pathway; and (iii) one or more genes encoding and expressing a reduction and dehydration pathway, comprising a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase, thereby converting the suitable monosaccharide or oligosaccharide to the commodity chemical.

[0253] Certain aspects also include recombinant microorganism that comprise (i) one or more genes encoding a biosynthesis pathway; (ii) one or more genes encoding and expressing a C—C ligation pathway; and (iii) one or more genes encoding and expressing a reduction and dehydration pathway, comprising a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase. Certain aspects also include recombinant microorganisms that comprise the above pathways individually or in certain combinations, such as recombinant microorganism that comprises one or more genes encoding a biosynthesis pathway, as described herein. Certain aspects may also include recombinant microorganisms that comprise one or more genes encoding and expressing a C—C ligation pathway, as described herein. Certain aspects may also include recombinant microorganisms that comprise one or more genes encoding and expressing a reduction and dehydration pathway, comprising a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase, as described herein.

[0254] As for recombinant microorganisms that comprise combinations of the above-noted pathways, certain aspects may include recombinant microorganisms that comprise (i) one or more genes encoding a biosynthesis pathway; and (ii) one or more genes encoding and expressing a C—C ligation pathway. Certain aspects may also include recombinant microorganisms that comprise (i) one or more genes encoding and expressing a C—C ligation pathway; and (ii) one or more genes encoding and expressing a reduction and dehydration pathway, comprising a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase.

[0255] Certain aspects may also include recombinant microorganisms that comprise one or more individual components of a dehydration and reduction pathway, such as a recombinant microorganism that comprises a diol dehydrogenase, a diol dehydratase, or a secondary alcohol dehydrogenase. These and other microorganisms may be utilized, for example, to convert a suitable polysaccharide to a first commodity chemical, or an intermediate thereof, or to convert a first commodity chemical, or an intermediate thereof, to a second commodity chemical.

[0256] Merely by way of illustration, a recombinant microorganism comprising a C—C ligation pathway may be utilized to convert butanal into a first commodity chemical, or an intermediate thereof, such as 5-hydroxy-4-octanone, which can then be converted into a second commodity chemical, or intermediate thereof, by any suitable pathway. As a further example, a recombinant microorganism comprising a C—C ligation pathway and a diol hydrogenase may be utilized for the sequential conversion of butanal into 5-hydroxy-4-octanone and then 4,5-octanonediol. Examples of recombinant microorganisms that comprise these and other various combinations of the individual pathways described herein, as well as various combinations of the individual components of those pathways, will be apparent to those skilled in the art, and may also be found in the Examples.

[0257] Also included are methods of converting a polysaccharide to a first commodity chemical, or an intermediate thereof, such as by utilizing a recombinant microorganism that comprises an aldehyde or ketone biosynthesis pathway. Also included are methods of converting a first commodity chemical, or intermediate thereof, to a second commodity chemical, such as by utilizing a recombinant microorganism that optionally comprises a biosynthesis pathway, optionally comprises C—C ligation pathway and/or optionally comprises one or more of the individual components of a dehydration and reduction pathway. Merely by way of illustration, a recombinant microorganism comprising an exogenous C—C ligase (e.g., benzaldehyde lyase from *Pseudomonas fluorescens*) could be utilized in a method to convert a first commodity chemical such as 3-methylbutanal to a second commodity chemical such as 2,7-dimethyl-5-hydroxy-4-octanone. Along this line of illustration, the same or different recombinant microorganism comprising a diol dehydrogenase could be utilized in a method to convert 2,7-dimethyl-5-hydroxy-4-octanone to another commodity chemical such as 2,7-dimethyl-4,5-octanediol (see Table 2 for other examples). As an additional illustrative example, a recombinant microorganism comprising an exogenous secondary alcohol dehydrogenase could be utilized in a method to convert a first commodity chemical such as 2,7-dimethyl-4-octanone to a second commodity chemical such as 2,7-dimethyloctanol.

[0258] Embodiments of a microbial system or isolated microorganism of the present application may include a naturally-occurring biosynthesis pathway, and/or an engineered, reconstructed, or re-designed biosynthesis pathway that has been optimized for improved functionality.

[0259] Embodiments of a microbial system or recombinant microorganism of the present invention may include a natural or reconstructed biosynthesis pathway, such as a butyraldehyde biosynthesis pathway, as found in such microorganisms as *Clostridium acetobutylicum* and *Streptomyces coelicolor*. In explanation, butyrate and butanol are the common fermentation products of certain bacterial species such as *Clostridia*, in which the production of butyrate and butanol is mediated by a synthetic thiolase dependent pathway characteristically similar to fatty acid degradation pathway. Such pathways may be initiated with the condensation of two molecules of acetyl-CoA to acetoacetyl-CoA, which is catalyzed by thiolase. Acetoacetyl-CoA is then reduced to β-hydroxy butyryl-CoA, which is catalyzed by NAD(P)H dependent β-hydroxy butyryl-CoA dehydrogenase (HBDH). Crotonase catalyzes dehydration from β-hydroxy butyryl-CoA to form crotonyl-CoA. Further reduction catalyzed by NADH-dependent butyryl-CoA dehydrogenase (BCDH) saturates the double bond at C2 of crotonyl-CoA to form butyryl-CoA.

[0260] In certain embodiments, thiolase, the first enzyme in this pathway, may be overexpressed to maximize production. In certain embodiments, thiolase may over-expressed in *E. coli*. In this regard, all three enzymes (e.g., HBDH, crotonase, and BCDH) catalyzing the following reaction steps are found in *Clostridium acetobutylicum* ATCC824. In certain embodiments, BDH, crotonase, and BCDH may be expressed or over-expressed in a suitable microorganism such as *E. coli*. Alternatively, a short-chain aliphatic acyl-CoA dehydrogenase derived from *Pseudomonas putida* KT2440 may be utilized in other embodiments of a microbial system or isolated microorganism of the present application.

[0261] Further to this end, butyryl-CoA in *Clostridia* may be readily converted to butanol and/or butyrate by at least a few different pathways. In one pathway, butyryl-CoA is directly reduced to butyraldehyde catalyzed by NADH dependent CoA-acylating aldehyde dehydrogenase (ALDH). Butyraldehyde may be further reduced to butanol by NADH-dependent butanol dehydrogenase. Although CoA-acylating ALDH catalyzes the one step reduction of butyryl-CoA to butyraldehyde, the incorporation of CoA-acylating ALDH to the microbial system may result in acetoaldehyde formation because of its promiscuous acetyl-CoA deacylating activity. In certain embodiments, the formation of acetoaldehyde may be minimized by functionally redesigning the relevant enzyme(s).

[0262] Butyryl-CoA in other biosynthesis pathways is deacylated to form butyryl phosphate catalyzed by phosphotransbutyrylase. Butyryl phosphate is then hydrolyzed by reversible butyryl phosphate kinase to form butyrate. This reaction is coupled with ATP generation from ADP. The butyrate formation through these enzymes is known to be significantly more specific. Certain embodiments may comprise phosphotransbutyrylase and butyryl phosphate kinase to the microbial system. In other embodiments, butyrate may be directly formed from butyryl-CoA by short chain acyl-CoA thioesterase.

[0263] Butyrate in *Clostridia* may also be sequentially reduced to butanol, which is catalyzed by a single alcohol/aldehyde dehydrogenase. Certain embodiments may comprise short chain aldehyde dehydrogenase from other bacteria such as *Pseudomonas putida* to complement the production of butyraldehyde in the microbial system. One potential concern in using short chain aldehyde dehydrogenase involves the possible formation of acetoaldehyde from acetate. Certain embodiments may be directed to minimizing the acetate formation in the microbial system, for example, by deleting several genes encoding enzymes involved in the acetate production.

[0264] Moreover, there are multiple routes in *E. coli* to form acetate, one of which is mediated by pyruvate oxygenase (POXB) from pyruvate, whereas another is mediated by phosphotransacetylase (PTA) and acetyl phosphate kinase (ACKA) from acetyl-CoA. The acetate production from *E. coli* mutant strains with $poxB^-$, pta^-, and $acka^-$ are significantly diminished. In addition, incorporation of acetyl-CoA synthase (ACS) which catalyses the acetyl-CoA formation from acetate is also known to significantly reduce the accumulation of acetate. Certain embodiments may comprise a microbial system or isolated microorganism with deleted POXB, PTA, and/or ACKA genes, and other embodiments may also comprise, separately or together with the deleted genes, one or more genes encoding and expressing ACS.

[0265] A microbial system or recombinant microorganism provided herein may also comprise a glutaraldehyde biosynthesis pathway. As one example, *Saccharomyces cerevisiae* has a lysine biosynthetic pathway in which acetyl-CoA is initially condensed to α-ketoglutarate, a common metabolite in citric acid cycle, to form homocitorate. This reaction is catalyzed by homocitrate synthase derived from Yeast, *Thermus thermophilus*, or *Deinococcus radiodurans*. Homoaconitase derived from Yeast, *Thermus thermophilus*, or *Deinococcus radiodurans* catalyzes the conversion between homocitrate and homoisocitrate. Homoisocitrate is then oxidatively decarboxylated to form 2-ketoadipate, which is catalyzed by homoisocitrate dehydrogenase derived from Yeast, *Thermus thermophilus*, or *Deinococcus radiodurans*. Homoisocitrate is also oxidatively decarboxylated to form glutaryl-CoA, which may be catalyzed by homoisocitrate dehydrogenase. Thus, certain embodiments may comprise a homocitrate synthase, a homoaconitase, and/or a homoisocitrate dehydrogenase.

[0266] Further to this end, in synthesizing 2-keto-adipic-semialdehyde, 2-ketoadipate is reduced to 2-keto-adipic-semialdehyde. This reaction can be catalyzed by dialdehyde dehydrogenase, which, for example, may be isolated from *Agrobacterium tumefaciens* C58. Thus, certain embodiments may incorporate dialdehyde dehydrogenases into a microbial system or recombinant microorganism.

[0267] In synthesizing glutaraldehyde, Acyl-CoA thioesterases (ACOT) may also catalyze the hydrolysis of glutaryl-CoA. The genes encoding (β-carboxylic acyl-CoA specific peroxisomal ACOTs are found in many mammalian species; both ACOT4 and ACOT8 derived from mice have been previously expressed in *E. coli* and shown that both enzymes are highly active on the hydrolysis of glutaryl-CoA to form glutarate. Certain embodiments may comprise one or more Acyl-CoA thioesterases.

[0268] Glutarate is sequentially reduced to glutaraldehyde. This reaction can be catalyzed by glutaraldehyde dehydrogenase (CpnE), which, for example, may be isolated from *Comomonas* sp. Strain NCIMB 9872. Certain embodiments may incorporate glutaraldehyde dehydrogenases such as CpnE into a microbial system or isolated microorganism. Other embodiments may comprise both ACOT and CpnE enzymes. Other embodiments may comprise CpnE enzymes redesigned to catalyze the reduction of 1-hydroxy propanoate and succinate to 1-hydroxy propanal and succinicaldehyde.

[0269] In certain aspects, the biosynthesis pathway may include an aldehyde biosynthesis pathway, a ketone biosynthesis pathway, or both. In certain aspects, the biosynthesis pathway may be include one or more of an acetoaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, 4-methylpentaldehyde, phenylacetoaldehyde, 2-phenyl acetoaldehyde, 2-(4-hydroxyphenyl)acetaldehyde, 2-Indole-3-acetoaldehyde, glutaraldehyde, 5-amino-pentaldehyde, succinate semialdehyde, and/or succinate 4-hydroxyphenyl acetaldehyde biosynthesis pathway, including various combinations thereof.

[0270] With regard to combinations of biosynthesis pathways, a biosynthesis pathway may comprise an acetoaldehyde biosynthesis pathway in combination with at least one of a propionaldehyde, butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, or phenylacetoaldehyde biosynthesis pathway. In certain aspects, a biosynthesis pathway may comprise a propionaldehyde biosynthesis pathway in combination with at least one of a butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, or phenylacetoaldehyde biosynthesis pathway. In certain aspects, a biosynthesis pathway may comprise a butyraldehyde biosynthesis pathway in combination with at least one of an isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, or phenylacetoaldehyde biosynthesis pathway. In certain aspects, a biosynthesis pathway may comprise an isobutyraldehyde biosynthesis pathway in combination with at least one of a 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, or phenylacetoaldehyde biosynthesis pathway. In certain aspects, a biosynthesis pathway may comprise a 2-methyl-butyraldehyde biosynthesis pathway in combination with at least one of a 3-methyl-butyraldehyde or a phenylacetoaldehyde biosynthesis pathway. In certain aspects, a biosynthesis pathway may comprise a 3-methyl-butyraldehyde biosynthesis pathway in combination with a phenylacetoaldehyde biosynthesis pathway.

[0271] In certain aspects, a propionaldehyde biosynthesis pathway may comprise a threonine deaminase (ilvA) gene from an organism such as *Escherichia coli* and a keto-isovalerate decarboxylase (kivd) gene from an organism such as *Lactococcus lactis*, and/or functional variants of these enzymes, including homologs or orthologs thereof, as well as optimized variants. These enzymes may be utilized generally to convert L-threonine to propionaldehyde.

[0272] In certain aspects, a butyraldehyde biosyntheis pathway may comprise at least one of a thiolase (atoB) gene from an organism such as *E. coli*, a β-hydroxy butyryl-CoA dehydrogenase (hbd) gene, a crotonase (crt) gene, a butyryl-CoA dehydrogenase (bcd) gene, an electron transfer flavoprotein A (etfA) gene, and/or an electron transfer flavoprotein B (etfB) gene from an organism such as *Clostridium acetobutyricum* (e.g., ATCC 824), as well as a coenzyme A-linked butyraldehyde dehydrogenase (ald) gene from an organism such as *Clostridium beijerinckii acetobutyricum* ATCC 824. In certain aspects, a coenzyme A-linked alcohol dehydrogenase (adhE2) gene from an organism such as *Clostridium acetobutyricum* ATCC 824 may be used as an alternative to an ald gene.

[0273] In certain aspects, an isobutyraldehyde biosynthetic pathway may comprise an acetolactate synthase (alsS) from an organism such as *Bacillus subtilis* or an als gene from an organism such as *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (codon usage may be optimized for *E. coli* protein expression). Such a pathway may also comprise acetolactate reductoisomerase (ilvC) and/or 2,3-dihydroxy-isovalerate dehydratase (ilvD) genes from an organism such as *E. coli*, as well as a keto-isovalerate decarboxylase (kivd) gene from an organism such as *Lactococcus* lactis.

[0274] In certain aspects, a 3-methylbutyraldehyde and 2-methylbutyraldehyde biosynthesis pathway may comprise an acetolactate synthase (alsS) gene from an organism such as *Bacillus subtilis* or an (als) gene from an organism such as *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (codon usage may be optimized for *E. coli* protein expression). Certain aspects of such a pathway may also comprise acetolactate reductoisomerase (ilvC), 2,3-dihydroxyisovalerate dehydratase (ilvD), isopropylmalate synthase (LeuA), isopropylmalate isomerase (LeuC and LeuD), and 3-isopropylmalate dehydrogenase (LeuB) genes from an organism such as *E. coli*, as well as a keto-isovalerate decarboxylase (kivd) from an organism such as *Lactococcus lactis.*

[0275] In certain aspects, a phenylacetoaldehyde and 4-hydroxyphenylacetoaldehyde biosynthesis pathway may comprise one or more of 3-deoxy-7-phosphoheptulonate synthase (aroF, aroG, and aroH), 3-dehydroquinate synthase (aroB), a 3-dehydroquinate dehydratase (aroD), dehydroshikimate reductase (aroE), shikimate kinase II (aroL), shikimate kinase I (aroK), 5-enolpyruvylshikimate-3-phosphate synthetase (aroA), chorismate synthase (aroC), fused chorismate mutase P/prephenate dehydratase (pheA), and/or fused chorismate mutase T/prephenate dehydrogenase (tyrA) genes from an organism such as *E. coli*, as well as a keto-isovalerate decarboxylase (kivd) from an organism such as *Lactococcus lactis.*

[0276] In certain aspects, such as for the ultimate production of 1,10-diamino-5-decanol and 1,10-dicarboxylic-5-decanol, a biosynthesis pathway may comprise one or more homocitrate synthase, homoaconitate hydratase, homoisocitrate dehydrogenase, and/or homoisocitrate dehydrogenase genes from an organism such as *Deinococcus radiodurans* and/or *Thermus thermophilus*, as well as a keto-adipate decarboxylase gene, a 2-aminoadipate transaminase gene, and a L-2-Aminoadipate-6-semialdehyde: NAD+6-oxidoreductase gene. Such a biosynthesis pathway would be able to convert α-ketoglutarate to 5-aminopentaldehyde.

[0277] In certain aspects, such as for one step in cyclopentanol production, a α-ketoadipate semialdehyde biosynthesis pathway may comprise homocitrate synthase (hcs), homoaconitate hydratase, and homoisocitrate dehydrogenase genes from an organism such as *Deinococcus radiodurans* and/or *Thermus thermophilus*, and an α-ketoadipate semialdehyde dehydrogenase gene. Such a biosynthesis pathway would be able to convert acetyl-CoA and α-ketoglutarate to α-ketoadipate semialdehyde.

[0278] For the production of certain commodity chemicals, such as 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and indole-3-ethanol, among other similar chemicals, a biosynthesis pathway (e.g., aldehyde biosynthesis pathway) may optionally or further comprise one or more genes encoding a carboxylase enzyme, such as an indole-3-pyruvate decarboxylase (IPDC). An IPDC may be obtained, for example, from such microorganisms as *Azospirillum brasilense* and *Paenibacillus polymyxa* E681. In this regard, an IPDC may be utilized to more efficiently catalyze the dexarboxylation of various carboxylic acids to form the corresponding aldehyde, which can be further converted to a commodity chemical by a reductase or dehydrogenase, as detailed herein.

[0279] In certain aspects, a 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and 2-(indole-3-)ethanol biosynthesis pathway may comprise a transketolase (tktA), a 3-deoxy-7-phosphoheptulonate synthase (aroF, aroG, and aroH), 3-dehydroquinate synthase (aroB), a 3-dehydroquinate dehydratase (aroD), a dehydroshikimate reductase (aroE), a shikimate kinase II (aroL), a shikimate kinase I (aroK), a 5-enolpyruvylshikimate-3-phosphate synthetase (aroA), a chorismate synthase (aroC, a fused chorismate mutase P/prephenate dehydratase (pheA), and a fused chorismate mutase T/prephenate dehydrogenase (tyrA) genes from *E. coli*, keto-isovalerate decarboxylase (kivd) from *Lactococcus* lactis, alcohol dehydrogenase (adh2) from *Saccharomyces cerevisiae*, Indole-3-pyruvate decarboxylase (ipdc) from *Azospirillum brasilense*, phenylethanol reductase (par) from *Rhodococcus* sp. ST-10, and abenzaldehyde lyase (bal) from *Pseudomonas fluorescence.*

[0280] As for all other pathways described herein, the components for each of the biosynthesis pathways described herein may be present in a recombinant microorganism either endogenously or exogenously. To improve the efficiency of a given biosynthesis pathway, endogenous genes, for example, may be up-regulated or over-expressed, such as by introducing an additional (i.e., exogenous) copy of that endogenous gene into the recombinant microorganism. Such pathways may also be optimized by altering via mutagenesis the endogenous version of a gene to improve functionality, followed by introduction of the altered gene into the microorganism. The expression of endogenous genes may be up or down-regulated, or even eliminated, according to known techniques in the art and described herein. Similarly, the expression levels of exogenously provided genes may be regulated as desired, such as by using various constitutive or inducible promoters. Such genes may also be "codon-optimized," as described herein and known in the art. Also included are functional naturally-occurring variants of the genes and enzymes described herein, including homologs or orthologs thereof.

[0281] Certain embodiments of a microbial system or isolated microorganism may comprise a CC-ligation pathway. In certain aspects, a CC-ligation pathway may comprise a ThDP-dependent enzyme, such as a C—C ligase, or an optimized C—C ligase. For example, eight-carbon unit molecules (butyroins) may be made from condensing together two four-carbon unit molecules (butyraldehydes). ThDP-dependent enzymes are a group of enzymes known to catalyze both breaking and formation of C—C bonds and have been utilized as catalysts in chemoenzymatic syntheses. The spectrum of chemical reactions that these enzymes catalyze ranges from decarboxylation of α-keto acids, oxidative decarboxylation, carboligation, and to the cleavage of C—C bonds.

[0282] To provide a few examples, benzaldehyde lyase (BAL) from *Pseudomonas fluorescens*, benzoylformate decarboxylase (BFD) from *Pseudomonas putida*, and pyruvate decarboxylase (PDC) from *Zymomonas mobilis* may catalyze a carboligation reaction between two aldehydes. BAL accepts the broadest spectrum of aldehydes as substrates among these three enzymes ranging from substituted benzaldehyde to acetoaldehyde, among others, as shown herein. BAL catalyzes stereospecific carboligation reaction between two aldehydes and forms α-hydroxy ketone swith over 99% ee for R-configuration. The benzoin formation from two benzaldehyde molecules is a favored reaction catalyzed by BAL and proceeds as fast as 320 μmol (benzoin) mg (protein)$^{-1}$ min^{-1}. The formation of α-hydroxy ketone may be carried out using many different aldehydes, including butyraldehyde.

[0283] BFD and PCD may also catalyze the carboligation reactions between two aldehyde molecules. BFD and PCD accept relatively larger and smaller aldehyde molecules, respectively. With the presence of benzaldehyde and acetoaldehyde, BFD catalyzes the formation of benzoin and (S)-α-hydroxy phenylpropanone (2S-HPP), whereas PCD catalyzes the formation of (R)-α-hydroxy phenylpropanone (2R-HPP) and (R)-α-hydroxy 2-butanone (acetoin). As detailed below, certain microbial systems or isolated microorganisms of the present application may comprise natural or optimized C—C ligases (ThDP-dependent enzymes) selected from benzaldehyde lyase (BAL) from *Pseudomonas fluorescens* benzoylformate decarboxylase (BFD) from *Pseudomonas putida*, and pyruvate decarboxylase (PDC) from *Zymomonas mobilis*. Other embodiments may comprise a benzaldehyde lyase (BAL) from *Pseudomonas fluorescens* (see SEQ ID NOS:143-144, showing the nucleotide and polypeptide sequences, respectively) including biologically active variants thereof, such as optimized variants.

[0284] A C—C ligation pathway of the present invention typically comprises one or more C—C ligases, such as a lyase enzyme. Exemplary lyases include, but are not limited to, acetoaldehyde lyases, propionaldehyde lyases, butyraldehyde lyases, isobutyraldehyde lyases, 2-methyl-butyraldehyde lyases, 3-methyl-butyraldehyde lyases (isoveraldehyde), phenylacetaldehyde lyases, α-keto adipate carboxylyases, pentaldehyde lyases, 4-methyl-pentaldehyde lyases, hexyldehyde lyases, heptaldehyde lyases, octaldehyde lyases, 4-hydroxyphenylacetaldehyde lyases, indoleacetaldehyde lyases, indolephenylacetaldehyde lyases. In certain aspects, a selected CC-ligase or lyase enzyme may have one or more of the above exemplified lyase activities, such as acetoaldehyde lyase activity, a propionaldehyde lyase activity, a butyraldehyde lyase activity, and/or an isobutyraldehyde lyase activity, among others.

[0285] As noted above, a C—C ligase may comprise a benzaldehyde lyase, such as a benzaldehyde lyase isolated from *Pseudomonas fluorescens* (SEQ ID NOS:143-144), as well as biologically active fragments or variants of this reference sequence, such as optimized variants of a benzaldehyde lyase. In this regard, certain aspects may comprise nucleotide sequences or polypeptide sequences having 80%, 85%, 90%, 95%, 97%, 98%, 99% sequence identity to SEQ ID NOS:143-144, and which are capable of catalyzing a carboligation reaction, or which possess C—C lyase activity, as described herein. In certain aspects, a BAL enzyme will comprise one or more conserved amino acid residues, including G27, E50, A57, G155, P162, P234, D271, G277, G422, G447, D448, and/or G512.

[0286] *Pseudomonas fluorescens* is able to grow on R-benzoin as the sole carbon and energy source because it harbours the enzyme benzaldehyde lyase that cleaves the acyloin linkage using thiamine diphosphate (ThDP) as a cofactor. In the reverse reaction, as utilized herein, benzaldehyde lyase catalyses the carboligation of two aldehydes with high substrate and stereospecificity. Structure-based comparisons with other proteins show that benzaldehyde lyase belongs to a group of closely related ThDP-dependent enzymes. The ThDP cofactors of these enzymes are fixed at their two ends in separate domains, suspending a comparatively mobile thiazolium ring between them. While the residues binding the two ends of ThDP are well conserved, the lining of the active

centre pocket around the thiazolium moiety varies greatly within the group. The active sites for BAL have been described, for example, in Kneen et al (*Biochimica et Biophysica Acta* 1753:263-271, 2005) and Brandt et al. (*Biochemistry* 47:7734-43, 2008). Benzaldehyde lyase derived from *Pseudomonas fluorescens* has been demonstrated herein to at least have an acetoaldehyde lyase activity, a propionaldehyde lyase activity, a butyraldehyde lyase activity, a 3-methyl-butyraldehyde lyase activity, a pentaldehyde lyase activity, a 4-methylpentaldehyde lyase activity, a hexyldehyde lyase activity, a phenylacetoaldehyde lyase activity, and an octaldehyde lyase activity (see Table 2), among other in vivo lyase activities (see FIGS. **48-55**).

[0287] In certain aspects, a C—C ligase, such as BAL derived from *Pseudomonas fluorescens*, BFD derived from *Pseudomonas putida*, or PDC derived from *Zymomonas* mobilis may comprise a lyase with a combination of lyase activities, such as a lyase having both a propionaldehyde lyase activity and a 3-methyl-butyraldehyde lyase activity, among other combinations and activities, such as those exemplary combinations detailed herein. Merely by way of illustration, a lyase having a combination of lyase activities may be referred to herein as a propionaldehyde/3-methyl-butyraldehyde lyase.

[0288] A dehydration and reduction pathway, comprising a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase, may be utilized to further convert an aldehyde, ketone, or corresponding alcohol, to a commodity chemical, such as a biofuel.

[0289] To this end, a dehydration and reduction pathway may comprise one or more diol dehydrogenases. A "diol dehydrogenase" refers generally to an enzyme that catalyzes the reversible reduction and oxidation of a α-hydroxy ketone and/or its corresponding diol. Certain embodiments of a microbial system or isolated microorganism may comprise genes encoding a diol dehydrogenase that specifically catalyzes the reduction of α-hydroxy-ketones, including, for example, a 4, 5, octanediol dehydrogenase. Diol dehydrogenases, such as 4, 5, octanediol dehydrogenase, may be isolated from a variety of organisms and incorporated into a microbial system or isolated microorganism. A particular group of alcohol dehydrogenases has a characteristic ability to oxidize various α-hydroxy alcohols and reduce various α-hydroxy ketones and α-keto ketones. As such, the recitation "diol dehydrogenase" may also encompass such alcohol dehydrogenases.

[0290] By way of example regarding diol dehydrogenases from exemplary organisms, glycerol dehydrogenase isolated from *Hansenula ofunaensis* has broad substrate specificity and is capable of catalyzing the oxidation of various α-hydroxy alcohols, including 1,2-octane, as well as the reduction of various α-hydroxy ketones and α-keto ketones, including 3-hydroxy-2-butanone and 3,4-hexanedione, with the activity comparable to its native substrates, glycerol and dihydroxyaceton, respectively (40-200%). As one further example, glycerol dehydrogenase discovered in *Hansenula polumorpha* DI-1 works similarly. In certain embodiments, a microbial system or recombinant microorganism may comprise a glycerol dehydrogenase gene isolated from *Hansenula ofunaensis*, a glycerol dehydrogenase isolated from *Hansenula polumorpha* DI-1 and/or a meso-2,3-butane diol dehydrogenase from *Klebsiella pneumoniae*. In other embodiments, a microbial system or isolated microorganism may comprise a 4, 5, octanediol dehydrogenase, among others detailed herein. Diol dehyodregnases may also be obtained from *Lactobaccilus brevis* ATCC 367, *Pseudomanas putida* KT2440, and *Klebsiella pneumoniae* MGH78578), as described herein (see Example 5).

[0291] Exemplary diol dehydrogenases include, but are not limited to, 2,3-butanediol dehydrogenase, 3,4-hexanediol dehydrogenase, 4,5-octanediol dehydrogenase, 5,6-decanediol dehydrogenase, 6,7-dodecanediol dehydrogenase, 7,8-tetradecanediol dehydrogenase, 8,9-hexadecanediol dehydrogenase, 2,5-dimethyl-3,4-hexanediol dehydrogenase, 3,6-dimethyl-4,5-octanediol dehydrogenase, 2,7-dimethyl-4,5-octanediol dehydrogenase, 2,9-dimethyl-5,6-decanediol dehydrogenase, 1,4-diphenyl-2,3-butanediol dehydrogenase, bis-1,4-(4-hydroxyphenyl)-2,3-butanediol dehydrogenase, 1,4-diindole-2,3-butanediol dehydrogenase, 1,2-cyclopentanediol dehydrogenase, 2,3-pentanediol dehydrogenase, 2,3-hexanediol dehydrogenase, 2,3-heptanediol dehydrogenase, 2,3-octanediol dehydrogenase, 2,3-nonanediol dehydrogenase, 4-methyl-2,3-pentanediol dehydrogenase, 4-methyl-2,3-hexanediol dehydrogenase, 5-methyl-2,3-hexanediol dehydrogenase, 6-methyl-2,3-heptanediol dehydrogenase, 1-phenyl-2,3-butanediol dehydrogenase, 1-(4-hydroxyphenyl)-2,3-butanediol dehydrogenase, 1-indole-2,3-butanediol dehydrogenase, 3,4-heptanediol dehydrogenase, 3,4-octanediol dehydrogenase, 3,4-nonanediol dehydrogenase, 3,4-decanediol dehydrogenase, 3,4-undecanediol dehydrogenase, 2-methyl-3,4-hexanediol dehydrogenase, 5-methyl-3,4-heptanediol dehydrogenase, 6-methyl-3,4-heptanediol dehydrogenase, 7-methyl-3,4-octanediol dehydrogenase, 1-phenyl-2,3-pentanediol dehydrogenase, 1-(4-hydroxyphenyl)-2,3-pentanediol dehydrogenase, 1-indole-2,3-pentanediol dehydrogenase, 4,5-nonanediol dehydrogenase, 4,5-decanediol dehydrogenase, 4,5-undecanediol dehydrogenase, 4,5-dodecanediol dehydrogenase, 2-methyl-3,4-heptanediol dehydrogenase, 3-methyl-4,5-octanediol dehydrogenase, 2-methyl-4,5-octanediol dehydrogenase, 8-methyl-4,5-nonanediol dehydrogenase, 1-phenyl-2,3-hexanediol dehydrogenase, 1-(4-hydroxyphenyl)-2,3-hexanediol dehydrogenase, 1-indole-2,3-hexanediol dehydrogenase, 5,6-undecanediol dehydrogenase, 5,6-undecanediol dehydrogenase, 5,6-tridecanediol dehydrogenase, 2-methyl-3,4-octanediol dehydrogenase, 3-methyl-4,5-nonanediol dehydrogenase, 2-methyl-4,5-nonanediol dehydrogenase, 2-methyl-5,6-decanediol dehydrogenase, 1-phenyl-2,3-heptanediol dehydrogenase, 1-(4-hydroxyphenyl)-2,3-heptanediol dehydrogenase, 1-indole-2,3-heptanediol dehydrogenase, 6,7-tridecanediol dehydrogenase, 6,7-tetradecanediol dehydrogenase, 2-methyl-3,4-nonanediol dehydrogenase, 3-methyl-4,5-decanediol dehydrogenase, 2-methyl-4,5-decanediol dehydrogenase, 2-methyl-5,6-undecanediol dehydrogenase, 1-phenyl-2,3-octanediol dehydrogenase, 1-(4-hydroxyphenyl)-2,3-octanediol dehydrogenase, 1-indole-2,3-octanediol dehydrogenase, 7,8-pentadecanediol dehydrogenase, 2-methyl-3,4-decanediol dehydrogenase, 3-methyl-4,5-undecanediol dehydrogenase, 2-methyl-4,5-undecanediol dehydrogenase, 2-methyl-5,6-dodecanediol dehydrogenase, 1-phenyl-2,3-nonanediol dehydrogenase, 1-(4-hydroxyphenyl)-2,3-nonanediol dehydrogenase, 1-indole-2,3-nonanediol dehydrogenase, 2-methyl-3,4-undecanediol dehydrogenase, 3-methyl-4,5-dodecanediol dehydrogenase, 2-methyl-4,5-dodecanediol dehydrogenase, 2-methyl-5,6-tridecanediol dehydrogenase, 1-phenyl-2,3-decanediol dehydrogenase, 1-(4-hydroxyphenyl)-2,3-decanediol dehydroge-

nase, 1-indole-2,3-decanediol dehydrogenase, 2,5-dimethyl-3,4-heptanediol dehydrogenase, 2,6-dimethyl-3,4-heptanediol dehydrogenase, 2,7-dimethyl-3,4-octanediol dehydrogenase, 1-phenyl-4-methyl-2,3-pentanediol dehydrogenase, 1-(4-hydroxyphenyl)-4-methyl-2,3-pentanediol dehydrogenase, 1-indole-4-methyl-2,3-pentanediol dehydrogenase, 2,6-dimethyl-4,5-octanediol dehydrogenase, 3,8-dimethyl-4,5-nonanediol dehydrogenase, 1-phenyl-4-methyl-2,3-hexanediol dehydrogenase, 1-(4-hydroxyphenyl)-4-methyl-2,3-hexanediol dehydrogenase, 1-indole-4-methyl-2,3-hexanediol dehydrogenase, 2,8-dimethyl-4,5-nonanediol dehydrogenase, 1-phenyl-5-methyl-2,3-hexanediol dehydrogenase, 1-(4-hydroxyphenyl)-5-methyl-2,3-hexanediol dehydrogenase, 1-indole-5-methyl-2,3-hexanediol dehydrogenase, 1-phenyl-6-methyl-2,3-heptanediol dehydrogenase, 1-(4-hydroxyphenyl)-6-methyl-2,3-heptanediol dehydrogenase, 1-indole-6-methyl-2,3-heptanediol dehydrogenase, 1-(4-hydroxyphenyl)-4-phenyl-2,3-butanediol dehydrogenase, 1-indole-4-phenyl-2,3-butanediol dehydrogenase, 1-indole-4-(4-hydroxyphenyl)-2,3-butanediol dehydrogenase, 1,10-diamino-5,6-decanediol dehydrogenase, 1,4-di(4-hydroxyphenyl)-2,3-butanediol, 2,3-hexanediol-1,6-dicarboxylic acid dehydrogenase, and the like.

[0292] In certain aspects, a selected diol dehydrogenase enzyme may have one or more of the above exemplified diol dehydrogenase activities, such as a 2,3-butanediol dehydrogenase activity, a 3,4-hexanediol dehydrogenase activity, and/or a 4,5-octanediol dehydrogenase activity, among others.

[0293] In certain aspects, a recombinant microorganism may comprise a diol dehydrogenase encoded by a nucleotide reference sequence selected from SEQ ID NO:97, 99, and 101, or an enzyme having a polyeptide sequence selected from SEQ ID NO:98, 100, and 102, including biologically active fragments or variants thereof, such as optimized variants. Certain aspects may also comprises nucleotide sequences or polypeptide sequences having 80%, 85%, 90%, 95%, 97%, 98%, 99% sequence identity to SEQ ID NOS:97-102.

[0294] Other embodiments may comprise re-designed diol dehydrogenases for reduction of 1-hydroxy propanal, succinicaldehyde, and glutaraldehyde to 1,3-propanediol, 1,4-butanediol, and 1,5 pentanediol, respectively, among others.

[0295] A dehydration and reduction pathway, as described herein, may comprise one or more diol dehydratases. A "diol dehydratase" refers generally to an enzyme that catalyzes the irreversible dehydration of diols. For instance, this enzyme may serve to dehydrate octanediol to form 4-octane. It has been recognized that there are at least two different types of diol dehydratases: a group dependent on and independent of coenzyme B12 for its catalysis. Coenzyme B12 dependent diol dehydratases are known to catalyze a radical mediated dehydration reaction from α-hydroxy alcohol to aldehydes or ketones. For example, a diol dehydratase from *Klebsiella pneumoniae* catalyzes the dehydration of glycerol to form β-hydroxypropyl aldehyde, accepts 2,3-butanediol as a substrate, and catalyzes the dehydration reaction to form 2-butanone.

[0296] As a further example, *Clostridium butylicum* contains coenzyme B12 independent diol dehydratases. FIG. **46** shows the in vivo biological activity of coenzyme B12 independent diol dehydratase (dhaB1) and activator (dhaB2) isolated from *Clostridium butylicum* (see Example 9). 46A shows the in vivo production of 1-propanol from 1,2-propanediol, FIG. **46**B shows the in vivo production of 2-butanol from meso-2,3 butanediol, and FIG. **46**C shows the in vivo production of cyclopentanone from trans-1,2-cyclopentanediol.

[0297] Thus, certain embodiments of the present invention may comprise optimized or redesigned diol dehydratases that accommodate various substrates, such as 4,5-octanediol as a substrate, and may include diol dehydratases isolated and/or optimized from *Klebsiella pneumoniae* and *Clostridium* butylicum, among other organisms described herein and known in the art.

[0298] Exemplary diol dehydratases include, but are not limited to, 2,3-butanediol dehydratase, 3,4-hexanediol dehydratase, 4,5-octanediol dehydratase, 5,6-decanediol dehydratase, 6,7-dodecanediol dehydratase, 7,8-tetradecanediol dehydratase, 8,9-hexadecanediol dehydratase, 2,5-dimethyl-3,4-hexanediol dehydratase, 3,6-dimethyl-4,5-octanediol dehydratase, 2,7-dimethyl-4,5-octanediol dehydratase, 2,9-dimethyl-5,6-decanediol dehydratase, 1,4-diphenyl-2,3-butanediol dehydratase, bis-1,4-(4-hydroxyphenyl)-2,3-butanediol dehydratase, 1,4-diindole-2,3-butanediol dehydratase, 1,2-cyclopentanediol dehydratase, 2,3-pentanediol dehydratase, 2,3-hexanediol dehydratase, 2,3-heptanediol dehydratase, 2,3-octanediol dehydratase, 2,3-nonanediol dehydratase, 4-methyl-2,3-pentanediol dehydratase, 4-methyl-2,3-hexanediol dehydratase, 5-methyl-2,3-hexanediol dehydratase, 6-methyl-2,3-heptanediol dehydratase, 1-phenyl-2,3-butanediol dehydratase, 1-(4-hydroxyphenyl)-2,3-butanediol dehydratase, 1-indole-2,3-butanediol dehydratase, 3,4-heptanediol dehydratase, 3,4-octanediol dehydratase, 3,4-nonanediol dehydratase, 3,4-decanediol dehydratase, 3,4-undecanediol dehydratase, 2-methyl-3,4-hexanediol dehydratase, 5-methyl-3,4-heptanediol dehydratase, 6-methyl-3,4-heptanediol dehydratase, 7-methyl-3,4-octanediol dehydratase, 1-phenyl-2,3-pentanediol dehydratase, 1-(4-hydroxyphenyl)-2,3-pentanediol dehydratase, 1-indole-2,3-pentanediol dehydratase, 4,5-nonanediol dehydratase, 4,5-decanediol dehydratase, 4,5-undecanediol dehydratase, 4,5-dodecanediol dehydratase, 2-methyl-3,4-heptanediol dehydratase, 3-methyl-4,5-octanediol dehydratase, 2-methyl-4,5-octanediol dehydratase, 8-methyl-4,5-nonanediol dehydratase, 1-phenyl-2,3-hexanediol dehydratase, 1-(4-hydroxyphenyl)-2,3-hexanediol dehydratase, 1-indole-2,3-hexanediol dehydratase, 5,6-undecanediol dehydratase, 5,6-undecanediol dehydratase, 5,6-tridecanediol dehydratase, 2-methyl-3,4-octanediol dehydratase, 3-methyl-4,5-nonanediol dehydratase, 2-methyl-4,5-nonanediol dehydratase, 2-methyl-5,6-decanediol dehydratase, 1-phenyl-2,3-heptanediol dehydratase, 1-(4-hydroxyphenyl)-2,3-heptanediol dehydratase, 1-indole-2,3-heptanediol dehydratase, 6,7-tridecanediol dehydratase, 6,7-tetradecanediol dehydratase, 2-methyl-3,4-nonanediol dehydratase, 3-methyl-4,5-decanediol dehydratase, 2-methyl-4,5-decanediol dehydratase, 2-methyl-5,6-undecanediol dehydratase, 1-phenyl-2,3-octanediol dehydratase, 1-(4-hydroxyphenyl)-2,3-octanediol dehydratase, 1-indole-2,3-octanediol dehydratase, 7,8-pentadecanediol dehydratase, 2-methyl-3,4-decanediol dehydratase, 3-methyl-4,5-undecanediol dehydratase, 2-methyl-4,5-undecanediol dehydratase, 2-methyl-5,6-dodecanediol dehydratase, 1-phenyl-2,3-nonanediol dehydratase, 1-(4-hydroxyphenyl)-2,3-nonanediol dehydratase, 1-indole-2,3-nonanediol dehydratase, 2-methyl-3,4-undecanediol dehydratase, 3-methyl-4,5-dodecanediol dehydratase, 2-methyl-4,5-dode-

canediol dehydratase, 2-methyl-5,6-tridecanediol dehydratase, 1-phenyl-2,3-decanediol dehydratase, 1-(4-hydroxyphenyl)-2,3-decanediol dehydratase, 1-indole-2,3-decanediol dehydratase, 2,5-dimethyl-3,4-heptanediol dehydratase, 2,6-dimethyl-3,4-heptanediol dehydratase, 2,7-dimethyl-3,4-octanediol dehydratase, 1-phenyl-4-methyl-2,3-pentanediol dehydratase, 1-(4-hydroxyphenyl)-4-methyl-2,3-pentanediol dehydratase, 1-indole-4-methyl-2,3-pentanediol dehydratase, 2,6-dimethyl-4,5-octanediol dehydratase, 3,8-dimethyl-4,5-nonanediol dehydratase, 1-phenyl-4-methyl-2,3-hexanediol dehydratase, 1-(4-hydroxyphenyl)-4-methyl-2,3-hexanediol dehydratase, 1-indole-4-methyl-2,3-hexanediol dehydratase, 2,8-dimethyl-4,5-nonanediol dehydratase, 1-phenyl-5-methyl-2,3-hexanediol dehydratase, 1-(4-hydroxyphenyl)-5-methyl-2,3-hexanediol dehydratase, 1-indole-5-methyl-2,3-hexanediol dehydratase, 1-phenyl-6-methyl-2,3-heptanediol dehydratase, 1-(4-hydroxyphenyl)-6-methyl-2,3-heptanediol dehydratase, 1-indole-6-methyl-2,3-heptanediol dehydratase, 1-(4-hydroxyphenyl)-4-phenyl-2,3-butanediol dehydratase, 1-indole-4-phenyl-2,3-butanediol dehydratase, 1-indole-4-(4-hydroxyphenyl)-2,3-butanediol dehydratase, 1,10-diamino-5,6-decanediol dehydratase, 1,4-di(4-hydroxyphenyl)-2,3-butanediol, 2,3-hexanediol-1,6-dicarboxylic acid dehydratase, and the like.

[0299] In certain aspects, a selected diol dehydratase enzyme may have one or more of the above exemplified diol dehydratase activities, such as a 2,3-butanediol dehydratase activity, a 3,4-hexanediol dehydratase activity, and/or a 4,5-octanediol dehydratase activity, among others.

[0300] In certain aspects, diol dehydratases may be obtained from *Klebsiella pneumoniae* MGH 78578, including from the pduCDE gene of this and other microorganisms. In certain aspects, a recombinant microorganism may comprise one or more diol dehydratases encoded by a nucleotide reference sequence selected from SEQ ID NO:103, 105, and 107, or an enzyme having a polyeptide sequence selected from SEQ ID NO:104, 106, and 108, including biologically active fragments or variants thereof, such as optimized variants. Certain aspects may also comprises nucleotide sequences or polypeptide sequences having 80%, 85%, 90%, 95%, 97%, 98%, 99% sequence identity to SEQ ID NOS:103-108. In certain aspects, polypeptides of SEQ ID NO:104 may comprise certain conserved amino acid residues, including those chosen from D149, P151, A155, A159, G165, E168, E170, A183, G189, G196, Q200, E208, G215, Y219, E221, T222, S224, Y226, G227, T228, F232, G235, D236, D237, T238, P239, S241, L245, Y249, S251, R252, G253, K255, R257, S260, E265, M268, G269, S275, Y278, L279, E280, C283, G291, Q293, G294, Q296, N297, G298, G312, E329, S341, R344, G356, D371, N372, F374, S377, R392, D393, R412, L477, A486, G499, D500, S516, N522, D523, Y524, G526, and G530.

[0301] In certain aspects, a diol dehydratase may include a polypeptide that comprises an amino acid sequence having 0%, 85%, 90%, 95%, 97%, 98%, 99% sequence identity to SEQ ID NOS:308-311. SEQ ID NO:308 shows the polypeptide sequence of PduG, a diol dehydratase reactivation large subunit derived from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578. SEQ ID NO:309 shows the polypeptide sequence of PduH, diol dehydratase reactivation small subunit derived from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578. SEQ ID NO:310 shows the polypeptide sequence of a B12-independent glycerol dehydratase from *Clostridium Butyricum*. SEQ ID NO:311 shows the polypeptide sequence of a glycerol dehydratase activator from *Clostridium Butyricum*. In certain aspects, a B12-independent glycerol dehydratase may comprise conserved amino acid residues, such as T36, G74, P87, E88, E97, W126, R221, A263, Q265, R287, D289, E309, R317, G335, G345, G346, N356, P374, R379, G399, G401, P403, D408, G432, C433, N452, C529, G533, G539, G540, S559, G603, N_{604}, A654, G658, R659, D676, N_{702}, Q735, N737, A747, P751, R760, V761, A762, G763, Q776, I780, and/or R782. In certain aspects, a B12-independent glycerol dehydratase activator may comprise certain conserved amino acid residues, including D19, G20, G22, R24, F28, G31, C32, C36, W38, C39, N41, P42, C58, C64, C96, G129, T132, G135, G136, D185, R187, N_{208}, R222, and/or R264.

[0302] A dehydration and reduction pathway, as described herein, may comprise one or more alcohol dehydrogenases or secondary alcohol dehydrogenases. An "alcohol dehydrogenase" or "secondary alcohol dehydrogenase" that is part of a dehydration and reduction pathway refers generally to an enzyme that catalyzes the conversion of aldehyde or ketone substituents to alcohols. For instance, 4-octanone may be reduced to 4-octanol by a secondary alcohol dehydrogenase one enzymatic step for the conversion of butyroin to a biofuel. *Pseudomonads* express at least one secondary alcohol dehydrogenase that oxidizes 4-octanol to 4-octanone using NAD+ as a co-factor. As another example, *Rhodococcus erythropolis* ATCC4277 catalyzes oxidation of medium to long chain secondary fatty alcohols using NADH as a co-factor, using an enzyme that also catalyzes the oxidation of 3-decanol and 4-decanol. In addition, *Norcadia fusca* AKU2123 contains an (S)-specific secondary alcohol dehydrogenase.

[0303] Genes encoding secondary alcohol dehydrogenases may be isolated from these and other organisms according to known techniques in the art and incorporated into the microbial systems recombinant organisms as described herein. In certain embodiments, a microbial system or isolated microorganism may comprise natural or optimized secondary alcohol dehydrogenases from *Pseudomonads, Rhodococcus erythropolis* ATCC4277, *Norcadia fusca* AKU2123, or other suitable organisms.

[0304] Examples of secondary alcohol dehydrogenases include, but are not limited to, 2-butanol dehydrogenase, 3-hexanol dehydrogenase, 4-octanol dehydrogenase, 5-decanol dehydrogenase, 6-dodecanol dehydrogenase, 7-tetradecanol dehydrogenase, 8-hexadecanol dehydrogenase, 2,5-dimethyl-3-hexanol dehydrogenase, 3,6-dimethyl-4-octanol dehydrogenase, 2,7-dimethyl-4-octanol dehydrogenase, 2,9-dimethyl-4-decanol dehydrogenase, 1,4-diphenyl-2-butanol dehydrogenase, bis-1,4-(4-hydroxyphenyl)-2-butanol dehydrogenase, 1,4-diindole-2-butanol dehydrogenase, cyclopentanol dehydrogenase, 2(or 3)-pentanol dehydrogenase, 2(or 3)-hexanol dehydrogenase, 2(or 3)-heptanol dehydrogenase, 2(or 3)-octanol dehydrogenase, 2(or 3)-nonanol dehydrogenase, 4-methyl-2(or 3)-pentanol dehydrogenase, 4-methyl-2(or 3)-hexanol dehydrogenase, 5-methyl-2(or 3)-hexanol dehydrogenase, 6-methyl-2(or 3)-heptanol dehydrogenase, 1-phenyl-2(or 3)-butanol dehydrogenase, 1-(4-hydroxyphenyl)-2(or 3)-butanol dehydrogenase, 1-indole-2(or 3)-butanol dehydrogenase, 3(or 4)-heptanol dehydrogenase, 3(or 4)-octanol dehydrogenase, 3(or 4)-nonanol dehydrogenase, 3(or 4)-decanol dehydrogenase, 3(or 4)-undecanol dehydrogenase, 2-methyl-3(or 4)-hexanol dehydrogenase, 5-methyl-3 (or 4)-heptanol dehydrogenase,

6-methyl-3 (or 4)-heptanol dehydrogenase, 7-methyl-3(or 4)-octanol dehydrogenase, 1-phenyl-2(or 3)-pentanol dehydrogenase, 1-(4-hydroxyphenyl)-2(or 3)-pentanol dehydrogenase, 1-indole-2(or 3)-pentanol dehydrogenase, 4(or 5)-nonanol dehydrogenase, 4(or 5)-decanol dehydrogenase, 4(or 5)-undecanol dehydrogenase, 4(or 5)-dodecanol dehydrogenase, 2-methyl-3(or 4)-heptanol dehydrogenase, 3-methyl-4(or 5)-octanol dehydrogenase, 2-methyl-4(or 5)-octanol dehydrogenase, 8-methyl-4(or 5)-nonanol dehydrogenase, 1-phenyl-2(or 3)-hexanol dehydrogenase, 1-(4-hydroxyphenyl)-2(or 3)-hexanol dehydrogenase, 1-indole-2(or 3)-hexanol dehydrogenase, 4(or 5)-undecanol dehydrogenase, 5(or 6)-undecanol dehydrogenase, 5(or 6)-tridecanol dehydrogenase, 2-methyl-3(or 4)-octanol dehydrogenase, 3-methyl-4(or 5)-nonanol dehydrogenase, 2-methyl-4(or 5)-nonanol dehydrogenase, 2-methyl-5(or 6)-decanol dehydrogenase, 1-phenyl-2(or 3)-heptanol dehydrogenase, 1-(4-hydroxyphenyl)-2(or 3)-heptanol dehydrogenase, 1-indole-2(or 3)-heptanol dehydrogenase, 6(or 7)-tridecanol dehydrogenase, 6(or 7)-tetradecanol dehydrogenase, 2-methyl-3(or 4)-nonanol dehydrogenase, 3-methyl-4(or 5)-decanol dehydrogenase, 2-methyl-4(or 5)-decanol dehydrogenase, 2-methyl-5(or 6)-undecanol dehydrogenase, 1-phenyl-2(or 3)-octanol dehydrogenase, 1-(4-hydroxyphenyl)-2(or 3)-octanol dehydrogenase, 1-indole-2(or 3)-octanol dehydrogenase, 7(or 8)-pentadecanol dehydrogenase, 2-methyl-3(or 4)-decanol dehydrogenase, 3-methyl-4(or 5)-undecanol dehydrogenase, 2-methyl-4(or 5)-undecanol dehydrogenase, 2-methyl-5 (or 6)-dodecanol dehydrogenase, 1-phenyl-2(or 3)-nonanol dehydrogenase, 1-(4-hydroxyphenyl)-2 (or 3)-nonanol dehydrogenase, 1-indole-2(or 3)-nonanol dehydrogenase, 2-methyl-3(or 4)-undecanol dehydrogenase, 3-methyl-4(or 5)-dodecanol dehydrogenase, 2-methyl-4(or 5)-dodecanol dehydrogenase, 2-methyl-5(or 6)-tridecanol dehydrogenase, 1-phenyl-2(or 3)-decanol dehydrogenase, 1-(4-hydroxyphenyl)-2 (or 3)-decanol dehydrogenase, 1-indole-2(or 3)-decanol dehydrogenase, 2,5-dimethyl-3(or 4)-heptanol dehydrogenase, 2,6-dimethyl-3(or 4)-heptanol dehydrogenase, 2,7-dimethyl-3(or 4)-octanol dehydrogenase, 1-phenyl-4-methyl-2(or 3)-pentanol dehydrogenase, 1-(4-hydroxyphenyl)-4-methyl-2(or 3)-pentanol dehydrogenase, 1-indole-4-methyl-2(or 3)-pentanol dehydrogenase, 2,6-dimethyl-4(or 5)-octanol dehydrogenase, 3,8-dimethyl-4(or 5)-nonanol dehydrogenase, 1-phenyl-4-methyl-2(or 3)-hexanol dehydrogenase, 1-(4-hydroxyphenyl)-4-methyl-2 (or 3)-hexanol dehydrogenase, 1-indole-4-methyl-2(or 3)-hexanol dehydrogenase, 2,8-dimethyl-4(or 5)-nonanol dehydrogenase, 1-phenyl-5-methyl-2(or 3)-hexanol dehydrogenase, 1-(4-hydroxyphenyl)-5-methyl-2(or 3)-hexanol dehydrogenase, 1-indole-5-methyl-2(or 3)-hexanol dehydrogenase, 1-phenyl-6-methyl-2(or 3)-heptanol dehydrogenase, 1-(4-hydroxyphenyl)-6-methyl-2(or 3)-heptanol dehydrogenase, 1-indole-6-methyl-2(or 3)-heptanol dehydrogenase, 1-(4-hydroxyphenyl)-4-phenyl-2(or 3)-butanol dehydrogenase, 1-indole-4-phenyl-2(or 3)-butanol dehydrogenase, 1-indole-4-(4-hydroxyphenyl)-2(or 3)-butanol dehydrogenase, 1,10-diamino-5-decanol dehydrogenase, 1,4-di(4-hydroxyphenyl)-2-butanol dehydrogenase, 2-hexanol-1,6-dicarboxylic acid dehydrogenase, phenylethanol dehydrogenase, 4-hydroxyphenylethanol dehydrogenase, Indole-3-ethanol dehydrogenase, and the like.

[0305] In certain aspects, a selected alcohol dehydrogenase or secondary alcohol dehydrogenase may have one or more of the above exemplified alcohol dehydrogenase activities, such as a 2-butanol dehydrogenase activity, 3-hexanol dehydrogenase activity, and/or a 4-octanol dehydrogenase activity, among others.

[0306] In certain aspects, a recombinant microorganism may comprise one or more secondary alcohol dehydrogenases encoded by a nucleotide reference sequence selected from SEQ ID NO:109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, and 141, or an enzyme having a polyeptide sequence selected from SEQ ID NO:110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, and 142, including biologically active fragments or variants thereof, such as optimized variants. Certain aspects may also comprises nucleotide sequences or polypeptide sequences having 80%, 85%, 90%, 95%, 97%, 98%, 99% sequence identity to SEQ ID NOS:109-142.

[0307] For the secondary alcohol dehydrogenase sequences referred to above, SEQ ID NO:109 is the nucleotide sequence and SEQ ID NO:110 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-1: PP_1946) isolated from *Pseudomonas putida* KT2440. SEQ ID NO:111 is the nucleotide sequence and SEQ ID NO:112 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-2: PP_1817) isolated from *Pseudomonas putida* KT2440.

[0308] SEQ ID NO:113 is the nucleotide sequence and SEQ ID NO:114 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-3: PP_1953) isolated from *Pseudomonas putida* KT2440. SEQ ID NO:115 is the nucleotide sequence and SEQ ID NO:116 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-4: PP_3037) isolated from *Pseudomonas putida* KT2440.

[0309] SEQ ID NO:117 is the nucleotide sequence and SEQ ID NO:118 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-5: PP_1852) isolated from *Pseudomonas putida* KT2440. SEQ ID NO:119 is the nucleotide sequence and SEQ ID NO:120 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-6: PP_2723) isolated from *Pseudomonas putida* KT2440.

[0310] SEQ ID NO:121 is the nucleotide sequence and SEQ ID NO:122 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-7: PP_2002) isolated from *Pseudomonas putida* KT2440. SEQ ID NO:123 is the nucleotide sequence and SEQ ID NO:124 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-8: PP_1914) isolated from *Pseudomonas putida* KT2440.

[0311] SEQ ID NO:125 is the nucleotide sequence and SEQ ID NO:126 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-9: PP_1914) isolated from *Pseudomonas putida* KT2440. SEQ ID NO:127 is the nucleotide sequence and SEQ ID NO:128 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-10: PP_3926) isolated from *Pseudomonas putida* KT2440.

[0312] SEQ ID NO:129 is the nucleotide sequence and SEQ ID NO:130 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-11: PFL_1756) isolated from *Pseudomonas fluorescens* Pf-5. SEQ ID NO:131 is the nucleotide sequence and SEQ ID NO:132 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-12: KPN_01694) isolated from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578.

[0313] SEQ ID NO:133 is the nucleotide sequence and SEQ ID NO:134 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-13: KPN_02061) isolated from *Kiebsiella pneumoniae* subsp. *pneumoniae* MGH

78578. SEQ ID NO:135 is the nucleotide sequence and SEQ ID NO:136 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-14: KPN_00827) isolated from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578.

[0314] SEQ ID NO:137 is the nucleotide sequence and SEQ ID NO:138 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-16: KPN_01350) isolated from *Kiebsiella pneumoniae* subsp. *pneumoniae* MGH 78578. SEQ ID NO:139 is the nucleotide sequence and SEQ ID NO:140 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-17: KPN_03369) isolated from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578. SEQ ID NO:141 is the nucleotide sequence and SEQ ID NO:142 is the polypeptide sequence of a secondary alcohol dehydrogenase (2adh-18: KPN_03363) isolated from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578.

[0315] In certain aspects, an alcohol dehydrogenase (e.g., DEHU hydrogenase), a secondary alcohol dehydrogenase (2ADH), a fragment, variant, or derivative thereof, or any other enzyme that utilizes such an active site, may comprise at least one of a nicotinamide adenine dinucleotide (NAD+), NADH, nicotinamide adenine dinucleotide phosphate (NADP+), or NADPH binding motif. In certain embodiments, the NAD+, NADH, NADP+, or NADPH binding motif may be selected from the group consisting of Y-X-G-G-X-Y, Y-X-X-G-G-X-Y, Y-X-X-X-G-G-X-Y, Y-X-G-X-X-Y, Y-X-X-G-G-X-X-Y, Y-X-X-X-G-X-X-Y, Y-X-G-X-Y, Y-X-X-G-X-Y, Y-X-X-X-G-X-Y, and Y-X-X-X-X-G-X-Y; wherein Y is independently selected from alanine, glycine, and serine, wherein G is glycine, and wherein X is independently selected from a genetically encoded amino acid.

[0316] As one example of a step in a reduction and dehydration pathway, α-hydroxy cyclopentanone may be reduced to 1,2-cyclopentanediol. For example, the glycerol dehydrogenase isolated from *Hansenula ofunaensis* favors the reduction of α-hydroxy ketones and α-keto ketones, and has very broad substrate specificity. The similar alcohol dehydrogenase derived from *Hansenula polumorpha* and meso-2,3-butanediol dehydrogenase has similar properties. Certain embodiments may incorporate a 1,2-cyclopentanediol dehydrogenase to the microbial system or isolated microorganism. Other embodiments may incorporate a glycerol dehydrogenase from *Hansenula ofunaensis, Hansenula polumorpha, Klebsiella pneumonia*, or any other suitable organism.

[0317] By way of example, a chemical or hydrocarbon such as 1,2-cyclopentanediol may be dehydrated to form cyclopentanone as one enzymatic step in a reduction and dehydration pathway. There are at least two different types of diol dehydratases that may catalyze dehydration of chemicals such as 1,2-cyclopentanediol. Certain embodiments of microbial system comprising a reduction and dehydration pathway will comprise diol dehydratases such as 1,2-cyclopentanediol dehydratase.

[0318] In the last enzymatic step for a reduction and dehydration pathway, the conversion of such exemplary chemicals as α-hydroxy cyclopentanone to cyclopentanol may include the reduction of cyclopentanone to cyclopentanol. This step may be catalyzed by cyclopentanol dehydrogenase, which is found in *Comomonas* sp. strain NCIMB 9872 and its gene (cpnA) has been isolated. Certain embodiments of a microbial system or isolated microorganism may comprise a cyclopentanol dehydrogenase, such as that expressed by cpnA in *Comomonas* sp. strain NCIMB 9872, among others described herein.

[0319] As detailed below, in certain embodiments, selected C—C ligation pathways may be utilized in combination with selected components or enzymes of a reduction and dehydration pathway to produce a commodity chemical, or intermediate thereof.

[0320] For example, certain embodiments include a method wherein the C—C ligation pathway may comprise an acetoaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,3-butanediol dehydrogenase, a 2,3-butanediol dehydratase, and a 2-butanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise a propionaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,4-hexanediol dehydrogenase, a 3,4-hexanediol dehydratase, and a 3-hexanol dehydrogenase.

[0321] Additional embodiments include a method wherein the C—C ligation pathway may comprise a butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 4,5-octanediol dehydrogenase, a 4,5-octanediol dehydratase, and a 4-octanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise a butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 5,6-decanediol dehydrogenase, a 5,6-decanediol dehydratase, and a 5-decanol dehydrogenase.

[0322] Additional embodiments include a method wherein the C—C ligation pathway may comprise a butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 6,7-dodecanediol dehydrogenase, a 6,7-dodecanediol dehydratase, and a 6-dodecanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise a butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 7,8-tetradecanediol dehydrogenase, a 7,8-tetradecanediol dehydratase, and a 7-tetradecanol dehydrogenase.

[0323] Additional embodiments include a method wherein the C—C ligation pathway may comprise a butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 8,9-hexadecanediol dehydrogenase, a 8,9-hexadecanediol dehydratase, and a 8-hexadecanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise an isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,5-dimethyl-3,4-hexanediol dehydrogenase, a 2,5-dimethyl-3,4-hexanediol dehydratase, and a 2,5-dimethyl-3-hexanol dehydrogenase.

[0324] Additional embodiments include a method wherein the C—C ligation pathway may comprise a 2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,6-dimethyl-4,5-octanediol dehydrogenase, a 3,6-dimethyl-4,5-octanediol dehydratase, and a 3,6-dimethyl-4-octanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise a 3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,7-dimethyl-4,5-octanediol dehydrogenase, a 2,7-dimethyl-4,5-octanediol dehydratase, and a 2,7-dimethyl-4-octanol dehydrogenase.

[0325] Additional embodiments include a method wherein the C—C ligation pathway may comprise a 3-methyl-butyraldehyde lyase and wherein the reduction and dehydration

pathway may comprise at least one of a 2,9-dimethyl-5,6-decanediol dehydrogenase, a 2,9-dimethyl-4,5-decanediol dehydratase, and a 2,9-dimethyl-4-decanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise a phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1,4-diphenyl-2,3-butanediol dehydrogenase, a 1,4-diphenyl-2,3-butanediol dehydratase, and a 1,4-diphenyl-2-butanol dehydrogenase.

[0326] Additional embodiments include a method wherein the C—C ligation pathway may comprise a phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a bis-1,4-(4-hydroxyphenyl)-2,3-butanediol dehydrogenase, a bis-1,4-(4-hydroxyphenyl)-2,3-butanediol dehydratase, and a bis-1,4-(4-hydroxyphenyl)-2-butanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise a phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1,4-diindole-2,3-butanediol dehydrogenase, a 1,4-diindole-2,3-butanediol dehydratase, and a 1,4-diindole-2-butanol dehydrogenase.

[0327] Additional embodiments include a method wherein the C—C ligation pathway may comprise an α-keto adipate carboxylyase, and wherein the reduction and dehydration pathway may comprise at least one of a 1,2-cyclopentanediol dehydrogenase, a 1,2-cyclopentanediol dehydratase, and a cyclopentanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an acetoaldehyde/propiondehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,3-pentanediol dehydrogenase, a 2,3-pentanediol dehydratase, and a 2(or 3)-pentanol dehydrogenase.

[0328] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an acetoaldehyde/butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,3-hexanediol dehydrogenase, a 2,3-hexanediol dehydratase, and a 2(or 3)-hexanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an acetoaldehyde/pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,3-heptanediol dehydrogenase, a 2,3-heptanediol dehydratase, and a 2(or 3)-heptanol dehydrogenase.

[0329] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an acetoaldehyde/hexyldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,3-octanediol dehydrogenase, a 2,3-octanediol dehydratase, and a 2(or 3)-octanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an acetoaldehyde/octaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,3-nonanediol dehydrogenase, a 2,3-nonanediol dehydratase, and a 2(or 3)-nonanol dehydrogenase.

[0330] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an acetoaldehyde/isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 4-methyl-2,3-pentanediol dehydrogenase, a 4-methyl-2,3-pentanediol dehydratase, and a 4-methyl-2(or 3)-pentanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an acetoaldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 4-methyl-2,3-hexanediol dehydrogenase, a 4-methyl-2,3-hexanediol dehydratase, and a 4-methyl-2(or 3)-hexanol dehydrogenase.

[0331] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an acetoaldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 5-methyl-2,3-hexanediol dehydrogenase, a 5-methyl-2,3-hexanediol dehydrogenase, and a 5-methyl-2(or 3)-hexanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an acetoaldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 6-methyl-2,3-heptanediol dehydrogenase, a 6-methyl-2,3-heptanediol dehydrogenase, and a 6-methyl-2(or 3)-heptanol dehydrogenase.

[0332] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an acetoaldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-2,3-butanediol dehydrogenase, a 1-phenyl-2,3-butanediol dehydratase, and a 1-phenyl-2(or 3)-butanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an acetoaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-2,3-butanediol dehydrogenase, a 1-(4-hydroxyphenyl)-2,3-butanediol dehydratase, and a 1-(4-hydroxyphenyl)-2(or 3)-butanol dehydrogenase.

[0333] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an acetoaldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-2,3-butanediol dehydrogenase, a 1-indole-2,3-butanediol dehydratase, and a 1-indole-2(or 3)-butanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a propionaldehyde/butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,4-heptanediol dehydrogenase, a 3,4-heptanediol dehydratase, and a 3(or 4)-heptanol dehydrogenase.

[0334] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a propionaldehyde/pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,4-octanediol dehydrogenase, a 3,4-octanediol dehydratase, and a 3(or 4)-octanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a propionaldehyde/hexyldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,4-nonanediol dehydrogenase, a 3,4-nonanediol dehydratase, and a 3(or 4)-nonanol dehydrogenase.

[0335] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a propionaldehyde/heptaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,4-decanediol dehydrogenase, a 3,4-decanediol dehydratase, and a 3(or 4)-decanol dehydrogenase. Additional

embodiments include a method wherein the C—C ligation pathway may comprise at least one of a propionaldehyde/octaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,4-undecanediol dehydrogenase, a 3,4-undecanediol dehydratase, and a 3(or 4)-undecanol dehydrogenase.

[0336] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a propionaldehyde/isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-3,4-hexanediol dehydrogenase, a 2-methyl-3,4-hexanediol dehydratase, and a 2-methyl-3(or 4)-hexanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a propionaldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 5-methyl-3,4-heptanediol dehydrogenase, a 5-methyl-3,4-heptanediol dehydratase, and a 5-methyl-3 (or 4)-heptanol dehydrogenase.

[0337] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a propionaldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 6-methyl-3,4-heptanediol dehydrogenase, a 6-methyl-3,4-heptanediol dehydratase, and a 6-methyl-3(or 4)-heptanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a propionaldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 7-methyl-3,4-octanediol dehydrogenase, a 7-methyl-3,4-octanediol dehydratase, and a 7-methyl-3(or 4)-octanol dehydrogenase.

[0338] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a propionaldehyde and a phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-2,3-pentanediol dehydrogenase, a 1-phenyl-2,3-pentanediol dehydratase, and a 1-phenyl-2(or 3)-pentanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a propionaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-2,3-pentanediol dehydrogenase, a 1-(4-hydroxyphenyl)-2,3-pentanediol dehydratase, and a 1-(4-hydroxyphenyl)-2(or 3)-pentanol dehydrogenase.

[0339] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a propionaldehyde/indoleacetoaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-2,3-pentanediol dehydrogenase, a 1-indole-2,3-pentanediol dehydratase, and a 1-indole-2(or 3)-pentanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a butyraldehyde/pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 4,5-nonanediol dehydrogenase, a 4,5-nonanediol dehydratase, and a 4(or 5)-nonanol dehydrogenase.

[0340] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a butyraldehyde/hexyldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 4,5-decanediol dehydrogenase, a 4,5-decanediol dehydratase, and a 4(or 5)-decanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a butyraldehyde/heptaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 4,5-undecanediol dehydrogenase, a 4,5-undecanediol dehydratase, and a 4(or 5)-undecanol dehydrogenase.

[0341] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a butyraldehyde/octaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 4,5-dodecanediol dehydrogenase, a 4,5-dodecanediol dehydratase, and a 4(or 5)-dodecanol dehydrogenase.

[0342] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a butyraldehyde/isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-3,4-heptanediol dehydrogenase, a 2-methyl-3,4-heptanediol dehydratase, and a 2-methyl-3(or 4)-heptanol dehydrogenase.

[0343] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a butyraldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3-methyl-4,5-octanediol dehydrogenase, a 3-methyl-4,5-octanediol dehydratase, and a 3-methyl-4(or 5)-octanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a butyraldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-4,5-octanediol dehydrogenase, a 2-methyl-4,5-octanediol dehydratase, and a 2-methyl-4(or 5)-octanol dehydrogenase.

[0344] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a butyraldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of an 8-methyl-4,5-nonanediol dehydrogenase, an 8-methyl-4,5-nonanediol dehydratase, and an 8-methyl-4(or 5)-nonanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a butyraldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-2,3-hexanediol dehydrogenase, a 1-phenyl-2,3-hexanediol dehydratase, and a 1-phenyl-2(or 3)-hexanol dehydrogenase.

[0345] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a butyraldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-2,3-hexanediol dehydrogenase, a 1-(4-hydroxyphenyl)-2,3-hexanediol dehydratase, and a 1-(4-hydroxyphenyl)-2(or 3)-hexanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a butyraldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-2,3-hexanediol dehydrogenase, a 1-indole-2,3-hexanediol dehydratase, and a 1-indole-2(or 3)-hexanol dehydrogenase.

[0346] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a pentaldehyde/hexyldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 5,6-undecanediol dehydrogenase, a 4,5-undecanediol dehy-

dratase, and a 4(or 5)-undecanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a pentaldehyde/heptaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 5,6-undecanediol dehydrogenase, a 5,6-undecanediol dehydratase, and a 5(or 6)-undecanol dehydrogenase.

[0347] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a pentaldehyde/octaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 5,6-tridecanediol dehydrogenase, a 5,6-tridecanediol dehydratase, and a 5(or 6)-tridecanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a pentaldehyde/isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-3,4-octanediol dehydrogenase, a 2-methyl-3,4-octanediol dehydratase, and a 2-methyl-3(or 4)-octanol dehydrogenase.

[0348] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a pentaldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3-methyl-4,5-nonanediol dehydrogenase, a 3-methyl-4,5-nonanediol dehydratase, and a 3-methyl-4(or 5)-nonanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a pentaldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-4,5-nonanediol dehydrogenase, a 2-methyl-4,5-nonanediol dehydratase, and a 2-methyl-4(or 5)-nonanol dehydrogenase.

[0349] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a pentaldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-5,6-decanediol dehydrogenase, a 2-methyl-5,6-decanediol dehydratase, and a 2-methyl-5(or 6)-decanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a pentaldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-2,3-heptanediol dehydrogenase, a 1-phenyl-2,3-heptanediol dehydratase, and a 1-phenyl-2(or 3)-heptanol dehydrogenase.

[0350] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a pentaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-2,3-heptanediol dehydrogenase, a 1-(4-hydroxyphenyl)-2,3-heptanediol dehydratase, and a 1-(4-hydroxyphenyl)-2(or 3)-heptanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a pentaldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-2,3-heptanediol dehydrogenase, a 1-indole-2,3-heptanediol dehydratase, and a 1-indole-2(or 3)-heptanol dehydrogenase.

[0351] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a hexyldehyde/heptaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 6,7-tridecanediol dehydrogenase, a 6,7-tridecanediol dehydratase, and a 6(or 7)-tridecanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a hexyldehyde/octaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 6,7-tetradecanediol dehydrogenase, a 6,7-tetradecanediol dehydratase, and a 6(or 7)-tetradecanol dehydrogenase.

[0352] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a hexyldehyde/isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-3,4-nonanediol dehydrogenase, a 2-methyl-3,4-nonanediol dehydratase, and a 2-methyl-3(or 4)-nonanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a hexyldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3-methyl-4,5-decanediol dehydrogenase, a 3-methyl-4,5-decanediol dehydratase, and a 3-methyl-4(or 5)-decanol dehydrogenase.

[0353] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a hexyldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-4,5-decanediol dehydrogenase, a 2-methyl-4,5-decanediol dehydratase, and a 2-methyl-4(or 5)-decanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a hexyldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-5,6-undecanediol dehydrogenase, a 2-methyl-5,6-undecanediol dehydratase, and a 2-methyl-5(or 6)-undecanol dehydrogenase.

[0354] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a hexyldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-2,3-octanediol dehydrogenase, a 1-phenyl-2,3-octanediol dehydratase, and a 1-phenyl-2(or 3)-octanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a hexyldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-2,3-octanediol dehydrogenase, a 1-(4-hydroxyphenyl)-2,3-octanediol dehydratase, and a 1-(4-hydroxyphenyl)-2(or 3)-octanol dehydrogenase.

[0355] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a hexyldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-2,3-octanediol dehydrogenase, a 1-indole-2,3-octanediol dehydratase, and a 1-indole-2(or 3)-octanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a heptaldehyde/octaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 7,8-pentadecanediol dehydrogenase, a 7,8-pentadecanediol dehydratase, and a 7(or 8)-pentadecanol dehydrogenase.

[0356] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a heptaldehyde/isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-3,4-decanediol dehydrogenase, a 2-methyl-3,4-de-

canediol dehydratase, and a 2-methyl-3(or 4)-decanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a heptaldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3-methyl-4,5-undecanediol dehydrogenase, a 3-methyl-4,5-undecanediol dehydratase, and a 3-methyl-4(or 5)-undecanol dehydrogenase.

[0357] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a heptaldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-4,5-undecanediol dehydrogenase, a 2-methyl-4,5-undecanediol dehydratase, and a 2-methyl-4(or 5)-undecanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a heptaldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-5,6-dodecanediol dehydrogenase, a 2-methyl-5,6-dodecanediol dehydratase, and a 2-methyl-5(or 6)-dodecanol dehydrogenase.

[0358] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a heptaldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-2,3-nonanediol dehydrogenase, a 1-phenyl-2, 3-nonanediol dehydratase, and a 1-phenyl-2(or 3)-nonanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a heptaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-2,3-nonanediol dehydrogenase, a 1-(4-hydroxyphenyl)-2,3-nonanediol dehydratase, and a 1-(4-hydroxyphenyl)-2 (or 3)-nonanol dehydrogenase.

[0359] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a heptaldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-2,3-nonanediol dehydrogenase, a 1-indole-2,3-nonanediol dehydratase, and a 1-indole-2(or 3)-nonanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an octaldehyde/isobutyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-3,4-undecanediol dehydrogenase, a 2-methyl-3,4-undecanediol dehydratase, and a 2-methyl-3(or 4)-undecanol dehydrogenase.

[0360] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an octaldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3-methyl-4,5-dodecanediol dehydrogenase, a 3-methyl-4,5-dodecanediol dehydratase, and a 3-methyl-4(or 5)-dodecanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an octaldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-4,5-dodecanediol dehydrogenase, a 2-methyl-4,5-dodecanediol dehydratase, and a 2-methyl-4(or 5)-dodecanol dehydrogenase.

[0361] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an octaldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2-methyl-5,6-tridecanediol dehydrogenase, a 2-methyl-5,6-tridecanediol dehydratase, and a 2-methyl-5(or 6)-tridecanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an octaldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-2,3-decanediol dehydrogenase, a 1-phenyl-2,3-decanediol dehydratase, and a 1-phenyl-2(or 3)-decanol dehydrogenase.

[0362] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an octaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-2,3-decanediol dehydrogenase, a 1-(4-hydroxyphenyl)-2,3-decanediol dehydratase, and a 1-(4-hydroxyphenyl)-2 (or 3)-decanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an octaldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-2,3-decanediol dehydrogenase, a 1-indole-2,3-decanediol dehydratase, and a 1-indole-2(or 3)-decanol dehydrogenase.

[0363] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an isobutyraldehyde/2-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,5-dimethyl-3,4-heptanediol dehydrogenase, a 2,5-dimethyl-3,4-heptanediol dehydratase, and a 2,5-dimethyl-3 (or 4)-heptanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an isobutyraldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,6-dimethyl-3,4-heptanediol dehydrogenase, a 2,6-dimethyl-3,4-heptanediol dehydratase, and a 2,6-dimethyl-3(or 4)-heptanol dehydrogenase.

[0364] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an isobutyraldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,7-dimethyl-3,4-octanediol dehydrogenase, a 2,7-dimethyl-3,4-octanediol dehydratase, and a 2,7-dimethyl-3 (or 4)-octanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an isobutyraldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-4-methyl-2,3-pentanediol dehydrogenase, a 1-phenyl-4-methyl-2,3-pentanediol dehydratase, and a 1-phenyl-4-methyl-2(or 3)-pentanol dehydrogenase.

[0365] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an isobutyraldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-4-methyl-2,3-pentanediol dehydrogenase, a 1-(4-hydroxyphenyl)-4-methyl-2,3-pentanediol dehydratase, and a 1-(4-hydroxyphenyl)-4-methyl-2(or 3)-pentanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an isobutyraldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a

1-indole-4-methyl-2,3-pentanediol dehydrogenase, a 1-indole-4-methyl-2,3-pentanediol dehydratase, and a 1-indole-4-methyl-2(or 3)-pentanol dehydrogenase.

[0366] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a 2-methyl-butyraldehyde/3-methyl-butyraldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,6-dimethyl-4,5-octanediol dehydrogenase, a 2,6-dimethyl-4,5-octanediol dehydratase, and a 2,6-dimethyl-4(or 5)-octanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a 2-methyl-butyraldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 3,8-dimethyl-4,5-nonanediol dehydrogenase, a 3,8-dimethyl-4,5-nonanediol dehydratase, and a 3,8-dimethyl-4(or 5)-nonanol dehydrogenase.

[0367] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a 2-methyl-butyraldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-4-methyl-2,3-hexanediol dehydrogenase, a 1-phenyl-4-methyl-2,3-hexanediol dehydratase, and a 1-phenyl-4-methyl-2(or 3)-hexanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a 2-methyl-butyraldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-4-methyl-2,3-hexanediol dehydrogenase, a 1-(4-hydroxyphenyl)-4-methyl-2,3-hexanediol dehydratase, and a 1-(4-hydroxyphenyl)-4-methyl-2 (or 3)-hexanol dehydrogenase.

[0368] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a 2-methyl-butyraldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-4-methyl-2,3-hexanediol dehydrogenase, a 1-indole-4-methyl-2,3-hexanediol dehydratase, and a 1-indole-4-methyl-2(or 3)-hexanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a 3-methyl-butyraldehyde/4-methyl-pentaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 2,8-dimethyl-4,5-nonanediol dehydrogenase, a 2,8-dimethyl-4,5-nonanediol dehydratase, and a 2,8-dimethyl-4(or 5)-nonanol dehydrogenase.

[0369] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a 3-methyl-butyraldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-5-methyl-2,3-hexanediol dehydrogenase, a 1-phenyl-5-methyl-2,3-hexanediol dehydratase, and a 1-phenyl-5-methyl-2(or 3)-hexanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a 3-methyl-butyraldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-5-methyl-2,3-hexanediol dehydrogenase, a 1-(4-hydroxyphenyl)-5-methyl-2,3-hexanediol dehydratase, and a 1-(4-hydroxyphenyl)-5-methyl-2(or 3)-hexanol dehydrogenase.

[0370] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a 3-methyl-butyraldehyde/indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-5-methyl-2,3-hexanediol dehydrogenase, a 1-indole-5-methyl-2,3-hexanediol dehydratase, and a 1-indole-5-methyl-2(or 3)-hexanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a 4-methyl-pentaldehyde/phenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-phenyl-6-methyl-2,3-heptanediol dehydrogenase, a 1-phenyl-6-methyl-2,3-heptanediol dehydratase, and a 1-phenyl-6-methyl-2(or 3)-heptanol dehydrogenase.

[0371] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a 4-methyl-pentaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-6-methyl-2,3-heptanediol dehydrogenase, a 1-(4-hydroxyphenyl)-6-methyl-2,3-heptanediol dehydratase, and a 1-(4-hydroxyphenyl)-6-methyl-2(or 3)-heptanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a 4-methyl-pentaldehyde/Indoleacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-6-methyl-2,3-heptanediol dehydrogenase, a 1-indole-6-methyl-2,3-heptanediol dehydratase, and a 1-indole-6-methyl-2(or 3)-heptanol dehydrogenase.

[0372] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a phenylacetaldehyde/4-hydroxyphenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-(4-hydroxyphenyl)-4-phenyl-2,3-butanediol dehydrogenase, a 1-(4-hydroxyphenyl)-4-phenyl-2,3-butanediol dehydratase, and a 1-(4-hydroxyphenyl)-4-phenyl-2(or 3)-butanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a phenylacetaldehyde/indolephenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-4-phenyl-2,3-butanediol dehydrogenase, a 1-indole-4-phenyl-2,3-butanediol dehydratase, and a 1-indole-4-phenyl-2(or 3)-butanol dehydrogenase.

[0373] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a 4-hydroxyphenylacetaldehyde/indolephenylacetaldehyde lyase and wherein the reduction and dehydration pathway may comprise at least one of a 1-indole-4-(4-hydroxyphenyl)-2,3-butanediol dehydrogenase, a 1-indole-4-(4-hydroxyphenyl)-2,3-butanediol dehydratase, and a 1-indole-4-(4-hydroxyphenyl)-2(or 3)-butanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise a 5-amino-pantaldehyde lyase, and wherein the reduction and dehydration pathway may comprise at least one of a 1,10-diamino-5,6-decanediol dehydrogenase, a 1,10-diamino-5,6-decanediol dehydratase, and a 1,10-diamino-5-decanol dehydrogenase.

[0374] Additional embodiments include a method wherein the C—C ligation pathway may comprise a 4-hydroxyphenyl acetaldehyde lyase, and wherein the reduction and dehydration pathway may comprise at least one of a 1,4-di(4-hydroxyphenyl)-2,3-butanediol, a 1,4-di(4-hydroxyphenyl)-2,3-butanediol dehydratase, and a 1,4-di(4-hydroxyphenyl)-2-butanol dehydrogenase. Additional embodiments include a method wherein the C—C ligation pathway may comprise a succinate semialdehyde lyase, and wherein the reduction and

dehydration pathway may comprise at least one of a 2,3-hexanediol-1,6-dicarboxylic acid dehydrogenase, a 2,3-hexanediol-1,6-dicarboxylic acid dehydratase, and a 2-hexanol-1,6-dicarboxylic dehydrogenase.

[0375] Certain embodiments of a microbial system or recombinant microorganism may comprise genes encoding enzymes that are able to catalyze (e.g., reduction and dehydration) the conversion of 4-octanol to octene or octane. Other embodiments may comprise redesigned or de novo designed enzymes for this reduction and dehydration pathway. For example, three redesigned enzymes could convert 4-octanone to either 3- and 4-octene. The first step could be catalyzed by redesigned isocitrate dehydrogenase. This enzyme could catalyze the formation of 4-hydroxy-3(or 5)-carboxylic octane. The 4-hydroxy group could be phosphorylated by redesigned kinase. Finally, redesigned mevalonate diphosphate decarboxylase catalyzes the formation of 3(or 4)-octene.

[0376] In other embodiments, several redesigned enzymes could convert 4-octanone to octane. For example, the 4-hydroxy-3(or 5)-carboxylic octane is sequentially reduced and dehydrated to form 3(or 5)-carboxylic octane. Redesigned enzymes involved in fatty acid metabolism can catalyze these reactions. The 3(or 5)-carboxylic octane can be reduced to corresponding aldehyde by aldehyde dehydrogenase and the product may be decarbonylated to form octane catalyzed by a redesigned decarbonylase.

[0377] As noted above, for the production of certain commodity chemicals, such as 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and indole-3-ethanol, among other similar chemicals, a biosynthesis pathway (e.g., aldehyde biosynthesis pathway) may optionally or further comprise one or more genes encoding a decarboxylase enzyme, such as an indole-3-pyruvate decarboxylase (IPDC), to produce an aldehyde. In certain aspects, an IPDC may comprise an amino acid sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO:312. An IDPC enzyme may comprise certain conserved amino acid residues, such as G24, D25, E48, A55, R60, G75, E89, H113, G252, G405, G413, G428, G430, and/or N456.

[0378] In these and other embodiments, a recombinant microorganism may comprise an aldehyde reductase, such as a phenylacetoaldehyde reductase (PAR), to convert an aldehyde to a commodity chemical. In certain aspects, a PAR may comprise an amino acid sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO:313, which shows the sequence of a PAR enzymed derived from *Rhodococcus* sp. ST-10. In certain aspects, a PAR enzyme may comprise at least one of a nicotinamide adenine dinucleotide (NAD+), NADH, nicotinamide adenine dinucleotide phosphate (NADP+), or NADPH binding motif. In certain embodiments, the NAD+, NADH, NADP+, or NADPH binding motif may be selected from the group consisting of Y-X-G-G-X-Y, Y-X-X-G-G-X-Y, Y-X-X-X-G-G-X-Y, Y-X-G-X-X-Y, Y-X-X-G-G-X-X-Y, Y-X-X-X-G-X-X-Y, Y-X-G-X-Y, Y-X-X-G-X-Y, Y-X-X-X-G-X-Y, and Y-X-X-X-X-G-X-Y; wherein Y is independently selected from alanine, glycine, and serine, wherein G is glycine, and wherein X is independently selected from a genetically encoded amino acid.

[0379] In certain embodiments, such a recombinant microorganism may also or alternatively comprise a secondary alcohol dehydrogenase having an activity selected from at least one of a phenylethanol dehydrogenase activity, a 4-hydroxyphenylethanol dehydrogenase activity, and an Indole-3-ethanol dehydrogenase activity, to reduce the aldehyde to its corresponding alcohol (e.g. 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and indole-3-ethanol).

[0380] Embodiments of the present invention also include methods for converting a suitable monosaccharide to a commodity chemical comprising, (a) obtaining a suitable monosaccharide; (b) contacting the suitable monosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide to the biofuel, wherein the microbial system comprises, (i) one or more genes encoding and expressing a fatty acid biosynthesis pathway, an amino acid biosynthetic pathway, and/or a short chain alcohol biosynthetic pathway; (ii) one or more genes encoding and expressing a keto-acid decarboxylase, aldehyde dehydrogenase, and/or alcohol dehydrogenase; and (iii) an enzymatic reduction pathway selected from (1) an enzymatic long chain alcohol reduction pathway, (2) an enzymatic decarbonylation pathway, (3) an enzymatic decarboxylation pathway, and (4) an enzymatic reduction pathway comprising (1), (2), and/or (3), thereby converting the suitable monosaccharide to the commodity chemical.

[0381] Embodiments of the present invention may comprise one or more genes encoding and expressing enzymes in a fatty acid synthesis pathway, which may be used, as one example, to produce biofuels in the form of alkanes, such as medium to long chain alkanes. In certain embodiments, the specificity of the fatty acid biosynthesis pathway in the microbial system may be recalibrated or redesigned. Merely by way of example, microorganisms generally produce a mixture of long chain fatty acids (e.g., *E. coli* naturally produce large quantities of long chain fatty acids (C16-C19: <95% in whole cells) and small quantity of medium chain fatty acids (C12: 2% and C14: 5% in whole cells)).

[0382] In certain embodiments, the recalibration or re-engineering may be directed to increasing production of medium chain alkanes, including, but not limited to, caprylate (C8), caprate (C10), laurate (C12), myristate (C14), and palmitate (C16), as alkanes produced from these fatty acids are major components of gasoline, diesels, and kerosene. In addition to these fatty acids, other embodiments may be directed to increased production of long chain fatty acids, including, but not limited to, stearate (C18), arachidonate (C20), behenate (C22) and longer fatty acids, as n-alkanes produced from these fatty acids are one of major components in heavy oils.

[0383] For example, *Cuphea* mainly accumulate medium chain fatty acids as major components in their seed oils, and these compositions alter depending on species. In particular, *Cuphea pulcherrima* accumulates caprylate (C8:0) 96%, *Cuphea koehneana* accumulates caprate (C10:0) 95.3%, and *Cuphea polymorpha* accumulates laurate (C12:0) 80.1%. Embodiments of the microbial systems or isolated microorganisms according to the present application may incorporate genes from various *Cuphea* species encoding enzymes involved in a fatty acid biosynthesis pathway, and these microorganisms may be directed in part to the production of middle chain fatty acids.

[0384] In other embodiments, acyl-acyl carrier protein (ACP) thioesterases (TEs) derived from various species including *Cuphea hookeriana, Cuphea palustris, Umbellularia californica*, and *Cinnamomum camphorum* may be over-expressed in such microorganisms as *E. coli*, wherein the specific activity for the formation of each medium chain

fatty acids, caprylate (C8), caprate (C10), laurate (C12), myristate (C14), and palmitate (C16) is improved over the wild type. Certain embodiments may include other enzyme components involved in fatty acid biosynthesis as known to a person skilled in the arts, including, but not limited to, ACP and β-ketoacyl ACP synthase (KAS) IV.

[0385] Microbial systems and isolated microorganisms of the present application may also incorporate fatty aldehyde dehydrogenases to reduce fatty acids to fatty aldehydes. Merely by way of explanation, the conversion of fatty acids to fatty aldehydes may be catalyzed by medium and/or long chain fatty aldehyde dehydrogenases isolated from various suitable organisms. Certain embodiments may incorporate, for example, a fatty aldehyde dehydrogenase derived from *Vibrio harveyi.*

[0386] Microbial systems and isolated microorganisms of the present application may also incorporate one or more enzymes that catalyze the conversion of fatty aldehydes to biofuels such as n-alkanes, including, for example, enzymes comprising an enzymatic long chain alcohol reduction pathway. Certain embodiments may incorporate genes from various other sources that encode enzymes capable of catalyzing the reduction and dehydration of fatty acids to biofuels, such as alkanes. For example, bacterial strain HD-1 is able to produce biofuels, such as n-alkanes, with various chain lengths, and also produces both odd and even numbered alkanes. Certain embodiments of the microbial systems and recombinant microorganisms provided herein may incorporate the HD-1 genes encoding the enzymes involved in this pathway.

[0387] Other embodiments may incorporate redesigned or de novo designed enzymes for this reduction pathway. For example, embodiments of the present invention may include a redesigned isocitrate dehydrogenase, which may catalyze the formation of 2-carboxy-1-alcohols. In certain embodiments, the 2-carboxy-1-alcohols may be sequentially reduced and dehydrated to form 2-carboxy-alkanes, which may be catalyzed by redesigned enzymes involved in fatty acid metabolism. The 2-carboxy-alkanes can be reduced to corresponding aldehyde by aldehyde dehydrogenase and then decarbonylated to form n-alkanes catalyzed by the redesigned decarbonylase as discussed below. Certain embodiments of these microbial systems may produce either even numbered n-alkanes, odd numbered n-alkanes, or both.

[0388] Certain embodiments of the present application may incorporate the genes encoding enzymes catalyzing decarbonylation, or an enzymatic decarbonylation pathway. Merely by way of example, green colonial alga *Botyrococcus braunii*, race A, produces linear odd-numbered C27, C29, and C31 hydrocarbons that total up to 32% of the alga's dry weight. Microsomal preparations of this organism have decarbonylation activity. This decarbonylase from *B. braunii* culture is a cobalt-protoporphyrin IX containing enzyme. Certain microbial systems of isolated microorganisms may incorporate the gene encoding fatty aldehyde decarbonylase from *Botyrococcus braunii.*

[0389] Other embodiments may include redesigned decarbonylase enzymes, for example, wherein the N-terminal membrane sequence is substituted. By way of explanation, the functional activity of a similar enzyme, cytochrome P450 containing Fe-protopolphyrin IX (heme), is improved by substituting N-terminal membrane associated sequence, and the functional activity of decarbonylases of the present microbial systems may comprise similar substitutions or improvements.

[0390] Other embodiments may incorporate the genes encoding a Co-porphyrin synthase. In explanation, decarbonylase enzymes may use Co-protoporphyrin IX as a co-factor, and *Clostridium tetranomorphum* is able to incorporate cobalt into incubated protopolphyrin IX. Certain embodiments may incorporate the Co-porphyrin synthase from *Clostridium tetranomorphum*, or from other suitable microorganisms. Other embodiments may incorporate de novo designed decarbonylation enzymes using inorganic metals such as Co^{2+}, Fe^{2+}, and Ni^{2+} as catalysts.

[0391] Certain embodiments may comprise genes encoding the enzymes responsible for the formation of alkenes, or an enzymatic decarboxylation pathway. These genes may be derived or isolated from various sources, such as higher plants and insects. For example, higher plants such as germinating safflower (*Carthamus tinctorius* L.) produce a number of odd numbered 1-alkenes, including 1-pentadecene, 1-heptadecene, 1,8-heptadecadiene and 1,8,11-heptadecatriene besides about 80-90% 1,8,11,14-heptadecatetraene by decarboxylation from their corresponding fatty acids. Certain embodiments may incorporate the genes from higher plants such as *Carthamus tinctorius.*

[0392] Other embodiments may incorporate the genes encoding the enzymes responsible for the formation of alkenes (e.g., an enzymatic decarboxylation pathway) from microorganisms, including, but not limited to, such as bacterial strain DH-1. By way of explanation, bacterial strain DH-1 produces n-alkenes in addition to n-alkanes.

[0393] Other embodiments may incorporate the genes from de novo designed enzymes for an enzymatic decarboxylation pathway. For example, these redesigned enzymes convert β-hydroxy fatty acids to n-alkenes. The first step is catalyzed by a redesigned kinase, which catalyzes the phosphorylation of a β-hydroxy group. A redesigned mevalonate diphosphate decarboxylase then catalyzes the formation of n-alkenes, such as n-1-alkene.

[0394] Any microorganism may be utilized according to the present invention. In certain aspects, a microorganism is a eukaryotic or prokaryotic microorganism. In certain aspects, a microrganism is a yeast, such as *S. cerevisiae.* In certain aspects, a microorganism is a bacteria, such as a gram-positive bacteria or a gram-negative bacteria. Given its rapid growth rate, well-understood genetics, the variety of available genetic tools, and its capability in producing heterologous proteins, genetically modified *E. coli* may be used in certain embodiments of a microbial system as described herein, whether for the degradation and metabolism of a polysaccharide, such as alginate or pectin, or the formation or biosynthesis of commodity chemicals, such as biofuels.

[0395] Other microorganisms may be used according to the present invention, based in part on the compatibility of enzymes and metabolites to host organisms. For example, other organisms such as *Acetobacter aceti, Achromobacter, Acidiphilium, Acinetobacter, Actinomadura, Actinoplanes, Aeropyrum pernix, Agrobacterium, Alcaligenes, Ananas comosus* (M), *Arthrobacter, Aspargillus niger, Aspargillus oryze, Aspergillus melleus, Aspergillus pulverulentus, Aspergillus saitoi, Aspergillus sojea, Aspergillus usamii, Bacillus alcalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus lentus, Bacillus licheniformis, Bacillus macerans, Bacillus stearo-*

thermophilus, Bacillus subtilis, Bifidobacterium, Brevibacillus brevis, Burkholderia cepacia, Candida cylindracea, Candida rugosa, Carica papaya (L), *Cellulosimicrobium, Cephalosporium, Chaetomium erraticum, Chaetomium gracile, Clostridium, Clostridium butyricum, Clostridium acetobutylicum, Clostridium thermocellum, Corynebacterium* (glutamicum), *Corynebacterium efficiens, Escherichia coli, Enterococcus, Erwina chrysanthemi, Gliconobacter, Gluconacetobacter, Haloarcula, Humicola insolens, Humicola nsolens, Kitasatospora setae, Klebsiella, Klebsiella oxytoca, Kluyveromyces, Kluyveromyces fragilis, Kluyveromyces lactis, Kocuria, Lactlactis, Lactobacillus, Lactobacillus fermentum, Lactobacillus sake, Lactococcus, Lactococcus lactis, Leuconostoc, Methylocystis, Methanolobus siciliae, Methanogenium organophilum, Methanobacterium bryantii, Microbacterium imperiale, Micrococcus lysodeikticus, Microlunatus, Mucor javanicus, Mycobacterium, Myrothecium, Nitrobacter, Nitrosomonas, Nocardia, Papaya carica, Pediococcus, Pediococcus halophilus, Penicillium, Penicillium camemberti, Penicillium citrinum, Penicillium emersonii, Penicillium roqueforti, Penicillum lilactinum, Penicillum multicolor, Paracoccus pantotrophus, Propionibacterium, Pseudomonas, Pseudomonas fluorescens, Pseudomonas denitrificans, Pyrococcus, Pyrococcus furiosus, Pyrococcus horikoshii, Rhizobium, Rhizomucor miehei, Rhizomucor pusillus Lindt, Rhizopus, Rhizopus delemar, Rhizopus japonicus, Rhizopus niveus, Rhizopus oryzae, Rhizopus oligosporus, Rhodococcus, Sccharomyces cerevisiae, Sclerotina libertina, Sphingobacterium multivorum, Sphingobium, Sphingomonas, Streptococcus, Streptococcus thermophilus* Y-1, *Streptomyces, Streptomyces griseus, Streptomyces lividans, Streptomyces murinus, Streptomyces rubiginosus, Streptomyces violaceoruber, Streptoverticillium mobaraense, Tetragenococcus, Thermus, Thiosphaera pantotropha, Trametes, Trichoderma, Trichoderma longibrachiatum, Trichoderma reesei, Trichoderma viride, Trichosporon penicillatum, Vibrio alginolyticus, Xanthomonas*, yeast, *Zygosaccharomyces rouxii, Zymomonas*, and *Zymomonus mobilis*, may be utilized as recombinant microorganisms provided herein, and, thus, may be utilized according to the various methods of the present invention.

[0396] The following Examples are offered by way of illustration, not limitation.

EXAMPLES

Example 1

Engineering E. Coli to Grow on Alginate as a Sole Source of Carbon

[0397] Wild type *E. coli* cannot use alginate polymer or degraded alginate as its sole carbon source (see FIG. **4**). *Vibrio splendidus*, however, is known to be able to metabolize alginate to support growth. To generate recombinant *E. coli* that use degraded alginate as its sole carbon source, a *Vibrio splendidus* fosmid library was constructed and cloned into *E. coli*.

[0398] To prepare the *Vibrio splendidus* fosmid library, genomic DNA was isolated from *Vibrio Splendidus* B01 (gift from Dr. Martin Polz, MIT) using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, Calif.). A fosmid library was then constructed using Copy Control Fosmid Library Production Kit (Epicentre, Madison, Wis.). This library consisted of random genomic fragments of approximately 40 kb inserted into the vector pCC1 FOS (Epicentre, Madison, Wis.).

[0399] The fosmid library was packaged into phage, and *E. coli* DH10B cells harboring a pDONR221 plasmid (Invitrogen, Carlsbad, Calif.) carrying certain *Vibrio splendidus* genes (V12B01_02425 to V12B01_02480; encoding a type II secretion apparatus; see SEQ ID NO:1) were transfected with the phage library. This secretome region encodes a type II secretion apparatus derived from *Vibrio splendidus*, which was cloned into a pDONR221 plasmid and introduced into *E. coli* strain DH10B (see Example 1).

[0400] Transformants were selected for chloroamphenicol resistance and then screened for their ability to grow on degraded alginate. The resultant transformants were screened for growth on degraded alginate media. Degraded alginate media was prepared by incubating 2% Alginate (Sigma-Aldrich, St. Louis, Mo.) 10 mM Na-Phosphate buffer, 50 mM KCl, 400 mM NaCl with alginate lyase from *Flavobacterium* sp. (Sigma-Aldrich, St. Louis, Mo.) at room temperature for at least one week. This degraded alginate was diluted to a concentration of 0.8% to make growth media that had a final concentration of 1×M9 salts, 2 mM $MgSO_4$, 100 μM CaCl2, 0.007% Leucine, 0.01% casamino acids, 1.5% NaCl (this includes all sources of sodium: M9, diluted alginate and added NaCl).

[0401] One fosmid-containing *E. coli* clone was isolated that grew well on this media. The fosmid DNA from this clone was isolated and prepared using FosmidMAX DNA Purification Kit (Epicentre, Madison, Wis.). This isolated fosmid was transferred back into DH10B cells, and these cells were tested for the ability to grown on alginate.

[0402] The results are illustrated in FIG. **4**, which shows that certain fosmid-containing *E. coli* clones are capable of growing on alginate as a sole source of carbon. *Agrobacterium tumefaciens* provides a positive control (see hatched circles). As a negative control, *E. coli* DH10B cells are not capable of growing on alginate (see immediate left of positive control).

[0403] These results also demonstrate that the sequences contained within this *Vibrio splendidus* derived fosmid clone are sufficient to confer on *E. coli* the ability to grow on degraded alginate as a sole source of carbon. Accordingly, the type II secretion machinery sequences contained within the pDONR221 vector (i.e., SEQ ID NO:1), which was harbored by the original DH10B cells, were not necessary for growth on degraded alginate.

[0404] The isolated fosmid sufficient to confer growth alginate as a sole source of carbon was sequenced by Elim Biopharmaceuticals (Hayward, Calif.) using the following primers: Uni R3—GGGCGGCCGCAAGGGGTTCGCGTTGGCCGA (SEQ ID NO:147) and PCC1FOS_uni_F—GGAGAAAATACCGCATCAGGCG (SEQ ID NO:148). Sequencing showed that the vector contained a genomic DNA section that contained the full length genes V12B01_24189 to V12B01_24249 (see SEQ ID NOS:2-64). SEQ ID NO:2 shows the nucleotide sequence of entire region between V12B01_24189 to V12B01_24249. SEQ ID NOS:3-64 show the individual putative genes contained within SEQ ID NO:2. In this sequence, there is a large gene before V12B01_24189 that is truncated in the fosmid clone. The large gene V12B01_24184 is a putative protein with similarity to autotransporters and belongs to COG3210, which is a cluster of orthologous proteins that include large exoproteins involved in heme utilization or adhesion. In the fosmid clone, V12B01_24184 is N-terminally truncated such that the first

5893 bp are missing from the predicted open reading frame (which is predicted to contain 22889 bp in total).

Example 2

Engineering E. Coli to Grow on Pectin as a Sole Source of Carbon

[0405] Wild type *E. coli* is not capable of growing on pectin, di-, or tri-galacturonates as a sole source of carbon. To identify the minimal components to confer on *E. coli* the capability of growing on pectin, di- and/or tri-galacturonates as a sole source of carbon, an *E. coli* strain BL21(DE3) harboring both the pBBRGal3P plasmid and the pTrcogl-kdgR plasmid was engineered and tested for the ability to grown on these polysaccharides.

[0406] The pBBRGal3P plasmid was engineered to contain certain genomic region of *Erwinia carotovora* subsp. *Atroseptica* SCR11043, comprising several genes (kdgF, kduI, kduD, pelW, togM, togN, toga, togB, kdgM, and paeX) encoding certain enzymes (kduI, kduD, ogl, pelW and paeX), transporters (togM, togN, togA, togB, and kdgM), and regulatory proteins (kdgR) responsible for the degradation of di- and trigalacturonate. SEQ ID NO:65 shows the nucleotide sequence of the kdgF-PaeX region from *Erwinia carotovora* subsp. *Atroseptica* SCR11043.

[0407] To construct this plasmid, the DNA sequence encoding kdgF, kduI, kduD, pelW, togM, togN, togA, togB, kdgM, paeX, ogl, and kdgR of *Erwinia carotovora* subsp. *Atroseptica* SCR11043 was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 6 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CGGGATCC AAGTTGCAGGATATGACGAAAGCG-3') (SEQ ID NO:149) and reverse (5'-GC TCTAGA AGATTATCCCTGTCTGCGGAAGCGG-3') (SEQ ID NO:150) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Erwinia carotovora* subsp. *Atroseptica* SCR11043 genome (ATCC) in 50 μl.

[0408] The vector pBBR1MCS-2 was then amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 2.5 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-GCTCTAGA GGGGTGCCTAATGAGTGAGCTAAC-3') (SEQ ID NO:151) and reverse (5'-CGGGATCC GCGTTAATATTTTGTTAAAATTCGC-3') (SEQ ID NO:152) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pBBR1MCS-2 in 50 μl. Both amplified DNA fragments were digested with BamHI and XbaI and ligated.

[0409] The pTrcogl-kdgR plasmid was engineered to contain certain genomic regions of *Erwinia carotovora* subsp. *Atroseptica* SCR11043, comprising two genes (ogl and kdgR) encoding an enzyme (ogl) and a regulatory protein (kdgR) responsible for degradation of di- and trigalacturonate. SEQ ID NO:66 shows the nucleotide sequence of ogl-kdgR from *Erwinia carotovora* subsp. *Atroseptica* SCR11043.

[0410] To prepare this construct, the DNA sequence encoding ogl and kdgR of *Erwinia carotovora* subsp. *Atroseptica* SCR11043 was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 4 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-GCTCTAGA GTTTATGTCGCACCCGCCGTTGG-3') (SEQ ID NO:153) and reverse (5'-CCCAAGC TTAGAAAGGGAAATTGTGGTAGCCC-3') (SEQ ID NO:154) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Erwinia* carotovora subsp. *Atroseptica* SCR11043 genome (ATCC) in 50 μl. The amplified DNA fragment was digested with XbaI and HindIII and ligated into pTrc99A pre-digested with the same restriction enzymes.

[0411] The plasmids pBBRGal3P and pTrcogl-kdgR were co-transformed into *E. coli* strain BL21(DE3). A single colony was inoculated into LB media containing 50 ug/ml kanamycin and 100 ug/ml ampicillin, and the culture was grown in incubation shaker with 200 rpm at 37 C. When culture reached OD 600 nm of 0.6, 500 ul of culture was transferred to eppendorf tube and centrifuged to pellet the cells. The cells were resuspended into 50 ul of M9 media containing 2 mM $MgSO_4$, 100 uM $CaCl_2$, 0.4% di- or trigalacturonate, and 5 ul of this solution was inoculated into 500 ul of fresh M9 media containing 2 mM $MgSO_4$, 100 uM $CaCl_2$, 0.4% di- or trigalacturonate. The culture was grown in incubation shaker with 200 rpm at 37 C.

[0412] The results in FIG. **5**A show that these two plasmids were sufficient to provide *E. coli* ability to grow on di- and trigalacturonate as sole source of carbon, but not pectin. In particular, these results show that the regions kdgF-paeX and ogl-kdgR were sufficient to confer this ability on *E. coli.*

[0413] Based on the information obtained from the above experiments, it was considered whether the introduction of pectate lyase, pectate acetylesterase, and methylesterase might confer *E. coli* capability of growing on pectin. To test this hypothesis, *E. coli* strain DH5α bacterial cells were engineered to contain both the pROU2 plasmid and the pPEL74 plasmid.

[0414] The pROU2 plasmid contains certain genomic regions of *Erwinia* chrysanthemi, comprising several genes (kdgF, kduI, kduD, pelW, togM, togN, togA, togB, kdgM, paeX, ogl, and kdgR) encoding enzymes (kduI, kduD, ogl, pelW, and paeX), transporters (togM, togN, togA, togB, and kdgM), and regulatory proteins (kdgR) responsible for degradation of di- and trigalacturonate.

[0415] The pPEL74 plasmid contains certain genomic regions of *Erwinia chrysanthemi*, comprising several genes (pelA, pelE, paeY, and pem) encoding pectate lyases (pelA and pelE), pectin acetylesterases (paeY), and pectin methylesterase (pem).

[0416] As shown in FIG. **5**B, *E. coli* DH5a engineered with pROU2 and pPEL74 was able to grow on pectin as a sole source of carbon, showing that the genes contained within these plasmids are sufficient to confer this property on an organism that is otherwise incapable of growing on pectin as a sole source of carbon.

Example 3

In Vitro Conversion of Alginate to Pyruvate and Glyceraldehyde-3-Phosphate

[0417] The ability of an enzyme mixture containing all required enzymes for alginate degradation and metabolism was investigated for its ability to produce pyruvate from alginate. In addition, various novel alcohol dehydrogenases (ADHs), such as ADH1-12 (see SEQ ID NOS:69-92), isolated from *Agrobacterium tumefaciens*, were tested for their ability to catalyze either DEHU or mannuronate hydrogenation.

[0418] A simplified metabolic pathway for alginate degradation and metabolism is shown in FIG. **2**. Alginate can be degraded by at least two different methodologies: enzymatic and chemical methodologies.

[0419] In enzymatic degradation, the degradation of alginate is catalyzed by a family of enzymes called alginate lyases. For this experiment, Atu3025 was used. Atu3025 is an exolytically acting enzyme and yields DEHU from alginate polymer. DEHU is converted to the common hexuronate metabolite, KDG. This reaction is catalyzed by alcohol dehydrogenases (e.g., DEHU hydrogenases).

[0420] Chemical degradation catalyzed by acid solution, such as formate, yields a monosaccharide mannuronate. Mannuronate is then converted to mannonate, which is catalyzed by enzymes with mannonate dehydrogenase (mannuronate reductase) activity. In bacteria, mannonate dehydratase (UxuA) catalyzes dehydration from mannuronate to form KDG.

[0421] KDG is readily metabolized to form of pyruvate and glyceraldehydes-3-phosphate (G3P). KDG is first phosphorylated to KDG-6-phosphate (KDGP), which is catalyzed by KDG kinase, and then broken down to pyruvate and G3P, which is catalyzed by KDGP aldolase.

[0422] Preparation of oligoalginate lyase Atu3025 derived from *Agrobacterium tumefaciens* C58. pETAtu3025 was constructed based on pET29 plasmid backbone (Novagen). The oligoalginate lyase Atu3025 was amplified by PCR: 98° C. for 10 sec, 55° C. for 15 sec, and 72° C. for 60 sec, repeated for 30 times. The reaction mixture contained 1× Phusion buffer, 2 mM dNTP, 0.5 μM forward (5'-GGAATTCCATATGCGTCCCTCTGCCCCGGCC-3') (SEQ ID NO:155) and reverse (5'-CGGGATCCTTAGAACTGCTTGGGAAGGGAG-3') (SEQ ID NO:156) primers, 2.5 U Phusion DNA polymerase (Finezyme), and an aliquot of *Agrobacterium tumefaciens* C58 (gift from Professor Eugene Nester, University of Washington) cells as a template in total volume of 100 μl. The amplified fragment was digested with NdeI and BamHI and ligated into pET29 pre-digested with the same enzymes using T4 DNA ligase to form pETAtu3025. The constructed plasmid was sequenced (Elim Biopharmaceuticals) and the DNA sequence of the insert was confirmed. The nucleotide sequence of the Atu3025 insert is provided in SEQ ID NO:67. The polypeptide sequence encoded by the Atu3025 insert is provided in SEQ ID NO:68.

[0423] The pETAtu3025 was transformed into *Escherichia coli* strain BL21(DE3). A colony of BL21(DE3) containing pETAtu3025 was inoculated into 50 ml of LB media containing 50 μg/ml kanamycin (Km^{50}). This strain was grown in an orbital shaker with 200 rpm at 37° C. The 0.2 mM IPTG was added to the culture when the $OD_{600\ nm}$ reached 0.6, and the induced culture was grown in an orbital shaker with 200 rpm at 20° C. 24 hours after the induction, the cells were harvested by centrifugation at 4,000 rpm×g for 10 min and the pellet was resuspended into 2 ml of Bugbuster (Novagen) containing 10 μl of Lysonase™ Bioprocessing Reagent (Novagen). The solution was again centrifuged at 4,000 rpm×g for 10 min and the supernatant was obtained.

[0424] Construction of pETADH1 through pETADH12. DNA sequences of ADH1-12 of *Agrobacterium tumefaciens* C58 were amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (Table 1) and reverse (Table 1) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Agrobacterium tumefaciens* C58 genome in 50 μl. Amplified DNA fragment was digested with NdeI and BamHI and ligated into pET28 pre-digested with the same restriction enzymes. For DNA sequences with internal NdeI or BamHI site, front and bottom half sequences of each ADH were first amplified using described method. The resulting two DNA fragments were gel purified and spliced by overlapping PCR.

TABLE 1

Primers used to amplify ADH1-12 from *Agrobacterium tumefaciens* C58.

Name	*A. tumefaciens* C58	Forward Primer	Reverse Primer
ADH1	Atu1557	GGAATTCCATATGTTCACAACGTCCGCCTA (SEQ ID NO:276)	GCTTGACGGCCATGTGGCCGAGGCCGC (SEQ ID NO:277)
		GCGGCCTCGGCCACATGGCCGTCAAGC (SEQ ID NO:278)	CGGGATCCTTAGGCGGCCTTCTGGCGCG (SEQ ID NO:279)
ADH2	Atu2022	GGAATTCCATATGGCTATTGCAAGAGGTTA (SEQ ID NO:280)	CGGGATCCTTAAGCGTCGAGCGAGGCCA (SEQ ID NO:281)
ADH3	Atu0626	GGAATTCCATATGACTAAAACAATGAAGGC (SEQ ID NO:282)	CACCGGGGCCGGGGTCCGGTATTGCCA (SEQ ID NO:283)
		TGGCAATACCGGACCCCGGCCCCGGTG (SEQ ID NO:284)	CGGGATCCTTAGGCGGCGAGATCCACGA (SEQ ID NO:285)
ADH4	Atu5240	GGAATTCCATATGACCGGGGCGAACCAGCC (SEQ ID NO:286)	ATAGCCGCTCATACGCCTCGGTTGCCT (SEQ ID NO:287)
		AGGCAACCGAGGCGTATGAGCGGCTAT (SEQ ID NO:288)	CGGGATCCTTAAGCGCCGTGCGGAAGGA (SEQ ID NO:289)
ADH5	Atu3163	GGAATTCCATATGACCATGCATGCCATTCA (SEQ ID NO:290)	CGGGATCCTTATTCGGCTGCAAATTGCA (SEQ ID NO:291)
ADH6	Atu2151	GGAATTCCATATGCGCGCGCTTTATTACGA (SEQ ID NO:292)	CGGGATCCTTATTCGAACCGGTCGATGA (SEQ ID NO:293)

TABLE 1-continued

Primers used to amplify ADH1-12 from *Agrobacterium tumefaciens* C58.

Name	*A. tumefaciens* C58	Forward Primer	Reverse Primer
ADH7	Atu2814	GGAATTCCATATGCTGGCGATTTTCTGTGA (SEQ ID NO:294)	CGGGATCCTTATGCGACCTCCACCATGC (SEQ ID NO:295)
ADH8	Atu5447	GGAATTCCATATGAAAGCCTTCGTCGTCGA (SEQ ID NO:296)	CGGGATCCTTAGGATGCGTATGTAACCA (SEQ ID NO:297)
ADH9	Atu4087	GGAATTCCATATGAAAGCGATTGTCGCCCA (SEQ ID NO:298)	CGGGATCCTTAGGAAAAGGCGATCTGCA (SEQ ID NO:299)
ADH10	Atu4289	GGAATTCCATATGCCGATGGCGCTCGGGCA (SEQ ID NO:300)	CGGGATCCTTAGAATTCGATGACTTGCC (SEQ ID NO:301)
ADH11	Atu3027	GGAATTCCATATGAAACATTCTCAGGACAA (SEQ ID NO:302)	GGGCGCCGATCATGTGGTGCGTTTCCG (SEQ ID NO:303)
		CGGAAACGCACCACATGATCGGCGCCC (SEQ ID NO:304)	CGGGATCCTTATGCCATACGTTCCATAT (SEQ ID NO:305)
ADH12	Atu3026	GGAATTCCATATGCAGCGTTTTACCAACAG (SEQ ID NO:306)	CGGGATCCTTAGGAAAACAGGACGCCGC (SEQ ID NO:307)

Expression and Purification of ADH 1-10.

[0425] All plasmids were transformed into *Escherichia coli* strain BL21(DE3). The single colonies of BL21(DE3) containing respective alcohol dehydrogenase (ADH) genes were inoculated into 50 ml of LB media containing 50 μg/ml kanamycin (Km^{50}). These strains were grown in an orbital shaker with 200 rpm at 37° C. The 0.2 mM IPTG was added to each culture when the $OD_{600\ nm}$ reached 0.6, and the induced culture was grown in an orbital shaker with 200 rpm at 20° C. 24 hours after the induction, the cells were harvested by centrifugation at 4,000 rpm×g for 10 min and the pellet was resuspended into 2 ml of Bugbuster (Novagen) containing 10 μl of Lysonase™ Bioprocessing Reagent (Novagen). The solution was again centrifuged at 4,000 rpm×g for 10 min and the supernatant was obtained.

Preparation of ~2% DEHU Solution by Enzymatic Degradation.

[0426] DEHU solution was enzymatically prepared. A 2% alginate solution was prepared by adding 10 g of low viscosity alginate into the 500 ml of 20 mM Tris-HCl (pH7.5) solution. An approximately 10 mg of alginate lyase derived from *Flavobacterium* sp. (purchased from Sigma-aldrich) was added to the alginate solution. 250 ml of this solution was then transferred to another bottle and the *E. coli* cell lysate containing Atu3025 prepared above section was added. The alginate degradation was carried out at room temperature over night. The resulting products were analyzed by thin layer chromatography, and DEHU formation was confirmed.

Preparation of D-Mannuronate Solution by Chemical Degradation.

[0427] D-mannuronate solution was chemically prepared based on the protocol previously described by Spoehr (Archive of Biochemistry, 14: pp 153-155). Fifty milligram of alginate was dissolved into 800 μL of ninety percent formate. This solution was incubated at 100° C. for over night. Formate was then evaporated and the residual substances were washed with absolute ethanol twice. The residual substance was again dissolved into absolute ethanol and filtrated. Ethanol was evaporated and residual substances were resuspended into 20 mL of 20 mM Tris-HCl (pH 8.0) and the solution was filtrated to make a D-mannuronate solution. This D-mannuronate solution was diluted 5-fold and used for assay.

Assay for DEHU Hydrogenase.

[0428] To identify DEHU hydrogenase, a NADPH dependent DEHU hydrogenation assay was performed. 20 μl of prepared cell lysate containing each ADH was added to 160 μl of 20-fold deluted DEHU solution prepared in the above section. 20 μl of 2.5 mg/ml of NADPH solution (20 mM Tris-HCl, pH 8.0) was added to initiate the hydrogenation reaction, as a preliminary study using cell lysate of *A. tumefaciens* C58 have shown that DEHU hydrogenation requires NADPH as a co-factor. The consumption of NADPH was monitored an absorbance at 340 nm for 30 min using the kinetic mode of ThermoMAX 96 well plate reader (Molecular Devises). *E. coli* cell lysate containing alcohol dehydrogenase (ADH) 10 lacking a portion of N-terminal domain was used in a control reaction mixture.

Assay for D-Mannuronate Hydrogenase.

[0429] To identify D-mannuronate hydrogenase, a NADPH dependent D-mannuronate hydrogenation assay was performed. 20 μl of prepared cell lysate containing each ADH was added to 160 μl of D-mannuronate solution prepared in the above section. 20 μl of 2.5 mg/ml of NADPH solution (20 mM Tris-HCl, pH 8.0) was added to initiate the hydrogenation reaction. The consumption of NADPH was monitored an absorbance at 340 nm for 30 min using the kinetic mode of ThermoMAX 96 well plate reader (Molecular Devises). *E. coli* cell lysate containing alcohol dehydrogenase (ADH) 10 lacking a portion of N-terminal domain was used in a control reaction mixture.

Construction of pETkdgK.

[0430] DNA sequence of kdgK of *Escherichia coli* encoding 2-keto-deoxy gluconate kinase was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction

mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-AGGTACGGTGAAATAA AGGAGG ATATACATATGTCCAAAAAGATTGCCGT-3') (SEQ ID NO:157) and reverse (5'-TTTTCCTTTT GCGGCCGCCCCGCTGGCATCGCCTCAC-3') (SEQ ID NO:158) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* DH10B genome in 50 μl. Amplified DNA fragment was digested with NdeI and NotI and ligated into pET29 pre-digested with the same restriction enzymes.

Construction of pETkd2A.

[0431] DNA sequence of kdgA Escherichi coli encoding 2-keto-deoxy gluconate-6-phosphate aldolase was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-GGCGATGCCAGCGTAA AGGAGG ATATA CATATGAAAAACTGGAAAACAAG-3') (SEQ ID NO:159) and reverse (5'-TTTTCCTTTT GCGGCCGCCCCAGCTTAGCGCCTTCTA-3') (SEQ ID NO:160) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* DH10B genome in 50 μl. Amplified DNA fragment was digested with NdeI and NotI and ligated into pET29 pre-digested with the same restriction enzymes.

Protein Expression and Purification.

[0432] All plasmids (pETAtu3025, pETADH11, pETADH12, pETkdgA, pETkdgK, and pETuxuA) were transformed into *Escherichia coli* strain BL21(DE3). The single colonies of BL21(DE3) containing respective plasmids were inoculated into 50 ml of LB media containing 50 μg/ml kanamycin (KM^{50}). These strains were grown in an orbital shaker with 200 rpm at 37° C. The 0.2 mM IPTG was added to each culture when the $OD_{600\ nm}$ reached 0.6, and the induced culture was grown in an orbital shaker with 200 rpm at 20° C. 24 hours after the induction, the cells were harvested by centrifugation at 4,000 rpm×g for 10 min and the pellet was resuspended into 2 ml of Bugbuster (Novagen) containing 10 μl of Lysonase™ Bioprocessing Reagent (Novagen) and suggested amount of protease inhibitor cocktail (SIGMA). The solution was again centrifuged at 4,000 rpm×g for 10 min and the supernatant was obtained. The supernatant was applied to Nickel-NTA spin column (Qiagen) to purify His-tagged proteins.

[0433] The results of the assays for DEHU hydrogenase activity and D-mannuronate hydrogenase activity of ADH1-10 are shown in FIGS. **7**A and **7**B. These results demonstrate that the novel enzymes ADH1 and ADH2 showed significant DEHU hydrogenase activity (FIG. **7**A), and that the novel enzymes ADH3, ADH4, and ADH9 showed significant mannuronate hydrogenase activity (FIG. **7**B).

In Vitro Pyruvate Formation.

[0434] The reaction mixture contained 1% alginate or ~0.5% mannuronate, ~5 ug of purified Atu3026 (ADH12) or Atu3027 (ADH11), and ~5 ug of purified oligoalginate lyase (Atu3025), UxuA, KdgK, and KdgA, 2 mM of ATP, and 0.6 mM of NADPH in 20 mM Tris-HCl pH7.0. The reaction was carried out over night and the pyruvate formation was monitored by the pyruvate assay kit (BioVision, Inc).

[0435] The results of in vitro pyruvate formation from alginate mediated by enzymatic and chemical degradation are shown in FIG. **6**B and FIG. **6**C, respectively. As can be seen in these figures, alginate was converted to pyruvate via the isolated enzymes. These results also show that each of Atu3026 (ADH12) and Atu3027 (ADH11) are capable of catalyzing both DEHU hydrogenase and mannuronate hydrogenase reactions.

Example 4

Construction and Biological Activity of Biosynthesis Pathways

Construction of Pathways:

[0436] A propionaldehyde biosynthetic pathway comprising a threonine deaminase (ilvA) gene from *Escherichia coli* and keto-isovalerate decarboxylase (kivd) from *Lactococcus lactis* is constructed and tested for the ability to convert L-threonine to propionaldehyde.

[0437] A butyraldehyde biosynthetic pathway comprising a thiolase (atoB) gene from *E. coli*, β-hydroxy butyryl-CoA dehydrogenase (hbd), crotonase (crt), butyryl-CoA dehydrogenase (bcd), electron transfer flavoprotein A (etfA), and electron transfer flavoprotein B (etfB)genes from *Clostridium acetobutyricum* ATCC 824, and a coenzyme A-linked butyraldehyde dehydrogenase (ald) gene from *Clostridium beijerinckii acetobutyricum* ATCC 824 was constructed in *E. coli* and tested for the ability to produce butyraldehyde. Also, a coenzyme A-linked alcohol dehydrogenase (adhE2) gene from *Clostridium acetobutyricum* ATCC 824 was used as an alternative to ald and tested for the ability to produce butanol.

[0438] An isobutyraldehyde biosynthetic pathway comprising an acetolactate synthase (alsS) from *Bacillus subtilis* or (als) from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (codon usage was optimized for *E. coli* protein expression) and acetolactate reductoisomerase (ilvC) and 2,3-dihydroxyisovalerate dehydratase (ilvD), genes from *E. coli* and keto-isovalerate decarboxylase (kivd) from *Lactococcus lactis* was constructed and tested for the ability to produce isobutyraldehyde, as measured by isobutanal production.

[0439] 3-methylbutyraldehyde and 2-methylbutyraldehyde biosynthesis pathways comprising an acetolactate synthase (alsS) from *Bacillus subtilis* or (als) from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (codon usage was optimized for *E. coli* protein expression), acetolactate reductoisomerase (ilvC), 2,3-dihydroxyisovalerate dehydratase (ilvD), isopropylmalate synthase (LeuA), isopropylmalate isomerase (LeuC and LeuD), and 3-isopropylmalate dehydrogenase (LeuB) genes from *E. coli* and keto-isovalerate decarboxylase (kivd) from *Lactococcus lactis* were constructed and tested for the ability to produce 3-isovaleraldehyde and 2-isovaleraldehyde.

[0440] Phenylacetoaldehyde and 4-hydroxyphenylacetoaldehyde biosynthesis pathways comprising a transketolase (tktA), a 3-deoxy-7-phosphoheptulonate synthase (aroF, aroG, and aroH), 3-dehydroquinate synthase (aroB), a 3-dehydroquinate dehydratase (aroD), a dehydroshikimate reductase (aroE), a shikimate kinase II (aroL), a shikimate kinase I (aroK), a 5-enolpyruvylshikimate-3-phosphate synthetase (aroA), a chorismate synthase (aroC), a fused chorismate mutase P/prephenate dehydratase (pheA), and a fused chorismate mutase T/prephenate dehydrogenase (tyrA) genes from

E. coli, keto-isovalerate decarboxylase (kivd) from *Lactococcus lactis* were constructed and tested for the ability to produce phenylacetoaldehyde and/or 4-hydroxyphenylacetoaldehyde.

[0441] A 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and 2-(indole-3-)ethanol biosynthesis pathway comprising a transketolase (tktA), a 3-deoxy-7-phosphoheptulonate synthase (aroF, aroG, and aroH), 3-dehydroquinate synthase (aroB), a 3-dehydroquinate dehydratase (aroD), a dehydroshikimate reductase (aroE), a shikimate kinase II (aroL), a shikimate kinase I (aroK), a 5-enolpyruvylshikimate-3-phosphate synthetase (aroA), a chorismate synthase (aroC), a fused chorismate mutase P/prephenate dehydratase (pheA), and a fused chorismate mutase T/prephenate dehydrogenase (tyrA) genes from *E. coli*, keto-isovalerate decarboxylase (kivd) from *Lactococcus* lactis, alcohol dehydrogenase (adh2) from *Saccharomyces cerevisiae*, Indole-3-pyruvate decarboxylase (ipdc) from *Azospirillum brasilense*, phenylethanol reductase (par) from *Rhodococcus* sp. ST-10, and benzaldehyde lyase (bal) from *Pseudomonas fluorescence* was constructed and tested for the ability to produce 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol and/or 2-(indole-3)ethanol.

Construction of pBADButP.

[0442] The DNA sequence encoding hbd, crt, bcd, etfA, and etfB of *Clostridium acetobutyricum* ATCC 824 was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 3 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCC GAGCTCTTAGGAGGATTAGTCATGGAAC-3') (SEQ ID NO:161) and reverse (5'-GCTCTAGA TTATTTTGAATAATCGTAGAAACC-3') (SEQ ID NO:162) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Clostridium acetobutyricum* ATCC 824 genome (ATCC) in 50 μl. Amplified DNA fragment was digested with BamHI and XbaI and ligated into pBAD33 pre-digested with the same restriction enzymes.

Construction of pBADButP-atoB.

[0443] The DNA sequence encoding atoB of *Escherichia coli* DH10B was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-GC TCTAGAGGAGGATATATATATGAAAAATTGTGTCATC GTC-3') (SEQ ID NO:163) and reverse (5'-AA CTGCAGTTAATTCAACCGTTCAATCACC-3') (SEQ ID NO:164) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* DH10B genome in 50 μl. Amplified DNA fragment was digested with XbaI and PstI and ligated into pBADButP pre-digested with the same restriction enzymes.

Construction of pBADatoB-ald.

[0444] The DNA sequence encoding atoB of *Escherichia coli* DH10B and ald from *Clostridium beijerinckii* were amplified separately by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CGAGCTC AGGAGGATATATATATGAAAAATTGTGTCATCGTCAGTG-3') (SEQ ID NO:165) for atoB and 5'-GGTTGAATTAAGGAGGATATATATATGAATAAAGACACACTAATACCTAC-3' for ald) (SEQ ID NO:166) and reverse (5'-GTCTTTATTCATATATATATCCTCCTTAATTCAACCGTTCAATCACCATC-3' (SEQ ID NO:146) for atoB and 5'-CCCAAGCTTAGCCGGCAAGTACACATCTTC-3' for ald) (SEQ ID NO:167) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* DH10B and *Clostridium beijerinckii* genome (ATCC) in 501, respectively. The amplified DNA fragments were gel purified and eluted into 30 ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 2 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CGAGCTC AGGAGGATATATATATGAAAAATTGTGTCATCGTCAGTG-3') (SEQ ID NO:168) and reverse (5'-CCCAAGCTTAGCCGGCAAGTACACATCTTC-3') (SEQ ID NO:169) primers, 1 U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was digested with SacI and HindIII and ligated into pBADButP pre-digested with the same restriction enzymes.

Construction of pBADButP-atoB-ALD.

[0445] The DNA fragment 1 encoding chloramphenicol acetyltransferase (CAT), P15 origin of replication, araBAD promoter, atoB of *Escherichia coli* DH10B and ald of *Clostridium beijerinckii* and the DNA fragment 2 encoding araBAD promoter, hbd, crt, bcd, etfA, and etfB of *Clostridium acetobutyricum* ATCC 824 were amplified separately by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 4 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-AAGGAAAAAA GCGGCCGCCCCTGAACCGACGACCGGGTCG-3') (SEQ ID NO:170) for fragment 1 and 5'-CGG GGTACCACTTTTCATACTCCCGCCATTCAG-3' (SEQ ID NO:274) for fragment 2, and reverse (5'-CGG GGTACCGCGGATACATATTTGAATGTATTTAG-3') (SEQ ID NO:171) for fragment 1 and (5'-AAGGAAAAAA GCGGCCGCGCGGATACATATTTGAATGTATTTAG-3') (SEQ ID NO:172) for fragment 2) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pBADatoB-ald and pBADButP in 50 μl, respectively. Amplified DNA fragments were digested with NotI and KpnI and ligated each other.

Construction of pBADilvCD.

[0446] The DNA fragments encoding ilvC and ilvD of *Escherichia coli* DH10B were amplified separately by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-GCTCTAGAGGAGGATATATATAT GGCTAACTACTTCAATACAC-3') (SEQ ID NO:173) for ilvC and 5'-TGCTGTTGCGGGTTAAGGAG-GATATATATATGCCTAAGTACCGTTCCGCC-3' for ilvD) (SEQ ID NO:174) and reverse (5'-AACGGTACTTAG-GCATATATATATCCTCCTTAACCCGCAA-CAGCAATACG-3') (SEQ ID NO:175) for ilvC and 5'-AC ATGCATGCTTAACCCCCCAGTTTCGATT-3') (SEQ ID NO:176) for ilvD) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* DH10B genome (ATCC) in 50 μl. The amplified DNA fragments were gel purified and eluted into 30 ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 2 min, repeated 30 times. The

reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-GCTCTAGAGGAGGATATATA TATGGCTAACTACTTCAATACAC-3') (SEQ ID NO:177) and reverse (5'-AC ATGCATGCTTAACCCCCCAGTTTCGATT-3') (SEQ ID NO:178) primers, 1 U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was digested with XbaI and SphI and ligated into pBAD33 pre-digested with the same restriction enzymes.

Construction of pBADals-ilvCD.

[0447] The DNA fragment encoding als of *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 of its codon usage optimized for over-expression in *E. coli* was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCCGAGCTCAGGAGGATATATATAT GGATAAACAGTATCCGGT-3') (SEQ ID NO:179) and reverse (5'-GC TCTAGATTACAGAATTTGACTCAGGT-3') (SEQ ID NO:180) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pETals in 50 μl. The amplified DNA fragment was digested with SacI and XbaI and ligated into pBADilvCD pre-digested with the same restriction enzymes.

Construction of pBADalsS-ilvCD.

[0448] The DNA fragments encoding front and bottom halves of alsS of *Bacillus subtilis* B26 were amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 0.5 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCCGAGCTCAGGAGGATATATATATG TTGACAAAAGCAACAAAAG-3') (SEQ ID NO:181) for front and 5'-CGGTACCCTTTCCAGAGATTTAGAG-3' (SEQ ID NO:275) for back halves, and reverse (5'-CTCTAAATCTCTGGAAAGGGTACCG-3') (SEQ ID NO:182) for front and (5'-GC TCTAGATTAGAGAGCTTTCGTTTTCATG-3' for back halves) (SEQ ID NO:183) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Bacillus subtilis* B26 genome (ATCC) in 50 μl. The amplified DNA fragments were gel purified and eluted into 30 ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCCGAGCTCAGGAGGAT ATATATATGTTGACAAAAGCAACAAAAG-3') (SEQ ID NO:184) and reverse (5'-GC TCTAGATTAGAGAGCTTTCGTTTTCATG-3') (SEQ ID NO:185) primers, 1 U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was internal XbaI site free and thus was digested with SacI and XbaI and ligated into pBADilvCD pre-digested with the same restriction enzymes.

Construction of pBADLeuABCD.

[0449] The DNA fragment encoding leuA, leuB, leuC, and leuD of *Escherichia coli* BL21(DE3) was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 3 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CGAGCTCAGGAGGATATATATATGAG CCAGCAAGTCATTATTTTCG-3') (SEQ ID NO:186) and reverse (5'-AAAA CTGCAGCGTTTGATGACGTGGACGATAGCGG-3') (SEQ ID NO:187) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* BL21(DE3) genome in 50 μl. The amplified DNA fragment was digested with SacI and XbaI and ligated into pBAD33 pre-digested with the same restriction enzymes.

Construction of pBADLeuABCD2.

[0450] The DNA fragment 1 encoding leuA and leuB and the DNA fragment 2 encoding leuC and leuD of *Escherichia coli* BL21 (DE3) were amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CG AGCTCAGGAGGATATATATATGAGCCAGCAAGTCAT TATTTTCG-3') (SEQ ID NO:188) for fragment 1 and (5'-AGGGGTGTAAGGAGGATATATATATG-GCTAAGACGTTATACGAAAAATTG-3') (SEQ ID NO:189) for fragment 2 and reverse (5'-CGTCTTAGC-CATATATATATCCTCCTTACACCCCT-TCTGCTACATAGCGG-3') (SEQ ID NO:190) for fragment 1 and (5'-AAAA CTGCAGCGTTTGATGACGTGGACGATAGCGG-3') (SEQ ID NO:191) for fragment 2 primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* BL21(DE3) genome in 50 μl, respectively. The amplified DNA fragments were gel purified and eluted into 30 ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 3 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CG AGCTCAGGAGGATATATATATGAGCCAGCAAGTCAT TATTTTCG-3') (SEQ ID NO:192) and reverse (5'-AAAA CTGCAGCGTTTGATGACGTGGACGATAGCGG-3') (SEQ ID NO:193) primers, 1 U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was digested with SacI and XbaI and ligated into pBAD33 pre-digested with the same restriction enzymes.

Construction of pBADLeuABCD4.

[0451] The DNA fragments encoding leuA, leuB, leuC and leuD of *Escherichia coli* BL21(DE3) were amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CGAGCTCAGGAGGATATATATATGAG CCAGCAAGTCATTATTTTCG-3') (SEQ ID NO:194) for leuA, (5'-GAAACCGTGTGAGGAGGATATATATAT-GTCGAAGAATTACCATATTGCCG-3') (SEQ ID NO:195) for leuB, (5'-AGGGGTGTAAGGAGGATATATATATG-GCTAAGACGTTATACGAAAAATTG-3') (SEQ ID NO:196) for leuC, and (5'-ACATTAAATAAGGAG-GATATATATATGGCAGAGAAATTTATCAAACACAC-3') (SEQ ID NO:197) for leuD and reverse (5'-ATTCTTCGA-CATATATATATCCTCCTCACACGGTTTC-CTTGTTGTTTTCG-3') (SEQ ID NO:198) for leuA, (5'-CGTCTTAGCCATATATATATCCTCCTTACACCCCTTCT GCTACATAGCGG-3') (SEQ ID NO:199) for leuB, (5'-TTTCTCTGCCATATATATATCCTCCT-TATTTAATGTTGCGAATGTCGGCG-3') (SEQ ID NO:200) for leuC, and (5'-AAAACTGCAGCGTTTGAT-GACGTGGACGATAGCGG-3') (SEQ ID NO:201) for leuD primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* BL21(DE3) genome in 50 μl, respectively. The amplified DNA fragments were gel purified and eluted into 30 ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98° C. for 10 sec, 60° C. for 15 sec, and

72° C. for 3 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CGAGCTCAGGAGGATATATATATGAGCCA GCAAGTCATTATTTTCG-3') (SEQ ID NO:202) and reverse (5'-AAAA CTGCAGCGTTTGATGACGTGGACGATAGCGG-3') (SEQ ID NO:203) primers, 1 U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was digested with SacI and XbaI and ligated into pBAD33 pre-digested with the same restriction enzymes.

Construction of pBADals-ilvCD-leuABCD, pBADals-ilvCD-leuABCD2 pBADals-ilvCD-leuABCD4 pBADalsS-ilvCD-leuABCD, pBADalsS-ilvCD-leuABCD2 pBADalsS-ilvCD-leuABCD4.

[0452] The DNA fragments 1 (for als) and 2 (for alsS) encoding chloramphenicol acetyltransferase (CAT), P15 origin of replication, araBAD promoter, als of *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 of its codon usage optimized for over-expression in *E. coli* or alsS of *Bacillus subtilis* B26 and ilvC and ilvD of *E. coli* DH 10B were amplified separately by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 4 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-AAG-GAAAAAA GCGGCCGCCCCTGAACCGACGACCGGGTCG-3') (SEQ ID NO:204) and reverse (5'-CGG GGTACCGCGGATACATATTTGAATGTATTTAG-3') (SEQ ID NO:205) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pBADals-ilvCD and pBAD-alsS-ilvCD in 50 μl, respectively.

[0453] To remove an internal SphI restriction enzyme site form leuC, overlap PCR was carried out. The front and bottom halves of DNA fragment 3 (for leuABCD), fragment 4 (for leuABCD2), and fragment 5 (for leuABCD4) encoding araBAD promoter, leuA, leuB, leuC, and leuD of *E. coli* BL21(DE3) were amplified separately by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 4 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-AAGGAAAAAA GCGGCCGCACTTTTCATACTCCCGCCATTCAG-3') (SEQ ID NO:206) for front and (5'-CAAAGGCCGTCTG-CACGCGCCGAAAGGCAAA-3') (SEQ ID NO:207) for back halves) and reverse (5'-TTTGCCTTTCGGCGCGTG-CAGACGGCCTTTG-3') (SEQ ID NO:208) for front and (5'-AC ATGCATGCCGTTTGATGACGTGGACGATAGCGG-3') (SEQ ID NO:209) for bottom halves, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pBA-DleuABCD, pBADleuABCD2, and pBADleuABCD4 in 50 μl, respectively. The amplified DNA fragments were gel purified and eluted into 30 ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 4 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-AAGGAAAAAA GCGGCCGCACTTTTCATACTCCCGCCATTCAG-3') (SEQ ID NO:210) and reverse (5'-AC ATGCATGCCGTTTGATGACGTGGACGATAGCGG-3') (SEQ ID NO:211) primers, 1 U Phusion High Fidelity DNA polymerase (NEB). The resulting fragment 3, 4, and 5 were digested with SphI and NotI and ligated into both fragment 1 and 2 pre-digested with the same restriction enzymes.

Construction of pBADaroG-tktA-aroBDE.

[0454] The DNA fragments encoding aroG, tktA, aroB, aroD, and aroE of *Escherichia coli* BL21(DE3) were amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCC GAGCTCAGGAGGATATATAT ATGAATTATCAGAAC-GACGATTTAC-3') (SEQ ID NO:212) for aroG, (5'-GCGTCGCGGGTAAGGAGGAAAATTTTAT-GTCCTCACGTAAAGAGCTTGCC-3') (SEQ ID NO:213) for tktA, (5'-GAACTGCTGTAAGGAGGTTAAAATTATG-GAGAGGATTGTCGTTACTCTCG-3') (SEQ ID NO:214) for aroB,}(5'-CAATCAGCGTAAGGAGGTATATATAAT-GAAAACCGTAACTGTAAAAGATC-3') (SEQ ID NO:215) for aroD, and (5'-TACACCAGGCATAAGGAG-GAATTAATTATGGAAACCTATGCTGTTTTTGG-3') (SEQ ID NO:216) for aroE and reverse (5'-TACGTGAGGA-CATAAAATTTTCCTCCTTACCCGC-GACGCGCTTTTACTGC-3') (SEQ ID NO:217) for aroG, (5'-CAATCCTCTCCATAATTTTAACCTCCT-TACAGCAGTTCTTTTGCTTTCGC-3') (SEQ ID NO:218) for tktA, (5'-CAATCAGCGTAAGGAGGTATATATAAT-GAAAACCGTAACTGTAAAAGATC-3') (SEQ ID NO:219) for aroB, (5'-TACGGTTTTCATTATATATACCTC-CTTACGCTGATTGACAATCGGCAATG-3') (SEQ ID NO:220) for aroD, and (5'-AC ATGCATGCTTACGCGGACAATTCCTCCTGCAA-3') (SEQ ID NO:221) for aroE, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* BL21(DE3) genome in 50 μl, respectively. The amplified DNA fragments were gel purified and eluted into 30 ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 3 min, repeated 30 times. The reaction mixture contained 1×Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCCGAGCTCAG GAGGATATATATATGAATTATCAGAACGACGATTTAC-3') (SEQ ID NO:222) and reverse (5'-AC ATGCATGCTTACGCGGACAATTCCTCCTGCAA-3') (SEQ ID NO:223) primers, 1 U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was digested with SacI and SphI and ligated into pBAD33 pre-digested with the same restriction enzymes.

Construction of pBADpheA-aroLAC.

[0455] The DNA fragments encoding pheA, aroL, aroA, and aroC of *Escherichia coli* DH10 were amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCCGAGCTCAGGAGGATATATATATG ACATCGGAAAACCCGTTACTGG-3') (SEQ ID NO:224) for pheA, (5'-GATCCAACCTAAGGAGGAAAATTTTAT-GACACAACCTCTTTTTCTGATCG-3') (SEQ ID NO:225) for aroL, (5'-GATCAATTGTTAAGGAGG-TATATATAATGGAATCCCTGACGTTACAACCC-3') (SEQ ID NO:226) for aroA, and (5'-CAGGCAGCCTAAG-GAGGAATTAATTATGGCTGGAAACA-CAATTGGACAAC-3') (SEQ ID NO:227) for aroC and reverse (5'-AGGTTGTGTCATAAAATTTTCCTCCT-TAGGTTGGATCAACAGGCACTACG-3') (SEQ ID NO:228) for pheA, (5'-CAGGGATTCCATTATATATAC-

CTCCTTAACAATTGATCGTCTGTGCCAGG-3') (SEQ ID NO:229) for aroL, (5'-GTTTCCAGCCATAATTAATTC-CTCCTTAGGCTGCCTGGCTAATCCGCGCC-3') (SEQ ID NO:230) for aroA, and (5'-AC ATGCATGCTTACCAGCGTGGAATATCAGTCTTC-3') (SEQ ID NO:231) for aroC primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* BL21(DE3) genome in 50 μl, respectively. The amplified DNA fragments were gel purified and eluted into 30 ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 4 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCC GAGCTCAGGAGGATATATATATGACATCGGAAAACC CGTTACTGG-3') (SEQ ID NO:232) and reverse (5'-AC ATGCATGCTTACCAGCGTGGAATATCAGTCTTC-3') (SEQ ID NO:233) primers, 1 U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was digested with SacI and SphI and ligated into pBAD33 pre-digested with the same restriction enzymes.

Construction of pBADtyrA-aroLAC.

[0456] The DNA fragments encoding pheA, aroL, aroA, and aroC of *Escherichia coli* DH10 were amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCCGAGCTCAGGAGGATATATATATGG TTGCTGAATTGACCGCATTAC-3') (SEQ ID NO:234) for tyrA, (5'-AATCGCCAGTAAGGAGGAAAATTTTAT-GACACAACCTCTTTTTCTGATCG-3') (SEQ ID NO:235) for aroL, (5'-GATCAATTGTTAAGGAGG-TATATATAATGGAATCCCTGACGTTACAACCC-3') (SEQ ID NO:236) for aroA, and (5'-CAGGCAGCCTAAG-GAGGAATTAATTATGGCTGGAAACA-CAATTGGACAAC-3') (SEQ ID NO:237) for aroC, and reverse (5'-GAGGTTGTGTCATAAAATTTTCCTCCT-TACTGGCGATTGTCATTCGCCTG-3') (SEQ ID NO:238) for tyrA, (5'-CAGGGATTCCATTATATATACCTCCT-TAACAATTGATCGTCTGTGCCAGG-3') (SEQ ID NO:239) for aroL, (5'-GTTTCCAGCCATAATTAATTC-CTCCTTAGGCTGCCTGGCTAATCCGCGCC-3') (SEQ ID NO:240) for aroA, and (5'-AC ATGCATGCTTACCAGCGTGGAATATCAGTCTTC-3') (SEQ ID NO:241) for aroC, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* BL21(DE3) genome in 50 μl, respectively. The amplified DNA fragments were gel purified and eluted into 30 ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 4 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCCGAGCTCAGG AGGATATATATATGGTTGCTGAATTGACCGCATTAC-3') (SEQ ID NO:242) and reverse (5'-AC ATGCATGCTTACCAGCGTGGAATATCAGTCTTC-3') (SEQ ID NO:243) primers, 1 U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was digested with SacI and SphI and ligated into pBAD33 pre-digested with the same restriction enzymes.

Construction of pBADpheA-aroLAC-aroG-tktA-aroBDE and pBADtyrA-aroLAC-aroG-tktA-aroBDE.

[0457] A DNA fragment 1 (for pheA) and 2 (for tyrA) encoding chloramphenicol acetyltransferase (CAT), P15 origin of replication, araBAD promoter, pheA or tyrA, aroL, aroA, aroC of *Escherichia coli* DH10B and a DNA fragment 3 encoding araBAD promoter, aroG, tktA, aroB, aroD, and aroE of *Escherichia coli* DH10B were amplified separately by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 4 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-AAGGAAAAAA GCGGCCGCCCCTGAACCGACGACCGGGTCG-3') (SEQ ID NO:244) for fragment 1 and 2 and (5'-GC TCTAGAACTTTTCATACTCCCGCCATTCAG-3') (SEQ ID NO:245) for fragment 3, and reverse (5'-GC TCTAGAGCGGATACATATTTGAATGTATTTAG-3') (SEQ ID NO:246) for fragment 1 and 2 and (5'-AAG-GAAAAAA GCGGCCGCGCGGATACATATTTGAATGTATTTAG-3') (SEQ ID NO:247) for fragment 3, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pBADpheA-aroLAC, pBADtyrA-aroLAC, and pBADaroG-tktA-aroBDE in 50 μl, respectively. Amplified DNA fragments 1 and 2 were digested with NotI and XbaI and ligated into fragment 3 pre-digested with the same restriction enzymes.

Construction of pTrcBAL.

[0458] A DNA sequence encoding benzaldehyde lyase (bal) of *Pseudomonas fluorescens* of its codon usage optimized for over-expression in *E. coli* was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CATG CCATGGCTATGATTACTGGTGG-3') (SEQ ID NO:248) and reverse (5'-CCCC GAGCTCTTACGCGCCGGATTGGAAATACA-3') (SEQ ID NO:249) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pETBAL in 50 μl. Amplified DNA fragment was digested with NcoI and SacI and ligated into pTrc99A pre-digested with the same restriction enzymes.

Construction of pTrcAdhE2.

[0459] A DNA sequence encoding Co-A linked alcohol/aldehyde dehydrogenase (adhE2) of *Clostridium acetobutyricum* ATCC824 was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CATGCCATGGCCAAAGTTACAAATCAAAAAG-3') (SEQ ID NO:250) and reverse (5'-C GAGCTCTTAAAATGATTTTATATAGATATCC-3') (SEQ ID NO:251) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Clostridium acetobutyricum* ATCC824 genome in 50 μl. Amplified DNA fragment was digested with NcoI and SacI and ligated into pTrc99A pre-digested with the same restriction enzymes.

Construction of pTrcAdh2.

[0460] A DNA sequence encoding alcohol dehydrogenase (adh2) of *Saccharomyces cerevisiae* was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CATG CCATGGGTATTCCAGAAACTCAAAAAG-3') (SEQ ID NO:252) and reverse (5'-CCC GAGCTCTTATTTAGAAGTGTCAACAACG-3') (SEQ ID NO:253) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng genome of *Saccharomyces cerevi-*

siae in 50 μl. Amplified DNA fragment was digested with NcoI and SacI and ligated into pTrc99A pre-digested with the same restriction enzymes.

Construction of pTrcBALD.

[0461] A DNA sequence encoding CoA-linked aldehyde dehydrogenase (ald) of *Clostridium beijerinckii* was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCCCGAGCTCAGGAGG ATATACATATGAATAAAGACACACTAATACC-3') (SEQ ID NO:254) and reverse (5'-CCC AAGCTTAGCCGGCAAGTACACATCTTC-3') (SEQ ID NO:255) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pETBAL in 50 μl. Amplified DNA fragment was digested with SacI and HndIII and ligated into pTrcBAL pre-digested with the same restriction enzymes.

Construction of pTrcBALK.

[0462] A DNA sequence encoding ketoisovalerate decarboxylase (kivd) of *Lactococcus lavtis* was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCCGAGCTCAGGAGGATATATATAT GTATACAGTAGGAGATTACC-3') (SEQ ID NO:256) and reverse (5'-GC TCTAGATTATGATTTATTTTGTTCAGCAAAT-3') (SEQ ID NO:257) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pETBAL in 50 μl. Amplified DNA fragment was digested with SacI and XbaI and ligated into pTrcBAL pre-digested with the same restriction enzymes.

Construction of pTrcAdh-Kivd.

[0463] A DNA sequence encoding ketoisovalerate decarboxylase (kivd) of *Lactococcus lavtis* was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCCGAGCTCAGGAGGATATATATATG TATACAGTAGGAGATTACC-3') (SEQ ID NO:258) and reverse (5'-GC TCTAGATTATGATTTATTTTGTTCAGCAAAT-3') (SEQ ID NO:259) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pETBAL in 50 μl. Amplified DNA fragment was digested with SacI and XbaI and ligated into pTrcAdh2 pre-digested with the same restriction enzymes.

Construction of pTrcBAL-DDH-2ADH.

[0464] To remove internal NcoI site, overlap PCR was carried out. DNA fragments encoding front and bottom halves of meso-2,3-butanedioldehydrogenase (ddh) of *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 and secondary alcohol dehydrogenase (2adh) of *Pseudomanas fluorescens* were amplified separately by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-C GAGCTCAGGAGGATATATATATGAAAAAAGTCGCAC TTGTTACCG-3') (SEQ ID NO:260) for front half of ddh, (5'-GGCCGGCGGCCGCGCGATGGCGGTGAAAGTG-3') (SEQ ID NO:261) for bottom half of ddh, (5'-AAC-TAATCTAGAGGAGGATATATATATGAG-CATGACGTTTTCCGGCCAGG-3') (SEQ ID NO:262) for front half of 2adh, and (5'-CCTTGCGGAGGGCTCGATG-GATGAGTTCGAC-3') (SEQ ID NO:263) for bottom half of 2adh, and reverse (5'-CACTTTCACCGCCATCGCGCGGC-CGCCGGCC-3') (SEQ ID NO:264) for front half of ddh, (5'-GCTCATATATATATCCTCCTCTAGATT-AGTTAAACACCATCCCGCCGTCG-3') (SEQ ID NO:265) for bottom half of ddh, (5'-GTCGAACTCATCCATCGAGC-CCTCCGCAAGG-3') (SEQ ID NO:266) for front half of 2adh, and (5'-CCC AAGCTTAGATCGCGGTGGCCCCGCCGTCG-3') (SEQ ID NO:267) for bottom half of 2adh, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Kiebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 for ddh and *Pseudomanas fluorescens* genome for 2adh in 50 μl, respectively. The amplified DNA fragments were gel purified and eluted into 30 ul of EB buffer (Qiagen). 5 ul from each DNA solution was combined and each DNA fragment was spliced by another round of PCR: 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 2 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CGAGCTCAGGAGGATATATATATGAAAA AAGTCGCACTTGTTACCG-3') (SEQ ID NO:268) and reverse (5'-CCC AAGCTTAGATCGCGGTGGCCCCGCCGTCG-3') (SEQ ID NO:269) primers, 1 U Phusion High Fidelity DNA polymerase (NEB). The spliced fragment was digested with SacI and HindIII and ligated into pTrcBAL pre-digested with the same restriction enzymes.

Construction of pBBRPduCDEGH.

[0465] A DNA sequence encoding propanediol dehydratase medium (pduD) and small (pdue) subunits and propanediol dehydratase reactivation large (pduG) and small (pduH) subunits of *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 2 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-GC TCTAGAGGAGGATTTAAAAATGGAAATTAACGAAA CGCTGC-3') (SEQ ID NO:270) and reverse (5'-TCC CCGCGGTTAAGCATGGCGATCCCGAAATGGAATCC CTTTGAC-3') (SEQ ID NO:271) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 in 50 μl. Amplified DNA fragment was digested with SacII and XbaI and ligated into pTrc99A pre-digested with the same restriction enzymes to form pBBRPduDEGH.

[0466] A DNA sequence encoding propanediol dehydratase large subunit (pduC) of *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCGCTCGAGGAGGATATATATATGAGATCGA AAAGATTTGAAGC-3') (SEQ ID NO:272) and reverse (5'-GCTCTAGATTAGCCAAGTTCATTGGGATCG-3') (SEQ ID NO:273) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Kiebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 in 50 μl. Amplified DNA fragment was digested with XhoI and XbaI and ligated into pBBRP-duDEGH pre-digested with the same restriction enzymes.

Construction of pTrcIpdc-Par.

[0467] A DNA sequence encoding indole-3-pyruvate (ipdc) of *Azospirillum brasilense* and phenylethanol reductase (par) of *Rhodococcus* sp. ST-10 were amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 1 min, repeated 30 times. The reaction

mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward primers (5'-CATG CCATGGGACTGGCTGAGGCACTGCTGC-3' (SEQ ID NO:314) for ipdc and 5'-CGAGCTCAGGAGGATATAT ATATGAAAGCTATCCAGTACACCCGTAT-3' (SEQ ID NO:315) for par, and reverse primers (5'-CGAGCTCTTAT-TCGCGCGGTGCCGCGTGCAGG-3' (SEQ ID NO:316) for ipdc and 5'-GC TCTAGATTACAGGCCCGGAACCACAACGGCGC-3' (SEQ ID NO:317) for par, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pTrcIpdc and pTrcPar, respectively, in 50 μl. Amplified DNA fragment of ipdc and par were digested with NcoI/SacI and SacI/XbaI, respectively, and were ligated into pTrc99A pre-digested with NcoI and XbaI.

Testing and Results:

[0468] To test the butyraldehyde biosynthesis pathway, DH10B harboring pBADButP-atoB/pTrcBALD and pBAD-ButP-atoB-ALD/pTrcB2DH/pBBRpduCDEGH were grown overnight in LB media containing 50 ug/ml chroramphenicol (Cm^{50}) and 100 ug/ml ampicillin (Amp^{100}) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm^{50} and Amp^{100} and was grown in incubation shaker at 37 C, 200 rpm. Three hours after inoculation, the cultures were induced with 13.3 mM arabinose and 1 mM IPTG and were grown for overnight. 700 ul of this culture was extracted with equal volume of ethylacetate and analyzed by GC-MS.

[0469] To test the isobutyeraldehyde biosynthesis pathway, DH10B cells harboring pBADals-ilvCD/pTrcBALK or pBADalsS-ilvCD/pTrcBALK were grown overnight in LB media containing 50 ug/ml chroramphenicol (Cm^{50}) and 100 ug/ml ampicillin (Amp^{100}) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm^{50} and Amp^{100} and was grown in incubation shaker at 37 C, 200 rpm. Three hours after inoculation, the cultures were induced with 13.3 mM arabinose and 1 mM IPTG and were grown for overnight. 700 ul of this culture was extracted with equal volume of ethylacetate and analyzed by GC-MS for the production of isobutyraldehyde. FIG. **8**B shows the production of isobutanal from these cultures.

[0470] To test the 3-methylbutyraldehyde and 2-methylbutyraldehyde biosynthesis pathways, DH10B harboring pBADals-ilvCD-LeuABCD/pTrcBALK, pBADals-ilvCD-LeuABCD2/pTrcBALK, pBADals-ilvCD-LeuABCD/pTrcBALK4, pBADalsS-LeuABCD/pTrcBALK, pBAD-alsS-LeuABCD2/pTrcBALK, or pBADalsS-LeuABCD4/pTrcBALK were grown overnight in LB media containing 50 ug/ml chroramphenicol (Cm^{50}) and 100 ug/ml ampicillin (Amp^{100}) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm^{50} and Amp^{100} and was grown in incubation shaker at 37 C, 200 rpm. Three hours after inoculation, the cultures were induced with 13.3 mM arabinose and 1 mM IPTG and were grown for overnight. 700 ul of this culture was extracted with equal volume of ethylacetate and analyzed by GC-MS. The production of 2-isovaleralcohol (2-methylpental) and 3-isovaleralcohol (3-methylpentanal) was monitored because 3-isovaleraldehyde and 2-isovaleraldehyde are spontaneously converted to their corresponding alcohols. FIG. **8**B shows the production of 2-methylpental and 3-methylpentanal from these cultures.

[0471] To test the phenylacetoaldehyde and 4-hydroxyphenylacetoaldehyde biosynthesis pathways, DH10B cells harboring pBADpheA-aroLAC/pTrcBALK, pBADtyrA-aroLAC/pTrcBALK, pBADaroG-tktA-aroBDE/pTrcBALK, pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcBALK, and pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcBALK were grown overnight in LB media containing 50 ug/ml chroramphenicol (Cm^{50}) and 100 ug/ml ampicillin (Amp^{100}) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm^{50} and Amp^{100} and was grown in incubation shaker at 37 C, 200 rpm. Three hours after inoculation, the cultures were induced with 13.3 mM arabinose and 1 mM IPTG and were grown for overnight. 700 ul of this culture was extracted with equal volume of ethylacetate and analyzed by GC-MS. The production of phenylacetoaldehyde, 4-hydroxyphenylaldehyde and their corresponding alcohols were monitored using GC-MS. FIG. **9**B shows the production of 4-hydroxyphenylethanol from these cultures.

[0472] To test the 2-phenylethanol, 2-(4-hydroxyphenyl) ethanol, and 2-(indole-3) ethanol biosynthesis pathways, DH10B harboring pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcBALK, pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcBALK, pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcAdh2-Kivd, pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcAdh2-Kivd, pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcIpdc-Par, and pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcIpdc-Par were grown overnight in LB media containing 50 ug/ml chroramphenicol (Cm^{50}) and 100 ug/ml ampicillin (Amp^{100}) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm^{50} and Amp^{100} and was grown in incubation shaker at 37 C, 200 rpm. Three hours after inoculation, the cultures were induced with 13.3 mM arabinose and 1 mM IPTG and were grown for overnight to a week. 700 ul of this culture was extracted with equal volume of ethylacetate and analyzed by GC-MS. The results are detailed below.

[0473] The production of 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol and/or 2-(indole-3-)ethanol was monitored using GC-MS. FIG. **42**A shows the production of 2-phenylethanol from these cultures at 24 hours. FIG. **42**B shows the production of 2-(4-hydroxyphenyl)ethanol from these cultures at 24 hours. FIG. **42**C shows the production of 2-(indole-3-)ethanol from these cultures at 24 hours.

[0474] FIG. **43**A shows the GC-MS chromatogram for control (pBAD33 and pTrc99A) at one week. FIG. **43**B shows the GC-MS chromatogram for 2-phenylethanol (5.97 min) production from pBADpheA-aroLAC-aroG-tktA-aroBDE and pTrcBALK at one week. FIG. **44** shows the GC-MS chromatogram for 2-(4-hydroxyphenyl)ethanol (9.36 min) and 2-(indole-3) ethanol (10.32 min) production from pBAD-tyrA-aroLAC-aroG-tktA-aroBDE and pTrcBALK at one week.

Example 5

Isolation and Biological Activity of Diol Dehydrogenases

[0475] Available substrates such as 3-hydroxy-2-butanone (acetoin), 4-hydroxy-3-hexanone (propioin), 5-hydroxy-4-octanone (butyroin), 6-hydroxy-5-decanone (valeroin), and 1,2-cyclopentanediol were used to measure the ability of diol dehydrogenases (ddh) to catalyze the reduction of large saturated α-hydroxyketones to produce a diol. All reagents were purchased from Sigma-Aldrich Co. and TCI America, unless otherwise stated.

[0476] For cloning and isolation of DDH polypeptides, genomic DNA from several species of bacteria were obtained from ATCC (Lactobaccilus brevis ATCC 367, Pseudomanas putida KT2440, and *Kiebsiella pneumoniae* MGH78578), PCR-amplified (using Phusion with polymerase with 1× Phusion buffer, 0.2 mM dNTP, 0.5 μL Phusion enzyme, 1.5 μM primers, and 20 pg template DNA in a 50 μL reaction) utilizing the following protocol: 30 cylces, 98° C./10 secs (denaturing), 60° C./15 secs (annealing), 72° C./30 secs (elongation). Polymerase chain reaction products were then digested using restriction enzymes NdeI and BamHI, then ligated into NdeI/BamHI digested pET28 vectors. Vectors containing ddh clones were transformed into BL21(DE3) competent cells for protein expression. Single colony was innoculated into LB media, and expression of 6×His-tagged proteins of interest was induced at OD_{600}=0.6 with 0.1 mM IPTG. Expression was allowed to proceed for 15 hours at 22° C. The 6×His-tagged enzymes were purified using Ni-NTA spin columns following suggested protocols by QIAGEN, yelding purified protein concentrations in the range of 1.1-6.5 mg/mL (determined by Bradford assay).

[0477] Diol dehydrogenase ddh 1 was isolated from *Lactobaccilus brevis* ATCC 367, diol dehydrogenase ddh2 was isolated from *Pseudomonas putida* KT2440, and diol dehydrogenase ddh3 was isolated from *Klebsiella pneumoniae* MGH78578. The nucleotide sequence encoding and polypeptide sequence of ddh 1 are shown in SEQ ID NOS:97 and 98, respectively; nucleotide sequence encoding and polypeptide sequence of ddh2 are shown in SEQ ID NOS:99 and 100, respectively; and nucleotide sequence encoding and polypeptide sequence of ddh3 are shown in SEQ ID NOS: 101 and 102, respectively.

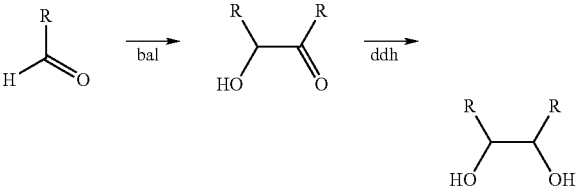
[0478] Reactions to measure biological activity of DDH polypeptides were performed in a final volume of 200 μL as follows: 25 mM substrate, 0.04 mg/mL DDH polypeptide, 0.25 mg/mL nicotinamide cofactor, 200 mM imidazole, 14 mM Tris-HCl, and 1.5% by volume DMSO. Biological activity was assayed using a Molecular Devices Thermomax 96 well plate reader, monitoring absorbance at 340 nm, which corresponds to NADH or NADPH concentration. For the kinetic studies, 0.04 mg/mL DDH polypeptide, 0.25 mg/mL NADH, 20 mM Tris HCl Buffer pH 6.5(red) or 9.0(ox), T=25 C, 100 uL total volume was used.

[0479] FIG. **12**A shows the biological activity of ddh1, ddh2, and ddh3 using butyroin as a substrate (triangles represent ddh3 activity). FIG. **12**B shows the oxidation activity of ddh3 towards 1,2-cyclopentanediol and 1,2-cyclohexanediol as measured by NADH production. FIG. **13** summarizes the results of kinetic studies for various substrates in the oxidation reactions catalyzed by the DDH polypeptides. These reactions were NAD+ dependent.

Example 6

Sequential In Vivo Biological Activity of CC-Ligases (Lyases) and Diol Dehydrogenases

[0480] The ability of a C—C lyase and a diol hydrogenase to perform the following sequential reaction was tested in *E. coli*:



[0481] For α-hydroxyketone and diol production, a pathway comprising a benzaldehyde lyase (bal) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) and meso-2,3-butanediol dehydrogenase (ddh) gene isolated from *Kiebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 was constructed in *E. coli* and tested for its ability to condensate the substrates detailed below in Table 2 (e.g., acetoaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, phenylacetaldehyde, and 4-hydroxyphenylacetaldehyde, or their corresponding alcohols) to form α-hydroxyketone and the corresponding diol in vivo. The production of various α-hydroxyketones and diols was monitored by gas chromatography-mass spectrometry (GC-MS).

TABLE 2

Summary of substrates and products.

Substrate	Produced α-hydroxyketone	Produced diol	Figures
Butanal	5-Hydroxy-4-octanone	4,5-Octanonediol	17A & B
n-Pentanal	6-Hydroxy-5-decanone	5,6-Decanediol	18A & B
3-Methylbutanal	2,7-Dimethyl-5-hydroxy-4-octanone	2,7-Dimethyl-4,5-octanediol	19A & B
n-Hexanal	7-Hydroxy-6-dodecanone	6,7-dodecanediol	20A & B
4-Methylpentanal	2,9-Dimethyl-6-hydroxy-5-decanone	2,9-Dimethyl-5,6-decanediol	21A & B
n-Octanal	9-Hydroxy-8-hexadecanone	8,9-hexadecanediol	22
Acetaldehyde	3-Hydroxy-2-butanone	2,3-Butanediol	23
n-Propanal	4-Hydroxy-3-hexanone	3,4-Hexanediol	24A & B
Phenylacetoaldehyde	1,4-Diphenyl-3-hydroxy-2-butanone	1,4-Diphenyl-2,3-butanediol	25

For Analysis of ≦C10.

[0482] *E. coli* harboring pTrcBAL-DDH-2ADH was grown for overnight in LB media containing 50 ug/ml Kanamycine (Km). This seed culture was innoculated into M9 media containing 3% (v/v) glycerol, 0.5% (g/v) and 50 ug/ml Km. 10 mL cultures were grown to $O.D._{600}$=0.7, then cultures were induced with 0.5 mM IPTG. The cells were allowed to express the enzymes of interest for 3 hours before various aldehydes were added to a concentration of 5-10 mM. After addition of aldehydes, the cultures were capped and incubated at 37° C. with shaking for 72 hours. Cultures were extracted with 2 mL ethyl acetate, and analyzed on GC-MS using the following protocol:

[0483] 1 μL injection w/50:1 split
[0484] Inlet temperature—150° C.
[0485] Initial oven temperature—50° C.
[0486] Temperature Ramp 1—10° C./min to 150° C.
[0487] Temperature Ramp 2—50° C./min to 300° C.
[0488] GC to MS transfer temp—250° C.
[0489] MS detection—full scan MW 35-200

For Analysis of ≧C12.

[0490] *E. coli* DH10B strains harboring pTrc99A (Ctrl vector) or pTrcBAL were inoculated into 0.75×M9/0.5% LB containing 0.1 mM $CaCl_2$, 2 mM $MgSO_4$, 1 mM KCl, 1% galacturonate, 5 μg/mL thiamine, Amp. The cultures were grown up to an optical density (600 n nm) of 0.8 and induced with 0.25 mM IPTG. The cells were allowed to express the proteins for 2.5 hours at 37° C., then aldehyde substrate was added to a concentration of 5 mM, the culture vial was capped tightly and incubated for 72 hours at 37° C. w/shaking 200 rpm. 1 mL of the final culture was extracted with 0.75 mL of ethyl acetate, centrifuged facilitate phase separation, then analyzed via GCMS using the following method.

[0491] 1 μL injection w/50:1 split
[0492] Inlet temperature—250° C.
[0493] Initial oven temperature—50° C.
[0494] Temperature Ramp 1—10° C./min to 125° C.
[0495] Temperature Ramp 2—30° C./min to 300° C.
[0496] Final Temperature 300° C.—1 minute
[0497] GC to MS transfer temp—250° C.
[0498] MS detection—full scan MW 40-260.

[0499] The results are depicted in FIGS. **17** through **25**. FIG. **17** shows the sequential conversion of butanal into 5-hydroxy-4-octanone and then 4,5-octanonediol. FIG. **18** shows the sequential conversion of n-pentanal into 6-hydroxy-5-decanone and then 5,6-decanediol. FIG. **19** shows the conversion of 3-methylbutanal into 2,7-dimethyl-5-hydroxy-4-octanone and then 2,7-Dimethyl-4,5-octanediol. FIG. **20** shows the sequential conversion of n-hexanal into 7-hydroxy-6-dodecanone and then 6,7-dodecanediol. FIG. **21** shows the conversion of 4-methylpentanal into 2,9-dimethyl-6-hydroxy-5-decanone and then 2,9-dimethyl-5,6-decanediol. FIG. **22** shows the conversion of n-octanal into 9-hydroxy-8-hexadecanone. FIG. **23** shows the conversion of acetaldehyde into 3-hydroxy-2-butanone. FIG. **24** shows the sequential conversion of n-propanal into 4-hydroxy-3-hexanone and then 3,4-hexanediol. FIG. **25** shows the conversion of phenylacetoaldehyde into 1,4-diphenyl-3-hydroxy-2-butanone.

[0500] Similar to above, a pathway comprising a benzaldehyde lyase (bal) gene isolated from *Pseudomonas fluorescens* (codon usage was optimized for *E. coli* protein expression) was constructed in *E. coli* and tested for its ability to catalyze the production of various α-hydroxyketones. The results, which show the broad spectrum of C—C ligase activity for the bal gene tested, are set forth in FIG. **48** through FIG. **55**.

Example 7

Sequential Biological Activity of Diol Dehydrogenases and Diol Dehydratases

[0501] To test the sequential biological activity of diol dehydrogenases and diol dehydratases in a dehydration and reduction pathway, butyroin was used as a substrate in a sequential reaction to produce 4-octanone. The enzyme diol dehydrogenase (e.g., ddh) catalyzes the reversible reduction and oxidation of α-hydroxy ketones and its corresponding diol, such as 5-hydroxy-4-octanone and 4,5-octanediol, and the enzyme diol dehydratase (e.g., pduCDE) catalyzes the irreversible dehydration of diols, such as 4,5-octanediol.

[0502] Diol dehydrogenase ddh from *Kiebsiella pneumoniae* MGH 78578 and diol dehydratase pduCDE from *Kiebsiella pneumoniae* MGH 78578 were cloned into a bacterial expression vector and expressed and purified on a Ni-NTA column, as described in Example X except that 1 mM of 1,2-propanediol was added at all time during the expression and purification of diol dehydratase. The large, medium, and small subunits of the pduCDE polyeptide are encoded by the nucleotide sequences of SEQ ID NOs:103, 105, and 107, respectively, and the polypeptide sequence are set forth in SEQ ID NOs: 104, 106, and 108, respectively.

[0503] The ddh3 and pduCDE polypeptides were incubated with butyroin and their appropriate cofactors, then assayed using gas chromatography-mass spectrometry (GC-MS) for their ability to perform sequential reactions resulting in the product 4-octanone. Reaction conditions are given in Table 3 below. The reaction mixture was incubated at 37° C. for 40 hours in a 0.6 mL eppendorf tube with minimal head space. The reaction product was extracted with an equivalent volume of ethyl acetate, stored in a glass vial, and sent to Thermo Fischer Scientific Instruments Division for compositional analysis by GC-MS.

TABLE 3

Reaction Conditions

Rxn Component	Concentration
5-hydroxy-4-octanone (butyroin)	8.4 mM
Adenosylcobalamin (coenzyme B_{12})	33.5 μM
KCl	9.6 mM
NADH	18 mM
dDH3 enzyme	0.19 mg/mL
dDOH1 enzyme mix	0.15 mg/mL
Reaction Buffer	10 mM Tris HCl pH 7.0

[0504] FIG. **26**A shows GC-MS data which confirms the presence of 4,5-octanediol in the sample extraction. The mass-spectra of the peaks, retention time, at 5.36 was identified as butyroin (substrate), and at 6.01, 6.09, and 6.12 min were identified as different isomers of 4,5-octanediol. This compound is the expected product resulting from the reduction of butyroin by ddh3.

[0505] FIG. **26**B shows GC-MS data confirming the presence of 4-octanone in the sample extraction. The mass-spectra of the peak, retention time, at 4.55 was identified as 4-octanone. This compound is the expected product resulting

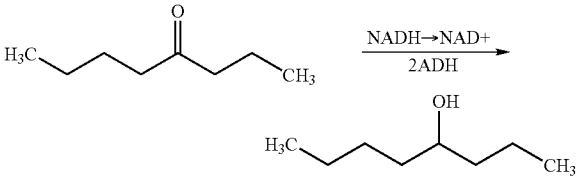
from the sequential dehydrogenation of butyroin and dehydration of 4,5-octanediol by ddh3 and pduCDE, respectively.

[0506] FIGS. **27**A and **27**B show comparisons between the sample extraction gas chromatograph/mass spectrum and the 4-octanone standard gas chromatograph/mass spectrum. These results demonstrate that 4-octanone was produced from butyroin using the enzymes diol dehydrogenase (ddh3) and a diol dehydratase (pduCDE). GC-MS analysis of the incubated reaction mixture confirmed starting material, intermediate and product, demonstrating that these enzymes can be reappropriated for these specific substrates.

Example 8

Isolation and Biological Activity of Secondary Alcohol Dehydrogenases

[0507] Substrates such as 4-octanone, 2,7-dimethyl-4-octanone, cyclopentanone and corresponding alcohols were utilized to measure the ability of secondary alcohol dehydrogenases (2ADHs) to catalyze the reduction of large saturated ketones to secondary alcohols. An example of a reaction catalyzed by secondary alcohol dehydrogenases is illustrated below (reduction of 4-octanone to 4-octanol is shown):



[0508] All enzymes and reagents were purchased from New England Biolabs and Sigma, respectively, unless otherwise stated.

[0509] Various secondary alcohol dehydrogenases (2ADHs) were isolated from *Pseudomonas putida* KT2440, *Pseudomonas fluorescens* Pf-5, and *Kiebsiella pneumoniae* MGH 78578. All vectors were transformed in BL21(DE3) competent cells and expression of the genes encoding the proteins of interest was induced with IPTG (via the T7 promoter). The cells were lysed, proteins were extracted and then purified on Ni-NTA columns. Final protein concentration in the Ni-NTA eluate was diluted to 0.15 mg/mL prior to assays.

[0510] NADPH/NADPH consumption and production assays were performed using a THERMOmax microplate reader in the kinetic mode, monitoring the NADPH absorbance peak at 340 nm until the reaction reached equilibrium. In the assay described in Table 2, 2ADH-2, 2ADH-5, 2ADH-8, and 2ADH-10 were tested for their ability to either catalyze the oxidation of 4-octanol or catalyze the reduction of 4-octanone. These reaction conditions are found in Table 4 below.

TABLE 4

Reaction Conditions for Various Enzyme Assays

Reaction Component	Final Concentration
NADH Production Assay (30° C.)	
2ADH enzyme	Approx. 0.058 μg/μL
4-octanol	5.55 mM
NAD+	Approx. 1.4 μg/μL
Imidizole (from Elution Buffer)	Approx. 280 mM

TABLE 4-continued

Reaction Conditions for Various Enzyme Assays

Reaction Component	Final Concentration
NADH Consumption Assay (30° C.)	
2ADH enzyme	Approx. 0.075 μg/μL
4-octanone	5.0 mM
NADH	Approx. 0.25 μg/μL
Imidizole (from Elution Buffer)	Approx. 250 mM
NADPH Production Assay (30° C.)	
2ADH enzyme	Approx. 0.058 μg/μL
4-octanol	5.55 mM
NADP+	Approx. 1.4 μg/μL
Imidizole (from Elution Buffer)	Approx. 280 mM

[0511] Further testing was performed, as described in Tables 5 below, in which 2ADH-2, 2ADH-11, 2ADH-12, 2ADH-13, 2ADH-14, 2ADH-15, 2ADH-16, 2ADH-17, and 2ADH-18 were tested for their ability to either catalyze the oxidation of 4-octanol, 2,7-dimethyl-4-octanonol, or cyclopentanol, or catalyze the reduction of 4-octanone, 2,7-dimethyl-4-octanonone, or cyclopentanone.

TABLE 5

Rxn Component	Final Concentration
Rxn Components for NADPH Consumption Assays (Reduction)	
Substrate	25 mM
Enzyme	0.04 mg/mL
Nicotinamide cofactor	0.25 mg/mL
Imidizole	200 mM
Tris HCl	14 mM
DMSO	1.5% by volume
Total Volume	200 μL
Rxn Components for NAD(P)H Production Assays (Oxidation)	
Substrate	5 mM
Enzyme	0.04 mg/mL
Nicotinamide cofactor	0.25 mg/mL
Imidizole	200 mM
Tris HCl	14 mM
Rxn Components for NAD(P)H Production Assay using 2,7-dimethyl-4-octanone as a substrate	
Substrate	50 mM
Enzyme	0.08 mg/mL
Nicotinamide cofactor	0.25 mg/mL
Imidizole	200 mM
Tris HCl	14 mM
DMSO	3% by volume

[0512] FIG. **30**A shows the results from the NADH Production Assay of Table 3, in which 2ADH-2 catalyzes the oxidation of 4-octanol in the presence of NAD+, as measured by NADH production. FIG. **30**B shows the results of the NADPH Production Assay of Table 3, in which 2ADH-5, 2ADH-8, and 2ADH-10 catalyze the oxidation of 4-octanol in the presence of NADP+, as measured by NADPH production.

[0513] FIG. **31** shows the oxidation of 4-octanol by 2ADH-11 (FIG. **31**A) and 2ADH-16 (FIG. **31**B), as measured by NADH and NADPH production, respectively. FIG. **32** shows the oxidation of 2,7-dimethyloctanol by 2ADH-11 and others (FIG. **32**A) and 2ADH-16 (FIG. **32**B), as measured by NADH and NADPH production, respectively.

[0514] FIG. **33**A shows the reduction of 2,7-dimethyl octanol by 2ADH 11 and 2ADH16 as monitored by NADPH consumption. FIG. **33**B shows the reduction activity of both 2ADH11 and 2ADH16 towards various substrates. FIG. **34** shows the oxidation (FIG. **34**A) and reduction (FIG. **34**B) of cyclopentanol by 2ADH-16.

[0515] Similar to above, kinetic testing for both oxidation and reduction reactions was performed on various substrates using 2ADH-16. The conditions for these studies were as follows: 0.04 mg/mL enzyme, 0.25 mg/mL cofactor, 20 mM Tris HCl Buffer pH 6.5(red) or 9.0(ox), T=25 C, 100 uL total volume was used. The calculated rate constants for the reduction reactions, along with the structures of the substrates, are summarized in FIG. **35**. The calculated rate constants for the oxidation reactions, along with the structures of the substrates, are summarized in FIG. **36**. These results show that 2ADH-16 is capable of catalyzing both the oxidation and reduction of a wide variety of substrates.

Example 9

Isolation and In Vitro and In Vivo Activity of Coenzyme B 12 Independent Diol Dehydratases

[0516] Substrates such as 1,2-propanediol, meso-2,3-butanediol, and trans-1,2-cyclopentanediol were utilized to test both the in vitro and in vivo biological activity of a B12 independent diol dehydratase in a dehydration and reduction pathway. Diol dehydratases catalyzes the irreversible dehydration of diols, such as 1,2-propanediol.

[0517] For in vitro activity, *E. coli* BL21(DE3) harboring pETPduCDE (diol dehydratase subunits) was inoculated into 100 mL LB media, grown to OD_{600}=0.7, induced with 0.15 mM IPTG, and incubated for 22 hours at 22° C. The cells were lysed and proteins of interest were purified on a Ni-NTA spin column. Purification of all three dehydratase subunits was accomplished by adding 5 mM 1,2-propanediol to the lysis and wash buffers. The Ni-NTA purification yielded approximately 660 μL of protein mixture at a concentration of 2.2 mg/mL. Protein concentration assays were conducted using a Bradford reagent protocol.

[0518] The purified PduCDE was used to set up in vitro diol dehydratase reactions. Three assays were conducted with 1,2-propanediol and meso-2,3-butanediol. Control reactions were also set up with elution buffer added in place of purifiedPduCDE. In vitro reactions were conducted under semi-anaerobic conditions in 2 mL screw cap glass vials. Reaction components and concentrations are given in Table 6.

TABLE 6

Reaction conditions for B_{12} dependent DDOH in vitro assay

Rxn Component	Concentration
Diol substrate	10 mM
Adenosylcobalamin (B_{12})	100 μg/mL
KCl	10 mM
dOH1 enzyme mix	0.08 mg/mL
Reaction Buffer	10 mM Tris HCl pH 7.5

[0519] After 48 hours, 1 mL of the reaction mixture was extracted with 0.5 mL of either ethylacetate or hexanol and analyzed by GCMS.

[0520] The following GCMS protocol was used for all experiments:

[0521] 1 μL injection w/50:1 split

[0522] Inlet temperature—250° C.

[0523] Initial oven temperature—50° C.

[0524] Temperature Ramp 1—10° C./min to 125° C.

[0525] Temperature Ramp 2—30° C./min to 300° C.

[0526] Final Temperature 300° C.—1 minute

[0527] GC to MS transfer temp—250° C.

[0528] MS detection—full scan MW 40-260

[0529] The results are shown in FIG. **45**. FIG. **45**A confirms the formation of 1-propanal from 1,2-propanediol, and FIG. **45**B confirms the formation of 2-butanone from meso-2,3-butanediol, both of which were catalyzed by B12 independent diol dehydratase.

[0530] For in vivo activity, the pBBRDhaB1/2 plasmid was constructed as follows: the DNA sequence encoding B12-independent glycerol dehydratase (dhaB1) and activator (dhaB2) of *Clostridium butyricum* was amplified by polymerase chain reaction (PCR): 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 2 min for dhaB1 and 1 min for dhaB2, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward primers (5'-CCGCTCGAGGAGGATATATATATGATTTCTAAAGG CTTTAGCACCC-3' (SEQ ID NO:318) for dhaB1 and 5'-ACGTGATGTAATCTAGAGGAGGATATATATATGAG CAAAGAAATTAAAGG-3' (SEQ ID NO:319) for dhaB2, and reverse primers (5'-TCTTTGCTCATATATATATCCTCC TCTAGATTACATCACGTGTTCAGTAC-3' (SEQ ID NO:320) for dhaB1 and 5'-C GAGCTCTTATTCGGCGCCAATGGTGCACGGG-3' (SEQ ID NO:321) for dhaB2, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pETdhaB1 and pET-dhaB2, respectively, in 50 μl. Amplified fragments were gel purified and spliced by another round of PCR: 98° C. for 10 sec, 60° C. for 15 sec, and 72° C. for 2.5 min, repeated 30 times. The reaction mixture contained 1× Phusion buffer (NEB), 2 mM dNTP, 0.5 μM forward (5'-CCG CTCGAGGAGGATATATATATGATTTCTAAAGGCTTTA GCACCC-3') (SEQ ID NO:322) and reverse primers (5'-C GAGCTCTTATTCGGCGCCAATGGTGCACGGG-3') (SEQ ID NO:323), 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng each fragment in 50 μl. Amplified DNA fragment was digested with XhoI and SacI and ligated into pBBR1MCS-2 pre-digested with the same restriction enzymes.

[0531] Two strains of *E. coli* DH10B harboring pBBR1MCS-2 or pBBRDhaB1/2 into TB media without glycerol were innoculated. Cultures were grown to OD_{600}=0.5 and the substrates 1,2-propanediol, meso-2,3-butanediol, and trans-1,2-cyclopentanediol were added to separate cultures to a concentration of 10 mM. 5 ug/ml of coenzyme S-adenosylmethionine was added before the culture is transferred to anaerobic environment. The cultures were incubated at 37 C for 48 hrs.

[0532] After 48 hours, 1 mL of culture was extracted with 0.5 mL of ethylacetate or hexanol and analyzed by GCMS, as described above. The results are shown in FIG. **46**. FIG. **46**A shows the in vivo production of 1-propanol from 1,2-propanediol. FIG. **46**B shows the in vivo production of 2-butanol from meso-2,3 butanediol. FIG. **46**C shows the in vivo production of cyclopentanone from trans-1,2-cyclopentanediol.

Example 10

Identification of Secreted Alginate Lyase and Genomic Regions Sufficient for Growth on Alginate as a Sole Source of Carbon

[0533] To identify secreted or external alginate lyases, and to identify genomic regions from *Vibrio splendidus* that are sufficient to confer growth in alginate as a sole source of carbon, the following clones were made using the gateway

system from Invitrogen (Carlsbad, Calif.). First, entry vectors were made by TOPO cloning PCR fragments into pENTR/D/TOPO. PCR fragments were generated using *Vibrio splendidus* B01 genomic DNA as a template and amplified with the following primer pairs:

[0534] Vs24214-24249: genomic region corresponding to gene id between V12B01_24214 and V12B01_24249 (see Example 1).

TABLE 7

24214 F	
cacc caagcgatagtttatatagcgt	(SEQ ID NO:324)
24249R	
gaaatgaacggatattacgt	(SEQ ID NO:325)

[0535] Vs24189-24209: genomic region corresponding to gene id between V12B01_24189 and V12B01_24209 (see Example 1).

TABLE 8

24189 R	
cggaacaggtgattgtggt	(SEQ ID NO:326)
24209 F	
cacc gcccacttcaagatgaagctgt	(SEQ ID NO:327)

[0536] Vs24214-24239: genomic region corresponding to gene id between V12B01_24214 and V12B01_24239 (see Example 1).

TABLE 9

24214 F	
cacc caagcgatagtttatatagcgt	(SEQ ID NO:328)
24239 R_1	
gtggctaagtacatgccggt	(SEQ ID NO:329)

[0537] The entry vectors were recombined with the destination vector pET-DEST42 (Invitrogen) using the LR recombinase enzyme (Invitrogen). These destination vectors were then put into electrocompetent DH10B or BL21 cells.

[0538] The alginate lyase clones were then made by digesting (using enzymes Nde I and Bam HI) the PCR products that were generated using *Vibrio splendidus* 12B01 genomic DNA as a template and amplified with the following primer pairs:

TABLE 10

GGAATTC CAT (SEQ ID NO:330)
atgacaaagaatatgacgactaaac
for
24214 ndeF
forward primer for V12B01_24214
CG GGATCC ttattatttcccctgccctgcagt (SEQ ID NO:331)
for reverse primer
24214 bamR
for V12B01_24214
24219 ndeF
GGAATTC CAT atgagctatcaaccacttttac (SEQ ID NO:332)
for forward
primer for V12B01_24219

TABLE 10-continued

CG GGATCC ttacagttgagcaaatgatcc (SEQ ID NO:333)
for reverse primer
24219 bamR
for V12B01_24219

[0539] The digested PCR products were then ligated into cut pET28 vector. Certain of the cloned genomic regions of *Vibrio splendidus* B01 were tested for the presence of secreted alginate lyases, and the above-described constructs were tested in various combinations for the ability to confer growth on alginate as a sole source of carbon.

[0540] The Vs24254 (SEQ ID NO: 32) region of *Vibro spendidus* encodes a functional external alginate lyase. BL21 cells expressing Vs24254 from the pET28 vector were capable of breaking down alginate in the growth medium. When grown on LB+2% alginate+0.1 mM Isopropyl β3-D-1-thiogalactopyranoside (IPTG), only cells expressing the Vs24254 gene give a positive TBA assay result of pink color. This assay was performed by spinning down an overnight culture grown on the above mentioned media. The media was then mixed in a 1:1 ratio with 0.8% thiobarbituric acid (TBA), heated for 10 min at 99 degrees Celsius, and assayed for pink coloration. FIG. **47** shows the results of this assay. The left tube in FIG. **47** represents media taken from an overnight culture of cells expressing Vs24254, while the right hand tube shows the TBA reaction using media from cells expressing Vs24259 (negative control). The lack of pink coloration in the negative control indicates that little or no cleavage of the alginate polymer has occurred. Wildtype *E. coli* cells not expressing any recombinant proteins show the same coloration as the negative control Vs24259 (data not shown).

[0541] To test the ability of recombinant *E. coli* to grow on alginate as a sole source of carbon, transformed cells were grown for 19 hours at 30 degrees Celsius with mild shaking in a 96-well plate. Each well held 222 μl of minimal media (see growth conditions for explanation of minimal media) with the 0.66% carbon source in the form of either degraded alginate or glucose (positive control for growth). All cells were either BL21 with no plasmid (BL21—negative control), one plasmid (Da or 3a), or two plasmids (Dk3a and Da3k). The plasmids are indicated by the lower case letter: "a" refers to the plasmid backbone pET-DEST42 and "k" refers to the pENTR/D/TOPO backbone. "D" indicates that the plasmid contains the genomic region Vs24214-24249, while "3" indicates that the plasmid contains the genomic region Vs24189-24209. Thus, Da would be pET-DEST42-Vs24214-24249, Da3k would be pET-DEST42-Vs24214-24249 and pENTR/D/TOPO-Vs24189-24209 and so on.

[0542] As shown in FIG. **56**A, the two vector-constructs pET-DEST42-Vs24214-24249 and pENTR/D/TOPO-Vs24189-24209 when combined in *E. coli* confer growth on degraded alginate as the sole carbon source. This same result is be observed when these genomic inserts are switched into the opposite vector (pET-DEST42-Vs24189-24209 and pENTR/D/TOPO-Vs24214-24249). FIG. **56**B shows growth on glucose as a positive control. Thus, the combined genomic regions of Vs24214-24249 and Vs24189-24209 from Vibro splendidus were sufficient to confer on *E. coli* the ability to grown on alginate as a sole source of carbon.

Example 11

Production of Ethanol from Alginate

[0543] The ability of recombinant *E. coli* to produce ethanol by growing on alginate on a source of carbon was tested.

To generate recombinant *E. coli*, DNA sequences encoding pyruvate decarboxylase (pdc), and two alcohol dehydrogenase (adhA and adhB) of *Zymomonas mobilis* were amplified by polymerase chain reaction (PCR). These amplified fragments were gel purified and spliced together by another round of PCR. The final amplified DNA fragment was digested with BamHI and XbaI ligated into pBBR1MCS-2 pre-digested with the same restriction enzymes. The resulting plasmid is referred to as pBBRPdc-AdhA/B.

[0544] *E. coli* was transformed with either pBBRPdc-AdhA/B or pBBRPdc-AdhA/B+1.5 Fos (fosmid clone containing genomic region between VI 2B01_24189 and V12B01_24249; these sequences confer on *E. coli* the ability to use alginate as a sole source of carbon, see Examples 1 and 10), grown in m9 media containing alginate, and tested for the production of ethanol. The results are shown in FIG. **57**, which demonstrates that the strain harboring pBBRPdc-AdhA/B+1.5 FOS showed significantly higher ethanol production when growing on alginate. These results indicate that the pBBRPdc-AdhA/B+1.5 FOS was able to utilize alginate as a source of carbon in the production of ethanol.

[0545] The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

[0546] These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

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SEQUENCE LISTING

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<213> ORGANISM: Vibrio splendidus

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        35                  40                  45

Glu Ile Val Ile Thr Ala Asn Lys Ser Glu Leu Val Leu Gly Val Gln
    50                  55                  60

Asn Asp Ser Ile Pro Val Ala Glu Asn Cys Val Gly Cys Val Asp Glu
65                  70                  75                  80

Asn Ala Ala Trp Val Asp Ala Pro Ile Ala Gly Glu Val Asn Phe Asp
                85                  90                  95

Leu Gln Gln Ala Asp Ala Glu Thr Phe Thr Glu Asp Asp Leu Ala Ala
            100                 105                 110

Ile Gln Glu Ala Ile Leu Gly Gly Ala Asp Pro Thr Gln Ile Leu Glu
        115                 120                 125

Ala Thr Ala Ala Gly Gly Gly Leu Gly Ser Ala Asn Ala Gly Phe Val
    130                 135                 140

Thr Ile Asp Tyr Asn Tyr Thr Glu Thr His Pro Ser Thr Phe Phe Glu
145                 150                 155                 160

Thr Ala Gly Leu Ala Glu Gln Thr Val Asp Glu Asp Arg Glu Glu Phe
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Thr Glu Gly Ser Ile Ser Gly Asn Thr Tyr Pro Gln Ser Val Thr Thr
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Thr Glu Thr Ile Ile Ala Gly Ser Leu Ala Leu Ala Pro Asn Ser Phe
    210                 215                 220

Ile Pro Glu Thr Leu Ser Leu Ala Ser Leu Leu Ser Glu Leu Asn Ser
225                 230                 235                 240

Asp Ile Thr Ser Ser Gly Gln Ser Val Ile Phe Thr Tyr Asp Ala Thr
                245                 250                 255

Thr Asn Ser Ile Val Gly Val Gln Asp Thr Asp Glu Val Leu Arg Ile
            260                 265                 270

Asp Ile Asp Ala Val Ser Val Gly Asn Asn Ile Glu Leu Ser Leu Thr
        275                 280                 285

Thr Thr Ile Ser Gln Pro Ile Asp His Val Pro Ser Val Gly Gly Gly
    290                 295                 300

Gln Val Ser Tyr Thr Gly Asp Gln Ile Asp Ile Ala Phe Asp Ile Gln
305                 310                 315                 320

Gly Glu Asp Thr Ala Gly Asn Pro Leu Ala Thr Pro Val Asn Ala Gln
                325                 330                 335

Val Ser Val Phe Asp Gly Ile Asp Pro Ser Val Glu Ser Val Asn Ile
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Thr Asn Val Glu Thr Ser Ser Ala Ala Ile Glu Gly Thr Phe Ser Asn
        355                 360                 365

Ile Gly Ser Asp Asn Leu Gln Ser Ala Val Phe Asp Ala Ser Ala Leu
    370                 375                 380

Asp Gln Phe Asp Gly Leu Leu Ser Asp Asn Gln Asn Thr Leu Ala Arg
385                 390                 395                 400

Leu Ser Asp Asp Gly Thr Thr Ile Thr Leu Ser Ile Gln Gly Arg Gly
                405                 410                 415

Glu Val Val Leu Thr Ile Ser Leu Asp Thr Asp Gly Thr Tyr Lys Phe
            420                 425                 430

Glu Gln Ser Asn Pro Ile Glu Gln Val Gly Thr Asp Ser Leu Thr Phe
        435                 440                 445

Ala Leu Pro Ile Thr Ile Thr Asp Phe Asp Gln Asp Val Val Thr Asn
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Thr Ile Asn Ile Ala Ile Thr Asp Gly Asp Ser Pro Val Ile Thr Asn
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Val Asp Ser Ile Asp Val Asp Glu Ala Gly Ile Val Gly Gly Ser Gln
                485                 490                 495

Glu Gly Thr Ala Pro Val Ser Gly Thr Gly Gly Ile Thr Ala Asp Ile
            500                 505                 510

Phe Glu Ser Asp Ile Ile Asp His Tyr Glu Leu Glu Pro Thr Glu Phe
        515                 520                 525

Asn Thr Asn Gly Thr Leu Val Ser Asn Gly Glu Ala Val Leu Leu Glu
    530                 535                 540

Leu Ile Asp Glu Thr Asn Gly Val Arg Thr Tyr Glu Gly Tyr Val Glu
545                 550                 555                 560

Val Asn Gly Ser Arg Ile Thr Val Phe Asp Val Lys Ile Asp Ser Pro
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Gly Ala Glu Asp Ala Leu Leu Thr Phe Ala Leu Pro Ile Tyr Ala Val
        595                 600                 605

Asp Ala Asp Gly Asp Arg Ser Ala Leu Ser Gly Gly Ser Asn Thr Pro
    610                 615                 620

Glu Ala Ala Glu Ile Leu Val Asn Val Lys Asp Asp Val Val Glu Leu
625                 630                 635                 640

Val Asp Lys Val Glu Ser Val Thr Glu Pro Thr Leu Ala Gly Asp Thr
                645                 650                 655

Ile Val Ser Tyr Asn Leu Phe Asn Phe Glu Gly Ala Asp Gly Ser Thr
            660                 665                 670

Ile Gln Ser Phe Asn Tyr Asp Gly Val Asp Tyr Ser Leu Asp Gln Ser
        675                 680                 685

Leu Leu Pro Asp Ala Thr Gln Ile Phe Ser Phe Thr Glu Gly Val Val
    690                 695                 700

Thr Ile Ser Leu Asn Gly Asp Phe Ser Phe Glu Val Ala Arg Asp Ile
705                 710                 715                 720

Asp His Ser Ser Ser Glu Thr Ile Val Lys Gln Phe Ser Phe Leu Ala
                725                 730                 735

Glu Asp Gly Asp Gly Asp Thr Asp Ser Ser Thr Leu Glu Leu Ser Ile
            740                 745                 750

Thr Asp Gly Gln Asp Pro Ile Ile Asp Leu Ile Pro Pro Val Thr Leu
        755                 760                 765

Ser Glu Thr Asn Leu Asn Asp Gly Ser Ala Pro Ser Gly Ser Thr Val
    770                 775                 780

Ser Ala Thr Glu Thr Ile Thr Phe Thr Ala Gly Ser Asp Asp Val Ala
785                 790                 795                 800

Ser Phe Arg Ile Glu Pro Thr Glu Phe Asn Val Gly Gly Ala Leu Lys
                805                 810                 815

Ser Asn Gly Phe Ser Val Glu Ile Lys Glu Asp Ser Ala Asn Pro Gly
            820                 825                 830

Thr Tyr Ile Gly Phe Ile Thr Asn Gly Ser Gly Ala Glu Ile Pro Val
        835                 840                 845

Phe Thr Ile Ala Phe Ser Thr Ser Thr Leu Gly Glu Tyr Thr Phe Thr
    850                 855                 860

Leu Leu Glu Ala Leu Asp His Val Asp Gly Leu Asp Lys Asn Asp Leu
865                 870                 875                 880

Ser Phe Asp Leu Pro Ile Tyr Ala Val Asp Thr Asp Gly Asp Asp Ser
                885                 890                 895

Leu Val Ser Gln Leu Asn Val Thr Ile Gly Asp Asp Val Gln Ile Met
            900                 905                 910

Gln Asp Gly Thr Leu Asp Ile Thr Glu Pro Asn Leu Ala Asp Gly Thr
        915                 920                 925

Ile Thr Thr Asn Thr Ile Asp Val Met Pro Asn Gln Ser Ala Asp Gly
    930                 935                 940

Ala Thr Ile Thr Arg Phe Thr Tyr Asp Gly Val Val Asn Thr Leu Asp
945                 950                 955                 960

Gln Ser Ile Ser Gly Glu Gln Gln Phe Ser Phe Thr Glu Gly Glu Leu
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Phe Ile Thr Leu Glu Gly Glu Val Arg Phe Glu Pro Asn Arg Asp Leu
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Asp His Ser Val Ser Glu Asp Ile Val Lys Ser Ile Val Val Thr Ser
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Ser Asp Phe Asp Asn Asp Pro Val Thr Ser Thr Ile Thr Leu Thr Ile
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Thr Asp Gly Asp Asn Pro Thr Ile Asp Val Ile Pro Ser Val Thr Leu
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Ser Glu Ile Asn Leu Ser Asp Gly Ser Ala Pro Ser Gly Ser Ala Val
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Ser Ser Thr Gln Thr Ile Thr Phe Thr Asn Gln Ser Asp Asp Val Val
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Arg Phe Arg Ile Glu Ser Thr Glu Phe Asn Thr Asn Asp Asp Leu Lys
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Ser Asn Gly Leu Ala Val Glu Leu Arg Glu Asp Pro Ala Gly Ser Gly
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Asp Tyr Ile Gly Phe Thr Thr Ser Ala Thr Asn Val Glu Thr Pro Val
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Phe Thr Leu Ser Phe Asn Ser Gly Ser Leu Gly Glu Tyr Thr Phe Thr
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Leu Ile Glu Ala Leu Asp His Gln Asp Ala Arg Gly Asn Asn Asp Leu
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Ser Phe Asp Leu Pro Val Tyr Ala Val Asp Ser Asp Gly Asp Asp Ser
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Leu Val Ser Pro Leu Asn Val Thr Ile Gly Asp Asp Val Gln Ile Met
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Gln Asp Ser Thr Leu Asp Ile Val Glu Pro Thr Val Ala Asp Leu Ala
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Ala Gly Thr Val Thr Thr Asn Thr Ile Asp Val Met Pro Asn Gln Ser
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Ser Ala Val Ser Ser Thr Gln Thr Ile Thr Tyr Thr Thr Gln Ser Asp
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Asp Val Thr Ser Phe Arg Ile Glu Pro Thr Glu Phe Asn Val Gly Gly
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Ala Leu Thr Ser Asn Gly Leu Ala Val Glu Leu Lys Ala Asp Pro Thr
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Thr Pro Gly Gly Tyr Ile Gly Phe Val Thr Asp Gly Ser Asn Val Glu
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Asn Asp Leu Thr Phe Asp Leu Pro Val Tyr Ala Val Asp Ser Asp Gly
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Asp Asp Ser Leu Val Ser Gln Leu Asn Val Thr Ile Gly Asp Asp Val
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Asp Gly Thr Ile Thr Thr Asn Thr Ile Asp Val Met Pro Glu Gln Ser
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    1490                1495                1500

Thr Leu Asp Gln Thr Asp Asn Gly Glu Gln Gln Phe Ser Phe Thr Glu
1505                1510                1515                1520

Gly Glu Leu Phe Ile Thr Leu Gln Gly Asp Val Arg Phe Glu Pro Asn
                1525                1530                1535

Arg Asn Leu Asp His Thr Ala Ser Glu Asp Ile Val Lys Ser Ile Val
            1540                1545                1550

Val Thr Ser Ser Asp Leu Asp Asn Asp Val Val Thr Ser Thr Val Thr
        1555                1560                1565

Leu Thr Ile Thr Asp Gly Asp Ile Pro Thr Ile Asp Ala Val Pro Ser
    1570                1575                1580

Val Thr Leu Ser Glu Ile Asn Leu Ser Asp Gly Ser Ala Pro Ser Gly
1585                1590                1595                1600

Thr Ala Val Ser Gln Thr Glu Thr Ile Thr Phe Thr Asn Gln Ser Asp
                1605                1610                1615

Asp Val Thr Ser Phe Arg Ile Glu Pro Ile Glu Phe Asn Val Gly Gly
            1620                1625                1630

Ala Leu Lys Ser Asn Gly Phe Ala Val Glu Ile Lys Glu Asp Ser Ala
        1635                1640                1645

Asn Pro Gly Thr Tyr Ile Gly Phe Ile Thr Asn Gly Ser Gly Ala Glu
    1650                1655                1660

Ile Pro Val Phe Thr Ile Ala Phe Ser Thr Ser Ser Leu Gly Glu Tyr
1665                1670                1675                1680

Thr Phe Thr Leu Leu Glu Ala Leu Asp His Val Asp Gly Leu Asp Lys
                1685                1690                1695

Asn Asp Leu Ser Phe Asp Leu Pro Val Tyr Ala Val Asp Thr Asp Gly
            1700                1705                1710

Asp Asp Ser Leu Val Ser Gln Leu Asn Val Thr Ile Gly Asp Asp Val
        1715                1720                1725

Gln Ile Met Gln Asp Gly Thr Leu Asp Ile Ile Glu Pro Asn Leu Ala
    1730                1735                1740

Asp Gly Thr Ile Thr Thr Ser Thr Ile Asp Val Met Pro Asn Gln Ser
1745                1750                1755                1760

Ala Asp Gly Ala Thr Ile Thr Gln Phe Thr Tyr Asp Gly Gln Leu Arg
                1765                1770                1775

Thr Leu Asp Gln Asn Asp Thr Gly Glu Gln Gln Phe Ser Phe Thr Glu
            1780                1785                1790
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Gly Glu Leu Phe Ile Thr Leu Glu Gly Glu Val Arg Phe Glu Pro Asn
        1795                1800                1805

Arg Asp Leu Asp His Thr Ala Ser Glu Asp Ile Val Lys Ser Ile Val
    1810                1815                1820

Val Thr Ser Ser Asp Phe Asp Asn Asp Ser Leu Thr Ser Thr Val Thr
1825                1830                1835                1840

Leu Thr Ile Thr Asp Gly Asp Asn Pro Thr Ile Asp Val Ile Pro Ser
                1845                1850                1855

Val Thr Leu Ser Glu Thr Asn Leu Ser Asp Gly Ser Ala Pro Ser Gly
            1860                1865                1870

Ser Ala Val Ser Ser Thr Gln Thr Ile Thr Phe Thr Asn Gln Ser Asp
        1875                1880                1885

Asp Val Val Arg Phe Arg Ile Glu Pro Thr Glu Phe Asn Thr Asn Asp
    1890                1895                1900

Asp Leu Lys Ser Asn Gly Leu Ala Val Glu Leu Arg Glu Asp Pro Ala
1905                1910                1915                1920

Gly Ser Gly Asp Tyr Ile Gly Phe Thr Thr Ser Ala Thr Asn Val Glu
                1925                1930                1935

Thr Thr Val Phe Thr Leu Ser Phe Ser Ser Thr Thr Leu Gly Glu Tyr
            1940                1945                1950

Thr Phe Thr Leu Leu Glu Ala Leu Asp His Gln Asp Ala Arg Gly Asn
        1955                1960                1965

Asn Asp Leu Ser Phe Glu Leu Pro Val Tyr Ala Val Asp Ser Asp Gly
    1970                1975                1980

Asp Asp Ser Leu Met Ser Pro Leu Asn Val Thr Ile Gly Asp Asp Val
1985                1990                1995                2000

Gln Ile Met Gln Asp Gly Thr Leu Asp Ile Val Glu Pro Thr Val Ala
                2005                2010                2015

Asp Leu Ala Ala Gly Ile Val Thr Thr Asn Thr Ile Asp Val Met Pro
            2020                2025                2030

Asn Gln Ser Ala Asp Gly Ala Thr Ile Thr Gln Phe Thr Tyr Asp Gly
        2035                2040                2045

Gln Leu Arg Thr Leu Asp Gln Asn Asp Asn Gly Glu Gln Gln Phe Ser
    2050                2055                2060

Phe Thr Glu Gly Glu Leu Phe Ile Thr Leu Glu Gly Glu Val Arg Phe
2065                2070                2075                2080

Glu Pro Asn Arg Asn Leu Asp His Thr Leu Asn Glu Asp Ile Val Lys
                2085                2090                2095

Ser Ile Val Val Thr Ser Ser Asp Ser Asp Asn Asp Val Leu Thr Ser
            2100                2105                2110

Thr Val Thr Leu Thr Ile Thr Asp Gly Asp Ile Pro Thr Ile Asp Asn
        2115                2120                2125

Val Pro Thr Val Ser Leu Ser Glu Thr Ser Leu Ser Asp Gly Ser Ser
    2130                2135                2140

Pro Ser Gly Ser Ala Val Ser Ser Thr Gln Thr Ile Thr Tyr Thr Thr
2145                2150                2155                2160

Gln Ser Asp Asp Val Thr Ser Phe Arg Ile Glu Pro Thr Glu Phe Asn
                2165                2170                2175

Val Gly Gly Ala Leu Lys Ser Asn Gly Leu Ala Val Glu Leu Lys Ala
            2180                2185                2190

Asp Pro Thr Thr Pro Gly Gly Tyr Ile Gly Phe Val Thr Asp Gly Ser
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        2195                2200                2205

Asn Val Glu Thr Asn Val Phe Thr Ile Ser Phe Ser Asp Thr Asn Leu
    2210                2215                2220

Gly Gln Tyr Thr Phe Thr Leu Leu Glu Ala Leu Asp His Ala Asp Ser
2225                2230                2235                2240

Leu Ala Asn Asn Asp Leu Ser Phe Asp Leu Pro Val Tyr Ala Val Asp
                2245                2250                2255

Ser Asp Gly Asp Asp Ser Leu Val Ser Gln Leu Asn Val Thr Ile Gly
            2260                2265                2270

Asp Asp Val Gln Ile Met Gln Gly Gly Thr Leu Asp Ile Thr Glu Pro
        2275                2280                2285

Asn Leu Ala Asp Gly Thr Thr Thr Thr Asn Thr Ile Asp Val Met Pro
    2290                2295                2300

Glu Gln Ser Ala Asp Gly Ala Thr Ile Thr Gln Phe Thr Tyr Asp Gly
2305                2310                2315                2320

Gln Val Arg Thr Leu Asp Gln Thr Asp Asn Gly Glu Gln Gln Phe Ser
                2325                2330                2335

Phe Thr Glu Gly Glu Leu Phe Ile Thr Leu Gln Gly Asp Val Arg Phe
            2340                2345                2350

Glu Pro Asn Arg Asn Leu Asp His Thr Ala Ser Glu Asp Ile Val Lys
        2355                2360                2365

Ser Ile Val Val Thr Ser Ser Asp Ser Asp Asn Asp Val Val Thr Ser
    2370                2375                2380

Thr Val Thr Leu Thr Ile Thr Asp Gly Asp Leu Pro Thr Ile Asp Ala
2385                2390                2395                2400

Val Pro Ser Val Thr Leu Ser Glu Thr Asn Leu Ser Asp Gly Ser Ala
                2405                2410                2415

Pro Ser Gly Ser Ala Val Ser Gln Thr Glu Thr Ile Thr Phe Thr Asn
            2420                2425                2430

Gln Ser Asp Asp Val Ala Ser Phe Arg Ile Glu Pro Thr Glu Phe Asn
        2435                2440                2445

Val Gly Gly Ala Leu Lys Ser Asn Gly Phe Ala Val Glu Ile Lys Glu
    2450                2455                2460

Asp Ser Ala Asn Pro Gly Thr Tyr Ile Gly Phe Ile Ala Asn Gly Ser
2465                2470                2475                2480

Ser Ala Glu Ile Pro Val Phe Thr Ile Ala Phe Ser Thr Ser Thr Leu
                2485                2490                2495

Gly Glu Tyr Thr Phe Thr Leu Leu Glu Ala Leu Asp His Ala Asp Gly
            2500                2505                2510

Leu Asp Lys Asn Asp Leu Ser Phe Glu Leu Pro Val Tyr Ala Val Asp
        2515                2520                2525

Thr Asp Gly Asp Asp Ser Leu Val Ser Gln Leu Asn Val Thr Ile Gly
    2530                2535                2540

Asp Asp Val Gln Ile Met Gln Asp Gly Thr Leu Asp Val Ile Glu Pro
2545                2550                2555                2560

Asn Leu Ala Asp Gly Thr Ile Thr Thr Asn Thr Ile Asp Val Met Pro
                2565                2570                2575

Glu Gln Ser Ala Asp Gly Ala Thr Ile Thr Gln Phe Thr Tyr Asp Gly
            2580                2585                2590

Gln Leu Arg Thr Leu Asp Gln Asn Asp Thr Gly Glu Gln Gln Phe Ser
        2595                2600                2605
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Phe Thr Glu Gly Glu Leu Phe Ile Thr Leu Glu Gly Glu Val Arg Phe
    2610                2615                2620

Glu Pro Asn Arg Asp Leu Asp His Ser Val Ser Glu Asp Ile Val Lys
2625                2630                2635                2640

Ser Ile Val Val Thr Ser Ser Asp Phe Asp Asn Asp Pro Val Thr Ser
                2645                2650                2655

Ala Ile Thr Leu Thr Ile Thr Asp Gly Asp Asn Pro Thr Ile Asp Ser
            2660                2665                2670

Val Pro Ser Val Val Leu Glu Glu Ala Asp Leu Thr Asp Gly Ser Ser
        2675                2680                2685

Pro Ser Gly Ser Ala Val Ser Gln Thr Glu Thr Ile Thr Phe Thr Asn
    2690                2695                2700

Gln Ser Asp Asp Val Glu Lys Phe Arg Leu Glu Pro Ser Glu Phe Asn
2705                2710                2715                2720

Thr Asn Asn Ala Leu Lys Ser Asp Gly Leu Ile Ile Glu Ile Arg Glu
                2725                2730                2735

Glu Pro Thr Gly Ser Gly Asn Tyr Ile Gly Phe Thr Thr Asp Ile Ser
            2740                2745                2750

Asn Val Glu Thr Thr Val Phe Thr Leu Asp Phe Ser Ser Thr Thr Leu
        2755                2760                2765

Gly Glu Tyr Thr Phe Thr Leu Leu Glu Ala Ile Asp His Thr Pro Val
    2770                2775                2780

Gln Gly Asn Asn Asp Leu Thr Phe Asn Leu Pro Val Tyr Ala Val Asp
2785                2790                2795                2800

Ser Asp Gly Asp Asp Ser Leu Met Ser Ser Leu Ser Val Thr Ile Thr
                2805                2810                2815

Asp Asp Val Gln Val Met Val Ser Gly Ser Leu Ser Ile Glu Glu Pro
            2820                2825                2830

Thr Val Ala Asp Leu Ala Ala Gly Thr Pro Thr Thr Ser Val Phe Asp
        2835                2840                2845

Val Leu Thr Ser Ala Ser Ala Asp Gly Ala Thr Ile Thr Gln Phe Thr
    2850                2855                2860

Tyr Asp Gly Gly Ala Val Leu Thr Leu Asp Gln Asn Asp Thr Gly Glu
2865                2870                2875                2880

Gln Lys Phe Val Val Ala Asp Gly Ala Leu Tyr Ile Thr Leu Gln Gly
                2885                2890                2895

Asp Ile Arg Phe Glu Pro Ser Arg Asn Leu Asp His Thr Gly Gly Asp
            2900                2905                2910

Ile Val Lys Ser Ile Val Val Thr Ser Ser Asp Ser Asp Ser Asp Leu
        2915                2920                2925

Val Ser Ser Thr Val Thr Leu Thr Ile Thr Asp Gly Asp Ile Pro Thr
    2930                2935                2940

Ile Asp Thr Val Pro Ser Val Thr Leu Ser Glu Thr Asn Leu Ser Asp
2945                2950                2955                2960

Gly Ser Ala Pro Asn Ala Ser Ala Val Ser Ser Thr Gln Thr Ile Thr
                2965                2970                2975

Phe Thr Asn Gln Ser Asp Asp Val Thr Ser Phe Arg Ile Glu Pro Thr
            2980                2985                2990

Asp Phe Asn Val Gly Gly Ala Leu Lys Ser Asn Gly Leu Ala Val Glu
        2995                3000                3005
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Leu Lys Ala Asp Pro Thr Thr Pro Gly Gly Tyr Ile Gly Phe Val Thr
    3010                3015                3020

Asp Gly Ser Asn Val Glu Thr Asn Val Phe Thr Ile Ser Phe Ser Asp
3025                3030                3035                3040

Thr Asn Leu Gly Gln Tyr Thr Phe Thr Leu Leu Glu Ala Leu Asp His
                3045                3050                3055

Val Asp Gly Leu Val Lys Asn Asp Leu Thr Phe Asp Leu Pro Val Tyr
            3060                3065                3070

Ala Val Asp Ser Asp Gly Asp Asp Ser Leu Val Ser Gln Leu Asn Val
        3075                3080                3085

Thr Ile Gly Asp Asp Val Gln Val Met Gln Asn Gln Ala Leu Asn Ile
    3090                3095                3100

Ile Glu Pro Thr Val Ala Asp Leu Ala Ala Gly Thr Pro Thr Thr Ala
3105                3110                3115                3120

Thr Val Asp Val Met Pro Ser Gln Ser Ala Asp Gly Ala Thr Ile Thr
                3125                3130                3135

Gln Phe Thr Tyr Asp Gly Gly Ala Ala Ile Thr Leu Asp Gln Asn Asp
            3140                3145                3150

Thr Gly Glu Gln Lys Phe Val Phe Thr Glu Gly Ser Leu Phe Ile Thr
        3155                3160                3165

Leu Gln Gly Glu Val Arg Phe Glu Pro Asn Arg Asn Leu Asn His Thr
    3170                3175                3180

Ala Ser Glu Asp Ile Val Lys Ser Ile Val Val Thr Ser Ser Asp Leu
3185                3190                3195                3200

Asp Asn Asp Val Leu Thr Ser Thr Val Thr Leu Thr Ile Thr Asp Gly
                3205                3210                3215

Asp Ile Pro Thr Ile Asp Ala Val Pro Ser Val Thr Leu Ser Glu Thr
            3220                3225                3230

Asn Leu Ser Asp Gly Ser Ala Pro Ser Ser Ser Ala Val Ser Gln Thr
        3235                3240                3245

Glu Thr Ile Thr Phe Ile Asn Gln Ser Asp Asp Val Ala Ser Phe Arg
    3250                3255                3260

Ile Glu Pro Thr Glu Phe Asn Val Gly Gly Ala Leu Lys Ser Asn Gly
3265                3270                3275                3280

Phe Ala Val Glu Ile Lys Glu Asp Ser Ala Asn Pro Gly Thr Tyr Ile
                3285                3290                3295

Gly Phe Ile Thr Asp Gly Ser Asn Thr Glu Val Pro Val Phe Thr Ile
            3300                3305                3310

Ala Phe Ser Thr Ser Thr Leu Gly Glu Tyr Thr Phe Thr Leu Leu Glu
        3315                3320                3325

Ala Leu Asp His Ala Asn Gly Leu Asp Lys Asn Asp Leu Ser Phe Asp
    3330                3335                3340

Leu Pro Val Tyr Ala Val Asp Ser Asp Gly Asp Asp Ser Leu Val Ser
3345                3350                3355                3360

Gln Leu Asn Val Thr Ile Gly Asp Asp Val Gln Ile Met Gln Asp Gly
                3365                3370                3375

Thr Leu Asp Ile Thr Glu Pro Asn Leu Ala Asp Gly Thr Ile Thr Thr
            3380                3385                3390

Asn Thr Ile Asp Val Met Pro Asn Gln Ser Ala Asp Gly Ala Thr Ile
        3395                3400                3405

Thr Glu Phe Ser Phe Gly Gly Ile Val Lys Thr Leu Asp Gln Ser Ile
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    3410                3415                3420

Val Gly Glu Gln Gln Phe Ser Phe Thr Glu Gly Glu Leu Phe Ile Thr
3425                3430                3435                3440

Leu Gln Gly Gln Val Arg Phe Glu Pro Asn Arg Asp Leu Asp His Ser
                3445                3450                3455

Ala Ser Glu Asp Ile Val Lys Ser Ile Val Val Thr Ser Ser Asp Phe
            3460                3465                3470

Asp Asn Asp Pro Val Thr Ser Thr Val Thr Leu Thr Ile Thr Asp Gly
        3475                3480                3485

Asp Ile Pro Thr Ile Asp Ala Val Pro Ser Val Thr Leu Ser Glu Thr
    3490                3495                3500

Asn Leu Ala Asp Gly Ser Ala Pro Ser Gly Ser Ala Val Ser Gln Thr
3505                3510                3515                3520

Glu Thr Ile Thr Phe Thr Asn Gln Ser Asp Asp Val Val Arg Phe Arg
                3525                3530                3535

Leu Glu Pro Thr Glu Phe Asn Thr Asn Asp Ala Leu Lys Ser Asn Gly
            3540                3545                3550

Leu Ala Val Glu Leu Arg Glu Glu Pro Gln Gly Ser Gly Gln Tyr Ile
        3555                3560                3565

Gly Phe Thr Thr Ser Ser Ser Asn Val Glu Thr Thr Val Phe Thr Leu
    3570                3575                3580

Asp Phe Asn Ser Gly Thr Leu Gly Glu Tyr Thr Phe Thr Leu Ile Glu
3585                3590                3595                3600

Ala Leu Asp His Gln Asp Ala Arg Gly Asn Asn Asp Leu Ser Phe Asn
                3605                3610                3615

Leu Pro Val Tyr Ala Val Asp Ser Asp Gly Asp Asp Ser Leu Val Ser
            3620                3625                3630

Gln Leu Gly Val Thr Ile Gly Asp Asp Val Gln Leu Met Gln Asp Gly
        3635                3640                3645

Thr Ile Thr Ser Arg Glu Pro Ala Ala Ser Val Glu Thr Ser Asn Thr
    3650                3655                3660

Phe Asp Val Met Pro Asn Gln Ser Ala Asp Gly Ala Lys Val Thr Ser
3665                3670                3675                3680

Phe Val Phe Asp Gly Lys Thr Ala Glu Ser Leu Asp Leu Asn Val Asn
                3685                3690                3695

Gly Glu Gln Glu Phe Val Phe Thr Glu Gly Ser Val Phe Ile Thr Thr
            3700                3705                3710

Glu Gly Glu Ile Arg Phe Glu Pro Val Arg Asn Gln Asn His Ala Gly
        3715                3720                3725

Gly Asp Ile Thr Lys Ser Ile Glu Val Thr Ser Val Asp Leu Asp Gly
    3730                3735                3740

Asp Ile Val Thr Ser Thr Val Thr Leu Lys Ile Val Asp Gly Asp Leu
3745                3750                3755                3760

Pro Thr Ile Asp Leu Val Pro Gly Ile Thr Leu Ser Glu Val Asp Leu
                3765                3770                3775

Ala Asp Gly Ser Val Pro Thr Gly Asn Pro Val Thr Met Thr Gln Thr
            3780                3785                3790

Ile Thr Tyr Thr Ala Gly Ser Asp Asp Val Ser His Phe Arg Ile Asp
        3795                3800                3805

Pro Thr Gln Phe Asn Thr Ser Gly Val Leu Lys Ser Asn Gly Leu Asp
    3810                3815                3820
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Val Glu Ile Lys Glu Gln Pro Ala Asn Ser Gly Asn Tyr Ile Gly Phe
3825                3830                3835                3840

Val Lys Asp Gly Ser Asn Val Glu Thr Asn Val Phe Thr Ile Ser Phe
                3845                3850                3855

Ser Thr Ser Asn Leu Gly Gln Tyr Thr Phe Thr Leu Leu Glu Ala Leu
            3860                3865                3870

Asp His Val Asp Gly Leu Gln Asn Asn Ile Leu Ser Phe Asp Val Pro
        3875                3880                3885

Val Leu Ala Val Asp Ala Asp Gly Asp Asp Ser Ala Met Ser Pro Met
    3890                3895                3900

Thr Val Ala Ile Thr Asp Asp Val Gln Gly Val Gln Asp Gly Thr Leu
3905                3910                3915                3920

Ser Ile Thr Glu Pro Ser Leu Ala Asp Leu Ala Ser Gly Thr Pro Pro
                3925                3930                3935

Thr Thr Ala Ile Ile Asp Val Met Pro Thr Gln Ser Ala Asp Gly Ala
            3940                3945                3950

Lys Val Thr Gln Phe Thr Tyr Asp Gly Gly Thr Ala Val Thr Leu Asp
        3955                3960                3965

Pro Ser Ile Ala Thr Glu Gln Val Phe Thr Val Thr Asp Gly Leu Leu
    3970                3975                3980

Tyr Ile Thr Ile Glu Gly Glu Val Arg Phe Glu Pro Ser Arg Asp Leu
3985                3990                3995                4000

Asp His Ser Ser Gly Asp Ile Val Arg Thr Ile Val Val Thr Thr Ser
                4005                4010                4015

Asp Phe Asp Asn Asp Thr Asp Thr Ala Asp Val Thr Leu Thr Ile Lys
            4020                4025                4030

Asp Gly Ile Asn Pro Val Ile Asn Val Val Pro Asp Val Asn Leu Ser
        4035                4040                4045

Glu Val Asn Leu Ala Asp Gly Ser Thr Pro Ser Gly Ser Ala Val Ser
    4050                4055                4060

Ser Thr His Thr Ile Thr Tyr Thr Glu Gly Ser Asp Asp Phe Ser His
4065                4070                4075                4080

Phe Arg Ile Ala Thr Asn Glu Phe Asn Pro Gly Asp Leu Leu Lys Ser
                4085                4090                4095

Ser Gly Leu Val Val Gln Leu Lys Glu Asp Pro Ala Ser Ala Gly Asp
            4100                4105                4110

Tyr Ile Gly Tyr Thr Asp Asp Gly Met Gly Asn Val Thr Asp Val Phe
        4115                4120                4125

Thr Ile Ser Phe Asp Ser Ala Asn Lys Ala Gln Phe Thr Phe Thr Leu
    4130                4135                4140

Ile Glu Ala Leu Asp His Leu Asp Gly Val Leu Tyr Asn Asp Leu Thr
4145                4150                4155                4160

Phe Arg Leu Pro Ile Tyr Ala Val Asp Thr Asp Asp Ser Glu Ser Thr
                4165                4170                4175

Lys Arg Asp Val Val Val Thr Ile Glu Asp Asp Ile Gln Gln Met Gln
            4180                4185                4190

Asp Gly Phe Leu Thr Ile Thr Glu Pro Asn Ser Gly Thr Pro Thr Thr
        4195                4200                4205

Thr Thr Val Asp Val Met Pro Ile Pro Ser Ala Asp Gly Ala Thr Ile
    4210                4215                4220
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Thr Gln Phe Thr Tyr Asp Gly Gly Ser Pro Ile Thr Leu Asn Gln Ser
4225                4230                4235                4240

Ile Ser Gly Glu Gln Glu Phe Val Phe Thr Glu Gly Ser Leu Phe Val
                4245                4250                4255

Thr Leu Asp Gly Asp Val Arg Phe Glu Pro Asn Arg Asn Leu Asp His
            4260                4265                4270

Ser Ala Gly Asp Ile Val Lys Ser Ile Val Phe Thr Ser Ser Asp Phe
        4275                4280                4285

Asp Asn Asp Ile Phe Ser Ser Lys Val Thr Leu Thr Ile Val Asp Gly
    4290                4295                4300

Asp Gly Pro Thr Ile Asp Val Val Pro Gly Val Ala Leu Ser Glu Ser
4305                4310                4315                4320

Leu Leu Ala Asp Gly Ser Thr Pro Ser Val Asn Pro Val Ser Met Thr
                4325                4330                4335

Gln Thr Ile Thr Ser Leu Ala Ser Ser Asp Asp Ile Ala Glu Ile Val
            4340                4345                4350

Val Glu Val Gly Leu Phe Asn Thr Asn Gly Ala Leu Lys Ser Asp Gly
        4355                4360                4365

Leu Ser Leu Ser Leu Arg Glu Asp Pro Val Asn Ser Gly Asp Tyr Ile
    4370                4375                4380

Ala Phe Thr Thr Asn Gly Ser Gly Val Glu Lys Val Ile Phe Thr Leu
4385                4390                4395                4400

Asp Phe Asp Asp Thr Asn Pro Ser Gln Tyr Thr Phe Thr Leu Leu Glu
                4405                4410                4415

Arg Leu Asp His Val Asp Gly Leu Gly Asn Asn Asp Leu Ser Phe Asp
            4420                4425                4430

Leu Ser Val Tyr Ala Glu Asp Thr Asp Gly Asp Ile Ser Ala Ser Lys
        4435                4440                4445

Pro Leu Thr Val Thr Ile Thr Asp Asp Val Gln Leu Met Gln Ser Gly
    4450                4455                4460

Ala Leu Asn Ile Thr Glu Pro Thr Thr Gly Thr Pro Thr Thr Ala Val
4465                4470                4475                4480

Phe Asp Val Met Pro Ala Gln Ser Ala Asp Gly Ala Thr Ile Thr Lys
                4485                4490                4495

Phe Thr Tyr Gly Ser Gln Pro Glu Glu Ser Leu Val Gln Thr Val Thr
            4500                4505                4510

Gly Glu Gln Glu Phe Val Phe Thr Glu Gly Ser Leu Phe Ile Asn Leu
        4515                4520                4525

Glu Gly Asp Val Arg Phe Glu Pro Asn Arg Asn Leu Asp His Ser Gly
    4530                4535                4540

Gly Asn Ile Val Lys Thr Ile Thr Val Thr Ser Glu Asp Lys Asp Gly
4545                4550                4555                4560

Asp Ile Val Thr Ser Thr Val Thr Leu Thr Ile Val Asp Gly Ala Pro
                4565                4570                4575

Pro Val Ile Asp Thr Val Pro Thr Val Ala Leu Glu Glu Ala Asn Leu
            4580                4585                4590

Val Asp Gly Ser Ser Pro Gly Leu Pro Val Ser Gln Thr Glu Ile Ile
        4595                4600                4605

Thr Phe Thr Ala Gly Ser Asp Asp Val Ser His Phe Arg Ile Asp Pro
    4610                4615                4620

Ala Gln Phe Asn Thr Ser Gly Asp Leu Lys Ala Asp Gly Leu Val Val
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4625                4630                4635                4640

Gln Leu Lys Glu Asp Pro Leu Asn Ser Asp Asn Tyr Ile Gly Tyr Val
                4645                4650                4655

Glu Ser Gly Gly Val Gln Thr Asp Ile Phe Thr Ile Thr Phe Ser Ser
            4660                4665                4670

Val Val Leu Gly Glu Tyr Thr Phe Thr Leu Leu Glu Glu Leu Asp His
        4675                4680                4685

Leu Pro Val Gln Gly Asn Asn Asp Gln Ile Phe Thr Leu Pro Val Ile
    4690                4695                4700

Ala Val Asp Lys Asp Asn Thr Asp Ser Ala Val Lys Pro Leu Thr Val
4705                4710                4715                4720

Thr Ile Thr Asp Asp Val Pro Thr Ile Thr Asp Thr Thr Gly Ala Ser
                4725                4730                4735

Thr Phe Val Val Asp Glu Asp Asp Leu Gly Thr Leu Ala Gln Ala Thr
            4740                4745                4750

Gly Ser Phe Val Thr Thr Glu Gly Ala Asp Gln Val Glu Val Tyr Glu
        4755                4760                4765

Leu Arg Asn Ile Ser Thr Leu Glu Ala Thr Leu Ser Ser Gly Ser Glu
    4770                4775                4780

Gly Ile Lys Ile Thr Glu Ile Thr Gly Ala Ala Asn Thr Thr Thr Tyr
4785                4790                4795                4800

Gln Gly Ala Thr Asp Pro Ser Gly Thr Pro Ile Phe Thr Leu Val Leu
                4805                4810                4815

Thr Asp Asp Gly Ala Tyr Thr Phe Thr Leu Leu Gly Pro Leu Asn His
            4820                4825                4830

Ala Thr Thr Pro Ser Asn Leu Asp Thr Leu Thr Ile Pro Phe Asp Val
        4835                4840                4845

Val Ala Val Asp Gly Asp Gly Asp Asp Ser Asn Gln Tyr Val Leu Pro
    4850                4855                4860

Ile Glu Val Leu Asp Asp Val Pro Val Met Thr Ala Pro Thr Gly Glu
4865                4870                4875                4880

Thr Val Val Asp Glu Asp Asp Leu Thr Gly Ile Gly Ser Asp Gln Ser
                4885                4890                4895

Glu Asp Thr Ile Ile Asn Gly Leu Phe Thr Val Asp Glu Gly Ala Asp
            4900                4905                4910

Gly Val Val Leu Tyr Glu Leu Val Asp Glu Asp Leu Val Leu Thr Gly
        4915                4920                4925

Leu Thr Ser Asp Gly Glu Ser Leu Glu Trp Leu Ala Val Ser Gln Asn
    4930                4935                4940

Gly Thr Thr Phe Thr Tyr Val Ala Gln Thr Ala Thr Ser Asn Glu Ala
4945                4950                4955                4960

Val Phe Glu Ile Ile Phe Asp Thr Ser Asp Asn Ser Tyr Gln Phe Glu
                4965                4970                4975

Leu Phe Lys Pro Leu Lys His Pro Asp Gly Ala Asn Glu Asn Ala Ile
            4980                4985                4990

Asp Leu Asp Phe Ser Ile Val Ala Glu Asp Phe Asp Gln Asp Gln Ser
        4995                5000                5005

Asp Ala Ile Gly Leu Lys Ile Thr Val Thr Asp Asp Val Pro Leu Val
    5010                5015                5020

Thr Thr Gln Ser Ile Thr Arg Leu Glu Gly Gln Gly Tyr Gly Asn Ser
5025                5030                5035                5040
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Lys Val Asp Met Phe Ala Asn Ala Thr Asp Val Gly Ala Asp Gly Ala
                5045                5050                5055

Val Leu Ser Arg Ile Glu Gly Ile Ser Asn Asn Gly Ala Asp Ile Val
            5060                5065                5070

Phe Arg Ser Gly Asn Asn Gly Pro Tyr Ser Ser Gly Phe Asp Leu Asn
        5075                5080                5085

Ser Gly Ser Gln Gln Val Arg Val Tyr Glu Gln Thr Asn Gly Gly Ala
    5090                5095                5100

Asp Thr Arg Glu Leu Gly Arg Leu Arg Ile Asn Ser Asn Gly Glu Val
5105                5110                5115                5120

Glu Phe Arg Ala Asn Gly Tyr Leu Asp His Asp Gly Asp Asp Thr Ile
                5125                5130                5135

Asp Phe Ser Ile Asn Val Ile Ala Thr Asp Gly Asp Leu Asp Thr Ser
            5140                5145                5150

Glu Thr Pro Leu Asp Ile Thr Ile Thr Asp Arg Asp Ser Thr Arg Ile
        5155                5160                5165

Ala Leu Lys Val Thr Thr Phe Glu Asp Ala Gly Arg Asp Ser Thr Ile
    5170                5175                5180

Pro Tyr Ala Thr Gly Asp Glu Pro Thr Leu Glu Asn Val Gln Asp Asn
5185                5190                5195                5200

Gln Asn Gly Leu Pro Asn Ala Pro Ala Gln Val Ala Leu Gln Val Ser
                5205                5210                5215

Leu Tyr Asp Gln Asp Asn Ala Glu Ser Ile Gly Gln Leu Thr Ile Lys
            5220                5225                5230

Ser Pro Asn Gly Gly Asp Ser His Gln Gly Thr Phe Tyr Tyr Phe Asp
        5235                5240                5245

Gly Ala Asp Tyr Ile Glu Leu Val Pro Glu Ser Asn Gly Ser Ile Ile
    5250                5255                5260

Phe Gly Ser Pro Glu Leu Glu Gln Ser Phe Ala Pro Asn Pro Ser Glu
5265                5270                5275                5280

Pro Arg Gln Thr Ile Ala Thr Ile Asp Asn Leu Phe Phe Val Pro Asp
                5285                5290                5295

Gln His Ala Ser Ser Asp Glu Thr Gly Gly Arg Val Arg Tyr Glu Leu
            5300                5305                5310

Glu Ile Glu Lys Asn Gly Ser Thr Asp His Thr Val Asn Ser Asn Phe
        5315                5320                5325

Arg Ile Glu Ile Glu Ala Val Ala Asp Ile Ala Thr Trp Asp Asp Ser
    5330                5335                5340

Asn Ser Thr Tyr Gln Tyr Gln Val Asn Glu Asp Glu Asp Asn Val Thr
5345                5350                5355                5360

Leu Gln Leu Asn Ala Glu Ser Gln Asp Asn Ser Asn Thr Glu Thr Ile
                5365                5370                5375

Thr Tyr Glu Leu Glu Ala Val Gln Gly Asp Gly Lys Phe Glu Leu Leu
            5380                5385                5390

Asp Gln Asn Gly Asn Val Leu Thr Pro Val Asn Gly Val Tyr Ile Ile
        5395                5400                5405

Ala Ser Ala Asp Ile Asn Ser Thr Val Val Asn Pro Ile Asp Asn Phe
    5410                5415                5420

Ser Gly Gln Ile Glu Phe Lys Ala Thr Ala Ile Thr Glu Glu Thr Leu
5425                5430                5435                5440
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Asn Pro Tyr Asp Asp Ser Asp Asn Gly Gly Ala Asn Asp Lys Thr Thr
                5445                5450                5455

Ala Arg Ser Val Glu Gln Ser Ile Val Ile Asp Val Thr Ala Asp Ala
            5460                5465                5470

Asp Pro Gly Thr Phe Ser Val Ser Arg Ile Gln Ile Asn Glu Asp Asn
        5475                5480                5485

Ile Asp Asp Pro Asp Tyr Val Gly Pro Leu Asp Asn Lys Asp Ala Phe
    5490                5495                5500

Thr Leu Asp Glu Val Ile Thr Met Thr Gly Ser Val Asp Ser Asp Ser
5505                5510                5515                5520

Ser Glu Glu Leu Phe Val Arg Ile Ser Asn Val Thr Glu Gly Ala Val
                5525                5530                5535

Leu Tyr Phe Leu Gly Thr Thr Thr Val Val Pro Thr Ile Thr Ile Asn
            5540                5545                5550

Gly Val Asp Tyr Gln Glu Ile Ala Tyr Ser Asp Leu Ala Asn Val Glu
        5555                5560                5565

Val Val Pro Thr Lys His Ser Asn Val Asp Phe Thr Phe Asp Val Thr
    5570                5575                5580

Gly Val Val Lys Asp Thr Ala Asn Leu Ser Thr Gly Ala Gln Ile Asp
5585                5590                5595                5600

Glu Glu Ile Leu Gly Thr Lys Thr Val Asn Val Glu Val Lys Gly Val
                5605                5610                5615

Ala Asp Thr Pro Tyr Gly Gly Thr Asn Gly Thr Ala Trp Ser Ala Ile
            5620                5625                5630

Thr Asp Gly Thr Thr Ser Gly Val Gln Thr Thr Ile Gln Glu Ser Gln
        5635                5640                5645

Asn Gly Asp Thr Phe Ala Glu Leu Asp Phe Thr Val Leu Ser Gly Glu
    5650                5655                5660

Arg Arg Pro Asp Thr Gly Thr Thr Pro Leu Ala Asp Asp Gly Ser Glu
5665                5670                5675                5680

Ser Ile Thr Val Ile Leu Ser Gly Ile Pro Asp Gly Val Val Leu Glu
                5685                5690                5695

Asp Gly Asp Gly Thr Val Ile Asp Leu Asn Phe Val Gly Tyr Glu Thr
            5700                5705                5710

Gly Pro Gly Gly Ser Pro Asp Leu Ser Lys Pro Ile Tyr Glu Ala Asn
        5715                5720                5725

Ile Thr Glu Ala Gly Lys Thr Ser Gly Ile Arg Ile Arg Pro Val Asp
    5730                5735                5740

Ser Ser Thr Glu Asn Ile His Ile Gln Gly Lys Val Ile Val Thr Glu
5745                5750                5755                5760

Asn Asp Gly His Thr Leu Thr Phe Asp Gln Glu Ile Arg Val Leu Val
                5765                5770                5775

Ile Pro Arg Ile Asp Thr Ser Ala Thr Tyr Val Asn Thr Thr Asn Gly
            5780                5785                5790

Asp Glu Asp Thr Ala Ile Asn Ile Asp Trp His Pro Glu Gly Thr Asp
        5795                5800                5805

Tyr Ile Asp Asp Asp Glu His Phe Thr Lys Ile Thr Ile Asn Gly Ile
    5810                5815                5820

Pro Leu Gly Val Thr Ala Val Val Asn Gly Asp Val Thr Val Asp Asp
5825                5830                5835                5840

Ser Thr Pro Gly Thr Leu Ile Ile Thr Pro Lys Asp Ala Ser Gln Thr
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               5845                5850                5855

Pro Glu Gln Phe Thr Gln Ile Ala Leu Ala Asn Asn Phe Ile Gln Met
            5860                5865                5870

Thr Pro Pro Ala Asp Ser Ser Ala Asp Phe Thr Leu Thr Thr Glu Leu
        5875                5880                5885

Lys Met Glu Glu Arg Asp His Glu Tyr Thr Ser Ser Gly Leu Glu Asp
    5890                5895                5900

Glu Asp Gly Gly Tyr Val Glu Ala Asp Pro Asp Ile Thr Gly Ile Ile
5905                5910                5915                5920

Asn Val Gln Val Arg Pro Val Val Glu Pro Gly Asp Ala Asp Asn Lys
                5925                5930                5935

Ile Val Val Ser Asn Glu Asp Gly Ser Gly Asp Leu Thr Thr Ile Thr
            5940                5945                5950

Ala Asp Ala Asn Gly Val Ile Lys Phe Thr Thr Asn Ser Asp Asn Gln
        5955                5960                5965

Thr Thr Asp Thr Asn Gly Asp Glu Ile Trp Asp Gly Glu Tyr Val Val
    5970                5975                5980

Arg Tyr Gln Glu Thr Asp Leu Ser Thr Val Glu Glu Gln Val Asp Glu
5985                5990                5995                6000

Val Ile Val Gln Leu Thr Asn Thr Asp Gly Ser Ala Leu Ser Asp Asp
                6005                6010                6015

Ile Leu Gly Gln Leu Leu Val Thr Gly Ala Ser Tyr Glu Gly Gly Gly
            6020                6025                6030

Arg Trp Val Val Thr Asn Glu Asp Ala Phe Ser Val Ser Ala Pro Asn
        6035                6040                6045

Gly Leu Asp Phe Thr Pro Ala Asn Asp Ala Asp Asp Val Ala Thr Asp
    6050                6055                6060

Phe Asn Asp Ile Lys Met Thr Ile Phe Thr Leu Val Ser Asp Pro Gly
6065                6070                6075                6080

Asp Ala Asn Asn Glu Thr Ser Ala Gln Val Gln Arg Thr Gly Glu Val
                6085                6090                6095

Thr Leu Ser Tyr Pro Glu Val Leu Thr Ala Pro Asp Lys Val Ala Ala
            6100                6105                6110

Asp Ile Ala Ile Val Pro Asp Ser Val Ile Asp Ala Val Glu Asp Thr
        6115                6120                6125

Gln Leu Asp Leu Gly Ala Ala Leu Asn Gly Ile Leu Ser Leu Thr Gly
    6130                6135                6140

Arg Asp Asp Ser Thr Asp Gln Val Thr Val Ile Ile Asp Gly Thr Leu
6145                6150                6155                6160

Val Ile Asp Ala Thr Thr Ser Phe Pro Ile Ser Leu Ser Gly Thr Ser
                6165                6170                6175

Asp Val Asp Phe Val Asn Gly Lys Tyr Val Tyr Glu Thr Thr Val Glu
            6180                6185                6190

Gln Gly Val Ala Val Asp Ser Ser Gly Leu Leu Leu Asn Leu Pro Pro
        6195                6200                6205

Asn Tyr Ser Gly Asp Phe Arg Leu Pro Met Thr Ile Val Thr Lys Asp
    6210                6215                6220

Leu Gln Ser Gly Asp Glu Lys Thr Leu Val Thr Glu Val Ile Ile Lys
6225                6230                6235                6240

Val Ala Pro Asp Ala Glu Thr Asp Pro Thr Ile Glu Val Asn Val Val
                6245                6250                6255
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Gly Ser Leu Asp Asp Ala Phe Asn Pro Val Asp Thr Asp Gly Gln Ala
            6260                6265                6270

Gly Gln Asp Pro Val Gly Tyr Glu Asp Thr Tyr Ile Gln Leu Asp Phe
        6275                6280                6285

Asn Ser Thr Ile Ser Asp Gln Val Ser Gly Val Glu Gly Gly Gln Glu
    6290                6295                6300

Ala Phe Thr Ser Ile Thr Leu Thr Leu Asp Asp Pro Ser Ile Gly Ala
6305                6310                6315                6320

Phe Tyr Asp Asn Thr Gly Thr Ser Leu Gly Thr Ser Val Thr Phe Asn
                6325                6330                6335

Gln Ala Glu Ile Ala Ala Gly Ala Leu Asp Asn Val Leu Phe Arg Ala
            6340                6345                6350

Ile Glu Asn Tyr Pro Thr Gly Asn Asp Ile Asn Gln Val Gln Val Asn
        6355                6360                6365

Val Ser Gly Thr Val Thr Asp Thr Ala Thr Tyr Asn Asp Pro Ala Ser
    6370                6375                6380

Pro Ala Gly Thr Ala Thr Asp Ser Asp Thr Phe Ser Thr Ser Val Ser
6385                6390                6395                6400

Phe Glu Val Val Pro Val Val Asp Asp Val Ser Val Thr Gly Pro Gly
                6405                6410                6415

Ser Asp Pro Asp Val Ile Glu Ile Thr Gly Asn Glu Asp Gln Leu Ile
            6420                6425                6430

Ser Leu Ser Gly Thr Gly Pro Val Ser Ile Ala Leu Thr Asp Leu Asp
        6435                6440                6445

Gly Ser Glu Gln Phe Val Ser Ile Lys Phe Thr Asp Val Pro Asp Gly
    6450                6455                6460

Phe Gln Met Arg Ala Asp Ala Gly Ser Thr Tyr Thr Val Lys Asn Asn
6465                6470                6475                6480

Gly Asn Gly Glu Trp Ser Val Gln Leu Pro Gln Ala Ser Gly Leu Ser
                6485                6490                6495

Phe Asp Leu Ser Glu Ile Ser Ile Leu Pro Pro Lys Asn Phe Ser Gly
            6500                6505                6510

Thr Ala Glu Phe Gly Val Glu Val Phe Thr Gln Glu Ser Leu Leu Gly
        6515                6520                6525

Val Pro Thr Ala Ala Ala Asn Leu Pro Ser Phe Lys Leu His Val Val
    6530                6535                6540

Pro Val Gly Asp Asp Val Asp Thr Asn Pro Thr Asp Ser Val Thr Gly
6545                6550                6555                6560

Asn Glu Gly Gln Asn Ile Asp Ile Glu Ile Asn Ala Thr Ile Leu Asp
                6565                6570                6575

Lys Glu Leu Ser Ala Thr Gly Ser Gly Thr Tyr Thr Glu Asn Ala Pro
            6580                6585                6590

Glu Thr Leu Arg Val Glu Val Ala Gly Val Pro Gln Asp Ala Ser Ile
        6595                6600                6605

Phe Tyr Pro Asp Gly Thr Thr Leu Ala Ser Tyr Asp Pro Ala Thr Gln
    6610                6615                6620

Leu Trp Thr Leu Asp Val Pro Ala Gln Ser Leu Asp Lys Ile Val Phe
6625                6630                6635                6640

Asn Ser Gly Glu His Asn Ser Asp Thr Gly Asn Val Leu Gly Ile Asn
                6645                6650                6655
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Gly Pro Leu Gln Ile Thr Val Arg Ser Val Asp Thr Asp Ala Asp Asn
            6660                6665                6670

Thr Glu Tyr Leu Gly Thr Pro Thr Ser Phe Asp Val Asp Leu Val Ile
        6675                6680                6685

Asp Pro Ile Asn Asp Gln Pro Ile Phe Val Asn Val Thr Asn Ile Glu
    6690                6695                6700

Thr Ser Glu Asp Ile Ser Val Ala Ile Asp Asn Phe Ser Ile Tyr Asp
6705                6710                6715                6720

Val Asp Ala Asn Phe Asp Asn Pro Asp Ala Pro Tyr Glu Leu Thr Leu
                6725                6730                6735

Lys Val Asp Gln Thr Leu Pro Gly Ala Gln Gly Val Phe Glu Phe Thr
            6740                6745                6750

Ser Ser Pro Asp Val Thr Phe Val Leu Gln Pro Asp Gly Ser Leu Val
        6755                6760                6765

Ile Thr Gly Lys Glu Ala Asp Ile Asn Thr Ala Leu Thr Asn Gly Ala
    6770                6775                6780

Val Thr Phe Lys Pro Asp Pro Asp Gln Asn Tyr Leu Asn Gln Thr Gly
6785                6790                6795                6800

Leu Val Thr Ile Asn Ala Thr Leu Asp Asp Gly Gly Asn Asn Gly Leu
                6805                6810                6815

Ile Asp Ala Val Asp Pro Asn Thr Ala Gln Thr Asn Gln Thr Thr Phe
            6820                6825                6830

Thr Ile Lys Val Thr Glu Val Asn Asp Ala Pro Val Ala Thr Asn Val
        6835                6840                6845

Asp Leu Gly Ser Ile Ala Glu Asp Ala Gln Ile Val Ile Val Glu Ser
    6850                6855                6860

Asp Leu Ile Ala Ala Ser Ser Asp Leu Glu Asn His Asn Leu Thr Val
6865                6870                6875                6880

Thr Gly Val Thr Leu Thr Gln Gly Gln Gly Gln Leu Thr Arg Tyr Glu
                6885                6890                6895

Asn Ala Gly Gly Ala Asp Asp Ala Ala Ile Thr Gly Pro Phe Trp Ile
            6900                6905                6910

Phe Ile Ala Asp Asn Asp Phe Asn Gly Asp Val Lys Phe Asn Tyr Ser
        6915                6920                6925

Ile Ile Asp Asp Gly Thr Thr Asn Gly Val Asp Asp Phe Lys Thr Asp
    6930                6935                6940

Ser Ala Glu Ile Ser Leu Val Val Thr Glu Val Asn Asp Gln Pro Val
6945                6950                6955                6960

Ala Ser Asn Ile Asp Leu Gly Thr Met Leu Glu Glu Gly Gln Leu Val
                6965                6970                6975

Ile Lys Glu Glu Asp Leu Ile Ser Ala Thr Thr Asp Pro Glu Asn Asp
            6980                6985                6990

Thr Ile Thr Val Asn Ser Leu Val Leu Asp Gln Gly Gln Gly Gln Leu
        6995                7000                7005

Gln Arg Phe Glu Asn Val Gly Gly Ala Asp Asp Ala Thr Ile Thr Gly
    7010                7015                7020

Pro Tyr Trp Val Phe Thr Ala Ala Asn Glu Tyr Asn Gly Asp Val Lys
7025                7030                7035                7040

Phe Thr Tyr Thr Val Glu Asp Asp Gly Thr Thr Asn Gly Ala Asp Asp
                7045                7050                7055

Phe Leu Thr Asp Thr Gly Glu Ile Ser Val Val Val Thr Glu Val Asn
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           7060                7065                7070

Asp Gln Pro Val Ala Thr Asp Ile Asp Leu Gly Asn Ile Leu Glu Glu
        7075                7080                7085

Gly Gln Leu Ile Ile Lys Glu Glu Asp Leu Ile Ala Ala Thr Ser Asp
    7090                7095                7100

Pro Glu Asn Asp Thr Ile Thr Val Thr Asn Leu Val Leu Asp Glu Gly
7105                7110                7115                7120

Gln Gly Gln Leu Gln Arg Phe Glu Asn Val Gly Gly Ala Asp Asp Ala
                7125                7130                7135

Met Ile Thr Gly Pro Tyr Trp Ile Phe Thr Ala Ala Asp Glu Tyr Asn
            7140                7145                7150

Gly Asn Val Lys Phe Thr Tyr Thr Val Glu Asp Asp Gly Thr Thr Asn
        7155                7160                7165

Gly Ala Asn Asp Phe Leu Thr Asp Thr Ala Glu Ile Thr Ala Ile Val
    7170                7175                7180

Asp Gly Val Asn Asp Thr Pro Val Val Asn Gly Asp Ser Val Thr Thr
7185                7190                7195                7200

Ile Val Asp Glu Asp Ala Gly Gln Leu Leu Ser Gly Ile Asn Val Ser
                7205                7210                7215

Asp Pro Asp Tyr Val Asp Ala Phe Ser Asn Asp Leu Met Thr Val Thr
            7220                7225                7230

Leu Thr Val Asp Tyr Gly Thr Leu Asn Val Ser Leu Pro Ala Val Thr
        7235                7240                7245

Thr Val Met Val Asn Gly Asn Asn Thr Gly Ser Val Ile Leu Val Gly
    7250                7255                7260

Thr Leu Ser Asp Leu Asn Ala Leu Ile Asp Thr Pro Thr Ser Pro Asn
7265                7270                7275                7280

Gly Val Tyr Leu Asp Ala Ser Leu Ser Pro Thr Asn Ser Ile Gly Leu
                7285                7290                7295

Glu Val Ile Ala Lys Asp Ser Gly Asn Pro Ser Gly Ile Ala Ile Glu
            7300                7305                7310

Thr Ala Pro Val Val Tyr Asn Ile Ala Val Thr Pro Val Ala Asn Ala
        7315                7320                7325

Pro Thr Leu Ser Ile Asp Pro Ala Phe Asn Tyr Val Arg Asn Ile Thr
    7330                7335                7340

Thr Ser Ser Ser Val Val Ala Asn Ser Gly Val Ala Leu Val Gly Ile
7345                7350                7355                7360

Val Ala Ala Leu Thr Asp Ile Thr Glu Glu Leu Thr Leu Lys Ile Ser
                7365                7370                7375

Asp Val Pro Asp Gly Val Asp Val Thr Ser Asp Val Gly Thr Val Ser
            7380                7385                7390

Leu Val Gly Asp Thr Trp Ile Ala Thr Ala Asp Ala Ile Asp Ser Leu
        7395                7400                7405

Arg Leu Val Glu Gln Ser Ser Leu Gly Lys Pro Leu Thr Pro Gly Asn
    7410                7415                7420

Tyr Thr Leu Lys Val Glu Ala Leu Ser Glu Glu Thr Asp Asn Asn Asp
7425                7430                7435                7440

Ile Ala Ile Ser Gln Asn Ile Asp Leu Asn Leu Asn Ile Val Ala Asn
                7445                7450                7455

Pro Ile Asp Leu Asp Leu Ser Ser Glu Thr Asp Asp Val Gln Leu Leu
            7460                7465                7470
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Ala Ser Asn Phe Asp Thr Asn Leu Thr Gly Gly Thr Gly Asn Asp Arg
        7475                7480                7485

Leu Val Gly Gly Ala Gly Asp Asp Thr Leu Val Gly Gly Asp Gly Asn
    7490                7495                7500

Asp Thr Leu Ile Gly Gly Gly Gly Ser Asp Ile Leu Thr Gly Gly Asn
7505                7510                7515                7520

Gly Met Asp Ser Phe Val Trp Leu Asn Ile Glu Asp Gly Val Glu Asp
                7525                7530                7535

Thr Ile Thr Asp Phe Ser Leu Ser Glu Gly Asp Gln Ile Asp Leu Arg
            7540                7545                7550

Glu Val Leu Pro Glu Leu Lys Asn Thr Ser Pro Asp Met Ser Ala Leu
        7555                7560                7565

Leu Gln Gln Ile Asp Ala Lys Val Glu Gly Asp Asp Ile Glu Leu Thr
    7570                7575                7580

Ile Lys Ser Asp Gly Leu Gly Thr Thr Glu Gln Val Ile Val Val Glu
7585                7590                7595                7600

Asp Leu Ala Pro Gln Leu Thr Leu Ser Gly Thr Met Pro Ser Asp Ile
                7605                7610                7615

Leu Asp Ala Leu Val Gln Gln Asn Val Ile Thr His Gly
            7620                7625


<210> SEQ ID NO 5
<211> LENGTH: 765
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 5

atgaaaaaaa catcactatt acttgcttcc attactctgg cactttctgg tgtagtacaa     60

gctgaccagc tagaagacat tcaaaaatca ggcacacttc gcgtcggcac cacaggcgac    120

tacaaacctt tttcttactt cgacggcaaa acctactctg gttatgacat tgacgtagcc    180

aaacatgttg cagagcagtt gggcgttgaa ttacagattg ttcgtaccac atggaaagat    240

ctactgaccg atctagacag cgataaatac gacatcgcga tgggcggtat cacgcgtaaa    300

atgcagcgtc agttaaacgc agaacaaact caaggttaca tgacctttgg caagtgtttc    360

ttagttgcga aaggcaaagc agaacaatac aacagcattg agaaagtgaa cctctcttct    420

gtgcgtgttg gcgtcaatat cggtgggact aatgagatgt ttgcggatgc taacttgcaa    480

gacgcgagct ttacgcgtta cgagaacaac ctagacgttc cgcaagccgt tgcggaaggt    540

aaagttgatg taatggtgac agaaactcct gaaggtctgt tctatcaagt gacggacgaa    600

cgtcttgaag cggcacgctg tgaaacaccg tttaccaaca gtcaattcgg ttacctgata    660

ccaaaaggtg aacaacgctt gttgaacaca gtgaacttca ttatggatga gatgaaattg    720

aaaggcgtcg aagaagagtt cctgatccac aactctctta agtaa                    765


<210> SEQ ID NO 6
<211> LENGTH: 254
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 6

Met Lys Lys Thr Ser Leu Leu Leu Ala Ser Ile Thr Leu Ala Leu Ser
1               5                  10                  15
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Gly Val Val Gln Ala Asp Gln Leu Glu Asp Ile Gln Lys Ser Gly Thr
            20                  25                  30

Leu Arg Val Gly Thr Thr Gly Asp Tyr Lys Pro Phe Ser Tyr Phe Asp
        35                  40                  45

Gly Lys Thr Tyr Ser Gly Tyr Asp Ile Asp Val Ala Lys His Val Ala
    50                  55                  60

Glu Gln Leu Gly Val Glu Leu Gln Ile Val Arg Thr Thr Trp Lys Asp
65                  70                  75                  80

Leu Leu Thr Asp Leu Asp Ser Asp Lys Tyr Asp Ile Ala Met Gly Gly
                85                  90                  95

Ile Thr Arg Lys Met Gln Arg Gln Leu Asn Ala Glu Gln Thr Gln Gly
            100                 105                 110

Tyr Met Thr Phe Gly Lys Cys Phe Leu Val Ala Lys Gly Lys Ala Glu
        115                 120                 125

Gln Tyr Asn Ser Ile Glu Lys Val Asn Leu Ser Ser Val Arg Val Gly
    130                 135                 140

Val Asn Ile Gly Gly Thr Asn Glu Met Phe Ala Asp Ala Asn Leu Gln
145                 150                 155                 160

Asp Ala Ser Phe Thr Arg Tyr Glu Asn Asn Leu Asp Val Pro Gln Ala
                165                 170                 175

Val Ala Glu Gly Lys Val Asp Val Met Val Thr Glu Thr Pro Glu Gly
            180                 185                 190

Leu Phe Tyr Gln Val Thr Asp Glu Arg Leu Glu Ala Ala Arg Cys Glu
        195                 200                 205

Thr Pro Phe Thr Asn Ser Gln Phe Gly Tyr Leu Ile Pro Lys Gly Glu
    210                 215                 220

Gln Arg Leu Leu Asn Thr Val Asn Phe Ile Met Asp Glu Met Lys Leu
225                 230                 235                 240

Lys Gly Val Glu Glu Glu Phe Leu Ile His Asn Ser Leu Lys
                245                 250


<210> SEQ ID NO 7
<211> LENGTH: 765
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 7

atgaaaaaaa catcactatt acttgcttcc attactctgg cactttctgg tgtagtacaa     60

gctgaccagc tagaagacat tcaaaaatca ggcacacttc gcgtcggcac cacaggcgac    120

tacaaacctt tttcttactt cgacggcaaa acctactctg gttatgacat tgacgtagcc    180

aaacatgttg cagagcagtt gggcgttgaa ttacagattg ttcgtaccac atggaaagat    240

ctactgaccg atctagacag cgataaatac gacatcgcga tgggcggtat cacgcgtaaa    300

atgcagcgtc agttaaacgc agaacaaact caaggttaca tgacctttgg caagtgtttc    360

ttagttgcga aaggcaaagc agaacaatac aacagcattg agaaagtgaa cctctcttct    420

gtgcgtgttg gcgtcaatat cggtgggact aatgagatgt ttgcggatgc taacttgcaa    480

gacgcgagct ttacgcgtta cgagaacaac ctagacgttc cgcaagccgt tgcggaaggt    540

aaagttgatg taatggtgac agaaactcct gaaggtctgt tctatcaagt gacggacgaa    600

cgtcttgaag cggcacgctg tgaaacaccg tttaccaaca gtcaattcgg ttacctgata    660

ccaaaaggtg aacaacgctt gttgaacaca gtgaacttca ttatggatga gatgaaattg    720
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aaaggcgtcg aagaagagtt cctgatccac aactctctta agtaa 765

<210> SEQ ID NO 8
<211> LENGTH: 588
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 8

Met Thr Ile Asp Thr Phe Val Val Leu Ala Tyr Phe Phe Phe Leu Ile
1 5 10 15

Ala Ile Gly Trp Met Phe Arg Lys Phe Thr Thr Ser Thr Ser Asp Tyr
20 25 30

Phe Arg Gly Gly Gly Lys Met Leu Trp Trp Met Val Gly Ala Thr Ala
35 40 45

Phe Met Thr Gln Phe Ser Ala Trp Thr Phe Thr Gly Ala Ala Gly Arg
50 55 60

Ala Phe Asn Asp Gly Phe Val Ile Val Ile Leu Phe Leu Ala Asn Ala
65 70 75 80

Phe Gly Tyr Phe Met Asn Tyr Met Tyr Phe Ala Pro Lys Phe Arg Gln
85 90 95

Leu Arg Val Val Thr Ala Ile Glu Ala Ile Arg Gln Arg Phe Gly Lys
100 105 110

Thr Ser Glu Gln Phe Phe Thr Trp Ala Gly Met Pro Asp Ser Leu Ile
115 120 125

Ser Ala Gly Ile Trp Leu Asn Gly Leu Ala Ile Phe Val Ala Ala Val
130 135 140

Phe Asn Ile Pro Met Glu Ala Thr Ile Val Val Thr Gly Met Val Leu
145 150 155 160

Val Leu Met Ala Val Thr Gly Gly Ser Trp Ala Val Val Ala Ser Asp
165 170 175

Phe Met Gln Met Leu Val Ile Met Ala Val Thr Ile Thr Cys Ala Val
180 185 190

Ala Ala Tyr Phe His Gly Gly Gly Leu Thr Asn Ile Val Ala Asn Phe
195 200 205

Asp Gly Asp Phe Met Leu Gly Asn Asn Leu Asn Tyr Met Ser Ile Phe
210 215 220

Val Leu Trp Val Val Phe Ile Phe Val Lys Gln Phe Gly Val Met Asn
225 230 235 240

Asn Ser Ile Asn Ala Tyr Arg Tyr Leu Cys Ala Lys Asp Ser Glu Asn
245 250 255

Ala Arg Lys Ala Ala Gly Leu Ala Cys Ile Leu Met Val Val Gly Pro
260 265 270

Leu Ile Trp Phe Leu Pro Pro Trp Tyr Val Ser Ala Phe Met Pro Asp
275 280 285

Phe Ala Leu Glu Tyr Ala Ser Met Gly Asp Lys Ala Gly Asp Ala Ala
290 295 300

Tyr Leu Ala Phe Val Gln Asn Val Met Pro Ala Gly Met Val Gly Leu
305 310 315 320

Leu Met Ser Ala Met Phe Ala Ala Thr Met Ser Ser Met Asp Ser Gly
325 330 335

Leu Asn Arg Asn Ala Gly Ile Phe Val Met Asn Phe Tyr Ser Pro Ile
340 345 350

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Leu Arg Gln Asn Ala Thr Gln Lys Glu Leu Val Ile Val Ser Lys Leu
        355                 360                 365

Thr Thr Ile Met Met Gly Ile Ile Ile Ile Ala Ile Gly Leu Phe Ile
    370                 375                 380

Asn Ser Leu Arg His Leu Ser Leu Phe Asp Ile Val Met Asn Val Gly
385                 390                 395                 400

Ala Leu Ile Gly Phe Pro Met Leu Ile Pro Val Leu Leu Gly Met Trp
                405                 410                 415

Ile Arg Lys Thr Pro Asp Trp Ala Gly Trp Ser Thr Leu Ile Val Gly
            420                 425                 430

Gly Phe Val Ser Tyr Ile Phe Gly Ile Ser Leu Gln Ala Glu Asp Ile
        435                 440                 445

Glu His Leu Phe Gly Met Glu Thr Ala Leu Thr Gly Arg Glu Trp Ser
    450                 455                 460

Asp Leu Lys Val Gly Leu Ser Leu Ala Ala His Val Val Phe Thr Gly
465                 470                 475                 480

Gly Tyr Phe Ile Leu Thr Ser Arg Phe Tyr Lys Gly Leu Ser Pro Glu
                485                 490                 495

Arg Glu Lys Glu Val Asp Gln Leu Phe Thr Asn Trp Asn Thr Pro Leu
            500                 505                 510

Val Ala Glu Gly Glu Glu Gln Gln Asn Leu Asp Thr Lys Gln Arg Ser
        515                 520                 525

Met Leu Gly Lys Leu Ile Ser Thr Ala Gly Phe Gly Ile Leu Ala Met
    530                 535                 540

Ala Leu Ile Pro Asn Glu Pro Thr Gly Arg Leu Leu Phe Leu Leu Cys
545                 550                 555                 560

Gly Ser Met Val Leu Thr Val Gly Ile Leu Leu Val Asn Ala Ser Lys
                565                 570                 575

Ala Pro Ala Lys Met Asn Asn Glu Ser Val Ala Lys
            580                 585
```

<210> SEQ ID NO 9
<211> LENGTH: 627
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 9

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atgacgacat taaatgaaca actagcaaac ctaaaagtaa ttcctgtaat cgcgatcaac     60

cgtgctgaag acgctatccc tctaggtaaa gcgttggttg aaaatggcat gccatgtgca    120

gaaattacac tacgtacaga atgtgcaatc gaagcgattc gcatcatgcg taaagaattc    180

ccagacatgc taatcggttc aggtactgta ctgactaacg agcaagttga cgcatctatc    240

gaagctggtg ttgatttcat cgtaagccca ggttttaacc cacgtactgt tcaatactgt    300

atcgataaag gtattgcaat cgtaccgggt gttaacaacc caagcctagt tgagcaagca    360

atggaaatgg gtcttcgcac gttgaagttc ttccctgctg agccttcagg cggtactggc    420

atgcttaaag cactaacagc agtttaccct gttaaattca tgcctactgg tggcgtaagc    480

ttgaagaatg ttgatgaata cctatcgatc ccttctgttc ttgcgtgtgg cggtacttgg    540

atggttccaa ctaaccttat cgatgaaggt aagtgggacg aactaggcaa gcttgttcgt    600

gacgcagttg atcacgttaa cgcttaa                                        627
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<210> SEQ ID NO 10
<211> LENGTH: 208
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 10

Met Thr Thr Leu Asn Glu Gln Leu Ala Asn Leu Lys Val Ile Pro Val
1 5 10 15

Ile Ala Ile Asn Arg Ala Glu Asp Ala Ile Pro Leu Gly Lys Ala Leu
20 25 30

Val Glu Asn Gly Met Pro Cys Ala Glu Ile Thr Leu Arg Thr Glu Cys
35 40 45

Ala Ile Glu Ala Ile Arg Ile Met Arg Lys Glu Phe Pro Asp Met Leu
50 55 60

Ile Gly Ser Gly Thr Val Leu Thr Asn Glu Gln Val Asp Ala Ser Ile
65 70 75 80

Glu Ala Gly Val Asp Phe Ile Val Ser Pro Gly Phe Asn Pro Arg Thr
85 90 95

Val Gln Tyr Cys Ile Asp Lys Gly Ile Ala Ile Val Pro Gly Val Asn
100 105 110

Asn Pro Ser Leu Val Glu Gln Ala Met Glu Met Gly Leu Arg Thr Leu
115 120 125

Lys Phe Phe Pro Ala Glu Pro Ser Gly Gly Thr Gly Met Leu Lys Ala
130 135 140

Leu Thr Ala Val Tyr Pro Val Lys Phe Met Pro Thr Gly Gly Val Ser
145 150 155 160

Leu Lys Asn Val Asp Glu Tyr Leu Ser Ile Pro Ser Val Leu Ala Cys
165 170 175

Gly Gly Thr Trp Met Val Pro Thr Asn Leu Ile Asp Glu Gly Lys Trp
180 185 190

Asp Glu Leu Gly Lys Leu Val Arg Asp Ala Val Asp His Val Asn Ala
195 200 205

<210> SEQ ID NO 11
<211> LENGTH: 933
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 11

atgaaatcat taaacatcgc ggtcattggc gagtgcatgg ttgagctaca aaagaaacaa 60

gacgggctta agcaaagttt tggtggcgat acgctgaata ctgcacttta cttgtcacgc 120

ttaacaaaag agcaagatat caacacgagc tacgtaactg cactaggcac tgacccattc 180

agtaccgaca tgttaaaaaa ttggcaagcg gaaggtatcg acacgagctt aattgctcag 240

ctggaccaca aacaaccagg gctttactac atcgagaccg atgaaactgg tgaacgcagt 300

ttccactact ggcgtagtga tgctgcagcg aagttcatgt ttgatcagga agacacgcct 360

gctcttcttg ataagctgtt ctcttttgac gcgatttact taagtggtat tacgctggca 420

atcttgacag aaaatggtcg cacgcagcta ttcaacttct tagacaaatt caaagctcaa 480

ggcggccaag tattcttcga caataactac cgacctaaac tttgggaaag ccaacaagaa 540

gcgatttctt ggtacttgaa aatgcttaag tacacagata cggctctgct gacgtttgat 600

gatgagcaag agctatacgg cgacgaaagc attgaacaat gtattacacg tacgtcagag 660

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tctggtgtga aagagatcgt cattaaacgt ggcgcgaaag actgcttagt ggttgaaagc    720

caaagcgctc aatacgttgc acccaaccct gtagacaaca tcgttgatac gactgccgct    780

ggcgactcgt tcagtgcagg cttcttggcc aagcgcttga gcggcggtag tgctcgtgat    840

gctgcatttg caggtcatat tgtggcagga accgtgattc agcatccagg tgctatcatt    900

cctctagaag cgacgcctga tctgtctcta taa                                 933


<210> SEQ ID NO 12
<211> LENGTH: 310
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 12

Met Lys Ser Leu Asn Ile Ala Val Ile Gly Glu Cys Met Val Glu Leu
1               5                   10                  15

Gln Lys Lys Gln Asp Gly Leu Lys Gln Ser Phe Gly Gly Asp Thr Leu
            20                  25                  30

Asn Thr Ala Leu Tyr Leu Ser Arg Leu Thr Lys Glu Gln Asp Ile Asn
        35                  40                  45

Thr Ser Tyr Val Thr Ala Leu Gly Thr Asp Pro Phe Ser Thr Asp Met
    50                  55                  60

Leu Lys Asn Trp Gln Ala Glu Gly Ile Asp Thr Ser Leu Ile Ala Gln
65                  70                  75                  80

Leu Asp His Lys Gln Pro Gly Leu Tyr Tyr Ile Glu Thr Asp Glu Thr
                85                  90                  95

Gly Glu Arg Ser Phe His Tyr Trp Arg Ser Asp Ala Ala Ala Lys Phe
            100                 105                 110

Met Phe Asp Gln Glu Asp Thr Pro Ala Leu Leu Asp Lys Leu Phe Ser
        115                 120                 125

Phe Asp Ala Ile Tyr Leu Ser Gly Ile Thr Leu Ala Ile Leu Thr Glu
    130                 135                 140

Asn Gly Arg Thr Gln Leu Phe Asn Phe Leu Asp Lys Phe Lys Ala Gln
145                 150                 155                 160

Gly Gly Gln Val Phe Phe Asp Asn Asn Tyr Arg Pro Lys Leu Trp Glu
                165                 170                 175

Ser Gln Gln Glu Ala Ile Ser Trp Tyr Leu Lys Met Leu Lys Tyr Thr
            180                 185                 190

Asp Thr Ala Leu Leu Thr Phe Asp Asp Glu Gln Glu Leu Tyr Gly Asp
        195                 200                 205

Glu Ser Ile Glu Gln Cys Ile Thr Arg Thr Ser Glu Ser Gly Val Lys
    210                 215                 220

Glu Ile Val Ile Lys Arg Gly Ala Lys Asp Cys Leu Val Val Glu Ser
225                 230                 235                 240

Gln Ser Ala Gln Tyr Val Ala Pro Asn Pro Val Asp Asn Ile Val Asp
                245                 250                 255

Thr Thr Ala Ala Gly Asp Ser Phe Ser Ala Gly Phe Leu Ala Lys Arg
            260                 265                 270

Leu Ser Gly Gly Ser Ala Arg Asp Ala Ala Phe Ala Gly His Ile Val
        275                 280                 285

Ala Gly Thr Val Ile Gln His Pro Gly Ala Ile Ile Pro Leu Glu Ala
    290                 295                 300
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Thr Pro Asp Leu Ser Leu
305 310

<210> SEQ ID NO 13
<211> LENGTH: 336
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 13

atgaactctt tctttatcct agatgaaaat ccatgggaag aacttggtgg cggcattaag 60

cgtaaaatcg ttgcttacac tgacgatcta atggcagtac acctatgctt tgataagggc 120

gcgattggcc accctcatac tcacgaaatt cacgaccaaa tcggttatgt tgttcgtggt 180

agcttcgaag ctgaaatcga cggcgagaag aaagtgctta aagaaggcga tgcttacttc 240

gctcgtaaac acatgatgca cggtgcagtt gctctagaac aagacagcat ccttcttgat 300

atcttcaatc ctgcgcgtga agatttccta aaataa 336

<210> SEQ ID NO 14
<211> LENGTH: 111
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 14

Met Asn Ser Phe Phe Ile Leu Asp Glu Asn Pro Trp Glu Glu Leu Gly
1 5 10 15

Gly Gly Ile Lys Arg Lys Ile Val Ala Tyr Thr Asp Asp Leu Met Ala
20 25 30

Val His Leu Cys Phe Asp Lys Gly Ala Ile Gly His Pro His Thr His
35 40 45

Glu Ile His Asp Gln Ile Gly Tyr Val Val Arg Gly Ser Phe Glu Ala
50 55 60

Glu Ile Asp Gly Glu Lys Lys Val Leu Lys Glu Gly Asp Ala Tyr Phe
65 70 75 80

Ala Arg Lys His Met Met His Gly Ala Val Ala Leu Glu Gln Asp Ser
85 90 95

Ile Leu Leu Asp Ile Phe Asn Pro Ala Arg Glu Asp Phe Leu Lys
100 105 110

<210> SEQ ID NO 15
<211> LENGTH: 2208
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 15

atgacgacta aaccagtatt gttgactgaa gctgaaatcg aacagcttca tcttgaagtg 60

ggccgttcta gcttaatggg caaaaccatt gcagcgaacg cgaaagacct agaagcattc 120

atgcgtttac ctattgatgt tccaggtcac ggtgaagctg ggggttacga acataaccgc 180

cacaagcaaa attacacgta catgaaccta gctggtcgca tgttcttgat cactaaagag 240

caaaaatacg ctgactttgt tacagaatta ctagaagagt acgcagacaa atatctaacg 300

tttgattacc acgtacagaa aaacaccaac ccaacaggtc gtttgttcca ccaaatccta 360

aacgaacact gctggttaat gttctcaagc ttagcttatt cttgtgttgc ttcaacactg 420

acacaagatc agcgtgacaa tattgagtct cgcatttttg aacccatgct agaaatgttc 480

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acggttaaat acgcacacga cttcgaccgt attcacaatc acggtatttg ggcagtagcc    540

gctgtgggta tctgtggtct tgctttaggc aaacgtgaat acctagaaat gtcagtgtac    600

ggcatcgacc gtaatgatac tggcggtttc ctagcgcaag tttctcagct atttgcacct    660

tctggctact acatggaagg tccttactac catcgttatg cgattcgccc aacgtgtgtg    720

ttcgctgaag tgattcaccg tcatatgcct gaagttgata tctacaacta caaaggcggc    780

gtgattggta acacagtaca agctatgctt gcgacagcgt acccgaacgg cgagttcccg    840

gctctgaatg atgcttctcg tactatgggt atcacagaca tgggtgttca ggttgcggtc    900

agtgtttaca gtaagcatta ctcttctgaa aacggtgtag accaaaacat tctgggtatg    960

gcgaagattc aagacgcagt atggatgcat ccatgtggtc ttgagctatc taaagcatac   1020

gaagccgcat ctgcagagaa agaaatcggc atgcctttct ggccaagtgt tgaattgaat   1080

gaaggccctc aaggtcacaa cggcgcgcaa ggctttatcc gtatgcagga taagaaaggc   1140

gacgtttctc aacttgtgat gaactacggc caacacggca tgggtcacgg caactttgat   1200

acgctgggta tttctttctt taaccgcggt caagaagtgc tacgtgaata cggcttctgt   1260

cgttgggtta acgttgagcc aaaattcggc ggccgttacc tagacgaaaa caaatcttac   1320

gctcgtcaaa cgattgctca caatgcagtt acgattgatg aaaaatgtca gaacaacttt   1380

gacgttgaac gtgcagactc agtacatggt ttacctcact tctttaaagt agaagacgat   1440

caaatcaacg gtatgagtgc atttgctaac gatcattacc aaggctttga catgcaacgc   1500

agcgtgttca tgctaaatct tgaagaatta gaatctccgt tattgttaga cctataccgc   1560

ttagattcta caaaaggcgg cgaaggcgag caccaatacg actattcaca ccaatatgcg   1620

ggtcagattg ttcgcactaa cttcgaatac caagcgaaca aagagctaaa cactctaggt   1680

gacgatttcg gttaccaaca tctatggaac gtcgcaagcg gtgaagtgaa gggcacagca   1740

attgtaagtt ggctacaaaa caacacctac tacacatggc taggtgcaac gtctaacgat   1800

aatgctgaag taatatttac tcgcactggc gctaacgacc caagtttcaa tctacgttca   1860

gagcctgcgt tcattctacg cagcaaaggc gaaacaacac tgtttgcttc tgttgttgaa   1920

acgcacggtt atttcaacga agaattcgag caatctgtca atgcacgtgg tgttgtgaaa   1980

gacatcaaag tcgtggctca caccaatgtc ggttcggtag ttgagatcac cacagagaaa   2040

tcaaacgtga cagtgatgat cagcaaccaa cttggcgcga ctgacagcac tgaacacaaa   2100

gtagaactga acggcaaagt atacagctgg aaaggcttct actcagtaga gacaacttta   2160

caagaaacga attcagaaga acttagcact gcagggcagg ggaaataa               2208
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<210> SEQ ID NO 16
<211> LENGTH: 735
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 16

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Met Thr Thr Lys Pro Val Leu Leu Thr Glu Ala Glu Ile Glu Gln Leu
1               5                  10                  15

His Leu Glu Val Gly Arg Ser Ser Leu Met Gly Lys Thr Ile Ala Ala
            20                  25                  30

Asn Ala Lys Asp Leu Glu Ala Phe Met Arg Leu Pro Ile Asp Val Pro
        35                  40                  45

Gly His Gly Glu Ala Gly Gly Tyr Glu His Asn Arg His Lys Gln Asn
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    50                  55                  60

Tyr Thr Tyr Met Asn Leu Ala Gly Arg Met Phe Leu Ile Thr Lys Glu
65                  70                  75                  80

Gln Lys Tyr Ala Asp Phe Val Thr Glu Leu Leu Glu Glu Tyr Ala Asp
                85                  90                  95

Lys Tyr Leu Thr Phe Asp Tyr His Val Gln Lys Asn Thr Asn Pro Thr
            100                 105                 110

Gly Arg Leu Phe His Gln Ile Leu Asn Glu His Cys Trp Leu Met Phe
        115                 120                 125

Ser Ser Leu Ala Tyr Ser Cys Val Ala Ser Thr Leu Thr Gln Asp Gln
    130                 135                 140

Arg Asp Asn Ile Glu Ser Arg Ile Phe Glu Pro Met Leu Glu Met Phe
145                 150                 155                 160

Thr Val Lys Tyr Ala His Asp Phe Asp Arg Ile His Asn His Gly Ile
                165                 170                 175

Trp Ala Val Ala Ala Val Gly Ile Cys Gly Leu Ala Leu Gly Lys Arg
            180                 185                 190

Glu Tyr Leu Glu Met Ser Val Tyr Gly Ile Asp Arg Asn Asp Thr Gly
        195                 200                 205

Gly Phe Leu Ala Gln Val Ser Gln Leu Phe Ala Pro Ser Gly Tyr Tyr
    210                 215                 220

Met Glu Gly Pro Tyr Tyr His Arg Tyr Ala Ile Arg Pro Thr Cys Val
225                 230                 235                 240

Phe Ala Glu Val Ile His Arg His Met Pro Glu Val Asp Ile Tyr Asn
                245                 250                 255

Tyr Lys Gly Gly Val Ile Gly Asn Thr Val Gln Ala Met Leu Ala Thr
            260                 265                 270

Ala Tyr Pro Asn Gly Glu Phe Pro Ala Leu Asn Asp Ala Ser Arg Thr
        275                 280                 285

Met Gly Ile Thr Asp Met Gly Val Gln Val Ala Val Ser Val Tyr Ser
    290                 295                 300

Lys His Tyr Ser Ser Glu Asn Gly Val Asp Gln Asn Ile Leu Gly Met
305                 310                 315                 320

Ala Lys Ile Gln Asp Ala Val Trp Met His Pro Cys Gly Leu Glu Leu
                325                 330                 335

Ser Lys Ala Tyr Glu Ala Ala Ser Ala Glu Lys Glu Ile Gly Met Pro
            340                 345                 350

Phe Trp Pro Ser Val Glu Leu Asn Glu Gly Pro Gln Gly His Asn Gly
        355                 360                 365

Ala Gln Gly Phe Ile Arg Met Gln Asp Lys Lys Gly Asp Val Ser Gln
    370                 375                 380

Leu Val Met Asn Tyr Gly Gln His Gly Met Gly His Gly Asn Phe Asp
385                 390                 395                 400

Thr Leu Gly Ile Ser Phe Phe Asn Arg Gly Gln Glu Val Leu Arg Glu
                405                 410                 415

Tyr Gly Phe Cys Arg Trp Val Asn Val Glu Pro Lys Phe Gly Gly Arg
            420                 425                 430

Tyr Leu Asp Glu Asn Lys Ser Tyr Ala Arg Gln Thr Ile Ala His Asn
        435                 440                 445

Ala Val Thr Ile Asp Glu Lys Cys Gln Asn Asn Phe Asp Val Glu Arg
    450                 455                 460
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Ala Asp Ser Val His Gly Leu Pro His Phe Phe Lys Val Glu Asp Asp
465                 470                 475                 480

Gln Ile Asn Gly Met Ser Ala Phe Ala Asn Asp His Tyr Gln Gly Phe
                485                 490                 495

Asp Met Gln Arg Ser Val Phe Met Leu Asn Leu Glu Glu Leu Glu Ser
            500                 505                 510

Pro Leu Leu Leu Asp Leu Tyr Arg Leu Asp Ser Thr Lys Gly Gly Glu
        515                 520                 525

Gly Glu His Gln Tyr Asp Tyr Ser His Gln Tyr Ala Gly Gln Ile Val
    530                 535                 540

Arg Thr Asn Phe Glu Tyr Gln Ala Asn Lys Glu Leu Asn Thr Leu Gly
545                 550                 555                 560

Asp Asp Phe Gly Tyr Gln His Leu Trp Asn Val Ala Ser Gly Glu Val
                565                 570                 575

Lys Gly Thr Ala Ile Val Ser Trp Leu Gln Asn Asn Thr Tyr Tyr Thr
            580                 585                 590

Trp Leu Gly Ala Thr Ser Asn Asp Asn Ala Glu Val Ile Phe Thr Arg
        595                 600                 605

Thr Gly Ala Asn Asp Pro Ser Phe Asn Leu Arg Ser Glu Pro Ala Phe
    610                 615                 620

Ile Leu Arg Ser Lys Gly Glu Thr Thr Leu Phe Ala Ser Val Val Glu
625                 630                 635                 640

Thr His Gly Tyr Phe Asn Glu Glu Phe Glu Gln Ser Val Asn Ala Arg
                645                 650                 655

Gly Val Val Lys Asp Ile Lys Val Val Ala His Thr Asn Val Gly Ser
            660                 665                 670

Val Val Glu Ile Thr Thr Glu Lys Ser Asn Val Thr Val Met Ile Ser
        675                 680                 685

Asn Gln Leu Gly Ala Thr Asp Ser Thr Glu His Lys Val Glu Leu Asn
    690                 695                 700

Gly Lys Val Tyr Ser Trp Lys Gly Phe Tyr Ser Val Glu Thr Thr Leu
705                 710                 715                 720

Gln Glu Thr Asn Ser Glu Glu Leu Ser Thr Ala Gly Gln Gly Lys
                725                 730                 735


<210> SEQ ID NO 17
<211> LENGTH: 2154
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 17

atgagctatc aaccactttt acttaacttt gatgaagcag ctgaacttcg taaagaactt     60

ggcaaggata gcctattagg taacgcactg actcgcgaca ttaaacaaac tgacgcttac    120

atggctgaag ttggcattga agtaccaggt cacggtgaag gcggcggtta cgagcacaac    180

cgtcataagc aaaactacat ccatatggat ctagcaggcc gtttgttcct tatcactgag    240

gaaacaaaat accgagatta catcgttgat atgctaacag cgtacgcgac ggtataccca    300

acacttgaaa gcaacgtaag ccgtgactct aaccctccgg gtaagctgtt ccaccaaacg    360

ttgaacgaga acatgtggat gctttacgct tcttgtgcgt acagctgcat ctaccacacg    420

atctctgaag agcaaaagcg tctgatcgaa gacgatcttc ttaagcaaat gatcgaaatg    480
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ttcgttgtga cttacgcaca cgacttcgat atcgtacaca accacggctt atgggcagtg 540

gcagcagtag gtatctgtgg ttacgcaatc aacgatcaag agtctgtaga caaagcacta 600

tacggcctga aactagacaa agtcagcggc ggtttcttag cgcaactaga ccaactgttt 660

tcgccagacg gctactacat ggaaggtcct tactaccacc gtttctctct gcgtccaatc 720

tacctgttcg cagaagcgat tgaacgtcgt cagcctgaag ttggtatcta tgaattcaac 780

gattcagtga tcaagacaac gtcttactct gtattcaaaa cggcattccc agacggtaca 840

ttgcctgctc tgaacgattc atcgaagaca atctctatca acgatgaagg cgttatcatg 900

gcaacgtctg tgtgttacca ccgttacgag caaactgaaa ctctacttgg tatggctaac 960

caccagcaaa acgtttgggt tcatgcttca ggtaaaacac tgtctgacgc ggttgatgca 1020

gcagacgaca tcaaagcatt caactggggt agcctgtttg taaccgacgg ccctgaaggc 1080

gaaaaaggcg gcgtaagcat ccttcgtcac cgtgacgaac aagatgacga cacgatggcg 1140

ttgatctggt ttggtcaaca cggttctgat caccagtacc actctgctct agaccacggt 1200

cactacgatg gcctgcacct aagcgtattt aaccgtggcc acgaagtgct gcacgatttc 1260

ggcttcggtc gctgggtaaa cgttgagcct aagtttggcg gtcgttacat cccagagaac 1320

aagtcttact gtaagcagac ggttgctcac aacacagtaa cggttgatca gaaaacgcag 1380

aacaacttca acacagcatt ggctgagtct aagtttggtc agaagcactt cttcgtagca 1440

gacgaccagt ctctacaagg catgagcggc acaatttctg agtactacac tggcgtagac 1500

atgcaacgca gcgtgattct tgctgaactt cctgagttcg agaagccact tgtaatcgac 1560

gtataccgca tcgaagctga cgctgaacac cagtacgacc tacccgttca ccactctggt 1620

cagatcatcc gtactgactt cgattacaac atggaaaaaa cgcttaagcc gctaggtgaa 1680

gacaacggtt accagcactt atggaacgtg gcttcaggca aagtgaacga agaaggttct 1740

ctagtaagct ggctacatga cagcagctac tacagcctag taaccagcgc gaatgcgggc 1800

agcgaagtga tttttgctcg cactggtgct aacgatccag acttcaacct taagagtgag 1860

cctgcgttca tcttacgtca gtctggtcaa aaccacgtgt ttgcttctgt actagaaacg 1920

catggttact ttaacgagtc tatcgaagcc tctgtaggcg ctcgtggtct agttaaatca 1980

gtatctgttg tgggccataa cagtgtcggg actgttgttc gcattcagac tacttctggc 2040

aacacttacc actacggtat ctcaaaccaa gctgaagaca cgcagcaagc aactcacact 2100

gttgagttcg cgggtgagac atactcgtgg gaaggatcat ttgctcaact gtaa 2154

<210> SEQ ID NO 18
<211> LENGTH: 717
<212> TYPE: PRT
<213> ORGANISM: Vibrio slpendidus

<400> SEQUENCE: 18

Met Ser Tyr Gln Pro Leu Leu Leu Asn Phe Asp Glu Ala Ala Glu Leu
1 5 10 15

Arg Lys Glu Leu Gly Lys Asp Ser Leu Leu Gly Asn Ala Leu Thr Arg
20 25 30

Asp Ile Lys Gln Thr Asp Ala Tyr Met Ala Glu Val Gly Ile Glu Val
35 40 45

Pro Gly His Gly Glu Gly Gly Gly Tyr Glu His Asn Arg His Lys Gln
50 55 60

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Asn Tyr Ile His Met Asp Leu Ala Gly Arg Leu Phe Leu Ile Thr Glu
65                  70                  75                  80

Glu Thr Lys Tyr Arg Asp Tyr Ile Val Asp Met Leu Thr Ala Tyr Ala
                85                  90                  95

Thr Val Tyr Pro Thr Leu Glu Ser Asn Val Ser Arg Asp Ser Asn Pro
            100                 105                 110

Pro Gly Lys Leu Phe His Gln Thr Leu Asn Glu Asn Met Trp Met Leu
        115                 120                 125

Tyr Ala Ser Cys Ala Tyr Ser Cys Ile Tyr His Thr Ile Ser Glu Glu
    130                 135                 140

Gln Lys Arg Leu Ile Glu Asp Asp Leu Leu Lys Gln Met Ile Glu Met
145                 150                 155                 160

Phe Val Val Thr Tyr Ala His Asp Phe Asp Ile Val His Asn His Gly
                165                 170                 175

Leu Trp Ala Val Ala Ala Val Gly Ile Cys Gly Tyr Ala Ile Asn Asp
            180                 185                 190

Gln Glu Ser Val Asp Lys Ala Leu Tyr Gly Leu Lys Leu Asp Lys Val
        195                 200                 205

Ser Gly Gly Phe Leu Ala Gln Leu Asp Gln Leu Phe Ser Pro Asp Gly
    210                 215                 220

Tyr Tyr Met Glu Gly Pro Tyr Tyr His Arg Phe Ser Leu Arg Pro Ile
225                 230                 235                 240

Tyr Leu Phe Ala Glu Ala Ile Glu Arg Arg Gln Pro Glu Val Gly Ile
                245                 250                 255

Tyr Glu Phe Asn Asp Ser Val Ile Lys Thr Thr Ser Tyr Ser Val Phe
            260                 265                 270

Lys Thr Ala Phe Pro Asp Gly Thr Leu Pro Ala Leu Asn Asp Ser Ser
        275                 280                 285

Lys Thr Ile Ser Ile Asn Asp Glu Gly Val Ile Met Ala Thr Ser Val
    290                 295                 300

Cys Tyr His Arg Tyr Glu Gln Thr Glu Thr Leu Leu Gly Met Ala Asn
305                 310                 315                 320

His Gln Gln Asn Val Trp Val His Ala Ser Gly Lys Thr Leu Ser Asp
                325                 330                 335

Ala Val Asp Ala Ala Asp Asp Ile Lys Ala Phe Asn Trp Gly Ser Leu
            340                 345                 350

Phe Val Thr Asp Gly Pro Glu Gly Glu Lys Gly Gly Val Ser Ile Leu
        355                 360                 365

Arg His Arg Asp Glu Gln Asp Asp Asp Thr Met Ala Leu Ile Trp Phe
    370                 375                 380

Gly Gln His Gly Ser Asp His Gln Tyr His Ser Ala Leu Asp His Gly
385                 390                 395                 400

His Tyr Asp Gly Leu His Leu Ser Val Phe Asn Arg Gly His Glu Val
                405                 410                 415

Leu His Asp Phe Gly Phe Gly Arg Trp Val Asn Val Glu Pro Lys Phe
            420                 425                 430

Gly Gly Arg Tyr Ile Pro Glu Asn Lys Ser Tyr Cys Lys Gln Thr Val
        435                 440                 445

Ala His Asn Thr Val Thr Val Asp Gln Lys Thr Gln Asn Asn Phe Asn
    450                 455                 460

Thr Ala Leu Ala Glu Ser Lys Phe Gly Gln Lys His Phe Phe Val Ala
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465                 470                 475                 480

Asp Asp Gln Ser Leu Gln Gly Met Ser Gly Thr Ile Ser Glu Tyr Tyr
                485                 490                 495

Thr Gly Val Asp Met Gln Arg Ser Val Ile Leu Ala Glu Leu Pro Glu
            500                 505                 510

Phe Glu Lys Pro Leu Val Ile Asp Val Tyr Arg Ile Glu Ala Asp Ala
        515                 520                 525

Glu His Gln Tyr Asp Leu Pro Val His His Ser Gly Gln Ile Ile Arg
    530                 535                 540

Thr Asp Phe Asp Tyr Asn Met Glu Lys Thr Leu Lys Pro Leu Gly Glu
545                 550                 555                 560

Asp Asn Gly Tyr Gln His Leu Trp Asn Val Ala Ser Gly Lys Val Asn
                565                 570                 575

Glu Glu Gly Ser Leu Val Ser Trp Leu His Asp Ser Ser Tyr Tyr Ser
            580                 585                 590

Leu Val Thr Ser Ala Asn Ala Gly Ser Glu Val Ile Phe Ala Arg Thr
        595                 600                 605

Gly Ala Asn Asp Pro Asp Phe Asn Leu Lys Ser Glu Pro Ala Phe Ile
    610                 615                 620

Leu Arg Gln Ser Gly Gln Asn His Val Phe Ala Ser Val Leu Glu Thr
625                 630                 635                 640

His Gly Tyr Phe Asn Glu Ser Ile Glu Ala Ser Val Gly Ala Arg Gly
                645                 650                 655

Leu Val Lys Ser Val Ser Val Val Gly His Asn Ser Val Gly Thr Val
            660                 665                 670

Val Arg Ile Gln Thr Thr Ser Gly Asn Thr Tyr His Tyr Gly Ile Ser
        675                 680                 685

Asn Gln Ala Glu Asp Thr Gln Gln Ala Thr His Thr Val Glu Phe Ala
    690                 695                 700

Gly Glu Thr Tyr Ser Trp Glu Gly Ser Phe Ala Gln Leu
705                 710                 715
```

<210> SEQ ID NO 19
<211> LENGTH: 825
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 19

```
atgaagtggt tattggcaat agttgcgatg tctggtgtcg cattggcggc agaaaataag     60

aatgttgagg tgagcagtga gcatttcgtc cgttatcaat accaagacaa aatcagctat    120

ggaaagctag acaatgacgc agtgttaccg gtcagcggcg atctctttgg cgaatattcg    180

gtagcaaaaa attcgatccc gttagagtcg gttgaggtgt tactaccgac aaaaccagag    240

aaagtcttcg ccgtcgggat gaacttcgct agccacttag cctcacctgc cgatgcacca    300

ccgccgatgt ttcttaaact tccttcttct ttgattctca cgggcgaagt gattcaagtg    360

ccaccaaaag caagaaatgt tcattttgaa ggcgagctgg tggttgtgat tggtagagag    420

ctcagtcaag ccagtgaaga agaagccgaa caagcgatct ttggcgtcac ggtgggcaac    480

gatattactg aaagaagttg gcaaggcgcc gatttacaat ggctccgagc gaaagcttcc    540

gatggttttg gcccggttgg caacacaatt gtgcgcggca ttgattacaa caatattgag    600

ttaaccactc gtgttaacgg taaagtggtt caacaagaaa atacttcgtt catgatccac    660
```

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aagccaagaa aagtcgtgag ctatttgagc tattatttta ccctcaaacc gggcgatcta 720

attttcatgg gcacgccagg tagaacttat gctctgtccg acaaagatca agtgagtgtc 780

acgattgaag gggtagggac tgtggtaaat gaagtgcggt tctga 825

<210> SEQ ID NO 20
<211> LENGTH: 274
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 20

Met Lys Trp Leu Leu Ala Ile Val Ala Met Ser Gly Val Ala Leu Ala
1 5 10 15

Ala Glu Asn Lys Asn Val Glu Val Ser Ser Glu His Phe Val Arg Tyr
20 25 30

Gln Tyr Gln Asp Lys Ile Ser Tyr Gly Lys Leu Asp Asn Asp Ala Val
35 40 45

Leu Pro Val Ser Gly Asp Leu Phe Gly Glu Tyr Ser Val Ala Lys Asn
50 55 60

Ser Ile Pro Leu Glu Ser Val Glu Val Leu Leu Pro Thr Lys Pro Glu
65 70 75 80

Lys Val Phe Ala Val Gly Met Asn Phe Ala Ser His Leu Ala Ser Pro
85 90 95

Ala Asp Ala Pro Pro Pro Met Phe Leu Lys Leu Pro Ser Ser Leu Ile
100 105 110

Leu Thr Gly Glu Val Ile Gln Val Pro Pro Lys Ala Arg Asn Val His
115 120 125

Phe Glu Gly Glu Leu Val Val Val Ile Gly Arg Glu Leu Ser Gln Ala
130 135 140

Ser Glu Glu Glu Ala Glu Gln Ala Ile Phe Gly Val Thr Val Gly Asn
145 150 155 160

Asp Ile Thr Glu Arg Ser Trp Gln Gly Ala Asp Leu Gln Trp Leu Arg
165 170 175

Ala Lys Ala Ser Asp Gly Phe Gly Pro Val Gly Asn Thr Ile Val Arg
180 185 190

Gly Ile Asp Tyr Asn Asn Ile Glu Leu Thr Thr Arg Val Asn Gly Lys
195 200 205

Val Val Gln Gln Glu Asn Thr Ser Phe Met Ile His Lys Pro Arg Lys
210 215 220

Val Val Ser Tyr Leu Ser Tyr Tyr Phe Thr Leu Lys Pro Gly Asp Leu
225 230 235 240

Ile Phe Met Gly Thr Pro Gly Arg Thr Tyr Ala Leu Ser Asp Lys Asp
245 250 255

Gln Val Ser Val Thr Ile Glu Gly Val Gly Thr Val Val Asn Glu Val
260 265 270

Arg Phe

<210> SEQ ID NO 21
<211> LENGTH: 717
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 21

-continued

atggctagca cttttaattc aatttcgggc tcgaagcgta gcctgcacgt gcaagtagca 60

cgcgaaatcg ctcgaggaat tttgtctggt gatctgccgc aaggttctat tattcctggt 120

gaaatggcgt tgtgtgaaca gtttggtatc agccgaacgg cacttcgtga agcagttaaa 180

ctactgacct ctaaaggtct gttagagtct cgccctaaaa ttggtactcg cgtagtcgac 240

cgcgcatact ggaacttcct tgatcctcaa ctgattgaat ggatggacgg actaaccgac 300

gtagaccaat tctgttctca gtttttaggc cttcgccgtg cgatcgagcc tgaagcgtgt 360

gcactggcgg caaaatttgc gacagctgaa caacgtatcg agctttcaga gatcttccaa 420

aagatggtcg aagtggatga agctgaagtg tttgaccaag aacgttggac agacattgat 480

actcgtttcc atagcttgat cttcaatgcg accggtaacg acttctatct accgttcggt 540

aatattctga ctactatgtt cgttaacttc atagtgcatt cttctgaaga gggaagcaca 600

tgcatcaatg aacaccgcag aatctatgaa gctatcatgg ccggtgattg tgacaaggct 660

agaattgctt ctgctgttca cttgcaagat gccaaccacc gtttggcaac agcataa 717

<210> SEQ ID NO 22
<211> LENGTH: 238
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 22

Met Ala Ser Thr Phe Asn Ser Ile Ser Gly Ser Lys Arg Ser Leu His
1 5 10 15

Val Gln Val Ala Arg Glu Ile Ala Arg Gly Ile Leu Ser Gly Asp Leu
20 25 30

Pro Gln Gly Ser Ile Ile Pro Gly Glu Met Ala Leu Cys Glu Gln Phe
35 40 45

Gly Ile Ser Arg Thr Ala Leu Arg Glu Ala Val Lys Leu Leu Thr Ser
50 55 60

Lys Gly Leu Leu Glu Ser Arg Pro Lys Ile Gly Thr Arg Val Val Asp
65 70 75 80

Arg Ala Tyr Trp Asn Phe Leu Asp Pro Gln Leu Ile Glu Trp Met Asp
85 90 95

Gly Leu Thr Asp Val Asp Gln Phe Cys Ser Gln Phe Leu Gly Leu Arg
100 105 110

Arg Ala Ile Glu Pro Glu Ala Cys Ala Leu Ala Ala Lys Phe Ala Thr
115 120 125

Ala Glu Gln Arg Ile Glu Leu Ser Glu Ile Phe Gln Lys Met Val Glu
130 135 140

Val Asp Glu Ala Glu Val Phe Asp Gln Glu Arg Trp Thr Asp Ile Asp
145 150 155 160

Thr Arg Phe His Ser Leu Ile Phe Asn Ala Thr Gly Asn Asp Phe Tyr
165 170 175

Leu Pro Phe Gly Asn Ile Leu Thr Thr Met Phe Val Asn Phe Ile Val
180 185 190

His Ser Ser Glu Glu Gly Ser Thr Cys Ile Asn Glu His Arg Arg Ile
195 200 205

Tyr Glu Ala Ile Met Ala Gly Asp Cys Asp Lys Ala Arg Ile Ala Ser
210 215 220

Ala Val His Leu Gln Asp Ala Asn His Arg Leu Ala Thr Ala
225 230 235

-continued

<210> SEQ ID NO 23
<211> LENGTH: 1779
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 23

atggaactca acacgattat tgtcggcatt tatttcctat tcttgattgc gataggttgg 60

atgtttagaa catttacaag tactactagt gactacttcc gcgggggcgg taacatgttg 120

tggtggatgg ttggtgcaac cgcctttatg acccagttta gtgcatggac attcaccggt 180

gcagcaggta aagcgtataa cgatggtttc gctgtagcgg tcatcttcgt agccaacgca 240

tttggttact tcatgaacta cgcgtacttc gcgccgaaat tccgtcaact tcgcgttgtt 300

acggtaatcg aagcgattcg tatgcgtttt ggtgcgacca acgaacaagt attcacttgg 360

tcttcaatgc caaactcagt ggtatctgcg ggtgtgtggt taaacgcatt ggcaatcatc 420

gcttcgggta tcttcggttt cgacatgaac atgactatct gggtgactgg cctagtggta 480

ttggcaatgt cggtaacagg tggttcatgg gcggtaatcg catctgactt catgcagatg 540

gttatcatca tggcggtaac ggtaacttgt gcggttgtag cggttgttca aggtggcggt 600

gttggtgaga ttgttaacaa cttcccagta caagatggtg gttcgttcct ttggggcaac 660

aacatcaact acctaagcat ctttacgatt tgggcattct tcatcttcgt taagcagttc 720

tcaatcacga acaacatgct taactcttac cgttacctag cggctaaaga ctcaaagaac 780

gctaagaaag ctgcactgct tgcttgtgtg ttgatgttgt gtggtgtgtt tatttggttc 840

atgccttctt ggttcattgc aggccaaggt gttgatttat cagcggctta cccgaatgca 900

ggtaaaaaag cgggtgactt tgcttaccta tacttcgtac aagagtacat gccagcaggt 960

atggttggtc tattagttgc cgcgatgttt gcagcgacaa tgtcttcaat ggactcaggt 1020

ctaaaccgta actcaggtat ttttgttaag aacttctacg aaacaatcgt tcgtaaaggt 1080

caagcatcag agaaagagct agtaaccgta tctaaaatta cttcagcggt atttggtttc 1140

gctattatcc taatcgcaca gttcatcaac tcattaaaag gcttaagcct gtttgatacg 1200

atgatgtacg taggtgcgtt aatcggcttc cctatgacga ttcctgcatt ccttggtttc 1260

ttcatcaaga agactccgga ctgggctggt tggggaacgc tagttgttgg tggtatcgta 1320

tcttatgtgg ttggttttgt tatcaacgcg gagatggtag cagcggcgtt tggtcttgat 1380

actctaacag gacgtgaatg gtctgatgtt aaagttgcga ttggtctgat tgctcacatc 1440

acgctaaccg gtggcttctt cgtactatct acgatgttct acaagcctct atcaaaagaa 1500

cgtcaagcgg atgttgataa gttctttggc aacttagata ccccattagt agctgaatcg 1560

gcagagcaaa aagtgttgga taacaaacaa cgtcaaatgc ttggtaaact gattgcggta 1620

gcgggtgttg gtattatgct gatggctctt ctgactaacc caatgtgggg gcgcctagtc 1680

ttcatcttat gtggtgtgat agtgggtggt gtcggtattc tacttgtgaa agcggtcgat 1740

gacggcggca agcaagcgaa agcagtaacc gaaagctaa 1779

<210> SEQ ID NO 24
<211> LENGTH: 592
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 24

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```
Met Glu Leu Asn Thr Ile Ile Val Gly Ile Tyr Phe Leu Phe Leu Ile
1               5                  10                  15

Ala Ile Gly Trp Met Phe Arg Thr Phe Thr Ser Thr Thr Ser Asp Tyr
            20                  25                  30

Phe Arg Gly Gly Gly Asn Met Leu Trp Trp Met Val Gly Ala Thr Ala
        35                  40                  45

Phe Met Thr Gln Phe Ser Ala Trp Thr Phe Thr Gly Ala Ala Gly Lys
    50                  55                  60

Ala Tyr Asn Asp Gly Phe Ala Val Ala Val Ile Phe Val Ala Asn Ala
65                  70                  75                  80

Phe Gly Tyr Phe Met Asn Tyr Ala Tyr Phe Ala Pro Lys Phe Arg Gln
                85                  90                  95

Leu Arg Val Val Thr Val Ile Glu Ala Ile Arg Met Arg Phe Gly Ala
            100                 105                 110

Thr Asn Glu Gln Val Phe Thr Trp Ser Ser Met Pro Asn Ser Val Val
        115                 120                 125

Ser Ala Gly Val Trp Leu Asn Ala Leu Ala Ile Ile Ala Ser Gly Ile
    130                 135                 140

Phe Gly Phe Asp Met Asn Met Thr Ile Trp Val Thr Gly Leu Val Val
145                 150                 155                 160

Leu Ala Met Ser Val Thr Gly Gly Ser Trp Ala Val Ile Ala Ser Asp
                165                 170                 175

Phe Met Gln Met Val Ile Ile Met Ala Val Thr Val Thr Cys Ala Val
            180                 185                 190

Val Ala Val Val Gln Gly Gly Gly Val Gly Glu Ile Val Asn Asn Phe
        195                 200                 205

Pro Val Gln Asp Gly Gly Ser Phe Leu Trp Gly Asn Asn Ile Asn Tyr
    210                 215                 220

Leu Ser Ile Phe Thr Ile Trp Ala Phe Phe Ile Phe Val Lys Gln Phe
225                 230                 235                 240

Ser Ile Thr Asn Asn Met Leu Asn Ser Tyr Arg Tyr Leu Ala Ala Lys
                245                 250                 255

Asp Ser Lys Asn Ala Lys Lys Ala Ala Leu Leu Ala Cys Val Leu Met
            260                 265                 270

Leu Cys Gly Val Phe Ile Trp Phe Met Pro Ser Trp Phe Ile Ala Gly
        275                 280                 285

Gln Gly Val Asp Leu Ser Ala Ala Tyr Pro Asn Ala Gly Lys Lys Ala
    290                 295                 300

Gly Asp Phe Ala Tyr Leu Tyr Phe Val Gln Glu Tyr Met Pro Ala Gly
305                 310                 315                 320

Met Val Gly Leu Leu Val Ala Ala Met Phe Ala Ala Thr Met Ser Ser
                325                 330                 335

Met Asp Ser Gly Leu Asn Arg Asn Ser Gly Ile Phe Val Lys Asn Phe
            340                 345                 350

Tyr Glu Thr Ile Val Arg Lys Gly Gln Ala Ser Glu Lys Glu Leu Val
        355                 360                 365

Thr Val Ser Lys Ile Thr Ser Ala Val Phe Gly Phe Ala Ile Ile Leu
    370                 375                 380

Ile Ala Gln Phe Ile Asn Ser Leu Lys Gly Leu Ser Leu Phe Asp Thr
385                 390                 395                 400
```

-continued

```
Met Met Tyr Val Gly Ala Leu Ile Gly Phe Pro Met Thr Ile Pro Ala
                405                 410                 415

Phe Leu Gly Phe Phe Ile Lys Lys Thr Pro Asp Trp Ala Gly Trp Gly
            420                 425                 430

Thr Leu Val Val Gly Gly Ile Val Ser Tyr Val Val Gly Phe Val Ile
        435                 440                 445

Asn Ala Glu Met Val Ala Ala Ala Phe Gly Leu Asp Thr Leu Thr Gly
    450                 455                 460

Arg Glu Trp Ser Asp Val Lys Val Ala Ile Gly Leu Ile Ala His Ile
465                 470                 475                 480

Thr Leu Thr Gly Gly Phe Phe Val Leu Ser Thr Met Phe Tyr Lys Pro
                485                 490                 495

Leu Ser Lys Glu Arg Gln Ala Asp Val Asp Lys Phe Phe Gly Asn Leu
            500                 505                 510

Asp Thr Pro Leu Val Ala Glu Ser Ala Glu Gln Lys Val Leu Asp Asn
        515                 520                 525

Lys Gln Arg Gln Met Leu Gly Lys Leu Ile Ala Val Ala Gly Val Gly
    530                 535                 540

Ile Met Leu Met Ala Leu Leu Thr Asn Pro Met Trp Gly Arg Leu Val
545                 550                 555                 560

Phe Ile Leu Cys Gly Val Ile Val Gly Gly Val Gly Ile Leu Leu Val
                565                 570                 575

Lys Ala Val Asp Asp Gly Gly Lys Gln Ala Lys Ala Val Thr Glu Ser
            580                 585                 590
```

<210> SEQ ID NO 25
<211> LENGTH: 2079
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 25

```
atgagcgacc aaaaatctct tgatgcaatc aggaagatga agctggaaaa cgatacttca     60

gcaggtaatc ttgtagacct actccctatc gaagttcaaa cacgtgactt cgacctatca    120

ttcctagaca ccttgagcga agcacgtccg cgtcttcttg ttcaagctga tcagctagaa    180

gaattcaaag caaaagtgaa agctgatcaa gctcactgta tgtttgatga tttctacaac    240

aactctaccg ttaagttcct tgagactgct cctttcgaag agcctcaagc gtacccagct    300

gagacggtag gtaaagcttc tctatggcgt ccttattggc gtcaaatgta cgttgattgc    360

caaatggcac tgaacgcgac acgtaaccta gcgattgctg gtgttgtaaa agaagacgaa    420

gcgctcattg cgaaagcaaa agcttggact ctaaaactgt ctacgtacga tccagaaggc    480

gtgacttctc gtggctataa cgatgaagcg gctttccgtg ttatcgctgc tatggcttgg    540

ggttacgatt ggctacacgg ctacttcacc gatgaagaac gccagcaagt tcaagatgct    600

ttgattgagc gtctagacga aatcatgcac cacctgaaag tgacggttga tctattgaac    660

aacccactaa atagccacgg tgttcgttct atctcttctg ctatcatccc aacgtgtatc    720

gcgctttacc acgatcaccc gaaagcaggc gagtacattg catacgcgct agaatactac    780

gcagtacatt acccaccatg gggcggtgta gacggcggtt gggctgaagg tcctgattac    840

tggaacacgc aaactgcatt cctaggcgaa gcattcgacc tattgaaagc atactgtggt    900

gtagacatgt ttaacaaaac attctacgaa aacacaggtg atttcccgct ttactgcatg    960
```

-continued

```
ccagttcact ctaagcgcgc gagcttctgt gaccagtctt caatcggcga tttcccaggt   1020

ttaaaactgg cttacaacat caagcactac gcaggtgtta accagaagcc tgagtacgtt   1080

tggtactata accagcttaa aggccgtgat actgaagcac acaccaaatt ctacaacttc   1140

ggttggtggg acttcggtta tgacgatctt cgttttaact tcctttggga tgcacctgaa   1200

gagaaagccc catcgaacga tccactgttg aaagtattcc caatcacggg ttgggctgca   1260

ttccacaaca agatgactga gcgtgataac catattcaca tggtattcaa atgttctccg   1320

tttggctcaa tcagccactc tcacggtgac caaaacgcat ttacgcttca cgcatttggt   1380

gaaacgctag cgtcagtaac aggttactat ggtggtttcg gtgtagacat gcacacgaaa   1440

tggcgtcgtc aaacgttctc taaaaacctg ccactatttg gcggtaaagg tcagtacggc   1500

gagaacaaga acacaggcta cgaaaaccac caagatcgct tttgtatcga agcgggcggc   1560

actatctctg acttcgacac tgaatctgat gtgaagatgg ttgaaggtga tgcaacggca   1620

tcttacaagt acttcgttcc tgaaatcgaa tcttacaagc gtaaagtctg gttcgttcaa   1680

ggtaaagtct tcgtaatgca agacaaggca acgctttctg aagagaaaga catgacttgg   1740

ctaatgcaca caactttcgc aaacgaagtg gcagacaagt ctttcactat ccgtggcgaa   1800

gttgcgcacc tagacgtaaa cttcatcaac gagtctgctg ataacatcac gtcagttaag   1860

aacgttgaag gctttggcga agttgaccca tacgagttca aagatcttga gatccaccgt   1920

cacgtggaag tggaattcaa gccatcgaaa gagcacaaca tcctgacgct tcttgttcct   1980

aataagaatg aaggcgagca agttgaagtg tttcacaagc ttgaaggcaa cacgctactg   2040

ctaaatgttg acggcgaaac ggtttcaatc gaactgtaa                          2079
```

<210> SEQ ID NO 26
<211> LENGTH: 692
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 26

```
Met Ser Asp Gln Lys Ser Leu Asp Ala Ile Arg Lys Met Lys Leu Glu
1               5                   10                  15

Asn Asp Thr Ser Ala Gly Asn Leu Val Asp Leu Leu Pro Ile Glu Val
            20                  25                  30

Gln Thr Arg Asp Phe Asp Leu Ser Phe Leu Asp Thr Leu Ser Glu Ala
        35                  40                  45

Arg Pro Arg Leu Leu Val Gln Ala Asp Gln Leu Glu Glu Phe Lys Ala
    50                  55                  60

Lys Val Lys Ala Asp Gln Ala His Cys Met Phe Asp Asp Phe Tyr Asn
65                  70                  75                  80

Asn Ser Thr Val Lys Phe Leu Glu Thr Ala Pro Phe Glu Glu Pro Gln
                85                  90                  95

Ala Tyr Pro Ala Glu Thr Val Gly Lys Ala Ser Leu Trp Arg Pro Tyr
            100                 105                 110

Trp Arg Gln Met Tyr Val Asp Cys Gln Met Ala Leu Asn Ala Thr Arg
        115                 120                 125

Asn Leu Ala Ile Ala Gly Val Val Lys Glu Asp Glu Ala Leu Ile Ala
    130                 135                 140

Lys Ala Lys Ala Trp Thr Leu Lys Leu Ser Thr Tyr Asp Pro Glu Gly
145                 150                 155                 160
```

-continued

```
Val Thr Ser Arg Gly Tyr Asn Asp Glu Ala Ala Phe Arg Val Ile Ala
                165                 170                 175

Ala Met Ala Trp Gly Tyr Asp Trp Leu His Gly Tyr Phe Thr Asp Glu
            180                 185                 190

Glu Arg Gln Gln Val Gln Asp Ala Leu Ile Glu Arg Leu Asp Glu Ile
        195                 200                 205

Met His His Leu Lys Val Thr Val Asp Leu Leu Asn Asn Pro Leu Asn
    210                 215                 220

Ser His Gly Val Arg Ser Ile Ser Ser Ala Ile Ile Pro Thr Cys Ile
225                 230                 235                 240

Ala Leu Tyr His Asp His Pro Lys Ala Gly Glu Tyr Ile Ala Tyr Ala
                245                 250                 255

Leu Glu Tyr Tyr Ala Val His Tyr Pro Pro Trp Gly Gly Val Asp Gly
            260                 265                 270

Gly Trp Ala Glu Gly Pro Asp Tyr Trp Asn Thr Gln Thr Ala Phe Leu
        275                 280                 285

Gly Glu Ala Phe Asp Leu Leu Lys Ala Tyr Cys Gly Val Asp Met Phe
    290                 295                 300

Asn Lys Thr Phe Tyr Glu Asn Thr Gly Asp Phe Pro Leu Tyr Cys Met
305                 310                 315                 320

Pro Val His Ser Lys Arg Ala Ser Phe Cys Asp Gln Ser Ser Ile Gly
                325                 330                 335

Asp Phe Pro Gly Leu Lys Leu Ala Tyr Asn Ile Lys His Tyr Ala Gly
            340                 345                 350

Val Asn Gln Lys Pro Glu Tyr Val Trp Tyr Tyr Asn Gln Leu Lys Gly
        355                 360                 365

Arg Asp Thr Glu Ala His Thr Lys Phe Tyr Asn Phe Gly Trp Trp Asp
    370                 375                 380

Phe Gly Tyr Asp Asp Leu Arg Phe Asn Phe Leu Trp Asp Ala Pro Glu
385                 390                 395                 400

Glu Lys Ala Pro Ser Asn Asp Pro Leu Leu Lys Val Phe Pro Ile Thr
                405                 410                 415

Gly Trp Ala Ala Phe His Asn Lys Met Thr Glu Arg Asp Asn His Ile
            420                 425                 430

His Met Val Phe Lys Cys Ser Pro Phe Gly Ser Ile Ser His Ser His
        435                 440                 445

Gly Asp Gln Asn Ala Phe Thr Leu His Ala Phe Gly Glu Thr Leu Ala
    450                 455                 460

Ser Val Thr Gly Tyr Tyr Gly Gly Phe Gly Val Asp Met His Thr Lys
465                 470                 475                 480

Trp Arg Arg Gln Thr Phe Ser Lys Asn Leu Pro Leu Phe Gly Gly Lys
                485                 490                 495

Gly Gln Tyr Gly Glu Asn Lys Asn Thr Gly Tyr Glu Asn His Gln Asp
            500                 505                 510

Arg Phe Cys Ile Glu Ala Gly Gly Thr Ile Ser Asp Phe Asp Thr Glu
        515                 520                 525

Ser Asp Val Lys Met Val Glu Gly Asp Ala Thr Ala Ser Tyr Lys Tyr
    530                 535                 540

Phe Val Pro Glu Ile Glu Ser Tyr Lys Arg Lys Val Trp Phe Val Gln
545                 550                 555                 560

Gly Lys Val Phe Val Met Gln Asp Lys Ala Thr Leu Ser Glu Glu Lys
```

-continued

```
               565                 570                 575

Asp Met Thr Trp Leu Met His Thr Thr Phe Ala Asn Glu Val Ala Asp
            580                 585                 590

Lys Ser Phe Thr Ile Arg Gly Glu Val Ala His Leu Asp Val Asn Phe
        595                 600                 605

Ile Asn Glu Ser Ala Asp Asn Ile Thr Ser Val Lys Asn Val Glu Gly
    610                 615                 620

Phe Gly Glu Val Asp Pro Tyr Glu Phe Lys Asp Leu Glu Ile His Arg
625                 630                 635                 640

His Val Glu Val Glu Phe Lys Pro Ser Lys Glu His Asn Ile Leu Thr
                645                 650                 655

Leu Leu Val Pro Asn Lys Asn Glu Gly Glu Gln Val Glu Val Phe His
            660                 665                 670

Lys Leu Glu Gly Asn Thr Leu Leu Leu Asn Val Asp Gly Glu Thr Val
        675                 680                 685

Ser Ile Glu Leu
    690
```

<210> SEQ ID NO 27
<211> LENGTH: 882
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 27

```
atgactaaac ctgtaatcgg tttcattggc ctaggtctta tgggcggcaa catggttgaa     60

aacctacaaa agcgcggcta ccacgtaaac gtaatggatc taagcgctga agctgttgct    120

cgcgtaacag atcgcggcaa cgcaactgca ttcacttctg ctaaagaact agctgctgca    180

agtgacatcg ttcagttttg tctgacaact tctgctgttg ttgaaaaaat cgtttacggc    240

gaagacggcg ttctagcggg catcaaagaa ggcgcagtac tagtagactt cggtacttct    300

atccctgctt ctactaagaa aatcggcgca gctcttgctg aaaaaggcgc gggcatgatc    360

gacgcacctc taggtcgtac tcctgcacac gctaaagatg gtcttctgaa catcatggct    420

gctggcgaca tggaaacttt caacaaagtt aaacctgttc ttgaagagca aggcgaaaac    480

gtattccacc taggggctct aggttctggt cacgtgacta agcttgtaaa caacttcatg    540

ggtatgacga ctgttgcgac tatgtctcaa gctttcgctg ttgctcaacg cgctggtgtt    600

gatggccaac aactgtttga catcatgtct gcaggtccat ctaactctcc gttcatgcaa    660

ttctgtaagt tctacgcggt agacggcgaa gagaagctag gtttctctgt tgctaacgca    720

aacaaagacc ttggttactt ccttgcactt tgtgaagagc taggtactga gtctctaatc    780

gctcaaggta ctgcaacaag cctacaagct gctgttgatg caggcatggg taacaacgac    840

gtaccagtaa tcttcgacta cttcgctaaa ctagagaagt aa                       882
```

<210> SEQ ID NO 28
<211> LENGTH: 293
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 28

```
Met Thr Lys Pro Val Ile Gly Phe Ile Gly Leu Gly Leu Met Gly Gly
1               5                   10                  15

Asn Met Val Glu Asn Leu Gln Lys Arg Gly Tyr His Val Asn Val Met
```

-continued

```
            20                  25                  30

Asp Leu Ser Ala Glu Ala Val Ala Arg Val Thr Asp Arg Gly Asn Ala
        35                  40                  45

Thr Ala Phe Thr Ser Ala Lys Glu Leu Ala Ala Ala Ser Asp Ile Val
    50                  55                  60

Gln Phe Cys Leu Thr Thr Ser Ala Val Val Glu Lys Ile Val Tyr Gly
65                  70                  75                  80

Glu Asp Gly Val Leu Ala Gly Ile Lys Glu Gly Ala Val Leu Val Asp
                85                  90                  95

Phe Gly Thr Ser Ile Pro Ala Ser Thr Lys Lys Ile Gly Ala Ala Leu
            100                 105                 110

Ala Glu Lys Gly Ala Gly Met Ile Asp Ala Pro Leu Gly Arg Thr Pro
        115                 120                 125

Ala His Ala Lys Asp Gly Leu Leu Asn Ile Met Ala Ala Gly Asp Met
    130                 135                 140

Glu Thr Phe Asn Lys Val Lys Pro Val Leu Glu Glu Gln Gly Glu Asn
145                 150                 155                 160

Val Phe His Leu Gly Ala Leu Gly Ser Gly His Val Thr Lys Leu Val
                165                 170                 175

Asn Asn Phe Met Gly Met Thr Thr Val Ala Thr Met Ser Gln Ala Phe
            180                 185                 190

Ala Val Ala Gln Arg Ala Gly Val Asp Gly Gln Gln Leu Phe Asp Ile
        195                 200                 205

Met Ser Ala Gly Pro Ser Asn Ser Pro Phe Met Gln Phe Cys Lys Phe
    210                 215                 220

Tyr Ala Val Asp Gly Glu Glu Lys Leu Gly Phe Ser Val Ala Asn Ala
225                 230                 235                 240

Asn Lys Asp Leu Gly Tyr Phe Leu Ala Leu Cys Glu Glu Leu Gly Thr
                245                 250                 255

Glu Ser Leu Ile Ala Gln Gly Thr Ala Thr Ser Leu Gln Ala Ala Val
            260                 265                 270

Asp Ala Gly Met Gly Asn Asn Asp Val Pro Val Ile Phe Asp Tyr Phe
        275                 280                 285

Ala Lys Leu Glu Lys
    290


<210> SEQ ID NO 29
<211> LENGTH: 1872
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 29

atggtagcgg tcgtcagttc tagtgctttg gcatttacga actggtttac gcttaacttg     60

gccactgaac aggtaaacca aacgatttat aacgagattg atcactcgct tacgatagaa    120

atcaatcaaa tagaaagtac cgttcagcgc accatcgata ccgttaactc tgttgcacaa    180

gagttcatga aatcccctta ccaagtgccg aatgaagcac tcatgcatta tgccgctaag    240

cttggtggca ttgacaagat tgtggtgggt tttgacgacg gccgttctta tacctctcgc    300

ccttcagagt ctttccctaa cggtgttgga ataaaagaaa aatacaatcc aaccactcga    360

ccttggtatc aacaagcgaa attgaaatca ggcttatctt ttagtggtct gtttttcact    420

aagagtactc aagtgcctat gatcggtgtg acctactcat accaagatcg tgtcatcatg    480
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gccgatatac gctttgacga tttggaaacg cagcttgaac agctggacag catctacgaa 540

gccaaaggca ttatcatcga cgaaaagggg atggtggtcg cttcaacaat cgaaaacgtg 600

cttccgcaaa ccaatatatc ttctgcagac actcaaatga aactcaacag tgccattgaa 660

cagcctgatc aattcattga gggtgtgatt gatggtaacc agagaatctt gatggccaag 720

aaagtggata ttggcagcca gaaagagtgg ttcatgatct ccagtattga ccctgaactc 780

gcgctcaatc agctgaatgg cgtgatgtcg agtgcgcgca tccttatcgt cgcttgtgta 840

cttggctcgg tgatattgat gattttactt ctgaatcgtt tctaccgccc aatcgtgtca 900

ctgcgcaaaa tcgtccacga tctatcacaa ggtaacggag acctcactca aaggcttgct 960

gagaagggga atgatgactt agggcatatc gccaaagaca tcaacttgtt cattatcggc 1020

ttacaagaga tggttaagga tgtgaaatac aagaactcgg atctcgatac caaggtactg 1080

agtattcgcg aaggttgtaa agaaaccagc gatgtactga aagttcatac tgatgaaacg 1140

gttcaagtgg tctctgcgat taacggcttg tctgaagcat caaacgaagt agagaagagt 1200

tctcagtcgg cggcagaagc agcaagagag gccgctgtgt tcagtgatga gacgaaacag 1260

attaacacgg tgacggaaac ctatatcagt gatcttgaga agcaagtctg caccacttct 1320

gatgacattc gctcaatggc caatgaaacg cagagcatcc agtctatcgt gtctgtgatt 1380

ggcggaattg cggaacaaac taatttgctg gcattgaatg cgtcaattga agcggcgagg 1440

gcgggtgaac atggtcgagg tttcgcggtg gttgctgatg aagtccgtgc gctagccaac 1500

cgaacgcaaa tcagtacctc tgaaattgat gaagcgttat ctggcttgca gtctaaatca 1560

gatggtttgg ttaaatctat tgagttgacc aaaagtaact gtgaactgac tcgcgctcaa 1620

gttgttcaag ctgtaaacat gttggcgaag ctaaccgagc agatggaaac agtaagtcgt 1680

tttaataatg acatttcggg ttcgtctgtt gagcaaaacg cccttattca gagcattgct 1740

aagaacatgc ataagattga aagctttgtt gaggagctta ataaactaag ccaagatcag 1800

ttaactgaat cagcagaaat caaaacactt aacggtagcg ttagtgaatt gatgagcagc 1860

tttaaggttt aa 1872

<210> SEQ ID NO 30
<211> LENGTH: 623
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 30

Met Val Ala Val Val Ser Ser Ser Ala Leu Ala Phe Thr Asn Trp Phe
1 5 10 15

Thr Leu Asn Leu Ala Thr Glu Gln Val Asn Gln Thr Ile Tyr Asn Glu
20 25 30

Ile Asp His Ser Leu Thr Ile Glu Ile Asn Gln Ile Glu Ser Thr Val
35 40 45

Gln Arg Thr Ile Asp Thr Val Asn Ser Val Ala Gln Glu Phe Met Lys
50 55 60

Ser Pro Tyr Gln Val Pro Asn Glu Ala Leu Met His Tyr Ala Ala Lys
65 70 75 80

Leu Gly Gly Ile Asp Lys Ile Val Val Gly Phe Asp Asp Gly Arg Ser
85 90 95

Tyr Thr Ser Arg Pro Ser Glu Ser Phe Pro Asn Gly Val Gly Ile Lys

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           100                 105                 110

Glu Lys Tyr Asn Pro Thr Thr Arg Pro Trp Tyr Gln Gln Ala Lys Leu
        115                 120                 125

Lys Ser Gly Leu Ser Phe Ser Gly Leu Phe Phe Thr Lys Ser Thr Gln
    130                 135                 140

Val Pro Met Ile Gly Val Thr Tyr Ser Tyr Gln Asp Arg Val Ile Met
145                 150                 155                 160

Ala Asp Ile Arg Phe Asp Asp Leu Glu Thr Gln Leu Glu Gln Leu Asp
                165                 170                 175

Ser Ile Tyr Glu Ala Lys Gly Ile Ile Ile Asp Glu Lys Gly Met Val
            180                 185                 190

Val Ala Ser Thr Ile Glu Asn Val Leu Pro Gln Thr Asn Ile Ser Ser
        195                 200                 205

Ala Asp Thr Gln Met Lys Leu Asn Ser Ala Ile Glu Gln Pro Asp Gln
    210                 215                 220

Phe Ile Glu Gly Val Ile Asp Gly Asn Gln Arg Ile Leu Met Ala Lys
225                 230                 235                 240

Lys Val Asp Ile Gly Ser Gln Lys Glu Trp Phe Met Ile Ser Ser Ile
                245                 250                 255

Asp Pro Glu Leu Ala Leu Asn Gln Leu Asn Gly Val Met Ser Ser Ala
            260                 265                 270

Arg Ile Leu Ile Val Ala Cys Val Leu Gly Ser Val Ile Leu Met Ile
        275                 280                 285

Leu Leu Leu Asn Arg Phe Tyr Arg Pro Ile Val Ser Leu Arg Lys Ile
    290                 295                 300

Val His Asp Leu Ser Gln Gly Asn Gly Asp Leu Thr Gln Arg Leu Ala
305                 310                 315                 320

Glu Lys Gly Asn Asp Asp Leu Gly His Ile Ala Lys Asp Ile Asn Leu
                325                 330                 335

Phe Ile Ile Gly Leu Gln Glu Met Val Lys Asp Val Lys Tyr Lys Asn
            340                 345                 350

Ser Asp Leu Asp Thr Lys Val Leu Ser Ile Arg Glu Gly Cys Lys Glu
        355                 360                 365

Thr Ser Asp Val Leu Lys Val His Thr Asp Glu Thr Val Gln Val Val
    370                 375                 380

Ser Ala Ile Asn Gly Leu Ser Glu Ala Ser Asn Glu Val Glu Lys Ser
385                 390                 395                 400

Ser Gln Ser Ala Ala Glu Ala Ala Arg Glu Ala Ala Val Phe Ser Asp
                405                 410                 415

Glu Thr Lys Gln Ile Asn Thr Val Thr Glu Thr Tyr Ile Ser Asp Leu
            420                 425                 430

Glu Lys Gln Val Cys Thr Thr Ser Asp Asp Ile Arg Ser Met Ala Asn
        435                 440                 445

Glu Thr Gln Ser Ile Gln Ser Ile Val Ser Val Ile Gly Gly Ile Ala
    450                 455                 460

Glu Gln Thr Asn Leu Leu Ala Leu Asn Ala Ser Ile Glu Ala Ala Arg
465                 470                 475                 480

Ala Gly Glu His Gly Arg Gly Phe Ala Val Val Ala Asp Glu Val Arg
                485                 490                 495

Ala Leu Ala Asn Arg Thr Gln Ile Ser Thr Ser Glu Ile Asp Glu Ala
            500                 505                 510
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Leu Ser Gly Leu Gln Ser Lys Ser Asp Gly Leu Val Lys Ser Ile Glu
        515                 520                 525

Leu Thr Lys Ser Asn Cys Glu Leu Thr Arg Ala Gln Val Val Gln Ala
    530                 535                 540

Val Asn Met Leu Ala Lys Leu Thr Glu Gln Met Glu Thr Val Ser Arg
545                 550                 555                 560

Phe Asn Asn Asp Ile Ser Gly Ser Ser Val Glu Gln Asn Ala Leu Ile
                565                 570                 575

Gln Ser Ile Ala Lys Asn Met His Lys Ile Glu Ser Phe Val Glu Glu
            580                 585                 590

Leu Asn Lys Leu Ser Gln Asp Gln Leu Thr Glu Ser Ala Glu Ile Lys
        595                 600                 605

Thr Leu Asn Gly Ser Val Ser Glu Leu Met Ser Ser Phe Lys Val
    610                 615                 620
```

<210> SEQ ID NO 31
<211> LENGTH: 1743
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 31

```
gtgaataagc caatctttgt cgtcgtactc gcttcgctta cgtatggctg cggtggaagc     60

agctccagtg actctagtga cccttctgat accaataact caggagcatc ttatggtgtt    120

gttgctccct atgatattgc caagtatcaa aacatccttt ccagctcaga tcttcaggtg    180

tctgatccta atggagagga gggcaataaa acctctgaag tcaaagatgg taacttcgat    240

ggttatgtca gtgattattt ttatgctgac gaagagacgg aaaatctgat cttcaaaatg    300

gcgaactaca agatgcgctc tgaagttcgt gaaggagaaa acttcgatat caatgaagca    360

ggcgtaagac gcagtctaca tgcggaaata agcctacctg atattgagca tgtaatggcg    420

agttctcccg cagatcacga tgaagtgacc gtgctacaga tccacaataa aggtacagac    480

gagagtggca cgggttatat ccctcatccg ctattgcgtg tggtttggga gcaagaacga    540

gatggcctca caggtcacta ctgggcagtc atgaaaaata atgccattga ctgtagcagt    600

gccgctgact cttcggattg ttatgccact tcatataatc gctacgattt gggagaggcg    660

gatctcgata acttcaccaa gtttgatctt tctgtttatg aaaataccct ttcgatcaaa    720

gtgaacgatg aagttaaagt cgacgaagac atcacctact ggcagcatct actgagttac    780

tttaaagcgg gtatctacaa tcaatttgaa aatggtgaag ccacggctca ctttcaggca    840

ctgcgataca ccaccacaca ggtcaacggc tcaaacgatt gggatattaa tgattggaag    900

ttgacgattc ctgcgagtaa agacacttgg tatggaagtg ggggtgacag tgcggctgaa    960

ctagaacctg agcgctgcga atcgagcaaa gaccttctcg ccaacgacag tgatgtctac   1020

gacagcgata ttggtctttc ttatttcaat accgatgaag ggagagtgca ctttagagcg   1080

gatatgggat atggcacctc taccgaaaat tctagctata ttcgctctga gctcagggag   1140

ttgtatcaaa gcagtgttca accggattgt agcaccagcg atgaagatac aagttggtat   1200

ttggacgaca ctagaacgaa cgctaccagt cacgagttaa ccgcaagctt acgaattgaa   1260

gactacccga acattaataa ccaagacccg aaagtggtgc ttgggcaaat acacggttgg   1320

aagatcaatc aagcattggt gaagttgtta tgggaaggcg agagtaagcc agtaagagtg   1380
```

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atactgaact ctgattttga gcgcaacaac caagactgta accattgtga cccgttcagt 1440

gtcgagttag gtacttattc ggcaagtgaa gagtggcgat atacgattcg agccaatcaa 1500

gacggtatct acttagcgac tcatgattta gatggaacta atacggtttc tcatttaatc 1560

ccttggggac aagattacac agataaagat ggggacacgg tctcgttgac gtcagattgg 1620

acatcgacag acatcgcttt ctatttcaaa gcgggcatct acccacaatt taagcctgat 1680

agcgactatg cgggtgaagt gtttgatgtg agctttagtt ctctaagagc agagcataac 1740

tga 1743

<210> SEQ ID NO 32
<211> LENGTH: 580
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 32

Met Asn Lys Pro Ile Phe Val Val Val Leu Ala Ser Leu Thr Tyr Gly
1 5 10 15

Cys Gly Gly Ser Ser Ser Ser Asp Ser Ser Asp Pro Ser Asp Thr Asn
20 25 30

Asn Ser Gly Ala Ser Tyr Gly Val Val Ala Pro Tyr Asp Ile Ala Lys
35 40 45

Tyr Gln Asn Ile Leu Ser Ser Ser Asp Leu Gln Val Ser Asp Pro Asn
50 55 60

Gly Glu Glu Gly Asn Lys Thr Ser Glu Val Lys Asp Gly Asn Phe Asp
65 70 75 80

Gly Tyr Val Ser Asp Tyr Phe Tyr Ala Asp Glu Glu Thr Glu Asn Leu
85 90 95

Ile Phe Lys Met Ala Asn Tyr Lys Met Arg Ser Glu Val Arg Glu Gly
100 105 110

Glu Asn Phe Asp Ile Asn Glu Ala Gly Val Arg Arg Ser Leu His Ala
115 120 125

Glu Ile Ser Leu Pro Asp Ile Glu His Val Met Ala Ser Ser Pro Ala
130 135 140

Asp His Asp Glu Val Thr Val Leu Gln Ile His Asn Lys Gly Thr Asp
145 150 155 160

Glu Ser Gly Thr Gly Tyr Ile Pro His Pro Leu Leu Arg Val Val Trp
165 170 175

Glu Gln Glu Arg Asp Gly Leu Thr Gly His Tyr Trp Ala Val Met Lys
180 185 190

Asn Asn Ala Ile Asp Cys Ser Ser Ala Ala Asp Ser Ser Asp Cys Tyr
195 200 205

Ala Thr Ser Tyr Asn Arg Tyr Asp Leu Gly Glu Ala Asp Leu Asp Asn
210 215 220

Phe Thr Lys Phe Asp Leu Ser Val Tyr Glu Asn Thr Leu Ser Ile Lys
225 230 235 240

Val Asn Asp Glu Val Lys Val Asp Glu Asp Ile Thr Tyr Trp Gln His
245 250 255

Leu Leu Ser Tyr Phe Lys Ala Gly Ile Tyr Asn Gln Phe Glu Asn Gly
260 265 270

Glu Ala Thr Ala His Phe Gln Ala Leu Arg Tyr Thr Thr Thr Gln Val
275 280 285

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```
Asn Gly Ser Asn Asp Trp Asp Ile Asn Asp Trp Lys Leu Thr Ile Pro
    290                 295                 300

Ala Ser Lys Asp Thr Trp Tyr Gly Ser Gly Gly Asp Ser Ala Ala Glu
305                 310                 315                 320

Leu Glu Pro Glu Arg Cys Glu Ser Ser Lys Asp Leu Leu Ala Asn Asp
                325                 330                 335

Ser Asp Val Tyr Asp Ser Asp Ile Gly Leu Ser Tyr Phe Asn Thr Asp
            340                 345                 350

Glu Gly Arg Val His Phe Arg Ala Asp Met Gly Tyr Gly Thr Ser Thr
        355                 360                 365

Glu Asn Ser Ser Tyr Ile Arg Ser Glu Leu Arg Glu Leu Tyr Gln Ser
    370                 375                 380

Ser Val Gln Pro Asp Cys Ser Thr Ser Asp Glu Asp Thr Ser Trp Tyr
385                 390                 395                 400

Leu Asp Asp Thr Arg Thr Asn Ala Thr Ser His Glu Leu Thr Ala Ser
                405                 410                 415

Leu Arg Ile Glu Asp Tyr Pro Asn Ile Asn Asn Gln Asp Pro Lys Val
            420                 425                 430

Val Leu Gly Gln Ile His Gly Trp Lys Ile Asn Gln Ala Leu Val Lys
        435                 440                 445

Leu Leu Trp Glu Gly Glu Ser Lys Pro Val Arg Val Ile Leu Asn Ser
    450                 455                 460

Asp Phe Glu Arg Asn Asn Gln Asp Cys Asn His Cys Asp Pro Phe Ser
465                 470                 475                 480

Val Glu Leu Gly Thr Tyr Ser Ala Ser Glu Glu Trp Arg Tyr Thr Ile
                485                 490                 495

Arg Ala Asn Gln Asp Gly Ile Tyr Leu Ala Thr His Asp Leu Asp Gly
            500                 505                 510

Thr Asn Thr Val Ser His Leu Ile Pro Trp Gly Gln Asp Tyr Thr Asp
        515                 520                 525

Lys Asp Gly Asp Thr Val Ser Leu Thr Ser Asp Trp Thr Ser Thr Asp
    530                 535                 540

Ile Ala Phe Tyr Phe Lys Ala Gly Ile Tyr Pro Gln Phe Lys Pro Asp
545                 550                 555                 560

Ser Asp Tyr Ala Gly Glu Val Phe Asp Val Ser Phe Ser Ser Leu Arg
                565                 570                 575

Ala Glu His Asn
            580
```

<210> SEQ ID NO 33
<211> LENGTH: 1569
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 33

```
atgaaacaaa ttactctaaa aactttactc gcttcttcta ttctacttgc ggttggttgt     60

gcgagcacga gcacgcctac tgctgatttt ccaaataaca aagaaactgg tgaagcgctt    120

ctgacgccag ttgctgtttc cgctagtagc catgatggta acggacctga tcgtctcgtt    180

gaccaagacc taactacacg ttggtcatct gcgggtgacg gcgagtgggc aacgctagac    240

tatggttcag tacaggagtt tgacgcggtt caggcatctt tcagtaaagg taatcagcgc    300

caatctaaat ttgatatcca agtgagtgtt gatggcgaaa gctggacaac ggtactagaa    360
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aaccaactaa gctcaggtaa agcgatcggc ctagagcgtt tccaatttga gccagtagtg 420

caagcacgct acgtaagata cgttggtcac ggtaacacca aaaacggttg gaacagtgtg 480

actggattag cggcggttaa ctgtagcatt aacgcatgtc ctgctagcca tatcatcact 540

tcagacgtgg ttgcagcaga agccgtgatt attgctgaaa tgaaagcggc agaaaaagca 600

cgtaaagatg cgcgcaaaga tctacgctct ggtaacttcg gtgtagcagc ggtttaccct 660

tgtgagacga ccgttgaatg tgacactcgc agtgcacttc cagttccgac aggcctgcca 720

gcgacaccag ttgcaggtaa ctcgccaagc gaaaactttg acatgacgca ttggtaccta 780

tctcaaccat ttgaccatga caaaaatggc aaacctgatg atgtgtctga gtggaacctt 840

gcaaacggtt accaacaccc tgaaatcttc tacacagctg atgacggcgg cctagtattc 900

aaagcttacg tgaaaggtgt acgtacctct aaaaacacta agtacgcgcg tacagagctt 960

cgtgaaatga tgcgtcgtgg tgatcagtct attagcacta aaggtgttaa taagaataac 1020

tgggtattct caagcgctcc tgaatctgac ttagagtcgg cagcgggtat tgacggcgtt 1080

ctagaagcga cgttgaaaat cgaccatgca acaacgacgg gtaatgcgaa tgaagtaggt 1140

cgctttatca ttggtcagat tcacgatcaa aacgatgaac caattcgttt gtactaccgt 1200

aaactgccaa accaagaaac gggtgcggtt tacttcgcac atgaaagcca agacgcaact 1260

aaagaggact tctaccctct agtgggcgac atgacggctg aagtgggtga cgatggtatc 1320

gcgcttggcg aagtgttcag ctaccgtatt gacgttaaag gcaacacgat gactgtaacg 1380

ctaatacgtg aaggcaaaga cgatgttgta caagtggttg atatgagcaa cagcggctac 1440

gacgcaggcg gcaagtacat gtacttcaaa gccggtgttt acaaccaaaa catcagcggc 1500

gacctagacg attactcaca agcgactttc tatcagctag atgtatcgca cgatcaatac 1560

aaaaagtaa 1569

<210> SEQ ID NO 34
<211> LENGTH: 522
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 34

Met Lys Gln Ile Thr Leu Lys Thr Leu Leu Ala Ser Ser Ile Leu Leu
1 5 10 15

Ala Val Gly Cys Ala Ser Thr Ser Thr Pro Thr Ala Asp Phe Pro Asn
20 25 30

Asn Lys Glu Thr Gly Glu Ala Leu Leu Thr Pro Val Ala Val Ser Ala
35 40 45

Ser Ser His Asp Gly Asn Gly Pro Asp Arg Leu Val Asp Gln Asp Leu
50 55 60

Thr Thr Arg Trp Ser Ser Ala Gly Asp Gly Glu Trp Ala Thr Leu Asp
65 70 75 80

Tyr Gly Ser Val Gln Glu Phe Asp Ala Val Gln Ala Ser Phe Ser Lys
85 90 95

Gly Asn Gln Arg Gln Ser Lys Phe Asp Ile Gln Val Ser Val Asp Gly
100 105 110

Glu Ser Trp Thr Thr Val Leu Glu Asn Gln Leu Ser Ser Gly Lys Ala
115 120 125

Ile Gly Leu Glu Arg Phe Gln Phe Glu Pro Val Val Gln Ala Arg Tyr

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    130                 135                 140

Val Arg Tyr Val Gly His Gly Asn Thr Lys Asn Gly Trp Asn Ser Val
145                 150                 155                 160

Thr Gly Leu Ala Ala Val Asn Cys Ser Ile Asn Ala Cys Pro Ala Ser
                165                 170                 175

His Ile Ile Thr Ser Asp Val Val Ala Ala Glu Ala Val Ile Ile Ala
            180                 185                 190

Glu Met Lys Ala Ala Glu Lys Ala Arg Lys Asp Ala Arg Lys Asp Leu
        195                 200                 205

Arg Ser Gly Asn Phe Gly Val Ala Ala Val Tyr Pro Cys Glu Thr Thr
    210                 215                 220

Val Glu Cys Asp Thr Arg Ser Ala Leu Pro Val Pro Thr Gly Leu Pro
225                 230                 235                 240

Ala Thr Pro Val Ala Gly Asn Ser Pro Ser Glu Asn Phe Asp Met Thr
                245                 250                 255

His Trp Tyr Leu Ser Gln Pro Phe Asp His Asp Lys Asn Gly Lys Pro
            260                 265                 270

Asp Asp Val Ser Glu Trp Asn Leu Ala Asn Gly Tyr Gln His Pro Glu
        275                 280                 285

Ile Phe Tyr Thr Ala Asp Asp Gly Gly Leu Val Phe Lys Ala Tyr Val
    290                 295                 300

Lys Gly Val Arg Thr Ser Lys Asn Thr Lys Tyr Ala Arg Thr Glu Leu
305                 310                 315                 320

Arg Glu Met Met Arg Arg Gly Asp Gln Ser Ile Ser Thr Lys Gly Val
                325                 330                 335

Asn Lys Asn Asn Trp Val Phe Ser Ser Ala Pro Glu Ser Asp Leu Glu
            340                 345                 350

Ser Ala Ala Gly Ile Asp Gly Val Leu Glu Ala Thr Leu Lys Ile Asp
        355                 360                 365

His Ala Thr Thr Thr Gly Asn Ala Asn Glu Val Gly Arg Phe Ile Ile
    370                 375                 380

Gly Gln Ile His Asp Gln Asn Asp Glu Pro Ile Arg Leu Tyr Tyr Arg
385                 390                 395                 400

Lys Leu Pro Asn Gln Glu Thr Gly Ala Val Tyr Phe Ala His Glu Ser
                405                 410                 415

Gln Asp Ala Thr Lys Glu Asp Phe Tyr Pro Leu Val Gly Asp Met Thr
            420                 425                 430

Ala Glu Val Gly Asp Asp Gly Ile Ala Leu Gly Glu Val Phe Ser Tyr
        435                 440                 445

Arg Ile Asp Val Lys Gly Asn Thr Met Thr Val Thr Leu Ile Arg Glu
    450                 455                 460

Gly Lys Asp Asp Val Val Gln Val Val Asp Met Ser Asn Ser Gly Tyr
465                 470                 475                 480

Asp Ala Gly Gly Lys Tyr Met Tyr Phe Lys Ala Gly Val Tyr Asn Gln
                485                 490                 495

Asn Ile Ser Gly Asp Leu Asp Asp Tyr Ser Gln Ala Thr Phe Tyr Gln
            500                 505                 510

Leu Asp Val Ser His Asp Gln Tyr Lys Lys
        515                 520
```

<210> SEQ ID NO 35

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<211> LENGTH: 1230
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 35

atgcaaattt ctaaagtcgc tacagctgtc gctctttcga caggtttatt atttggttgt 60

aacagtgatg gtttacctat tccaacagat ccaggcggaa cagaccctgt tgaacctgtt 120

gaagtttact ctatagaaaa cgtctattgg gatctgacag gtggtgctgt tgctgcacag 180

tcactcagcg gaacttcacc atatcgcttt gataataatg aggaaggtac tcgtgctcta 240

agcatttaca gtggagacgt agctaatggc ttcacttttg agagttcaat atatactgct 300

gaagaagaag gtgttgtttc ctttgaaggt aaggactgta cttacacagt gactgagcaa 360

cagctagata tgacctgtga aaaagatgac gtagaaacag cttactcagc aacagagatt 420

acagatgaat ctgttataac tgcattagaa aatgccgatg atggaaaacc taaatcagtc 480

gatgatgtga acgctgcgat tgcatcagca gaagatggcg cgattattga tttatcatct 540

gaaggtacgt ttgataccgg tgttattgag ctaaataaag ctgtcacaat tgatggtgct 600

ggtttagcaa ccattaccgg agatgcttgt attgatgtca ctgcacccgg tgcaggtatc 660

aaaaacatga cttttgctaa cgacaatttg gccgggtgtt ttggtaggga gtcagctggt 720

acttcagata atgaaactgg tgcgatcgtt attggtaaaa ttggtaaaga ttcagatcct 780

gtagcacttg aaaacctaaa gttcgatgca aacggcatta ccgaagatga tctaggtact 840

aaaaaagcaa gttggttatt ctctcgaggt tactttacat tagacaatag cgaatttgtc 900

ggtttaagtg gcagtttcca aaataatgca attcgtatta actgtagtag tgacaacggg 960

cgatttggtt cacaaatcac aaataataca ttcactatta actctggtgg tagtgatgtg 1020

ggcggaatta aagttggtga ttctagcagt gccgtcataa agaatagtga tgataacctt 1080

ggctgtaatg tcactattga aagcaatacg ttcaatggtt acaaaaccct actttcagct 1140

gacaacggta aagatataag aaatacagcc atctacgcac aaccatctgc agtgaacact 1200

gcggcaggta aagaaaatat cttgaactaa 1230

<210> SEQ ID NO 36
<211> LENGTH: 409
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 36

Met Gln Ile Ser Lys Val Ala Thr Ala Val Ala Leu Ser Thr Gly Leu
1 5 10 15

Leu Phe Gly Cys Asn Ser Asp Gly Leu Pro Ile Pro Thr Asp Pro Gly
20 25 30

Gly Thr Asp Pro Val Glu Pro Val Glu Val Tyr Ser Ile Glu Asn Val
35 40 45

Tyr Trp Asp Leu Thr Gly Gly Ala Val Ala Ala Gln Ser Leu Ser Gly
50 55 60

Thr Ser Pro Tyr Arg Phe Asp Asn Asn Glu Glu Gly Thr Arg Ala Leu
65 70 75 80

Ser Ile Tyr Ser Gly Asp Val Ala Asn Gly Phe Thr Phe Glu Ser Ser
85 90 95

Ile Tyr Thr Ala Glu Glu Glu Gly Val Val Ser Phe Glu Gly Lys Asp
100 105 110

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Cys Thr Tyr Thr Val Thr Glu Gln Gln Leu Asp Met Thr Cys Glu Lys
        115                 120                 125

Asp Asp Val Glu Thr Ala Tyr Ser Ala Thr Glu Ile Thr Asp Glu Ser
    130                 135                 140

Val Ile Thr Ala Leu Glu Asn Ala Asp Asp Gly Lys Pro Lys Ser Val
145                 150                 155                 160

Asp Asp Val Asn Ala Ala Ile Ala Ser Ala Glu Asp Gly Ala Ile Ile
                165                 170                 175

Asp Leu Ser Ser Glu Gly Thr Phe Asp Thr Gly Val Ile Glu Leu Asn
            180                 185                 190

Lys Ala Val Thr Ile Asp Gly Ala Gly Leu Ala Thr Ile Thr Gly Asp
        195                 200                 205

Ala Cys Ile Asp Val Thr Ala Pro Gly Ala Gly Ile Lys Asn Met Thr
    210                 215                 220

Phe Ala Asn Asp Asn Leu Ala Gly Cys Phe Gly Arg Glu Ser Ala Gly
225                 230                 235                 240

Thr Ser Asp Asn Glu Thr Gly Ala Ile Val Ile Gly Lys Ile Gly Lys
                245                 250                 255

Asp Ser Asp Pro Val Ala Leu Glu Asn Leu Lys Phe Asp Ala Asn Gly
            260                 265                 270

Ile Thr Glu Asp Asp Leu Gly Thr Lys Lys Ala Ser Trp Leu Phe Ser
        275                 280                 285

Arg Gly Tyr Phe Thr Leu Asp Asn Ser Glu Phe Val Gly Leu Ser Gly
    290                 295                 300

Ser Phe Gln Asn Asn Ala Ile Arg Ile Asn Cys Ser Ser Asp Asn Gly
305                 310                 315                 320

Arg Phe Gly Ser Gln Ile Thr Asn Asn Thr Phe Thr Ile Asn Ser Gly
                325                 330                 335

Gly Ser Asp Val Gly Gly Ile Lys Val Gly Asp Ser Ser Ser Ala Val
            340                 345                 350

Ile Lys Asn Ser Asp Asp Asn Leu Gly Cys Asn Val Thr Ile Glu Ser
        355                 360                 365

Asn Thr Phe Asn Gly Tyr Lys Thr Leu Leu Ser Ala Asp Asn Gly Lys
    370                 375                 380

Asp Ile Arg Asn Thr Ala Ile Tyr Ala Gln Pro Ser Ala Val Asn Thr
385                 390                 395                 400

Ala Ala Gly Lys Glu Asn Ile Leu Asn
                405
```

<210> SEQ ID NO 37
<211> LENGTH: 861
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 37

```
atgaattctg ttacaaaaat tgctgcagct gttgcatgta ctcttttagc gggcacagct     60

gctggtgcat ctcttgatta tcgttacgag tatcgtgctg cgacggatta tacaaagact    120

aatggtgata cggctcacgt agacgctcgc catcaacacc gagttaagct aggtgaaagc    180

tttaagctgt cagacaagtg gaagcactct actggtctag aacttaagtt ccacggtgat    240

gactcttact atgatgaaga ttcaggttct gttaaatcag caaacagcca gagtttttac    300
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gatggcaatt ggtacatcta tggtatggag atcgataaca ctgcgacata caaaatagac 360

aataattggt atctacaaat gggtatgcct attgcttggg attgggatga gcctaatgct 420

aacgatggcg actggaagat gaaaaaggtt acgtttaaac ctcagttccg cgttggctat 480

aaagcagata tgggtttaac aactgctatt cgttaccgtc atgaatatgc tgacttccgt 540

aaccacacac aatttggcga caaagattct gaaactggcg agcgtttaga atcagctcaa 600

aagtctaaag ttacactgac gggctcttac aaaattgaat ctctacctaa gcttggcctt 660

tcttacgaag caaactatgt aaaatctttg gataacgtac ttctttataa tagtgatgac 720

tgggaatggg atgctggctt aaaggtaaac tacaagttcg gttcttggaa acctttttgct 780

gaaatctggt cttctgatat cagttcatct tcaaaagatc gtgaagctaa ataccgtgtt 840

ggtattgctt actcattcta a 861

<210> SEQ ID NO 38
<211> LENGTH: 286
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 38

Met Asn Ser Val Thr Lys Ile Ala Ala Ala Val Ala Cys Thr Leu Leu
1 5 10 15

Ala Gly Thr Ala Ala Gly Ala Ser Leu Asp Tyr Arg Tyr Glu Tyr Arg
20 25 30

Ala Ala Thr Asp Tyr Thr Lys Thr Asn Gly Asp Thr Ala His Val Asp
35 40 45

Ala Arg His Gln His Arg Val Lys Leu Gly Glu Ser Phe Lys Leu Ser
50 55 60

Asp Lys Trp Lys His Ser Thr Gly Leu Glu Leu Lys Phe His Gly Asp
65 70 75 80

Asp Ser Tyr Tyr Asp Glu Asp Ser Gly Ser Val Lys Ser Ala Asn Ser
85 90 95

Gln Ser Phe Tyr Asp Gly Asn Trp Tyr Ile Tyr Gly Met Glu Ile Asp
100 105 110

Asn Thr Ala Thr Tyr Lys Ile Asp Asn Asn Trp Tyr Leu Gln Met Gly
115 120 125

Met Pro Ile Ala Trp Asp Trp Asp Glu Pro Asn Ala Asn Asp Gly Asp
130 135 140

Trp Lys Met Lys Lys Val Thr Phe Lys Pro Gln Phe Arg Val Gly Tyr
145 150 155 160

Lys Ala Asp Met Gly Leu Thr Thr Ala Ile Arg Tyr Arg His Glu Tyr
165 170 175

Ala Asp Phe Arg Asn His Thr Gln Phe Gly Asp Lys Asp Ser Glu Thr
180 185 190

Gly Glu Arg Leu Glu Ser Ala Gln Lys Ser Lys Val Thr Leu Thr Gly
195 200 205

Ser Tyr Lys Ile Glu Ser Leu Pro Lys Leu Gly Leu Ser Tyr Glu Ala
210 215 220

Asn Tyr Val Lys Ser Leu Asp Asn Val Leu Leu Tyr Asn Ser Asp Asp
225 230 235 240

Trp Glu Trp Asp Ala Gly Leu Lys Val Asn Tyr Lys Phe Gly Ser Trp
245 250 255

-continued

Lys Pro Phe Ala Glu Ile Trp Ser Ser Asp Ile Ser Ser Ser Ser Lys
260 265 270

Asp Arg Glu Ala Lys Tyr Arg Val Gly Ile Ala Tyr Ser Phe
275 280 285

<210> SEQ ID NO 39
<211> LENGTH: 1038
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 39

atgtttaaga aaaacatatt agcagtggcg ttattagcga ctgtgccaat ggttactttc 60

gcaaataacg gtgtttctta ccccgtacct gccgataaat tcgatatgca taattggaaa 120

ataaccatac cttcagatat taatgaagat ggtcgcgttg atgaaataga aggggtcgct 180

atgatgagct actcacatag tgatttcttc catcttgata aagacggcaa ccttgtattt 240

gaagtgcaga accaagcgat tacgacgaaa aactcgaaga atgcgcgttc tgagttacgc 300

cagatgccaa gaggcgcaga tttctctatc gatacggctg ataaaggaaa ccagtgggca 360

ctgtcgagtc acccagcggc tagtgaatac agtgctgtgg gcggaacatt agaagcgaca 420

ttaaaagtga atcacgtctc agttaacgct aagttcccag aaaaataccc agctcattct 480

gttgtggttg gtcagattca tgctaaaaaa cacaacgagc taatcaaagc tggaaccggt 540

tatgggcatg gtaatgaacc actaaagatc ttctataaga agtttcctga ccaagaaatg 600

ggttcagtat tctggaacta tgaacgtaac ctagagaaaa aagatcctaa ccgtgccgat 660

atcgcttatc cagtgtgggg taacacgtgg gaaaaccctg cagagccggg tgaagccggt 720

attgctcttg gtgaagagtt tagctacaaa gtggaagtga aaggcaccat gatgtaccta 780

acgtttgaaa ccgagcgtca cgataccgtt aagtatgaaa tcgacctgag taagggcatc 840

gatgaacttg actcaccaac gggctatgct gaagatgatt tttactacaa agcgggcgca 900

tacggccaat gtagcgtgag cgattctcac cctgtatggg ggcctggttg tggcggtact 960

ggcgatttcg ctgtcgataa aaagaatggc gattacaaca gtgtgacttt ctctgcgctt 1020

aagttaaacg gtaaatag 1038

<210> SEQ ID NO 40
<211> LENGTH: 345
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 40

Met Phe Lys Lys Asn Ile Leu Ala Val Ala Leu Leu Ala Thr Val Pro
1 5 10 15

Met Val Thr Phe Ala Asn Asn Gly Val Ser Tyr Pro Val Pro Ala Asp
20 25 30

Lys Phe Asp Met His Asn Trp Lys Ile Thr Ile Pro Ser Asp Ile Asn
35 40 45

Glu Asp Gly Arg Val Asp Glu Ile Glu Gly Val Ala Met Met Ser Tyr
50 55 60

Ser His Ser Asp Phe Phe His Leu Asp Lys Asp Gly Asn Leu Val Phe
65 70 75 80

Glu Val Gln Asn Gln Ala Ile Thr Thr Lys Asn Ser Lys Asn Ala Arg
85 90 95

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Ser Glu Leu Arg Gln Met Pro Arg Gly Ala Asp Phe Ser Ile Asp Thr
            100                 105                 110

Ala Asp Lys Gly Asn Gln Trp Ala Leu Ser Ser His Pro Ala Ala Ser
        115                 120                 125

Glu Tyr Ser Ala Val Gly Gly Thr Leu Glu Ala Thr Leu Lys Val Asn
    130                 135                 140

His Val Ser Val Asn Ala Lys Phe Pro Glu Lys Tyr Pro Ala His Ser
145                 150                 155                 160

Val Val Val Gly Gln Ile His Ala Lys Lys His Asn Glu Leu Ile Lys
                165                 170                 175

Ala Gly Thr Gly Tyr Gly His Gly Asn Glu Pro Leu Lys Ile Phe Tyr
            180                 185                 190

Lys Lys Phe Pro Asp Gln Glu Met Gly Ser Val Phe Trp Asn Tyr Glu
        195                 200                 205

Arg Asn Leu Glu Lys Lys Asp Pro Asn Arg Ala Asp Ile Ala Tyr Pro
    210                 215                 220

Val Trp Gly Asn Thr Trp Glu Asn Pro Ala Glu Pro Gly Glu Ala Gly
225                 230                 235                 240

Ile Ala Leu Gly Glu Glu Phe Ser Tyr Lys Val Glu Val Lys Gly Thr
                245                 250                 255

Met Met Tyr Leu Thr Phe Glu Thr Glu Arg His Asp Thr Val Lys Tyr
            260                 265                 270

Glu Ile Asp Leu Ser Lys Gly Ile Asp Glu Leu Asp Ser Pro Thr Gly
        275                 280                 285

Tyr Ala Glu Asp Asp Phe Tyr Tyr Lys Ala Gly Ala Tyr Gly Gln Cys
    290                 295                 300

Ser Val Ser Asp Ser His Pro Val Trp Gly Pro Gly Cys Gly Gly Thr
305                 310                 315                 320

Gly Asp Phe Ala Val Asp Lys Lys Asn Gly Asp Tyr Asn Ser Val Thr
                325                 330                 335

Phe Ser Ala Leu Lys Leu Asn Gly Lys
            340                 345
```

<210> SEQ ID NO 41
<211> LENGTH: 897
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 41

```
atggataact ctccggtgct gagccgattt ttagagaatg gatttttact ccagcagaaa     60

ctgagccttg ttctttgttg tgtgttgatc gcagcttctg catggatttt aggacagctt    120

gcatggttta ttgaacctgc tgagcaaacc gtcgtgccat ggacagcaac ggcttcctcg    180

tcttcaacgc ctcaatcgac tcttgatatc tcttctttgc agcagagcaa catgtttggt    240

gcttataacc caaccacgcc tgctgtggtt gagcagcaag ttatccaaga tgcgccaaag    300

acgcgactga acctcgtttt agtgggtgca gtagccagtt ctaatccaaa gctgagcttg    360

gctgtgattg ccaatcgcgg cacacaagca acctacggca ttaatgaaga gatcgaaggt    420

acgcgagcta agttaaaagc ggtattagtc gatcgcgtga ttattgataa ctcaggtcga    480

gacgaaacct tgatgcttga aggcattgag tacaagcgtt tgtctgtatc agcacctgcg    540

ccacctcgta cctcttcttc tgtgcgtggc aacaacccag cttctgcaga agagaagcta    600
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gatgaaatta aagcgaagat aatgaaagat ccgcaacaaa tcttccaata tgttcgactg 660

tctcaggtga aacgcgacga taaagtgatt ggttatcgtg tgagccctgg caaagattca 720

gaacttttta actctgttgg gctccaaaac ggagatattg ccactcagtt aaatggacaa 780

gacctgacag accctgctgc tatgggcaac atattccgtt ctatctcaga gctgacagag 840

ctaaacctcg tcgtcgagag agatggtcaa caacatgaag tgtttattga attttag 897

<210> SEQ ID NO 42
<211> LENGTH: 298
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 42

Met Asp Asn Ser Pro Val Leu Ser Arg Phe Leu Glu Asn Gly Phe Leu
1 5 10 15

Leu Gln Gln Lys Leu Ser Leu Val Leu Cys Cys Val Leu Ile Ala Ala
20 25 30

Ser Ala Trp Ile Leu Gly Gln Leu Ala Trp Phe Ile Glu Pro Ala Glu
35 40 45

Gln Thr Val Val Pro Trp Thr Ala Thr Ala Ser Ser Ser Ser Thr Pro
50 55 60

Gln Ser Thr Leu Asp Ile Ser Ser Leu Gln Gln Ser Asn Met Phe Gly
65 70 75 80

Ala Tyr Asn Pro Thr Thr Pro Ala Val Val Glu Gln Gln Val Ile Gln
85 90 95

Asp Ala Pro Lys Thr Arg Leu Asn Leu Val Leu Val Gly Ala Val Ala
100 105 110

Ser Ser Asn Pro Lys Leu Ser Leu Ala Val Ile Ala Asn Arg Gly Thr
115 120 125

Gln Ala Thr Tyr Gly Ile Asn Glu Glu Ile Glu Gly Thr Arg Ala Lys
130 135 140

Leu Lys Ala Val Leu Val Asp Arg Val Ile Ile Asp Asn Ser Gly Arg
145 150 155 160

Asp Glu Thr Leu Met Leu Glu Gly Ile Glu Tyr Lys Arg Leu Ser Val
165 170 175

Ser Ala Pro Ala Pro Pro Arg Thr Ser Ser Ser Val Arg Gly Asn Asn
180 185 190

Pro Ala Ser Ala Glu Glu Lys Leu Asp Glu Ile Lys Ala Lys Ile Met
195 200 205

Lys Asp Pro Gln Gln Ile Phe Gln Tyr Val Arg Leu Ser Gln Val Lys
210 215 220

Arg Asp Asp Lys Val Ile Gly Tyr Arg Val Ser Pro Gly Lys Asp Ser
225 230 235 240

Glu Leu Phe Asn Ser Val Gly Leu Gln Asn Gly Asp Ile Ala Thr Gln
245 250 255

Leu Asn Gly Gln Asp Leu Thr Asp Pro Ala Ala Met Gly Asn Ile Phe
260 265 270

Arg Ser Ile Ser Glu Leu Thr Glu Leu Asn Leu Val Val Glu Arg Asp
275 280 285

Gly Gln Gln His Glu Val Phe Ile Glu Phe
290 295

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<210> SEQ ID NO 43
<211> LENGTH: 2025
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 43

gtgaagcatt ggtttaagaa aagtgcatgg ttattggcag gaagcttaat ctgcacaccc 60

gcagccatcg cgagtgattt tagtgccagc tttaaaggca ctgatattca agagtttatt 120

aatattgttg gtcgtaacct agagaagacg atcatcgttg acccttcggt gcgcggaaaa 180

atcgatgtac gcagctacga cgtactcaat gaagagcaat actacagctt cttcctaaac 240

gtattggaag tgtatggcta cgcggttgtc gaaatggact cgggtgttct taagatcatc 300

aaggccaaag attcgaaaac atcggcaatt ccagtcgttg gagacagtga cacgatcaaa 360

ggcgacaatg tggtgacacg tgttgtgacg gttcgtaatg tctcggtgcg tgaactttct 420

cctctgcttc gtcaactaaa cgacaatgca ggcgcgggta acgttgtgca ctacgaccca 480

gccaacatca tccttattac aggccgagcg gcggtagtaa accgtttagc tgaaatcatc 540

aagcgtgttg accaagcggg tgataaagag attgaagtcg ttgagctaaa gaatgcttct 600

gcggcagaaa tggtacgtat cgttgatgcg ttaagcaaaa ccactgatgc gaaaaacaca 660

cctgcatttc tacaacctaa attagttgcc gatgaacgta ccaatgcgat tcttatctca 720

ggcgacccta aagtacgtag ccgtttaaga aggctgattg aacagcttga tgttgaaatg 780

gcaaccaagg gcaataacca agttatttac cttaaatatg caaaagccga agatctagtt 840

gatgtgctga aaggcgtgtc ggacaaccta caatcagaga agcagacatc aaccaaagga 900

agttcatcgc agcgtaacca agtgatgatc tcagctcaca gtgacaccaa ctctttagtg 960

attaccgcac agccggacat catgaatgcg cttcaagatg tgatcgcaca gctggatatt 1020

cgtcgtgctc aagtattgat tgaagcactg attgtcgaaa tggccgaagg tgacggcgtt 1080

aaccttggtg tgcagtgggg taaccttgaa acgggtgcca tgattcagta cagcaacact 1140

ggcgcttcca ttggcggtgt gatggttggt ttagaagaag cgaaagacag cgaaacgaca 1200

accgctgttt atgattcaga cggtaaattc ttacgtaatg aaaccacgac ggaagaaggt 1260

gactattcaa cattagcttc cgcactttct ggtgttaatg gtgcggcaat gagtgtggta 1320

atgggtgact ggaccgcctt gatcagtgca gtagcgaccg attcaaattc aaatatccta 1380

tcttctccaa gtatcaccgt gatggataac ggcgaagcgt cattcattgt gggtgaagag 1440

gtgcctgttc taaccggttc tacagcaggc tcaagtaacg acaacccatt ccaaacagtt 1500

gaacgtaaag aagtgggtat caagcttaaa gtggtgccgc aaatcaatga aggtgattcg 1560

gttcaactgc aaatagaaca agaagtatcg aacgtattag gcgccaatgg tgcggttgat 1620

gtgcgttttg ctaagcgaca gctaaataca tcagtgattg ttcaagacgg tcaaatgctg 1680

gtgttgggtg gcttgattga cgagcgagca ttggaaagtg aatctaaggt gccgttcttg 1740

ggagatattc ctgtgcttgg acacttgttc aaatcaacca gtactcaggt tgagaaaaag 1800

aacctaatgg tcttcatcaa accaaccatt attcgtgatg gtatgacagc cgatggtatc 1860

acgcagcgta aatacaactt catccgtgct gagcagttgt acaaggctga gcaaggactg 1920

aagttaatgg cagacgataa catcccagta ttgcctaaat ttggtgccga catgaatcac 1980

ccggctgaaa ttcaagcctt catcgatcaa atggaacaag aataa 2025

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<210> SEQ ID NO 44
<211> LENGTH: 674
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 44

Met Lys His Trp Phe Lys Lys Ser Ala Trp Leu Leu Ala Gly Ser Leu
1 5 10 15

Ile Cys Thr Pro Ala Ala Ile Ala Ser Asp Phe Ser Ala Ser Phe Lys
20 25 30

Gly Thr Asp Ile Gln Glu Phe Ile Asn Ile Val Gly Arg Asn Leu Glu
35 40 45

Lys Thr Ile Ile Val Asp Pro Ser Val Arg Gly Lys Ile Asp Val Arg
50 55 60

Ser Tyr Asp Val Leu Asn Glu Glu Gln Tyr Tyr Ser Phe Phe Leu Asn
65 70 75 80

Val Leu Glu Val Tyr Gly Tyr Ala Val Val Glu Met Asp Ser Gly Val
85 90 95

Leu Lys Ile Ile Lys Ala Lys Asp Ser Lys Thr Ser Ala Ile Pro Val
100 105 110

Val Gly Asp Ser Asp Thr Ile Lys Gly Asp Asn Val Val Thr Arg Val
115 120 125

Val Thr Val Arg Asn Val Ser Val Arg Glu Leu Ser Pro Leu Leu Arg
130 135 140

Gln Leu Asn Asp Asn Ala Gly Ala Gly Asn Val Val His Tyr Asp Pro
145 150 155 160

Ala Asn Ile Ile Leu Ile Thr Gly Arg Ala Ala Val Val Asn Arg Leu
165 170 175

Ala Glu Ile Ile Lys Arg Val Asp Gln Ala Gly Asp Lys Glu Ile Glu
180 185 190

Val Val Glu Leu Lys Asn Ala Ser Ala Ala Glu Met Val Arg Ile Val
195 200 205

Asp Ala Leu Ser Lys Thr Thr Asp Ala Lys Asn Thr Pro Ala Phe Leu
210 215 220

Gln Pro Lys Leu Val Ala Asp Glu Arg Thr Asn Ala Ile Leu Ile Ser
225 230 235 240

Gly Asp Pro Lys Val Arg Ser Arg Leu Arg Arg Leu Ile Glu Gln Leu
245 250 255

Asp Val Glu Met Ala Thr Lys Gly Asn Asn Gln Val Ile Tyr Leu Lys
260 265 270

Tyr Ala Lys Ala Glu Asp Leu Val Asp Val Leu Lys Gly Val Ser Asp
275 280 285

Asn Leu Gln Ser Glu Lys Gln Thr Ser Thr Lys Gly Ser Ser Ser Gln
290 295 300

Arg Asn Gln Val Met Ile Ser Ala His Ser Asp Thr Asn Ser Leu Val
305 310 315 320

Ile Thr Ala Gln Pro Asp Ile Met Asn Ala Leu Gln Asp Val Ile Ala
325 330 335

Gln Leu Asp Ile Arg Arg Ala Gln Val Leu Ile Glu Ala Leu Ile Val
340 345 350

Glu Met Ala Glu Gly Asp Gly Val Asn Leu Gly Val Gln Trp Gly Asn
355 360 365

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Leu Glu Thr Gly Ala Met Ile Gln Tyr Ser Asn Thr Gly Ala Ser Ile
    370                 375                 380

Gly Gly Val Met Val Gly Leu Glu Glu Ala Lys Asp Ser Glu Thr Thr
385                 390                 395                 400

Thr Ala Val Tyr Asp Ser Asp Gly Lys Phe Leu Arg Asn Glu Thr Thr
                405                 410                 415

Thr Glu Glu Gly Asp Tyr Ser Thr Leu Ala Ser Ala Leu Ser Gly Val
            420                 425                 430

Asn Gly Ala Ala Met Ser Val Val Met Gly Asp Trp Thr Ala Leu Ile
        435                 440                 445

Ser Ala Val Ala Thr Asp Ser Asn Ser Asn Ile Leu Ser Ser Pro Ser
    450                 455                 460

Ile Thr Val Met Asp Asn Gly Glu Ala Ser Phe Ile Val Gly Glu Glu
465                 470                 475                 480

Val Pro Val Leu Thr Gly Ser Thr Ala Gly Ser Ser Asn Asp Asn Pro
                485                 490                 495

Phe Gln Thr Val Glu Arg Lys Glu Val Gly Ile Lys Leu Lys Val Val
            500                 505                 510

Pro Gln Ile Asn Glu Gly Asp Ser Val Gln Leu Gln Ile Glu Gln Glu
        515                 520                 525

Val Ser Asn Val Leu Gly Ala Asn Gly Ala Val Asp Val Arg Phe Ala
    530                 535                 540

Lys Arg Gln Leu Asn Thr Ser Val Ile Val Gln Asp Gly Gln Met Leu
545                 550                 555                 560

Val Leu Gly Gly Leu Ile Asp Glu Arg Ala Leu Glu Ser Glu Ser Lys
                565                 570                 575

Val Pro Phe Leu Gly Asp Ile Pro Val Leu Gly His Leu Phe Lys Ser
            580                 585                 590

Thr Ser Thr Gln Val Glu Lys Lys Asn Leu Met Val Phe Ile Lys Pro
        595                 600                 605

Thr Ile Ile Arg Asp Gly Met Thr Ala Asp Gly Ile Thr Gln Arg Lys
    610                 615                 620

Tyr Asn Phe Ile Arg Ala Glu Gln Leu Tyr Lys Ala Glu Gln Gly Leu
625                 630                 635                 640

Lys Leu Met Ala Asp Asp Asn Ile Pro Val Leu Pro Lys Phe Gly Ala
                645                 650                 655

Asp Met Asn His Pro Ala Glu Ile Gln Ala Phe Ile Asp Gln Met Glu
            660                 665                 670

Gln Glu
```

<210> SEQ ID NO 45
<211> LENGTH: 1503
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 45

```
atggctgaat tggtaggggc ggcacgtact tatcagcgct tgccgtttag ctttgcgaat     60

cgctacaaga tggtgttgga ataccaacat ccagagcgcg caccgatact ttattatgtt    120

gagccactga aatcggcggc gatcattgaa gtgagtcgtg ttgtgaaaaa tggtttcacg    180

ccacaagcga ttactctcga tgagtttgat aaaaaactaa ccgatgctta tcagcgtgac    240

tcgtcagaag ctcgtcagct catggaagac attggtgctg atagtgatga tttcttctca    300
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ctagcggaag aactgcctca agacgaagac ttacttgaat cagaagatga tgcaccaatc 360

atcaagttaa tcaatgcgat gctgggtgag gcgatcaaag agggtgcttc ggatatacac 420

atcgaaacct ttgaaaagtc actttgtatc cgtttccgag ttgatggtgt gctgcgtgat 480

gttctagcgc caagccgtaa actggctccg ctattggttt cacgtgtcaa ggttatggct 540

aaactggata ttgcggaaaa acgcgtgcca caagatggtc gtatttctct gcgtattggt 600

ggccgagcgg ttgatgttcg tgtttcaacc atgccttctt cgcatggtga gcgtgtggta 660

atgcgtctgt tggacaaaaa tgccactcgt ctagacttgc acagtttagg tatgacagcc 720

gaaaaccatg aaaacttccg taagctgatt cagcgcccac atggcattat cttggtgacc 780

ggcccgacag gttcaggtaa atcgacgacc ttgtacgcag gtctgcaaga actcaacagc 840

aatgaacgaa acattttaac cgttgaagac ccaatcgaat tcgatatcga tggcattggt 900

caaacacaag tgaaccctaa ggttgatatg acctttgcgc gtggtttacg tgccattctt 960

cgtcaagatc ctgatgttgt tatgattggt gagatccgtg acttggagac cgcagagatt 1020

gctgtccagg cctctttgac aggtcactta gttatgtcga ctctgcatac caatactgcc 1080

gtcggtgcga ttacacgtct acgtgatatg ggcattgaac ctttcttgat ctcttcttcg 1140

ctgctgggtg ttttggctca gcgcttggtt cgtactttat gtaacgaatg taaagaacct 1200

tatgaagccg ataaagagca gaagaaactg tttgggttga agaagaaaga aagcttgacg 1260

ctttaccatg ccaaaggttg tgaagagtgt ggccataagg gttatcgagg tcgtacgggt 1320

attcatgagc tgttgatgat tgatgattca gtacaagagc tgattcacag tgaagcgggt 1380

gagcaggcga ttgataaagc aattcgtggc acaacaccaa gtattcgaga tgatggcttg 1440

agcaaagttc tgaaaggggt aacgtcccta gaagaagtga tgcgcgtgac caaggaagtc 1500

tag 1503

<210> SEQ ID NO 46
<211> LENGTH: 500
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 46

Met Ala Glu Leu Val Gly Ala Ala Arg Thr Tyr Gln Arg Leu Pro Phe
1 5 10 15

Ser Phe Ala Asn Arg Tyr Lys Met Val Leu Glu Tyr Gln His Pro Glu
20 25 30

Arg Ala Pro Ile Leu Tyr Tyr Val Glu Pro Leu Lys Ser Ala Ala Ile
35 40 45

Ile Glu Val Ser Arg Val Val Lys Asn Gly Phe Thr Pro Gln Ala Ile
50 55 60

Thr Leu Asp Glu Phe Asp Lys Lys Leu Thr Asp Ala Tyr Gln Arg Asp
65 70 75 80

Ser Ser Glu Ala Arg Gln Leu Met Glu Asp Ile Gly Ala Asp Ser Asp
85 90 95

Asp Phe Phe Ser Leu Ala Glu Glu Leu Pro Gln Asp Glu Asp Leu Leu
100 105 110

Glu Ser Glu Asp Asp Ala Pro Ile Ile Lys Leu Ile Asn Ala Met Leu
115 120 125

Gly Glu Ala Ile Lys Glu Gly Ala Ser Asp Ile His Ile Glu Thr Phe

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    130                 135                 140

Glu Lys Ser Leu Cys Ile Arg Phe Arg Val Asp Gly Val Leu Arg Asp
145                 150                 155                 160

Val Leu Ala Pro Ser Arg Lys Leu Ala Pro Leu Leu Val Ser Arg Val
                165                 170                 175

Lys Val Met Ala Lys Leu Asp Ile Ala Glu Lys Arg Val Pro Gln Asp
            180                 185                 190

Gly Arg Ile Ser Leu Arg Ile Gly Gly Arg Ala Val Asp Val Arg Val
        195                 200                 205

Ser Thr Met Pro Ser Ser His Gly Glu Arg Val Val Met Arg Leu Leu
    210                 215                 220

Asp Lys Asn Ala Thr Arg Leu Asp Leu His Ser Leu Gly Met Thr Ala
225                 230                 235                 240

Glu Asn His Glu Asn Phe Arg Lys Leu Ile Gln Arg Pro His Gly Ile
                245                 250                 255

Ile Leu Val Thr Gly Pro Thr Gly Ser Gly Lys Ser Thr Thr Leu Tyr
            260                 265                 270

Ala Gly Leu Gln Glu Leu Asn Ser Asn Glu Arg Asn Ile Leu Thr Val
        275                 280                 285

Glu Asp Pro Ile Glu Phe Asp Ile Asp Gly Ile Gly Gln Thr Gln Val
    290                 295                 300

Asn Pro Lys Val Asp Met Thr Phe Ala Arg Gly Leu Arg Ala Ile Leu
305                 310                 315                 320

Arg Gln Asp Pro Asp Val Val Met Ile Gly Glu Ile Arg Asp Leu Glu
                325                 330                 335

Thr Ala Glu Ile Ala Val Gln Ala Ser Leu Thr Gly His Leu Val Met
            340                 345                 350

Ser Thr Leu His Thr Asn Thr Ala Val Gly Ala Ile Thr Arg Leu Arg
        355                 360                 365

Asp Met Gly Ile Glu Pro Phe Leu Ile Ser Ser Ser Leu Leu Gly Val
    370                 375                 380

Leu Ala Gln Arg Leu Val Arg Thr Leu Cys Asn Glu Cys Lys Glu Pro
385                 390                 395                 400

Tyr Glu Ala Asp Lys Glu Gln Lys Lys Leu Phe Gly Leu Lys Lys Lys
                405                 410                 415

Glu Ser Leu Thr Leu Tyr His Ala Lys Gly Cys Glu Glu Cys Gly His
            420                 425                 430

Lys Gly Tyr Arg Gly Arg Thr Gly Ile His Glu Leu Leu Met Ile Asp
        435                 440                 445

Asp Ser Val Gln Glu Leu Ile His Ser Glu Ala Gly Glu Gln Ala Ile
    450                 455                 460

Asp Lys Ala Ile Arg Gly Thr Thr Pro Ser Ile Arg Asp Asp Gly Leu
465                 470                 475                 480

Ser Lys Val Leu Lys Gly Val Thr Ser Leu Glu Glu Val Met Arg Val
                485                 490                 495

Thr Lys Glu Val
            500
```

<210> SEQ ID NO 47
<211> LENGTH: 1221
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

-continued

<400> SEQUENCE: 47

atggcggcat ttgaatacaa agcactggat gccaaaggca aaagtaaaaa aggctcaatt 60

gaagcagata atgctcgtca ggctcgccaa agaataaaag agcttggctt gatgccggtt 120

gagatgaccg aggctaaagc aaaaacagca aaaggtgctc agccatcgac cagctttaaa 180

cgcggcatca gtacgcctga tcttgcgctt attactcgtc aaatatccac gctcgttcaa 240

tctggtatgc cgctagaaga gtgtttgaaa gccgttgccg aacagtctga gaaacctcgt 300

attcgcacca tgctactcgc ggtgagatct aaggtgactg aaggttattc gttagcagac 360

agcttgtctg attatcccca tatcttcgat gagctattca gagccatggt tgctgctggt 420

gagaagtcag ggcatctaga tgcggtattg gaacgattgg ctgactacgc agaaaaccgt 480

cagaagatgc gttctaagtt gctgcaagcg atgatctacc ccatcgtgct ggtggtgttt 540

gcggtgacga ttgtgtcgtt cctactggca acggtagtgc cgaagatcgt tgagcctatt 600

atccaaatgg gacaagagct ccctcagtcg acacaatttt tattagcatc gagtgaattt 660

atccagaatt ggggcatcca attactggtg ttgaccattg gtgtgattgt gttggttaag 720

actgcgctga aaaagccggg cgttcgcatg agctgggatc gcaaattatt gagcatcccg 780

ctgataggca agatagcgaa agggatcaac acctctcgtt ttgcacgaac actttctatc 840

tgtacctcta gtgcgattcc tatccttgaa gggatgaagg tcgcggtaga tgtgatgtcg 900

aatcatcacg tgaaacaaca agtattacag gcatcagata gcgttagaga aggggcaagc 960

ctgcgtaaag cgcttgatca aaccaaactc tttcccccga tgatgctgca tatgatcgcc 1020

agtggtgagc agagtggcca attggaacag atgctgacaa gagcggcaga taatcaggat 1080

caaagctttg aatcgaccgt taatatcgcg ttaggcattt ttaccccagc gcttattgcg 1140

ttgatggctg gcttagtgct gtttatcgtg atggcgacgc tgatgccaat gcttgaaatg 1200

aacaatttaa tgagtggtta a 1221

<210> SEQ ID NO 48
<211> LENGTH: 406
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 48

Met Ala Ala Phe Glu Tyr Lys Ala Leu Asp Ala Lys Gly Lys Ser Lys
1 5 10 15

Lys Gly Ser Ile Glu Ala Asp Asn Ala Arg Gln Ala Arg Gln Arg Ile
20 25 30

Lys Glu Leu Gly Leu Met Pro Val Glu Met Thr Glu Ala Lys Ala Lys
35 40 45

Thr Ala Lys Gly Ala Gln Pro Ser Thr Ser Phe Lys Arg Gly Ile Ser
50 55 60

Thr Pro Asp Leu Ala Leu Ile Thr Arg Gln Ile Ser Thr Leu Val Gln
65 70 75 80

Ser Gly Met Pro Leu Glu Glu Cys Leu Lys Ala Val Ala Glu Gln Ser
85 90 95

Glu Lys Pro Arg Ile Arg Thr Met Leu Leu Ala Val Arg Ser Lys Val
100 105 110

Thr Glu Gly Tyr Ser Leu Ala Asp Ser Leu Ser Asp Tyr Pro His Ile
115 120 125

-continued

```
Phe Asp Glu Leu Phe Arg Ala Met Val Ala Ala Gly Glu Lys Ser Gly
    130                 135                 140

His Leu Asp Ala Val Leu Glu Arg Leu Ala Asp Tyr Ala Glu Asn Arg
145                 150                 155                 160

Gln Lys Met Arg Ser Lys Leu Leu Gln Ala Met Ile Tyr Pro Ile Val
                165                 170                 175

Leu Val Val Phe Ala Val Thr Ile Val Ser Phe Leu Leu Ala Thr Val
            180                 185                 190

Val Pro Lys Ile Val Glu Pro Ile Ile Gln Met Gly Gln Glu Leu Pro
        195                 200                 205

Gln Ser Thr Gln Phe Leu Leu Ala Ser Ser Glu Phe Ile Gln Asn Trp
    210                 215                 220

Gly Ile Gln Leu Leu Val Leu Thr Ile Gly Val Ile Val Leu Val Lys
225                 230                 235                 240

Thr Ala Leu Lys Lys Pro Gly Val Arg Met Ser Trp Asp Arg Lys Leu
                245                 250                 255

Leu Ser Ile Pro Leu Ile Gly Lys Ile Ala Lys Gly Ile Asn Thr Ser
            260                 265                 270

Arg Phe Ala Arg Thr Leu Ser Ile Cys Thr Ser Ser Ala Ile Pro Ile
        275                 280                 285

Leu Glu Gly Met Lys Val Ala Val Asp Val Met Ser Asn His His Val
    290                 295                 300

Lys Gln Gln Val Leu Gln Ala Ser Asp Ser Val Arg Glu Gly Ala Ser
305                 310                 315                 320

Leu Arg Lys Ala Leu Asp Gln Thr Lys Leu Phe Pro Pro Met Met Leu
                325                 330                 335

His Met Ile Ala Ser Gly Glu Gln Ser Gly Gln Leu Glu Gln Met Leu
            340                 345                 350

Thr Arg Ala Ala Asp Asn Gln Asp Gln Ser Phe Glu Ser Thr Val Asn
        355                 360                 365

Ile Ala Leu Gly Ile Phe Thr Pro Ala Leu Ile Ala Leu Met Ala Gly
    370                 375                 380

Leu Val Leu Phe Ile Val Met Ala Thr Leu Met Pro Met Leu Glu Met
385                 390                 395                 400

Asn Asn Leu Met Ser Gly
                405
```

<210> SEQ ID NO 49
<211> LENGTH: 444
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 49

```
atgaaaaata aaatgaaaaa acaatcaggc tttaccctat tagaagtcat ggttgttgtc     60

gttatccttg gtgttctagc aagttttgtt gtacctaacc tgttgggcaa caaagagaag    120

gcggatcaac aaaaagccat cactgatatt gtggcgctag agaacgcgct cgacatgtac    180

aaactggata acagcgttta cccaacaacg gatcaaggcc tggacgggtt ggtgacaaag    240

ccaagcagtc cagagcctcg taactaccga gacggcggtt acatcaagcg tctacctaac    300

gacccatggg gcaatgagta ccaataccta agtcctggtg ataacggcac aattgatatc    360

ttcactcttg gcgcagatgg tcaagaaggt ggtgaaggta ttgctgcaga tatcggcaac    420
```

-continued

tggaacatgc aggacttcca ataa 444

<210> SEQ ID NO 50
<211> LENGTH: 146
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 50

Lys Asn Lys Met Lys Lys Gln Ser Gly Phe Thr Leu Leu Glu Val Met
1 5 10 15

Val Val Val Val Ile Leu Gly Val Leu Ala Ser Phe Val Val Pro Asn
20 25 30

Leu Leu Gly Asn Lys Glu Lys Ala Asp Gln Gln Lys Ala Ile Thr Asp
35 40 45

Ile Val Ala Leu Glu Asn Ala Leu Asp Met Tyr Lys Leu Asp Asn Ser
50 55 60

Val Tyr Pro Thr Thr Asp Gln Gly Leu Asp Gly Leu Val Thr Lys Pro
65 70 75 80

Ser Ser Pro Glu Pro Arg Asn Tyr Arg Asp Gly Gly Tyr Ile Lys Arg
85 90 95

Leu Pro Asn Asp Pro Trp Gly Asn Glu Tyr Gln Tyr Leu Ser Pro Gly
100 105 110

Asp Asn Gly Thr Ile Asp Ile Phe Thr Leu Gly Ala Asp Gly Gln Glu
115 120 125

Gly Gly Glu Gly Ile Ala Ala Asp Ile Gly Asn Trp Asn Met Gln Asp
130 135 140

Phe Gln
145

<210> SEQ ID NO 51
<211> LENGTH: 594
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 51

gtgaaaacta agcaaacaca gccaggtttc accttgattg agattctttt ggtgttggta 60

ttactgtcag tatcggcggt cgcggtgatc tcgaccatcc ctaccaatag caaagatgtt 120

gctaaaaaat acgctcaaag cttttatcag cgaattcagc tactcaatga agaggctatt 180

ttgagtggct tagattttgg tgttcgtgtt gatgaaaaaa aatcgactta cgttctgatg 240

actttgaagt ctgatggctg gcaagaaacg gagttcgaaa agatcccttc ttcaactgaa 300

ttaccggaag aactggcact gtcgctgaca ttaggtggtg gcgcgtggga agacgatgat 360

cggttgttca atccaggaag cttatttgat gaagatatgt ttgctgatct tgaagaggaa 420

aagaagccga aaccaccaca gatctacatc ttgtcgagtg ctgaaatgac gccatttgta 480

ctgtcgtttt acccaaatac cggtgacaca atacaagatg tttggcgcat tcgagtattg 540

gataatggtg tgattcgatt actcgagccg ggagaagaag atgaagaaga ataa 594

<210> SEQ ID NO 52
<211> LENGTH: 197
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 52

-continued

```
Met Lys Thr Lys Gln Thr Gln Pro Gly Phe Thr Leu Ile Glu Ile Leu
1               5                   10                  15

Leu Val Leu Val Leu Leu Ser Val Ser Ala Val Ala Val Ile Ser Thr
            20                  25                  30

Ile Pro Thr Asn Ser Lys Asp Val Ala Lys Lys Tyr Ala Gln Ser Phe
        35                  40                  45

Tyr Gln Arg Ile Gln Leu Leu Asn Glu Glu Ala Ile Leu Ser Gly Leu
    50                  55                  60

Asp Phe Gly Val Arg Val Asp Glu Lys Lys Ser Thr Tyr Val Leu Met
65                  70                  75                  80

Thr Leu Lys Ser Asp Gly Trp Gln Glu Thr Glu Phe Glu Lys Ile Pro
                85                  90                  95

Ser Ser Thr Glu Leu Pro Glu Glu Leu Ala Leu Ser Leu Thr Leu Gly
            100                 105                 110

Gly Gly Ala Trp Glu Asp Asp Asp Arg Leu Phe Asn Pro Gly Ser Leu
        115                 120                 125

Phe Asp Glu Asp Met Phe Ala Asp Leu Glu Glu Glu Lys Lys Pro Lys
    130                 135                 140

Pro Pro Gln Ile Tyr Ile Leu Ser Ser Ala Glu Met Thr Pro Phe Val
145                 150                 155                 160

Leu Ser Phe Tyr Pro Asn Thr Gly Asp Thr Ile Gln Asp Val Trp Arg
                165                 170                 175

Ile Arg Val Leu Asp Asn Gly Val Ile Arg Leu Leu Glu Pro Gly Glu
            180                 185                 190

Glu Asp Glu Glu Glu
        195
```

<210> SEQ ID NO 53
<211> LENGTH: 396
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 53

```
atgaagaaga ataaccgttc tccttatcgt tctcgcggta tgcctcttgg ttctcgagga     60

atgactctgc ttgaagtatt ggttgcgctg gctatcttcg ctacggcggc gatcagtgtg    120

attcgtgctg tcacccagca catcaatacg ctcagttatc tcgaagaaaa aaccttcgcg    180

gcgatggtcg ttgataatca aatggcccta gtcatgctac atcctgagat gcttaaaaaa    240

gcgcagggca cgcaagagtt agcgggaaga gaatggttct ggaaggtgac tcccatcgat    300

accagcgata atttattaaa ggcgtttgat gtgagtgcgg caaccagtaa gaaagcgtct    360

ccagtcgtta cggtgcgcag ttatgtggtt aattaa                              396
```

<210> SEQ ID NO 54
<211> LENGTH: 131
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 54

```
Met Lys Lys Asn Asn Arg Ser Pro Tyr Arg Ser Arg Gly Met Pro Leu
1               5                   10                  15

Gly Ser Arg Gly Met Thr Leu Leu Glu Val Leu Val Ala Leu Ala Ile
            20                  25                  30
```

-continued

```
Phe Ala Thr Ala Ala Ile Ser Val Ile Arg Ala Val Thr Gln His Ile
        35                  40                  45

Asn Thr Leu Ser Tyr Leu Glu Glu Lys Thr Phe Ala Ala Met Val Val
    50                  55                  60

Asp Asn Gln Met Ala Leu Val Met Leu His Pro Glu Met Leu Lys Lys
65                  70                  75                  80

Ala Gln Gly Thr Gln Glu Leu Ala Gly Arg Glu Trp Phe Trp Lys Val
                85                  90                  95

Thr Pro Ile Asp Thr Ser Asp Asn Leu Leu Lys Ala Phe Asp Val Ser
            100                 105                 110

Ala Ala Thr Ser Lys Lys Ala Ser Pro Val Val Thr Val Arg Ser Tyr
        115                 120                 125

Val Val Asn
    130
```

<210> SEQ ID NO 55
<211> LENGTH: 804
<212> TYPE: DNA
<213> ORGANISM: Vibrio slpendidus

<400> SEQUENCE: 55

```
atgtggttaa ttaagagaat gtggtcaatt aagagcatgt tattaattaa gaacagctcg     60

ctaactaaga gcgtgtcgct aactaagagc atgtcggaaa ataagcgtac gccgcgtaaa    120

caaggtctac cttcaaaagg gagaggcttt accttaattg aagtcttggt ctcgattgct    180

atctttgcca cgctaagtat ggcggcttat caggtggtta atcaggtgca gcgaagcaac    240

gagatctcta ttgagcgcag tgctcgtttg aaccaactgc aacgcagttt agtcatttta    300

gataatgatt ttcgccagat ggcggtgcga aaatttcgta ccaacggtga agaagcatca    360

tctaagctga tcttaatgaa agagtattta ttggactccg acagtgtagg catcatgttt    420

actcgtctag gttggcacaa cccacaacag cagtttcctc gcggtgaagt cacgaaggtt    480

ggctaccgta ttaaagaaga aacacttgag cgtgtatggt ggcgttatcc cgatacacct    540

tcaggccaag aaggtgtgat tacccctctg cttgatgatg ttgaaagctt ggaattcgag    600

ttttatgacg gaagccgctg ggggaaagag tggcaaaccg ataaatcact gccgaaagcg    660

gtgaggctta agctgacact gaaagactat ggtgagatag agcgtgttta tctcactccc    720

ggtggcaccc tagatcaggc cgatgattct tcaaacagtg actcttcagg cagtagtgag    780

gggaataatg actcatcgaa ctaa                                           804
```

<210> SEQ ID NO 56
<211> LENGTH: 267
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 56

```
Met Trp Leu Ile Lys Arg Met Trp Ser Ile Lys Ser Met Leu Leu Ile
1               5                   10                  15

Lys Asn Ser Ser Leu Thr Lys Ser Val Ser Leu Thr Lys Ser Met Ser
            20                  25                  30

Glu Asn Lys Arg Thr Pro Arg Lys Gln Gly Leu Pro Ser Lys Gly Arg
        35                  40                  45

Gly Phe Thr Leu Ile Glu Val Leu Val Ser Ile Ala Ile Phe Ala Thr
    50                  55                  60
```

-continued

```
Leu Ser Met Ala Ala Tyr Gln Val Val Asn Gln Val Gln Arg Ser Asn
65                  70                  75                  80

Glu Ile Ser Ile Glu Arg Ser Ala Arg Leu Asn Gln Leu Gln Arg Ser
                85                  90                  95

Leu Val Ile Leu Asp Asn Asp Phe Arg Gln Met Ala Val Arg Lys Phe
            100                 105                 110

Arg Thr Asn Gly Glu Glu Ala Ser Ser Lys Leu Ile Leu Met Lys Glu
        115                 120                 125

Tyr Leu Leu Asp Ser Asp Ser Val Gly Ile Met Phe Thr Arg Leu Gly
    130                 135                 140

Trp His Asn Pro Gln Gln Gln Phe Pro Arg Gly Glu Val Thr Lys Val
145                 150                 155                 160

Gly Tyr Arg Ile Lys Glu Glu Thr Leu Glu Arg Val Trp Trp Arg Tyr
                165                 170                 175

Pro Asp Thr Pro Ser Gly Gln Glu Gly Val Ile Thr Pro Leu Leu Asp
            180                 185                 190

Asp Val Glu Ser Leu Glu Phe Glu Phe Tyr Asp Gly Ser Arg Trp Gly
        195                 200                 205

Lys Glu Trp Gln Thr Asp Lys Ser Leu Pro Lys Ala Val Arg Leu Lys
    210                 215                 220

Leu Thr Leu Lys Asp Tyr Gly Glu Ile Glu Arg Val Tyr Leu Thr Pro
225                 230                 235                 240

Gly Gly Thr Leu Asp Gln Ala Asp Asp Ser Ser Asn Ser Asp Ser Ser
                245                 250                 255

Gly Ser Ser Glu Gly Asn Asn Asp Ser Ser Asn
            260                 265
```

<210> SEQ ID NO 57
<211> LENGTH: 1050
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 57

```
atgactcatc gaactaataa gcgtttagcg acaaggtcag ccttgggacg taaacaacgt     60

ggtgtcgcgc tgatcattat tttgatgcta ttggcgatca tggcaaccat tgctggcagc    120

atgtccgagc gtttgtttac gcaattcaag cgcgttggta accaactgaa ttaccaacag    180

gcttactggt acagcattgg tgtggaagcg cttgtgcaaa acggtattag gcaaagttac    240

aaagacagtg ataccgtgaa cctaagccaa ccatgggcgt tagaagagca ggtataccca    300

ttggattatg gccaagttaa gggccgcatt gttgatgctc aggcatgttt taatcttaat    360

gccttagccg gagtggcgac cacttcaagt aaccagactc cttatttaat cacggtttgg    420

caaaccttat tggaaaacca agacgttgag ccttatcagg ctgaggttat cgcaaattca    480

acgtgggaat ttgttgatgc ggatacacga accacctctt cgtctggtgt agaagacagc    540

acgtatgaag cgatgaagcc ctcttatttg gcggcgaatg gcttaatggc cgatgaatcc    600

gagctacgag cggtttatca agtcactggt gaagtgatga ataaggttcg ccccttttgtt    660

tgcgctctgc caaccgatga tttccgcttg aatgtgaata ctctcacgga aaaacaagca    720

ccgttattgg aagcgatgtt tgcgccaggc ttaagtgaat cggatgccaa acagctgata    780

gataaacgcc catttgatgg ctgggatacg gtagatgctt tcatggctga acctgccatt    840
```

-continued

gttggtgtaa gtgccgaagt cagcaagaaa gcgaaagcat atttaactgt agatagcgcc 900

tattttgagc tagatgcaga ggtattagtt gagcagtcac gtgtacgtat acggacgctt 960

ttctatagta gtaatcgaga aacagtgacg gtagtacgcc gtcgttttgg aggaatcagt 1020

gagcgagttt ctgaccgttc gactgagtag 1050

<210> SEQ ID NO 58
<211> LENGTH: 349
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 58

Met Thr His Arg Thr Asn Lys Arg Leu Ala Thr Arg Ser Ala Leu Gly
1 5 10 15

Arg Lys Gln Arg Gly Val Ala Leu Ile Ile Ile Leu Met Leu Leu Ala
20 25 30

Ile Met Ala Thr Ile Ala Gly Ser Met Ser Glu Arg Leu Phe Thr Gln
35 40 45

Phe Lys Arg Val Gly Asn Gln Leu Asn Tyr Gln Gln Ala Tyr Trp Tyr
50 55 60

Ser Ile Gly Val Glu Ala Leu Val Gln Asn Gly Ile Arg Gln Ser Tyr
65 70 75 80

Lys Asp Ser Asp Thr Val Asn Leu Ser Gln Pro Trp Ala Leu Glu Glu
85 90 95

Gln Val Tyr Pro Leu Asp Tyr Gly Gln Val Lys Gly Arg Ile Val Asp
100 105 110

Ala Gln Ala Cys Phe Asn Leu Asn Ala Leu Ala Gly Val Ala Thr Thr
115 120 125

Ser Ser Asn Gln Thr Pro Tyr Leu Ile Thr Val Trp Gln Thr Leu Leu
130 135 140

Glu Asn Gln Asp Val Glu Pro Tyr Gln Ala Glu Val Ile Ala Asn Ser
145 150 155 160

Thr Trp Glu Phe Val Asp Ala Asp Thr Arg Thr Thr Ser Ser Ser Gly
165 170 175

Val Glu Asp Ser Thr Tyr Glu Ala Met Lys Pro Ser Tyr Leu Ala Ala
180 185 190

Asn Gly Leu Met Ala Asp Glu Ser Glu Leu Arg Ala Val Tyr Gln Val
195 200 205

Thr Gly Glu Val Met Asn Lys Val Arg Pro Phe Val Cys Ala Leu Pro
210 215 220

Thr Asp Asp Phe Arg Leu Asn Val Asn Thr Leu Thr Glu Lys Gln Ala
225 230 235 240

Pro Leu Leu Glu Ala Met Phe Ala Pro Gly Leu Ser Glu Ser Asp Ala
245 250 255

Lys Gln Leu Ile Asp Lys Arg Pro Phe Asp Gly Trp Asp Thr Val Asp
260 265 270

Ala Phe Met Ala Glu Pro Ala Ile Val Gly Val Ser Ala Glu Val Ser
275 280 285

Lys Lys Ala Lys Ala Tyr Leu Thr Val Asp Ser Ala Tyr Phe Glu Leu
290 295 300

Asp Ala Glu Val Leu Val Glu Gln Ser Arg Val Arg Ile Arg Thr Leu
305 310 315 320

-continued

```
Phe Tyr Ser Ser Asn Arg Glu Thr Val Thr Val Val Arg Arg Arg Phe
                325                 330                 335

Gly Gly Ile Ser Glu Arg Val Ser Asp Arg Ser Thr Glu
            340                 345
```

<210> SEQ ID NO 59
<211> LENGTH: 1248
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 59

```
gtgagcgagt ttctgaccgt tcgactgagt agcgaaccac aaagccctgt gcagtggtta     60

gtttggtcga caagccaaca agaagtgata gcaagcggtg aactgtctag ctgggaacag    120

cttgacgagt taacgcctta cgctgaaaag cgcagctgta tcgctttatt gccgggaagt    180

gaatgcttaa ttaagcgtgt tgagatcccg aaaggtgctg ctcgccagtt tgattctatg    240

ctgccgttct tattagaaga cgaagtcgca caagatatcg aagacttaca cctgactatt    300

ttagataaag atgccactca cgctaccgtg tgtggtgtgg atcgtgaatg gctaaaacaa    360

gctttagacc tgtttcgcga agccaatata atcttccgta aggtgctacc agatacacta    420

gccgtgcctt ttgaagaaca aggcatcagt gcgttgcaga tagatcagca ttggttattg    480

cgccaaggtc actctcaacg tcaaggtcac tatcaagccg tatcgatcag tgaagcatgg    540

ttaccgatgt ttttgcaaag tgattgggtt gtcgctggtg aggaagagca agcgacgact    600

atcttcagct ataccgcgat gccgagcgac gacgttcaac agcaaagcgg cctcgagtgg    660

caagcaaagc ctgcggaatt ggtgatgtct ttattgagtc agcaagcgat cacaagcggc    720

gtaaatttac tgactggcac ctttaaaacc aaatcttcat tcagtaaata ttggcgtgtt    780

tggcagaaag tggcgattgc tgcttgtttg ctggtggccg tgattgtgac tcagcaagtg    840

ttgaaggttc agcaatacga agcgcaagca caagcctacc gcatggagag tgagcgtatc    900

tttagagctg tgctgcctgg caaacaacgc attccgaccg tgagttacct caagcgtcag    960

atgaatgatg aagctaagaa atacggtggt tcaggcgaag gtgattcttt acttggttgg   1020

ttagctttgc tgcctgaaac cttagggcaa gtgaagacga tcgaagttga aagcattcgc   1080

tacgatggca accgttctga ggttcgactg caggctaaaa gttctgactt ccaacacttt   1140

gagaccgcaa gggtgaagct cgaagagaag tttgtcgttg agcaagggcc attgaaccgt   1200

aatggcgatg ccgtatttgg cagttttact cttaaacccc atcaataa                1248
```

<210> SEQ ID NO 60
<211> LENGTH: 415
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 60

```
Met Ser Glu Phe Leu Thr Val Arg Leu Ser Ser Glu Pro Gln Ser Pro
1               5                   10                  15

Val Gln Trp Leu Val Trp Ser Thr Ser Gln Gln Glu Val Ile Ala Ser
            20                  25                  30

Gly Glu Leu Ser Ser Trp Glu Gln Leu Asp Glu Leu Thr Pro Tyr Ala
        35                  40                  45

Glu Lys Arg Ser Cys Ile Ala Leu Leu Pro Gly Ser Glu Cys Leu Ile
    50                  55                  60
```

-continued

```
Lys Arg Val Glu Ile Pro Lys Gly Ala Ala Arg Gln Phe Asp Ser Met
65                  70                  75                  80

Leu Pro Phe Leu Leu Glu Asp Glu Val Ala Gln Asp Ile Glu Asp Leu
                85                  90                  95

His Leu Thr Ile Leu Asp Lys Asp Ala Thr His Ala Thr Val Cys Gly
            100                 105                 110

Val Asp Arg Glu Trp Leu Lys Gln Ala Leu Asp Leu Phe Arg Glu Ala
        115                 120                 125

Asn Ile Ile Phe Arg Lys Val Leu Pro Asp Thr Leu Ala Val Pro Phe
    130                 135                 140

Glu Glu Gln Gly Ile Ser Ala Leu Gln Ile Asp Gln His Trp Leu Leu
145                 150                 155                 160

Arg Gln Gly His Ser Gln Arg Gln Gly His Tyr Gln Ala Val Ser Ile
                165                 170                 175

Ser Glu Ala Trp Leu Pro Met Phe Leu Gln Ser Asp Trp Val Val Ala
            180                 185                 190

Gly Glu Glu Glu Gln Ala Thr Thr Ile Phe Ser Tyr Thr Ala Met Pro
        195                 200                 205

Ser Asp Asp Val Gln Gln Gln Ser Gly Leu Glu Trp Gln Ala Lys Pro
    210                 215                 220

Ala Glu Leu Val Met Ser Leu Leu Ser Gln Gln Ala Ile Thr Ser Gly
225                 230                 235                 240

Val Asn Leu Leu Thr Gly Thr Phe Lys Thr Lys Ser Ser Phe Ser Lys
                245                 250                 255

Tyr Trp Arg Val Trp Gln Lys Val Ala Ile Ala Ala Cys Leu Leu Val
            260                 265                 270

Ala Val Ile Val Thr Gln Gln Val Leu Lys Val Gln Gln Tyr Glu Ala
        275                 280                 285

Gln Ala Gln Ala Tyr Arg Met Glu Ser Glu Arg Ile Phe Arg Ala Val
    290                 295                 300

Leu Pro Gly Lys Gln Arg Ile Pro Thr Val Ser Tyr Leu Lys Arg Gln
305                 310                 315                 320

Met Asn Asp Glu Ala Lys Lys Tyr Gly Gly Ser Gly Glu Gly Asp Ser
                325                 330                 335

Leu Leu Gly Trp Leu Ala Leu Leu Pro Glu Thr Leu Gly Gln Val Lys
            340                 345                 350

Thr Ile Glu Val Glu Ser Ile Arg Tyr Asp Gly Asn Arg Ser Glu Val
        355                 360                 365

Arg Leu Gln Ala Lys Ser Ser Asp Phe Gln His Phe Glu Thr Ala Arg
    370                 375                 380

Val Lys Leu Glu Glu Lys Phe Val Val Glu Gln Gly Pro Leu Asn Arg
385                 390                 395                 400

Asn Gly Asp Ala Val Phe Gly Ser Phe Thr Leu Lys Pro His Gln
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<210> SEQ ID NO 61
<211> LENGTH: 489
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 61

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cgattagtca ttggttgttc tattttattg atactgggcg ttgtctattg gggattaata 120

caaccactta gccaacgagc cgagcttgca caaagccgca ttcaaagtga gaagcaactt 180

ctggcttggg taacggacaa agcgaatcaa gtggttgaac tacgaggcag tggtggcatc 240

agtgccagtc agcctttgaa ccaatctgtg cctgcttcta tgcgccgttt taacatcgag 300

ctgatacgcg tgcaaccacg cggtgagatg ctgcaagttt ggattaagcc tgtgccattt 360

aataagttcg ttgactggct gacatacctg aaagaaaagc agggtgttga ggttgagttt 420

atggatattg atcgctctga tagccctggg gttattgaga tcaaccgact acagtttaaa 480

cgaggttaa 489

<210> SEQ ID NO 62
<211> LENGTH: 162
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 62

Met Arg Asn Met Ile Glu Pro Leu Gln Ala Trp Trp Ala Ser Ile Ser
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Gln Arg Glu Gln Arg Leu Val Ile Gly Cys Ser Ile Leu Leu Ile Leu
20 25 30

Gly Val Val Tyr Trp Gly Leu Ile Gln Pro Leu Ser Gln Arg Ala Glu
35 40 45

Leu Ala Gln Ser Arg Ile Gln Ser Glu Lys Gln Leu Leu Ala Trp Val
50 55 60

Thr Asp Lys Ala Asn Gln Val Val Glu Leu Arg Gly Ser Gly Gly Ile
65 70 75 80

Ser Ala Ser Gln Pro Leu Asn Gln Ser Val Pro Ala Ser Met Arg Arg
85 90 95

Phe Asn Ile Glu Leu Ile Arg Val Gln Pro Arg Gly Glu Met Leu Gln
100 105 110

Val Trp Ile Lys Pro Val Pro Phe Asn Lys Phe Val Asp Trp Leu Thr
115 120 125

Tyr Leu Lys Glu Lys Gln Gly Val Glu Val Glu Phe Met Asp Ile Asp
130 135 140

Arg Ser Asp Ser Pro Gly Val Ile Glu Ile Asn Arg Leu Gln Phe Lys
145 150 155 160

Arg Gly

<210> SEQ ID NO 63
<211> LENGTH: 780
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 63

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gtgcgtggtt taagcattga aggcgttgag ggcaccgttt ggcaaggtcg cgctaacaat 180

atcgcgtggc agcgtgtcaa ttacggctca gtgcagtggg acttccagtt ctctaaacta 240

ttccaagcca aagcagaact tgcggttcgc tttggccgca acagcgacat gaacttatca 300

ggtaaaggac gtgtcggata tagcatgagt ggtgcttacg cggaaaactt agtggcatca 360

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gggcaagttg aactgacgat caaacatgcg gttcatgctc aaccttggtg tcaatcaggt 480

gaaggtacgc ttgcttggtc tggtgcagca gtcgactcgc cagtgggttc gttagacctt 540

ggccctgtga ttgcggacat aacgtgtgaa gacagcacaa ttgcagccaa aggcactcag 600

aagagcgatc aggtagacag cgagttctca gcgagcgtaa cacctaacca acgctacacc 660

tcggcagcat ggtttaagcc aggcgctgaa ttcccgccag caatgcagag tcagcttaag 720

tggttgggca atcctgatag ccaaggtaaa taccaattta cttatcaagg ccgcttttag 780

<210> SEQ ID NO 64
<211> LENGTH: 259
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 64

Met Lys Arg Gly Leu Ser Phe Lys Tyr Gly Leu Leu Phe Ser Val Ile
1 5 10 15

Phe Ile Val Phe Phe Ser Val Ser Leu Leu Leu His Leu Pro Ala Ala
20 25 30

Phe Ala Leu Lys His Ala Pro Val Val Arg Gly Leu Ser Ile Glu Gly
35 40 45

Val Glu Gly Thr Val Trp Gln Gly Arg Ala Asn Asn Ile Ala Trp Gln
50 55 60

Arg Val Asn Tyr Gly Ser Val Gln Trp Asp Phe Gln Phe Ser Lys Leu
65 70 75 80

Phe Gln Ala Lys Ala Glu Leu Ala Val Arg Phe Gly Arg Asn Ser Asp
85 90 95

Met Asn Leu Ser Gly Lys Gly Arg Val Gly Tyr Ser Met Ser Gly Ala
100 105 110

Tyr Ala Glu Asn Leu Val Ala Ser Met Pro Ala Ser Asn Val Met Lys
115 120 125

Tyr Ala Pro Ala Ile Pro Val Pro Val Ser Ile Ala Gly Gln Val Glu
130 135 140

Leu Thr Ile Lys His Ala Val His Ala Gln Pro Trp Cys Gln Ser Gly
145 150 155 160

Glu Gly Thr Leu Ala Trp Ser Gly Ala Ala Val Asp Ser Pro Val Gly
165 170 175

Ser Leu Asp Leu Gly Pro Val Ile Ala Asp Ile Thr Cys Glu Asp Ser
180 185 190

Thr Ile Ala Ala Lys Gly Thr Gln Lys Ser Asp Gln Val Asp Ser Glu
195 200 205

Phe Ser Ala Ser Val Thr Pro Asn Gln Arg Tyr Thr Ser Ala Ala Trp
210 215 220

Phe Lys Pro Gly Ala Glu Phe Pro Pro Ala Met Gln Ser Gln Leu Lys
225 230 235 240

Trp Leu Gly Asn Pro Asp Ser Gln Gly Lys Tyr Gln Phe Thr Tyr Gln
245 250 255

Gly Arg Phe

<210> SEQ ID NO 65
<211> LENGTH: 10967
<212> TYPE: DNA

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<213> ORGANISM: Erwinia carotovora subsp. Atroseptica SCRI1043

<400> SEQUENCE: 65

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aaaccacgcc gtgcatctcg tgtttcaccg ccagataggc gtcgcccgtt ttcagggtgc 180

gtttttcacc ttcgatcacg acttcaaagc tgccagcggc aacataagca atctggtcgt 240

gaatctcatg gaagtgcggc gtaccaatcg caccttttatc aaagtgcacg taaaccatca 300

tcagctcatc gctccatgtc atgattttac gtttaatgcc accgcccagc tcttcccatg 360

gcgtttcatc atcaataaag tatcttctca tcatctctct cctctaacgc tctttttgcc 420

cataccttct attgcgtcaa caaaccgtgt acgacaacga atgcatggct atggattgcg 480

acattttagc cacatcagta ccagaagaaa cataaaataa gcaaaaccat gacggccctc 540

aagaaataaa taaaacatta tttcattttt attgaattcg catctcatcc aaactatcat 600

cccgcataac aagaaagaac cgggcatgtt gaggaacagg tgacgttgtc actgccacgc 660

aacatcatct gtttcgcccg gcgctttcgc caggaacgat tcctcttctt ggaacggcgc 720

ctgatttttg tttttctctg aaagagaggc taagaaatgc aagttcgtca aagcattcac 780

agcgatcacg cgaagcagct agatacagca ggcctgcgtc gtgaattcct gatcgaacag 840

atttttttctg ccgatgccta cactatgacc tatagccaca tcgaccgaat catcgtcggt 900

ggcatcatgc ccgtacacag cgccgtaacg attggcggtg aagtgggtaa acaactcggc 960

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tttggtcacg ttgacattct ggtcaacaac gccggtatca tccgtcgtga agatgctatc 1980

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gattcccccc tcattccctt tgtcaggtga tctatcatga ttgttcgttc tctgcttgtc 9960

ggggccatta tgatgtctgt aaatggatta agttacgcac aacctgtttt ctctgtctgg 10020

ccacacggtg aagcaccggg tgcctcttct tcaacggcac agccgcaagt ggtcgaacgg 10080

agtaaagatc cttctcttcc cgatcgagcc gcaacgggta ttcgcagccc tgaaattacc 10140

gtttatccgg cagagaaacc caatggcatg gcattactca ttacgccggg cggttcttat 10200

cagcgcgtcg tgctagataa agaaggcagc gatctagccc ctttctttaa tcaacaaggc 10260

tacacccttt tcgtgatgac ctatcgtatg cccggtgaag gccataaaga aggcgctgac 10320

gctccgctag ccgatgccca acgagccatc agaacactga gagccaacgc cgaaaagtgg 10380

cacattaacc cgcagcgcat cggtattatg gggttctccg ccggtggtca cgttgccgcc 10440

agccttggaa cccgattcgc acagtccgtt taccccgcga tggacgccgt tgataacgta 10500

agcgcacgcc ctgacttcat ggtgttgatg taccccgtaa tttctatgca ggcagatatt 10560

gcgcacgccg gttcacgtaa acagttaatc ggcgagcaac cgatggaagt acaagcggta 10620

cgttattctc ctgagaaaca ggttactgat cagactcccc ccacgttttt ggtgcatgcg 10680

gttgacgatc cgtcagtgtc ggttgataac agcctggtga tgtttagcgc gctgcgggca 10740

aagcagattc cggtcgaaat gcatctcttt gagaaaggta aacacggctt cggtctccgc 10800

ggcaccaagg ggcttcctgc cgctgcctgg cctcaactgc tggacaactg gctacgcgct 10860

ttacctgcaa gcaacgaatt gccgaaagcc gcgccataag gtatagcaaa catcgtaacc 10920

gaaataaatc gttacgccgt caccgcttcc gcagacaggg ataatct 10967

<210> SEQ ID NO 66
<211> LENGTH: 2582
<212> TYPE: DNA
<213> ORGANISM: Erwinia carotovora subsp. Atroseptica SCRI1043

<400> SEQUENCE: 66

ccaacggcgg gtgcgacata aacataagcg aatcgaagcg ctgcgctccg gtgagtatct 60

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gaagtaattt acgatagttt ctttccaaag gcccattcgg gcctttgtta tttcagcgtt 120

tattgattca tcaaacctgc gctttctctg ctcgaatgtt ttcactagat ctgaaacagg 180

tggtgaaaac atgaagaatg ttttataaaa taaaaccacg atcacggaaa aatgaaacat 240

tgtttctata ataccgatat gacaggcgtc tcgcgtgaga tttgtggcct gatttttgaa 300

caaccggtgt cggggtgacc gattcgtcgg acgttcagta atgtcaggtt atcgaagcgt 360

atgcgtgtgt ggcgtcaaat tcttcatgat aagttctaag gatttacgga tggccaaagg 420

taataagatc cccctaacgt ttcataccta ccaggatgca gcaaccggca ccgaagttgt 480

gcgtttaacc ccgcccgatg ttatctgcca ccggaattat ttctaccaga agtgtttctt 540

caatgacggt agcaagctgc tgtttggcgc tgcatttgat ggcccatgga actactatct 600

gctggattta aaagagcaga acgccacaca gttgacggaa ggcaaaggcg acaatacttt 660

tggtggtttc ctgtctccga atgacgatgc gctatattac gttaaaaata cccgtaattt 720

gatgcgtgtc gatctgacta cgctggaaga gaaaacgatt tatcaggtgc ctgacgattg 780

ggtcggctac ggtacttggg ttgccaactc cgattgcacc aaaatggtcg gtattgagat 840

caagaaagaa gactggaagc cactgaccga ttggaaaaaa ttccaggagt tctacttcac 900

taatccttgc tgtcgtctga ttcgcgtcga tttggtaacg ggcgaagcgg agactatcct 960

tcaggaaaac cagtggctgg gtcacccaat ctaccgtcca ggtgatgaca acacggttgc 1020

tttctgtcac gaaggcccgc atgacctggt tgatgctcgt atgtggttca tcaacgaaga 1080

tggcaccaac atgcgcaaag tgaaagagca tgcagaaggc gaaagctgca cccacgaatt 1140

ttgggtgccg gatggctccg cgatgattta tgtctcttat cttaaagacg ataccaaccg 1200

ttatattcgc agcatcgatc ccgttacgct ggaagatcgc caactgcgtg taatgccgcc 1260

gtgttctcac ctgatgagta actatgatgg cacactgttg gtcggtgatg gttccgatgc 1320

accggtcgac gtgcaggatg atggtggcta caaaattgag aacgatccgt tcctgtatgt 1380

tttcaacctg aaaactggca aagaacatcg tattgcgcag cacaatacat cctgggaagt 1440

gttggaaggg gaccgtcagg tcactcaccc gcacccgtct ttcacgccgg ataataaaca 1500

agttctgttt acttctgacg tagatggaaa acctgcgttg tatctggcga aggttcctga 1560

ttcagtctgg aactaataat actaataaat ccgcgtcacg tttcatggcg cggattattt 1620

taaaatattt acttacatat tattttatta agtctctgac gcggttattt ctcaaactta 1680

acttgattat cgttgttgct ccattgccat aatcaaagcg ttccctttat actaaaacca 1740

ttgttctatt ttttttaaaa caaaaaaacc tgagtagggt aaccacaaaa atggctagtg 1800

cagatttaga taaacaaccc gattccgtgt cgtccgtttt aaaggttttt ggtattttgc 1860

aggcattagg tgaagagaga gaaattggta ttaccgagct ttctcagcga gtcatgatgt 1920

ctaagagtac cgtttaccgt ttcttgcaga cgatgaaatc cctgggctat gtcgcgcagg 1980

aaggtgaatc agagaagtat tcgctaacgc tcaagttgtt tgaacttggt gcaaaagcat 2040

tgcagaacgt agacttaatc cgcagtgcgg atatacagat gcgcgagttg tctgtgctga 2100

cgcgggaaac gattcacctt ggcgcgttgg atgaagacgg catcgtttat atccacaaga 2160

ttgattctat gtataacctg cgtatgtatt cgcgcatcgg tcgccgtaat ccactacaca 2220

gtaccgcaat tggtaaagtg ttgctggctt ggcgcgatcg cggtgaagtg gaagaggttc 2280

tgtcgactgt cgaattcacg cgtagtacgc cacacacatt gtgtactgct gaagatcttc 2340

-continued

tcaatcaact ggatgtcgtg cgtgagcaag gctacgggga agataaagaa gagcaggaag 2400

aagggctgcg ttgtatcgct gtgccagtat tcgatcgttt tggtgtggtg attgccggcc 2460

tcagtatttc cttcccaacg attcgttttt cagaagaaaa caaacacgaa tatgtggcca 2520

tgctgcacac cgcagctaga aatatctctg agcaaatggg ctaccacaat ttccctttct 2580

ga 2582

<210> SEQ ID NO 67
<211> LENGTH: 2331
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium tumefaciens

<400> SEQUENCE: 67

atgcgtccct ctgccccggc catctccaga cagacacttc tcgatgaacc ccgcccgggc 60

tcattgacca ttggctacga gccgagcgaa gaagcacaac cgacggagaa ccctccgcgc 120

ttttcatggc tacccgatat tgacgacggc gcgcgttacg tgctgcgcat ttcgaccgat 180

cccggtttta cagacaaaaa aacgctcgtc ttcgaggatc tcgcctggaa tttcttcacc 240

ccggatgaag cactgccgga cggccattat cactggtgtt atgcgctatg ggatcagaaa 300

tccgcaacag cgcattccaa ctggagcacc gtacgcagtt tcgagatcag tgaagcactg 360

ccgaaaacgc cgctgcccgg caggtctgcc cgccatgctg ccgcgcaaac cagccaccct 420

cggctgtggc tcaactccga gcaattgagt gccttcgccg atgccgttgc gaaggacccc 480

aaccattgtg gctgggccga gttttacgaa aaatcggtcg agccgtggct cgagcggccg 540

gtcatgccgg aaccgcagcc ctatcccaac aacacgcgtg tcgccacgct ctggcggcag 600

atgtatatag actgccagga agtgatctat gcgatccggc acctggccat tgccggccgc 660

gtgctcggac gcgacgacct tctcgatgca tcccgcaaat ggctgctggc cgtcgccgcc 720

tgggacacga aaggtgcgac ctcacgcgcc tataatgacg aggcggggtt ccgcgtcgtc 780

gtcgcactcg cctggggtta tgactggctg tacgaccatc tgagcgaaga cgaacgcagg 840

accgtgcgat ccgttcttct cgaacggacg cgggaagttg ccgatcatgt catcgcacac 900

gcccgcattc acgtctttcc ctatgacagc catgcggtgc gctcgctttc ggctgtattg 960

acgccggcct gcatcgcact tcagggagaa agcgacgagg ctggcgaatg gctcgactat 1020

accgtcgaat tccttgccac gctctattct ccctgggcgg gaaccgatgg tggttgggcg 1080

gaaggtccgc attactggat gaccggcatg gcctatctca tcgaggccgc caatctgatc 1140

cgctcctata ttggttatga cctctatcaa cggccgtttt tccagaatac cggtcgcttc 1200

ccgctttaca ccaaggcgcc gggaacccgc cgcgccaact tcggcgacga ctccaccctt 1260

ggcgaccttc ccggcctgaa gctgggatac aacgtccggc aattcgccgg cgtcaccggc 1320

aatggccatt accagtggta tttcgatcac atcaaggccg atgcgacagg cacggaaatg 1380

gccttttaca attacggctg gtgggacctc aacttcgacg atctcgtcta tcgccacgat 1440

tacccgcagg tggaagccgt gtctcccgcc gacctgccgg cactcgccgt tttcgatgat 1500

attggttggg cgaccatcca aaaagacatg gaagacccgg accggcacct gcagttcgtc 1560

ttcaaatcca gcccttacgg ttcgctcagc cacagtcacg gcgaccagaa tgcctttgtg 1620

ctttatgccc atggcgagga tctggcgatc cagtccggtt attacgtggc gttcaattcg 1680

cagatgcatc tgaattggcg gcgtcagaca cggtcgaaaa atgccgtgct gatcggcggc 1740

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```
aaaggccaat atgcggaaaa ggacaaggcg cttgcacgcc gcgccgccgg ccgcatcgtc   1800

tcggtggagg aacagcccgg ccatgttcgt atcgtcggcg atgcaaccgc cgcctaccag   1860

gttgcgaacc cgctggttca aaaggtgctg cgcgaaaccc acttcgttaa tgacagctat   1920

ttcgtgattg tcgacgaagt cgaatgttcg gaaccccagg aactgcaatg gctttgccat   1980

acactcggag cgccgcagac cggcaggtca agcttccgct acaatggccg gaaagccggt   2040

ttctacggac agttcgttta ctcttcgggc ggcacgccgc aaatcagcgc cgtggagggt   2100

tttcccgata tcgacccgaa agaattcgaa gggctcgaca tacaccacca tgtctgcgcc   2160

acggttccgg ccgccacccg gcatcgcctt gtcacccttc tggtgcctta cagcctgaag   2220

gagccgaagc gcattttcag cttcatcgat gatcagggtt tttccaccga catctacttc   2280

agtgatgtcg atgacgagcg tttcaagctc tcccttccca agcagttcta a            2331
```

<210> SEQ ID NO 68
<211> LENGTH: 776
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens

<400> SEQUENCE: 68

```
Met Arg Pro Ser Ala Pro Ala Ile Ser Arg Gln Thr Leu Leu Asp Glu
1               5                   10                  15

Pro Arg Pro Gly Ser Leu Thr Ile Gly Tyr Glu Pro Ser Glu Glu Ala
            20                  25                  30

Gln Pro Thr Glu Asn Pro Pro Arg Phe Ser Trp Leu Pro Asp Ile Asp
        35                  40                  45

Asp Gly Ala Arg Tyr Val Leu Arg Ile Ser Thr Asp Pro Gly Phe Thr
    50                  55                  60

Asp Lys Lys Thr Leu Val Phe Glu Asp Leu Ala Trp Asn Phe Phe Thr
65                  70                  75                  80

Pro Asp Glu Ala Leu Pro Asp Gly His Tyr His Trp Cys Tyr Ala Leu
                85                  90                  95

Trp Asp Gln Lys Ser Ala Thr Ala His Ser Asn Trp Ser Thr Val Arg
            100                 105                 110

Ser Phe Glu Ile Ser Glu Ala Leu Pro Lys Thr Pro Leu Pro Gly Arg
        115                 120                 125

Ser Ala Arg His Ala Ala Ala Gln Thr Ser His Pro Arg Leu Trp Leu
    130                 135                 140

Asn Ser Glu Gln Leu Ser Ala Phe Ala Asp Ala Val Ala Lys Asp Pro
145                 150                 155                 160

Asn His Cys Gly Trp Ala Glu Phe Tyr Glu Lys Ser Val Glu Pro Trp
                165                 170                 175

Leu Glu Arg Pro Val Met Pro Glu Pro Gln Pro Tyr Pro Asn Asn Thr
            180                 185                 190

Arg Val Ala Thr Leu Trp Arg Gln Met Tyr Ile Asp Cys Gln Glu Val
        195                 200                 205

Ile Tyr Ala Ile Arg His Leu Ala Ile Ala Gly Arg Val Leu Gly Arg
    210                 215                 220

Asp Asp Leu Leu Asp Ala Ser Arg Lys Trp Leu Leu Ala Val Ala Ala
225                 230                 235                 240

Trp Asp Thr Lys Gly Ala Thr Ser Arg Ala Tyr Asn Asp Glu Ala Gly
                245                 250                 255
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Phe Arg Val Val Val Ala Leu Ala Trp Gly Tyr Asp Trp Leu Tyr Asp
            260                 265                 270

His Leu Ser Glu Asp Glu Arg Arg Thr Val Arg Ser Val Leu Leu Glu
        275                 280                 285

Arg Thr Arg Glu Val Ala Asp His Val Ile Ala His Ala Arg Ile His
    290                 295                 300

Val Phe Pro Tyr Asp Ser His Ala Val Arg Ser Leu Ser Ala Val Leu
305                 310                 315                 320

Thr Pro Ala Cys Ile Ala Leu Gln Gly Glu Ser Asp Glu Ala Gly Glu
                325                 330                 335

Trp Leu Asp Tyr Thr Val Glu Phe Leu Ala Thr Leu Tyr Ser Pro Trp
            340                 345                 350

Ala Gly Thr Asp Gly Gly Trp Ala Glu Gly Pro His Tyr Trp Met Thr
        355                 360                 365

Gly Met Ala Tyr Leu Ile Glu Ala Ala Asn Leu Ile Arg Ser Tyr Ile
    370                 375                 380

Gly Tyr Asp Leu Tyr Gln Arg Pro Phe Phe Gln Asn Thr Gly Arg Phe
385                 390                 395                 400

Pro Leu Tyr Thr Lys Ala Pro Gly Thr Arg Arg Ala Asn Phe Gly Asp
                405                 410                 415

Asp Ser Thr Leu Gly Asp Leu Pro Gly Leu Lys Leu Gly Tyr Asn Val
            420                 425                 430

Arg Gln Phe Ala Gly Val Thr Gly Asn Gly His Tyr Gln Trp Tyr Phe
        435                 440                 445

Asp His Ile Lys Ala Asp Ala Thr Gly Thr Glu Met Ala Phe Tyr Asn
    450                 455                 460

Tyr Gly Trp Trp Asp Leu Asn Phe Asp Asp Leu Val Tyr Arg His Asp
465                 470                 475                 480

Tyr Pro Gln Val Glu Ala Val Ser Pro Ala Asp Leu Pro Ala Leu Ala
                485                 490                 495

Val Phe Asp Asp Ile Gly Trp Ala Thr Ile Gln Lys Asp Met Glu Asp
            500                 505                 510

Pro Asp Arg His Leu Gln Phe Val Phe Lys Ser Ser Pro Tyr Gly Ser
        515                 520                 525

Leu Ser His Ser His Gly Asp Gln Asn Ala Phe Val Leu Tyr Ala His
    530                 535                 540

Gly Glu Asp Leu Ala Ile Gln Ser Gly Tyr Tyr Val Ala Phe Asn Ser
545                 550                 555                 560

Gln Met His Leu Asn Trp Arg Arg Gln Thr Arg Ser Lys Asn Ala Val
                565                 570                 575

Leu Ile Gly Gly Lys Gly Gln Tyr Ala Glu Lys Asp Lys Ala Leu Ala
            580                 585                 590

Arg Arg Ala Ala Gly Arg Ile Val Ser Val Glu Glu Gln Pro Gly His
        595                 600                 605

Val Arg Ile Val Gly Asp Ala Thr Ala Ala Tyr Gln Val Ala Asn Pro
    610                 615                 620

Leu Val Gln Lys Val Leu Arg Glu Thr His Phe Val Asn Asp Ser Tyr
625                 630                 635                 640

Phe Val Ile Val Asp Glu Val Glu Cys Ser Glu Pro Gln Glu Leu Gln
                645                 650                 655

Trp Leu Cys His Thr Leu Gly Ala Pro Gln Thr Gly Arg Ser Ser Phe
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            660                 665                 670

Arg Tyr Asn Gly Arg Lys Ala Gly Phe Tyr Gly Gln Phe Val Tyr Ser
        675                 680                 685

Ser Gly Gly Thr Pro Gln Ile Ser Ala Val Glu Gly Phe Pro Asp Ile
    690                 695                 700

Asp Pro Lys Glu Phe Glu Gly Leu Asp Ile His His His Val Cys Ala
705                 710                 715                 720

Thr Val Pro Ala Ala Thr Arg His Arg Leu Val Thr Leu Leu Val Pro
                725                 730                 735

Tyr Ser Leu Lys Glu Pro Lys Arg Ile Phe Ser Phe Ile Asp Asp Gln
            740                 745                 750

Gly Phe Ser Thr Asp Ile Tyr Phe Ser Asp Val Asp Asp Glu Arg Phe
        755                 760                 765

Lys Leu Ser Leu Pro Lys Gln Phe
    770                 775


<210> SEQ ID NO 69
<211> LENGTH: 1068
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium temefaciens C58

<400> SEQUENCE: 69

atgttcacaa cgtccgccta tgcctgcgat gacggctctt cgccgatgaa gctcgcgacc     60

atcaggcgcc gcgatcccgg tccgcgcgat gtcgaaatcg agatagaatt ctgtggcgtc    120

tgccactcgg acatccatac ggcccgcagc gaatggccgg gctccctcta cccttgcgtc    180

cccggccacg aaatcgtcgg ccgtgtcggt cgggtgggcg cgcaagtcac ccggttcaag    240

acgggtgacc gcgtcggtgt cggctgtatc gtcgatagct gccgcgaatg cgcaagctgc    300

gccgaagggc tggagcaata ttgcgaaaac ggcatgaccg gcacctataa ctcccctgac    360

aaggcgatgg gcggcggcgc gcatacgctt ggcggctatt ccgcccatgt ggtggtggat    420

gaccgctatg tgctcaatat tcccgaaggg ctcgatccgg cggcagcagc accgctactc    480

tgcgctggta tcaccaccta ctcgccgctg cgccactgga atgccggccc cggcaaacgc    540

gtcggcgtcg tcggtctggg cggcctcggc catatggccg tcaagctcgc caatgccatg    600

ggtgcgactg tcgtgatgat caccacctcg cccggcaagg cggaggatgc caaaaaactc    660

ggcgcacacg aggtgatcat ctcccgcgat gcggagcaga tgaagaaggc tacctcgagc    720

ctcgatctca tcatcgatgc tgtcgccgcc gaccacgaca tcgacgccta tctggcgctg    780

ctgaaacgcg atggcgcgct ggtgcaggtg ggcgcgccgg aaaagccact ttcggtgatg    840

gccttcagcc tcatccccgg ccgcaagacc tttgccggct cgatgatcgg cggtattccc    900

gagactcagg aaatgctgga tttctgcgcc gaaaaaggca tcgccggcga aatcgagatg    960

atcgatatcg atcagatcaa tgacgcttat gaacgcatga taaaaagcga tgtgcgttat   1020

cgtttcgtca ttgatatgaa gagcctgccg cgccagaagg ccgcctga                1068


<210> SEQ ID NO 70
<211> LENGTH: 355
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 70

Met Phe Thr Thr Ser Ala Tyr Ala Cys Asp Asp Gly Ser Ser Pro Met
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```
1               5                   10                  15

Lys Leu Ala Thr Ile Arg Arg Arg Asp Pro Gly Pro Arg Asp Val Glu
            20                  25                  30

Ile Glu Ile Glu Phe Cys Gly Val Cys His Ser Asp Ile His Thr Ala
        35                  40                  45

Arg Ser Glu Trp Pro Gly Ser Leu Tyr Pro Cys Val Pro Gly His Glu
    50                  55                  60

Ile Val Gly Arg Val Gly Arg Val Gly Ala Gln Val Thr Arg Phe Lys
65                  70                  75                  80

Thr Gly Asp Arg Val Gly Val Gly Cys Ile Val Asp Ser Cys Arg Glu
                85                  90                  95

Cys Ala Ser Cys Ala Glu Gly Leu Glu Gln Tyr Cys Glu Asn Gly Met
            100                 105                 110

Thr Gly Thr Tyr Asn Ser Pro Asp Lys Ala Met Gly Gly Gly Ala His
        115                 120                 125

Thr Leu Gly Gly Tyr Ser Ala His Val Val Val Asp Asp Arg Tyr Val
    130                 135                 140

Leu Asn Ile Pro Glu Gly Leu Asp Pro Ala Ala Ala Ala Pro Leu Leu
145                 150                 155                 160

Cys Ala Gly Ile Thr Thr Tyr Ser Pro Leu Arg His Trp Asn Ala Gly
                165                 170                 175

Pro Gly Lys Arg Val Gly Val Val Gly Leu Gly Gly Leu Gly His Met
            180                 185                 190

Ala Val Lys Leu Ala Asn Ala Met Gly Ala Thr Val Val Met Ile Thr
        195                 200                 205

Thr Ser Pro Gly Lys Ala Glu Asp Ala Lys Lys Leu Gly Ala His Glu
    210                 215                 220

Val Ile Ile Ser Arg Asp Ala Glu Gln Met Lys Lys Ala Thr Ser Ser
225                 230                 235                 240

Leu Asp Leu Ile Ile Asp Ala Val Ala Ala Asp His Asp Ile Asp Ala
                245                 250                 255

Tyr Leu Ala Leu Leu Lys Arg Asp Gly Ala Leu Val Gln Val Gly Ala
            260                 265                 270

Pro Glu Lys Pro Leu Ser Val Met Ala Phe Ser Leu Ile Pro Gly Arg
        275                 280                 285

Lys Thr Phe Ala Gly Ser Met Ile Gly Gly Ile Pro Glu Thr Gln Glu
    290                 295                 300

Met Leu Asp Phe Cys Ala Glu Lys Gly Ile Ala Gly Glu Ile Glu Met
305                 310                 315                 320

Ile Asp Ile Asp Gln Ile Asn Asp Ala Tyr Glu Arg Met Ile Lys Ser
                325                 330                 335

Asp Val Arg Tyr Arg Phe Val Ile Asp Met Lys Ser Leu Pro Arg Gln
            340                 345                 350

Lys Ala Ala
        355
```

<210> SEQ ID NO 71
<211> LENGTH: 1047
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 71

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atggctattg caagaggtta tgctgcgacc gacgcgtcga agccgcttac cccgttcacc 60

ttcgaacgcc gcgagccgaa tgatgacgac gtcgtcatcg atatcaaata tgccggcatc 120

tgccactcgg acatccacac cgtccgcaac gaatggcaca atgccgttta cccgatcgtt 180

ccgggccacg aaatcgccgg tgtcgtgcgg gccgttggtt ccaaggtcac gcggttcaag 240

gtcggcgacc atgtcggcgt cggctgcttt gtcgattcct gcgttggctg cgccacccgc 300

gatgtcgaca atgagcagta tatgccgggt ctcgtgcaga cctacaattc cgttgaacgg 360

gacggcaaga gcgcgaccca gggcggttat tccgaccata tcgtggtcag ggaagactac 420

gtcctgtcca tcccggacaa cctgccgctc gatgcctccg cgccgcttct ctgcgccggc 480

atcacgctct attcgccgct gcagcactgg aatgcaggcc ccggcaagaa agtggctatc 540

gtcggcatgg gtggccttgg ccacatgggc gtgaagatcg gctcggccat gggcgctgat 600

atcaccgttc tctcgcagac gctgtcgaag aaggaagacg gcctcaagct cggcgcgaag 660

gaatattacg ccaccagcga cgcctcgacc tttgagaaac tcgccggcac cttcgacctg 720

atcctgtgca cagtctcggc cgaaatcgac tggaacgcct acctcaacct gctcaaggtc 780

aacggcacga tggttctgct cggcgtgccg gaacatgcga tcccggtgca cgcattctcg 840

gtcattcccg cccgccgttc gctcgccggt tcgatgatcg gctcgatcaa ggaaacccag 900

gaaatgctgg atttctgcgg caagcacgac atcgtttcgg aaatcgaaac gatcggcatc 960

aaggacgtca acgaagccta tgagcgcgtg ctgaagagcg acgtgcgtta ccgcttcgtc 1020

atcgacatgg cctcgctcga cgcttga 1047

<210> SEQ ID NO 72
<211> LENGTH: 348
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 72

Met Ala Ile Ala Arg Gly Tyr Ala Ala Thr Asp Ala Ser Lys Pro Leu
1 5 10 15

Thr Pro Phe Thr Phe Glu Arg Arg Glu Pro Asn Asp Asp Asp Val Val
20 25 30

Ile Asp Ile Lys Tyr Ala Gly Ile Cys His Ser Asp Ile His Thr Val
35 40 45

Arg Asn Glu Trp His Asn Ala Val Tyr Pro Ile Val Pro Gly His Glu
50 55 60

Ile Ala Gly Val Val Arg Ala Val Gly Ser Lys Val Thr Arg Phe Lys
65 70 75 80

Val Gly Asp His Val Gly Val Gly Cys Phe Val Asp Ser Cys Val Gly
85 90 95

Cys Ala Thr Arg Asp Val Asp Asn Glu Gln Tyr Met Pro Gly Leu Val
100 105 110

Gln Thr Tyr Asn Ser Val Glu Arg Asp Gly Lys Ser Ala Thr Gln Gly
115 120 125

Gly Tyr Ser Asp His Ile Val Val Arg Glu Asp Tyr Val Leu Ser Ile
130 135 140

Pro Asp Asn Leu Pro Leu Asp Ala Ser Ala Pro Leu Leu Cys Ala Gly
145 150 155 160

Ile Thr Leu Tyr Ser Pro Leu Gln His Trp Asn Ala Gly Pro Gly Lys
165 170 175

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Lys Val Ala Ile Val Gly Met Gly Gly Leu Gly His Met Gly Val Lys
            180                 185                 190

Ile Gly Ser Ala Met Gly Ala Asp Ile Thr Val Leu Ser Gln Thr Leu
        195                 200                 205

Ser Lys Lys Glu Asp Gly Leu Lys Leu Gly Ala Lys Glu Tyr Tyr Ala
    210                 215                 220

Thr Ser Asp Ala Ser Thr Phe Glu Lys Leu Ala Gly Thr Phe Asp Leu
225                 230                 235                 240

Ile Leu Cys Thr Val Ser Ala Glu Ile Asp Trp Asn Ala Tyr Leu Asn
                245                 250                 255

Leu Leu Lys Val Asn Gly Thr Met Val Leu Leu Gly Val Pro Glu His
            260                 265                 270

Ala Ile Pro Val His Ala Phe Ser Val Ile Pro Ala Arg Arg Ser Leu
        275                 280                 285

Ala Gly Ser Met Ile Gly Ser Ile Lys Glu Thr Gln Glu Met Leu Asp
    290                 295                 300

Phe Cys Gly Lys His Asp Ile Val Ser Glu Ile Glu Thr Ile Gly Ile
305                 310                 315                 320

Lys Asp Val Asn Glu Ala Tyr Glu Arg Val Leu Lys Ser Asp Val Arg
                325                 330                 335

Tyr Arg Phe Val Ile Asp Met Ala Ser Leu Asp Ala
            340                 345


<210> SEQ ID NO 73
<211> LENGTH: 1029
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 73

atgactaaaa caatgaaggc ggcggttgtc cgcgcatttg gaaaaccgct gaccatcgag     60

gaagtggcaa taccggatcc cggccccggt gaaattctca tcaactacaa ggcgacgggc    120

gtttgccaca ccgacctgca cgccgcaacg ggggattggc cggtcaagcc caacccgccc    180

ttcattcccg gacatgaagg tgcaggttac gtcgccaaga tcggcgctgg cgtcaccggc    240

atcaaggagg gcgaccgcgc cggcacgccc tggctctaca ccgcctgcgg atgctgcatt    300

ccctgccgta ccggctggga aaccctgtgc ccgagccaga agaactcagg ttattccgtc    360

aacggcagct ttgccgaata tggccttgcc gatccgaaat tcgtcggccg cctgcctgac    420

aatctcgatt tcggcccagc cgcacccgtg ctctgcgccg gcgttacagt ctataagggc    480

ctgaaggaaa ccgaagtcag gcccggtgaa tgggtggtca tttcaggcat tggcgggctt    540

ggccacatgg ccgtgcaata tgcgaaagcc atgggcatgc atgtggttgc cgccgatatt    600

ttcgacgaca agctggcgct tgccaaaaag ctcggagccg acgtcgtcgt caacggccgc    660

gcgcctgacg cggtggagca agtgcaaaag gcaaccggcg gcgtccatgg cgcgctggtg    720

acggcggttt caccgaaggc catggagcag gcttatggct tcctgcgctc caagggcacg    780

atggcgcttg tcggtctgcc gccgggcttc atctccattc cggtgttcga cacggtgctg    840

aagcgcatca cggtgcgtgg ctccatcgtc ggcacgcggc aggatctgga ggaggcgttg    900

accttcgccg gtgaaggcaa ggtggccgcc cacttctcgt gggacaagct cgaaaacatc    960

aatgatatct tccatcgcat ggaagagggc aagatcgacg gccgtatcgt cgtggatctc   1020
```

-continued

gccgcctga 1029

<210> SEQ ID NO 74
<211> LENGTH: 342
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 74

Met Thr Lys Thr Met Lys Ala Ala Val Val Arg Ala Phe Gly Lys Pro
1 5 10 15

Leu Thr Ile Glu Glu Val Ala Ile Pro Asp Pro Gly Pro Gly Glu Ile
20 25 30

Leu Ile Asn Tyr Lys Ala Thr Gly Val Cys His Thr Asp Leu His Ala
35 40 45

Ala Thr Gly Asp Trp Pro Val Lys Pro Asn Pro Pro Phe Ile Pro Gly
50 55 60

His Glu Gly Ala Gly Tyr Val Ala Lys Ile Gly Ala Gly Val Thr Gly
65 70 75 80

Ile Lys Glu Gly Asp Arg Ala Gly Thr Pro Trp Leu Tyr Thr Ala Cys
85 90 95

Gly Cys Cys Ile Pro Cys Arg Thr Gly Trp Glu Thr Leu Cys Pro Ser
100 105 110

Gln Lys Asn Ser Gly Tyr Ser Val Asn Gly Ser Phe Ala Glu Tyr Gly
115 120 125

Leu Ala Asp Pro Lys Phe Val Gly Arg Leu Pro Asp Asn Leu Asp Phe
130 135 140

Gly Pro Ala Ala Pro Val Leu Cys Ala Gly Val Thr Val Tyr Lys Gly
145 150 155 160

Leu Lys Glu Thr Glu Val Arg Pro Gly Glu Trp Val Val Ile Ser Gly
165 170 175

Ile Gly Gly Leu Gly His Met Ala Val Gln Tyr Ala Lys Ala Met Gly
180 185 190

Met His Val Val Ala Ala Asp Ile Phe Asp Asp Lys Leu Ala Leu Ala
195 200 205

Lys Lys Leu Gly Ala Asp Val Val Val Asn Gly Arg Ala Pro Asp Ala
210 215 220

Val Glu Gln Val Gln Lys Ala Thr Gly Gly Val His Gly Ala Leu Val
225 230 235 240

Thr Ala Val Ser Pro Lys Ala Met Glu Gln Ala Tyr Gly Phe Leu Arg
245 250 255

Ser Lys Gly Thr Met Ala Leu Val Gly Leu Pro Pro Gly Phe Ile Ser
260 265 270

Ile Pro Val Phe Asp Thr Val Leu Lys Arg Ile Thr Val Arg Gly Ser
275 280 285

Ile Val Gly Thr Arg Gln Asp Leu Glu Glu Ala Leu Thr Phe Ala Gly
290 295 300

Glu Gly Lys Val Ala Ala His Phe Ser Trp Asp Lys Leu Glu Asn Ile
305 310 315 320

Asn Asp Ile Phe His Arg Met Glu Glu Gly Lys Ile Asp Gly Arg Ile
325 330 335

Val Val Asp Leu Ala Ala
340

-continued

<210> SEQ ID NO 75
<211> LENGTH: 1008
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 75

atgaccgggg cgaaccagcc ttgggaggtt caagaggttc ccgttccgaa ggcagagcca 60

ggacttgtcc ttgttaaaat ccacgcctcc ggcatgtgct acacggacgt gtgggcgacg 120

cagggtgccg gtggcgacat ctatccgcag acccccggcc atgaggttgt cggcgagatc 180

atcgaggtcg gcgcgggcgt tcatacgcgc aaggtgggag accgggtcgg caccacctgg 240

gtgcagtcct cttgtggacg atgctcctac tgccgccaga accgtccgtt gaccggccag 300

acagccatga actgcgattc acccaggaca acggggttcg cgacgcaagg cgggcacgca 360

gagtacatcg cgatctctgc tgaaggcaca gtgttattac ccgacgggct cgactacacg 420

gatgccgcac ccatgatgtg cgcaggctac acgacctgga gcggcttgcg cgacgccgag 480

cccaaacctg gtgacagaat tgcggtactt ggcatcggcg ggctggggca cgtcgccgtg 540

cagttctcca aagccttggg gtttgagacc atcgcgatca cgcattcacc cgacaagcac 600

aagttggcca ccgatcttgg tgcagacatc gtcgtcgccg atggcaaaga gttattggag 660

gccggcggtg cggacgttct tctggttacg accaacgact tcgacaccgc cgaaaaagcg 720

atggcgggcg taaggcctga cgggcgcatc gttctttgcg cgctcgactt cagcaagccg 780

ttctcgatcc cgtccgacgg caagccgttc cacatgatgc gccaacgcgt ggttgggtcc 840

acgcatggcg gacagcacta tctcgccgaa atcctcgatc tcgccgccaa gggcaaggtc 900

aagccgattg tcgagacctt cgccctcgag caggcaaccg aggcatatga gcggctatcc 960

accgggaaga tgcgcttccg gggcgtgttc cttccgcacg gcgcttga 1008

<210> SEQ ID NO 76
<211> LENGTH: 335
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 76

Met Thr Gly Ala Asn Gln Pro Trp Glu Val Gln Glu Val Pro Val Pro
1 5 10 15

Lys Ala Glu Pro Gly Leu Val Leu Val Lys Ile His Ala Ser Gly Met
20 25 30

Cys Tyr Thr Asp Val Trp Ala Thr Gln Gly Ala Gly Gly Asp Ile Tyr
35 40 45

Pro Gln Thr Pro Gly His Glu Val Val Gly Glu Ile Ile Glu Val Gly
50 55 60

Ala Gly Val His Thr Arg Lys Val Gly Asp Arg Val Gly Thr Thr Trp
65 70 75 80

Val Gln Ser Ser Cys Gly Arg Cys Ser Tyr Cys Arg Gln Asn Arg Pro
85 90 95

Leu Thr Gly Gln Thr Ala Met Asn Cys Asp Ser Pro Arg Thr Thr Gly
100 105 110

Phe Ala Thr Gln Gly Gly His Ala Glu Tyr Ile Ala Ile Ser Ala Glu
115 120 125

Gly Thr Val Leu Leu Pro Asp Gly Leu Asp Tyr Thr Asp Ala Ala Pro
130 135 140

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Met Met Cys Ala Gly Tyr Thr Thr Trp Ser Gly Leu Arg Asp Ala Glu
145                 150                 155                 160

Pro Lys Pro Gly Asp Arg Ile Ala Val Leu Gly Ile Gly Gly Leu Gly
                165                 170                 175

His Val Ala Val Gln Phe Ser Lys Ala Leu Gly Phe Glu Thr Ile Ala
            180                 185                 190

Ile Thr His Ser Pro Asp Lys His Lys Leu Ala Thr Asp Leu Gly Ala
        195                 200                 205

Asp Ile Val Val Ala Asp Gly Lys Glu Leu Leu Glu Ala Gly Gly Ala
    210                 215                 220

Asp Val Leu Leu Val Thr Thr Asn Asp Phe Asp Thr Ala Glu Lys Ala
225                 230                 235                 240

Met Ala Gly Val Arg Pro Asp Gly Arg Ile Val Leu Cys Ala Leu Asp
                245                 250                 255

Phe Ser Lys Pro Phe Ser Ile Pro Ser Asp Gly Lys Pro Phe His Met
            260                 265                 270

Met Arg Gln Arg Val Val Gly Ser Thr His Gly Gly Gln His Tyr Leu
        275                 280                 285

Ala Glu Ile Leu Asp Leu Ala Ala Lys Gly Lys Val Lys Pro Ile Val
    290                 295                 300

Glu Thr Phe Ala Leu Glu Gln Ala Thr Glu Ala Tyr Glu Arg Leu Ser
305                 310                 315                 320

Thr Gly Lys Met Arg Phe Arg Gly Val Phe Leu Pro His Gly Ala
                325                 330                 335
```

<210> SEQ ID NO 77
<211> LENGTH: 1017
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 77

```
atgaccatgc atgccattca attcgtcgag aagggacgcg ccgtgctggc ggaactcccc     60

gtcgccgatc tgccgccggg ccatgcgctc gtgcgggtca aggcttcggg gctttgccat    120

accgatatcg acgtgctgca tgcgcgttat ggcgacggtg cgttccccgt cattccgggg    180

catgaatatg ctggcgaagt cgcagccgtg gcttccgatg tgacagtctt caaggctggc    240

gaccgggttg tcgtcgatcc caatctgccc tgtggcacct gcgccagctg caggaaaggg    300

ctgaccaacc tttgcagcac attgaaagct tacggcgttt cccacaatgg cggctttgcg    360

gagttcagtg tggtgcgtgc cgatcacctg cacggtatcg gttcgatgcc ctatcacgtc    420

gcggcgctgg ctgagccgct tgcctgtgtt gtcaatggca tgcagagtgc gggtattggc    480

gagagtggcg tggtgccgga gaatgcgctt gttttcggtg ctgggcccat cggcctgctg    540

cttgccctgt cgctgaaatc acgcggcatt gcgacggtga cgatggccga tatcaatgaa    600

agcaggctgg cctttgccca ggacctcggg cttcagacgg cggtatccgg ctcggaagcg    660

ctctcgcggc agcggaagga gttcgatttc gtggccgatg cgacgggtat tgccccggtc    720

gccgaggcga tgatcccgct ggttgcggat ggcggcacgg cgctattctt cggcgtctgc    780

gcgccggatg cccgtatttc ggtggcaccg tttgaaatct tccggcgcca gctgaaactt    840

gtcggctcgc attcgctgaa ccgcaacata ccgcaggcgc ttgccattct ggagacggat    900

ggcgaggtca tggcgcggct cgtttcgcac cgcttgccgc tttcggagat gctgccgttc    960
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tttacgaaaa aaccgtctga tccggcgacg atgaaagtgc aatttgcagc cgaatga 1017

<210> SEQ ID NO 78
<211> LENGTH: 338
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 78

Met Thr Met His Ala Ile Gln Phe Val Glu Lys Gly Arg Ala Val Leu
1 5 10 15

Ala Glu Leu Pro Val Ala Asp Leu Pro Pro Gly His Ala Leu Val Arg
20 25 30

Val Lys Ala Ser Gly Leu Cys His Thr Asp Ile Asp Val Leu His Ala
35 40 45

Arg Tyr Gly Asp Gly Ala Phe Pro Val Ile Pro Gly His Glu Tyr Ala
50 55 60

Gly Glu Val Ala Ala Val Ala Ser Asp Val Thr Val Phe Lys Ala Gly
65 70 75 80

Asp Arg Val Val Val Asp Pro Asn Leu Pro Cys Gly Thr Cys Ala Ser
85 90 95

Cys Arg Lys Gly Leu Thr Asn Leu Cys Ser Thr Leu Lys Ala Tyr Gly
100 105 110

Val Ser His Asn Gly Gly Phe Ala Glu Phe Ser Val Val Arg Ala Asp
115 120 125

His Leu His Gly Ile Gly Ser Met Pro Tyr His Val Ala Ala Leu Ala
130 135 140

Glu Pro Leu Ala Cys Val Val Asn Gly Met Gln Ser Ala Gly Ile Gly
145 150 155 160

Glu Ser Gly Val Val Pro Glu Asn Ala Leu Val Phe Gly Ala Gly Pro
165 170 175

Ile Gly Leu Leu Leu Ala Leu Ser Leu Lys Ser Arg Gly Ile Ala Thr
180 185 190

Val Thr Met Ala Asp Ile Asn Glu Ser Arg Leu Ala Phe Ala Gln Asp
195 200 205

Leu Gly Leu Gln Thr Ala Val Ser Gly Ser Glu Ala Leu Ser Arg Gln
210 215 220

Arg Lys Glu Phe Asp Phe Val Ala Asp Ala Thr Gly Ile Ala Pro Val
225 230 235 240

Ala Glu Ala Met Ile Pro Leu Val Ala Asp Gly Gly Thr Ala Leu Phe
245 250 255

Phe Gly Val Cys Ala Pro Asp Ala Arg Ile Ser Val Ala Pro Phe Glu
260 265 270

Ile Phe Arg Arg Gln Leu Lys Leu Val Gly Ser His Ser Leu Asn Arg
275 280 285

Asn Ile Pro Gln Ala Leu Ala Ile Leu Glu Thr Asp Gly Glu Val Met
290 295 300

Ala Arg Leu Val Ser His Arg Leu Pro Leu Ser Glu Met Leu Pro Phe
305 310 315 320

Phe Thr Lys Lys Pro Ser Asp Pro Ala Thr Met Lys Val Gln Phe Ala
325 330 335

Ala Glu

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<210> SEQ ID NO 79
<211> LENGTH: 1044
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 79

atgcgcgcgc tttattacga acgattcggc gagacccctg tagtcgcgtc cctgcctgat 60

ccggcaccga gcgatggcgg cgtggtgatt gcggtgaagg caaccggcct ctgccgcagc 120

gactggcatg gctggatggg acatgacacg gatatccgtc tgccgcatgt gcccggccac 180

gagttcgccg gcgtcatctc cgcagtcggc agaaacgtca cccgcttcaa gacgggtgat 240

cgcgttaccg tgcctttcgt ctccggctgc ggccattgcc atgagtgccg ctccggcaat 300

cagcaggtct gcgaaacgca gttccagccc ggcttcaccc attggggttc cttcgccgaa 360

tatgtcgcca tcgactatgc cgatcagaac ctcgtgcacc tgccggaatc gatgagttac 420

gccaccgccg ccggcctcgg ttgccgtttc gccacctcct tccgggcggt gacggatcag 480

ggacgcctga agggcggcga atggctggct gtccatggct gcggcggtgt cggtctctcc 540

gccatcatga tcggcgccgg cctcggcgca caggtcgtcg ccatcgatat tgccgaagac 600

aagctcgaac tcgcccggca actgggtgca accgcaacca tcaacagccg ctccgttgcc 660

gatgtcgccg aagcggtgcg cgacatcacc ggtggcggcg cgcatgtgtc ggtggatgcg 720

cttggccatc cgcagacctg ctgcaattcc atcagcaacc tgcgccggcg cggacgccat 780

gtgcaggtgg ggctgatgct ggcagaccat gccatgccgg ccattcccat ggcccgggtg 840

atcgctcatg agctggagat ctatggcagc cacggcatgc aggcatggcg ttacgaggac 900

atgctggcca tgatcgaaag cggcaggctt gcgccggaaa agctgattgg ccgccatatc 960

tcgctgaccg aagcggccgt cgccctgccc ggaatggata ggttccagga gagcggcatc 1020

agcatcatcg accggttcga atag 1044

<210> SEQ ID NO 80
<211> LENGTH: 357
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 80

Met Asn Leu Arg Thr Asn Asp Glu Ala Met Met Arg Ala Leu Tyr Tyr
1 5 10 15

Glu Arg Phe Gly Glu Thr Pro Val Val Ala Ser Leu Pro Asp Pro Ala
20 25 30

Pro Ser Asp Gly Gly Val Val Ile Ala Val Lys Ala Thr Gly Leu Cys
35 40 45

Arg Ser Asp Trp His Gly Trp Met Gly His Asp Thr Asp Ile Arg Leu
50 55 60

Pro His Val Pro Gly His Glu Phe Ala Gly Val Ile Ser Ala Val Gly
65 70 75 80

Arg Asn Val Thr Arg Phe Lys Thr Gly Asp Arg Val Thr Val Pro Phe
85 90 95

Val Ser Gly Cys Gly His Cys His Glu Cys Arg Ser Gly Asn Gln Gln
100 105 110

Val Cys Glu Thr Gln Phe Gln Pro Gly Phe Thr His Trp Gly Ser Phe
115 120 125

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```
Ala Glu Tyr Val Ala Ile Asp Tyr Ala Asp Gln Asn Leu Val His Leu
    130                 135                 140

Pro Glu Ser Met Ser Tyr Ala Thr Ala Ala Gly Leu Gly Cys Arg Phe
145                 150                 155                 160

Ala Thr Ser Phe Arg Ala Val Thr Asp Gln Gly Arg Leu Lys Gly Gly
                165                 170                 175

Glu Trp Leu Ala Val His Gly Cys Gly Gly Val Gly Leu Ser Ala Ile
            180                 185                 190

Met Ile Gly Ala Gly Leu Gly Ala Gln Val Val Ala Ile Asp Ile Ala
        195                 200                 205

Glu Asp Lys Leu Glu Leu Ala Arg Gln Leu Gly Ala Thr Ala Thr Ile
    210                 215                 220

Asn Ser Arg Ser Val Ala Asp Val Ala Glu Ala Val Arg Asp Ile Thr
225                 230                 235                 240

Gly Gly Gly Ala His Val Ser Val Asp Ala Leu Gly His Pro Gln Thr
                245                 250                 255

Cys Cys Asn Ser Ile Ser Asn Leu Arg Arg Arg Gly Arg His Val Gln
            260                 265                 270

Val Gly Leu Met Leu Ala Asp His Ala Met Pro Ala Ile Pro Met Ala
        275                 280                 285

Arg Val Ile Ala His Glu Leu Glu Ile Tyr Gly Ser His Gly Met Gln
    290                 295                 300

Ala Trp Arg Tyr Glu Asp Met Leu Ala Met Ile Glu Ser Gly Arg Leu
305                 310                 315                 320

Ala Pro Glu Lys Leu Ile Gly Arg His Ile Ser Leu Thr Glu Ala Ala
                325                 330                 335

Val Ala Leu Pro Gly Met Asp Arg Phe Gln Glu Ser Gly Ile Ser Ile
            340                 345                 350

Ile Asp Arg Phe Glu
        355
```

<210> SEQ ID NO 81
<211> LENGTH: 1011
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 81

```
atgctggcga ttttctgtga cactcccggt caattaaccg ccaaggatct gccgaacccc     60

gtgcgcggcg aaggtgaagt cctggtacgt attcgccgga ttggcgtttg cggcacggat    120

ctgcacatct ttaccggcaa ccagccctat ctttcctatc cgcggatcat gggtcacgaa    180

ctttccggca cggttgagga ggcacccgct ggcagccacc tttccgctgg cgatgtggtg    240

accataattc cctatatgtc ctgcgggaaa tgcaatgcct gcctgaaggg taagagcaat    300

tgctgccgca atatcggtgt gcttggcgtt catcgcgatg gcggcatggt ggaatatctg    360

agcgtgccgc agcaattcgt gctgaaggcg gaggggctga gcctcgacca ggcagccatg    420

acggaatttc tggcgatcgg tgcccatgcg gtgcgtcgcg gtgccgtcga aaaagggcaa    480

aaggtcctga tcgtcggtgc cggcccgatc ggcatggcgg ttgctgtctt tgcggttctc    540

gatggcacgg aagtgacgat gatcgacggt cgcaccgacc ggctggattt ctgcaaggac    600

cacctcggtg tcgctcatac agtcgccctc ggcgacggtg acaaagatcg tctgtccgac    660

attaccggtg gcaatttctt cgatgcggtg tttgatgcga ccggcaatcc gaaagccatg    720
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gagcgcggtt tctccttcgt cggtcacggc ggctcctatg ttctggtgtc catcgtcgcc 780

agcgatatca gcttcaacga cccggaattt cacaagcgtg agacgacgct gctcggcagc 840

cgcaacgcga cggctgatga tttcgagcgg gtgcttcgcg ccttgcgcga agggaaagtg 900

ccggaggcac taatcaccca tcgcatgaca cttgccgatg ttccctcgaa gttcgccggc 960

ctgaccgatc cgaaagccgg agtcatcaag ggcatggtgg aggtcgcatg a 1011

<210> SEQ ID NO 82
<211> LENGTH: 336
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 82

Met Leu Ala Ile Phe Cys Asp Thr Pro Gly Gln Leu Thr Ala Lys Asp
1 5 10 15

Leu Pro Asn Pro Val Arg Gly Glu Gly Glu Val Leu Val Arg Ile Arg
20 25 30

Arg Ile Gly Val Cys Gly Thr Asp Leu His Ile Phe Thr Gly Asn Gln
35 40 45

Pro Tyr Leu Ser Tyr Pro Arg Ile Met Gly His Glu Leu Ser Gly Thr
50 55 60

Val Glu Glu Ala Pro Ala Gly Ser His Leu Ser Ala Gly Asp Val Val
65 70 75 80

Thr Ile Ile Pro Tyr Met Ser Cys Gly Lys Cys Asn Ala Cys Leu Lys
85 90 95

Gly Lys Ser Asn Cys Cys Arg Asn Ile Gly Val Leu Gly Val His Arg
100 105 110

Asp Gly Gly Met Val Glu Tyr Leu Ser Val Pro Gln Gln Phe Val Leu
115 120 125

Lys Ala Glu Gly Leu Ser Leu Asp Gln Ala Ala Met Thr Glu Phe Leu
130 135 140

Ala Ile Gly Ala His Ala Val Arg Arg Gly Ala Val Glu Lys Gly Gln
145 150 155 160

Lys Val Leu Ile Val Gly Ala Gly Pro Ile Gly Met Ala Val Ala Val
165 170 175

Phe Ala Val Leu Asp Gly Thr Glu Val Thr Met Ile Asp Gly Arg Thr
180 185 190

Asp Arg Leu Asp Phe Cys Lys Asp His Leu Gly Val Ala His Thr Val
195 200 205

Ala Leu Gly Asp Gly Asp Lys Asp Arg Leu Ser Asp Ile Thr Gly Gly
210 215 220

Asn Phe Phe Asp Ala Val Phe Asp Ala Thr Gly Asn Pro Lys Ala Met
225 230 235 240

Glu Arg Gly Phe Ser Phe Val Gly His Gly Gly Ser Tyr Val Leu Val
245 250 255

Ser Ile Val Ala Ser Asp Ile Ser Phe Asn Asp Pro Glu Phe His Lys
260 265 270

Arg Glu Thr Thr Leu Leu Gly Ser Arg Asn Ala Thr Ala Asp Asp Phe
275 280 285

Glu Arg Val Leu Arg Ala Leu Arg Glu Gly Lys Val Pro Glu Ala Leu
290 295 300

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Ile Thr His Arg Met Thr Leu Ala Asp Val Pro Ser Lys Phe Ala Gly
305                 310                 315                 320

Leu Thr Asp Pro Lys Ala Gly Val Ile Lys Gly Met Val Glu Val Ala
                325                 330                 335


<210> SEQ ID NO 83
<211> LENGTH: 1005
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 83

gtgaaagcct tcgtcgtcga caagtacaag aagaagggcc cgctgcgtct ggccgacatg     60

cccaatccgg tcatcggcgc caatgatgtg ctggttcgca tccatgccac tgccatcaat    120

cttctcgact ccaaggtgcg cgacggggaa ttcaagctgt tcctgcccta tcgtcctccc    180

ttcattctcg gtcatgatct ggccggaacg gtcatccgcg tcggcgcgaa tgtacggcag    240

ttcaagacag gcgacgaggt tttcgctcgc ccgcgtgatc accgggtcgg aaccttcgca    300

gaaatgattg cggtcgatgc cgcagacctt gcgctgaagc caacgagcct gtccatggag    360

caggcagcgt cgatcccgct cgtcggactg actgcctggc aggcgcttat cgaggttggc    420

aaggtcaagt ccggccagaa ggttttcatc caggccggtt ccggcggtgt cggcaccttc    480

gccatccagc ttgccaagca tctcggcgct accgtggcca cgaccaccag cgccgcgaat    540

gccgaactgg tcaaaagcct cggcgcagat gtggtgatcg actacaagac gcaggacttc    600

gaacaggtgc tgtccggcta cgatctcgtc ctgaacagcc aggatgccaa gacgctggaa    660

aagtcgttga acgtgctgag accgggcgga aagctcattt cgatctccgg tccgccggat    720

gttgcctttg ccagatcgtt gaaactgaat ccgctcctgc gttttgtcgt cagaatgctg    780

agccgtggtg tcctgaaaaa ggcaagcaga cgcggtgtcg attactcttt cctgttcatg    840

cgcgccgaag gtcagcaatt gcatgagatc gccgaactga tcgatgccgg caccatccgt    900

ccggtcgtcg acaaggtgtt tcaatttgcg cagacgcccg acgccctggc ctatgtcgag    960

accggacggg caaggggcaa ggttgtggtt acatacgcat cctag                   1005


<210> SEQ ID NO 84
<211> LENGTH: 359
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 84

Met Pro Ser Leu Cys Arg Lys Pro Trp Leu Ser Ser Leu Pro Asp Leu
1               5                   10                  15

Ile Asn Val Ser His Trp Arg Lys Pro Val Lys Ala Phe Val Val Asp
            20                  25                  30

Lys Tyr Lys Lys Lys Gly Pro Leu Arg Leu Ala Asp Met Pro Asn Pro
        35                  40                  45

Val Ile Gly Ala Asn Asp Val Leu Val Arg Ile His Ala Thr Ala Ile
    50                  55                  60

Asn Leu Leu Asp Ser Lys Val Arg Asp Gly Glu Phe Lys Leu Phe Leu
65                  70                  75                  80

Pro Tyr Arg Pro Pro Phe Ile Leu Gly His Asp Leu Ala Gly Thr Val
                85                  90                  95

Ile Arg Val Gly Ala Asn Val Arg Gln Phe Lys Thr Gly Asp Glu Val
            100                 105                 110
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Phe Ala Arg Pro Arg Asp His Arg Val Gly Thr Phe Ala Glu Met Ile
        115                 120                 125

Ala Val Asp Ala Ala Asp Leu Ala Leu Lys Pro Thr Ser Leu Ser Met
    130                 135                 140

Glu Gln Ala Ala Ser Ile Pro Leu Val Gly Leu Thr Ala Trp Gln Ala
145                 150                 155                 160

Leu Ile Glu Val Gly Lys Val Lys Ser Gly Gln Lys Val Phe Ile Gln
                165                 170                 175

Ala Gly Ser Gly Gly Val Gly Thr Phe Ala Ile Gln Leu Ala Lys His
            180                 185                 190

Leu Gly Ala Thr Val Ala Thr Thr Thr Ser Ala Ala Asn Ala Glu Leu
        195                 200                 205

Val Lys Ser Leu Gly Ala Asp Val Val Ile Asp Tyr Lys Thr Gln Asp
    210                 215                 220

Phe Glu Gln Val Leu Ser Gly Tyr Asp Leu Val Leu Asn Ser Gln Asp
225                 230                 235                 240

Ala Lys Thr Leu Glu Lys Ser Leu Asn Val Leu Arg Pro Gly Gly Lys
                245                 250                 255

Leu Ile Ser Ile Ser Gly Pro Pro Asp Val Ala Phe Ala Arg Ser Leu
            260                 265                 270

Lys Leu Asn Pro Leu Leu Arg Phe Val Val Arg Met Leu Ser Arg Gly
        275                 280                 285

Val Leu Lys Lys Ala Ser Arg Arg Gly Val Asp Tyr Ser Phe Leu Phe
    290                 295                 300

Met Arg Ala Glu Gly Gln Gln Leu His Glu Ile Ala Glu Leu Ile Asp
305                 310                 315                 320

Ala Gly Thr Ile Arg Pro Val Val Asp Lys Val Phe Gln Phe Ala Gln
                325                 330                 335

Thr Pro Asp Ala Leu Ala Tyr Val Glu Thr Gly Arg Ala Arg Gly Lys
            340                 345                 350

Val Val Val Thr Tyr Ala Ser
        355


<210> SEQ ID NO 85
<211> LENGTH: 1032
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 85

atgaaagcga ttgtcgccca cggggcaaag gatgtgcgca tcgaagaccg gccggaggaa     60

aagccgggtc cgggcgaggt gcggctccgt ctggcgaggg gcgggatctg cggcagtgat    120

ctgcattatt acaatcatgg cggtttcggc gccgtgcggc ttcgtgaacc catggtgctg    180

ggccatgagg tttccgccgt catcgaggaa ctgggcgaag gcgttgaggg gctgaagatc    240

ggcggtctgg tggcggtttc gccgtcgcgc ccatgccgaa cctgccgctt ctgccaggag    300

ggtctgcaca atcagtgcct caacatgcgg ttttatggca gcgccatgcc tttcccgcat    360

attcagggcg cgttccggga aatttctggtg gcggacgccc tgcaatgcgt gccggccgat    420

ggtctcagcg ccggggaagc cgccatggcg gaaccgctgg cggtgacgct gcatgccaca    480

cgccgggccg gcgatttgct gggaaaacgt gtgctcgtca cgggttgcgg ccccatcggc    540

attctctcca ttctggctgc gcgccgggcg ggtgctgctg aaatcgtcgc caccgacctt    600
```

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tccgatttca cgctcggcaa ggcgcgtgaa gcgggggcgg accgtgtcat caacagcaag 660

gatgagcccg atgcgctcgc cgcttatggt gcaaacaagg gaaccttcga cattctctat 720

gaatgctcgg gtgcggccgt ggcgcttgcc ggcggcatta cggcactgcg gccgcgcggc 780

atcatcgtcc agctcgggct cggcggcgat atgagcctgc cgatgatggc gatcacagcc 840

aaggaactcg acctgcgtgg ttcctttcgc ttccacgagg aattcgccac cggcgtcgag 900

ctgatgcgca agggcctgat cgacgtcaaa cccttcatca cccagaccgt cgatcttgcc 960

gacgccatct cggccttcga attcgcctcg gatcgcagcc gcgccatgaa ggtgcagatc 1020

gccttttcct aa 1032

<210> SEQ ID NO 86
<211> LENGTH: 343
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 86

Met Lys Ala Ile Val Ala His Gly Ala Lys Asp Val Arg Ile Glu Asp
1 5 10 15

Arg Pro Glu Glu Lys Pro Gly Pro Gly Glu Val Arg Leu Arg Leu Ala
20 25 30

Arg Gly Gly Ile Cys Gly Ser Asp Leu His Tyr Tyr Asn His Gly Gly
35 40 45

Phe Gly Ala Val Arg Leu Arg Glu Pro Met Val Leu Gly His Glu Val
50 55 60

Ser Ala Val Ile Glu Glu Leu Gly Glu Gly Val Glu Gly Leu Lys Ile
65 70 75 80

Gly Gly Leu Val Ala Val Ser Pro Ser Arg Pro Cys Arg Thr Cys Arg
85 90 95

Phe Cys Gln Glu Gly Leu His Asn Gln Cys Leu Asn Met Arg Phe Tyr
100 105 110

Gly Ser Ala Met Pro Phe Pro His Ile Gln Gly Ala Phe Arg Glu Ile
115 120 125

Leu Val Ala Asp Ala Leu Gln Cys Val Pro Ala Asp Gly Leu Ser Ala
130 135 140

Gly Glu Ala Ala Met Ala Glu Pro Leu Ala Val Thr Leu His Ala Thr
145 150 155 160

Arg Arg Ala Gly Asp Leu Leu Gly Lys Arg Val Leu Val Thr Gly Cys
165 170 175

Gly Pro Ile Gly Ile Leu Ser Ile Leu Ala Ala Arg Arg Ala Gly Ala
180 185 190

Ala Glu Ile Val Ala Thr Asp Leu Ser Asp Phe Thr Leu Gly Lys Ala
195 200 205

Arg Glu Ala Gly Ala Asp Arg Val Ile Asn Ser Lys Asp Glu Pro Asp
210 215 220

Ala Leu Ala Ala Tyr Gly Ala Asn Lys Gly Thr Phe Asp Ile Leu Tyr
225 230 235 240

Glu Cys Ser Gly Ala Ala Val Ala Leu Ala Gly Gly Ile Thr Ala Leu
245 250 255

Arg Pro Arg Gly Ile Ile Val Gln Leu Gly Leu Gly Gly Asp Met Ser
260 265 270

-continued

```
Leu Pro Met Met Ala Ile Thr Ala Lys Glu Leu Asp Leu Arg Gly Ser
        275                 280                 285

Phe Arg Phe His Glu Glu Phe Ala Thr Gly Val Glu Leu Met Arg Lys
    290                 295                 300

Gly Leu Ile Asp Val Lys Pro Phe Ile Thr Gln Thr Val Asp Leu Ala
305                 310                 315                 320

Asp Ala Ile Ser Ala Phe Glu Phe Ala Ser Asp Arg Ser Arg Ala Met
                325                 330                 335

Lys Val Gln Ile Ala Phe Ser
            340
```

<210> SEQ ID NO 87
<211> LENGTH: 939
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 87

```
atgccgatgg cgctcgggca cgaagcggcg ggcgtcgtcg aggcattggg cgaaggcgtg     60

cgcgatcttg agcccggcga tcatgtggtc atggtcttca tgcccagttg cggacattgc    120

ctgccctgtg cggaaggcag gcccgctctg tgcgagccgg gcgccgccgc caatgcagca    180

ggcaggctgt tgggtggcgc cacccgcctg aactatcatg gcgaggtcgt ccatcatcac    240

cttggtgtgt cggcctttgc cgaatatgcc gtggtgtcgc gcaattcgct ggtcaagatc    300

gaccgcgatc ttccatttgt cgaggcggca ctcttcggct gcgcggttct caccggcgtc    360

ggcgccgtcg tgaatacggc aagggtcagg accggctcga ctgcggtcgt catcggactt    420

ggcggtgtgg gccttgccgc ggttctcgga gcccgggcgg ccggtgccag caagatcgtc    480

gccgtcgacc tttcgcagga aaagcttgca ctcgccagcg aactgggcgc gaccgccatc    540

gtgaacggac gcgatgagga tgccgtcgag caggtccgcg agctcacttc cggcggtgcc    600

gattatgcct tcgagatggc agggtctatt cgcgccctcg aaaacgcctt caggatgacc    660

aaacgtggcg gcaccaccgt taccgccggt ctgccaccgc cgggtgcggc cctgccgctc    720

aacgtcgtgc agctcgtcgg cgaggagcgg acactcaagg gcagctatat cggcacctgt    780

gtgcctctcc gggatattcc gcgcttcatc gccctttatc gcgacggccg gttgccggtg    840

aaccgccttc tgagcggaag gctgaagcta gaagacatca atgaagggtt cgaccgcctg    900

cacgacggaa gcgccgttcg gcaagtcatc gaattctga                            939
```

<210> SEQ ID NO 88
<211> LENGTH: 312
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 88

```
Met Pro Met Ala Leu Gly His Glu Ala Ala Gly Val Val Glu Ala Leu
1               5                   10                  15

Gly Glu Gly Val Arg Asp Leu Glu Pro Gly Asp His Val Val Met Val
            20                  25                  30

Phe Met Pro Ser Cys Gly His Cys Leu Pro Cys Ala Glu Gly Arg Pro
        35                  40                  45

Ala Leu Cys Glu Pro Gly Ala Ala Ala Asn Ala Ala Gly Arg Leu Leu
    50                  55                  60

Gly Gly Ala Thr Arg Leu Asn Tyr His Gly Glu Val Val His His His
```

-continued

65 70 75 80

Leu Gly Val Ser Ala Phe Ala Glu Tyr Ala Val Val Ser Arg Asn Ser
85 90 95

Leu Val Lys Ile Asp Arg Asp Leu Pro Phe Val Glu Ala Ala Leu Phe
100 105 110

Gly Cys Ala Val Leu Thr Gly Val Gly Ala Val Val Asn Thr Ala Arg
115 120 125

Val Arg Thr Gly Ser Thr Ala Val Val Ile Gly Leu Gly Gly Val Gly
130 135 140

Leu Ala Ala Val Leu Gly Ala Arg Ala Ala Gly Ala Ser Lys Ile Val
145 150 155 160

Ala Val Asp Leu Ser Gln Glu Lys Leu Ala Leu Ala Ser Glu Leu Gly
165 170 175

Ala Thr Ala Ile Val Asn Gly Arg Asp Glu Asp Ala Val Glu Gln Val
180 185 190

Arg Glu Leu Thr Ser Gly Gly Ala Asp Tyr Ala Phe Glu Met Ala Gly
195 200 205

Ser Ile Arg Ala Leu Glu Asn Ala Phe Arg Met Thr Lys Arg Gly Gly
210 215 220

Thr Thr Val Thr Ala Gly Leu Pro Pro Pro Gly Ala Ala Leu Pro Leu
225 230 235 240

Asn Val Val Gln Leu Val Gly Glu Glu Arg Thr Leu Lys Gly Ser Tyr
245 250 255

Ile Gly Thr Cys Val Pro Leu Arg Asp Ile Pro Arg Phe Ile Ala Leu
260 265 270

Tyr Arg Asp Gly Arg Leu Pro Val Asn Arg Leu Leu Ser Gly Arg Leu
275 280 285

Lys Leu Glu Asp Ile Asn Glu Gly Phe Asp Arg Leu His Asp Gly Ser
290 295 300

Ala Val Arg Gln Val Ile Glu Phe
305 310

<210> SEQ ID NO 89
<211> LENGTH: 1035
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 89

atgaaacatt ctcaggacaa accacgcctg ctgattgcga tgcgtagcga gcttccagaa 60

ggcttcttcg gtccgcgcga atgggcaagg ctgaatgccg tagcggacat tattccgggc 120

tttccccata cggatttcga cacggcgaac ggtgccgagg ctctcgccga agcggatatt 180

ctgctcgctg cctggggtac gccatccctg acacgcgaac gactttcacg cgcgccgcgg 240

ctgaaaatgc tggcctatgc ggcatcatcg gtgcggatgg ttgcgcccgc agaattctgg 300

gagacgtcgg atattctggt cacgacagca gcttccgcca tggccgtgcc ggttgccgaa 360

ttcacctatg cggcaatcat catgtgcggc aaggatgtgt ttcgattgcg ggatgaacat 420

agaacagagc gcggcaccgg cgtttttggc agcaggcgcg gcagaagcct gccctatctt 480

ggcaatcatg cccgcaaggt tggcattgtc ggcgcctcgc gcatcgggcg gctggtgatg 540

gagatgctgg cgcgcggcac attcgagatt gccgtttacg atcccctttct gtcggcggaa 600

gaggccgcat cccttggcgc gaagaaagcc gaactggacg agcttctcgc atggtccgat 660

-continued

gtggtctcgc tgcacgcgcc gatcctgccg gaaacgcacc atatgatcgg cgcccgcgaa 720

ctggcgctga tggcggacca tgccatcttc atcaacacgg cgcggggctg gctggtcgac 780

cacgatgcat tgctgactga agcgatttcc ggacggctgc gcattctgat tgacacgccc 840

gaacccgagc ccctgcccac ggacagcccg ttttacgatc tgcccaatgt cgttctaacc 900

ccccatatag ccggggcgct gggcaatgaa ttgcgcgcac tttccgatct ggccattacc 960

gaaattgaac gtttcgtggc gggacttgcg cccctccacc cggtccacaa gcaggatatg 1020

gaacgtatgg catga 1035

<210> SEQ ID NO 90
<211> LENGTH: 331
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 90

Met Arg Ser Glu Leu Pro Glu Gly Phe Phe Gly Pro Arg Glu Trp Ala
1 5 10 15

Arg Leu Asn Ala Val Ala Asp Ile Ile Pro Gly Phe Pro His Thr Asp
20 25 30

Phe Asp Thr Ala Asn Gly Ala Glu Ala Leu Ala Glu Ala Asp Ile Leu
35 40 45

Leu Ala Ala Trp Gly Thr Pro Ser Leu Thr Arg Glu Arg Leu Ser Arg
50 55 60

Ala Pro Arg Leu Lys Met Leu Ala Tyr Ala Ala Ser Ser Val Arg Met
65 70 75 80

Val Ala Pro Ala Glu Phe Trp Glu Thr Ser Asp Ile Leu Val Thr Thr
85 90 95

Ala Ala Ser Ala Met Ala Val Pro Val Ala Glu Phe Thr Tyr Ala Ala
100 105 110

Ile Ile Met Cys Gly Lys Asp Val Phe Arg Leu Arg Asp Glu His Arg
115 120 125

Thr Glu Arg Gly Thr Gly Val Phe Gly Ser Arg Arg Gly Arg Ser Leu
130 135 140

Pro Tyr Leu Gly Asn His Ala Arg Lys Val Gly Ile Val Gly Ala Ser
145 150 155 160

Arg Ile Gly Arg Leu Val Met Glu Met Leu Ala Arg Gly Thr Phe Glu
165 170 175

Ile Ala Val Tyr Asp Pro Phe Leu Ser Ala Glu Glu Ala Ala Ser Leu
180 185 190

Gly Ala Lys Lys Ala Glu Leu Asp Glu Leu Leu Ala Trp Ser Asp Val
195 200 205

Val Ser Leu His Ala Pro Ile Leu Pro Glu Thr His His Met Ile Gly
210 215 220

Ala Arg Glu Leu Ala Leu Met Ala Asp His Ala Ile Phe Ile Asn Thr
225 230 235 240

Ala Arg Gly Trp Leu Val Asp His Asp Ala Leu Leu Thr Glu Ala Ile
245 250 255

Ser Gly Arg Leu Arg Ile Leu Ile Asp Thr Pro Glu Pro Glu Pro Leu
260 265 270

Pro Thr Asp Ser Pro Phe Tyr Asp Leu Pro Asn Val Val Leu Thr Pro
275 280 285

-continued

```
His Ile Ala Gly Ala Leu Gly Asn Glu Leu Arg Ala Leu Ser Asp Leu
    290                 295                 300

Ala Ile Thr Glu Ile Glu Arg Phe Val Ala Gly Leu Ala Pro Leu His
305                 310                 315                 320

Pro Val His Lys Gln Asp Met Glu Arg Met Ala
                325                 330
```

<210> SEQ ID NO 91
<211> LENGTH: 750
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 91

```
atgcagcgtt ttaccaacag aaccatcgtt gtcgccgggg ccggccggga tatcggccgg     60

gcatgcgcca tccgtttcgc acaggaaggc gccaatgtcg ttcttaccta taatggcgcg    120

gcagagggcg cggccacagc cgttgccgaa atcgaaaagc ttggtcgttc ggctctggcg    180

atcaaggcgg atctcacaaa cgccgccgaa gtcgaggctg ccatatctgc ggctgcggac    240

aagtttgggg agatccacgg cctcgtccat gttgccggcg gcctgatcgc ccgcaagaca    300

atcgcagaaa tggatgaagc cttctggcat caggtcctcg acgtcaatct gacatcgctg    360

ttcctgacgg ccaagaccgc attgccgaag atggccaagg gcggcgcgat cgtcactttc    420

tcgtcgcagg ccggccgtga tggcggcggc ccgggcgctc ttgcctatgc cacttccaag    480

ggtgccgtga tgaccttcac ccgcggactt gccaaagaag tcggccccaa aatccgcgtc    540

aacgccgttt gccccggtat gatctccacc accttccacg ataccttcac caagccggag    600

gtgcgcgaac gggtggccgg cgcgacgtcg ctcaagcgcg aagggtcgag cgaagacgtc    660

gccggtctgg tggccttcct cgcgtctgac gatgccgctt atgtcaccgg cgcctgctac    720

gacatcaatg gcggcgtcct gttttcctga                                      750
```

<210> SEQ ID NO 92
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 92

```
Met Gln Arg Phe Thr Asn Arg Thr Ile Val Val Ala Gly Ala Gly Arg
1               5                   10                  15

Asp Ile Gly Arg Ala Cys Ala Ile Arg Phe Ala Gln Glu Gly Ala Asn
            20                  25                  30

Val Val Leu Thr Tyr Asn Gly Ala Ala Glu Gly Ala Ala Thr Ala Val
        35                  40                  45

Ala Glu Ile Glu Lys Leu Gly Arg Ser Ala Leu Ala Ile Lys Ala Asp
    50                  55                  60

Leu Thr Asn Ala Ala Glu Val Glu Ala Ala Ile Ser Ala Ala Ala Asp
65                  70                  75                  80

Lys Phe Gly Glu Ile His Gly Leu Val His Val Ala Gly Gly Leu Ile
                85                  90                  95

Ala Arg Lys Thr Ile Ala Glu Met Asp Glu Ala Phe Trp His Gln Val
            100                 105                 110

Leu Asp Val Asn Leu Thr Ser Leu Phe Leu Thr Ala Lys Thr Ala Leu
        115                 120                 125
```

-continued

```
Pro Lys Met Ala Lys Gly Gly Ala Ile Val Thr Phe Ser Ser Gln Ala
    130                 135                 140

Gly Arg Asp Gly Gly Gly Pro Gly Ala Leu Ala Tyr Ala Thr Ser Lys
145                 150                 155                 160

Gly Ala Val Met Thr Phe Thr Arg Gly Leu Ala Lys Glu Val Gly Pro
                165                 170                 175

Lys Ile Arg Val Asn Ala Val Cys Pro Gly Met Ile Ser Thr Thr Phe
            180                 185                 190

His Asp Thr Phe Thr Lys Pro Glu Val Arg Glu Arg Val Ala Gly Ala
        195                 200                 205

Thr Ser Leu Lys Arg Glu Gly Ser Ser Glu Asp Val Ala Gly Leu Val
    210                 215                 220

Ala Phe Leu Ala Ser Asp Asp Ala Ala Tyr Val Thr Gly Ala Cys Tyr
225                 230                 235                 240

Asp Ile Asn Gly Gly Val Leu Phe Ser
                245
```

<210> SEQ ID NO 93
<211> LENGTH: 930
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli DH10B

<400> SEQUENCE: 93

```
atgtccaaaa agattgccgt gattggcgaa tgcatgattg agctttccga gaaaggcgcg     60

gacgttaagc gcggtttcgg cggcgatacc ctgaacactt ccgtctatat cgcccgtcag    120

gtcgatcctg cggcattaac cgttcattac gtaacggcgc tgggaacgga cagttttagc    180

cagcagatgc tggacgcctg gcacggcgag aacgttgata cttccctgac ccaacggatg    240

gaaaaccgtc tgccgggcct ttactacatt gaaaccgaca gcaccggcga gcgtacgttc    300

tactactggc ggaacgaagc cgccgccaaa ttctggctgg agagtgagca gtctgcggcg    360

atttgcgaag agctggcgaa tttcgattat ctctacctga gcgggattag cctggcgatc    420

ttaagcccga ccagccgcga aaagctgctt tccctgctgc gcgaatgccg cgccaacggc    480

ggaaaagtga ttttcgacaa taactatcgt ccgcgcctgt gggccagcaa agaagagaca    540

cagcaggtgt accaacaaat gctggaatgc acggatatcg ccttcctgac gctggacgac    600

gaagacgcgc tgtggggtca acagccggtg gaagacgtca ttgcgcgcac ccataacgcg    660

ggcgtgaaag aagtggtggt gaaacgcggg gcggattctt gcctggtgtc cattgctggc    720

gaagggttag tggatgttcc ggcggtgaaa ctgccgaaag aaaaagtgat cgataccacc    780

gcagctggcg actctttcag tgccggttat ctggcggtac gtctgacagg cggcagcgcg    840

gaagacgcgg cgaaacgtgg gcacctgacc gcaagtaccg ttattcagta tcgcggcgcg    900

attatcccgc gtgaggcgat gccagcgtaa                                     930
```

<210> SEQ ID NO 94
<211> LENGTH: 309
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli DH10B

<400> SEQUENCE: 94

```
Met Ser Lys Lys Ile Ala Val Ile Gly Glu Cys Met Ile Glu Leu Ser
1               5                   10                  15

Glu Lys Gly Ala Asp Val Lys Arg Gly Phe Gly Gly Asp Thr Leu Asn
```

-continued

```
            20                  25                  30

Thr Ser Val Tyr Ile Ala Arg Gln Val Asp Pro Ala Ala Leu Thr Val
        35                  40                  45

His Tyr Val Thr Ala Leu Gly Thr Asp Ser Phe Ser Gln Gln Met Leu
    50                  55                  60

Asp Ala Trp His Gly Glu Asn Val Asp Thr Ser Leu Thr Gln Arg Met
65                  70                  75                  80

Glu Asn Arg Leu Pro Gly Leu Tyr Tyr Ile Glu Thr Asp Ser Thr Gly
                85                  90                  95

Glu Arg Thr Phe Tyr Tyr Trp Arg Asn Glu Ala Ala Ala Lys Phe Trp
            100                 105                 110

Leu Glu Ser Glu Gln Ser Ala Ala Ile Cys Glu Glu Leu Ala Asn Phe
        115                 120                 125

Asp Tyr Leu Tyr Leu Ser Gly Ile Ser Leu Ala Ile Leu Ser Pro Thr
    130                 135                 140

Ser Arg Glu Lys Leu Leu Ser Leu Leu Arg Glu Cys Arg Ala Asn Gly
145                 150                 155                 160

Gly Lys Val Ile Phe Asp Asn Asn Tyr Arg Pro Arg Leu Trp Ala Ser
                165                 170                 175

Lys Glu Glu Thr Gln Gln Val Tyr Gln Gln Met Leu Glu Cys Thr Asp
            180                 185                 190

Ile Ala Phe Leu Thr Leu Asp Asp Glu Asp Ala Leu Trp Gly Gln Gln
        195                 200                 205

Pro Val Glu Asp Val Ile Ala Arg Thr His Asn Ala Gly Val Lys Glu
    210                 215                 220

Val Val Val Lys Arg Gly Ala Asp Ser Cys Leu Val Ser Ile Ala Gly
225                 230                 235                 240

Glu Gly Leu Val Asp Val Pro Ala Val Lys Leu Pro Lys Glu Lys Val
                245                 250                 255

Ile Asp Thr Thr Ala Ala Gly Asp Ser Phe Ser Ala Gly Tyr Leu Ala
            260                 265                 270

Val Arg Leu Thr Gly Gly Ser Ala Glu Asp Ala Ala Lys Arg Gly His
        275                 280                 285

Leu Thr Ala Ser Thr Val Ile Gln Tyr Arg Gly Ala Ile Ile Pro Arg
    290                 295                 300

Glu Ala Met Pro Ala
305


<210> SEQ ID NO 95
<211> LENGTH: 642
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli DH10B

<400> SEQUENCE: 95

atgaaaaact ggaaaacaag tgcagaatca atcctgacca ccggcccggt tgtaccggtt     60

atcgtggtaa aaaaactgga acacgcggtg ccgatggcaa aagcgttggt tgctggtggg    120

gtgcgcgttc tggaagtgac tctgcgtacc gagtgtgcag ttgacgctat ccgtgctatc    180

gccaaagaag tgcctgaagc gattgtgggt gccggtacgg tgctgaatcc acagcagctg    240

gcagaagtca ctgaagcggg tgcacagttc gcaattagcc cgggtctgac cgagccgctg    300

ctgaaagctg ctaccgaagg gactattcct ctgattccgg ggatcagcac tgtttccgaa    360
```

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ctgatgctgg gtatggacta cggtttgaaa gagttcaaat tcttcccggc tgaagctaac 420

ggcggcgtga aagccctgca ggcgatcgcg ggtccgttct cccaggtccg tttctgcccg 480

acgggtggta tttctccggc taactaccgt gactacctgg cgctgaaaag cgtgctgtgc 540

atcggtggtt cctggctggt tccggcagat gcgctggaag cgggcgatta cgaccgcatt 600

actaagctgg cgcgtgaagc tgtagaaggc gctaagctgt aa 642

<210> SEQ ID NO 96
<211> LENGTH: 213
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli DH10B

<400> SEQUENCE: 96

Met Lys Asn Trp Lys Thr Ser Ala Glu Ser Ile Leu Thr Thr Gly Pro
1 5 10 15

Val Val Pro Val Ile Val Val Lys Lys Leu Glu His Ala Val Pro Met
20 25 30

Ala Lys Ala Leu Val Ala Gly Gly Val Arg Val Leu Glu Val Thr Leu
35 40 45

Arg Thr Glu Cys Ala Val Asp Ala Ile Arg Ala Ile Ala Lys Glu Val
50 55 60

Pro Glu Ala Ile Val Gly Ala Gly Thr Val Leu Asn Pro Gln Gln Leu
65 70 75 80

Ala Glu Val Thr Glu Ala Gly Ala Gln Phe Ala Ile Ser Pro Gly Leu
85 90 95

Thr Glu Pro Leu Leu Lys Ala Ala Thr Glu Gly Thr Ile Pro Leu Ile
100 105 110

Pro Gly Ile Ser Thr Val Ser Glu Leu Met Leu Gly Met Asp Tyr Gly
115 120 125

Leu Lys Glu Phe Lys Phe Phe Pro Ala Glu Ala Asn Gly Gly Val Lys
130 135 140

Ala Leu Gln Ala Ile Ala Gly Pro Phe Ser Gln Val Arg Phe Cys Pro
145 150 155 160

Thr Gly Gly Ile Ser Pro Ala Asn Tyr Arg Asp Tyr Leu Ala Leu Lys
165 170 175

Ser Val Leu Cys Ile Gly Gly Ser Trp Leu Val Pro Ala Asp Ala Leu
180 185 190

Glu Ala Gly Asp Tyr Asp Arg Ile Thr Lys Leu Ala Arg Glu Ala Val
195 200 205

Glu Gly Ala Lys Leu
210

<210> SEQ ID NO 97
<211> LENGTH: 780
<212> TYPE: DNA
<213> ORGANISM: Lactobaccilus brevis ATCC 367

<400> SEQUENCE: 97

atggcatcaa atggaaaagt agcaatggtt accggtggcg gacaaggaat tggtgaagcc 60

atctcgaaac ggttagctaa cgacggcttt gctgtggcaa ttgctgattt gaacttggac 120

aatgccaaca aggtcgtttc tgatattgaa gctgctggtg gcaaggccat tgcggtcaag 180

accgatgtct ctgatcgtga tagcgtgttt gctgcggtta atgaagcggc cgacaagctg 240

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ggcggctttg acgttatcgt taataacgcc ggccttggcc caaccacgcc aattgacacc 300

atcacccaag aacagtttga tacggtttat cacgttaacg tgggtggggt tctttggggc 360

attcaagcag cccatgcgaa gttcaaggaa ttgggtcatg gtgggaagat catttccgcg 420

acgtctcaag ccggggttgt tggtaacccg aacttagctc tgtacagtgg aactaagttt 480

gccattcgtg gtgtgaccca agttgcggcg cgtgacttag ccgctgaagg tatcacggtc 540

aatgcttatg cacccgggat tgttaagaca ccaatgatgt ttgacatcgc tcacaaggtt 600

ggtcaaaatg ctggtaaaga cgacgaatgg gggatgcaaa ccttctcaaa ggacatcgct 660

ttatgtcgat tgtcagaacc agaagatgtg gctaacgggg tggctttctt agccggtccc 720

gattctaact acattacggg tcaaacactt gaagttgatg gtgggatgca gttccactaa 780

<210> SEQ ID NO 98
<211> LENGTH: 259
<212> TYPE: PRT
<213> ORGANISM: Lactobaccilus brevis ATCC 367

<400> SEQUENCE: 98

Met Ala Ser Asn Gly Lys Val Ala Met Val Thr Gly Gly Gly Gln Gly
1 5 10 15

Ile Gly Glu Ala Ile Ser Lys Arg Leu Ala Asn Asp Gly Phe Ala Val
            20 25 30

Ala Ile Ala Asp Leu Asn Leu Asp Asn Ala Asn Lys Val Val Ser Asp
        35 40 45

Ile Glu Ala Ala Gly Gly Lys Ala Ile Ala Val Lys Thr Asp Val Ser
    50 55 60

Asp Arg Asp Ser Val Phe Ala Ala Val Asn Glu Ala Ala Asp Lys Leu
65 70 75 80

Gly Gly Phe Asp Val Ile Val Asn Asn Ala Gly Leu Gly Pro Thr Thr
                85 90 95

Pro Ile Asp Thr Ile Thr Gln Glu Gln Phe Asp Thr Val Tyr His Val
            100 105 110

Asn Val Gly Gly Val Leu Trp Gly Ile Gln Ala Ala His Ala Lys Phe
        115 120 125

Lys Glu Leu Gly His Gly Gly Lys Ile Ile Ser Ala Thr Ser Gln Ala
    130 135 140

Gly Val Val Gly Asn Pro Asn Leu Ala Leu Tyr Ser Gly Thr Lys Phe
145 150 155 160

Ala Ile Arg Gly Val Thr Gln Val Ala Ala Arg Asp Leu Ala Ala Glu
                165 170 175

Gly Ile Thr Val Asn Ala Tyr Ala Pro Gly Ile Val Lys Thr Pro Met
            180 185 190

Met Phe Asp Ile Ala His Lys Val Gly Gln Asn Ala Gly Lys Asp Asp
        195 200 205

Glu Trp Gly Met Gln Thr Phe Ser Lys Asp Ile Ala Leu Cys Arg Leu
    210 215 220

Ser Glu Pro Glu Asp Val Ala Asn Gly Val Ala Phe Leu Ala Gly Pro
225 230 235 240

Asp Ser Asn Tyr Ile Thr Gly Gln Thr Leu Glu Val Asp Gly Gly Met
                245 250 255

Gln Phe His

-continued

<210> SEQ ID NO 99
<211> LENGTH: 1089
<212> TYPE: DNA
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 99

atgaatgacc tgagccacac ccacatgcgc gcggccgtct ggcatggccg ccacgatatt 60

cgtgtcgaac aggtaccttt gccggccgac cctgcgccgg gctgggtgca gatcaaggtg 120

gactggtgcg gcatctgcgg ctccgacctg cacgaatatg ttgccggccc ggtgttcatc 180

ccggtagagg ccccgcaccc gctgaccggc attcagggcc agtgcatcct cggccacgaa 240

ttctgcggcc acatcgccaa gcttggcgaa ggcgtggaag gctatgccgt aggcgacccg 300

gtggcggcag acgcgtgcca gcattgtggt acctgctatt actgcaccca tggcctgtac 360

aacatctgcg aacgcctggc gttcaccggc ctgatgaaca acggtgcctt cgccgagctg 420

gtcaacgtgc ccgccaacct gctctaccgg ctgccgcagg gcttccctgc cgaagccggg 480

gcactgatcg agccgctggc ggtgggtatg cacgcggtga aaaaggccgg cagcctgctt 540

gggcaaaccg ttgtagtggt tggggccggc accatcggcc tgtgcaccat catgtgcgcc 600

aaggctgcag gtgcggcaca ggtcatcgcc cttgagatgt cctctgcgcg caaagccaag 660

gccaaggaag cgggcgccaa cgtggtgctg gaccccagcc agtgcgatgc cctggcggaa 720

atccgcgcac tgactgctgg gctgggcgcc gatgtgagtt ttgagtgcat cggcaacaaa 780

catacggcca agctggccat cgacaccatc cgcaaagcag gcaagtgcgt gctggtgggt 840

attttcgaag agcccagcga gttcaacttc ttcgagctgg tgtccaccga gaagcaagtg 900

ctgggggcgt tggcgtacaa cggcgagttt gctgacgtga ttgccttcat tgctgatggt 960

cggctggata ttcgcccgct ggtaaccggc cggatcggat tggagcagat tgtcgagctg 1020

ggcttcgagg aactggtgaa caacaaagag gagaacgtga agatcatcgt ttcaccaggt 1080

gtgcgctga 1089

<210> SEQ ID NO 100
<211> LENGTH: 362
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 100

Met Asn Asp Leu Ser His Thr His Met Arg Ala Ala Val Trp His Gly
1 5 10 15

Arg His Asp Ile Arg Val Glu Gln Val Pro Leu Pro Ala Asp Pro Ala
20 25 30

Pro Gly Trp Val Gln Ile Lys Val Asp Trp Cys Gly Ile Cys Gly Ser
35 40 45

Asp Leu His Glu Tyr Val Ala Gly Pro Val Phe Ile Pro Val Glu Ala
50 55 60

Pro His Pro Leu Thr Gly Ile Gln Gly Gln Cys Ile Leu Gly His Glu
65 70 75 80

Phe Cys Gly His Ile Ala Lys Leu Gly Glu Gly Val Glu Gly Tyr Ala
85 90 95

Val Gly Asp Pro Val Ala Ala Asp Ala Cys Gln His Cys Gly Thr Cys
100 105 110

Tyr Tyr Cys Thr His Gly Leu Tyr Asn Ile Cys Glu Arg Leu Ala Phe

-continued

```
        115                 120                 125

Thr Gly Leu Met Asn Asn Gly Ala Phe Ala Glu Leu Val Asn Val Pro
    130                 135                 140

Ala Asn Leu Leu Tyr Arg Leu Pro Gln Gly Phe Pro Ala Glu Ala Gly
145                 150                 155                 160

Ala Leu Ile Glu Pro Leu Ala Val Gly Met His Ala Val Lys Lys Ala
                165                 170                 175

Gly Ser Leu Leu Gly Gln Thr Val Val Val Val Gly Ala Gly Thr Ile
            180                 185                 190

Gly Leu Cys Thr Ile Met Cys Ala Lys Ala Ala Gly Ala Ala Gln Val
        195                 200                 205

Ile Ala Leu Glu Met Ser Ser Ala Arg Lys Ala Lys Ala Lys Glu Ala
    210                 215                 220

Gly Ala Asn Val Val Leu Asp Pro Ser Gln Cys Asp Ala Leu Ala Glu
225                 230                 235                 240

Ile Arg Ala Leu Thr Ala Gly Leu Gly Ala Asp Val Ser Phe Glu Cys
                245                 250                 255

Ile Gly Asn Lys His Thr Ala Lys Leu Ala Ile Asp Thr Ile Arg Lys
            260                 265                 270

Ala Gly Lys Cys Val Leu Val Gly Ile Phe Glu Glu Pro Ser Glu Phe
        275                 280                 285

Asn Phe Phe Glu Leu Val Ser Thr Glu Lys Gln Val Leu Gly Ala Leu
    290                 295                 300

Ala Tyr Asn Gly Glu Phe Ala Asp Val Ile Ala Phe Ile Ala Asp Gly
305                 310                 315                 320

Arg Leu Asp Ile Arg Pro Leu Val Thr Gly Arg Ile Gly Leu Glu Gln
                325                 330                 335

Ile Val Glu Leu Gly Phe Glu Glu Leu Val Asn Asn Lys Glu Glu Asn
            340                 345                 350

Val Lys Ile Ile Val Ser Pro Gly Val Arg
        355                 360
```

<210> SEQ ID NO 101
<211> LENGTH: 771
<212> TYPE: DNA
<213> ORGANISM: Klebsiella pneumoniae MGH78578

<400> SEQUENCE: 101

```
atgaaaaaag tcgcacttgt taccggcgcc ggccagggga ttggtaaagc tatcgccctt     60

cgtctggtga aggatggatt tgccgtggcc attgccgatt ataacgacgc caccgccaaa    120

gcggtcgcct cggaaatcaa ccaggccggc ggacacgccg tggcggtgaa agtggatgtc    180

tccgaccgcg atcaggtatt tgccgccgtt gaacaggcgc gcaaaacgct gggcggcttc    240

gacgtcatcg tcaataacgc cggtgtggca ccgtctacgc cgatcgagtc cattaccccg    300

gagattgtcg acaaagtcta caacatcaac gtcaaagggg tgatctgggg tattcaggcg    360

gcggtcgagg cctttaagaa agaggggcac ggcgggaaaa tcatcaacgc ctgttcccag    420

gccggccacg tcggcaaccc ggagctggcg gtgtatagct ccagtaaatt cgcggtacgc    480

ggcttaaccc agaccgccgc tcgcgacctc gcgccgctgg gcatcacggt caacggctac    540

tgcccgggga ttgtcaaaac gccaatgtgg gccgaaattg accgccaggt gtccgaagcc    600

gccggtaaac cgctgggcta cggtaccgcc gagttcgcca aacgcatcac tctcggtcgt    660
```

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ctgtccgagc cggaagatgt cgccgcctgc gtctcctatc ttgccagccc ggattctgat 720

tacatgaccg gtcagtcgtt gctgatcgac ggcgggatgg tatttaacta a 771

<210> SEQ ID NO 102
<211> LENGTH: 256
<212> TYPE: PRT
<213> ORGANISM: Klebsiella pneumoniae MGH78578

<400> SEQUENCE: 102

Met Lys Lys Val Ala Leu Val Thr Gly Ala Gly Gln Gly Ile Gly Lys
1 5 10 15

Ala Ile Ala Leu Arg Leu Val Lys Asp Gly Phe Ala Val Ala Ile Ala
20 25 30

Asp Tyr Asn Asp Ala Thr Ala Lys Ala Val Ala Ser Glu Ile Asn Gln
35 40 45

Ala Gly Gly His Ala Val Ala Val Lys Val Asp Val Ser Asp Arg Asp
50 55 60

Gln Val Phe Ala Ala Val Glu Gln Ala Arg Lys Thr Leu Gly Gly Phe
65 70 75 80

Asp Val Ile Val Asn Asn Ala Gly Val Ala Pro Ser Thr Pro Ile Glu
85 90 95

Ser Ile Thr Pro Glu Ile Val Asp Lys Val Tyr Asn Ile Asn Val Lys
100 105 110

Gly Val Ile Trp Gly Ile Gln Ala Ala Val Glu Ala Phe Lys Lys Glu
115 120 125

Gly His Gly Gly Lys Ile Ile Asn Ala Cys Ser Gln Ala Gly His Val
130 135 140

Gly Asn Pro Glu Leu Ala Val Tyr Ser Ser Ser Lys Phe Ala Val Arg
145 150 155 160

Gly Leu Thr Gln Thr Ala Ala Arg Asp Leu Ala Pro Leu Gly Ile Thr
165 170 175

Val Asn Gly Tyr Cys Pro Gly Ile Val Lys Thr Pro Met Trp Ala Glu
180 185 190

Ile Asp Arg Gln Val Ser Glu Ala Ala Gly Lys Pro Leu Gly Tyr Gly
195 200 205

Thr Ala Glu Phe Ala Lys Arg Ile Thr Leu Gly Arg Leu Ser Glu Pro
210 215 220

Glu Asp Val Ala Ala Cys Val Ser Tyr Leu Ala Ser Pro Asp Ser Asp
225 230 235 240

Tyr Met Thr Gly Gln Ser Leu Leu Ile Asp Gly Gly Met Val Phe Asn
245 250 255

<210> SEQ ID NO 103
<211> LENGTH: 1665
<212> TYPE: DNA
<213> ORGANISM: Klebsiella pneumoniae MGH78578

<400> SEQUENCE: 103

atgagatcga aaagatttga agcactggcg aaacgccctg tgaatcagga tggtttcgtt 60

aaggagtgga ttgaagaggg ctttatcgcg atggaaagcc ctaacgatcc caaaccttct 120

atccgcatcg tcaacggcgc ggtgaccgaa ctcgacgata aaccggttga gcagttcgac 180

ctgattgacc actttatcgc gcgctacggc attaatctcg cccgggccga agaagtgatg 240

-continued

gccatggatt cggttaagct cgccaacatg ctctgcgacc cgaacgttaa acgcagcgac 300

atcgtgccgc tcactaccgc gatgaccccg gcgaaaatcg tggaagtggt gtcgcatatg 360

aacgtggtcg agatgatgat ggcgatgcaa aaaatgcgcg cccgccgcac gccgtcccag 420

caggcgcatg tcactaatat caaagataat ccggtacaga ttgccgccga cgccgctgaa 480

ggcgcatggc gcggctttga cgagcaggag accaccgtcg ccgtggcgcg ctacgcgccg 540

ttcaacgcca tcgccctgct ggtcggttca caggttggcc gccccggcgt cctcacccag 600

tgttcgctgg aagaagccac cgagctgaaa ctgggcatgc tgggccacac ctgctatgcc 660

gaaaccattt cggtatacgg tacggaaccg gtgtttaccg atggcgatga caccccgtgg 720

tcgaaaggct tcctcgcctc ctcctacgcc tcgcgcggcc tgaaaatgcg ctttacctcc 780

ggttccggct cggaggtgca gatgggctat gccgaaggca aatcgatgct ttatctcgaa 840

gcgcgctgca tctacatcac caaagccgcc ggggtgcaag gcctgcagaa tggctccgtc 900

agctgtatcg gcgtgccgtc cgccgtgccg tccgggatcc gcgccgtact ggcggaaaac 960

ctgatctgct cagcgctgga tctggagtgc gcctccagca acgatcaaac ctttacccac 1020

tcggatatgc ggcgtaccgc gcgtctgctg atgcagttcc tgccaggtac cgactttatc 1080

tcctccggtt actcggcggt gccgaactac gacaacatgt tcgccggttc caacgaagat 1140

gccgaagact tcgatgacta caacgtgatc cagcgcgacc tgaaggtcga tggcggcctg 1200

cggccggtgc gtgaagagga cgtgatcgcc attcgcaaca aagccgcccg cgcgctgcag 1260

gcggtatttg ccggcatggg tttgccgcct attacggatg aagaagtaga agccgccacc 1320

tacgcccacg gttcaaaaga tatgcctgag cgcaatatcg tcgaggacat caagtttgct 1380

caggagatca tcaacaagaa ccgcaacggc ctggaggtgg tgaaagccct ggcgaaaggc 1440

ggcttccccg atgtcgccca ggacatgctc aatattcaga aagccaagct caccggcgac 1500

tacctgcata cctccgccat cattgttggc gagggccagg tgctctcggc cgtgaatgac 1560

gtgaacgatt atgccggtcc ggcaacaggc taccgcctgc aaggcgagcg ctgggaagag 1620

attaaaaata tcccgggcgc gctcgatccc aatgaacttg gctaa 1665

<210> SEQ ID NO 104
<211> LENGTH: 554
<212> TYPE: PRT
<213> ORGANISM: Klebsiella pneumoniae MGH78578

<400> SEQUENCE: 104

Met Arg Ser Lys Arg Phe Glu Ala Leu Ala Lys Arg Pro Val Asn Gln
1 5 10 15

Asp Gly Phe Val Lys Glu Trp Ile Glu Glu Gly Phe Ile Ala Met Glu
20 25 30

Ser Pro Asn Asp Pro Lys Pro Ser Ile Arg Ile Val Asn Gly Ala Val
35 40 45

Thr Glu Leu Asp Asp Lys Pro Val Glu Gln Phe Asp Leu Ile Asp His
50 55 60

Phe Ile Ala Arg Tyr Gly Ile Asn Leu Ala Arg Ala Glu Glu Val Met
65 70 75 80

Ala Met Asp Ser Val Lys Leu Ala Asn Met Leu Cys Asp Pro Asn Val
85 90 95

Lys Arg Ser Asp Ile Val Pro Leu Thr Thr Ala Met Thr Pro Ala Lys

-continued

```
           100                 105                 110

Ile Val Glu Val Val Ser His Met Asn Val Val Glu Met Met Met Ala
        115                 120                 125

Met Gln Lys Met Arg Ala Arg Arg Thr Pro Ser Gln Gln Ala His Val
    130                 135                 140

Thr Asn Ile Lys Asp Asn Pro Val Gln Ile Ala Ala Asp Ala Ala Glu
145                 150                 155                 160

Gly Ala Trp Arg Gly Phe Asp Glu Gln Glu Thr Thr Val Ala Val Ala
                165                 170                 175

Arg Tyr Ala Pro Phe Asn Ala Ile Ala Leu Leu Val Gly Ser Gln Val
            180                 185                 190

Gly Arg Pro Gly Val Leu Thr Gln Cys Ser Leu Glu Glu Ala Thr Glu
        195                 200                 205

Leu Lys Leu Gly Met Leu Gly His Thr Cys Tyr Ala Glu Thr Ile Ser
    210                 215                 220

Val Tyr Gly Thr Glu Pro Val Phe Thr Asp Gly Asp Asp Thr Pro Trp
225                 230                 235                 240

Ser Lys Gly Phe Leu Ala Ser Ser Tyr Ala Ser Arg Gly Leu Lys Met
                245                 250                 255

Arg Phe Thr Ser Gly Ser Gly Ser Glu Val Gln Met Gly Tyr Ala Glu
            260                 265                 270

Gly Lys Ser Met Leu Tyr Leu Glu Ala Arg Cys Ile Tyr Ile Thr Lys
        275                 280                 285

Ala Ala Gly Val Gln Gly Leu Gln Asn Gly Ser Val Ser Cys Ile Gly
    290                 295                 300

Val Pro Ser Ala Val Pro Ser Gly Ile Arg Ala Val Leu Ala Glu Asn
305                 310                 315                 320

Leu Ile Cys Ser Ala Leu Asp Leu Glu Cys Ala Ser Ser Asn Asp Gln
                325                 330                 335

Thr Phe Thr His Ser Asp Met Arg Arg Thr Ala Arg Leu Leu Met Gln
            340                 345                 350

Phe Leu Pro Gly Thr Asp Phe Ile Ser Ser Gly Tyr Ser Ala Val Pro
        355                 360                 365

Asn Tyr Asp Asn Met Phe Ala Gly Ser Asn Glu Asp Ala Glu Asp Phe
    370                 375                 380

Asp Asp Tyr Asn Val Ile Gln Arg Asp Leu Lys Val Asp Gly Gly Leu
385                 390                 395                 400

Arg Pro Val Arg Glu Glu Asp Val Ile Ala Ile Arg Asn Lys Ala Ala
                405                 410                 415

Arg Ala Leu Gln Ala Val Phe Ala Gly Met Gly Leu Pro Pro Ile Thr
            420                 425                 430

Asp Glu Glu Val Glu Ala Ala Thr Tyr Ala His Gly Ser Lys Asp Met
        435                 440                 445

Pro Glu Arg Asn Ile Val Glu Asp Ile Lys Phe Ala Gln Glu Ile Ile
    450                 455                 460

Asn Lys Asn Arg Asn Gly Leu Glu Val Val Lys Ala Leu Ala Lys Gly
465                 470                 475                 480

Gly Phe Pro Asp Val Ala Gln Asp Met Leu Asn Ile Gln Lys Ala Lys
                485                 490                 495

Leu Thr Gly Asp Tyr Leu His Thr Ser Ala Ile Ile Val Gly Glu Gly
            500                 505                 510
```

-continued

```
Gln Val Leu Ser Ala Val Asn Asp Val Asn Asp Tyr Ala Gly Pro Ala
        515                 520                 525

Thr Gly Tyr Arg Leu Gln Gly Glu Arg Trp Glu Glu Ile Lys Asn Ile
    530                 535                 540

Pro Gly Ala Leu Asp Pro Asn Glu Leu Gly
545                 550
```

<210> SEQ ID NO 105
<211> LENGTH: 690
<212> TYPE: DNA
<213> ORGANISM: Klebsiella pneumoniae MGH78578

<400> SEQUENCE: 105

```
atggaaatta acgaaacgct gctgcgccag attatcgaag aggtgctgtc ggagatgaaa     60

tcaggcgcag ataagccggt ctcctttagc gcgcctgcgg cttctgtcgc ctctgccgcg    120

ccggtcgccg ttgcgcctgt gtccggcgac agcttcctga cggaaatcgg cgaagccaaa    180

cccggcacgc agcaggatga agtcattatt gccgtcgggc cagcgtttgg tctggcgcaa    240

accgccaata tcgtcggcat tccgcataaa aatattctgc gcgaagtgat cgccggcatt    300

gaggaagaag gcatcaaagc ccgggtgatc cgctgcttta agtcttctga cgtcgccttc    360

gtggcagtgg aaggcaaccg cctgagcggc tccggcatct cgatcggtat tcagtcgaaa    420

ggcaccaccg tcatccacca gcgcggcctg ccgccgcttt ccaatctgga actcttcccg    480

caggcgccgc tgctgacgct ggaaacctac cgtcagattg gcaaaaacgc cgcgcgctac    540

gccaaacgcg agtcgccgca gccggtgccg acgcttaacg atcagatggc tcgtcccaaa    600

taccaggcga agtcggccat tttgcacatt aaagagacca aatacgtggt gacgggcaaa    660

aacccgcagg aactgcgcgt ggcgctttaa                                     690
```

<210> SEQ ID NO 106
<211> LENGTH: 229
<212> TYPE: PRT
<213> ORGANISM: Klebsiella pneumoniae MGH78578

<400> SEQUENCE: 106

```
Met Glu Ile Asn Glu Thr Leu Leu Arg Gln Ile Ile Glu Glu Val Leu
1               5                   10                  15

Ser Glu Met Lys Ser Gly Ala Asp Lys Pro Val Ser Phe Ser Ala Pro
            20                  25                  30

Ala Ala Ser Val Ala Ser Ala Ala Pro Val Ala Val Ala Pro Val Ser
        35                  40                  45

Gly Asp Ser Phe Leu Thr Glu Ile Gly Glu Ala Lys Pro Gly Thr Gln
    50                  55                  60

Gln Asp Glu Val Ile Ile Ala Val Gly Pro Ala Phe Gly Leu Ala Gln
65                  70                  75                  80

Thr Ala Asn Ile Val Gly Ile Pro His Lys Asn Ile Leu Arg Glu Val
                85                  90                  95

Ile Ala Gly Ile Glu Glu Glu Gly Ile Lys Ala Arg Val Ile Arg Cys
            100                 105                 110

Phe Lys Ser Ser Asp Val Ala Phe Val Ala Val Glu Gly Asn Arg Leu
        115                 120                 125

Ser Gly Ser Gly Ile Ser Ile Gly Ile Gln Ser Lys Gly Thr Thr Val
    130                 135                 140
```

-continued

```
Ile His Gln Arg Gly Leu Pro Pro Leu Ser Asn Leu Glu Leu Phe Pro
145                 150                 155                 160

Gln Ala Pro Leu Leu Thr Leu Glu Thr Tyr Arg Gln Ile Gly Lys Asn
                165                 170                 175

Ala Ala Arg Tyr Ala Lys Arg Glu Ser Pro Gln Pro Val Pro Thr Leu
            180                 185                 190

Asn Asp Gln Met Ala Arg Pro Lys Tyr Gln Ala Lys Ser Ala Ile Leu
        195                 200                 205

His Ile Lys Glu Thr Lys Tyr Val Val Thr Gly Lys Asn Pro Gln Glu
    210                 215                 220

Leu Arg Val Ala Leu
225
```

<210> SEQ ID NO 107
<211> LENGTH: 525
<212> TYPE: DNA
<213> ORGANISM: Klebsiella pneumoniae MGH78578

<400> SEQUENCE: 107

```
atgaataccg acgcaattga atccatggta cgcgacgtgc tgagccggat gaacagccta     60

caggacggga taacgcccgc gccagccgcg ccgacaaacg acaccgttcg ccagccaaaa    120

gttagcgact acccgttagc gacccgccat ccggagtggg tcaaaaccgc taccaataaa    180

acgctcgatg acctgacgct ggagaacgta ttaagcgatc gcgttacggc gcaggacatg    240

cgcatcactc cggaaacgct gcgtatgcag gcggcgatcg cccaggatgc cggacgcgat    300

cggctggcga tgaactttga gcgggccgca gagctcaccg cggttcccga cgaccgaatc    360

cttgagatct acaacgccct gcgcccatac cgttccaccc aggcggagct actggcgatc    420

gctgatgacc tcgagcatcg ctaccaggca cgactctgtg ccgcctttgt tcgggaagcg    480

gccgggctgt acatcgagcg taagaagctg aaaggcgacg attaa                    525
```

<210> SEQ ID NO 108
<211> LENGTH: 174
<212> TYPE: PRT
<213> ORGANISM: Klebsiella pneumoniae MGH78578

<400> SEQUENCE: 108

```
Met Asn Thr Asp Ala Ile Glu Ser Met Val Arg Asp Val Leu Ser Arg
1               5                   10                  15

Met Asn Ser Leu Gln Asp Gly Ile Thr Pro Ala Pro Ala Ala Pro Thr
            20                  25                  30

Asn Asp Thr Val Arg Gln Pro Lys Val Ser Asp Tyr Pro Leu Ala Thr
        35                  40                  45

Arg His Pro Glu Trp Val Lys Thr Ala Thr Asn Lys Thr Leu Asp Asp
    50                  55                  60

Leu Thr Leu Glu Asn Val Leu Ser Asp Arg Val Thr Ala Gln Asp Met
65                  70                  75                  80

Arg Ile Thr Pro Glu Thr Leu Arg Met Gln Ala Ala Ile Ala Gln Asp
                85                  90                  95

Ala Gly Arg Asp Arg Leu Ala Met Asn Phe Glu Arg Ala Ala Glu Leu
            100                 105                 110

Thr Ala Val Pro Asp Asp Arg Ile Leu Glu Ile Tyr Asn Ala Leu Arg
        115                 120                 125
```

-continued

```
Pro Tyr Arg Ser Thr Gln Ala Glu Leu Leu Ala Ile Ala Asp Asp Leu
    130                 135                 140

Glu His Arg Tyr Gln Ala Arg Leu Cys Ala Ala Phe Val Arg Glu Ala
145                 150                 155                 160

Ala Gly Leu Tyr Ile Glu Arg Lys Lys Leu Lys Gly Asp Asp
                165                 170
```

<210> SEQ ID NO 109
<211> LENGTH: 789
<212> TYPE: DNA
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 109

```
atgacagtca attatgattt ttccggaaaa gtcgtgctgg ttaccggcgc tggctctggt     60

attggccgtg ccactgcgct tgccttcgcg cagtcgggcg catccgttgc ggtcgcagac    120

atctcgactg accacggttt gaaaaccgta gagttggtca aagccgaagg aggcgaggcg    180

accttcttcc atgtcgatgt aggctctgaa cccagcgtcc agtcgatgct ggctggtgtc    240

gtggcgcatt acggcggcct ggacattgcg cacaacaacg ccggcattga ggccaatatc    300

gtgccgctgg ccgagctgga ctccgacaac tggcgtcgtg tcatcgatgt gaacctttcc    360

tcggtgttct attgcctgaa aggtgaaatc cctctgatgc tgaaaagggg cggcggcgcc    420

attgtgaata ccgcatcggc ctccgggctg attggcggct atcgcctttc cgggtatacc    480

gccacgaagc acggcgtagt ggggctgact aaggctgctg ctatcgatta tgcaaaccag    540

aatatccgga ttaatgccgt gtgccctggt ccagttgact ccccattcct ggctgacatg    600

ccgcaaccca tgcgcgatcg acttctcttt ggcactccaa ttggacgatt ggccaccgca    660

gaggagatcg cgcgttcggt tctgtggctg tgttctgacg atgcaaaata cgtggtgggc    720

cattcgatgt cagtcgacgg tggcgtggca gtgactgcgg ttggtactcg aatggatgat    780

ctcttttaa                                                            789
```

<210> SEQ ID NO 110
<211> LENGTH: 262
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 110

```
Met Thr Val Asn Tyr Asp Phe Ser Gly Lys Val Val Leu Val Thr Gly
1               5                   10                  15

Ala Gly Ser Gly Ile Gly Arg Ala Thr Ala Leu Ala Phe Ala Gln Ser
            20                  25                  30

Gly Ala Ser Val Ala Val Ala Asp Ile Ser Thr Asp His Gly Leu Lys
        35                  40                  45

Thr Val Glu Leu Val Lys Ala Glu Gly Gly Glu Ala Thr Phe Phe His
    50                  55                  60

Val Asp Val Gly Ser Glu Pro Ser Val Gln Ser Met Leu Ala Gly Val
65                  70                  75                  80

Val Ala His Tyr Gly Gly Leu Asp Ile Ala His Asn Asn Ala Gly Ile
                85                  90                  95

Glu Ala Asn Ile Val Pro Leu Ala Glu Leu Asp Ser Asp Asn Trp Arg
            100                 105                 110

Arg Val Ile Asp Val Asn Leu Ser Ser Val Phe Tyr Cys Leu Lys Gly
```

-continued

```
        115                 120                 125

Glu Ile Pro Leu Met Leu Lys Arg Gly Gly Gly Ala Ile Val Asn Thr
    130                 135                 140

Ala Ser Ala Ser Gly Leu Ile Gly Gly Tyr Arg Leu Ser Gly Tyr Thr
145                 150                 155                 160

Ala Thr Lys His Gly Val Val Gly Leu Thr Lys Ala Ala Ala Ile Asp
                165                 170                 175

Tyr Ala Asn Gln Asn Ile Arg Ile Asn Ala Val Cys Pro Gly Pro Val
            180                 185                 190

Asp Ser Pro Phe Leu Ala Asp Met Pro Gln Pro Met Arg Asp Arg Leu
        195                 200                 205

Leu Phe Gly Thr Pro Ile Gly Arg Leu Ala Thr Ala Glu Glu Ile Ala
    210                 215                 220

Arg Ser Val Leu Trp Leu Cys Ser Asp Asp Ala Lys Tyr Val Val Gly
225                 230                 235                 240

His Ser Met Ser Val Asp Gly Gly Val Ala Val Thr Ala Val Gly Thr
                245                 250                 255

Arg Met Asp Asp Leu Phe
            260
```

<210> SEQ ID NO 111
<211> LENGTH: 762
<212> TYPE: DNA
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 111

```
atgagcatga ccttttctgg ccaggtagcc ctggtgaccg gcgcgggtgc cggcatcggc     60

cgggcaaccg ccctggcgtt cgcccacgag ggcatgaaag tggtggtggc ggacctcgac    120

ccggtcggcg gcgaggccac cgtggcgcag atccacgcgg caggcggcga agcgctgttc    180

attgcctgcg acgtgacccg cgacgccgag gtgcgccagt tgcatgagcg cctgatggcc    240

gcctacggcc ggctggacta cgccttcaac aacgccggga tcgagatcga gcaacaccgc    300

ctggccgaag gcagcgaagc ggagttcgat gccatcatgg gcgtgaacgt gaagggcgtg    360

tggttgtgca tgaagtatca gttgcccttg ttgctggccc aaggcggtgg ggccatcgtc    420

aataccgcgt cggtggcggg gctaggggcg gcgccaaaga tgagcatcta cagcgccagc    480

aagcatgcgg tcatcggtct gaccaagtcg gcggccatcg agtacgccaa gaagggcatc    540

cgcgtgaacg ccgtgtgccc ggccgtgatc gacaccgaca tgttccgccg cgcttaccag    600

gccgacccgc gcaaggccga gttcgccgca gccatgcacc cggtagggcg cattggcaag    660

gtcgaggaaa tcgccagcgc cgtgctgtat ctgtgcagtg acggcgcggc gtttaccacc    720

gggcattgcc tgacggtgga tggtggggct acggcgatct ga                       762
```

<210> SEQ ID NO 112
<211> LENGTH: 253
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 112

```
Met Ser Met Thr Phe Ser Gly Gln Val Ala Leu Val Thr Gly Ala Gly
1               5                   10                  15

Ala Gly Ile Gly Arg Ala Thr Ala Leu Ala Phe Ala His Glu Gly Met
            20                  25                  30
```

-continued

```
Lys Val Val Val Ala Asp Leu Asp Pro Val Gly Gly Glu Ala Thr Val
        35                  40                  45

Ala Gln Ile His Ala Ala Gly Gly Glu Ala Leu Phe Ile Ala Cys Asp
    50                  55                  60

Val Thr Arg Asp Ala Glu Val Arg Gln Leu His Glu Arg Leu Met Ala
65                  70                  75                  80

Ala Tyr Gly Arg Leu Asp Tyr Ala Phe Asn Asn Ala Gly Ile Glu Ile
                85                  90                  95

Glu Gln His Arg Leu Ala Glu Gly Ser Glu Ala Glu Phe Asp Ala Ile
            100                 105                 110

Met Gly Val Asn Val Lys Gly Val Trp Leu Cys Met Lys Tyr Gln Leu
        115                 120                 125

Pro Leu Leu Leu Ala Gln Gly Gly Gly Ala Ile Val Asn Thr Ala Ser
    130                 135                 140

Val Ala Gly Leu Gly Ala Ala Pro Lys Met Ser Ile Tyr Ser Ala Ser
145                 150                 155                 160

Lys His Ala Val Ile Gly Leu Thr Lys Ser Ala Ala Ile Glu Tyr Ala
                165                 170                 175

Lys Lys Gly Ile Arg Val Asn Ala Val Cys Pro Ala Val Ile Asp Thr
            180                 185                 190

Asp Met Phe Arg Arg Ala Tyr Gln Ala Asp Pro Arg Lys Ala Glu Phe
        195                 200                 205

Ala Ala Ala Met His Pro Val Gly Arg Ile Gly Lys Val Glu Glu Ile
    210                 215                 220

Ala Ser Ala Val Leu Tyr Leu Cys Ser Asp Gly Ala Ala Phe Thr Thr
225                 230                 235                 240

Gly His Cys Leu Thr Val Asp Gly Gly Ala Thr Ala Ile
                245                 250
```

<210> SEQ ID NO 113
<211> LENGTH: 810
<212> TYPE: DNA
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 113

```
atgtcttttc aaaacaaaat cgttgtgctc acaggcgcag cttctggcat cggcaaagcg     60

acagcacagc tgctagtgga gcagggcgcc catgtggttg ccatggatct taaaagcgac    120

ttgcttcaac aagcattcgg cagtgaggag cacgttctgt gcatccctac cgacgtcagc    180

gatagcgaag ccgtgcgagc cgccttccag gcagtggacg cgaaatttgg ccgtgtcgac    240

gtgattatta acgccgcggg catcaacgca cctacgcgag aagccaacca gaaaatggtt    300

gatgccaacg tcgctgccct cgatgccatg aagagcgggc gggcgcccac tttcgacttc    360

ctggccgata cctcggatca ggatttccgg cgcgtaatgg aagtcaattt gttcagccag    420

ttttactgca ttcgagaggg tgttccgctg atgcgccgag cgggtggcgg cagcatcgtc    480

aacatctcca gcgtggcagc gctcctgggc gtggcaatgc cactttacta ccccgcctcc    540

aaggcggcgg tgctgggcct cacccgtgca gcggcagctg agttggcacc ttacaacatt    600

cgtgtgaatg ccatcgctcc aggctctgtc gacacaccat tgatgcatga gcaaccaccg    660

gaagtcgttc agttcctggt cagcatgcaa cccatcaagc ggctggccca acccgaggag    720

cttgcccaaa gcatcctgtt ccttgccggt gagcattcgt ccttcatcac cggacagacg    780
```

-continued

```
ctttctccca acggcgggat gcacatgtaa                                      810


<210> SEQ ID NO 114
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 114

Met Ser Phe Gln Asn Lys Ile Val Val Leu Thr Gly Ala Ala Ser Gly
1               5                   10                  15

Ile Gly Lys Ala Thr Ala Gln Leu Leu Val Glu Gln Gly Ala His Val
            20                  25                  30

Val Ala Met Asp Leu Lys Ser Asp Leu Leu Gln Gln Ala Phe Gly Ser
        35                  40                  45

Glu Glu His Val Leu Cys Ile Pro Thr Asp Val Ser Asp Ser Glu Ala
    50                  55                  60

Val Arg Ala Ala Phe Gln Ala Val Asp Ala Lys Phe Gly Arg Val Asp
65                  70                  75                  80

Val Ile Ile Asn Ala Ala Gly Ile Asn Ala Pro Thr Arg Glu Ala Asn
                85                  90                  95

Gln Lys Met Val Asp Ala Asn Val Ala Ala Leu Asp Ala Met Lys Ser
            100                 105                 110

Gly Arg Ala Pro Thr Phe Asp Phe Leu Ala Asp Thr Ser Asp Gln Asp
        115                 120                 125

Phe Arg Arg Val Met Glu Val Asn Leu Phe Ser Gln Phe Tyr Cys Ile
    130                 135                 140

Arg Glu Gly Val Pro Leu Met Arg Arg Ala Gly Gly Gly Ser Ile Val
145                 150                 155                 160

Asn Ile Ser Ser Val Ala Ala Leu Leu Gly Val Ala Met Pro Leu Tyr
                165                 170                 175

Tyr Pro Ala Ser Lys Ala Ala Val Leu Gly Leu Thr Arg Ala Ala Ala
            180                 185                 190

Ala Glu Leu Ala Pro Tyr Asn Ile Arg Val Asn Ala Ile Ala Pro Gly
        195                 200                 205

Ser Val Asp Thr Pro Leu Met His Glu Gln Pro Pro Glu Val Val Gln
    210                 215                 220

Phe Leu Val Ser Met Gln Pro Ile Lys Arg Leu Ala Gln Pro Glu Glu
225                 230                 235                 240

Leu Ala Gln Ser Ile Leu Phe Leu Ala Gly Glu His Ser Ser Phe Ile
                245                 250                 255

Thr Gly Gln Thr Leu Ser Pro Asn Gly Gly Met His Met
            260                 265


<210> SEQ ID NO 115
<211> LENGTH: 771
<212> TYPE: DNA
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 115

atgacccttg aaggcaaaac tgcactcgtc accggttcca ccagcggcat tggcctgggc     60

atcgcccagg tattggcccg ggctggcgcc aacatcgtgc tcaacggctt tggtgacccg    120

ggccccgcca tggcggaaat tgcccggcac ggggtgaagg ttgtgcacca cccggccgac    180
```

-continued

```
ctgtcggatg tggtccagat cgaggctttg ttcaacctgg ccgaacgcga gttcggcggc    240

gtcgacatcc tggtcaacaa cgccggtatc cagcatgtgg caccggttga gcagttcccg    300

ccagaaagct gggacaagat catcgccctg aacctgtcgg ccgtattcca tggcacgcgc    360

ctggcgctgc cgggcatgcg cacgcgcaac tgggggcgca tcatcaatat cgcttcggtg    420

catggcctgg tcggctcgat tggcaaggca gcctacgtgg cagccaagca tggcgtgatc    480

ggcctgacca aggtggtcgg cctggaaacc gccaccagtc atgtcacctg caatgccata    540

tgcccgggct gggtgctgac accgctggtg caaaagcaga tcgacgatcg tgcggccaag    600

ggtggcgatc ggctgcaagc gcagcacgat ctgctggcag aaaagcaacc gtcgctggct    660

ttcgtcaccc ccgaacacct cggtgagctg gtactctttc tgtgcagcga ggccggtagc    720

caggttcgcg gcgccgcctg gaacgtcgat ggtggctggt tggcccagtg a              771


<210> SEQ ID NO 116
<211> LENGTH: 256
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 116

Met Thr Leu Glu Gly Lys Thr Ala Leu Val Thr Gly Ser Thr Ser Gly
1               5                   10                  15

Ile Gly Leu Gly Ile Ala Gln Val Leu Ala Arg Ala Gly Ala Asn Ile
            20                  25                  30

Val Leu Asn Gly Phe Gly Asp Pro Gly Pro Ala Met Ala Glu Ile Ala
        35                  40                  45

Arg His Gly Val Lys Val Val His His Pro Ala Asp Leu Ser Asp Val
    50                  55                  60

Val Gln Ile Glu Ala Leu Phe Asn Leu Ala Glu Arg Glu Phe Gly Gly
65                  70                  75                  80

Val Asp Ile Leu Val Asn Asn Ala Gly Ile Gln His Val Ala Pro Val
                85                  90                  95

Glu Gln Phe Pro Pro Glu Ser Trp Asp Lys Ile Ile Ala Leu Asn Leu
            100                 105                 110

Ser Ala Val Phe His Gly Thr Arg Leu Ala Leu Pro Gly Met Arg Thr
        115                 120                 125

Arg Asn Trp Gly Arg Ile Ile Asn Ile Ala Ser Val His Gly Leu Val
    130                 135                 140

Gly Ser Ile Gly Lys Ala Ala Tyr Val Ala Ala Lys His Gly Val Ile
145                 150                 155                 160

Gly Leu Thr Lys Val Val Gly Leu Glu Thr Ala Thr Ser His Val Thr
                165                 170                 175

Cys Asn Ala Ile Cys Pro Gly Trp Val Leu Thr Pro Leu Val Gln Lys
            180                 185                 190

Gln Ile Asp Asp Arg Ala Ala Lys Gly Gly Asp Arg Leu Gln Ala Gln
        195                 200                 205

His Asp Leu Leu Ala Glu Lys Gln Pro Ser Leu Ala Phe Val Thr Pro
    210                 215                 220

Glu His Leu Gly Glu Leu Val Leu Phe Leu Cys Ser Glu Ala Gly Ser
225                 230                 235                 240

Gln Val Arg Gly Ala Ala Trp Asn Val Asp Gly Gly Trp Leu Ala Gln
                245                 250                 255
```

-continued

<210> SEQ ID NO 117
<211> LENGTH: 750
<212> TYPE: DNA
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 117

atgtccaagc aacttacact cgaaggcaaa gtggccctgg ttcagggcgg ttcccgaggc 60

attggcgcag ctatcgtaag gcgcctggcc cgcgaaggcg cgcaagtggc cttcacctat 120

gtcagctctg ccggcccggc tgaagaactg gctcgggaaa ttaccgagaa cggcggcaaa 180

gccttggccc tgcgggctga cagcgctgat gccgcggccg tgcagctggc ggttgatgac 240

accgagaaag ccttgggccg gctggatatc ctggtcaaca acgccggtgt gctggcagtg 300

gccccagtga cagagttcga cctggccgac ttcgatcata tgctggccgt gaacgtacgc 360

agcgtgttcg tcgccagcca ggccgcggca cgctatatgg gccagggcgg tcgtatcatc 420

aacattggca gcaccaacgc cgagcgcatg ccgtttgccg gtggtgcacc gtacgccatg 480

agcaagtcgg cactggttgg tctgacccgc ggcatggcac gcgacctcgg gccgcagggc 540

attaccgtga acaacgtgca gcccggcccg gtggacaccg acatgaaccc ggccagtggc 600

gagtttgccg agagcctgat tccgctgatg gccattgggc gatatggcga gccggaggag 660

attgccagct tcgtggctta cctggcaggg cctgaagccg ggtatatcac cggggccagc 720

ctgactgtag atggtgggtt tgcagcctga 750

<210> SEQ ID NO 118
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 118

Met Ser Lys Gln Leu Thr Leu Glu Gly Lys Val Ala Leu Val Gln Gly
1 5 10 15

Gly Ser Arg Gly Ile Gly Ala Ala Ile Val Arg Arg Leu Ala Arg Glu
20 25 30

Gly Ala Gln Val Ala Phe Thr Tyr Val Ser Ser Ala Gly Pro Ala Glu
35 40 45

Glu Leu Ala Arg Glu Ile Thr Glu Asn Gly Gly Lys Ala Leu Ala Leu
50 55 60

Arg Ala Asp Ser Ala Asp Ala Ala Ala Val Gln Leu Ala Val Asp Asp
65 70 75 80

Thr Glu Lys Ala Leu Gly Arg Leu Asp Ile Leu Val Asn Asn Ala Gly
85 90 95

Val Leu Ala Val Ala Pro Val Thr Glu Phe Asp Leu Ala Asp Phe Asp
100 105 110

His Met Leu Ala Val Asn Val Arg Ser Val Phe Val Ala Ser Gln Ala
115 120 125

Ala Ala Arg Tyr Met Gly Gln Gly Gly Arg Ile Ile Asn Ile Gly Ser
130 135 140

Thr Asn Ala Glu Arg Met Pro Phe Ala Gly Gly Ala Pro Tyr Ala Met
145 150 155 160

Ser Lys Ser Ala Leu Val Gly Leu Thr Arg Gly Met Ala Arg Asp Leu
165 170 175

Gly Pro Gln Gly Ile Thr Val Asn Asn Val Gln Pro Gly Pro Val Asp

-continued

```
            180                 185                 190

Thr Asp Met Asn Pro Ala Ser Gly Glu Phe Ala Glu Ser Leu Ile Pro
        195                 200                 205

Leu Met Ala Ile Gly Arg Tyr Gly Glu Pro Glu Glu Ile Ala Ser Phe
    210                 215                 220

Val Ala Tyr Leu Ala Gly Pro Glu Ala Gly Tyr Ile Thr Gly Ala Ser
225                 230                 235                 240

Leu Thr Val Asp Gly Gly Phe Ala Ala
                245
```

<210> SEQ ID NO 119
<211> LENGTH: 858
<212> TYPE: DNA
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 119

```
atgagcgact accctacccc tccattccca tcccaaccgc aaagcgttcc cggttcccag     60

cgcaagatgg atccgtatcc ggactgcggt gagcagagct acaccggcaa caatcgcctc    120

gcaggcaaga tcgccttgat aaccggtgct gacagcggca tcgggcgtgc ggtggcgatt    180

gcctatgccc gagaaggcgc tgacgttgcc attgcctatc tgaatgaaca cgacgatgcg    240

caggaaaccg cgcgctgggt caaagcggct ggccgccagt gcctgctgct gcccggcgac    300

ctggcacaga aacagcactg ccacgacatc gtcgacaaga ccgtggcgca gtttggtcgc    360

atcgatatcc tggtcaacaa cgccgcgttc cagatggccc atgaaagcct ggacgacatt    420

gatgacgatg aatgggtgaa gaccttcgat accaacatca ccgccatttt ccgcatttgc    480

cagcgcgctt tgccctcgat gccaaagggc ggttcgatca tcaacaccag ttcggtcaac    540

tctgacgacc cgtcacccag cctgttggcc tatgccgcga ccaaaggggc tattgccaat    600

ttcactgcag gccttgcgca actgctgggc aagcagggca ttcgcgtcaa cagcgtcgca    660

cccggcccga tctggacccc gctgatcccg gccaccatgc ctgatgaggc ggtgagaaac    720

ttcggttccg gttacccgat gggacggccg ggtcaacctg tggaggtggc gccaatctat    780

gtcttgctgg ggtccgatga agccagctac atctcgggtt cgcgttacgc cgtgacggga    840

ggcaaaccta ttctgtga                                                  858
```

<210> SEQ ID NO 120
<211> LENGTH: 285
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 120

```
Met Ser Asp Tyr Pro Thr Pro Pro Phe Pro Ser Gln Pro Gln Ser Val
1               5                   10                  15

Pro Gly Ser Gln Arg Lys Met Asp Pro Tyr Pro Asp Cys Gly Glu Gln
            20                  25                  30

Ser Tyr Thr Gly Asn Asn Arg Leu Ala Gly Lys Ile Ala Leu Ile Thr
        35                  40                  45

Gly Ala Asp Ser Gly Ile Gly Arg Ala Val Ala Ile Ala Tyr Ala Arg
    50                  55                  60

Glu Gly Ala Asp Val Ala Ile Ala Tyr Leu Asn Glu His Asp Asp Ala
65                  70                  75                  80

Gln Glu Thr Ala Arg Trp Val Lys Ala Ala Gly Arg Gln Cys Leu Leu
```

-continued

```
               85                  90                  95

Leu Pro Gly Asp Leu Ala Gln Lys Gln His Cys His Asp Ile Val Asp
            100                 105                 110

Lys Thr Val Ala Gln Phe Gly Arg Ile Asp Ile Leu Val Asn Asn Ala
        115                 120                 125

Ala Phe Gln Met Ala His Glu Ser Leu Asp Asp Ile Asp Asp Asp Glu
    130                 135                 140

Trp Val Lys Thr Phe Asp Thr Asn Ile Thr Ala Ile Phe Arg Ile Cys
145                 150                 155                 160

Gln Arg Ala Leu Pro Ser Met Pro Lys Gly Gly Ser Ile Ile Asn Thr
                165                 170                 175

Ser Ser Val Asn Ser Asp Asp Pro Ser Pro Ser Leu Leu Ala Tyr Ala
            180                 185                 190

Ala Thr Lys Gly Ala Ile Ala Asn Phe Thr Ala Gly Leu Ala Gln Leu
        195                 200                 205

Leu Gly Lys Gln Gly Ile Arg Val Asn Ser Val Ala Pro Gly Pro Ile
    210                 215                 220

Trp Thr Pro Leu Ile Pro Ala Thr Met Pro Asp Glu Ala Val Arg Asn
225                 230                 235                 240

Phe Gly Ser Gly Tyr Pro Met Gly Arg Pro Gly Gln Pro Val Glu Val
                245                 250                 255

Ala Pro Ile Tyr Val Leu Leu Gly Ser Asp Glu Ala Ser Tyr Ile Ser
            260                 265                 270

Gly Ser Arg Tyr Ala Val Thr Gly Gly Lys Pro Ile Leu
        275                 280                 285


<210> SEQ ID NO 121
<211> LENGTH: 774
<212> TYPE: DNA
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 121

atgatcgaaa tcagcggcag caccccgggc cacaatggcc gggtagcctt ggtcacgggc     60

gccgcccgcg gcatcggtct gggcattgcc gcatggctga tctgcgaagg ctggcaagtg    120

gtgctgagtg atctggaccg ccagcgtggt accaaagtgg ccaaggcgtt gggcgacaac    180

gcctggttca tcaccatgga cgttgccgac gaggcccagg tcagtgccgg cgtgtccgaa    240

gtgctcgggc agttcggccg gctggacgcg ctggtgtgca atgcggccat tgccaacccg    300

cacaaccaga cgctggaaag cctgagcctg gcacaatgga accgggtgct gggggtcaac    360

ctcagcggcc ccatgctgct ggccaagcat tgtgcgccgt acctgcgtgc gcacaatggg    420

gcgatcgtca acctgacctc tacccgtgct cggcagtccg aacccgacac cgaggcttac    480

gcggcaagca agggcggcct ggtggctttg acccatgccc tggccatgag cctgggcccg    540

gagattcgcg tcaatgcggt gagcccgggc tggatcgatg cccgtgatcc gtcgcagcgc    600

cgtgccgagc cgttgagcga agctgaccat gcccagcatc caacgggcag ggtagggacc    660

gtggaagatg tcgcggccat ggttgcctgg ttgctgtcac gccaggcggc atttgtcacc    720

ggccaggagt ttgtggtcga tggcggcatg acccgcaaga tgatctatac ctga          774


<210> SEQ ID NO 122
<211> LENGTH: 257
<212> TYPE: PRT
```

-continued

<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 122

Met Ile Glu Ile Ser Gly Ser Thr Pro Gly His Asn Gly Arg Val Ala
1 5 10 15

Leu Val Thr Gly Ala Ala Arg Gly Ile Gly Leu Gly Ile Ala Ala Trp
20 25 30

Leu Ile Cys Glu Gly Trp Gln Val Val Leu Ser Asp Leu Asp Arg Gln
35 40 45

Arg Gly Thr Lys Val Ala Lys Ala Leu Gly Asp Asn Ala Trp Phe Ile
50 55 60

Thr Met Asp Val Ala Asp Glu Ala Gln Val Ser Ala Gly Val Ser Glu
65 70 75 80

Val Leu Gly Gln Phe Gly Arg Leu Asp Ala Leu Val Cys Asn Ala Ala
85 90 95

Ile Ala Asn Pro His Asn Gln Thr Leu Glu Ser Leu Ser Leu Ala Gln
100 105 110

Trp Asn Arg Val Leu Gly Val Asn Leu Ser Gly Pro Met Leu Leu Ala
115 120 125

Lys His Cys Ala Pro Tyr Leu Arg Ala His Asn Gly Ala Ile Val Asn
130 135 140

Leu Thr Ser Thr Arg Ala Arg Gln Ser Glu Pro Asp Thr Glu Ala Tyr
145 150 155 160

Ala Ala Ser Lys Gly Gly Leu Val Ala Leu Thr His Ala Leu Ala Met
165 170 175

Ser Leu Gly Pro Glu Ile Arg Val Asn Ala Val Ser Pro Gly Trp Ile
180 185 190

Asp Ala Arg Asp Pro Ser Gln Arg Arg Ala Glu Pro Leu Ser Glu Ala
195 200 205

Asp His Ala Gln His Pro Thr Gly Arg Val Gly Thr Val Glu Asp Val
210 215 220

Ala Ala Met Val Ala Trp Leu Leu Ser Arg Gln Ala Ala Phe Val Thr
225 230 235 240

Gly Gln Glu Phe Val Val Asp Gly Gly Met Thr Arg Lys Met Ile Tyr
245 250 255

Thr

<210> SEQ ID NO 123
<211> LENGTH: 741
<212> TYPE: DNA
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 123

atgagcctgc aaggtaaagt tgcactggtt accggcgcca gccgtggcat tggccaggcc 60

atcgccctcg agctgggccg ccagggcgcg accgtgatcg gtaccgccac gtcggcgtcc 120

ggtgccgagc gcatcgctgc caccctgaaa gaacacggca ttaccggcac tggcatggag 180

ctgaacgtga ccagcgccga atcggttgaa gccgtactgg ccgccattgg cgagcagttc 240

ggcgcgccgg ccatcttggt caacaatgcc ggtatcaccc gcgacaacct catgctgcgc 300

atgaaagacg acgagtggtt tgatgtcatc gacaccaacc tgaacagcct ctaccgtctg 360

tccaagggcg tgctgcgtgg catgaccaag gcgcgttggg gtcgtatcat cagcatcggc 420

-continued

tcggtcgttg gtgccatggg taacgcaggt caggccaact acgcggctgc caaggccggt 480

ctggaaggtt tcagccgcgc cctggcgcgt gaagtgggtt cgcgtggtat caccgtcaac 540

tcggtgaccc caggcttcat cgataccgac atgacccgcg agctgccaga agctcagcgc 600

gaagccctgc agacccagat tccgctgggc cgcctgggcc aggctgacga aattgccaag 660

gtggtttcgt tcctggcatc cgacggcgcc gcctacgtga ccggcgctac cgtgccggtc 720

aacggcggga tgtacatgta a 741

<210> SEQ ID NO 124
<211> LENGTH: 246
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 124

Met Ser Leu Gln Gly Lys Val Ala Leu Val Thr Gly Ala Ser Arg Gly
1 5 10 15

Ile Gly Gln Ala Ile Ala Leu Glu Leu Gly Arg Gln Gly Ala Thr Val
20 25 30

Ile Gly Thr Ala Thr Ser Ala Ser Gly Ala Glu Arg Ile Ala Ala Thr
35 40 45

Leu Lys Glu His Gly Ile Thr Gly Thr Gly Met Glu Leu Asn Val Thr
50 55 60

Ser Ala Glu Ser Val Glu Ala Val Leu Ala Ala Ile Gly Glu Gln Phe
65 70 75 80

Gly Ala Pro Ala Ile Leu Val Asn Asn Ala Gly Ile Thr Arg Asp Asn
85 90 95

Leu Met Leu Arg Met Lys Asp Asp Glu Trp Phe Asp Val Ile Asp Thr
100 105 110

Asn Leu Asn Ser Leu Tyr Arg Leu Ser Lys Gly Val Leu Arg Gly Met
115 120 125

Thr Lys Ala Arg Trp Gly Arg Ile Ile Ser Ile Gly Ser Val Val Gly
130 135 140

Ala Met Gly Asn Ala Gly Gln Ala Asn Tyr Ala Ala Ala Lys Ala Gly
145 150 155 160

Leu Glu Gly Phe Ser Arg Ala Leu Ala Arg Glu Val Gly Ser Arg Gly
165 170 175

Ile Thr Val Asn Ser Val Thr Pro Gly Phe Ile Asp Thr Asp Met Thr
180 185 190

Arg Glu Leu Pro Glu Ala Gln Arg Glu Ala Leu Gln Thr Gln Ile Pro
195 200 205

Leu Gly Arg Leu Gly Gln Ala Asp Glu Ile Ala Lys Val Val Ser Phe
210 215 220

Leu Ala Ser Asp Gly Ala Ala Tyr Val Thr Gly Ala Thr Val Pro Val
225 230 235 240

Asn Gly Gly Met Tyr Met
245

<210> SEQ ID NO 125
<211> LENGTH: 738
<212> TYPE: DNA
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 125

-continued

atgactcaga aaatagctgt cgtgaccggc ggcagtcgcg gcattggcaa gtccatcgtg 60

ctggccctgg ccggcgcggg ttatcaggtt gccttcagtt atgtccgtga cgaggcgtca 120

gccgctgcct tgcaggcgca ggtcgaaggg ctcggccggg actgcctggc cgtgcagtgt 180

gatgtcaagg aagcgccgag cattcaggcg ttttttgaac gggtcgagca acgtttcgag 240

cgtatcgact tgttggtcaa caacgccggt attacccgtg acggtttgct cgccacgcaa 300

tcgttgaacg acatcaccga ggtcatccag accaacctgg tcggcacgtt gttgtgctgt 360

cagcaggtgc tgccctgcat gatgcgccaa cgcagcgggt gcatcgtcaa cctcagttcg 420

gtggccgcgc aaaagcccgg caagggccag agcaactacg ccgccgccaa aggcggtgta 480

gaagcattga cacgcgcact ggcggtggag ttggcgccgc gcaacatccg ggtcaacgcg 540

gtggcgcccg gcatcgtcag caccgacatg agccaagccc tggtcggcgc ccatgagcag 600

gaaatccagt cgcggctgtt gatcaaacgg ttcgcccggc ctgaagaaat tgccgacgcg 660

gtgctgtatc tggccgagcg cggcctgtac atcacgggcg aagtcctgtc cgtcaacggc 720

ggattgaaaa tgccatga 738

<210> SEQ ID NO 126
<211> LENGTH: 245
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 126

Met Thr Gln Lys Ile Ala Val Val Thr Gly Gly Ser Arg Gly Ile Gly
1 5 10 15

Lys Ser Ile Val Leu Ala Leu Ala Gly Ala Gly Tyr Gln Val Ala Phe
20 25 30

Ser Tyr Val Arg Asp Glu Ala Ser Ala Ala Ala Leu Gln Ala Gln Val
35 40 45

Glu Gly Leu Gly Arg Asp Cys Leu Ala Val Gln Cys Asp Val Lys Glu
50 55 60

Ala Pro Ser Ile Gln Ala Phe Phe Glu Arg Val Glu Gln Arg Phe Glu
65 70 75 80

Arg Ile Asp Leu Leu Val Asn Asn Ala Gly Ile Thr Arg Asp Gly Leu
85 90 95

Leu Ala Thr Gln Ser Leu Asn Asp Ile Thr Glu Val Ile Gln Thr Asn
100 105 110

Leu Val Gly Thr Leu Leu Cys Cys Gln Gln Val Leu Pro Cys Met Met
115 120 125

Arg Gln Arg Ser Gly Cys Ile Val Asn Leu Ser Ser Val Ala Ala Gln
130 135 140

Lys Pro Gly Lys Gly Gln Ser Asn Tyr Ala Ala Ala Lys Gly Gly Val
145 150 155 160

Glu Ala Leu Thr Arg Ala Leu Ala Val Glu Leu Ala Pro Arg Asn Ile
165 170 175

Arg Val Asn Ala Val Ala Pro Gly Ile Val Ser Thr Asp Met Ser Gln
180 185 190

Ala Leu Val Gly Ala His Glu Gln Glu Ile Gln Ser Arg Leu Leu Ile
195 200 205

Lys Arg Phe Ala Arg Pro Glu Glu Ile Ala Asp Ala Val Leu Tyr Leu
210 215 220

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```
Ala Glu Arg Gly Leu Tyr Ile Thr Gly Glu Val Leu Ser Val Asn Gly
225                 230                 235                 240

Gly Leu Lys Met Pro
                245
```

<210> SEQ ID NO 127
<211> LENGTH: 768
<212> TYPE: DNA
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 127

```
atgtccaaga cccacctgtt cgacctcgac ggcaagattg cctttgtttc cggcgccagc     60

cgtggcatcg gcgaggccat cgcccacttg ctcgcgcagc aaggggccca tgtgatcgtt    120

tccagccgca agcttgacgg gtgccagcag gtggccgacg ccatcattgc cgccggcggc    180

aaggccacgg ctgtggcctg ccacattggt gagctggaac agattcagca ggtgttcgcc    240

ggcattcgcg aacagttcgg gcgactggac gtgctggtca acaatgcagc caccaacccg    300

caattctgca atgtgctgga caccgaccca ggggcgttcc agaagaccgt ggacgtgaac    360

atccgtggtt acttcttcat gtcggtggag gctggcaagc tgatgcgcga gaacggcggc    420

ggcagcatca tcaacgtggc gtcgatcaac ggtgtttcac ccgggctgtt ccaaggcatc    480

tactcggtga ccaaggcggc ggtcatcaac atgaccaagg tgttcgccaa agagtgtgca    540

cccttcggta ttcgctgcaa cgcgctactg ccggggctga ccgataccaa gttcgcttcg    600

gcattggtga agaacgaagc catcctcaac gccgccttgc agcagatccc cctcaaacgc    660

gtggccgacc ccaaggaaat ggcgggtgcg gtgctgtacc tggccagcga tgcctccagc    720

tacaccaccg gcaccacgct caatgtcgac ggtggcttcc tgtcctga                 768
```

<210> SEQ ID NO 128
<211> LENGTH: 255
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 128

```
Met Ser Lys Thr His Leu Phe Asp Leu Asp Gly Lys Ile Ala Phe Val
1               5                   10                  15

Ser Gly Ala Ser Arg Gly Ile Gly Glu Ala Ile Ala His Leu Leu Ala
            20                  25                  30

Gln Gln Gly Ala His Val Ile Val Ser Ser Arg Lys Leu Asp Gly Cys
        35                  40                  45

Gln Gln Val Ala Asp Ala Ile Ile Ala Ala Gly Gly Lys Ala Thr Ala
    50                  55                  60

Val Ala Cys His Ile Gly Glu Leu Glu Gln Ile Gln Gln Val Phe Ala
65                  70                  75                  80

Gly Ile Arg Glu Gln Phe Gly Arg Leu Asp Val Leu Val Asn Asn Ala
                85                  90                  95

Ala Thr Asn Pro Gln Phe Cys Asn Val Leu Asp Thr Asp Pro Gly Ala
            100                 105                 110

Phe Gln Lys Thr Val Asp Val Asn Ile Arg Gly Tyr Phe Phe Met Ser
        115                 120                 125

Val Glu Ala Gly Lys Leu Met Arg Glu Asn Gly Gly Gly Ser Ile Ile
    130                 135                 140

Asn Val Ala Ser Ile Asn Gly Val Ser Pro Gly Leu Phe Gln Gly Ile
```

-continued

```
145                 150                 155                 160

Tyr Ser Val Thr Lys Ala Ala Val Ile Asn Met Thr Lys Val Phe Ala
                165                 170                 175

Lys Glu Cys Ala Pro Phe Gly Ile Arg Cys Asn Ala Leu Leu Pro Gly
            180                 185                 190

Leu Thr Asp Thr Lys Phe Ala Ser Ala Leu Val Lys Asn Glu Ala Ile
        195                 200                 205

Leu Asn Ala Ala Leu Gln Gln Ile Pro Leu Lys Arg Val Ala Asp Pro
    210                 215                 220

Lys Glu Met Ala Gly Ala Val Leu Tyr Leu Ala Ser Asp Ala Ser Ser
225                 230                 235                 240

Tyr Thr Thr Gly Thr Thr Leu Asn Val Asp Gly Gly Phe Leu Ser
                245                 250                 255


<210> SEQ ID NO 129
<211> LENGTH: 762
<212> TYPE: DNA
<213> ORGANISM: Pseudomonas fluorescens Pf-5

<400> SEQUENCE: 129

atgagcatga cgttttccgg ccaggtggcc ctagtgaccg gcgcagccaa tggtatcggc     60

cgcgccaccg cccaggcatt tgccgcacaa ggcttgaagg tggtggtggc ggacctggac    120

acggcggggg gcgagggcac cgtggcgctg atccgcgagg ccggtggcga ggcattgttc    180

gtgccgtgca acgttaccct ggaggcggat gtgcaaagcc tcatggcccg caccatcgaa    240

gcctatgggc gcctggatta cgccttcaac aatgccggta tcgagatcga aaagggccgc    300

cttgcggagg gctccatgga tgagttcgac gccatcatgg gggtcaacgt caaaggggtc    360

tggctgtgca tgaagtacca gttgccgctg ctgctggccc agggcggtgg ggcgatcgtc    420

aacaccgcct cggtggcggg cctgggcgcg gcgccgaaga tgagcatcta tgcggcctcc    480

aagcatgcgg tgatcggcct gaccaagtcg gcggccatcg aatatgcgaa gaagaaaatc    540

cgcgtgaacg cggtatgccc ggcggtgatc gacaccgaca tgttccgccg tgcctacgag    600

gcggacccga agaaggccga gttcgccgcg gccatgcacc cggtggggcg catcggcaag    660

gtcgaggaga tcgccagtgc ggtgctctac ctgtgcagcg atggcgcggc ctttaccacc    720

ggccatgcac tggcggtcga cggcggggcc accgcgatct ga                       762


<210> SEQ ID NO 130
<211> LENGTH: 253
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas fluorscens Pf-5

<400> SEQUENCE: 130

Met Ser Met Thr Phe Ser Gly Gln Val Ala Leu Val Thr Gly Ala Ala
1               5                   10                  15

Asn Gly Ile Gly Arg Ala Thr Ala Gln Ala Phe Ala Ala Gln Gly Leu
            20                  25                  30

Lys Val Val Val Ala Asp Leu Asp Thr Ala Gly Gly Glu Gly Thr Val
        35                  40                  45

Ala Leu Ile Arg Glu Ala Gly Gly Glu Ala Leu Phe Val Pro Cys Asn
    50                  55                  60

Val Thr Leu Glu Ala Asp Val Gln Ser Leu Met Ala Arg Thr Ile Glu
65                  70                  75                  80
```

-continued

```
Ala Tyr Gly Arg Leu Asp Tyr Ala Phe Asn Asn Ala Gly Ile Glu Ile
                85                  90                  95

Glu Lys Gly Arg Leu Ala Glu Gly Ser Met Asp Glu Phe Asp Ala Ile
            100                 105                 110

Met Gly Val Asn Val Lys Gly Val Trp Leu Cys Met Lys Tyr Gln Leu
        115                 120                 125

Pro Leu Leu Leu Ala Gln Gly Gly Gly Ala Ile Val Asn Thr Ala Ser
    130                 135                 140

Val Ala Gly Leu Gly Ala Ala Pro Lys Met Ser Ile Tyr Ala Ala Ser
145                 150                 155                 160

Lys His Ala Val Ile Gly Leu Thr Lys Ser Ala Ala Ile Glu Tyr Ala
                165                 170                 175

Lys Lys Lys Ile Arg Val Asn Ala Val Cys Pro Ala Val Ile Asp Thr
            180                 185                 190

Asp Met Phe Arg Arg Ala Tyr Glu Ala Asp Pro Lys Lys Ala Glu Phe
        195                 200                 205

Ala Ala Ala Met His Pro Val Gly Arg Ile Gly Lys Val Glu Glu Ile
    210                 215                 220

Ala Ser Ala Val Leu Tyr Leu Cys Ser Asp Gly Ala Ala Phe Thr Thr
225                 230                 235                 240

Gly His Ala Leu Ala Val Asp Gly Gly Ala Thr Ala Ile
                245                 250
```

<210> SEQ ID NO 131
<211> LENGTH: 735
<212> TYPE: DNA
<213> ORGANISM: Klebsiella pneumoniae subsp. pneumoniae MGH78578

<400> SEQUENCE: 131

```
atgaaacttg ccagtaaaac cgccattgtc accggcgccg cacgcggtat cggctttggc     60

attgcccagg tgcttgcgcg ggaaggcgcg cgagtgatta tcgccgatcg tgatgcacac    120

ggcgaagccg ccgccgcttc cctgcgcgaa tcgggcgcac aggcgctgtt tatcagctgc    180

aatatcgctg aaaaaacgca ggtcgaagcc ctgtattccc aggccgaaga ggcgtttggc    240

ccggtagaca ttctggtgaa taacgccgga atcaaccgcg acgccatgct gcacaaatta    300

acggaagcgg actgggacac ggttatcgac gttaacctga aaggcacttt cctctgtatg    360

cagcaggccg ctatccgcat gcgcgagcgc ggtgcgggcc gcattatcaa tatcgcttcc    420

gccagttggc ttggcaacgt cgggcaaacc aactattcgg cgtcaaaagc cggcgtggtg    480

ggaatgacca aaaccgcctg ccgcgaactg gcgaaaaaag gtgtcacggt gaatgccatc    540

tgcccgggct ttatcgatac cgacatgacg cgcggcgtac cggaaaacgt ctggcaaatc    600

atggtcagca aaattcccgc gggttacgcc ggcgaggcga aagacgtcgg cgagtgtgtg    660

gcgtttctgg cgtccgatgg cgcgcgctat atcaatggtg aagtgattaa cgtcggcggc    720

ggcatggtgc tgtaa                                                     735
```

<210> SEQ ID NO 132
<211> LENGTH: 253
<212> TYPE: PRT
<213> ORGANISM: Klebsiella pneumoniae subsp. pneumoniae MGH78578

<400> SEQUENCE: 132

-continued

```
Met Ser Met Thr Phe Ser Gly Gln Val Ala Leu Val Thr Gly Ala Ala
1               5                  10                  15

Asn Gly Ile Gly Arg Ala Thr Ala Gln Ala Phe Ala Ala Gln Gly Leu
            20                  25                  30

Lys Val Val Val Ala Asp Leu Asp Thr Ala Gly Gly Glu Gly Thr Val
        35                  40                  45

Ala Leu Ile Arg Glu Ala Gly Gly Glu Ala Leu Phe Val Pro Cys Asn
    50                  55                  60

Val Thr Leu Glu Ala Asp Val Gln Ser Leu Met Ala Arg Thr Ile Glu
65                  70                  75                  80

Ala Tyr Gly Arg Leu Asp Tyr Ala Phe Asn Asn Ala Gly Ile Glu Ile
                85                  90                  95

Glu Lys Gly Arg Leu Ala Glu Gly Ser Met Asp Glu Phe Asp Ala Ile
            100                 105                 110

Met Gly Val Asn Val Lys Gly Val Trp Leu Cys Met Lys Tyr Gln Leu
        115                 120                 125

Pro Leu Leu Leu Ala Gln Gly Gly Gly Ala Ile Val Asn Thr Ala Ser
    130                 135                 140

Val Ala Gly Leu Gly Ala Ala Pro Lys Met Ser Ile Tyr Ala Ala Ser
145                 150                 155                 160

Lys His Ala Val Ile Gly Leu Thr Lys Ser Ala Ala Ile Glu Tyr Ala
                165                 170                 175

Lys Lys Lys Ile Arg Val Asn Ala Val Cys Pro Ala Val Ile Asp Thr
            180                 185                 190

Asp Met Phe Arg Arg Ala Tyr Glu Ala Asp Pro Lys Lys Ala Glu Phe
        195                 200                 205

Ala Ala Ala Met His Pro Val Gly Arg Ile Gly Lys Val Glu Glu Ile
    210                 215                 220

Ala Ser Ala Val Leu Tyr Leu Cys Ser Asp Gly Ala Ala Phe Thr Thr
225                 230                 235                 240

Gly His Ala Leu Ala Val Asp Gly Gly Ala Thr Ala Ile
                245                 250
```

<210> SEQ ID NO 133
<211> LENGTH: 750
<212> TYPE: DNA
<213> ORGANISM: Klebsiella pneumoniae subsp. pneumoniae MGH78578

<400> SEQUENCE: 133

```
atgttattga aagataaagt cgccattatt actggcgcgg cctccgcacg cggtttgggc     60

ttcgcgactg cgaaattatt cgccgaaaac ggcgcgaaag tggtcattat cgacctcaat    120

ggcgaagcca gtaaaaccgc cgcggcggca ttaggcgaag accatctcgg cctggcggcc    180

aacgtcgctg atgaagtgca ggtgcaggcg gccatcgaac agatcctggc gaaatacggt    240

cgggttgatg tactggtcaa taacgccggg attacccagc cgctgaagct gatggatatc    300

aagcgcgcca actatgacgc ggtgcttgat gttagcctgc gcggcacgct gctgatgtcg    360

caggcggtta tccccaccat gcgggcgcaa aaatccggca gcatcgtctg catctcgtcc    420

gtctccgccc agcgcggcgg cggtattttc ggcggaccgc actacagcgc ggcaaaagcc    480

ggggtgctgg gtctggcgcg ggcgatggcg cgcgagcttg gcccggataa cgtccgcgtt    540

aactgcatca ccccggggct gattcagacc gacattaccg ccggcaagct gactgatgac    600
```

-continued

atgacggcca acattcttgc cggcattccg atgaaccgcc ttggcgacgc gatagacatc 660

gcgcgcgccg cgctgttcct cggcagcgat ctttcctcct actccaccgg catcaccctg 720

gacgttaacg gcggcatgtt aattcactaa 750

<210> SEQ ID NO 134
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Klebsiella pneumoniae subsp. pneumoniae MGH78578

<400> SEQUENCE: 134

Met Leu Leu Lys Asp Lys Val Ala Ile Ile Thr Gly Ala Ala Ser Ala
1 5 10 15

Arg Gly Leu Gly Phe Ala Thr Ala Lys Leu Phe Ala Glu Asn Gly Ala
20 25 30

Lys Val Val Ile Ile Asp Leu Asn Gly Glu Ala Ser Lys Thr Ala Ala
35 40 45

Ala Ala Leu Gly Glu Asp His Leu Gly Leu Ala Ala Asn Val Ala Asp
50 55 60

Glu Val Gln Val Gln Ala Ala Ile Glu Gln Ile Leu Ala Lys Tyr Gly
65 70 75 80

Arg Val Asp Val Leu Val Asn Asn Ala Gly Ile Thr Gln Pro Leu Lys
85 90 95

Leu Met Asp Ile Lys Arg Ala Asn Tyr Asp Ala Val Leu Asp Val Ser
100 105 110

Leu Arg Gly Thr Leu Leu Met Ser Gln Ala Val Ile Pro Thr Met Arg
115 120 125

Ala Gln Lys Ser Gly Ser Ile Val Cys Ile Ser Ser Val Ser Ala Gln
130 135 140

Arg Gly Gly Gly Ile Phe Gly Gly Pro His Tyr Ser Ala Ala Lys Ala
145 150 155 160

Gly Val Leu Gly Leu Ala Arg Ala Met Ala Arg Glu Leu Gly Pro Asp
165 170 175

Asn Val Arg Val Asn Cys Ile Thr Pro Gly Leu Ile Gln Thr Asp Ile
180 185 190

Thr Ala Gly Lys Leu Thr Asp Asp Met Thr Ala Asn Ile Leu Ala Gly
195 200 205

Ile Pro Met Asn Arg Leu Gly Asp Ala Ile Asp Ile Ala Arg Ala Ala
210 215 220

Leu Phe Leu Gly Ser Asp Leu Ser Ser Tyr Ser Thr Gly Ile Thr Leu
225 230 235 240

Asp Val Asn Gly Gly Met Leu Ile His
245

<210> SEQ ID NO 135
<211> LENGTH: 750
<212> TYPE: DNA
<213> ORGANISM: Klebsiella pneumoniae subsp. pneumoniae MGH78578

<400> SEQUENCE: 135

atgttattga aagataaagt cgccattatt actggcgcgg cctccgcacg cggtttgggc 60

ttcgcgactg cgaaattatt cgccgaaaac ggcgcgaaag tggtcattat cgacctcaat 120

ggcgaagcca gtaaaaccgc cgcggcggca ttaggcgaag accatctcgg cctggcggcc 180

-continued

aacgtcgctg atgaagtgca ggtgcaggcg gccatcgaac agatcctggc gaaatacggt 240

cgggttgatg tactggtcaa taacgccggg attacccagc cgctgaagct gatggatatc 300

aagcgcgcca actatgacgc ggtgcttgat gttagcctgc gcggcacgct gctgatgtcg 360

caggcggtta tccccaccat gcgggcgcaa aaatccggca gcatcgtctg catctcgtcc 420

gtctccgccc agcgcggcgg cggtattttc ggcggaccgc actacagcgc ggcaaaagcc 480

ggggtgctgg gtctggcgcg ggcgatggcg cgcgagcttg gcccggataa cgtccgcgtt 540

aactgcatca ccccggggct gattcagacc gacattaccg ccggcaagct gactgatgac 600

atgacggcca acattcttgc cggcattccg atgaaccgcc ttggcgacgc gatagacatc 660

gcgcgcgccg cgctgttcct cggcagcgat ctttcctcct actccaccgg catcaccctg 720

gacgttaacg gcggcatgtt aattcactaa 750

<210> SEQ ID NO 136
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Klebsiella pneumoniae subsp. pneumoniae MGH78578

<400> SEQUENCE: 136

Met Leu Leu Lys Asp Lys Val Ala Ile Ile Thr Gly Ala Ala Ser Ala
1 5 10 15

Arg Gly Leu Gly Phe Ala Thr Ala Lys Leu Phe Ala Glu Asn Gly Ala
20 25 30

Lys Val Val Ile Ile Asp Leu Asn Gly Glu Ala Ser Lys Thr Ala Ala
35 40 45

Ala Ala Leu Gly Glu Asp His Leu Gly Leu Ala Ala Asn Val Ala Asp
50 55 60

Glu Val Gln Val Gln Ala Ala Ile Glu Gln Ile Leu Ala Lys Tyr Gly
65 70 75 80

Arg Val Asp Val Leu Val Asn Asn Ala Gly Ile Thr Gln Pro Leu Lys
85 90 95

Leu Met Asp Ile Lys Arg Ala Asn Tyr Asp Ala Val Leu Asp Val Ser
100 105 110

Leu Arg Gly Thr Leu Leu Met Ser Gln Ala Val Ile Pro Thr Met Arg
115 120 125

Ala Gln Lys Ser Gly Ser Ile Val Cys Ile Ser Ser Val Ser Ala Gln
130 135 140

Arg Gly Gly Gly Ile Phe Gly Gly Pro His Tyr Ser Ala Ala Lys Ala
145 150 155 160

Gly Val Leu Gly Leu Ala Arg Ala Met Ala Arg Glu Leu Gly Pro Asp
165 170 175

Asn Val Arg Val Asn Cys Ile Thr Pro Gly Leu Ile Gln Thr Asp Ile
180 185 190

Thr Ala Gly Lys Leu Thr Asp Asp Met Thr Ala Asn Ile Leu Ala Gly
195 200 205

Ile Pro Met Asn Arg Leu Gly Asp Ala Ile Asp Ile Ala Arg Ala Ala
210 215 220

Leu Phe Leu Gly Ser Asp Leu Ser Ser Tyr Ser Thr Gly Ile Thr Leu
225 230 235 240

Asp Val Asn Gly Gly Met Leu Ile His
245

-continued

<210> SEQ ID NO 137
<211> LENGTH: 714
<212> TYPE: DNA
<213> ORGANISM: Klebsiella pneumoniae subsp. pneumoniae MGH78578

<400> SEQUENCE: 137

atgacagcgt ttcacaacaa atcagtgctg gttttaggcg ggagtcgggg aattggcgcg 60

gcgatcgtca ggcgttttgt cgccgatggc gcgtcggtgg tgtttagcta ttccggttcg 120

ccggaagcgg ccgagcggct ggcggcagag accggcagca cggcggtgca ggcggacagc 180

gccgatcgcg atgcggtgat aagcctggtc cgcgacagcg gcccgctgga cgtgttagtg 240

gtcaatgccg ggatcgcgct tttcggtgac gctctcgagc aggacagcga tgcaatcgat 300

cgcctgttcc acatcaatat tcacgccccc taccatgcct ccgtcgaagc ggcgcgccgc 360

atgccggaag gcgggcgcat tattgtcatc ggctcagtca atggcgatcg catgccgttg 420

ccgggaatgg cggcctatgc gctcagcaaa tcggccctgc aggggctggc gcgcggcctg 480

gcgcgggatt ttggcccgcg cggcatcacg gtcaacgtcg tccagcccgg cccaattgat 540

accgacgcca acccggagaa cggcccgatg aaagagctga tgcacagctt tatggccatt 600

aagcgccatg gccgtccgga agaggtggcg ggaatggtgg cgtggctggc cggtccggag 660

gcgtcgtttg tcactggcgc catgcacacc atcgacggag cgtttggcgc ctga 714

<210> SEQ ID NO 138
<211> LENGTH: 237
<212> TYPE: PRT
<213> ORGANISM: Klebsiella pneumoniae subsp. pneumoniae MGH78578

<400> SEQUENCE: 138

Met Thr Ala Phe His Asn Lys Ser Val Leu Val Leu Gly Gly Ser Arg
1 5 10 15

Gly Ile Gly Ala Ala Ile Val Arg Arg Phe Val Ala Asp Gly Ala Ser
20 25 30

Val Val Phe Ser Tyr Ser Gly Ser Pro Glu Ala Ala Glu Arg Leu Ala
35 40 45

Ala Glu Thr Gly Ser Thr Ala Val Gln Ala Asp Ser Ala Asp Arg Asp
50 55 60

Ala Val Ile Ser Leu Val Arg Asp Ser Gly Pro Leu Asp Val Leu Val
65 70 75 80

Val Asn Ala Gly Ile Ala Leu Phe Gly Asp Ala Leu Glu Gln Asp Ser
85 90 95

Asp Ala Ile Asp Arg Leu Phe His Ile Asn Ile His Ala Pro Tyr His
100 105 110

Ala Ser Val Glu Ala Ala Arg Arg Met Pro Glu Gly Gly Arg Ile Ile
115 120 125

Val Ile Gly Ser Val Asn Gly Asp Arg Met Pro Leu Pro Gly Met Ala
130 135 140

Ala Tyr Ala Leu Ser Lys Ser Ala Leu Gln Gly Leu Ala Arg Gly Leu
145 150 155 160

Ala Arg Asp Phe Gly Pro Arg Gly Ile Thr Val Asn Val Val Gln Pro
165 170 175

Gly Pro Ile Asp Thr Asp Ala Asn Pro Glu Asn Gly Pro Met Lys Glu
180 185 190

-continued

```
Leu Met His Ser Phe Met Ala Ile Lys Arg His Gly Arg Pro Glu Glu
        195                 200                 205

Val Ala Gly Met Val Ala Trp Leu Ala Gly Pro Glu Ala Ser Phe Val
    210                 215                 220

Thr Gly Ala Met His Thr Ile Asp Gly Ala Phe Gly Ala
225                 230                 235


<210> SEQ ID NO 139
<211> LENGTH: 750
<212> TYPE: DNA
<213> ORGANISM: Klebsiella pneumoniae subp. pneumoniae MGH78578

<400> SEQUENCE: 139

atgaacggcc tgctaaacgg taaacgtatt gtcgtcaccg gtgcggcgcg cggtctcggg     60

taccactttg ccgaagcctg cgccgctcag ggcgcgacgg tggtgatgtg cgacatcctg    120

cagggagagc tggcggaaag cgctcatcgc ctgcagcaga agggctatca ggtcgaatct    180

cacgccatcg atcttgccag tcaagcatcg atcgagcagg tcttcagcgc catcggcgcg    240

caggggtcta tcgatggctt agtcaataac gcagcgatgg ccaccggcgt cggcggaaaa    300

aatatgatcg attacgatcc ggatctgtgg gatcgggtaa tgacggtcaa cgttaaaggc    360

acctggttgg tgacccgcgc ggcggtaccg ctgctgcgcg aaggggcggc gatcgtcaac    420

gtcgcttcgg ataccgcgct gtggggcgcg ccgcggctga tggcctatgt cgccagtaag    480

ggcgcggtga ttgcgatgac ccgctccatg gcccgcgagc tgggtgaaaa gcggatccgt    540

atcaacgcca tcgcgccggg actgacccgc gttgaggcca cggaatacgt tcccgccgag    600

cgtcatcagc tgtatgagaa cggccgcgcg ctcagcggcg cgcagcagcc ggaagatgtc    660

accggcagcg tggtctggct gctgagcgat ctttcgcgct ttatcaccgg ccaactgatc    720

ccggtcaacg gcggttttgt ctttaactaa                                     750


<210> SEQ ID NO 140
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Klebsiella pneumoniae subsp. pneumoinae MGH78578

<400> SEQUENCE: 140

Met Asn Gly Leu Leu Asn Gly Lys Arg Ile Val Val Thr Gly Ala Ala
1               5                   10                  15

Arg Gly Leu Gly Tyr His Phe Ala Glu Ala Cys Ala Ala Gln Gly Ala
            20                  25                  30

Thr Val Val Met Cys Asp Ile Leu Gln Gly Glu Leu Ala Glu Ser Ala
        35                  40                  45

His Arg Leu Gln Gln Lys Gly Tyr Gln Val Glu Ser His Ala Ile Asp
    50                  55                  60

Leu Ala Ser Gln Ala Ser Ile Glu Gln Val Phe Ser Ala Ile Gly Ala
65                  70                  75                  80

Gln Gly Ser Ile Asp Gly Leu Val Asn Asn Ala Ala Met Ala Thr Gly
                85                  90                  95

Val Gly Gly Lys Asn Met Ile Asp Tyr Asp Pro Asp Leu Trp Asp Arg
            100                 105                 110

Val Met Thr Val Asn Val Lys Gly Thr Trp Leu Val Thr Arg Ala Ala
        115                 120                 125

Val Pro Leu Leu Arg Glu Gly Ala Ala Ile Val Asn Val Ala Ser Asp
```

-continued

```
    130                 135                 140

Thr Ala Leu Trp Gly Ala Pro Arg Leu Met Ala Tyr Val Ala Ser Lys
145                 150                 155                 160

Gly Ala Val Ile Ala Met Thr Arg Ser Met Ala Arg Glu Leu Gly Glu
                165                 170                 175

Lys Arg Ile Arg Ile Asn Ala Ile Ala Pro Gly Leu Thr Arg Val Glu
            180                 185                 190

Ala Thr Glu Tyr Val Pro Ala Glu Arg His Gln Leu Tyr Glu Asn Gly
        195                 200                 205

Arg Ala Leu Ser Gly Ala Gln Gln Pro Glu Asp Val Thr Gly Ser Val
    210                 215                 220

Val Trp Leu Leu Ser Asp Leu Ser Arg Phe Ile Thr Gly Gln Leu Ile
225                 230                 235                 240

Pro Val Asn Gly Gly Phe Val Phe Asn
                245
```

<210> SEQ ID NO 141
<211> LENGTH: 795
<212> TYPE: DNA
<213> ORGANISM: Klebsiella pneumoniae subsp. pneumoniae MGH78578

<400> SEQUENCE: 141

```
atgaatgcac aaattgaagg gcgcgtcgcg gtagtcaccg gcggttcgtc aggaatcggc     60

tttgaaacgc tgcgcctgct gctgggcgaa ggggcgaaag tcgccttttg cggccgcaac    120

ccggatcggc ttgccagcgc ccatgcggcg ttgcaaaacg aatatccaga aggtgaggtg    180

ttctcctggc gctgtgacgt actgaacgaa gctgaagttg aggcgttcgc cgccgcggtc    240

gccgcgcgtt tcggcggcgt cgatatgctg attaataacg ccggccaggg ctatgtcgcc    300

cacttcgccg atacgccacg tgaggcctgg ctgcacgaag ccgaactgaa actgttcggc    360

gtgattaacc cggtaaaggc ctttcagtcc ctgctagagg cgtcggatat cgcctcgatt    420

acctgtgtga actcgctgct ggcgttacag ccggaagagc acatgatcgc cacctctgcc    480

gcccgcgccg cgctgctcaa tatgacgctg actctgtcga aagagctggt ggataaaggt    540

attcgtgtga attccattct gctggggatg gtggagtccg ggcagtggca gcgccgtttt    600

gagagccgaa gcgataagag ccagagttgg cagcagtgga ccgccgatat cgcccgtaag    660

cgggggatcc cgatggcgcg tctcggtaag ccgcaggagc cagcgcaagc gctgctattc    720

ctcgcttcgc cgctggcctc ctttaccacc ggcgcggcgc tggacgtttc cggcggtttc    780

tgtcgccatc tgtaa                                                     795
```

<210> SEQ ID NO 142
<211> LENGTH: 264
<212> TYPE: PRT
<213> ORGANISM: Klebsiella pneumoniae subsp. pneumoniae MGH78578

<400> SEQUENCE: 142

```
Met Asn Ala Gln Ile Glu Gly Arg Val Ala Val Val Thr Gly Gly Ser
1               5                   10                  15

Ser Gly Ile Gly Phe Glu Thr Leu Arg Leu Leu Leu Gly Glu Gly Ala
            20                  25                  30

Lys Val Ala Phe Cys Gly Arg Asn Pro Asp Arg Leu Ala Ser Ala His
        35                  40                  45
```

-continued

```
Ala Ala Leu Gln Asn Glu Tyr Pro Glu Gly Glu Val Phe Ser Trp Arg
    50                  55                  60

Cys Asp Val Leu Asn Glu Ala Glu Val Glu Ala Phe Ala Ala Ala Val
65                  70                  75                  80

Ala Ala Arg Phe Gly Gly Val Asp Met Leu Ile Asn Asn Ala Gly Gln
                85                  90                  95

Gly Tyr Val Ala His Phe Ala Asp Thr Pro Arg Glu Ala Trp Leu His
            100                 105                 110

Glu Ala Glu Leu Lys Leu Phe Gly Val Ile Asn Pro Val Lys Ala Phe
        115                 120                 125

Gln Ser Leu Leu Glu Ala Ser Asp Ile Ala Ser Ile Thr Cys Val Asn
    130                 135                 140

Ser Leu Leu Ala Leu Gln Pro Glu Glu His Met Ile Ala Thr Ser Ala
145                 150                 155                 160

Ala Arg Ala Ala Leu Leu Asn Met Thr Leu Thr Leu Ser Lys Glu Leu
                165                 170                 175

Val Asp Lys Gly Ile Arg Val Asn Ser Ile Leu Leu Gly Met Val Glu
            180                 185                 190

Ser Gly Gln Trp Gln Arg Arg Phe Glu Ser Arg Ser Asp Lys Ser Gln
        195                 200                 205

Ser Trp Gln Gln Trp Thr Ala Asp Ile Ala Arg Lys Arg Gly Ile Pro
    210                 215                 220

Met Ala Arg Leu Gly Lys Pro Gln Glu Pro Ala Gln Ala Leu Leu Phe
225                 230                 235                 240

Leu Ala Ser Pro Leu Ala Ser Phe Thr Thr Gly Ala Ala Leu Asp Val
                245                 250                 255

Ser Gly Gly Phe Cys Arg His Leu
            260
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<210> SEQ ID NO 143
<211> LENGTH: 1795
<212> TYPE: DNA
<213> ORGANISM: Pseudomonas fluorescens

<400> SEQUENCE: 143

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cgccaagcaa tcgggctttg gggcagaatt gggtcgcgaa gggcttgagg agtttgccca     60

gtccaagatc atcaacgccg cgctataaat taaaggatcc cccatggcga tgattacagg    120

cggcgaactg gttgttcgca ccctaataaa ggctggggtc gaacatctgt tcggcctgca    180

cggcgcgcat atcgatacga tttttcaagc ctgtctcgat catgatgtgc cgatcatcga    240

cacccgccat gaggccgccg cagggcatgc ggccgagggc tatgcccgcg ctggcgccaa    300

gctgggcgtg gctggtcacg gcgggcgggg gatttaccaa tgcggtcacg cccattgcca    360

acgcttggct ggatcgcaag gccggtgtat tcctcacccg ggatcgggcg cgctgcgtga    420

tgatgaaacc aacacgttgc aggcggggat tgatcaggtc gccatggcgg cgcccattac    480

caaatgggcg catcgggtga tggcaaccga gcatatccca cggctggtga tgcaggcgat    540

ccgcgccgcg ttgagcgcgc cacgcgggcc ggtgttgctg gatctgccgt gggatattct    600

gatgaaccag attgatgagg atagcgtcat tatccccgat ctggtcttgt ccgcgcatgg    660

ggccagaccc gaccctgccg atctggatca ggctctcgcg cttttgcgca aggcggagcg    720

gccggtcatc gtgctcggct cagaagcctc gcggacagcg cgcaagacgg cgcttagcgc    780
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cttcgtggcg gcgactggcg tgccggtgtt tgccgattat gaagggctaa gcatgctctc 840

ggggctgccc gatgctatgc ggggcgggct ggtgcaaaac ctctattctt ttgccaaagc 900

cgatgccgcg ccagatctcg tgctgatgct gggggcgcgc tttggcctta acaccgggca 960

tggatctggg cagttgatcc cccatagcgc gcaggtcatt caggtcgacc ctgatgcctg 1020

cgagctggga cgcctgcagg gcatcgctct gggcattgtg gccgatgtgg gtgggaccat 1080

cgaggctttg gcgcaggcca ccgcgcaaga tgcggcttgg ccggatcgcg gcgactggtg 1140

cgccaaagtg acggatctgg cgcaagagcg ctatgccagc atcgctgcga aatcgagcag 1200

cgagcatgcg ctccaccccct ttcacgcctc gcaggtcatt gccaaacacg tcgatgcagg 1260

ggtgacggtg gtagcggatg gtgcgctgac ctatctctgg ctgtccgaag tgatgagccg 1320

cgtgaaaccc ggcggttttc tctgccacgg ctatctaggc tcgatgggcg tgggcttcgg 1380

cacggcgctg ggcgcgcaag tggccgatct tgaagcaggc cgccgcacga tccttgtgac 1440

cggcgatggc tcggtgggct atagcatcgg tgaatttgat acgctggtgc gcaaacaatt 1500

gccgctgatc gtcatcatca tgaacaacca aagctggggg gcgacattgc atttccagca 1560

attggccgtc ggccccaatc gcgtgacggg cacccgtttg gaaaatggct cctatcacgg 1620

ggtggccgcc gcctttggcg cggatggcta tcatgtcgac agtgtggaga gcttttctgc 1680

ggctctggcc caagcgctcg cccataatcg ccccgcctgc atcaatgtcg cggtcgcgct 1740

cgatccgatc ccgcccgaag aactcattct gatcggcatg gacccttcg catga 1795

<210> SEQ ID NO 144
<211> LENGTH: 563
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas fluorescens

<400> SEQUENCE: 144

Met Ala Met Ile Thr Gly Gly Glu Leu Val Val Arg Thr Leu Ile Lys
1 5 10 15

Ala Gly Val Glu His Leu Phe Gly Leu His Gly Ala His Ile Asp Thr
20 25 30

Ile Phe Gln Ala Cys Leu Asp His Asp Val Pro Ile Ile Asp Thr Arg
35 40 45

His Glu Ala Ala Ala Gly His Ala Ala Glu Gly Tyr Ala Arg Ala Gly
50 55 60

Ala Lys Leu Gly Val Ala Gly His Gly Gly Arg Gly Ile Tyr Gln Cys
65 70 75 80

Gly His Ala His Cys Gln Arg Leu Ala Gly Ser Gln Gly Arg Cys Ile
85 90 95

Pro His Pro Gly Ser Gly Ala Leu Arg Asp Asp Glu Thr Asn Thr Leu
100 105 110

Gln Ala Gly Ile Asp Gln Val Ala Met Ala Ala Pro Ile Thr Lys Trp
115 120 125

Ala His Arg Val Met Ala Thr Glu His Ile Pro Arg Leu Val Met Gln
130 135 140

Ala Ile Arg Ala Ala Leu Ser Ala Pro Arg Gly Pro Val Leu Leu Asp
145 150 155 160

Leu Pro Trp Asp Ile Leu Met Asn Gln Ile Asp Glu Asp Ser Val Ile
165 170 175

Ile Pro Asp Leu Val Leu Ser Ala His Gly Ala Arg Pro Asp Pro Ala

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            180                 185                 190

Asp Leu Asp Gln Ala Leu Ala Leu Leu Arg Lys Ala Glu Arg Pro Val
        195                 200                 205

Ile Val Leu Gly Ser Glu Ala Ser Arg Thr Ala Arg Lys Thr Ala Leu
    210                 215                 220

Ser Ala Phe Val Ala Ala Thr Gly Val Pro Val Phe Ala Asp Tyr Glu
225                 230                 235                 240

Gly Leu Ser Met Leu Ser Gly Leu Pro Asp Ala Met Arg Gly Gly Leu
                245                 250                 255

Val Gln Asn Leu Tyr Ser Phe Ala Lys Ala Asp Ala Ala Pro Asp Leu
            260                 265                 270

Val Leu Met Leu Gly Ala Arg Phe Gly Leu Asn Thr Gly His Gly Ser
        275                 280                 285

Gly Gln Leu Ile Pro His Ser Ala Gln Val Ile Gln Val Asp Pro Asp
    290                 295                 300

Ala Cys Glu Leu Gly Arg Leu Gln Gly Ile Ala Leu Gly Ile Val Ala
305                 310                 315                 320

Asp Val Gly Gly Thr Ile Glu Ala Leu Ala Gln Ala Thr Ala Gln Asp
                325                 330                 335

Ala Ala Trp Pro Asp Arg Gly Asp Trp Cys Ala Lys Val Thr Asp Leu
            340                 345                 350

Ala Gln Glu Arg Tyr Ala Ser Ile Ala Ala Lys Ser Ser Ser Glu His
        355                 360                 365

Ala Leu His Pro Phe His Ala Ser Gln Val Ile Ala Lys His Val Asp
    370                 375                 380

Ala Gly Val Thr Val Val Ala Asp Gly Ala Leu Thr Tyr Leu Trp Leu
385                 390                 395                 400

Ser Glu Val Met Ser Arg Val Lys Pro Gly Gly Phe Leu Cys His Gly
                405                 410                 415

Tyr Leu Gly Ser Met Gly Val Gly Phe Gly Thr Ala Leu Gly Ala Gln
            420                 425                 430

Val Ala Asp Leu Glu Ala Gly Arg Arg Thr Ile Leu Val Thr Gly Asp
        435                 440                 445

Gly Ser Val Gly Tyr Ser Ile Gly Glu Phe Asp Thr Leu Val Arg Lys
    450                 455                 460

Gln Leu Pro Leu Ile Val Ile Ile Met Asn Asn Gln Ser Trp Gly Ala
465                 470                 475                 480

Thr Leu His Phe Gln Gln Leu Ala Val Gly Pro Asn Arg Val Thr Gly
                485                 490                 495

Thr Arg Leu Glu Asn Gly Ser Tyr His Gly Val Ala Ala Ala Phe Gly
            500                 505                 510

Ala Asp Gly Tyr His Val Asp Ser Val Glu Ser Phe Ser Ala Ala Leu
        515                 520                 525

Ala Gln Ala Leu Ala His Asn Arg Pro Ala Cys Ile Asn Val Ala Val
    530                 535                 540

Ala Leu Asp Pro Ile Pro Pro Glu Glu Leu Ile Leu Ile Gly Met Asp
545                 550                 555                 560

Pro Phe Ala


<210> SEQ ID NO 145
<211> LENGTH: 9
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: A polypeptide that is similar to an autotransporter adhesion or type I secretion target repeat.
<220> FEATURE:
<221> NAME/KEY: VARIANT
<222> LOCATION: 3, 5, 7, 8, 9
<223> OTHER INFORMATION: Xaa = Any Amino Acid

<400> SEQUENCE: 145

Gly Gly Xaa Gly Xaa Asp Xaa Xaa Xaa
1 5

<210> SEQ ID NO 146
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 146

gtctttattc atatatatat cctccttaat tcaaccgttc aatcaccatc 50

<210> SEQ ID NO 147
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 147

gggcggccgc aaggggttcg cgttggccga 30

<210> SEQ ID NO 148
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 148

ggagaaaata ccgcatcagg cg 22

<210> SEQ ID NO 149
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 149

cgggatccaa gttgcaggat atgacgaaag cg 32

<210> SEQ ID NO 150
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 150

gctctagaag attatccctg tctgcggaag cgg 33

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<210> SEQ ID NO 151
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 151

gctctagagg ggtgcctaat gagtgagcta ac 32

<210> SEQ ID NO 152
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 152

cgggatccgc gttaatattt tgttaaaatt cgc 33

<210> SEQ ID NO 153
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 153

gctctagagt ttatgtcgca cccgccgttg g 31

<210> SEQ ID NO 154
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 154

cccaagctta gaaagggaaa ttgtggtagc cc 32

<210> SEQ ID NO 155
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 155

ggaattccat atgcgtccct ctgccccggc c 31

<210> SEQ ID NO 156
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 156

cgggatcctt agaactgctt gggaagggag 30

<210> SEQ ID NO 157
<211> LENGTH: 50
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 157

aggtacggtg aaataaagga ggatatacat atgtccaaaa agattgccgt 50

<210> SEQ ID NO 158
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 158

ttttccttttt gcggccgccc cgctggcatc gcctcac 37

<210> SEQ ID NO 159
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 159

ggcgatgcca gcgtaaagga ggatatacat atgaaaaact ggaaaacaag 50

<210> SEQ ID NO 160
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 160

ttttccttttt gcggccgccc cagcttagcg ccttcta 37

<210> SEQ ID NO 161
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 161

cccgagctct taggaggatt agtcatggaa c 31

<210> SEQ ID NO 162
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 162

gctctagatt attttgaata atcgtagaaa cc 32

<210> SEQ ID NO 163
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

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<400> SEQUENCE: 163

gctctagagg aggatatata tatgaaaaat tgtgtcatcg tc 42

<210> SEQ ID NO 164
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 164

aactgcagtt aattcaaccg ttcaatcacc 30

<210> SEQ ID NO 165
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 165

cgagctcagg aggatatata tatgaaaaat tgtgtcatcg tcagtg 46

<210> SEQ ID NO 166
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 166

ggttgaatta aggaggatat atatatgaat aaagacacac taatacctac 50

<210> SEQ ID NO 167
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 167

cccaagctta gccggcaagt acacatcttc 30

<210> SEQ ID NO 168
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 168

cgagctcagg aggatatata tatgaaaaat tgtgtcatcg tcagtg 46

<210> SEQ ID NO 169
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 169

cccaagctta gccggcaagt acacatcttc 30

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<210> SEQ ID NO 170
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 170

aaggaaaaaa gcggccgccc ctgaaccgac gaccgggtcg 40

<210> SEQ ID NO 171
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 171

cggggtaccg cggatacata tttgaatgta tttag 35

<210> SEQ ID NO 172
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 172

aaggaaaaaa gcggccgcgc ggatacatat ttgaatgtat ttag 44

<210> SEQ ID NO 173
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 173

gctctagagg aggatatata tatggctaac tacttcaata cac 43

<210> SEQ ID NO 174
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 174

tgctgttgcg ggttaaggag gatatatata tgcctaagta ccgttccgcc 50

<210> SEQ ID NO 175
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 175

aacggtactt aggcatatat atatcctcct taacccgcaa cagcaatacg 50

<210> SEQ ID NO 176
<211> LENGTH: 30
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 176

acatgcatgc ttaacccccc agtttcgatt 30

<210> SEQ ID NO 177
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 177

gctctagagg aggatatata tatggctaac tacttcaata cac 43

<210> SEQ ID NO 178
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 178

acatgcatgc ttaacccccc agtttcgatt 30

<210> SEQ ID NO 179
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 179

cccgagctca ggaggatata tatatggata aacagtatcc ggt 43

<210> SEQ ID NO 180
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 180

gctctagatt acagaatttg actcaggt 28

<210> SEQ ID NO 181
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 181

cccgagctca ggaggatata tatatgttga caaaagcaac aaaag 45

<210> SEQ ID NO 182
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

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<400> SEQUENCE: 182

ctctaaatct ctggaaaggg taccg 25

<210> SEQ ID NO 183
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 183

gctctagatt agagagcttt cgttttcatg 30

<210> SEQ ID NO 184
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 184

cccgagctca ggaggatata tatatgttga caaaagcaac aaaag 45

<210> SEQ ID NO 185
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 185

gctctagatt agagagcttt cgttttcatg 30

<210> SEQ ID NO 186
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 186

cgagctcagg aggatatata tatgagccag caagtcatta ttttcg 46

<210> SEQ ID NO 187
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 187

aaaactgcag cgtttgatga cgtggacgat agcgg 35

<210> SEQ ID NO 188
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 188

cgagctcagg aggatatata tatgagccag caagtcatta ttttcg 46

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<210> SEQ ID NO 189
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 189

aggggtgtaa ggaggatata tatatggcta agacgttata cgaaaaattg 50

<210> SEQ ID NO 190
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 190

cgtcttagcc atatatatat cctccttaca ccccttctgc tacatagcgg 50

<210> SEQ ID NO 191
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 191

aaaactgcag cgtttgatga cgtggacgat agcgg 35

<210> SEQ ID NO 192
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 192

cgagctcagg aggatatata tatgagccag caagtcatta ttttcg 46

<210> SEQ ID NO 193
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 193

aaaactgcag cgtttgatga cgtggacgat agcgg 35

<210> SEQ ID NO 194
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 194

cgagctcagg aggatatata tatgagccag caagtcatta ttttcg 46

<210> SEQ ID NO 195
<211> LENGTH: 50
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 195

gaaaccgtgt gaggaggata tatatatgtc gaagaattac catattgccg 50

<210> SEQ ID NO 196
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 196

aggggtgtaa ggaggatata tatatggcta agacgttata cgaaaaattg 50

<210> SEQ ID NO 197
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 197

acattaaata aggaggatat atatatggca gagaaattta tcaaacacac 50

<210> SEQ ID NO 198
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 198

attcttcgac atatatatat cctcctcaca cggtttcctt gttgttttcg 50

<210> SEQ ID NO 199
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 199

cgtcttagcc atatatatat cctccttaca ccccttctgc tacatagcgg 50

<210> SEQ ID NO 200
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 200

tttctctgcc atatatatat cctccttatt taatgttgcg aatgtcggcg 50

<210> SEQ ID NO 201
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

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<400> SEQUENCE: 201

aaaactgcag cgtttgatga cgtggacgat agcgg 35

<210> SEQ ID NO 202
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 202

cgagctcagg aggatatata tatgagccag caagtcatta ttttcg 46

<210> SEQ ID NO 203
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 203

aaaactgcag cgtttgatga cgtggacgat agcgg 35

<210> SEQ ID NO 204
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 204

aaggaaaaaa gcggccgccc ctgaaccgac gaccgggtcg 40

<210> SEQ ID NO 205
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 205

cggggtaccg cggatacata tttgaatgta tttag 35

<210> SEQ ID NO 206
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 206

aaggaaaaaa gcggccgcac ttttcatact cccgccattc ag 42

<210> SEQ ID NO 207
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 207

caaaggccgt ctgcacgcgc cgaaaggcaa a 31

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<210> SEQ ID NO 208
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 208

tttgcctttc ggcgcgtgca gacggccttt g 31

<210> SEQ ID NO 209
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<400> SEQUENCE: 209

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<210> SEQ ID NO 210
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<400> SEQUENCE: 210

aaggaaaaaa gcggccgcac ttttcatact cccgccattc ag 42

<210> SEQ ID NO 211
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 211

acatgcatgc cgtttgatga cgtggacgat agcgg 35

<210> SEQ ID NO 212
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 212

cccgagctca ggaggatata tatatgaatt atcagaacga cgatttac 48

<210> SEQ ID NO 213
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 213

gcgtcgcggg taaggaggaa aattttatgt cctcacgtaa agagcttgcc 50

<210> SEQ ID NO 214
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<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 214

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<210> SEQ ID NO 215
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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caatcagcgt aaggaggtat atataatgaa aaccgtaact gtaaaagatc 50

<210> SEQ ID NO 216
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<400> SEQUENCE: 216

tacaccaggc ataaggagga attaattatg gaaacctatg ctgtttttgg 50

<210> SEQ ID NO 217
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tacgtgagga cataaaattt tcctccttac ccgcgacgcg cttttactgc 50

<210> SEQ ID NO 218
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<400> SEQUENCE: 218

caatcctctc cataatttta acctccttac agcagttctt ttgctttcgc 50

<210> SEQ ID NO 219
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<400> SEQUENCE: 219

caatcagcgt aaggaggtat atataatgaa aaccgtaact gtaaaagatc 50

<210> SEQ ID NO 220
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<400> SEQUENCE: 220

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<210> SEQ ID NO 221
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 221

acatgcatgc ttacgcggac aattcctcct gcaa 34

<210> SEQ ID NO 222
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 222

cccgagctca ggaggatata tatatgaatt atcagaacga cgatttac 48

<210> SEQ ID NO 223
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 223

acatgcatgc ttacgcggac aattcctcct gcaa 34

<210> SEQ ID NO 224
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 224

cccgagctca ggaggatata tatatgacat cggaaaaccc gttactgg 48

<210> SEQ ID NO 225
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<400> SEQUENCE: 225

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<210> SEQ ID NO 226
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<210> SEQ ID NO 227
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<400> SEQUENCE: 227

caggcagcct aaggaggaat taattatggc tggaaacaca attggacaac 50

<210> SEQ ID NO 228
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<220> FEATURE:
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<400> SEQUENCE: 228

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<210> SEQ ID NO 229
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 229

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<210> SEQ ID NO 230
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 230

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<210> SEQ ID NO 231
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 231

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<210> SEQ ID NO 232
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 232

cccgagctca ggaggatata tatatgacat cggaaaaccc gttactgg 48

<210> SEQ ID NO 233
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<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 233

acatgcatgc ttaccagcgt ggaatatcag tcttc 35

<210> SEQ ID NO 234
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 234

cccgagctca ggaggatata tatatggttg ctgaattgac cgcattac 48

<210> SEQ ID NO 235
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 235

aatcgccagt aaggaggaaa attttatgac acaacctctt tttctgatcg 50

<210> SEQ ID NO 236
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 236

gatcaattgt taaggaggta tatataatgg aatccctgac gttacaaccc 50

<210> SEQ ID NO 237
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 237

caggcagcct aaggaggaat taattatggc tggaaacaca attggacaac 50

<210> SEQ ID NO 238
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 238

gaggttgtgt cataaaattt tcctccttac tggcgattgt cattcgcctg 50

<210> SEQ ID NO 239
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

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<400> SEQUENCE: 239

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<210> SEQ ID NO 240
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 240

gtttccagcc ataattaatt cctccttagg ctgcctggct aatccgcgcc 50

<210> SEQ ID NO 241
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 241

acatgcatgc ttaccagcgt ggaatatcag tcttc 35

<210> SEQ ID NO 242
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 242

cccgagctca ggaggatata tatatggttg ctgaattgac cgcattac 48

<210> SEQ ID NO 243
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 243

acatgcatgc ttaccagcgt ggaatatcag tcttc 35

<210> SEQ ID NO 244
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 244

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<210> SEQ ID NO 245
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 245

gctctagaac ttttcatact cccgccattc ag 32

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<210> SEQ ID NO 246
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 246

gctctagagc ggatacatat ttgaatgtat ttag 34

<210> SEQ ID NO 247
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 247

aaggaaaaaa gcggccgcgc ggatacatat ttgaatgtat ttag 44

<210> SEQ ID NO 248
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 248

catgccatgg ctatgattac tggtgg 26

<210> SEQ ID NO 249
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 249

ccccgagctc ttacgcgccg gattggaaat aca 33

<210> SEQ ID NO 250
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 250

catgccatgg ccaaagttac aaatcaaaaa g 31

<210> SEQ ID NO 251
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 251

cgagctctta aaatgatttt atatagatat cc 32

<210> SEQ ID NO 252
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<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 252

catgccatgg gtattccaga aactcaaaaa g 31

<210> SEQ ID NO 253
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 253

cccgagctct tatttagaag tgtcaacaac g 31

<210> SEQ ID NO 254
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 254

ccccgagctc aggaggatat acatatgaat aaagacacac taatacc 47

<210> SEQ ID NO 255
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 255

cccaagctta gccggcaagt acacatcttc 30

<210> SEQ ID NO 256
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 256

cccgagctca ggaggatata tatatgtata cagtaggaga ttacc 45

<210> SEQ ID NO 257
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 257

gctctagatt atgatttatt ttgttcagca aat 33

<210> SEQ ID NO 258
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

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<400> SEQUENCE: 258

cccgagctca ggaggatata tatatgtata cagtaggaga ttacc 45

<210> SEQ ID NO 259
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 259

gctctagatt atgatttatt ttgttcagca aat 33

<210> SEQ ID NO 260
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 260

cgagctcagg aggatatata tatgaaaaaa gtcgcacttg ttaccg 46

<210> SEQ ID NO 261
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 261

ggccggcggc cgcgcgatgg cggtgaaagt g 31

<210> SEQ ID NO 262
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 262

aactaatcta gaggaggata tatatatgag catgacgttt tccggccagg 50

<210> SEQ ID NO 263
<211> LENGTH: 31
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 263

ccttgcggag ggctcgatgg atgagttcga c 31

<210> SEQ ID NO 264
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 264

cactttcacc gccatcgcgc ggccgccggc c 31

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<210> SEQ ID NO 265
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 265

gctcatatat atatcctcct ctagattagt taaacaccat cccgccgtcg 50

<210> SEQ ID NO 266
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 266

gtcgaactca tccatcgagc cctccgcaag g 31

<210> SEQ ID NO 267
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 267

cccaagctta gatcgcggtg gccccgccgt cg 32

<210> SEQ ID NO 268
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 268

cgagctcagg aggatatata tatgaaaaaa gtcgcacttg ttaccg 46

<210> SEQ ID NO 269
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 269

cccaagctta gatcgcggtg gccccgccgt cg 32

<210> SEQ ID NO 270
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 270

gctctagagg aggatttaaa aatggaaatt aacgaaacgc tgc 43

<210> SEQ ID NO 271
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<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 271

tccccgcggt taagcatggc gatcccgaaa tggaatccct ttgac 45

<210> SEQ ID NO 272
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 272

ccgctcgagg aggatatata tatgagatcg aaaagatttg aagc 44

<210> SEQ ID NO 273
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 273

gctctagatt agccaagttc attgggatcg 30

<210> SEQ ID NO 274
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 274

cggggtacca cttttcatac tcccgccatt cag 33

<210> SEQ ID NO 275
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 275

cggtaccctt tccagagatt tagag 25

<210> SEQ ID NO 276
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 276

ggaattccat atgttcacaa cgtccgccta 30

<210> SEQ ID NO 277
<211> LENGTH: 27
<212> TYPE: DNA
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<400> SEQUENCE: 277

gcttgacggc catgtggccg aggccgc 27

<210> SEQ ID NO 278
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 278

gcggcctcgg ccacatggcc gtcaagc 27

<210> SEQ ID NO 279
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 279

cgggatcctt aggcggcctt ctggcgcg 28

<210> SEQ ID NO 280
<211> LENGTH: 30
<212> TYPE: DNA
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<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 280

ggaattccat atggctattg caagaggtta 30

<210> SEQ ID NO 281
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 281

cgggatcctt aagcgtcgag cgaggcca 28

<210> SEQ ID NO 282
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 282

ggaattccat atgactaaaa caatgaaggc 30

<210> SEQ ID NO 283
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 283

caccggggcc ggggtccggt attgcca 27

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<210> SEQ ID NO 284
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 284

tggcaatacc ggaccccggc cccggtg 27

<210> SEQ ID NO 285
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 285

cgggatcctt aggcggcgag atccacga 28

<210> SEQ ID NO 286
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 286

ggaattccat atgaccgggg cgaaccagcc 30

<210> SEQ ID NO 287
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<212> TYPE: DNA
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atagccgctc atacgcctcg gttgcct 27

<210> SEQ ID NO 288
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 288

aggcaaccga ggcgtatgag cggctat 27

<210> SEQ ID NO 289
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 289

cgggatcctt aagcgccgtg cggaagga 28

<210> SEQ ID NO 290
<211> LENGTH: 30
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 290

ggaattccat atgaccatgc atgccattca 30

<210> SEQ ID NO 291
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 291

cgggatcctt attcggctgc aaattgca 28

<210> SEQ ID NO 292
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 292

ggaattccat atgcgcgcgc tttattacga 30

<210> SEQ ID NO 293
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<212> TYPE: DNA
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<400> SEQUENCE: 293

cgggatcctt attcgaaccg gtcgatga 28

<210> SEQ ID NO 294
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 294

ggaattccat atgctggcga ttttctgtga 30

<210> SEQ ID NO 295
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 295

cgggatcctt atgcgacctc caccatgc 28

<210> SEQ ID NO 296
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

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<400> SEQUENCE: 296

ggaattccat atgaaagcct tcgtcgtcga 30

<210> SEQ ID NO 297
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 297

cgggatcctt aggatgcgta tgtaacca 28

<210> SEQ ID NO 298
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 298

ggaattccat atgaaagcga ttgtcgccca 30

<210> SEQ ID NO 299
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 299

cgggatcctt aggaaaaggc gatctgca 28

<210> SEQ ID NO 300
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 300

ggaattccat atgccgatgg cgctcgggca 30

<210> SEQ ID NO 301
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 301

cgggatcctt agaattcgat gacttgcc 28

<210> SEQ ID NO 302
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 302

ggaattccat atgaaacatt ctcaggacaa 30

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<210> SEQ ID NO 303
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 303

gggcgccgat catgtggtgc gtttccg 27

<210> SEQ ID NO 304
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 304

cggaaacgca ccacatgatc ggcgccc 27

<210> SEQ ID NO 305
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 305

cgggatcctt atgccatacg ttccatat 28

<210> SEQ ID NO 306
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 306

ggaattccat atgcagcgtt ttaccaacag 30

<210> SEQ ID NO 307
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 307

cgggatcctt aggaaaacag gacgccgc 28

<210> SEQ ID NO 308
<211> LENGTH: 610
<212> TYPE: PRT
<213> ORGANISM: Klebsiella pneumoniae subsp. pneumoniae MGH 78578

<400> SEQUENCE: 308

Met Arg Tyr Ile Ala Gly Ile Asp Ile Gly Asn Ser Ser Thr Glu Val
1 5 10 15

Ala Leu Ala Thr Val Asp Asp Ala Gly Val Leu Asn Ile Arg His Ser
20 25 30

Ala Leu Ala Glu Thr Thr Gly Ile Lys Gly Thr Leu Arg Asn Val Phe
35 40 45

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```
Gly Ile Gln Glu Ala Leu Thr Gln Ala Ala Lys Ala Ala Gly Ile Gln
    50                  55                  60

Leu Ser Asp Ile Ser Leu Ile Arg Ile Asn Glu Ala Thr Pro Val Ile
65                  70                  75                  80

Gly Asp Val Ala Met Glu Thr Ile Thr Glu Thr Ile Ile Thr Glu Ser
                85                  90                  95

Thr Met Ile Gly His Asn Pro Lys Thr Pro Gly Gly Val Gly Leu Gly
            100                 105                 110

Val Gly Ile Thr Ile Thr Pro Glu Ala Leu Leu Ser Cys Ser Ala Asp
        115                 120                 125

Thr Pro Tyr Ile Leu Val Val Ser Ser Ala Phe Asp Phe Ala Asp Val
    130                 135                 140

Ala Ala Met Val Asn Ala Ala Thr Ala Ala Gly Tyr Gln Ile Thr Gly
145                 150                 155                 160

Ile Ile Leu Gln Gln Asp Asp Gly Val Leu Val Asn Asn Arg Leu Gln
                165                 170                 175

Gln Pro Leu Pro Val Ile Asp Glu Val Gln His Ile Asp Arg Ile Pro
            180                 185                 190

Leu Gly Met Leu Ala Ala Val Glu Val Ala Leu Pro Gly Lys Ile Ile
        195                 200                 205

Glu Thr Leu Ser Asn Pro Tyr Gly Ile Ala Thr Val Phe Asp Leu Asn
    210                 215                 220

Ala Glu Glu Thr Lys Asn Ile Val Pro Met Ala Arg Ala Leu Ile Gly
225                 230                 235                 240

Asn Arg Ser Ala Val Val Val Lys Thr Pro Ser Gly Asp Val Lys Ala
                245                 250                 255

Arg Ala Ile Pro Ala Gly Asn Leu Leu Leu Ile Ala Gln Gly Arg Ser
            260                 265                 270

Val Gln Val Asp Val Ala Ala Gly Ala Glu Ala Ile Met Lys Ala Val
        275                 280                 285

Asp Gly Cys Gly Lys Leu Asp Asn Val Ala Gly Glu Ala Gly Thr Asn
    290                 295                 300

Ile Gly Gly Met Leu Glu His Val Arg Gln Thr Met Ala Glu Leu Thr
305                 310                 315                 320

Asn Lys Pro Ala Gln Glu Ile Arg Ile Gln Asp Leu Leu Ala Val Asp
                325                 330                 335

Thr Ala Val Pro Val Ser Val Thr Gly Gly Leu Ala Gly Glu Phe Ser
            340                 345                 350

Leu Glu Gln Ala Val Gly Ile Ala Ser Met Val Lys Ser Asp Arg Leu
        355                 360                 365

Gln Met Ala Leu Ile Ala Arg Glu Ile Glu His Lys Leu Gln Ile Ala
    370                 375                 380

Val Gln Val Gly Gly Ala Glu Ala Glu Ala Ala Ile Leu Gly Ala Leu
385                 390                 395                 400

Thr Thr Pro Gly Thr Thr Arg Pro Leu Ala Ile Leu Asp Leu Gly Ala
                405                 410                 415

Gly Ser Thr Asp Ala Ser Ile Ile Asn Ala Gln Gly Glu Ile Ser Ala
            420                 425                 430

Thr His Leu Ala Gly Ala Gly Asp Met Val Thr Met Ile Ile Ala Arg
        435                 440                 445
```

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```
Glu Leu Gly Leu Glu Asp Arg Tyr Leu Ala Glu Glu Ile Lys Lys Tyr
    450                 455                 460

Pro Leu Ala Lys Val Glu Ser Leu Phe His Leu Arg His Glu Asp Gly
465                 470                 475                 480

Ser Val Gln Phe Phe Pro Ser Ala Leu Pro Pro Ala Val Phe Ala Arg
                485                 490                 495

Val Cys Val Val Lys Pro Asp Glu Leu Val Pro Leu Pro Gly Asp Leu
            500                 505                 510

Pro Leu Glu Lys Val Arg Ala Ile Arg Arg Ser Ala Lys Ser Arg Val
        515                 520                 525

Phe Val Thr Asn Ala Leu Arg Ala Leu Arg Gln Val Ser Pro Thr Gly
    530                 535                 540

Asn Ile Arg Asp Ile Pro Phe Val Val Leu Val Gly Gly Ser Ser Leu
545                 550                 555                 560

Asp Phe Glu Ile Pro Gln Leu Val Thr Asp Ala Leu Ala His Tyr Arg
                565                 570                 575

Leu Val Ala Gly Arg Gly Asn Ile Arg Gly Cys Glu Gly Pro Arg Asn
            580                 585                 590

Ala Val Ala Ser Gly Leu Leu Leu Ser Trp Gln Lys Gly Gly Thr His
        595                 600                 605

Gly Glu
    610


<210> SEQ ID NO 309
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Klebsiella pneumoniae subsp. pneumoniae MGH78578

<400> SEQUENCE: 309

Met Glu Ser Ser Val Val Ala Pro Ala Ile Val Ile Ala Val Thr Asp
1               5                   10                  15

Glu Cys Ser Glu Gln Trp Arg Asp Val Leu Leu Gly Ile Glu Glu Glu
            20                  25                  30

Gly Ile Pro Phe Val Leu Gln Pro Gln Thr Gly Gly Asp Leu Ile His
        35                  40                  45

His Ala Trp Gln Ala Ala Gln Arg Ser Pro Leu Gln Val Gly Ile Ala
    50                  55                  60

Cys Asp Arg Glu Arg Leu Ile Val His Tyr Lys Asn Leu Pro Ala Ser
65                  70                  75                  80

Thr Pro Leu Phe Ser Leu Met Tyr His Gln Asn Arg Leu Ala Arg Arg
                85                  90                  95

Asn Thr Gly Asn Asn Ala Ala Arg Leu Val Lys Gly Ile Pro Phe Arg
            100                 105                 110

Asp Arg His Ala
        115


<210> SEQ ID NO 310
<211> LENGTH: 787
<212> TYPE: PRT
<213> ORGANISM: Clostridium butyricum

<400> SEQUENCE: 310

Met Ile Ser Lys Gly Phe Ser Thr Gln Thr Glu Arg Ile Asn Ile Leu
1               5                   10                  15
```

-continued

```
Lys Ala Gln Ile Leu Asn Ala Lys Pro Cys Val Glu Ser Glu Arg Ala
            20                  25                  30

Ile Leu Ile Thr Glu Ser Phe Lys Gln Thr Glu Gly Gln Pro Ala Ile
        35                  40                  45

Leu Arg Arg Ala Leu Ala Leu Lys His Ile Leu Glu Asn Ile Pro Ile
    50                  55                  60

Thr Ile Arg Asp Gln Glu Leu Ile Val Gly Ser Leu Thr Lys Glu Pro
65                  70                  75                  80

Arg Ser Ser Gln Val Phe Pro Glu Phe Ser Asn Lys Trp Leu Gln Asp
                85                  90                  95

Glu Leu Asp Arg Leu Asn Lys Arg Thr Gly Asp Ala Phe Gln Ile Ser
            100                 105                 110

Glu Glu Ser Lys Glu Lys Leu Lys Asp Val Phe Glu Tyr Trp Asn Gly
        115                 120                 125

Lys Thr Thr Ser Glu Leu Ala Thr Ser Tyr Met Thr Glu Glu Thr Arg
    130                 135                 140

Glu Ala Val Asn Cys Asp Val Phe Thr Val Gly Asn Tyr Tyr Tyr Asn
145                 150                 155                 160

Gly Val Gly His Val Ser Val Asp Tyr Gly Lys Val Leu Arg Val Gly
                165                 170                 175

Phe Asn Gly Ile Ile Asn Glu Ala Lys Glu Gln Leu Glu Lys Asn Arg
            180                 185                 190

Ser Ile Asp Pro Asp Phe Ile Lys Lys Glu Lys Phe Leu Asn Ser Val
        195                 200                 205

Ile Ile Ser Cys Glu Ala Ala Ile Thr Tyr Val Asn Arg Tyr Ala Lys
    210                 215                 220

Lys Ala Lys Glu Ile Ala Asp Asn Thr Ser Asp Ala Lys Arg Lys Ala
225                 230                 235                 240

Glu Leu Asn Glu Ile Ala Lys Ile Cys Ser Lys Val Ser Gly Glu Gly
                245                 250                 255

Ala Lys Ser Phe Tyr Glu Ala Cys Gln Leu Phe Trp Phe Ile His Ala
            260                 265                 270

Ile Ile Asn Ile Glu Ser Asn Gly His Ser Ile Ser Pro Ala Arg Phe
        275                 280                 285

Asp Gln Tyr Met Tyr Pro Tyr Tyr Glu Asn Asp Lys Asn Ile Thr Asp
    290                 295                 300

Lys Phe Ala Gln Glu Leu Ile Asp Cys Ile Trp Ile Lys Leu Asn Asp
305                 310                 315                 320

Ile Asn Lys Val Arg Asp Glu Ile Ser Thr Lys His Phe Gly Gly Tyr
                325                 330                 335

Pro Met Tyr Gln Asn Leu Ile Val Gly Gly Gln Asn Ser Glu Gly Lys
            340                 345                 350

Asp Ala Thr Asn Lys Val Ser Tyr Met Ala Leu Glu Ala Ala Val His
        355                 360                 365

Val Lys Leu Pro Gln Pro Ser Leu Ser Val Arg Ile Trp Asn Lys Thr
    370                 375                 380

Pro Asp Glu Phe Leu Leu Arg Ala Ala Glu Leu Thr Arg Glu Gly Leu
385                 390                 395                 400

Gly Leu Pro Ala Tyr Tyr Asn Asp Glu Val Ile Ile Pro Ala Leu Val
                405                 410                 415

Ser Arg Gly Leu Thr Leu Glu Asp Ala Arg Asp Tyr Gly Ile Ile Gly
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```
            420                 425                 430

Cys Val Glu Pro Gln Lys Pro Gly Lys Thr Glu Gly Trp His Asp Ser
        435                 440                 445

Ala Phe Phe Asn Leu Ala Arg Ile Val Glu Leu Thr Ile Asn Ser Gly
    450                 455                 460

Phe Asp Lys Asn Lys Gln Ile Gly Pro Lys Thr Gln Asn Phe Glu Glu
465                 470                 475                 480

Met Lys Ser Phe Asp Glu Phe Met Lys Ala Tyr Lys Ala Gln Met Glu
                485                 490                 495

Tyr Phe Val Lys His Met Cys Cys Ala Asp Asn Cys Ile Asp Ile Ala
            500                 505                 510

His Ala Glu Arg Ala Pro Leu Pro Phe Leu Ser Ser Met Val Asp Asn
        515                 520                 525

Cys Ile Gly Lys Gly Lys Ser Leu Gln Asp Gly Gly Ala Glu Tyr Asn
    530                 535                 540

Phe Ser Gly Pro Gln Gly Val Gly Val Ala Asn Ile Gly Asp Ser Leu
545                 550                 555                 560

Val Ala Val Lys Lys Ile Val Phe Asp Glu Asn Lys Ile Thr Pro Ser
                565                 570                 575

Glu Leu Lys Lys Thr Leu Asn Asn Asp Phe Lys Asn Ser Glu Glu Ile
            580                 585                 590

Gln Ala Leu Leu Lys Asn Ala Pro Lys Phe Gly Asn Asp Ile Asp Glu
        595                 600                 605

Val Asp Asn Leu Ala Arg Glu Gly Ala Leu Val Tyr Cys Arg Glu Val
    610                 615                 620

Asn Lys Tyr Thr Asn Pro Arg Gly Gly Asn Phe Gln Pro Gly Leu Tyr
625                 630                 635                 640

Pro Ser Ser Ile Asn Val Tyr Phe Gly Ser Leu Thr Gly Ala Thr Pro
                645                 650                 655

Asp Gly Arg Lys Ser Gly Gln Pro Leu Ala Asp Gly Val Ser Pro Ser
            660                 665                 670

Arg Gly Cys Asp Val Ser Gly Pro Thr Ala Ala Cys Asn Ser Val Ser
        675                 680                 685

Lys Leu Asp His Phe Ile Ala Ser Asn Gly Thr Leu Phe Asn Gln Lys
    690                 695                 700

Phe His Pro Ser Ala Leu Lys Gly Asp Asn Gly Leu Met Asn Leu Ser
705                 710                 715                 720

Ser Leu Ile Arg Ser Tyr Phe Asp Gln Lys Gly Phe His Val Gln Phe
                725                 730                 735

Asn Val Ile Asp Lys Lys Ile Leu Leu Ala Ala Gln Lys Asn Pro Glu
            740                 745                 750

Lys Tyr Gln Asp Leu Ile Val Arg Val Ala Gly Tyr Ser Ala Gln Phe
        755                 760                 765

Ile Ser Leu Asp Lys Ser Ile Gln Asn Asp Ile Ile Ala Arg Thr Glu
    770                 775                 780

His Val Met
785


<210> SEQ ID NO 311
<211> LENGTH: 304
<212> TYPE: PRT
<213> ORGANISM: Clostridium buyricum
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-continued

<400> SEQUENCE: 311

Met Ser Lys Glu Ile Lys Gly Val Leu Phe Asn Ile Gln Lys Phe Ser
1 5 10 15

Leu His Asp Gly Pro Gly Ile Arg Thr Ile Val Phe Phe Lys Gly Cys
20 25 30

Ser Met Ser Cys Leu Trp Cys Ser Asn Pro Glu Ser Gln Asp Ile Lys
35 40 45

Pro Gln Val Met Phe Asn Lys Asn Leu Cys Thr Lys Cys Gly Arg Cys
50 55 60

Lys Ser Gln Cys Lys Ser Ala Ala Ile Asp Met Asn Ser Glu Tyr Arg
65 70 75 80

Ile Asp Lys Ser Lys Cys Thr Glu Cys Thr Lys Cys Val Asp Asn Cys
85 90 95

Leu Ser Gly Ala Leu Val Ile Glu Gly Arg Asn Tyr Ser Val Glu Asp
100 105 110

Val Ile Lys Glu Leu Lys Lys Asp Ser Val Gln Tyr Arg Arg Ser Asn
115 120 125

Gly Gly Ile Thr Leu Ser Gly Gly Glu Val Leu Leu Gln Pro Asp Phe
130 135 140

Ala Val Glu Leu Leu Lys Glu Cys Lys Ser Tyr Gly Trp His Thr Ala
145 150 155 160

Ile Glu Thr Ala Met Tyr Val Asn Ser Glu Ser Val Lys Lys Val Ile
165 170 175

Pro Tyr Ile Asp Leu Ala Met Ile Asp Ile Lys Ser Met Asn Asp Glu
180 185 190

Ile His Arg Lys Phe Thr Gly Val Ser Asn Glu Ile Ile Leu Gln Asn
195 200 205

Ile Lys Leu Ser Asp Glu Leu Ala Lys Glu Ile Ile Ile Arg Ile Pro
210 215 220

Val Ile Glu Gly Phe Asn Ala Asp Leu Gln Ser Ile Gly Ala Ile Ala
225 230 235 240

Gln Phe Ser Lys Ser Leu Thr Asn Leu Lys Arg Ile Asp Leu Leu Pro
245 250 255

Tyr His Asn Tyr Gly Glu Asn Lys Tyr Gln Ala Ile Gly Arg Glu Tyr
260 265 270

Ser Leu Lys Glu Leu Lys Ser Pro Ser Lys Asp Lys Met Glu Arg Leu
275 280 285

Lys Ala Leu Val Glu Ile Met Gly Ile Pro Cys Thr Ile Gly Ala Glu
290 295 300

<210> SEQ ID NO 312
<211> LENGTH: 545
<212> TYPE: PRT
<213> ORGANISM: Azospirillum brasilense

<400> SEQUENCE: 312

Met Lys Leu Ala Glu Ala Leu Leu Arg Ala Leu Lys Asp Arg Gly Ala
1 5 10 15

Gln Ala Met Phe Gly Ile Pro Gly Asp Phe Ala Leu Pro Phe Phe Lys
20 25 30

Val Ala Glu Glu Thr Gln Ile Leu Pro Leu His Thr Leu Ser His Glu
35 40 45

-continued

```
Pro Ala Val Gly Phe Ala Ala Asp Ala Ala Ala Arg Tyr Ser Ser Thr
    50                  55                  60

Leu Gly Val Ala Ala Val Thr Tyr Gly Ala Gly Ala Phe Asn Met Val
65                  70                  75                  80

Asn Ala Val Ala Gly Ala Tyr Ala Glu Lys Ser Pro Val Val Val Ile
                85                  90                  95

Ser Gly Ala Pro Gly Thr Thr Glu Gly Asn Ala Gly Leu Leu Leu His
            100                 105                 110

His Gln Gly Arg Thr Leu Asp Thr Gln Phe Gln Val Phe Lys Glu Ile
        115                 120                 125

Thr Val Ala Gln Ala Arg Leu Asp Asp Pro Ala Lys Ala Pro Ala Glu
    130                 135                 140

Ile Ala Arg Val Leu Gly Ala Ala Arg Ala Gln Ser Arg Pro Val Tyr
145                 150                 155                 160

Leu Glu Ile Pro Arg Asn Met Val Asn Ala Glu Val Glu Pro Val Gly
                165                 170                 175

Asp Asp Pro Ala Trp Pro Val Asp Arg Asp Ala Leu Ala Ala Cys Ala
            180                 185                 190

Asp Glu Val Leu Ala Ala Met Arg Ser Ala Thr Ser Pro Val Leu Met
        195                 200                 205

Val Cys Val Glu Val Arg Arg Tyr Gly Leu Glu Ala Lys Val Ala Glu
    210                 215                 220

Leu Ala Gln Arg Leu Gly Val Pro Val Val Thr Thr Phe Met Gly Arg
225                 230                 235                 240

Gly Leu Leu Ala Asp Ala Pro Thr Pro Pro Leu Gly Thr Tyr Ile Gly
                245                 250                 255

Val Ala Gly Asp Ala Glu Ile Thr Arg Leu Val Glu Glu Ser Asp Gly
            260                 265                 270

Leu Phe Leu Leu Gly Ala Ile Leu Ser Asp Thr Asn Phe Ala Val Ser
        275                 280                 285

Gln Arg Lys Ile Asp Leu Arg Lys Thr Ile His Ala Phe Asp Arg Ala
    290                 295                 300

Val Thr Leu Gly Tyr His Thr Tyr Ala Asp Ile Pro Leu Ala Gly Leu
305                 310                 315                 320

Val Asp Ala Leu Leu Glu Arg Leu Pro Pro Ser Asp Arg Thr Thr Arg
                325                 330                 335

Gly Lys Glu Pro His Ala Tyr Pro Thr Gly Leu Gln Ala Asp Gly Glu
            340                 345                 350

Pro Ile Ala Pro Met Asp Ile Ala Arg Ala Val Asn Asp Arg Val Arg
        355                 360                 365

Ala Gly Gln Glu Pro Leu Leu Ile Ala Ala Asp Met Gly Asp Cys Leu
    370                 375                 380

Phe Thr Ala Met Asp Met Ile Asp Ala Gly Leu Met Ala Pro Gly Tyr
385                 390                 395                 400

Tyr Ala Gly Met Gly Phe Gly Val Pro Ala Gly Ile Gly Ala Gln Cys
                405                 410                 415

Val Ser Gly Gly Lys Arg Ile Leu Thr Val Val Gly Asp Gly Ala Phe
            420                 425                 430

Gln Met Thr Gly Trp Glu Leu Gly Asn Cys Arg Arg Leu Gly Ile Asp
        435                 440                 445
```

-continued

```
Pro Ile Val Ile Leu Phe Asn Asn Ala Ser Trp Glu Met Leu Arg Thr
    450                 455                 460

Phe Gln Pro Glu Ser Ala Phe Asn Asp Leu Asp Asp Trp Arg Phe Ala
465                 470                 475                 480

Asp Met Ala Ala Gly Met Gly Gly Asp Gly Val Arg Val Arg Thr Arg
                485                 490                 495

Ala Glu Leu Lys Ala Ala Leu Asp Lys Ala Phe Ala Thr Arg Gly Arg
            500                 505                 510

Phe Gln Leu Ile Glu Ala Met Ile Pro Arg Gly Val Leu Ser Asp Thr
        515                 520                 525

Leu Ala Arg Phe Val Gln Gly Gln Lys Arg Leu His Ala Ala Pro Arg
    530                 535                 540

Glu
545


<210> SEQ ID NO 313
<211> LENGTH: 348
<212> TYPE: PRT
<213> ORGANISM: Rhodococcus sp. ST-10

<400> SEQUENCE: 313

Met Lys Ala Ile Gln Tyr Thr Arg Ile Gly Ala Glu Pro Glu Leu Thr
1               5                   10                  15

Glu Ile Pro Lys Pro Glu Pro Gly Pro Gly Glu Val Leu Leu Glu Val
            20                  25                  30

Thr Ala Ala Gly Val Cys His Ser Asp Asp Phe Ile Met Ser Leu Pro
        35                  40                  45

Glu Glu Gln Tyr Thr Tyr Gly Leu Pro Leu Thr Leu Gly His Glu Gly
    50                  55                  60

Ala Gly Lys Val Ala Ala Val Gly Glu Gly Val Glu Gly Leu Asp Ile
65                  70                  75                  80

Gly Thr Asn Val Val Val Tyr Gly Pro Trp Gly Cys Gly Asn Cys Trp
                85                  90                  95

His Cys Ser Gln Gly Leu Glu Asn Tyr Cys Ser Arg Ala Gln Glu Leu
            100                 105                 110

Gly Ile Asn Pro Pro Gly Leu Gly Ala Pro Gly Ala Leu Ala Glu Phe
        115                 120                 125

Met Ile Val Asp Ser Pro Arg His Leu Val Pro Ile Gly Asp Leu Asp
    130                 135                 140

Pro Val Lys Thr Val Pro Leu Thr Asp Ala Gly Leu Thr Pro Tyr His
145                 150                 155                 160

Ala Ile Lys Arg Ser Leu Pro Lys Leu Arg Gly Gly Ser Tyr Ala Val
                165                 170                 175

Val Ile Gly Thr Gly Gly Leu Gly His Val Ala Ile Gln Leu Leu Arg
            180                 185                 190

His Leu Ser Ala Ala Thr Val Ile Ala Leu Asp Val Ser Ala Asp Lys
        195                 200                 205

Leu Glu Leu Ala Thr Lys Val Gly Ala His Glu Val Val Leu Ser Asp
    210                 215                 220

Lys Asp Ala Ala Glu Asn Val Arg Lys Ile Thr Gly Ser Gln Gly Ala
225                 230                 235                 240

Ala Leu Val Leu Asp Phe Val Gly Tyr Gln Pro Thr Ile Asp Thr Ala
                245                 250                 255
```

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Met Ala Val Ala Gly Val Gly Ser Asp Val Thr Ile Val Gly Ile Gly
260 265 270

Asp Gly Gln Ala His Ala Lys Val Gly Phe Phe Gln Ser Pro Tyr Glu
275 280 285

Ala Ser Val Thr Val Pro Tyr Trp Gly Ala Arg Asn Glu Leu Ile Glu
290 295 300

Leu Ile Asp Leu Ala His Ala Gly Ile Phe Asp Ile Ser Val Glu Thr
305 310 315 320

Phe Ser Leu Asp Asn Gly Ala Glu Ala Tyr Arg Arg Leu Ala Ala Gly
325 330 335

Thr Leu Ser Gly Arg Ala Val Val Val Pro Gly Leu
340 345

<210> SEQ ID NO 314
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 314

catgccatgg gactggctga ggcactgctg c 31

<210> SEQ ID NO 315
<211> LENGTH: 47
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 315

cgagctcagg aggatatata tatgaaagct atccagtaca cccgtat 47

<210> SEQ ID NO 316
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 316

cgagctctta ttcgcgcggt gccgcgtgca gg 32

<210> SEQ ID NO 317
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 317

gctctagatt acaggcccgg aaccacaacg gcgc 34

<210> SEQ ID NO 318
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 318

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ccgctcgagg aggatatata tatgatttct aaaggcttta gcaccc 46

<210> SEQ ID NO 319
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 319

acgtgatgta atctagagga ggatatatat atgagcaaag aaattaaagg 50

<210> SEQ ID NO 320
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 320

tctttgctca tatatatatc ctcctctaga ttacatcacg tgttcagtac 50

<210> SEQ ID NO 321
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 321

cgagctctta ttcggcgcca atggtgcacg gg 32

<210> SEQ ID NO 322
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 322

ccgctcgagg aggatatata tatgatttct aaaggcttta gcaccc 46

<210> SEQ ID NO 323
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 323

cgagctctta ttcggcgcca atggtgcacg gg 32

<210> SEQ ID NO 324
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 324

cacccaagcg atagtttata tagcgt 26

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<210> SEQ ID NO 325
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 325

gaaatgaacg gatattacgt 20

<210> SEQ ID NO 326
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 326

cggaacaggt gattgtggt 19

<210> SEQ ID NO 327
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 327

caccgcccac ttcaagatga agctgt 26

<210> SEQ ID NO 328
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 328

cacccaagcg atagtttata tagcgt 26

<210> SEQ ID NO 329
<211> LENGTH: 20
<212> TYPE: DNA
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1. A method for converting a polysaccharide to a commodity chemical, comprising:

(a) contacting the polysaccharide, wherein the polysaccharide is optionally derived from biomass, with a polysaccharide degrading or depolymerizing metabolic system, wherein the metabolic system is selected from;

(i) enzymatic or chemical catalysis, and

(ii) a microbial system, wherein the microbial system comprises a recombinant microorganism, wherein the recombinant microorganism comprises one or exogenous genes that allow it to grow on the polysaccharide as a sole source of carbon,

thereby converting the polysaccharide to a suitable monosaccharide or oligosaccharide; and

(b) contacting the suitable monosaccharide or oligosaccharide with commodity chemical biosynthesis pathway, wherein the commodity chemical biosynthesis pathway comprises an aldehyde or ketone biosynthesis pathway,

thereby converting the polysaccharide to the commodity chemical

2. The method of claim **1**, wherein the biomass is selected from marine biomass and vegetable/fruit/plant biomass.

3. The method of claim **2**, wherein the marine biomass is selected from kelp, giant kelp, sargasso, seaweed, algae, marine microflora, microalgae, and sea grass.

4. The method of claim **2**, wherein the vegetable/fruit/plant biomass comprises plant peel or pomace.

5. The method of claim **2**, wherein the vegetable/fruit/plant biomass is selected from citrus, potato, tomato, grape, gooseberry, carrot, mango, sugar-beet, apple, switchgrass, wood, and stover.

6. The method of claim **1**, wherein the polysaccharide is selected from alginate, agar, carrageenan, fucoidan, pectin, polygalacturonate, cellulose, hemicellulose, xylan, arabinan, and mannan.

7. The method of claim **1**, wherein the suitable monosaccharide or oligosaccharide is selected from 2-keto-3-deoxy D-gluconate (KDG), D-mannitol, guluronate, mannuronate, mannitol, lyxose, glycerol, xylitol, glucose, mannose, galactose, xylose, arabinose, glucuronate, galacturonates, and rhamnose.

8. The method of claim **1**, wherein the commodity chemical is selected from methane, methanol, ethane, ethene, ethanol, n-propane, 1-propene, 1-propanol, propanal, acetone, propionate, n-butane, 1-butene, 1-butanol, butanal, butanoate, isobutanal, isobutanol, 2-methylbutanal, 2-methylbutanol, 3-methylbutanal, 3-methylbutanol, 2-butene, 2-butanol, 2-butanone, 2,3-butanediol, 3-hydroxy-2-butanone, 2,3-butanedione, ethylbenzene, ethenylbenzene, 2-phenylethanol, phenylacetaldehyde, 1-phenylbutane, 4-phenyl-1-butene, 4-phenyl-2-butene, 1-phenyl-2-butene, 1-phenyl-2-butanol, 4-phenyl-2-butanol, 1-phenyl-2-butanone, 4-phenyl-2-butanone, 1-phenyl-2,3-butandiol, 1-phenyl-3-hydroxy-2-butanone, 4-phenyl-3-hydroxy-2-butanone, 1-phenyl-2,3-butanedione, n-pentane, ethylphenol, ethenylphenol, 2-(4-hydroxyphenyl)ethanol, 4-hydroxyphenylacetaldehyde, 1-(4-hydroxyphenyl) butane, 4-(4-hydroxyphenyl)-1-butene, 4-(4-hydroxyphenyl)-2-butene, 1-(4-hydroxyphenyl)-1-butene, 1-(4-hydroxyphenyl)-2-butanol, 4-(4-hydroxyphenyl)-2-butanol, 1-(4-hydroxyphenyl)-2-butanone, 4-(4-hydroxyphenyl)-2-butanone, 1-(4-hydroxyphenyl)-2,3-butandiol, 1-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 4-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-2,3-butanonedione, indolylethane, indolylethene, 2-(indole-3-)ethanol, n-pentane, 1-pentene, 1-pentanol, pentanal, pentanoate, 2-pentene, 2-pentanol, 3-pentanol, 2-pentanone, 3-pentanone, 4-methylpentanal, 4-methylpentanol, 2,3-pentanediol, 2-hydroxy-3-pentanone, 3-hydroxy-2-pentanone, 2,3-pentanedione, 2-methylpentane, 4-methyl-1-pentene, 4-methyl-2-pentene, 4-methyl-3-

pentene, 4-methyl-2-pentanol, 2-methyl-3-pentanol, 4-methyl-2-pentanone, 2-methyl-3-pentanone, 4-methyl-2,3-pentanediol, 4-methyl-2-hydroxy-3-pentanone, 4-methyl-3-hydroxy-2-pentanone, 4-methyl-2,3-pentanedione, 1-phenylpentane, 1-phenyl-1-pentene, 1-phenyl-2-pentene, 1-phenyl-3-pentene, 1-phenyl-2-pentanol, 1-phenyl-3-pentanol, 1-phenyl-2-pentanone, 1-phenyl-3-pentanone, 1-phenyl-2,3-pentanediol, 1-phenyl-2-hydroxy-3-pentanone, 1-phenyl-3-hydroxy-2-pentanone, 1-phenyl-2,3-pentanedione, 4-methyl-1-phenylpentane, 4-methyl-1-phenyl-1-pentene, 4-methyl-1-phenyl-2-pentene, 4-methyl-1-phenyl-3-pentene, 4-methyl-1-phenyl-3-pentanol, 4-methyl-1-phenyl-2-pentanol, 4-methyl-1-phenyl-3-pentanone, 4-methyl-1-phenyl-2-pentanone, 4-methyl-1-phenyl-2,3-pentanediol, 4-methyl-1-phenyl-2,3-pentanedione, 4-methyl-1-phenyl-3-hydroxy-2-pentanone, 4-methyl-1-phenyl-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl) pentane, 1-(4-hydroxyphenyl)-1-pentene, 1-(4-hydroxyphenyl)-2-pentene, 1-(4-hydroxyphenyl)-3-pentene, 1-(4-hydroxyphenyl)-2-pentanol, 1-(4-hydroxyphenyl)-3-pentanol, 1-(4-hydroxyphenyl)-2-pentanone, 1-(4-hydroxyphenyl)-3-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanediol, 1-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-1-(4-hydroxyphenyl) pentane, 4-methyl-1-(4-hydroxyphenyl)-2-pentene, 4-methyl-1-(4-hydroxyphenyl)-3-pentene, 4-methyl-1-(4-hydroxyphenyl)-1-pentene, 4-methyl-1-(4-hydroxyphenyl)-3-pentanol, 4-methyl-1-(4-hydroxyphenyl)-2-pentanol, 4-methyl-1-(4-hydroxyphenyl)-3-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2,3-pentanediol, 4-methyl-1-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, 1-indole-3-pentane, 1-(indole-3)-1-pentene, 1-(indole-3)-2-pentene, 1-(indole-3)-3-pentene, 1-(indole-3)-2-pentanol, 1-(indole-3)-3-pentanol, 1-(indole-3)-2-pentanone, 1-(indole-3)-3-pentanone, 1-(indole-3)-2,3-pentanediol, 1-(indole-3)-2-hydroxy-3-pentanone, 1-(indole-3)-3-hydroxy-2-pentanone, 1-(indole-3)-2,3-pentanedione, 4-methyl-1-(indole-3-)pentane, 4-methyl-1-(indole-3)-2-pentene, 4-methyl-1-(indole-3)-3-pentene, 4-methyl-1-(indole-3)-1-pentene, 4-methyl-2-(indole-3)-3-pentanol, 4-methyl-1-(indole-3)-2-pentanol, 4-methyl-1-(indole-3)-3-pentanone, 4-methyl-1-(indole-3)-2-pentanone, 4-methyl-1-(indole-3)-2,3-pentanediol, 4-methyl-1-(indole-3)-2,3-pentanedione, 4-methyl-1-(indole-3)-3-hydroxy-2-pentanone, 4-methyl-1-(indole-3)-2-hydroxy-3-pentanone, n-hexane, 1-hexene, 1-hexanol, hexanal, hexanoate, 2-hexene, 3-hexene, 2-hexanol, 3-hexanol, 2-hexanone, 3-hexanone, 2,3-hexanediol, 2,3-hexanedione, 3,4-hexanediol, 3,4-hexanedione, 2-hydroxy-3-hexanone, 3-hydroxy-2-hexanone, 3-hydroxy-4-hexanone, 4-hydroxy-3-hexanone, 2-methylhexane, 3-methylhexane, 2-methyl-2-hexene, 2-methyl-3-hexene, 5-methyl-1-hexene, 5-methyl-2-hexene, 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1,4-diphenylbutane, 1,4-diphenyl-1-butene, 1,4-diphenyl-2-butene, 1,4-diphenyl-2-butanol, 1,4-diphenyl-2-butanone, 1,4-diphenyl-2,3-butanediol, 1,4-diphenyl-3-hydroxy-2-butanone, 1-(4-hydeoxyphenyl)-4-phenylbutane, 1-(4-hydeoxyphenyl)-4-phenyl-1-butene, 1-(4-hydeoxyphenyl)-4-phenyl-2-butene, 1-(4-hydeoxyphenyl)-4-phenyl-2-butanol, 1-(4-hydeoxyphenyl)-4-phenyl-2-butanone, 1-(4-hydeoxyphenyl)-4-phenyl-2,3-butanediol, 1-(4-hydeoxyphenyl)-4-phenyl-3-hydroxy-2-butanone, 1-(indole-3)-4-phenylbutane, 1-(indole-3)-4-phenyl-1-butene, 1-(indole-3)-4-phenyl-2-butene, 1-(indole-3)-4-phenyl-2-butanol, 1-(indole-3)-4-phenyl-2-butanone, 1-(indole-3)-4-phenyl-2,3-butanediol, 1-(indole-3)-4-phenyl-3-hydroxy-2-butanone, 4-hydroxyphenylacetoaldehyde, 1,4-di(4-hydroxyphenyl)butane, 1,4-di(4-hydroxyphenyl)-1-butene, 1,4-di(4-hydroxyphenyl)-2-butene, 1,4-di(4-hydroxyphenyl)-2-butanol, 1,4-di(4-hydroxyphenyl)-2-butanone, 1,4-di(4-hydroxyphenyl)-2,3-butanediol, 1,4-di(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3-)butane, 1-(4-hydroxyphenyl)-4-(indole-3)-1-butene, 1-di(4-hydroxyphenyl)-4-(indole-3)-2-butene, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanol, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3)-2,3-butanediol, 1-(4-hydroxyphenyl-4-(indole-3)-3-hydroxy-2-butanone, indole-3-acetoaldehyde, 1,4-di(indole-3-)butane, 1,4-di(indole-3)-1-butene, 1,4-di(indole-3)-2-butene, 1,4-di(indole-3)-2-butanol, 1,4-di(indole-3)-2-butanone, 1,4-di(indole-3)-2,3-butanediol, 1,4-di(indole-3)-3-hydroxy-2-butanone, succinate semialdehyde, hexane-1,8-dicarboxylic acid, 3-hexene-1,8-dicarboxylic acid, 3-hydroxy-hexane-1,8-dicarboxylic acid, 3-hexanone-1,8-dicarboxylic acid, 3,4-hexanediol-1,8-dicarboxylic acid, 4-hydroxy-3-hexanone-1,8-dicarboxylic acid, fucoidan, iodine, chlorophyll, carotenoid, calcium, magnesium, iron, sodium, potassium, and phosphate.

9. A method for converting a polysaccharide to a suitable monosaccharide or oligosaccharide, comprising:

(a) contacting the polysaccharide, wherein the polysaccharide is optionally obtained from biomass, with a microbial system for a time sufficient to convert the polysaccharide to a suitable monosaccharide or oligosaccharide, wherein the microbial system comprises,

(i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase, wherein the lyase and/or hydrolase optionally comprises at least one signal peptide or at least one autotransporter domain;

(ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, a polysaccharide transporter, and a superchannel; and

(iii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase,

thereby converting the polysaccharide to a suitable monosaccharide or oligosaccharide.

10. A method for converting a polysaccharide to a suitable monosaccharide or oligosaccharide, comprising:

(a) contacting the polysaccharide, wherein the polysaccharide is optionally obtained from biomass, with a chemical or enzymatic catalysis pathway for a time sufficient to convert the polysaccharide to a first monosaccharide or oligosaccharide; and

(b) contacting the first monosaccharide or oligosaccharide with a microbial system for a time sufficient to convert the first monosaccharide or oligosaccharide to the suitable monosaccharide or oligosaccharide, wherein the microbial system comprises,

(i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase

(ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, a polysaccharide transporter, and a superchannel; and

(ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase,

thereby converting the polysaccharide to the suitable monosaccharide or oligosaccharide.

11. The method of claim **9**, wherein the lyase is selected from an alginate lyase, a pectate lyase, a polymannuronate lyase, a polygluronate lyase, a polygalacturonate lyase and a rhamnogalacturonate lyase.

12. The method of claim **9**, wherein the hydrolase is selected from an alginate hydrolase, a rhamnogalacturonate hydrolase, a polymannuronate hydrolase, a pectin hydrolase, and a polygalacturonate hydrolase.

13. The method of claim **9**, wherein the transporter is selected from an ABC transporter, a symporter, and an outer membrane porin.

14. The method of claim **13**, wherein the ABC transporter is selected from Atu3021, Atu3022, Atu3023, Atu3024, algM1, algM2, AlgQ1, AlgQ2, AlgS, OG2516_05558, OG2516_05563, OG2516_05568, OG2516_05573, TogM, TogN, TogA, TogB, and functional variants thereof.

15. The method of claim **13**, wherein the symporter is selected from V12B01_24239 (SEQ ID NO:26), V12B01_24194 (SEQ ID NO:8), and TogT, and functional variants thereof.

16. The method of claim **13**, wherein the outermembrane porin comprises a porin selected from V12B01_24269, KdgM, and KdgN, and functional variants thereof.

17. A recombinant microorganism that is capable of growing on a polysaccharide as a sole source of carbon, wherein the polysaccharide is selected from alginate, pectin, tri-galacturonate, di-galacturonate, cellulose, and hemi-cellulose.

18. The recombinant microorganism of claim **17**, wherein the polysaccharide is alginate.

19. The recombinant microorganism of claim **17**, wherein the polysaccharide is pectin.

20. The recombinant microorganism of claim **17**, wherein the polysaccharide is tri-galacturonate.

21. A recombinant microrganism, comprising (i) at least one gene encoding and expressing an enzyme selected from a lyase and a hydrolase, wherein the lyase or hydrolase optionally comprises at least one signal peptide or at least one autotransporter domain; (ii) at least one gene encoding and expressing an enzyme selected from a monosaccharide transporter, a disaccharide transporter, a trisaccharide transporter, an oligosaccharide transporter, a polysaccharide transporter, and a superchannel; and (iii) at least one gene encoding and expressing an enzyme selected from a monosaccharide dehydrogenase, an isomerase, a dehydratase, a kinase, and an aldolase.

22. The recombinant microorganism of claim **21**, wherein the microorganism is capable of growing on a polysaccharide as a sole source of carbon.

23. The recombinant microorganism of claim **22**, wherein the polysaccharide is selected from alginate, pectin, and tri-galacturonate.

24. A method for converting a suitable monosaccharide or oligosaccharide to a first commodity chemical comprising,

(a) contacting the suitable monosaccharide or oligosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide or oligosaccharide to the commodity chemical, wherein the microbial system comprises a recombinant microorganism, wherein the microorganism comprises a commodity chemical biosynthesis pathway,

thereby converting the suitable monosaccharide or oligosaccharide to the first commodity chemical.

25. The method of claim **24**, wherein the commodity chemical pathway comprises one or more genes encoding an aldehyde or ketone biosynthesis pathway.

26. The method of claim **25**, wherein the aldehyde or ketone biosynthesis pathway is selected from one or more of an acetoaldehyde, a propionaldehyde, a butyraldehyde, an isobutyraldehyde, a 2-methyl-butyraldehyde, a 3-methyl-butyraldehyde, a 2-phenyl acetaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, a 2-Indole-3-acetoaldehyde, a glutaraldehyde, a 5-amino-pentaldehyde, a succinate semialdehyde, and a succinate 4-hydroxyphenyl acetaldehyde biosynthesis pathway.

27. The method of claim **26**, wherein the aldehyde or ketone biosynthesis pathway comprises an acetoaldehyde biosynthesis pathway and a biosynthesis pathway selected from a propionaldehyde, butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, a 2-phe-

nyl acetoaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

28. The method of claim **26**, wherein the aldehyde or ketone biosynthesis pathway comprises a propionaldehyde biosynthesis pathway and a biosynthesis pathway selected from a butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, and phenylacetoaldehyde biosynthesis pathway.

29. The method of claim **26**, wherein the aldehyde or ketone biosynthesis pathway comprises a butyraldehyde biosynthesis pathway and a biosynthesis pathway selected from an isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl)acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

30. The method of claim **26**, wherein the aldehyde or ketone biosynthesis pathway comprises an isobutyraldehyde biosynthesis pathway and a biosynthesis pathway selected from a 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl)acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

31. The method of claim **26**, wherein the aldehyde or ketone biosynthesis pathway comprises a 2-methyl-butyraldehyde biosynthesis pathway and a biosynthesis pathway selected from a 3-methyl-butyraldehyde, a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl)acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

32. The method of claim **26**, wherein the aldehyde or ketone biosynthesis pathway comprises a 3-methyl-butyraldehyde biosynthesis pathway and a biosynthesis pathway selected from a 2-phenyl acetoaldehyde, a 2-(4-hydroxyphenyl) acetaldehyde, and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

33. The method of claim **26**, wherein the aldehyde or ketone biosynthesis pathway comprises a 2-phenyl acetoaldehyde biosynthesis pathway and a biosynthesis pathway selected from a 2-(4-hydroxyphenyl)acetaldehyde and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

34. The method of claim **26**, wherein the aldehyde or ketone biosynthesis pathway comprises a 2-(4-hydroxyphenyl)acetaldehyde biosynthesis pathway and a 2-Indole-3-acetoaldehyde biosynthesis pathway.

35. The method of claim **24**, wherein the first commodity chemical is further enzymatically and/or chemically reduced and dehydrated to a second commodity chemical.

36. A method for converting a suitable monosaccharide or oligosaccharide to a commodity chemical comprising,

(a) contacting the suitable monosaccharide or oligosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide or oligosaccharide to the commodity chemical, wherein the microbial system comprises;

(i) one or more genes encoding and expressing an aldehyde biosynthesis pathway, wherein the aldehyde biosynthesis pathway comprises one or more genes encoding and expressing a decarboxylase enzyme; and

(ii) one or more genes encoding and expressing an aldehyde reductase,

thereby converting the suitable monosaccharide or oligosaccharide to the commodity chemical.

37. The method of claim **36**, wherein the decarboxylase enzyme is an indole-3-pyruvate decarboxylase (IPDC).

38. The method of claim **37**, wherein the IPDC comprises an amino acid sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 312

39. The method of claim **36**, wherein the aldehyde reductase enzyme is a phenylacetaldehyde reductase (PAR).

40. The method of claim **39**, wherein the PAR comprises an amino acid sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the amino acid sequence set forth in SEQ ID NO: 313.

41. The method of claim **36**, wherein the commodity chemical is selected from 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and indole-3-ethanol.

42. A recombinant microorganism, comprising (i) one or more genes encoding and expressing an aldehyde biosynthesis pathway, wherein the aldehyde biosynthesis pathway comprises one or more genes encoding and expressing a decarboxylase enzyme; and (ii) one or more genes encoding and expressing an aldehyde reductase.

43. The recombinant microorganism of claim **42**, wherein the aldehyde biosynthesis pathway further comprises one or more genes encoding and expressing an enzyme selected from a CoA-linked aldehyde dehydrogenase, an aldehyde dehydrogenase, and an alcohol dehydrogenase.

44. The recombinant microorganism of claim **42**, wherein the decarboxylase enzyme is an indole-3-pyruvate decarboxylase (IPDC).

45. The recombinant microorganism of claim **42**, wherein the aldehyde reductase enzyme is a phenylacetoaldehyde reductase (PAR).

46. The recombinant microorganism of claim **42**, wherein the microorganism is capable of converting a suitable monosaccharide or oligosaccharide to a commodity chemical.

47. The recombinant microorganism of claim **46**, wherein the commodity chemical is selected from 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and indole-3-ethanol.

48. The recombinant microorganism of claim **17**, wherein the microorganism comprises reduced ethanol production capability compared to a wild-type microorganism.

49. The recombinant microorganism of claim **48**, wherein the microorganism comprises a reduction or inhibition in the conversion of acetyl-coA to ethanol.

50. The recombinant microorganism of claim **48**, wherein the recombinant microorganism comprises a reduction of an ethanol dehydrogenase, thereby providing a reduced ethanol production capability.

51. The recombinant microorganism of claim **50**, wherein the ethanol dehydrogenase is an adhE, homolog or variant thereof.

52. The recombinant microorganism of claim **50**, wherein the microorganism comprises a deletion or knockout of an adhE, homolog or variant thereof.

53. The recombinant microorganism of claim **17** or **42**, wherein the recombinant microorganism comprises one or more deletions or knockouts in a gene encoding an enzyme selected from an enzyme that catalyzes the conversion of acetyl-coA to ethanol, an enzyme that catalyzes the conversion of pyruvate to lactate, an enzyme that catalyzes the conversion of fumarate to succinate, an enzyme that catalyzes the conversion of acetyl-coA and phosphate to coA and acetyl phosphate, an enzyme that catalyzes the conversion of acetyl-

coA and formate to coA and pyruvate, and an enzyme that catalyzes the conversion of alpha-keto acid to branched chain amino acids.

54. The microbial system or recombinant microorganism of claim **1**, wherein the recombinant microorganism or microbial system comprises a microorganism selected from *Acetobacter aceti, Achromobacter, Acidiphilium, Acinetobacter, Actinomadura, Actinoplanes, Aeropyrum pernix, Agrobacterium, Alcaligenes, Ananas comosus* (M), *Arthrobacter, Aspargillus niger, Aspargillus oryze, Aspergillus melleus, Aspergillus pulverulentus, Aspergillus saitoi, Aspergillus sojea, Aspergillus usamii, Bacillus alcalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus lentus, Bacillus licheniformis, Bacillus macerans, Bacillus stearothermophilus, Bacillus subtilis, Bifidobacterium, Brevibacillus brevis, Burkholderia cepacia, Candida cylindracea, Candida rugosa, Carica papaya* (L), *Cellulosimicrobium, Cephalosporium, Chaetomium erraticum, Chaetomium gracile, Clostridium, Clostridium butyricum, Clostridium acetobutylicum, Clostridium thermocellum, Corynebacterium* (glutamicum), *Corynebacterium efficiens, Escherichia coli, Enterococcus, Erwina chrysanthemi, Gliconobacter, Gluconacetobacter, Haloarcula, Humicola insolens, Humicola nsolens, Kitasatospora setae, Klebsiella, Klebsiella oxytoca, Kluyveromyces, Kluyveromyces fragilis, Kluyveromyces lactis, Kocuria, Lactlactis, Lactobacillus, Lactobacillus fermentum, Lactobacillus sake, Lactococcus, Lactococcus lactis, Leuconostoc, Methylocystis, Methanolobus siciliae, Methanogenium organophilum, Methanobacterium bryantii, Microbacterium imperiale, Micrococcus lysodeikticus, Microlunatus, Mucor javanicus, Mycobacterium, Myrothecium, Nitrobacter, Nitrosomonas, Nocardia, Papaya carica, Pediococcus, Pediococcus halophilus, Penicillium, Penicillium camemberti, Penicillium citrinum, Penicillium emersonii, Penicillium roqueforti, Penicillum lilactinum, Penicillum multicolor, Paracoccus pantotrophus, Propionibacterium, Pseudomonas, Pseudomonas fluorescens, Pseudomonas denitrificans, Pyrococcus, Pyrococcus furiosus, Pyrococcus horikoshii, Rhizobium, Rhizomucor miehei, Rhizomucor pusillus Lindt, Rhizopus, Rhizopus delemar, Rhizopus japonicus, Rhizopus niveus, Rhizopus oryzae, Rhizopus oligosporus, Rhodococcus, Sccharomyces cerevisiae, Sclerotina libertina, Sphingobacterium multivorum, Sphingobium, Sphingomonas, Streptococcus, Streptococcus thermophilus* Y-1, *Streptomyces, Streptomyces griseus, Streptomyces lividans, Streptomyces murinus, Streptomyces rubiginosus, Streptomyces violaceoruber, Streptoverticillium mobaraense, Tetragenococcus, Thermus, Thiosphaera pantotropha, Trametes, Trichoderma, Trichoderma longibrachiatum, Trichoderma reesei, Trichoderma viride, Trichosporon penicillatum, Vibrio alginolyticus, Xanthomonas*, yeast, *Zygosaccharomyces rouxii, Zymomonas*, and *Zymomonus mobilis.*

55. A commodity chemical produced by the method of claim **1**.

56. A blended commodity chemical comprising the commodity chemical of claim **55** and a refinery-produced petroleum product.

57. The blended commodity chemical of claim **56**, wherein the commodity chemical is selected from a C10-C12 hydrocarbon, 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and indole-3-ethanol.

58. The blended commodity chemical of claim **57**, wherein the C10-C12 hydrocarbon is selected from 2,7-dimethyloctane and 2,9-dimethyldecane.

59. The blended commodity chemical of claim **56**, wherein the refinery-produced petroleum product is selected from jet fuel and diesel fuel.

60. A method of producing a commodity chemical enriched refinery-produced petroleum product, comprising

(a) blending the refinery-produced petroleum product with the commodity chemical produced by the method of claim **1**,

thereby producing the commodity chemical enriched refinery-produced petroleum product.

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