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(54) **BIOFUEL PRODUCTION**

Related U.S. Application Data

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(73) Assignees: **BIO ARCHITECTURE LAB, INC.**, Seattle, WA (US); **UNIVERSITY OF WASHINGTON**, Seattle, WA (US)

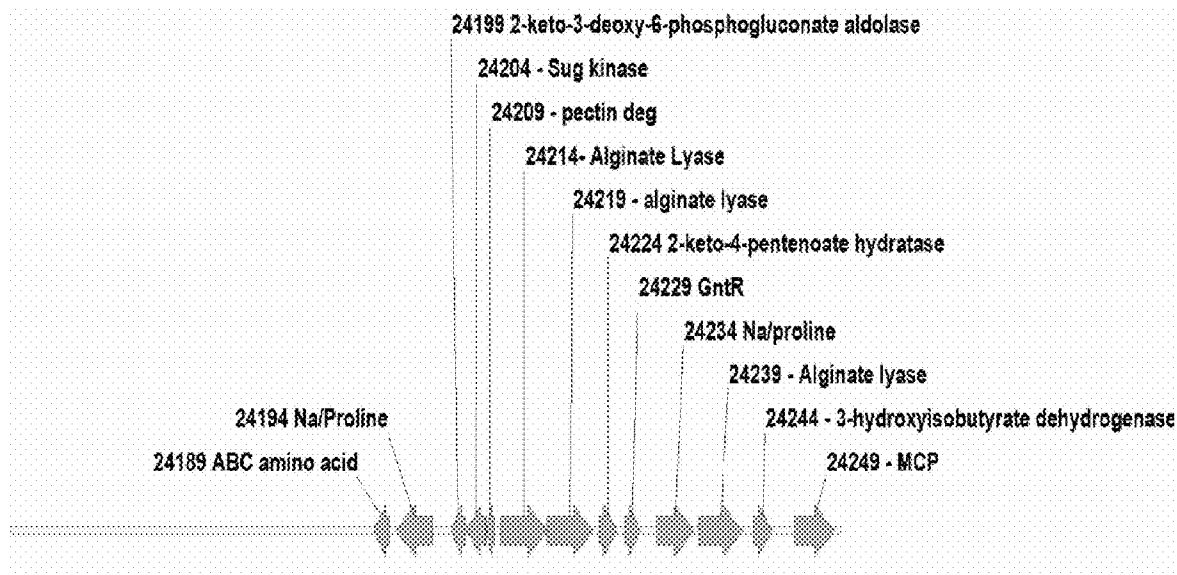
(57) **ABSTRACT**

(21) Appl. No.: **12/245,540**

(22) Filed: **Oct. 3, 2008**

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



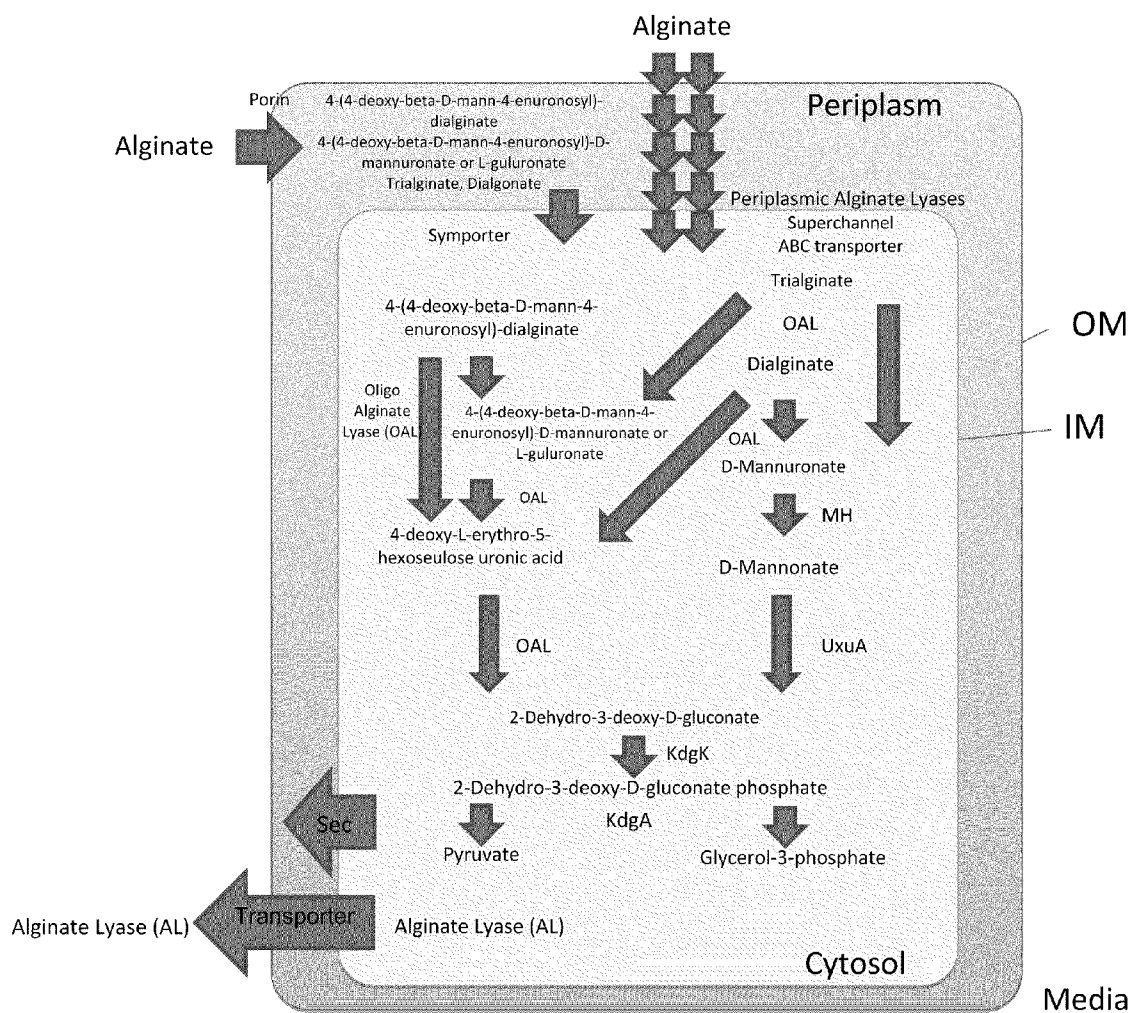


Figure 2

Figure 3

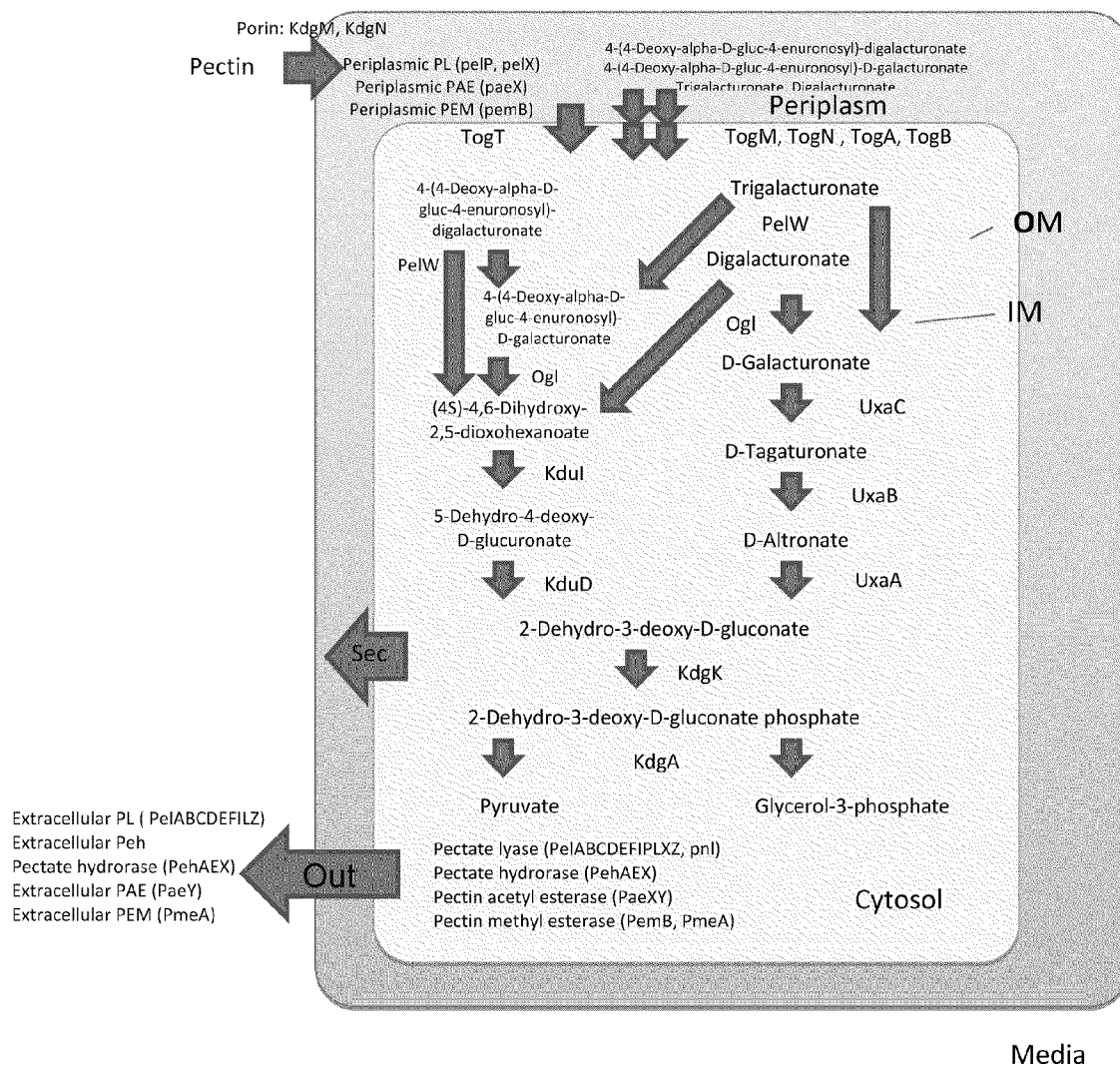


Figure 4
E. coli Growing on Alginate

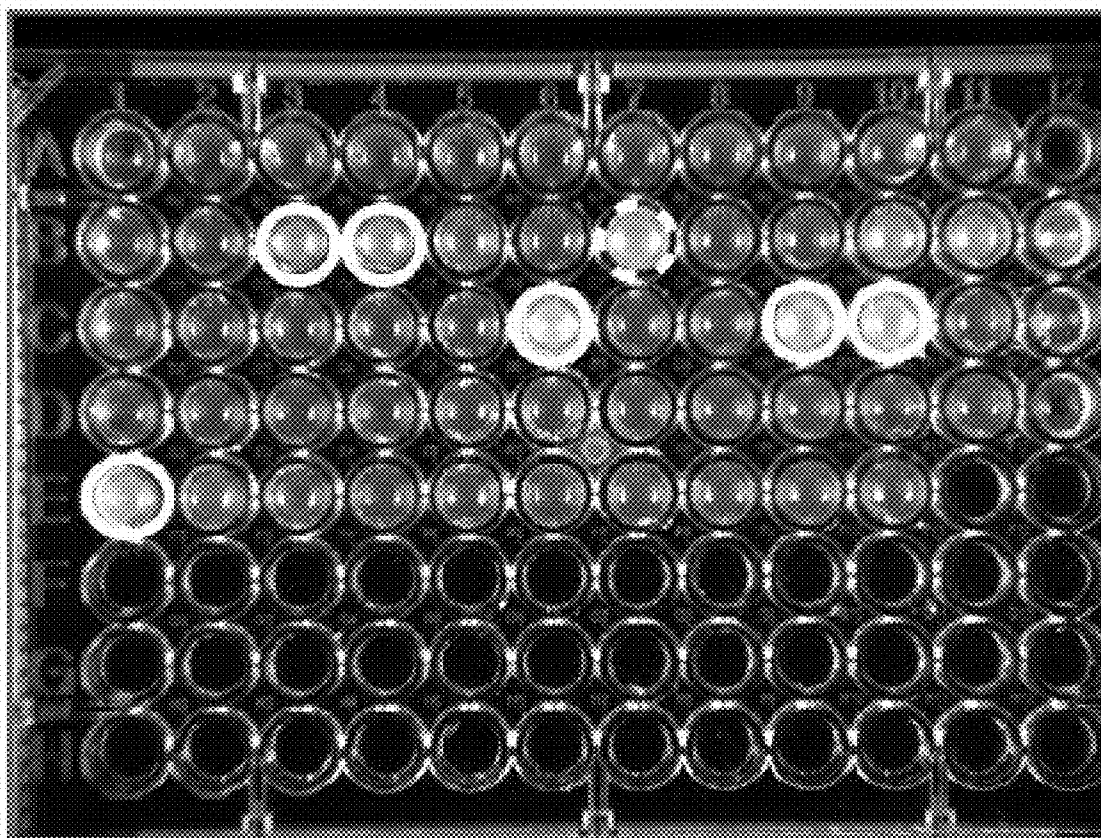


Figure 5A

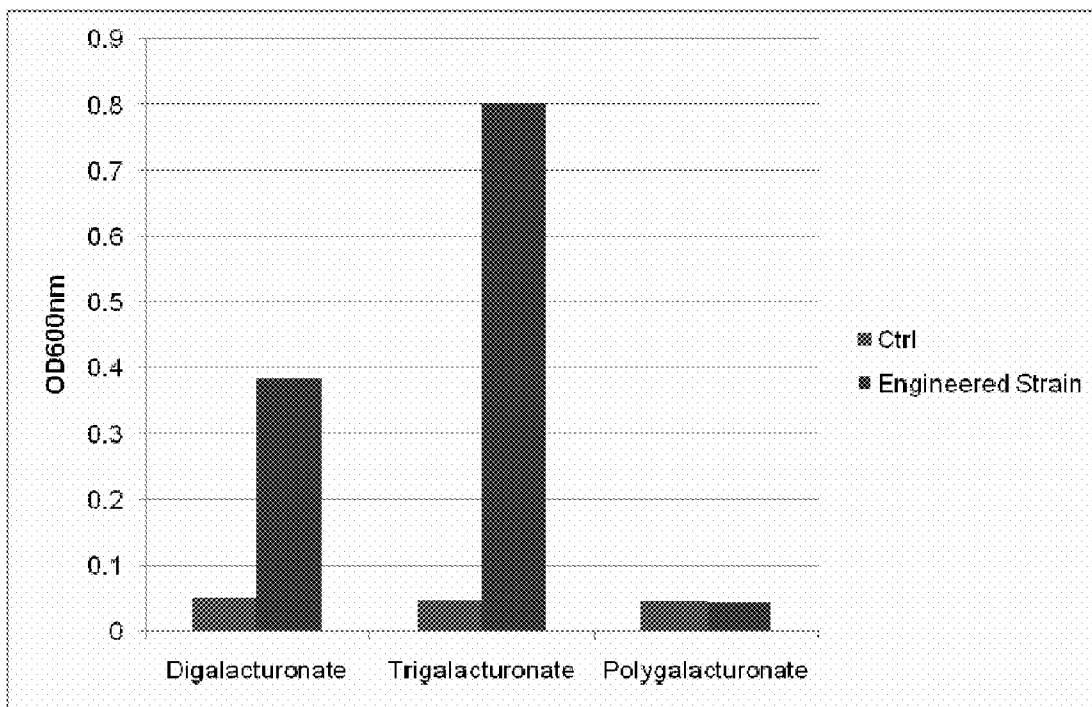
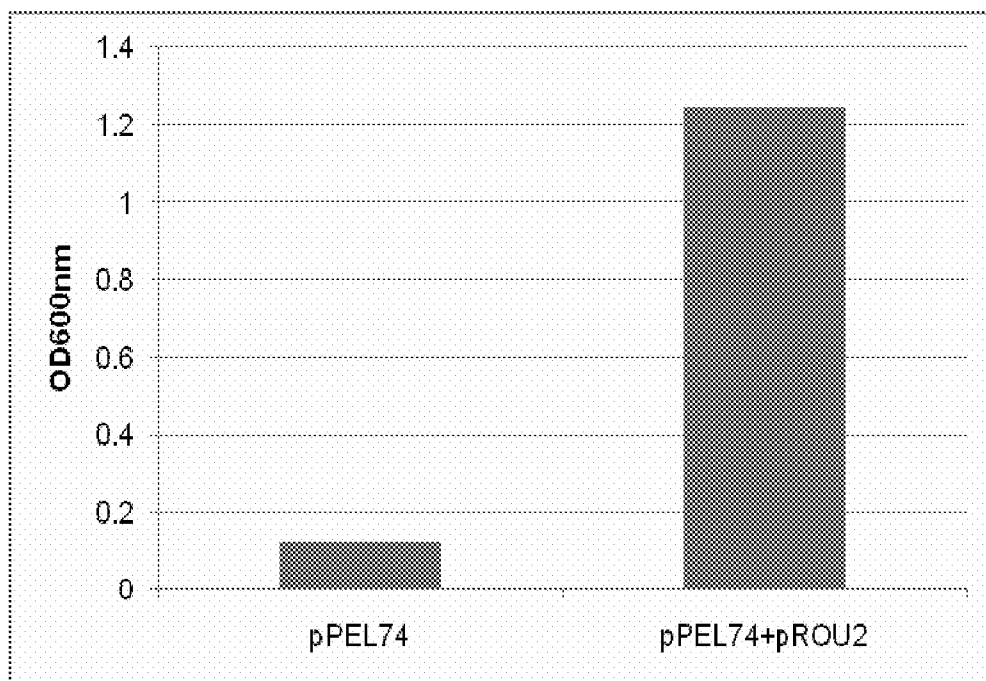


Figure 5B



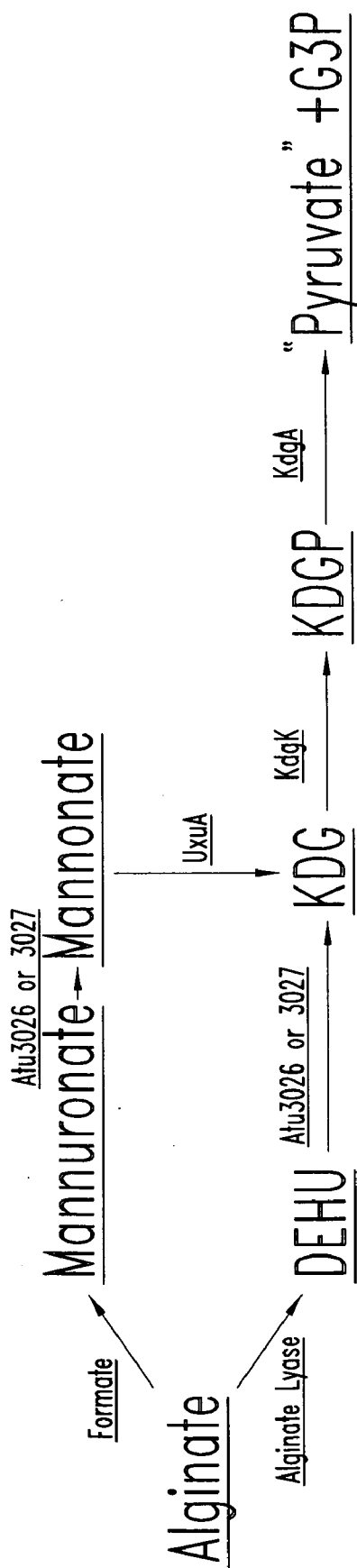


FIG. 6A

Figure 6B

Pyruvate formation from alginate (enzymatic degradation route)

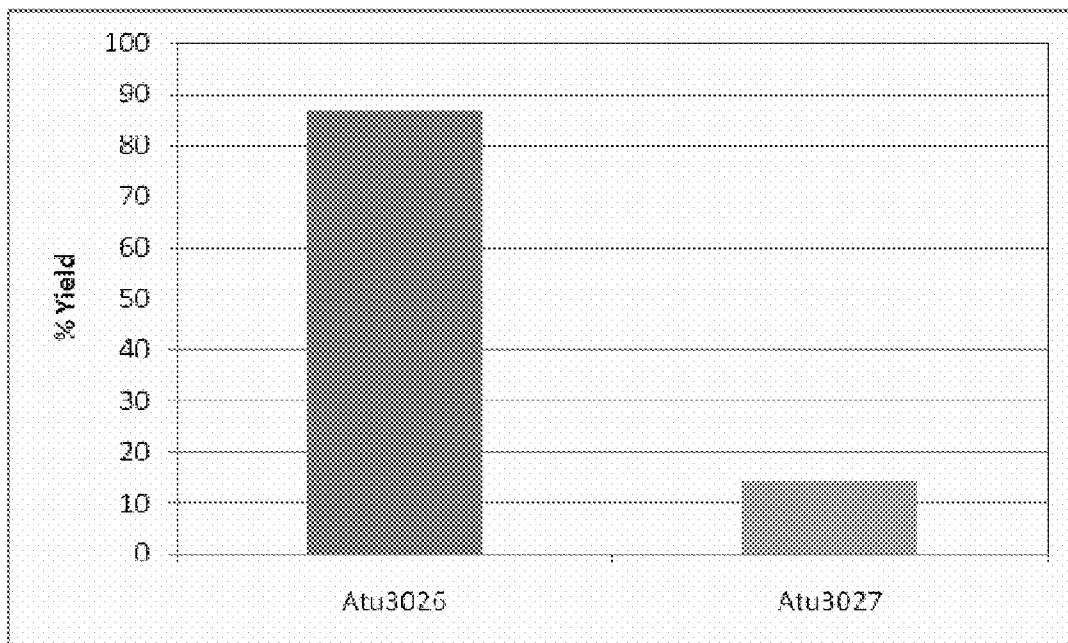
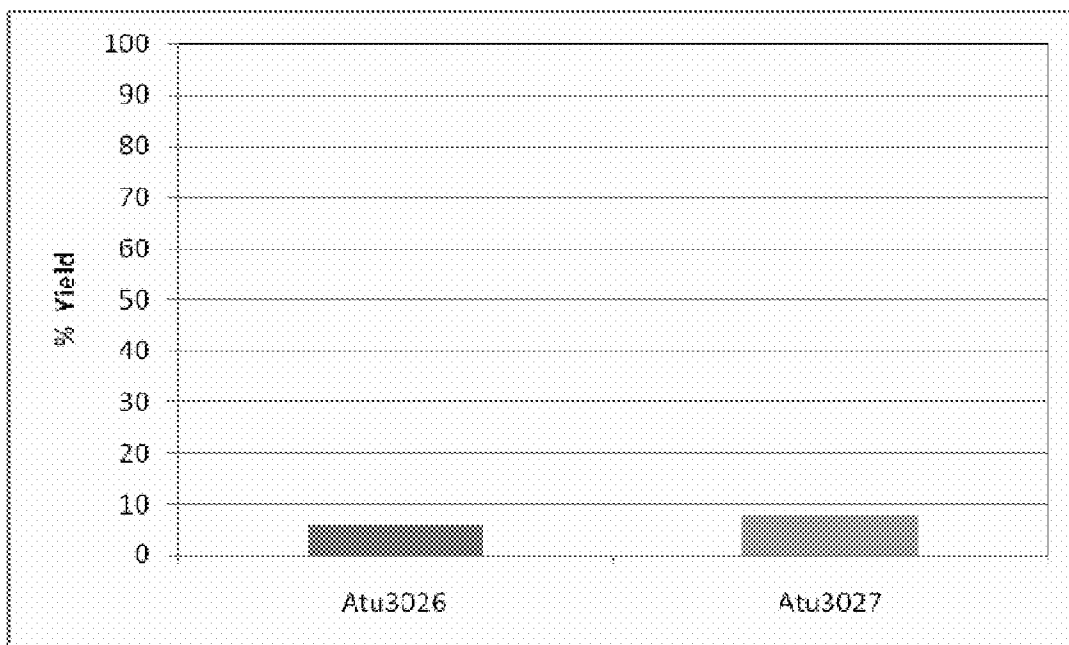


Figure 6C

Pyruvate formation from alginate (chemical degradation route).



GC-MS chromatogram of control

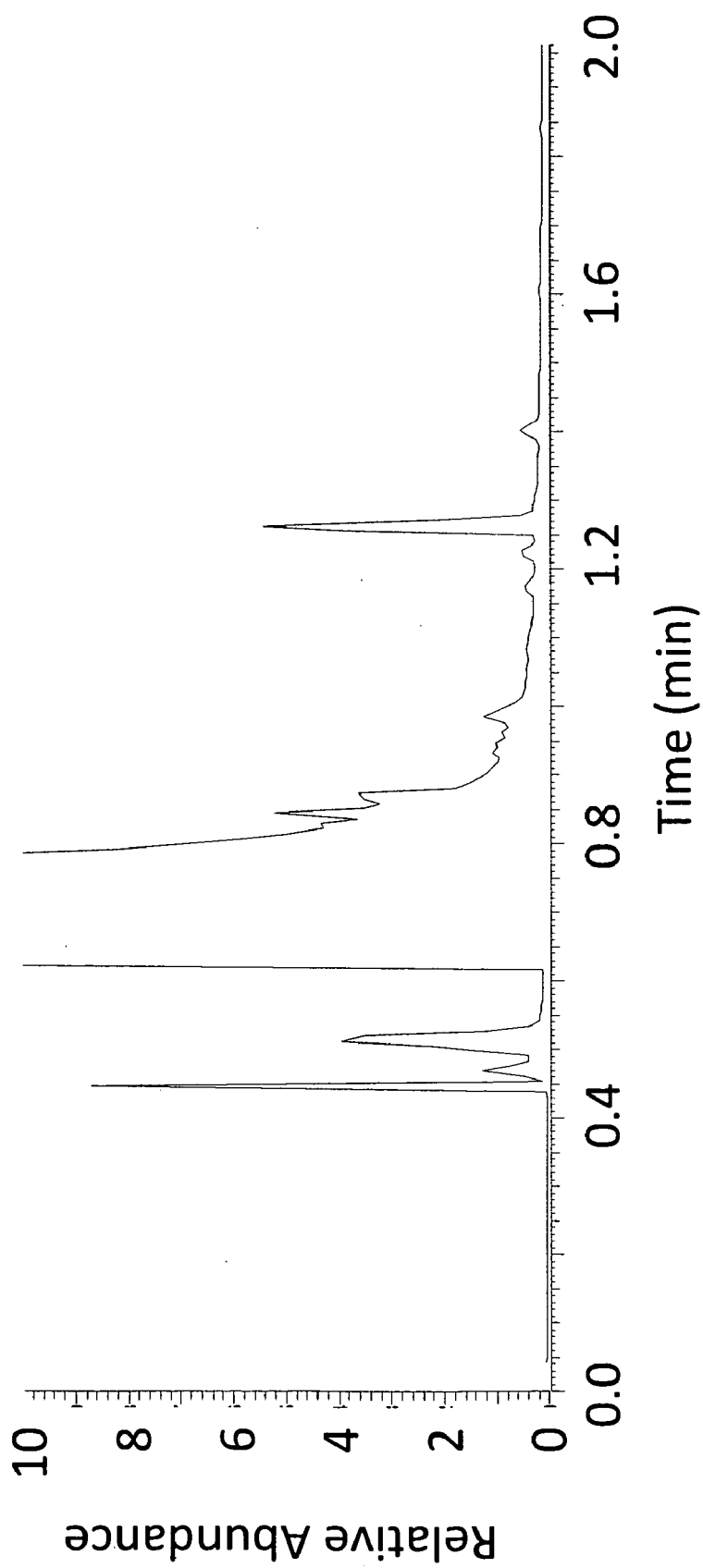


FIG. 8A

GC-MS chromatogram for isobutyraldehyde, 3-methylpentanol, and 2-methylpentanol production from pBADalsS-ilvCD-leuABCD2

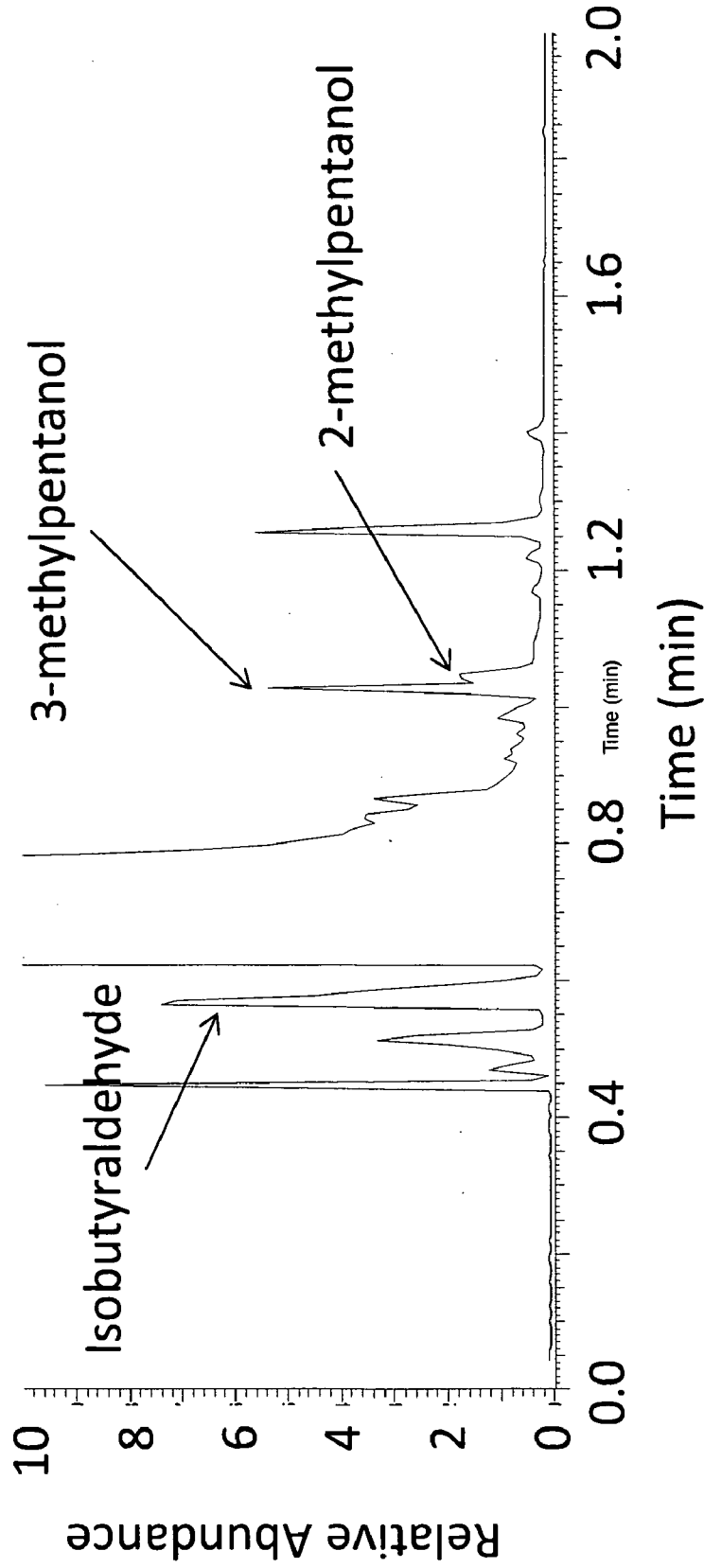


FIG. 8B

GC-MS chromatogram of control

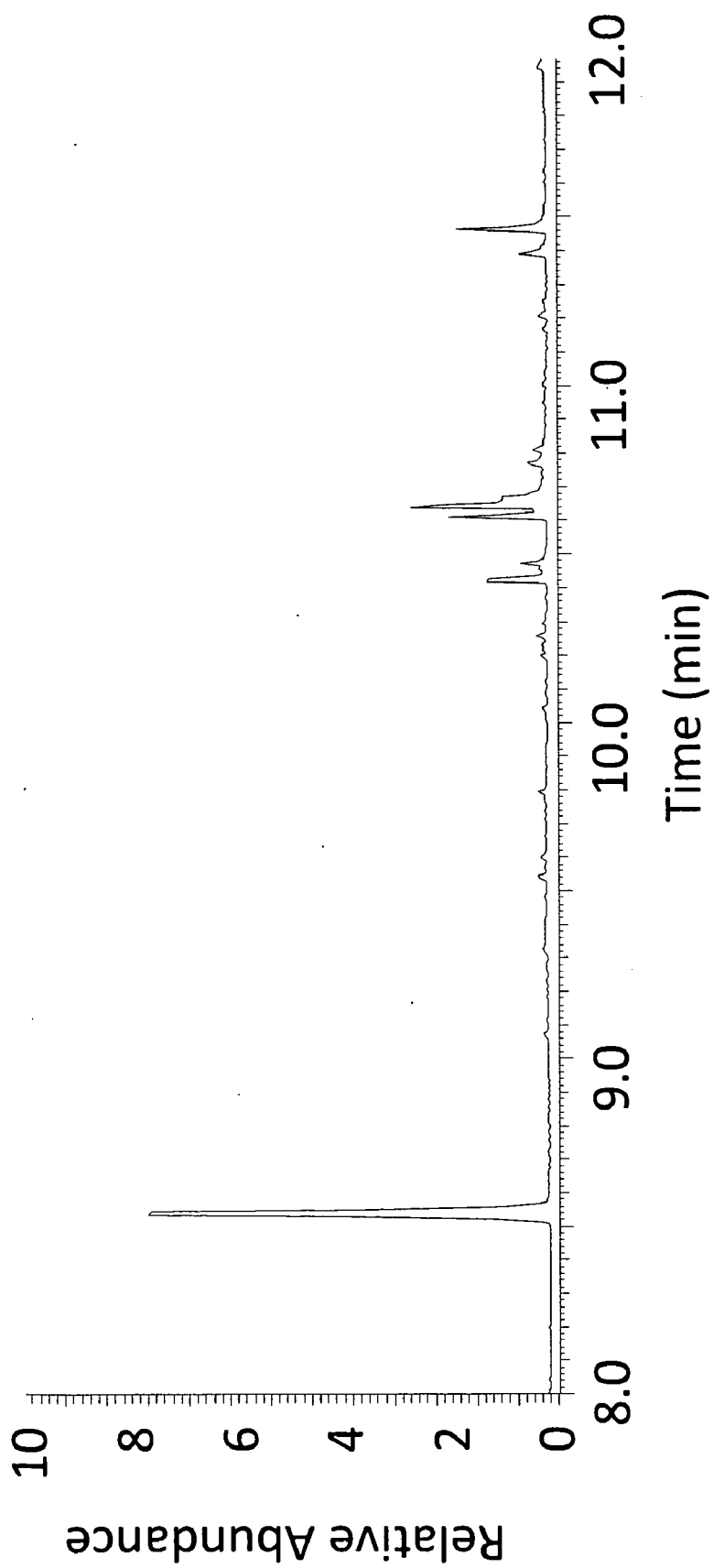


FIG. 9A

GC-MS chromatogram for 4-hydroxy-2-phenylethanol and indole-3-ethanol production from pBADtyrA-aroLAC-aroG-kttA-aroBDE and pTrcBALK

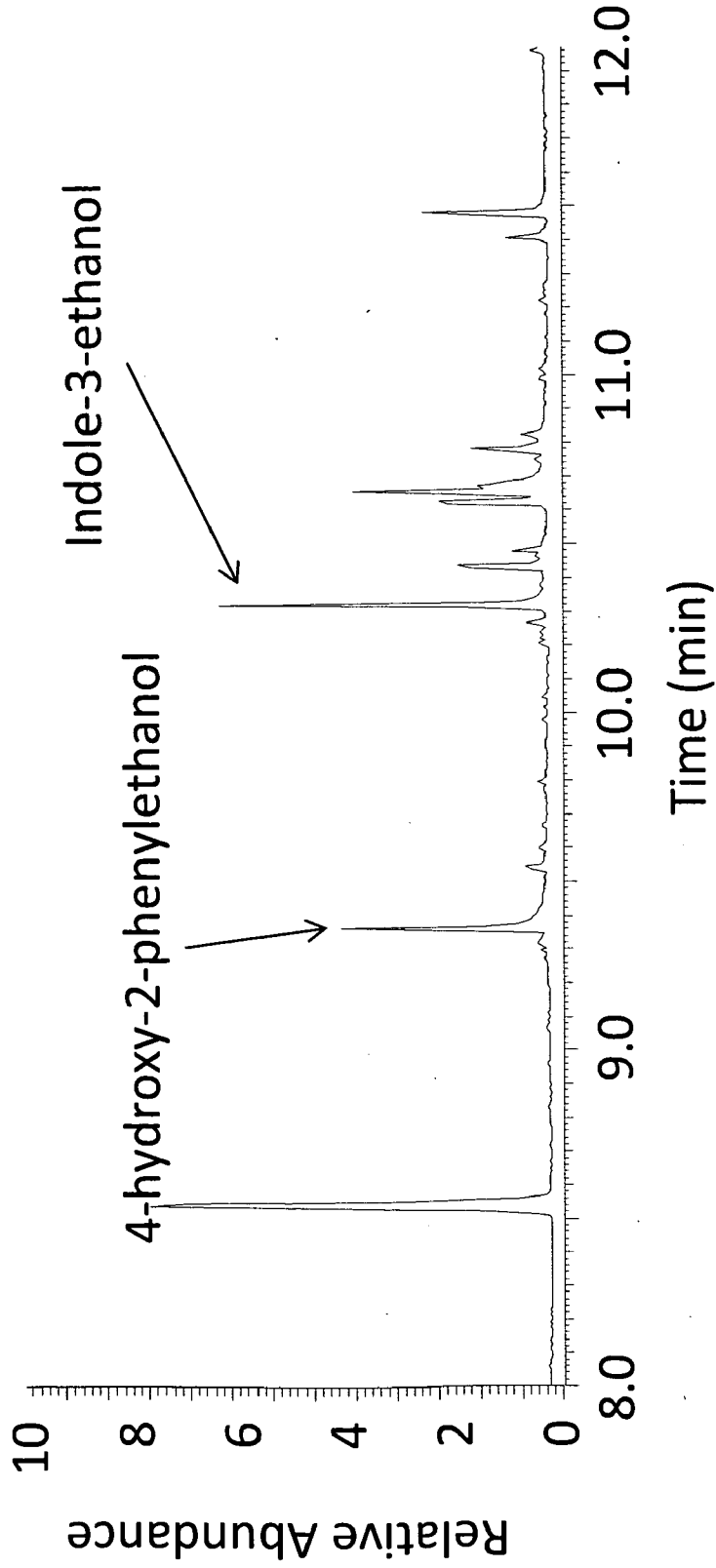


FIG. 9B

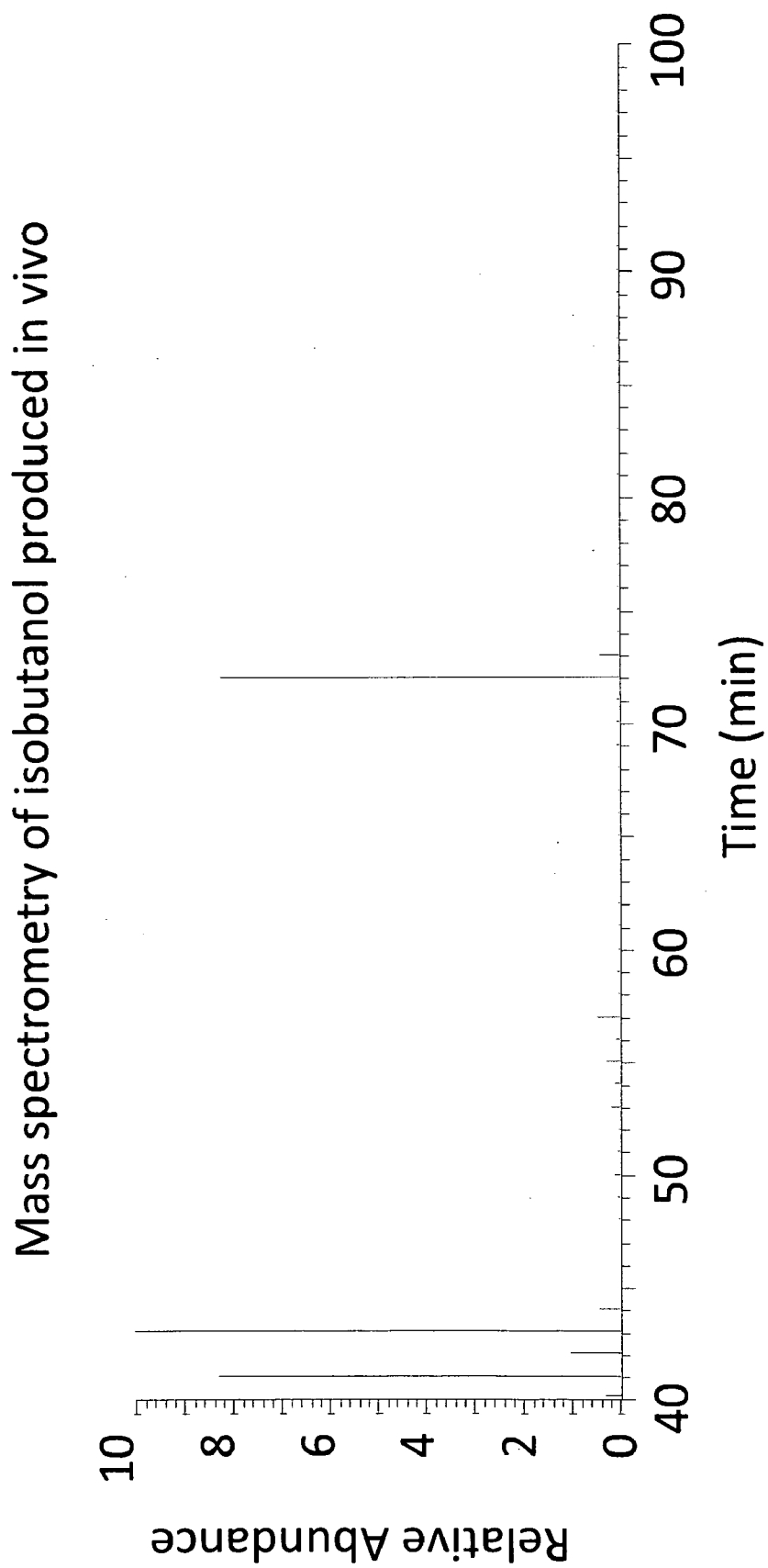


FIG. 10A

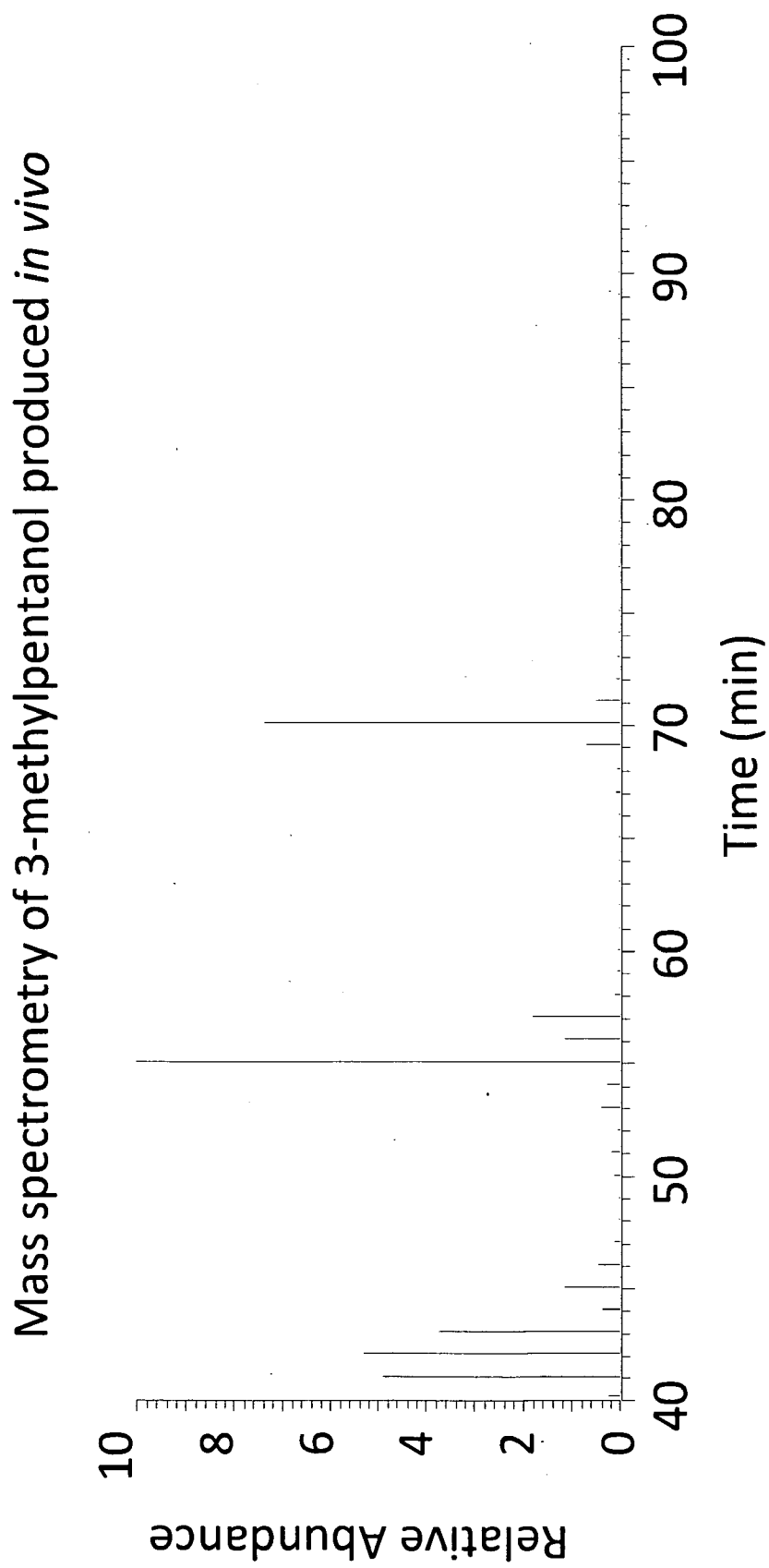


FIG. 10B

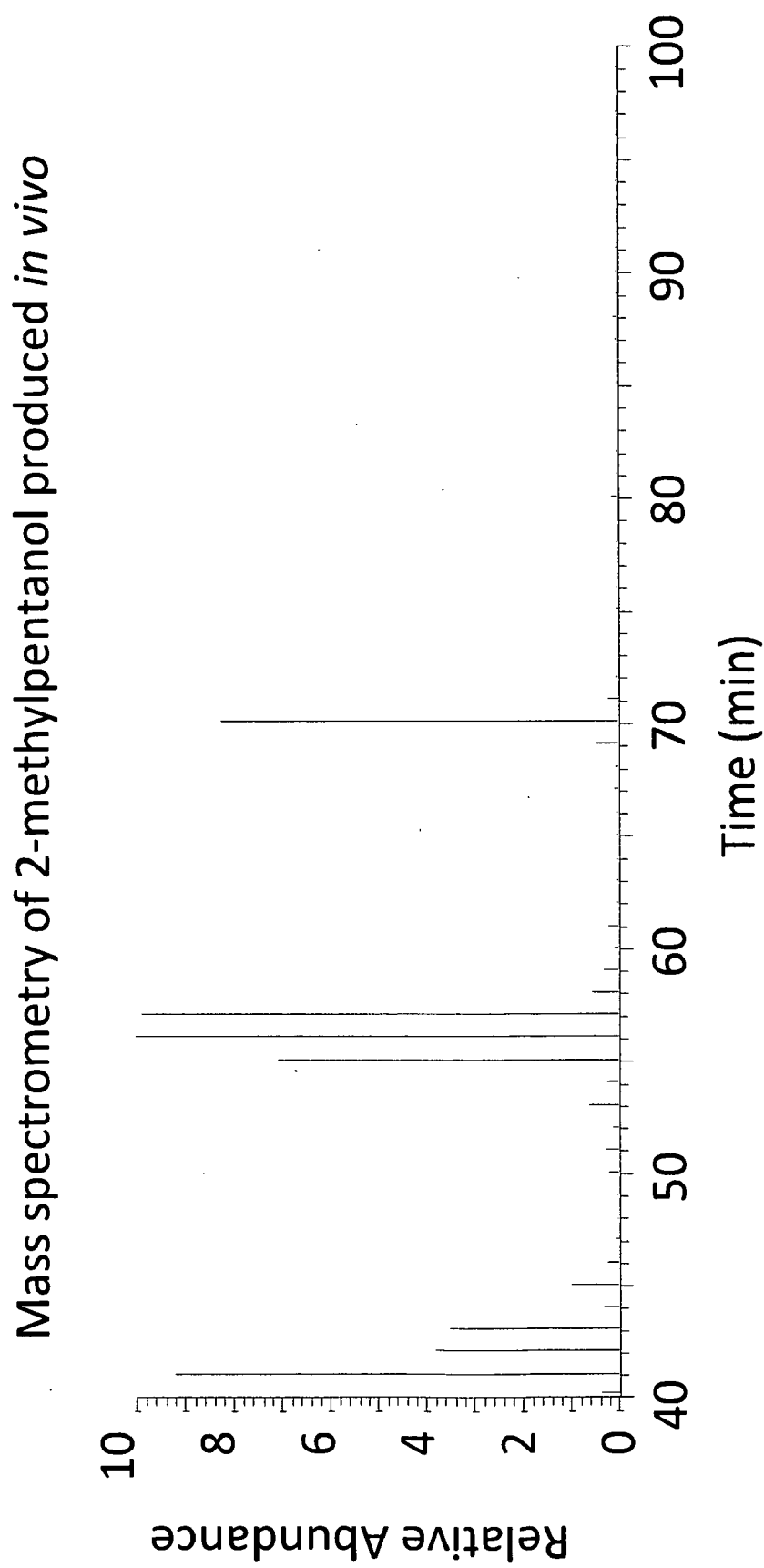


FIG. 10C

Mass spectrometry of 2-phenylethanol produced *in vivo*

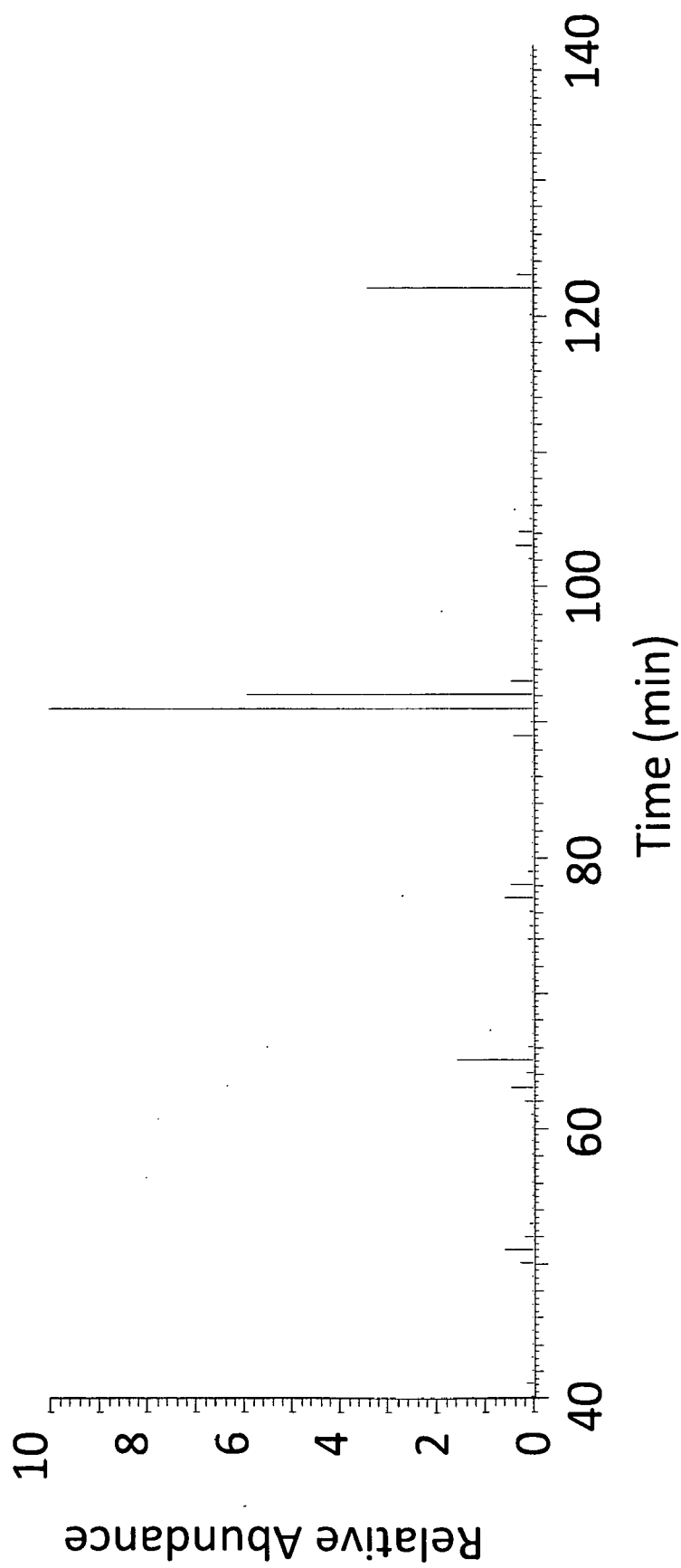


FIG. 11A

Mass spectrometry of 4-hydroxyphenylethanol produced *in vivo*

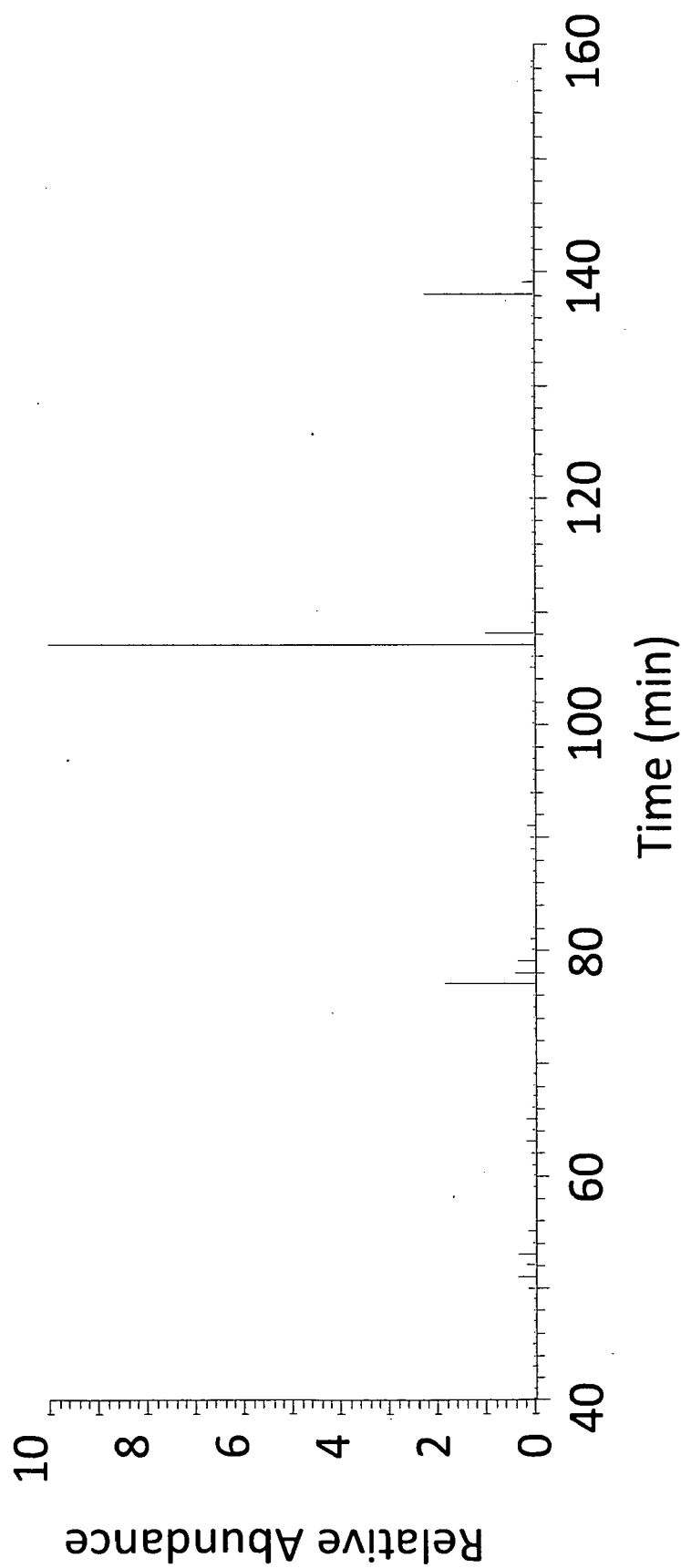


FIG. 11B

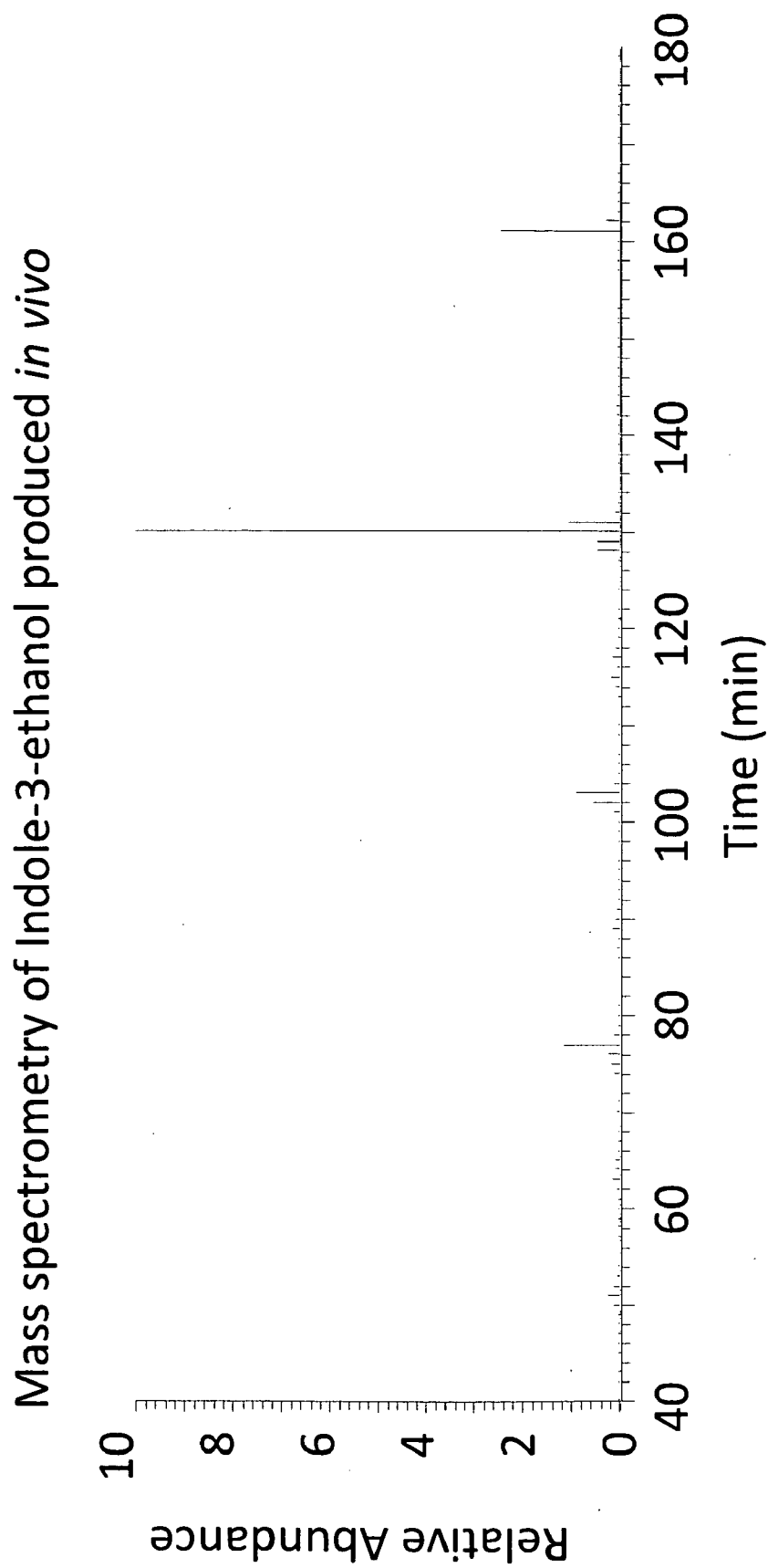


FIG. 11C

Figure 12A

Reduction of butyoin by *ddh1*, *ddh2*, and *ddh3* monitored by NADH consumption.

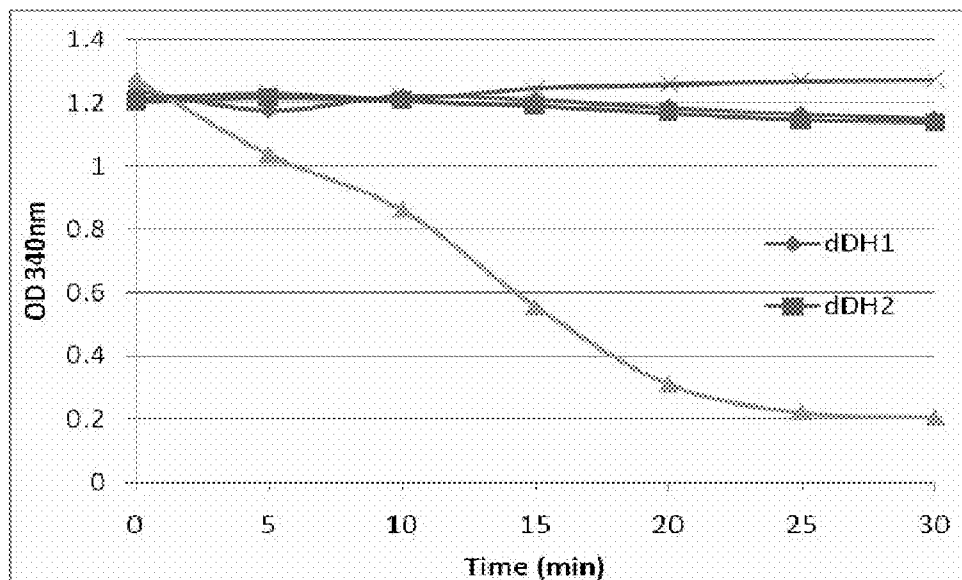
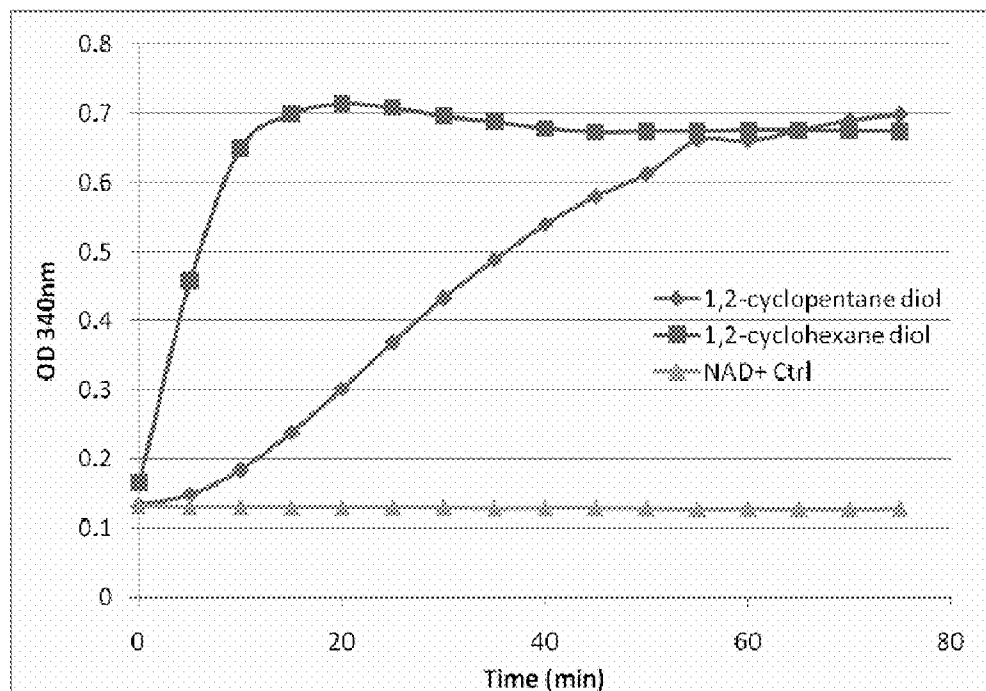


Figure 12B

Oxidation activity of *ddh3* towards 1,2-cyclopentane diol and 1,2-cyclohexane diol as measured by NADH production.



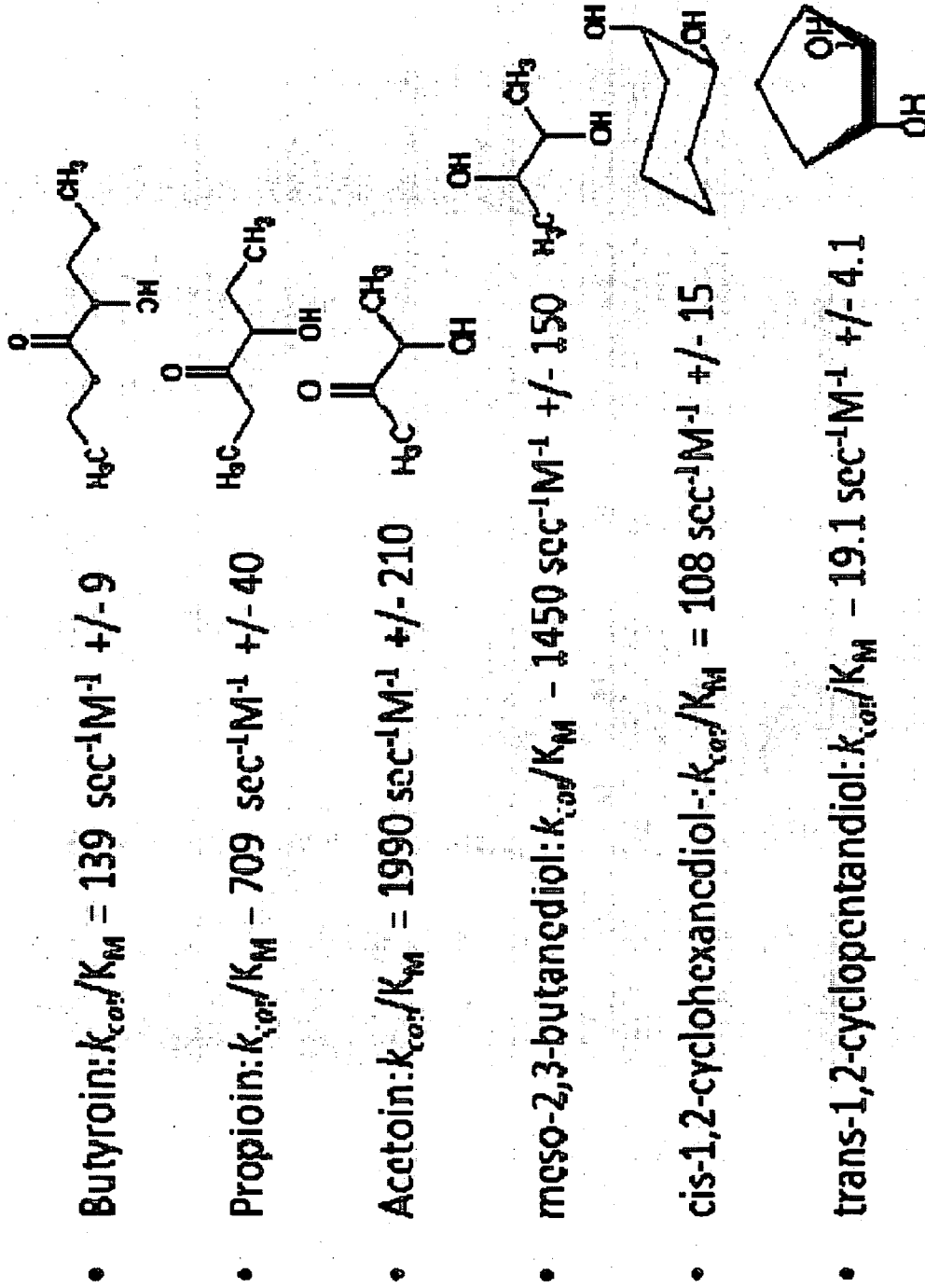


FIG. 13

Figure 14A

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

ATGGCATCAAATGGAAAAGTAGCAATGGTTACCGGTGGCGGACAAGGAATTGGTGAAGC
CATCTCGAAACGGTTAGCTAACGACGGCTTTGCTGTGGCAATTGCTGATTTGAACTTGG
ACAATGCCAACAAAGGTCGTTTCTGATATTGAAGCTGCTGGTGGCAAGGCCATTGCGGTC
AAGACCGATGTCTCTGATCGTGATAGCGTGTTTGGCTGCGGTTAATGAAGCGGCCGACAA
GCTGGGCGGCTTTGACGTTATCGTTAATAACGCCGGCCTTGGCCAACCACGCCAATTG
ACACCATCACCCAAGAACAGTTTGATACGGTTTATCACGTTAACGTGGGTGGGGTTCTT
TGGGGCATTCAAGCAGCCCATGCGAAGTTCAAGGAATTGGGTCATGGTGGGAAGATCAT
TTCCGCGACGTCTCAAGCCGGGGTTGTTGGTAACCCGAACTTAGCTCTGTACAGTGGAA
CTAAGTTTGCCATTCGTGGTGTGACCCAAGTTGCGGCGCGTGACTTAGCCGCTGAAGGT
ATCACGGTCAATGCTTATGCACCCGGGATTGTTAAGACACCAATGATGTTTGACATCGC
TCACAAGGTTGGTCAAAATGCTGGTAAAGACGACGAATGGGGGATGCAAACCTTCTCAA
AGGACATCGCTTTATGTCGATTGTCAGAACCAGAAGATGTGGCTAACGGGGTGGCTTTC
TTAGCCGGTCCCGATTCTAACTACATTACGGGTCAAACACTTGAAGTTGATGGTGGGAT
GCAGTTCCACTAA (SEQ ID NO:97)

Figure 14B

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

MASNGKVAMVTGGGQGI GEAI SKRLANDGFAVAIADLNLDNANKVVS DIEAAGGKAI AV
KTDVSDRDSVFAAVNEAADKLG GFDVIVN NAGLGPTT PIDTITQE QFDTVYHVNVGGVL
WGIQAAHAKFKELGHGGKII SATSQAGVVG NPNLALYS GTKFAIRGVTQVAARDLAAEG
ITVNAYAPGIVKTPMMFDIAHKV GQNAGKDDEWGMQTF SKDIALCRLSEPEDVANGVAF
LAGPDSNYITGQTLEVDGGMQFH (SEQ ID NO:98)

Figure 15A

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

ATGAATGACCTGAGCCACACCCACATGCGCGCGGCCGTCTGGCATGGCCGCCACGATAT
TCGTGTGGAACAGGTACCTTTGCCGGCCGACCCTGCGCCGGGCTGGGTGCAGATCAAGG
TGGACTGGTGCGGCATCTGCGGCTCCGACCTGCACGAATATGTTGCCGGCCCGGTGTTT
ATCCCGGTAGAGGCCCGCACCCGCTGACCGGCATTCAGGGCCAGTGCATCCTCGGCCA
CGAATTCTGCGGCCACATCGCCAAGCTTGGCGAAGGCGTGGAAGGCTATGCCGTAGGCG
ACCCGGTGGCGGCAGACGCGTGCCAGCATTGTGGTACCTGCTATTACTGCACCCATGGC
CTGTACAACATCTGCGAACGCCTGGCGTTACCGGCCTGATGAACAACGGTGCCTTCGC
CGAGCTGGTCAACGTGCCCGCCAACCTGCTCTACCGGCTGCCGCAGGGCTTCCCTGCCG
AAGCCGGGGCACTGATCGAGCCGCTGGCGGTGGGTATGCACGCGGTGAAAAAGGCCGGC
AGCCTGCTTGGGCAAACCGTTGTAGTGGTTGGGGCCGGCACCATCGGCCTGTGCACCAT
CATGTGCGCCAAGGCTGCAGGTGCGGCACAGGTCATCGCCCTTGAGATGTCCTCTGCGC
GCAAAGCCAAGGCCAAGGAAGCGGGCGCCAACGTGGTGCTGGACCCAGCCAGTGCAT
GCCCTGGCGGAAATCCGCGCACTGACTGCTGGGCTGGGCGCCGATGTGAGTTTTGAGTG
CATCGGCAACAAACATACGGCCAAGCTGGCCATCGACACCATCCGCAAAGCAGGCAAGT
GCGTGCTGGTGGGTATTTTCGAAGAGCCCAGCGAGTTCAACTTCTTCGAGCTGGTGTCC
ACCGAGAAGCAAGTGCTGGGGCGTGGCGTACAACGGCGAGTTTGCTGACGTGATTGC
CTTCATTGCTGATGGTCCGGCTGGATATTCGCCCCTGGTAACCGGCCGGATCGGATTGG
AGCAGATTGTCGAGCTGGGCTTCGAGGAACCTGGTGAACAACAAAGAGGAGAACGTGAAG
ATCATCGTTTTACCAGGTGTGCGCTGA (SEQ ID NO:99)

Figure 15B

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

MNDLSHTHMRAAVVWHGRHDIRVEQVPLPADPAPGWVQIKVDWCGICGSDLHEYVAGPVF
IPVEAPHPLTGIQGCILGHEFCGHI AKLGEGVEGYAVGDPVAADACQHCCTCYCTHG
LYNICERLAFTGLMNNGAFAELVNVPANLLYRLPQGFPAEAGALIEPLAVGMHAVKKAG
SLLGQTVVVVVGAGTIGLCTIMCAKAAGAAQVIALEMSSARKAKAKEAGANVVLDPDQCD
ALAEIRALTAGLGADVSEFECIGNKHTAKLAIDTIRKAGKCVLVGIFEEPSEFNFFELVS
TEKQVLGALAYNGEFADVIAFIADGRDIRPLVTGRIGLEQIVELGFEELVNNKEENVK
IIVSPGVR (SEQ ID NO:100)

Figure 16A

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

ATGAAAAAAGTCGCACTTGTACCGGCGCCGGCCAGGGGATTGGTAAAGCTATCGCCCT
TCGTCTGGTGAAGGATGGATTTGCCGTGGCCATTGCCGATTATAACGACGCCACCGCCA
AAGCGGTGCCTCGGAAATCAACCAGGCCGGCGGACACGCCGTGGCGGTGAAAGTGGAT
GTCTCCGACCGCGATCAGGTATTTGCCGCCGTGAAACAGGCGCGCAAAACGCTGGGCGG
CTTCGACGTCATCGTCAATAACGCCGGTGTGGCACCGTCTACGCCGATCGAGTCCATTA
CCCCGGAGATTGTCGACAAAGTCTACAACATCAACGTCAAAGGGGTGATCTGGGGTATT
CAGGCGGCGGTTCGAGGCCTTTAAGAAAGAGGGGCACGGCGGGAAAATCATCAACGCCTG
TTCCCAGGCCGGCCACGTCGGCAACCCGGAGCTGGCGGTGTATAGCTCCAGTAAATTCG
CGGTACGCGGCTTAACCCAGACCGCCGCTCGCGACCTCGCGCCGCTGGGCATCACGGTC
AACGGCTACTGCCCGGGGATTGTCAAACGCCAATGTGGGCCGAAATTGACCGCCAGGT
GTCCGAAGCCCGCGGTAAACCGCTGGGCTACGGTACCGCCGAGTTCGCCAAACGCATCA
CTCTCGGTCTGTCTGTCGAGCCGGAAGATGTCGCCGCCTGCGTCTCCTATCTTGCCAGC
CCGGATTCTGATTACATGACCGGTTCAGTCGTTGCTGATCGACGGCGGGATGGTATTTAA
CTAA (SEQ ID NO:101)

Figure 16B

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

MKKVALVTGAGQIGKAIALRLVKDGFVAIAIADYNDATAKAVASEINQAGGHAVAVKVD
VSDRDQVFAAVEQARKTLGGFDVIVNAGVAPSTPIESITPEIVDKVYNINVKGVIWGI
QAAVEAFKKEGHGGKIINACSQAGHVGNPVELAVYSSSKFAVRGLTQTAARDLAPLGITV
NGYCPGIVKTPMWAEIDRQVSEAAGKPLGYGTAEFAKRITLGRLEPEDVAACVSYLAS
PDSYMTGQSLLIDGGMVFN (SEQ ID NO:102)

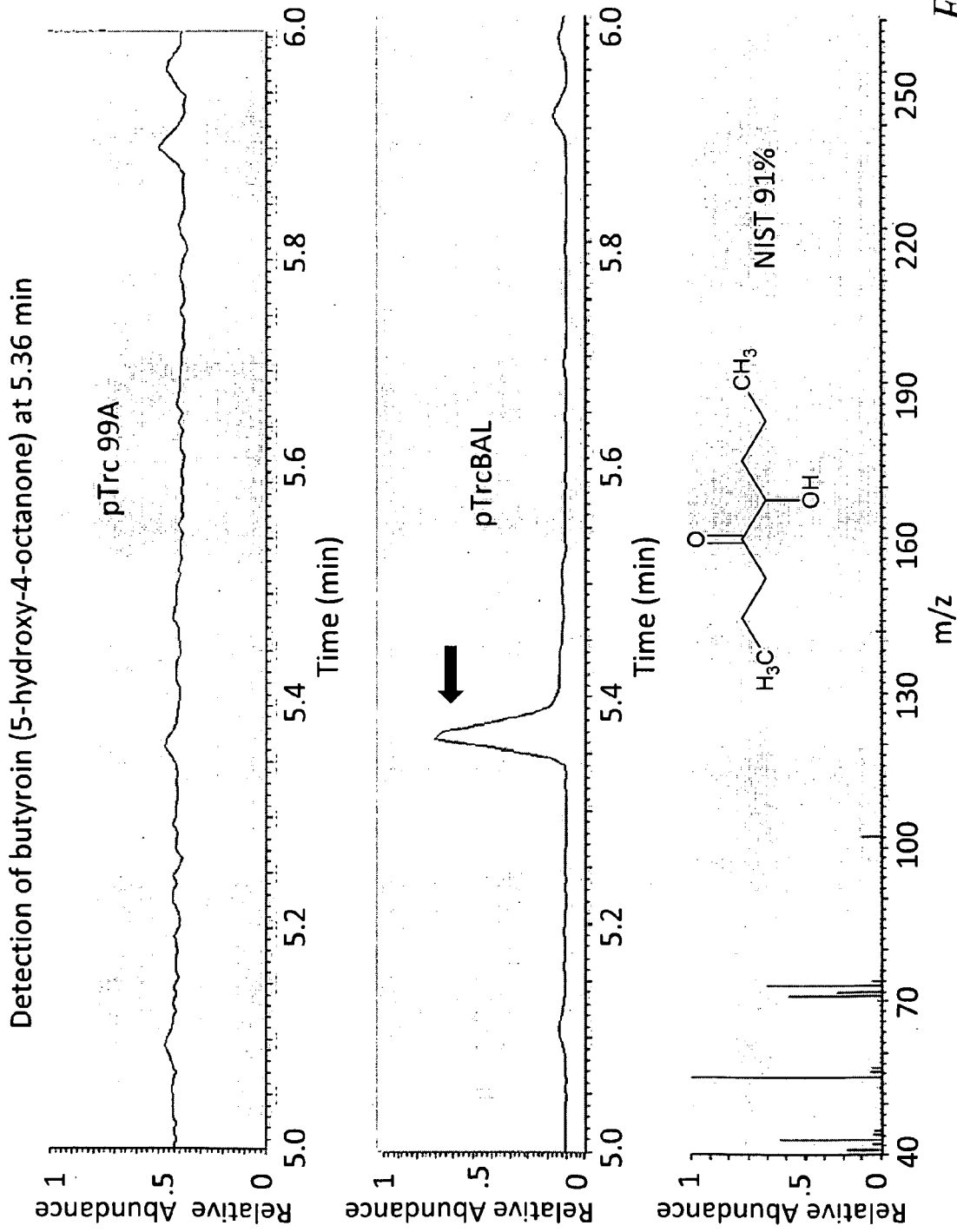


FIG. 17A

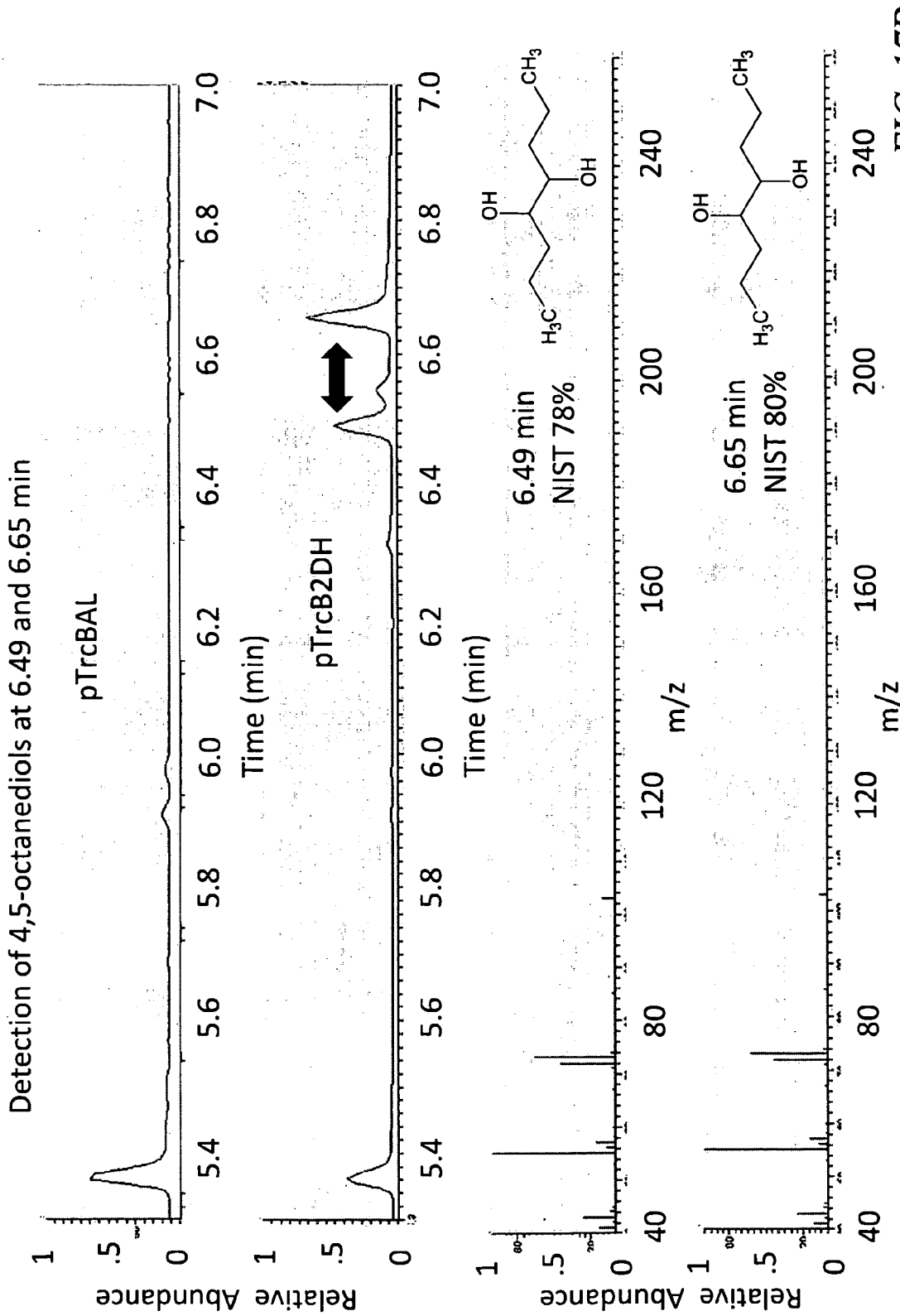


FIG. 17B

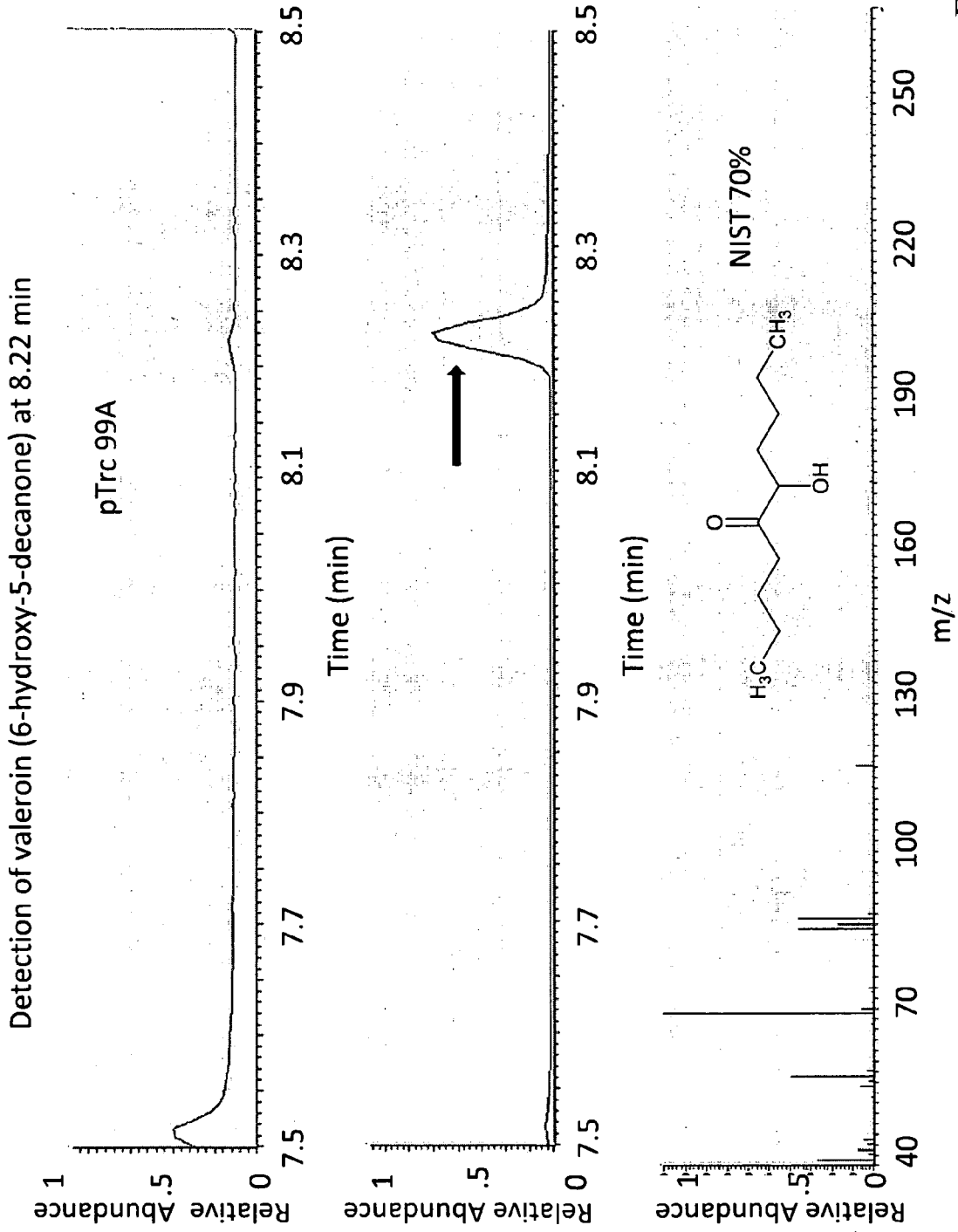


FIG. 18A

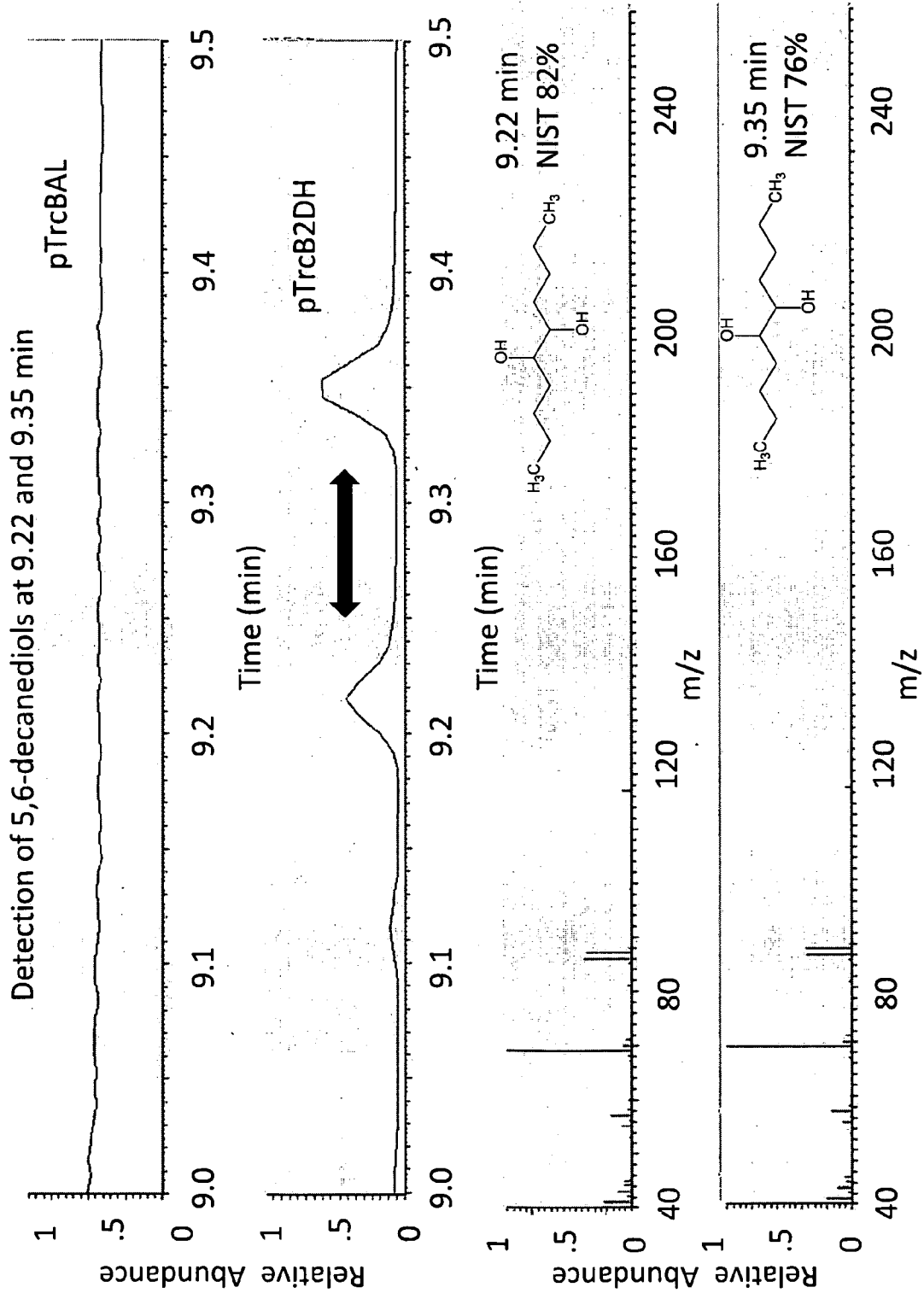


FIG. 18B

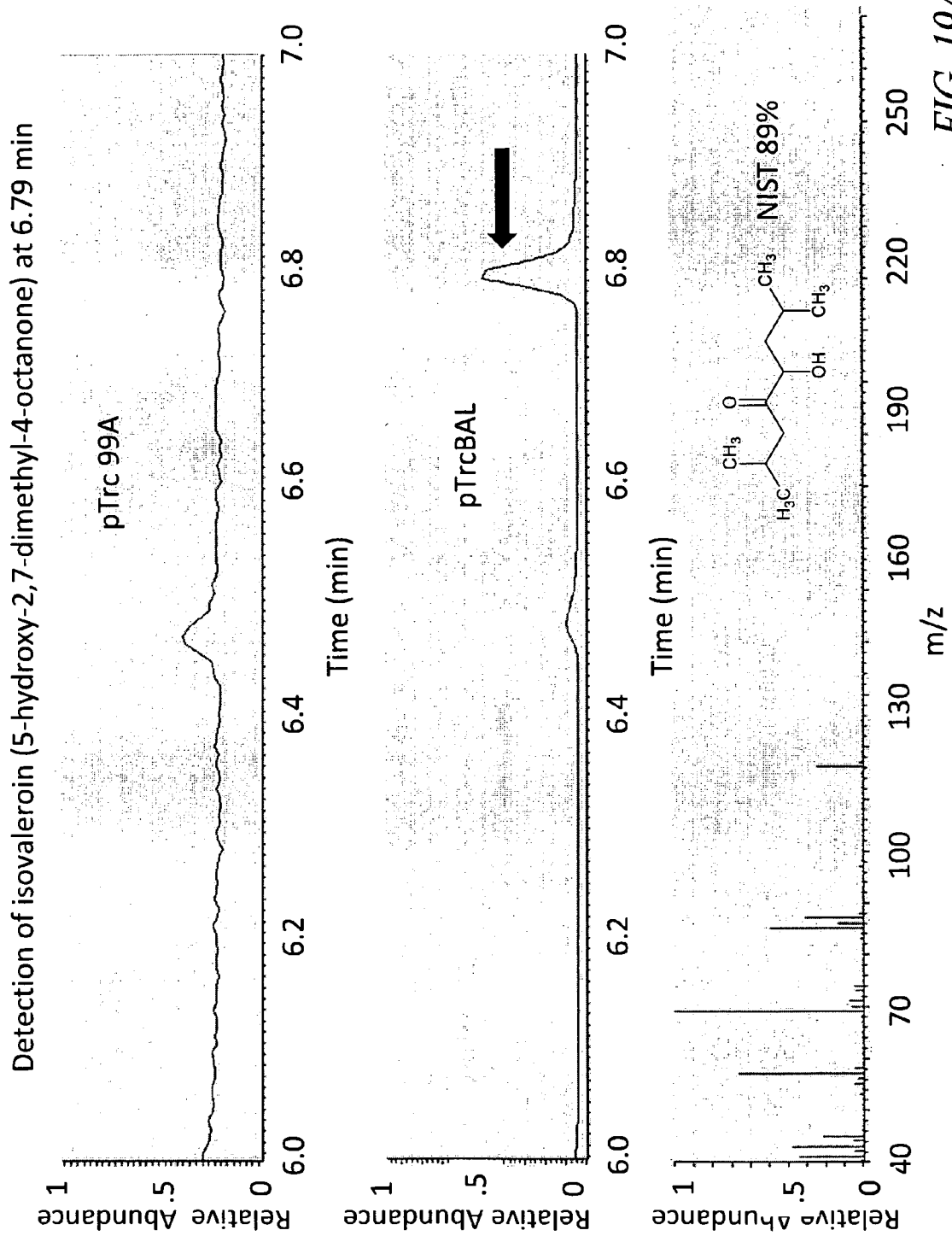


FIG. 19A

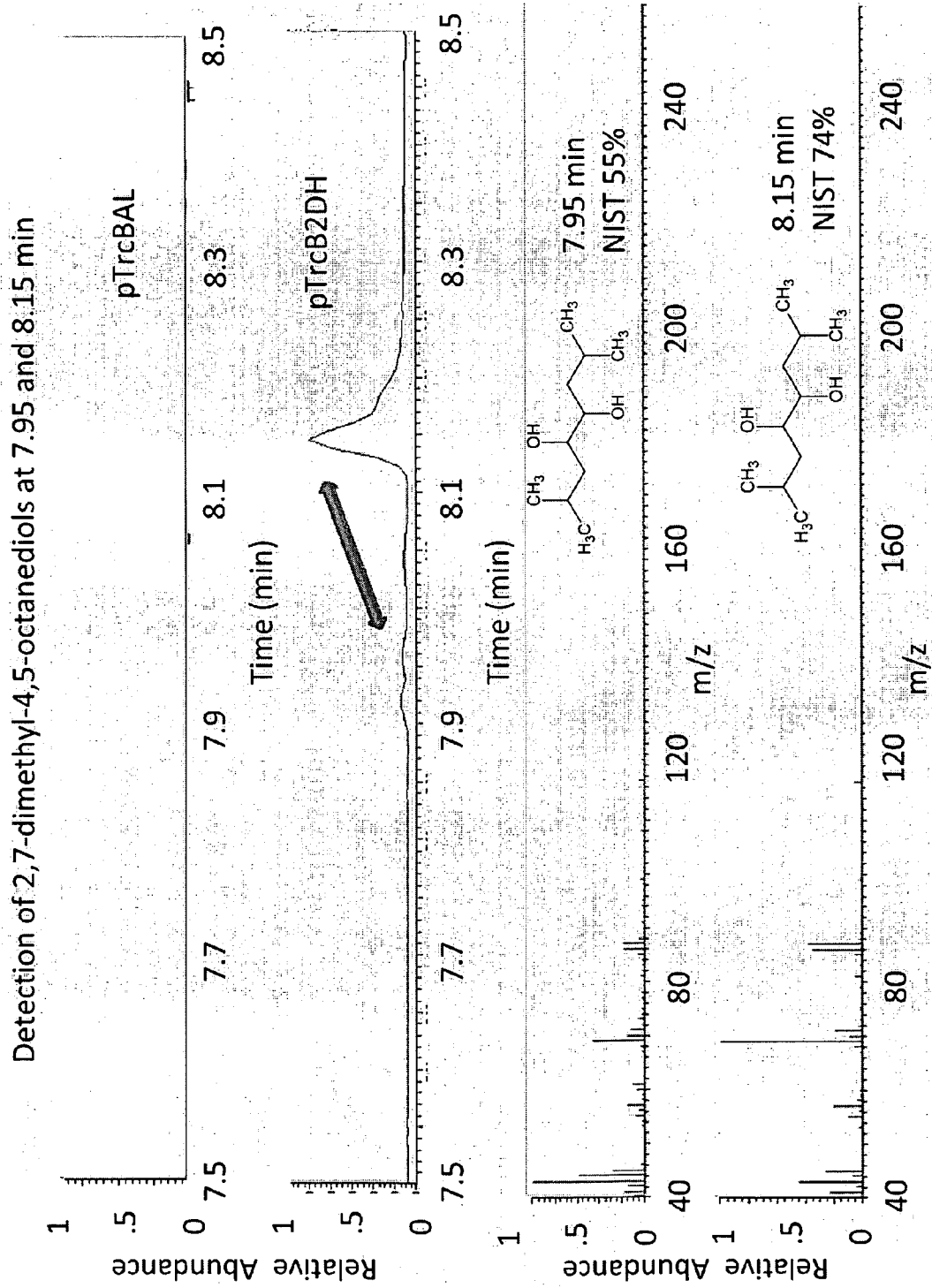


FIG. 19B

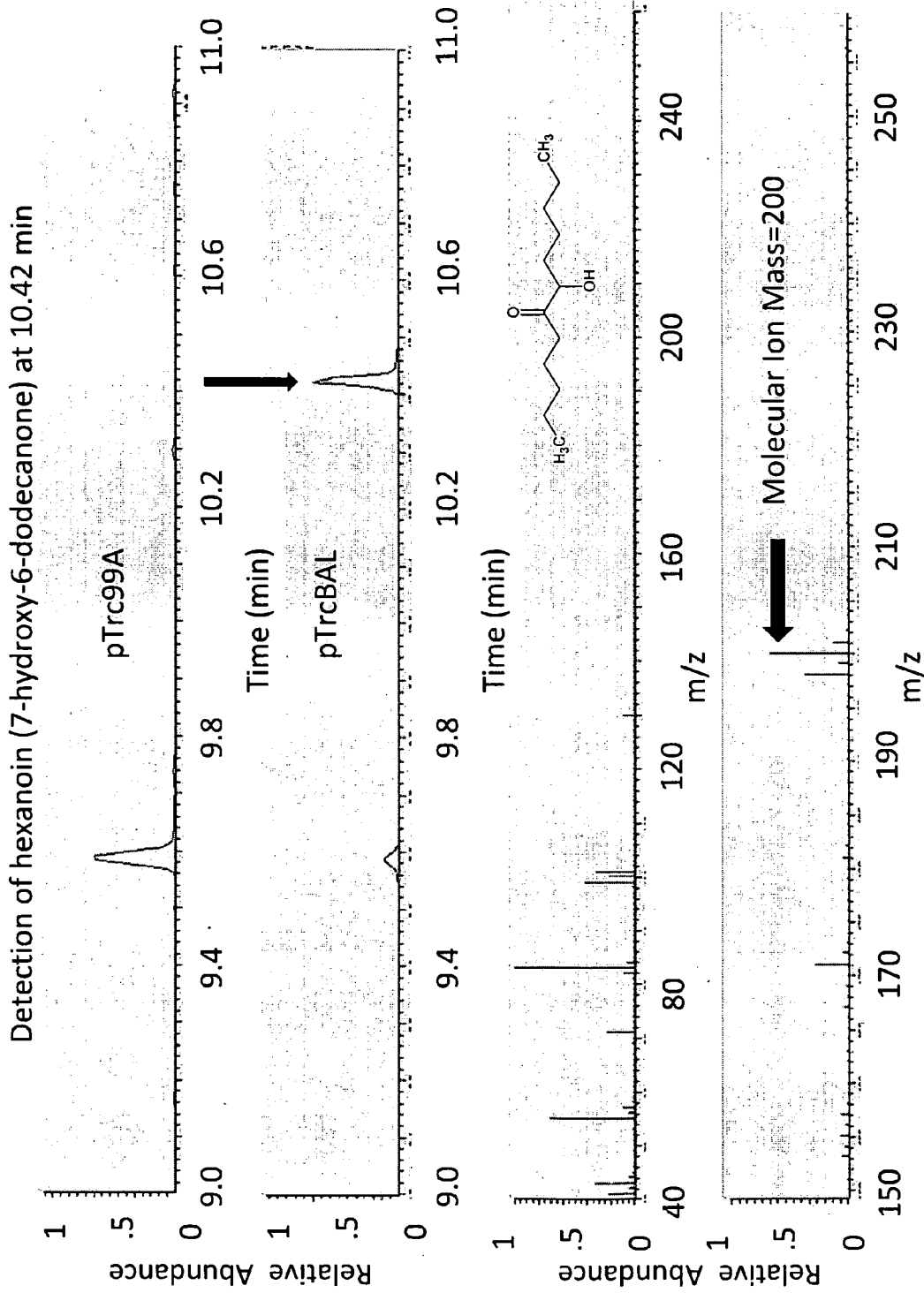


FIG. 20A

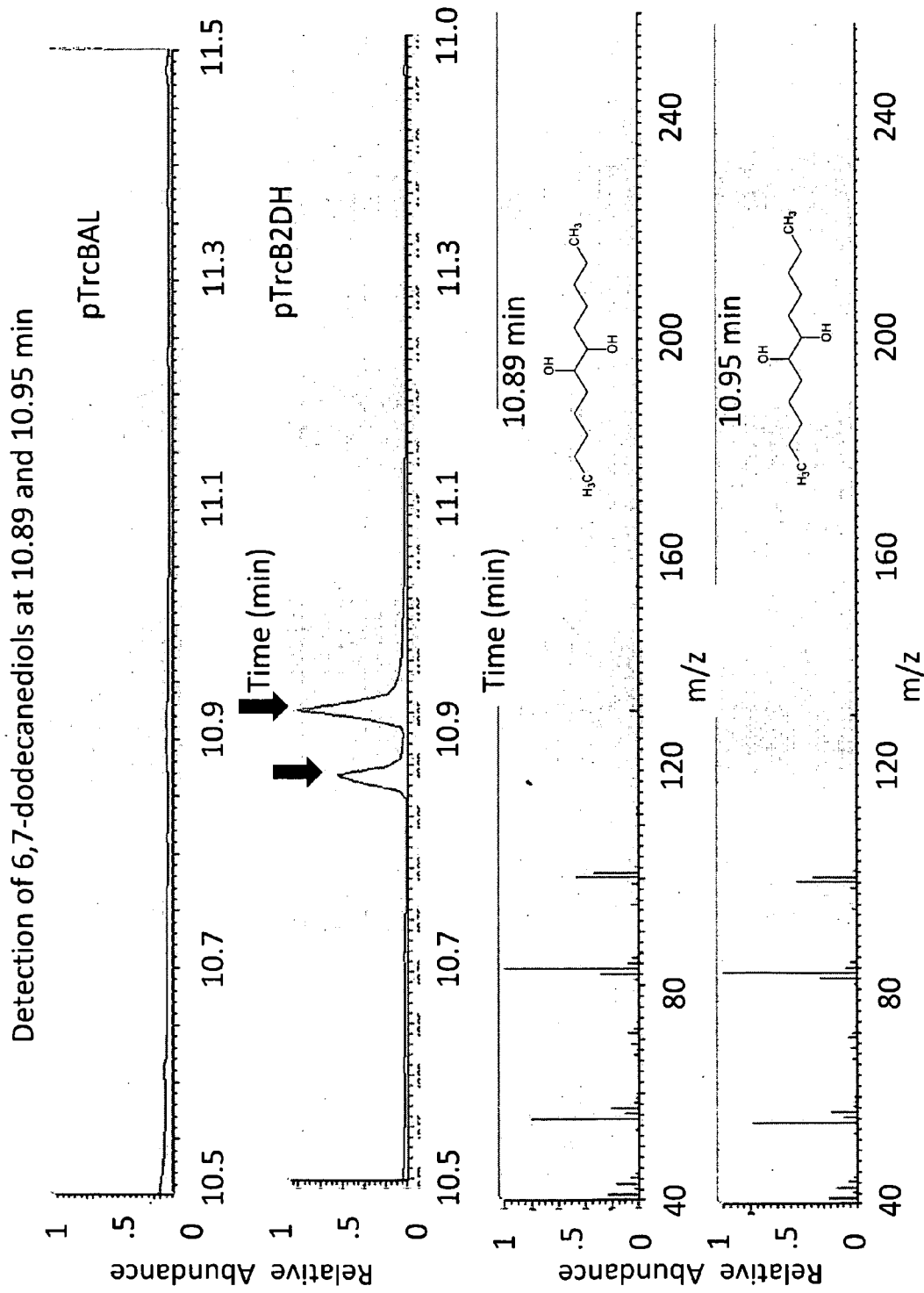


FIG. 20B

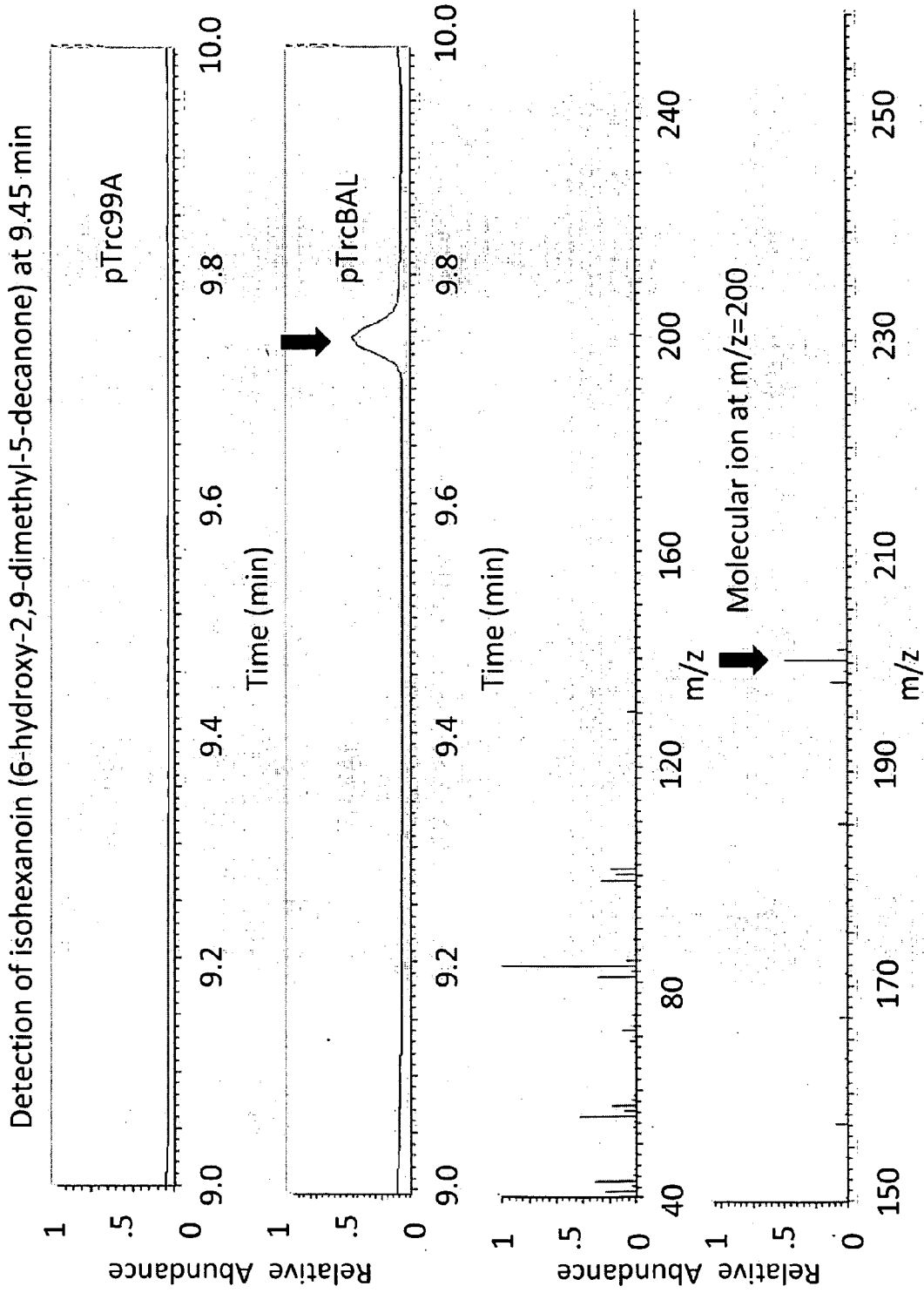


FIG. 21A

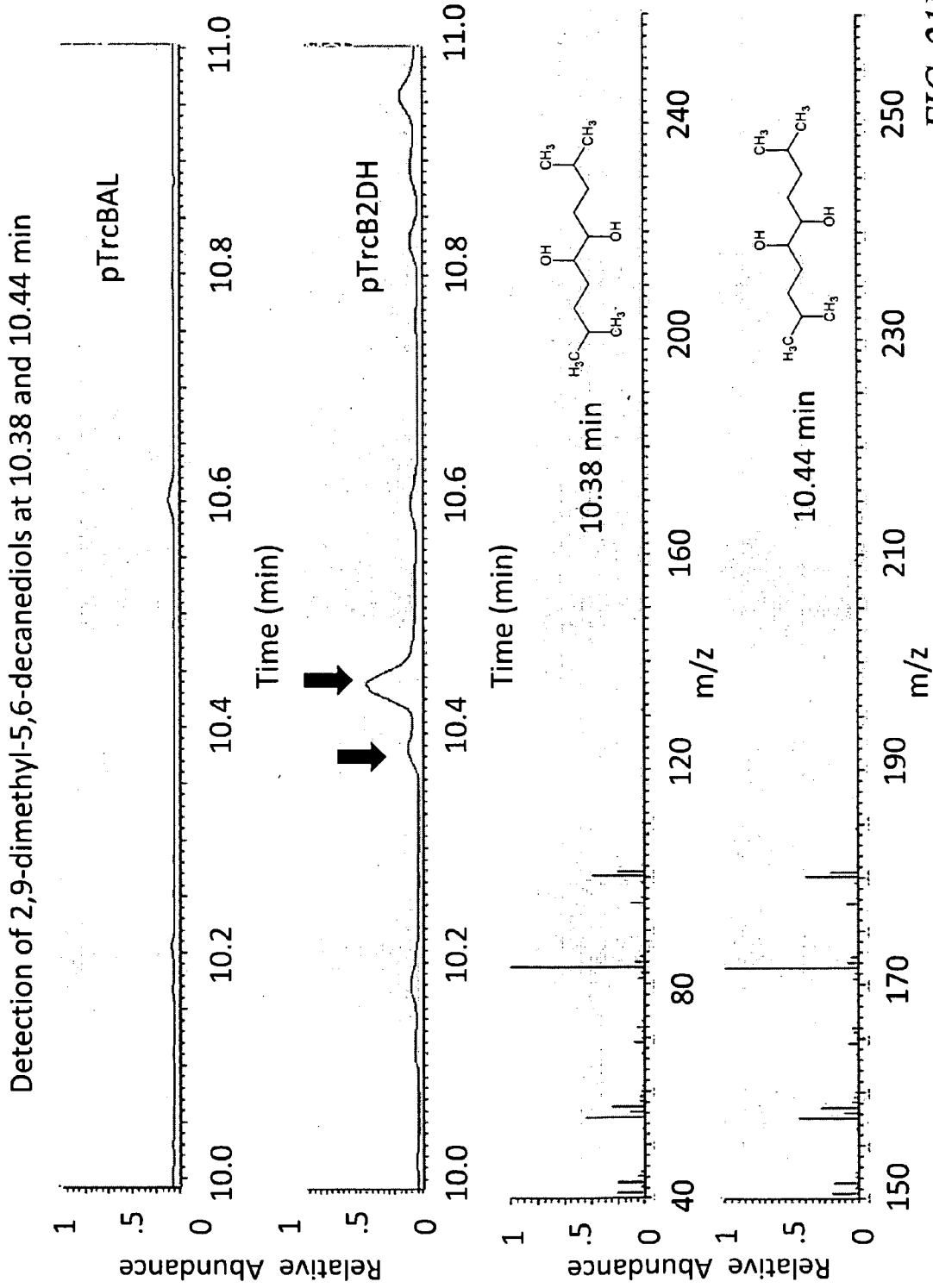


FIG. 21B

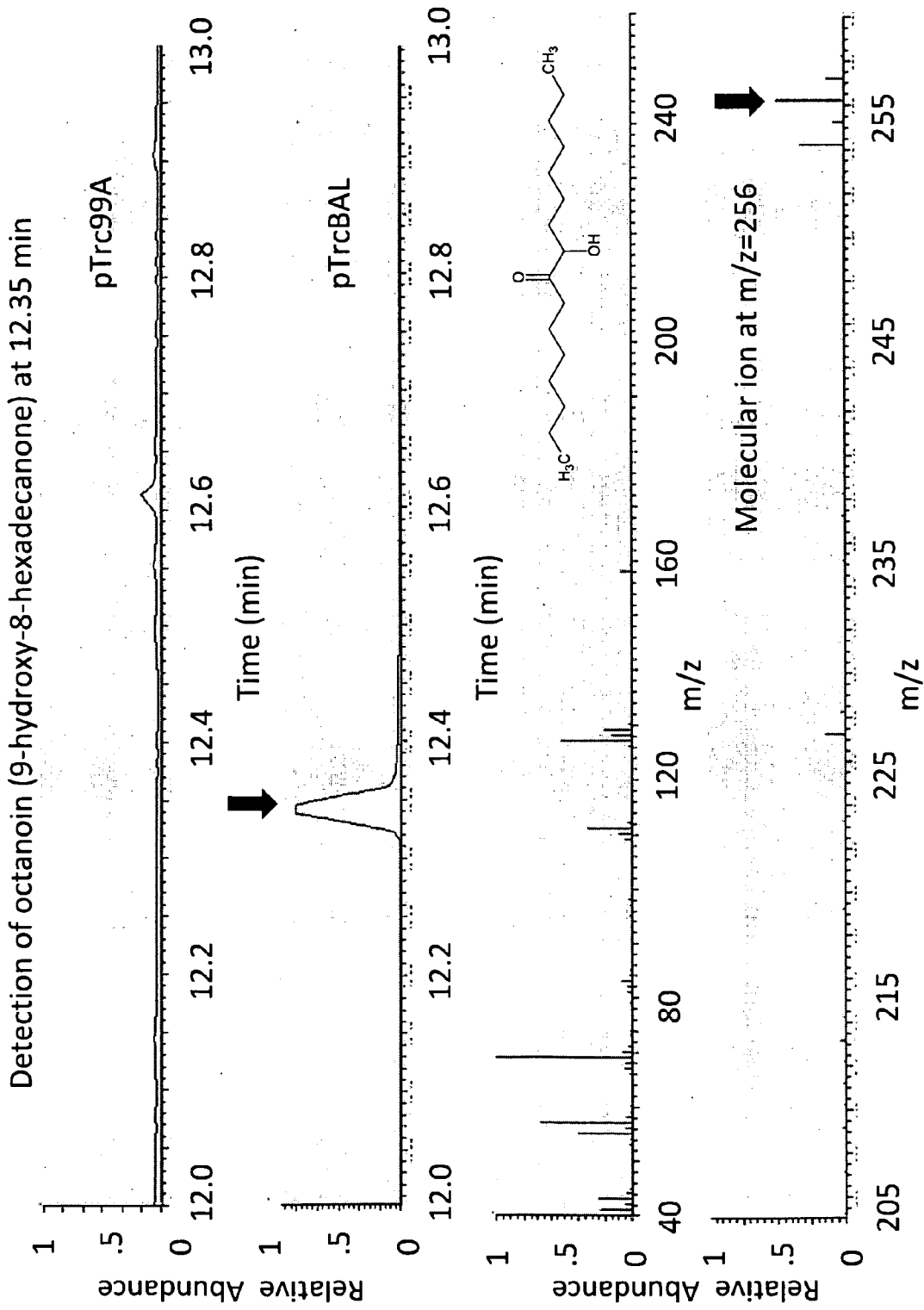


FIG. 22

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

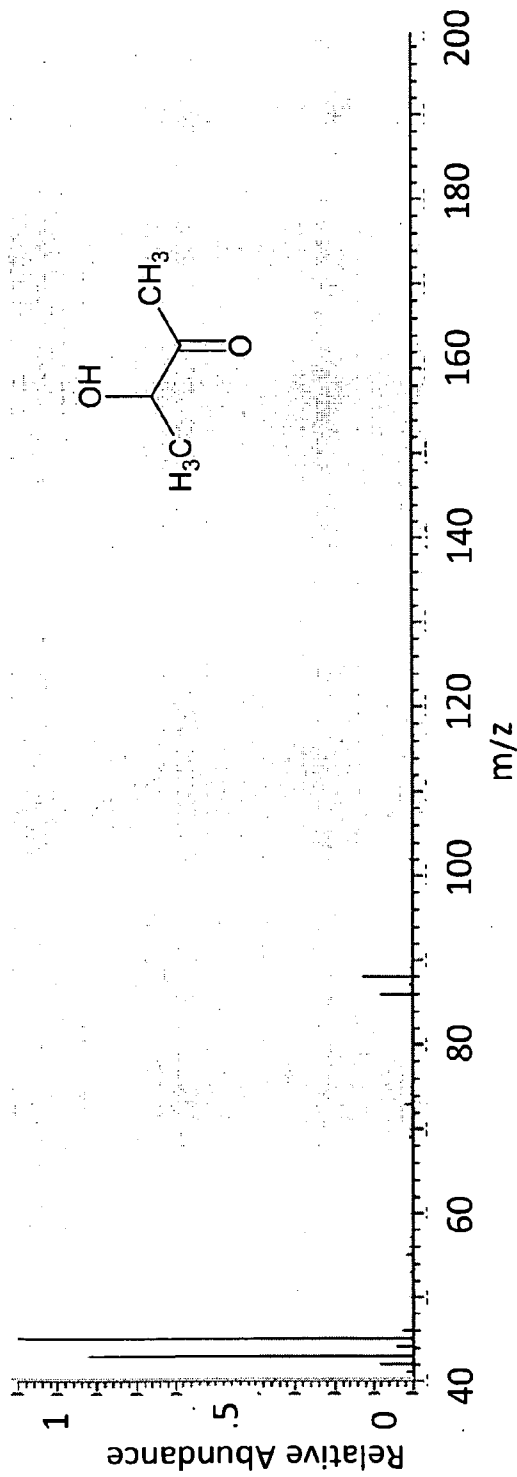
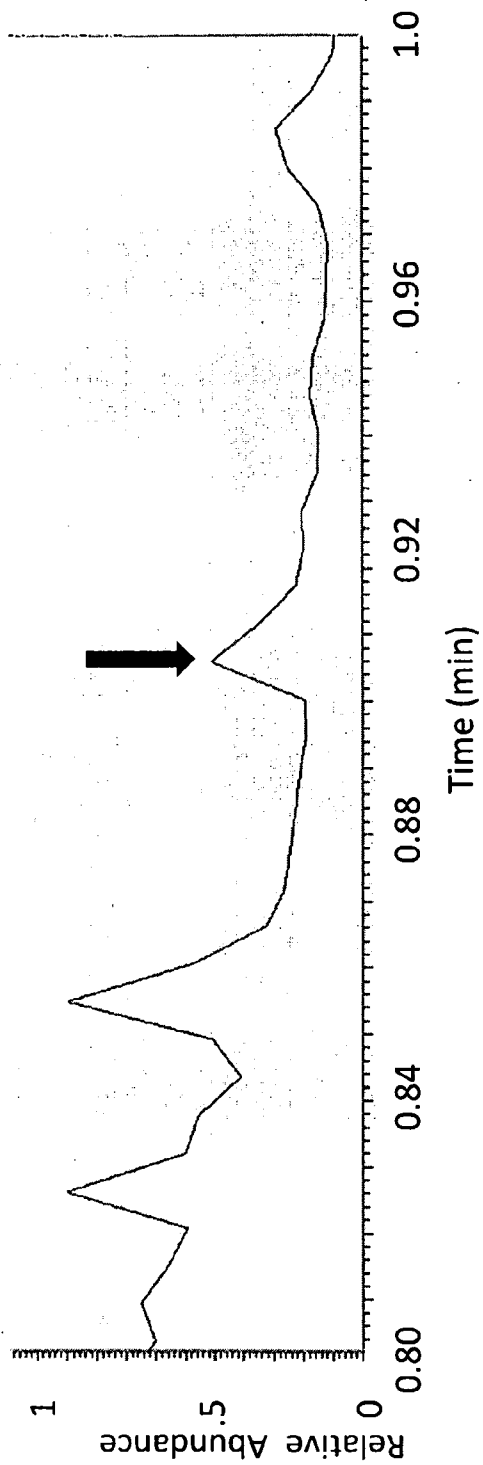
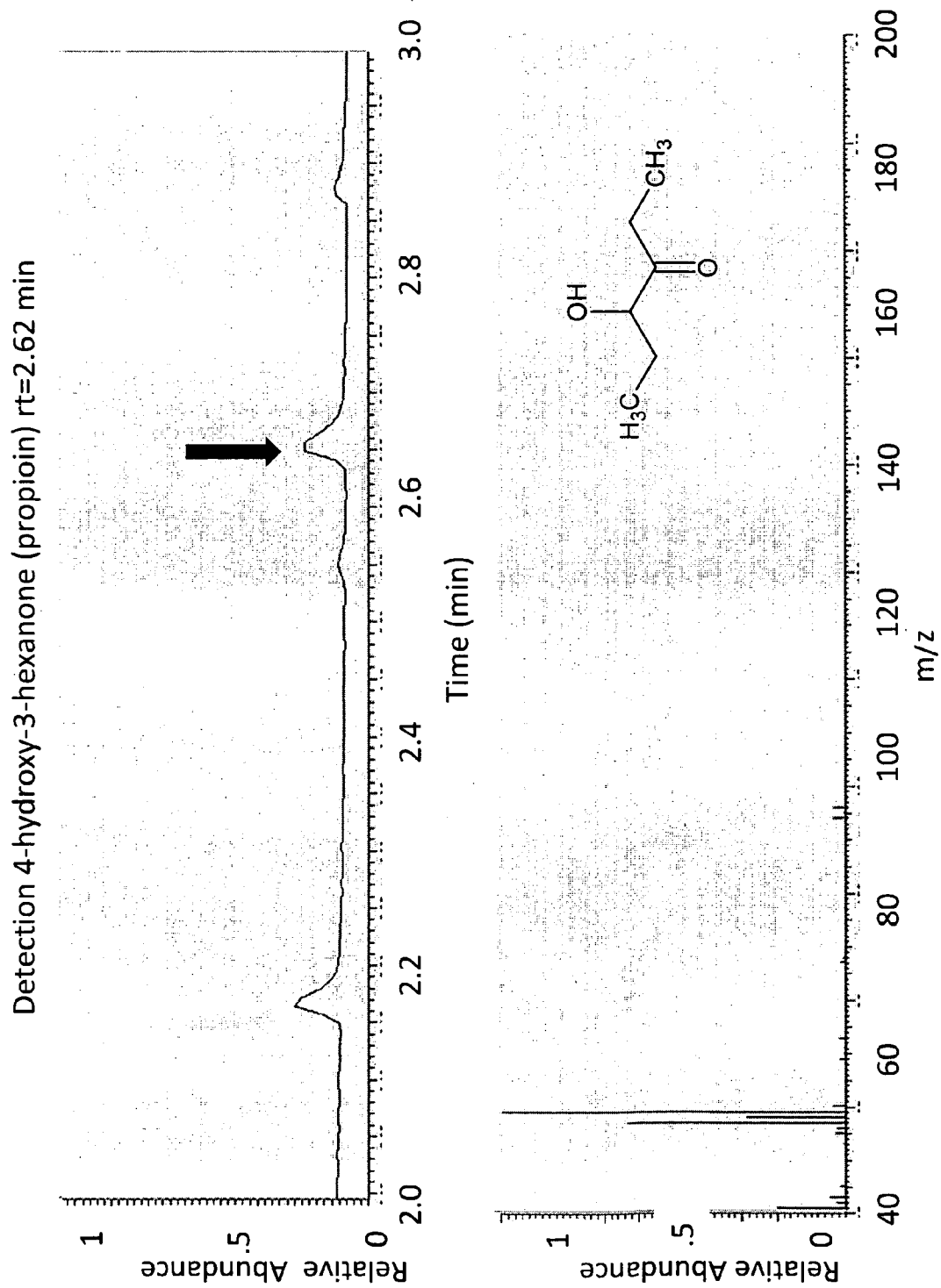


FIG. 23



Detection 3,4-hexanediol rt=3.79 min

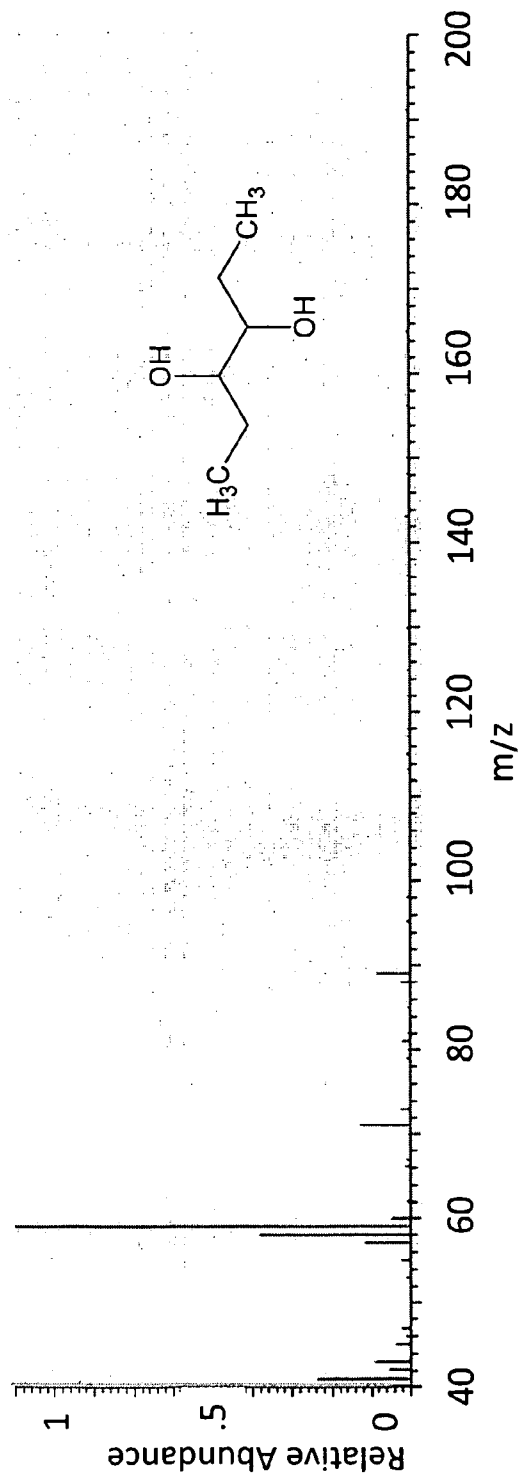
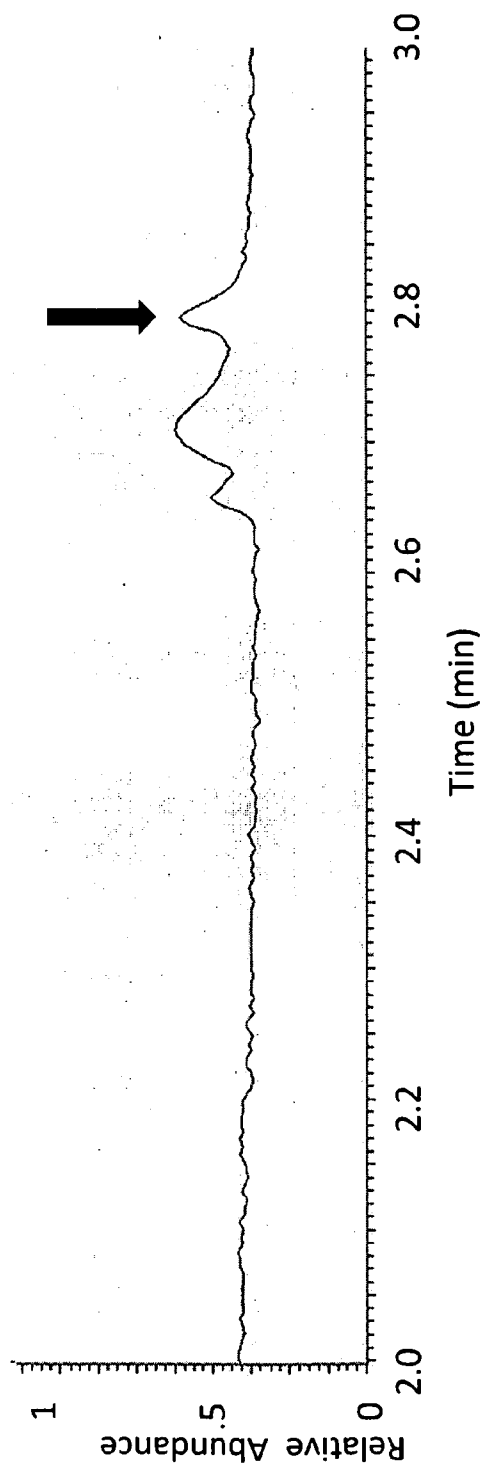


FIG. 24B

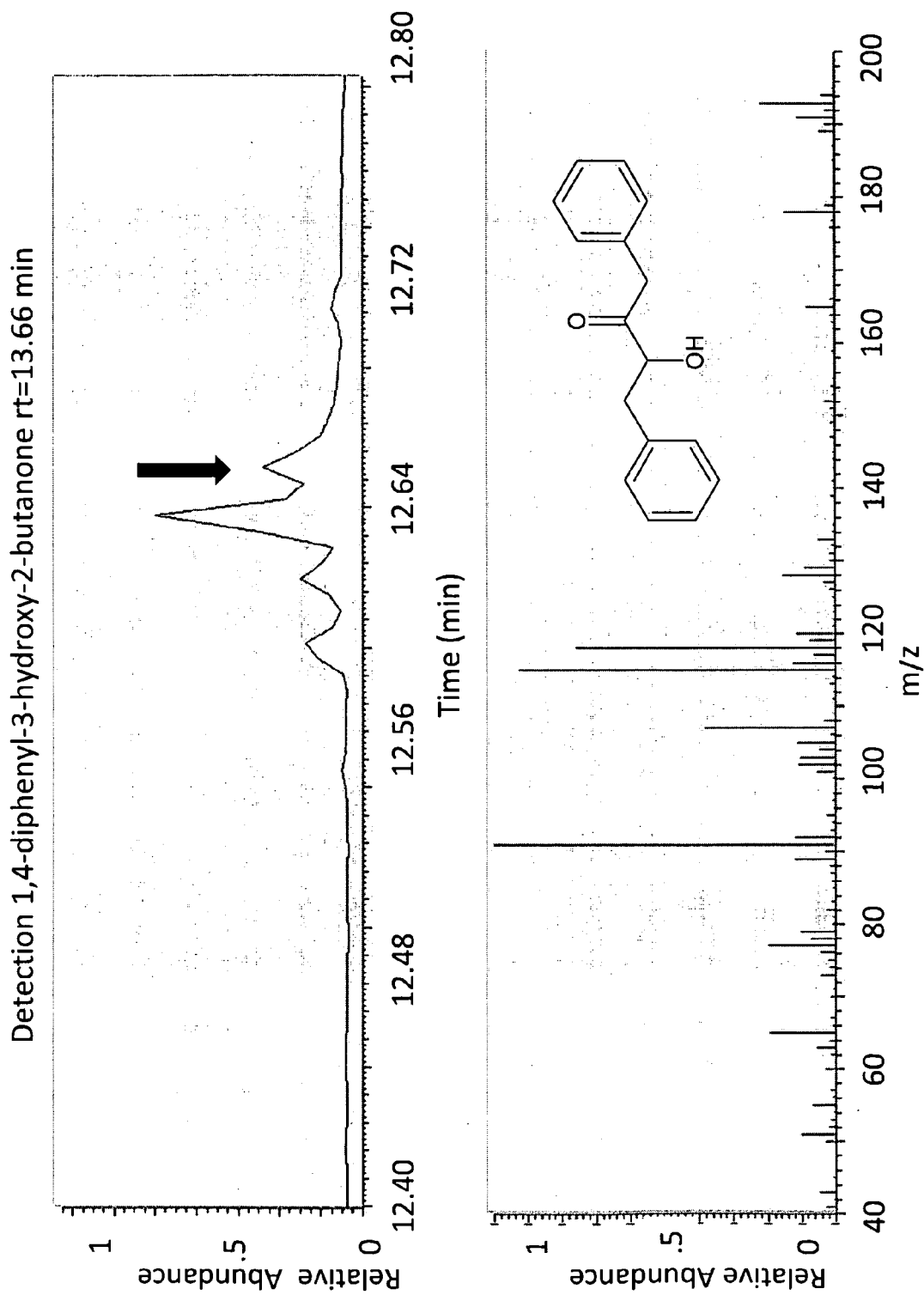


FIG. 25

GCMS data confirming the presence of 4,5-octanediols in the sample extraction

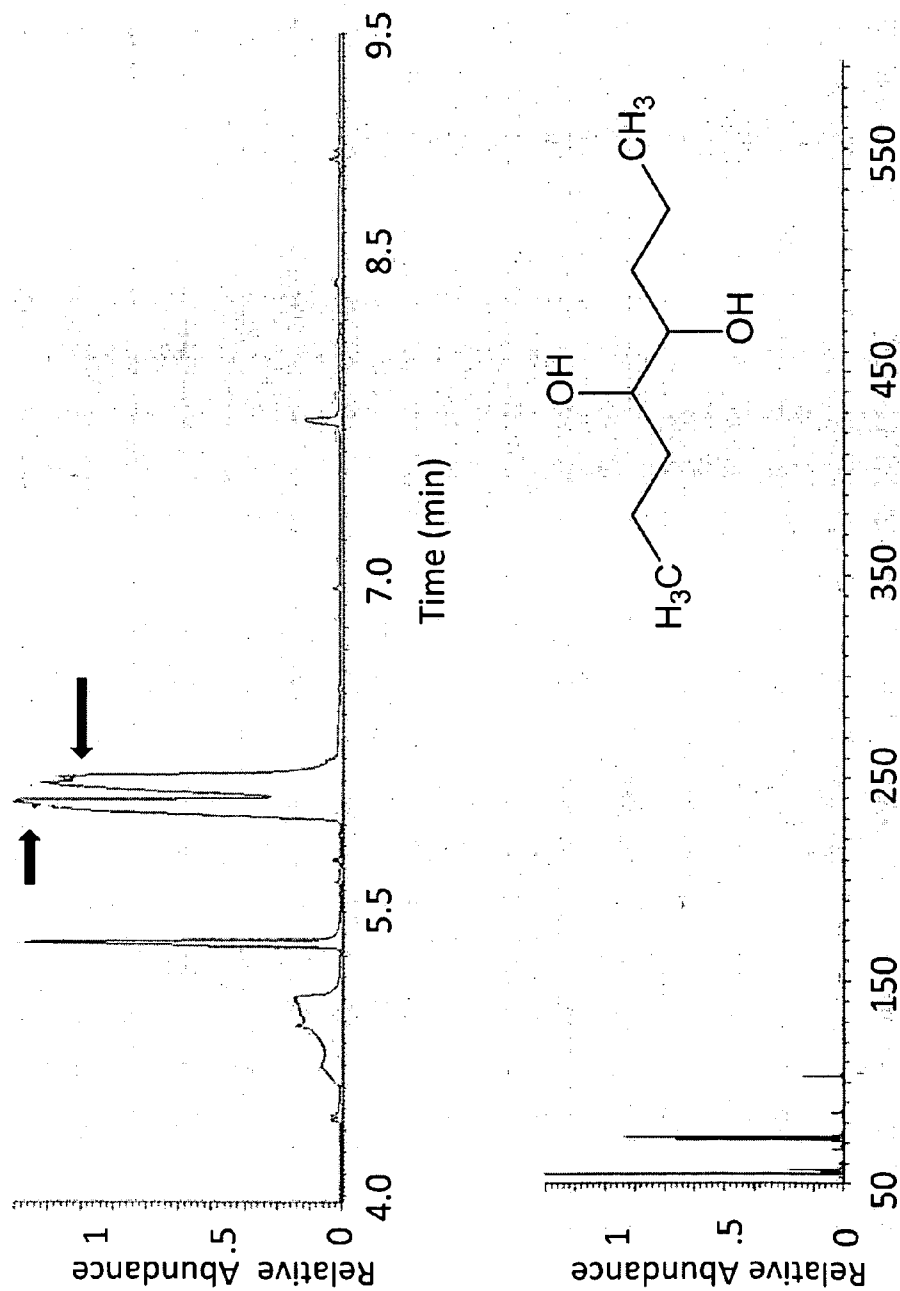


FIG. 26A

GCMS data confirming the presence of 4-octanone in the sample extraction

Sample Solution - Peak at 4.55 identified as 4-octanone

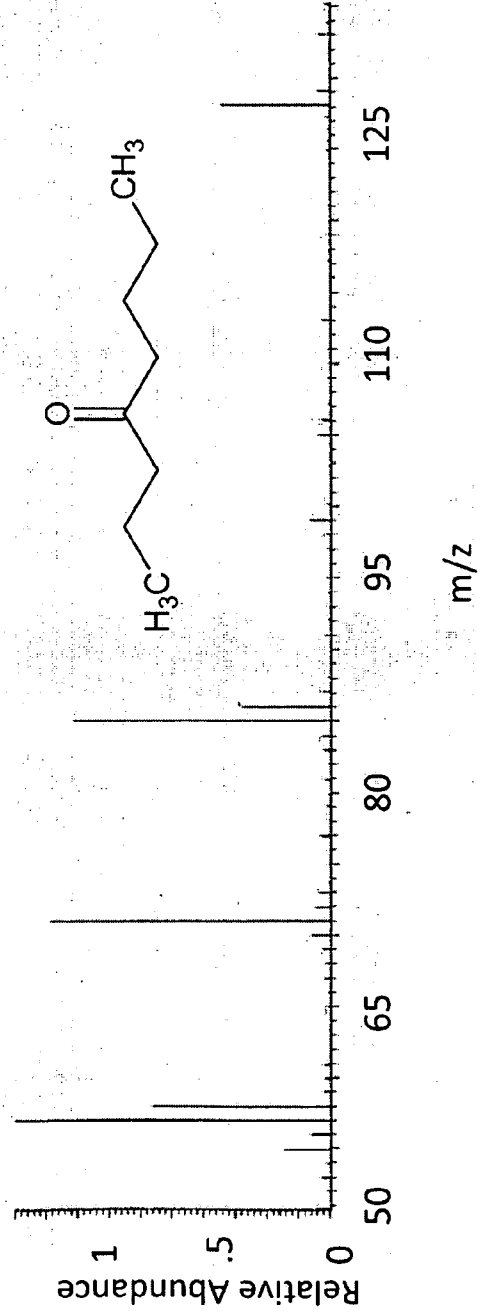
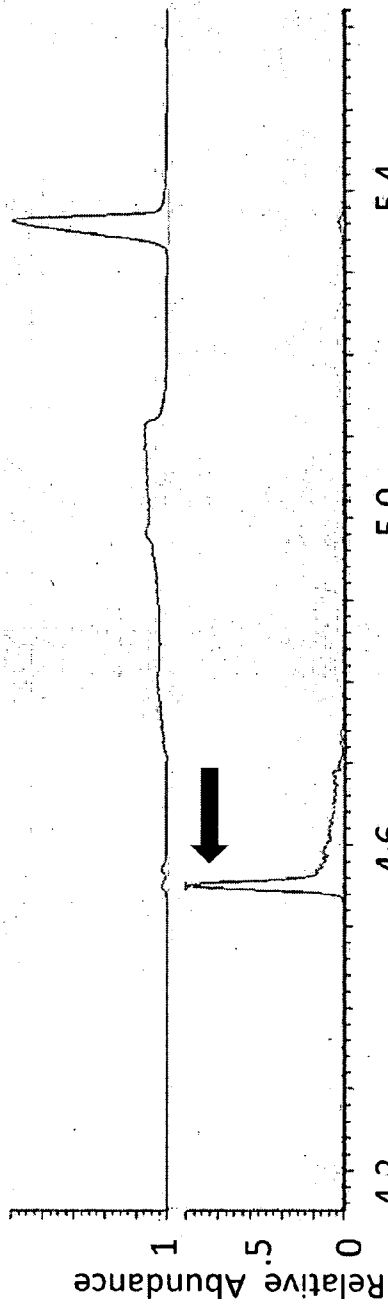


FIG. 26B

Comparison between the sample extraction gas chromatograph (top) and the 4-octanone standard gas chromatograph (bottom)

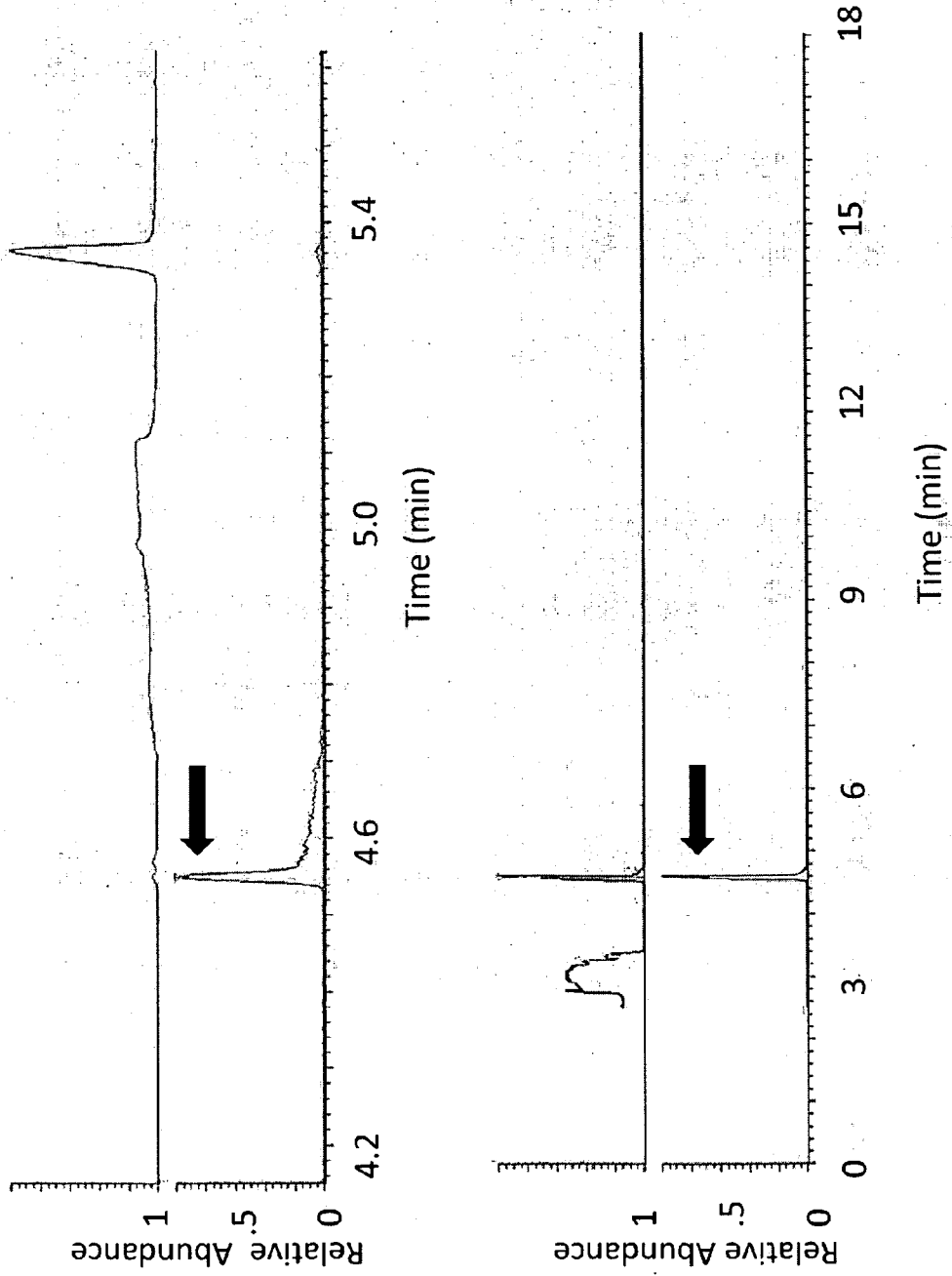


FIG. 27A

Comparison between the sample mass spectrum (top) and the 4-octanone standard mass spectrum (bottom)

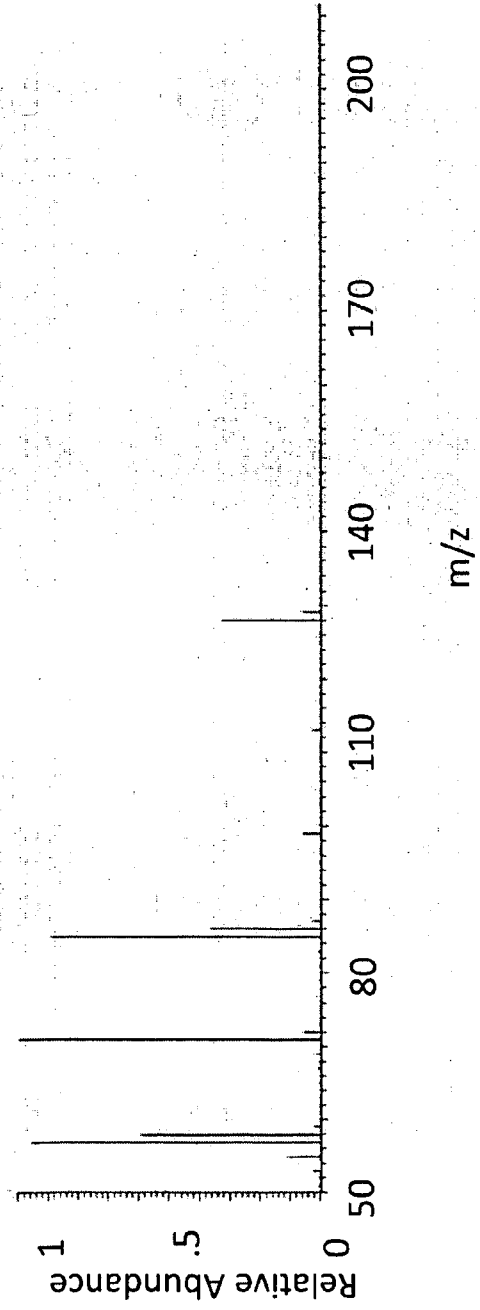
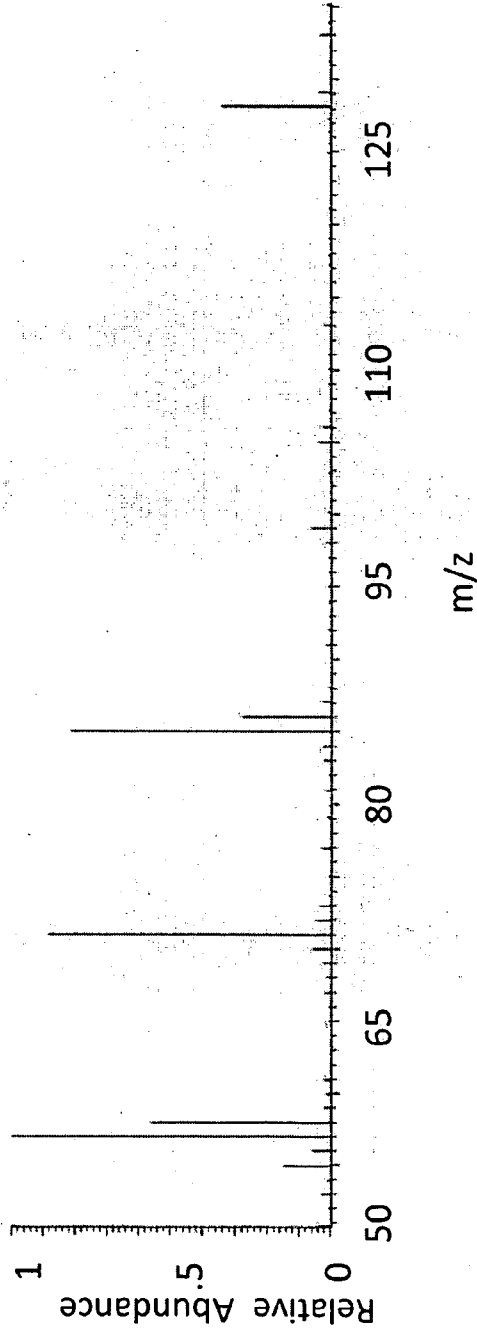


FIG. 27B

Figure 28A

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

ATGAGATCGAAAAGATTTGAAGCACTGGCGAAACGCCCTGTGAATCAGGATGGTTTCGTTAAGGA
GTGGATTGAAGAGGGCTTTATCGCGATGGAAAGCCCTAACGATCCCAAACCTTCTATCCGCATCG
TCAACGGCGCGGTGACCGAACTCGACGATAAACC GGTTGAGCAGTTCGACCTGATTGACCACTTT
ATCGCGCGCTACGGCATTAAATCTCGCCC GGGCCGAAGAAGTGATGGCCATGGATTTCGGTTAAGCT
CGCCAACATGCTCTGCGACCCGAACGTTAAACGCAGCGACATCGTGCCGCTCACTACCGCGATGA
CCCCGGCGAAAATCGTGGAAGTGGTGTGCGATATGAACGTGGTTCGAGATGATGATGGCGATGCAA
AAAATGCGCGCCCCGCCGCACGCCGTCCCAGCAGGCGCATGTCACTAATATCAAAGATAATCCGGT
ACAGATTGCCGCCGACGCCGCTGAAGGCGCATGGCGCGGCTTTGACGAGCAGGAGACCACCGTCG
CCGTGGCGCGCTACGCGCCGTTCAACGCCATCGCCCTGCTGGTTCGGTTCACAGGTTGGCCGCCCC
GGGTCCTCACCCAGTGTTTCGCTGGAAGAAGCCACCGAGCTGAAACTGGGCATGCTGGGCCACAC
CTGCTATGCCGAAACCAATTCGGTATACGGTACGGAACCGGTGTTTACCGATGGCGATGACACCC
CGTGGTTCGAAAGGCTTCCTCGCCTCCTCCTACGCCTCGCGCGGCCTGAAAATGCGCTTTACCTCC
GGTTCGGCTCGGAGGTGCAGATGGGCTATGCCGAAGGCAAATCGATGCTTTATCTCGAAGCGCG
CTGCATCTACATCACCAAAGCCGCCGGGTGCAAGGCCTGCAGAATGGCTCCGTCAGCTGTATCG
GCGTGCCGTCCGCCGTGCCGTCCGGGATCCGCGCCGCTACTGGCGGAAAACCTGATCTGCTCAGCG
CTGGATCTGGAGTGCGCCTCCAGCAACGATCAAACCTTTACCCACTCGGATATGCGGGCGTACCGC
GCGTCTGCTGATGCAGTTCCTGCCAGGTACCGACTTTATCTCCTCCGGTTACTCGGCGGTGCCGA
ACTACGACAACATGTTCCGCCGTTCCAACGAAGATGCCGAAGACTTCGATGACTACAACGTGATC
CAGCGCGACCTGAAGGTCGATGGCGGCCTGCGGCCGGTGCGTGAAGAGGACGTGATCGCCATTTCG
CAACAAAGCCCGCCCGCGCTGCAGGCGGTATTTGCCGGCATGGGTTTGCCGCCATTACGGATG
AAGAAGTAGAAGCCGCCACCTACGCCACGGTTCAAAAGATATGCCTGAGCGCAATATCGTTCGAG
GACATCAAGTTTGCTCAGGAGATCATCAACAAGAACC GCAACGGCCTGGAGGTGGTCAAAGCCCT
GGCGAAAGGCGGCTTCCCCGATGTCGCCAGGACATGCTCAATATTCAGAAAGCCAAGCTCACCG
GCGACTACCTGCATACCTCCGCCATCATGTTGGCGAGGGCCAGGTGCTCTCGGCCGTGAATGAC
GTGAACGATTATGCCGGTCCGGCAACAGGCTACCGCCTGCAAGGCGAGCGCTGGGAAGAGATTAA
AAATATCCCGGGCGCGCTCGATCCCAATGAACTTGCTAA (SEQ ID NO:103)

Figure 28B

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

MRSKRFEALAKRPVNQDGFVKEWIEEGFIAMESPNDPKPSIRIVNGAVTELDKPVQFDLIDHF
IARYGINLARAEEVMAMDSVKLANMLCDPNVKRSDIVPLTTAMTPAKIVEVVSHMNVVEMMMAMQ
KMRARRTPSQAHVTNIKDNPVQIAADAAEGAWRGFDEQETTVAVARYAPFNAIALLVGSQVGRP
GVLTCQSLEEATELKLGLMLGHTCYAETISVYGTEPVFTDGD DTPWSKGF LASSYASRGLKMRFTS
GSGSEVQMGYAEGKSMLYLEARCIYITKAAGVQGLQNGSVSCIGVPSAVPSGIRAVLAENLICSA
LDLECASSNDQTFTHSDMRRTARLLMQFLPGTDFI SSGYSAVPNYDNMFAGSNEDAEDFDDYNVI
QRDLKVDGGLRPVREEDVIAIRNKAARALQAVFAGMGLPPI TDEEVEAATYAHGSKDMPERNIVE
DIKFAQEIINKNRNGLEVVKALAKGGFPDVAQDMLNIQKAKLTGDYLHTSAIIVGEGQVLSAVND
VNDYAGPATGYRLQGERWEEIKNIPGALDPNELG (SEQ ID NO:104)

Figure 29A

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

ATGGAATTAACGAAACGCTGCTGCGCCAGATTATCGAAGAGGTGCTGTCGGAGATGAAATCAGG
CGCAGATAAGCCGGTCTCCTTTAGCGCGCCTGCGGCTTCTGTGCGCTCTGCCGCGCCGGTCGCCG
TTGCGCCTGTGTCCGGCGACAGCTTCCTGACGGAAATCGGGCAAGCCAAACCCGGCACGCAGCAG
GATGAAGTCATTATTGCCGTCCGGCCAGCGTTTGGTCTGGCGCAAACCGCCAATATCGTCGGCAT
TCCGCATAAAAAATATTCTGCGCGAAGTGATCGCCGGCATTGAGGAAGAAGGCATCAAAGCCCGGG
TGATCCGCTGCTTTAAGTCTTCTGACGTGCGCTTCGTGGCAGTGGAAGGCAACCGCCTGAGCGGC
TCCGGCATCTCGATCGGTATTCAGTCGAAAGGCACCACCGTCATCCACCAGCGCGGCCCTGCCGCC
GCTTCCAATCTGGAACCTTCCCGCAGGCGCCGCTGCTGACGCTGGAAACCTACCGTCAGATTG
GCAAAAACGCCGCGCTACGCCAAACGCGAGTCGCCGACCCGGTGCCGACGCTTAACGATCAG
ATGGCTCGTCCCAAATACCAGGCGAAGTCGGCCATTTTGCACATTAAGAGACCAAATACGTGGT
GACGGGCAAAAACCCGCAGGAAGTGCAGCGTGGCGCTTTAA (SEQ ID NO:105)

Figure 29B

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

MEINETLLRQIIIEEVLSEMKSADKPVSFSAAPAASVASAAPVAVAPVSGDSFLTEIGEAKPGTQQ
DEVI IAVGPAFGLAQTANIVGIPHKNILREVIAGIEEEGIKARVIRCFKSSDVAFFVAVEGNRLSG
SGISIGIQSKGTTVIHQRLPPLSNLELFPQAPLLTLETYRQIGKNAARYAKRESPQPVPTLNDQ
MARPKYQAKSAILHIKETKYVVTGKNPQELRVAL (SEQ ID NO:106)

Figure 29C

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

ATGAATACCGACGCAATTGAATCCATGGTACGCGACGTGCTGAGCCGGATGAACAGCCTACAGGA
CGGGATAACGCCCGCGCCAGCCGCGCCGACAAACGACACCGTTCCGCCAGCCAAAAGTTAGCGACT
ACCCGTTAGCGACCCGCCATCCGGAGTGGGTCAAACCGCTACCAATAAACGCTCGATGACCTG
ACGCTGGAGAACGTATTAAGCGATCGCGTTACGGCGCAGGACATGCGCATCACTCCGGAAACGCT
GCGTATGCAGGCGGCGATCGCCCAGGATGCCGGACGCGATCGGCTGGCGATGAACTTTGAGCGGG
CCGCAGAGCTCACCGCGGTTCCCGACGACCGAATCCTTGAGATCTACAACGCCCTGCGCCCATAC
CGTTCACCCAGGCGGAGCTACTGGCGATCGCTGATGACCTCGAGCATCGCTACCAGGCACGACT
CTGTGCCGCTTTGTTCCGGGAAGCGCGCGGGCTGTACATCGAGCGTAAGAAGCTGAAAGCGGACG
ATTAA (SEQ ID NO:107)

Figure 29D

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

MNTDAIESMVRDVL SRMNSLQDGI TPAPAAPTNDTVRQPKVSDYPLATRHPEWVK TATNKTLDL
TLENVLSDRVTAQDMRITPETLRMQAAIAQDAGRDR LAMNFERAAELTAVPDDRILEIYNALRPY
RSTQAE LLA IADDLEHRYQARLCAAFVREAAGLYIERK KLGDD (SEQ ID NO:108)

Figure 32A

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

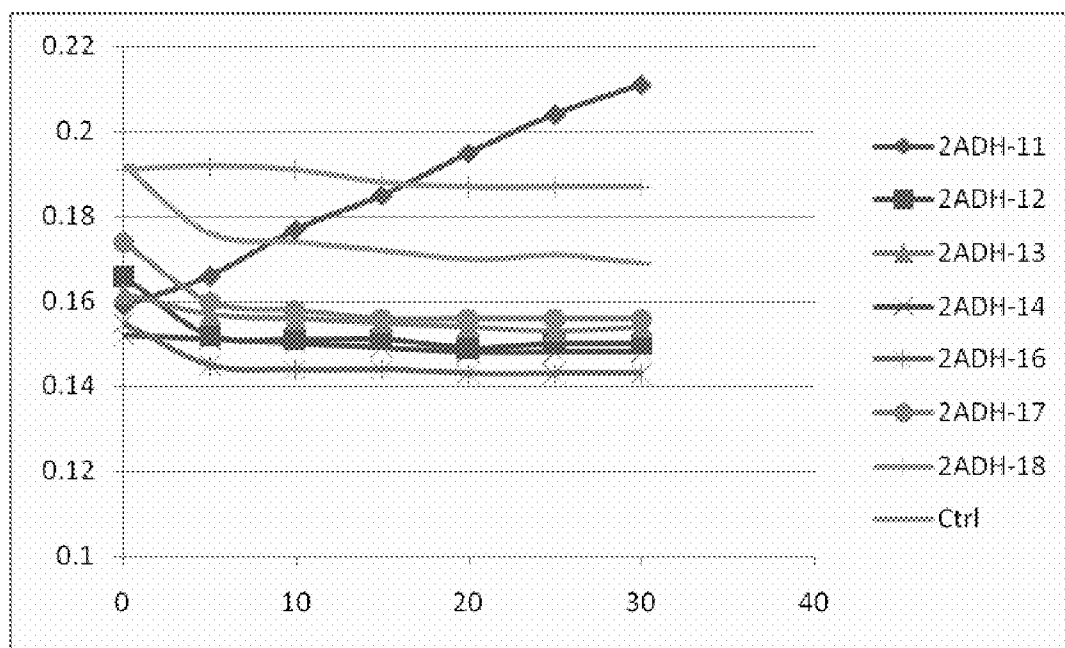


Figure 32B

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

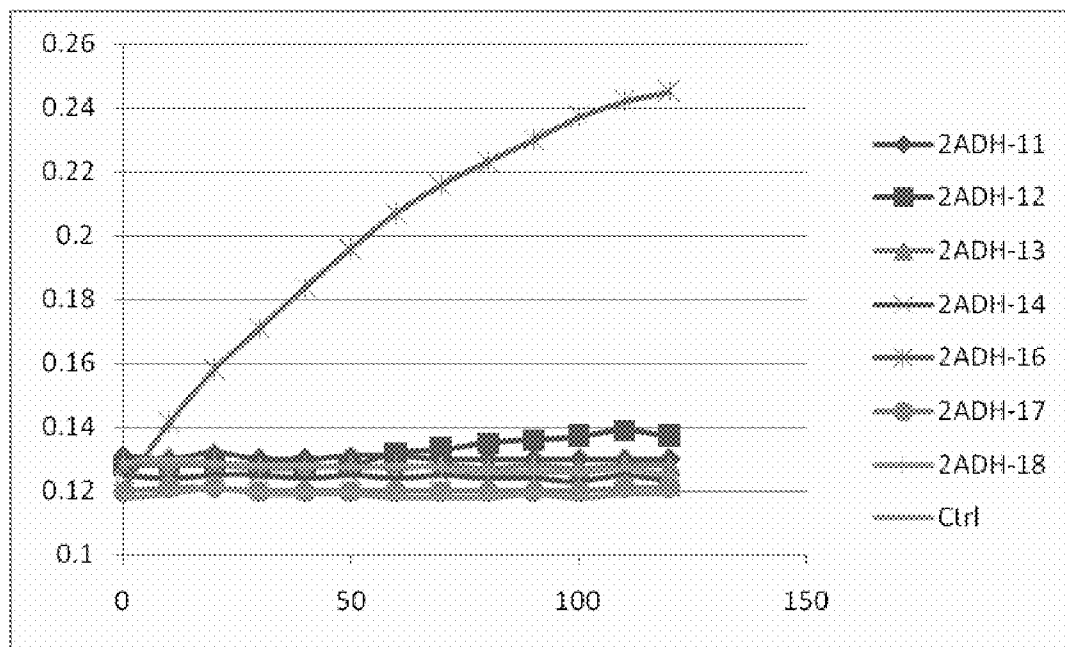


Figure 33A
Reduction of of 2,7-dimethyl octanol monitored by NADPH consumption

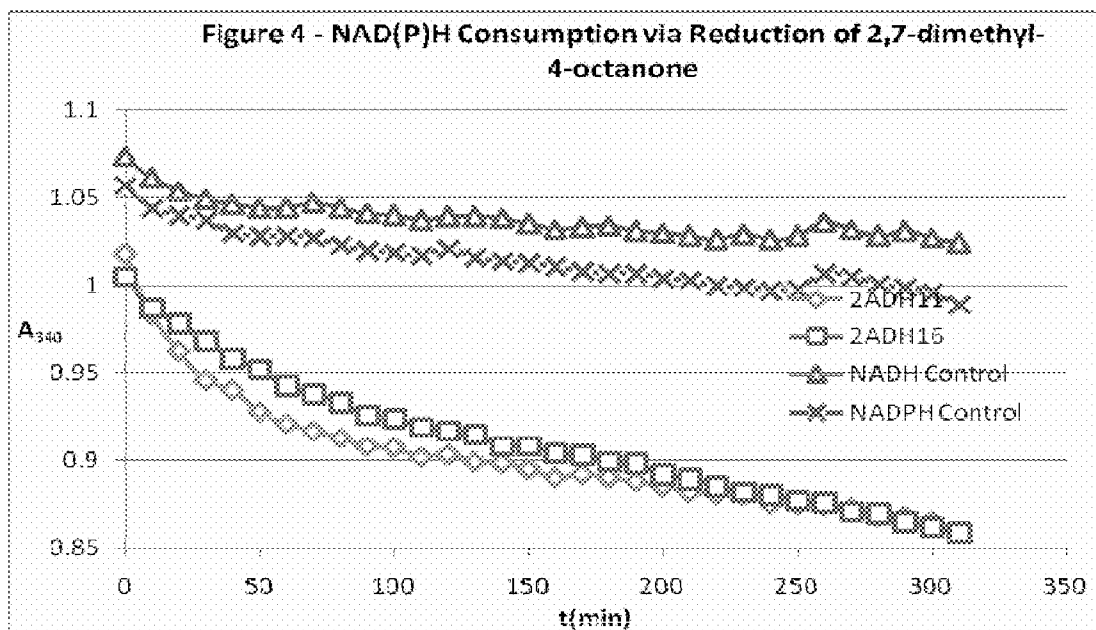


Figure 33B
Activity of 2ADH11 and 2ADH16 Towards Various Substrates

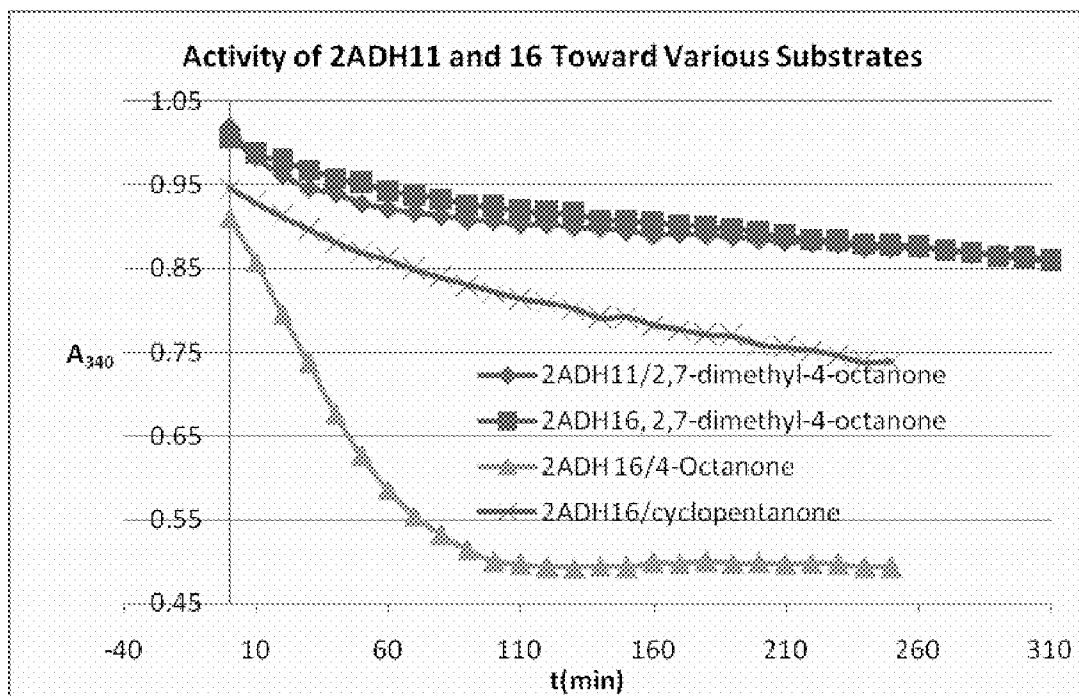


Figure 34A

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

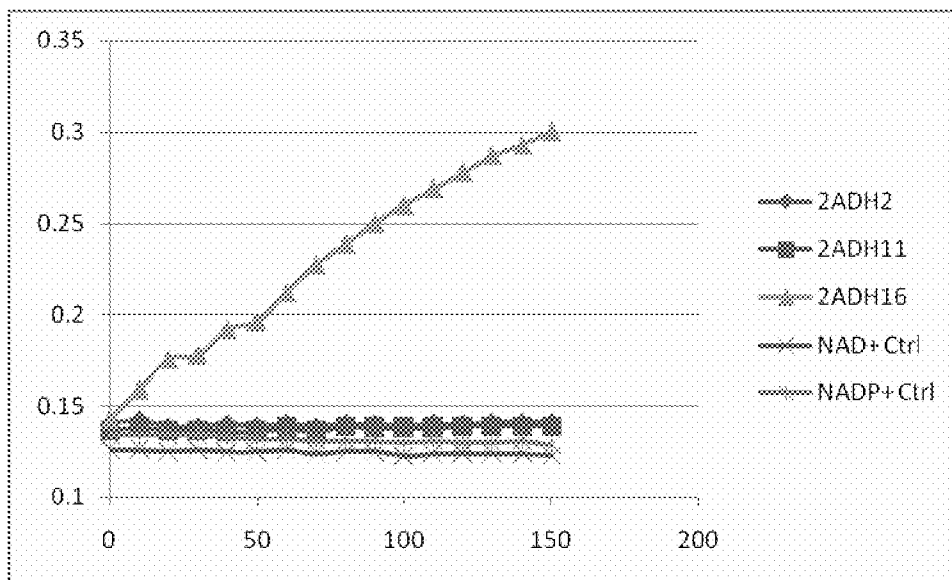
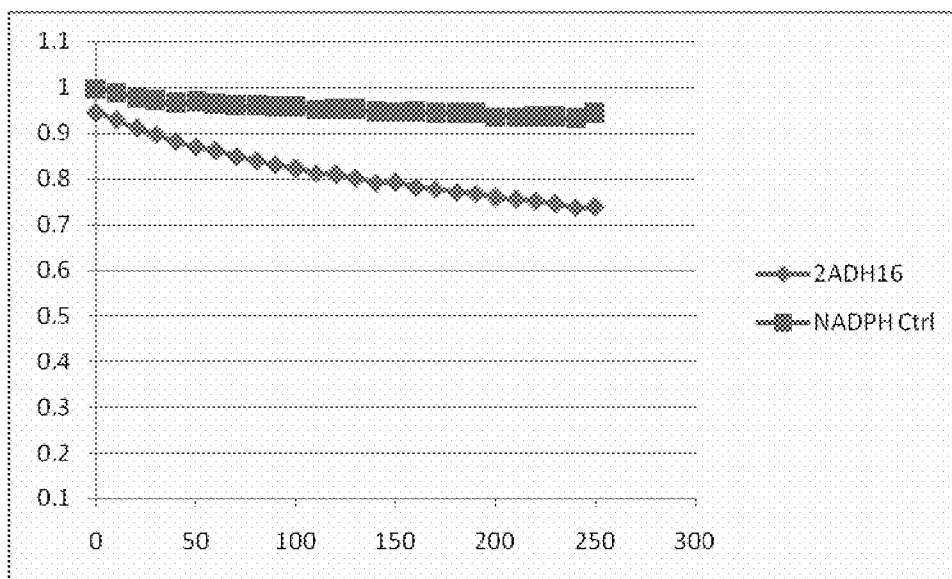


Figure 34B

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



Calculated Rate Constants for Reduction Reactions of 2ADH16

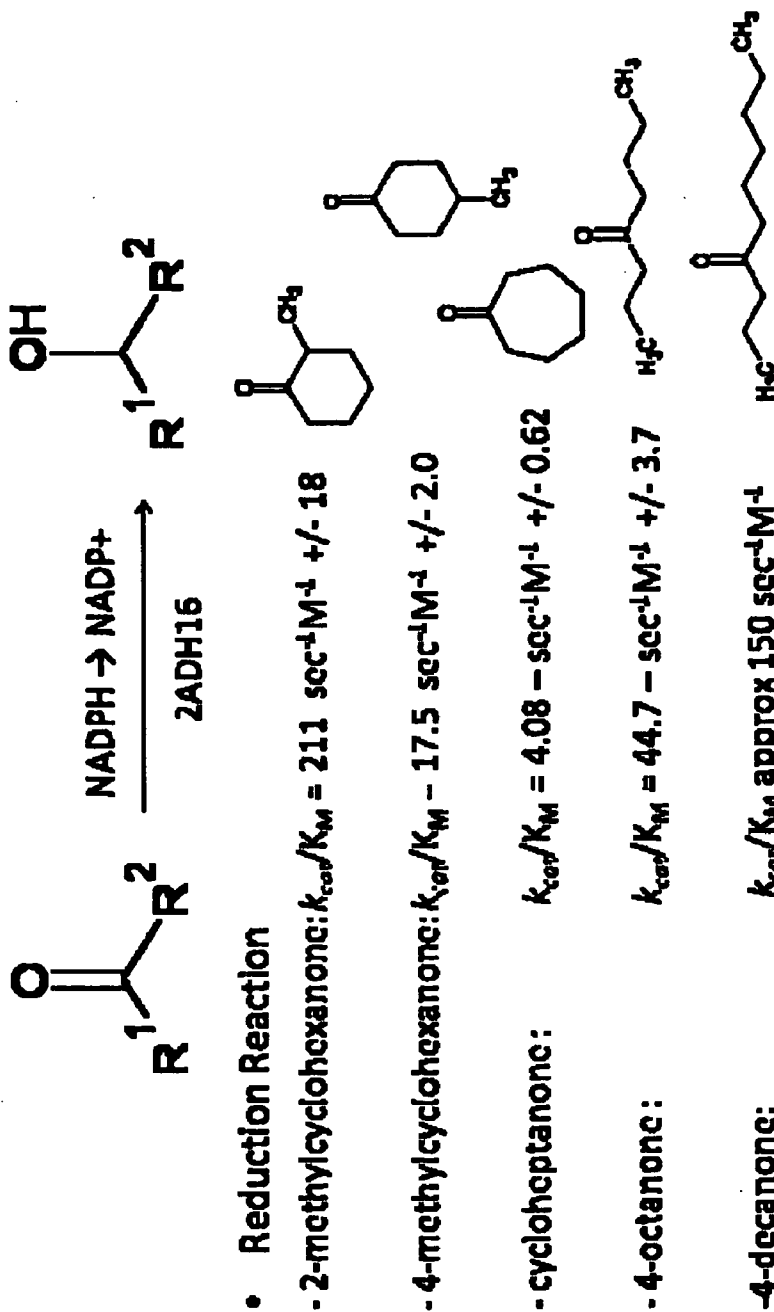
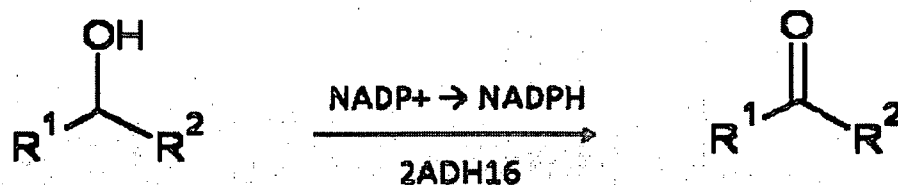


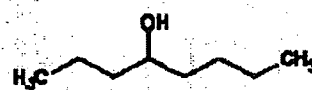
FIG. 35

Calculated Rate Constants for Oxidation Reactions of 2ADH16

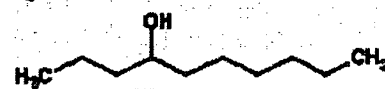


• Oxidation Reaction

- 4-octanol: $k_{\text{cat}}/K_M = 1430 \text{ sec}^{-1}\text{M}^{-1} \pm 200$



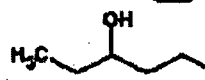
- 4-decanol: $k_{\text{cat}}/K_M = 1260 \text{ sec}^{-1}\text{M}^{-1} \pm 290$



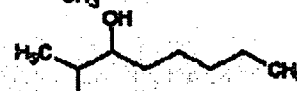
- cycloheptanol: $k_{\text{cat}}/K_M = 198 \text{ sec}^{-1}\text{M}^{-1} \pm 19$



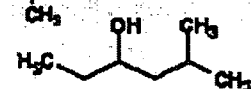
- 3-hexanol: $k_{\text{cat}}/K_M = 148 \text{ sec}^{-1}\text{M}^{-1} \pm 16$



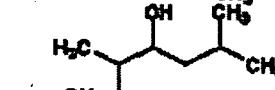
- 2-methyl-3-octanol: $k_{\text{cat}}/K_M = 123 \text{ sec}^{-1}\text{M}^{-1} \pm 16$



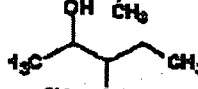
- 5-methyl-3-hexanol: $k_{\text{cat}}/K_M = 17.9 \text{ sec}^{-1}\text{M}^{-1} \pm 3.5$



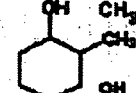
- 2,5-dimethyl-3-hexanol: $k_{\text{cat}}/K_M = 1.55 \text{ sec}^{-1}\text{M}^{-1} \pm .24$



- 3-methyl-2-pentanol: $k_{\text{cat}}/K_M = 8.54 \text{ sec}^{-1}\text{M}^{-1} \pm 2.04$



- 2-methylcyclohexanol: $k_{\text{cat}}/K_M = 97.4 \text{ sec}^{-1}\text{M}^{-1} \pm 14.4$



- 4-methylcyclohexanol: $k_{\text{cat}}/K_M = 10.4 \text{ sec}^{-1}\text{M}^{-1} \pm 21.1$

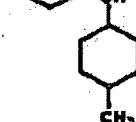


FIG. 36

Figure 37A
Alginate Lyases

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

Figure 37B
Alginate Lyases

Protein	Organism	GenBank	GenPept
Family 7			
poly(α -L-guluronate) lyase (AlyVGI;AlyVG1)	<u>Vibrio halioticoli IAM14596T</u>	<u>AF114039</u>	<u>AAF22512.1</u>
alginate lyase / poly-mannuronate lyase (AlyVOA)	<u>Vibrio sp. O2</u>	<u>DQ235160</u>	<u>ABB36771.1</u>
alginate lyase / poly-mannuronate lyase (AlyVOB)	<u>Vibrio sp. O2</u>	<u>DQ235161</u>	<u>ABB36772.1</u>
alginate lyase (AlyVI)	<u>Vibrio sp. QY101</u>	<u>AY221030</u>	<u>AAP45155.1</u>
exo-oligoalginate lyase (HdAlex;HdAlex-1)	<u>Haliotis discus hannai</u>	<u>AB234872</u>	<u>BAE81787.1</u>
alginate lyase (HdAly)	<u>Haliotis discus hannai</u>	<u>AB110094</u>	<u>BAC87758.1</u>
polysaccharide lyase acting on glucuronic acid (vAL-1)	<u>Chlorella virus CVK2</u>	<u>AB044791</u>	<u>BAB19127.1</u>
alginate lyase (AlyII)	<u>Pseudomonas sp. OS-ALG-9</u>	<u>AB003330</u>	<u>BAA19848.1</u>
Family18			
alginate lyase	<u>Pseudoalteromonas sp. 272</u>		
alginate lyase (Aly)	<u>Pseudoalteromonas sp. IAM14594</u>	<u>AF082561</u>	<u>AAD16034.1</u>
Family15			
exotype alginate lyase (Atu3025)	<u>Agrobacterium tumefaciens str. C58</u>	<u>AE009232</u>	<u>AAL43841.1</u>
		<u>NC 003305</u>	<u>NP 533525.1</u>
exotype alginate lyase (AGR_L_3558p)	<u>Agrobacterium tumefaciens str. C58 (Cereon)</u>	<u>AE008381</u>	<u>AAK90358.1</u>
		<u>NC 003063</u>	<u>NP 357573.1</u>
oligo alginate lyase (A1-IV)	<u>Sphingomonas sp. A1</u>	<u>AB011415</u>	<u>BAB03319.1</u>
alginate lyase (A1-IV')	<u>Sphingomonas sp. A1</u>	<u>AB176667</u>	<u>BAD90006.1</u>

Figure 38A
Pectate Lyases

Protein	Organism	GenBank/GenPept	
Family1			
pectate lyase	<u>Bacillus agaradhaerens</u>	-	<u>AAE59745.1</u>
		-	<u>AAS29292.1</u>
pectate lyase	<u>Bacillus halodurans</u>	-	<u>AAE59748.1</u>
		-	<u>AAS29295.1</u>
pectate lyase (BH3819)	<u>Bacillus halodurans C-125</u>	<u>AP001520</u>	<u>BAB07538.1</u>
		<u>NC 002570</u>	<u>NP 244686.1</u>
pectate lyase (BH0698)	<u>Bacillus halodurans C-125</u>	<u>AP001509</u>	<u>BAB04417.1</u>
		<u>NC 002570</u>	<u>NP 241564.1</u>
pectate lyase (PelA)	<u>Bacillus licheniformis 14A</u>	-	<u>AAE59746.1</u>
		-	<u>AAN26179.1</u>
		<u>AJ517194</u>	<u>CAD56882.1</u>
		-	<u>AAS29293.1</u>
BLi04129 or BL00947 (PelII)	<u>Bacillus licheniformis DSM 13 ATCC 14580</u>	<u>CP000002</u>	<u>AAU25568.1</u>
		<u>AE017333</u>	<u>AAU42942.1</u>
pectate lyase (Pel)	<u>Bacillus licheniformis RN1</u>	<u>AB428424</u>	<u>BAG12908.1</u>
pectate lyase (PelB)	<u>Bacillus pumilus DKS1</u>	<u>EU652988</u>	<u>ACD11362.1</u>
pectate lyase (fragment)	<u>Bacillus sp. KSM-P7</u>	<u>AB015043</u>	<u>BAA76884.1</u>
pectate lyase	<u>Bacillus sp. AAI12</u>	-	<u>AAE59747.1</u>
		-	<u>AAS29294.1</u>
pectate lyase	<u>Bacillus sp. I534</u>	-	<u>AAE59749.1</u>
		-	<u>AAR65348.1</u>
		-	<u>AAS29296.1</u>
pectate lyase (Pel-103)	<u>Bacillus sp. KSM-P103</u>	<u>AB015044</u>	<u>BAA76885.1</u>
pectate lyase (Pel-34K)	<u>Bacillus sp. P-2850</u>	<u>AB080666</u>	<u>BAC11008.1</u>
pectate lyase (Pel-4A)	<u>Bacillus sp. P-4-N</u>	<u>AB041769</u>	<u>BAA96477.1</u>
pectate lyase (Pel-4B)	<u>Bacillus sp. P-4-N</u>	<u>AB042100</u>	<u>BAA96478.1</u>
pectate lyase (PI47)	<u>Bacillus sp. TS-47</u>	<u>AB045986</u>	<u>BAB40336.1</u>
pectate lyase (PelK)	<u>Bacillus sp. YA-14</u>	<u>D26349</u>	<u>BAA05383.1</u>
		<u>AX601431</u>	<u>CAD67509.1</u>
		<u>AX601436</u>	<u>CAD67510.1</u>
		<u>AX601448</u>	<u>CAD67511.1</u>
pectate lyase	<u>Bacillus subtilis</u>	<u>AX951870</u>	<u>CAF05441.1</u>
pectate lyase (Pel)	<u>Bacillus subtilis AC327</u>	<u>D86417</u>	<u>BAA22313.1</u>

Figure 38B
Pectate Lyases

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

Figure 38C
Pectate Lyases

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

Figure 38D
Pectate Lyases

Protein	Organism	GenBank	GenPept
pectate lyase (PelB;PI;Pstru-3)	<u>Xanthomonas campestris pv. malvacearum strain B414</u>	<u>L38573</u>	<u>AAC41522.1</u>
pectin lyase (AN2331.2)	<u>Aspergillus nidulans FGSC A4</u>	<u>DQ490478</u>	<u>ABF50854.1</u>
		<u>AACD01000038</u>	<u>EAA64442.1</u>
pectin lyase (AN2569.2)	<u>Aspergillus nidulans FGSC A4</u>	<u>AACD01000043</u>	<u>EAA64674.1</u>
		<u>DQ490480</u>	<u>ABF50856.1</u>
pectate lyase (PelA;AN0741.2)	<u>Aspergillus nidulans FGSC A4</u>	<u>U05592</u>	<u>AAA80568.1</u>
		<u>DQ490468</u>	<u>ABF50844.1</u>
		<u>EF452421</u>	<u>ABO38859.1</u>
		<u>AACD01000012</u>	<u>EAA65383.1</u>
pectate lyase (AN7646.2)	<u>Aspergillus nidulans FGSC A4</u>	<u>AACD01000130</u>	<u>EAA61832.1</u>
		<u>DQ490513</u>	<u>ABF50889.1</u>
pectin lyase A (PelA) - PI1A	<u>Aspergillus niger CBS 120.49 / N400</u>	<u>X55784</u>	<u>CAA39305.1</u>
		<u>X60724</u>	<u>CAA43130.1</u>
pectin lyase C (PelC)	<u>Aspergillus niger CBS 120.49 / N400</u>	<u>AY839647</u>	<u>AAW03313.1</u>
pectin lyase F (PelF)	<u>Aspergillus niger CBS 120.49 / N400</u>	<u>AJ489943</u>	<u>CAD34589.1</u>
pectate lyase A (PlyA)	<u>Aspergillus niger CBS 120.49 / N400</u>	<u>AJ276331</u>	<u>CAC33162.1</u>
pectin lyase B (PelB)	<u>Aspergillus niger CBS 120.49 / N400</u>	<u>A12248</u>	<u>CAA01023.1</u>
		<u>X65552</u>	<u>CAA46521.1</u>
An14g04370 (PelA)	<u>Aspergillus niger CBS 513.88</u>	<u>AM270321</u>	<u>CAK48529.1</u>
An03g00190 (PelB)	<u>Aspergillus niger CBS 513.88</u>	<u>AM270043</u>	<u>CAK37997.1</u>
An15g07160 (PelF)	<u>Aspergillus niger CBS 513.88</u>	<u>AM270351</u>	<u>CAK48551.1</u>
An19g00270 (PelD)	<u>Aspergillus niger CBS 513.88</u>	<u>AM270415</u>	<u>CAK47350.1</u>
pectate lyase I (PlyA;An10g00870)	<u>Aspergillus niger CBS 513.88</u>	<u>AM270216</u>	<u>CAK40523.1</u>
pectin lyase D (PelD)	<u>Aspergillus niger N756</u>	<u>M55657</u>	<u>AAA32701.1</u>
pectin lyase 2 (Pel2)	<u>Aspergillus oryzae KBN616</u>	<u>AB029323</u>	<u>BAB82468.1</u>
pectin lyase 1 (Pel1)	<u>Aspergillus oryzae KBN616</u>	<u>AB029322</u>	<u>BAB82467.1</u>
pectin lyase 1 (Pel1;AO090010000504)	<u>Aspergillus oryzae RIB 40</u>	<u>EF452419</u>	<u>ABO38857.1</u>
		<u>AP007175</u>	<u>BAE66352.1</u>
pectin lyase 2 (Pel2;AO090010000030)	<u>Aspergillus oryzae RIB 40</u>	<u>AP007175</u>	<u>BAE65949.1</u>

Figure 38E
Pectate Lyases

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

Figure 39A
Rhamnogalacturonases

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

Figure 39B
Rhamnogalacturonate Hydrolases

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

Figure 40A
Pectate Methyl Esterases

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

Figure 40B
Pectate Methyl Esterases

Protein	Organism	GenBank	GenPept
pectin methylesterase (PME1)	<u>Cochliobolus carbonum</u>	<u>AF159252</u>	<u>AA43340.1</u>
pectin methylesterase	<u>Daucus carota</u>		
pectinesterase FaPE1	<u>Fragaria x ananassa</u>	<u>AY324809</u>	<u>AAQ21124.1</u>
pectin methyl-esterase (Pef1)	<u>Medicago truncatula</u>	<u>AJ249611</u>	<u>CAB65291.1</u>
pectin methyl-esterase (Per)	<u>Medicago truncatula</u>	<u>AJ249611</u>	<u>CAB65290.2</u>
pectin methylesterase	<u>Nicotiana benthamiana</u>	<u>AY238968</u>	<u>AAO85706.1</u>
pectin methylesterase	<u>Nicotiana plumbaginifolia</u>	<u>Z71752</u>	<u>CAA96434.1</u>
pectin methylesterase (NtPME1)	<u>Nicotiana tabacum</u>	<u>AY772945</u>	<u>AAX13972.1</u>
pectin methylesterase	<u>Nicotiana tabacum</u>	<u>AJ401158</u>	<u>CAB95025.1</u>
pectin methylesterase (PME1) (fragment)	<u>Orobanche cumana</u>	<u>AY072720</u>	<u>AAL66865.1</u>
pectin methylesterase (fragment)	<u>Orobanche cumana</u>	<u>AF333068</u>	<u>AAG49395.1</u>
pectin methylesterase	<u>Petunia inflata</u>	<u>L27101</u>	<u>AAA33714.1</u>
pectin methyl esterase (PttPME1)	<u>Populus tremula x Populus tremuloides</u>	<u>AJ277547</u>	<u>CAC01624.1</u>
pectin methylesterase PME1 (fragment)	<u>Prunus armeniaca</u>	<u>AF184079</u>	<u>AAG12248.1</u>
pectin methylesterase (SgPME1)	<u>Salix gilgiana</u>	<u>AB029461</u>	<u>BAA89480.1</u>
pectin methylesterase	<u>Sitophilus oryzae</u>	<u>AY841894</u>	<u>AAW28928.1</u>
		<u>U50985</u>	<u>AAB67739.1</u>
		-	<u>AAQ71552.1</u>
		<u>A15983</u>	<u>CAA01257.1</u>
		<u>A17011</u>	<u>CAA01315.1</u>
		<u>X07910</u>	<u>CAA30746.1</u>
pectin methylesterase 2	<u>Solanum lycopersicum</u>	<u>X74639</u>	<u>CAA52704.1</u>
		<u>U49330</u>	<u>AAD09283.1</u>
pectin methylesterase (PmeU1)	<u>Solanum lycopersicum</u>	<u>AY046596</u>	<u>AAL02367.1</u>
		<u>U50986</u>	<u>AAB67740.1</u>
		<u>A17010</u>	<u>CAA01314.1</u>
pectin methylesterase 1 (PME1.9)	<u>Solanum lycopersicum</u>	<u>X74638</u>	<u>CAA52703.1</u>
pectin methyl esterase (Pest1)	<u>Solanum tuberosum</u>	<u>AF152171</u>	<u>AAF23891.1</u>
pectin methyl esterase (Pest2)	<u>Solanum tuberosum</u>	<u>AF152172</u>	<u>AAF23892.1</u>
pectin methylesterase isoform alpha (PME2) (fragment)	<u>Vigna radiata</u>	<u>AF229849</u>	<u>AAF35897.1</u>
pectin methylesterase (PME)	<u>Vitis riparia</u>	<u>AF178989</u>	<u>AAD51853.1</u>

Figure 41
Pectate Acetyl Esterases

Protein	Organism	GenBank/GenPept	
Family12			
acetyl xylan esterase (Rgae;BH1115)	<u>Bacillus halodurans C-125</u>	<u>AP001511</u>	<u>BAB04834.1</u>
		<u>NC_002570</u>	<u>NP_241981.1</u>
cephalosporin C deacetylase	<u>Bacillus sp. KCCM10143</u>	<u>AF184175</u>	<u>AAF25818.1</u>
acetyl xylan esterase (YesT;BSU07020)	<u>Bacillus subtilis subsp. subtilis str. 168</u>	<u>Z99107</u>	<u>CAB12521.1</u>
		<u>NC_000964</u>	<u>NP_388583.1</u>
ECA3252 (PaeY)	<u>Erwinia carotovora subsp. atroseptica SCR11043</u>	<u>BX950851</u>	<u>CAG76150.1</u>
pectin acetylesterase (PaeY)	<u>Erwinia chrysanthemi 3937</u>	<u>Y09828</u>	<u>CAA70971.1</u>
rhamnogalacturonan acetylesterase (Rha1)	<u>Aspergillus aculeatus KSM 510</u>	<u>X89714</u>	<u>CAA61858.1</u>
rhamnogalacturonan acetylesterase (AN2528.2)	<u>Aspergillus nidulans FGSC A4</u>	<u>DQ490479</u>	<u>ABF50855.1</u>
		<u>AACD01000043</u>	<u>EAA64633.1</u>
pectin acetylesterase	<u>Vigna radiata Wilzeck</u>	<u>X99348</u>	<u>CAA67728.1</u>

Production of 2-phenyl ethanol (24 hrs)

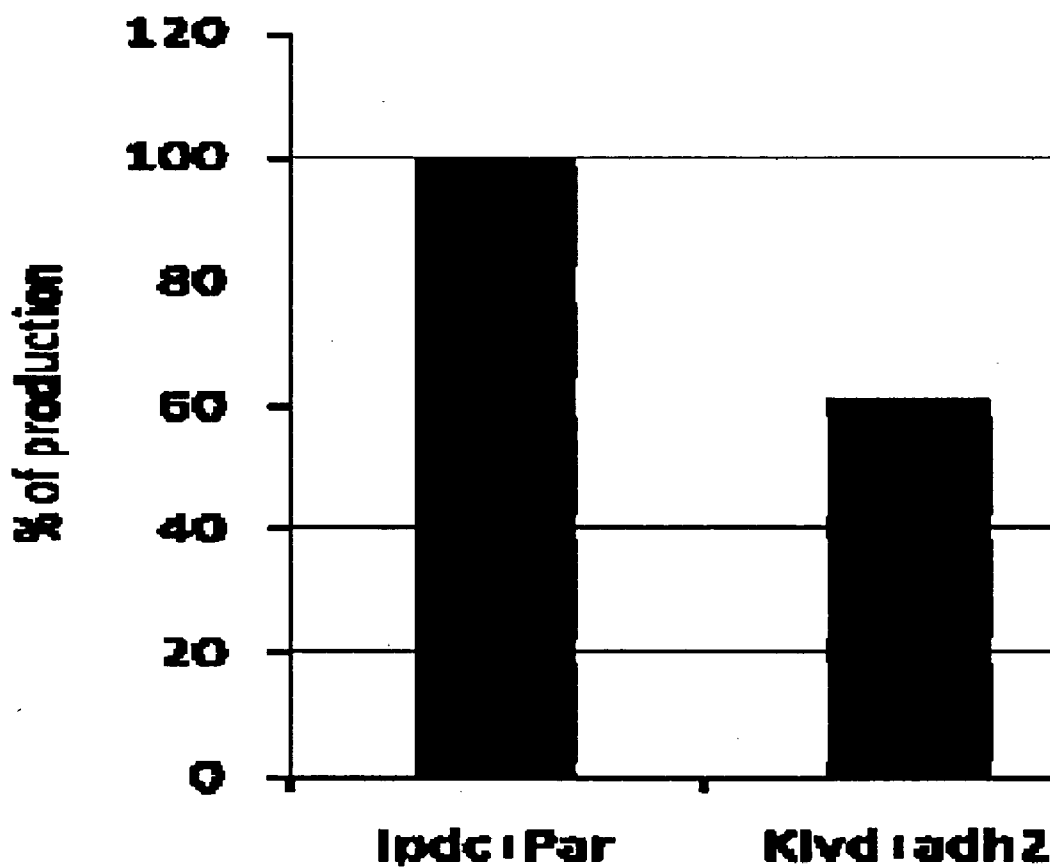


FIG. 42A

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

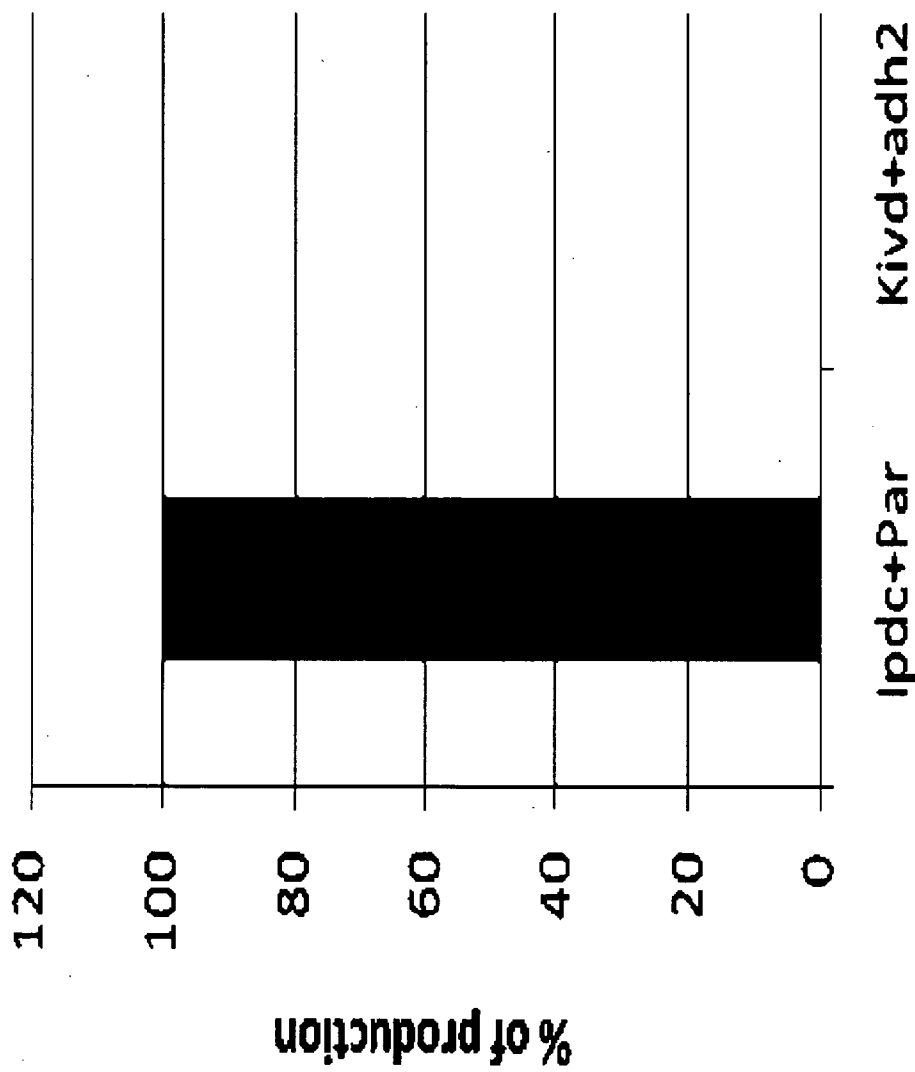


FIG. 42B

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

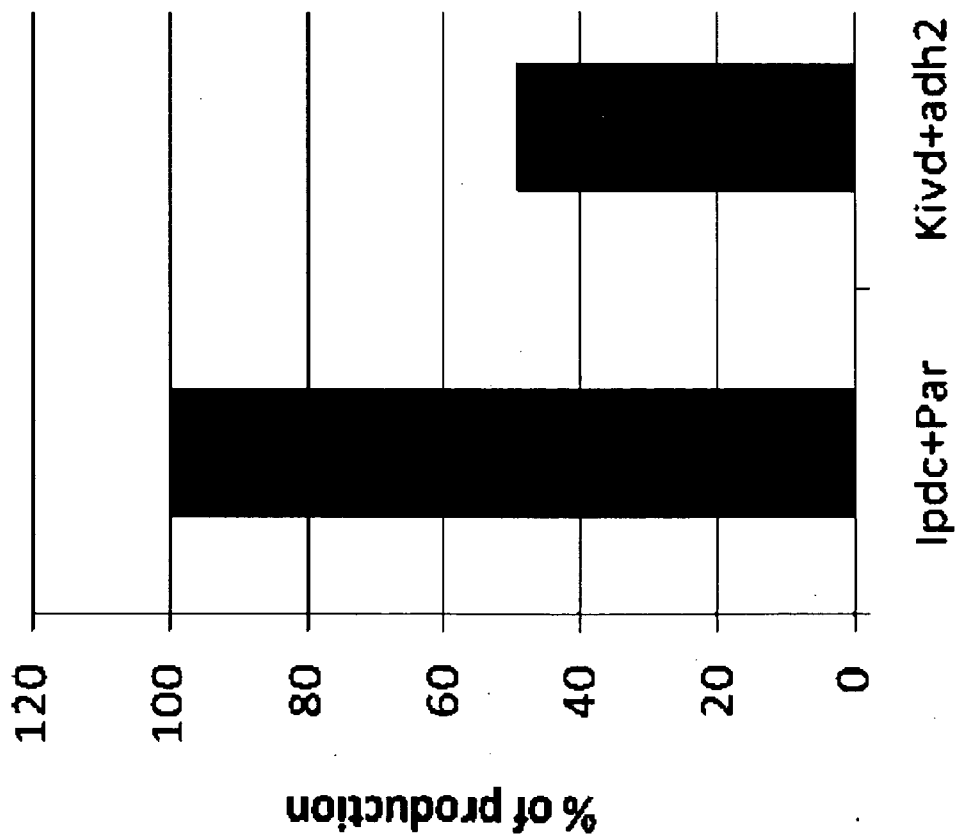


FIG. 42C

GC-MS chromatogram of control

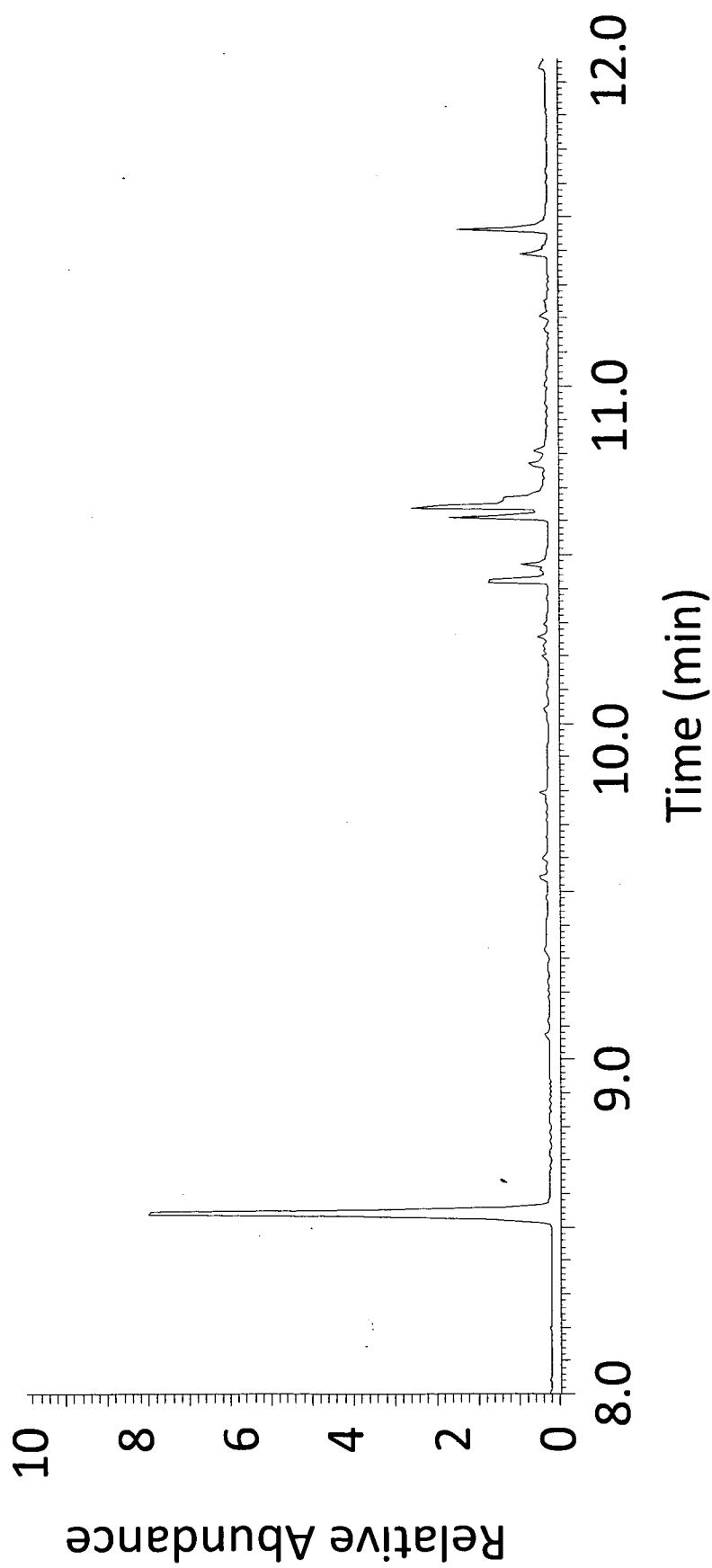


FIG. 43A

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

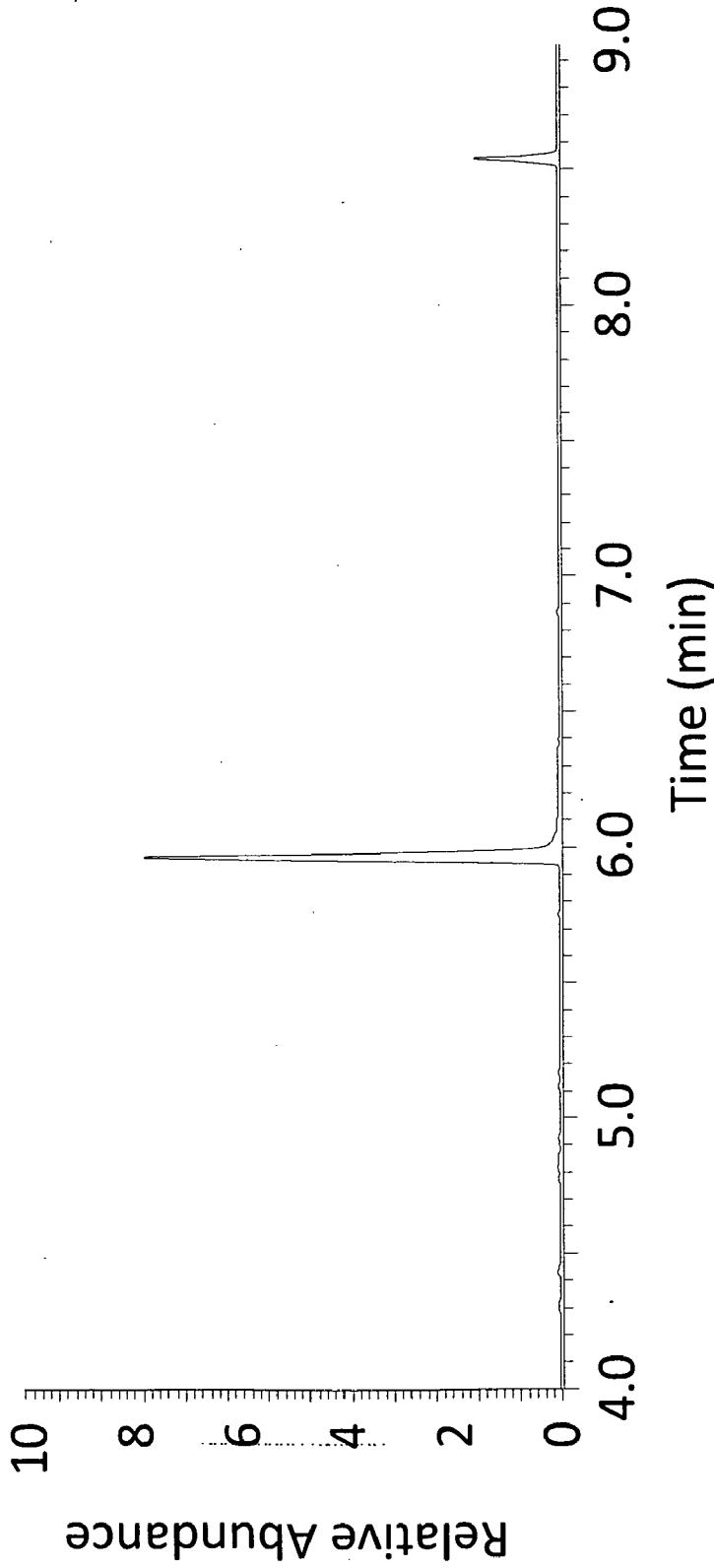


FIG. 43B

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

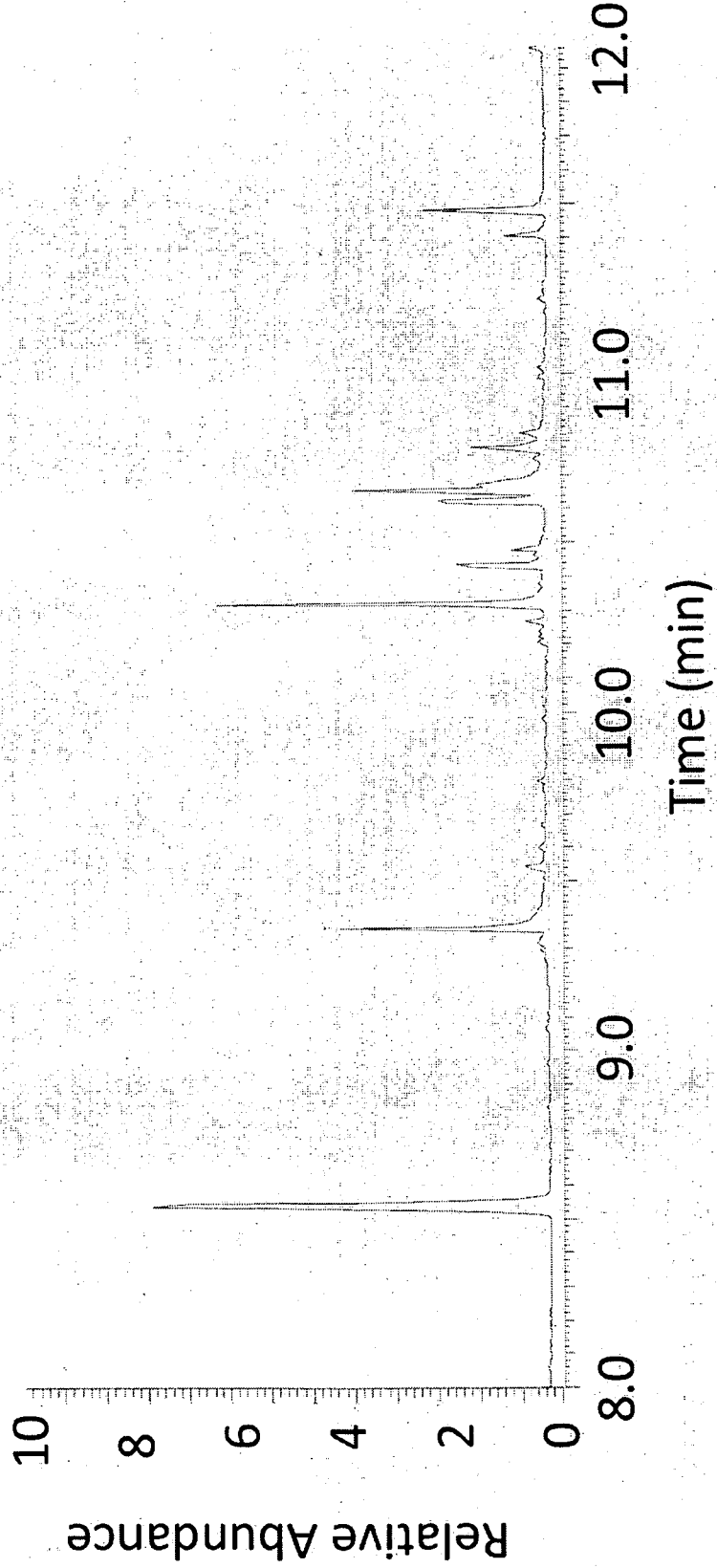


FIG. 44

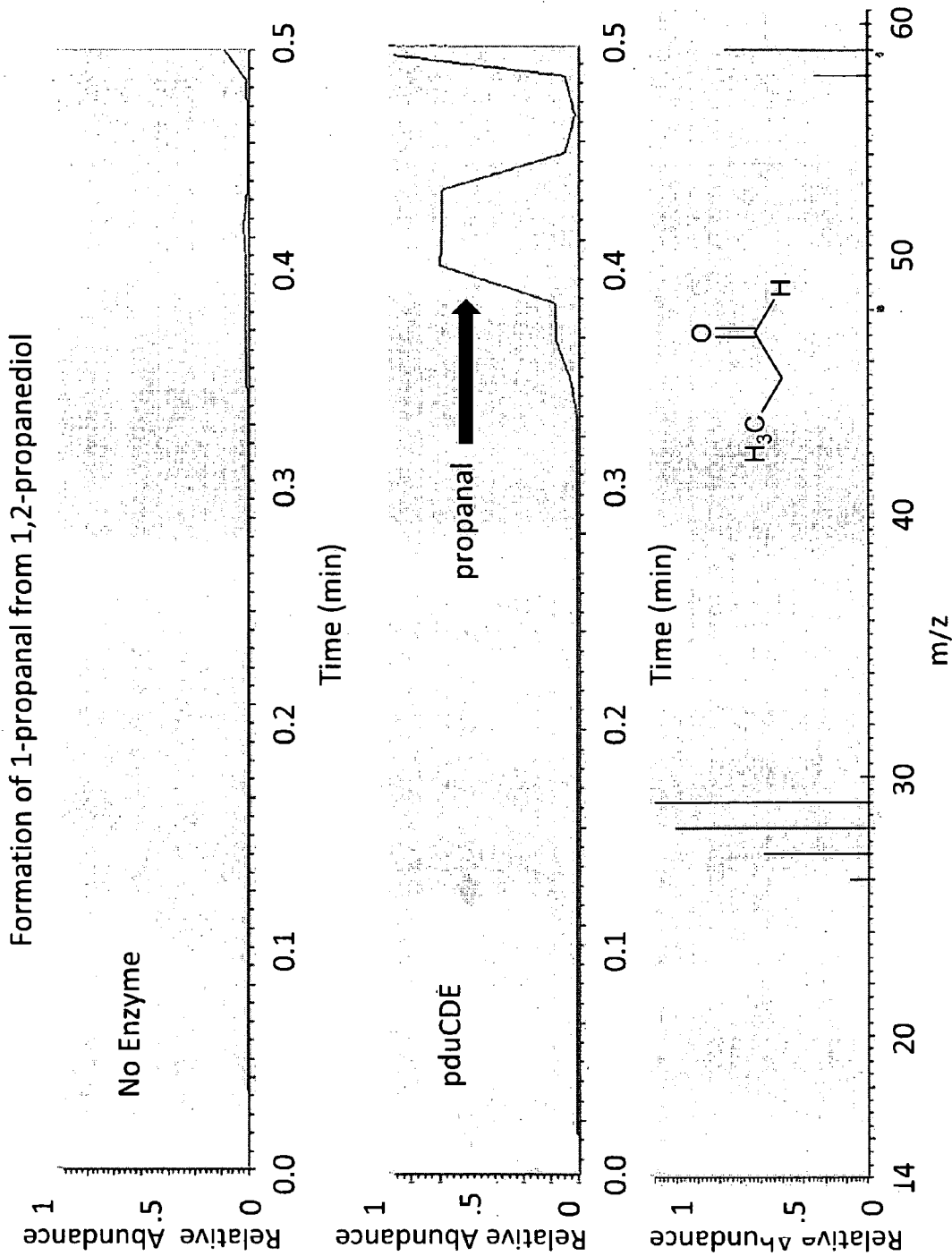


FIG. 45

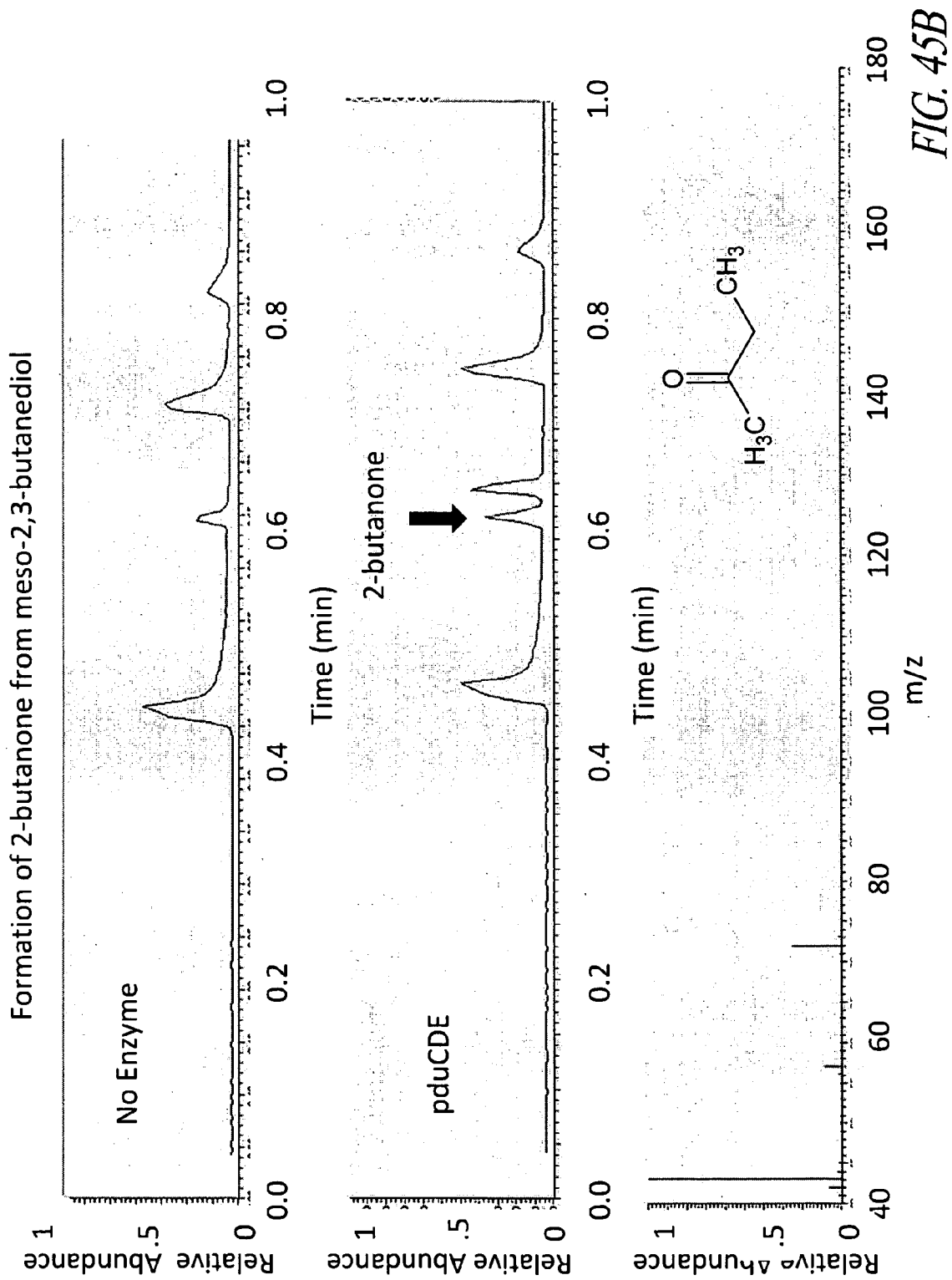


FIG. 45B

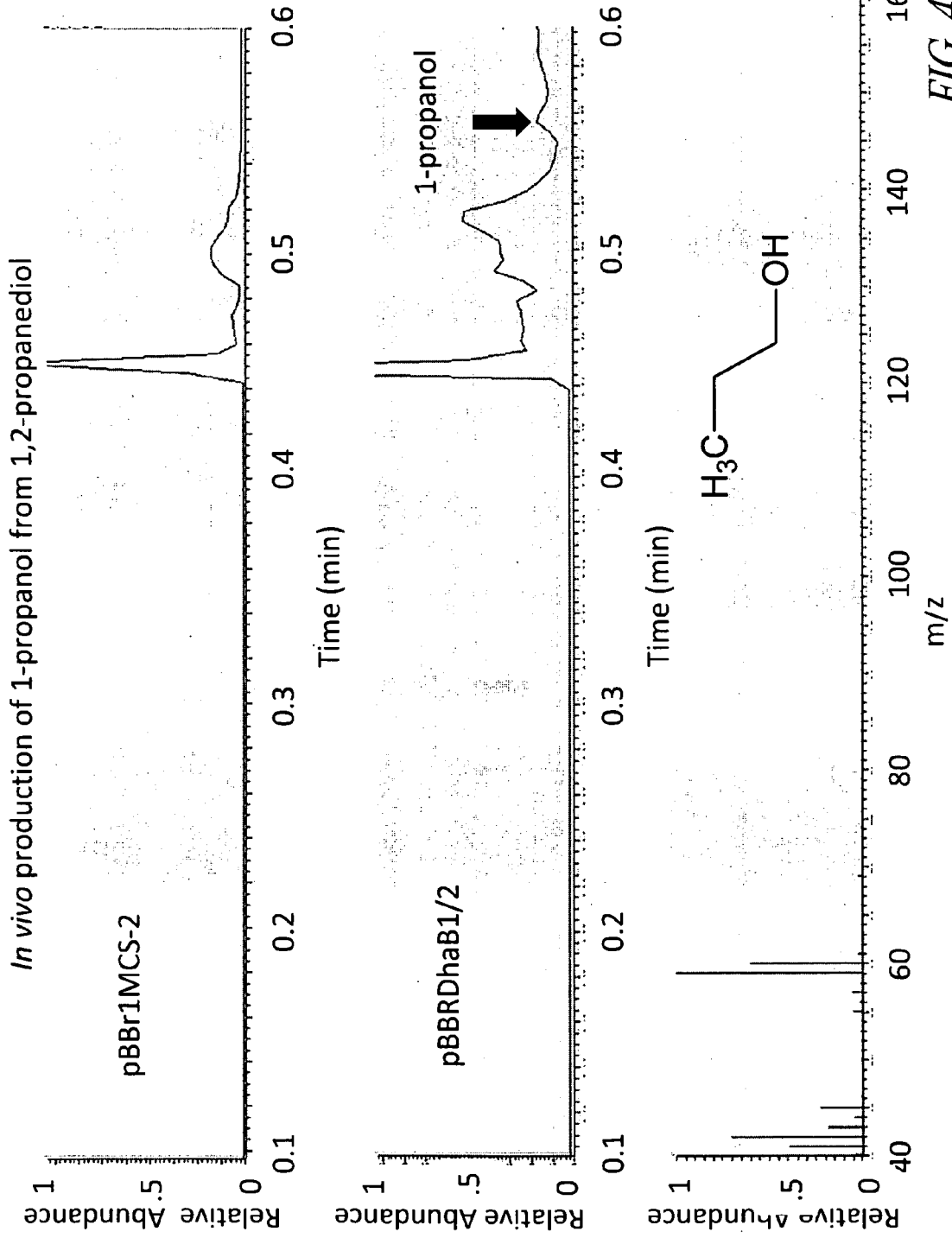
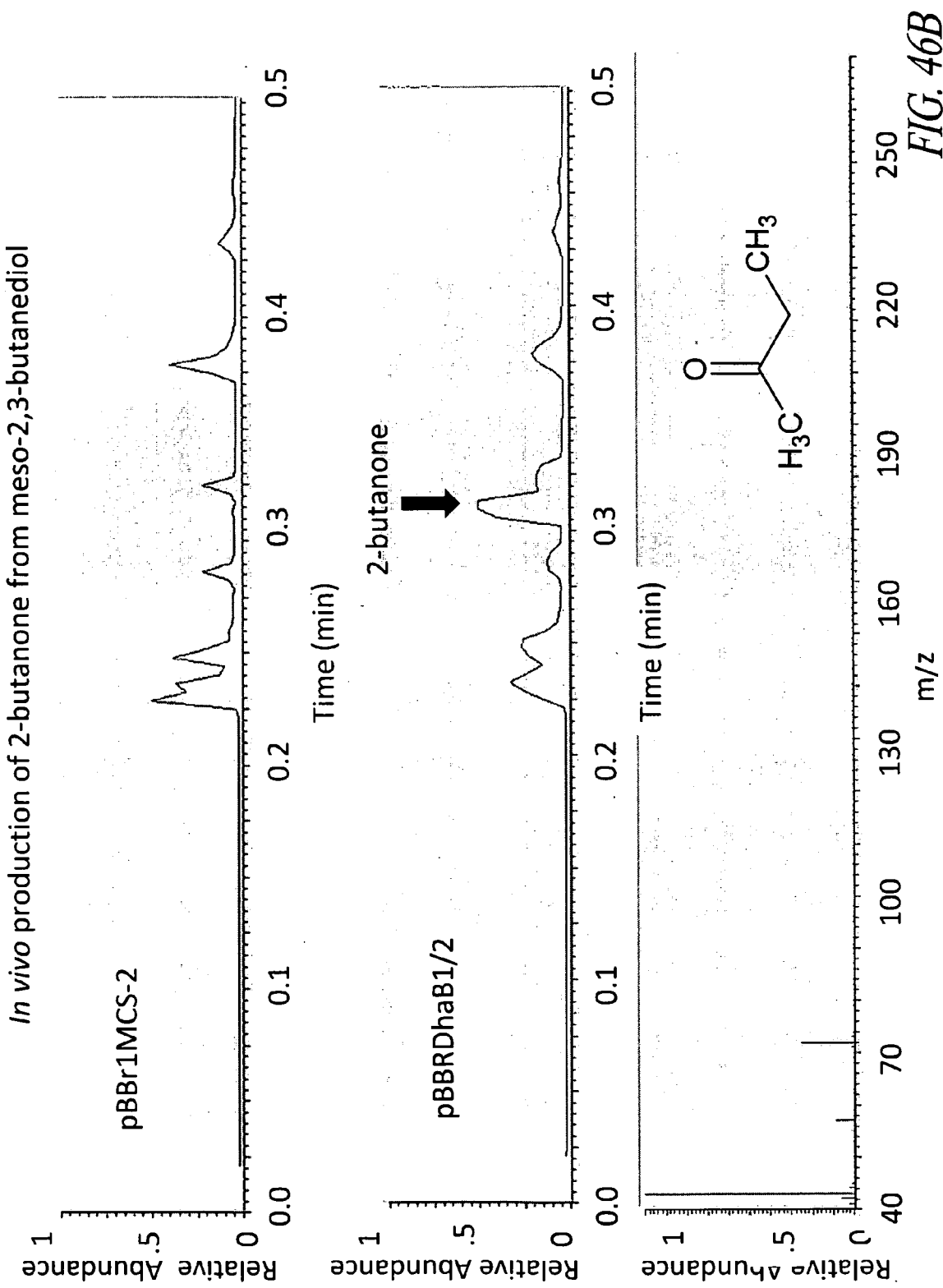


FIG. 46A



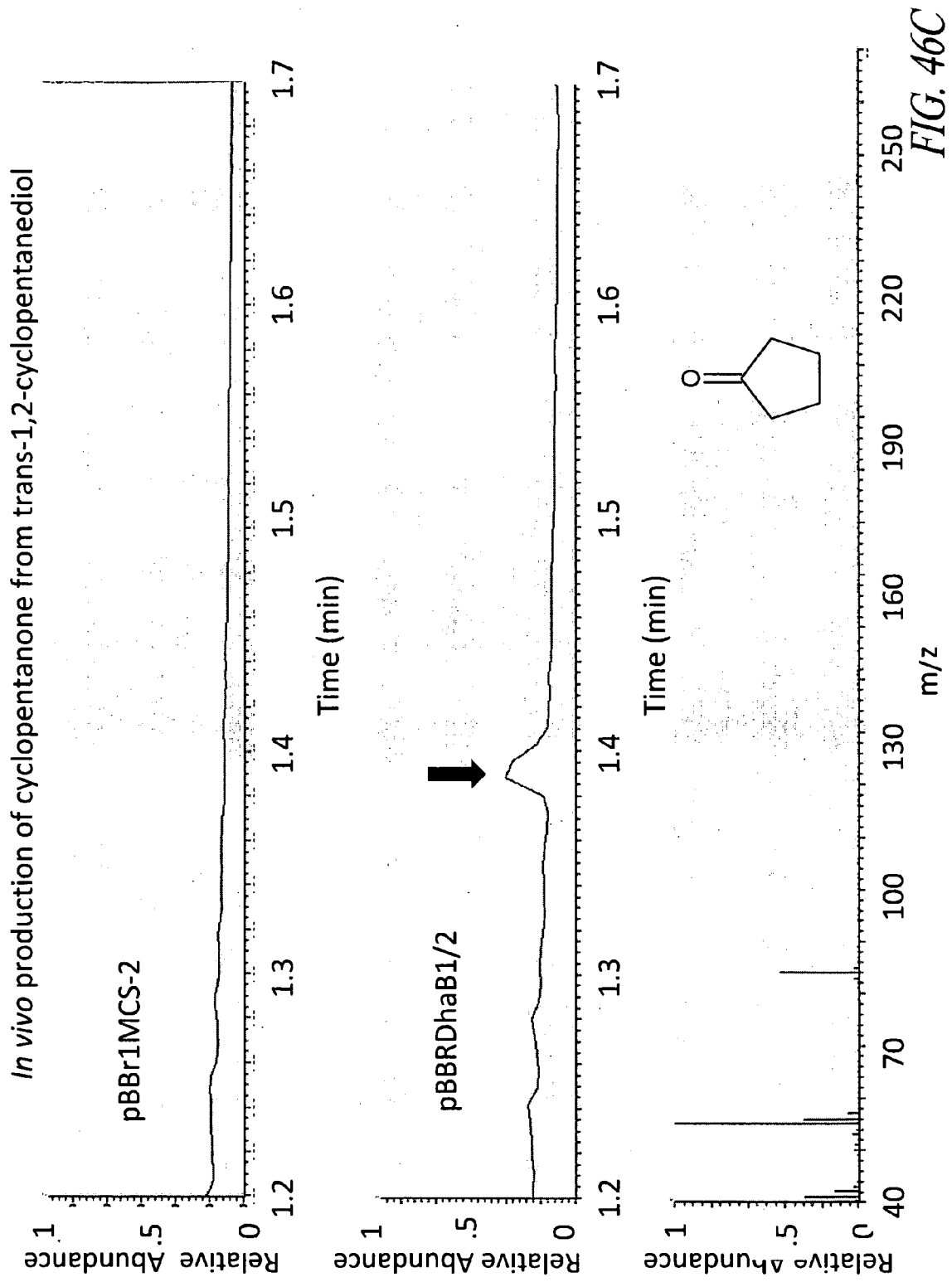
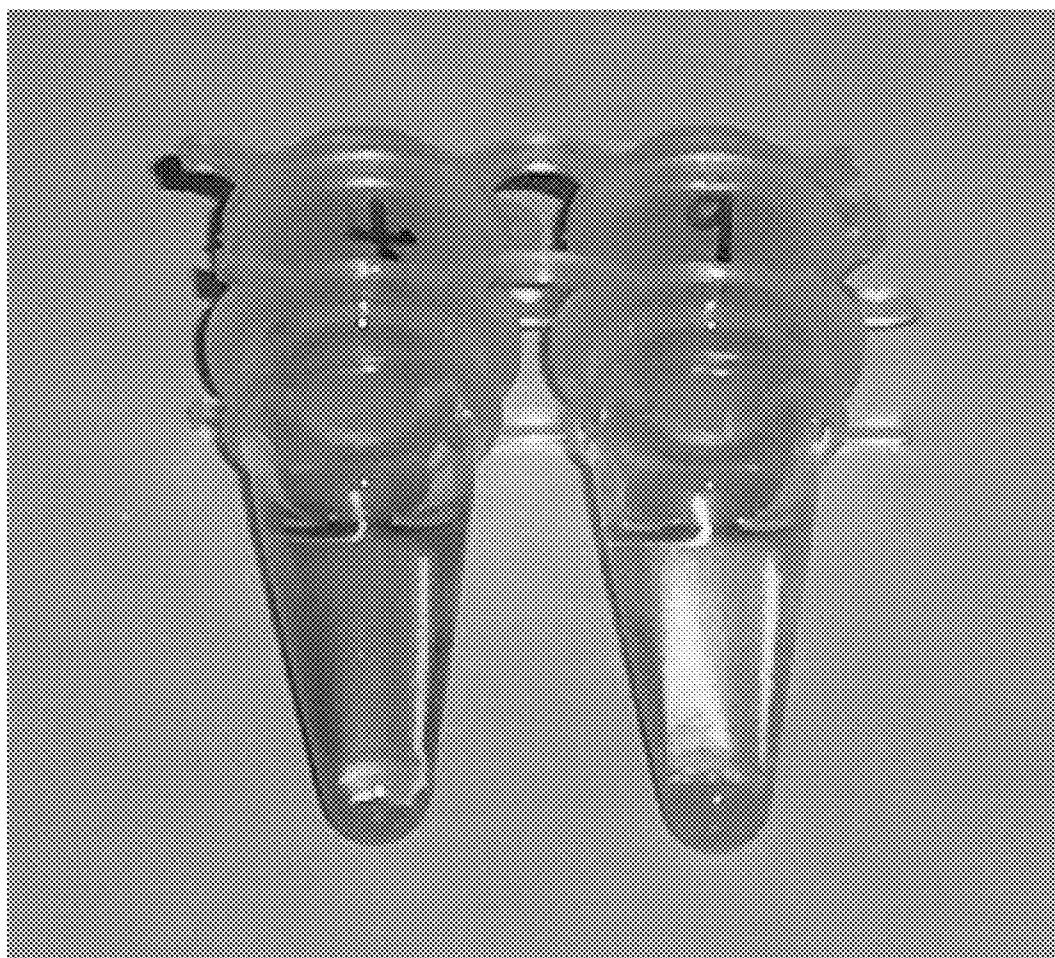


Figure 47
Thiobarbituric acid (TBA) Assay



In vivo production of 3-hydroxy-2-pentanone and 2-hydroxy-3-pentanone from ligation reaction between acetaldehyde and propionaldehyde catalyzed by BAL enzyme

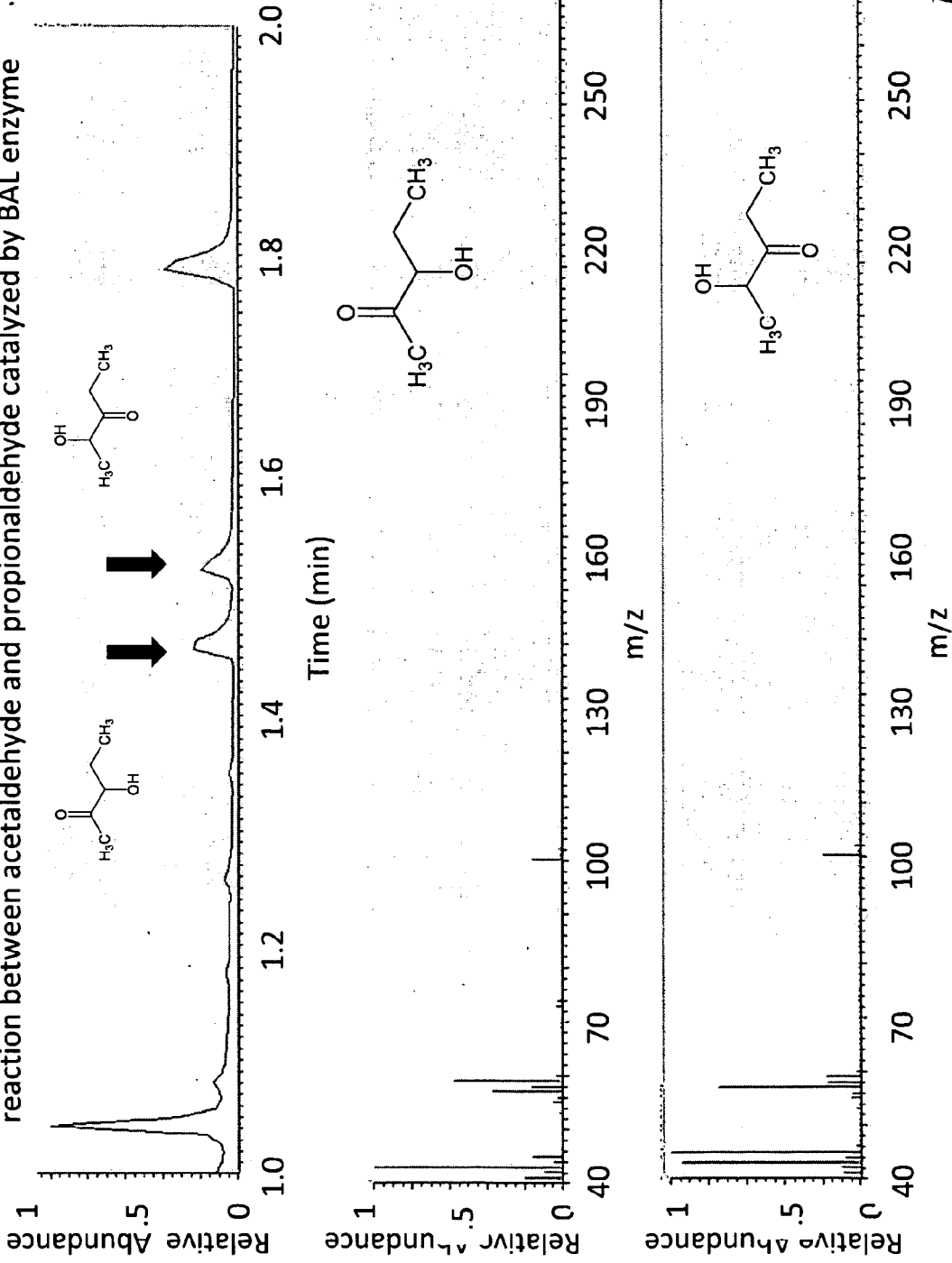


FIG. 48A

In vivo production of 4-hydroxy-3-heptanone and 3-hydroxy-4-heptanone from ligation reaction between propionaldehyde and butyraldehyde catalyzed by BAL enzyme

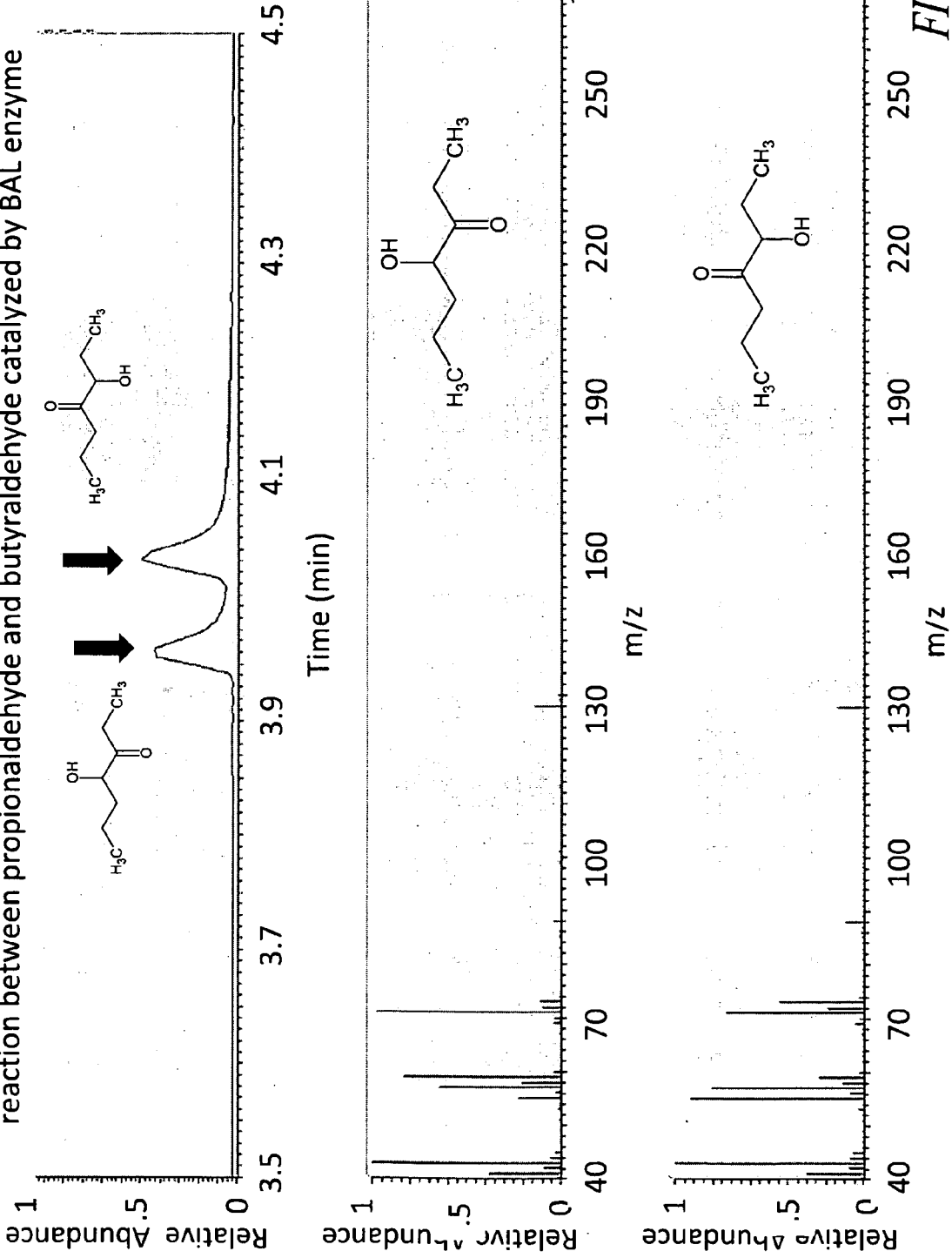


FIG. 48B

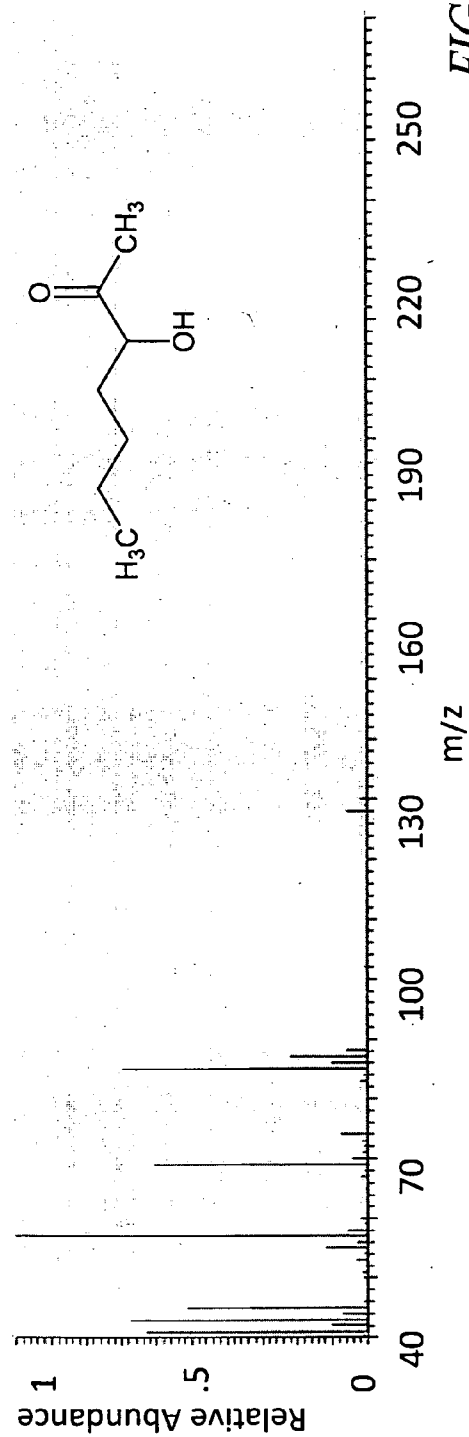
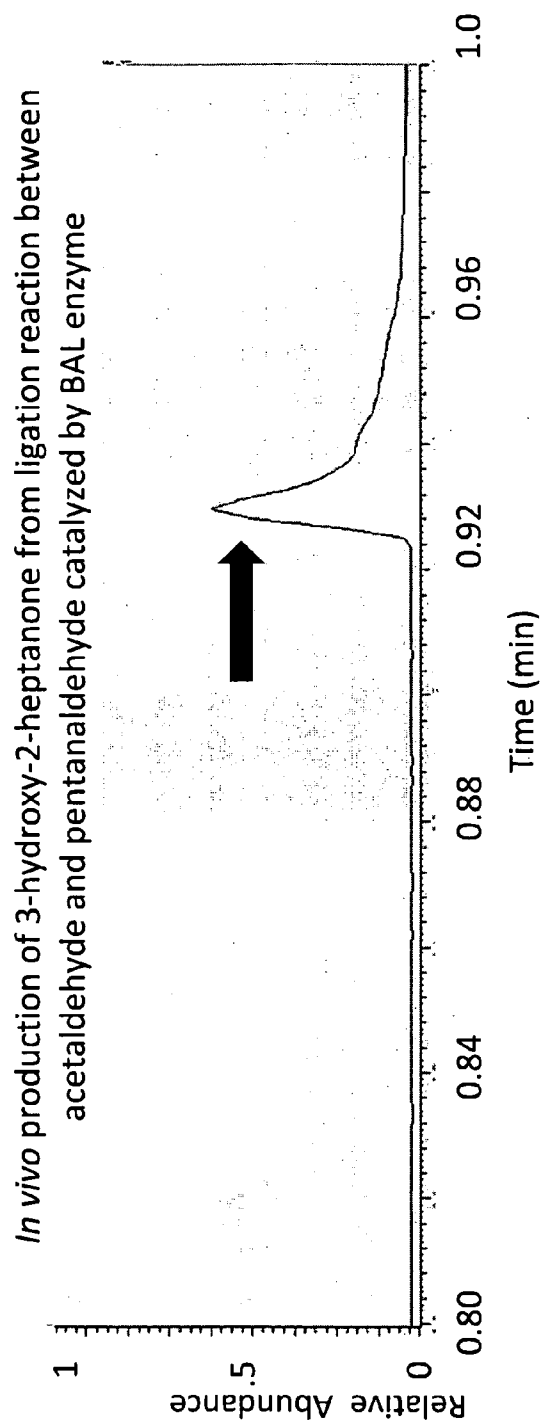


FIG. 49A

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

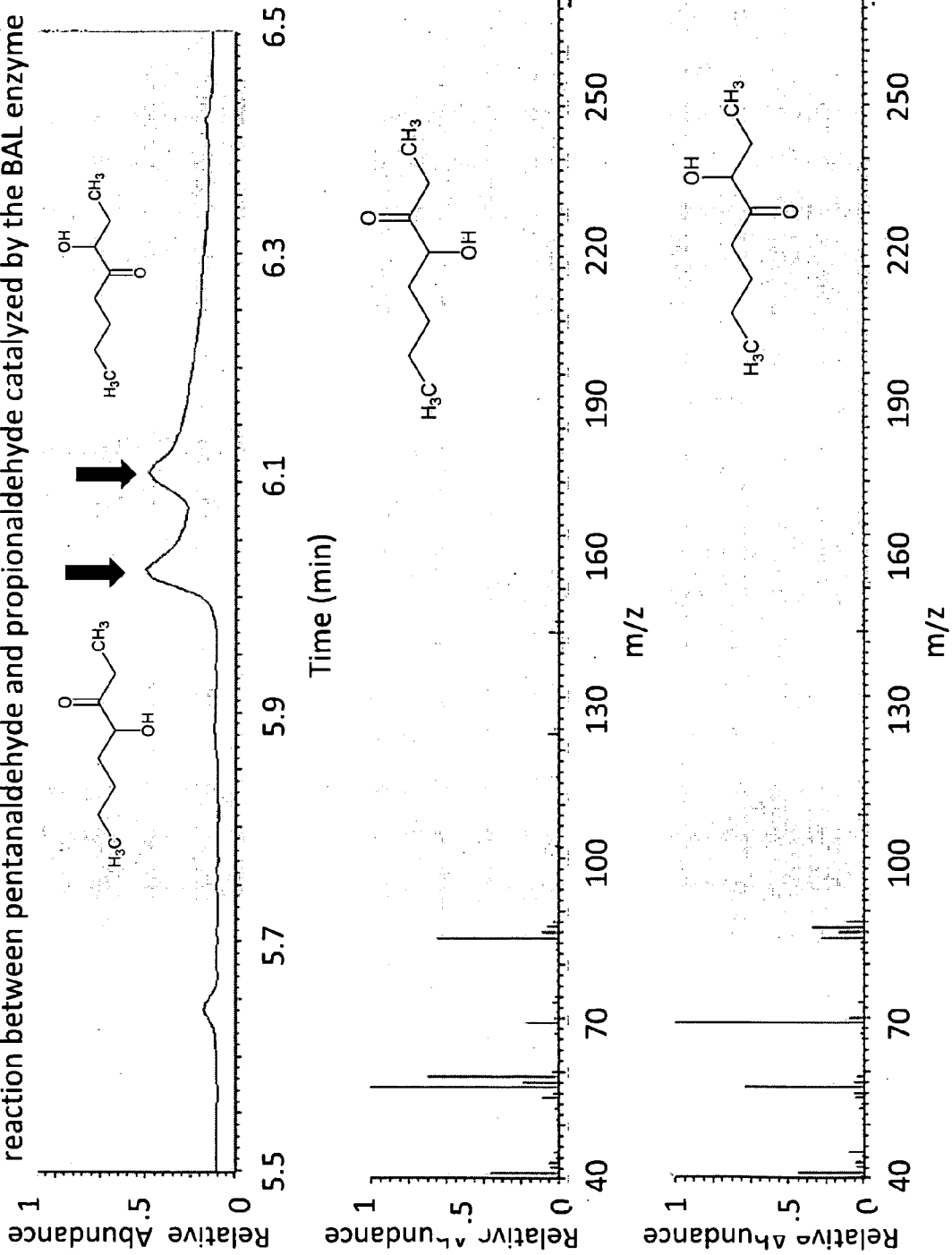


FIG. 49B

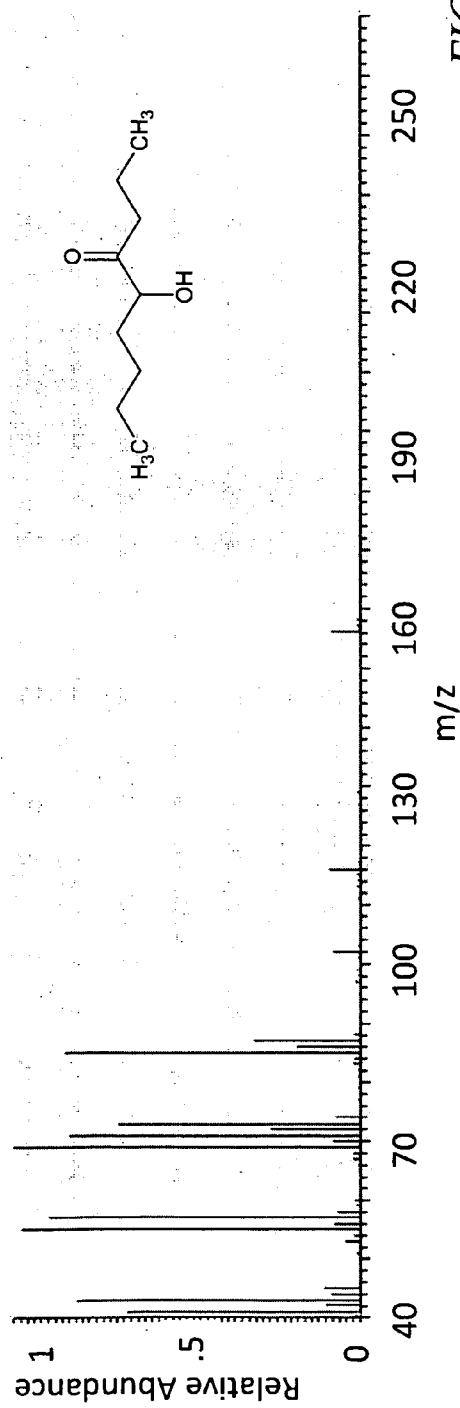
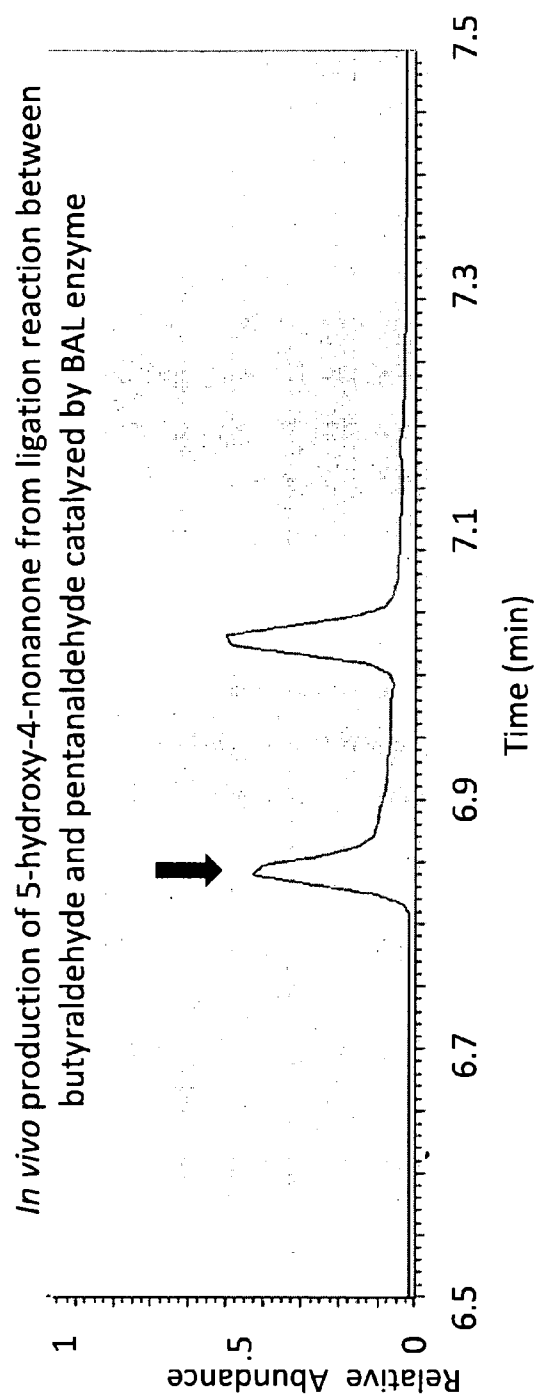


FIG. 50A

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

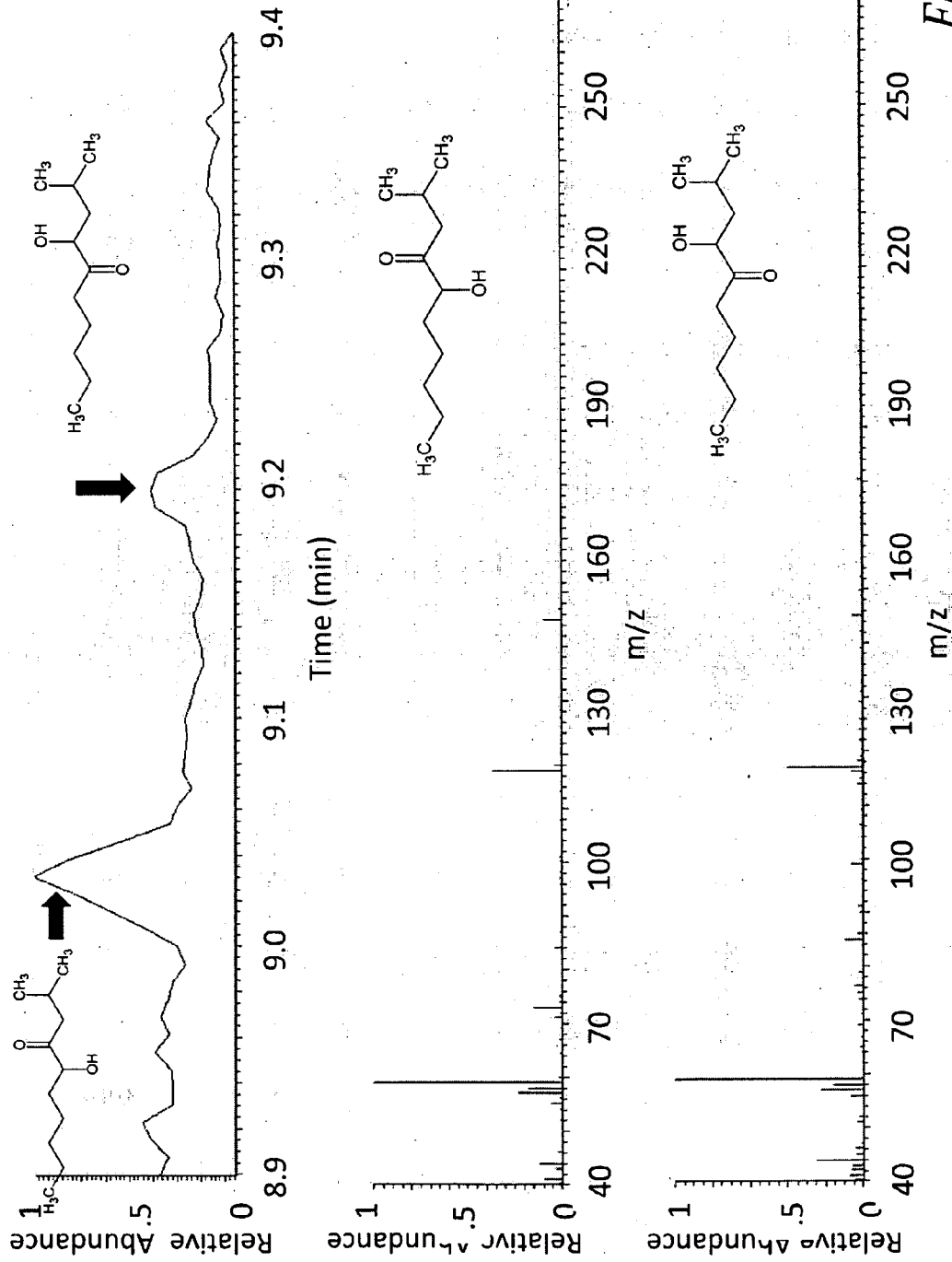


FIG. 50B

In vivo production of 6-methyl-3-hydroxy-2-heptanone from ligation reaction between acetaldehyde and 4-methylhexanal catalyzed by BAL enzyme

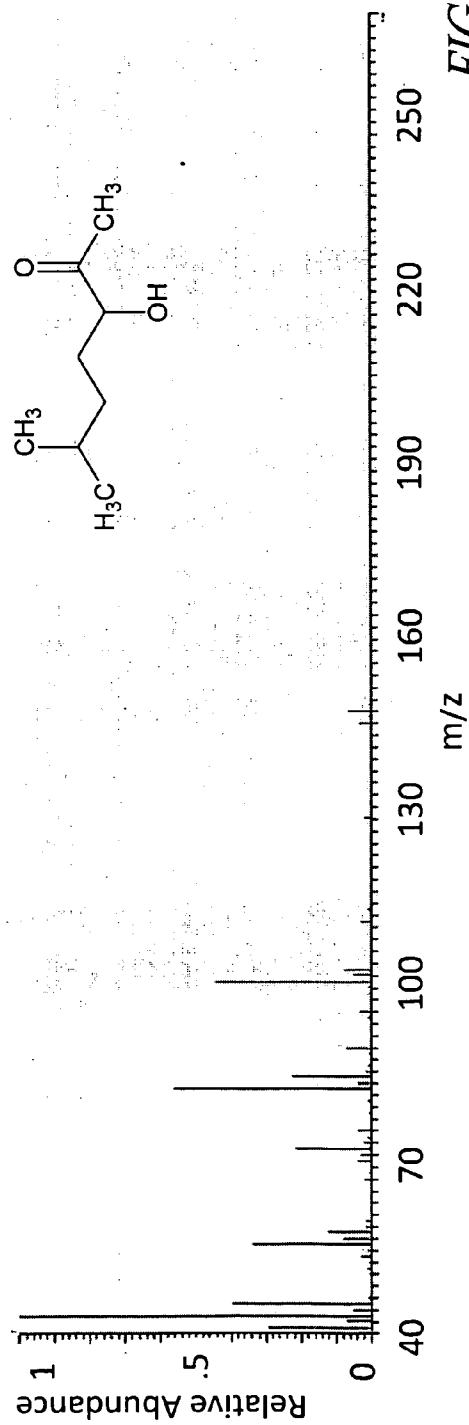
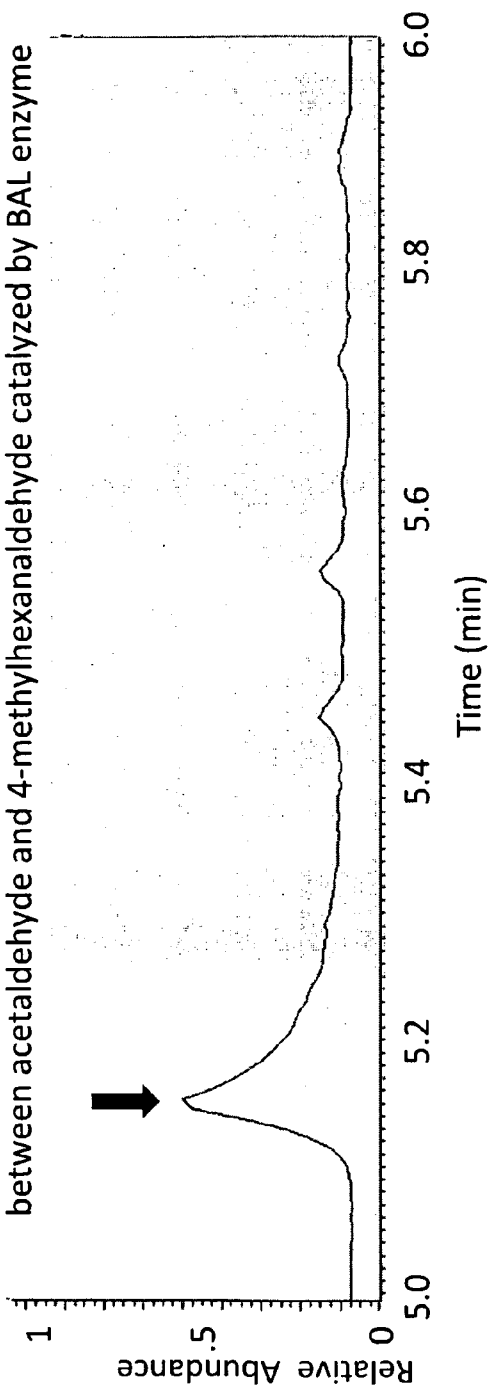


FIG. 51A

In vivo production of 7-methyl-4-hydroxy-3-octanone from ligation reaction between 4-methylhexanaldehyde and propionaldehyde catalyzed by BAL enzyme

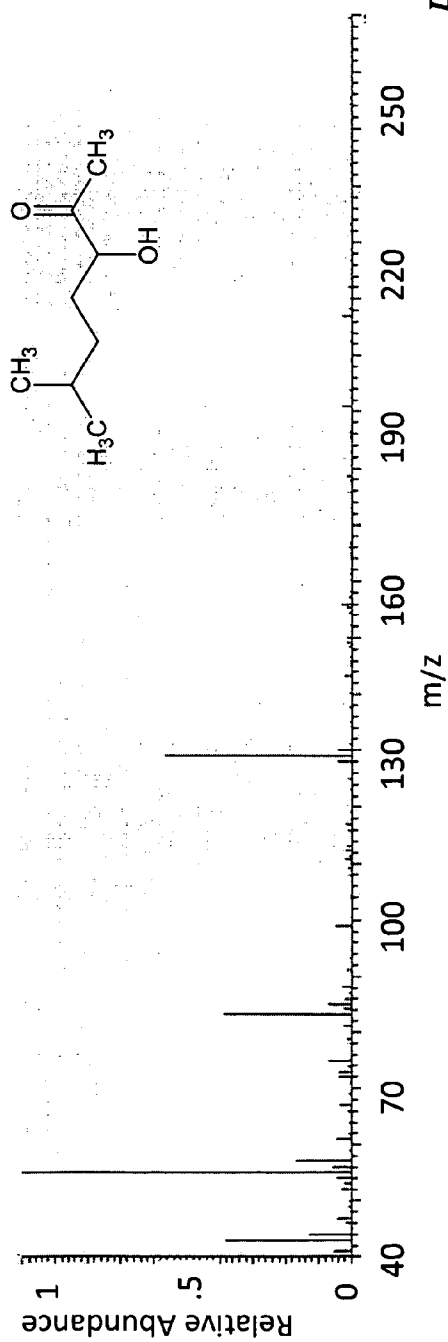
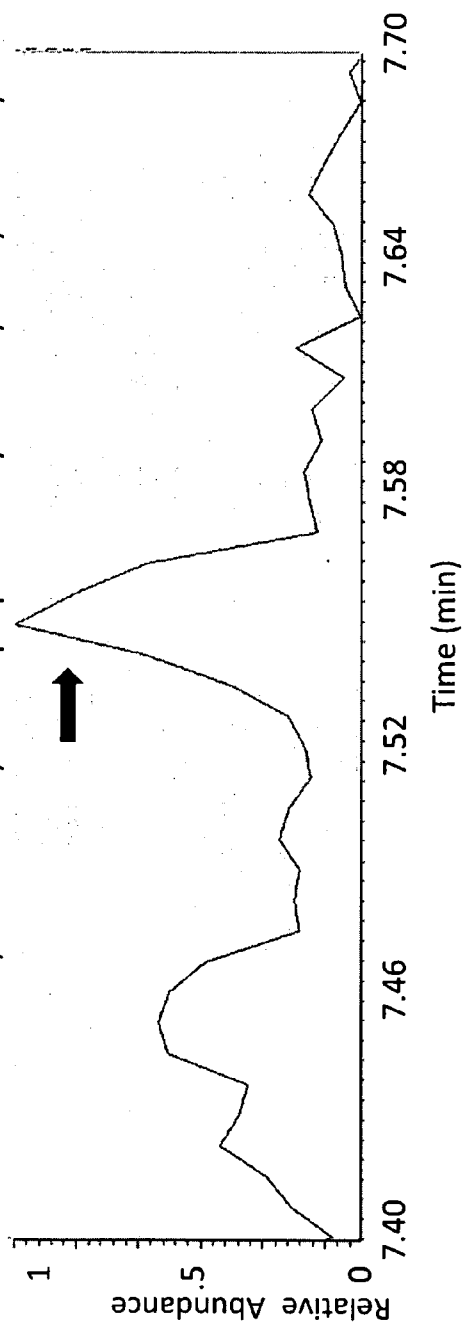


FIG. 51B

In vivo production of 8-methyl-5-hydroxy-4-nonanone from ligation reaction between 4-methylhexanaldehyde and butyraldehyde catalyzed by BAL enzyme

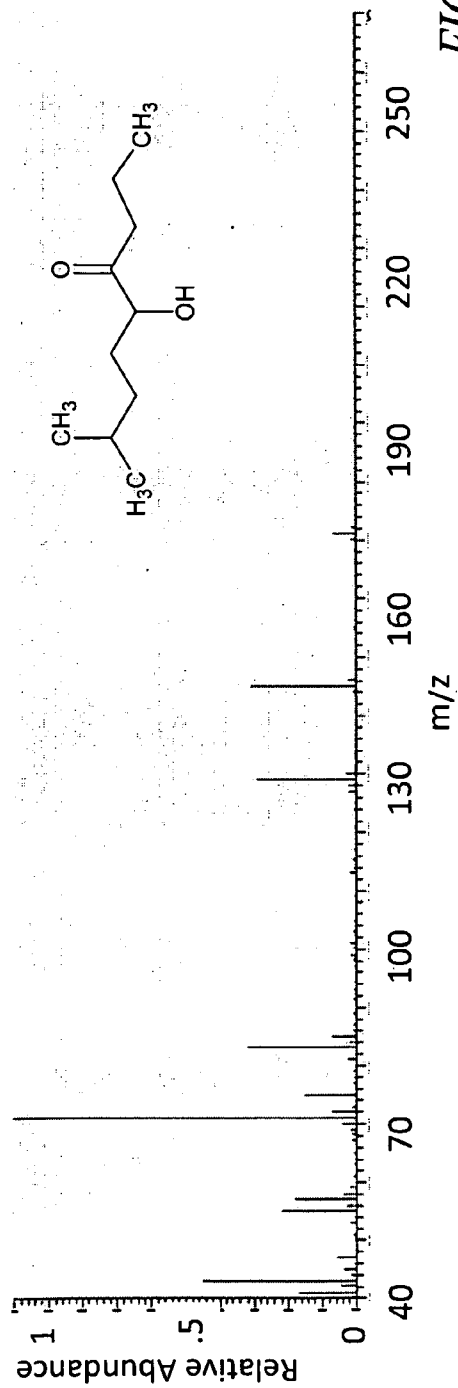
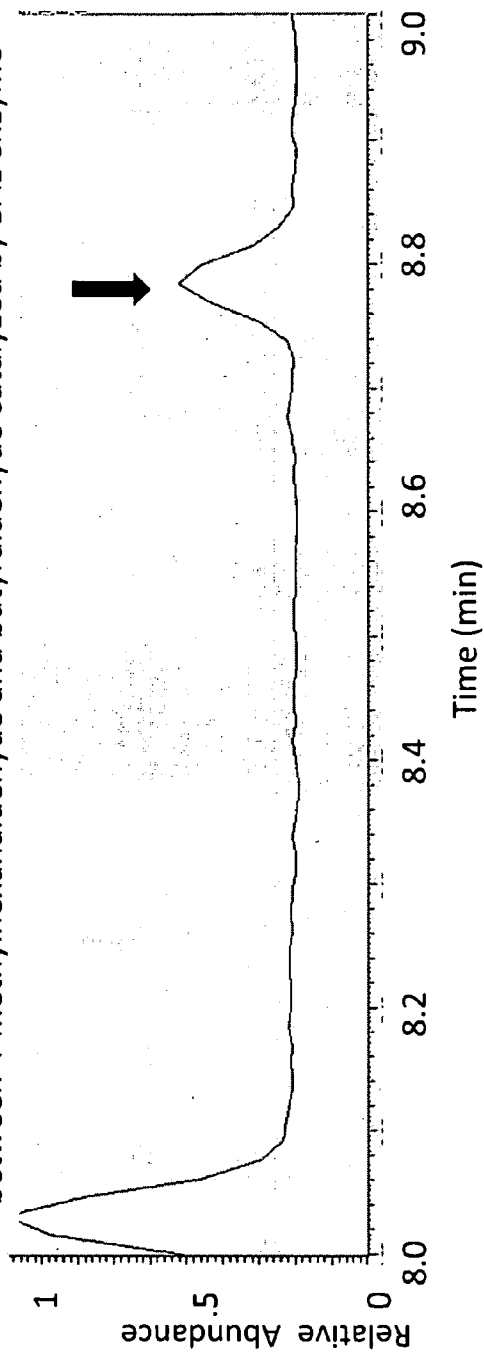


FIG. 52A

In vivo production 3-hydroxy-2-decanone from ligation reaction between acetaldehyde and octanal catalyzed by BAL

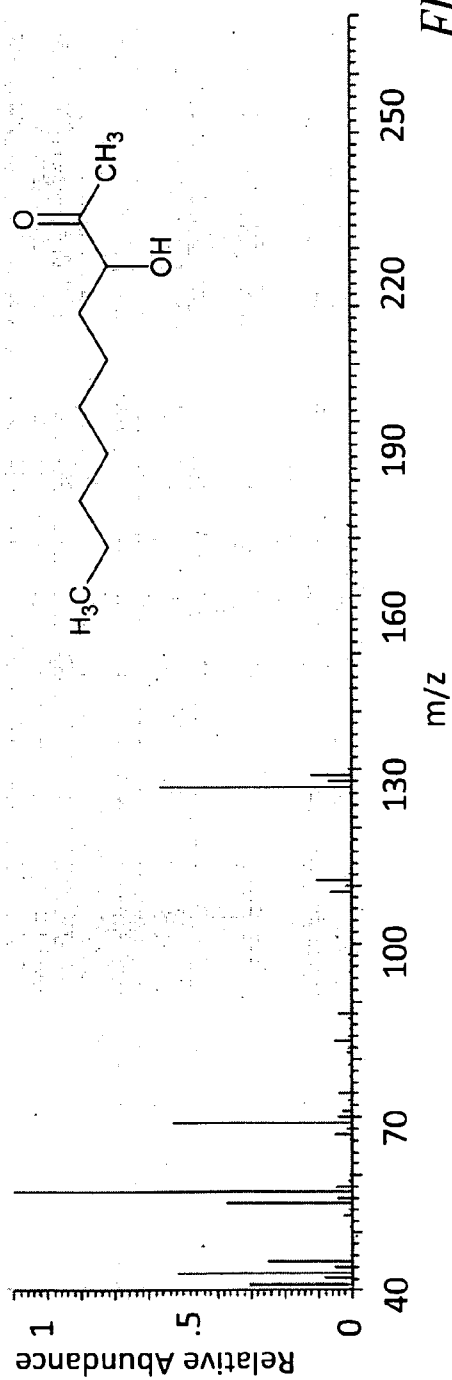
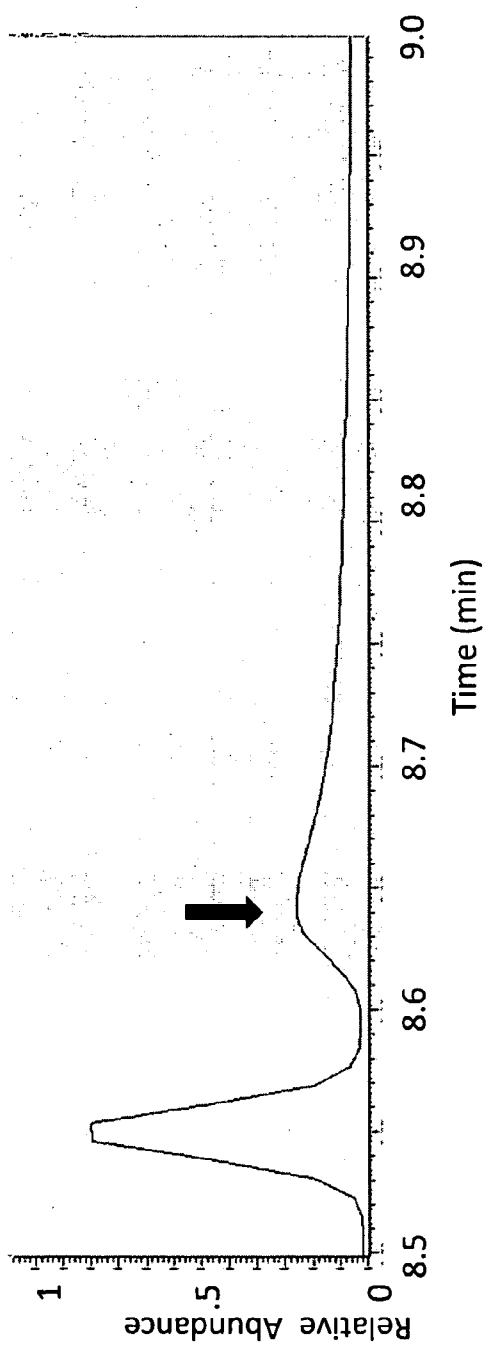


FIG. 52B

In vivo production 4-hydroxy-3-undecanone from ligation reaction between octanal and propionaldehyde catalyzed by BAL

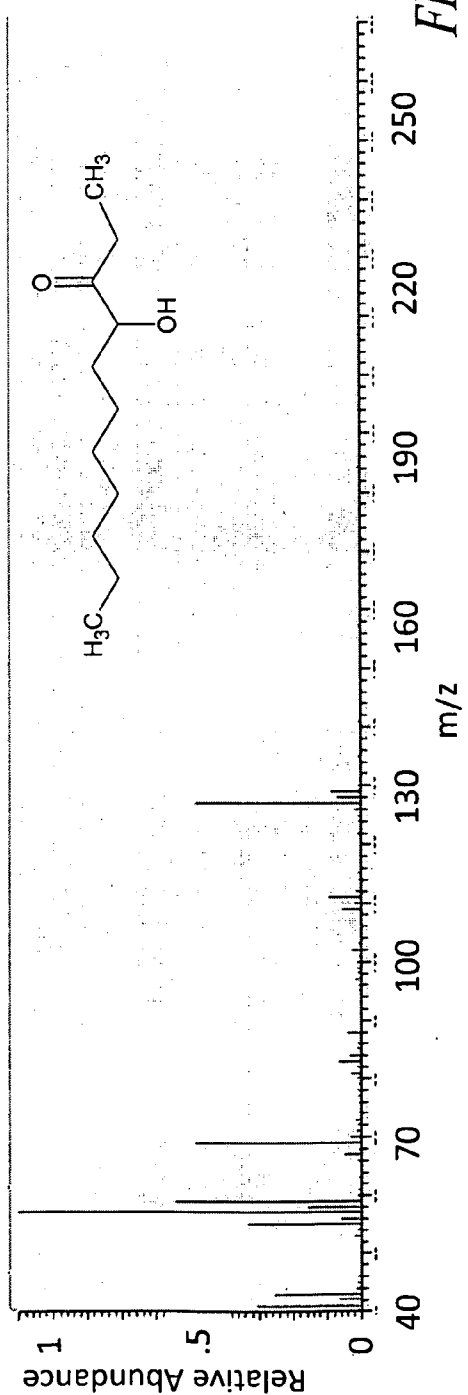
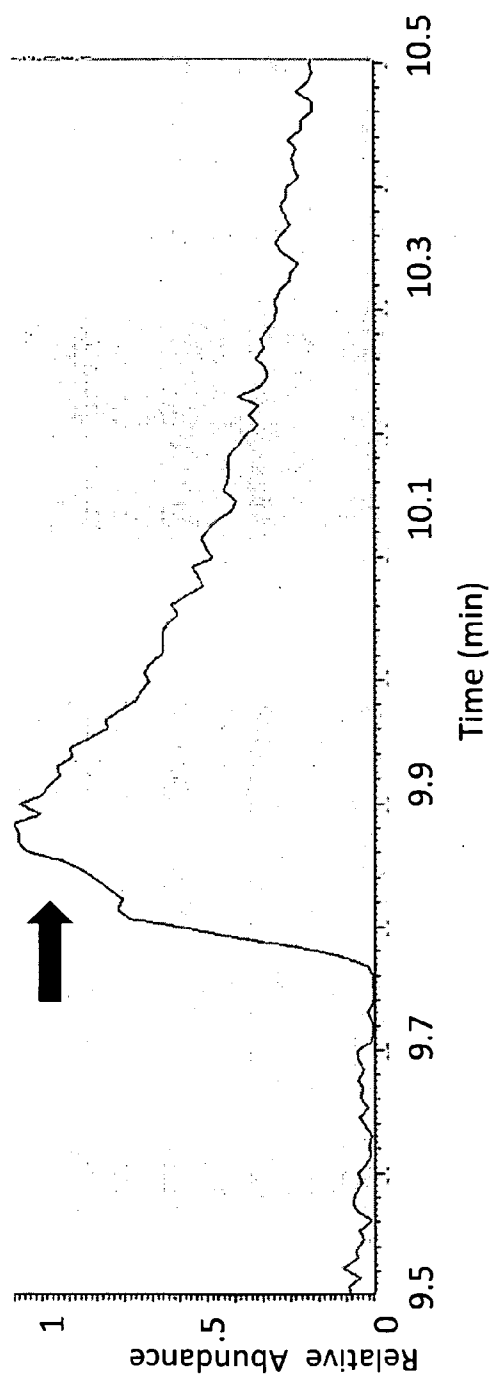


FIG. 53A

In vivo production 5-hydroxy-4-dodecanone from ligation reaction between octanal and butyraldehyde catalyzed by BAL.

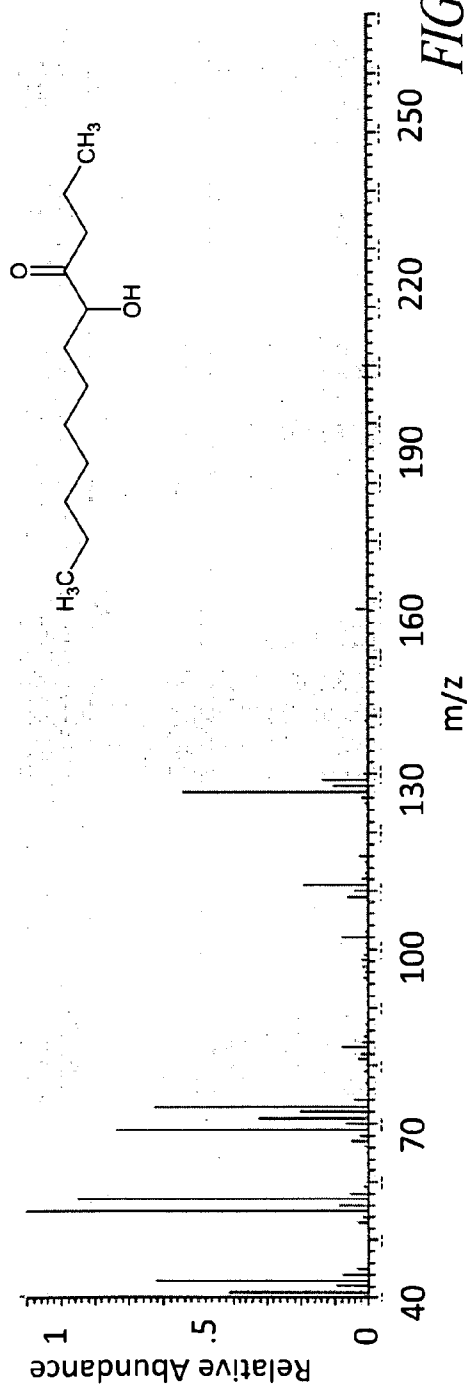
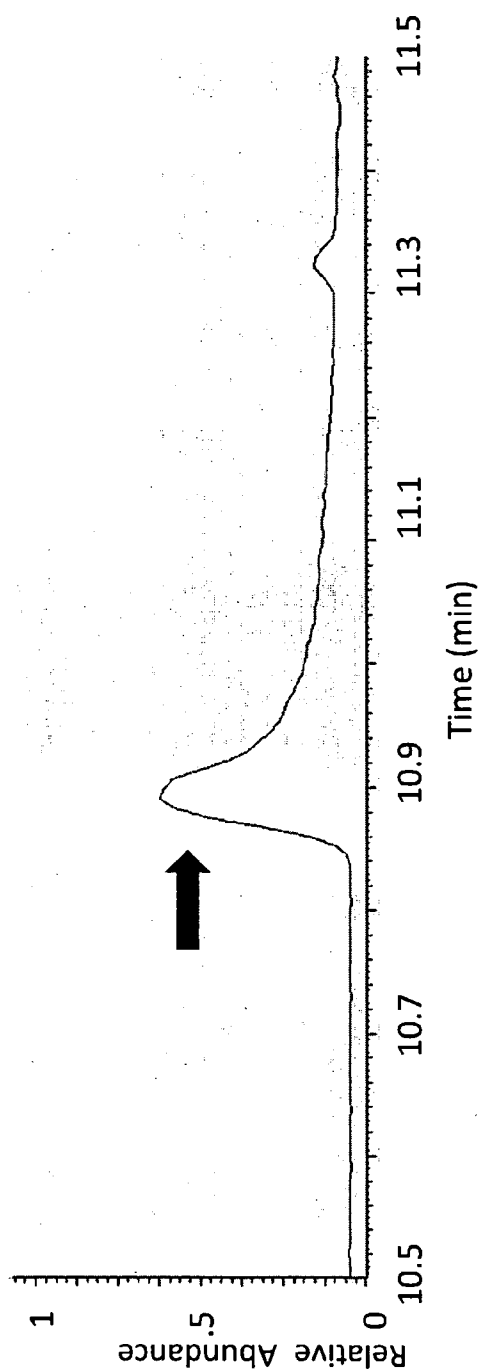


FIG. 53B

In vivo production of 6-hydroxy-5-tridecanone from ligation reaction between octanaldehyde and pentanaldehyde catalyzed by BAL enzyme

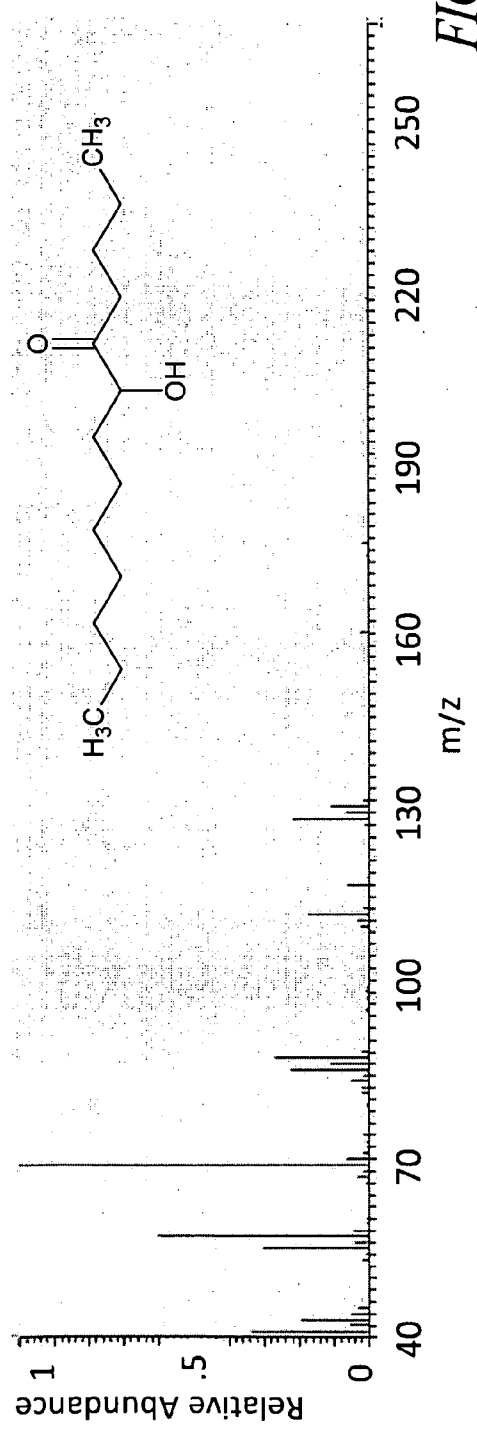
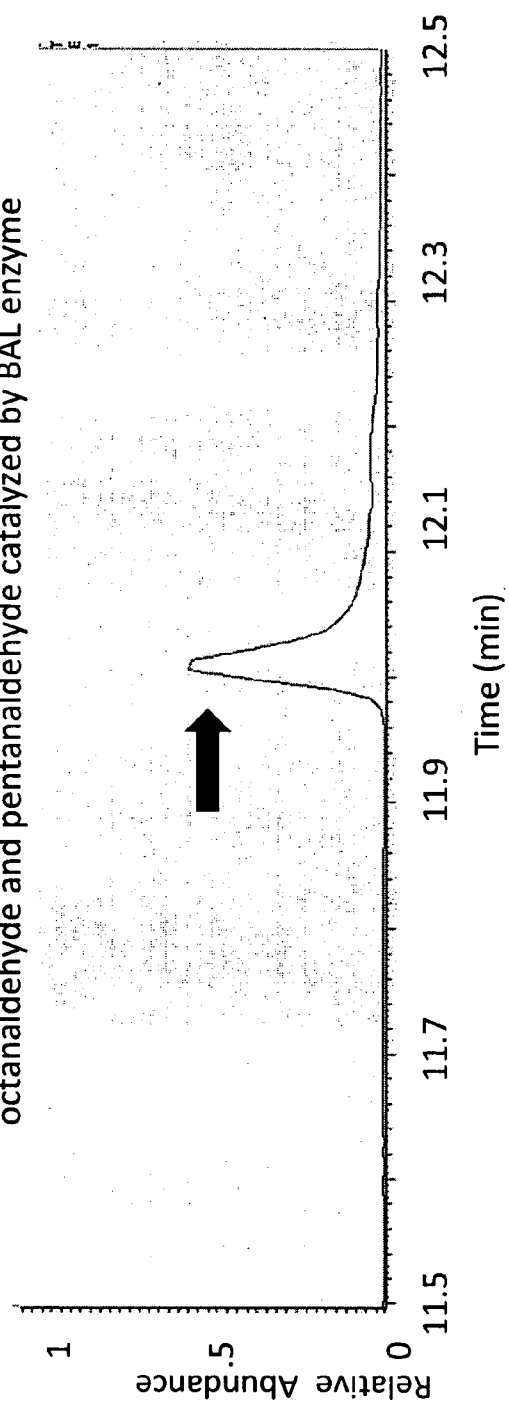


FIG. 54A

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

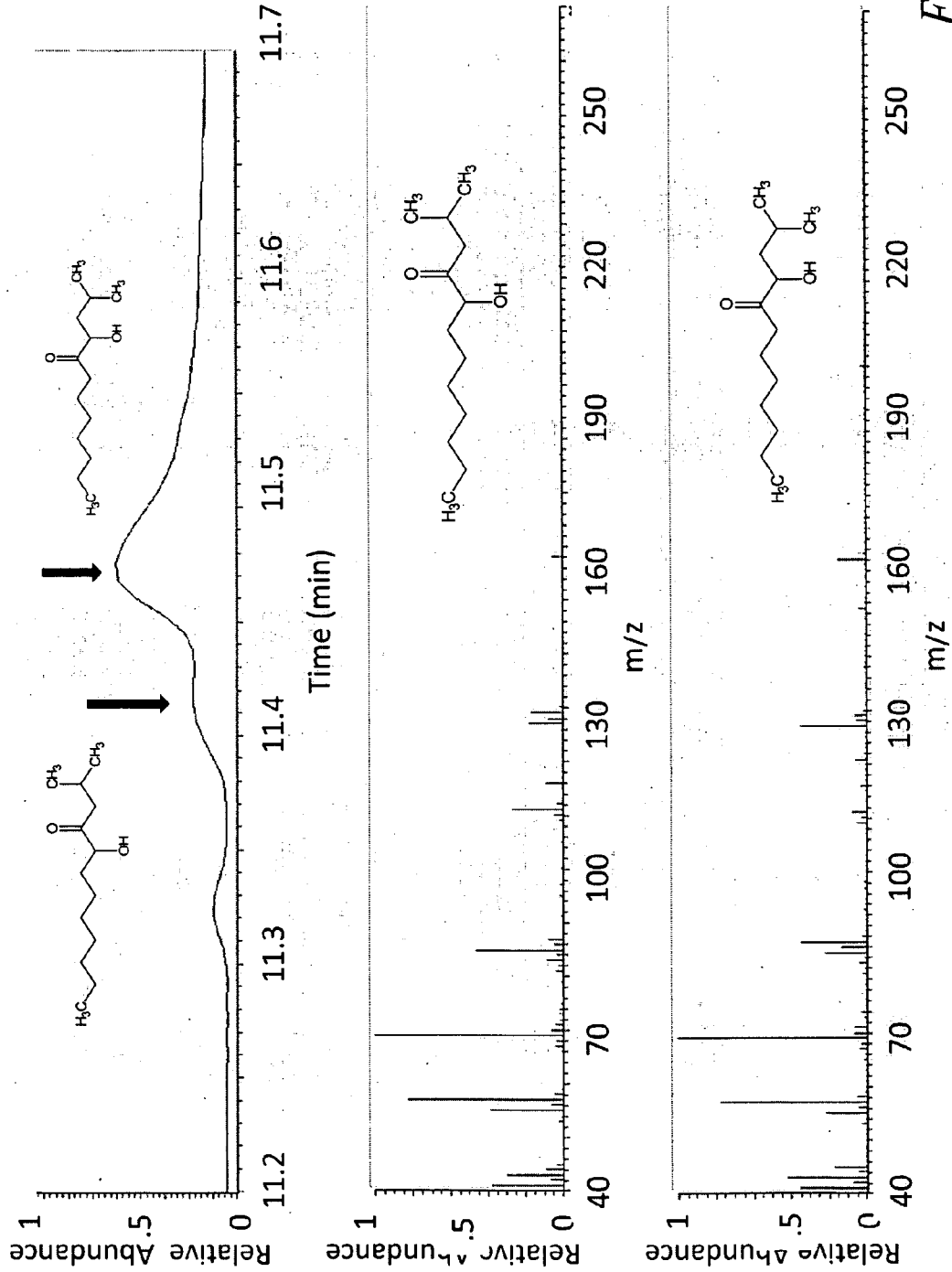


FIG. 54B

In vivo production 2-methyl-6-hydroxy-5-tridecanone from ligation reaction between octanaldehyde and 4-methylpentanal catalyzed by BAL enzyme

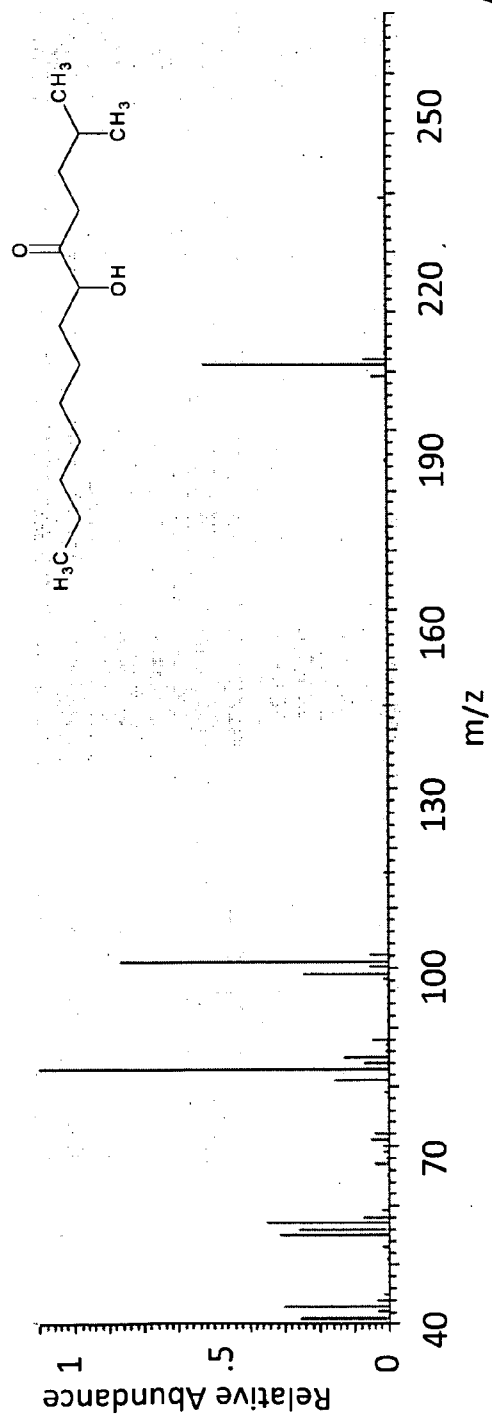
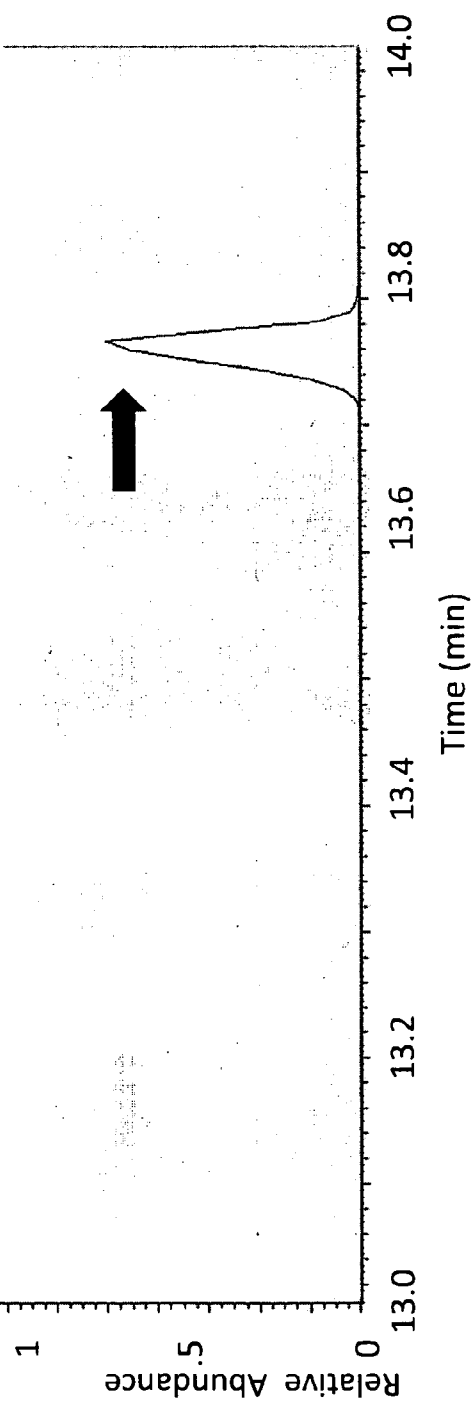


FIG. 55

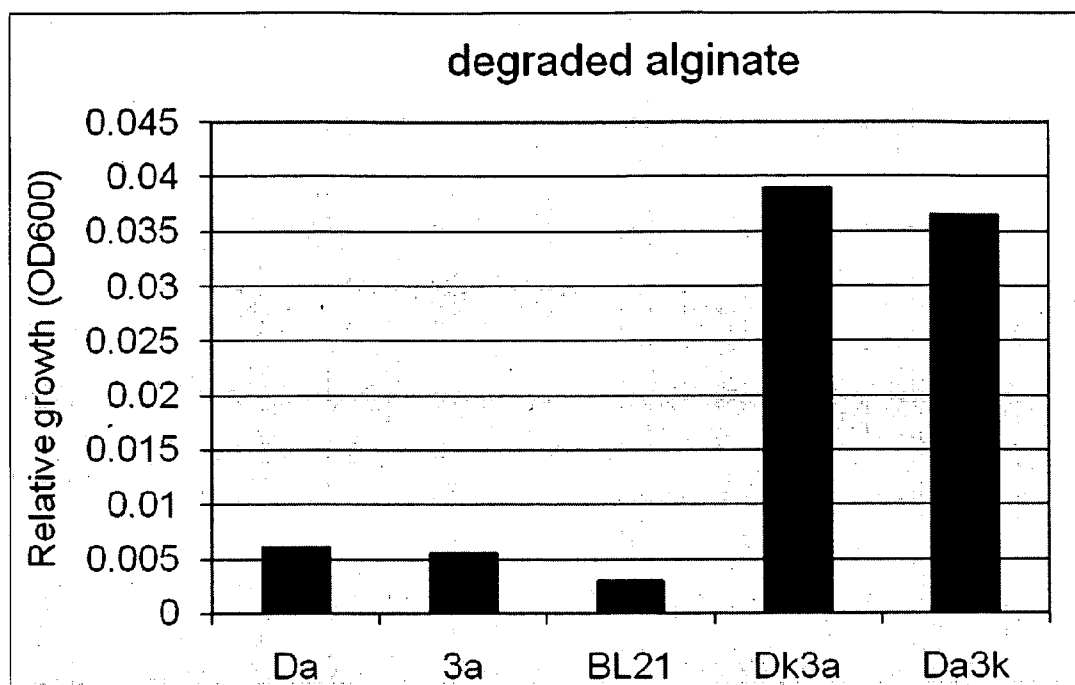


FIG. 56A

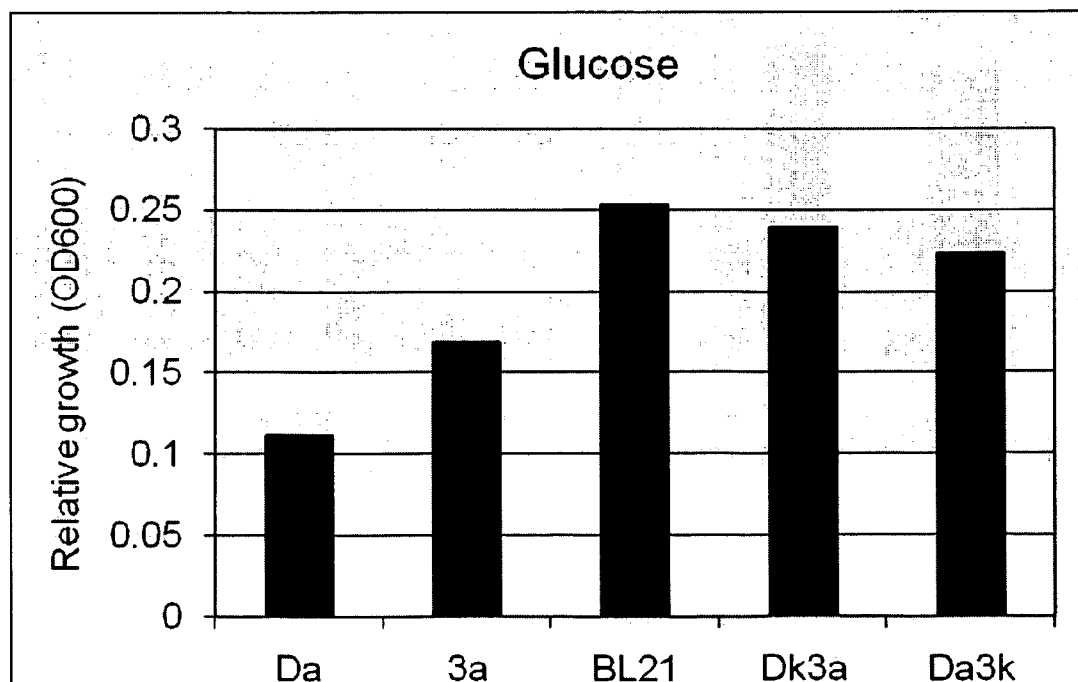
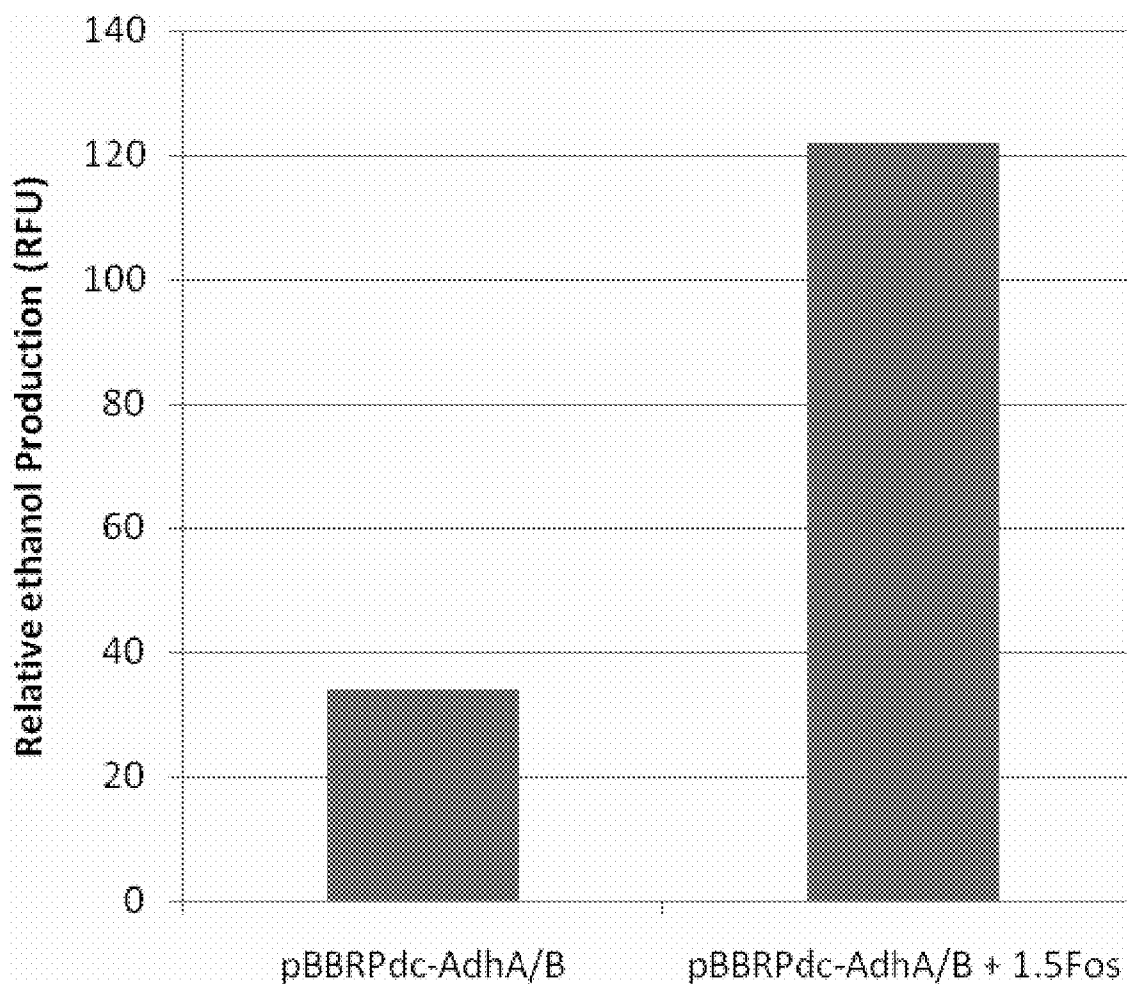


FIG. 56B

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_40102_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 CO₂ 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 lignocellulosic biomass, and there are many problems associated with using this process. Large-scale cultivation of lignocellulosic 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 lignocellulosic 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. 5A shows the growth of *E. coli* on various lengths of galacturonate after 24 hr. The recombinant strain in FIG. 5A 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. 5B shows the growth of recombinant *E. coli* on pectin after 3-4 days. The recombinant strain in FIG. 5B 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. 6A illustrates a simplified metabolic pathway for alginate degradation and metabolism. FIG. 6B shows the results of in vitro degradation of alginate to form pyruvate by an enzymatic degradation route. FIG. 6C 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. 7A shows DEHU hydrogenase activity as monitored by NADPH consumption, and FIG. 7B shows mannuronate hydrogenase activity as monitored by NADPH consumption.

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

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

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

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

[0020] FIG. 12 shows the biological activity of diol dehydratases. FIG. 12A shows the reduction of butyoin by ddh1, ddh2, and ddh3 as monitored by NADH consumption. FIG. 12B 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. 14A) (SEQ ID NO:97) and polypeptide sequence (FIG. 14B) (SEQ ID NO:98) of diol dehydrogenase DDH1 isolated from *Lactobacillus brevis* ATCC 367.

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

[0024] FIG. 16 shows the nucleotide sequence (FIG. 16A) (SEQ ID NO:101) and polypeptide sequence (FIG. 16B) (SEQ ID NO:102) of diol dehydrogenase DDH3 isolated from *Klebsiella 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-octanediol. FIG. 17A shows the detection of butyrolin (5-hydroxy-4-octanone) at 5.36 minutes, and FIG. 17B 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. 18A shows the detection of valeroin (6-hydroxy-5-decanone) at 8.22 minutes, and FIG. 18B 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. 19A shows the detection of isoveraloin (2,7-dimethyl-5-hydroxy-4-octanone) at 6.79 minutes, and FIG. 19B 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. 20A shows the detection of hexanoin (7-hydroxy-6-decanone) at 10.42 minutes, and FIG. 20B 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. 21A shows the detec-

tion of isohexanoin (2,9-Dimethyl-6-hydroxy-5-decanone) at 9.45 minutes, and FIG. 21B 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 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-propional into 4-hydroxy-3-hexanone and then 3,4-hexanediol. FIG. 24A shows the detection of propioin (4-hydroxy-3-hexanone) at rt=2.62 minutes, and FIG. 24B 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 phenylacetaldehyde 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 *Klebsiella pneumoniae* MGH 78578 (DDH3) and a diol dehydratase pduCDE from *Klebsiella pneumoniae* MGH 78578. FIG. 26A 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 butyrolin by ddh3. FIG. 26B 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 butyrolin 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 *Klebsiella pneumoniae* MGH 78578 (DDH3) and a diol dehydratase pduCDE from *Klebsiella pneumoniae* MGH 78578. FIGS. 27A and 27B 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 butyrolin using the enzymes diol dehydrogenase (ddh3) and a diol dehydratase (pduCDE).

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

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

isolated from *Klebsiella pneumoniae* MGH78578 (pduD), in addition to the nucleotide sequence (FIG. 29C) (SEQ ID NO:107) and polypeptide sequence (FIG. 29D) (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. 30A) and NADPH production (FIG. 30B).

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

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

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

[0042] FIG. 34 shows the oxidation and reduction of cyclopentanol by secondary alcohol dehydrogenases. FIG. 34A shows the oxidation of cyclopentanol as monitored by NADH or NADPH formation. FIG. 34B 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. 39A shows a list of rhamnogalacturonan lyase genes/proteins that may be utilized according to the methods and recombinant microorganisms described herein. FIG. 39B 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. 42A), 2-(4-hydroxyphenyl)ethanol (FIG. 42B), and 2-(indole-3-)ethanol (FIG. 42C) 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. 43B) at one week from the recombinant microorganisms described in Example 4 (pBADpheA-aroLAC-aroG-tktA-aroBDE and pTrcBALK). FIG. 43A 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. 45A), and the formation of 2-butanone from meso-2,3-butanediol (FIG. 45B), both of which were catalyzed in vitro by an isolated B12 independent diol dehydratase, as described in Example 9.

[0054] FIG. 46A shows the in vivo production of 1-propanol from 1,2-propanediol. FIG. 46B shows the in vivo production of 2-butanol from meso-2,3 butanediol. FIG. 46C 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. 48A), 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. 48B).

[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. 49A), 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. 49B).

[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. 50A), 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. 50B).

[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. 51A), and catalyzed the in vivo production of 7-methyl-4-hydroxy-3-octanone from a ligation reaction between 4-methylhexanal and propionaldehyde (FIG. 51B).

[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. 52A), and catalyzed the in vivo production of 3-hydroxy-2-decanone from a ligation reaction between acetaldehyde and octanal (FIG. 52B).

[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. 53A), and catalyzed the in vivo production of 5-hydroxy-4-dodecanone from a ligation reaction between octanal and butyraldehyde (FIG. 53B).

[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. 54A) 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. 54B).

[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. 56A), as described in Example 10. Growth on glucose (FIG. 56B) 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] Certain embodiments of the present invention relate to methods for converting a suitable monosaccharide or oligosaccharide to a commodity chemical, comprising: (a) contacting the suitable monosaccharide or oligosaccharide with a commodity chemical biosynthesis pathway, wherein the commodity chemical biosynthesis pathway comprises an aldehyde or ketone biosynthesis pathway, a C—C ligation pathway, and/or a dehydration and reduction pathway, thereby converting the suitable monosaccharide or oligosaccharide 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 suitable monosaccharide or oligosaccharide is obtained from a biomass-derived polysaccharide, wherein 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) gluconate, mannuronate, mannitol, lyxose, glycerol, xylitol, glucose, mannose, galactose, xylose, arabinose, glucuronate, galacturonates, and rhamnose, and D-mannitol.

[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, isobutanol, isobutanal, 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-butanediol, 1-phenyl-2,3-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-butanediol, 1-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 4-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-2,3-butanedione, indolyethane, indolyethene, 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-pentanedione, 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-pentanedione, 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-pentanedione, 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-pentanedione, 4-methyl-1-phenyl-3-hydroxy-2-pentanone, 4-methyl-1-phenyl-2-hydroxy-3-pen-

ethyl-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-hydroxy-2-butanone, 2,3-butanediol, 1,5-

pentane diol, homocitrate, homoisocitrate, 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, phenylacetaldehyde, 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-hydroxyphenyl)-4-phenylbutane, 1-(4-hydroxyphenyl)-4-phenyl-1-butene, 1-(4-hydroxyphenyl)-4-phenyl-2-butene, 1-(4-hydroxyphenyl)-4-phenyl-2-butanol, 1-(4-hydroxyphenyl)-4-phenyl-2-butanone, 1-(4-hydroxyphenyl)-4-phenyl-2,3-butanediol, 1-(4-hydroxyphenyl)-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-hydroxyphenylacetaldehyde, 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-acetaldehyde, 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 suitable monosaccharide or oligosaccharide to a commodity chemical comprising, (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 and expressing a biosynthesis pathway; (ii) one or more genes encoding and expressing a C—C ligation pathway; and (iii) a reduction and dehydration pathway, comprising one or more genes encoding and expressing an enzyme selected from a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase, thereby converting the suitable monosaccharide or oligosaccharide to the commodity chemical. In certain aspects, the biosynthesis pathway is selected from (a) an aldehyde biosynthesis pathway, (b) a ketone synthesis pathway, and (c) both (a) and (b).

[0071] In certain aspects, the biosynthesis pathway comprises an acetaldehyde biosynthesis pathway, and wherein

the acetoaldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to an acetoaldehyde. In certain aspects, the biosynthesis pathway comprises a propionaldehyde biosynthesis pathway, and wherein the propionaldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a propionaldehyde. In certain aspects, the biosynthesis pathway comprises a butyraldehyde biosynthesis pathway, and wherein the butyraldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a butyraldehyde.

[0072] In certain aspects, the biosynthesis pathway comprises a isobutyraldehyde biosynthesis pathway, and wherein the isobutyraldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a isobutyraldehyde. In certain aspects, the biosynthesis pathway comprises a 2-methylbutyraldehyde biosynthesis pathway, and wherein the 2-methylbutyraldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a 2-methylbutyraldehyde. In certain aspects, the biosynthesis pathway comprises a 3-methylbutyraldehyde biosynthesis pathway, and wherein the 3-methylbutyraldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a 3-methylbutyraldehyde.

[0073] In certain aspects, the biosynthesis pathway comprises a 4-methylpentanaldehyde biosynthesis pathway, and wherein the 4-methylpentanaldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a 4-methylpentanaldehyde. In certain aspects, the biosynthesis pathway comprises a phenylacetaldehyde biosynthesis pathway, and wherein the phenylacetaldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a phenylacetaldehyde. In certain aspects, the biosynthesis pathway comprises a 5-amino pentanaldehyde biosynthesis pathway, and wherein the 5-amino pentanaldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a 5-amino pentanaldehyde.

[0074] In certain aspects, the biosynthesis pathway comprises a 2-(4-hydroxyphenyl)acetaldehyde biosynthesis pathway, and wherein the 2-(4-hydroxyphenyl)acetaldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a 2-(4-hydroxyphenyl)acetaldehyde. In certain aspects, the biosynthesis pathway comprises a 2-(Indole-3-)acetaldehyde biosynthesis pathway, and wherein the 2-(Indole-3-)acetaldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a 2-(Indole-3-)acetaldehyde.

[0075] In certain aspects, the C—C ligation pathway comprises at least one enzyme selected from an acetoaldehyde lyase, a propionaldehyde lyase, a butyraldehyde lyase, an isobutyraldehyde lyase, a 2-methyl-butyr aldehyde lyase, a 3-methyl-butyr aldehyde lyase, a phenylacetaldehyde lyase, an oxaloacetate decarboxylase, an α -keto glutarate decarboxylase, an α -keto adipate decarboxylase, a pentanaldehyde lyase, a 4-methyl-pentanaldehyde lyase, a hexaldehyde lyase, a heptaldehyde lyase, an octaldehyde lyase, a 4-hydroxyphenylacetaldehyde lyase, an indoleacetaldehyde lyase, an indolephenylacetaldehyde lyase, a benzaldehyde lyase, a pyruvate decarboxylase, a benzformate lyase, and a 2-keto isovalerate decarboxylase. In certain aspects, the C—C ligation pathway comprises a C—C ligase or an optimized C—C ligase.

[0076] In certain aspects, the C—C ligase or optimized C—C ligase comprises at least one enzymatic activity selected from an acetoaldehyde lyase activity, a propionalde-

hyde lyase activity, a butyraldehyde lyase activity, an isobutyraldehyde lyase activity, a 2-methyl-butyr aldehyde lyase activity, a 3-methyl-butyr aldehyde lyase activity, a phenylacetaldehyde lyase activity, an oxaloacetate decarboxylase activity, an α -keto glutarate decarboxylase activity, an α -keto adipate decarboxylase activity, a pentanaldehyde lyase activity a 4-methyl-pentanaldehyde lyase activity, a hexaldehyde lyase activity, a heptaldehyde lyase activity, an octaldehyde lyase activity, a 4-hydroxyphenylacetaldehyde lyase activity, an indoleacetaldehyde lyase activity, an indolephenylacetaldehyde lyase activity, a benzaldehyde lyase activity, a pyruvate decarboxylase activity, a benzformate lyase activity, and a 2-keto isovalerate decarboxylase activity.

[0077] In certain aspects, the C—C ligation pathway comprises a benzaldehyde lyase, or a biologically active variant or fragment thereof. In certain aspects, the benzaldehyde lyase is derived from *Pseudomonas fluorescens*. In certain aspects, the benzaldehyde lyase comprises a polypeptide having 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: 144. In certain aspects, the amino acid sequence of the benzaldehyde lyase comprises one or more conserved residues selected from G27, E50, A57, G155, P162, P234, D271, G277, G422, G447, D448, and G512.

[0078] In certain aspects, the dehydration and reduction pathway comprises a diol dehydrogenase selected from 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 dehydrogenase, 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, and 2,3-hexanediol-1,6-dicarboxylic acid dehydrogenase. In certain aspects, the diol dehydrogenase comprises a polypeptide having 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 NOS:98, 100, or 102.

[0079] In certain aspects, the dehydration and reduction pathway comprises a diol dehydratase selected from 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-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, 1-phenyl-2,3-dodecanediol dehydratase, 1-(4-hydroxyphenyl)-2,3-dodecanediol dehydratase, 1-indole-2,3-dodecanediol dehydratase, 2-methyl-3,4-undecanediol dehydratase, 3-methyl-4,5-dodecanediol dehydratase, 2-methyl-4,5-dodecanediol 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, 1-(4-hydroxyphenyl)-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, and 2,3-hexanediol-1,6-dicarboxylic acid dehydratase.

[0080] In certain aspects, the diol dehydratase comprises a polypeptide having 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 NOS:104, 106, 108, 308, 309, 310, or 311. In certain aspects, the polypeptide of SEQ ID NO: 104 comprises one or more conserved residues selected 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.

[0081] In certain aspects, the polypeptide of SEQ ID NO:310 comprises one or more conserved residues selected from 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, N604, A654, G658, R659, D676, N702, Q735, N737, A747, P751, R760, V761, A762, G763, Q776, I780, and R782. In certain aspects, the polypeptide of SEQ ID NO:311 comprises one or more conserved residues selected from D19, G20, G22, R24, F28, G31, C32, C36, W38, C39, N41, P42, C58, C64, C96, G129, T132, G135, G136, D185, R187, N208, R222, and R264.

[0082] In certain aspects, the dehydration and reduction pathway comprises a secondary alcohol dehydrogenase selected from 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, and Indole-3-ethanol dehydrogenase.

[0083] In certain aspects, the secondary alcohol dehydrogenase comprises a polypeptide having 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 NOS: 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, and 142.

[0084] In certain aspects, the secondary alcohol dehydrogenase comprises at least one of a nicotinamide adenine dinucleotide (NAD⁺), a NADH, nicotinamide adenine dinucleotide phosphate (NADP⁺), or a NADPH binding motif. In certain aspects, the NAD⁺, NADH, NADP⁺, or NADPH binding motif is 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.

[0085] Certain embodiments include a recombinant microorganism, comprising (i) one or more genes encoding and expressing an aldehyde and/or ketone biosynthesis pathway; (ii) one or more genes encoding and expressing a C—C ligation pathway; and (iii) a reduction and dehydration pathway, comprising one or more genes encoding and expressing an enzyme selected from a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase. In certain aspects, the microorganism is capable of converting a suitable monosaccharide or suitable oligosaccharide to a commodity chemical, or an intermediate thereof.

[0086] In certain aspects, the one or more genes encoding the biosynthesis pathway encode a pathway selected from an acetoaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, 4-methylpentaldehyde, phenyl acetaldehyde, glutaraldehyde, 5-amino-pentaldehyde, succinate semialdehyde, succinate 4-hydroxyphenyl acetaldehyde, and an indole-3-acetaldehyde biosynthesis pathway, and combinations thereof.

[0087] In certain aspects, the one or more genes encoding and expressing the C—C ligation pathway comprise a nucleotide sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the nucleotide sequence set forth in SEQ ID NO:143. In certain aspects, the one or more genes encoding the diol dehydrogenase comprise a nucleotide sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the nucleotide sequence set forth in SEQ ID NOS:97, 99, or 101. In certain aspects, the one or more genes encoding the diol dehydratase comprise a nucleotide sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the nucleotide sequence set forth in SEQ ID NOS: 103, 105, or 107. In certain aspects, the one or more genes encoding a secondary alcohol dehydrogenase comprise a nucleotide sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the nucleotide sequence set forth in SEQ ID NOS: 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, or 141.

[0088] Certain embodiments include a recombinant microorganism, comprising one or more genes encoding and expressing an aldehyde or ketone biosynthesis pathway, wherein the pathway comprises at least one exogenous gene. Certain embodiments include a recombinant microorganism, comprising one or more exogenous genes encoding and expressing one or more enzymes selected from a C—C ligase, a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase. In certain aspects, the one or more enzymes comprise a C—C ligase and a diol dehydrogenase. In certain aspects, the one or more enzymes comprise a diol dehydrogenase and a diol dehydratase.

[0089] In certain aspects, the recombinant 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.

[0090] In certain aspects, the microorganism is a bacteria. In certain aspects, the microorganism is a gram-negative bacteria. In certain aspects, the microorganism is a eukaryote. In certain aspects, the eukaryote is a fungus. In certain aspects, the fungus is a yeast.

[0091] Certain embodiments include methods for converting a suitable monosaccharide to a commodity chemical comprising, (a) contacting the suitable monosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide to the commodity chemical, wherein the microbial system comprises, (i) one or more genes encoding and expressing a pathway selected from a fatty acid biosynthesis pathway, an amino acid biosynthetic pathway, and 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 or oligosaccharide to the commodity chemical.

[0092] Certain embodiments include a recombinant microorganism, comprising (i) one or more genes encoding and expressing a pathway selected from a fatty acid biosynthesis pathway, an amino acid biosynthetic pathway, and 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).

[0093] In certain aspects, the recombinant microorganism or microbial systems described herein comprise a microorganism selected from *Acetobacter aceti*, *Achromobacter*, *Acidiphilium*, *Acinetobacter*, *Actinomadura*, *Actinoplanes*, *Aeropyrum pernix*, *Agrobacterium*, *Alcaligenes*, *Ananas comosus* (M), *Arthrobacter*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus melleus*, *Aspergillus pulverulentus*, *Aspergillus saitoi*, *Aspergillus sojae*, *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*, *Erwinia chrysanthemi*, *Gliconobacter*, *Gluconacetobacter*, *Haloarcula*, *Humicola insolens*, *Humi-*

cola insolens, Kitasatospora setae, Klebsiella, Klebsiella oxytoca, Kluyveromyces, Kluyveromyces fragilis, Kluyveromyces lactis, Kocuria, Lactolactis, Lactobacillus, Lactobacillus fermentum, Lactobacillus sake, Lactococcus, Lactococcus lactis, Leuconostoc, Methylocystis, Methanobolus siciliae, Methanogenium organophilum, Methanobacterium bryantii, Microbacterium imperiale, Micrococcus lysodeikticus, Microlophus, Mucor javanicus, Mycobacterium, Myrothecium, Nitrobacter, Nitrosomonas, Nocardia, Papaya carica, Pediococcus, Pediococcus halophilus, Penicillium, Penicillium camemberti, Penicillium citrinum, Penicillium emersonii, Penicillium roqueforti, Penicillium lilactinum, Penicillium 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, Saccharomyces cerevisiae, Sclerotinia libertina, Sphingobacterium multivorum, Sphingobium, Sphingomonas, Streptococcus, Streptococcus thermophilus Y-1, Streptomyces, Streptomyces griseus, Streptomyces lividans, Streptomyces murinus, Streptomyces rubiginosus, Streptomyces violaceoruber, Streptovercillium mobaraense, Tetragenococcus, Thermus, Thiosphaera pantotrophica, Trametes, Trichoderma, Trichoderma longibrachiatum, Trichoderma reesei, Trichoderma viride, Trichosporon penicillatum, Vibrio alginolyticus, Xanthomonas, yeast, Zygosaccharomyces rouxii, Zymomonas, and Zymomonas mobilis.

[0094] 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.

[0095] 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

[0096] 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.

[0097] 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.

[0098] As an additional advantage, and in contrast to lignocellulosic 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 lignocellulosic 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).

[0099] As an additional example, biomass from plants such as fruit, certain plants, 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.

[0100] 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.

[0101] 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.

[0102] 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.

[0103] 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 blend-stock for jet and diesel fuels, refineries may reduce Green House Gas emissions by more than 80%.

[0104] 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 a 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.

[0105] 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

[0106] 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.

[0107] 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.

[0108] 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, 15, 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.

[0109] 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.

[0110] 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.

[0111] 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.

[0112] 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.

[0113] 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.

[0114] 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.

[0115] 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.

[0116] 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.

[0117] 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.

[0118] 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.

[0119] 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.

[0120] As used herein, the terms “function” and “functional” and the like refer to a biological or enzymatic function.

[0121] 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).

[0122] “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.

[0123] 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 microorganism.

[0124] 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.

[0125] 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.

[0126] 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*.

[0127] 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., CO₂ concentration, nutrient levels, light, heat). In the absence of that condition, inducible promoters typically do not allow significant or measurable levels of transcriptional activity.

[0128] 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.

[0129] 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.

[0130] 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.

[0131] 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.

[0132] 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.

[0133] 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.

[0134] 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 dehydrogenase, pectate lyase, alginate lyase, diol dehydratase, transporter, etc.).

[0135] 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.

[0136] 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.

[0137] 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 NaHPO₄ (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 NaHPO₄ (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).

[0138] “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 NaHPO₄ (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 NaHPO₄ (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.

[0139] “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 NaHPO₄ (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 NaHPO₄ (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.

[0140] 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.

[0141] 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.

[0142] 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).

[0143] In general, the T_m of a perfectly matched duplex of DNA may be predicted as an approximation by the formula: $T_m = 81.5 + 16.6 (\log_{10} M) + 0.41 (\% G+C) - 0.63 (\% \text{formamide}) - (600/\text{length})$ 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 $T_m - 15^\circ \text{C.}$ for high stringency, or $T_m - 30^\circ \text{C.}$ for moderate stringency.

[0144] 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% polyvinylpyrrolidone 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.

[0145] 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.

[0146] 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*.

[0147] 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.

[0148] 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).

[0149] "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.

[0150] 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.

[0151] 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 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.

[0152] 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 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.

[0153] A "recombinant" microorganism typically comprises one or more exogenous nucleotide sequences, such as in a plasmid or vector.

[0154] 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.

[0155] 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.

[0156] "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.

[0157] 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.

[0158] 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.

[0159] 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).

[0160] 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.

[0161] 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.

[0162] Certain examples of alginate-derived polysaccharides include saturated polysaccharides, such as β -D-mannuronate, α -L-gluronate, dialginate, trialginate, pentalginate, hexalginate, heptalginate, octalginate, nonalginate, decalginate, undecalginate, dodecalginate and polyalginate, as well as unsaturated polysaccharides such as 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)-hexalginate, 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.

[0163] 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.

[0164] 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).

[0165] 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, hemicellulose 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.

[0166] 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.

[0167] 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, ethylbenzene, 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-butanediol, 1-phenyl-3-hydroxy-2-butanone, 4-phenyl-3-hydroxy-2-butanone, 1-phenyl-2,3-butanedione, n-pentane, ethylphenol, ethylphenol, 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-butanediol, 1-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 4-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-2,3-butanedione, indolyethane, indolyethene,

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-2-pentanol, 4-methyl-1-phenyl-3-pentanol, 4-methyl-1-phenyl-2-pentanone, 4-methyl-1-phenyl-3-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)-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)-3-pentanol, 4-methyl-1-(4-hydroxyphenyl)-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-3-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-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)-3-hexanol, 5-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-hy-

droxy-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-hydroxy-2-butanone, 2,3-butanediol, 1,5-pentane diol, homocitrate, homoisocitrate, 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, phenylacetaldehyde, 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-hydroxyphenyl)-4-phenylbutane, 1-(4-hydroxyphenyl)-4-phenyl-1-butene, 1-(4-hydroxyphenyl)-4-phenyl-2-butene, 1-(4-hydroxyphenyl)-4-phenyl-2-butanol, 1-(4-hydroxyphenyl)-4-phenyl-2-butanone, 1-(4-hydroxyphenyl)-4-phenyl-2,3-butanediol, 1-(4-hydroxyphenyl)-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-hydroxyphenylacetaldehyde, 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)-2,3-butanediol, 1-(4-hydroxyphenyl)-4-(indole-3)-3-hydroxy-2-butanone, indole-3-acetaldehyde, 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.

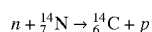
[0168] The recitation “optimized” as used herein refers to a pathway, gene, polypeptide, enzyme, or other molecule having an altered biological activity, such as by the genetic alteration of a polypeptide’s amino acid sequence or by the alteration/modification of the polypeptide’s surrounding cellular 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.

[0169] 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.

[0170] 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 integers 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.

[0171] 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:



[0172] 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.

[0173] 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.

[0174] 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.

[0175] 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.

[0176] 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.

[0177] 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.

[0178] 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.

[0179] ALs are mainly classified into two distinctive sub-families 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.

[0180] 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 AI include five endo-acting ALs, AI-I, AI-II, AI-II', AI-III, and AI-IV' and an exo-acting AL, AI-IV.

[0181] Typically, AI-I, AI-II, and AI-III have molecular weights of 66 kDa, 25 kDa, and 40 kDa, respectively. AI-II and AI-III are self-splicing products of AI-I. AI-II may be more specific to G and AI-III may be specific to M. AI-I may have high activity for both M and G. AI-IV has molecular weight of about 85 kDa and catalyzes exo-lytic depolymerization of oligoalginate. Although both AI-II' and AI-IV' are functional homologues of AI-II and AI-IV. AI-II' has endolytic activity and may have no preference to M or G. AI-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 AI-I, AI-II', AI-IV, and Atu3025, and may include optimal codon usage for the suitable host organisms, such as *E. coli*.

[0182] 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 polypeptide (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 enzymes described in FIG. 37, as well as the secreted alginate lyase encoded by Vs24254 from *Vibrio splendidus*.

[0183] 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.

[0184] 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.

[0185] 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.

[0186] 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.

[0187] 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*.

[0188] 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.

[0189] 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.

[0190] 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.

[0191] A microbial system or recombinant microorganism according to the present invention may also comprise permeases/transporters/superchannels/porins that catalyze the transport of polysaccharides and monosaccharides (e.g., D-mannuronate and D-lyxose) from the 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.

[0192] 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-mannuronate 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 improved transport of certain monosaccharides, such as D-mannuronate, DEHU, and D-lyxose.

[0193] Certain aspects may employ a recombinant microorganism that comprises a "superchannel," by which aquatic or marine-biomass polysaccharides such as alginate poly-

mers, 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.

[0194] As one particular example of a microorganism comprising a superchannel, *Sphingomonas* sp. strain A1 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 AlgQ2), 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.

[0195] 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.

[0196] 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).

[0197] 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 At1557 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:71 shows the nucleotide and SEQ ID NO:72 shows the polypeptide sequence of ADH2 At2022 isolated from *Agrobacterium tumefaciens* C58. SEQ ID NO:73 shows the nucle-

otide and SEQ ID NO:74 shows the polypeptide sequence of ADH3 Atu0626 isolated from *Agrobacterium tumefaciens* C58.

[0198] 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.

[0199] 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.

[0200] SEQ ID NO:87 shows the nucleotide and SEQ ID NO:88 shows the polypeptide sequence of ADH10 Atu4289 isolated from *Agrobacterium tumefaciens* C58. 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.

[0201] 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.

[0202] 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.

[0203] 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.

[0204] Certain embodiments may include a microbial system or isolated microorganism with enhanced efficiency for converting monosaccharides such as D-mannuronate and D-xy-

lulose into monosaccharides suitable for a biofuel biosynthesis pathway such as KDG. Merely by way of explanation, D-mannuronate and D-xylulose are metabolites in microbes such as *E. coli*. D-mannuronate is converted by a D-mannuronate dehydratase to KDG. D-xylulose enters the pentose phosphate pathway. Thus, to increase conversion of D-mannuronate to KDG, an exogenous or endogenous D-mannuronate dehydratase (e.g., *uxuA*) gene may be over-expressed in 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.

[0205] 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 dehydratase, 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.

[0206] 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.

[0207] 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.

[0208] 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 ggxgdxxxx (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 ggxgdxxxx (SEQ ID NO:145) repeat.

[0209] 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.

[0210] 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.

[0211] 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.

[0212] 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-deoxygluconokinase.

[0213] 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.

[0214] 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.

[0215] 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.

[0216] 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.

[0217] 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.

[0218] 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.

[0219] 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.

[0220] 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.

[0221] 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.

[0222] 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.

[0223] 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.

[0224] 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.

[0225] 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.

[0226] 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.

[0227] FIG. 32 shows the nucleotide coding sequence and polypeptide sequence of putative protein V12B01_02425. FIG. 32A shows the nucleotide sequence that encodes the putative protein V12B01_02425 (SEQ ID NO:41). FIG. 32B shows the polypeptide sequence of putative protein V12B01_02425 (SEQ ID NO:42), which is similar to a type II secretory pathway component EpsC.

[0228] 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.

[0229] 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.

[0230] 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.

[0231] 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.

[0232] 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.

[0233] 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.

[0234] 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.

[0235] 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.

[0236] 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.

[0237] 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.

[0238] 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.

[0239] 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 relatively 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).

[0240] 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.

[0241] 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.

[0242] 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.

[0243] Polygalacturonases, rhamnogalacturonan lyases, and rhamnogalacturonan hydrolases 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 rhamnogalacturonan lyases (i.e., rhamnogalacturonases) described in FIG. 39A. Examples of rhamnogalacturonate hydrolases 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. 39B.

[0244] 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 methyl 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.

[0245] 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.

[0246] Di- and tri-galacturonates 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 transporter systems, such as TogT and/or TogMNAB.

[0247] 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.

[0248] In certain aspects, a recombinant microorganism, such as *E. coli*, that is able to grow 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* SCR11043. SEQ ID NO:66 shows the nucleotide sequence of ogl-kdgR from *Erwinia carotovora* subsp. *Atroseptica* SCR11043.

[0249] In certain aspects, a recombinant microorganism, such as *E. coli*, that is able to grow 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 acetylsterases (paeY), and pectin methylsterase (pem) (see Example 2).

[0250] Additional examples of isomerases that may be utilized herein include gluconate isomerases, such as those in the family uxaC, as well as 4-deoxy-L-threo-5-hexulose 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 dehydratases 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.

[0251] Certain aspects may 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-phosphate-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.

[0252] 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 grow on alginate may be engineered to produce an increased amount of ethanol from alginate as compared to *E. coli* that is not engineered to grow 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 gene, 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*.

[0253] 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.

[0254] 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.

[0255] An enzymatic pathway may utilize one or more enzymes described herein that are capable of catalyzing the degradation of polysaccharides, such as alginate or pectin.

[0256] 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.

[0257] 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.

[0258] 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.

[0259] 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.

[0260] 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.

[0261] 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.

[0262] 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.

[0263] 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.

[0264] 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-octanediol. 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.

[0265] 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.

[0266] 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.

[0267] 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.

[0268] 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 uti-

lized in other embodiments of a microbial system or isolated microorganism of the present application.

[0269] 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 acetaldehyde formation because of its promiscuous acetyl-CoA deacylating activity. In certain embodiments, the formation of acetaldehyde may be minimized by functionally redesigning the relevant enzyme(s).

[0270] 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.

[0271] 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 acetaldehyde 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.

[0272] 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.

[0273] 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 homocitrate. This reaction is catalyzed by homocitrate synthase derived from Yeast, *Thermus thermophilus*, or *Deinococcus radiodurans*. Homocitrate derived from Yeast, *Thermus thermophilus*, or *Deinococcus radiodurans* catalyzes the conversion between homocitrate and homoisocitrate. Homo-isocitrate is then oxi-

datively 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.

[0274] 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.

[0275] In synthesizing glutaraldehyde, Acyl-CoA thioesterases (ACOT) may also catalyze the hydrolysis of glutaryl-CoA. The genes encoding ω -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.

[0276] 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 succinaldehyde.

[0277] 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 include one or more of an acetoaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, 4-methyl-pentaldehyde, 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.

[0278] 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.

[0279] 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.

[0280] In certain aspects, a butyraldehyde biosynthesis 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.

[0281] 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-dihydroxyisovalerate 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*.

[0282] 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*.

[0283] 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*.

[0284] 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.

[0285] In certain aspects, such as for one step in cyclopentanol production, a α -keto adipate 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 -keto adipate semialdehyde dehydrogenase gene. Such a biosynthesis pathway would be able to convert acetyl-CoA and α -ketoglutarate to α -keto adipate semialdehyde.

[0286] 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 decarboxylation 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.

[0287] In certain aspects, a 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and 2-(indole-3-yl)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 a benzaldehyde lyase (bal) from *Pseudomonas fluorescens*.

[0288] 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.

[0289] 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 (butyroids) 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, carbonylation, and to the cleavage of C—C bonds.

[0290] 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 carbonylation reaction between two aldehydes. BAL accepts the broadest spectrum of aldehydes as substrates among these three enzymes ranging from substituted benzaldehyde to acetaldehyde, among others, as shown herein. BAL catalyzes stereospecific carbonylation reaction between two aldehydes and forms α -hydroxy ketone with 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.

[0291] BFD and PCD may also catalyze the carbonylation reactions between two aldehyde molecules. BFD and PCD accept relatively larger and smaller aldehyde molecules, respectively. With the presence of benzaldehyde and acetaldehyde, 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.

[0292] 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, acetaldehyde lyases, propionaldehyde lyases, butyraldehyde lyases, isobutyraldehyde lyases, 2-methyl-butyraldehyde lyases, 3-methyl-butyraldehyde lyases (isovaldehyde), phenylacetaldehyde lyases, α -keto adipate carboxylases, pentaldehyde lyases, 4-methyl-pentaldehyde lyases, hexaldehyde lyases, heptaldehyde lyases, octaldehyde lyases, 4-hydroxyphenylacetaldehyde lyases, indoleac-

etaldehyde 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.

[0293] 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 carbonylation 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.

[0294] *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 carbonylation 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 hexaldehyde lyase activity, a phenylacetaldehyde lyase activity, and an octaldehyde lyase activity (see Table 2), among other in vivo lyase activities (see FIGS. 48-55).

[0295] 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.

[0296] 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.

[0297] 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.

[0298] 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 dihydroxyacetone, respectively (40-200%). As one further example, glycerol dehydrogenase discovered in *Hansenula polymorpha* 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 polymorpha* 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 dehydrogenases may also be obtained from *Lactobacillus brevis* ATCC 367, *Pseudomonas putida* KT2440, and *Klebsiella pneumoniae* MGH78578), as described herein (see Example 5).

[0299] 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 dehydroge-

nase, 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 dehydrogenase, 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.

[0300] 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.

[0301] 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 polypeptide 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 comprise nucleotide sequences or polypeptide sequences having 80%, 85%, 90%, 95%, 97%, 98%, 99% sequence identity to SEQ ID NOS:97-102.

[0302] Other embodiments may comprise re-designed diol dehydrogenases for reduction of 1-hydroxy propanal, succinaldehyde, and glutaraldehyde to 1,3-propanediol, 1,4-butanediol, and 1,5-pentanediol, respectively, among others.

[0303] 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.

[0304] 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). FIG. 46A shows the in vivo production of 1-propanol from 1,2-propanediol, FIG. 46B shows the in vivo production of 2-butanol from meso-2,3-butanediol, and FIG. 46C shows the in vivo production of cyclopentanone from trans-1,2-cyclopentanediol.

[0305] 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.

[0306] 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-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.

[0307] 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.

[0308] 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 polypeptide 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 comprise 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.

[0309] 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, N604, A654, G658, R659, D676, N702, 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, N208, R222, and/or R264.

[0310] 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 butyrolin 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.

[0311] 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.

[0312] 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)-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-hydroxyph-

nyl)-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.

[0313] 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.

[0314] 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 polypeptide 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 comprise nucleotide sequences or polypeptide sequences having 80%, 85%, 90%, 95%, 97%, 98%, 99% sequence identity to SEQ ID NOS:109-142.

[0315] 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.

[0316] 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.

[0317] 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.

[0318] 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.

[0319] 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.

[0320] 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.

[0321] 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 *Klebsiella 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.

[0322] 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 *Klebsiella 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.

[0323] 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.

[0324] 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 polymorpha* 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 polymorpha*, *Klebsiella pneumoniae*, or any other suitable organism.

[0325] 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.

[0326] 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.

[0327] 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.

[0328] For example, certain embodiments include a method wherein the C—C ligation pathway may comprise an acetaldehyde 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.

[0329] 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.

[0330] 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.

[0331] 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.

[0332] 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.

[0333] 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.

[0334] 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.

[0335] Additional embodiments include a method wherein the C—C ligation pathway may comprise an α -keto adipate carboxylase, 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/propionaldehyde 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.

[0336] 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.

[0337] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an acetoaldehyde/hexaldehyde 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.

[0338] 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.

[0339] 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 dehydratase, 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 dehydratase, and a 6-methyl-2(or 3)-heptanol dehydrogenase.

[0340] 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.

[0341] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of an acetaldehyde/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.

[0342] 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/hexaldehyde 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.

[0343] 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.

[0344] 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.

[0345] 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.

[0346] 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.

[0347] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a propionaldehyde/indoleacetaldehyde 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.

[0348] Additional embodiments include a method wherein the C—C ligation pathway may comprise at least one of a butyraldehyde/hexaldehyde 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.

[0349] 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. 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.

[0350] 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 dehydroge-

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.

[0380] 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.

[0381] 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.

[0382] 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.

[0383] 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.

[0384] As noted above, for the production of certain commodity chemicals, such as 2-phenylethanol, 2-(4-hydrox-

ylphenyl)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.

[0385] In these and other embodiments, a recombinant microorganism may comprise an aldehyde reductase, such as a phenylacetaldehyde 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-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.

[0386] 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).

[0387] 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.

[0388] 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)).

[0389] 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.

[0390] 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.

[0391] 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.

[0392] 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*.

[0393] 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.

[0394] 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.

[0395] 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 *Botryococcus 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 *Botryococcus braunii*.

[0396] 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-protoporphyrin 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.

[0397] 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 protoporphyrin 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.

[0398] 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*.

[0399] 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 bacte-

rial strain DH-1. By way of explanation, bacterial strain DH-1 produces n-alkenes in addition to n-alkanes.

[0400] 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.

[0401] 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 microorganism 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.

[0402] 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 oryzae*, *Aspargillus melleus*, *Aspargillus pulverulentus*, *Aspargillus saitoi*, *Aspargillus sojae*, *Aspargillus usamii*, *Bacillus alcalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus macerans*, *Bacillus steartophilus*, *Bacillus subtilis*, *Bifidobacterium*, *Brevibacterium 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 insolens*, *Kitasatospora setae*, *Klebsiella*, *Klebsiella oxytoca*, *Kluyveromyces*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Kocuria*, *Lactolactis*, *Lactobacillus*, *Lactobacillus fermentum*, *Lactobacillus sake*, *Lactococcus*, *Lactococcus lactis*, *Leuconostoc*, *Methylocystis*, *Methanolobus siciliae*, *Methanogenium organophilum*, *Methanobacterium bryantii*, *Microbacterium imperiale*, *Micrococcus lysodeikticus*, *Micrococcus*, *Mucor javanicus*, *Mycobacterium*, *Myrothecium*, *Nitrobacter*, *Nitrosomonas*, *Nocardia*, *Papaya carica*, *Pediococcus*, *Pediococcus halophilus*, *Penicillium*, *Penicillium camemberti*, *Penicillium citrinum*, *Penicillium emersonii*, *Penicillium roqueforti*, *Penicillium lilactinum*, *Penicillium multicolor*, *Paracoccus pantotrophus*, *Propionibacterium*, *Pseudomonas*, *Pseudomonas fluorescens*, *Pseudomonas denitrificans*, *Pyrococcus*, *Pyrococcus furiosus*, *Pyrococcus horikoshii*, *Rhizomucor*, *Rhizomucor miehei*, *Rhizomucor pusillus* Lindt, *Rhizopus*, *Rhizopus delemar*, *Rhizopus japonicus*, *Rhizopus niveus*, *Rhizopus oryzae*, *Rhizopus oligosporus*, *Rhodococcus*, *Sccharomyces cerevisiae*, *Sclerotinia 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 pantotrophia*, *Trametes*, *Trichoderma*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, *Trichoderma viride*, *Trichosporon penicillatum*, *Vibrio alginolyticus*, *Xanthomonas*, yeast, *Zygosaccharomyces rouxii*, *Zymomonas*, and *Zymomonas mobilis*, may be utilized as recombinant microorganisms provided herein, and, thus, may be utilized according to the various methods of the present invention.

[0403] 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

[0404] 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*.

[0405] 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 pCC1FOS (Epicentre, Madison, Wis.).

[0406] 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).

[0407] 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 1xM9 salts, 2 mM MgSO₄, 100 μ M CaCl₂, 0.007% Leucine, 0.01% casamino acids, 1.5% NaCl (this includes all sources of sodium: M9, diluted alginate and added NaCl).

[0408] 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.

[0409] 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).

[0410] 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.

[0411] 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—GGGCGGCCGCAAGGGGTTTCGCGTTGGCCGA (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

[0412] 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 grow on these polysaccharides.

[0413] The pBBRGal3P plasmid was engineered to contain certain genomic region of *Erwinia carotovora* subsp. *Atroseptica* SCRI 1043, 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* SCRI 1043.

[0414] 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* SCRI 1043 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 AAGTTGCAGGATATGAC-GAAAGCG-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* SCRI 1043 genome (ATCC) in 50 μl.

[0415] 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 GGGGTGCCTAATGAGT-GAGCTAAC-3') (SEQ ID NO:151) and reverse (5'-CGG-GATCC GCGTTAATATTTGTAAAATTCGC-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.

[0416] The pTrcogl-kdgR plasmid was engineered to contain certain genomic regions of *Erwinia carotovora* subsp. *Atroseptica* SCRI 1043, 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* SCRI 1043.

[0417] To prepare this construct, the DNA sequence encoding ogl and kdgR of *Erwinia carotovora* subsp. *Atroseptica* SCRI 1043 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* SCRI 1043 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.

[0418] 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 μg/ml kanamycin and 100 μg/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 μl of culture was transferred to eppendorf tube and centrifuged to pellet the cells. The cells were resuspended into 50 μl of M9 media containing 2 mM MgSO₄, 100 μM CaCl₂, 0.4% di- or trigalacturonate, and 5 μl of this solution was inoculated into 500 μl of fresh M9 media containing 2 mM MgSO₄, 100 μM CaCl₂, 0.4% di- or trigalacturonate. The culture was grown in incubation shaker with 200 rpm at 37° C.

[0419] The results in FIG. 5A 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*.

[0420] Based on the information obtained from the above experiments, it was considered whether the introduction of pectate lyase, pectate acetyltransferase, and methyltransferase might confer *E. coli* capability of growing on pectin. To test

this hypothesis, *E. coli* strain DH5 α bacterial cells were engineered to contain both the pROU2 plasmid and the pPEL74 plasmid.

[0421] 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.

[0422] 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 acetyltransferases (paeY), and pectin methyl-esterase (pem).

[0423] As shown in FIG. 5B, *E. coli* DH5 α 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

[0424] 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.

[0425] 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.

[0426] 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).

[0427] 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.

[0428] KDG is readily metabolized to form of pyruvate and glyceraldehydes-3-phosphate (G3P). KDG is first phospho-

rylated 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.

[0429] 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 \times Phusion buffer, 2 mM dNTP, 0.5 μ M forward (5'-GGAATCCATATGCGTCCCTTGCCCCGGCC-3') (SEQ ID NO:155) and reverse (5'-CGGGATCCTTAGAACTGCTTGGAAGG-GAG-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 μ l. 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.

[0430] 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 μ g/ml kanamycin (Km⁵⁰). 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_{600nm} 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 \times g for 10 min and the pellet was resuspended into 2 ml of Bugbuster (Novagen) containing 10 μ l of LysonaseTM Bioprocessing Reagent (Novagen). The solution was again centrifuged at 4,000 rpm \times g for 10 min and the supernatant was obtained.

[0431] 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M 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 μ l. 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 <i>Agrobacterium tumefaciens</i> C58.			
A. tumefaciens			
Name	C58	Forward Primer	Reverse Primer
ADH1	Atu1557	GGAATTCATATGTTTCAACAACGTCGCCCTA (SEQ ID NO:276) GCGGCCTCGGCCACATGGCCGTC AAGC (SEQ ID NO:278)	GCTTGACGGCCATGTGGCCGAGGCCGC (SEQ ID NO:277) CGGGATCCTTAGCGCCCTTCTGGCCGC (SEQ ID NO:279)

TABLE 1-continued

Primers used to amplify ADH1-12 from <i>Agrobacterium tumefaciens</i> C58.			
Name	<i>A. tumefaciens</i> C58	Forward Primer	Reverse Primer
ADH2	Atu2022	GGAATTCATATGGCTATTGCAAGAGGTTA (SEQ ID NO:280)	CGGGATCCTTAAGCGTCGAGCGAGGCCA (SEQ ID NO:281)
ADH3	Atu0626	GGAATTCATATGACTAAAACAATGAAGGC (SEQ ID NO:282) TGGCAATACCGGACCCCGCCCGGTG (SEQ ID NO:284)	CACCGGGCCGGGTCCGGTATTGCCA (SEQ ID NO:283) CGGGATCCTTAGCGCGAGATCCACGA (SEQ ID NO:285)
ADH4	Atu5240	GGAATTCATATGACCGGGCGAACCAGCC (SEQ ID NO:286) AGGCAACCGAGCGTATGAGCGGCTAT (SEQ ID NO:288)	ATAGCCGCTCATACGCCTCGGTTGCCT (SEQ ID NO:287) CGGGATCCTTAAGCGCGTGCGAAGGA (SEQ ID NO:289)
ADH5	Atu3163	GGAATTCATATGACCATGCATGCCATTCA (SEQ ID NO:290)	CGGGATCCTTATTCGGCTGCAAATTGCA (SEQ ID NO:291)
ADH6	Atu2151	GGAATTCATATGCGCGCTTTATTACGA (SEQ ID NO:292)	CGGGATCCTTATTCGAACCGGTCGATGA (SEQ ID NO:293)
ADH7	Atu2814	GGAATTCATATGCTGGCGATTTTCTGTGA (SEQ ID NO:294)	CGGGATCCTTATGCGACCTCCACCATGC (SEQ ID NO:295)
ADH8	Atu5447	GGAATTCATATGAAAGCCTTCGTCGTCGA (SEQ ID NO:296)	CGGGATCCTTAGGATGCGTATGTAACCA (SEQ ID NO:297)
ADH9	Atu4087	GGAATTCATATGAAAGCGATTGTGCCCCA (SEQ ID NO:298)	CGGGATCCTTAGGAAAAGCGATCTGCA (SEQ ID NO:299)
ADH10	Atu4289	GGAATTCATATGCCGATGGCGCTCGGGCA (SEQ ID NO:300)	CGGGATCCTTAGAATTCGATGACTTGCC (SEQ ID NO:301)
ADH11	Atu3027	GGAATTCATATGAAACATTCTCAGGACAA (SEQ ID NO:302) CGGAAACGCACCACATGATCGGCGCCC (SEQ ID NO:304)	GGGCGCGATCATGTGGTGCCTTCCG (SEQ ID NO:303) CGGGATCCTTATGCCATACGTTCCATAT (SEQ ID NO:305)
ADH12	Atu3026	GGAATTCATATGCAGCGTTTTACCAACAG (SEQ ID NO:306)	CGGGATCCTTAGGAAAACAGGACGCCGC (SEQ ID NO:307)

Expression and Purification of ADH1-10.

[0432] 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 (Km⁵⁰). 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_{600nm} 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.

[0433] 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.

[0434] 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.

[0435] To identify DEHU hydrogenase, a NADPH dependent DEHU hydrogenation assay was performed. 20 μ l of prepared cell lysate containing each ADH was added to 160 μ l of 20-fold deluted DEHU solution prepared in the above section. 20 μ l 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 Devices). *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.

[0436] To identify D-mannuronate hydrogenase, a NADPH dependent D-mannuronate hydrogenation assay was performed. 20 μ l of prepared cell lysate containing each ADH was added to 160 μ l of D-mannuronate solution prepared in the above section. 20 μ l 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 Devices). *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.

[0437] 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-AGGTACGGTGAAATAA AGGAGG ATATACATATGTCCAAAAAGATTGCCGT-3') (SEQ ID NO:157) and reverse (5'-TTTTCCTTTT GCGGCCGCCCGCTGGCATCGCCTCAC-3') (SEQ ID NO:158) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* DH10B genome in 50 μ l. Amplified DNA fragment was digested with NdeI and NotI and ligated into pET29 pre-digested with the same restriction enzymes.

Construction of pETkdgA.

[0438] DNA sequence of kdgA *Escherichia 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-GGCGATGCCAGCGTAA AGGAGG ATATA CATATGAAAACTGGAAAACAAG-3') (SEQ ID NO:159) and reverse (5'-TTTTCCTTTT GCGGCCGCCCGCTTAGCGCCTTCTA-3') (SEQ ID NO:160) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* DH10B genome in 50 μ l. Amplified DNA fragment was digested with NdeI and NotI and ligated into pET29 pre-digested with the same restriction enzymes.

Protein Expression and Purification.

[0439] All plasmids (pETAtu3025, pETADH11, pETADH12, pETkdgA, pETkdgK, and pETXuA) 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 μ g/ml kanamycin (Km⁵⁰). 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_{600nm} 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 \times g for 10 min and the pellet was resuspended into 2 ml of Bugbuster (Novagen) containing 10 μ l of Lysonase™ Bioprocessing Reagent (Novagen) and suggested amount of protease inhibitor cocktail (SIGMA). The solution was again centrifuged at 4,000 rpm \times g for 10 min and the supernatant was obtained. The supernatant was applied to Nickel-NTA spin column (Qiagen) to purify His-tagged proteins.

[0440] The results of the assays for DEHU hydrogenase activity and D-mannuronate hydrogenase activity of ADH1-10 are shown in FIGS. 7A and 7B. These results demonstrate that the novel enzymes ADH1 and ADH2 showed significant DEHU hydrogenase activity (FIG. 7A), and that the novel enzymes ADH3, ADH4, and ADH9 showed significant mannuronate hydrogenase activity (FIG. 7B).

In Vitro Pyruvate Formation.

[0441] The reaction mixture contained 1% alginate or ~0.5% mannuronate, ~5 μ g of purified Atu3026 (ADH12) or Atu3027 (ADH11), and ~5 μ g 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).

[0442] The results of in vitro pyruvate formation from alginate mediated by enzymatic and chemical degradation are shown in FIG. 6B and FIG. 6C, 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:

[0443] 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.

[0444] 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.

[0445] 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.

[0446] 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.

[0447] Phenylacetaldehyde and 4-hydroxyphenylacetaldehyde biosynthesis pathways comprising a transketolase (tktA), a 3-deoxy-7-phosphoheptulonate synthase (aroF, aroG, and aroH), 3-dehydroquininate synthase (aroB), a 3-dehydroquininate 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 phenylacetaldehyde and/or 4-hydroxyphenylacetaldehyde.

[0448] A 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and 2-(indole-3-yl)ethanol biosynthesis pathway comprising a transketolase (tktA), a 3-deoxy-7-phosphoheptulonate synthase (aroF, aroG, and aroH), 3-dehydroquininate synthase (aroB), a 3-dehydroquininate 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 fluorescens* was constructed and tested for the ability to produce 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol and/or 2-(indole-3-yl)ethanol.

[0449] Construction of pBADButP.

[0450] 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 TTATTTGAATAATCGTAGAAACC-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.

[0451] Construction of pBADButP-atoB.

[0452] 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'-GCTCTAGAGGAGGATATATATGAAAAATTGTGTCATC 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.

[0453] Construction of pBADatoB-ald.

[0454] 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 AGGAGGATATATATGAAAAATTGTGTCATCGTCAGTG-3') (SEQ ID NO:165) for atoB and 5'-GGTTGAATTAAGGAGGATATATATAT-GAATAAAGACACACTAATACCTAC-3' for ald (SEQ ID NO:166) and reverse (5'-GTCTTTATTCATATATATATC-TCCTTAATTCAACCGTTCAATCACCATC-3' (SEQ ID NO:146) for atoB and 5'-CCCAAGCTTAGCCGCAAG-TACACATCTTC-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 50 μl, respectively. The amplified DNA fragments were gel purified and eluted into 30 μl of EB buffer (Qiagen). 5 μl 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 AGGAGGATATATATGAAAAATTGTGTCATCGTCAGTG-3') (SEQ ID NO:168) and reverse (5'-CCCAAGCTTAGCCGCAAGTACACATCTTC-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.

[0455] Construction of pBADButP-atoB-ALD.

[0456] 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 μ M forward (5'-AAGGAAAAA GCGGCCGCCCTGAACCGACGACCGGGTCG-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'-AAGGAAAAA GCGGCCGCCCGGATAACATATTTGAATGTATTTAG-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 μ l, respectively. Amplified DNA fragments were digested with NotI and KpnI and ligated each other.

[0457] Construction of pBADilvCD.

[0458] 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-GC TCTAGAGGAGGATATATATATGGCTAACTACTTCAAT ACAC-3') (SEQ ID NO:173) for ilvC and 5'-TGCTGT-TGCGGGTTAAGGAGGATATATATATGC-CTAAGTACCGTTCCGCC-3' for ilvD) (SEQ ID NO:174) and reverse (5'-AACGGTACTTAGGCATATATATATCCTC-CTTAACCCGCAACAGCAATACG-3') (SEQ ID NO:175) for ilvC and 5'-AC ATGCATGCTTAACCCCGAGTTTCGATT-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 μ l. The amplified DNA fragments were gel purified and eluted into 30 μ l of EB buffer (Qiagen). 5 μ l 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-G C TCTAGAGGAGGATATATATATGGCTAACTACTTCAAT ACAC-3') (SEQ ID NO:177) and reverse (5'-AC ATGCATGCTTAACCCCGAGTTTCGATT-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.

[0459] Construction of pBADals-ilvCD.

[0460] 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-CCC GAGCTCAGGAGGATATATATATGGATAAACAGTATCC GGT-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 μ l. The amplified DNA fragment was digested with SacI and XbaI and ligated into pBADilvCD pre-digested with the same restriction enzymes.

[0461] Construction of pBADalsS-ilvCD.

[0462] 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-CCC GAGCTCAGGAGGATATATATATGTTGACAAAAGCAA CAAAAG-3') (SEQ ID NO:181) for front and 5'-CGGTAC-CCTTTCCAGAGATTTAGAG-3' (SEQ ID NO:275) for back halves, and reverse (5'-CTCTAAATCTCTG-GAAAGGGTACCG-3') (SEQ ID NO:182) for front and (5'-GCTCTAGATTAGAGAGCTTTTCGTTTTCATG-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 μ l. The amplified DNA fragments were gel purified and eluted into 30 μ l of EB buffer (Qiagen). 5 μ l 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-CCC GAGCTCAGGAGGATATATATATGTTGACAAAAGCAA CAAAAG-3') (SEQ ID NO:184) and reverse (5'-GC TCTAGATTAGAGAGCTTTTCGTTTTCATG-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.

[0463] Construction of pBADLeuABCD.

[0464] 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-C GAGCTCAGGAGGATATATATATGAGCCAGCAAGTCA TTATTTTCG-3') (SEQ ID NO:186) and reverse (5'-AAAA CTGCAGCGTTTGTATGACGTGGACGATAGCGG-3') (SEQ ID NO:187) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Escherichia coli* BL21 (DE3) genome in 50 μ l. The amplified DNA fragment was digested with SacI and XbaI and ligated into pBAD33 pre-digested with the same restriction enzymes.

[0465] Construction of pBADLeuABCD2.

[0466] 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M 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 CTGCAGCGTTTGTATGACGTGGACGATAGCGG-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 μ l, respectively. The amplified DNA fragments were gel purified and eluted into 30 μ l of EB buffer (Qiagen). 5 μ l 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-CG AGCTCAGGAGGATATATATATGAGCCAGCAAGTCAT TATTTTCG-3')

(SEQ ID NO:192) and reverse (5'-AAAA CTGCAGCGTTTGTATGACGTGGACGATAGCGG-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.

[0467] Construction of pBADLeuABCD4.

[0468] 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'-CG AGCTCAGGAGGATATATATATGAGCCAGCAAGTCAT TATTTTCG-3') (SEQ ID NO:194) for leuA, (5'-GAAAC-CGTGTGAGGAGGATATATATATGTCGAA-GAATTACCATATTGCCG-3') (SEQ ID NO:195) for leuB, (5'-AGGGGTGTAAGGAGGATATATATATG-GCTAAGACGTTATACGAAAAATTG-3') (SEQ ID NO:196) for leuC, and (5'-ACATTAATAAGGAG-GATATATATATGGCAGAGAAATTTATCAAACACAC-3') (SEQ ID NO:197) for leuD and reverse (5'-ATTCTTCGA-CATATATATATCCTCCTCACACGGTTTC-CTTGTTGTTTTTCG-3') (SEQ ID NO:198) for leuA, (5'-CGTCTTAGCCATATATATATCCTCCTTACACCCCTTCT GCTACATAGCGG-3') (SEQ ID NO:199) for leuB, (5'-TTTCTCTGCCATATATATATCCTCCT-TATTTAATGTTGCCAATGTCGGCG-3') (SEQ ID NO:200) for leuC, and (5'-AAAACCTGCAGCGTTTGAT-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'-CG AGCTCAGGAGGATATATATGAGCCAGCAAGTCAT TATTTTCG-3') (SEQ ID NO:202) and reverse (5'-AAAA CTGCAGCGTTTGTATGACGTGGACGATAGCGG-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.

[0469] Construction of pBADals-ilvCD-leuABCD, pBADals-ilvCD-leuABCD2, pBADals-ilvCD-leuABCD4, pBADalsS-ilvCD-leuABCD, pBADalsS-ilvCD-leuABCD2, pBADalsS-ilvCD-leuABCD4.

[0470] 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* 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'-AAGGAAAAA GCGGCCGCCCTGAACCGACGACCGGGTCG-3') (SEQ ID NO:204) and reverse (5'-CGG GGTACCGCGGATACATATTTGAATGTATTAG-3') (SEQ ID NO:205) primers, 1 U Phusion High Fidelity DNA

polymerase (NEB), and 50 ng pBADals-ilvCD and pBADalsS-ilvCD in 50 μl, respectively.

[0471] 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'-AAGGAAAAA GCGGCCGCCACTTTTCATACTCCCGCCATTCAG-3') (SEQ ID NO:206) for front and (5'-CAAAGGCCGTCTG-CACGCGCCGAAAGGCAAA-3') (SEQ ID NO:207) for back halves) and reverse (5'-TTTGCCTTTCGCGCGGTG-CAGACGGCCTTTG-3') (SEQ ID NO:208) for front and (5'-AC ATGCAITGCCGTTTGTATGACGTGGACGATAGCGG-3') (SEQ ID NO:209) for bottom halves, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pBADleuABCD, 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'-AAGGAAAAA GCGGCCGCCACTTTTCATACTCCCGCCATTCAG-3') (SEQ ID NO:210) and reverse (5'-AC ATGCAITGCCGTTTGTATGACGTGGACGATAGCGG-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.

[0472] Construction of pBADaroG-ktkA-aroBDE.

[0473] 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 GAGCTCAGGAGGATATAT ATGAATTATCAGAAC-GACGATTTAC-3') (SEQ ID NO:212) for aroG, (5'-GCGTCCGCGGGTAAGGAGGAAAATTTTAT-GTCCTCACGTAAAGAGCTTGCC-3') (SEQ ID NO:213) for tktA, (5'-GAACTGCTGTAAGGAGGTTAAAATTATG-GAGAGGATTGTCGTTACTCTCG-3') (SEQ ID NO:214) for aroB, (5'-CAATCAGCGTAAGGAGGTATATATAAT-GAAAACCGTAACTGTAAGAGATC-3') (SEQ ID NO:215) for aroD, and (5'-TACACCAGGCATAAGGAG-GAATTAATTATGAAAACCTATGCTGTTTTTGG-3') (SEQ ID NO:216) for aroE and reverse (5'-TACGTGAGGA-CATAAAATTTTCTCCTTACCCGC-GACGCGCTTTTACTGC-3') (SEQ ID NO:217) for aroG, (5'-CAATCCTCTCCATAATTTAACCTCCT-TACAGCAGTTCTTTTGCTTTTCGC-3') (SEQ ID NO:218) for tktA, (5'-CAATCAGCGTAAGGAGGTATATATAAT-GAAAACCGTAACTGTAAGAGATC-3') (SEQ ID NO:219) for aroB, (5'-TACGGTTTTTCAATTATATACCTC-CTTACGCTGATTGACAATCGGCAATG-3') (SEQ ID NO:220) for aroD, and (5'-AC ATGCAITGCTTACGCGGACAATTCCTCCTGCAA-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 μ l, respectively. The amplified DNA fragments were gel purified and eluted into 30 μ l of EB buffer (Qiagen). 5 μ l 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-CCC GAGCTCAGGAGGATATATATATGAATTATCAGAACG ACGATTTAC-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.

[0474] Construction of pBADp*heA*-*aroLAC*.

[0475] 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-CCC GAGCTCAGGAGGATATATATATGACATCGGAAAACC CGTTACTGG-3') (SEQ ID NO:224) for *pheA*, (5'-GATC-CAACCTAAGGAGGAAAATTTTATGACA-CAACCTCTTTTTCTGATCG-3') (SEQ ID NO:225) for *aroL*, (5'-GATCAATTGTTAAGGAGG-TATATATAATGGAATCCCTGACGTTACAACCC-3') (SEQ ID NO:226) for *aroA*, and (5'-CAGGCAGCCTAAG-GAGGAATTAATTATGGCTGGAACA-CAATTGGACAAC-3') (SEQ ID NO:227) for *aroC* and reverse (5'-AGGTTGTGTCATAAAATTTTCCTCT-TAGGTTGGATCAACAGGCACTACG-3') (SEQ ID NO:228) for *pheA*, (5'-CAGGATTCATATATATAC-CTCCTTAAACAATTGATCGTCTGTGCCAGG-3') (SEQ ID NO:229) for *aroL*, (5'-GTTTCCAGCCATAATTAATTC-CTCCTTAGGCTGCCTGGCTAATCCGCGCC-3') (SEQ ID NO:230) for *aroA*, and (5'-AC ATGCATGCTTACCAGCGTGGAAATATCAGTCTTC-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 μ l, respectively. The amplified DNA fragments were gel purified and eluted into 30 μ l of EB buffer (Qiagen). 5 μ l 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-CCC GAGCTCAGGAGGATATATATATGACATCGGAAAACC CGTTACTGG-3') (SEQ ID NO:232) and reverse (5'-AC ATGCATGCTTACCAGCGTGGAAATATCAGTCTTC-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.

[0476] Construction of pBAD*tyrA*-*aroLAC*.

[0477] 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-CCC GAGCTCAGGAGGATATATATATGAGTTGCTGAATTGA

CCGCATTAC-3') (SEQ ID NO:234) for *tyrA*, (5'-AATCGC-CAGTAAGGAGGAAAATTTTATGACA-CAACCTCTTTTTCTGATCG-3') (SEQ ID NO:235) for *aroL*, (5'-GATCAATTGTTAAGGAGG-TATATATAATGGAATCCCTGACGTTACAACCC-3') (SEQ ID NO:236) for *aroA*, and (5'-CAGGCAGCCTAAG-GAGGAATTAATTATGGCTGGAACA-CAATTGGACAAC-3') (SEQ ID NO:237) for *aroC*, and reverse (5'-GAGGTTGTGTCATAAAATTTTCCTCT-TACTGGCGATTGTCATTCGCGCTG-3') (SEQ ID NO:238) for *tyrA*, (5'-CAGGGATTCCATTATATATACCTCT-TAACAATTGATCGTCTGTGCCAGG-3') (SEQ ID NO:239) for *aroL*, (5'-GTTTCCAGCCATAATTAATTC-CTCCTTAGGCTGCCTGGCTAATCCGCGCC-3') (SEQ ID NO:240) for *aroA*, and (5'-AC ATGCATGCTTACCAGCGTGGAAATATCAGTCTTC-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 μ l, respectively. The amplified DNA fragments were gel purified and eluted into 30 μ l of EB buffer (Qiagen). 5 μ l 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-CCC GAGCTCAGGAGGATATATATATGGTTGCTGAATTGA CCGCATTAC-3') (SEQ ID NO:242) and reverse (5'-AC ATGCATGCTTACCAGCGTGGAAATATCAGTCTTC-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.

[0478] Construction of pBAD*pheA*-*aroLAC*-*aroG*-*tktA*-*aroBDE* and pBAD*tyrA*-*aroLAC*-*aroG*-*tktA*-*aroBDE*.

[0479] 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-AAGGAAAAA GCGGCCGCCCTGAACCGACGACCGGGTTCG-3') (SEQ ID NO:244) for fragment 1 and 2 and (5'-GC TCTAGAACTTTTCATACTCCCGCAITCAG-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-GAAAAA GCGGCCGCCCGGATACATATTTGAATGTATTTAG-3') (SEQ ID NO:247) for fragment 3, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng pBAD*pheA*-*aroLAC*, pBAD*tyrA*-*aroLAC*, and pBAD*aroG*-*tktA*-*aroBDE* in 50 μ l, 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.

[0480] Construction of pTrcBAL.

[0481] 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.

[0482] Construction of pTrcAdhE2.

[0483] 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'-CATG CCATGGCCAAAGTTACAAATCAAAAAG-3') (SEQ ID NO:250) and reverse (5'-C GAGCTCTTAAAATGATTTATATAGATATCC-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.

[0484] Construction of pTrcAdh2.

[0485] 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 CCATGGGTATTCCAGAACTCAAAAAG-3') (SEQ ID NO:252) and reverse (5'-CCC GAGCTCTTATTAGAAGTGTCAACAACG-3') (SEQ ID NO:253) primers, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng genome of *Saccharomyces cerevisiae* in 50 μl. Amplified DNA fragment was digested with NcoI and SacI and ligated into pTrc99A pre-digested with the same restriction enzymes.

[0486] Construction of pTrcBALD.

[0487] 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.

[0488] Construction of pTrcBALK.

[0489] A DNA sequence encoding ketoisovalerate decarboxylase (kivd) of *Lactococcus lactis* 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'-CCC GAGCTCAGGAGGATATATATATGTATACAGTAGAGA TTACC-3') (SEQ ID NO:256) and reverse (5'-GC TCTAGATTATGATTTATTTGTTTCAGCAAAT-3') (SEQ ID NO:257) primers, 1 U Phusion High Fidelity DNA poly-

merase (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.

[0490] Construction of pTrcAdh-Kivd.

[0491] A DNA sequence encoding ketoisovalerate decarboxylase (kivd) of *Lactococcus lactis* 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'-CCC GAGCTCAGGAGGATATATATGTATACAGTAGGAGA TTACC-3') (SEQ ID NO:258) and reverse (5'-GC TCTAGATTATGATTTATTTGTTTCAGCAAAT-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.

[0492] Construction of pTrcBAL-DDH-2ADH.

[0493] To remove internal NcoI site, overlap PCR was carried out. DNA fragments encoding front and bottom halves of meso-2,3-butanediol dehydrogenase (ddh) of *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 and secondary alcohol dehydrogenase (2adh) of *Pseudomonas 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'-GGCCGGCGGCCGCGCATGGCGGTGAAAGTG-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-GATGAGTTTCGAC-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 AAGCTTAGATCGCGGTGGCCCCGCGTCG-3') (SEQ ID NO:267) for bottom half of 2adh, 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 for ddh and *Pseudomonas 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'-C GAGCTCAGGAGGATATATATATGAAAAAAGTCGCAC TTGTTACCG-3') (SEQ ID NO:268) and reverse (5'-CCC AAGCTTAGATCGCGGTGGCCCCGCGTCG-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.

[0494] Construction of pBBRPduCDEGH.

[0495] A DNA sequence encoding propanediol dehydratase medium (pduD) and small (pduE) subunits and pro-

panediol 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 TCTAGAGGAGGATTTAAAAATGGAAATTAACGAAACGCTGC-3') (SEQ ID NO:270) and reverse (5'-TCC CCGCGGTTAAGCATGGCGATCCCGAAATGGAATCCCTTTGAC-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.

[0496] 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'-CCG CTCGAGGAGGATATATATATGAGATCGAAAAGATTTGAAGC-3') (SEQ ID NO:272) and reverse (5'-GC TCTAGATTAGCCAAGTTCATTGGGATCG-3') (SEQ ID NO:273) 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 XhoI and XbaI and ligated into pBBRpduDEGH pre-digested with the same restriction enzymes.

[0497] Construction of pTrcIpd-Par.

[0498] 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'-C GAGCTCAGGAGGATATATATATGAAAGCTATCCAGTACACCCGAT-3' (SEQ ID NO:315) for par, and reverse primers (5'-CGAGCTCTTATTCGCGCGGTGCCGCGTG-CAGG-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 pTrcIpd 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:

[0499] 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 chloramphenicol (Cm⁵⁰) and 100 ug/ml ampicillin (Amp¹⁰⁰) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm⁵⁰ and Amp¹⁰⁰ 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.

[0500] To test the isobutyraldehyde biosynthesis pathway, DH10B cells harboring pBADals-ilmvCD/pTrcBALK or pBADalsS-ilmvCD/pTrcBALK were grown overnight in LB media containing 50 ug/ml chloramphenicol (Cm⁵⁰) and 100 ug/ml ampicillin (Amp¹⁰⁰) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm⁵⁰ and Amp¹⁰⁰ 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. 8B shows the production of isobutanal from these cultures.

[0501] To test the 3-methylbutyraldehyde and 2-methylbutyraldehyde biosynthesis pathways, DH10B harboring pBADals-ilmvCD-LeuABCD/pTrcBALK, pBADals-ilmvCD-LeuABCD2/pTrcBALK, pBADals-ilmvCD-LeuABCD/pTrcBALK4, pBADalsS-LeuABCD/pTrcBALK, pBADalsS-LeuABCD2/pTrcBALK, or pBADalsS-LeuABCD4/pTrcBALK were grown overnight in LB media containing 50 ug/ml chloramphenicol (Cm⁵⁰) and 100 ug/ml ampicillin (Amp¹⁰⁰) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm⁵⁰ and Amp¹⁰⁰ 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-methylpentanal) and 3-isovaleralcohol (3-methylpentanal) was monitored because 3-isovaleraldehyde and 2-isovaleraldehyde are spontaneously converted to their corresponding alcohols. FIG. 8B shows the production of 2-methylpentanal and 3-methylpentanal from these cultures.

[0502] To test the phenylacetaldehyde and 4-hydroxyphenylacetaldehyde 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 chloramphenicol (Cm⁵⁰) and 100 ug/ml ampicillin (Amp¹⁰⁰) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm⁵⁰ and Amp¹⁰⁰ 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 phenylacetaldehyde, 4-hydroxyphenylaldehyde and their corresponding alcohols were monitored using GC-MS. FIG. 9B shows the production of 4-hydroxyphenylethanol from these cultures.

[0503] 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/pTrcIpd-Par, and pBADpheA-aroLAC-aroG-tktA-aroBDE/pTrcIpd-Par were grown overnight in LB media containing 50 ug/ml chloramphenicol (Cm⁵⁰) and 100 ug/ml ampicillin (Amp¹⁰⁰) at 37 C, 200 rpm. An aliquot of each seed culture was inoculated into fresh TB media containing Cm⁵⁰ and

Amp¹⁰⁰ 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.

[0504] The production of 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol and/or 2-(indole-3-)ethanol was monitored using GC-MS. FIG. 42A shows the production of 2-phenylethanol from these cultures at 24 hours. FIG. 42B shows the production of 2-(4-hydroxyphenyl)ethanol from these cultures at 24 hours. FIG. 42C shows the production of 2-(indole-3-)ethanol from these cultures at 24 hours.

[0505] FIG. 43A shows the GC-MS chromatogram for control (pBAD33 and pTrc99A) at one week. FIG. 43B 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

[0506] Available substrates such as 3-hydroxy-2-butanone (acetoin), 4-hydroxy-3-hexanone (propioin), 5-hydroxy-4-octanone (butyroin), 6-hydroxy-5-decanone (valeroiin), 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.

[0507] For cloning and isolation of DDH polypeptides, genomic DNA from several species of bacteria were obtained from ATCC (*Lactobacillus brevis* ATCC 367, *Pseudomonas putida* KT2440, and *Klebsiella pneumoniae* MGH78578), PCR-amplified (using Phusion with polymerase with 1 \times Phusion buffer, 0.2 mM dNTP, 0.5 μ L Phusion enzyme, 1.5 μ M primers, and 20 pg template DNA in a 50 μ L reaction) utilizing the following protocol: 30 cycles, 98 $^{\circ}$ C./10 secs (denaturing), 60 $^{\circ}$ C./15 secs (annealing), 72 $^{\circ}$ 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 inoculated into LB media, and expression of 6 \times His-tagged proteins of interest was induced at OD₆₀₀=0.6 with 0.1 mM IPTG. Expression was allowed to proceed for 15 hours at 22 $^{\circ}$ C. The 6 \times His-tagged enzymes were purified using Ni-NTA spin columns following suggested protocols by QIAGEN, yielding purified protein concentrations in the range of 1.1-6.5 mg/mL (determined by Bradford assay).

[0508] Diol dehydrogenase ddh1 was isolated from *Lactobacillus 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 ddh1 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.

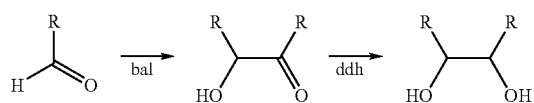
[0509] Reactions to measure biological activity of DDH polypeptides were performed in a final volume of 200 μ L as follows: 25 mM substrate, 0.04 mg/mL DDH polypeptide, 0.25 mg/mL nicotinamide cofactor, 200 mM imidazole, 14 mM Tris-HCl, and 1.5% by volume DMSO. Biological activity was assayed using a 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.

[0510] FIG. 12A shows the biological activity of ddh1, ddh2, and ddh3 using butyroin as a substrate (triangles represent ddh3 activity). FIG. 12B 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

[0511] The ability of a C—C lyase and a diol hydrogenase to perform the following sequential reaction was tested in *E. coli*:



[0512] 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 *Klebsiella 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., acetaldehyde, 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	FIGS.
Butanal	5-Hydroxy-4-octanone	4,5-Octanediol	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
Phenylacetaldehyde	1,4-Diphenyl-3-hydroxy-2-butanone	1,4-Diphenyl-2,3-butanediol	25

For Analysis of \cong C10.

[0513] *E. coli* harboring pTrcBAL-DDH-2ADH was grown for overnight in LB media containing 50 ug/ml Kanamycine (Km). This seed culture was inoculated 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:

- [0514] 1 μ L injection w/ 50:1 split
- [0515] Inlet temperature—150° C.
- [0516] Initial oven temperature—50° C.
- [0517] Temperature Ramp 1—10° C./min to 150° C.
- [0518] Temperature Ramp 2—50° C./min to 300° C.
- [0519] GC to MS transfer temp—250° C.
- [0520] MS detection—full scan MW 35-200

For Analysis of \cong C12.

[0521] *E. coli* DH10B strains harboring pTrc99A (Ctrl vector) or pTrcBAL were inoculated into 0.75xM9/0.5% LB containing 0.1 mM CaCl₂, 2 mM MgSO₄, 1 mM KCl, 1% galacturonate, 5 μ g/mL thiamine, Amp. The cultures were grown up to an optical density (600 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.

- [0522] 1 μ L injection w/ 50:1 split
- [0523] Inlet temperature—250° C.
- [0524] Initial oven temperature—50° C.
- [0525] Temperature Ramp 1—10° C./min to 125° C.
- [0526] Temperature Ramp 2—30° C./min to 300° C.
- [0527] Final Temperature 300° C.—1 minute
- [0528] GC to MS transfer temp—250° C.
- [0529] MS detection—full scan MW 40-260.
- [0530] 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-octanediol. 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 phenylacetaldehyde into 1,4-diphenyl-3-hydroxy-2-butanone.

[0531] 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

[0532] To test the sequential biological activity of diol dehydrogenases and diol dehydratases in a dehydration and reduction pathway, butyrolin 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.

[0533] Diol dehydrogenase ddh from *Klebsiella pneumoniae* MGH 78578 and diol dehydratase pduCDE from *Klebsiella 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 polypeptide 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.

[0534] The *ddh3* and *pduCDE* polypeptides were incubated with butyrolin 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 (butyrolin)	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

[0535] FIG. 26A 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 butyrolin (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 butyrolin by *ddh3*.

[0536] FIG. 26B 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 butyrolin and dehydration of 4,5-octanediol by *ddh3* and *pduCDE*, respectively.

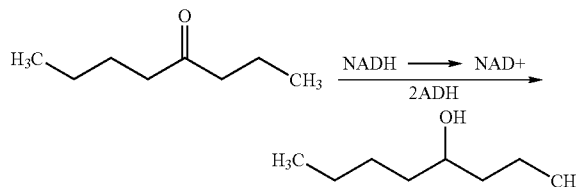
[0537] FIGS. 27A and 27B show comparisons between the sample extraction gas chromatograph/mass spectrum and the 4-octanone standard gas chromatograph/mass spectrum. These results demonstrate that 4-octanone was produced from butyrolin using the enzymes diol dehydrogenase (*ddh3*) and a diol dehydratase (*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

[0538] 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):



[0539] All enzymes and reagents were purchased from New England Biolabs and Sigma, respectively, unless otherwise stated.

[0540] Various secondary alcohol dehydrogenases (2ADHs) were isolated from *Pseudomonas putida* KT2440, *Pseudomonas fluorescens* Pf-5, and *Klebsiella 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.

[0541] 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
<u>NADH Production Assay (30° C.)</u>	
2ADH enzyme	Approx. 0.058 μg/μL
4-octanol	5.55 mM
NAD+	Approx. 1.4 μg/μL
Imidazole (from Elution Buffer)	Approx. 280 mM
<u>NADH Consumption Assay (30° C.)</u>	
2ADH enzyme	Approx. 0.075 μg/μL
4-octanone	5.0 mM
NADH	Approx. 0.25 μg/μL
Imidazole (from Elution Buffer)	Approx. 250 mM
<u>NADPH Production Assay (30° C.)</u>	
2ADH enzyme	Approx. 0.058 μg/μL
4-octanol	5.55 mM
NADP+	Approx. 1.4 μg/μL
Imidazole (from Elution Buffer)	Approx. 280 mM

[0542] 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-octanol, or cyclopentanol, or catalyze the reduction of 4-octanone, 2,7-dimethyl-4-octanone, 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
Imidazole	200 mM
Tris HCl	14 mM
DMSO	15% by volume
Total Volume	200 μ L
Rxn Components for NAD(P)H Production Assays (Oxidation)	
Substrate	5 mM
Enzyme	0.04 mg/mL
Nicotinamide cofactor	0.25 mg/mL
Imidazole	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
Imidazole	200 mM
Tris HCl	14 mM
DMSO	3% by volume

[0543] FIG. 30A 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. 30B 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.

[0544] FIG. 31 shows the oxidation of 4-octanol by 2ADH-11 (FIG. 31A) and 2ADH-16 (FIG. 31B), as measured by NADH and NADPH production, respectively. FIG. 32 shows the oxidation of 2,7-dimethyloctanol by 2ADH-11 and others (FIG. 32A) and 2ADH-16 (FIG. 32B), as measured by NADH and NADPH production, respectively.

[0545] FIG. 33A shows the reduction of 2,7-dimethyl octanol by 2ADH11 and 2ADH16 as monitored by NADPH consumption. FIG. 33B shows the reduction activity of both 2ADH11 and 2ADH16 towards various substrates. FIG. 34 shows the oxidation (FIG. 34A) and reduction (FIG. 34B) of cyclopentanol by 2ADH-16.

[0546] 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 μ L 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

[0547] 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 catalyze the irreversible dehydration of diols, such as 1,2-propanediol.

[0548] For in vitro activity, *E. coli* BL21(DE3) harboring pETPduCDE (diol dehydratase subunits) was inoculated into 100 mL LB media, grown to OD₆₀₀=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 μ L of protein mixture at a concentration of 2.2 mg/mL. Protein concentration assays were conducted using a Bradford reagent protocol.

[0549] 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 purified PduCDE. In vitro reactions were conducted under semi-aerobic 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 μ g/mL
KCl	10 mM
dOH1 enzyme mix	0.08 mg/mL
Reaction Buffer	10 mM Tris HCl pH 7.5

[0550] After 48 hours, 1 mL of the reaction mixture was extracted with 0.5 mL of either ethylacetate or hexanol and analyzed by GCMS.

[0551] The following GCMS protocol was used for all experiments:

[0552] 1 μ L injection w/ 50:1 split

[0553] Inlet temperature—250° C.

[0554] Initial oven temperature—50° C.

[0555] Temperature Ramp 1—10° C./min to 125° C.

[0556] Temperature Ramp 2—30° C./min to 300° C.

[0557] Final Temperature 300° C.—1 minute

[0558] GC to MS transfer temp—250° C.

[0559] MS detection—full scan MW 40-260

[0560] The results are shown in FIG. 45. FIG. 45A confirms the formation of 1-propanal from 1,2-propanediol, and FIG. 45B confirms the formation of 2-butanone from meso-2,3-butanediol, both of which were catalyzed by B12 independent diol dehydratase.

[0561] 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 \times Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward primers (5'-CCG

CTCGAGGAGGATATATATATGATTTCTAAAGGCTTTA
GCACCC-3' (SEQ ID NO:318) for dhaB1 and 5'-ACGT-
GATGTAA

TCTAGAGGAGGATATATATATGAGCAAAGAAATTA

AGG-3' (SEQ ID NO:319) for dhaB2, and reverse primers (5'-TCTTTGCTCATATATATATCCTCC TCTAGATTACATCACGTGTTTCAGTAC-3' (SEQ ID NO:320) for dhaB1 and 5'-C GAGCTCTTATTTCGGCGCCAATGGTGCACGGG-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 μ l. 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 1x Phusion buffer (NEB), 2 mM dNTP, 0.5 μ M forward (5'-CCG CTCGAGGAGGATATATATATGATTCTAAAGGCTTACGACCC-3') (SEQ ID NO:322) and reverse primers (5'-C GAGCTCTTATTTCGGCGCCAATGGTGCACGGG-3') (SEQ ID NO:323), 1 U Phusion High Fidelity DNA polymerase (NEB), and 50 ng each fragment in 50 μ l. Amplified DNA fragment was digested with XhoI and SacI and ligated into pBBR1MCS-2 pre-digested with the same restriction enzymes.

[0562] Two strains of *E. coli* DH10B harboring pBBR1MCS-2 or pBBRDhaB1/2 into TB media without glycerol were inoculated. Cultures were grown to OD₆₀₀=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 μ g/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.

[0563] 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. 46A shows the in vivo production of 1-propanol from 1,2-propanediol. FIG. 46B shows the in vivo production of 2-butanol from meso-2,3 butanediol. FIG. 46C 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

[0564] 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:

[0565] Vs24214-24249: genomic region corresponding to gene id between V12B01_24214 and V12B01_24249 (see Example 1).

TABLE 7

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

[0566] 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 gcccaacttcaagatgaagctgt (SEQ ID NO:327)

[0567] 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)

[0568] 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.

[0569] 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

24214 ndeF	GGAATTC CAT atgacaaagaatgatgacgactaaac (SEQ ID NO:330) for forward primer for V12B01_24214
24214 bamR	CG GGATCC ttattatttcccctgcctgcagt (SEQ ID NO:331) for reverse primer for V12B01_24214
24219 ndeF	GGAATTC CAT atgagctatcaaccacttttac (SEQ ID NO:332) for forward primer for V12B01_24219
24219 bamR	CG GGATCC ttacagttgagcaaatgatcc (SEQ ID NO:333) for reverse primer for V12B01_24219

[0570] 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.

[0571] The Vs24254 (SEQ ID NO: 32) region of *Vibrio splendidus* 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 β -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).

[0572] 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 μ l 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.

[0573] As shown in FIG. 56A, 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 observed when these genomic inserts are switched into the opposite vector (pET-DEST42-Vs24189-24209 and pENTR/D/TOPO-Vs24214-24249). FIG. 56B shows growth on glucose as a positive control. Thus, the combined genomic regions of Vs24214-24249 and Vs24189-24209 from *Vibrio splendidus* were sufficient to confer on *E. coli* the ability to grow on alginate as a sole source of carbon.

Example 11

Production of Ethanol from Alginate

[0574] 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.

[0575] *E. coli* was transformed with either pBBRPdc-AdhA/B or pBBRPdc-AdhA/B+1.5 Fos (fosmid clone containing genomic region between V12B01_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.

[0576] 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.

[0577] 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.

[0578] The following publications are herein incorporated by reference in their entirety.

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Arg	Ser	Ile	Thr	Arg	Ser	Ser	Gly	Gly	Gln	Ser	Ile	Ser	Glu	Thr	Leu
180					185					190					
Thr	Glu	Gly	Ser	Ile	Ser	Gly	Asn	Thr	Tyr	Pro	Gln	Ser	Val	Thr	Thr
195					200					205					
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
340					345					350					
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

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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
 965 970 975
 Phe Ile Thr Leu Glu Gly Glu Val Arg Phe Glu Pro Asn Arg Asp Leu
 980 985 990
 Asp His Ser Val Ser Glu Asp Ile Val Lys Ser Ile Val Val Thr Ser
 995 1000 1005
 Ser Asp Phe Asp Asn Asp Pro Val Thr Ser Thr Ile Thr Leu Thr Ile
 1010 1015 1020
 Thr Asp Gly Asp Asn Pro Thr Ile Asp Val Ile Pro Ser Val Thr Leu
 1025 1030 1035 1040
 Ser Glu Ile Asn Leu Ser Asp Gly Ser Ala Pro Ser Gly Ser Ala Val
 1045 1050 1055
 Ser Ser Thr Gln Thr Ile Thr Phe Thr Asn Gln Ser Asp Asp Val Val
 1060 1065 1070
 Arg Phe Arg Ile Glu Ser Thr Glu Phe Asn Thr Asn Asp Asp Leu Lys
 1075 1080 1085
 Ser Asn Gly Leu Ala Val Glu Leu Arg Glu Asp Pro Ala Gly Ser Gly
 1090 1095 1100
 Asp Tyr Ile Gly Phe Thr Thr Ser Ala Thr Asn Val Glu Thr Pro Val
 1105 1110 1115 1120
 Phe Thr Leu Ser Phe Asn Ser Gly Ser Leu Gly Glu Tyr Thr Phe Thr
 1125 1130 1135
 Leu Ile Glu Ala Leu Asp His Gln Asp Ala Arg Gly Asn Asn Asp Leu
 1140 1145 1150
 Ser Phe Asp Leu Pro Val Tyr Ala Val Asp Ser Asp Gly Asp Asp Ser
 1155 1160 1165
 Leu Val Ser Pro Leu Asn Val Thr Ile Gly Asp Asp Val Gln Ile Met
 1170 1175 1180
 Gln Asp Ser Thr Leu Asp Ile Val Glu Pro Thr Val Ala Asp Leu Ala
 1185 1190 1195 1200
 Ala Gly Thr Val Thr Thr Asn Thr Ile Asp Val Met Pro Asn Gln Ser
 1205 1210 1215
 Ala Asp Gly Ala Thr Val Thr Gln Phe Thr Tyr Asp Gly Gln Leu Arg
 1220 1225 1230

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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
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

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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
 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

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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
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

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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
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

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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 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

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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	
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				

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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	
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

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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
 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

-continued

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
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

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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
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

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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
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

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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
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

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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
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
Asn Ser Gly Glu His	Asn Ser Asp Thr Gly	Asn Val Leu Gly Ile Asn
6645	6650	6655
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
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
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
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

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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
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

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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
 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

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atgaaaaaaaa catcactatt acttgcttcc attactctgg cactttctgg tgtagtacaa    60
gctgaccagc tagaagacat tcaaaaatca ggcacacttc gcgtcggcac cacaggcgac    120
tacaaacctt tttcttactt cgacggcaaa acctactctg gttatgacat tgacgtagcc    180
aaacatggtt cagagcagtt gggcgttgaa ttacagattg ttcgtaccac atggaaagat    240

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ctactgaccg atctagacag cgataaatac gacatcgcgga tgggctgtat cacgcgtaaa 300
atgcagcgtc agttaaacgc agaacaact caaggttaca tgaccttgg caagtgtttc 360
ttagttgcga aaggcaaacg agaacaatac aacagcattg agaaagtga cctctcttct 420
gtgctgtgtt gcgtcaatat cgggtgggact aatgagatgt ttgcggatgc taacttgcaa 480
gacgcgagct ttacgcgtta cgagaacaac ctagacgttc cgcaagccgt tgcggaaggt 540
aaagttgatg taatggtgac agaaactcct gaaggtctgt tctatcaagt gacggacgaa 600
cgtcttgaag cggcacgctg tgaacacccg tttaccaaca gtcaattcgg ttacctgata 660
ccaaaagggtg aacaacgctt gttgaacaca gtgaacttca ttatggatga gatgaaattg 720
aaagcgtcgc aagaagagtt cctgatccac aactctctta agtaa 765

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<210> SEQ ID NO 6

<211> LENGTH: 254

<212> TYPE: PRT

<213> ORGANISM: *Vibrio splendidus*

<400> SEQUENCE: 6

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Met Lys Lys Thr Ser Leu Leu Leu Ala Ser Ile Thr Leu Ala Leu Ser
1           5           10           15

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

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<210> SEQ ID NO 7

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<211> LENGTH: 765
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 7
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gctgaccagc tagaagacat tcaaaaatca ggcacacttc gcgtcggcac cacaggcgac    120
tacaaaacctt tttcttactt cgacggcaaa acctactctg gttatgacat tgacgtagcc    180
aaacatggtt cagagcagtt gggcggttgaa ttacagattg ttcgtaccac atggaaagat    240
ctactgaccg atctagacag cgataaatac gacatcgcga tgggcggtat cacgcgtaaa    300
atgcagcgtc agttaaaccg agaacaaact caaggttaca tgaccttgg caagtgtttc    360
ttagttgcga aaggcaaagc agaacaatac aacagcattg agaaagtga cctctcttct    420
gtgcgtggtt gcgtcaatat cgggtgggact aatgagatgt ttgcggatgc taacttgcaa    480
gacgcgagct ttacgcgtta cgagaacaac ctacagcttc 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

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<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

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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	
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						

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<210> SEQ ID NO 9
<211> LENGTH: 627
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 9

atgacgacat taaatgaaca actagcaaac ctaaaagtaa ttctgtaat cgcgatcaac    60
cgtgctgaag acgctatccc tctaggtaaa gcggttggtg aaaatggcat gccatgtgca    120
gaaattacac tacgtacaga atgtgcaatc gaagcgattc gcatcatgcg taaagaattc    180
ccagacatgc taatcggttc aggtactgta ctgactaacg agcaagttga cgcactctatc    240
gaagctggty ttgatttcat cgtaagccca ggttttaacc cacgtactgt tcaatactgt    300
atcgataaag gtattgcaat cgtacgggtt gttaacaacc caagcctagt tgagcaagca    360
atggaaatgg gtcttcgcac gttgaagttc ttcctctctg agccttcagg cggtaactggc    420
atgcttaaag cactaacagc agtttacctt gttaaattca tgcctactgg tggcgttaagc    480
ttgaagaatg ttgatgaata cctatcgatc ccttctgttc ttgcgtgtgg cggtaactgg    540
atggttccaa ctaaccttat cgatgaaggt aagtgggacg aactaggcaa gcttgttctgt    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

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<210> SEQ ID NO 11
<211> LENGTH: 933
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 11
atgaaatcat taaacatcgc ggtcattggc gagtgcattgg ttgagctaca aaagaaacaa    60
gacgggctta agcaaagttt tgggtggcgat acgctgaata ctgcacttta cttgtcacgc    120
ttaacaaaag agcaagatat caacacgagc tacgtaactg cactaggcac tgaccattc    180
agtaccgaca tgtaaaaaa ttggcaagcg gaaggtatcg acacgagctt aattgctcag    240
ctggaccaca aacaaccagg gctttactac atcgagaccg atgaaactgg tgaacgcagt    300
ttccactact ggcgtagtga tgctgcagcg aagttcatgt ttgatcagga agacacgcct    360
gctcttcttg ataagctggt ctcttttgac gcgatttact taagtggat tacgctggca    420
atcttgacag aaaatggtcg cacgcagcta ttcaacttct tagacaaatt caaagctcaa    480
ggcgccaag tattcttcga caataactac cgacctaac tttgggaaag ccaacaagaa    540
gcgatttctt ggtacttgaa aatgcttaag tacacagata cggtctgct gacgtttgat    600
gatgagcaag agctatacgg cgacgaaagc attgaacaat gtattacacg tacgtcagag    660
tctggttga aagagatcgt cattaacgt ggcgcgaaag actgcttagt ggttgaagc    720
caaagcgctc aatacgttgc acccaacct gtagacaaca tcgttgatac gactgcccgt    780
ggcgactcgt tcagtcagc cttcttggcc aagcgcttga gcgcggtag tgctcgtgat    840
gctgcatttg caggtcatat tgtggcagga accgtgattc agcatccagg tgctatcatt    900
cctctagaag cgacgcctga tctgtctcta taa                                933

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<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

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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: <i>Vibrio splendidus</i>			
<400> SEQUENCE: 15			
atgacgacta aaccagtatt gttgactgaa gctgaaatcg aacagcttca tcttgaagtg			60
ggcggttcta 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
aaacgaacct gctggtaaat gttctcaagc ttagcttatt cttgtgttgc ttcaacctg			420
acacaagatc agcgtgacaa tattgagtct cgcatttttg aacctatgct agaaatgttc			480
acggttaaat acgcacacga cttcgacctt attcacaatc acggtatttg ggcagtagcc			540
gctgtgggta tctgtggtct tgctttaggc aaactgtaat acctagaaat gtcagtgtac			600
ggcatcgacc gtaatgatac tggcggtttc ctagcgaag tttctcagct atttgcacct			660
tctggctact acatggaagg tccttactac catcgttatg cgattcgecc aacgtgtgtg			720
ttcggtgaag 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
gcgaagatc aagacgcagt atggatgcat ccatgtggtc ttgagctatc taaagcatac			1020
gaagccgcat ctgcagagaa agaaatcgcc atgcctttct ggccaagtgt tgaattgaat			1080
gaaggccctc aaggtcacia cggcgcgcaa ggctttatcc gtatgcagga taagaaaggc			1140
gacgtttctc aacttgtgat gaactacggc caacacggca tgggtcacgg caactttgat			1200
acgctgggta tttctttctt taaccgcggt caagaagtgc tacgtgaata cggcttctgt			1260
cgttgggta acgttgagcc aaaattcggc ggccgttacc tagacgaaaa caaatcttac			1320
gctcgtcaaa cgattgtcga caatgcagtt acgattgatg aaaaatgtca gaacaacttt			1380
gacgttgaac gtgcagactc agtacatggt ttacctcact tctttaaagt agaagacgat			1440
caaatcaacg gtatgagtgc atttgctaac gatcattacc aaggccttga catgcaacgc			1500
agcgtgttca tgctaaatct tgaagaatta gaatctccgt tattgttaga cctataccgc			1560
ttagattcta caaaaggcgg cgaaggcgag caccaatcag actattcaca ccaatatgcy			1620
ggtcagattg ttcgcactaa cttcgaatac caagcgaaca aagagctaaa cactctaggt			1680
gacgatttcg gttaccaaca tctatggaac gtcgcaagcg gtgaagtga gggcacagca			1740
attgtaagtt ggctacaaaa caacacctac tacacatggc taggtgcaac gtctaacgat			1800
aatgtgaag taatatttac tcgcaactggc gctaacgacc caagtttcaa tctacgttca			1860
gagcctgcgt tcattctacg cagcaaaaggc gaaacaacac tgtttgcttc tgttgtgaa			1920

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acgcacgggtt atttcaacga agaattcgag caatctgtca atgcacgtgg tgttgtgaaa 1980
gacatcaaag tcgtggctca caccaatgtc gggtcggtag ttgagatcac cacagagaaa 2040
tcaaactgga cagtgatgat cagcaaccaa cttggcgcga ctgacagcac tgaacacaaa 2100
gtagaactga acggcaaagt atacagctgg aaaggcttct actcagtaga gacaacttta 2160
caagaaacga attcagaaga acttagcact gcagggcagg ggaataaa 2208

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<210> SEQ ID NO 16
<211> LENGTH: 735
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

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<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
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

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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	
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				

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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

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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 tactactgag    240
gaaacaaaat accgagatta catcgttgat atgctaacag cgtacgcgac ggtataacca    300
acacttgaaa gaaacgtaag ccgtgactct aacctccgg gtaagctggt ccaccaaacg    360
ttgaacgaga acatgtggat gctttacgct tcttgtgctg acagctgcat ctaccacacg    420
atctctgaag agcaaaagcg tctgatcgaa gacgatcttc ttaagcaaat gatcgaaatg    480
ttcgttgatg 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 tgaacgatcc atcgaagaca atctctatca acgatgaagg cgttatcatg    900
gcaacgtctg tgtgttacca ccggttacgag caaactgaaa ctctacttgg tatggctaac    960
caccagcaaa acgtttgggt tcatgcttca ggtaaaacac tgtctgacgc ggttgatgca   1020
gcagacgaca tcaaagcatt caactggggg agcctggttg taaccgacgg ccctgaaggc   1080
gaaaaaggcg gcgtaagcat ccttcgtcac cgtgacgaac aagatgacga cacgatggcg   1140
ttgatctggt ttggtcaaca cggttctgat caccagtacc actctgctct agaccacggt   1200
cactacgatg gcctgcacct aagcgtatct aaccgtggcc acgaagtgct gcacgatctc   1260
ggcttcggtc gctgggtaaa cgttgagcct aagtttgccg gtcgttacat ccagagaaac   1320
aagtcttact gtaagcagac ggttgctcac aacacagtaa cggttgatca gaaaacgcag   1380
aacaacttca acacagcatt ggctgagtct aagtttggtc agaagcactt ctctgtagca   1440
gacgaccagt ctctacaagg catgagcggc acaatttctg agtactacac tggcgtagac   1500
atgcaacgca gcgtgattct tgctgaactt cctgagttcg agaagccact tgtaatcgac   1560
gtataaccgca tcgaagctga cgctgaacac cagtacgacc taccogttca ccactctggt   1620
cagatcatcc gtactgactt cgattacaac atggaaaaaa cgcttaagcc gctaggtgaa   1680
gacaacgggt accagcactt atggaacgtg gcttcaggca aagtgaacga agaaggttct   1740
ctagtaagct ggctacatga cagcagctac tacagcctag taaccagcgc gaatgcgggc   1800
agcgaagtga tttttgctcg cactgggtgct aacgatccag acttcaacct taagagtgag   1860
cctcgttcca tcttacgcca gtctgggtcaa aaccacgtgt ttgcttctgt actagaacg   1920
  
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catggttact ttaacgagtc tatcgaagcc tctgtaggcg ctcgtggtct agttaaataca 1980
gtatctgttg tgggccataa cagtgtcggg actggtgttc gcattcagac tacttctggc 2040
aacacttacc actacggtat ctcaaacc aa gctgaagaca cgcagcaagc aactcacact 2100
gttgagttcg cgggtgagac atactcgtgg gaaggatcat ttgctcaact gtaa 2154

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<210> SEQ ID NO 18
<211> LENGTH: 717
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

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<400> SEQUENCE: 18

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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 Tyr Glu His Asn Arg His Lys Gln
50          55          60
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

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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															
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					

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<210> SEQ ID NO 19
<211> LENGTH: 825
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

<400> SEQUENCE: 19
atgaagtgtt tattggcaat agttgcgatg tctggtgtcg cattggcggc agaaaataag    60
aatggtgagg tgagcagtga gcatttcgtc cgttatcaat accaagacaa aatcagctat    120
ggaaagctag acaatgacgc agtgttaccg gtcagcggcg atctctttgg cgaatattcg    180
gtagcaaaaa attcgatecc gttagagtcg gttgaggtgt tactaccgac aaaaccagag    240
aaagtcttcg ccgtcgggat gaacttcgct agccacttag cctcacctgc cgatgcacca    300
ccgcgatgtt ttcttaaaact tccttcttct ttgattctca cggcggaagt gattcaagtg    360
ccacaaaaag caagaaatgt tcattttgaa ggcgagctgg tggttgtgat tggtagagag    420
ctcagtcaag ccagtgaaga agaagccgaa caagcgatct ttggcgtcac ggtgggcaac    480
gatattactg aaagaagttg gcaaggcgcc gatttacaat ggctccgagc gaaagcttcc    540
gatggttttg gcccggttgg caacacaatt gtgcgcgcca ttgattacaa caatattgag    600
ttaaccactc gtgtaacgg taaagtggtt caacaagaaa atacttcggt catgatccac    660
aagccaagaa aagtcgtgag ctatttgagc tattatttta ccctcaaacc gggcgatcta    720
attttcatgg gcacgccagg tagaacttat gctctgtccg acaaatgatca agtgagtgtc    780
acgattgaag gggtagggac tgtggtaaat gaagtgcggt tctga                    825

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<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

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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

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atggctagca cttttaattc aatttcgggc tcgaagcgta gcctgcacgt gcaagtagca    60
cgcgaaatcg ctcgaggaat tttgtctggt gatctgcegc aaggttctat tattcctggt    120
gaaatggcgt tgtgtgaaca gtttggtatc agccgaacgg cacttcgtga agcagttaaa    180
ctactgacct ctaaaggctc gtttagagtct cgccctaaaa ttggtactcg cgtagtcgac    240
cgcgcatact ggaacttctc tgatcctcaa ctgattgaat ggatggacgg actaacggac    300
gtagaccaat tctgtttcct 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 ctactatggt cgtaacttc atagtgcatt cttctgaaga ggggaagcaca    600
tgcatcaatg aacaccgcag aatctatgaa gctatcatgg ccggtgattg tgacaaggct    660
agaattgctt ctgctgttca cttgcaagat gcccaaccacc gtttggaac agcataa    717

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<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

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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

<210> SEQ ID NO 23

<211> LENGTH: 1779

<212> TYPE: DNA

<213> ORGANISM: *Vibrio splendidus*

<400> SEQUENCE: 23

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atggaactca acacgattat tgtcggcatt tatttcctat tcttgattgc gataggttgg      60
atgtttagaa catttacaag tactactagt gactacttcc gcgggggcgg taacatgttg      120
tggtggatgg ttggtgcaac cgcccttatg acccagttta gtgcatggac attcaccggt      180
gcagcaggta aagcgtataa cgatggtttc gctgtagcgg tcatcttcgt agccaacgca      240
tttggttact tcatgaacta cgcgtacttc ggcgcgaaat tccgtcaact tcgcgttggt      300
acggaatcgc aagcgattcg tatgcgtttt ggtgcgacca acgaacaagt attcacttgg      360
tcttcaatgc caaactcagt ggtatctgcg ggtgtgtggt taaacgcatt ggcaatcatc      420
gcttcgggta tcttcgggtt cgacatgaac atgactatct gggtgactgg cctagtggta      480
ttggcaatgt cggtaacagg tggttcatgg gcggtaatcg catctgactt catgcagatg      540
gttatcatca tggcggtaac ggtaacttgt gcggtttag cggttgttca aggtggcggg      600
gttggtgaga ttgtaacaa cttcccagta caagatggtg gttcgttcct ttggggcaac      660
aacatcaact acctaagcat ctttaogatt tgggcattct tcatcttcgt taagcagttc      720
tcaatcacga acaacatgct taactcttac cgttacctag cggctaaga ctcaaagaac      780
gctaagaaag ctgcaactgt tgcttgtgtg ttgatgttgt gtggtgtggt tatttggttc      840
atgccttctt ggttcattgc aggccaaagt gttgatttat cagcggctta cccgaatgca      900
ggtaaaaaag cgggtgactt tgcttaccta tacttcgtac aagagtacat gccagcaggt      960
atggttggtc tattagtgtc cgcgatggtt gcagcgacaa tgtcttcaat ggactcaggt     1020
ctaaaccgta actcaggtat ttttgtaag aacttctacg aaacaatcgt tcgtaaaggt     1080
caagcatcag agaaagagct agtaaccgta tctaaaatta cttcagcggg atttggtttc     1140

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gctattatcc taatcgcaac gttcatcaac tcattaaaag gcttaagcct gtttgatacg 1200
atgatgtacg taggtgcggt aatcggcttc cctatgacga ttctgcatt ccttggtttc 1260
ttcatcaaga agactccgga ctgggctggt tggggaacgc tagttgttg tggtatcgta 1320
tcttatgtgg ttggttttgt tatcaacgcg gagatggtag cagcggcggt ttggtcttgat 1380
actctaacag gacgtgaatg gtctgatgtt aaagttgcga ttggtctgat tgctcacatc 1440
acgctaaceg gtggcttctt cgtactatct acgatgttct acaagcctct atcaaaagaa 1500
cgtaaacggg atggtgataa gttctttggc aacttagata cccattagt agctgaatcg 1560
gcagagcaaa aagtgttggg taacaaacaa cgtcaaatgc ttggtaaact gattgcbgta 1620
gcbggtgttg gtattatgct gatggctctt ctgactaacc caatgtgggg gcgcctagtc 1680
ttcatcttat gtggtgtgat agtgggtggt gtcggtattc tacttgtgaa agcggctgat 1740
gacggcggca agcaagcgaa agcagtaacc gaaagctaa 1779

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<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

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ttcctagaca ccttgagcga agcacgtccg cgtcttcttg ttcaagctga tcagctagaa 180
gaattcaaaag caaaagtgaag agctgatcaa gctcactgta tgtttgatga tttctacaac 240
aactctaccg ttaagttcct tgagactgct cctttcgaag agcctcaagc gtaccagct 300
gagacggtag gtaaagcttc tctatggcgt ccttattggc gtcaaatgta cgttgattgc 360
caaatggcac tgaacgcgac acgtaacctc gcgattgctg gtgttgtaaa agaagacgaa 420
gcgctcattg cgaagcaaaa agcttgact ctaaaactgt ctacgtacga tccagaaggc 480
gtgacttctc gtggctataa cgatgaagcg gctttcctg tttatcgctgc tatggcttgg 540
ggttacgatt ggctacacgg ctacttcacc gatgaagaac gccagcaagt tcaagatgct 600
ttgattgagc gtctagacga aatcatgcac cacctgaaag tgacgggtga tctattgaac 660
aaccactaa atagccacgg tgttcgttct atctctctg ctatcatccc aacgtgtatc 720
gcgctttacc acgatcacc gaaagcagcg gagtacattg catacgcgct agaatactac 780
gcagtacatt acccaccatg gggcgggtga gacggcggtt gggctgaagg tctgattac 840
tggaacacgc aaactgcatt cctagcgcaa gcattcgacc tattgaaagc atactgtggt 900
gtagacatgt ttaacaaaac attctacgaa aacacaggtg atttcccgt ttaactgcac 960
ccagttcact ctaagcgcgc gagcttctgt gaccagtctt caatcggcga tttcccaggt 1020
ttaaaactgg cttacaacat caagcactac gcaggtgtta accagaagcc tgagtacgtt 1080
tggtactata accagcttaa aggccgtgat actgaagcac acaccaaatt ctacaacttc 1140
ggttggtggg acttcgggta tgacgatctt cgttttaact tcctttggga tgcacctgaa 1200
gagaaagccc catcgaacga tccactgttg aaagtattcc caatcacggg ttgggctgca 1260
ttccacaaca agatgactga gcggtataac catattcaca tggatttcaa atgttctccg 1320
tttggctcaa tcagccactc tcacggtgac caaaacgcat ttacgcttca cgcatttgg 1380
gaaacgctag cgtcagtaac aggttactat ggtggtttcg gtgtagacat gcacacgaaa 1440
tggcgtcgtc aaacgttctc taaaaacctg cactatttg gcggtaaagg tcagtacggc 1500
gagaacaaga acacaggcta cgaaaaccac caagatcgtc tttgtatcga agcgggccc 1560
actatctctg acttcgacac tgaatctgat gtgaagatgg ttgaaggtga tgcaacggca 1620
tcttacaagt acttcgttcc tgaatcga tcttacaagc gtaaagtctg gttcgttcaa 1680
ggtaaagtct tcgtaatgca agacaaggca acgctttctg aagagaaaga catgacttgg 1740
ctaatacaca caactttcgc aaacgaagtg gcagacaagt ctttactat ccgtggcgaa 1800
ggtgcgcacc tagacgtaaa cttcatcaac gactctgctg ataacatcac gtcagttaag 1860
aacgttgaag gctttggcga agttgaccca tacgagttca aagatcttga gatccaccgt 1920
cacgtggaag tggaattcaa gccatogaaa gagcacaaca tctgacgct tctgttctc 1980
aataagaatg aaggcgagca agttgaagtg tttcacaagc ttgaaggcaa cacgtactg 2040
ctaaatggtg acggcgaaaac ggtttcaatc gaactgtaa 2079

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<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

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1	5	10	15
Asn Asp Thr Ser Ala 20	Gly 25	Asn Leu Val Asp 30	Leu Leu Pro Ile Glu Val 35
Gln Thr Arg Asp Phe 35	Asp 40	Leu Ser Phe Leu 45	Asp Thr Leu Ser Glu Ala 50
Arg Pro Arg Leu Leu 50	Val 55	Gln Ala Asp Gln 60	Leu Glu Glu Phe Lys Ala 65
Lys Val Lys Ala Asp 65	Gln 70	Ala His Cys Met 75	Phe Asp Asp Phe Tyr Asn 80
Asn Ser Thr Val Lys 85	Phe 90	Leu Glu Thr Ala 95	Pro Phe Glu Glu Pro Gln 100
Ala Tyr Pro Ala Glu 100	Thr 105	Val Gly Lys Ala 110	Ser Leu Trp Arg Pro Tyr 115
Trp Arg Gln Met Tyr 115	Val 120	Asp Cys Gln Met 125	Ala Leu Asn Ala Thr Arg 130
Asn Leu Ala Ile Ala 130	Gly 135	Val Val Lys Glu 140	Asp Glu Ala Leu Ile Ala 145
Lys Ala Lys Ala Trp 145	Thr 150	Leu Lys Leu Ser 155	Thr Tyr Asp Pro Glu Gly 160
Val Thr Ser Arg Gly 165	Tyr 170	Asn Asp Glu Ala 175	Ala Phe Arg Val Ile Ala 180
Ala Met Ala Trp Gly 180	Tyr 185	Asp Trp Leu His 190	Gly Tyr Phe Thr Asp Glu 195
Glu Arg Gln Gln Val 195	Gln 200	Asp Ala Leu Ile 205	Glu Arg Leu Asp Glu Ile 210
Met His His Leu Lys 210	Val 215	Thr Val Asp Leu 220	Leu Asn Asn Pro Leu Asn 225
Ser His Gly Val Arg 225	Ser 230	Ile Ser Ser Ala 235	Ile Ile Pro Thr Cys Ile 240
Ala Leu Tyr His Asp 245	His 250	Pro Lys Ala Gly 255	Glu Tyr Ile Ala Tyr Ala 260
Leu Glu Tyr Tyr Ala 260	Val 265	His Tyr Pro Pro 270	Trp Gly Gly Val Asp Gly 275
Gly Trp Ala Glu Gly 275	Pro 280	Asp Tyr Trp Asn 285	Thr Gln Thr Ala Phe Leu 290
Gly Glu Ala Phe Asp 290	Leu 295	Leu Lys Ala Tyr 300	Cys Gly Val Asp Met Phe 305
Asn Lys Thr Phe Tyr 305	Glu 310	Asn Thr Gly Asp 315	Phe Pro Leu Tyr Cys Met 320
Pro Val His Ser Lys 325	Arg 330	Ala Ser Phe Cys 335	Asp Gln Ser Ser Ile Gly 340
Asp Phe Pro Gly Leu 340	Lys 345	Leu Ala Tyr Asn 350	Ile Lys His Tyr Ala Gly 355
Val Asn Gln Lys Pro 355	Glu 360	Tyr Val Trp Tyr 365	Tyr Asn Gln Leu Lys Gly 370
Arg Asp Thr Glu Ala 370	His 375	Thr Lys Phe Tyr 380	Asn Phe Gly Trp Trp Asp 385
Phe Gly Tyr Asp Asp 385	Leu 390	Arg Phe Asn Phe 395	Leu Trp Asp Ala Pro Glu 400
Glu Lys Ala Pro Ser 405	Asn 410	Asp Pro Leu Leu 415	Lys Val Phe Pro Ile Thr

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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
 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

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atgactaaac ctgtaatcgg tttcattggc ctaggcttta tgggcggcaa catggttgaa    60
aacctacaaa agcgcgggcta ccacgtaaac gtaatggatc taagcgtga agctgttgct    120
cgcgtaacag atcgcgggcaa cgcaactgca ttcacttctg ctaagaact agctgtgca    180
agtgacatcg ttcagttttg tctgacaact tctgctgttg ttgaaaaaat cgtttacggc    240
gaagacggcg ttctagcggg catcaaagaa ggcgcagtac tagtagactt cggctacttct    300
atccctgctt ctactaagaa aatcggcgca gctcttctg aaaaaggcgc gggcatgatc    360
gacgcacctc taggtcgtac tctgtcacac gctaaagatg gtcttctgaa catcatggct    420

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gctggcgaca tggaaacttt caacaaagtt aaacctgttc ttgaagagca aggcgaaaac 480
gtattccacc taggggctct aggttctggt cacgtgacta agcttgtaaa caacttcatg 540
ggatagacga ctggttgcgac tatgtctcaa gctttcgctg ttgctcaacg cgctggtggt 600
gatggccaac aactgtttga catcatgtct gcagggtccat ctaactctcc gttcatgcaa 660
ttctgtaagt tctacggcgt agacggcgaa gagaagctag gtttctctgt tgctaacgca 720
aacaagacc ttggttactt ccttgcactt tgtgaagagc taggtactga gtctctaadc 780
gctcaaggta ctgcaacaag cctacaagct gctgttgatg caggcatggg taacaacgac 840
gtaccagtaa tcttcgacta cttcgctaaa ctagagaagt aa 882

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<210> SEQ ID NO 28
<211> LENGTH: 293
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

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<400> SEQUENCE: 28

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```

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
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

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 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 tgttgcaaaa    180
gagttcatga aatcccccta ccaagtgcgc aatgaagcac tcatgcatta tgcgctaag    240
cttgggtggca ttgacaagat tgtggtgggt tttgacgacg gccgttctta tacctctcgc    300
ccttcagagt ctttccctaa cgggtgttga ataaaagaaa aatacaatcc aacctctga    360
ccttggtatc aacaagcgaa attgaaatca ggcttatctt ttagtggctc gtttttact    420
aagagtactc aagtgcctat gatcgggtg acctactcat accaagatcg tgcacatcg    480
gccgatatac gctttgacga tttgaaaacg cagcttgaac agctggacag catctacgaa    540
gccaaaggca ttatcatcga cgaaaagggg atggtggtcg cttcaacaat cgaaaacgtg    600
cttcgcgaaa ccaatatatc ttctgcgac actcaaatga aactcaacag tgccattgaa    660
cagcctgac aattcattga ggggtgtgatt gatggtaacc agagaatctt gatggccaag    720
aaagtggata ttggcagcca gaaagagtgg ttcattgatc ccagtattga ccctgaactc    780
gcgctcaatc agctgaatgg cgtgatgtcg agtgcgcgca tccttatcgt cgtttgtgta    840
cttggctcgg tgatattgat gattttactt ctgaatcgtt tctaccgccc aatcgtgtca    900
ctgcgcaaaa tcgtccacga tctatcacia ggtaacggag acctcactca aaggcttgct    960
gagaagggga atgatgactt agggcatatc gccaaagaca tcaacttgtt cattatcggc    1020
ttacaagaga tggttaagga tgtgaaatca aagaactcgg atctcgatac caaggtactg    1080
agtattcgcg aaggttgtaa agaaaccagc gatgtactga aagttcatac tgatgaaacg    1140
gttcaagtgg tctctgcgat taacggcttg tctgaagcat caaacgaagt agagaagagt    1200
tctcagtcgg cggcagaagc agcaagagag gccgctgtgt tcagtgatga gacgaaacg    1260
attaacacgg tgacgaaac ctatatcagt gatcttgaga agcaagctcg caccacttct    1320
gatgacatc gctcaatggc caatgaaaac cagagcatcc agtctatcgt gctctgtgatt    1380
ggcggaaatt cggacaacaa taatttgcgt gcattgaatg cgtcaattga agcggcgagg    1440
gcgggtgaac atggtcgagg tttcgcggtg gttgctgatg aagtcgctgc gctagccaac    1500
cgaaacgaaa tcagtacctc tgaaattgat gaagcgttat ctggcttgcg gtetaaatca    1560
gatggtttgg ttaaacttat tgagttgacc aaaagtaact gtgaactgac tcgogctcaa    1620
gttgttcaag ctgtaaacat gttggcgaag ctaaccgagc agatggaaac agtaagtcgt    1680
tttaataatg acatttcggg ttcgtctggt gagcaaaaac cccttattca gagcattgct    1740
aagaacatgc ataagattga aagctttggt gagagctta ataaactaag ccaagatcag    1800
ttaactgaat cagcagaaat caaacactt aacggtagcg ttagtgaatt gatgagcagc    1860

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-continued

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
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

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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					
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 ccctctgat accaataact caggagcadc ttatggtggt    120
gttgctccct atgatattgc caagtatcaa aacatccttt ccagctcaga tcttcaggtg    180
tctgatccta atggagagga gggcaataaa acctctgaag tcaaagatgg taacttcgat    240
ggttatgtca gtgattatgt ttatgctgac gaagagacgg aaaatctgat cttcaaaatg    300
gcgaactaca agatgcgctc tgaagttcgt gaaggagaaa acttcgatat caatgaagca    360
ggcgtaagac gcagctetaca tgcggaaata agcctacctg atattgagca tgtaatggcg    420
agttctcccg cagatcacga tgaagtgacc gtgctacaga tccacaataa aggtacagac    480
gagagtggca cgggttatat ccctcatccg ctattgcgtg tggtttggga gcaagaacga    540

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gatggcctca caggctacta 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 aatgggtgaag ccacggctca ctttcaggca    840
ctcgatatac ccaccacaca ggtcaacggc tcaaacgatt gggatattaa tgattggaag    900
ttgacgattc ctgcgagtaa agacacttgg tatggaagtg ggggtgacag tgcggctgaa    960
ctagaacctg agcgtcgca atcgagcaaa gaccttctcg ccaacgacag tgatgtctac   1020
gacagcgata ttggtctttc ttatttcaat accgatgaag ggagagtga ctttagagcg   1080
gatatgggat atggcacctc taccgaaaat tctagctata ttcgctctga gctcagggag   1140
ttgtatcaaa gcagtgttca accggattgt agcaccagcg atgaagatac aagtgggtat   1200
ttggacgaca ctagaacgaa cgctaccagt cacgagttaa ccgcaagctt acgaattgaa   1260
gactaccga acattaataa ccaagaccg aaagtgggtc ttgggcaaat acacggttg   1320
aagatcaatc aagcattggt gaagtgttga tgggaaggcg agagtaagcc agtaagagt   1380
atactgaact ctgattttga gcgcaacaac caagactgta accattgtga cccgttcagt   1440
gtcgagttag gtacttattc ggcaagtga gagtggcgat atacgattcg agccaatcaa   1500
gacggtatct acttagcgac tcatgattta gatggaacta atacggttcc tcatttaatc   1560
ccttggggac aagattacac agataaagat ggggacacgg tctcgttgac gtcagattgg   1620
acatcgacag acatcgcttt ctatttcaaa gcgggcatct acccaaat taagcctgat   1680
agcgactatg cgggtgaagt gtttgatgtg agctttagtt ctctaagagc agagcataac   1740
tga

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<210> SEQ ID NO 32
<211> LENGTH: 580
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

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<400> SEQUENCE: 32

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```

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

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-continued

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 cgctagttag catgatggtg acggacctga tegtctcgtt    180
gaccaagacc taactacacg ttggtcatct gcgggtgacg gcgagtgggc aacgctagac    240
tatggttcag tacaggagtt tgacgcggtt caggcatctt tcagtaaagg taatcagcgc    300
caatctaaat ttgatatcca agtgagtgtt gatggcgaac gctggacaac ggtactagaa    360
aaccaactaa gctcaggtaa agcgcctggc ctgagcgtt tccaatttga gccagtagtg    420
caagcacgct acgtaagata cgttggctac ggtaacacca aaaacggttg gaacagtgtg    480
actggattag cggcggttaa ctgtagcatt aacgcattgc ctgctagcca tatcatcaact    540
tcagacgtgg ttgcagcaga agccgtgatt attgctgaaa tgaagcggc agaaaaagca    600
cgtaaagatg cgcgcaaaga tctacgctct ggtaacttcg gtgtagcagc ggtttaccct    660
tgtgagacga cgttgaatg tgacactcgc agtgcacttc cagttccgac aggcctgcca    720
gcgacaccag ttgcaggtaa ctgcgcaagc gaaaactttg acatgacgca ttggtaccta    780
tctcaacccat ttgacctaga caaaaatggc aaacctgatg atgtgtctga gtggaacctt    840
gcaaacgggtt accaacaccc tgaaatcttc tacacagctg atgacggcgg cctagtattc    900
aaagcttacg tgaaggtgtg acgtacctct aaaaactacta agtacgcgcg tacagagctt    960
cgtgaaatga tgcgtcgtgg tgatcagtct attagcacta aaggtgttaa taagaataac   1020
tgggtattct caagcgctcc tgaatctgac ttagagtcgg cagcgggtat tgacggcggt   1080
ctagaagcga cgttgaaat cgaccatgca acaacgacgg gtaatgcgaa tgaagtaggt   1140
cgctttatca ttggtcagat tcacgatcaa aacgatgaac caattcgttt gtactaccgt   1200
aaactgcca accaagaaac gggctcgggt tacttcgcac atgaaagcca agacgcaact   1260
aaagaggact tctacctct agtggggcag atgacggctg aagtgggtga cgatggtatc   1320
gcgcttgcg aagtgttcag ctaccgtatt gacgttaaag gcaacacgat gactgtaacg   1380
ctaatacgtg aaggcaaga cgatgttgta caagtgggtg atatgagcaa cagcggctac   1440
gacgcaggcg gcaagtacat gtacttcaaa gccggtgttt acaacaaaa catcagcggc   1500
gacctagacg attactcaca agcgacttct tatcagctag atgtatcgca cgatcaatac   1560
aaaaagtaa                                     1569

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<210> SEQ ID NO 34
 <211> LENGTH: 522
 <212> TYPE: PRT

-continued

<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
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

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-continued

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

<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 tgaacctggt    120
gaagtttact ctatagaaaa cgtctattgg gatctgacag gtggtgctgt tgctgcacag    180
tcactcagcg gaacttcacc atatcgcttt gataataatg aggaaggtag tcgtgctcta    240
agcatttaca gtggagacgt agctaattgg ttcacttttg agagttcaat atatactgct    300
gaagaagaag gtggtgtttc ctttgaagg aaggactgta cttacacagt gactgagcaa    360
cagctagata tgacctgtga aaaagatgac gtagaaacag cttactcagc aacagagatt    420
acagatgaat ctgttataac tgcattagaa aatgccgatg atggaaaacc taaatcagtc    480
gatgatgtga acgctgcgat tgcacagca gaagatggcg cgattattga tttatcatct    540
gaaggtacgt ttgataccgg tgttattgag ctaaataaag ctgtcacaat tgatggtgct    600
ggttttagca ccattaccgg agatgcttgt attgatgtca ctgcaccogg tgcaggtatc    660
aaaaacatga cttttgctaa cgacaatttg gccgggtggt ttggtagga gtcagctggt    720
acttcagata atgaaactgg tgcgatcgtt attggtaaaa ttggtaaaga ttcagatcct    780
gtagcacttg aaaacctaaa gttcagatgca aacggcatta ccgaagatga tctaggtact    840
aaaaaagcaa gttggttatt ctctcgaggt tactttacat tagacaatag cgaatttgtc    900
ggtttaagtg gcagtttcca aaataatgca atctgtatta actgtagtag tgacaacggg    960
cgatttggtt cacaaatcac aaataatgca ttcactatta actctggtgg tagtgatgtg   1020
ggcggaaatta aagttggtga ttctagcagt gccgtcataa agaatagtga tgataacctt   1080
ggctgtaaty tcaactattg aagcaatagc ttcaatggtt acaaaaccct actttcagct   1140
gacaacggta aagatataag aaatacagcc atctacgcac aaccatctgc agtgaacctt   1200
gcggcaggta aagaaaatat cttgaactaa                               1230

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<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
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

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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												
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 agcagtgggc ttattagcga ctgtgccaat gggtactttc    60
gcaaataaacg gtgtttctta ccccgtagct gccgataaat tcgatatgca taattggaaa    120
ataaccatac cttcagatat taatgaagat ggtagcgttg atgaaataga aggggtcgct    180
atgatgagct actcacatag tgatttcttc catcttgata aagacggcaa ccttgatttt    240
gaagtgcaga accaagcgat tacgacgaaa aactcgaaga atgtagcgttc tgagttacgc    300
cagatgccaa gaggcgcaga tttctctatc gatacggctg ataaaggaaa ccagtgggca    360
ctgtcgagtc acccagcggc tagtgaatac agtgctgtgg gcggaacatt agaagcgaca    420
ttaaagtga atcacgtctc agttaacgct aagttcccag aaaaataccc agctcattct    480
gttgtaggttg gtcagattca tgctaaaaaa cacaacgagc taatcaaagc tggaaaccggt    540
tatgggcatg gtaatgaacc actaaagatc ttctataaga agtttctga ccaagaaatg    600
ggttcagtat tctggaacta tgaacgtaac ctagagaaaa aagatcctaa ccgtgccgat    660
atcgcttacc cagtgtgggg taacacgtgg gaaaaccctg cagagccggg tgaagccggt    720
attgctcttg gtgaagagtt tagctacaaa gtggaagtga aaggcaccat gatgtaccta    780
acgtttgaaa ccgagcgtca cgataccggt aagtatgaaa tcgacctgag taagggcacc    840
gatgaacttg actcaccaac gggctatgct gaagatgatt tttactacaa agcgggagca    900
tacggccaat gttagcgtgag cgattctcac cctgtatggg ggctggttg tggcggtaact    960
ggcgatttcg ctgtcgataa aaagaatggc gattacaaca gtgtgacttt ctctgcgctt   1020

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aagttaaacg gtaaataag

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

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

-continued

<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 tcttgatata tcttctttgc agcagagcaa catgtttggg    240
gcttataacc caaccacgcc tgctgtggtt gagcagcaag ttatccaaga tgcgccaag    300
acgcgactga acctcgtttt agtgggtgca gtagccagtt ctaatccaaa gctgagcttg    360
gctgtgattg ccaatcgccg cacacaagca acctacggca ttaatgaaga gatcgaaggt    420
acgcgagcta agttaaagc ggtattagtc gatcgctgta ttattgataa ctcaggtcga    480
gacgaaacct tgatgcttga aggcattgag tacaagcgtt tgtctgtatc agcacctgag    540
ccacctcgta cctcttcttc tgtgctgggc aacaaccag cttctgcaga agagaagcta    600
gatgaaatta aagcgaagat aatgaaagat ccgcaacaaa tcttccaata tgttcgactg    660
tctcaggtga aacgcgacga taaagtatt gggtatctg tgagccctgg caaagattca    720
gaacttttta actctgttgg gctccaaaac ggagatattg ccaactcagtt aaatggacaa    780
gacctgacag acctgctgac tatgggcaac atattcggtt ctatctcaga gctgacagag    840
ctaaacctcg tcgtcgagag agatgggtcaa 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
  
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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

<210> SEQ ID NO 43

<211> LENGTH: 2025

<212> TYPE: DNA

<213> ORGANISM: *Vibrio splendidus*

<400> SEQUENCE: 43

```

gtgaagcatt ggtttaagaa aagtgcattg ttattggcag gaagcttaat ctgcacaccc 60
gcagccatcg cgagtgattt tagtgccagc tttaaaggca ctgatattca agagtttatt 120
aatattggtg gtcgtaacct agagaagacg atcatcgttg acccttcggg ggcgggaaaa 180
atcgatgtac gcagctacga cgtactcaat gaagagcaat actacagctt cttcctaaac 240
gtattggaag tgtatggcta cgcggttgtc gaaatggact cgggtgttct taagatcatc 300
aaggcctaaag attcgaatac atcggcaatt ccagtcgttg gagacagtga cacgatcaaa 360
ggcgacaatg tgggtgacac gtttgtagac gttcgtaatg tctcgggtgc tgaactttct 420
cctctgcttc gtcaactaaa cgacaatgca ggccgggta acggttgca ctacgacca 480
gccaacatca tccttattac aggccgagcg gcggtagtaa accggttagc tgaatcattc 540
aagcgtgttg accaagcggg tgataaagag attgaagtcg ttgagctaaa gaatgcttct 600
gcggcagaaa tggtagctat cgttgatgcy ttaagcaaaa cactgatgc gaaaaacaca 660
cctgcatttc tacaacctaa attagttgcc gatgaacgta ccaatgcgat tcttatctca 720
ggcgacccta aagtacgtag ccgtttaaga aggctgattg aacagcttga tgttgaatg 780
gcaaccaagg gcaataacca agttatttac cttaaatatg caaagccga agatctagtt 840
gatgtgctga aaggcgtgct ggacaacctc caatcagaga agcagacatc aaccaaaagg 900
agttcatcgc agcgtaacca agtgatgatc tcagctcaca gtgacaccaa ctcttttagt 960
attaccgcac agccggacat catgaatgcy cttcaagatg tgatcgaca gctggatatt 1020
cgtcgtgctc aagtattgat tgaagcactg attgtcgaaa tggccgaagg tgacggcggt 1080
aaccttggtg tgcagtgggg taaccttgaa acgggtgcca tgattcagta cagcaacact 1140
ggcgcttcca ttggcggtgt gatggttggg ttagaagaag cgaagacag cgaaacgaca 1200
  
```

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accgctgttt atgattcaga cggtaaattc ttacgtaatg aaaccacgac ggaagaaggt 1260
gactattcaa cattagcttc cgcactttct ggtgttaatg gtgcggcaat gagggtggta 1320
atgggtgact ggaccgcctt gatcagtgca gtagcgaccg attcaaattc aaatataccta 1380
tcttctccaa gtatcacctg gatggataac ggccaagcgt cattcattgt ggggtgaagag 1440
gtgcctgttc taaccggttc tacagcaggc tcaagtaacg acaaccatt ccaaacagtt 1500
gaacgtaaag aagtggtgat caagcttaaa gtggtgccgc aatcaatga aggtgattcg 1560
gttcaactgc aaatagaaca agaagtatcg aacgtattag gcgccaatgg tgcggttgat 1620
gtgcgttttg ctaagcgaca gctaaataca tcagtattg ttcaagacgg tcaaatgctg 1680
gtgttggtg gcttgattga cgagcgagca ttgaaaagt aatctaaggt gccgttcttg 1740
ggagatattc ctgtgcttgg acactgttgc aaatcaacca gtactcaggt tgagaaaaag 1800
aacctaattg tcttcatcaa accaaccatt attcgtgatg gtatgacagc cgatggatc 1860
acgcagccta aatacaactt catccgtgct gagcagttgt acaaggctga gcaaggactg 1920
aagttaatgg cagacgataa catcccagta ttgcctaaat ttggtgccga catgaatcac 1980
ccggtgaaa ttcaagcctt catcgatcaa atggaacaag aataa 2025

```

<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

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-continued

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
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

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<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
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

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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*

<400> SEQUENCE: 47

```

atggcggcat ttgaatacaa agcactggat gccaaaggca aaagtaaaaa aggctcaatt    60
gaagcagata atgctcgtca ggctcgccaa agaataaaag agcttggtt gatgccggtt    120
gagatgaccg aggctaaagc aaaaacagca aaagtgctc agccatcgac cagctttaa    180
cgcggcacga gtacgcctga tcttgcgctt attactcgtc aaatatccac gctcgttcaa    240
tctggtatgc cgctagaaga gtgtttgaaa gccgttgccg aacagtctga gaaacctcgt    300
attcgcacca tgctactcgc ggtgagatct aaggtgactg aaggttattc gttagcagac    360
agcttgctcg attatcccca tatcttcgat gagctattca gagccatggt tctgctggt    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 acacaathtt tattagcatc gagtgaatht    660
atccagaatt ggggcatcca attactggtg ttgaccattg gtgtgattgt gttggttaag    720
actgcgctga aaaagccggg cgttcgcatg agctgggacg gcaaattatt gagcatcccg    780
ctgataggca agatagcgaa agggatcaac acctctcgtt ttgcacgaac actttctatc    840
tgtacctcta gtgcgattcc tacccttgaa gggatgaagg tcgcggtaga tgtgatgtcg    900
aatcatcacg tgaacaaca agtattacag gcatcagata gcgttagaga aggggcaagc    960
ctgcgtaaag cgcttgatca aaccaaaact tttccccga tgatgctgca tatgatcgcc    1020
agtggtgagc agagtggcca attggaacag atgctgacaa gagcggcaga taatcaggat    1080
caaagctttg aatcgaccgt taatcgcgct ttaggcattt ttaccccagc gcttattgctg    1140
ttgatggctg gcttagtgct gtttatcgtg atggcgagcg tgatgccaat gcttgaatg    1200
aacaatttaa tgatgggta a                                1221
    
```

<210> SEQ ID NO 48

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<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

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

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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 aaatgaaaa acaatcaggc tttaccctat tagaagtcac ggttgtgtgc    60
gttatccttg gtgttctagc aagttttggt gtacctaacc tgttgggcaa caaagagaag    120
gcggatcaac aaaaagccat cactgatatt gtggcgctag agaacgcgct cgacatgtac    180
aaactggata acagcgttta cccaacaacg gatcaaggcc tggacggggt ggtgacaaaag    240
ccaagcagtc cagagcctcg taactaccga gacggcggtt acatcaagcg tctacctaac    300
gacccatggg gcaatgagta ccaataccta agtcctggtg ataacggcac aattgatatc    360
ttcactcttg gcgcagatgg tcaagaaggt ggtgaaggta ttgctgcaga tatcggaac    420
tggaacatgc aggacttcca ataa                                           444

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<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
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

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<210> SEQ ID NO 51
<211> LENGTH: 594
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

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<400> SEQUENCE: 51

```

gtgaaaaacta agcaaacaca gccaggtttc accttgattg agattctttt ggtggttgta      60
ttactgtcag tatcggcggt cgcggtgacg tcgacccatcc ctaccaatag caaagatggt      120
gctaaaaaat acgctcaaag cttttatcag cgaattcagc tactcaatga agaggctatt      180
ttgagtggct tagattttgg tgttcgtggt gatgaaaaaa aatcgactta cgttctgatg      240
actttgaagt ctgatggctg gcaagaaaac gagttcgaag agatcccttc ttcaactgaa      300
ttaccggaag aactggcact gtcgctgaca ttagtggtg ggcgctggga agacgatgat      360
cggttgttca atccaggaag cttatttgat gaagatatgt ttgctgatct tgaagaggaa      420
aagaagccga aaccaccaca gatctacatc ttgtcgagtg ctgaaatgac gccatttgta      480
ctgtcgtttt acccaataac cggtgacaca atacaagatg tttggcgcac tcgagtattg      540
gataatgggtg tgattcgatt actcgagccg ggagaagaag atgaagaaga ataa          594

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<210> SEQ ID NO 52

<211> LENGTH: 197

<212> TYPE: PRT

<213> ORGANISM: *Vibrio splendidus*

<400> SEQUENCE: 52

```

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

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```

atgaagaaga ataaccgttc tccttatcgt tctcgcggta tgctcttgg ttctcgagga    60
atgactctgc ttgaagtatt ggttgcgctg gctatcttcg ctacggcggc gatcagtggtg    120
attcgtgctg tcaccagca catcaatcag ctcagttatc tcgaagaaaa aaccttcgctg    180
gcgatggctg ttgataatca aatggcccta gtcagcttac atcctgagat gcttaaaaaa    240
gcgaggggca cgcaagagtt agcgggaaga gaatggttct ggaaggtgac tcccatcgat    300
accagcgata atttattaa ggcgtttgat gtgagtgctg caaccagtaa gaaagcgtct    360
ccagtcgtta cggtgcgag ttatgtggtt aattaa                                396

```

```

<210> SEQ ID NO 54
<211> LENGTH: 131
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

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```

<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
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

```

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<210> SEQ ID NO 55
<211> LENGTH: 804
<212> TYPE: DNA
<213> ORGANISM: Vibrio splendidus

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<400> SEQUENCE: 55

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```

atgtgggtaa ttaagagaat gtggtaatt aagagcatgt tattaattaa gaacagctcg    60
ctaactaaga gcgtgtcgtc aactaagagc atgtcggaaa ataagcgtac gccgcgtaaa    120
caaggtctac cttcaaaagg gagaggcttt accttaattg aagtcttggc ctcgattgct    180
atctttgcca cgtaagtat gccggcttat caggtgggta atcaggtgca gcaagcaac    240
gagatctcta ttgagcgcag tgctcgtttg aaccaactgc aacgcagttt agtcatttta    300
gataatgatt ttcgccagat gccggtgcga aaatttcgta ccaacggtga agaagcatca    360
tctaagctga tcttaatgaa agagtattta ttggactcgg acagtgtagg catcatgttt    420
actcgtctag gttggcacia ccacaacag cagtttcctc gcggtgaagt cacgaaggtt    480
ggctaccgta ttaaagaaga aaccttgag cgtgtatggt gccgttatcc cgatacacct    540

```

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tcaggccaag aaggtgtgat taccctctg cttgatgatg ttgaaagctt ggaattcgag   600
ttttatgacg gaagccgctg ggggaaagag tggcaaaccg ataatcact gccgaaagcg   660
gtgaggctta agctgacact gaaagactat ggtgagatag agcgtgttta tctcactccc   720
ggtggcaccc tagatcagcg cgatgattct tcaaacagtg actcttcagg cagtagtgag   780
gggaataatg actcatcgaa ctaa                                           804

```

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<210> SEQ ID NO 56
<211> LENGTH: 267
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

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```

<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
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

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<400> SEQUENCE: 57

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atgactcatc gaactaataa gcgtttagcg acaaggctcag ccttgggacg taaacaacgt    60
gggtgcgcgc tgatcattat tttgatgcta ttggcgatca tggcaaccat tgctggcagc   120
atgtccgagc gtttgtttac gcaattcaag cgcgttggtg accaactgaa ttaccaacag   180
gcttactggt acagcattgg tgtggaagcg cttgtgcaaa acggtattag gcaaagttag   240
aaagacagtg ataccgtgaa cctaagccaa ccatggggcgt tagaagagca ggtataccca   300
ttggattatg gccaaagtaa gggccgcatt gttgatgctc aggcattgtt 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 agtcaactgg gaagtgatga ataaggttcg cccctttggt   660
tgcgctctgc caaccgatga tttccgcttg aatgtgaata ctctcacgga aaaacaagca   720
ccgttattgg aagcgatggt tgcgccaggc ttaagtgaat cggatgcca acagctgata   780
gataaacgcc catttgatgg ctgggatacg gtagatgctt tcattggctga acctgccatt   840
gttgggtgtaa gtgccgaagt cagcaagaaa gcgaaagcat atttaactgt agatagcgcc   900
tattttgagc tagatgcaga ggtattagtt gagcagtcac gtgtacgtat acggacgctt   960
ttctatagta gtaatcgaga aacagtgcag 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

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ttagctttgc tgccctgaaac cttagggcaa gtgaagacga tcgaagttga aagcattcgc 1080
tacgatggca accgtttctga ggttcgactg caggctaaaa gttctgactt ccaacacttt 1140
gagaccgcaa gggatgaagct cgaagagaag tttgtcgttg agcaagggcc attgaaccgt 1200
aatggcgatg ccgtatttgg cagttttact cttaaaccoc atcaataa 1248

```

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<210> SEQ ID NO 60
<211> LENGTH: 415
<212> TYPE: PRT
<213> ORGANISM: Vibrio splendidus

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<400> SEQUENCE: 60

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```

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
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

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-continued

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
405 410 415

<210> SEQ ID NO 61
<211> LENGTH: 489
<212> TYPE: DNA
<213> ORGANISM: *Vibrio splendidus*

<400> SEQUENCE: 61

atgagaaata tgattgaacc actccaagcg tgggtgggctt caataagtca gcggaacaa 60
cgattagtca ttggttggtc tattttatg atactgggcg ttgtctattg gggattaata 120
caaccactta gccaacgagc cgagcttgca caaagccgca ttcaaagtga gaagcaactt 180
ctggcttggg taacggacaa agcgaatcaa gtggttgaac tacgaggcag tgggtggcatc 240
agtgccagtc agcctttgaa ccaatctgtg cctgcttcta tgcgccgttt taacatcgag 300
ctgatacgcg tgcaaccacg cggtgagatg ctgcaagttt ggattaagcc tgtgccattt 360
aataagttcg ttgactggct gacatacctg aaagaaaagc aggggtgttga 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
1 5 10 15

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

-continued

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

```

gtgaaacgcg gtttatcttt caaatacggc ctgttattca gcgtcatttt tatcgttttt    60
ttctcggtaa gcttgtgtgt gcatttgctt gccgcttttg ctctcaagca tgcacccgtc    120
gtgcgtggtt taagcattga aggcgttgag ggcaccgttt ggcaaggctc cgctaacaat    180
atcgcggtgc agcgtgtcaa ttacggctca gtgcagtggg acttcagtt ctctaaacta    240
ttccaagcca aagcagaact tgcggttcgc tttggcgcga acagcgacat gaacttatca    300
ggtaaaggac gtgtcggata tagcatgagt ggtgcttacg cggaaaactt agtggcatca    360
atgccagcca gcaacgtgat gaaatagcgc ccagctatcc cagtgcctgt gtctattgca    420
gggcaagttg aactgacgat caaacatgcg gttcatgctc aaccttggtg tcaatcaggt    480
gaaggtacgc ttgcttggtc tgggtgcagca gtcgactcgc cagtgggttc gttagacctt    540
ggcctgtga ttgcgacat aacgtgtgaa gacagcacia ttgcagccaa aggcactcag    600
aagagcgatc aggtagacag cgagtcttca gcgagcgtaa cacctaacca acgctacacc    660
tcggcagcat ggtttaagcc aggcgtgaa ttcccgccag caatgcagag tcagcttaag    720
tggttgggca atcctgatag ccaaggtaaa taccaattta cttatcaagg ccgcttttag    780

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<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

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ctgtggaaca	ccatgccttg	tcatacgc	at	gagcgccgga	tggaagtcta	tttctat	1380
gatatggatg	aggaaacggc	cgttttccac	at	atgatggggc	aaccgcagga	aaccgcgtcac	1440
atagttatta	aaaacgagca	ggcggtgatt	tc	accgagct	ggtcgattca	ttccggtggt	1500
ggcaccagac	gctacacctt	tatctggggc	at	ggttggcg	agaatcaagt	ttccggtgac	1560
atggatcacg	tcaaggttag	cgagttacgt	ta	atcgcttt	caaccggaat	taccggtggt	1620
ccctacagta	acagctaacg	actaagtatt	gt	cgcttata	gagagattat	tgatatgatt	1680
ttaaattcct	ttgatttgca	aggtaaagtt	gct	ccttata	cggttga	tacgggttta	1740
ggtcagggta	tggctatcgg	tctggcacia	gct	ggctgtg	atcctgttg	cgccaacatc	1800
gttgaaccia	aagataccat	cgaaaaagtt	acc	gcactgg	gacgccgttt	cctcagcctg	1860
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<212> TYPE: DNA

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<211> LENGTH: 2331
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium tumefaciens

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<400> SEQUENCE: 67

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accgtcgaat tccttgccac gctctattct ccctgggicgg gaaccgatgg tggttgggicg   1080
gaaggtccgc attactggat gaccggcatg gcctatctca tcgaggccgc caatctgatc   1140
cgctcctata ttggttatga cctctatcaa cggccgtttt tccagaatac cggtcgcttc   1200
ccgctttaca ccaaggcgcc gggaaaccgc cgcgccaaact tcggcgacga ctccaccctt   1260
ggcgaccttc ccggcctgaa gctgggatac aacgtccggc aatcgcgcgg cgtcaccggc   1320
aatggccatt accagtggta tttcgatcac atcaaggccg atgcgacagg cacggaaatg   1380
gccttttaca attacggctg gtgggacctc aacttcgacg atctcgtcta tcgccacgat   1440
taccgcagg tggaaaccgt gtctcccgcc gacctgcggc cactcgcctt tttcgatgat   1500
attggttggg cgaccatcca aaaagacatg gaagaccggc accggcacct gcagttcgtc   1560
ttcaaatcca gcccttacgg ttcgctcagc cacagtcacg gcgaccagaa tgcctttgtg   1620
ctttatgccc atggcgagga tctggcgatc cagtccggtt attacgtggc gttcaattcg   1680
cagatgcate tgaattggcg gcgtcagaca cggtcgaaaa atgccgtgct gatcggcggc   1740
aaaggccaat atgcggaaaa ggacaaggcg cttgcacgcc gcgccgcggc ccgcatcgtc   1800
tcggtggagg aacagcccg ccatgttctg atcgtcggcg atgcaaccgc cgctaccag   1860
gttgcgaaac cgctggttca aaagtgctg cgcgaaaccc acttcgttaa tgacagctat   1920
ttcgtgattg tcgacgaagt cgaatgttcg gaaccccagg aactgcaatg gctttgccat   1980
acactcggag cgcgcagac cggcaggta agcttcgctt acaatggccg gaaagccggt   2040
ttctacggac agttcgttta ctcttcgggc ggcacgccgc aatcagcgc cgtggagggt   2100
tttcccata tcgacccgaa agaattcgaa gggctcgaca tacaccacca tgtctgcgcc   2160
acggttcggc ccgccaccg gcategcctt gtcacccttc tgggtcctta 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: PR

<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

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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
 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 tumefaciens C58

<400> SEQUENCE: 69

atgttcacaa cgtccgccta tgccctgcgat gacggctctt cgccgatgaa gctcgcgacc 60
 atcaggcgcc gcgatcccg tccgcgcgat gtcgaaatcg agatagaatt ctgtggcgtc 120
 tgccactcgg acatccatac ggcccgcagc gaatggcgg gctccctcta ccettgcgtc 180
 cccggccacg aaatcgtcgg ccgtgtcgg cggtggggcg cgcaagtcac ccggttcaag 240
 acgggtgacc gcgtcggtgt cggctgtatc gtcgatagct gcccggaatg cgcaagctgc 300
 gccgaagggc tggagcaata ttgcgaaaac ggcatgaccg gcacctataa cteccctgac 360
 aaggcgatgg gggcgggcgc gcatacgtt ggcggctatt ccgcccattg ggtggtggat 420
 gaccgctatg tgctcaatat tcccgaagg ctcgatccgg cggcagcagc accgctactc 480

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tgcgctggta tcaccaccta ctgcgcgctg cgccactgga atgccggccc cggcaaacgc   540
gtcggcgctcg 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 ggtgcagggtg ggcgcgcggg aaaagccact ttcggtgatg   840
gccttcagcc tcatccccgg ccgcaagacc tttgccggct cgatgatcgg cggatttccc   900
gagactcagg aaatgctgga tttctgcgcc gaaaaaggca tcgccggcga aatcgagatg   960
atcgatatcg atcagatcaa tgacgcttat gaacgcatga taaaaagcga tgtgcgcttat 1020
cgtttcgtca ttgatatgaa gagcctgccg cgccagaagg ccgcctga   1068

```

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<210> SEQ ID NO 70
<211> LENGTH: 355
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

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<400> SEQUENCE: 70

```

```

Met Phe Thr Thr Ser Ala Tyr Ala Cys Asp Asp Gly Ser Ser Pro Met
 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 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

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-continued

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

```

atggctattg caagaggtta tgctgcgacc gacgcgtcga agccgcttac cccgttcacc    60
ttcgaacgcc gcgagccgaa tgatgacgac gtcgtcatcg atatcaaata tgccggcadc    120
tgccactcgg acatccacac cgtccgcaac gaatggcaca atgccgttta cccgatcgtt    180
ccgggccacg aaatcgccgg tgctgtgctg gccgttggtt ccaaggcac gcggttcaag    240
gtcggcgacc atgtcgccgt cggctgcttt gtcgattect gcgttggtcg cgcaccccg    300
gatgtcgaca atgagcagta tatgccgggt ctctgtcaga cctacaattc cgttgaacgg    360
gacggcaaga gcgcgaccca gggcggttat tccgaccata tcgtggtcag ggaagactac    420
gtctctgcca tcccggacaa cctgcgctc gatgcctcgg cgccgcttct ctgcgcccgc    480
atcacgctct attcgcgct gcagcactgg aatgcaggcc ccggcaagaa agtggctatc    540
gtcggcatgg gtggccttgg ccacatgggc gtgaagatcg gctcggccat gggcgctgat    600
atcaccgttc tctcgagac gctgtogaag aaggaagacg gcctcaagct cggcgcgaag    660
gaatattacg ccaccagcga cgctcgacc tttgagaaac tcgcccgcac cttcgacctg    720
atcctgtgca cagtctcggc cgaaatcgac tggaacgcct acctcaacct gctcaaggtc    780
aacggcacga tggttctgct cggcgtgccc gaacatgcca tcccgggtgca cgcattctcg    840
gtcattcccc cccgcccgtc gctcgcgggt tcgatgatcg gctcgatcaa ggaaccacg    900
gaaatgctgg atttctgctg caagcacgac atcgtttcgg aaatcgaaac gatcggcacc    960
aaggacgtca acgaagccta tgagcgcgtg ctgaagagcg acgtgcgtta ccgcttcgtc   1020
atcgacatgg cctcgcctga 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

-continued

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					
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	caatgaagc	ggcggttgtc	cgcgatttg	gaaaaccgct	gaccatcgag	60
gaagtggcaa	taccgatcc	cgccccggt	gaaattctca	tcaactacaa	ggcgacgggc	120
gtttgccaca	ccgacctgca	cgccgcaacg	ggggattggc	cggtcaagcc	caaccgccc	180

-continued

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ttcattcccc gacatgaagg tgcaggttac gtcgccaaga tggcgctgg cgtcaccggc 240
atcaaggagg gcgaccgcgc cggcacgccc tggctctaca ccgctcggg atgctgcatt 300
ccctgccgta ccggctggga aaccctgtgc ccgagccaga agaactcagg ttattccgtc 360
aacggcagct ttgccgaata tggccttgcc gatccgaaat tcgtcggccg cctgcctgac 420
aatctcgatt tcggcccagc cgcacccgtg ctctgcgccg gcgttacagt ctataagggc 480
ctgaaggaaa ccgaagttag gcccggtgaa tgggtggtca tttcaggeat tggcgggctt 540
ggccacatgg cegtgaata tgcgaaagcc atgggcatgc atgtggttgc cgcgatatt 600
ttcgacgaca agctggcgct tgccaaaaag ctccggagccg acgtcgtcgt caacggccgc 660
gcgctgacg cggtaggaca agtgcaaaa gcaaccggcg gcgtccatgg cgcgctggtg 720
acggcggttt caccgaaggc catggagcag gcttatggct tcctgcgctc caagggcacc 780
atggcgcttg tcggtctgcc gccgggcttc atctccattc cgggtgtcga cacggtgctg 840
aagcgcacga cggtagctgg ctccatcgtc ggcacgcggc aggatctgga ggaggcgttg 900
accttcgccc gtgaaggcaa ggtggcccgc cacttctcgt gggacaagct cgaaaacatc 960
aatgatatct tccatcgcac ggaagagggc aagatcgacg gccgtatcgt cgtggatctc 1020
gccgcctga 1029

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<210> SEQ ID NO 74
<211> LENGTH: 342
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

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<400> SEQUENCE: 74

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```

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

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<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
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 aagggacgcy ccgtgctggc ggaactcccc 60

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gtcgccgate tgcgcgagg ccacgcgctc gtgcgggtca aggettcggg gctttgccat 120
accgatatcg acgtgctgca tgcgcgttat ggcgacggtg cgttccccgt cattccgggg 180
catgaatatg ctggcggaagt cgcagccgtg gcttccgatg tgacagtctt caaggctggc 240
gaccgggttg tcgtcgatcc caatctgccc tgtggcacct gcgccagctg caggaaaggg 300
ctgaccaacc tttgcagcac attgaaagct tacggcgctt cccacaatgg cggtttgctg 360
gagttcagtg tgggtgcgtc cgatcacctg cacggtatcg gttcgatgcc ctatcacgtc 420
gcgggcgttg ctgagccgct tgcctgtgtt gtcaatggca tgcagagtgc gggatttggc 480
gagagtggcg tgggtgcgga gaatgcgctt gtttccggtg ctgggcccac cgccctgctg 540
cttgccctgt cgctgaaatc acgcggcatt gcgacggtga cgatggccga tatcaatgaa 600
agcaggctgg cctttgccc gacccctggg cttcagacgg cggtatccgg ctcggaagcg 660
ctctcgcggc agcggaaagga gttcgatttc gtggccgatg cgacgggtat tgccccggtc 720
gcccaggcga tgatccccgt ggttcgggat ggcggcacgg cgctattctt cggcgtctgc 780
gcgccggatg cccgtatttc ggtggcacgg tttgaaatct tccggcgcca gctgaaactt 840
gtcggctcgc attcgtgtaa ccgcaacata ccgcaggcgc ttgccattct ggagacggat 900
ggcgaggtea tggcgcggtt cgtttcgcac cgcttgccgc tttcggagat gctgcccgtt 960
tttacgaaaa aaccgtctga tccggcgacg atgaaagtgc aatttcgagc 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

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-continued

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

<210> SEQ ID NO 79
 <211> LENGTH: 1044
 <212> TYPE: DNA
 <213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 79

```

atgcgcgcg tttattacga acgattcggc gagaccectg tagtcgcgtc cctgcctgat    60
ccggcaccga gcgatggcgg cgtggtgatt gcggtgaagg caaccggcct ctgccgcagc    120
gactggcatg gctggatggg acatgacacg gatatccgtc tgccgcatgt gcccggccac    180
gagttcgccg gcgtcatctc cgcagtcggc agaaacgtca cccgctcaa gacgggtgat    240
cgcggttaccg tgcccttctg ctccggctgc ggccattgcc atgagtgccg ctccggcaat    300
cagcaggtct gcgaaacgca gttccagccc ggcttcaccc attggggttc cttcgccgaa    360
tatgtcgcca tcgactatgc cgatcagaac ctcgtgcacc tgccggaatc gatgagttac    420
gccaccgccc ccggcctcgg ttgccgttcc gccacctcct tccgggcggg gacggatcag    480
ggacgcctga agggcggcga atggctggct gtccatggct gcggcggtgt cggctctctc    540
gccatcatga tcggcgccgg cctcggcgca caggtcgtcg ccatacgatat tgccgaagac    600
aagctcgaac tcgcccggca actgggtgca accgcaacca tcaacagccg ctccgttgcc    660
gatgtcgccg aagcgggtgc cgacatcacc ggtggcggcg cgcattgtgc ggtggatgcg    720
cttggccate cgcagacctg ctgcaattcc atcagcaacc tgcgcccggcg cggacgccat    780
gtgcaggtgg ggctgatgct ggcagaccat gccatgccgg ccattcccat ggcccgggtg    840
atcgctcatg agctggagat ctatggcagc cacggcatgc aggcattggcg ttaacaggac    900
atgctggcca tgatcgaaag cggcaggctt gcgcccggaaa agctgattgg ccgccatata    960
tcgctgaccg aagcggccgt cgcctctccc ggaatggata ggttccagga gagcggcatc   1020
agcatcatcg accggttcga atag                                         1044

```

<210> SEQ ID NO 80
 <211> LENGTH: 357

-continued

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<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

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

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-continued

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<211> LENGTH: 1011
<212> TYPE: DNA
<213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 81
atgctggcga ttttctgtga cactcccggc caattaaccg ccaaggatct gccgaacccc   60
gtgcgcggcg aaggtgaagt cctggtacgt attcgccgga ttggcgtttg cggcacggat   120
ctgcacatct ttaccggcaa ccagccctat ctttcctatc cgccggatcat gggtcacgaa   180
ctttccggca cggttgagga ggcacccgct ggcagccacc tttccgctgg cgatgtggtg   240
accataattc cctatatgtc ctgcgggaaa tgcaatgcct gcctgaaggg taagagcaat   300
tgctgccgca atategggtg gcttggcggt catcgcgatg gcggcatggt ggaatatctg   360
agcgtgccgc agcaattcgt gctgaaggcg gaggggctga gcctcgacca ggcagccatg   420
acggaatttc tggcgatcgg tgcccacgcg gtgcgtcgcg gtgccgtcga aaaagggcaa   480
aaggtcctga tcgtcggtgc cggcccgatc ggcacggcgg ttgctgtctt tgcggttctc   540
gatggcacgg aagtgacgat gatcgacggt cgcaccgacc ggcctggattt ctgcaaggac   600
cacctcggty tcgctcatac agtcgccctc ggcgacggtg acaaagatcg tctgtccgac   660
attaccggty gcaatttctt cgatcggtg tttgatgcga ccggcaatcc gaaagccatg   720
gagcgcggtt tctcctctgt cggtcacggc ggctcctatg ttctggtgtc catcgctgcc   780
agcgatatca gtttcaacga cccggaattt cacaagcgtg agacgacgct gctcggcagc   840
cgcaacgcga cggctgatga tttcgagcgg gtgcttcgcg ccttgcgcga agggaaagtg   900
ccggaggcac taatcaccca tcgatgaca cttgccgatg ttcctcga gttcggccggc   960
ctgaccgatc cgaagccgg agtcatcaag ggcacgtggt aggtcgcgatg a           1011

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<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

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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	
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 tcatcgggcgc caatgatgtg ctggttcgca tccatgccac tgccatcaat    120
cttctcgact ccaaggtgcg cgacggggaa ttcaagctgt tcctgcccta tcgtcctccc    180
ttcattctcg gtcgatgatc ggccggaaac gtcacccgcg tcggcgcgaa tgtacggcag    240
ttcaagacag ggcagcaggt tttcgctcgc ccgctgatc accgggtcgg aaccttcgca    300
gaaatgattg cggtcgatgc cgcagacctt gcgctgaagc caacgagcct gtccatggag    360
caggcagcgt cgatcccgtc cgtcggactg actgectggc aggcgcttat cgaggttggc    420
aaggtcaagt ccggccagaa ggttttcatc caggccggtt ccggcgggtg cggcaccttc    480
gccatccagc ttgccaagca tctcggcgct accgtggcca cgaccaccag cgccgccaat    540
gccgaactgg tcaaaagcct cggcgcagat gtggtgatcg actacaagac gcaggacttc    600
gaacaggtgc tgtccggcta cgatctctgc ctgaacagcc aggatgccaa gacgctggaa    660
aagtcggtga acgtgctgag accgggcgga aagctcattt cgatctccgg tccgcccgat    720
gttgcccttg ccagatcgtt gaaaatgaat ccgctcctgc gttttgtcgt cagaatgctg    780
agccgtgggt tcctgaaaaa ggcaagcaga cgcggtgtcg attactcttt cctgttcatg    840
cgcgccgaag gtcagcaatt gcatgagatc gccgaactga tcgatgccgg caccatccgt    900
ccggtcgtcg acaaggtggt tcaatctgag cagacgcccg acgcccgggc ctatgtcgag    960
accggacggg caaggggcaa ggttgtggtt acatagcgcg cctag                                1005
    
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<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

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

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355

<210> SEQ ID NO 85
 <211> LENGTH: 1032
 <212> TYPE: DNA
 <213> ORGANISM: Agrobacterium tumefaciens C58

<400> SEQUENCE: 85

```

atgaaagcga ttgtcgccca cggggcaaaag gatgtgcgca tcgaagaccg gccggaggaa      60
aagccggggtc cgggcgaggt gcggtccctg ctggcgaggg gcgggatctg cggcagtgat      120
ctgcattatt acaatcatgg cggtttcggc gccgtgcggc ttcgtgaacc catggtgctg      180
ggccatgagg tttccgccgt catcgaggaa ctgggcgaag gcgttgaggg gctgaagatc      240
ggcggtctgg tggcggttcc gccgtgcgca ccatgccgaa cctgccgctt ctgccaggag      300
ggtctgcaca atcagtgcct caacatgcgg ttttatggca gcgccatgcc tttcccgcat      360
attcagggcg cgttccggga aattctggtg gcggacgccc tgcaatgcgt gccggccgat      420
ggtctcagcg ccggggaagc cgccatggcg gaaccgctgg cggtgacgct gcatgccaca      480
cgccgggccc gcgatttgcg gggaaaacgt gtgctcgtca cgggttgcgg ccccatcggc      540
attctctcca ttctggctgc gcgcggggcg ggtgctgctg aaatcgtcgc caccgacctt      600
tccgatttca cgctcgccaa ggccgctgaa gcggggggcg accgtgtcat caacagcaag      660
gatgagcccg atgcgctcgc cgcttatggt gcaaacaagg gaaccttoga cattctctat      720
gaatgctcgg gtgcggccgt ggcgcttgcc ggccgatta cggcaactgc gccgcgcggc      780
atcatcgtcc agctcgggct cggcgcgcat atgagcctgc cgatgatggc gatcacagcc      840
aaggaactcg acctgcgtgg ttcctttcgc ttccacgagg aattcgccac cggcgtcgag      900
ctgatgcgca agggcctgat cgacgtcaaa cccttcatca cccagaccgt cgatcttgcc      960
gacgccatct cggccttoga attcgcctcg gatcgcagcc gcgccatgaa ggtgcagatc     1020
gccttttctt 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
  
```

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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	
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 caccgcctg aactatcatg gcgaggctgt ccatcatcac    240
cttgggtgtg cggcctttgc cgaatatgcc gtggtgtcgc gcaattcgtt ggtcaagatc    300
gaccgcgata ttccatttgt cgaggcggca ctcttcggct gcgcggttct caccggcgtc    360
ggcgccgtcg tgaatacggc aagggtcagg accggctega ctgcggctgt catcgactt    420
ggcgggtgtg gccttgccgc ggttctcgga gcccgggcgg ccggtgccag caagatcgtc    480
gccgtcgacc tttcgcagga aaagcttgca ctcgccagcg aactgggcgc gaccgccatc    540
gtgaacggac gcgatgagga tgccgtcgag caggtccgcg agctcacttc cggcgggtgc    600
gattatgcct tcgagatggc aggtctatt cgcgccctcg aaaacgcctt caggatgacc    660
aaacgtggcg gcaccacgtt taccgcccgt ctgccaccgc cgggtgcggc cctgccgcctc    720

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aacgtcgtgc agctcgtcgg cgaggagcgg acaactcaagg gcagctatat cggcacctgt 780
gtgcctctcc gggatattcc gcgcttcac gccctttatc gcgacggccg gttgcccgtg 840
aacccgcttc tgagcgggaag gctgaagcta gaagacatca atgaagggtt cgaccgcctg 900
cacgacggaa gcgcccgttc gcaagtcatc gaattctga 939

```

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<210> SEQ ID NO 88
<211> LENGTH: 312
<212> TYPE: PRT
<213> ORGANISM: Agrobacterium tumefaciens C58

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```

<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
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

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<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 ggcacatcgc gtgcggatgg ttgcgccgcg agaattctgg    300
gagacgtcgg atattctggt cacgacagca gcttccgccca tggccgtgcc ggttgccgaa    360
ttcacctatg cggcaatcat catgtgcggc aaggatgtgt ttcgattgcg ggatgaacat    420
agaacagagc gcggcaccgg cgtttttggc agcaggcgcg gcagaagcct gccctatctt    480
ggcaatcatg cccgcaaggc tggcattgtc ggcgcctcgc gcacggggcg gctggtgatg    540
gagatgctgg cgcgcggcac attcgagatt gccgtttacg atccctttct gtcggcggaa    600
gaggccgcac cccttggcgc gaagaaagcc gaactggacg agcttctcgc atggctccgat    660
gtggtctcgc tgcacgcgcc gatcctgccg gaaacgcacc atatgatcgg cgcgcccgaa    720
ctggcgctga tggcggacca tgccatcttc atcaacacgg cgcggggctg gctggtcgcac    780
cacgatgcat tgctgactga agcgtattcc ggacggctgc gcattctgat tgacacgccc    840
gaacccgagc ccctgccacc ggacagcccg ttttacgacg tgcccaatgt cgttctaacc    900
ccccatagag cggggcgctg gggcaatgaa ttgcgcgcac tttccgatct ggccattacc    960
gaaattgaac gtttcgtggc gggacttgcg cccctccacc cggtccacaa gcaggatatg   1020
gaacgtatgg catga                                     1035

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```

<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

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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

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 tatcgcccg 60
gcatgcgcca tcggtttcgc acaggaaggc gccaatgtcg ttcttaccta taatggcgcg 120
gcagagggcg cgccacagc cgttgcgaa atcgaaaagc ttggtcgctc ggctctggcg 180
atcaaggcgg atctcacaaa cgccgccgaa gtcgaggctg ccatatctgc ggctgaggac 240
aagtttgggg agatccacgg cctcgtccat gttgccggcg gcctgatcgc ccgcaagaca 300
atcgcagaaa tggatgaagc cttctggcat caggtcctcg acgtcaatct gacatcgctg 360
ttctgacgg ccaagaccgc attgcogaag atggccaagg gcggcgcgat cgtcactttc 420
tcgtcgcagg ccggcgtgta tggcggcggc ccgggcgctc ttgectatgc cacttccaag 480
ggtgccgtga tgaccttcac ccgcgggactt gccaaagaag tcggcccca aatccgcgctc 540
aacgcccgtt gccccggtat gatctccacc accttccacg ataccttcac caagccggag 600
gtgcgcgaac ggggtggcgg cgcgacgtcg ctcaagcgcg aagggtcgag cgaagacgctc 660
gccggtctgg tggccttct cgcgtctgac gatgccgctt atgtcacgg cgctgctac 720
gacatcaatg gcggcgtcct gtttctctga 750
    
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<210> SEQ ID NO 92
 <211> LENGTH: 249

-continued

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<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
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

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<210> SEQ ID NO 93
<211> LENGTH: 930
<212> TYPE: DNA
<213> ORGANISM: Escherichia coli DH10B

<400> SEQUENCE: 93

atgtccaaaa agattgccgt gattggcgaa tgcattgattg agctttccga gaaaggcgcg      60
gacgttaagc gcggtttcgg cgccgatacc ctgaacctt ccgctatat cgcccgtcag      120
gtcgatcctg cggcattaac cgttcattac gtaacggcgc tgggaacgga cagttttagc      180
cagcagatgc tggacgctg gcacggcgag aacgttgata ctccctgac ccaacggatg      240
gaaaaccgtc tgccgggctt ttactacatt gaaaccgaca gcaccggcga gcgtacgttc      300
tactactggc ggaacgaagc cgccgccaaa ttctggctgg agagttagca gtctgctggc      360
atttgcaag agctggcgaa ttctgattat ctctacctga gctggattag cctggcgatc      420
ttaagcccca ccagcccgca aaagtgtctt tcctgctgc gcgaatgccg cgccaacggc      480

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ggaaaagtga ttttcgacaa taactatcgt ccgcgctgt gggccagcaa agaagagaca 540
cagcaggtgt accaacaat gctggaatgc acggatatcg ccttcctgac gctggacgac 600
gaagacgcgc tgtggggta acagccgggtg gaagacgtca ttgctgcgcac ccataacgcg 660
ggcgtgaaag aagtgggtgt gaaacgcggg gcggattctt gcctggtgtc cattgctggc 720
gaagggttag tggatgttcc ggcggtgaaa ctgccgaaag aaaaagtgat cgataaccacc 780
gcagctggcg actctttcag tgccggttat ctggcggtag gtctgacagg cggcagcgcg 840
gaagacgcgg cgaacgtgg gcacctgacc gcaagtaccg ttattcagta tcgctgcgcg 900
attatccgcg gtgagggcat gccagcgtaa 930

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<210> SEQ ID NO 94
<211> LENGTH: 309
<212> TYPE: PRT
<213> ORGANISM: Escherichia coli DH10B

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<400> SEQUENCE: 94

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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
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

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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

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atgaaaaact ggaaaacaag tgcagaatca atcctgacca cggccccggt tgtaccggtt    60
atcgtggtaa aaaaactgga acacgcggtg ccgatggcaa aagcgttggg tctgtgtggg    120
gtgcgcttc tggaagtgc tctgcgtacc gagtgtgcag ttgacgctat cctgtctatc    180
gccaaagaag tgcctgaagc gattgtgggt gccggtacgg tgetgaatcc acagcagctg    240
gcagaagtca ctgaagcggg tgcacagttc gcaattagcc cgggtctgac cgagccgctg    300
ctgaaagctg ctaccgaagg gactattcct ctgattccgg ggatcagcac tgtttccgaa    360
ctgatgctgg gtatggacta cggtttgaag gagttcaaat tcttcccggc tgaagctaac    420
ggcgccgtga aagcctgca gccgatcgcg ggtccggtct cccagggtccg tttctgcccg    480
acgggtggta tttctccggc taactaccgt gactacctgg cgctgaaaag cgtgctgtgc    540
atcgggtggt cctggctggt tccggcagat gcgctggaag cgggcgatta cgaccgcatt    600
actaagctgg cgcgtgaagc tgtagaaggc gctaagctgt aa                          642

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<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

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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: Lactobacillus brevis ATCC 367

<400> SEQUENCE: 97

atggcatcaa atggaaaagt agcaatggtt accggtggcg gacaaggaat tggatgaagcc 60
 atctcgaaac ggtagctaa cgacggcttt gctgtggcaa ttgctgattt gaacttggac 120
 aatgccaaca aggtcgtttc tgatattgaa gctgctggcg gcaaggccat tgcgggcaag 180
 accgatgtct ctgatcgtga tagcgtgttt gctgcgggta atgaagcggc cgacaagctg 240
 ggcggctttg acgttatcgt taataacgcc ggccttgccc caaccacgcc aattgacacc 300
 atcacccaag aacagtttga tacggtttat cacgttaacg tgggtggggg tctttggggc 360
 attcaagcag cccatgcgaa gttcaaggaa ttgggtcatg gtgggaagat catttccgcg 420
 acgtctcaag cgggggttgt tgtaaccgc aacttagctc tgtacagtgg aactaagttt 480
 gccattcgtg gtgtgaccca agttgcggcg cgtgacttag ccgctgaagg tatcacggtc 540
 aatgcttatg caccgggat tgtaagaca ccaatgatgt ttgacatcgc tcacaagggt 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: Lactobacillus 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

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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

<210> SEQ ID NO 99
 <211> LENGTH: 1089
 <212> TYPE: DNA
 <213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 99

```

atgaatgacc tgagccacac ccacatgcgc gcgccgtct ggcatggccg ccacgatatt 60
cgtgtcgaac aggtaccttt gccggccgac cctgcgccgg gctgggtgca gatcaagggtg 120
gactggtgcg gcatctgcgg ctccgacctg cacgaatatg ttgccggccc ggtgttcac 180
ccggtagagg ccccgcaccc gctgaccggc attcagggcc agtgcaccc cggccacgaa 240
ttctgcggcc acatgcgcaa gcttggcgaa ggcgtggaag gctatgccgt aggcgacccg 300
gtggcggcag acgcgtgcca gcattgtggt acctgctatt actgcacca tggcctgtac 360
aacatctgcg aacgcctggc gttcaccggc ctgatgaaca acggtgcctt cgccgagctg 420
gtcaactgtc ccgccaacct gctctaccgg ctgccgcagg gcttcctgc cgaagccggg 480
gcactgatcg agccgctggc ggtgggatg caccggtga aaaaggccgg cagcctgctt 540
gggcaaaccg ttgtagtgtt tggggccggc accatcgcc tgtgcacccat catgtgcgcc 600
aaggctgcag gtgcggcaca ggtcatcgcc cttgagatgt cctctgcgcg caaagccaag 660
gccaaaggaag cgggcgccaa cgtggtgctg gaccccgacc agtgcgatgc cctggcgcaa 720
atccgcgcac tgactgctgg gctggggcgc gatgtgagtt ttgagtgcac cggcaaaaa 780
catacggcca agctggccat cgacaaccat cgcaaagcag gcaagtgcgt gctggtgggt 840
atcttcgaag agcccagcga gttcaacttc ttcgagctgg tgtccaccga gaagcaagt 900
ctgggggctg tggcgtacaa cggcgagttt gctgacgtga ttgccttcat tgetgatggt 960
cggctggata ttcgcccctt ggtaaccggc cggatcggat tggagcagat tgtcgagctg 1020
ggcttcgagg aactggtgaa caacaagag gagaactgga agatcatcgt ttcaccaggt 1080
gtgcgctga 1089
  
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<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
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

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<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 ggacacggcg tggcggtgaa agtggatgtc    180
tccgaccgcg atcaggtatt tgccgccgtt gaacaggcgc gcaaaacgct gggcggcttc    240
gacgtcatcg tcaataacgc cgggtgtggc cegtctaegc cgatcgagtc cattaccccg    300
gagattgtcg acaaagtcta caacatcaac gtcaaagggg tgatctgggg tattcaggcg    360
gcggtcgagg cctttaagaa agagggggc ggcgggaaaa tcatcaacgc ctgttcccag    420
gccggccaeg tcggcaaccc ggagctggcg gtgtatagct ccagtaaatt cgcggtacgc    480
ggcttaaccc agaccgccgc tcgcgacctc gcgccgctgg gcatacgggt caacggctac    540
tgcccgggga ttgtcaaaac gccaatgtgg gccgaaattg accgccaggt gtccgaagcc    600
gccggtaaac cgctgggcta cggtagccgc gagttcgcca aacgcatcac tctcggtcgt    660
ctgtccgagc cggaagatgt cgcgcctgc gtctcctatc ttgccagccc ggattctgat    720
tacaatgacc gtcagtcggt gctgatcgac ggcgggatgg tatttaacta a          771

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<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

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 attaaaaata tcccgggcgc gctcgatccc aatgaacttg gctaa 1665

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<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
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

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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

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

```

atggaatta acgaaacgct gctgcccag attatcgaag aggtgctgtc ggagatgaaa    60
tcaggcgcag ataagccggt ctcctttagc ggcctgceg cttctgtgc ctctgcccgg    120
ccggtcgcgc ttgcccctgt gtcggcgac agcttcctga cggaaatcgg cgaagccaaa    180
cccggcacgc agcaggatga agtcattatt gccgtcgggc cagcgtttgg tctggcgcaa    240
accgccaata tcgtcgccat tccgcataaa aatattctgc gcgaagtgat cgcggcatt    300
gaggaagaag gcatcaaagc ccgggtgatc cgctgcttta agtcttctga cgtgccttc    360
gtggcagtgg aaggcaaccg cctgagcggc tccggcatct cgatcggtat tcagtcgaaa    420
ggcaccacgc tcatccacca gcgcccctg ccgcccgttt ccaatctgga actcttcccg    480
caggcggcgc tgctgagcct gaaaacctac cgtcagattg gcaaaaacgc cgcgcgctac    540
gccaaaacgc agtcgcccga gccgggtgcc acgcttaacg atcagatggc tcgtccc aaa    600
taccaggcga agtcggccat tttgcacatt aaagagacca aatcgtggt gacgggcaaa    660
aaccgcagg aactgcgcgt ggcgctttaa    690
    
```

<210> SEQ ID NO 106
 <211> LENGTH: 229
 <212> TYPE: PRT
 <213> ORGANISM: Klebsiella pneumoniae MGH78578

-continued

<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
 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 cgcacaaaacg acaccgttcg ccagccaaaa 120
 gttagcgact acccgttagc gacccgccat cggagtgagg tcaaaaccgc taccaataaa 180
 acgctcgatg acctgacgct ggagaacgta ttaagcgatc gcgttacggc gcaggacatg 240
 cgcatactc cggaaacgct gcgtatgcag gcggcgatcg cccaggatgc cggacgcgat 300
 cgggtggcga tgaactttga gcgggcccga gagctcaccg cggttcccga cgaccgaatc 360
 cttgagatct acaacgccct gcgccatcac cgttccaccc aggcggagct actggcgatc 420
 gctgatgacc tcgagcatcg ctaccaggca cgactctgtg ccgcctttgt tcgggaagcg 480
 gccgggctgt acatcgagcg taagaagctg aaaggcgacg attaa 525

<210> SEQ ID NO 108

-continued

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<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

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 attatgatatt ttccgaaaaa gtcgtgctgg ttaccggcgc tggctctggt    60
attggcgcgt ccaactgcgct tgccttcgcg cagtcgggcg catccgttgc ggtcgcagac    120
atctcgactg accacggttt gaaaaccgta gagttggtca aagccgaagg aggcgagggcg    180
accttcttcc atgctgatgt aggctctgaa cccagcgtcc agtcgatgct ggctggtgtc    240
gtggcgcaat acggcggcct ggacattgcg cacaacaacg ccggcattga ggccaatata    300
gtgccgctgg ccgagctgga ctccgacaac tggcgtcgtg tcatcgatgt gaacctttcc    360
tcggtgttct attgcctgaa aggtgaaatc cctctgatgc tgaaggggg cggcggcgcc    420
attgtgaata ccgcatcgcc ctccgggctg attggcggct atcgctttc cgggtatacc    480
gccacgaagc acggcgtagt ggggctgact aaggctgctg ctatcgatta tgcaaaccag    540
aatatccgga ttaatgccgt gtgccctggt ccagttgact cccattcct ggctgacatg    600
ccgcaaccca tgccgatcgc acttctcttt ggcaactcaa ttggacgatt ggccaccgca    660
gaggagatcg cgcgctcggt tctgtggctg tgttctgacg atgcaaaata cgtggtgggc    720
cattcgatgt cagtcgacgg tggcgtggca gtgactgcgg ttggtactcg aatggatgat    780
ctcttttaa                                     789

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<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
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 gcgcggtgc cgcatcggc   60
cgggcaaccg cctggcggtt cgcccacgag ggcataaaag tgggtggtggc ggacctcgac   120
cgggtcggcg gcgagggcac cgtggcgcag atccacggcg caggcggcga agegctgttc   180
attgcctgcg acgtgaccgg cgacgccgag gtgcgccagt tgcatagagc cctgatggcc   240
gcctacggcc ggctggacta cgccttcaac aacgccggga tcgagatcga gcaaacaccg   300

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-continued

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ctggccgaag gcagcgaagc ggagttcgat gccatcatgg gcgtgaacgt gaagggcgtg 360
tggttgtgca tgaagatca gttgcocctg ttgetggccc aaggcgtgg ggccatcgtc 420
aataccgcgt cggtgccggg gctagggggc gcgccaaaga tgagcatcta cagcgcagc 480
aagcatgcgg tcatcggtct gaccaagtcg gcggccatcg agtacgcaa gaagggcatc 540
cgcgtgaacg ccgtgtgccc ggccgtgatc gacaccgaca tgttccgccg cgcttaccag 600
gccgaccgcg gcaaggccga gttcgccgca gccatgcacc cggtagggcg cattggcaag 660
gtcgaggaaa tcgccagcgc cgtgctgtat ctgtgcagtg acggcggcgc gtttaccacc 720
gggcattgcc tgacggtgga tgggtgggct acggcgatct ga 762

```

```

<210> SEQ ID NO 112
<211> LENGTH: 253
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas putida KT2440

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```

<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
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

```

-continued

<213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 113

```

atgtcttttc aaaacaaaat cgttggtgctc acaggcgcag cttctggcat cggcaaagcg    60
acagcacagc tgctagtgga gcaggggccc catgtggttg ccatggatct taaaagcgac    120
ttgcttcaac aagcattcgg cagtgaggag cacgttctgt gcattccctac cgacgtcagc    180
gatagcgaag cegtgcgagc cgccttccag gcagtggacg cghaaatttg cegtgtcagc    240
gtgattatta acgccggggg catcaacgca cctacgcgag aagccaacca gaaaatgggt    300
gatgccaaag tcgctgcctc cgatgccatg aagagcgggc gggcgccac ttcgacttc    360
ctggccgata cctcggatca ggatttccgg cgcgtaatgg aagcaattt gttcagccag    420
ttttactgca ttcgagaggg tgttccgctg atgcgccgag cgggtggcgg cagcatcgtc    480
aacatctcca gcgtggcagc gctcctgggc gtggcaatgc cactttacta ccccgctcc    540
aaggcggcgg tgctgggctc caccctgca ggcgcagctg agttggcacc ttacaacatt    600
cgtgtgaatg ccatcgctcc aggtctgtc gacacacat tgatgcatga gcaaccaccg    660
gaagtcgttc agttcctggt cagcatgcaa cccatcaagc ggctggccca acccgaggag    720
cttgcccaaa gcatcctggt ccttgccggt gagcattcgt ccttcatcac cggacagacg    780
ctttctcca 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

```

-continued

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 aaggcaaac tgcactcgtc accggttcca ccagcggcat tggcctgggc      60
atgcccagg tattggccc ggctggcgcc aacatcgtgc tcaacggctt tggtagcccg      120
ggccccgcca tgggggaaat tgcccggcac ggggtgaagg ttgtgcacca cccggccgac      180
ctgtcggatg tggtcagat cgaggctttg ttcaacctgg ccgaacgcca gttcggcggc      240
gtcgacatcc tggtaacaaa cgccggatc cagcatgtgg caccggttga gcagttcccg      300
ccagaaagct gggacaagat catcgccctg aacctgtcgg ccgtattcca tggcacgcgc      360
ctggcgctgc cgggcatgcg cacgcgcaac tggggggcga tcatcaatat cgcttcggtg      420
catggcctgg tgggtcgcg tggcaaggca gcctacgtgg cagccaagca tggcgtgatc      480
ggcctgacca aggtggtcgg cctggaaacc gccaccagtc atgtcacctg caatgccata      540
tgcccgggct ggggtcgtgac accgctggtg caaaagcaga tgcacgatcg tgcggccaag      600
ggtggcgatc ggctgcaagc gcagcacgat ctgctggcag aaaagcaacc gtcgctggct      660
ttcgtcacc ccgaacacct cggtgagctg gtactctttc tgtgcagcga ggcggtagc      720
caggttcgcg gcgccgctg 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

-continued

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
 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 acctacccc tccattccca tcccaaccgc aaagcgttcc cgttcccag 60
 cgcaagatgg atccgtatcc ggactgcggt gagcagagct acaccgcaa caatcgctc 120
 gcaggcaaga tcgccttgat aaccggtgct gacagcggca tcggcgctgc ggtggcgatt 180
 gcctatgccc gagaaggcgc tgacgttgcc attgcctatc tgaatgaaca cgaogatgag 240
 caggaaaccg cgcgctgggt caaagcggct ggccgccagt gcctgctgct gcccgcgac 300
 ctggcacaga aacagcaactg ccacgacatc gtcgacaaga ccgtggcgca gtttggtcgc 360
 atcgatatcc tggtaacaaa cgcccggttc cagatggccc atgaaagcct ggaogacatt 420
 gatgacgatg aatgggtgaa gacctcgat accaacaatca ccgccatttt ccgcatttgc 480
 cagcgcgctt tgccctcgat gccaaagggc ggttcgatca tcaacaccag ttcggtaaac 540
 tctgacgacc cgtcaccag cctgttgccc tatgccgca ccaaaggggc tattgccaat 600
 ttcaactgca gcttgcgca actgctgggc aagcagggca ttcgctcaa cagcgtcgca 660
 cccggcccga tctggacccc gctgatccc gccaccatgc ctgatgagc ggtgagaaac 720
 ttcggttccc gttaccgat gggacggccg ggtcaacctg tggaggtggc gccaatctat 780

-continued

```
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
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 caccocgggc cacaatggcc gggtagcctt ggtcacgggc 60
```

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```

gccgcccgcg gcatcggtct gggcattgcc gcatggctga tctgcgaagg ctggcaagtg 120
gtgtgtagtg atctggaccg ccagcgtggt accaaagtgg ccaaggcgtt gggcgacaac 180
gcctggttca tcaccatgga cgttgccgac gaggcccagg tcagtgccgg cgtgtccgaa 240
gtgctcgggc agttcggcgc gctggacgcg ctggtgtgca atgcggccat tgccaaccgc 300
cacaaccaga cgctgaaaag cctgagcctg gcacaatgga accgggtgct ggggggtcaac 360
ctcagcggcc ccattgctgt ggccaagcat tgtgcgccgt acctgcgtgc gcacaatggg 420
gcgatcgtca acctgacctc tacccgtgct eggcagtcg aaccgcacac cgaggcttac 480
gcggcaagca agggcggcct ggtggctttg acccatgccc tggccatgag cctggggccc 540
gagattcgcg tcaatgcggt gagccgggc tggatcgatg cccgtgatcc gtcgcagcgc 600
cgtgccgagc cgttgagcga agctgacat gccagcatc caacgggcag ggtagggacc 660
gtggaagatg tcggggccat ggttgctcgg ttgctgtcac gccaggcggc atttgtcacc 720
ggccaggagt ttgtggtcga tggcggcatg acccgcaaga tgatctatac ctga 774

```

<210> SEQ ID NO 122

<211> LENGTH: 257

<212> TYPE: PRT

<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

```

-continued

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 tgcactgggt accggcgcca gccgtggcat tggccaggcc	60
atcgccctcg agctgggccc ccaggggcgc accgtgatcg gtaccgccac gtcggcgctc	120
ggtgccgagc gcatcgctgc caccctgaaa gaacacggca ttaccggcac tggcatggag	180
ctgaacgtga ccagcgcga atcggttgaa gccgtactgg ccgccattgg cgagcagttc	240
ggcgcgccgg ccatcttggt caacaatgcc ggtatcacc cgcacaacct catgctgcgc	300
atgaaagacg acgagtgggt tgatgtcatc gacaccaacc tgaacagcct ctaccgtctg	360
tccaaggcgc tgctgcgtgg catgaccaag gcgcgttggg gtcgtatcat cagcatcggc	420
tcggtcgttg tgccatggg taacgcaggt caggccaact acgcggctgc caaggccggt	480
ctggaagggt tcagccgcgc cctggcgcgt gaagtgggtt cgcgtggtat caccgtcaac	540
tcggtgacc caggttcat cgataccgac atgacccgcg agctgccaga agctcagcgc	600
gaagccctgc agaccagat tccgctgggc cgcctgggcc aggctgacga aattgccaaag	660
gtggtttcgt tcttgcatc 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

-continued

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

```

atgactcaga aatagctgt cgtgaccggc ggcagtcgcg gcattggcaa gtccatcgtg    60
ctggccctgg ccggcgccgg ttatcagggt gccttcagtt atgtccgtga cgaggcgtea    120
gccgctgcct tgcaggcgca ggtcgaaggg ctggccgggg actgectggc cgtgcagtgt    180
gatgtcaagg aagcgccgag cttcaggcgc ttttttgaac gggtcgagca acgtttcgag    240
cgtatcgact tgttggtcaa caacgccggt attaccctgtg acggtttgct cgccacgcaa    300
tcgttgaacg acatcaccga ggtcatccag accaacctgg tcggcacggt gttgtgctgt    360
cagcaggtgc tgccttgcac gatgcgccaa cgcagcgggt gcatcgtaaa cctcagttcg    420
gtggccgccc aaaagcccgg caagggccag agcaactacg ccgcccgaaa aggcggtgta    480
gaagcattga cagcgcact ggcggtggag ttggcgccgc gcaacatccg ggtcaacgcg    540
gtggcgcccc gcatcgtcag caccgacatg agccaagccc tggtcggcgc ccatgagcag    600
gaaatccagt cgcggctgtt gatcaaacgg ttcgcccggc ctgaagaaat tgcgacgcg    660
gtgtgtatc tggccgagcg cggcctgtac atcacgggcg aagtctgtc 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

-continued

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	
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 cccacctggt cgacctcgac ggcaagattg cctttgtttc cggcgccagc    60
cgtggcatcg gcgagggccat cgcccacttg ctgcgcgacg aaggggcccc tgtgatcggt    120
tccagccgca agcttgacgg gtgccagcag gtggccgacg ccatcattgc cgccggcgcc    180
aaggccacgg ctgtggcctg ccacattggt gagctggaac agattcagca ggtgttcgcc    240
ggcattcgcg aacagttcgg gcgactggac gtgctggtea acaatgcagc caccaaccgg    300
caattctgca atgtgctgga caccgaccca ggggcgttcc agaagaccgt ggacgtgaac    360
atccgtggtt acttcttcat gtcggtggag gctggcaagc tgatgcgcca gaacggcgcc    420
ggcagcatca tcaacgtggc gtcgatcaac ggtgtttcac ccgggctggt ccaaggcadc    480
tactcgggtg ccaaggcgcc ggtcatcaac atgaccaagg tgttcgccc aagagtgtgca    540
cccttcggta ttcgctgcaa cgcgctactg ccggggctga ccgataccaa gttcgtctcg    600
gcattgtgta agaacgaagc catcctcaac gccgccttgc agcagatccc cctcaaaccg    660
gtggccgacc ccaaggaaat ggcgggtgcg gtgctgtacc tggccagcga tgccctccagc    720
tacaccaccg gcaccacgct caatgtcgac ggtggcttcc tgtctctga                    768

```

<210> SEQ ID NO 128
 <211> LENGTH: 255
 <212> TYPE: PRT
 <213> ORGANISM: Pseudomonas putida KT2440

<400> SEQUENCE: 128

-continued

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
 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 ggcagccaa tggatcggc 60
cgcgccaccg cccaggcatt tgccgcacaa ggcttgaagg tgggtggtggc ggacctggac 120
acggcggggg gcgagggcac cgtggcgctg atccgcgagg ccggtggcga ggcattgttc 180
gtgocgtgca acgttaccct ggaggcggat gtgcaaagcc tcattggccc caccatcgaa 240
gcctatgggc gcctggatta cgccttcaac aatgccggtg tcgagatcga aaagggccgc 300
cttgcgagg gctccatgga tgagttcgac gccatcatgg gggccaactg caaaggggtc 360
tggctgtgca tgaagtacca gttgcoctg ctgctggccc agggcggtgg ggcgatcgtc 420
aacaccgcct cgggtggcgg cctgggcgcg gcgccaaga tgagcatcta tgcggcctcc 480
aagcatgagg tgatcgccct gaccaagtgc gcggccatcg aatatgcgaa gaagaaaatc 540
cgcgtaacg cggtatgccc ggcggtgatc gacaccgaca tgttccgcgc tgcctacgag 600

```

-continued

```

gcggaccgga agaaggccga gttcgccgcg gccatgcacc cggtagggcg catcgcaag 660
gtcgaggaga tcgccagtgc ggtgctctac ctgtgcagcg atggcgcggc ctttaccacc 720
ggccatgcac tggcggtcga cggcggggccc accgcgatct ga 762

```

```

<210> SEQ ID NO 130
<211> LENGTH: 253
<212> TYPE: PRT
<213> ORGANISM: Pseudomonas fluorescens PF-5

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```

<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
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 tgcttgccg ggaaggcgcg cgagtgatta tcgccgatcg tgatgcacac 120
ggcgaagccg ccgccgcttc cctgcgcgaa tcgggcgcac aggcgctggt taccagctgc 180

```

-continued

```

aatatcgctg aaaaaacgca ggtcgaagcc ctgtattccc aggccgaaga ggcgtttggc 240
ccggtagaca ttctggtgaa taacgccgga atcaaccgcg acgccatgct gcacaaatta 300
acggaagcgg actgggacac ggttatcgac gttaacctga aaggcacttt cctctgtatg 360
cagcaggcgg ctatccgcat gcgcgagcgc ggtgcgggcc gcattatcaa tatcgcttcc 420
gccagttggc ttggcaactg cgggcaaac aactattcgg cgtcaaaagc cggcgtggtg 480
ggaatgacca aaaccgcctg ccgcgaactg gcgaaaaag gtgtcacggt gaatgccatc 540
tgcccgggct ttatcgatgc cgacatgacg cgcggcgctac 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

```

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

```


-continued

```

<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 cgccgcgcca ttaggcgaag accatctcgg cctggcggcc  180
aacgtcgcctg atgaagtgca ggtgcaggcg gccatcgaac agatcctggc gaaatacggc  240
cgggttgatg tactggtcaa taacgccggg attaccagc cgctgaagct gatggatgc  300
aagcgcgcca actatgacgc ggtgcttgat gttagcctgc gcggcacgct gctgatgctg  360
caggcgggta tccccacat gcgggcgcaa aaatccggca gcacgtctg catctcgtcc  420
gtctccgccc agcgcggcgg cggtatttcc ggcggaccgc actacagcgc ggcaaaagcc  480
gggggtgctg gtctggcgcg ggcgatggcg cgcgagcttg gcccgataa cgtccgcggt  540
aactgcatac ccccggggct gattcagacc gacattaccg ccggcaagct gactgatgac  600
atgacggcca acattcttgc cggcattccg atgaaccgcc ttggcgacgc gatagacatc  660
gcgcgcgceg cgctgttctc cggcagcgat ctttctctct actccaccgg catcacctg  720
gacgttaacg gcggcatggt 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

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-continued

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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 actggcgcgcg cctccgcacg cggtttgggg      60
ttcgcgactg cgaattatt cgccgaaaac ggcgcgaaag tggtcattat cgacctcaat      120
ggcgaagcca gtaaaccgc cgccggcgga ttaggcgaag accatctcgg cctggcgggc      180
aacgtcgctg atgaagtgca ggtgcaggcg gccatcgaac agatcctggc gaaatacggg      240
cgggttgatg tactggtcaa taacgccggg attaccacgc cgctgaagct gatggatc      300
aagcgcgcca actatgacgc ggtgcttgat gttagcctgc gcggcacgct gctgatgtcg      360
caggcgggta tccccaccat gcggcgcaaa aatccggca gcatcgtctg catctcgtcc      420
gtctccgccc agcgcggcgg cggatatttc ggcggaaccg actacagcgc ggcaaaagcc      480
gggggtgctg gtctggcgcg ggcgatggcg cgcgagcttg gcccgataa cgtccgcggt      540
aactgcatca ccccggggct gattcagacc gacattaccg ccggcaagct gactgatgac      600
atgacggcca acattcttgc cggcattccg atgaaccgcc ttggcgacgc gatagacatc      660
gcgcgcgccc cgctgttctc cggcagcgat ctttctctct actccaccgg catcacctcg      720
gacgttaacg gcggcatggt 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

```


-continued

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	
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 subsp. pneumoniae MGH78578

<400> SEQUENCE: 139

```

atgaacggcc tgctaaacgg taaacgtatt gtcgtcaccg gtgcggcgcg cggctctcggg    60
taccactttg ccgaagcctg cgccgctcag ggcgcgacgg tggatgatgtg cgacatcctg    120
cagggagagc tggcgaaaag cgctcatcgc ctgcagcaga agggctatca ggtcgaatct    180
cacgccatcg atcttgccag tcaagcatcg atcgagcagg tcttcagcgc catcggcgcg    240
caggggtcta tcgatggctt agtcaataac gcagcgatgg ccaccggcgt cggcggaaaa    300
aatatgatcg attacgatcc ggatctgtgg gatcgggtaa tgacgggtcaa cgttaaaggc    360
acctggttgg tgaccgcgcg gccggtaccg ctgctgcgcg aaggggcggc gatcgtcaac    420
gtcgtctcgg ataccgcgct gtggggcgcg ccgcggctga tggcctatgt cgccagtaag    480
ggcgcggtga ttgcgatgac ccgctccatg gcccgcgagc tgggtgaaaa gcggatccgt    540
atcaacgcca tcgcgcgggg actgaccgcg gttgaggcca cggaaatcgt tccgcgag    600
cgtcatcagc tgtatgagaa cggccgcgcg ctcagcggcg cgcagcagcc ggaagatgtc    660
accggcagcg tggctctggct gctgagcgat ctttcgcgct ttatcaccgg ccaactgatc    720
ccggtaaacg gcggttttgt cttaactaa                                     750

```

<210> SEQ ID NO 140
 <211> LENGTH: 249
 <212> TYPE: PRT

-continued

<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 15Arg Gly Leu Gly Tyr His Phe Ala Glu Ala Cys Ala Ala Gln Gly Ala
20 25 30Thr Val Val Met Cys Asp Ile Leu Gln Gly Glu Leu Ala Glu Ser Ala
35 40 45His Arg Leu Gln Gln Lys Gly Tyr Gln Val Glu Ser His Ala Ile Asp
50 55 60Leu Ala Ser Gln Ala Ser Ile Glu Gln Val Phe Ser Ala Ile Gly Ala
65 70 75 80Gln Gly Ser Ile Asp Gly Leu Val Asn Asn Ala Ala Met Ala Thr Gly
85 90 95Val Gly Gly Lys Asn Met Ile Asp Tyr Asp Pro Asp Leu Trp Asp Arg
100 105 110Val Met Thr Val Asn Val Lys Gly Thr Trp Leu Val Thr Arg Ala Ala
115 120 125Val Pro Leu Leu Arg Glu Gly Ala Ala Ile Val Asn Val Ala Ser Asp
130 135 140Thr Ala Leu Trp Gly Ala Pro Arg Leu Met Ala Tyr Val Ala Ser Lys
145 150 155 160Gly Ala Val Ile Ala Met Thr Arg Ser Met Ala Arg Glu Leu Gly Glu
165 170 175Lys Arg Ile Arg Ile Asn Ala Ile Ala Pro Gly Leu Thr Arg Val Glu
180 185 190Ala Thr Glu Tyr Val Pro Ala Glu Arg His Gln Leu Tyr Glu Asn Gly
195 200 205Arg Ala Leu Ser Gly Ala Gln Gln Pro Glu Asp Val Thr Gly Ser Val
210 215 220Val Trp Leu Leu Ser Asp Leu Ser Arg Phe Ile Thr Gly Gln Leu Ile
225 230 235 240Pro 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 aggcgcttcg cgccgcggtc 240

gccgcgcggt tcggcggcgt cgatatgctg attaataacg ccggccaggg ctatgtcgcc 300

cacttcgccc atacgccacg tgaggcctgg ctgcacgaag ccgaactgaa actgttcggc 360

gtgattaacc cggtaaagc ctttcagtcc ctgctagagg cgtcggatat cgcctcgatt 420

acctgtgtga actcgtgctg gccgttacag ccggaagagc acatgatcgc cacctctgcc 480

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gcccgcgcgcg cgctgctcaa tatgacgctg actctgtcga aagagctggt ggataaaggt 540
attcgtgtga attccattct gctggggatg gtggagtcgc gccagtgcca gcgccgtttt 600
gagagccgaa gcgataagag ccagagttgg cagcagtggc ccgccgatat cgcccgtaag 660
cgggggatcc cgatggcgcg tctcggttaag ccgcaggagc cagcgcaagc gctgctattc 720
ctcgcttcgc cgctggcctc ctttaccacc ggcgcggcgc tggacgtttc cgcgcgtttc 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
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

```

<210> SEQ ID NO 143

<211> LENGTH: 1795

<212> TYPE: DNA

<213> ORGANISM: *Pseudomonas fluorescens*

-continued

<400> SEQUENCE: 143

```

cgccaagcaa tcgggctttg gggcagaatt gggtcgcgaa gggcttgagg agtttgccca    60
gtccaagatc atcaacgccg cgctataaat taaaggatcc cccatggcga tgattacagg    120
cggcgaactg gttgttcgca ccctaataaa ggctggggtc gaacatctgt tcggcctgca    180
cggcgcgcac atcgatacga tttttcaagc ctgtctcgat catgatgtgc cgatcatcga    240
caccgcccat gaggccgccg cagggcatgc ggccgagggc tatgcccgcg ctggcgccaa    300
gctgggcgctg gctggtcacg gcgggcgggg gatttaccaa tgcggtcacg cccattgcca    360
acgcttggtt ggatcgaag gccgggtgat tctcaccgcg ggatcgggcg cgctgcgtga    420
tgatgaaacc aacacgttgc aggcggggat tgatcaggtc gccatggcgg cgcccattac    480
caaatgggag catcgggtga tggcaaccga gcatatccca cggctggtga tgcaggcgat    540
ccgcgccgcg ttgagcgcgc cacgcggggc ggtgttgctg gatctgccgt gggatattct    600
gatgaaccag attgatgagg atagcgtcat tatccccgat ctggtcttgt ccgcgcattg    660
ggccagaccc gaccctgccg atctggatca ggctctcgcg cttttgcgca aggcggagcg    720
gccggtcacc gtgctcggct cagaagcctc gcggacagcg cgcaagacgg cgcttagcgc    780
cttcgtggcg gcgactggcg tgccgggtgt tgccgattat gaagggctaa gcatgctctc    840
ggggctgccc gatgctatgc ggggcgggct ggtgcaaac ctctattctt ttgccaaagc    900
cgatgcccgc ccagatctcg tctgatgctc gggggcgcgc tttggcctta acaccgggca    960
tggatctggg cagttgatcc cccatagcgc gcaggtcatt caggctgacc ctgatgcctg   1020
cgagctggga cgcctgcagg gcatcgctct gggcattgtg gccgatgtgg gtgggacct   1080
cgaggctttg gcgcaggcca ccgcgcaaga tgcggcttgg ccggatcgcg gcgactggtg   1140
cgccaaagtg acggatctgg cgcaagagcg ctatgccagc atcgctgcga aatcgagcag   1200
cgagcatgcg ctccaccctt tcaecgcctc gcaggtcatt gccaaacacg tcgatgcagg   1260
ggtagcgggt gtagcggatg gtgcctgac ctatctctgg ctgtccgaag tgatgagccg   1320
cgtgaaaccc gccggttttc tctgccacgg ctatctaggc tcgatggcg tgggcttcgg   1380
cacggcgctg ggcgcgcaag tggccgatct tgaagcaggc cgccgcacga tccttgtgac   1440
cggcgatggc tcggtgggct atagcatcgg tgaatttgat acgctggtgc gcaacaatt   1500
gccgctgacg gtcacatca tgaacaacca aagctggggg gcgacattgc atttccagca   1560
attggcgcgt gcccccattc gcgtgacggg cacccgtttg gaaaatggct cctatcacgg   1620
ggtagccgcc gcctttggcg cggatggcta tcatgtcgac agtgtggaga gcttttctgc   1680
ggctctggcc caagcgcctc ccataatcg ccccgctgc atcaatgtcg cggtcgcgct   1740
cgatccgate ccgccgaag aactcattct gatcggcatg gacccttcg catga       1795

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<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

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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
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					

-continued

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
 <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

gtctttatc 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

gggcgccgc aaggggttcg cgttggccga 30

<210> SEQ ID NO 148
 <211> LENGTH: 22
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Primer

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<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

<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 ggtgcctaataat 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 gttaatatatt tgtaaaatt 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 cccgcogttg 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 ttgtgtagc cc 32

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<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 gggaaggag 30

<210> SEQ ID NO 157
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 157

aggtagcgtg 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

ttttcctttt ggggcccgc cgtggcatc 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 ggaaaaacaag 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

ttttcctttt ggggcccgc cagcttagcg ccttcta 37

<210> SEQ ID NO 161
<211> LENGTH: 31

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<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

<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

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<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 aggatata 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

<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 gcggccgcc 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 ttgaaatgta 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 ttgaaatgat tttag 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 aggatata tatggctaac tacttcaata cac 43

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<210> SEQ ID NO 174
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 174

tgctggtgcg ggtaaggag 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 aggcataatat atatcctcct taaccgcaa cagcaatacg 50

<210> SEQ ID NO 176
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 176

acatgcatgc ttaaccccc 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 aggatata tataatgctaact 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 ttaaccccc 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

cccagctca ggaggatata tatatggata aacagtatcc ggt 43

<210> SEQ ID NO 180
<211> LENGTH: 28

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<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

cccagctca ggaggatata tatatggtga caaaagcaac aaaag 45

<210> SEQ ID NO 182
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<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

cccagctca ggaggatata tatatggtga 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

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 187
aaaactgcag cgtttgatga cgtggacgat agcgg 35

<210> SEQ ID NO 188
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 188
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 189
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 190
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<400> SEQUENCE: 191
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<400> SEQUENCE: 194

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<400> SEQUENCE: 195

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<210> SEQ ID NO 196
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<212> TYPE: DNA
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<400> SEQUENCE: 196

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<210> SEQ ID NO 197
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<400> SEQUENCE: 197

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<210> SEQ ID NO 198
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<220> FEATURE:
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<400> SEQUENCE: 198

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<210> SEQ ID NO 199
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<212> TYPE: DNA
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<400> SEQUENCE: 199

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<212> TYPE: DNA
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<400> SEQUENCE: 201

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<210> SEQ ID NO 202
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<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 202

cgagctcagg aggatatata tatgagccag caagtcatta ttttcg 46

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<212> TYPE: DNA
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<400> SEQUENCE: 203

aaaactgcag cgtttgatga cgtggacgat agcgg 35

<210> SEQ ID NO 204
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<212> TYPE: DNA
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<400> SEQUENCE: 204

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<210> SEQ ID NO 205
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<212> TYPE: DNA
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<400> SEQUENCE: 205
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<210> SEQ ID NO 206
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 206
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<210> SEQ ID NO 207
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 207
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 208
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 209
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<210> SEQ ID NO 210
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 210
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<210> SEQ ID NO 211
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<220> FEATURE:
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<400> SEQUENCE: 211
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<210> SEQ ID NO 212
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<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:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 213

gcgtcgcggg taaggaggaa aattttatgt cctcacgtaa agagcttgcc 50

<210> SEQ ID NO 214
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<212> TYPE: DNA
<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
<220> FEATURE:
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<400> SEQUENCE: 215

caatcagcgt aaggaggat atataatgaa aaccgtaact gtaaaagatc 50

<210> SEQ ID NO 216
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 216

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<210> SEQ ID NO 217
<211> LENGTH: 50
<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 217

tacgtgagga cataaaattt tcctccttac cgcgcgcgcg cttttactgc 50

<210> SEQ ID NO 218
<211> LENGTH: 50

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 218

caatcctctc cataatttta acctccttac agcagttctt ttgctttcgc 50

<210> SEQ ID NO 219
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 219

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<210> SEQ ID NO 220
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 220

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<210> SEQ ID NO 221
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 221

acatgcatgc ttacgaggac aattcctcct gcaa 34

<210> SEQ ID NO 222
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 222

cccagctca ggaggatata tatatgaatt atcagaacga cgatttac 48

<210> SEQ ID NO 223
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 223

acatgcatgc ttacgaggac aattcctcct gcaa 34

<210> SEQ ID NO 224
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

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<400> SEQUENCE: 224
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<210> SEQ ID NO 225
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 225
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<210> SEQ ID NO 226
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 226
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<210> SEQ ID NO 227
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 227
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<210> SEQ ID NO 228
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<212> TYPE: DNA
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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:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 229
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<210> SEQ ID NO 230
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 230
gtttccagcc ataattaatt cctccttagg ctgcctgget aatccgcgcc 50

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<210> SEQ ID NO 231
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 231

acatgcatgc ttaccagcgt ggaatcag tcttc 35

<210> SEQ ID NO 232
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 232

cccagctca ggaggatata tatatgacat cggaaaacc gttactgg 48

<210> SEQ ID NO 233
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 233

acatgcatgc ttaccagcgt ggaatcag tcttc 35

<210> SEQ ID NO 234
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 234

cccagctca ggaggatata tatatggtg ctgaattgac cgcattac 48

<210> SEQ ID NO 235
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 235

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<210> SEQ ID NO 236
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 236

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<210> SEQ ID NO 237
<211> LENGTH: 50

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 237

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<210> SEQ ID NO 238
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 238

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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<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:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 240

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<210> SEQ ID NO 241
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 241

acatgcatgc ttaccagcgt ggaatcacg 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

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<210> SEQ ID NO 243
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

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<400> SEQUENCE: 243
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<210> SEQ ID NO 244
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<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
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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
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<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
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 248
catgccatgg ctatgattac tgggtg 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
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<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 aatcaaaaa 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 aatgatttt atatagatat cc 32

<210> SEQ ID NO 252
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 252

catgccatgg gtattccaga aactcaaaaa g 31

<210> SEQ ID NO 253
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 253

cccgagctct tatttagaag tgtcaacaac g 31

<210> SEQ ID NO 254
<211> LENGTH: 47
<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
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<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
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 258

cccgagctca ggaggatata tatatgtata cagtaggaga ttacc 45

<210> SEQ ID NO 259
<211> LENGTH: 33
<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
<211> LENGTH: 46
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 260

cgagctcagg aggatata tatgaaaaa 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 cgcgcatgg cgggtgaaagt g 31

<210> SEQ ID NO 262
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<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

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<400> SEQUENCE: 262
aactaatcta gaggaggata tatatatgag catgacgttt tccggccagg 50

<210> SEQ ID NO 263
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 263
ccttgccgag 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 ggcccgcggc c 31

<210> SEQ ID NO 265
<211> LENGTH: 50
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 265
gctcatatat atatcctcct ctagattagt taaacacccat cccgccgtcg 50

<210> SEQ ID NO 266
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 266
gtcgaactca tccatcgagc cctccgcaag g 31

<210> SEQ ID NO 267
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<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
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 268
cgagctcagg aggatatata tatgaaaaa gtcgcacttg ttaccg 46

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<210> SEQ ID NO 269
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<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 269

cccaagctta gatcgcggtg gccccgccgt cg 32

<210> SEQ ID NO 270
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 270

gctctagagg aggatttaa aatggaaatt aacgaaacgc tgc 43

<210> SEQ ID NO 271
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 271

tccccgcggt taagcatggc gatccccgaaa 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
<211> LENGTH: 30
<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
<211> LENGTH: 25

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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
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 276

ggaattccat atgttcacaa cgtccgccta 30

<210> SEQ ID NO 277
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 277

gcttgacggc catgtggcgg aggccgc 27

<210> SEQ ID NO 278
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<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:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 279

cgggatcctt aggcggcctt ctggcgcg 28

<210> SEQ ID NO 280
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<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

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<400> SEQUENCE: 281
cgggatcctt aagcgtcgag cgaggcca 28

<210> SEQ ID NO 282
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 282
ggaattccat atgactaaaa caatgaaggc 30

<210> SEQ ID NO 283
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 283
caccggggcc ggggtccggt attgcca 27

<210> SEQ ID NO 284
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 284
tggcaatacc ggacccggc cccggtg 27

<210> SEQ ID NO 285
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 285
cgggatcctt aggcggcgag atccacga 28

<210> SEQ ID NO 286
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 286
ggaattccat atgaccgggg cgaaccagcc 30

<210> SEQ ID NO 287
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 287
atagccgctc atacgcctcg gttgcct 27

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<210> SEQ ID NO 288
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 288

aggcaaccga ggcgtatgag cggctat

27

<210> SEQ ID NO 289
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 289

cgggatcctt aagcgccgtg cggaagga

28

<210> SEQ ID NO 290
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 290

ggaattccat atgaccatgc atgccattca

30

<210> SEQ ID NO 291
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<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
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 292

ggaattccat atgcgcgcgc tttattacga

30

<210> SEQ ID NO 293
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 293

cgggatcctt attcgaaccg gtcgatga

28

<210> SEQ ID NO 294
<211> LENGTH: 30

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 294

ggaattccat atgctggcga tttctgtga 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

<400> SEQUENCE: 296

ggaattccat atgaaagcct tcgtcgtga 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 ttgtcgcca 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 aggaaaagc gatctgca 28

<210> SEQ ID NO 300
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

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<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

<210> SEQ ID NO 303
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 303
ggggccgat catgtggtgc gtttcg 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 atgccatagc 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

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<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
 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

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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

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

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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
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					

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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

<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

-continued

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
 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

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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
 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

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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
 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

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47

<210> SEQ ID NO 316
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<400> SEQUENCE: 316

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<210> SEQ ID NO 318
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<400> SEQUENCE: 318

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<210> SEQ ID NO 319
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<400> SEQUENCE: 319

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<400> SEQUENCE: 320

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<400> SEQUENCE: 321

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<400> SEQUENCE: 322
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<210> SEQ ID NO 323
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 323
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<210> SEQ ID NO 324
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 324
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<210> SEQ ID NO 325
<211> LENGTH: 20
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<213> ORGANISM: Artificial Sequence
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<400> SEQUENCE: 325
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<210> SEQ ID NO 326
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 326
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<210> SEQ ID NO 327
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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<400> SEQUENCE: 327
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<210> SEQ ID NO 328
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<212> TYPE: DNA
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<400> SEQUENCE: 328
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<210> SEQ ID NO 329
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<220> FEATURE:
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<400> SEQUENCE: 329

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 330

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<210> SEQ ID NO 331
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 331

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<210> SEQ ID NO 332
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<212> TYPE: DNA
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<220> FEATURE:
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<400> SEQUENCE: 332

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<210> SEQ ID NO 333
<211> LENGTH: 29
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 333

cgggatcctt acagttgagc aatgatcc            29

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1. A method for converting a suitable monosaccharide or oligosaccharide to a commodity chemical, comprising:

- (a) contacting the suitable monosaccharide or oligosaccharide with a commodity chemical biosynthesis pathway, wherein the commodity chemical biosynthesis pathway comprises an aldehyde or ketone biosynthesis pathway, a C—C ligation pathway, and/or a dehydration and reduction pathway, thereby converting the suitable monosaccharide or oligosaccharide 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 suitable monosaccharide or oligosaccharide is obtained from a biomass-derived polysaccharide, 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) gluconate, mannuronate, mannitol, xylose, glycerol, xylitol, glucose, mannose, galactose, xylose, arabinose, glucuronate, galacturonates, and rhamnose, and D-mannitol.

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, 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-butanedione, 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-hexanedione, 5-methyl-1-phenyl-3-hydroxy-2-hexanone, 5-methyl-1-phenyl-2-hydroxy-3-hexanone, 4-methyl-1-phenyl-2-hydroxy-3-hexanone, 4-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-methyl-4-octene, 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-

octanediol, 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-decanedione, 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-hydroxy-2-butanone, 2,3-butanediol, 1,5-pentane diol, homocitrate, homoisocitrate, 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-diaminododecane, 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, phenylacetaldehyde, 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-hydroxyphenyl)-4-phenylbutane, 1-(4-hydroxyphenyl)-4-phenyl-1-butene, 1-(4-hydroxyphenyl)-4-phenyl-2-butene, 1-(4-hydroxyphenyl)-4-phenyl-2-butanone, 1-(4-hydroxyphenyl)-4-phenyl-2,3-butanediol, 1-(4-hydroxyphenyl)-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-butanone, 1-(indole-3)-4-phenyl-2,3-butanediol, 1-(indole-3)-4-phenyl-3-hydroxy-2-butanone, 4-hydroxyphenylacetaldehyde, 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-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-butanone, 1-(4-hydroxyphenyl)-4-(indole-3)-2,3-butanediol, 1-(4-hydroxyphenyl)-4-(indole-3)-3-hydroxy-2-butanone, indole-3-acetaldehyde, 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-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 suitable monosaccharide or oligosaccharide to a commodity chemical comprising,

(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 and expressing a biosynthesis pathway;
- (ii) one or more genes encoding and expressing a C—C ligation pathway; and
- (iii) a reduction and dehydration pathway, comprising one or more genes encoding and expressing an enzyme selected from a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase,

thereby converting the suitable monosaccharide or oligosaccharide to the commodity chemical.

10. The method of claim **9**, wherein the biosynthesis pathway is selected from (a) an aldehyde biosynthesis pathway, (b) a ketone synthesis pathway, and (c) both (a) and (b).

11. The method of claim **9**, wherein the biosynthesis pathway comprises an acetaldehyde biosynthesis pathway, and wherein the acetaldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to an acetaldehyde.

12. The method of claim **9**, wherein the biosynthesis pathway comprises a propionaldehyde biosynthesis pathway, and

wherein the propionaldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a propionaldehyde.

13. The method of claim **9**, wherein the biosynthesis pathway comprises a butyraldehyde biosynthesis pathway, and wherein the butyraldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a butyraldehyde.

14. The method of claim **9**, wherein the biosynthesis pathway comprises an isobutyraldehyde biosynthesis pathway, and wherein the isobutyraldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to an isobutyraldehyde.

15. The method of claim **9**, wherein the biosynthesis pathway comprises a 2-methylbutyraldehyde biosynthesis pathway, and wherein the 2-methylbutyraldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a 2-methylbutyraldehyde.

16. The method of claim **9**, wherein the biosynthesis pathway comprises a 3-methylbutyraldehyde biosynthesis pathway, and wherein the 3-methylbutyraldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a 3-methylbutyraldehyde.

17. The method of claim **9**, wherein the biosynthesis pathway comprises a 4-methylpentaldehyde biosynthesis pathway, and wherein the 4-methylpentaldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a 4-methylpentaldehyde.

18. The method of claim **9**, wherein the biosynthesis pathway comprises a phenylacetaldehyde biosynthesis pathway, and wherein the phenylacetaldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a phenylacetaldehyde.

19. The method of claim **9**, wherein the biosynthesis pathway comprises a 5-amino pentaldehyde biosynthesis pathway, and wherein the 5-amino pentaldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a 5-amino pentaldehyde.

20. The method of claim **9**, wherein the biosynthesis pathway comprises a 2-(4-hydroxyphenyl)acetaldehyde biosynthesis pathway, and wherein the 2-(4-hydroxyphenyl)acetaldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a 2-(4-hydroxyphenyl)acetaldehyde.

21. The method of claim **9**, wherein the biosynthesis pathway comprises a 2-(Indole-3-)acetaldehyde biosynthesis pathway, and wherein the 2-(Indole-3-)acetaldehyde biosynthesis pathway converts the suitable monosaccharide or oligosaccharide to a 2-(Indole-3-)acetaldehyde.

22. The method of claim **9**, wherein the C—C ligation pathway comprises at least one enzyme selected from an acetaldehyde lyase, a propionaldehyde lyase, a butyraldehyde lyase, an isobutyraldehyde lyase, a 2-methyl-butyr-aldehyde lyase, a 3-methyl-butyr-aldehyde lyase, a phenylacetaldehyde lyase, an oxaloacetate decarboxylase, an α -keto glutarate decarboxylase, an α -keto adipate decarboxylase, a pentaldehyde lyase, a 4-methyl-pentaldehyde lyase, a hexaldehyde lyase, a heptaldehyde lyase, an octaldehyde lyase, a 4-hydroxyphenylacetaldehyde lyase, an indoleacetaldehyde lyase, an indolephenylacetaldehyde lyase, a benzaldehyde lyase, a pyruvate decarboxylase, a benzformate lyase, and a 2-keto isovalerate decarboxylase.

23. The method of claim 9, wherein the C—C ligation pathway comprises a C—C ligase or an optimized C—C ligase.

24. The method of claim 23, wherein the C—C ligase or optimized C—C ligase comprises at least one enzymatic activity selected from an acetaldehyde lyase activity, a propionaldehyde lyase activity, a butyraldehyde lyase activity, an isobutyraldehyde lyase activity, a 2-methyl-butyraldehyde lyase activity, a 3-methyl-butyraldehyde lyase activity, a phenylacetaldehyde lyase activity, an oxaloacetate decarboxylase activity, an α -keto glutarate decarboxylase activity, an α -keto adipate decarboxylase activity, a pentaldehyde lyase activity, a 4-methyl-pentaldehyde lyase activity, a hexaldehyde lyase activity, a heptaldehyde lyase activity, an octaldehyde lyase activity, a 4-hydroxyphenylacetaldehyde lyase activity, an indoleacetaldehyde lyase activity, an indolephenylacetaldehyde lyase activity, a benzaldehyde lyase activity, a pyruvate decarboxylase activity, a benzformate lyase activity, and a 2-keto isovalerate decarboxylase activity.

25. The method of claim 9, wherein the C—C ligation pathway comprises a benzaldehyde lyase, or a biologically active variant or fragment thereof.

26. The method of claim 25, wherein the benzaldehyde lyase is derived from *Pseudomonas fluorescens*.

27. The method of claim 26, wherein the benzaldehyde lyase comprises a polypeptide having 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: 144.

28. The method of claim 27, wherein the amino acid sequence of the benzaldehyde lyase comprises one or more conserved residues selected from G27, E50, A57, G155, P162, P234, D271, G277, G422, G447, D448, and G512.

29. The method of claim 9, wherein the dehydration and reduction pathway comprises a diol dehydrogenase selected from 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 dehydrogenase, 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, 1,10-diamino-5,6-decanediol dehydrogenase, 1,4-di(4-hydroxyphenyl)-2,3-butanediol, and 2,3-hexanediol-1,6-dicarboxylic acid dehydrogenase.

30. The method of claim 9, wherein the diol dehydrogenase comprises a polypeptide having 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 NOS: 98, 100, or 102.

31. The method of claim 9, wherein the dehydration and reduction pathway comprises a diol dehydratase selected from 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-dodecanediol 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, and 2,3-hexanediol-1,6-dicarboxylic acid dehydratase.

32. The method of claim **9**, wherein the diol dehydratase comprises a polypeptide having 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 NOS:104, 106, 108, 308, 309, 310, or 311.

33. The method of claim **32**, wherein the polypeptide of SEQ ID NO:104 comprises one or more conserved residues selected 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.

34. The method of claim **32**, wherein the polypeptide of SEQ ID NO:310 comprises one or more conserved residues selected from 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, N604, A654, G658, R659, D676, N702, Q735, N737, A747, P751, R760, V761, A762, G763, Q776, I780, and R782.

35. The method of claim **32**, wherein the polypeptide of SEQ ID NO:311 comprises one or more conserved residues selected from D19, G20, G22, R24, F28, G31, C32, C36, W38, C39, N41, P42, C58, C64, C96, G129, T132, G135, G136, D185, R187, N208, R222, and R264.

36. The method of claim **9**, wherein the dehydration and reduction pathway comprises a secondary alcohol dehydrogenase selected from 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, cyclopentanediol 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-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)-heptanol 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, and Indole-3-ethanol dehydrogenase.

37. The method of claim **9**, wherein the secondary alcohol dehydrogenase comprises a polypeptide having 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 NOS:110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, and 142.

38. The method of claim **37**, wherein the secondary alcohol dehydrogenase comprises at least one of a nicotinamide adenine dinucleotide (NAD⁺), a NADH, nicotinamide adenine dinucleotide phosphate (NADP⁺), or a NADPH binding motif.

39. The method of claim **38**, wherein the NAD⁺, NADH, NADP⁺, or NADPH binding motif is 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-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.

40. A recombinant microorganism, comprising (i) one or more genes encoding and expressing an aldehyde and/or ketone biosynthesis pathway; (ii) one or more genes encoding and expressing a C—C ligation pathway; and (iii) a reduction and dehydration pathway, comprising one or more genes encoding and expressing an enzyme selected from a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase.

41. The recombinant microorganism of claim **40**, wherein the microorganism is capable of converting a suitable monosaccharide or suitable oligosaccharide to a commodity chemical, or an intermediate thereof.

42. The recombinant microorganism of claim **40**, wherein the one or more genes encoding the biosynthesis pathway encode a pathway selected from an acetaldehyde, propionaldehyde, butyraldehyde, isobutyraldehyde, 2-methyl-butyraldehyde, 3-methyl-butyraldehyde, 4-methyl-pentaldehyde, phenyl acetaldehyde, glutaraldehyde, 5-amino-pentaldehyde, succinate semialdehyde, succinate 4-hydroxyphenyl acetaldehyde, and an indole-3-acetaldehyde biosynthesis pathway, and combinations thereof.

43. The recombinant microorganism of claim **40**, wherein the one or more genes encoding and expressing the C—C ligation pathway comprise a nucleotide sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the nucleotide sequence set forth in SEQ ID NO: 143.

44. The recombinant microorganism of claim **40**, wherein the one or more genes encoding the diol dehydrogenase comprise a nucleotide sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the nucleotide sequence set forth in SEQ ID NOS:97, 99, or 101.

45. The recombinant microorganism of claim **40**, wherein the one or more genes encoding the diol dehydratase comprise a nucleotide sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the nucleotide sequence set forth in SEQ ID NOS: 103, 105, or 107.

46. The recombinant microorganism of claim **40**, wherein the one or more genes encoding a secondary alcohol dehydrogenase comprise a nucleotide sequence that is at least 80%, 90%, 95%, 98%, or 99% identical to the nucleotide sequence set forth in SEQ ID NOS: 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, or 141.

47. A recombinant microorganism, comprising one or more genes encoding and expressing an aldehyde or ketone biosynthesis pathway, wherein the pathway comprises at least one exogenous gene.

48. A recombinant microorganism, comprising one or more exogenous genes encoding and expressing one or more enzymes selected from a C—C ligase, a diol dehydrogenase, a diol dehydratase, and a secondary alcohol dehydrogenase.

49. The recombinant microorganism of claim 47, wherein the one or more enzymes comprise a C—C ligase and a diol dehydrogenase.

50. The recombinant microorganism of claim 47, wherein the one or more enzymes comprise a diol dehydrogenase and a diol dehydratase.

51. The recombinant microorganism of claim 40, wherein the microorganism comprises reduced ethanol production capability compared to a wild-type microorganism.

52. The recombinant microorganism of claim 40, wherein the microorganism comprises a reduction or inhibition in the conversion of acetyl-coA to ethanol.

53. The recombinant microorganism of claim 40, wherein the recombinant microorganism comprises a reduction of an ethanol dehydrogenase, thereby providing a reduced ethanol production capability.

54. The recombinant microorganism of claim 53, wherein the ethanol dehydrogenase is an adhE, homolog or variant thereof.

55. The recombinant microorganism of claim 54, wherein the microorganism comprises a deletion or knockout of an adhE, homolog or variant thereof.

56. The recombinant microorganism of claim 40, 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.

57. The recombinant microorganism of claim 40, wherein the microorganism is a bacteria.

58. The recombinant microorganism of claim 40, wherein the microorganism is a gram-negative bacteria.

59. The recombinant microorganism of claim 40, wherein the microorganism is a eukaryote.

60. The recombinant microorganism of claim 59, wherein the eukaryote is a fungus.

61. The recombinant microorganism of claim 60, wherein the fungus is a yeast.

62. A method for converting a suitable monosaccharide to a commodity chemical comprising,

(a) contacting the suitable monosaccharide with a microbial system for a time sufficient to convert to the suitable monosaccharide to the commodity chemical, wherein the microbial system comprises,

(i) one or more genes encoding and expressing a pathway selected from a fatty acid biosynthesis pathway, an amino acid biosynthetic pathway, and 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 or oligosaccharide to the commodity chemical.

63. A recombinant microorganism, comprising (i) one or more genes encoding and expressing a pathway selected from a fatty acid biosynthesis pathway, an amino acid biosynthetic pathway, and 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).

64. The recombinant microorganism of claim 1, wherein the microorganism is selected from *Acetobacter acetii*, *Achromobacter*, *Acidiphilium*, *Acinetobacter*, *Actinomadura*, *Actinoplanes*, *Aeropyrum pernix*, *Agrobacterium*, *Alcaligenes*, *Ananas comosus* (M), *Arthrobacter*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus melleus*, *Aspergillus pulverulentus*, *Aspergillus saitoi*, *Aspergillus sojae*, *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 insolens*, *Kitasatospora setae*, *Klebsiella*, *Klebsiella oxytoca*, *Kluyveromyces*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Kocuria*, *Lactolactis*, *Lactobacillus*, *Lactobacillus fermentum*, *Lactobacillus sake*, *Lactococcus*, *Lactococcus lactis*, *Leuconostoc*, *Methylocystis*, *Methanobacillus 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*, *Penicillium lilactinum*, *Penicillium 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*, *Secharomyces cerevisiae*, *Sclerotinia 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 *Zymomonas mobilis*.

65. A commodity chemical produced by the method of claim 1.

66. A blended commodity chemical comprising the commodity chemical of claim **65** and a refinery-produced petroleum product.

67. The blended commodity chemical of claim **66**, wherein the commodity chemical is selected from a C10-C12 hydrocarbon, 2-phenylethanol, 2-(4-hydroxyphenyl)ethanol, and indole-3-ethanol.

68. The blended commodity chemical of claim **67**, wherein the C10-C12 hydrocarbon is selected from 2,7-dimethyldecane and 2,9-dimethyldecane.

69. The blended commodity chemical of claim **66**, wherein the refinery-produced petroleum product is selected from jet fuel and diesel fuel.

70. 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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