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(54) **PRODUCTION OF FATTY ACID DERIVATIVES**

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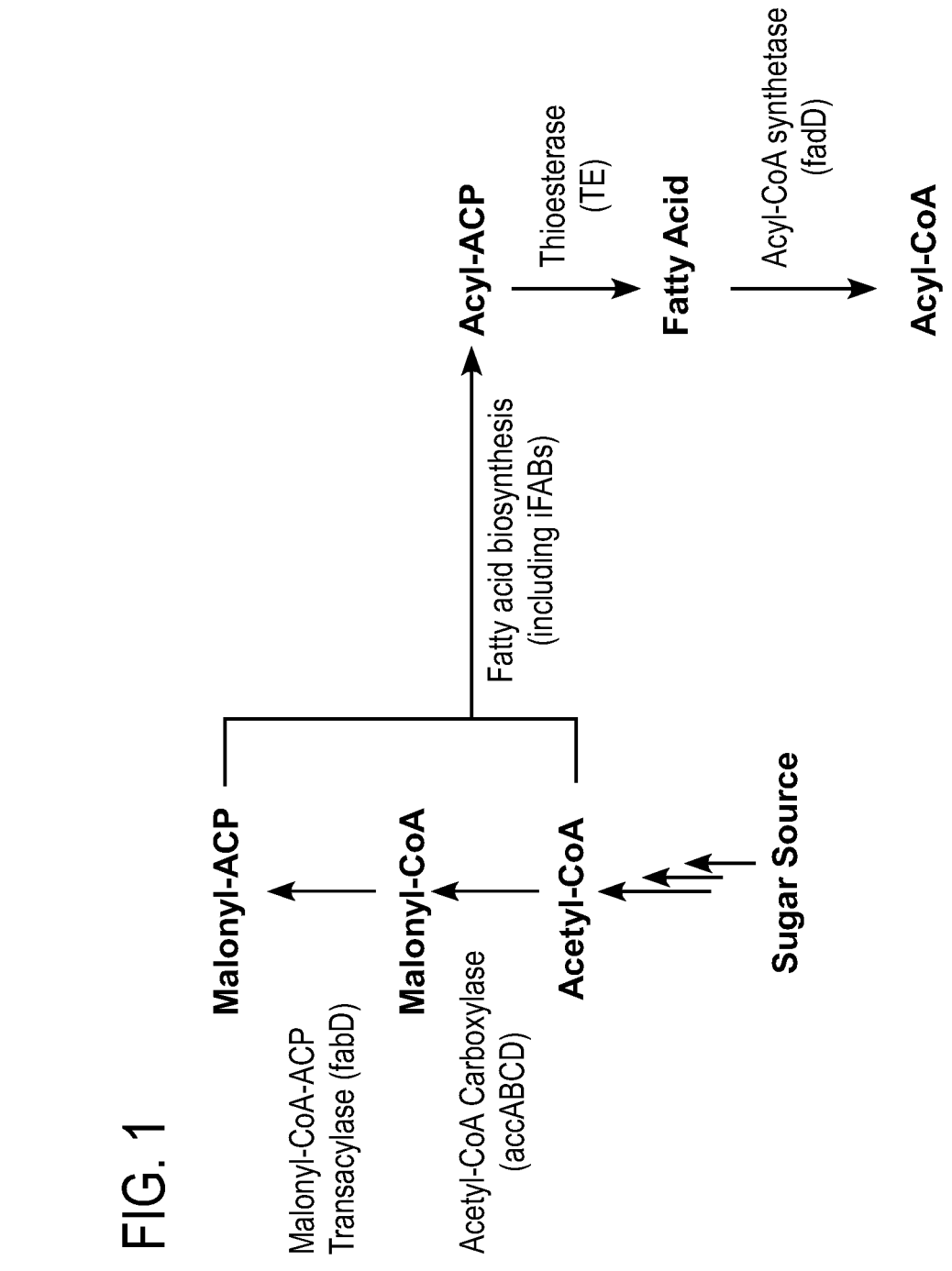
(57) **ABSTRACT**

The invention relates to compositions and methods, including polynucleotide sequences, amino acid sequences, recombinant host cells and recombinant host cell cultures engineered to produce fatty acid derivative compositions comprising fatty acids, fatty alcohols, fatty aldehydes, fatty esters, alkanes, terminal olefins, internal olefins or ketones. The fatty acid derivative composition is produced extracellularly with a higher titer, yield or productivity than the corresponding wild type or non-engineered host cell.

FIG. 1


Malonyl-ACP
Malonyl-CoA-ACP Transacylase (fabD)
Malonyl-CoA
Acetyl-CoA Carboxylase (accABCD)
Acetyl-CoA
Sugar Source
Fatty acid biosynthesis (including iFABs)
Acyl-ACP
Thioesterase (TE)
Fatty Acid
Acyl-CoA synthetase (fadD)
Acyl-CoA

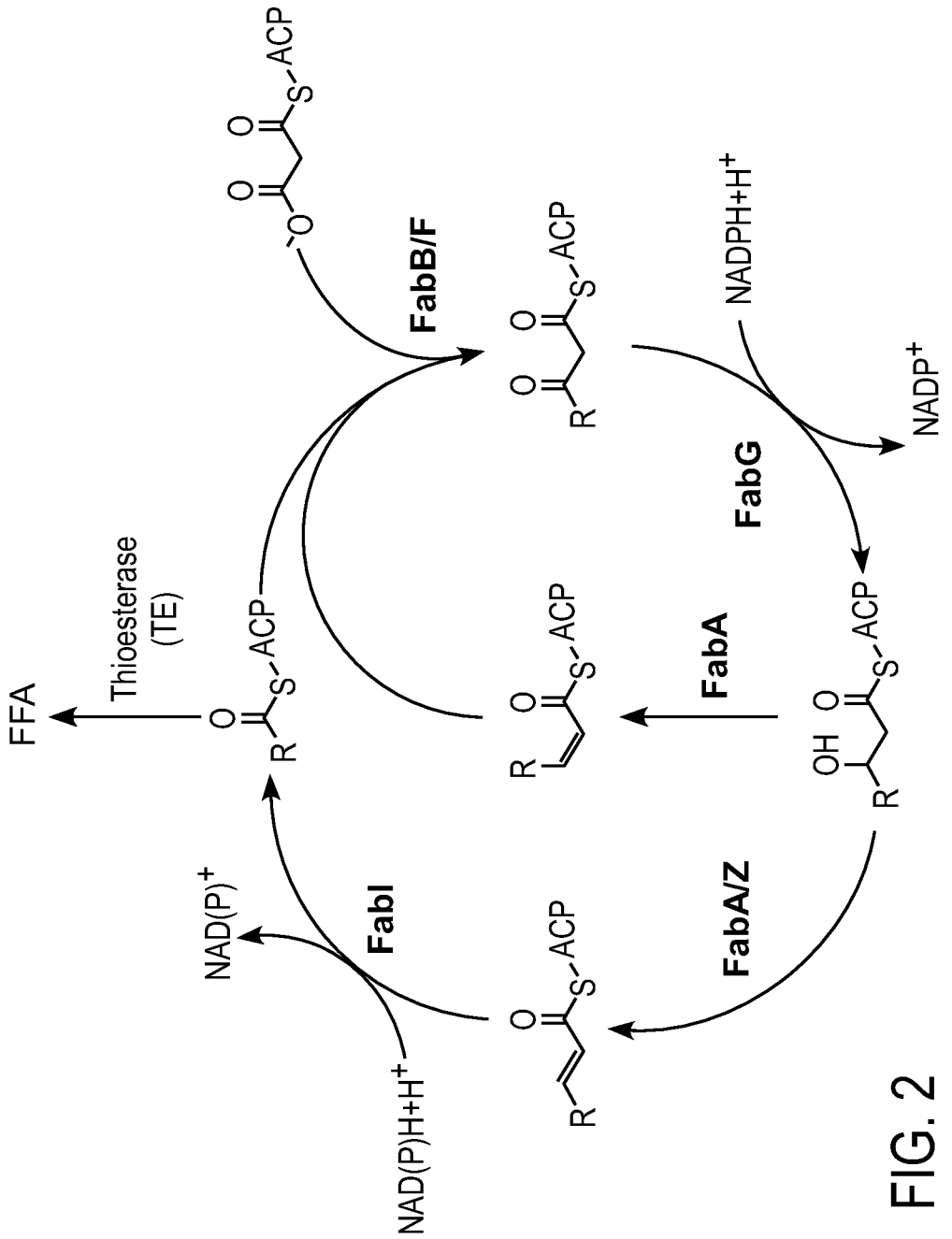

FFA
Thioesterase
(TE)
ACP
FabB/F
NADPH+H+
NADP+
FabG
FabA
FabA/Z
FabI
NAD(P)+
NAD(P)H+H+

FIG. 2

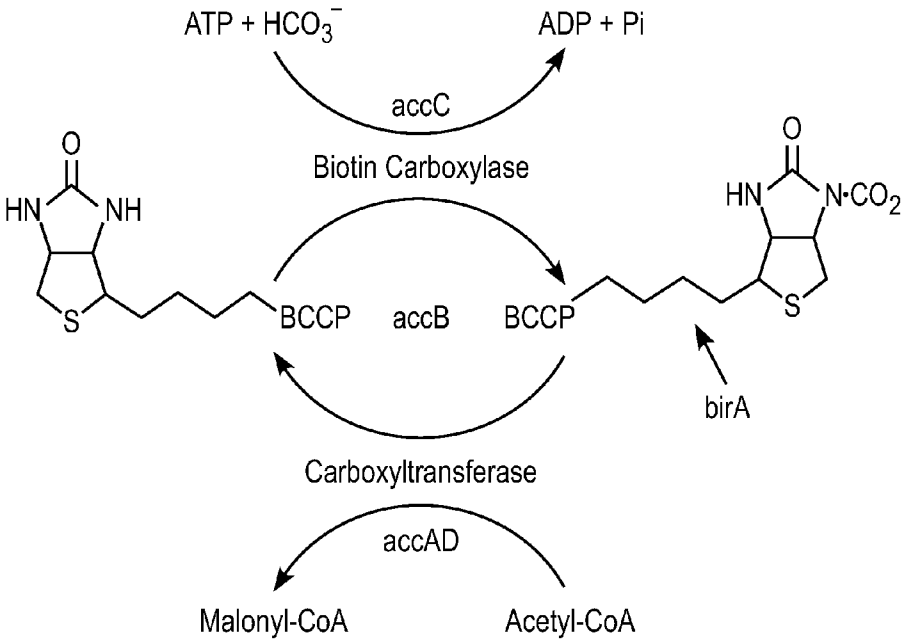

ATP + HCO3−
ADP + Pi
accC
Biotin Carboxylase
HN
NH
O
S
BCCP
accB
BCCP
HN
N·CO2
O
S
birA
Carboxyltransferase
accAD
Malonyl-CoA
Acetyl-CoA

FIG. 3

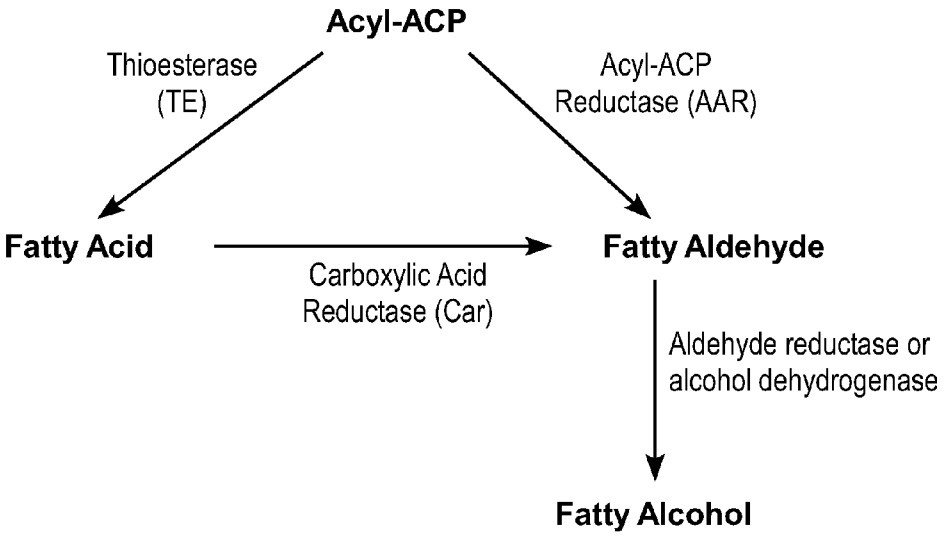

Acyl-ACP
Thioesterase
(TE)
Acyl-ACP
Reductase (AAR)
Fatty Acid
Carboxylic Acid
Reductase (Car)
Fatty Aldehyde
Aldehyde reductase or
alcohol dehydrogenase
Fatty Alcohol

FIG. 4

FIG. 5

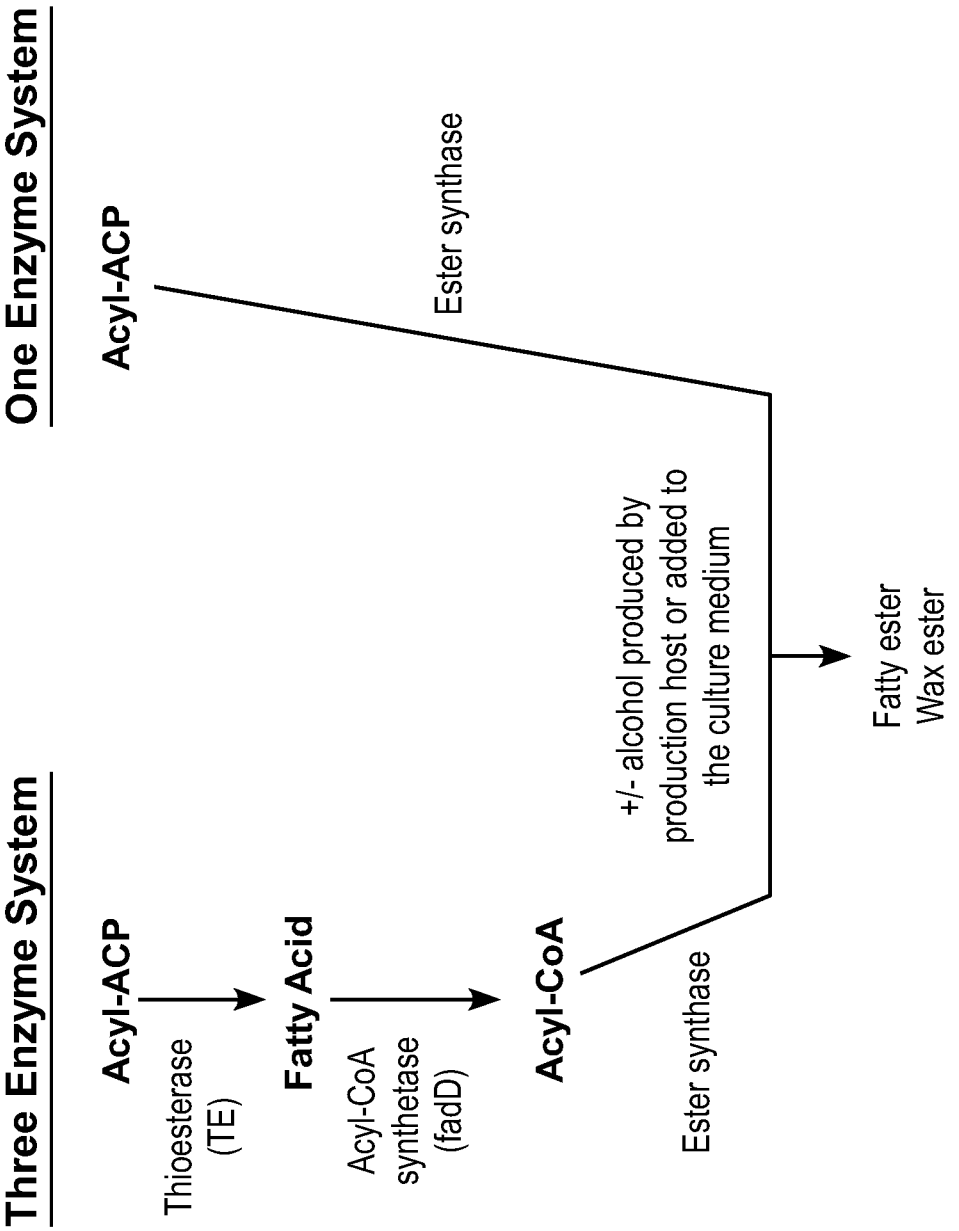
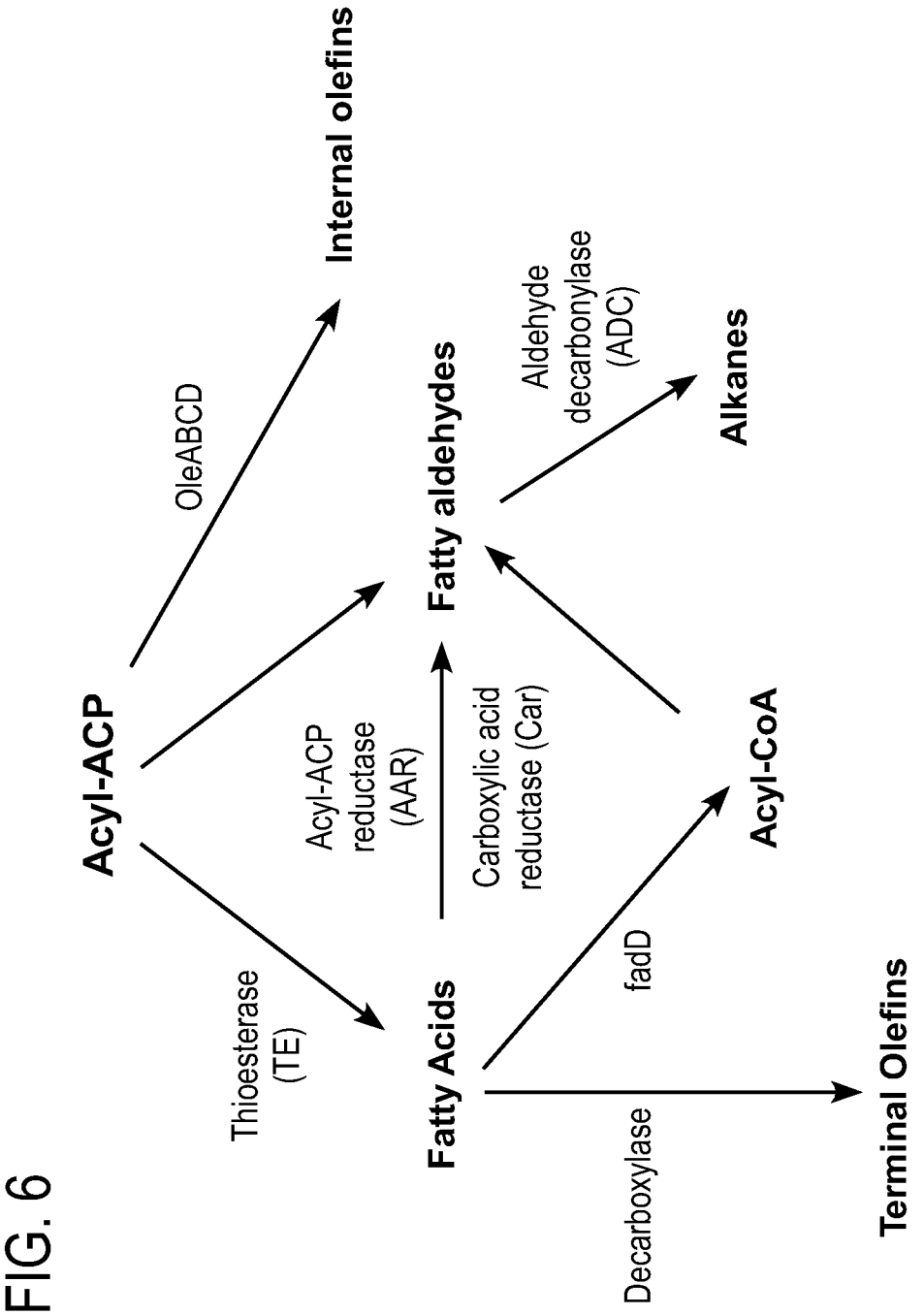

Three Enzyme System
Acyl-ACP
Thioesterase (TE)
Fatty Acid
Acyl-CoA synthetase (fadD)
Acyl-CoA
Ester synthase
+/- alcohol produced by production host or added to the culture medium
Fatty ester
Wax ester
One Enzyme System
Acyl-ACP
Ester synthase

FIG. 6


Acyl-ACP
OleABCD
Internal olefins
Thioesterase (TE)
Acyl-ACP reductase (AAR)
Fatty aldehydes
Aldehyde decarbonylase (ADC)
Alkanes
Carboxylic acid reductase (Car)
Fatty Acids
fadD
Acyl-CoA
Decarboxylase
Terminal Olefins

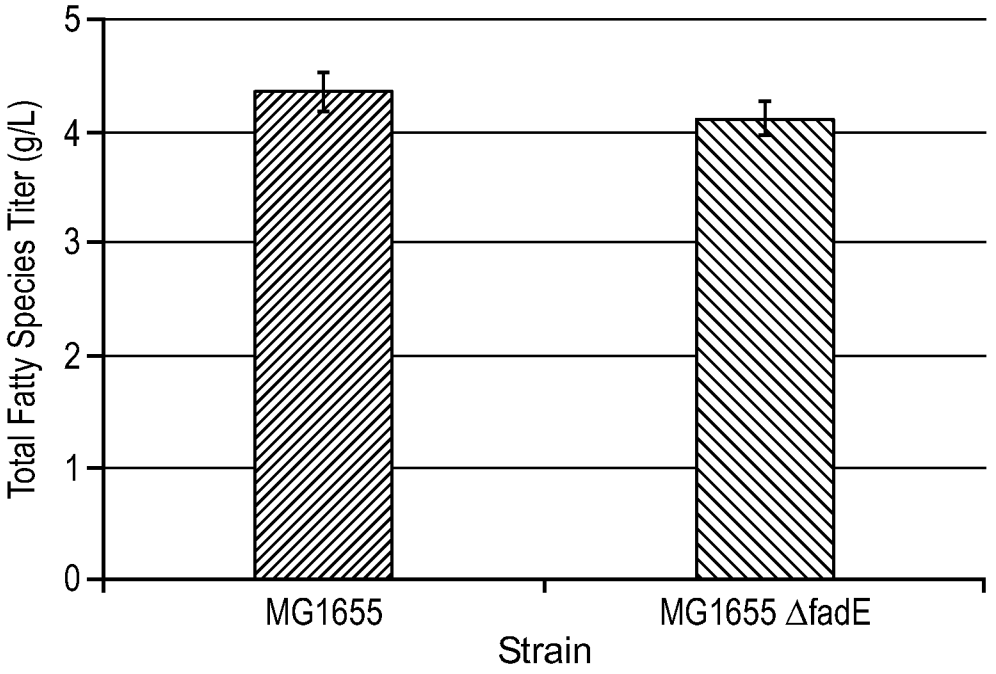

Total Fatty Species Titer (g/L)
5
4
3
2
1
0
MG1655
MG1655 ΔfadE
Strain

FIG. 7

FIG. 8

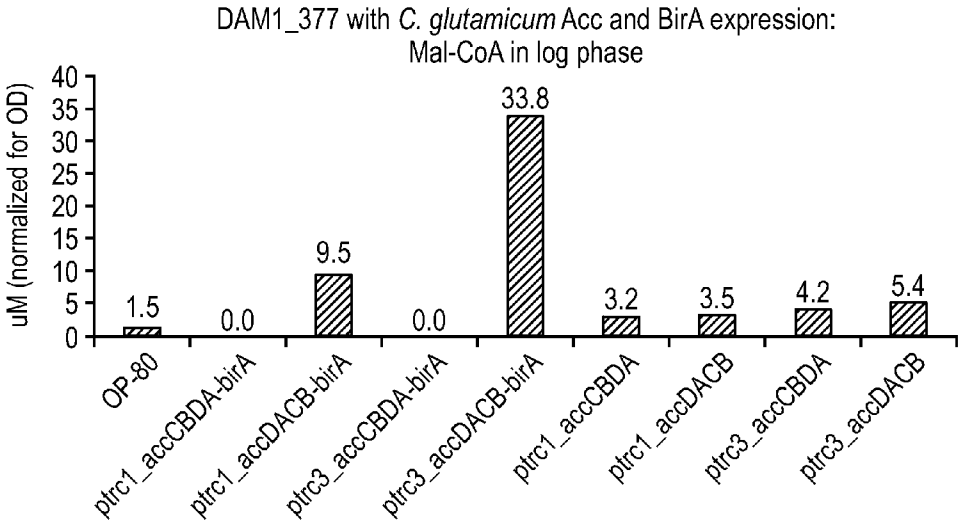
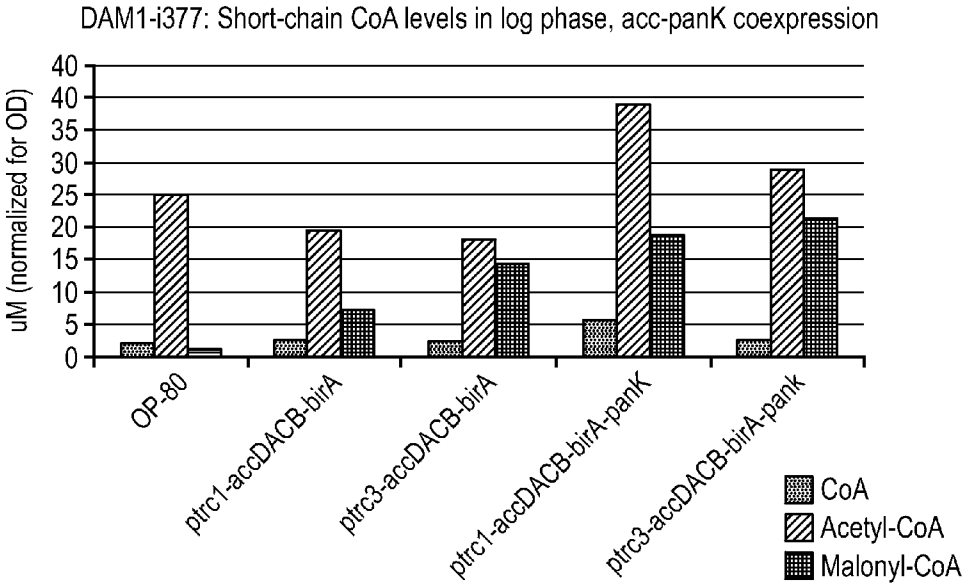

DAM1_377 with C. glutamicum Acc and BirA expression:
Mal-CoA in log phase
uM (normalized for OD)
40
35
30
25
20
15
10
5
0
1.5
0.0
9.5
0.0
33.8
3.2
3.5
4.2
5.4
OP-80
ptrc1_accCBDA-birA
ptrc1_accDACB-birA
ptrc3_accCBDA-birA
ptrc3_accDACB-birA
ptrc1_accCBDA
ptrc1_accDACB
ptrc3_accCBDA
ptrc3_accDACB

FIG. 9


DAM1-i377: Short-chain CoA levels in log phase, acc-panK coexpression
uM (normalized for OD)
40
40
35
30
25
20
15
10
5
0
OP-80
ptrc1-accDACB-birA
ptrc3-accDACB-birA
ptrc1-accDACB-birA-panK
ptrc3-accDACB-birA-pank
CoA
Acetyl-CoA
Malonyl-CoA

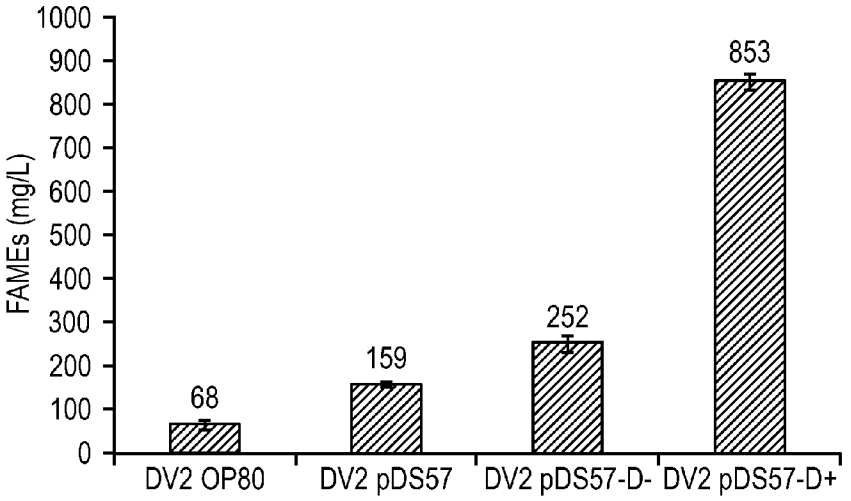

1000
900
800
700
600
500
400
300
200
100
0
FAMEs (mg/L)
68
159
252
853
DV2 OP80
DV2 pDS57
DV2 pDS57-D-
DV2 pDS57-D+

FIG. 10

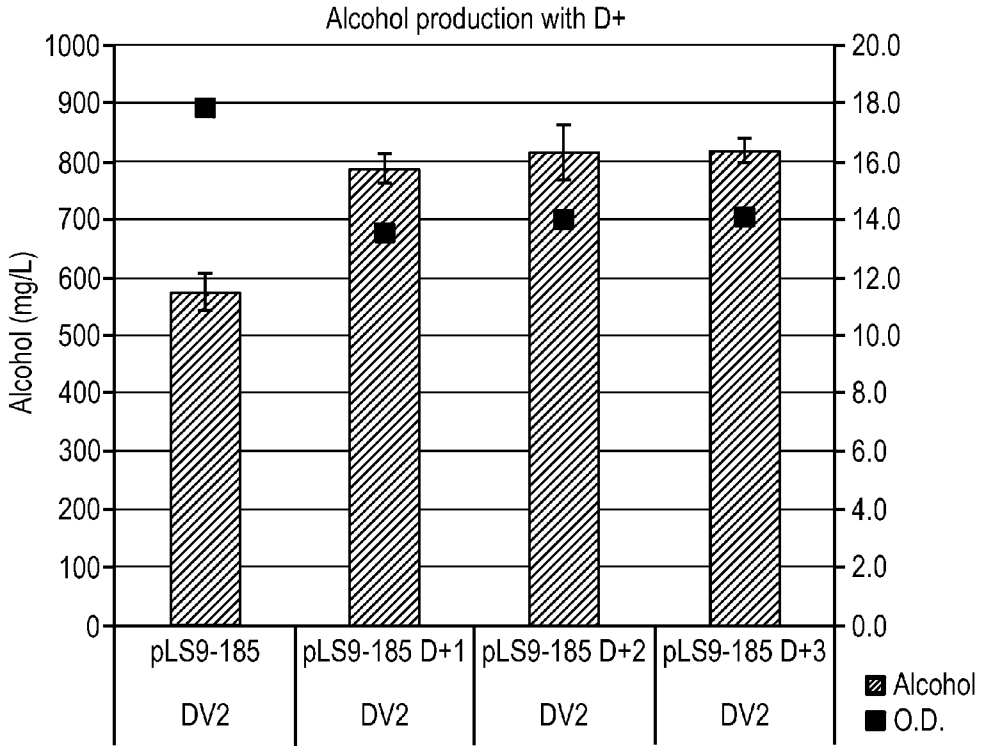
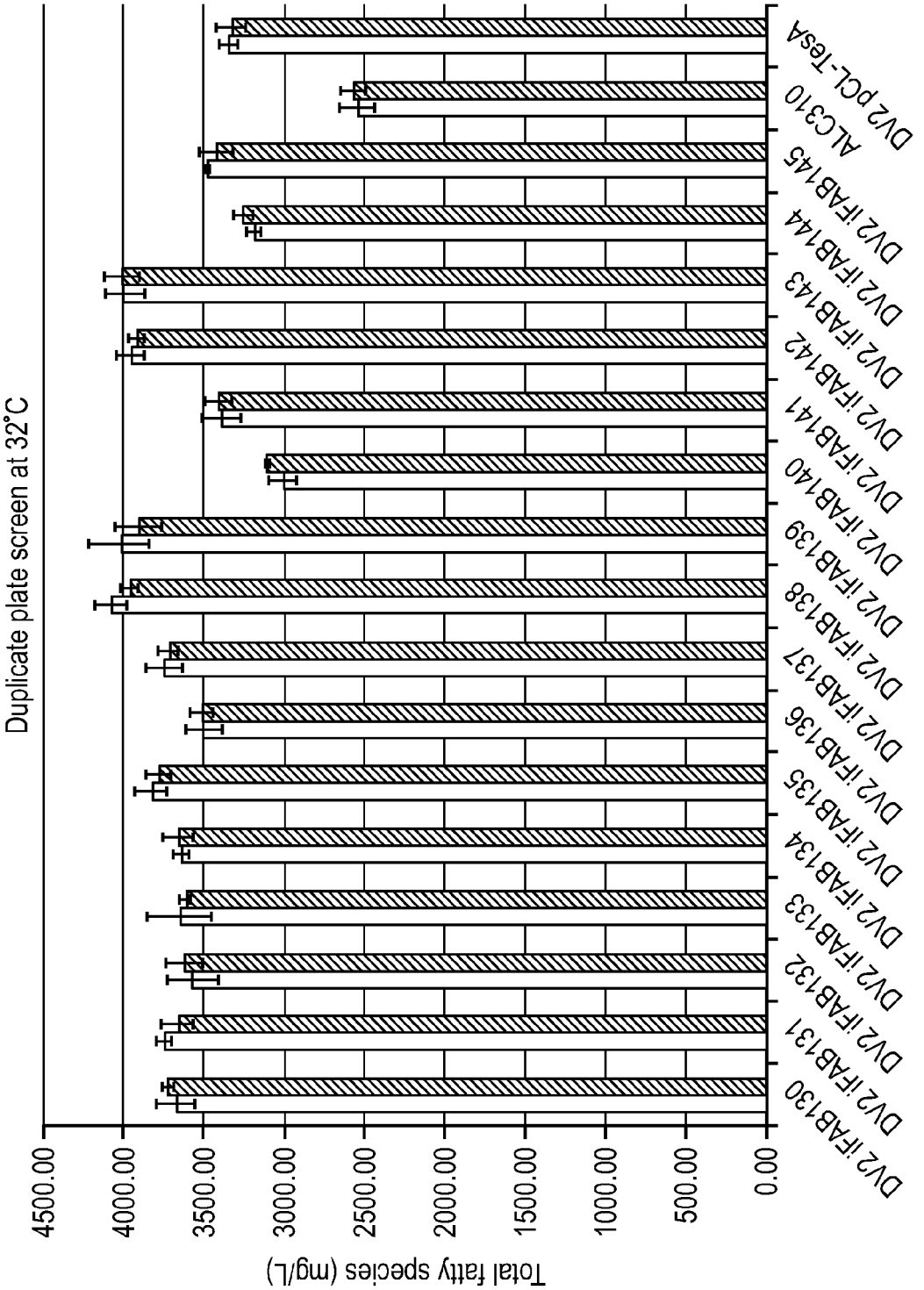

Alcohol production with D+
1000
900
800
700
600
500
400
300
200
100
0
Alcohol (mg/L)
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
pLS9-185 DV2
pLS9-185 D+1 DV2
pLS9-185 D+2 DV2
pLS9-185 D+3 DV2
Alcohol
O.D.

FIG. 11

FIG. 12A


Duplicate plate screen at 32°C
Total fatty species (mg/L)
4500.00
4000.00
3500.00
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
DV2 iFAB130
DV2 iFAB131
DV2 iFAB132
DV2 iFAB133
DV2 iFAB134
DV2 iFAB135
DV2 iFAB136
DV2 iFAB137
DV2 iFAB138
DV2 iFAB139
DV2 iFAB140
DV2 iFAB141
DV2 iFAB142
DV2 iFAB143
DV2 iFAB144
DV2 iFAB145
ALC310
DV2 pCL-TesA

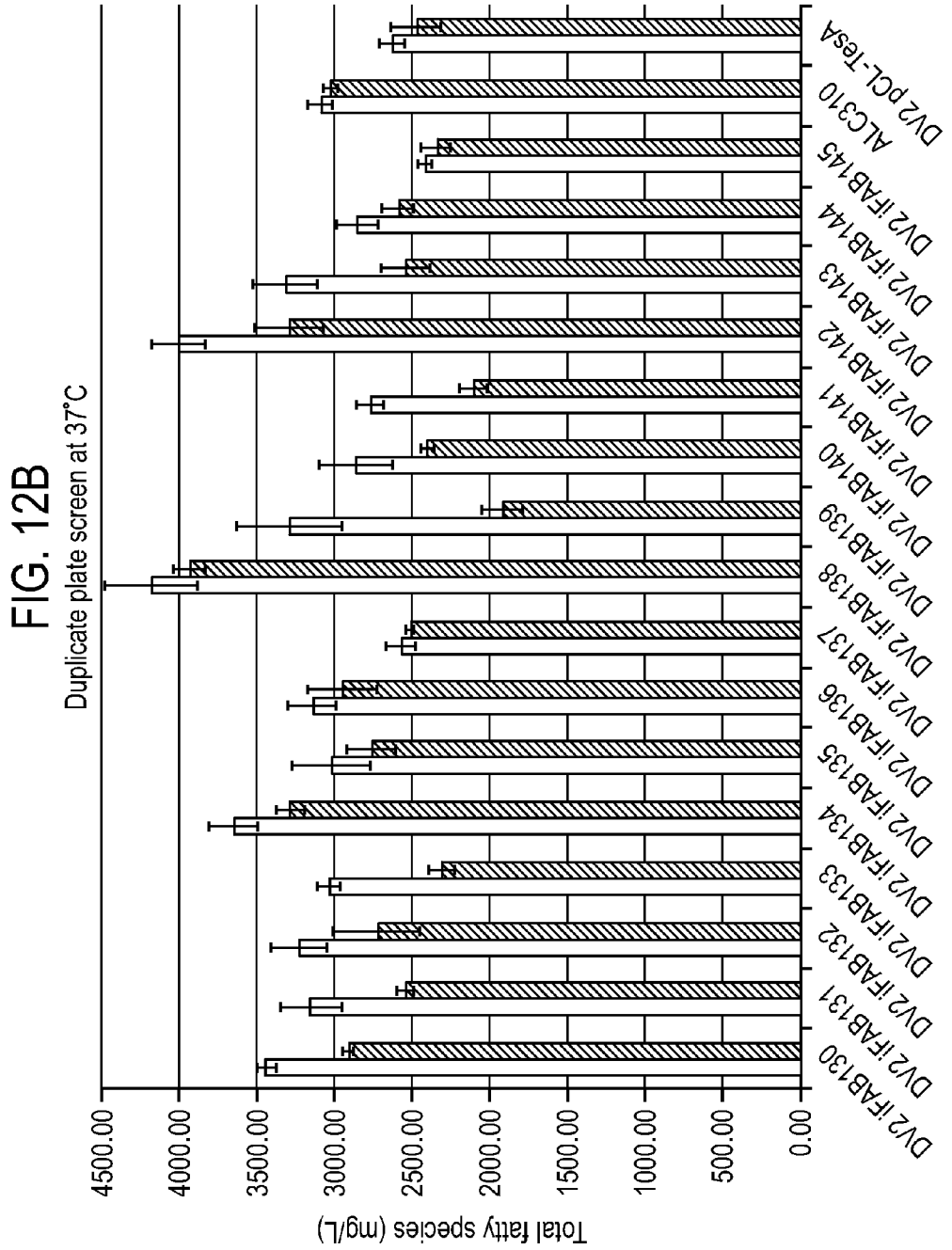

Duplicate plate screen at 37°C
Total fatty species (mg/L)
4500.00
4000.00
3500.00
3000.00
2500.00
2000.00
1500.00
1000.00
500.00
0.00
DV2 iFAB130
DV2 iFAB131
DV2 iFAB132
DV2 iFAB133
DV2 iFAB134
DV2 iFAB135
DV2 iFAB136
DV2 iFAB137
DV2 iFAB138
DV2 iFAB139
DV2 iFAB140
DV2 iFAB141
DV2 iFAB142
DV2 iFAB143
DV2 iFAB144
DV2 iFAB145
ALC310
DV2 pCL-TesA

FIG. 12B

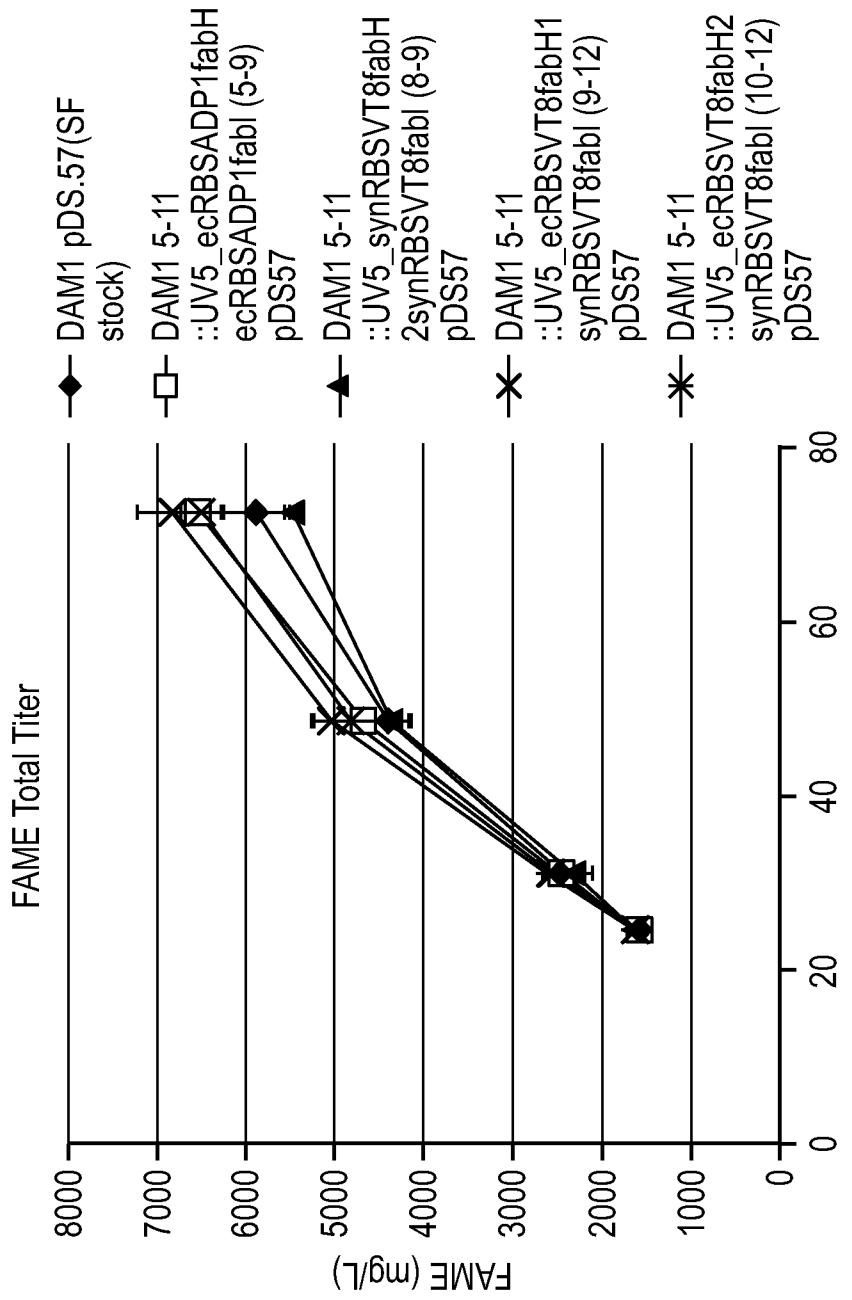

FAME Total Titer
FAME (mg/L)
Time (Total Elapsed)
0
1000
2000
3000
4000
5000
6000
7000
8000
0
20
40
60
80
DAM1 pDS.57(SF stock)
DAM1 5-11 ::UV5_ecRBSADP1fabH ecRBSADP1fabI (5-9) pDS57
DAM1 5-11 ::UV5_synRBSVT8fabH 2synRBSVT8fabI (8-9) pDS57
DAM1 5-11 ::UV5_ecRBSVT8fabH1 synRBSVT8fabI (9-12) pDS57
DAM1 5-11 ::UV5_ecRBSVT8fabH2 synRBSVT8fabI (10-12) pDS57

FIG. 13

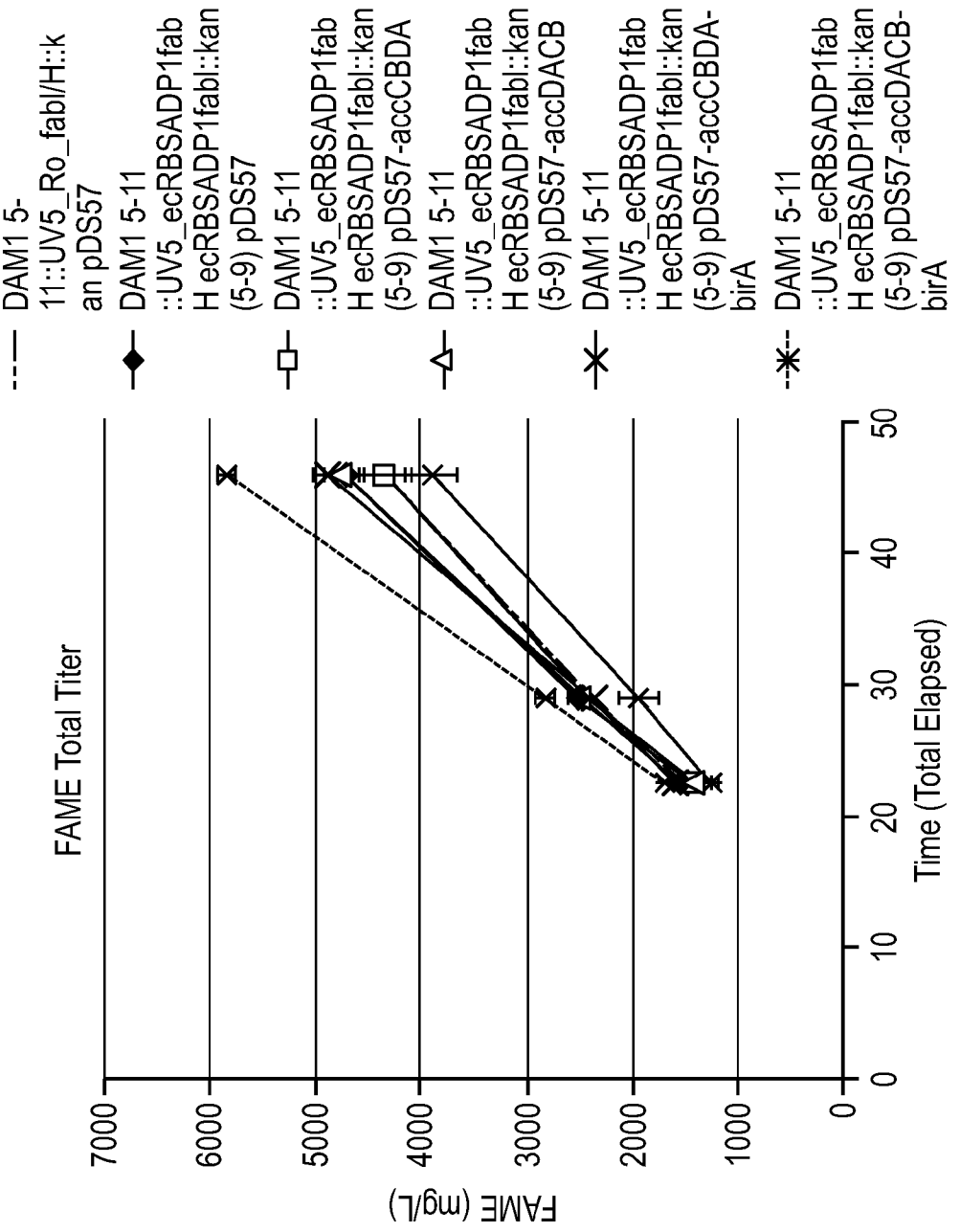

FAME Total Titer
FAME (mg/L)
Time (Total Elapsed)
0
1000
2000
3000
4000
5000
6000
7000
0
10
20
30
40
50
DAM1 5-11::UV5_Ro_fabI/H::kan pDS57
DAM1 5-11::UV5_ecRBSADP1fabH ecRBSADP1fabI::kan (5-9) pDS57
DAM1 5-11::UV5_ecRBSADP1fabH ecRBSADP1fabI::kan (5-9) pDS57-accCBDA
DAM1 5-11::UV5_ecRBSADP1fabH ecRBSADP1fabI::kan (5-9) pDS57-accDACB
DAM1 5-11::UV5_ecRBSADP1fabH ecRBSADP1fabI::kan (5-9) pDS57-accCBDA-birA
DAM1 5-11::UV5_ecRBSADP1fabH ecRBSADP1fabI::kan (5-9) pDS57-accDACB-birA

FIG. 14

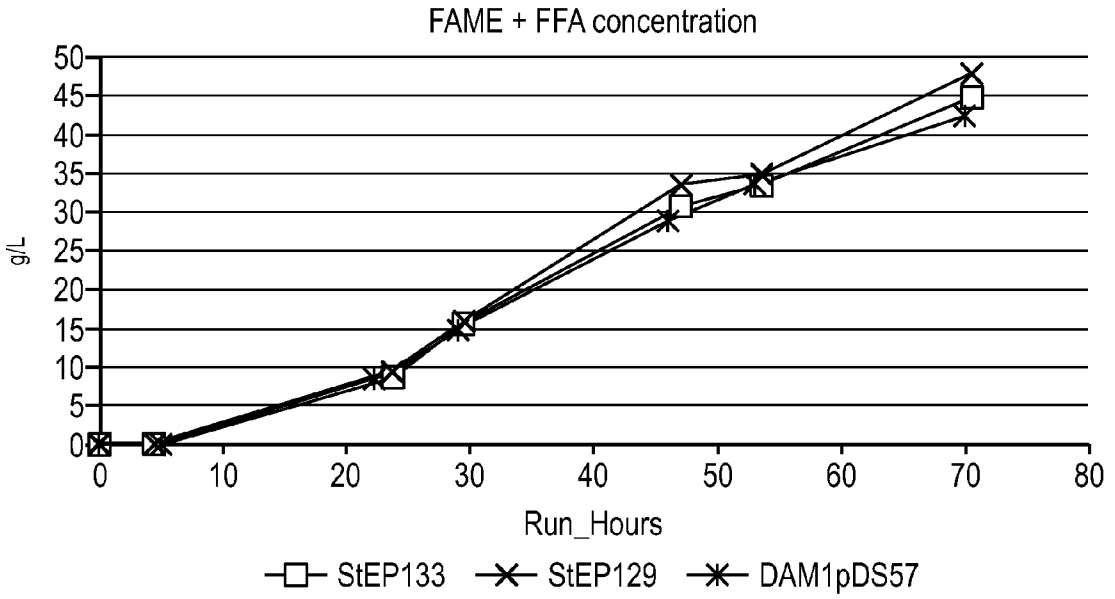

FAME + FFA concentration
g/L
50
45
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
Run_Hours
StEP133
StEP129
DAM1pDS57

FIG. 15

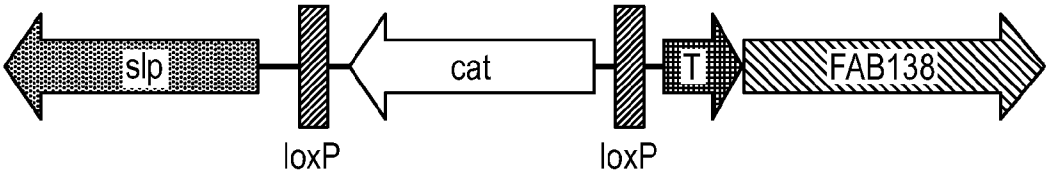


FIG. 16A

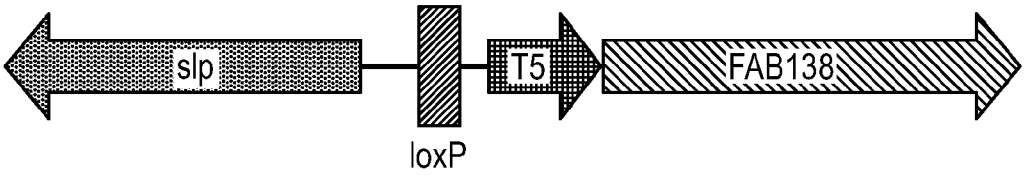


FIG. 16B

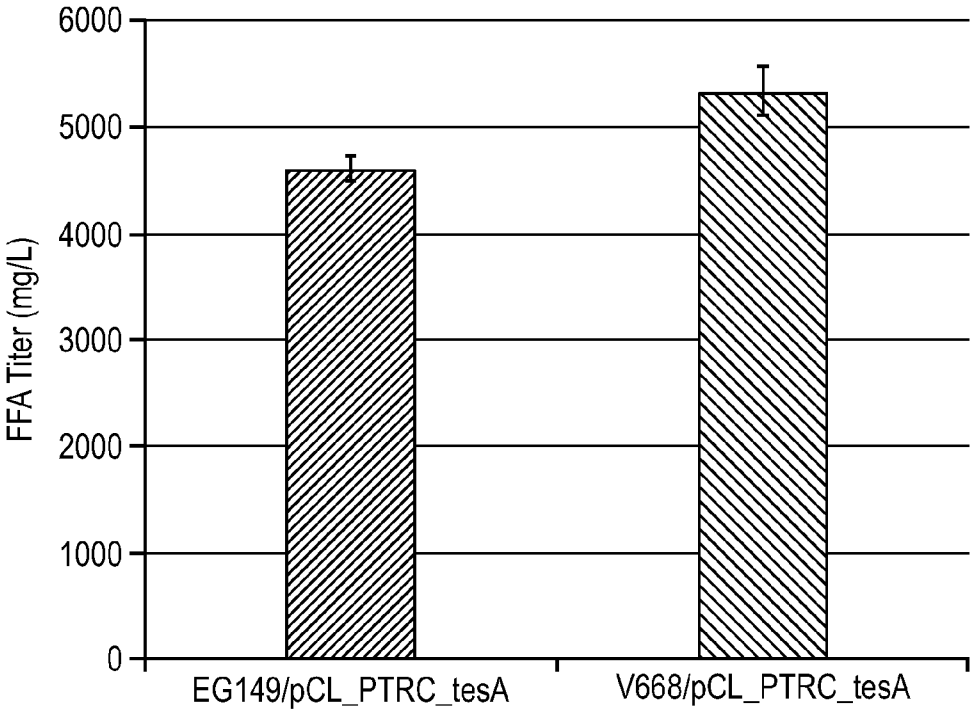
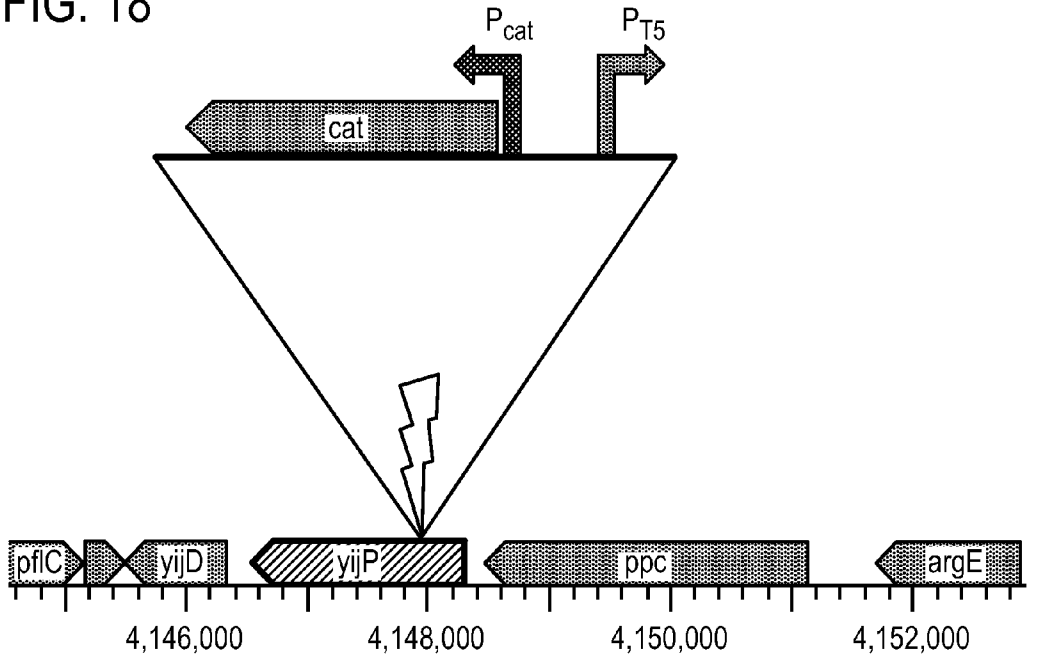

6000
5000
4000
3000
2000
1000
0
FFA Titer (mg/L)
EG149/pCL_PTRC_tesA
V668/pCL_PTRC_tesA

FIG. 17

FIG. 18


P_{cat}
P_{T5}
cat
pflC
yijD
yijP
ppc
argE
4,146,000
4,148,000
4,150,000
4,152,000

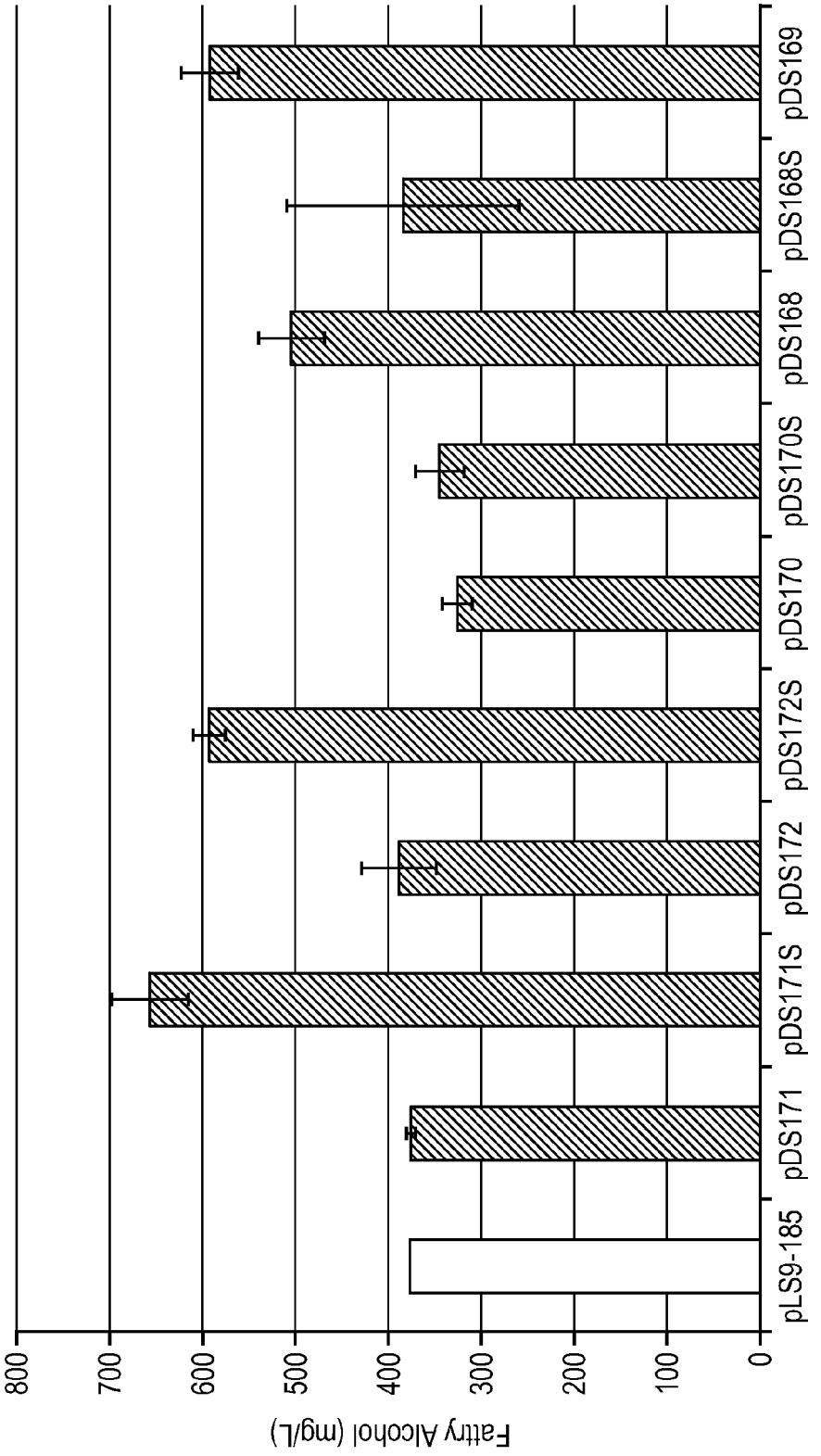

Fatty Alcohol (mg/L)
800
700
600
500
400
300
200
100
0
pLS9-185
pDS171
pDS171S
pDS172
pDS172S
pDS170
pDS170S
pDS168
pDS168S
pDS169
Strain

FIG. 19

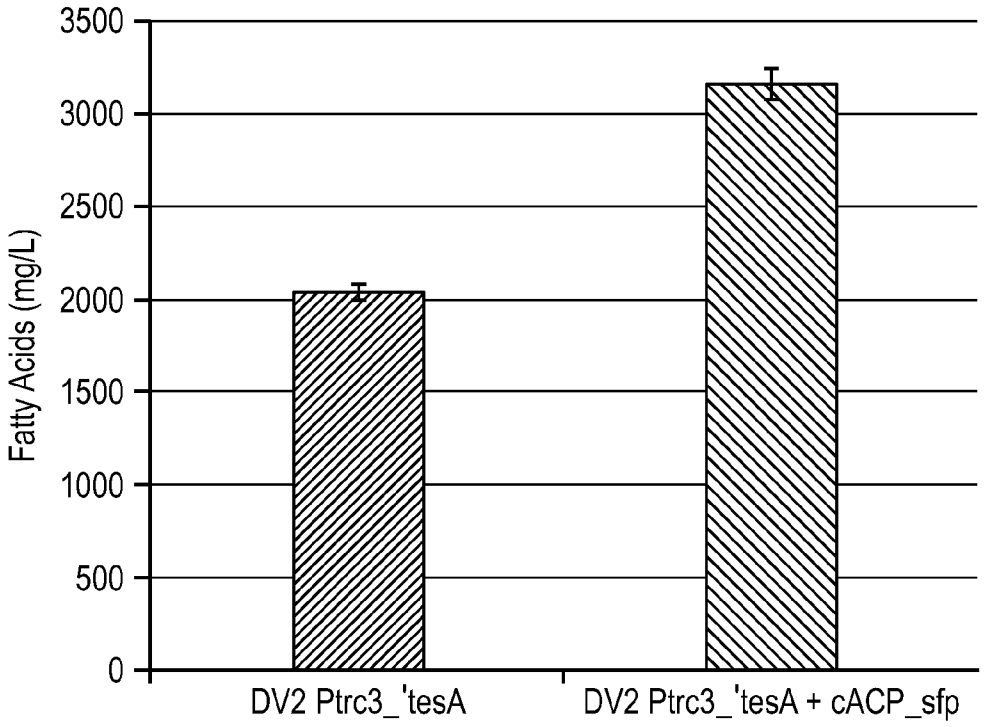

3500
3000
2500
2000
1500
1000
500
0
Fatty Acids (mg/L)
DV2 Ptrc3_'tesA
DV2 Ptrc3_'tesA + cACP_sfp

FIG. 20

PRODUCTION OF FATTY ACID DERIVATIVES

FIELD OF THE INVENTION

[0001] The invention relates to engineered host cells together with vector and strain modifications effective to improve the titer, yield and productivity of fatty acid derivatives relative to "wild-type" or non-engineered host cells. The invention further relates to methods of making and using such modified vectors and strains for the fermentative production of fatty acid derivatives and fatty acid derivative compositions.

BACKGROUND OF THE INVENTION

[0002] Fatty acid derivatives including fatty aldehydes, fatty alcohols, hydrocarbons (alkanes and olefins), fatty esters (e.g., waxes, fatty acid esters, or fatty esters) and ketones comprise important categories of industrial chemicals and fuels. These molecules and their derivatives have numerous applications including use as surfactants, lubricants, plasticizers, solvents, emulsifiers, emollients, thickeners, flavors, fragrances, and fuels.

[0003] Crude petroleum is currently a primary source of raw materials for producing petrochemicals and fuels. The two main classes of raw materials derived from petroleum are short chain olefins (e.g., ethylene and propylene) and aromatics (e.g., benzene and xylene isomers). These raw materials are derived from longer chain hydrocarbons in crude petroleum by cracking it at considerable expense using a variety of methods, such as catalytic cracking, steam cracking, or catalytic reforming. These raw materials can be used to make petrochemicals such as monomers, solvents, detergents, and adhesives, which otherwise cannot be directly refined from crude petroleum.

[0004] Petrochemicals, in turn, can be used to make specialty chemicals, such as plastics, resins, fibers, elastomers, pharmaceuticals, lubricants, and gels. Particular specialty chemicals that can be produced from petrochemical raw materials include fatty acids, hydrocarbons, fatty aldehydes, fatty alcohols, esters, ketones, etc.

[0005] Hydrocarbons have many commercial uses. For example, shorter chain alkanes and alkenes are used in transportation fuels. Longer chain alkenes are used in plastics, lubricants, and synthetic lubricants. In addition, alkenes are used as a feedstock to produce alcohols, esters, plasticizers, surfactants, tertiary amines, enhanced oil recovery agents, fatty acids, thiols, alkenylsuccinic anhydrides, epoxides, chlorinated alkanes, chlorinated alkenes, waxes, fuel additives, and drag flow reducers.

[0006] Esters have many commercial uses. For example, biodiesel, an alternative fuel, is comprised of esters (e.g., fatty acid methyl ester, fatty acid ethyl esters, etc.). Some low molecular weight esters are volatile with a pleasant odor which makes them useful as fragrances or flavoring agents. In addition, esters are used as solvents for lacquers, paints, and varnishes. Furthermore, some naturally occurring substances, such as waxes, fats, and oils are comprised of esters. Esters are also used as softening agents in resins and plastics, plasticizers, flame retardants, and additives in gasoline and oil. In addition, esters can be used in the manufacture of polymers, films, textiles, dyes, and pharmaceuticals.

[0007] Aldehydes are used to produce many specialty chemicals. For example, aldehydes are used to produce polymers, resins (e.g., Bakelite), dyes, flavorings, plasticizers, perfumes, pharmaceuticals, and other chemicals, some of which may be used as solvents, preservatives, or disinfectants. In addition, certain natural and synthetic compounds, such as vitamins and hormones, are aldehydes, and many sugars contain aldehyde groups. Fatty aldehydes can be converted to fatty alcohols by chemical or enzymatic reduction.

[0008] Fatty alcohols have many commercial uses. The shorter chain fatty alcohols are used in the cosmetic and food industries as emulsifiers, emollients, and thickeners. Due to their amphiphilic nature, fatty alcohols behave as nonionic surfactants, which are useful in personal care and household products, such as, for example, detergents. In addition, fatty alcohols are used in waxes, gums, resins, pharmaceutical salves and lotions, lubricating oil additives, textile antistatic and finishing agents, plasticizers, cosmetics, industrial solvents, and solvents for fats.

[0009] Fatty alcohols are aliphatic alcohols consisting of a chain of 8 to 22 carbon atoms. Fatty alcohols usually have even number of carbon atoms and a single alcohol group (—OH) attached to the terminal carbon. Some are unsaturated and some are branched. They are widely used in industrial chemistry. Most fatty alcohols in nature are found as waxes which are esters with fatty acids and fatty alcohols. They are produced by bacteria, plants and animals.

[0010] Currently, fatty alcohols are produced via catalytic hydrogenation of fatty acids produced from natural sources, such as coconut oil, palm oil, palm kernel oil, tallow and lard, or by chemical hydration of alpha-olefins produced from petrochemical feedstocks. Fatty alcohols derived from natural sources have varying chain lengths. The chain length of fatty alcohols is important and specific to particular applications. Dehydration of fatty alcohols to alpha-olefins can also be accomplished by chemical catalysis.

[0011] Due to the inherent challenges posed by exploring, extracting, transporting and refining petroleum for use in chemical and fuel products, there is a need for a an alternate source which can be produced economically and used for chemical and fuel production. There is also a need for a petroleum replacement that does not cause the type of environmental damage created by the exploring, extracting, transporting and refining petroleum and the burning of petroleum-based fuels.

[0012] One method of producing renewable petroleum is by engineering host cells to produce renewable petroleum products. Biologically derived fuels and chemicals offer advantages over petroleum based fuels. Biologically derived chemicals such as hydrocarbons (e.g., alkanes, alkenes, or alkynes), fatty alcohols, esters, fatty acids, fatty aldehydes, and ketones are directly converted from biomass to the desired chemical product.

[0013] In order for the use of biologically-derived fatty acid derivatives from fermentable sugars or biomass to be commercially viable as a source for production of renewable chemicals and fuels, the process must be optimized for efficient conversion and recovery of product. The development of biologically derived fuels and chemicals has been a focus of research and development in recent years, however, there remains a need for improvement in the relevant processes and products in order for biologically derived fuels and chemicals to become a commercially viable option. Areas for improvement include the energy efficiency of the production process and product yield. The current invention addresses this need.

SUMMARY OF THE INVENTION

[0014] The present invention provides novel genetically engineered host cells which produce fatty acid derivative compositions at a high titer, yield or productivity; cell cultures comprising such novel genetically engineered host cells and methods of using the same. The invention also provides methods of making compositions comprising fatty acid derivatives by culturing the genetically engineered host cells of the invention, compositions made by such methods, and other features apparent upon further review.

[0015] In one embodiment, the invention provides a cultured genetically engineered host cell comprising (a) a polynucleotide sequence encoding one or more of: (i) an acetyl-CoA carboxylase (EC 6.4.1.2) polypeptide, (ii) a FadR polypeptide, (iii) a heterologous iFAB polypeptide, (iv) a sequence having a transposon insertion in the yijP gene, and (v) a heterologous ACP protein; as well as (b) a polynucleotide sequence encoding a fatty acid derivative biosynthetic polypeptide, wherein the genetically engineered host cell produces a fatty acid derivative composition at a higher titer, yield or productivity when cultured in medium containing a carbon source under conditions effective to overexpress the polynucleotide(s) relative to a corresponding wild type host cell propagated under the same conditions as the genetically engineered host cell.

[0016] The fatty acid derivative composition includes one or more of a fatty acid, a fatty aldehyde, a fatty alcohol, a fatty ester, an alkane, a terminal olefin, an internal olefin and a ketone.

[0017] In one embodiment, the genetically engineered host cell produces a fatty acid derivative composition with a titer, yield or productivity that is at least 3 times greater, at least 5 times greater, at least 8 times greater, or at least 10 times greater than the titer of a fatty acid derivative composition produced by a corresponding wild type (non-engineered) host cell propagated under the same conditions as the genetically engineered host cell (e.g., a titer of from 30 g/L to 250 g/L, a yield of from 10% to 40%, or a productivity of 0.7 mg/L/hr to 3 g/L/hr).

[0018] In some embodiments, the fatty acid derivative composition is produced extracellularly.

[0019] In other embodiments, the host cell is further engineered to comprise a heterologous acp sequence with or without an introduced sfp gene.

[0020] The polynucleotide sequence encoding a fatty acid derivative biosynthetic polypeptide is selected from the group consisting of a polypeptide:

[0021] (a) having thioesterase activity, wherein the recombinant host cell synthesizes fatty acids;

[0022] (b) having thioesterase activity and carboxylic acid reductase ("CAR") activity, wherein the recombinant host cell synthesizes fatty aldehydes and fatty alcohols;

[0023] (c) having thioesterase activity, carboxylic acid reductase activity and alcohol dehydrogenase activity wherein the recombinant host cell synthesizes fatty alcohols;

[0024] (d) having acyl-CoA reductase ("AAR") activity wherein the recombinant host cell synthesizes fatty aldehydes and fatty alcohols;

[0025] (e) having acyl-CoA reductase ("AAR") activity and alcohol dehydrogenase activity wherein the recombinant host cell synthesizes fatty alcohols;

[0026] (f) having fatty alcohol forming acyl-CoA reductase ("FAR") activity, wherein the recombinant host cell synthesizes fatty alcohols;

[0027] (g) having thioesterase activity, carboxylic acid reductase activity and aldehyde decarbonylase activity, wherein the recombinant host cell synthesizes alkanes;

[0028] (h) having acyl-CoA reductase ("AAR") activity and aldehyde decarbonylase activity, wherein the recombinant host cell synthesizes alkanes;

[0029] (i) having ester synthase activity wherein the recombinant host cell synthesizes fatty esters;

[0030] (j) having thioesterase activity, acyl-CoA synthase activity and ester synthase activity wherein the recombinant host cell synthesizes fatty esters;

[0031] (k) having OleA activity, wherein the recombinant host cell synthesizes aliphatic ketones;

[0032] (l) having OleABCD activity, wherein the recombinant host cell synthesizes internal olefins; and

[0033] (m) having thioesterase activity and decarboxylase activity, wherein the recombinant host cell synthesizes terminal olefins.

[0034] These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] FIG. **1** presents an exemplary biosynthetic pathway for use in production of acyl CoA as a precursor to fatty acid derivatives in a recombinant microorganism. The cycle is initiated by condensation of malonyl-ACP and acetyl-CoA.

[0036] FIG. **2** presents an exemplary fatty acid biosynthetic cycle, where malonyl-ACP is produced by the transacylation of malonyl-CoA to malonyl-ACP (catalyzed by malonyl-CoA:ACP transacylase; fabD), then β-ketoacyl-ACP synthase III (fabH) initiates condensation of malonyl-ACP with acetyl-CoA. Elongation cycles begin with the condensation of malonyl-ACP and an acyl-ACP catalyzed by β-ketoacyl-ACP synthase I (fabB) and β-ketoacyl-ACP synthase II (fabF) to produce a β-keto-acyl-ACP, then the β-keto-acyl-ACP is reduced by β-ketoacyl-ACP reductase (fabG) to produce a β-hydroxy-acyl-ACP, which is dehydrated to a trans-2-enoyl-acyl-ACP by β-hydroxyacyl-ACP dehydratase (fabA or fabZ). FabA can also isomerize trans-2-enoyl-acyl-ACP to cis-3-enoyl-acyl-ACP, which can bypass fabI and can used by fabB (typically for up to an aliphatic chain length of C16) to produce β-keto-acyl-ACP. The final step in each cycle is catalyzed by enoyl-ACP reductase (fabI) that converts trans-2-enoyl-acyl-ACP to acyl-ACP. In the methods described herein, termination of fatty acid synthesis occurs by thioesterase removal of the acyl group from acyl-ACP to release free fatty acids (FFA). Thioesterases (e.g., tesA) hydrolyze thioester bonds, which occur between acyl chains and ACP through sulfhydryl bonds.

[0037] FIG. **3** illustrates the structure and function of the acetyl-CoA carboxylase (accABCD) enzyme complex.

[0038] FIG. **4** presents an overview of an exemplary biosynthetic pathway for production of fatty alcohol starting with acyl-ACP, where the production of fatty aldehyde is catalyzed by the enzymatic activity of acyl-ACP reductase (AAR) or thioesterase and carboxylic acid reductase (Car). The fatty aldehyde is converted to fatty alcohol by aldehyde reductase (also referred to as alcohol dehydrogenase).

[0039] FIG. **5** presents an overview of two exemplary biosynthetic pathways for production of fatty esters starting with acyl-ACP, where the production of fatty esters is accomplished by a one enzyme system or a three enzyme system.

[0040] FIG. **6** presents an overview of exemplary biosynthetic pathways for production of hydrocarbons starting with acyl-ACP, where the production of ketones is catalyzed by the enzymatic activity of OleA; the production of internal olefins is catalyzed by the enzymatic activity of OleABCD; the production of alkanes is catalyzed by the enzymatic conversion of fatty aldehydes to alkanes by way of aldehyde decarbonylase to; and the production of terminal olefins is catalyzed by the enzymatic conversion of fatty acids to terminal olefins by a decarboxylase

[0041] FIG. **7** illustrates fatty acid derivative ("Total Fatty Species") production by the MG1655 *E. coli* strain with the fadE gene attenuated (i.e., deleted) compared to fatty acid derivative production by *E. coli* MG1655. The data presented in FIG. **7** shows that attenuation of the fadE gene did not affect fatty acid derivative production.

[0042] FIG. **8** shows malonyl-CoA levels in DAM1_i377 in log phase expressing eight different *C. glutamicum* acetyl-CoA carboxylase (Acc) operon constructs.

[0043] FIG. **9** shows intracellular short chain-CoA levels in *E. coli* DAM1$_{\pm 377}$ in log phase expressing ptrc1/3_accDACB-birA±panK operon constructs. "accDACB+birA" is also referred to herein as "accD+".

[0044] FIG. **10** shows fatty acid methyl ester (FAME) production in *E. coli* strain DV2 expressing ester synthase 9 from *M. hydrocarbonoclasticus* and components of an acetyl-CoA carboxylase complex from *C. glutamicum.*

[0045] FIG. **11** shows production of fatty alcohols by *E. coli* expressing the *Synechococcus elongatus* PCC7942 AAR together with the accD+operon" from *C. glutamicum* on a pCL plasmid. Triplicate samples are shown for the accD+ strains.

[0046] FIGS. **12**A and B show data for production of "Total Fatty Species" (mg/L) from duplicate plate screens when plasmid pCL-WT TRC WT TesA was transformed into each of the iFAB-containing strains shown in the figures and a fermentation was run in FA2 media with 20 hours from induction to harvest at both 32° C. (FIG. **12**A) and 37° C. (FIG. **12**B).

[0047] FIG. **13** shows FAME production of *E. coli* DAM1 with plasmid pDS57 and integrated fabHI operons. The fabH/I genes are from *Marinobacter aquaeoli* VT8 or from *Acinetobacter baylyi* ADP1. See Table 7 for a more details on the fabH/I operons in these strains.

[0048] FIG. **14** shows FAME production of *E. coli* DAM 1 with plasmid pDS57 and different configurations of the *C. glutamicum* acc genes as well as integrated fabHI operons. The strains contain the fabH/I genes from *Rhodococcus opacus* or *Acinetobacter baylyi* ADP1. See Table 7 for more details on the fabH/I and acc operons.

[0049] FIG. **15** shows FAME and FFA titers of two *E. coli* DAM1 pDS57 strains with integrated fabH/I genes strains selected from FIG. **13** compared to the control strain *E. coli* DAM1 pDS57.

[0050] FIG. **16** is a diagrammatic depiction of the iFAB 138 locus, including a diagram of cat-loxP-T5 promoter integrated in front of FAB 138 (**16**A); and a diagram of iT5_138 (**16**B). The sequence of cat-loxP-T5 promoter integrated in front of FAB138 with 50 base pair of homology shown on each side of cat-loxP-T5 promoter region is provided as SEQ ID NO:1 and the sequence of the iT5_138 promoter region with 50 base pair homology on each side is provided as SEQ ID NO:2.

[0051] FIG. **17** shows that correcting the rph and ilvG genes in the EG149 strain allows for a higher level of FFA production than in the V668 strain where the rph and ilvG genes were not corrected.

[0052] FIG. **18** is a diagrammatic depiction of a transposon cassette insertion in the yijP gene of strain LC535 (transposon hit 68F11). Promoters internal to the transposon cassette are shown, and may have effects on adjacent gene expression.

[0053] FIG. **19** illustrates fatty alcohol production in *E. coli* DV2 expressing *Synechococcus elongatus* acyl-ACP reductase (AAR) and coexpressing various cyanobacterial acyl carrier proteins (ACPs). (Details regarding the source of the ACPs are provided in Table 13).

[0054] FIG. **20** illustrates fatty acid production in *E. coli* DV2 expressing leaderless *E. coli* thioesterase 'tesA and coexpressing a cyanobacterial acyl carrier protein (cACP) and *B. subtilis* sfp.

DETAILED DESCRIPTION OF THE INVENTION

[0055] The invention is based, at least in part, on the discovery that modification of various aspects of the fatty acid biosynthetic pathway in a recombinant host cell facilitates enhanced production of fatty acid derivatives by the host cell.

[0056] The invention relates to compositions of fatty acid derivatives having desired characteristics and methods for producing the same. Further, the invention relates to recombinant host cells (e.g., microorganisms), cultures of recombinant host cells, methods of making and using recombinant host cells, for example, use of cultured recombinant host cells in the fermentative production of fatty acid derivatives having desired characteristics.

[0057] All patents, publications, and patent applications cited in this specification are herein incorporated by reference as if each individual patent, publication, or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

DEFINITIONS

[0058] As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a recombinant host cell" includes two or more such recombinant host cells, reference to "a fatty alcohol" includes one or more fatty alcohols, or mixtures of fatty alcohols, reference to "a nucleic acid coding sequence" includes one or more nucleic acid coding sequences, reference to "an enzyme" includes one or more enzymes, and the like.

[0059] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although other methods and materials similar, or equivalent, to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

[0060] In describing and claiming the present invention, the following terminology will be used in accordance with the definitions set out below.

[0061] Accession Numbers: Sequence Accession numbers throughout this description were obtained from databases provided by the NCBI (National Center for Biotechnology Information) maintained by the National Institutes of Health, U.S.A. (which are identified herein as "NCBI Accession

Numbers" or alternatively as "GenBank Accession Numbers"), and from the UniProt Knowledgebase (UniProtKB) and Swiss-Prot databases provided by the Swiss Institute of Bioinformatics (which are identified herein as "UniProtKB Accession Numbers").

[0062] Enzyme Classification (EC) Numbers: EC numbers are established by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB), description of which is available on the IUBMB Enzyme Nomenclature website on the World Wide Web. EC numbers classify enzymes according to the reaction catalyzed.

[0063] As used herein, the term "nucleotide" refers to a monomeric unit of a polynucleotide that consists of a heterocyclic base, a sugar, and one or more phosphate groups. The naturally occurring bases (guanine, (G), adenine, (A), cytosine, (C), thymine, (T), and uracil (U)) are typically derivatives of purine or pyrimidine, though it should be understood that naturally and non-naturally occurring base analogs are also included. The naturally occurring sugar is the pentose (five-carbon sugar) deoxyribose (which forms DNA) or ribose (which forms RNA), though it should be understood that naturally and non-naturally occurring sugar analogs are also included. Nucleic acids are typically linked via phosphate bonds to form nucleic acids or polynucleotides, though many other linkages are known in the art (e.g., phosphorothioates, boranophosphates, and the like).

[0064] As used herein, the term "polynucleotide" refers to a polymer of ribonucleotides (RNA) or deoxyribonucleotides (DNA), which can be single-stranded or double-stranded and which can contain non-natural or altered nucleotides. The terms "polynucleotide," "nucleic acid sequence," and "nucleotide sequence" are used interchangeably herein to refer to a polymeric form of nucleotides of any length, either RNA or DNA. These terms refer to the primary structure of the molecule, and thus include double- and single-stranded DNA, and double- and single-stranded RNA. The terms include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs and modified polynucleotides such as, though not limited to methylated and/or capped polynucleotides. The polynucleotide can be in any form, including but not limited to, plasmid, viral, chromosomal, EST, cDNA, mRNA, and rRNA.

[0065] As used herein, the terms "polypeptide" and "protein" are used interchangeably to refer to a polymer of amino acid residues. The term "recombinant polypeptide" refers to a polypeptide that is produced by recombinant techniques, wherein generally DNA or RNA encoding the expressed protein is inserted into a suitable expression vector that is in turn used to transform a host cell to produce the polypeptide.

[0066] As used herein, the terms "homolog," and "homologous" refer to a polynucleotide or a polypeptide comprising a sequence that is at least about 50% identical to the corresponding polynucleotide or polypeptide sequence. Preferably homologous polynucleotides or polypeptides have polynucleotide sequences or amino acid sequences that have at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or at least about 99% homology to the corresponding amino acid sequence or polynucleotide sequence. As used herein the terms sequence "homology" and sequence "identity" are used interchangeably.

[0067] One of ordinary skill in the art is well aware of methods to determine homology between two or more sequences. Briefly, calculations of "homology" between two sequences can be performed as follows. The sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a first sequence that is aligned for comparison purposes is at least about 30%, preferably at least about 40%, more preferably at least about 50%, even more preferably at least about 60%, and even more preferably at least about 70%, at least about 80%, at least about 90%, or about 100% of the length of a second sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions of the first and second sequences are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent homology between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps and the length of each gap, that need to be introduced for optimal alignment of the two sequences.

[0068] The comparison of sequences and determination of percent homology between two sequences can be accomplished using a mathematical algorithm, such as BLAST (Altschul et al., J. Mol. Biol., 215(3): 403-410 (1990)). The percent homology between two amino acid sequences also can be determined using the Needleman and Wunsch algorithm that has been incorporated into the GAP program in the GCG software package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6 (Needleman and Wunsch, J. Mol. Biol., 48: 444-453 (1970)). The percent homology between two nucleotide sequences also can be determined using the GAP program in the GCG software package, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. One of ordinary skill in the art can perform initial homology calculations and adjust the algorithm parameters accordingly. A preferred set of parameters (and the one that should be used if a practitioner is uncertain about which parameters should be applied to determine if a molecule is within a homology limitation of the claims) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. Additional methods of sequence alignment are known in the biotechnology arts (see, e.g., Rosenberg, BMC Bioinformatics, 6: 278 (2005); Altschul, et al., FEBS J., 272(20): 5101-5109 (2005)).

[0069] 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 Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Aqueous and non-aqueous methods are described in that reference and either method can be used. Specific hybridization conditions referred to herein are as follows: 1) low stringency hybridization conditions—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); 2) medium stringency hybridization conditions—6×SSC at about 45° C., followed by one or more washes in 0.2×SSC, 0.1% SDS at

60° C.; 3) high stringency hybridization conditions—6×SSC at about 45° C., followed by one or more washes in 0.2.×SSC, 0.1% SDS at 65° C.; and 4) very high stringency hybridization conditions—0.5M sodium phosphate, 7% SDS at 65° C., followed by one or more washes at 0.2×SSC, 1% SDS at 65° C. Very high stringency conditions (4) are the preferred conditions unless otherwise specified.

[0070] An "endogenous" polypeptide refers to a polypeptide encoded by the genome of the parental microbial cell (also termed "host cell") from which the recombinant cell is engineered (or "derived").

[0071] An "exogenous" polypeptide refers to a polypeptide which is not encoded by the genome of the parental microbial cell. A variant (i.e., mutant) polypeptide is an example of an exogenous polypeptide.

[0072] The term "heterologous" as used herein typically refers to a nucleotide sequence or a protein not naturally present in an organism. For example, a polynucleotide sequence endogenous to a plant can be introduced into a host cell by recombinant methods, and the plant polynucleotide is then a heterologous polynucleotide in a recombinant host cell.

[0073] As used herein, the term "fragment" of a polypeptide refers to a shorter portion of a full-length polypeptide or protein ranging in size from four amino acid residues to the entire amino acid sequence minus one amino acid residue. In certain embodiments of the invention, a fragment refers to the entire amino acid sequence of a domain of a polypeptide or protein (e.g., a substrate binding domain or a catalytic domain).

[0074] As used herein, the term "mutagenesis" refers to a process by which the genetic information of an organism is changed in a stable manner. Mutagenesis of a protein coding nucleic acid sequence produces a mutant protein. Mutagenesis also refers to changes in non-coding nucleic acid sequences that result in modified protein activity.

[0075] As used herein, the term "gene" refers to nucleic acid sequences encoding either an RNA product or a protein product, as well as operably-linked nucleic acid sequences affecting the expression of the RNA or protein (e.g., such sequences include but are not limited to promoter or enhancer sequences) or operably-linked nucleic acid sequences encoding sequences that affect the expression of the RNA or protein (e.g., such sequences include but are not limited to ribosome binding sites or translational control sequences).

[0076] Expression control sequences are known in the art and include, for example, promoters, enhancers, polyadenylation signals, transcription terminators, internal ribosome entry sites (IRES), and the like, that provide for the expression of the polynucleotide sequence in a host cell. Expression control sequences interact specifically with cellular proteins involved in transcription (Maniatis et al., Science, 236: 1237-1245 (1987)). Exemplary expression control sequences are described in, for example, Goeddel, Gene Expression Technology: Methods in Enzymology, Vol. 185, Academic Press, San Diego, Calif. (1990).

[0077] In the methods of the invention, an expression control sequence is operably linked to a polynucleotide sequence. By "operably linked" is meant that a polynucleotide sequence and an expression control sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the expression control sequence(s). Operably linked promoters are located upstream of the selected polynucleotide sequence in terms of the direction of transcription and translation. Operably linked enhancers can be located upstream, within, or downstream of the selected polynucleotide.

[0078] As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid, i.e., a polynucleotide sequence, to which it has been linked One type of useful vector is an episome (i.e., a nucleic acid capable of extra-chromosomal replication). Useful vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors." In general, expression vectors of utility in recombinant DNA techniques are often in the form of "plasmids," which refer generally to circular double stranded DNA loops that, in their vector form, are not bound to the chromosome. The terms "plasmid" and "vector" are used interchangeably herein, in as much as a plasmid is the most commonly used form of vector. However, also included are such other forms of expression vectors that serve equivalent functions and that become known in the art subsequently hereto.

[0079] In some embodiments, a recombinant vector further comprises a promoter operably linked to the polynucleotide sequence. In some embodiments, the promoter is a developmentally-regulated, an organelle-specific, a tissue-specific, an inducible, a constitutive, or a cell-specific promoter. The recombinant vector typically comprises at least one sequence selected from the group consisting of (a) an expression control sequence operatively coupled to the polynucleotide sequence; (b) a selection marker operatively coupled to the polynucleotide sequence; (c) a marker sequence operatively coupled to the polynucleotide sequence; (d) a purification moiety operatively coupled to the polynucleotide sequence; (e) a secretion sequence operatively coupled to the polynucleotide sequence; and (f) a targeting sequence operatively coupled to the polynucleotide sequence. In certain embodiments, the nucleotide sequence is stably incorporated into the genomic DNA of the host cell, and the expression of the nucleotide sequence is under the control of a regulated promoter region.

[0080] The expression vectors described herein include a polynucleotide sequence described herein in a form suitable for expression of the polynucleotide sequence in a host cell. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of polypeptide desired, etc. The expression vectors described herein can be introduced into host cells to produce polypeptides, including fusion polypeptides, encoded by the polynucleotide sequences as described herein. Expression of genes encoding polypeptides in prokaryotes, for example, *E. coli*, is most often carried out with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion polypeptides. Fusion vectors add a number of amino acids to a polypeptide encoded therein, usually to the amino- or carboxy-terminus of the recombinant polypeptide. Such fusion vectors typically serve one or more of the following three purposes: (1) to increase expression of the recombinant polypeptide; (2) to increase the solubility of the recombinant polypeptide; and (3) to aid in the purification of the recombinant polypeptide by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant polypeptide. This enables

separation of the recombinant polypeptide from the fusion moiety after purification of the fusion polypeptide. In certain embodiments, a polynucleotide sequence of the invention is operably linked to a promoter derived from bacteriophage T5.

[0081] In certain embodiments, the host cell is a yeast cell, and the expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari et al., EMBO J., 6: 229-234 (1987)), pMFa (Kurjan et al., Cell, 30: 933-943 (1982)), pJRY88 (Schultz et al., Gene, 54: 113-123 (1987)), pYES2 (Invitrogen Corp., San Diego, Calif.), and picZ (Invitrogen Corp., San Diego, Calif.).

[0082] In other embodiments, the host cell is an insect cell, and the expression vector is a baculovirus expression vector. Baculovirus vectors available for expression of proteins in cultured insect cells (e.g., Sf9 cells) include, for example, the pAc series (Smith et al., Mol. Cell. Biol., 3: 2156-2165 (1983)) and the pVL series (Lucklow et al., Virology, 170: 31-39 (1989)).

[0083] In yet another embodiment, the polynucleotide sequences described herein can be expressed in mammalian cells using a mammalian expression vector. Other suitable expression systems for both prokaryotic and eukaryotic cells are well known in the art; see, e.g., Sambrook et al., "Molecular Cloning: A Laboratory Manual," second edition, Cold Spring Harbor Laboratory, (1989).

[0084] As used herein "acyl-CoA" refers to an acyl thioester formed between the carbonyl carbon of alkyl chain and the sulfhydryl group of the 4'-phosphopantethionyl moiety of coenzyme A (CoA), which has the formula R—C(O)S—CoA, where R is any alkyl group having at least 4 carbon atoms.

[0085] As used herein "acyl-ACP" refers to an acyl thioester formed between the carbonyl carbon of alkyl chain and the sulfhydryl group of the phosphopantetheinyl moiety of an acyl carrier protein (ACP). The phosphopantetheinyl moiety is post-translationally attached to a conserved serine residue on the ACP by the action of holo-acyl carrier protein synthase (ACPS), a phosphopantetheinyl transferase. In some embodiments an acyl-ACP is an intermediate in the synthesis of fully saturated acyl-ACPs. In other embodiments an acyl-ACP is an intermediate in the synthesis of unsaturated acyl-ACPs. In some embodiments, the carbon chain will have about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or 26 carbons. Each of these acyl-ACPs are substrates for enzymes that convert them to fatty acid derivatives such as those described in FIGS. **4-6**

[0086] As used herein, the term "fatty acid derivative" means a "fatty acid" or a "fatty acid derivative", which may be referred to as a "fatty acid or derivative thereof". The term "fatty acid" means a carboxylic acid having the formula RCOOH. R represents an aliphatic group, preferably an alkyl group. R can comprise between about 4 and about 22 carbon atoms. Fatty acids can be saturated, monounsaturated, or polyunsaturated. A "fatty acid derivative" is a product made in part from the fatty acid biosynthetic pathway of the production host organism. "Fatty acid derivatives" includes products made in part from acyl-ACP or acyl-ACP derivatives. Exemplary fatty acid derivatives include, for example, acyl-CoA, fatty acids, fatty aldehydes, short and long chain alcohols, fatty alcohols, hydrocarbons, esters (e.g., waxes, fatty acid esters, or fatty esters), terminal olefins, internal olefins, and ketones.

[0087] A "fatty acid derivative composition" as referred to herein is produced by a recombinant host cell and typically comprises a mixture of fatty acid derivative. In some cases, the mixture includes more than one type of product (e.g., fatty acids and fatty alcohols, fatty acids and fatty acid esters or alkanes and olefins). In other cases, the fatty acid derivative compositions may comprise, for example, a mixture of fatty alcohols (or another fatty acid derivative) with various chain lengths and saturation or branching characteristics. In still other cases, the fatty acid derivative composition comprises a mixture of both more than one type of product and products with various chain lengths and saturation or branching characteristics.

[0088] As used herein, the term "fatty acid biosynthetic pathway" means a biosynthetic pathway that produces fatty acids and derivatives thereof. The fatty acid biosynthetic pathway may include additional enzymes to produce fatty acids derivatives having desired characteristics.

[0089] As used herein, "fatty aldehyde" means an aldehyde having the formula RCHO characterized by a carbonyl group (C═O). In some embodiments, the fatty aldehyde is any aldehyde made from a fatty alcohol. In certain embodiments, the R group is at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, or at least 19, carbons in length. Alternatively, or in addition, the R group is 20 or less, 19 or less, 18 or less, 17 or less, 16 or less, 15 or less, 14 or less, 13 or less, 12 or less, 11 or less, 10 or less, 9 or less, 8 or less, 7 or less, or 6 or less carbons in length. Thus, the R group can have an R group bounded by any two of the above endpoints. For example, the R group can be 6-16 carbons in length, 10-14 carbons in length, or 12-18 carbons in length. In some embodiments, the fatty aldehyde is a C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17, C18, C19, C20, C21, C22, C23, C24, C25, or a C26 fatty aldehyde. In certain embodiments, the fatty aldehyde is a C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17, or C18 fatty aldehyde.

[0090] As used herein, "fatty alcohol" means an alcohol having the formula ROH. In some embodiments, the R group is at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, or at least 19, carbons in length. Alternatively, or in addition, the R group is 20 or less, 19 or less, 18 or less, 17 or less, 16 or less, 15 or less, 14 or less, 13 or less, 12 or less, 11 or less, 10 or less, 9 or less, 8 or less, 7 or less, or 6 or less carbons in length. Thus, the R group can have an R group bounded by any two of the above endpoints. For example, the R group can be 6-16 carbons in length, 10-14 carbons in length, or 12-18 carbons in length. In some embodiments, the fatty alcohol is a C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17, C18, C19, C20, C21, C22, C23, C24, C25, or a C26 fatty alcohol. In certain embodiments, the fatty alcohol is a C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17, or C18 fatty alcohol.

[0091] The R group of a fatty acid derivative, for example a fatty alcohol, can be a straight chain or a branched chain. Branched chains may have more than one point of branching and may include cyclic branches. In some embodiments, the branched fatty acid, branched fatty aldehyde, or branched fatty alcohol is a C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17, C18, C19, C20, C21, C22, C23, C24, C25, or a C_{2-6} branched fatty acid, branched fatty aldehyde, or branched fatty alcohol. In particular embodiments, the

branched fatty acid, branched fatty aldehyde, or branched fatty alcohol is a C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16, C17, or C18 branched fatty acid, branched fatty aldehyde, or branched fatty alcohol. In certain embodiments, the hydroxyl group of the branched fatty acid, branched fatty aldehyde, or branched fatty alcohol is in the primary (C1) position.

[0092] In certain embodiments, the branched fatty acid derivative is an iso-fatty acid derivative, for example an iso-fatty aldehyde, an iso-fatty alcohol, or an antesio-fatty acid derivative, an anteiso-fatty aldehyde, or an anteiso-fatty alcohol. In exemplary embodiments, the branched fatty acid derivative is selected from iso-C7:0, iso-C8:0, iso-C9:0, iso-C10:0, iso-C11:0, iso-C12:0, iso-C13:0, iso-C14:0, iso-C15:0, iso-C16:0, iso-C17:0, iso-C18:0, iso-C19:0, anteiso-C7:0, anteiso-C8:0, anteiso-C9:0, anteiso-C10:0, anteiso-C11:0, anteiso-C12:0, anteiso-C13:0, anteiso-C14:0, anteiso-C15:0, anteiso-C16:0, anteiso-C17:0, anteiso-C18:0, and an anteiso-C19:0 branched fatty alcohol.

[0093] The R group of a branched or unbranched fatty acid derivative can be saturated or unsaturated. If unsaturated, the R group can have one or more than one point of unsaturation. In some embodiments, the unsaturated fatty acid derivative is a monounsaturated fatty acid derivative. In certain embodiments, the unsaturated fatty acid derivative is a C6:1, C7:1, C8:1, C9:1, C10:1, C11:1, C12:1, C13:1, C14:1, C15:1, C16:1, C17:1, C18:1, C19:1, C20:1, C21:1, C22:1, C23:1, C24:1, C25:1, or a C26:1 unsaturated fatty acid derivative. In certain embodiments, the unsaturated fatty acid derivative, is a C10:1, C12:1, C14:1, C16:1, or C18:1 unsaturated fatty acid derivative. In other embodiments, the unsaturated fatty acid derivative is unsaturated at the omega-7 position. In certain embodiments, the unsaturated fatty acid derivative comprises a cis double bond.

[0094] As used herein, a recombinant or engineered "host cell" is a host cell, e.g., a microorganism used to produce one or more of fatty acid derivatives include, for example, acyl-CoA, fatty acids, fatty aldehydes, short and long chain alcohols, hydrocarbons, fatty alcohols, esters (e.g., waxes, fatty acid esters, or fatty esters), terminal olefins, internal olefins, and ketones.

[0095] In some embodiments, the recombinant host cell comprises one or more polynucleotides, each polynucleotide encoding a polypeptide having fatty acid biosynthetic enzyme activity, wherein the recombinant host cell produces a fatty acid derivative composition when cultured in the presence of a carbon source under conditions effective to express the polynucleotides.

[0096] As used herein, the term "clone" typically refers to a cell or group of cells descended from and essentially genetically identical to a single common ancestor, for example, the bacteria of a cloned bacterial colony arose from a single bacterial cell.

[0097] As used herein, the term "culture" typical refers to a liquid media comprising viable cells. In one embodiment, a culture comprises cells reproducing in a predetermined culture media under controlled conditions, for example, a culture of recombinant host cells grown in liquid media comprising a selected carbon source and nitrogen.

[0098] "Culturing" or "cultivation" refers to growing a population of recombinant host cells under suitable conditions in a liquid or solid medium. In particular embodiments, culturing refers to the fermentative bioconversion of a substrate to an end-product. Culturing media are well known and individual components of such culture media are available from commercial sources, e.g., under the Difco™ and BBL™ trademarks. In one non-limiting example, the aqueous nutrient medium is a "rich medium" comprising complex sources of nitrogen, salts, and carbon, such as YP medium, comprising 10 g/L of peptone and 10 g/L yeast extract of such a medium.

[0099] The host cell can be additionally engineered to assimilate carbon efficiently and use cellulosic materials as carbon sources according to methods described in U.S. Pat. Nos. 5,000,000; 5,028,539; 5,424,202; 5,482,846; 5,602,030; WO 2010127318. In addition, in some embodiments the host cell is engineered to express an invertase so that sucrose can be used as a carbon source.

[0100] As used herein, the term "under conditions effective to express said heterologous nucleotide sequence(s)" means any conditions that allow a host cell to produce a desired fatty acid derivative. Suitable conditions include, for example, fermentation conditions.

[0101] As used herein, "modified" or an "altered level of" activity of a protein, for example an enzyme, in a recombinant host cell refers to a difference in one or more characteristics in the activity determined relative to the parent or native host cell. Typically differences in activity are determined between a recombinant host cell, having modified activity, and the corresponding wild-type host cell (e.g., comparison of a culture of a recombinant host cell relative to the corresponding wild-type host cell). Modified activities can be the result of, for example, modified amounts of protein expressed by a recombinant host cell (e.g., as the result of increased or decreased number of copies of DNA sequences encoding the protein, increased or decreased number of mRNA transcripts encoding the protein, and/or increased or decreased amounts of protein translation of the protein from mRNA); changes in the structure of the protein (e.g., changes to the primary structure, such as, changes to the protein's coding sequence that result in changes in substrate specificity, changes in observed kinetic parameters); and changes in protein stability (e.g., increased or decreased degradation of the protein). In some embodiments, the polypeptide is a mutant or a variant of any of the polypeptides described herein. In certain instances, the coding sequence for the polypeptides described herein are codon optimized for expression in a particular host cell. For example, for expression in *E. coli*, one or more codons can be optimized as described in, e.g., Grosjean et al., Gene 18:199-209 (1982).

[0102] The term "regulatory sequences" as used herein typically refers to a sequence of bases in DNA, operably-linked to DNA sequences encoding a protein that ultimately controls the expression of the protein. Examples of regulatory sequences include, but are not limited to, RNA promoter sequences, transcription factor binding sequences, transcription termination sequences, modulators of transcription (such as enhancer elements), nucleotide sequences that affect RNA stability, and translational regulatory sequences (such as, ribosome binding sites (e.g., Shine-Dalgarno sequences in prokaryotes or Kozak sequences in eukaryotes), initiation codons, termination codons).

[0103] As used herein, the phrase "the expression of said nucleotide sequence is modified relative to the wild type nucleotide sequence," means an increase or decrease in the level of expression and/or activity of an endogenous nucle-

otide sequence or the expression and/or activity of a heterologous or non-native polypeptide-encoding nucleotide sequence.

[0104] As used herein, the term "express" with respect to a polynucleotide is to cause it to function. A polynucleotide which encodes a polypeptide (or protein) will, when expressed, be transcribed and translated to produce that polypeptide (or protein). As used herein, the term "overexpress" means to express or cause to be expressed a polynucleotide or polypeptide in a cell at a greater concentration than is normally expressed in a corresponding wild-type cell under the same conditions.

[0105] The terms "altered level of expression" and "modified level of expression" are used interchangeably and mean that a polynucleotide, polypeptide, or hydrocarbon is present in a different concentration in an engineered host cell as compared to its concentration in a corresponding wild-type cell under the same conditions.

[0106] As used herein, the term "titer" refers to the quantity of fatty acid derivative produced per unit volume of host cell culture. In any aspect of the compositions and methods described herein, a fatty acid derivative is produced at a titer of about 25 mg/L, about 50 mg/L, about 75 mg/L, about 100 mg/L, about 125 mg/L, about 150 mg/L, about 175 mg/L, about 200 mg/L, about 225 mg/L, about 250 mg/L, about 275 mg/L, about 300 mg/L, about 325 mg/L, about 350 mg/L, about 375 mg/L, about 400 mg/L, about 425 mg/L, about 450 mg/L, about 475 mg/L, about 500 mg/L, about 525 mg/L, about 550 mg/L, about 575 mg/L, about 600 mg/L, about 625 mg/L, about 650 mg/L, about 675 mg/L, about 700 mg/L, about 725 mg/L, about 750 mg/L, about 775 mg/L, about 800 mg/L, about 825 mg/L, about 850 mg/L, about 875 mg/L, about 900 mg/L, about 925 mg/L, about 950 mg/L, about 975 mg/L, about 1000 mg/L, about 1050 mg/L, about 1075 mg/L, about 1100 mg/L, about 1125 mg/L, about 1150 mg/L, about 1175 mg/L, about 1200 mg/L, about 1225 mg/L, about 1250 mg/L, about 1275 mg/L, about 1300 mg/L, about 1325 mg/L, about 1350 mg/L, about 1375 mg/L, about 1400 mg/L, about 1425 mg/L, about 1450 mg/L, about 1475 mg/L, about 1500 mg/L, about 1525 mg/L, about 1550 mg/L, about 1575 mg/L, about 1600 mg/L, about 1625 mg/L, about 1650 mg/L, about 1675 mg/L, about 1700 mg/L, about 1725 mg/L, about 1750 mg/L, about 1775 mg/L, about 1800 mg/L, about 1825 mg/L, about 1850 mg/L, about 1875 mg/L, about 1900 mg/L, about 1925 mg/L, about 1950 mg/L, about 1975 mg/L, about 2000 mg/L (2 g/L), 3 g/L, 5 g/L, 10 g/L, 20 g/L, 30 g/L, 40 g/L, 50 g/L, 60 g/L, 70 g/L, 80 g/L, 90 g/L, 100 g/L or a range bounded by any two of the foregoing values. In other embodiments, a fatty acid derivative is produced at a titer of more than 100 g/L, more than 200 g/L, more than 300 g/L, or higher, such as 500 g/L, 700 g/L, 1000 g/L, 1200 g/L, 1500 g/L, or 2000 g/L. The preferred titer of fatty acid derivative produced by a recombinant host cell according to the methods of the invention is from 5 g/L to 200 g/L, 10 g/L to 150 g/L, 20 g/L to 120 g/L and 30 g/L to 100 g/L. The titer may refer to a particular fatty acid derivative or a combination of fatty acid derivatives produced by a given recombinant host cell culture.

[0107] As used herein, the "yield of fatty acid derivative produced by a host cell" refers to the efficiency by which an input carbon source is converted to product (i.e., fatty alcohol or fatty aldehyde) in a host cell. Host cells engineered to produce fatty acid derivatives according to the methods of the invention have a yield of at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 21%, at least 22%, at least 23%, at least 24%, at least 25%, at least 26%, at least 27%, at least 28%, at least 29%, or at least 30% or a range bounded by any two of the foregoing values. In other embodiments, a fatty acid derivative or derivatives is produced at a yield of more than 30%, 40%, 50%, 60%, 70%, 80%, 90% or more. Alternatively, or in addition, the yield is about 30% or less, about 27% or less, about 25% or less, or about 22% or less. Thus, the yield can be bounded by any two of the above endpoints. For example, the yield of a fatty acid derivative or derivatives produced by the recombinant host cell according to the methods of the invention can be 5% to 15%, 10% to 25%, 10% to 22%, 15% to 27%, 18% to 22%, 20% to 28%, or 20% to 30%. The yield may refer to a particular fatty acid derivative or a combination of fatty acid derivatives produced by a given recombinant host cell culture.

[0108] As used herein, the term "productivity" refers to the quantity of a fatty acid derivative or derivatives produced per unit volume of host cell culture per unit time. In any aspect of the compositions and methods described herein, the productivity of a fatty acid derivative or derivatives produced by a recombinant host cell is at least 100 mg/L/hour, at least 200 mg/L/hour, at least 300 mg/L/hour, at least 400 mg/L/hour, at least 500 mg/L/hour, at least 600 mg/L/hour, at least 700 mg/L/hour, at least 800 mg/L/hour, at least 900 mg/L/hour, at least 1000 mg/L/hour, at least 1100 mg/L/hour, at least 1200 mg/L/hour, at least 1300 mg/L/hour, at least 1400 mg/L/hour, at least 1500 mg/L/hour, at least 1600 mg/L/hour, at least 1700 mg/L/hour, at least 1800 mg/L/hour, at least 1900 mg/L/hour, at least 2000 mg/L/hour, at least 2100 mg/L/hour, at least 2200 mg/L/hour, at least 2300 mg/L/hour, at least 2400 mg/L/hour, or at least 2500 mg/L/hour. For example, the productivity of a fatty acid derivative or derivatives produced by a recombinant host cell according to the methods of the may be from 500 mg/L/hour to 2500 mg/L/hour, or from 700 mg/L/hour to 2000 mg/L/hour. The productivity may refer to a particular fatty acid derivative or a combination of fatty acid derivatives produced by a given recombinant host cell culture.

[0109] As used herein, the term "total fatty species" and "total fatty acid product" may be used interchangeably herein with reference to the amount of fatty alcohols, fatty aldehydes and fatty acids, as evaluated by GC-FID as described in International Patent Application Publication WO 2008/119082. The same terms may be used to mean fatty esters and free fatty acids when referring to a fatty ester analysis.

[0110] As used herein, the term "glucose utilization rate" means the amount of glucose used by the culture per unit time, reported as grams/liter/hour (g/L/hr).

[0111] As used herein, the term "carbon source" refers to a substrate or compound suitable to be used as a source of carbon for prokaryotic or simple eukaryotic cell growth. Carbon sources can be in various forms, including, but not limited to polymers, carbohydrates, acids, alcohols, aldehydes, ketones, amino acids, peptides, and gases (e.g., CO and CO_2). Exemplary carbon sources include, but are not limited to, monosaccharides, such as glucose, fructose, mannose, galactose, xylose, and arabinose; oligosaccharides, such as fructo-oligosaccharide and galacto-oligosaccharide; polysaccharides such as starch, cellulose, pectin, and xylan; disaccharides, such as sucrose, maltose, cellobiose, and turanose; cellulosic material and variants such as hemicelluloses, methyl cellulose and sodium carboxymethyl cellulose; satu-

rated or unsaturated fatty acids, succinate, lactate, and acetate; alcohols, such as ethanol, methanol, and glycerol, or mixtures thereof. The carbon source can also be a product of photosynthesis, such as glucose. In certain preferred embodiments, the carbon source is biomass. In other preferred embodiments, the carbon source is glucose. In other preferred embodiments the carbon source is sucrose.

[0112] As used herein, the term "biomass" refers to any biological material from which a carbon source is derived. In some embodiments, a biomass is processed into a carbon source, which is suitable for bioconversion. In other embodiments, the biomass does not require further processing into a carbon source. The carbon source can be converted into a biofuel. An exemplary source of biomass is plant matter or vegetation, such as corn, sugar cane, or switchgrass. Another exemplary source of biomass is metabolic waste products, such as animal matter (e.g., cow manure). Further exemplary sources of biomass include algae and other marine plants. Biomass also includes waste products from industry, agriculture, forestry, and households, including, but not limited to, fermentation waste, ensilage, straw, lumber, sewage, garbage, cellulosic urban waste, and food leftovers. The term "biomass" also can refer to sources of carbon, such as carbohydrates (e.g., monosaccharides, disaccharides, or polysaccharides).

[0113] As used herein, the term "isolated," with respect to products (such as fatty acids and derivatives thereof) refers to products that are separated from cellular components, cell culture media, or chemical or synthetic precursors. The fatty acids and derivatives thereof produced by the methods described herein can be relatively immiscible in the fermentation broth, as well as in the cytoplasm. Therefore, the fatty acids and derivatives thereof can collect in an organic phase either intracellularly or extracellularly.

[0114] As used herein, the terms "purify," "purified," or "purification" mean the removal or isolation of a molecule from its environment by, for example, isolation or separation. "Substantially purified" molecules are at least about 60% free (e.g., at least about 70% free, at least about 75% free, at least about 85% free, at least about 90% free, at least about 95% free, at least about 97% free, at least about 99% free) from other components with which they are associated. As used herein, these terms also refer to the removal of contaminants from a sample. For example, the removal of contaminants can result in an increase in the percentage of fatty acid derivatives in a sample. For example, when a fatty acid derivative is produced in a recombinant host cell, the fatty acid derivative can be purified by the removal of host cell proteins. After purification, the percentage of fatty acid derivative in the sample is increased. The terms "purify," "purified," and "purification" are relative terms which do not require absolute purity. Thus, for example, when a fatty acid derivative is produced in recombinant host cells, a purified fatty acid derivative is a fatty acid derivative that is substantially separated from other cellular components (e.g., nucleic acids, polypeptides, lipids, carbohydrates, or other hydrocarbons).

General Overview of the Invention

[0115] In the compositions and methods of the invention, the production of a desired fatty acid derivative composition (e.g., acyl-CoA, fatty acids, fatty aldehydes, short and long chain alcohols, hydrocarbons, fatty alcohols, esters (e.g., waxes, fatty acid esters, or fatty esters), terminal olefins, internal olefins, and ketones is enhanced by modifying the expression of one or more genes involved in a biosynthetic pathway for fatty acid, fatty ester, alkane, alkene, olefin, or fatty alcohol, production, degradation and/or secretion.

[0116] The invention provides recombinant host cells which have been engineered to provide enhanced fatty acid biosynthesis relative to non-engineered or native host cells (for example by strain improvements, as further described herein below).

[0117] The disclosure identifies polynucleotides useful in the recombinant host cells, methods, and compositions of the invention; however it will be recognized that absolute sequence identity to such polynucleotides is not necessary. For example, changes in a particular polynucleotide sequence can be made and the encoded polypeptide screened for activity. Such changes typically comprise conservative mutations and silent mutations (such as, for example, codon optimization). Modified or mutated (i.e., mutant) polynucleotides and encoded variant polypeptides can be screened for a desired function, such as, an improved function compared to the parent polypeptide, including but not limited to increased catalytic activity, increased stability, or decreased inhibition (e.g., decreased feedback inhibition), using methods known in the art. The disclosure identifies enzymatic activities involved in various steps (i.e., reactions) of the fatty acid biosynthetic pathways described herein according to Enzyme Classification (EC) number, and provides exemplary polypeptides (i.e., enzymes) categorized by such EC numbers, and exemplary polynucleotides encoding such polypeptides. Such exemplary polypeptides and polynucleotides, which are identified herein by Accession Numbers and/or Sequence Identifier Numbers (SEQ ID NOs), are useful for engineering fatty acid pathways in parental host cells to obtain the recombinant host cells described herein. It is to be understood, however, that polypeptides and polynucleotides described herein are exemplary and non-limiting. The sequences of homologues of exemplary polypeptides described herein are available to those of skill in the art using databases such as, for example, the Entrez databases provided by the National Center for Biotechnology Information (NCBI), the ExPasy databases provided by the Swiss Institute of Bioinformatics, the BRENDA database provided by the Technical University of Braunschweig, and the KEGG database provided by the Bioinformatics Center of Kyoto University and University of Tokyo, all which are available on the World Wide Web.

[0118] A variety of host cells can be modified to contain a fatty acid biosynthetic pathway such as those described herein, resulting in recombinant host cells suitable for the production of fatty acid derivatives. It is understood that a variety of cells can provide sources of genetic material, including polynucleotide sequences that encode polypeptides suitable for use in a recombinant host cell provided herein.

Strain Improvements

[0119] In order generate a high titer, yield, and productivity of fatty acid derivatives, a number of modifications were made to the production host cells.

[0120] FadR is a key regulatory factor involved in fatty acid degradation and fatty acid biosynthetic pathways (Cronan et al., Mol. Microbiol., 29(4): 937-943 (1998)). The *E. coli* ACS enzyme FadD and the fatty acid transport protein FadL are essential components of a fatty acid uptake system. FadL mediates transport of fatty acids into the bacterial cell, and FadD mediates formation of acyl-CoA esters. When no other carbon source is available, exogenous fatty acids are taken up

by bacteria and converted to acyl-CoA esters, which can bind to the transcription factor FadR and derepress the expression of the fad genes that encode proteins responsible for fatty acid transport (FadL), activation (FadD), and β-oxidation (FadA, FadB, FadE, and FadH). When alternative sources of carbon are available, bacteria synthesize fatty acids as acyl-ACPs, which are used for phospholipid synthesis, but are not substrates for β-oxidation. Thus, acyl-CoA and acyl-ACP are both independent sources of fatty acids can result in different end-products (Caviglia et al., J. Biol. Chem., 279(12): 1163-1169 (2004)). U.S. Provisional Application No. 61/470,989 describes improved methods of producing fatty acid derivatives in a host cell which is genetically engineered to have an altered level of expression of a FadR polypeptide as compared to the level of expression of the FadR polypeptide in a corresponding wild-type host cell.

[0121] There are conflicting reports in the literature as to factors that can limit fatty acid biosynthesis in host cells, such as *E. coli*. One suggestion is that a limitation of the main precursors for fatty acid biosynthesis, for example, acetyl-CoA and malonyl-CoA can result in decreased synthesis of fatty acid derivatives. One approach to increasing the flux through fatty acid biosynthesis is to manipulate various enzymes in the pathway (FIGS. **1-2**). Example 2 describes studies which show construction of fab operons that encode enzymes in the biosynthetic pathway for conversion of malonyl-CoA into acyl-ACPs and integration into the chromosome of an *E. coli* host cell as a means to increase the flux of fatty acid biosynthesis.

[0122] The supply of acyl-ACPs from acetyl-CoA via the acetyl-CoA carboxylase (acc) complex and fatty acid biosynthetic (fab) pathway is another step that may limit the rate of fatty acid derivative production (FIG. **3**). In a study detailed in Example 3, the effect of overexpression of an optimized version of *E. coli Corynebacterium glutamicum* accABCD (±birA) demonstrated that such genetic modifications can lead to increased production of acetyl-coA and malonyl-CoA in *E. coli*.

[0123] In yet another approach, mutations in the rph and ilvG genes in the *E. coli* host cell were shown to result in higher free fatty acid (FFA) production, which translated into higher production of fatty alcohol. See Example 4.

[0124] In still another approach, transposon mutagenesis and high-throughput screening was carried out to find beneficial mutations that increase the titer or yield. Example 5 describes studies where it was observed that a transposon insertion in the yijP gene can improve the fatty alcohol yield in shake flask and fed-batch fermentations.

Generation of Fatty Acid Derivative by Recombinant Host Cells

[0125] This disclosure provides numerous examples of polypeptides (i.e., enzymes) having activities suitable for use in the fatty acid biosynthetic pathways described herein. Such polypeptides are collectively referred to herein as "fatty acid biosynthetic polypeptides" or "fatty acid biosynthetic enzymes". Non-limiting examples of fatty acid pathway polypeptides suitable for use in recombinant host cells of the invention are provided herein.

[0126] In some embodiments, the invention includes a recombinant host cell comprising a polynucleotide sequence (also referred to herein as a "fatty acid biosynthetic polynucleotide" sequence) which encodes a fatty acid biosynthetic polypeptide.

[0127] The polynucleotide sequence, which comprises an open reading frame encoding a fatty acid biosynthetic polypeptide and operably-linked regulatory sequences, can be integrated into a chromosome of the recombinant host cells, incorporated in one or more plasmid expression systems resident in the recombinant host cell, or both. In the Examples, both plasmid expression systems and integration into the host genome are used to illustrate different embodiments of the present invention.

[0128] In some embodiments, a fatty acid biosynthetic polynucleotide sequence encodes a polypeptide which is endogenous to the parental host cell of the recombinant cell being engineered. Some such endogenous polypeptides are overexpressed in the recombinant host cell. An "endogenous polypeptide", as used herein, refers to a polypeptide which is encoded by the genome of the parental (e.g., wild-type) cell that is engineered to produce the recombinant host cell.

[0129] In some embodiments, the fatty acid biosynthetic polynucleotide sequence encodes an exogenous or heterologous polypeptide. In other words, the polypeptide encoded by the polynucleotide is exogenous to the parental host cell. An "exogenous" (or "heterologous") polypeptide, as used herein, refers to a polypeptide not encoded by the genome of the parental (e.g., wild-type) host cell that is being engineered to produce the recombinant host cell. Such a polypeptide can also be referred to as a "non-native" polypeptide. A variant (that is, a mutant) polypeptide is an example of a heterologous polypeptide.

[0130] In certain embodiments, the genetically modified host cell overexpresses a gene encoding a polypeptide (protein) that increases the rate at which the host cell produces the substrate of a fatty acid biosynthetic enzyme, i.e., a fatty acyl-thioester substrate. In certain embodiments, the enzyme encoded by the over expressed gene is directly involved in fatty acid biosynthesis.

[0131] Such recombinant host cells may be further engineered to comprise a polynucleotide sequence encoding one or more "fatty acid biosynthetic polypeptides", (enzymes involved in fatty acid biosynthesis), for example, a polypeptide:

[0132] (1) having thioesterase activity, wherein the recombinant host cell synthesizes fatty acids;

[0133] (2) having thioesterase activity and carboxylic acid reductase ("CAR") activity, wherein the recombinant host cell synthesizes fatty aldehydes and fatty alcohols;

[0134] (3) having thioesterase activity, carboxylic acid reductase activity and alcohol dehydrogenase activity wherein the recombinant host cell synthesizes fatty alcohols;

[0135] (4) having acyl-CoA reductase ("AAR") activity wherein the recombinant host cell synthesizes fatty aldehydes and fatty alcohols;

[0136] (5) having acyl-CoA reductase ("AAR") activity and alcohol dehydrogenase activity wherein the recombinant host cell synthesizes fatty alcohols;

[0137] (6) having fatty alcohol forming acyl-CoA reductase ("FAR") activity, wherein the recombinant host cell synthesizes fatty alcohols;

[0138] (7) having thioesterase activity, carboxylic acid reductase activity and aldehyde decarbonylase activity, wherein the recombinant host cell synthesizes alkanes;

[0139] (8) having acyl-CoA reductase activity and aldehyde decarbonylase activity, wherein the recombinant host cell synthesizes alkanes

[0140] (9) having ester synthase activity wherein the recombinant host cell synthesizes fatty esters ("one enzyme system"; FIG. **5**);

[0141] (10) having thioesterase activity, acyl-CoA synthase activity and ester synthase activity wherein the recombinant host cell synthesizes fatty esters ("three enzyme system"; FIG. **5**);

[0142] (11) having OleA activity, wherein the recombinant host cell synthesizes aliphatic ketones;

[0143] (12) having OleABCD activity, wherein the recombinant host cell synthesizes internal olefins, or

[0144] (13) having thioesterase activity and decarboxylase activity, wherein the recombinant host cell synthesizes terminal olefins.

[0145] In some embodiments, at least one polypeptide encoded by a fatty acid biosynthetic polynucleotide is an exogenous (or heterologous) polypeptide (for example, a polypeptide originating from an organism other than the parental host cell, or, a variant of a polypeptide native to the parental microbial cell) or an endogenous polypeptide (that is, a polypeptide native to the parental host cell) wherein the endogenous polypeptide is overexpressed in the recombinant host cell.

[0146] Table 1 provides a listing of exemplary proteins which can be expressed in recombinant host cells to facilitate production of particular fatty acid derivatives.

TABLE 1

Gene Designations					
Gene Designation	Source Organism	Enzyme Name	Accession No.	EC Number	Exemplary Use
1. Fatty Acid Production Increase/Product Production Increase					
accA	*E. coli*, Lactococci	Acetyl-CoA carboxylase, subunit A (carboxyltransferase alpha)	AAC73296, NP_414727	6.4.1.2	increase Malonyl-CoA production
accB	*E. coli*, Lactococci	Acetyl-CoA carboxylase, subunit B (BCCP: biotin carboxyl carrier protein)	NP_417721	6.4.1.2	increase Malonyl-CoA production
accC	*E. coli*, Lactococci	Acetyl-CoA carboxylase, subunit C (biotin carboxylase)	NP_417722	6.4.1.2, 6.3.4.14	increase Malonyl-CoA production
accD	*E. coli*, Lactococci	Acetyl-CoA carboxylase, subunit D (carboxyltransferase beta)	NP_416819	6.4.1.2	increase Malonyl-CoA production
fadD	*E. coli* W3110	acyl-CoA synthase	AP_002424	2.3.1.86, 6.2.1.3	increase Fatty acid production
fabA	*E. coli* K12	β-hydroxydecanoyl thioester dehydratase/isomerase	NP_415474	4.2.1.60	increase fatty acyl-ACP/CoA production
fabB	*E. coli*	3-oxoacyl-[acyl-carrier-protein] synthase I	BAA16180	2.3.1.41	increase fatty acyl-ACP/CoA production
fabD	*E. coli K12*	[acyl-carrier-protein] S-malonyltransferase	AAC74176	2.3.1.39	increase fatty acyl-ACP/CoA production
fabF	*E. coli* K12	3-oxoacyl-[acyl-carrier-protein] synthase II	AAC74179	2.3.1.179	increase fatty acyl-ACP/CoA production
fabG	*E. coli* K12	3-oxoacyl-[acyl-carrier protein] reductase	AAC74177	1.1.1.100	increase fatty acyl-ACP/CoA production
fabH	*E. coli* K12	3-oxoacyl-[acyl-carrier-protein] synthase III	AAC74175	2.3.1.180	increase fatty acyl-ACP/CoA production
fabI	*E. coli* K12	enoyl-[acyl-carrier-protein] reductase	NP_415804	1.3.1.9	increase fatty acyl-ACP/CoA production
fabR	*E. coli* K12	Transcriptional Repressor	NP_418398	none	modulate unsaturated fatty acid production

TABLE 1-continued

Gene Designations					
Gene Designation	Source Organism	Enzyme Name	Accession No.	EC Number	Exemplary Use
fabV	*Vibrio cholerae*	enoyl-[acyl-carrier-protein] reductase	YP_001217283	1.3.1.9	increase fatty acyl-ACP/CoA production
fabZ	*E. coli* K12	(3R)-hydroxymyristol acyl carrier protein dehydratase	NP_414722	4.2.1.—	increase fatty acyl-ACP/CoA production
fadE	*E. coli* K13	acyl-CoA dehydrogenase	AAC73325	1.3.99.3, 1.3.99.—	reduce fatty acid degradation
fadR	*E. coli*	transcriptional regulatory protein	NP_415705	none	Block or reverse fatty acid degradation
2. Chain Length Control					
tesA (with or without leader sequence)	*E. coli*	thioesterase - leader sequence is amino acids 1-26	P0ADA1	3.1.2.—, 3.1.1.5	C18 Chain Length
tesA (without leader sequence)	*E. coli*	thioesterase	AAC73596, NP_415027	3.1.2.—, 3.1.1.5	C18:1 Chain Length
tesA (mutant of *E. coli* thioesterase I complexed with octanoic acid)	*E. coli*	thioesterase	L109P	3.1.2.—, 3.1.1.5	<C18 Chain Length
fatB1	*Umbellularia californica*	thioesterase	Q41635	3.1.2.14	C12:0 Chain Length
fatB2	*Cuphea hookeriana*	thioesterase	AAC49269	3.1.2.14	C8:0-C10:0 Chain Length
fatB3	*Cuphea hookeriana*	thioesterase	AAC72881	3.1.2.14	C14:0-C16:0 Chain Length
fatB	*Cinnamomumcamphora*	thioesterase	Q39473	3.1.2.14	C14:0 Chain Length
fatB	*Arabidopsis thaliana*	thioesterase	CAA85388	3.1.2.14	C16:1 Chain Length
fatA1	*Helianthus annuus*	thioesterase	AAL79361	3.1.2.14	C18:1 Chain Length
atfata	*Arabidopsis thaliana*	thioesterase	NP_189147, NP_193041	3.1.2.14	C18:1 Chain Length
fatA	*Brassica juncea*	thioesterase	CAC39106	3.1.2.14	C18:1 Chain Length
fatA	*Cuphea hookeriana*	thioesterase	AAC72883	3.1.2.14	C18:1 Chain Length
tes	*Photbacterium profundum*	thioesterase	YP_130990	3.1.2.14	Chain Length
tesB	*E. coli*	thioesterase	NP_414986	3.1.2.14	Chain Length
fadM	*E. coli*	thioesterase	NP_414977	3.1.2.14	Chain Length
yciA	*E. coli*	thioesterase	NP_415769	3.1.2.14	Chain Length
ybgC	*E. coli*	thioesterase	NP_415264	3.1.2.14	Chain Length
3. Saturation Level Control*					
Sfa	*E. coli*	Suppressor of fabA	AAN79592, AAC44390	none	increase monounsaturated fatty acids
fabA	*E. coli* K12	β-hydroxydecanoyl thioester dehydratase/isomerase	NP_415474	4.2.1.60	produce unsaturated fatty acids
GnsA	*E. coli*	suppressors of the secG null mutation	ABD18647.1	none	increase unsaturated fatty acid esters
GnsB	*E. coli*	suppressors of the secG null mutation	AAC74076.1	none	increase unsaturated fatty acid esters

TABLE 1-continued

Gene Designations					
Gene Designation	Source Organism	Enzyme Name	Accession No.	EC Number	Exemplary Use
fabB	*E. coli*	3-oxoacyl-[acyl-carrier-protein] synthase I	BAA16180	2.3.1.41	modulate unsaturated fatty acid production
des	*Bacillus subtilis*	D5 fatty acyl desaturase	O34653	1.14.19	modulate unsaturated fatty acid production
4. Product Output: wax production					
AT3G51970	*Arabidopsis thaliana*	long-chain-alcohol O-fatty-acyltransferase	NP_190765	2.3.1.26	wax production
ELO1	*Pichia angusta*	Fatty acid elongase	BAD98251	2.3.1.—	produce very long chain length fatty acids
plsC	*Saccharomyces cerevisiae*	acyltransferase	AAA16514	2.3.1.51	wax production
DAGAT/DGAT	*Arabidopsis thaliana*	diacylglycerol acyltransferase	AAF19262	2.3.1.20	wax production
hWS	*Homo sapiens*	acyl-CoA wax alcohol acyltransferase	AAX48018	2.3.1.20	wax production
aft1	*Acinetobacter* sp. ADP1	bifunctional wax ester synthase/acyl-CoA:diacylglycerol acyltransferase	AAO17391	2.3.1.20	wax production
ES9	*Marinobacter hydrocarbonoclasticus*	wax ester synthase	ABO21021	2.3.1.20	wax production
mWS	*Simmondsia chinensis*	wax ester synthase	AAD38041	2.3.1.—	wax production
5. Product Output: Fatty Alcohol Output					
		thioesterases (see above)			increase fatty acid/fatty alcohol production
BmFAR	*Bombyxmori*	FAR (fatty alcohol forming acyl-CoA reductase)	BAC79425	1.1.1.—	convert acyl-CoA to fatty alcohol
acr1	*Acinetobacter* sp. ADP1	acyl-CoA reductase	YP_047869	1.2.1.42	reduce fatty acyl-CoA to fatty aldehydes
yqhD	*E. coli* W3110	alcohol dehydrogenase	AP_003562	1.1.—.—	reduce fatty aldehydes to fatty alcohols; increase fatty alcohol production
alrA	*Acinetobacter* sp. ADP1	alcohol dehydrogenase	CAG70252	1.1.—.—	reduce fatty aldehydes to fatty alcohols
BmFAR	*Bombyxmori*	FAR (fatty alcohol forming acyl-CoA reductase)	BAC79425	1.1.1.—	reduce fatty acyl-CoA to fatty alcohol
GTNG_1865	*Geobacillusthermodenitrificans* NG80-2	Long-chain aldehyde dehydrogenase	YP_001125970	1.2.1.3	reduce fatty aldehydes to fatty alcohols
AAR	*Synechococcus elongatus*	Acyl-ACP reductase	YP_400611	1.2.1.42	reduce fatty acyl-ACP/CoA to fatty aldehydes
carB	*Mycobacterium smegmatis*	carboxylic acid reductase protein	YP_889972	6.2.1.3, 1.2.1.42	reduce fatty acids to fatty aldehyde
FadD	*E. coli* K12	acyl-CoA synthetase	NP_416319	6.2.1.3	activates fatty acids to fatty acyl-CoAs
atoB	*Erwinia carotovora*	acetyl-CoA acetyltransferase	YP_049388	2.3.1.9	production of butanol

TABLE 1-continued

Gene Designations

Gene Designation	Source Organism	Enzyme Name	Accession No.	EC Number	Exemplary Use
hbd	*Butyrivibrio fibrisolvens*	Beta-hydroxybutyryl-CoA dehydrogenase	BAD51424	1.1.1.157	production of butanol
CPE0095	*Clostridium perfringens*	crotonasebutyryl-CoA dehydryogenase	BAB79801	4.2.1.55	production of butanol
bcd	*Clostridium beijerinckii*	butyryl-CoA dehydryogenase	AAM14583	1.3.99.2	production of butanol
ALDH	*Clostridium beijerinckii*	coenzyme A-acylating aldehyde dehydrogenase	AAT66436	1.2.1.3	production of butanol
AdhE	*E. coli* CFT073	aldehyde-alcohol dehydrogenase	AAN80172	1.1.1.1 1.2.1.10	production of butanol
		6. Fatty Alcohol Acetyl Ester Output			
		thioesterases (see above)			modify output
acr1	*Acinetobacter* sp. ADP1	acyl-CoA reductase	YP_047869	1.2.1.42	modify output
yqhD	*E. Coli* K12	alcohol dehydrogenase	AP_003562	1.1.—.—	modify output
AAT	*Fragaria* x *ananassa*	alcohol O-acetyltransferase	AAG13130	2.3.1.84	modify output
		7. Product Export			
AtMRP5	*Arabidopsis thaliana*	*Arabidopsis thaliana* multidrug resistance-associated	NP_171908	none	modify product export amount
AmiS2	*Rhodococcus* sp.	ABC transporter AmiS2	JC5491	none	modify product export amount
AtPGP1	*Arabidopsis thaliana*	*Arabidopsis thaliana* p glycoprotein 1	NP_181228	none	modify product export amount
AcrA	*Candidatus Protochlamydia amoebophila* UWE25	putative multidrug-efflux transport protein acrA	CAF23274	none	modify product export amount
AcrB	*Candidatus Protochlamydia amoebophila* UWE25	probable multidrug-efflux transport protein, acrB	CAF23275	none	modify product export amount
TolC	*Francisella tularensis* subsp. *novicida*	Outer membrane protein [Cell envelope biogenesis,	ABD59001	none	modify product export amount
AcrE	*Shigella sonnei* Ss046	transmembrane protein affects septum formation and cell membrane permeability	YP_312213	none	modify product export amount
AcrF	*E. coli*	Acriflavine resistance protein F	P24181	none	modify product export amount
tll1619	*Thermosynechococcus elongatus* [BP-1]	multidrug efflux transporter	NP_682409.1	none	modify product export amount
tll0139	*Thermosynechococcus elongatus* [BP-1]	multidrug efflux transporter	NP_680930.1	none	modify product export amount
		8. Fermentation			
replication checkpoint genes					increase output efficiency
umuD	*Shigella sonnei* Ss046	DNA polymerase V, subunit	YP_310132	3.4.21.—	increase output efficiency

TABLE 1-continued

Gene Designations

Gene Designation	Source Organism	Enzyme Name	Accession No.	EC Number	Exemplary Use
umuC	*E. coli*	DNA polymerase V, subunit	ABC42261	2.7.7.7	increase output efficiency
pntA, pntB	*Shigella flexneri*	NADH:NADPH transhydrogenase (alpha and beta subunits) 9. Other	P07001, P0AB70	1.6.1.2	increase output efficiency
fabK	*Streptococcus pneumoniae*	trans-2-enoyl-ACP reductase II	AAF98273	1.3.1.9	Contributes to fatty acid biosynthesis
fabL	*Bacillus licheniformis* DSM 13	enoyl-(acyl carrier protein) reductase	AAU39821	1.3.1.9	Contributes to fatty acid biosynthesis
fabM	*Streptococcus mutans*	trans-2,cis-3-decenoyl-ACP isomerase	DAA05501	4.2.1.17	Contributes to fatty acid biosynthesis

Production of Fatty Acids

[0147] The recombinant host cells may comprise one or more polynucleotide sequences that comprise an open reading frame encoding a thioesterase, e.g., having an Enzyme Commission number of EC 3.1.1.5 or EC 3.1.2.—(for example, EC 3.1.2.14), together with operably-linked regulatory sequences that facilitate expression of the protein in the recombinant host cells. In the recombinant host cells, the open reading frame coding sequences and/or the regulatory sequences are modified relative to the corresponding wild-type gene encoding the thioesterase. The activity of the thioesterase in the recombinant host cell is modified relative to the activity of the thioesterase expressed from the corresponding wild-type gene in a corresponding host cell. In some embodiments, a fatty acid derivative composition comprising fatty acids is produced by culturing a recombinant cell in the presence of a carbon source under conditions effective to express the thioesterase.

[0148] In related embodiments, the recombinant host cell comprises a polynucleotide encoding a polypeptide having thioesterase activity, and one or more additional polynucleotides encoding polypeptides having other fatty acid biosynthetic enzyme activities. In some such instances, the fatty acid produced by the action of the thioesterase is converted by one or more enzymes having a different fatty acid biosynthetic enzyme activity to another fatty acid derivative, such as, for example, a fatty ester, fatty aldehyde, fatty alcohol, or a hydrocarbon.

[0149] The chain length of a fatty acid, or a fatty acid derivative made therefrom, can be selected for by modifying the expression of particular thioesterases. The thioesterase will influence the chain length of fatty acid derivatives produced. The chain length of a fatty acid derivative substrate can be selected for by modifying the expression of selected thioesterases (EC 3.1.2.14 or LC 3, 1.1.5). Hence, host cells can be engineered to express, overexpress, have attenuated expression, or not express one or more selected thioesterases to increase the production of a preferred fatty acid derivative substrate. For example, C_{10} fatty acids can be produced by expressing a thioesterase that has a preference for producing C_{10} fatty acids and attenuating thioesterases that have a preference for producing fatty acids other than C_{10} fatty acids (e.g., a thioesterase which prefers to produce C_{14} fatty acids). This would result in a relatively homogeneous population of fatty acids that have a carbon chain length of 10. In other instances, C_{14} fatty acids can be produced by attenuating endogenous thioesterases that produce non-C_{14} fatty acids and expressing the thioesterases that use C_{14}-ACP. In some situations, C_{12} fatty acids can be produced by expressing thioesterases that use C_{12}-ACP and attenuating thioesterases that produce non-C_{12} fatty acids. For example, C12 fatty acids can be produced by expressing a thioesterase that has a preference for producing C12 fatty acids and attenuating thioesterases that have a preference for producing fatty acids other than C12 fatty acids. This would result in a relatively homogeneous population of fatty acids that have a carbon chain length of 12. The fatty acid derivatives are recovered from the culture medium with substantially all of the fatty acid derivatives produced extracellularly. The fatty acid derivative composition produced by a recombinant host cell can be analyzed using methods known in the art, for example, GC-FID, in order to determine the distribution of particular fatty acid derivatives as well as chain lengths and degree of saturation of the components of the fatty acid derivative composition. Acetyl-CoA, malonyl-CoA, and fatty acid overproduction can be verified using methods known in the art, for example, by using radioactive precursors, HPLC, or GC-MS subsequent to cell lysis.

[0150] Additional non-limiting examples of thioesterases and polynucleotides encoding them for use in the fatty acid pathway are provided in PCT Publication No. WO 2010/075483, expressly incorporated by reference herein.

Production of Fatty Aldehydes

[0151] In one embodiment, the recombinant host cell produces a fatty aldehyde. In some embodiments, a fatty acid produced by the recombinant host cell is converted into a fatty aldehyde. In some embodiments, the fatty aldehyde produced by the recombinant host cell is then converted into a fatty alcohol or a hydrocarbon.

[0152] In some embodiments, native (endogenous) fatty aldehyde biosynthetic polypeptides, such as aldehyde reduc-

tases, are present in the host cell (e.g., *E. coli*) and are effective to convert fatty aldehydes to fatty alcohols. In other embodiments, a native (endogenous) fatty aldehyde biosynthetic polypeptide is overexpressed. In still other embodiments, an exogenous fatty aldehyde biosynthetic polypeptide is introduced into a recombinant host cell and expressed or overexpressed.

[0153] A native or recombinant host cell may comprise a polynucleotide encoding an enzyme having fatty aldehyde biosynthesis activity (also referred to herein as a "fatty aldehyde biosynthetic polypeptide" or a "fatty aldehyde biosynthetic polypeptide" or enzyme). A fatty aldehyde is produced when the fatty aldehyde biosynthetic enzyme is expressed or overexpressed in the host cell.

[0154] A recombinant host cell engineered to produce a fatty aldehyde will typically convert some of the fatty aldehyde to a fatty alcohol.

[0155] In some embodiments, a fatty aldehyde is produced by expressing or overexpressing in the recombinant host cell a polynucleotide encoding a polypeptide having fatty aldehyde biosynthetic activity such as carboxylic acid reductase (CAR) activity.

[0156] The terms "carboxylic acid reductase," and "CAR," are used interchangeably herein with respect to a "fatty aldehyde biosynthetic polypeptide". CarB, is an exemplary carboxylic acid reductase. In practicing the invention, a gene encoding a carboxylic acid reductase polypeptide may be expressed or overexpressed in the host cell. In some embodiments, the CarB polypeptide has the amino acid sequence of SEQ ID NO: 7. In other embodiments, the CarB polypeptide is a variant or mutant of SEQ ID NO: 7.

[0157] Examples of carboxylic acid reductase (CAR) polypeptides and polynucleotides encoding them include, but are not limited to FadD9 (EC 6.2.1.-, UniProtKB Q50631, GenBank NP_217106, SEQ ID NO: 34), CarA (GenBank ABK75684), CarB (GenBank YP889972; SEQ ID NO: 33) and related polypeptides described in PCT Publication No. WO 2010/042664 and U.S. Pat. No. 8,097,439, each of which is expressly incorporated by reference herein. In some embodiments the recombinant host cell further comprises a polynucleotide encoding a thioesterase.

[0158] In some embodiments, the fatty aldehyde is produced by expressing or overexpressing in the recombinant host cell a polynucleotide encoding a fatty aldehyde biosynthetic polypeptide, such as a polypeptide having acyl-ACP reductase (AAR) activity. Expression of acyl-ACP reductase in a recombinant host cell results in the production of fatty aldehydes and fatty alcohols. (See FIG. **4**.) Native (endogenous) aldehyde reductases present in a recombinant host cell (e.g., *E. coli*), can convert fatty aldehydes into fatty alcohols. Exemplary acyl-ACP reductase polypeptides are described in PCT Publication Nos. WO2009/140695 and WO/2009/140696, both of which are expressly incorporated by reference herein.

[0159] A composition comprising fatty aldehydes ("a fatty aldehyde composition") is produced by culturing a host cell in the presence of a carbon source under conditions effective to express the fatty aldehyde biosynthetic enzyme. In some embodiments, the fatty aldehyde composition comprises fatty aldehydes and fatty alcohols. Typically, the fatty aldehyde composition is recovered from the extracellular environment of the recombinant host cell, i.e., the cell culture medium.

Production of Fatty Alcohols

[0160] In some embodiments, the recombinant host cell comprises a polynucleotide encoding a polypeptide (an enzyme) having fatty alcohol biosynthetic activity (also referred to herein as a "fatty alcohol biosynthetic polypeptide" or a "fatty alcohol biosynthetic enzyme"), and a fatty alcohol is produced by the recombinant host cell. A composition comprising fatty alcohols ("a fatty alcohol composition") may be produced by culturing the recombinant host cell in the presence of a carbon source under conditions effective to express a fatty alcohol biosynthetic enzyme. In some embodiments, the fatty alcohol composition comprises fatty alcohols, however, a fatty alcohol composition may comprise other fatty acid derivatives. Typically, the fatty alcohol composition is recovered from the extracellular environment of the recombinant host cell, i.e., the cell culture medium.

[0161] In one approach, recombinant host cells have been engineered to produce fatty alcohols by expressing a thioesterase, which catalyzes the conversion of acyl-ACPs into free fatty acids (FFAs) and a carboxylic acid reductase (CAR), which converts free fatty acids into fatty aldehydes. Native (endogenous) aldehyde reductases present in the host cell (e.g., *E. coli*) can convert the fatty aldehydes into fatty alcohols.

[0162] In some embodiments, native (endogenous) fatty aldehyde biosynthetic polypeptides, such as aldehyde reductases present in the host cell, may be sufficient to convert fatty aldehydes to fatty alcohols. However, in other embodiments, a native (endogenous) fatty aldehyde biosynthetic polypeptide is overexpressed and in still other embodiments, an exogenous fatty aldehyde biosynthetic polypeptide is introduced into a recombinant host cell and expressed or overexpressed.

[0163] In some embodiments, the fatty alcohol is produced by expressing or overexpressing in the recombinant host cell a polynucleotide encoding a polypeptide having fatty alcohol biosynthetic activity which converts a fatty aldehyde to a fatty alcohol. For example, an "alcohol dehydrogenase" (also referred to herein as an "aldehyde reductase", e.g., EC 1.1.1.1), may be used in practicing the invention. As used herein, the term "alcohol dehydrogenase" refers to a polypeptide capable of catalyzing the conversion of a fatty aldehyde to an alcohol (e.g., a fatty alcohol). One of ordinary skill in the art will appreciate that certain alcohol dehydrogenases are capable of catalyzing other reactions as well, and these non-specific alcohol dehydrogenases also are encompassed by the term "alcohol dehydrogenase." Examples of alcohol dehydrogenase polypeptides useful in accordance with the invention include, but are not limited to A1rA of *Acinetobacter* sp. M-1 (SEQ ID NO: 3) or A1rA homologs such as AlrAadpl (SEQ ID NO: 4) and endogenous *E. coli* alcohol dehydrogenases such as YjgB, (AAC77226) (SEQ ID NO: 5), DkgA (NP_417485), DkgB (NP_414743), YdjL (AAC74846), YdjJ (NP_416288), AdhP (NP_415995), YhdH (NP_417719), YahK (NP_414859), YphC (AAC75598), YqhD (446856) and YbbO [AAC73595.1]. Additional examples are described in International Patent Application Publication Nos. WO 2007/136762, WO2008/119082 and WO 2010/062480, each of which is expressly incorporated by reference herein. In certain embodiments, the fatty alcohol biosynthetic polypeptide has aldehyde reductase or alcohol dehydrogenase activity (EC 1.1.1.1).

[0164] In another approach, recombinant host cells have been engineered to produce fatty alcohols by expressing fatty

alcohol forming acyl-CoA reductases or fatty acyl reductases ("FARs") which convert fatty acyl-thioester substrates (e.g., fatty acyl-CoA or fatty acyl-ACP) to fatty alcohols. In some embodiments, the fatty alcohol is produced by expressing or overexpressing a polynucleotide encoding a polypeptide having fatty alcohol forming acyl-CoA reductase (FAR) activity in a recombinant host cell. Examples of FAR polypeptides useful in accordance with this embodiment are described in PCT Publication No. WO 2010/062480 which is expressly incorporated by reference herein.

[0165] Fatty alcohol may be produced via an acyl-CoA dependent pathway utilizing fatty acyl-ACP and fatty acyl-CoA intermediates and an acyl-CoA independent pathway utilizing fatty acyl-ACP intermediates but not a fatty acyl-CoA intermediate. In particular embodiments, the enzyme encoded by the over expressed gene is selected from a fatty acid synthase, an acyl-ACP thioesterase, a fatty acyl-CoA synthase and an acetyl-CoA carboxylase. In some embodiments, the protein encoded by the over expressed gene is endogenous to the host cell. In other embodiments, the protein encoded by the overexpressed gene is heterologous to the host cell.

[0166] Fatty alcohols are also made in nature by enzymes that are able to reduce various acyl-ACP or acyl-CoA molecules to the corresponding primary alcohols. See also, U.S. Patent Publication Nos. 20100105963, and 20110206630 and U.S. Pat. No. 8,097,439, expressly incorporated by reference herein.

[0167] Strategies to increase production of fatty alcohols by recombinant host cells include increased flux through the fatty acid biosynthetic pathway by overexpression of native fatty acid biosynthetic genes and/or expression of exogenous fatty acid biosynthetic genes from different organisms in the production host such that fatty alcohol biosynthesis is increased.

Production of Esters

[0168] As used herein, the term "fatty ester" may be used with reference to an ester. A fatty ester as referred to herein can be any ester made from a fatty acid, for example a fatty acid ester. In some embodiments, a fatty ester contains an A side and a B side. As used herein, an "A side" of an ester refers to the carbon chain attached to the carboxylate oxygen of the ester. As used herein, a "B side" of an ester refers to the carbon chain comprising the parent carboxylate of the ester. In embodiments where the fatty ester is derived from the fatty acid biosynthetic pathway, the A side is contributed by an alcohol, and the B side is contributed by a fatty acid.

[0169] Any alcohol can be used to form the A side of the fatty esters. For example, the alcohol can be derived from the fatty acid biosynthetic pathway. Alternatively, the alcohol can be produced through non-fatty acid biosynthetic pathways. Moreover, the alcohol can be provided exogenously. For example, the alcohol can be supplied in the fermentation broth in instances where the fatty ester is produced by an organism. Alternatively, a carboxylic acid, such as a fatty acid or acetic acid, can be supplied exogenously in instances where the fatty ester is produced by an organism that can also produce alcohol.

[0170] The carbon chains comprising the A side or B side can be of any length. In one embodiment, the A side of the ester is at least about 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, or 18 carbons in length. When the fatty ester is a fatty acid methyl ester, the A side of the ester is 1 carbon in length. When the fatty ester is a fatty acid ethyl ester, the A side of the ester is 2 carbons in length. The B side of the ester can be at least about 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26 carbons in length. The A side and/or the B side can be straight or branched chain. The branched chains can have one or more points of branching. In addition, the branched chains can include cyclic branches. Furthermore, the A side and/or B side can be saturated or unsaturated. If unsaturated, the A side and/or B side can have one or more points of unsaturation.

[0171] In one embodiment, the fatty ester is produced biosynthetically. In this embodiment, first the fatty acid is "activated." Non-limiting examples of "activated" fatty acids are acyl-CoA, acyl ACP, and acyl phosphate. Acyl-CoA can be a direct product of fatty acid biosynthesis or degradation. In addition, acyl-CoA can be synthesized from a free fatty acid, a CoA, and an adenosine nucleotide triphosphate (ATP). An example of an enzyme which produces acyl-CoA is acyl-CoA synthase.

[0172] In some embodiments, the recombinant host cell comprises a polynucleotide encoding a polypeptide, e.g., an enzyme having ester synthase activity, (also referred to herein as an "ester synthase polypeptide" or an "ester synthase"). A fatty ester is produced by a reaction catalyzed by the ester synthase polypeptide expressed or overexpressed in the recombinant host cell. In some embodiments, a composition comprising fatty esters (also referred to herein as a "fatty ester composition") comprising fatty esters is produced by culturing the recombinant cell in the presence of a carbon source under conditions effective to express an ester synthase. In some embodiments, the fatty ester composition is recovered from the cell culture.

[0173] Ester synthase polypeptides include, for example, an ester synthase polypeptide classified as EC 2.3.1.75, or any other polypeptide which catalyzes the conversion of an acyl-thioester to a fatty ester, including, without limitation, a thioesterase, an ester synthase, an acyl-CoA:alcohol transacylase, an acyltransferase, or a fatty acyl-CoA:fatty alcohol acyltransferase. For example, the polynucleotide may encode wax/dgat, a bifunctional ester synthase/acyl-CoA:diacylglycerol acyltransferase from *Simmondsia chinensis, Acinetobacter* sp. Strain ADP, *Alcanivorax borkumensis, Pseudomonas aeruginosa, Fundibacter jadensis, Arabidopsis thaliana*, or *Alkaligenes eutrophus*. In a particular embodiment, the ester synthase polypeptide is an *Acinetobacter* sp. diacylglycerol O-acyltransferase (wax-dgaT; UniProtKB Q8GGG1, GenBank AA017391) or *Simmondsia chinensis* wax synthase (UniProtKB Q9XGY6, GenBank AAD38041. In another embodiment, the ester synthase polypeptide is for example ES9 (a wax ester synthase from *Marinobacter hydrocarbonoclasticus* DSM 8798, UniProtKB A3RE51 (SEQ ID NO: 6); ES8 of *Marinobacter hydrocarbonoclasticus* DSM8789 (GenBank Accession No. AB021021; SEQ ID NO:7); GenBank AB021021, encoded by the ws2 gene; or ES376 (another wax ester synthase derived from *Marinobacter hydrocarbonoclasticus* DSM 8798, UniProtKB A3RE50, GenBank AB021020, encoded by the ws1 gene. In a particular embodiment, the polynucleotide encoding the ester synthase polypeptide is overexpressed in the recombinant host cell.

[0174] In some embodiments, a fatty acid ester is produced by a recombinant host cell engineered to express three fatty acid biosynthetic enzymes: a thioesterase enzyme, an acyl-CoA synthetase (fadD) enzyme and an ester synthase enzyme ("three enzyme system"; FIG. **5**).

[0175] In other embodiments, a fatty acid ester is produced by a recombinant host cell engineered to express one fatty acid biosynthetic enzyme, an ester synthase enzyme ("one enzyme system"; FIG. **5**).

[0176] Non-limiting examples of ester synthase polypeptides and polynucleotides encoding them suitable for use in these embodiments include those described in PCT Publication Nos. WO 2007/136762 and WO2008/119082, and WO/2011/038134 ("three enzyme system") and WO/2011/038132 ("one enzyme system"), each of which is expressly incorporated by reference herein.

[0177] The recombinant host cell may produce a fatty ester, such as a fatty acid methyl ester, a fatty acid ethyl ester or a wax ester in the extracellular environment of the host cells.

Production of Hydrocarbons

[0178] This aspect of the invention is based, at least in part, on the discovery that altering the level of expression of a fatty aldehyde biosynthetic polypeptide, for example, an acyl-ACP reductase polypeptide (EC 6.4.1.2) and a hydrocarbon biosynthetic polypeptide, e.g., a decarbonylase in a recombinant host cell facilitates enhanced production of hydrocarbons by the recombinant host cell.

[0179] In one embodiment, the recombinant host cell produces a hydrocarbon, such as an alkane or an alkene (e.g., a terminal olefin or an internal olefin) or a ketone. In some embodiments, a fatty aldehyde produced by a recombinant host cell is converted by decarboxylation, removing a carbon atom to form a hydrocarbon. In other embodiments, a fatty acid produced by a recombinant host cell is converted by decarboxylation, removing a carbon atom to form a terminal olefin. In some embodiments, an acyl-ACP intermediate is converted by decarboxylation, removing a carbon atom to form an internal olefin or a ketone. See, FIG. **6**.

[0180] In some embodiments, the recombinant host cell comprises a polynucleotide encoding a polypeptide (an enzyme) having hydrocarbon biosynthetic activity (also referred to herein as a "hydrocarbon biosynthetic polypeptide" or a "hydrocarbon biosynthetic enzyme"), and the hydrocarbon is produced by expression or overexpression of the hydrocarbon biosynthetic enzyme in a recombinant host cell.

[0181] An alkane biosynthetic pathway from cyanobacteria consisting of an acyl-acyl carrier protein reductase (AAR) and an aldehyde decarbonylase (ADC), which together convert intermediates of fatty acid metabolism to alkanes and alkenes has been used to engineer recombinant host cells for the production of hydrocarbons (FIG. **6**). The second of two reactions in the pathway through which saturated acyl-ACPs are converted to alkanes in cyanobacteria entails scission of the C1-C2 bond of a fatty aldehyde intermediate by the enzyme aldehyde decarbonylase (ADC), a ferritin-like protein with a binuclear metal cofactor of unknown composition.

[0182] In some embodiments, the hydrocarbon is produced by expressing or overexpressing in the recombinant host cell a polynucleotide encoding a polypeptide having hydrocarbon biosynthetic activity such as an aldehyde decarbonylase (ADC) activity (e.g., EC 4.1.99.5). exemplary polynucleotides encoding an aldehyde decarbonylase useful in accordance with this embodiment include, but are not limited to, those described in PCT Publication Nos. WO 2008/119082 and WO 2009/140695 which are expressly incorporated by reference herein and those sequences presented in Table 2, below. In some embodiments the recombinant host cell further comprises a polynucleotide encoding a fatty aldehyde biosynthesis polypeptide. In some embodiments the recombinant host cell further comprises a polynucleotide encoding an acyl-ACP reductase. See, for example Table 2, below.

TABLE 2

Exemplary Hydrocarbon Biosynthetic Polynucleotides and Polypeptides.

Protein name	Polypeptide sequence	Nucleotide sequence	Sequence
Decarbonylase (ADC)	SEQ ID NO: 35	SEQ ID NO: 36	*Synechococcus elongatus* PCC7942 YP.sub.--400610 (Synpcc7942.sub.--1593)
Acyl-ACP Reducatase (AAR)	SEQ ID NO: 37	SEQ ID NO: 38	*Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594)
Decarbonylase (ADC)	SEQ ID NO: 39	SEQ ID NO: 40	*Prochlorococcus mariunus* CCMP1986 PMM0532
Acyl-ACP Reducatase (AAR)	SEQ ID NO: 41	SEQ ID NO: 42	*Prochlorococcus marinus* CCMP1986 PMM0533 (NP_892651)

[0183] In some embodiments, a composition comprising hydrocarbons (also referred to herein as a "hydrocarbon composition") is produced by culturing the recombinant cell in the presence of a carbon source under conditions effective to express the Acyl-CoA reductase and decarbonylase polynucleotides. In some embodiments, the hydrocarbon composition comprises saturated and unsaturated hydrocarbons, however, a hydrocarbon composition may comprise other fatty acid derivatives. Typically, the hydrocarbon composition is recovered from the extracellular environment of the recombinant host cell, i.e., the cell culture medium.

[0184] As used herein, the term "alkane" means saturated hydrocarbons or compounds that consist only of carbon (C) and hydrogen (H), wherein these atoms are linked together by single bonds (i.e., they are saturated compounds).

[0185] The terms "olefin" and "alkene" are used interchangeably herein, and refer to hydrocarbons containing at least one carbon-to-carbon double bond (i.e., they are unsaturated compounds).

[0186] The terms "terminal olefin," "α-olefin", "terminal alkene" and "1-alkene" are used interchangeably herein with reference to α-olefins or alkenes with a chemical formula CxH2x, distinguished from other olefins with a similar molecular formula by linearity of the hydrocarbon chain and the position of the double bond at the primary or alpha position.

[0187] In some embodiments, a terminal olefin is produced by expressing or overexpressing in the recombinant host cell a polynucleotide encoding a hydrocarbon biosynthetic polypeptide, such as a polypeptide having decarboxylase activity as described, for example, in PCT Publication No. WO 2009/085278 which is expressly incorporated by reference herein. In some embodiments the recombinant host cell further comprises a polynucleotide encoding a thioesterase.

[0188] In other embodiments, a ketone is produced by expressing or overexpressing in the recombinant host cell a polynucleotide encoding a hydrocarbon biosynthetic polypeptide, such as a polypeptide having OleA activity as described, for example, in PCT Publication No. WO 2008/147781, which is expressly incorporated by reference herein.

[0189] In related embodiments, an internal olefin is produced by expressing or overexpressing in the recombinant host cell a polynucleotide encoding a hydrocarbon biosyn-

thetic polypeptide, such as a polypeptide having OleCD or OleBCD activity together with a polypeptide having OleA activity as described, for example, in PCT Publication No. WO 2008/147781, expressly incorporated by reference herein.

Recombinant Host Cells and Cell Cultures

[0190] Strategies to increase production of fatty acid derivatives by recombinant host cells include increased flux through the fatty acid biosynthetic pathway by overexpression of native fatty acid biosynthetic genes and expression of exogenous fatty acid biosynthetic genes from different organisms in the production host.

[0191] As used herein, the term "recombinant host cell" or "engineered host cell" refers to a host cell whose genetic makeup has been altered relative to the corresponding wild-type host cell, for example, by deliberate introduction of new genetic elements and/or deliberate modification of genetic elements naturally present in the host cell. The offspring of such recombinant host cells also contain these new and/or modified genetic elements. In any of the aspects of the invention described herein, the host cell can be selected from the group consisting of a plant cell, insect cell, fungus cell (e.g., a filamentous fungus, such as *Candida* sp., or a budding yeast, such as *Saccharomyces* sp.), an algal cell and a bacterial cell. In one preferred embodiment, recombinant host cells are "recombinant microorganisms."

[0192] Examples of host cells that are microorganisms, include but are not limited to cells from the genus *Escherichia, Bacillus, Lactobacillus, Zymomonas, Rhodococcus, Pseudomonas, Aspergillus, Trichoderma, Neurospora, Fusarium, Humicola, Rhizomucor, Kluyveromyces, Pichia, Mucor, Myceliophtora, Penicillium, Phanerochaete, Pleurotus, Trametes, Chrysosporium, Saccharomyces, Stenotrophamonas, Schizosaccharomyces, Yarrowia*, or *Streptomyces*. In some embodiments, the host cell is a Gram-positive bacterial cell. In other embodiments, the host cell is a Gram-negative bacterial cell.

[0193] In some embodiments, the host cell is an *E. coli* cell.

[0194] In other embodiments, the host cell is a *Bacillus lentus* cell, a *Bacillus brevis* cell, a *Bacillus stearothermophilus* cell, a *Bacillus lichenoformis* cell, a *Bacillus alkalophilus* cell, a *Bacillus coagulans* cell, a *Bacillus circulans* cell, a *Bacillus pumilis* cell, a *Bacillus thuringiensis* cell, a *Bacillus clausii* cell, a *Bacillus megaterium* cell, a *Bacillus subtilis* cell, or a *Bacillus amyloliquefaciens* cell.

[0195] In other embodiments, the host cell is a *Trichoderma koningii* cell, a *Trichoderma viride* cell, a *Trichoderma reesei* cell, a *Trichoderma longibrachiatum* cell, an *Aspergillus awamori* cell, an *Aspergillus fumigates* cell, an *Aspergillus foetidus* cell, an *Aspergillus nidulans* cell, an *Aspergillus niger* cell, an *Aspergillus oryzae* cell, a *Humicola insolens* cell, a *Humicola lanuginose* cell, a *Rhodococcus opacus* cell, a *Rhizomucor miehei* cell, or a *Mucor michei* cell.

[0196] In yet other embodiments, the host cell is a *Streptomyces lividans* cell or a *Streptomyces murinus* cell.

[0197] In yet other embodiments, the host cell is an Actinomycetes cell.

[0198] In some embodiments, the host cell is a *Saccharomyces cerevisiae* cell.

[0199] In other embodiments, the host cell is a cell from a eukaryotic plant, algae, cyanobacterium, green-sulfur bacterium, green non-sulfur bacterium, purple sulfur bacterium, purple non-sulfur bacterium, extremophile, yeast, fungus, an engineered organism thereof, or a synthetic organism. In some embodiments, the host cell is light-dependent or fixes carbon. In some embodiments, the host cell has autotrophic activity. In some embodiments, the host cell has photoautotrophic activity, such as in the presence of light. In some embodiments, the host cell is heterotrophic or mixotrophic in the absence of light. In certain embodiments, the host cell is a cell from *Arabidopsis thaliana, Panicum virgatum, Miscanthus giganteus, Zea mays, Botryococcuse braunii, Chlamydomonas reinhardtii, Dunaliela sauna, Synechococcus* Sp. PCC 7002, *Synechococcus* Sp. PCC 7942, *Synechocystis* Sp. PCC 6803, *Thermosynechococcus elongates* BP-1, *Chlorobium tepidum, Chlorojlexus auranticus, Chromatiumm vinosum, Rhodospirillum rubrum, Rhodobacter capsulatus, Rhodopseudomonas palusris, Clostridium ljungdahlii, Clostridiuthermocellum, Penicillium chrysogenum, Pichia pastoris, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Pseudomonas fluorescens*, or *Zymomonas mobilis*.

Production of Fatty Acid Derivative Compositions by Recombinant Host Cells

[0200] A large variety of fatty acid derivatives can be produced by recombinant host cells comprising strain improvements as described herein, including, but not limited to, fatty acids, acyl-CoA, fatty aldehydes, short and long chain alcohols, hydrocarbons (e.g., alkanes, alkenes or olefins, such as terminal or internal olefins), fatty alcohols, esters (e.g., wax esters, fatty acid esters (e.g., methyl or ethyl esters)), and ketones.

[0201] In some embodiments of the present invention, the higher titer of fatty acid derivatives in a particular composition is a higher titer of a particular type of fatty acid derivative (e.g., fatty alcohols, fatty acid esters, or hydrocarbons) produced by a recombinant host cell culture relative to the titer of the same fatty acid derivatives produced by a control culture of a corresponding wild-type host cell. In such cases, the fatty acid derivative compositions may comprise, for example, a mixture of the fatty alcohols with a variety of chain lengths and saturation or branching characteristics.

[0202] In other embodiments of the present invention, the higher titer of fatty acid derivatives in a particular compositions is a higher titer of a combination of different fatty acid derivatives (for example, fatty aldehydes and alcohols, or fatty acids and esters) relative to the titer of the same fatty acid derivative produced by a control culture of a corresponding wild-type host cell.

Engineering Host Cells

[0203] In some embodiments, a polynucleotide (or gene) sequence is provided to the host cell by way of a recombinant vector, which comprises a promoter operably linked to the polynucleotide sequence. In certain embodiments, the promoter is a developmentally-regulated, an organelle-specific, a tissue-specific, an inducible, a constitutive, or a cell-specific promoter.

[0204] In some embodiments, the recombinant vector comprises at least one sequence selected from the group consisting of (a) an expression control sequence operatively coupled to the polynucleotide sequence; (b) a selection marker operatively coupled to the polynucleotide sequence; (c) a marker sequence operatively coupled to the polynucleotide sequence; (d) a purification moiety operatively coupled to the polynucleotide sequence; (e) a secretion sequence opera-

tively coupled to the polynucleotide sequence; and (f) a targeting sequence operatively coupled to the polynucleotide sequence.

[0205] The expression vectors described herein include a polynucleotide sequence described herein in a form suitable for expression of the polynucleotide sequence in a host cell. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of polypeptide desired, etc. The expression vectors described herein can be introduced into host cells to produce polypeptides, including fusion polypeptides, encoded by the polynucleotide sequences as described herein.

[0206] Expression of genes encoding polypeptides in prokaryotes, for example, *E. coli*, is most often carried out with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion polypeptides. Fusion vectors add a number of amino acids to a polypeptide encoded therein, usually to the amino- or carboxy-terminus of the recombinant polypeptide. Such fusion vectors typically serve one or more of the following three purposes: (1) to increase expression of the recombinant polypeptide; (2) to increase the solubility of the recombinant polypeptide; and (3) to aid in the purification of the recombinant polypeptide by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant polypeptide. This enables separation of the recombinant polypeptide from the fusion moiety after purification of the fusion polypeptide. Examples of such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin, and enterokinase. Exemplary fusion expression vectors include pGEX (Pharmacia Biotech, Inc., Piscataway, N.J.; Smith et al., *Gene,* 67: 31-40 (1988)), pMAL (New England Biolabs, Beverly, Mass.), and pRITS (Pharmacia Biotech, Inc., Piscataway, N.J.), which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant polypeptide.

[0207] Examples of inducible, non-fusion *E. coli* expression vectors include pTrc (Amann et al., *Gene* (1988) 69:301-315) and pET 11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gni). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident 2 prophage harboring a T7 gni gene under the transcriptional control of the lacUV 5 promoter.

[0208] Suitable expression systems for both prokaryotic and eukaryotic cells are well known in the art; see, e.g., Sambrook et al., "Molecular Cloning: A Laboratory Manual," second edition, Cold Spring Harbor Laboratory, (1989). Examples of inducible, non-fusion *E. coli* expression vectors include pTrc (Amann et al., *Gene,* 69: 301-315 (1988)) and PET 11 d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif., pp. 60-89 (1990)). In certain embodiments, a polynucleotide sequence of the invention is operably linked to a promoter derived from bacteriophage T5.

[0209] In one embodiment, the host cell is a yeast cell. In this embodiment, the expression vector is a yeast expression vector.

[0210] Vectors can be introduced into prokaryotic or eukaryotic cells via a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell. Suitable methods for transforming or transfecting host cells can be found in, for example, Sambrook et al. (supra).

[0211] For stable transformation of bacterial cells, it is known that, depending upon the expression vector and transformation technique used, only a small fraction of cells will take-up and replicate the expression vector. In order to identify and select these transformants, a gene that encodes a selectable marker (e.g., resistance to an antibiotic) can be introduced into the host cells along with the gene of interest. Selectable markers include those that confer resistance to drugs such as, but not limited to, ampicillin, kanamycin, chloramphenicol, or tetracycline. Nucleic acids encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a polypeptide described herein or can be introduced on a separate vector. Cells stably transformed with the introduced nucleic acid can be identified by growth in the presence of an appropriate selection drug.

Host Cells

[0212] As used herein, an engineered or recombinant "host cell" is a cell used to produce a fatty acid derivative composition as further described herein.

[0213] A host cell is referred to as an "engineered host cell" or a "recombinant host cell" if the expression of one or more polynucleotides or polypeptides in the host cell are altered or modified as compared to their expression in a corresponding wild-type (or "native") host cell under the same conditions.

[0214] In any of the aspects of the invention described herein, the host cell can be selected from the group consisting of a eukaryotic plant, algae, cyanobacterium, green-sulfur bacterium, green non-sulfur bacterium, purple sulfur bacterium, purple non-sulfur bacterium, extremophile, yeast, fungus, engineered organisms thereof, or a synthetic organism. In some embodiments, the host cell is light dependent or fixes carbon. In some embodiments, the host cell has autotrophic activity.

[0215] Various host cells can be used to produce fatty acid derivatives, as described herein.

Mutants or Variants

[0216] In some embodiments, the polypeptide is a mutant or a variant of any of the polypeptides described herein. The terms "mutant" and "variant" as used herein refer to a polypeptide having an amino acid sequence that differs from a wild-type polypeptide by at least one amino acid. For example, the mutant can comprise one or more of the following conservative amino acid substitutions: replacement of an aliphatic amino acid, such as alanine, valine, leucine, and isoleucine, with another aliphatic amino acid; replacement of a serine with a threonine; replacement of a threonine with a serine; replacement of an acidic residue, such as aspartic acid and glutamic acid, with another acidic residue; replacement of a residue bearing an amide group, such as asparagine and glutamine, with another residue bearing an amide group; exchange of a basic residue, such as lysine and arginine, with another basic residue; and replacement of an aromatic residue, such as phenylalanine and tyrosine, with another aro-

matic residue. In some embodiments, the mutant polypeptide has about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more amino acid substitutions, additions, insertions, or deletions.

[0217] Preferred fragments or mutants of a polypeptide retain some or all of the biological function (e.g., enzymatic activity) of the corresponding wild-type polypeptide. In some embodiments, the fragment or mutant retains at least 75%, at least 80%, at least 90%, at least 95%, or at least 98% or more of the biological function of the corresponding wild-type polypeptide. In other embodiments, the fragment or mutant retains about 100% of the biological function of the corresponding wild-type polypeptide. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without affecting biological activity may be found using computer programs well known in the art, for example, LASERGENE™ software (DNASTAR, Inc., Madison, Wis.).

[0218] In yet other embodiments, a fragment or mutant exhibits increased biological function as compared to a corresponding wild-type polypeptide. For example, a fragment or mutant may display at least a 10%, at least a 25%, at least a 50%, at least a 75%, or at least a 90% improvement in enzymatic activity as compared to the corresponding wild-type polypeptide. In other embodiments, the fragment or mutant displays at least 100% (e.g., at least 200%, or at least 500%) improvement in enzymatic activity as compared to the corresponding wild-type polypeptide.

[0219] It is understood that the polypeptides described herein may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on the polypeptide function. Whether or not a particular substitution will be tolerated (i.e., will not adversely affect desired biological function, such as carboxylic acid reductase activity) can be determined as described in Bowie et al. (Science, 247: 1306-1310 (1990)). A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine), and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[0220] Variants can be naturally occurring or created in vitro. In particular, such variants can be created using genetic engineering techniques, such as site directed mutagenesis, random chemical mutagenesis, Exonuclease III deletion procedures, or standard cloning techniques. Alternatively, such variants, fragments, analogs, or derivatives can be created using chemical synthesis or modification procedures.

[0221] Methods of making variants are well known in the art. These include procedures in which nucleic acid sequences obtained from natural isolates are modified to generate nucleic acids that encode polypeptides having characteristics that enhance their value in industrial or laboratory applications. In such procedures, a large number of variant sequences having one or more nucleotide differences with respect to the sequence obtained from the natural isolate are generated and characterized. Typically, these nucleotide differences result in amino acid changes with respect to the polypeptides encoded by the nucleic acids from the natural isolates.

For example, variants can be prepared by using random and site-directed mutagenesis. Random and site-directed mutagenesis are described in, for example, Arnold, Curr. Opin. Biotech., 4: 450-455 (1993).

[0222] Random mutagenesis can be achieved using error prone PCR (see, e.g., Leung et al., Technique, 1: 11-15 (1989); and Caldwell et al., PCR Methods Applic., 2: 28-33 (1992)). In error prone PCR, PCR is performed under conditions where the copying fidelity of the DNA polymerase is low, such that a high rate of point mutations is obtained along the entire length of the PCR product. Briefly, in such procedures, nucleic acids to be mutagenized (e.g., a polynucleotide sequence encoding a carboxylic reductase enzyme) are mixed with PCR primers, reaction buffer, $MgCl_2$, $MnCl_2$, Taq polymerase, and an appropriate concentration of dNTPs for achieving a high rate of point mutation along the entire length of the PCR product. For example, the reaction can be performed using 20 fmoles of nucleic acid to be mutagenized, 30 pmole of each PCR primer, a reaction buffer comprising 50 mM KCl, 10 mM Tris HCl (pH 8.3), 0.01% gelatin, 7 mM $MgCl_2$, 0.5 mM $MnCl_2$, 5 units of Taq polymerase, 0.2 mM dGTP, 0.2 mM dATP, 1 mM dCTP, and 1 mM dTTP. PCR can be performed for 30 cycles of 94° C. for 1 min, 45° C. for 1 min, and 72° C. for 1 min. However, it will be appreciated that these parameters can be varied as appropriate. The mutagenized nucleic acids are then cloned into an appropriate vector, and the activities of the polypeptides encoded by the mutagenized nucleic acids are evaluated.

[0223] Site-directed mutagenesis can be achieved using oligonucleotide-directed mutagenesis to generate site-specific mutations in any cloned DNA of interest. Oligonucleotide mutagenesis is described in, for example, Reidhaar-Olson et al., Science, 241: 53-57 (1988). Briefly, in such procedures a plurality of double stranded oligonucleotides bearing one or more mutations to be introduced into the cloned DNA are synthesized and inserted into the cloned DNA to be mutagenized (e.g., a polynucleotide sequence encoding a CAR polypeptide). Clones containing the mutagenized DNA are recovered, and the activities of the polypeptides they encode are assessed.

[0224] Another method for generating variants is assembly PCR. Assembly PCR involves the assembly of a PCR product from a mixture of small DNA fragments. A large number of different PCR reactions occur in parallel in the same vial, with the products of one reaction priming the products of another reaction. Assembly PCR is described in, for example, U.S. Pat. No. 5,965,408.

[0225] Still another method of generating variants is sexual PCR mutagenesis. In sexual PCR mutagenesis, forced homologous recombination occurs between DNA molecules of different, but highly related, DNA sequences in vitro as a result of random fragmentation of the DNA molecule based on sequence homology. This is followed by fixation of the crossover by primer extension in a PCR reaction. Sexual PCR mutagenesis is described in, for example, Stemmer, Proc. Natl. Acad. Sci., U.S.A., 91: 10747-10751 (1994).

[0226] Variants can also be created by in vivo mutagenesis. In some embodiments, random mutations in a nucleic acid sequence are generated by propagating the sequence in a bacterial strain, such as an *E. coli* strain, which carries mutations in one or more of the DNA repair pathways. Such

"mutator" strains have a higher random mutation rate than that of a wild-type strain. Propagating a DNA sequence (e.g., a polynucleotide sequence encoding a CAR polypeptide) in one of these strains will eventually generate random mutations within the DNA. Mutator strains suitable for use for in vivo mutagenesis are described in, for example, International Patent Application Publication No. WO 1991/016427.

[0227] Variants can also be generated using cassette mutagenesis. In cassette mutagenesis, a small region of a double-stranded DNA molecule is replaced with a synthetic oligonucleotide "cassette" that differs from the native sequence. The oligonucleotide often contains a completely and/or partially randomized native sequence.

[0228] Recursive ensemble mutagenesis can also be used to generate variants. Recursive ensemble mutagenesis is an algorithm for protein engineering (i.e., protein mutagenesis) developed to produce diverse populations of phenotypically related mutants whose members differ in amino acid sequence. This method uses a feedback mechanism to control successive rounds of combinatorial cassette mutagenesis. Recursive ensemble mutagenesis is described in, for example, Arkin et al., Proc. Natl. Acad. Sci., U.S.A., 89: 7811-7815 (1992).

[0229] In some embodiments, variants are created using exponential ensemble mutagenesis. Exponential ensemble mutagenesis is a process for generating combinatorial libraries with a high percentage of unique and functional mutants, wherein small groups of residues are randomized in parallel to identify, at each altered position, amino acids which lead to functional proteins. Exponential ensemble mutagenesis is described in, for example, Delegrave et al., Biotech. Res, 11: 1548-1552 (1993).

[0230] In some embodiments, variants are created using shuffling procedures wherein portions of a plurality of nucleic acids that encode distinct polypeptides are fused together to create chimeric nucleic acid sequences that encode chimeric polypeptides as described in, for example, U.S. Pat. Nos. 5,965,408 and 5,939,250.

[0231] Insertional mutagenesis is mutagenesis of DNA by the insertion of one or more bases. Insertional mutations can occur naturally, mediated by virus or transposon, or can be artificially created for research purposes in the lab, e.g., by transposon mutagenesis. When exogenous DNA is integrated into that of the host, the severity of any ensuing mutation depends entirely on the location within the host's genome wherein the DNA is inserted. For example, significant effects may be evident if a transposon inserts in the middle of an essential gene, in a promoter region, or into a repressor or an enhancer region. Transposon mutagenesis and high-throughput screening was done to find beneficial mutations that increase the titer or yield of a fatty acid derivative or derivatives.

Culture Recombinant Host Cells and Cell Cultures/Fermentation

[0232] As used herein, the term "fermentation" broadly refers to the conversion of organic materials into target substances by host cells, for example, the conversion of a carbon source by recombinant host cells into fatty acids or derivatives thereof by propagating a culture of the recombinant host cells in a media comprising the carbon source.

[0233] As used herein, the term "conditions permissive for the production" means any conditions that allow a host cell to produce a desired product, such as a fatty acid or a fatty acid derivative. Similarly, the term "conditions in which the polynucleotide sequence of a vector is expressed" means any conditions that allow a host cell to synthesize a polypeptide. Suitable conditions include, for example, fermentation conditions. Fermentation conditions can comprise many parameters, including but not limited to temperature ranges, levels of aeration, feed rates and media composition. Each of these conditions, individually and in combination, allows the host cell to grow. Fermentation can be aerobic, anaerobic, or variations thereof (such as micro-aerobic). Exemplary culture media include broths or gels. Generally, the medium includes a carbon source that can be metabolized by a host cell directly. In addition, enzymes can be used in the medium to facilitate the mobilization (e.g., the depolymerization of starch or cellulose to fermentable sugars) and subsequent metabolism of the carbon source.

[0234] For small scale production, the engineered host cells can be grown in batches of, for example, about 100 mL, 500 mL, 1 L, 2 L, 5 L, or 10 L; fermented; and induced to express a desired polynucleotide sequence, such as a polynucleotide sequence encoding a CAR polypeptide. For large scale production, the engineered host cells can be grown in batches of about 10 L, 100 L, 1000 L, 10,000 L, 100,000 L, and 1,000,000 L or larger; fermented; and induced to express a desired polynucleotide sequence. Alternatively, large scale fed-batch fermentation may be carried out.

[0235] The fatty acid derivative compositions described herein are found in the extracellular environment of the recombinant host cell culture and can be readily isolated from the culture medium. A fatty acid derivative may be secreted by the recombinant host cell, transported into the extracellular environment or passively transferred into the extracellular environment of the recombinant host cell culture. The fatty acid derivative is isolated from a recombinant host cell culture using routine methods known in the art.

Products Derived from Recombinant Host Cells

[0236] As used herein, "fraction of modem carbon" or fM has the same meaning as defined by National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs4990B and 4990C, known as oxalic acids standards HOxI and HOxII, respectively. The fundamental definition relates to 0.95 times the 14C/12C isotope ratio HOxI (referenced to AD 1950). This is roughly equivalent to decay-corrected pre-Industrial Revolution wood. For the current living biosphere (plant material), fM is approximately 1.1.

[0237] Bioproducts (e.g., the fatty acid derivatives produced in accordance with the present disclosure) comprising biologically produced organic compounds, and in particular, the fatty acid derivatives produced using the fatty acid biosynthetic pathway herein, have not been produced from renewable sources and, as such, are new compositions of matter. These new bioproducts can be distinguished from organic compounds derived from petrochemical carbon on the basis of dual carbon-isotopic fingerprinting or ^{14}C dating. Additionally, the specific source of biosourced carbon (e.g., glucose vs. glycerol) can be determined by dual carbon-isotopic fingerprinting (see, e.g., U.S. Pat. No. 7,169,588, which is herein incorporated by reference).

[0238] The ability to distinguish bioproducts from petroleum based organic compounds is beneficial in tracking these materials in commerce. For example, organic compounds or chemicals comprising both biologically based and petroleum based carbon isotope profiles may be distinguished from organic compounds and chemicals made only of petroleum

based materials. Hence, the bioproducts herein can be followed or tracked in commerce on the basis of their unique carbon isotope profile.

[0239] Bioproducts can be distinguished from petroleum based organic compounds by comparing the stable carbon isotope ratio ($^{13}C/^{12}C$) in each sample. The $^{13}C/^{12}C$ ratio in a given bioproduct is a consequence of the $^{13}C/^{12}C$ ratio in atmospheric carbon dioxide at the time the carbon dioxide is fixed.

[0240] It also reflects the precise metabolic pathway. Regional variations also occur. Petroleum, C3 plants (the broadleaf), C4 plants (the grasses), and marine carbonates all show significant differences in $^{13}C/^{12}C$ and the corresponding $\delta^{13}C$ values. Furthermore, lipid matter of C3 and C4 plants analyze differently than materials derived from the carbohydrate components of the same plants as a consequence of the metabolic pathway. Within the precision of measurement, ^{13}C shows large variations due to isotopic fractionation effects, the most significant of which for bioproducts is the photosynthetic mechanism. The major cause of differences in the carbon isotope ratio in plants is closely associated with differences in the pathway of photosynthetic carbon metabolism in the plants, particularly the reaction occurring during the primary carboxylation (i.e., the initial fixation of atmospheric CO_2). Two large classes of vegetation are those that incorporate the "C3" (or Calvin-Benson) photosynthetic cycle and those that incorporate the "C4" (or Hatch-Slack) photosynthetic cycle.

[0241] In C3 plants, the primary CO_2 fixation or carboxylation reaction involves the enzyme ribulose-1,5-diphosphate carboxylase, and the first stable product is a 3-carbon compound. C3 plants, such as hardwoods and conifers, are dominant in the temperate climate zones.

[0242] In C4 plants, an additional carboxylation reaction involving another enzyme, phosphoenolpyruvate carboxylase, is the primary carboxylation reaction. The first stable carbon compound is a 4-carbon acid that is subsequently decarboxylated. The CO_2 thus released is refixed by the C3 cycle. Examples of C4 plants are tropical grasses, corn, and sugar cane.

[0243] Both C4 and C3 plants exhibit a range of $^{13}C/^{12}C$ isotopic ratios, but typical values are about −7 to about −13 per mil for C4 plants and about −19 to about −27 per mil for C3 plants (see, e.g., Stuiver et al., Radiocarbon 19:355 (1977)). Coal and petroleum fall generally in this latter range. The 13C measurement scale was originally defined by a zero set by Pee Dee Belemnite (PDB) limestone, where values are given in parts per thousand deviations from this material. The "613C" values are expressed in parts per thousand (per mil), abbreviated, % o, and are calculated as follows:

$$\delta^{13}C(\%)=[(^{13}C/^{12}C)\text{sample}-(^{13}C/^{12}C)\text{standard}]/(^{13}C/^{12}C)\text{ standard}\times 1000$$

[0244] Since the PDB reference material (RM) has been exhausted, a series of alternative RMs have been developed in cooperation with the IAEA, USGS, NIST, and other selected international isotope laboratories. Notations for the per mil deviations from PDB is $\delta^{13}C$. Measurements are made on CO_2 by high precision stable ratio mass spectrometry (IRMS) on molecular ions of masses 44, 45, and 46.

[0245] The compositions described herein include bioproducts produced by any of the methods described herein, including, for example, fatty aldehyde and alcohol products. Specifically, the bioproduct can have a $\delta^{13}C$ of about −28 or greater, about −27 or greater, −20 or greater, −18 or greater, −15 or greater, −13 or greater, −10 or greater, or −8 or greater. For example, the bioproduct can have a $\delta^{13}C$ of about −30 to about −15, about −27 to about −19, about −25 to about −21, about −15 to about −5, about −13 to about −7, or about −13 to about −10. In other instances, the bioproduct can have a $\delta^{13}C$ of about −10, −11, −12, or −12.3.

[0246] Bioproducts produced in accordance with the disclosure herein, can also be distinguished from petroleum based organic compounds by comparing the amount of ^{14}C in each compound. Because ^{14}C has a nuclear half-life of 5730 years, petroleum based fuels containing "older" carbon can be distinguished from bioproducts which contain "newer" carbon (see, e.g., Currie, "Source Apportionment of Atmospheric Particles", Characterization of Environmental Particles, J. Buffle and H. P. van Leeuwen, Eds., 1 of Vol. I of the IUPAC Environmental Analytical Chemistry Series (Lewis Publishers, Inc.) 3-74, (1992)).

[0247] The basic assumption in radiocarbon dating is that the constancy of ^{14}C concentration in the atmosphere leads to the constancy of ^{14}C in living organisms. However, because of atmospheric nuclear testing since 1950 and the burning of fossil fuel since 1850, ^{14}C has acquired a second, geochemical time characteristic. Its concentration in atmospheric CO_2, and hence in the living biosphere, approximately doubled at the peak of nuclear testing, in the mid-1960s. It has since been gradually returning to the steady-state cosmogenic (atmospheric) baseline isotope rate ($^{14}C/^{12}C$) of about 1.2×10-12, with an approximate relaxation "half-life" of 7-10 years. (This latter half-life must not be taken literally; rather, one must use the detailed atmospheric nuclear input/decay function to trace the variation of atmospheric and biospheric ^{14}C since the onset of the nuclear age.)

[0248] It is this latter biospheric ^{14}C time characteristic that holds out the promise of annual dating of recent biospheric carbon. ^{14}C can be measured by accelerator mass spectrometry (AMS), with results given in units of "fraction of modern carbon" (fM). fM is defined by National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs) 4990B and 4990C. As used herein, "fraction of modern carbon" or "fM" has the same meaning as defined by National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs) 4990B and 4990C, known as oxalic acids standards HOxI and HOxII, respectively. The fundamental definition relates to 0.95 times the $^{14}C/^{12}C$ isotope ratio HOxI (referenced to AD 1950). This is roughly equivalent to decay-corrected pre-Industrial Revolution wood. For the current living biosphere (plant material), fM is approximately 1.1.

[0249] The compositions described herein include bioproducts that can have an fM ^{14}C of at least about 1. For example, the bioproduct of the invention can have an fM ^{14}C of at least about 1.01, an fM ^{14}C of about 1 to about 1.5, an fM ^{14}C of about 1.04 to about 1.18, or an fM ^{14}C of about 1.111 to about 1.124.

[0250] Another measurement of ^{14}C is known as the percent of modern carbon (pMC). For an archaeologist or geologist using ^{14}C dates, AD 1950 equals "zero years old". This also represents 100 pMC. "Bomb carbon" in the atmosphere reached almost twice the normal level in 1963 at the peak of thermo-nuclear weapons. Its distribution within the atmosphere has been approximated since its appearance, showing values that are greater than 100 pMC for plants and animals living since AD 1950. It has gradually decreased over time with today's value being near 107.5 pMC. This means that a

fresh biomass material, such as corn, would give a ^{14}C signature near 107.5 pMC. Petroleum based compounds will have a pMC value of zero. Combining fossil carbon with present day carbon will result in a dilution of the present day pMC content. By presuming 107.5 pMC represents the ^{14}C content of present day biomass materials and 0 pMC represents the ^{14}C content of petroleum based products, the measured pMC value for that material will reflect the proportions of the two component types. For example, a material derived 100% from present day soybeans would give a radiocarbon signature near 107.5 pMC. If that material was diluted 50% with petroleum based products, it would give a radiocarbon signature of approximately 54 pMC.

[0251] A biologically based carbon content is derived by assigning "100%" equal to 107.5 pMC and "0%" equal to 0 pMC. For example, a sample measuring 99 pMC will give an equivalent biologically based carbon content of 93%. This value is referred to as the mean biologically based carbon result and assumes all the components within the analyzed material originated either from present day biological material or petroleum based material.

[0252] A bioproduct comprising one or more fatty acid derivatives as described herein can have a pMC of at least about 50, 60, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or 100. In other instances, a fatty acid derivative described herein can have a pMC of between about 50 and about 100; about 60 and about 100; about 70 and about 100; about 80 and about 100; about 85 and about 100; about 87 and about 98; or about 90 and about 95. In yet other instances, a fatty acid derivative described herein can have a pMC of about 90, 91, 92, 93, 94, or 94.2.

Screening Fatty Acid Derivative Compositions Produced by Recombinant Host Cells

[0253] To determine if conditions are sufficient to allow expression, a host cell can be cultured, for example, for about 4, 8, 12, 24, 36, or 48 hours. During and/or after culturing, samples can be obtained and analyzed to determine if the conditions allow expression. For example, the host cells in the sample or the medium in which the host cells were grown can be tested for the presence of a desired product. When testing for the presence of a product, assays, such as, but not limited to, TLC, HPLC, GC/FID, GC/MS, LC/MS, MS, can be used. Recombinant host cell cultures are screened at the 96 well plate level, 1 liter and 5 liter tank level and in a 1000 L pilot plant using a GC/FID assay for "total fatty species".

Utility of Fatty Acid Derivative Compositions

[0254] A fatty acid is a carboxylic acid with a long aliphatic tail (chain), which is either saturated or unsaturated. Most naturally occurring fatty acids have a chain of an even number of carbon atoms, from 4 to 28. Fatty acids are usually derived from triglycerides. When they are not attached to other molecules, they are known as "free" fatty acids. Fatty acids are usually produced industrially by the hydrolysis of triglycerides, with the removal of glycerol.

[0255] Palm, soybean, rapeseed, coconut oil and sunflower oil are currently the most common sources of fatty acids. The majority of fatty acids derived from such sources are used in human food products. Coconut oil and palm kernel oil (consist mainly of 12 and 14 carbon fatty acids). These are particularly suitable for further processing to surfactants for washing and cleansing agents as well as cosmetics. Palm, soybean, rapeseed, and sunflower oil, as well as animal fats such as tallow, contain mainly long-chain fatty acids (e.g., C18, saturated and unsaturated) which are used as raw materials for polymer applications and lubricants. Ecological and toxicological studies suggest that fatty acid-derived products based on renewable resources have more favorable properties than petrochemical-based substances.

[0256] Fatty aldehydes are used to produce many specialty chemicals. For example, aldehydes are used to produce polymers, resins (e.g., Bakelite), dyes, flavorings, plasticizers, perfumes, pharmaceuticals, and other chemicals, some of which may be used as solvents, preservatives, or disinfectants. In addition, certain natural and synthetic compounds, such as vitamins and hormones, are aldehydes, and many sugars contain aldehyde groups. Fatty aldehydes can be converted to fatty alcohols by chemical or enzymatic reduction.

[0257] Fatty alcohols have many commercial uses. Worldwide annual sales of fatty alcohols and their derivatives are in excess of U.S. $1 billion. The shorter chain fatty alcohols are used in the cosmetic and food industries as emulsifiers, emollients, and thickeners. Due to their amphiphilic nature, fatty alcohols behave as nonionic surfactants, which are useful in personal care and household products, such as, for example, detergents. In addition, fatty alcohols are used in waxes, gums, resins, pharmaceutical salves and lotions, lubricating oil additives, textile antistatic and finishing agents, plasticizers, cosmetics, industrial solvents, and solvents for fats.

[0258] The invention also provides a surfactant composition or a detergent composition comprising a fatty alcohol produced by any of the methods described herein. One of ordinary skill in the art will appreciate that, depending upon the intended purpose of the surfactant or detergent composition, different fatty alcohols can be produced and used. For example, when the fatty alcohols described herein are used as a feedstock for surfactant or detergent production, one of ordinary skill in the art will appreciate that the characteristics of the fatty alcohol feedstock will affect the characteristics of the surfactant or detergent composition produced. Hence, the characteristics of the surfactant or detergent composition can be selected for by producing particular fatty alcohols for use as a feedstock.

[0259] A fatty alcohol-based surfactant and/or detergent composition described herein can be mixed with other surfactants and/or detergents well known in the art. In some embodiments, the mixture can include at least about 10%, at least about 15%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, or a range bounded by any two of the foregoing values, by weight of the fatty alcohol. In other examples, a surfactant or detergent composition can be made that includes at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or a range bounded by any two of the foregoing values, by weight of a fatty alcohol that includes a carbon chain that is 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22 carbons in length. Such surfactant or detergent compositions also can include at least one additive, such as a microemulsion or a surfactant or detergent from non-microbial sources such as plant oils or petroleum, which can be present in the amount of at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at

least about 85%, at least about 90%, at least about 95%, or a range bounded by any two of the foregoing values, by weight of the fatty alcohol.

[0260] Esters have many commercial uses. For example, biodiesel, an alternative fuel, is comprised of esters (e.g., fatty acid methyl esters, fatty acid ethyl esters, etc.). Some low molecular weight esters are volatile with a pleasant odor, which makes them useful as fragrances or flavoring agents. In addition, esters are used as solvents for lacquers, paints, and varnishes. Furthermore, some naturally occurring substances, such as waxes, fats, and oils are comprised of esters. Esters are also used as softening agents in resins and plasticizers, flame retardants, and additives in gasoline and oil. In addition, esters can be used in the manufacture of polymers, films, textiles, dyes, and pharmaceuticals.

[0261] Hydrocarbons have many commercial uses. For example, shorter chain alkanes are used as fuels. Longer chain alkanes (e.g., from five to sixteen carbons) are used as transportation fuels (e.g., gasoline, diesel, or aviation fuel). Alkanes having more than sixteen carbon atoms are important components of fuel oils and lubricating oils. Even longer alkanes, which are solid at room temperature, can be used, for example, as a paraffin wax. In addition, longer chain alkanes can be cracked to produce commercially valuable shorter chain hydrocarbons.

[0262] Like short chain alkanes, short chain alkenes are used in transportation fuels. Longer chain alkenes are used in plastics, lubricants, and synthetic lubricants. In addition, alkenes are used as a feedstock to produce alcohols, esters, plasticizers, surfactants, tertiary amines, enhanced oil recovery agents, fatty acids, thiols, alkenylsuccinic anhydrides, epoxides, chlorinated alkanes, chlorinated alkenes, waxes, fuel additives, and drag flow reducers.

[0263] Ketones are used commercially as solvents. For example, acetone is frequently used as a solvent, but it is also a raw material for making polymers. Ketones are also used in lacquers, paints, explosives, perfumes, and textile processing. In addition, ketones are used to produce alcohols, alkenes, alkanes, imines, and enamines.

[0264] Lubricants are typically composed of olefins, particularly polyolefins and alpha-olefins. Lubricants can either be refined from crude petroleum or manufactured using raw materials refined from crude petroleum. Obtaining these specialty chemicals from crude petroleum requires a significant financial investment as well as a great deal of energy. It is also an inefficient process because frequently the long chain hydrocarbons in crude petroleum are cracked to produce smaller monomers. These monomers are then used as the raw material to manufacture the more complex specialty chemicals.

[0265] The invention is further illustrated by the following examples. The examples are provided for illustrative purposes only. They are not to be construed as limiting the scope or content of the invention in any way.

EXAMPLES

Example 1

Production Host Modifications—Attenuation of Acyl-CoA Dehydrogenase

[0266] This example describes the construction of a genetically engineered host cell wherein the expression of a fatty acid degradation enzyme is attenuated.

[0267] The fadE gene of *E. coli* MG1655 (an *E. coli* K strain) was deleted using the Lambda Red (also known as the Red-Driven Integration) system described by Datsenko et al., Proc. Natl. Acad. Sci. USA 97: 6640-6645 (2000), with the following modifications:

[0268] The following two primers were used to create the deletion of fadE:

```
Del-fadE-F
                                                    (SEQ ID NO: 9)
5'-AAAAACAGCAACAATGTGAGCTTTGTTGTAATTATATTGTAAACATA

TTGATTCCGGGGATCCGTCGACC;
and

Del-fadE-R
                                                    (SEQ ID NO: 10)
5'-AAACGGAGCCTTTCGGCTCCGTTATTCATTTACGCGGCTTCAACTTT

CCTGTAGGCTGGAGCTGCTTC
```

[0269] The Del-fadE-F and Del-fadE-R primers were used to amplify the kanamycin resistance (KmR) cassette from plasmid pKD13 (described by Datsenko et al., supra) by PCR. The PCR product was then used to transform electrocompetent *E. coli* MG1655 cells containing pKD46 (described in Datsenko et al., supra) that had been previously induced with arabinose for 3-4 hours. Following a 3-hour outgrowth in a super optimal broth with catabolite repression (SOC) medium at 37° C., the cells were plated on Luria agar plates containing 50 μg/mL of Kanamycin. Resistant colonies were identified and isolated after an overnight incubation at 37° C. Disruption of the fadE gene was confirmed by PCR amplification using primers fadE-L2 and fadE-R1, which were designed to flank the *E. coli* fadE gene.

[0270] The fadE deletion confirmation primers were:

```
                                                    (SEQ ID NO: 11)
fadE-L2 5'-CGGGCAGGTGCTATGACCAGGAC;
and

                                                    (SEQ ID NO: 12)
fadE-R1 5'-CGCGGCGTTGACCGGCAGCCTGG
```

[0271] After the fadE deletion was confirmed, a single colony was used to remove the KmR marker using the pCP20 plasmid as described by Datsenko et al., supra. The resulting MG1655 *E. coli* strain with the fadE gene deleted and the KmR marker removed was named *E. coli* MG1655 AfadE, or *E. coli* MG 1655 D1.

[0272] Fatty acid derivative ("total fatty species") production by the MG1655 *E. coli* strain with the fadE gene deleted was compared to fatty acid derivative production by *E. coli* MG1655. The data presented in FIG. **7** shows that deletion of the fadE gene did not affect fatty acid derivative production.

[0273] A number of exemplary host cell strains are described herein, examples of which are described below in Table 3.

TABLE 3

Genetic Characterization of *E. coli* strains.

Strain	Genetic Characterization
DV2	MG1655 F-, λ-, ilvG-, rfb-50, rph-1, ΔfhuA::FRT, ΔfadE::FRT

TABLE 3-continued

Genetic Characterization of *E. coli* strains.

Strain	Genetic Characterization
DV2.1	DV2 fabB::fabB[A329V]
D178	DV2.1 entD::loxP_P_{T5}_entD
EG149	D178 insH-11::(P_{LACUV5}-V_{cho}_fabV-S_{typ}_(fabHDG)-S_{typ}_fabA-C_{ace}_fabF_FRT), iFAB138
V642	EG149 rph+
SL313	V642 lacIZ::P_{A1}_'tesA/pDG109
V668	V642 ilvG$^+$
LC397	V668 lacIZ::P_{TRC}_12H08_kan
SL571	V668 lacIZ:: P_{TRC}_12H08_FRT
V324	D178 lacIZ::P_{TRC}_tesA
ALC310	D178/pALC310
V928	LC397/pV869
LC341	LC397/pLC308
V940	LC397/pV171.1
LC434	LC397/pLC274
EG442	V642 Tn7::P_{TRC}-ABR lacIZ::P_{T5O}-ABR
D851	SL571 yijP::Tn5-cat/pV171.1
D859	SL571 yijP::Tn5-cat/pEP55
BD64	DV2 ifab138 iT5fadR
Shu.002	Isogenic to BD64 except that it contains the T5 promoter controlling expression of the FAB138 operon
Shu.034	Isogenic to shu2 except that it also contains the yijP::Tn5-cat transposon cassette

ABR denotes the operon alrAadp1-fabB[A329G]-fadR,
P_{T5O} is an inducible T5 promoter containing a lacO binding site, and
$P_{TRC\text{-}AT}$ is the TRC promoter with the anti-termination region removed.

Example 2

Increased Flux Through the Fatty Acid Synthesis Pathway—Acetyl CoA Carboxylase Mediated

A. Fatty Ester Production.

[0274] The main precursors for fatty acid biosynthesis are malonyl-CoA and acetyl-CoA (FIG. **1**). It has been suggested that these precursors limit the rate of fatty acid biosynthesis in *E. coli*. In this study, synthetic acc operons [*Corynebacterium glutamicum* accABCD (±birA)] were overexpressed and the genetic modifications led to increased acetyl-coA and malonyl-CoA production in *E. coli*.

[0275] In one approach, in order to increase malonyl-CoA levels, an acetyl-CoA carboxylase enzyme complex from *Corynebacterium glutamicum* ("*C. glutamicum*") was overexpressed. Acetyl-CoA carboxylase ("acc") consists of four discrete subunits, accA, accB, accC and accD (FIG. **3**). The advantage of *C. glutamicum* acc is that two subunits are expressed as fusion proteins, accCB and accDA, respectively, which facilitates its balanced expression. Additionally, *C. glutamicum* birA, which biotinylates the accB subunit (FIG. **3**). Exemplary *C. glutamicum* bir DNA sequences are presented as SEQ ID NO:55 and SEQ ID NO:56. A *C. glutamicum* bir protein sequence is presented as SEQ ID NO:57.

[0276] The synthetic operons of the *C. glutamicum* acc genes were cloned in the following way in OP80: Ptrc1-accDACB, Ptrc3-accDACB, Ptrc1-accCBDA and Ptrc3-CBDA. Ptrc1 and Ptrc3 are derivatives of the commonly used Ptrc promoter, which allow attenuated transcription of target genes. Note that the native sequences were amplified from the chromosomal DNA as they showed favorable codon usage (only the codon for Arg6 in accCB was changed). The *C. glutamicum* birA gene was codon optimized and obtained by gene synthesis. It was cloned then downstream of the acc genes in all four operon constructs. Below we refer to the operon configuration accDACB as accD- and the operon configuration accDACB+birA as accD+.

[0277] The resulting plasmids were transformed into *E. coli* DAM1_i377, which contains integrated copies (i) of leaderless thioesterase 'tesA and acyl-CoA synthetase fadD from *E. coli* and Ester synthase 9 (ES9) from *Marinobacter hydrocarbonoclasticus*. All genes are controlled by Ptrc promoters. The strains were grown in 5NBT media in shake flasks and were analyzed for malonyl-CoA using the method described above. FIG. **9** shows that six of the eight *C. glutamicum* acc±birA constructs showed elevated levels of malonyl-CoA in logarithmic phase demonstrating their functionality in *E. coli*. It was noted that coexpression of birA further increased malonyl-CoA levels in the ptrc1/3_accDACB strains, in particular with the plasmid containing the Ptrc3-accDACB-birA operon configuration (plasmid pAS119.50D; SEQ ID NO:62).

[0278] In order to test the effect of combining panK and acc-birA overexpression, the optimized panK gene was cloned downstream of birA in ptrc1/3_accDACB-birA. Pantothenate kinase panK (or CoaA) catalyzes the first step in the biosynthesis of coenzyme A, an essential cofactor that is involved in many reactions, e.g. the formation of acetyl-CoA, the substrate for acetyl-CoA carboxylase. The resulting plasmids were transformed into DAM1_i377, grown in 5NBT (+TVS1) media in shake flasks, and the strains were analyzed for short-chain-CoAs using the method described above.

[0279] As shown in FIG. **9**, in log phase panK coexpression further increased malonyl-CoA levels and also increased acetyl-CoA levels demonstrating that panK can further increase the malonyl-CoA levels

[0280] The impact of coexpressing an acetyl-CoA carboxylase enzyme complex on fatty ester production was evaluated by expressing ester synthase 9 (SEQ ID NO:6) with and without acc genes in another *E. coli* production host. More specifically, plasmids OP80 (vector control), pDS57 (with ES9), pDS57-accD- (with ES9 and accDACB) or pDS57-accD+(with ES9 and accDACB-birA; SEQ ID NO:63) were transformed into *E. coli* strain DV2 and the corresponding transformants were selected on LB plates supplemented with 100 mg/L of spectinomycin.

[0281] Two transformants of each plasmid were independently inoculated into LB medium supplemented with 100 mg/L of spectinomycin and grown for 5-8 hours at 32C. The cultures were diluted 30-fold into a minimal medium with the following composition: 0.5 g/L NaCl, 1 mM MgSO4×7H2O, 0.1 mM CaCl2, 2 g/L NH4C1, 3 g/L KH2PO4, 6 g/L Na2HPO4, 1 mg/L thiamine, 1× trace metal solution, 10 mg/L ferric citrate, 100 mM Bis-Tris (pH7.0), 30 g/L glucose and 100 mg/L spectinomycin. After over-night growth at 32 C, the cultures were diluted 10-fold in quadruplicate into minimal medium of the same composition except that the media contained 1 g/L instead of 2 g/L NH4Cl and was supplemented with 1 mM IPTG and 2% (v/v) methanol. The resulting cultures were then grown at 32° C. in a shaker.

[0282] The production of fatty acid methyl esters (FAMEs) was analyzed by gas chromatography with flame ionization detector (GC-FID). The samples were extracted with butyl acetate in a ratio of 1:1 vol/vol. After strong vortexing, the samples were centrifuged, and the organic phase was analyzed by gas chromatography (GC). The analysis conditions were as follows: instrument: Trace GC Ultra, Thermo Electron Corporation with Flame ionization detector (FID) detec-

tor; column: DB-1 (1% diphenyl siloxane; 99% dimethyl siloxane) CO1 UFM 1/0.1/5 01 DET from Thermo Electron Corporation, phase pH 5, FT: 0.4 μm, length 5 m, id: 0.1 mm; inlet conditions: 250° C. splitless, 3.8 m 1/25 split method used depending upon sample concentration with split flow of 75 mL/m; carrier gas, flow rate: Helium, 3.0 mL/m; block temperature: 330° C.; oven temperature: 0.5 m hold at 50° C., 100° C./m to 330° C., 0.5 m hold at 330° C.; detector temperature: 300° C.; injection volume: 2 μL; run time/flow rate: 6.3 m/3.0 mL/m (splitless method), 3.8 m/1.5 mL/m (split 1/25 method), 3.04 m/1.2 mL/m (split 1/50 method).

[0283] FAMEs produced are shown in FIG. **10**. The expression of ES9 by itself in *E. coli* DV2 led to FAME production above the control DV20P80. Coexpression of the *C. glutamicum* acetyl-CoA carboxylase complex led to an approx. 1.5-fold increase in FAMEs and the additional expression of the *C. glutamicum* biotin protein ligase led to an approx. 5-fold increase in FAMEs. These results suggest that the increased supply of malonyl-CoA improves the ability of ES9 to convert intermediates of the fatty acid biosynthetic machinery to fatty acid methyl esters in *E. coli*.

B. Fatty Alcohol Production

[0284] The impact of coexpressing an acetyl-CoA carboxylase enzyme complex on Fatty alcohol production was evaluated by expressing the Acyl-ACP reductase (AAR) from *Synechococcus elongatus* with and without acc genes in *E. coli* DV2. The accD+operon configuration was selected as it gave the best results when coexpressed with ester synthase (see previous example).

[0285] The accDABC-birA operon was cloned downstream from the aar gene in pLS9185, a pCL1920 derivative) using Infusion technology, the resulting plasmid was transformed into *E. coli* DV2 and the corresponding transformants were selected on LB plates supplemented with 100 mg/L of spectinomycin.

[0286] Fatty alcohols produced are shown in FIG. **11**. The coexpression of AAR and accD+led to a ca. 1.5-fold increase in fatty alcohol titers as compared to the AAR only control (PLS9185). The data were reproducible (triplicate samples were shown). These results demonstrate that increasing malonyl-CoA levels lead to improved fatty acid production when this acyl-ACP reductase is used.

[0287] In addition, Example 3 describes co-expression of acc genes together with entire fab operons.

Example 3

Increased Flux Through the Fatty Acid Synthesis Pathway—iFABs

A. Fatty Acid Derivative Production

[0288] Strategies to increase the flux through the fatty acid synthesis pathway in recombinant host cells include both overexpression of native *E. coli* fatty acid biosynthesis genes and expression of exogenous fatty acid biosynthesis genes from different organisms in *E. coli*.

[0289] In this study, exogenous fatty acid biosynthesis genes from different organisms were combined in the genome of *E. coli* DV2. *E. coli* DV2 has the following genetic characterization: F-, λ-, ilvG-, rfb-50, rph-1, ΔfhuA::FRT, ΔfadE::FRT.

[0290] Sixteen strains containing iFABs 130-145 were evaluated. The detailed structure of iFABs 130-145 is presented in iFABs Table 4, below.

TABLE 4

Components found in iFABs 130-145.

Abbreviation	Full Description
St_fabD	*Salmonella typhimurium* fabD gene
nSt_fabH	*Salmonella typhimurium* fabH gene with the native RBS
sSt_fabH	*Salmonella typhimurium* fabH gene with a synthetic RBS
Cac_fabF	*Clostridium acetobutylicum* (ATCC824) fabF gene
St_fabG	*Salmonella typhimurium* fabG gene
St_fabA	*Salmonella typhimurium* fabA gene
St_fabZ	*Salmonella typhimurium* fabZ gene
BS_fabI	*Bacillus subtilis* fabI gene
BS_fabL	*Bacillus subtilis* fabL gene
Vc_FabV	*Vibrio chorlerae* fabV gene
Ec_FabI	*Escherichia coli* fabI gene

[0291] Each "iFAB" comprises various components in the following order: BS_fabI, BS_FabL, Vc_FabV, or Ec_FabI. All constructs contain St_H, St_D, and St_G, yet half of them have a synthetic RBS in front of St_H. All constructs contain either St_fabA or St_fabZ.

[0292] All constructs include Cac_fabF. See Table 4, below for the specific composition of iFABs 130-145.

TABLE 4

Compostion of iFABs 130-145.

Strain Name	BS_fabI	BS_fabL	Vc_fabV	Ec_fabI	nSt_fabH	sSt_fabH	St_fabD	St_fabG	St_fabA	St_fabZ	Cac_fabF
DV2ifab130	1	0	0	0	1	0	1	1	1	0	1
DV2iFab131	1	0	0	0	1	0	1	1	0	1	1
DV2iFab132	1	0	0	0	0	1	1	1	1	0	1
DV2iFab133	1	0	0	0	0	1	1	1	0	1	1
DV2ifab134	0	1	0	0	1	0	1	1	1	0	1
DV2iFab135	0	1	0	0	1	0	1	1	0	1	1
DV2iFab136	0	1	0	0	0	1	1	1	1	0	1
DV2iFab137	0	1	0	0	0	1	1	1	0	1	1
DV2iFab138	0	0	1	0	1	0	1	1	1	0	1
DV2iFab139	0	0	1	0	1	0	1	1	0	1	1
DV2iFab140	0	0	1	0	0	1	1	1	1	0	1
DV2iFab141	0	0	1	0	0	1	1	1	0	1	1
DV2ifab142	0	0	0	1	1	0	1	1	1	0	1
DV2iFab143	0	0	0	1	1	0	1	1	0	1	1
DV2iFab144	0	0	0	1	0	1	1	1	1	0	1
DV2iFab145	0	0	0	1	0	1	1	1	0	1	1

[0293] The plasmid pCL-WT TRC WT TesA was transformed into each of the strains shown above and a fermentation was run in FA2 media with 20 hours from induction to harvest at both 32° C. and 37° C. Data for production of "Total Fatty Species" from duplicate plate screens is shown in FIGS. **12**A and **12**B.

[0294] From this screen the best construct was determined to be DV2 with iFAB138. The iFAB138 construct was transferred into strain D178 to make strain EG149. This strain was used for further engineering. The sequence of iFAB138 in the genome of EG149 is presented as SEQ ID NO:19. (LOCUS integrated_pDS138 8029 bp ds-DNA linear15-JUL-2010).

B. Fatty Ester Production

[0295] A full synthetic fab operon was integrated into the *E. coli* chromosome and evaluated for increased FAME production by expression in *E. coli* DAM1 pDS57. In addition, four synthetic acc operons from *Corynebaterium glutamicum* were coexpressed and evaluated for improved FAME productivity. Several strains were obtained that produced FAMEs at a faster rate and higher titers.

[0296] Sixteen different fab operons were constructed (either assembled in vitro or as plasmid-based intermediates) as summarized in Table 5. The fab operons were put under the control of the lacUV5 promoter and integrated into the IS5-11 site of *E. coli* DAM1. These strains were named ifab130 to 145. They were transformed either with pDS57 (containing ester synthase 377) or pDS57 coexpressing different versions of acc operons, see above) for evaluation of FAME production. Exemplary plasmids are described in Table 5.

TABLE 5

Genotype of integrated fab operons.

Strain	Genotype of additional fab operon
DAM1-ifab130	IS5-11::PlacUV5 BsfabI (natRBS) StfabHDG StfabA CacfabF::FRT
DAM1-ifab131	IS5-11::PlacUV5 BsfabI (natRBS) StfabHDG StfabZ CacfabF::FRT
DAM1-ifab132	IS5-11::PlacUV5 BsfabI (synRBS) StfabHDG StfabART CacfabF::F
DAM1-ifab133	IS5-11::PlacUV5 BsfabI (synRBS) StfabHDG StfabZ CacfabF::FRT
DAM1-ifab134	IS5-11::PlacUV5 BsfabL (natRBS) StfabHDG StfabA CacfabF::FRT
DAM1-ifab135	IS5-11::PlacUV5 BsfabL (natRBS) StfabHDG StfabZ CacfabF::FRT
DAM1-ifab136	IS5-11::PlacUV5 BsfabL (synRBS) StfabHDG StfabA CacfabF::FRT
DAM1-ifab137	IS5-11::PlacUV5 BsfabL (synRBS) StfabHDG StfabZ CacfabF::FRT
DAM1-ifab138	IS5-11::PlacUV5 VcfabV (natRBS) StfabHDG StfabA CacfabF::FRT
DAM1-ifab139	IS5-11::PlacUV5 VcfabV (natRBS) StfabHDG StfabZ CacfabF::FRT
DAM1-ifab140	IS5-11::PlacUV5 VcfabV (synRBS) StfabHDG StfabA CacfabF::FRT
DAM1-ifab141	IS5-11::PlacUV5 VcfabV (synRBS) StfabHDG StfabZ CacfabF::FRT
DAM1-ifab142	IS5-11::PlacUV5 EcfabI (natRBS) StfabHDG StfabA CacfabF::FRT
DAM1-ifab143	IS5-11::PlacUV5 EcfabI (natRBS) StfabHDG StfabZ CacfabF::FRT

TABLE 5-continued

Genotype of integrated fab operons.

Strain	Genotype of additional fab operon
DAM1-ifab144	IS5-11::PlacUV5 EcfabI (synRBS) StfabHDG StfabA CacfabF::FRT
DAM1-ifab145	IS5-11::PlacUV5 EcfabI (synRBS) StfabHDG StfabZ CacfabF::FRT

Bs: *Bacillus subtilis*;
St: *Salmonella typhimurium*;
Cac: *Clostridium acetobutylicum*;
Vc: *Vibrio cholera*;
Ec: *Escherichia coli*.

TABLE 6

Plasmids containing ester synthase ES9 (from *Marinobacter hydrocarbonclasticus*) and synthetic acc operons (from *Corynebactrium glutamicum*)

Plasmid	Genes
pTB.071	pDS57-accCBDA
pTB.072	pDS57-ES9-accCBDA-birA
pTB.073	pDS57-ES9-accDACB
pTB.074	pDS57-ES9-accDACB-birA

pDS57 = pCL_ptrc-ES9

[0297] The DAM1 ifab strains were analyzed in 96-well plates (4NBT medium), shake flasks (5NBT medium) and in fermenters at 32° C. The best results were obtained in 96-well plates and in shake flasks, where several DAM1 ifab strains with pDS57-acc-birA plasmids showed higher FAME titers. In particular, DAM1 ifab131, ifab135, ifab137, ifab138 and ifab143 with pDS57-accDACB-birA showed 20-40% improved titers indicating that in these strains a higher flux through the fatty acid pathway was achieved, which apparently resulted in a better product formation rate (these results were reproducible in several independent experiments).

[0298] It was also observed that the FAMEs produced by some of the outperforming DAM1 ifab/acc strains showed a shift towards higher chain length. In particular, DAM1 ifab138 pDS57-accDACB-birA showed a significantly higher C16 and C18 to C8-C14 FAME ratio than the control. These results suggest that a stronger pull by tesA/fadD/WS377 may further improve FAME production.

C. Effect of Overexpressing fabH and fabI on Fatty Acid Methyl Ester (FAME) Production

[0299] Strategies to increase the flux through the fatty acid synthesis pathway in recombinant host cells include both overexpression of native fatty acid biosynthesis genes and expression of heterologous fatty acid biosynthesis genes. FabH and fabI are two fatty acid biosynthetic enzymes that have been shown to be feedback inhibited. A study was conducted to determine if FabH and FabI might be limiting the rate of FAME production.

[0300] FabH and fabI homologues (from *E. coli, B. subtilis, Acinetobacter baylyi* ADP1, *Marinobacter aquaeoli* VT8, and *Rhodococcus opacus*) were overexpressed as a synthetic operon and evaluated in *E. coli* DAM1 pDS57 (a strain observed to be a good FAME producer).

[0301] In one approach, fabHfabI operons were constructed from organisms that accumulate waxes (*A. baylyi, M. aquaeoli*) or triacylglycerides (*R. opacus*) and integrated into the chromosome of *E. coli* DAM1 pDS57. In a related

approach, a synthetic acc operons from *C. glutamicum* were co-expressed (as described in Example 2, above).

[0302] Eleven different fabHI operons were constructed (assembled in vitro) as summarized in Table 7. The fabHI operons were put under the control of IPTG inducible lacUV5 promoter and integrated into the IS5-11 site of *E. coli* DAM1. These strains were named as shown in the table below. They were transformed either with pDS57 (containing ester synthase 377) or pDS57 coexpressing different versions of acc operons for evaluation of FAME production.

D. Effect of Inserting a Strong Promoter in Front of Operon FAB138 on Fatty Acid Methyl Ester (FAME) Production

[0306] The lacUV5 promoter of FAB138 was replaced by a T5 promoter leading to higher levels of expression of FAB138, as confirmed by mRNA analysis. The expression of FAB138 from the T5 promoter resulted in a higher titer, yield and productivity of fatty esters.

[0307] Strain BD64 is DV2 ifab138 iT5_fadR. Strain shu. 002 is isogenic to strain BD64 except that it contains the T5 promoter controlling expression of the FAB138 operon.

TABLE 7

Genotype of integrated fabHI operons

Strain	Genotype of additional fab operon	plasmid
StEP117	DAM 1 IS5-11 ::PlacUV5 (synRBS) EcfabH (synRBS) bsfabI::kan	pDS57
StEP118	DAM 1 IS5-11 ::PlacUV5 (synRBS) EcfabH (synRBS) BsfabL::kan	pDS57
StEP127	DAM 1 IS5-11 ::PlacUV5 (ecRBS) ecfabH (ecRBS) bsfabI::kan	pDS57
StEP128	DAM 1 IS5-11 ::PlacUV5 (ecRBS) EcfabH (ecRBS) BsfabL::kan	pDS57
StEP129	DAM 1 IS5-11 ::PlacUV5 (ecRBS) ADP1fabH (ecRBS) ADP1fabI::kan	pDS57
StEP130	DAM 1 IS5-11 ::PlacUV5 (synRBS) ADP1fabH (synRBS) ADP1fabI::kan	pDS57
StEP131	DAM 1 IS5-11 ::PlacUV5 (synRBS) VT8fabH1 (synRBS) VT8fabI::kan	pDS57
StEP132	DAM 1 IS5-11 ::PlacUV5 (synRBS) VT8fabH2 (synRBS) VT8fabI::kan	pDS57
StEP133	DAM 1 IS5-11 ::PlacUV5 (ecRBS) VT8fabH1 (synRBS) VT8fabI::kan	pDS57
StEP134	DAM 1 IS5-11 ::PlacUV5 (ecRBS) VT8fabH2 (synRBS) VT8fabI::kan	pDS57
StEP151	DAM 1 IS5 11::PlacUV5 (synRBS)RofabI (synRBS) RofabH::kan	pDS57
StEP153	DAM 1 IS5-11 ::PlacUV5 (ecRBS) ADP1fabH (ecRBS) ADP1fabI::kan	pDS57-accCBDA
StEP154	DAM 1 IS5-11 ::PlacUV5 (ecRBS) ADP1fabH (ecRBS) ADP1fabI::kan	pDS57-accDACB
StEP155	DAM 1 IS5-11 ::PlacUV5 (ecRBS) ADP1fabH (ecRBS) ADP1fabI::kan	pDS57-accCBDA-birA
StEP156	DAM 1 IS5-11 ::PlacUV5 (ecRBS) ADP1fabH (ecRBS) ADP1fabI::kan	pDS57-accDACB-birA
StEP157	DAM 1 IS5-11 ::PlacUV5 (synRBS) ecfabH (synRBS) bsfabI::kan	pDS57-accCBDA
StEP158	DAM 1 IS5-11 ::PlacUV5 (synRBS) ecfabH (synRBS) bsfabI::kan	pDS57-accCBDA-birA
StEP159	DAM 1 IS5-11 ::PlacUV5 (ecRBS) ecfabH (synRBS) bsfabI::kan	pDS57-accCBDA
StEP160	DAM 1 IS5-11 ::PlacUV5 (ecRBS) ecfabH (synRBS) bsfabI::kan	pDS57-accCBDA-birA
StEP161	DAM 1 IS5-11 ::PlacUV5 (ecRBS) VT8fabH1 (synRBS) VT8fabI::kan	pDS57-accCBDA
StEP162	DAM 1 IS5-11 ::PlacUV5 (ecRBS) VT8fabH1 (synRBS) VT8fabI::kan	pDS57-accCBDA-birA
StEP163	DAM 1 IS5-11 ::PlacUV5 (ecRBS) VT8fabH2 (synRBS) VT8fabI::kan	pDS57-accCBDA
StEP164	DAM 1 IS5-11 ::PlacUV5 (ecRBS) VT8fabH2 (synRBS) VT8fabI::kan	pDS57-accCBDA-birA

Bs: *Bacillus subtilis*;
Ec: *Escherichia coli*,
ADP1: *Acinetobacter* sp. ADP1,
VT8: *Marinobacter aquaeolei* VT8,
Ro; *Rhodococcus opacus* B4

[0303] The DAM1 ifabHI strains were analyzed in 96-well plates (4NBT medium), shake flasks (5NBT medium) and in fermenters at 32° C.

[0304] In shake flask, a number of the ifabHI strains carrying pDS57 plasmid performed better than the control DAM1 pDS57strain, reaching 10 to 15% higher FAME titers (FIG. **13**). Additional increase in FAME titers was obtained when ifabHI strains were transformed with pDS57-acc-birA plasmids, in particular an increase of 50% in FAME titers was observed in strain StEP156 (DAM1 5-11::UV5(ecRBS) ADP1fabH (ecRBS)ADP1fabI pDS57-accDACB-birA) (FIG. **14**).

[0305] Some of the ifabHI strains were also run in fermenters, where an increase in FAME titers, specific productivity and yield were also observed (FIG. **15**), indicating that in these strains a higher flux through the fatty acid pathway was achieved, which resulted in a better product formation rate. In particular stEP129 (DAM1 5-11::UV5(ecRBS)ADP1fabH (ecRBS)ADP1fabI pDS57) showed higher FAME titers and yield in several independent fermentation runs. Other combinations of fabH and fabI may be used to achieve similar effects. Although FAME is exemplified here, this approach increases the flux through the fatty acid biosynthetic pathway and is therefore a useful approach to increase production of any fatty acid derivative.

TABLE 8

Primers used to Generate iT5 138 Cassette and Verify its Insertion in New Strains

Primer Name	SEQ ID NO	Sequence
DG405	20	TTGTCCATCTTTATATAATTTGGGGGTAGGGTGTT CTTTATGTAAAAAAAACgtttTAGGATGCATATGG CGGCC
DG406	21	GATAAATCCACGAATTTTAGGTTTGATGATCATTG GTCTCCTCCTGCAGGTGCGTGTTCGTCGTCATCGC AATTG
DG422	22	ACTCACCGCATTGGTGTAGTAAGGCGCACC
DG423	23	TGAATGTCATCACGCAGTTCCCAGTCATCC
DG744	24	CCATCTTCTTTGTACAGACGTTGACTGAACATG
DG749	24	GCACCATAGCCGTAATCCCACAGGTTATAG
oTREE047	26	TGTCATTAATGGTTAATAATGTTGA

[0308] Primers DG405 and DG406 were used to amplify a cat-loxP and T5 promoter cassette adding 50 bp homology to each end of the PCR product, such that it could be integrated into any strain replacing the lacUV5 promoter regulating expression of the FAB138 operon. The cat-loxP-T5 promoter was transformed into BD64/pKD46 strain. Transformants were recovered on LB+chloramphenicol plates at 37° C. overnight, patched to a fresh LB+chloramphenicol plate, and verified by colony PCR using primers DG422 and DG423. Plasmid pJW168 was transformed into strain BD64 i-cat-loxP-T5_138 and selected on LB+carbenicillin plates at 32° C. In order to remove the cat marker, expression of the cre-recombinase was induced by IPTG. The plasmid pHW168 was removed by growing cultures at 42° C. Colonies were patched on LB+chloramphenicol and LB+carbenicillin to verify loss of pJW168 and removal of cat marker, respectively. The colony was also patched into LB as a positive control, all patched plates were incubated at 32° C. The removal of the cat marker was confirmed by colony PCR using primers DG422 and DG423. The resulting PCR product was verified by sequencing with primers EG744, EG749 and oTREE047, the strain was called shu.002. FIGS. **16**A and B provides a map of the strains generated.

[0309] FIG. **16** shows the FAB138 locus: a diagram of the cat-loxP-T5 promoter integrated in front of FAB138 (FIG. **16**A) and a diagram of the iT5_138 promoter region (FIG. **16**B).

[0310] The sequence of the cat-loxP-T5 promoter integrated in front of FAB138 with 50 base pair of homology shown in each side of cat-loxP-T5 promoter region is presented as SEQ ID NO:1 and the sequence of the iT5_138 promoter region with 50 base pair homology in each side is presented as SEQ ID NO:2.

[0311] There are a number of conditions that can lead to increased fatty acid flux. In this example increased fatty acid flux was achieved by altering the promoter strength of operon FAB138. The expression of FAB138 from the T5 promoter was beneficial, however, when this promoter change was combined with the insertion of yijP::Tn5 cassette further improvements were observed in titer, yield and productivity of fatty acid esters and other fatty acid derivatives. (See Example 5).

Example 4

Increasing the Amount of Free Fatty Acid (FFA) Product by Repairing the Rph and ilvG Mutations

[0312] The ilvG and rph mutations were corrected in this strain resulting in higher production of FFA. Strains EG149 (D178 is 5-11::iFAB138) and V668 (EG149 rph+ilvG+) were transformed with pCL-tesA obtained from D191. Fermentation was run at 32° C. in FA2 media for 40 hours to compare the FFA production of strains D178, EG149, and V668 with pCL-tesA. Fermentation and extraction was run according to a standard FALC fermentation protocol exemplified by the following.

[0313] A frozen cell bank vial of the selected *E. coli* strain was used to inoculate 20 mL of LB broth in a 125 mL baffled shake flask containing spectinomycin antibiotic at a concentration of 115 μg/mL. This shake flask was incubated in an orbital shaker at 32° C. for approximately six hours, then 1.25 mL of the broth was transferred into 125 mL of low P FA2 seed media (2 g/L NH_4C1, 0.5 g/L NaCl, 3 g/L KH_2PO_4, 0.25 g/L $MgSO_4$-7H2O, 0.015 g/L mM $CaCl_2$-2H2O, 30 g/L glucose, 1 mL/L of a trace minerals solution (2 g/L of $ZnCl_2$. $4H_2O$, 2 g/L of $CaCl_2$. $6H_2O$, 2 g/L of Na_2MoO_4. $2H_2O$, 1.9 g/L of $CuSO_4.5H_2O$, 0.5 g/L of H_3BO_3, and 10 mL/L of concentrated HCl), 10 mg/L of ferric citrate, 100 mM of Bis-Tris buffer (pH 7.0), and 115 μg/mL of spectinomycin), in a 500 mL baffled Erlenmeyer shake flask, and incubated on a shaker overnight at 32° C.

[0314] 100 mL of this low P FA2 seed culture was used to inoculate a 5 L Biostat Aplus bioreactor (Sartorius BBI), initially containing 1.9 L of sterilized F1 bioreactor fermentation medium. This medium is initially composed of 3.5 g/L of KH_2PO_4, 0.5 g/L of $(NH_4)_2SO_4$, 0.5 g/L of $MgSO_4$ heptahydrate, 10 g/L of sterile filtered glucose, 80 mg/L ferric citrate, 5 g/L Casamino acids, 10 mL/L of the sterile filtered trace minerals solution, 1.25 mL/L of a sterile filtered vitamin solution (0.42 g/L of riboflavin, 5.4 g/L of pantothenic acid, 6 g/L of niacin, 1.4 g/L of pyridoxine, 0.06 g/L of biotin, and 0.04 g/L of folic acid), and the spectinomycin at the same concentration as utilized in the seed media. The pH of the culture was maintained at 6.9 using 28% w/v ammonia water, the temperature at 33° C., the aeration rate at 1 lpm (0.5 v/v/m), and the dissolved oxygen tension at 30% of saturation, utilizing the agitation loop cascaded to the DO controller and oxygen supplementation. Foaming was controlled by the automated addition of a silicone emulsion based antifoam (Dow Corning 1410).

[0315] A nutrient feed composed of 3.9 g/L $MgSO_4$ heptahydrate and 600 g/L glucose was started when the glucose in the initial medium was almost depleted (approximately 4-6 hours following inoculation) under an exponential feed rate of 0.3 hr-1 to a constant maximal glucose feed rate of 10-12 g/L/hr, based on the nominal fermentation volume of 2 L. Production of fatty alcohol in the bioreactor was induced when the culture attained an OD of 5 AU (approximately 3-4 hours following inoculation) by the addition of a 1M IPTG stock solution to a final concentration of 1 mM. The bioreactor was sampled twice per day thereafter, and harvested approximately 72 hours following inoculation.

[0316] A 0.5 mL sample of the well-mixed fermentation broth was transferred into a 15 mL conical tube (VWR), and thoroughly mixed with 5 mL of butyl acetate. The tube was inverted several times to mix, then vortexed vigorously for approximately two minutes. The tube was then centrifuged for five minutes to separate the organic and aqueous layers, and a portion of the organic layer transferred into a glass vial for gas chromatographic analysis.

[0317] Correcting the rph and ilvG mutations resulted in a 116% increase in the FFA production of the base strain with pCL-tesA. As seen in FIG. **17**, V668/pCL-tesA produces more FFA than the D178/pCL-tesA, or the EG149/pCL-tesA control. Since FFA is a precursor to the LS9 products, higher FFA production is a good indicator that the new strain can product higher levels of LS9 products.

[0318] It has been demonstrated that expression of many genes, not limited to, fabA, B, Z, G, H, D, and fadR can lead to increased fatty acid production. Further strain improvements are likely to result in higher titers, yields and productivity of fatty acid derivatives such as FALC by recombinant host cells.

Example 5

Increased Production of Fatty Acid Derivatives by Transposon Mutagenesis—yijP

A. Fatty Alcohol Production

[0319] To improve the titer, yield, productivity of fatty alcohol production by *E. coli*, transposon mutagenesis and

high-throughput screening was carried out and beneficial mutations were sequenced. A transposon insertion in the yijP strain was shown to improve the strain's fatty alcohol yield in both shake flask and fed-batch fermentations.

[0320] The SL313 strain produces fatty alcohols. The genotype of this strain is provided in Table **.

[0321] The genotype of this strain is MG1655 (ΔfadE::FRT ΔfhuA::FRT fabBA329V ΔentD::T5-entD ΔinsH-11::PlacUV5 fab138 rph+lacI::PA1_tesA) containing the plasmid pDG109 (pCL1920_PTRC_carBopt_12H08_alrAadp1_fabB[A329G]_fadR).

[0322] Briefly, transposon mutagenesis was carried out by preparation of transposon DNA was prepared by cloning a DNA fragment into the plasmid EZ-Tn5™ pMOD™<R6K ori/MCS> (Epicentre Biotechnologies). The DNA fragment contains a T5 promoter and the cat gene flanked by loxP sites. The resulting plasmid was named p100.38 and the sequence is listed in Appendix I. This plasmid was digested with PshAI restriction enzyme, incubated with EZ-Tn5™ Transposase enzyme (Epicentre Biotechnologies), and electroporated into electrocompetent SL313 cells as per the manufacturer's instructions. The resulting colonies contained the transposon DNA inserted randomly into the chromosome of SL313.

[0323] Transposon clones were then subjected to high-throughput screening to measure production of fatty alcohols. Briefly, colonies were picked into deep-well plates containing LB, grown overnight, inoculated into fresh LB and grown for 3 hours, inoculated into fresh FA-2.1 media, grown for 16 hours, then extracted using butyl acetate. The crude extract was derivatized with BSTFA (N,O-bis[Trimethylsilyl]trifluoroacetamide) and analyzed using GC/FID. Spectinomycin (100 ug/mL) was included in all media to maintain selection of the pDG109 plasmid.

[0324] Hits were selected by choosing clones that produced a similar total fatty species as the control strain SL313, but that had a higher percent of fatty alcohol species and a lower percent of free fatty acids than the control. Strain 68F11 was identified as a hit and was validated in a shake flask fermentation, according to the shake flask fermentation method described below. A comparison of transposon hit 68F11 to control strain SL313 indicated that 68F11 produces a higher percentage of fatty alcohol species than the control, while both strains produce similar titers of total fatty species.

[0325] A single colony of hit 68F11, named LC535, was sequenced to identify the location of the transposon insertion. Sequencing was performed according to previous transposon IDFs. Briefly, genomic DNA was purified from a 10 mL overnight LB culture using the kit ZR Fungal/Bacterial DNA MiniPrep™ (Zymo Research) according to the manufacturer's instructions. The purified genomic DNA was sequenced outward from the transposon using primers internal to the transposon:

DG150
(SEQ ID NO: 27)
5'-GCAGTTATTGGTGCCCTTAAACGCCTGGTTGCTACGCCTG-3'

DG131
(SEQ ID NO: 28)
5'-GAGCCAATATGCGAGAACACCCGAGAA-3'

[0326] Strain LC535 was determined to have a transposon insertion in the yijP gene (FIG. **18**). yijP encodes a conserved inner membrane protein whose function is unclear. The yijP gene is in an operon and co-transcribed with the ppc gene, encoding phosphoenolpyruvate carboxylase, and the yijO gene, encoding a predicted DNA-binding transcriptional regulator of unknown function. Promoters internal to the transposon likely have effects on the level and timing of transcription of yijP, ppc and yijO, and may also have effects on adjacent genes frwD, pflC, pfld, and argE. Promoters internal to the transposon cassette are shown, and may have effects on adjacent gene expression.

[0327] Strain LC535 was evaluated in a fed-batch fermentation on two different dates. Both fermentations demonstrated that LC535 produced fatty alcohols with a higher yield than control SL313, and the improvement was 1.3-1.9% absolute yield based on carbon input.

[0328] The yijP transposon cassette was further evaluated in a different strain V940, which produces fatty alcohol at a higher yield than strain SL313. The yijP::Tn5-cat cassette was amplified from strain LC535 using primers:

LC277
(SEQ ID NO: 29)
5'-CGCTGAACGTATTGCAGGCCGAGTTGCTGCACCGCTCCCGCCAGGCA
G-3'

LC278
(SEQ ID NO: 30)
5'-GGAATTGCCACGGTGCGGCAGGCTCCATACGCGAGGCCAGGTTATCC
AACG-3'

This linear DNA was electroporated into strain SL571 and integrated into the chromosome using the lambda red recombination system. Colonies were screened using primers outside the transposon region:

(SEQ ID NO: 31)
DG407 5'-AATCACCAGCACTAAAGTGCGCGGTTCGTTACCCG-3'

(SEQ ID NO: 32)
DG408 5'-ATCTGCCGTGGATTGCAGAGTCTATTCAGCTACG-3'

[0329] A colony with the correct yijP transposon cassette was transformed with the production plasmid pV171.1 to produce strain D851. D851 (V940 yijP::Tn5-cat) was tested in a shake-flask fermentation against isogenic strain V940 that does not contain the yijP transposon cassette. The result of this fermentation showed that the yijP transposon cassette confers production of a higher percent of fatty alcohol by the D851 strain relative to the V940 strain and produces similar titers of total fatty species as the V940 control strain.

[0330] Strain D851 was evaluated in a fed-batch fermentation on two different dates. Data from these fermentations is shown in Table 9 which illustrates that in 5-liter fed-batch fermentations, strains with the yijP::Tn5-cat transposon insertion had an increased total fatty species ("FAS") yield and an increase in percent fatty alcohol ("FALC").

[0331] The terms "total fatty species" and "total fatty acid product" may be used interchangeably herein with reference to the amount of fatty alcohols, fatty aldehydes and free fatty acids, as evaluated by GC-FID as described in International Patent Application Publication WO 2008/119082. The same terms may be used to mean fatty esters and free fatty acids when referring to a fatty ester analysis. As used herein, the term "fatty esters" includes beta hydroxy esters.

TABLE 9

Effect of yijp transposon insertion on titer and yield of FAS and FALC.

Strain	FAS Titer	FAS Yield	Percent FALC	FALC Yield
V940	68 g/L	18.7%	95.0%	17.8%
D851	70 g/L	19.4%	96.1%	18.6%
V940	64 g/L	18.4%	91.9%	16.9%
D851	67 g/L	19.0%	94.0%	17.8%

Shake Flask Fermentation Method

[0332] To assess production of fatty acid esters in tank a glycerol vial of desired strain was used to inoculate 20 mL LB+spectinomycin in shake flask and incubated at 32° C. for approximately six hours. 4 mL of LB culture was used to inoculate 125 mL Low PFA Seed Media (below), which was then incubated at 32° C. shaker overnight. 50 mL of the overnight culture was used to inoculate 1 L of Tank Media. Tanks were run at pH 7.2 and 30.5° C. under pH stat conditions with a maximum feed rate of 16 g/L/hr (glucose or methanol).

TABLE 10

Low P FA Seed Media

Component	Concentration
NH4Cl	2 g/L
NaCl	0.5 g/L
KH2PO4	1 g/L
MgSO4—7H2O	0.25 g/L
CaCl2—2H2O	0.015 g/L
Glucose	20 g/L
TM2 Trace Minerals solution	1 mL/L
Ferric citrate	10 mg/L
Bis Tris buffer (pH 7.0)	100 mM
Spectinomycin	115 mg/L

TABLE 11

Tank Media

Component	Concentration
(NH4)2SO4	0.5 g/L
KH2PO4	3.0 g/L
Ferric Citrate	0.034 g/L
TM2 Trace Minerals Solution	10 mL/L
Casamino acids	5 g/L
Post sterile additions	
MgSO4—7H2O	2.2 g/L
Trace Vitamins Solution	1.25 mL/L
Glucose	5 g/L
Inoculum	50 mL/L

[0333] Further studies suggest that the improved titer and yield of FAS and FALC in strains with the yijP transposon insertion is due to reduction in the activity of phosphoenolpyruvate carboxylase (ppc). A ppc enzyme assay was carried out in-vitro in the following strains to evaluate this hypothesis.

[0334] 1) Δppc=DG14 (LC942 Δppc::cat-sacB/pLC56)

[0335] 2) wt-ppc=DG16 (LC942/pLC56)

[0336] 3) yijP::Tn5=DG18 (LC942 yijP::Tn5-cat/pCL56)

[0337] Ppc activity was measured in cells grown in a shake flask fermentation (as detailed above) and harvested 12-16 hours after induction. Approximately 5 mL of cells were centrifuged and the cell paste was suspended in BugBuster Protein Extraction Reagent (Novagen) with a protease inhibitor cocktail solution. The cell suspension was incubated with gentle shaking on a shaker for 20 min. Insoluble cell debris was removed by centrifugation at 16,000×g for 20 min at 4° C. followed by transferring the supernatant to a new tube. Ppc activity in the cell lysate was determined by a coupling reaction with citrate synthase using following reaction mixture: 0.4 mM acetyl-CoA, 10 mM phosphoenolpyruvate, 0.5 mM monobromobimane, 5 mM $MgCl_2$, 10 mM $NaHCO_3$, and 10 units citrate synthase from porcine heart in 100 mM Tris-HCl (pH 8.0). The formation of CoA in the reaction with citrate synthase using oxaloacetate and acetyl-CoA was monitored photometrically using fluorescent derivatization of CoA with monobromobimane.

[0338] The Ppc assay results showed that the yijP::Tn5-cat transposon cassette decreased the Ppc activity in the cell. The results also indicate that the highest yield of fatty alcohol production requires a level of Ppc expression lower than the wild-type level.

[0339] Proteomics data was also collected to assess the abundance of the Ppc protein in two strains with and without the yijP::Tn5-cat transposon cassette. Protein samples were collected from strains V940 and D851 grown in bioreactors under standard fatty alcohol production conditions. Samples were taken at three different time points: 32, 48, 56 hours and prepared for analysis.

[0340] Sample collection and protein isolation was carried out as follows:

[0341] 1. 20 ml of fermentation broth were collected from each bioreactor at each time point. Samples were quenched with ice-cold PBS and harvested by centrifugation (4500 rpm/10 min) at 4° C. Cell pellet was washed with ice-cold PBS and centrifuged one more time and stored at −80° C. for further processing.

[0342] 2. Total protein extraction was performed using a French press protocol. Briefly, cell pellets were resuspended in 7 ml of ice-cold PBS and French pressed at 2000 psi twice to ensure complete lysing of the bacteria. Samples were centrifuged for 20 min at 10000 rpm at 4° C. to separate non-lysed cells and cell debris from the protein fraction. Total protein concentration of clear lysate was determined using BCA Protein Assay Reagent. Samples were diluted to 2 mg proteins/ml concentration and frozen at −80° C.

[0343] 3. Samples were resuspended in the appropriate buffer and trypsinized overnight at 37° C. and lyophilized. Fragmented protein samples were labeled with isotopically enriched methylpiperazine acetic acid at room temperature for 30 min. Labeled samples were separated using cation exchange liquid chromatography and subjected to mass spectroscopy analysis using an ion trap mass spectrometer. Raw data was normalized using background subtraction and bias correction.

[0344] Proteomics data showed a significant reduction in the relative abundance of Ppc protein in D851 strain when compared to V940 at 36 hours and 48 hours). These data show that the yijP::Tn5-cat transposon cassette results in a significant reduction in Ppc abundance in the cell. This suggests that

the observed benefits to fatty alcohol production by strains harboring the yijP::Tn5-cat transposon hit is due to reducing the amount of Ppc protein.

[0345] These results suggest that altering ppc activity can improve the yield of fatty acid derivatives. There are a number of ways to alter the expression of the ppc gene, and the yijP transposon insertion is one way to accomplish this. While the mechanism is not part of the invention, if the effect of reducing phosphoenolpyruvate carboxylase activity is to limit the flow of carbon through the TCA cycle, one could achieve similar results by decreasing the activity of citrate synthase (gltA) or slowing the TCA cycle by decreasing the activity of any of the enzymes involved in the TCA cycle.

B. Fatty Ester Production

[0346] Additional strains with a transposon insertion in yijP were evaluated for production of fatty acid esters. Strains containing a transposon insertion in yijP were shown to produce higher yields of fatty acid esters and maintain the glucose utilization rate for a longer time in tanks.

[0347] A strain designated, "shu.010" was developed which is isogenic to strain BD64 except that it contains the yijP::Tn5-cat transposon cassette. The cassette containing the yijP::(Tn5) transposon DNA was amplified from strain DG851 using primers DG408 and DG407 (Table 12). The cassette was transformed into BD64/pKD46. Transformants were recovered on LB+chloramphenicol plates at 37° C. overnight, patched to a fresh LB+chloramphenicol plate, and verified by colony PCR using primers DG131, DG407, and DG408.

TABLE 12

Primers used to amplify yijP::Tn5 cassette and verify its insertion in new strains

Name	SEQ ID NO:	Sequence	Description
DG131	28	GAGCCAATATGCGAGAACACCCGAGAA	Primer in Tn5
DG407	31	AATCACCAGCACTAAAGTGCGCGGTTCGTTACCCG	Primer 568 bp of Tn5 insertion site
DG408	32	ATCTGCCGTGGATTGCAGAGTCTATTCAGCTACG	Forward primer 464 bp of Tn5 insertion site
	Expected in transformants	Wild-type	Primer Pair
Product Size:	572 bp		DG131/DG408
Product size:		1101 bp	DG407/DG408

[0348] Plasmid pKEV022 was transformed into shu.010. After selection in LB+spectinomycin plates, one colony was selected and called shu.015. Strain shu.015 was grown in tanks using standard conditions (see Appendix I for media and tank conditions). The tank performance of shu.015 was compared to strains KEV006.1 (BD64 pKEV018) and KEV075 (BD64 pKEV022) for Total Fatty Acid Product, Total Product Yield and glucose utilization rate.

[0349] The yield of total fatty acid products for all strains was similar, however, shu.015 was able to sustain higher glucose utilization rates for a longer time than either KEV006.1 or KEV075, suggesting that yijP::Tn5 was responsible for the improvement.

Example 6

Increased Flux Through the Fatty Acid Synthesis Pathway—Acyl Carrier Protein (ACP) Mediated Fatty Alcohol Production

[0350] When terminal pathway enzymes from sources other than *E. coli* are expressed in *E. coli* as the heterologous host to convert fatty acyl-ACPs to products, limitations may exist in the recognition, affinity and/or turnover of the recombinant pathway enzyme towards the *E. coli* fatty acyl-ACPs. Note that although ACP proteins are conserved to some extent in all organisms, their primary sequence can differ significantly.

[0351] To test this hypothesis the acp genes from several cyanobacteria were cloned downstream from the *Synechococcus elongatus* PCC7942 acyl-ACP reductase (AAR) present in pLS9-185, which is a pCL1920 derivative (3-5 copies/cell). In addition, the sfp gene (Accession no. X63158; SEQ ID NO:53) from *Bacillus subtilis*, encoding a phosphopantetheinyl transferase with broad substrate specificity, was cloned downstream of the respective acp genes. This enzyme is involved in conversion of the inactive apo-ACP to the active holo-ACP. The plasmids constructed are described in Table 13.

TABLE 13

Plasmids coexpressing cyanobacterial ACP with and without *B. subtilis* sfp downstream from *S. elongatus* PCC7942 AAR.

Base plasmid	ACP Source	ACP SEQ ID NO. (DNA/ Polypeptide)	Without sfp	With sfp
pLS9-185	*Synechococcus elongatus* 7942	49/50	pDS168	pDS168S
pLS9-185	*Synechocystis* sp. 6803	45/46	pDS169	not available
pLS9-185	*Prochlorococcus marinus* MED4	47/48	pDS170	pDS170S
pLS9-185	*Nostoc punctiforme* 73102	43/44	pDS171	pDS171S
pLS9-185	*Nostoc* sp. 7120	51/52	pDS172	pDS172S

[0352] All the acp genes were cloned with a synthetic RBS into the EcoRI site immediately downstream of the aar gene in pLS9-185 using InFusion technology. The EcoRI site was reconstructed downstream of the acp gene. Similarly, the *B. subtilis* sfp gene was InFusion cloned into this EcoRI site along with a synthetic RBS. All plasmids were transformed into *E. coli* MG1655 DV2. The control for these experiments was the expression of AAR alone (pLS9-185).

[0353] The results from standard shake flask fermentation experiments are shown in FIG. **19**. Significant improvement in fatty alcohol titers were observed in strains containing the plasmids pDS171S, pDS 172S, pDS 168 and pDS169demonstrating that ACP overexpression can be beneficial for fatty alcohol production, in this case presumably by aiding in the recognition, affinity and/or turnover of acyl-ACPs by the heterologous terminal pathway enzyme. (See Table 13 for the source of the ACPs and presence or absence of sfp.)

Fatty Acid Production.

[0354] In order to evaluate if the overexpression of an ACP can also increase free fatty acid production, one cyanobacterial ACP gene with sfp was amplified from pDS171s (Table 13) and cloned downstream from 'tesA into a pCL vector. The resulting operon was under the control of the Ptrc3 promoter, which provides slightly lower transcription levels than the Ptrc wildtype promoter. The construct was cloned into *E. coli* DV2 and evaluated for fatty acid production. The control strain contained the identical plasmid but without cyanobacterial ACP and *B. subtilis* sfp.

[0355] The results from a standard microtiter plate fermentation experiment are shown in FIG. **20**. Significant improvement in fatty acid titer was observed in the strain coexpressing the heterologous ACP demonstrating that ACP overexpression can be beneficial for fatty acid production, in this case presumably by increasing the flux through the fatty acid biosynthetic pathway.

[0356] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention. It is to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting. Preferred embodiments of this invention are described herein. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0357] The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein.

TABLE 14

Table of Sequences.

SEQ ID	Designation	Sequence
1	cat-loxP-T5 (in front of iFAB138)	TTGTCCATCTTTATATAATTTGGGGGTAGGGTGTTCTTTATGTAAAAAAAACgtttTAGGATGCATATG GCGGCCGCataacttcgtataGCATACATtatacgaagttaTCTAGAGTTGCATGCCTGCAGGtccgct tattatcacttattcaggcgtagcAaccaggcgtttaagggcaccaataactgccttaaaaaaattacg ccccgccctgccactcatcgcagtactgttgtaattcattaagcattctgccgacatggaagccatcac aaacggcatgatgaacctgaatcgccagcggcatcagcaccttgtcgccttgcgtataatatttgccca tggtgaaaacgggggcgaagaagttgtccatattggccacgtttaaatcaaaactggtgaaactcaccc agggattggctgagacgaaaaacatattctcaataaaccctttagggaaataggccaggttttcaccgt aacacgccacatcttgcgaatatatgtgtagaaactgccggaaatcgtcgtggtattcactccagagcg atgaaaacgtttcagtttgctcatggaaaacggtgtaacaagggtgaacactatcccatatcaccagct caccgtctttcattgccatacggaattccggatgagcattcatcaggcgggcaagaatgtgaataaagg ccggataaaacttgtgcttatttttctttacggtctttaaaaaggccgtaatatccagctgaacggtct ggttataggtacattgagcaactgactgaaatgcctcaaaatgttctttacgatgccattgggatatat caacggtggtatatccagtgatttttttctccattttagcttccttagctcctgaaaatctcgataact caaaaaatacgcccggtagtgatcttatttcattatggtgaaagttggaacctcttacgtgccgatcaa cgtctcattttcgccaaaagttggcccagggcttcccggtatcaacagggacaccaggatttatttatt ctgcgaagtgatcttccgtcacaggtatttattcGACTCTAGataacttcgtataGCATACATTATACG AAGTTATGGATCCAGCTTATCGATACCGTCaaacAAATCATAAAAAATTTATTTGCTTTcaggaaaatt tttctgTATAATAGATTCAATTGCGATGACGACGAACACGCACCTGCAGGAGGAGACCAATGATCATCA AACCTAAAATTCGTGGATTTATC
2	T5 (in front of iFAB138)	TTGTCCATCTTTATATAATTTGGGGGTAGGGTGTTCTTTATGTAAAAAAAACgtttTAGGATGCATATG GCGGCCGCataacttcgtataGCATACATTATACGAAGTTATGGATCCAGCTTATCGATACCGTCaaac AAATCATAAAAAATTTATTTGCTTTcaggaaaatttttctgTATAATAGATTCAATTGCGATGACGACG AACACGCACCTGCAGGAGGAGACCAATGATCATCAAACCTAAAATTCGTGGATTTATC

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
3	AlrA *Acinetobacter* sp. M-1	MSNHQIRAYAAMQAGEQVVPYQFDAGELKAHQVEVKVEYCGLCHSDLSVINNEWQSSVYPAVAGHEIIG TIIALGSEAKGLKLGQRVGIGWTAETCQACDPCIGGNQVLCTGEKKATIIGHAGGFADKVRAGWQWVIP LPDDLDPESAGPLLCGGITVLDPLLKHKIQATHHVGVIGIGGLGHIAIKLLKAWGCEITAFSSNPDKTE ELKANGADQVVNSRDAQAIKGTRWKLIILSTANGTLNVKAYLNTLAPKGSLHFLGVTLEPIPVSVGAIM GGAKSVTSSPTGSPLALRQLLQFAARKNIAPQVELFPMSQLNEAIERLHSGQARYRIVLKADFD
4	AlrAadp1	MATTNVIHAYAAMQAGEALVPYSFDAGELQPHQVEVKVEYCGLCHSDVSVLNNEWHSSVYPVVAGHEVI GTITQLGSEAKGLKIGQRVGIGWTAESCQACDQCISGQQVLCTGENTATIIGHAGGFADKVRAGWQWVI PLPDELDPTSAGPLLCGGITVFDPILKHQIQATHHVAVIGIGGLGHMAIKLLKAWGCEITAFSSNPNKT DELKAMGADHVVNSRDDAEIKSQQGKFDLLLSTVNVPLNWNAYLNTLAPNGTFHFLGVVMEPIPVPVGA LLGGAKSLTASPTGSPAALRKLLEFAARKNIAPQIEMY
5	yjgB	atgTCGATGATAAAAAGCTATGCCGCAAAAGAAGCGGGCGGCGAACTGGAAGTTTATGAGTACGATCCC GGTGAGCTGAGGCCACAAGATGTTGAAGTGCAGGTGGATTACTGCGGGATCTGCCATTCCGATCTGTCG ATGATCGATAACGAATGGGGATTTTCACAATATCCGCTGGTTGCCGGGCATGAGGTGATTGGGCGCGTG GTGGCACTCGGGAGCGCCGCGCAGGATAAAGGTTTGCAGGTCGGTCAGCGTGTCGGGATTGGCTGGACG GCGCGTAGCTGTGGTCACTGCGACGCCTGTATTAGCGGTAATCAGATCAACTGCGAGCAAGGTGCGGTG CCGACGATTATGAATCGCGGTGGCTTTGCCGAGAAGTTGCGTGCGGACTGGCAATGGGTGATTCCACTG CCAGAAAATATTGATATCGAGTCCGCCGGGCCGCTGTTGTGCGGCGGTATCACGGTCTTTAAACCACTG TTGATGCACCATATCACTGCTACCAGCCGCGTTGGGGTAATTGGTATTGGCGGGCTGGGGCATATCGCT ATAAAACTTCTGCACGCAATGGGATGCGAGGTGACAGCCTTTAGTTCTAATCCGGCGAAAGAGCAGGAA GTGCTGGCGATGGGTGCCGATAAAGTGGTGAATAGCCGCGATCCGCAGGCACTGAAAGCACTGGCGGGG CAGTTTGATCTCATTATCAACACCGTCAACGTCAGCCTCGACTGGCAGCCCTATTTTGAGGCGCTGACC TATGGCGGTAATTTCCATACGGTCGGTGCGGTTCTCACGCCGCTGTCTGTTCCGGCCTTTACGTTAATT GCGGGCGATCGCAGCGTCTCTGGTTCTGCTACCGGCACGCCTTATGAGCTGCGTAAGCTGATGCGTTTT GCCGCCCGCAGCAAGGTTGCGCCGACCACCGAACTGTTCCCGATGTCGAAAATTAACGACGCCATCCAG CATGTGCGCGACGGTAAGGCGCGTTACCGCGTGGTGTTGAAAGCCGATTTTtga
6	ES9 of *Marinobacter hydrocarbonoclasticus* DSM8789 protein	1 MKRLGTLDAS WLAVESEDTP MHVGTLQIFS LPEGAPETFL RDMVTRMKEA GDVAPPWGYK 61 LAWSGFLGRV IAPAWKVDKD IDLDYHVRHS ALPRPGGERE LGILVSRLHS NPLDFSRPLW 121 ECHVIEGLEN NRFALYTKMH HSMIDGISGV RLMQRVLTTD PERCNMPPPW TVRPHQRRGA 181 KTDKEASVPA AVSQAMDALK LQADMAPRLW QAGNRLVHSV RHPEDGLTAP FTGPVSVLNH 241 RVTAQRRFAT QHYQLDRLKN LAHASGGSLN DIVLYLCGTA LRRFLAEQNN LPDTPLTAGI 301 PVNIRPADDE GTGTQISFMI ASLATDEADP LNRLQQIKTS TRRAKEHLQK LPKSALTQYT 361 MLLMSPYILQ LMSGLGGRMR PVFNVTISNV PGPEGTLYYE GARLEAMYPV SLIAHGGALN 421 ITCLSYAGSL NFGFTGCRDT LPSMQKLAVY TGEALDELES LILPPKKRAR TRK
7	ES8 of *Marinobacter hydrocarbonoclasticus* DSM8789(GenBank Accession No. ABO21021) protein	MTPLNPTDQLFLWLEKRQQPMHVGGLQLFSFPEGAPDDYVAQLADQLRQK TEVTAPFNQRLSYRLGQPVWVEDEHLDLEHHFRFEALPTPGRIRELLSFV SAEHSHLMDRERPMWEVHLIEGLKDRQFALYTKVHHSLVDGVSAMRMATR MLSENPDEHGMPPIWDLPCLSRDRGESDGHSLWRSVTHLLGLSDRQLGTI PTVAKELLKTINQARKDPAYDSIFHAPRCMLNQKITGSRRFAAQSWCLKR IRAVCEAYGTTVNDVVTAMCAAALRTYLMNQDALPEKPLVAFVPVSLRRD DSSGGNQVGVILASLHTDVQDAGERLLKIHHGMEEAKQRYRHMSPEEIVN YTALTLAPAAFHLLTGLAPKWQTFNVVISNVPGPSRPLYWNGAKLEGMYP VSIDMDRLALNMTLTSYNDQVEFGLIGCRRTLPSLQRMLDYLEQGLAELE LNAGL
8	ester synthase AtfA1 from *Alcanivorax borkumensis* SK2 (YP.sub.-694462) protein	MKALSPVDQLFLWLEKRQQPMHVGGLQLFSFPEGAGPKYVSELAQQMRDY CHPVAPFNQRLTRRLGQYYWTRDKQFDIDHHFRHEALPKPGRIRELLSLV SAEHSNLLDRERPMWEAHLIEGIRGRQFALYYKIHHSVMDGISAMRIASK TLSTDPSEREMAPAWAFNTKKRSRSLPSNPVDMASSMARLTASISKQAAT VPGLAREVYKVTQKAKKDENYVSIFQAPDTILNNTITGSRRFAAQSFPLP RLKVIAKAYNCTINTVVLSMCGHALREYLISQHALPDEPLIAMVPMSLRQ DDSTGGNQIGMILANLGTHICDPANRLRVIHDSVEEAKSRFSQMSPEEIL NFTALTMAPTGLNLLTGLAPKWRAFNVVISNIPGPKEPLYWNGAQLQGVY PVSIALDRIALNITLTSYVDQMEFGLIACRRTLPSMQRLLDYLEQSIREL EIGAGIK
9	Del-fadE-F	AAAAACAGCAACAATGTGAGCTTTGTTGTAATTATATTGTAAACATATTGATTCCGGGGATCCGTCGACC
10	Del-fadE-R	AAACGGAGCCTTTCGGCTCCGTTATTCATTTACGCGGCTTCAACTTTCCTGTAGGCTGGAGCTGCTTC
11	fadE-L2	CGGGCAGGTGCTATGACCAGGAC
12	fadE-R1	CGCGGCGTTGACCGGCAGCCTGG
13	D+ F1	CCTTGGCATTGGCAATTTGAGAATTCGAGGAGGAAAACTAAATGACCATTTCCTCACCTT
14	D+ R1	TTTTGTTCGGGCCCAAGCTTTTATTGCAAACGCAGATGCGTGATTTCACCCGCATTCAGC

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
15	D+ R2	CGGGCCCAAGCTTCGAATTCTTATTGCAAACGCAGATGCGTGATTTCACCCGCATTCAGC
16	D+ F3	GAATAGCGCCGTCGACGAGGAGGAAAACTAAATGACCATTTCCTCACCTTTGATTGACGT
17	D+ R3	TGATGATGATGATGGTCGACTTATTGCAAACGCAGATGCGTGATTTCACCCGCATTCAGC
18	D+ operon	GAGGAGGAAAACTAAATGACCATTTCCTCACCTTTGATTGACGTCGCCAACCTTCCAGACATCAACACC ACTGCCGGCAAGATCGCCGACCTTAAGGCTCGCCGCGCGGAAGCCCATTTCCCCATGGGTGAAAAGGCA GTAGAGAAGGTCCACGCTGCTGGACGCCTCACTGCCCGTGAGCGCTTGGATTACTTACTCGATGAGGGC TCCTTCATCGAGACCGATCAGCTGGCTCGCCACCGCACCACCGCTTTCGGCCTGGGCGCTAAGCGTCCT GCAACCGACGGCATCGTGACCGGCTGGGGCACCATTGATGGACGCGAAGTCTGCATCTTCTCGCAGGAC GGCACCGTATTCGGTGGCGCGCTTGGTGAGGTGTACGGCGAAAAGATGATCAAGATCATGGAGCTGGCA ATCGACACCGGCCGCCCATTGATCGGTCTTTACGAAGGCGCTGGCGCTCGTATTCAGGACGGCGCTGTC TCCCTGGACTTCATTTCCCAGACCTTCTACCAAAACATTCAGGCTTCTGGCGTTATCCCACAGATCTCC GTCATCATGGGCGCATGTGCAGGTGGCAACGCTTACGGCCCAGCTCTGACCGACTTCGTGGTCATGGTG GACAAGACCTCCAAGATGTTCGTTACCGGCCCAGACGTGATCAAGACCGTCACCGGCGAGGAAATCACC CAGGAAGAGCTTGGCGGAGCAACCACCCACATGGTGACCGCTGGTAACTCCCACTACACCGCTGCGACC GATGAGGAAGCACTGGATTGGGTACAGGACCTGGTGTCCTTCCTCCCATCCAACAATCGCTCCTACGCA CCGATGGAAGACTTCGACGAGGAAGAAGGCGGCGTTGAAGAAAACATCACCGCTGACGATCTGAAGCTC GACGAGATCATCCCAGATTCCGCGACCGTTCCTTACGACGTCCGCGATGTCATCGAATGCCTCACCGAC GATGGCGAATACCTGGAAATCCAGGCAGACCGCGCAGAAAACGTTGTTATTGCATTCGGCCGCATCGAA GGCCAGTCCGTTGGCTTTGTTGCCAACCAGCCAACCCAGTTCGCTGGCTGCCTGGACATCGACTCCTCT GAGAAGGCAGCTCGCTTCGTCCGCACCTGCGACGCGTTCAACATCCCAATCGTCATGCTTGTCGACGTC CCCGGCTTCCTCCCAGGCGCAGGCCAGGAGTACGGTGGCATTCTGCGTCGTGGCGCAAAGCTGCTCTAC GCATACGGCGAAGCAACCGTTCCAAAGATCACCGTCACCATGCGTAAGGCTTACGGCGGAGCGTACTGC GTGATGGGTTCCAAGGGCTTGGGCTCTGACATCAACCTTGCATGGCCAACCGCACAGATCGCCGTCATG GGCGCTGCTGGCGCAGTTGGATTCATCTACCGCAAGGAGCTCATGGCAGCTGATGCCAAGGGCCTCGAT ACCGTAGCTCTGGCTAAGTCCTTCGAGCGCGAGTATGAAGACCACATGCTCAACCCGTACCACGCTGCA GAACGTGGCCTGATCGACGCCGTGATCCTGCCAAGCGAAACCCGCGGACAGATTTCCCGCAACCTTCGC CTGCTCAAGCACAAGAACGTCACTCGCCCTGCTCGCAAGCACGGCAACATGCCACTGTAAGGAGGAAAA CTAAATGTCAGTCGAGACTCGCAAGATCACCAAGGTTCTTGTCGCTAACCGTGGTGAGATTGCAATCCG CGTGTTCCGTGCAGCTCGAGATGAAGGCATCGGATCTGTCGCCGTCTACGCAGAGCCAGATGCAGATGC ACCATTCGTGTCATATGCAGACGAGGCTTTTGCCCTCGGTGGCCAAACATCCGCTGAGTCCTACCTTGT CATTGACAAGATCATCGATGCGGCCCGCAAGTCCGGCGCCGACGCCATCCACCCCGGCTACGGCTTCCT CGCAGAAAACGCTGACTTCGCAGAAGCAGTCATCAACGAAGGCCTGATCTGGATTGGACCTTCACCTGA GTCCATCCGCTCCCTCGGCGACAAGGTCACCGCTCGCCACATCGCAGATACCGCCAAGGCTCCAATGGC TCCTGGCACCAAGGAACCAGTAAAAGACGCAGCAGAAGTTGTGGCTTTCGCTGAAGAATTCGGTCTCCC AATCGCCATCAAGGCAGCTTTCGGTGGCGGCGGACGTGGCATGAAGGTTGCCTACAAGATGGAAGAAGT CGCTGACCTCTTCGAGTCCGCAACCCGTGAAGCAACCGCAGCGTTCGGCCGCGGCGAGTGCTTCGTGGA GCGCTACCTGGACAAGGCACGCCACGTTGAGGCTCAGGTCATCGCCGATAAGCACGGCAACGTTGTTGT CGCCGGAACCCGTGACTGCTCCCTGCAGCGCCGTTTCCAGAAGCTCGTCGAAGAAGCACCAGCACCATT CCTCACCGATGACCAGCGCGAGCGTCTCCACTCCTCCGCGAAGGCTATCTGTAAGGAAGCTGGCTACTA CGGTGCAGGCACCGTTGAGTACCTCGTTGGCTCCGACGGCCTGATCTCCTTCCTCGAGGTCAACACCCG CCTCCAGGTGGAACACCCAGTCACCGAAGAGACCACCGGCATCGACCTGGTCCGCGAAATGTTCCGCAT CGCAGAAGGCCACGAGCTCTCCATCAAGGAAGATCCAGCTCCACGCGGCCACGCATTCGAGTTCCGCAT CAACGGCGAAGACGCTGGCTCCAACTTCATGCCTGCACCAGGCAAGATCACCAGCTACCGCGAGCCACA GGGCCCAGGCGTCCGCATGGACTCCGGTGTCGTTGAAGGTTCCGAAATCTCCGGACAGTTCGACTCCAT GCTGGCAAAGCTGATCGTTTGGGGCGACACCCGCGAGCAGGCTCTCCAGCGCTCCCGCCGTGCACTTGC AGAGTACGTTGTCGAGGGCATGCCAACCGTTATCCCATTCCACCAGCACATCGTGGAAAACCCAGCATT CGTGGGCAACGACGAAGGCTTCGAGATCTACACCAAGTGGATCGAAGAGGTTTGGGATAACCCAATCGC ACCTTACGTTGACGCTTCCGAGCTCGACGAAGATGAGGACAAGACCCCAGCACAGAAGGTTGTTGTGGA GATCAACGGCCGTCGCGTTGAGGTTGCACTCCCAGGCGATCTGGCACTCGGTGGCACCGCTGGTCCTAA GAAGAAGGCCAAGAAGCGTCGCGCAGGTGGTGCAAAGGCTGGCGTATCCGGCGATGCAGTGGCAGCTCC AATGCAGGGCACTGTCATCAAGGTCAACGTCGAAGAAGGCGCTGAAGTCAACGAAGGCGACACCGTTGT TGTCCTCGAGGCTATGAAGATGGAAAACCCTGTGAAGGCTCATAAGTCCGGAACCGTAACCGGCCTTAC TGTCGCTGCAGGCGAGGGTGTCAACAAGGGCGTTGTTCTCCTCGAGATCAAGTAATCTAGAGGAGGAAA ACTAAATGAATGTTGACATTAGCCGCTCTCGTGAACCGTTGAACGTGGAACTGTTGAAAGAAAAACTGC TGCAGAACGGTGATTTCGGTCAAGTGATCTACGAGAAGGTCACCGGCTCTACCAATGCGGACCTGCTGG CTCTGGCGGGCAGCGGCGCTCCAAACTGGACCGTCAAGACTGTTGAATTTCAGGACCACGCCCGTGGCC GTCTGGGTCGTCCGTGGAGCGCACCGGAGGGTTCCCAAACCATCGTCAGCGTTCTGGTCCAACTGAGCA TTGATCAGGTGGACCGTATTGGTACGATCCCGCTGGCCGCAGGCTTGGCTGTTATGGATGCGCTGAATG ATCTGGGCGTGGAGGGTGCAGGCCTGAAATGGCCGAACGATGTTCAGATCCACGGTAAGAAGTTGTGCG GTATTCTGGTTGAAGCAACCGGCTTCGACTCCACTCCGACCGTGGTTATCGGTTGGGGTACGAATATCT CGTTGACGAAAGAAGAGCTGCCGGTCCCGCACGCGACCAGCCTGGCCCTGGAGGGTGTTGAAGTTGACC GTACGACGTTCCTGATTAACATGCTGACCCATCTGCATACCCGTCTGGATCAGTGGCAGGGTCCGTCTG TGGACTGGCTGGATGACTATCGCGCGGTTTGTAGCAGCATTGGCCAAGATGTGCGTGTCCTGCTGCCTG GTGACAAAGAGCTGCTGGGCGAGGCGATTGGCGTGGCGACCGGTGGTGAGATCCGTGTGCGCGACGCCA GCGGCACGGTCCACACGCTGAATGCGGGTGAAATCACGCATCTGCGTTTGCAATAA
19	iFAB138	TGTAGGCTGGAGCTGCTTCGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCGGAATAGGAACTTCGA ACTGCAGGTCGACGGATCCCCGGAATATTTAAATCATTTGTACTTTTTGAACAGCAGAGTCGCATTATG GCCACCGAAGCCCAGGCTGTTGGACAGAACGTAGTTGACTTCTGCATTACGGCCCTCGTTAGGAACGTA

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
		ATCCAGGTCGCATTCCGGATCCGCCTCTTTGTAGCCGATGGTCGGCGGAATGAAACCCTCTTCAATAGC
		TTTGGCACAGATAATCGCTTCGACTGCACCGCCAGCGCCCAGCAGGTGGCCGGTCATGCTCTTGGTGCT
		AGACACCGGCACTTTGTAGGCGTATTCACCCAGGACCGTCTTGATCGCTTGGGTTTCGAAGCTGTCATT
		GTACGCCGTGCTCGTACCGTGCGCGTTGATATAGGAAATGTCCTCTGGGCGGACATTATCTTCTTCCAT
		TGCCAGTTTCATTGCACGTGCACCACCTTCACCATTCGGCGCTGGGCTCGTGATATGATATGCGTCGCA
		GGTCGCACCATAGCCAACGATCTCGGCATAGATTTTGGCACCACGCTTCAGCGCGTGCTCCAACTCTTC
		CAAGATAACGATACCGCTGCCCTCGCCCATCACAAAACCGCTGCGATCCTTATCGAACGGGATGCTGGC
		GCGCTTCGGGTCCTCAGATTTGGTCACGGCCTTCATCGAGGCAAAACCCGCCAGGCTCAACGGGGTGAT
		ACCTGCTTCGCTACCACCAGAGATCATAACGTCGCTATAACCAAACTTAATGTTACGGAAGGACTCACC
		AATGCTGTTGTTCGCGCTCGCACATGCGGTGACAATGGTCGTGCAAATACCTTTAGCGCCATAACGAAT
		CGCCAGATTACCGCTTGCCATATTCGCAATGATCATCGGAATAGTCATAGGGCTCACACGACCCGGACC
		TTTGGTAATCAGCTTTTCATCCTGCTTCTCAATGGTGCCGATGCCGCCAATGCCGCTACCAACAATGAC
		GCCGAAACGATTCTTATCAATCGACTCCAGGTCCAGTTTGCTGTCCTTGATTGCCTCATCCGCCGCAAC
		GATCGCAAACTGGCTAAAACGGTCCATACGGTTCGCCTCACGCTTGTCGATAAAGTCCTCCGGGGTGAA
		GTCCTTCACTTCGGCAGCCAGCTTAACTTTGAAATCGGTTGCGTCAAACGCTTTGATCTTGTCAATGCC
		ACATTTACCCTCTTTGATGCTGCACCAGAAGCTATCAGCGTTGTTACCCACCGGCGTCACTGCACCAAT
		ACCCGTAATGACAACGCGGCGATTCATtttgttgcctccttTTAgaacgcggaagtatcctggaacaaa
		ccgactttcaaatcgtgtgcggtatagatcaggcgaccatccaccagaacctcaccgtccgccaggccc
		atgatcaggcgacggtttacgatacgtttgaaatgaatacgataggtgactttcctggctgtcggcaga
		acctggccggtaaatttcacttcgcccacgcccagagcgcggcctttgccttcgccgcccaaccagccc
		aggtagaatcccaccaattgccacatagcatccagacccagacaaccgggcatcaccggatcgccgata
		aagtggcatccgaagaaccatagatccggattgatatccagctcggcttcgacatagcctttgtcgaaa
		ttgccgcccgtttcggtcatcttaacgacgcggtccatcatcagcatgttcggtgcagggagttgcggc
		cctttagcgccaaacagttcaccacgaccagaggcaagaaggtcttcttttgtataggattcgcgttta
		tctaccatgttttatgtaaaccttaaaaTTAAACCATGTACATTCCGCCGTTGACGTGCAGAGTCTCAC
		CAGTGATGTAACTCGCTTCGTCAGAGGCTAAAAATGCAACCGCACTGGCGATTTCCTGAGCGCCGCCGA
		GGCGACCCGCAGGCACCTGCGCCAGGATACCCGCACGCTGATCGTCAGACAGCGCACGCGTCATGTCCG
		TTTCAATAAAACCCGGAGCCACAACATTGACAGTAATACCACGGGACGCAACTTCACGCGCCAGTGATT
		TACTGAAACCGATCAGGCCCGCTTTCGCCGCAGCGTAGTTTGCCTGACCTGCATTTCCCATGGTACCAA
		CCACAGAACCAATAGTGATAATGCGACCACAACGCTTTTTCATCATAGCGCGCATTACCGCTTTTGACA
		GGCGGAAAACGGATGATAAGTTGGTTTCGATAATATCGTTCCACTCATCATCTTTCATTCGCATCAACA
		GATTATCACGAGTGATACCGGCATTATTAACCAGGATATCCACTTCACCAAATTCTGCGCGAATATTTT
		CCAGAACAGATTCAATAGATGCAGGATCGGTCACATTCAACATCAAACCTTTCCCGTTAGCACCTAAAT
		AGTCGCTAATGTTCTTCGCACCATTTTCACTGGTCGCAGTCCCGATAACTTTCGCGCCGCGGGCAACGA
		GAGTCTCTGCAATTGCGCGGCCTATGCCACGGCTTGCACCAGTCACCAGCGCAATCTTTCCTTCAAAGC
		TCATGGTTTTCCTCTTTTATTGCGTAAGTGCCGCAGACAGCGCCGCCGGCTCGTTCAGCGCCGACGCTG
		TCAGGGTGTCGACAATACGTTTCGTCAGACCAGTGAGGACTTTACCTGGACCCACTTCATAAAGATGTT
		CAACGCCCTGCGCCGCGATAAATTCCACGCTCTTCGTCCACTGTACCGGATTGTACAACTGGCGAACCA
		GCGCATCGCGGATAGCGGCGGCATCGGTTTCACATTTCACGTCAACGTTGTTCACTACCGGCACCGTTG
		GCGCGCTAAAGGTAATTTTGGCTAATTCAACCGCCAGCTTATCTGCCGCTGGTTTCATCAGCGCGCAGT
		GCGACGGTACGCTCACCGGCAGCGGCAGCGCGCGTTTCGCGCCAGCGGCTTTACAGGCTGCGCCCGCAC
		GTTCTACCGCCTCTTTATGCCCGGCGATAACCACCTGTCCCGGCGAGTTAAAGTTAACCGGCGAAACAA
		CCTGCCCTTCGGCAGATTCTTCACAGGCTTTAGCAATAGAGGCATCATCCAGCCCGATGATCGCAGACA
		TGCCGCCAGTGCCTTCCGGAACCGCTTCCTGCATGAATTTACCGCGCATTTCCACCAGACGAACGGCAT
		CAGCAAAGTTGATGACGCCAGCGCAAACCAGCGCGGAATATTCGCCCAGGCTGTGACCTGCCATTAACG
		CAGGCATTTTACCGCCCTGCTGCTGCCAAACGCGCCAAAGCGCGACGGAAGCGGTTAATAACGCCGGCT
		GCGTCTGCCAGGTTTTATTCAGTTCTTCCGCTGGACCTTGCTGGGTGAGCGCCCACAGATCATATCCCA
		GAGCCGCAGAAGCTTCAGCAAACGTTTCTTCTACGATAGGGTAATTTGCCGCCATCTCGGCCAACATCC
		CAACGCTCTGAGAACCCTGACCGGGGAACACAAATGCAAATTGCGTCATGTTTAAATCCTTATACTAGA
		AACGAATCAGCGCGGAGCCCCAGGTGAATCCACCCCCGAAGGCTTCAAGCAATACCAGCTGACCGGCTT
		TAATTCGCCCGTCACGCACGGCTTCATCCAGCGCGCACGGCACAGAAGCCGCGGAGGTATTGCCGTGCC
		TGTCCAGCGTGACGACGACATTGTCCATCGACATGCCGAGTTTTTTCGCTGTCGCGCTAATGATACGCA
		GGTTAGCCTGATGCGGCACCAGCCAATCGAGTTCTGAGCGATCCAGGTTATTAGCCGCCAGCGTCTCAT
		CGACAATATGCGCCAGTTCAGTGACCGCCACTTTAAAGACTTCATTGCCCGCCATTGTCAGGTAAATCG
		GGTTATCCGGATTTACGCGATCGGCATTCGGCAGGGTCAGTAATTCACCGTAACGGCCATCGGCATGAA
		GATGAGTGGAGATAATACCCGGTTCTTCAGAAGCGCTCAGTACGGCCGCGCCTGCGCCATCGCCGAAAA
		TAATGATCGTACCGCGATCGCCAGGATCGCAAGTGCGGGCTAATACATCGGAACCGACCACCAGCGCGT
		GTTTAACCGCGCCGGATTTAACGTACTGGTCGGCGATGCTTAACGCGTAGGTGAAACCTGCGCACGCTG
		CCGCGACATCAAACGCCGGGCAACCTTTAATACCGAGCATACTTTGAATCTGACATGCCGCGCTTGGAA
		ATGCATGCGTTGCTGATGTGGTAGCCACCACAATCAAGCCAATTTGGTCTTTATCGATCCCCGCCATCT
		CAATCGCGCGATTCGCAGCGGTAAAGCCCATCGTCGCGACAGTTTCATTCGGCGCGGCGATATGGCGTT
		TACGAATACCTGTACGAGTGACAATCCACTCGTCAGAGGTCTCAACCATTTTTTCCAGATCGGCGTTAG
		TCCGCACTTGTTCGGGCAGATAGCTGCCAGTACCAATAATCTTCGTATACATGTACGCTCAGTCACTaa
		aTTACTCGATATCAATCACATCAAATTCGACTTCTGGATTGACGTCAGCATCGTAATCAATGCCTTCAA
		TGCCAAAGCCAAACAGCTTGATGAACTCTTCTTTGTACATGTCGTAATCGGTCAGCTCACGCAGGTTCT
		CTGTGGTGATTTGTGGCCACAGATCACGGCAGTGCTGCTGAATGTCATCACGCAGTTCCCAGTCATCCA
		AACGCAGACGATTGTGATCATCCACTTCCGGCGCTGAACCATCT

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
20	DG405	TTGTCCATCTTTATATAATTTGGGGGTAGGGTGTTCTTTATGTAAAAAAAACgtttTAGGATGCATATG GCGGCC
21	DG406	GATAAATCCACGAATTTTAGGTTTGATGATCATTGGTCTCCTCCTGCAGGTGCGTGTTCGTCGTCATCG CAATTG
22	DG422	ACTCACCGCATTGGTGTAGTAAGGCGCACC
23	DG423	TGAATGTCATCACGCAGTTCCCAGTCATCC
24	DG744	CCATCTTCTTTGTACAGACGTTGACTGAACATG
25	DG749	GCACCATAGCCGTAATCCCACAGGTTATAG
26	oTREE047	TGTCATTAATGGTTAATAATGTTGA
27	DG150	GCAGTTATTGGTGCCCTTAAACGCCTGGTTGCTACGCCTG
28	DG131	GAGCCAATATGCGAGAACACCCGAGAA
29	LC277	CGCTGAACGTATTGCAGGCCGAGTTGCTGCACCGCTCCCGCCAGGCAG
30	LC278	GGAATTGCCACGGTGCGGCAGGCTCCATACGCGAGGCCAGGTTATCCAACG
31	DG407	AATCACCAGCACTAAAGTGCGCGGTTCGTTACCCG
32	DG408	ATCTGCCGTGGATTGCAGAGTCTATTCAGCTACG
33	carA	MTIETREDRFNRRIDHLFETDPQFAAARPDEAISAAAADPELRLPAAVKQILAGYADRPALGKRAVEFV TDEEGRTTAKLLPRFDTITYRQLAGRIQAVTNAWHNHPVNAGDRVAILGFTSVDYTTIDIALLELGAVS VPLQTSAPVAQLQPIVAETEPKVIASSVDFLADAVALVESGPAPSRLVVFDYSHEVDDQREAFEAAKGK LAGTGVVVETITDALDRGRSLADAPLYVPDEADPLTLLIYTSGSTGTPKGAMYPESKTATMWQAGSKAR WDETLGVMPSITLNFMPMSHVMGRGILCSTLASGGTAYFAARSDLSTFLEDLALVRPTQLNFVPRIWDM LFQEYQSRLDNRRAEGSEDRAEAAVLEEVRTQLLGGRFVSALTGSAPISAEMKSWVEDLLDMHLLEGYG STEAGAVFIDGQIQRPPVIDYKLVDVPDLGYFATDRPYPRGELLVKSEQMFPGYYKRPEITAEMFDEDG YYRTGDIVAELGPDHLEYLDRRNNVLKLSQGEFVTVSKLEAVFGDSPLVRQTYVYGNSARSYLLAVVVP TEEALSRWDGDELKSRISDSLQDAARAAGLQSYEIPRDFLVETTPFTLENGLLTGIRKLARPKLKAHYG ERLEQLYTDLAEGQANELRELRRNGADRPVVETVSRAAVALLGASVTDLRSDAHFTDLGGDSLSALSFS NLLHEIFDVDVPVGVIVSPATDLAGVAAYIEGELRGSKRPTYASVHGRDATEVRARDLALGKFIDAKTL SAAPGLPRSGTEIRTVLLTGATGFLGRYLALEWLERMDLVDGKVICLVRARSDDEARARLDATFDTGDA TLLEHYRALAADHLEVIAGDKGEADLGLDHDTWQRLADTVDLIVDPAALVNHVLPYSQMFGPNALGTAE LIRIALTTTIKPYVYVSTIGVGQGISPEAFVEDADIREISATRRVDDSYANGYGNSKWAGEVLLREAHD WCGLPVSVFRCDMILADTTYSGQLNLPDMFTRLMLSLVATGIAPGSFYELDADGNRQRAHYDGLPVEFI AEAISTIGSQVTDGFETFHVMNPYDDGIGLDEYVDWLIEAGYPVHRVDDYATWLSRFETALRALPERQR QASLLPLLHNYQQPSPPVCGAMAPTDRFRAAVQDAKIGPDKDIPHVTADVIVKYISNLQMLGLL*
34	FadD9	MSINDQRLTRRVEDLYASDAQFAAASPNEAITQAIDQPGVALPQLIRMVMEGYADRPALGQRALRFVTD PDSGRTMVELLPRFETITYRELWARAGTLATALSAEPAIRPGDRVCVLGFNSVDYTTIDIALIRLGAVS VPLQTSAPVTGLRPIVTETEPTMIATSIDNLGDAVEVLAGHAPARLVVFDYHGKVDTHREAVEAARARL AGSVTIDTLAELIERGRALPATPIADSADDALALLIYTSGSTGAPKGAMYRESQVMSFWRKSSGWFEPS GYPSITLNFMPMSHVGGRQVLYGTLSNGGTAYFVAKSDLSTLFEDLALVRPTELCFVPRIWDMVFAEFH SEVDRRLVDGADRAALEAQVKAELRENVLGGRFVMALTGSAPISAEMTAWVESLLADVHLVEGYGSTEA GMVLNDGMVRRPAVIDYKLVDVPELGYFGTDQPYPRGELLVKTQTMFPGYYQRPDVTAEVFDPDGFYRT GDIMAKVGPDQFVYLDRRNNVLKLSQGEFIAVSKLEAVFGDSPLVRQIFIYGNSARAYPLAVVVPSGDA LSRHGIENLKPVISESLQEVARAAGLQSYEIPRDFIIETTPFTLENGLLTGIRKLARPQLKKFYGERLE RLYTELADSQSNELRELRQSGPDAPVLPTLCRAAAALLGSTAADVRPDAHFADLGGDSLSALSLANLLH EIFGVDVPVGVIVSPASDLRALADHIEAARTGVRRPSFASIHGRSATEVHASDLTLDKFIDAATLAAAP NLPAPSAQVRTVLLTGATGFLGRYLALEWLDRMDLVNGKLICLVRARSDEEAQARLDATFDSGDPYLVR HYRELGAGRLEVLAGDKGEADLGLDRVTWQRLADTVDLIVDPAALVNHVLPYSQLFGPNAAGTAELLRL ALTGKRKPYIYTSTIAVGEQIPPEAFTEDADIRAISPTRRIDDSYANGYANSKWAGEVLLREAHEQCGL PVTVFRCDMILADTSYTGQLNLPDMFTRLMLSLAATGIAPGSFYELDAHGNRQRAHYDGLPVEFVAEAI CTLGTHSPDRFVTYHVMNPYDDGIGLDEFVDWLNSPTSGSGCTIQRIADYGEWLQRFETSLRALPDRQR HASLLPLLHNYREPAKPICGSIAPTDQFRAAVQEAKIGPDKDIPHLTAAIIAKYISNLRLLGLL*

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
35	*Synechococcus elongatus* PCC7942 YP.sub.--400610 (Synpcc7942.sub.--1593)(decarbonylase DNA)	atgccgcagc ttgaagccag ccttgaactg gactttcaaa gcgagtccta caaagacgct 60 tacagccgca tcaacgcgat cgtgattgaa ggcgaacaag aggcgttcga caactacaat 120 cgccttgctg agatgctgcc cgaccagcgg gatgagcttc acaagctagc caagatggaa 180 cagcgccaca tgaaaggctt tatggcctgt ggcaaaaatc tctccgtcac tcctgacatg 240 ggttttgccc agaaattttt cgagcgcttg cacgagaact tcaaagcggc ggctgcggaa 300 ggcaaggtcg tcacctgcct actgattcaa tcgctaatca tcgagtgctt tgcgatcgcg 360 gcttacaaca tctacatccc agtggcggat gcttttgccc gcaaaatcac ggaggggggtc 420 gtgcgcgacg aatacctgca ccgcaacttc ggtgaagagt ggctgaaggc gaattttgat 480 gcttccaaag ccgaactgga agaagccaat cgtcagaacc tgcccttggt ttggctaatg 540 ctcaacgaag tggccgatga tgctcgcgaa ctcgggatgg agcgtgagtc gctcgtcgag 600 gactttatga ttgcctacgg tgaagctctg gaaaacatcg gcttcacaac gcgcgaaatc 660 atgcgtatgt ccgcctatgg ccttgcggcc gtttga 696
36	*Synechococcus elongatus* PCC7942 YP.sub.--400610 (Synpcc7942.sub.--1593)(decarbonylase polypeptide)	Met Pro Gln Leu Glu Ala Ser Leu Glu Leu Asp Phe Gln Ser Glu Ser 1 5 10 15 Tyr Lys Asp Ala Tyr Ser Arg Ile Asn Ala Ile Val Ile Glu Gly Glu 20 25 30 Gln Glu Ala Phe Asp Asn Tyr Asn Arg Leu Ala Glu Met Leu Pro Asp 35 40 45 Gln Arg Asp Glu Leu His Lys Leu Ala Lys Met Glu Gln Arg His Met 50 55 60 Lys Gly Phe Met Ala Cys Gly Lys Asn Leu Ser Val Thr Pro Asp Met 65 70 75 80 Gly Phe Ala Gln Lys Phe Phe Glu Arg Leu His Glu Asn Phe Lys Ala 85 90 95 Ala Ala Ala Glu Gly Lys Val Val Thr Cys Leu Leu Ile Gln Ser Leu 100 105 110 Ile Ile Glu Cys Phe Ala Ile Ala Ala Tyr Asn Ile Tyr Ile Pro Val 115 120 125 Ala Asp Ala Phe Ala Arg Lys Ile Thr Glu Gly Val Val Arg Asp Glu 130 135 140 Tyr Leu His Arg Asn Phe Gly Glu Glu Trp Leu Lys Ala Asn Phe Asp 145 150 155 160 Ala Ser Lys Ala Glu Leu Glu Glu Ala Asn Arg Gln Asn Leu Pro Leu 165 170 175 Val Trp Leu Met Leu Asn Glu Val Ala Asp Asp Ala Arg Glu Leu Gly 180 185 190 Met Glu Arg Glu Ser Leu Val Glu Asp Phe Met Ile Ala Tyr Gly Glu 195 200 205 Ala Leu Glu Asn Ile Gly Phe Thr Thr Arg Glu Ile Met Arg Met Ser 210 215 220 Ala Tyr Gly Leu Ala Ala Val 225 230
37	*Synecho-coccus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (AAR DNA)	atgttcggtc ttatcggtca tctcaccagt ttggagcagg cccgcgacgt ttctcgcagg 60 atgggctacg acgaatacgc cgatcaagga ttggagtttt ggagtagcgc tcctcctcaa 120 atcgttgatg aaatcacagt caccagtgcc acaggcaagg tgattcacgg tcgctacatc 180 gaatcgtgtt tcttgccgga aatgctggcg gcgcgccgct tcaaaacagc cacgcgcaaa 240 gttctcaatg ccatgtccca tgcccaaaaa cacggcatcg acatctcggc cttgggggggc 300 tttacctcga ttattttcga gaatttcgat ttggccagtt tgcggcaagt gcgcgacact 360 accttggagt ttgaacggtt caccaccggc aatactcaca cggcctacgt aatctgtaga 420

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
		caggtggaag ccgctgctaa aacgctgggc atcgacatta cccaagcgac agtagcggtt 480
		gtcggcgcga ctggcgatat cggtagcgct gtctgccgct ggctcgacct caaactgggt 540
		gtcggtgatt tgatcctgac ggcgcgcaat caggagcgtt tggataacct gcaggctgaa 600
		ctcggccggg gcaagattct gcccttggaa gccgctctgc cggaagctga ctttatcgtg 660
		tgggtcgcca gtatgcctca gggcgtagtg atcgacccag caaccctgaa gcaaccctgc 720
		gtcctaatcg acgggggcta ccccaaaaac ttgggcagca aagtccaagg tgagggcatc 780
		tatgtcctca atggcggggt agttgaacat tgcttcgaca tcgactggca gatcatgtcc 840
		gctgcagaga tggcgcggcc cgagcgccag atgtttgcct gctttgccga ggcgatgctc 900
		ttggaatttg aaggctggca tactaacttc tcctggggcc gcaaccaaat cacgatcgag 960
		aagatggaag cgatcggtga ggcatcggtg cgccacggct tccaaccctt ggcattggca 1020
		atttga
38	*Synecho-coccus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (AAR polypeptide)	Met Phe Gly Leu Ile Gly His Leu Thr Ser Leu Glu Gln Ala Arg Asp 1 5 10 15
		Val Ser Arg Arg Met Gly Tyr Asp Glu Tyr Ala Asp Gln Gly Leu Glu 20 25 30
		Phe Trp Ser Ser Ala Pro Pro Gln Ile Val Asp Glu Ile Thr Val Thr 35 40 45
		Ser Ala Thr Gly Lys Val Ile His Gly Arg Tyr Ile Glu Ser Cys Phe 50 55 60
		Leu Pro Glu Met Leu Ala Ala Arg Arg Phe Lys Thr Ala Thr Arg Lys 65 70 75 80
		Val Leu Asn Ala Met Ser His Ala Gln Lys His Gly Ile Asp Ile Ser 85 90 95
		Ala Leu Gly Gly Phe Thr Ser Ile Ile Phe Glu Asn Phe Asp Leu Ala 100 105 110
		Ser Leu Arg Gln Val Arg Asp Thr Thr Leu Glu Phe Glu Arg Phe Thr 115 120 125
		Thr Gly Asn Thr His Thr Ala Tyr Val Ile Cys Arg Gln Val Glu Ala 130 135 140
		Ala Ala Lys Thr Leu Gly Ile Asp Ile Thr Gln Ala Thr Val Ala Val 145 150 155 160
		Val Gly Ala Thr Gly Asp Ile Gly Ser Ala Val Cys Arg Trp Leu Asp 165 170 175
		Leu Lys Leu Gly Val Gly Asp Leu Ile Leu Thr Ala Arg Asn Gln Glu 180 185 190
		Arg Leu Asp Asn Leu Gln Ala Glu Leu Gly Arg Gly Lys Ile Leu Pro 195 200 205
		Leu Glu Ala Ala Leu Pro Glu Ala Asp Phe Ile Val Trp Val Ala Ser 210 215 220
		Met Pro Gln Gly Val Val Ile Asp Pro Ala Thr Leu Lys Gln Pro Cys 225 230 235 240
		Val Leu Ile Asp Gly Gly Tyr Pro Lys Asn Leu Gly Ser Lys Val Gln 245 250 255
		Gly Glu Gly Ile Tyr Val Leu Asn Gly Gly Val Val Glu His Cys Phe 260 265 270
		Asp Ile Asp Trp Gln Ile Met Ser Ala Ala Glu Met Ala Arg Pro Glu 275 280 285
		Arg Gln Met Phe Ala Cys Phe Ala Glu Ala Met Leu Leu Glu Phe Glu 290 295 300
		Gly Trp His Thr Asn Phe Ser Trp Gly Arg Asn Gln Ile Thr Ile Glu 305 310 315 320
		Lys Met Glu Ala Ile Gly Glu Ala Ser Val Arg His Gly Phe Gln Pro 325 330 335
		Leu Ala Leu Ala Ile 340

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
39	*Prochlorococcus mariunus* CCMP1986 PMM0532 (decarbonylase DNA)	atgcaaacac tcgaatctaa taaaaaaact aatctagaaa attctattga tttacccgat 60 tttactactg attcttacaa agacgcttat agcaggataa atgcaatagt tattgaaggt 120 gaacaagagg ctcatgataa ttacatttcc ttagcaacat taattcctaa cgaattagaa 180 gagttaacta aattagcgaa aatggagctt aagcacaaaa gaggctttac tgcatgtgga 240 agaaatctag gtgttcaagc tgacatgatt tttgctaaag aattcttttc caaattacat 300 ggtaattttc aggttgcgtt atctaatggc aagacaacta catgcctatt aatacaggca 360 attttaattg aagcttttgc tatatccgcg tatcacgttt acataagagt tgctgatcct 420 ttcgcgaaaa aaattaccca aggtgttgtt aaagatgaat atcttcattt aaattatgga 480 caagaatggc taaaagaaaa tttagcgact tgtaaagatg agctaatgga agcaaataag 540 gttaaccttc cattaatcaa gaagatgtta gatcaagtct cggaagatgc ttcagtacta 600 gctatggata gggaagaatt aatggaagaa ttcatgattg cctatcagga cactctcctt 660 gaaataggtt tagataatag agaaattgca agaatggcaa tggctgctat agtttaa 717
40	*Prochlorococcus mariunus* CCMP1986 PMM0532 (decarbonylase polypeptide)	Met Gln Thr Leu Glu Ser Asn Lys Lys Thr Asn Leu Glu Asn Ser Ile 1 5 10 15 Asp Leu Pro Asp Phe Thr Thr Asp Ser Tyr Lys Asp Ala Tyr Ser Arg 20 25 30 Ile Asn Ala Ile Val Ile Glu Gly Glu Gln Glu Ala His Asp Asn Tyr 35 40 45 Ile Ser Leu Ala Thr Leu Ile Pro Asn Glu Leu Glu Glu Leu Thr Lys 50 55 60 Leu Ala Lys Met Glu Leu Lys His Lys Arg Gly Phe Thr Ala Cys Gly 65 70 75 80 Arg Asn Leu Gly Val Gln Ala Asp Met Ile Phe Ala Lys Glu Phe Phe 85 90 95 Ser Lys Leu His Gly Asn Phe Gln Val Ala Leu Ser Asn Gly Lys Thr 100 105 110 Thr Thr Cys Leu Leu Ile Gln Ala Ile Leu Ile Glu Ala Phe Ala Ile 115 120 125 Ser Ala Tyr His Val Tyr Ile Arg Val Ala Asp Pro Phe Ala Lys Lys 130 135 140 Ile Thr Gln Gly Val Val Lys Asp Glu Tyr Leu His Leu Asn Tyr Gly 145 150 155 160 Gln Glu Trp Leu Lys Glu Asn Leu Ala Thr Cys Lys Asp Glu Leu Met 165 170 175 Glu Ala Asn Lys Val Asn Leu Pro Leu Ile Lys Lys Met Leu Asp Gln 180 185 190 Val Ser Glu Asp Ala Ser Val Leu Ala Met Asp Arg Glu Glu Leu Met 195 200 205 Glu Glu Phe Met Ile Ala Tyr Gln Asp Thr Leu Leu Glu Ile Gly Leu 210 215 220 Asp Asn Arg Glu Ile Ala Arg Met Ala Met Ala Ala Ile Val
41	*Prochlorococcus marinu* CCMP1986 PMM0533 (NP_892651)(DNA)	atgtttgggc ttataggtca ttcaactagt tttgaagatg caaaaagaaa ggcttcatta 60 ttgggctttg atcatattgc ggatggtgat ttagatgttt ggtgcacagc tccacctcaa 120 ctagttgaaa atgtagaggt taaaagtgct ataggtatat caattgaagg ttcttatatt 180 gattcatgtt tcgttcctga aatgctttca agatttaaaa cggcaagaag aaaagtatta 240 aatgcaatgg aattagctca aaaaaaaggt attaatatta ccgctttggg ggggttcact 300 tctatcatct ttgaaaattt taatctcctt caacataagc agattagaaa cacttcacta 360 gagtgggaaa ggtttacaac tggtaatact catactgcgt gggttatttg caggcaatta 420 gagatgaatg ctcctaaaat aggtattgat cttaaaagcg caacagttgc tgtagttggt 480

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
		gctactggag atataggcag tgctgtttgt cgatggttaa tcaataaaac aggtattggg 540
		gaacttcttt tggtagctag gcaaaaggaa cccttggatt ctttgcaaaa ggaattagat 600
		ggtggaacta tcaaaaatct agatgaagca ttgcctgaag cagatattgt tgtatgggta 660
		gcaagtatgc caaagacaat ggaaatcgat gctaataatc ttaaacaacc atgtttaatg 720
		attgatggag gttatccaaa gaatctagat gaaaaatttc aaggaaataa tatacatgtt 780
		gtaaaaggag gtatagtaag attcttcaat gatataggtt ggaatatgat ggaactagct 840
		gaaatgcaaa atccccagag agaaatgttt gcatgctttg cagaagcaat gattttagaa 900
		tttgaaaaat gtcatacaaa ctttagctgg ggaagaaata atatatctct cgagaaaatg 960
		gagtttattg gagctgcttc tgtaaagcat ggcttctctg caattggcct agataagcat 1020
		ccaaaagtac tagcagtttg a 1041
42	*Prochloro-coccus marinu* CCMP1986 PMM0533 (NP_892651) (polypeptide)	Met Phe Gly Leu Ile Gly His Ser Thr Ser Phe Glu Asp Ala Lys Arg 1 5 10 15
		Lys Ala Ser Leu Leu Gly Phe Asp His Ile Ala Asp Gly Asp Leu Asp 20 25 30
		Val Trp Cys Thr Ala Pro Pro Gln Leu Val Glu Asn Val Glu Val Lys 35 40 45
		Ser Ala Ile Gly Ile Ser Ile Glu Gly Ser Tyr Ile Asp Ser Cys Phe 50 55 60
		Val Pro Glu Met Leu Ser Arg Phe Lys Thr Ala Arg Arg Lys Val Leu 65 70 75 80
		Asn Ala Met Glu Leu Ala Gln Lys Lys Gly Ile Asn Ile Thr Ala Leu 85 90 95
		Gly Gly Phe Thr Ser Ile Ile Phe Glu Asn Phe Asn Leu Leu Gln His 100 105 110
		Lys Gln Ile Arg Asn Thr Ser Leu Glu Trp Glu Arg Phe Thr Thr Gly 115 120 125
		Asn Thr His Thr Ala Trp Val Ile Cys Arg Gln Leu Glu Met Asn Ala 130 135 140
		Pro Lys Ile Gly Ile Asp Leu Lys Ser Ala Thr Val Ala Val Val Gly 145 150 155 160
		Ala Thr Gly Asp Ile Gly Ser Ala Val Cys Arg Trp Leu Ile Asn Lys 165 170 175
		Thr Gly Ile Gly Glu Leu Leu Leu Val Ala Arg Gln Lys Glu Pro Leu 180 185 190
		Asp Ser Leu Gln Lys Glu Leu Asp Gly Gly Thr Ile Lys Asn Leu Asp 195 200 205
		Glu Ala Leu Pro Glu Ala Asp Ile Val Val Trp Val Ala Ser Met Pro 210 215 220
		Lys Thr Met Glu Ile Asp Ala Asn Asn Leu Lys Gln Pro Cys Leu Met 225 230 235 240
		Ile Asp Gly Gly Tyr Pro Lys Asn Leu Asp Glu Lys Phe Gln Gly Asn 245 250 255
		Asn Ile His Val Val Lys Gly Gly Ile Val Arg Phe Phe Asn Asp Ile 260 265 270
		Gly Trp Asn Met Met Glu Leu Ala Glu Met Gln Asn Pro Gln Arg Glu 275 280 285
		Met Phe Ala Cys Phe Ala Glu Ala Met Ile Leu Glu Phe Glu Lys Cys 290 295 300
		His Thr Asn Phe Ser Trp Gly Arg Asn Asn Ile Ser Leu Glu Lys Met 305 310 315 320
		Glu Phe Ile Gly Ala Ala Ser Val Lys His Gly Phe Ser Ala Ile Gly 325 330 335
		Leu Asp Lys His Pro Lys Val Leu Ala Val 340 345

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
43	*Nostoc punctiforme* PCC 73102_acp Accession# YP_001867863 (DNA)	ATGAGCCAAACGGAACTTTTTGAAAAGGTCAAGAAAATCGTCATCGAACAACTGAGTGTTGAAGATGCTT CCAAAATCACTCCACAAGCTAAGTTTATGGAAGATTTAGGAGCTGATTCCCTGGATACTGTTGAACTCGT GATGGCTTTGGAAGAAGAATTTGATATCGAAATTCCCGACGAAGCTGCCGAGCAGATTGTATCGGTTCAA GACGCAGTAGATTACATCAATAACAAAGTTGCTGCATCAGCTTAA
44	*Nostoc punctiforme* PCC 73102_acp Accession# YP_001867863 (polypeptide)	MSQTELPEKVKKIVIEQLSVEDASKITPQAKFMEDLGADSLDTVELVMALEEEFDIEIPDEAAEQIVSVQ DAVDYINNKVAASA
45	*Synechocystis* sp. PCC 6803_acp Accession # NP_440632.1 (DNA)	ATGAATCAGGAAATTTTTGAAAAAGTAAAAAAAAATCGTCGTGGAACAGTTGGAAGTGGATCCTGACAAAG TGACCCCCGATGCCACCTTTGCCGAAGATTTAGGGGCTGATTCCCTCGATACAGTGGAATTGGTCATGGC CCTGGAAGAAGAGTTTGATATTGAAATTCCCGATGAAGTGGCGGAAACCATTGATACCGTGGGCAAAGCC GTTGAGCATATCGAAAGTAAATAA
46	*Synechocystis* sp. PCC 6803_acp Accession # NP_440632.1 (polypeptide)	MNQEIFEKVKKIVVEQLEVDPDKVTPDATFAEDLGADSLDTVEL VMALEEEFDIEIPDEVAETIDTVGKAVEHIESK
47	*Prochlorococcus marinu*; subsp. *pastoris* str. CCMP1986_acp Accession# NP_893725. (DNA)	ATGTCACAAGAAGAAATCCTTCAAAAAGTATGCTCTATTGTTTCTGAGCAACTAAGTGTTGAATCAGCCG AAGTAAAATCTGATTCAAACTTTCAAAATGATTTAGGTGCAGACTCCCTAGACACCGTAGAGCTAGTTAT GGCTCTTGAAGAAGCATTTGATATCGAGATACCTGATGAAGCAGCTGAAGGTATCGCAACAGTAGGAGAT GCTGTTAAATTCATCGAAGAAAAAAAAGGTTAA
48	*Prochlorococcus marinu*; subsp. *pastoris* str. CCMP1986_acp Accession# NP_893725. (polypeptide)	MSQEEILQKVCSIVSEQLSVESAEVKSDSNFQNDLGADSLDTVELVMALEEAFDIEIPDEAAEGIATVGD AVKFIEEKKG
49	*Synechococcus elongatu* PCC 7942_acp Accession# YP_399555 (DNA)	ATGAGCCAAGAAGACATCTTCAGCAAAGTCAAAGACATTGTGGCTGAGCAGCTGAGTGTGGATGTGGCTG AAGTCAAGCCAGAATCCAGCTTCCAAAACGATCTGGGAGCGGACTCGCTGGACACCGTGGAACTGGTGAT GGCTCTGGAAGAGGCTTTCGATATCGAAATCCCCGATGAAGCCGCTGAAGGCATTGCGACCGTTCAAGAC GCCGTCGATTTCATCGCTAGCAAAGCTGCCTAG
50	*Synechococcus elongatu* PCC 7942_acp Accession# YP_399555 (polypeptide)	MSQEDIFSKVKDIVAEQLSVDVAEVKPESSFQNDLGADSLDTVELVMALEEAFDIEIPDEAAEGIATVQD AVDFIASKAA
51	*Nostoc* sp. PCC 7120_acp Accession# NP_487382.1 (DNA)	ATGAGCCAATCAGAAACTTTTGAAAAAGTCAAAAAAATTGTTATCGAACAACTAAGTGTGGAGAACCCTG ACACAGTAACTCCAGAAGCTAGTTTTGCCAACGATTTACAGGCTGATTCCCTCGATACAGTAGAACTAGT AATGGCTTTGGAAGAAGAATTTGATATCGAAATTCCCGATGAAGCCGCAGAGAAAATTACCACTGTTCAA GAAGCGGTGGATTACATCAATAACCAAGTTGCCGCATCAGCTTAA
52	*Nostoc* sp. PCC 7120_acp Accession# NP_487382.1 (polypeptide)	MSQSETFEKVKKIVIEQLSVENPDTVTPEASFANDLQADSLDTVELVMALEEEFDIEIPDEAAEKITTVQ EAVDYINNQV AASA
53	*B.subtilis* sfp (synthesized)as in accession# X63158.1 (DNA)	ATGAAGATTTACGGAATTTATATGGACCGCCCGCTTTCACAGGAAGAAAATGAACGGTTCATGACTTTCA TATCACCTGAAAAACGGGAGAAATGCCGGAGATTTTATCATAAAGAAGATGCTCACCGCACCCTGCTGGG AGATGTGCTCGTTCGCTCAGTCATAAGCAGGCAGTATCAGTTGGACAAATCCGATATCCGCTTTAGCACG CAGGAATACGGGAAGCCGTGCATCCCTGATCTTCCCGACGCTCATTTCAACATTTCTCACTCCGGCCGCT GGGTCATTGGTGCGTTTGATTCACAGCCGATCGGCATAGATATCGAAAAAACGAAACCGATCAGCCTTGA GATCGCCAAGCGCTTCTTTTCAAAAACAGAGTACAGCGACCTTTTAGCAAAAGACAAGGACGAGCAGACA GACTATTTTTATCATCTATGGTCAATGAAAGAAAGCTTTATCAAACAGGAAGGCAAAGGCTTATCGCTTC CGCTTGATTCCTTTTCAGTGCGCCTGCATCAGGACGGACAAGTATCCATTGAGCTTCCGGACAGCCATTC CCCATGCTATATCAAAACGTATGAGGTCGATCCCGGCTACAAAATGGCTGTATGCGCCGCACACCCTGAT TTCCCCGAGGATATCACAATGGTCTCGTACGAAGAGCTTTTATAA

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
54	*B.subtilis* sfp (synthesized)as in accession# X63158.1 (polypeptide)	MKIYGIYMDRPLSQEENERFMTFISPEKREKCRRFYHKEDAHRTLLGDVLVRSVISRQYQLDKSDIRFSTC EYGKPCIPD LPDAHFNISHSGRWVIGAFDSQPIGIDIEKTKPISLEIAKRFFSKTEYSDLLAKDKDEQTDYFYHLWSMKE SFIKQEGKG LSLPLDSFSVRLHQDGQVSIELPDSHSPCYIKTYEVDPGYKMAVCAAHPDFPEDITMVSYEELL
55	birA from *Corynebacterium glutamicum* (YP_224991), DNA	1 ttgggcgtgt cgcccttaaa gcgcgctttt cgacgcgacc ccactacatt ggcttccatg 61 aacgttgaca tttcacgatc cagagagccg ctaaacgttg agctcctgaa ggaaaaattg 121 ctccaaaacg gtgactttgg ccaggtcatt tacgaaaaag tgacaggctc cactaatgct 181 gacttgctgg cacttgcagg ttctggcgct ccaaactgga cggtgaaaac tgtcgagttt 241 caagatcatg cgcgtgggcg actcggccgc ccgtggtctg cccctgaggg ttcccaaaca 301 atcgtgtctg tgctcgttca actatctatt gatcaagtgg accggattgg cactattcca 361 ctcgcggcgg gactcgctgt catggatgcg ttgaatgacc tcggtgtgga aggtgccgga 421 ctgaaatggc ccaacgatgt tcaaatccac ggcaagaaac tctgcggcat cctggtggaa 481 gccaccggct ttgattccac cccaacagtt gtcatcggtt ggggcactaa tatcagcctg 541 actaaagagg agcttcctgt tcctcatgca acttccctcg cattggaagg tgttgaagtc 601 gacagaacca cattccttat taatatgctc acacatctgc atactcgact ggaccagtgg 661 cagggtccaa gtgtggattg gctcgatgat taccgtgcgg tatgttccag tattggccaa 721 gatgttcgag tgcttctacc tggggataaa gaactcttag gtgaagcgat cggtgtcgcg 781 actggcggag aaattcgtgt tcgcgatgct tcgggcaccg ttcacaccct caacgccggt 841 gaaattacgc accttcgcct gcagtaa
56	birA from birA from *Corynebacterium glutamicum* (YP_224991), synthetic DNA	1 ttgggcgtgt cgcccttaaa gcgcgctttt cgacgcgacc ccactacatt ggcttccatg 61 aacgttgaca tttcacgatc cagagagccg ctaaacgttg agctcctgaa ggaaaaattg 121 ctccaaaacg gtgactttgg ccaggtcatt tacgaaaaag tgacaggctc cactaatgct 181 gacttgctgg cacttgcagg ttctggcgct ccaaactgga cggtgaaaac tgtcgagttt 241 caagatcatg cgcgtgggcg actcggccgc ccgtggtctg cccctgaggg ttcccaaaca 301 atcgtgtctg tgctcgttca actatctatt gatcaagtgg accggattgg cactattcca 361 ctcgcggcgg gactcgctgt catggatgcg ttgaatgacc tcggtgtgga aggtgccgga 421 ctgaaatggc ccaacgatgt tcaaatccac ggcaagaaac tctgcggcat cctggtggaa 481 gccaccggct ttgattccac cccaacagtt gtcatcggtt ggggcactaa tatcagcctg 541 actaaagagg agcttcctgt tcctcatgca acttccctcg cattggaagg tgttgaagtc 601 gacagaacca cattccttat taatatgctc acacatctgc atactcgact ggaccagtgg 661 cagggtccaa gtgtggattg gctcgatgat taccgtgcgg tatgttccag tattggccaa 721 gatgttcgag tgcttctacc tggggataaa gaactcttag gtgaagcgat cggtgtcgcg 781 actggcggag aaattcgtgt tcgcgatgct tcgggcaccg ttcacaccct caacgccggt 841 gaaattacgc accttcgcct gcagtaa
57	*Corynebacterium glutamicum* (YP_224991), Protein	1 MNVDISRSRE PLNVELLKEK LLQNGDFGQV IYEKVTGSTN ADLLALAGSG APNWTVKTVE 61 FQDHARGRLG RPWSAPEGSQ TIVSVLVQLS IDQVDRIGTI PLAAGLAVMD ALNDLGVEGA 121 GLKWPNDVQI HGKKLCGILV EATGFDSTPT VVIGWGTNIS LTKEELPVPH ATSLALEGVE 181 VDRTTFLINM LTHLHTRLDQ WQGPSVDWLD DYRAVCSSIG QDVRVLLPGD KELLGEAIGV 241 ATGGEIRVRD ASGTVHTLNA GEITHLRLQ.
58	accDA1 (dtsR) from *Corynebacterium glutamicum* (YP_224991), DNA	1 ATGACCATTT CCTCACCTTT GATTGACGTC GCCAACCTTC CAGACATCAA CACCACTGCC 61 GGCAAGATCG CCGACCTTAA GGCTCGCCGC GCGGAAGCCC ATTTCCCCAT GGGTGAAAAG 121 GCAGTAGAGA AGGTCCACGC TGCTGGACGC CTCACTGCCC GTGAGCGCTT GGATTACTTA 181 CTCGATGAGG GCTCCTTCAT CGAGACCGAT CAGCTGGCTC GCCACCGCAC CACCGCTTTC 241 GGCCTGGGCG CTAAGCGTCC TGCAACCGAC GGCATCGTGA CCGGCTGGGG CACCATTGAT 301 GGACGCGAAG TCTGCATCTT CTCGCAGGAC GGCACCGTAT TCGGTGGCGC GCTTGGTGAG 361 GTGTACGGCG AAAAGATGAT CAAGATCATG GAGCTGGCAA TCGACACCGG CCGCCCATTG 421 ATCGGTCTTT ACGAAGGCGC TGGCGCTCGT ATTCAGGACG GCGCTGTCTC CCTGGACTTC 481 ATTTCCCAGA CCTTCTACCA AAACATTCAG GCTTCTGGCG TTATCCCACA GATCTCCGTC 541 ATCATGGGCG CATGTGCAGG TGGCAACGCT TACGGCGAAG CTCTGACCGA CTTCGTGGTC 601 ATGGTGGACA AGACCTCCAA GATGTTCGTT ACCGGCCCAG ACGTGATCAA GACCGTCACC 661 GGCGAGGAAA TCACCCAGGA AGAGCTTGGC GGAGCAACCA CCCACATGGT GACCGCTGGT 721 AACTCCCACT ACACCGCTGC GACCGATGAG GAAGCACTGG ATTGGGTACA GGACCTGGTG 781 TCCTTCCTCC CATCCAACAA TCGCTCCTAC GCACCGATGG AAGACTTCGA CGAGGAAGAA 841 GGCGGCGTTG AAGAAAACAT CACCGCTGAC GATCTGAAGC TCGACGAGAT CATCCCAGAT 901 TCCGCGACCG TTCCTTACGA CGTCCGCGAT GTCATCGAAT GCCTCACCGA CGATGGCGAA 961 TACCTGGAAA TCCAGGCAGA CCGCGCAGAA AACGTTGTTA TTGCATTCGG CCGCATCGAA 1021 GGCCAGTCCG TTGGCTTTGT TGCCAACCAG CCAACCCAGT TCGCTGGCTG CCTGGACATC 1081 GACTCCTCTG AGAAGGCAGC TCGCTTCGTC CGCACCTGCG ACGCGTTCAA CATCCCAATC 1141 GTCATGCTTG TCGACGTCCC CGGCTTCCTC CCAGGCGCAG GCCAGGAGTA CGGTGGCATT 1201 CTGCGTCGTG GCGCAAAGCT GCTCTACGCA TACGGCGAAG CAACCGTTCC AAAGATCACC 1261 GTCACCATGC GTAAGGCTTA CGGCGGAGCG TACTGCGTGA TGGGTTCCAA GGGCTTGGGC 1321 TCTGACATCA ACCTTGCATG GCCAACCGCA CAGATCGCCG TCATGGGCGC TGCTGGCGCA 1381 GTTGGATTCA TCTACCGCAA GGAGCTCATG GCAGCTGATG CCAAGGGCCT CGATACCGTA

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
		1441 GCTCTGGCTA AGTCCTTCGA GCGCGAGTAT GAAGACCACA TGCTCAACCC GTACCACGCT 1501 GCAGAACGTG GCCTGATCGA CGCCGTGATC CTGCCAAGCG AAACCCGCGG ACAGATTTCC 1561 CGCAACCTTC GCCTGCTCAA GCACAAGAAC GTCACTCGCC CTGCTCGCAA GCACGGCAAC 1621 ATGCCACTGT AA
59	accDA1 (dtsR) from *Corynebacterium glutamicum* (YP_224991), Protein	1 MTISSPLIDV ANLPDINTTA GKIADLKARR AEAHFPMGEK AVEKVHAAGR LTARERLDYL 61 LDEGSFIETD QLARHRTTAF GLGAKRPATD GIVTGWGTID GREVCIFSQD GTVFGGALGE 121 VYGEKMIKIM ELAIDTGRPL IGLYEGAGAR IQDGAVSLDF ISQTFYQNIQ ASGVIPQISV 181 IMGACAGGNA YGPALTDFVV MVDKTSKMFV TGPDVIKTVT GEEITQEELG GATTHMVTAG 241 NSHYTAATDE EALDWVQDLV SFLPSNNRSY APMEDFDEEE GGVEENITAD DLKLDEIIPD 301 SATVPYDVRD VIECLTDDGE YLEIQADRAE NVVIAFGRIE GQSVGFVANQ PTQFAGCLDI 361 DSSEKAARFV RTCDAFNIPI VMLVDVPGFL PGAGQEYGGI LRRGAKLLYA YGEATVPKIT 421 VTMRKAYGGA YCVMGSKGLG SDINLAWPTA QIAVMGAAGA VGFIYRKELM AADAKGLDTV 481 ALAKSFEREY EDHMLNPYHA AERGLIDAVI LPSETRGQIS RNLRLLKHKN VTRPARKHGN 541 MPL
60	accCB from *Corynebacterium glutamicum* (YP_224991, DNA	1 atgtcagtcg agactcgcaa gatcaccaag gttcttgtcg ctaaccgtgg tgagattgca 61 atccgcgtgt tccgtgcagc tcgagatgaa ggcatcggat ctgtcgccgt ctacgcagag 121 ccagatgcag atgcaccatt cgtgtcatat gcagacgagg cttttgccct cggtggccaa 181 acatccgctg agtcctacct tgtcattgac aagatcatcg atgcggcccg caagtccggc 241 gccgacgcca tccaccccgg ctacggcttc ctcgcagaaa acgctgactt cgcagaagca 301 gtcatcaacg aaggcctgat ctggattgga ccttcacctg agtccatccg ctccctcggc 361 gacaaggtca ccgctcgcca catcgcagat accgccaagg ctccaatggc tcctggcacc 421 aaggaaccag taaaagacgc agcagaagtt gtggctttcg ctgaagaatt cggtctccca 481 atcgccatca aggcagcttt cggtggcggc ggacgtggca tgaaggttgc ctacaagatg 541 gaagaagtcg ctgacctctt cgagtccgca acccgtgaag caaccgcagc gttcggccgc 601 ggcgagtgct tcgtggagcg ctacctggac aaggcacgcc acgttgaggc tcaggtcatc 661 gccgataagc acggcaacgt tgttgtcgcc ggaacccgtg actgctccct gcagcgccgt 721 ttccagaagc tcgtcgaaga agcaccagca ccattcctca ccgatgacca gcgcgagcgt 781 ctccactcct ccgcgaaggc tatctgtaag gaagctggct actacggtgc aggcaccgtt 841 gagtacctcg ttggctccga cggcctgatc tccttcctcg aggtcaacac ccgcctccag 901 gtggaacacc cagtcaccga agagaccacc ggcatcgacc tggtccgcga aatgttccgc 961 atcgcagaag gccacgagct ctccatcaag gaagatccag ctccacgcgg ccacgcattc 1021 gagttccgca tcaacggcga agacgctggc tccaacttca tgcctgcacc aggcaagatc 1081 accagctacc gcgagccaca gggcccaggc gtccgcatgg actccggtgt cgttgaaggt 1141 tccgaaatct ccggacagtt cgactccatg ctggcaaagc tgatcgtttg gggcgacacc 1201 cgcgagcagg ctctccagcg ctcccgccgt gcacttgcag agtacgttgt cgagggcatg 1261 ccaaccgtta tcccattcca ccagcacatc gtggaaaacc cagcattcgt gggcaacgac 1321 gaaggcttcg agatctacac caagtggatc gaagaggttt gggataaccc aatcgcacct 1381 tacgttgacg cttccgagct cgacgaagat gaggacaaga ccccagcaca gaaggttgtt 1441 gtggagatca acggccgtcg cgttgaggtt gcactcccag gcgatctggc actcggtggc 1501 accgctggtc ctaagaagaa ggccaagaag cgtcgcgcag gtggtgcaaa ggctggcgta 1561 tccggcgatg cagtggcagc tccaatgcag ggcactgtca tcaaggtcaa cgtcgaagaa 1621 ggcgctgaag tcaacgaagg cgacaccgtt gttgtcctcg aggctatgaa gatggaaaac 1681 cctgtgaagg ctcataagtc cggaaccgta accggcctta ctgtcgctgc aggcgagggt 1741 gtcaacaagg gcgttgttct cctcgagatc aagtaa
61	accCB from *Corynebacterium glutamicum* (YP_224991, Protein	1 MSVETRKITK VLVANRGEIA IRVFRAARDE GIGSVAVYAE PDADAPFVSY ADEAFALGGQ 61 TSAESYLVID KIIDAARKSG ADAIHPGYGF LAENADFAEA VINEGLIWIG PSPESIRSLG 121 DKVTARHIAD TAKAPMAPGT KEPVKDAAEV VAFAEEFGLP IAIKAAFGGG GRGMKVAYKM 181 EEVADLFESA TREATAAFGR GECFVERYLD KARHVEAQVI ADKHGNVVVA GTRDCSLQRR 241 FQKLVEEAPA PFLTDDQRER LHSSAKAICK EAGYYGAGTV EYLVGSDGLI SFLEVNTRLQ 301 VEHPVTEETT GIDLVREMFR IAEGHELSIK EDPAPRGHAF EFRINGEDAG SNFMPAPGKI 361 TSYREPQGPG VRMDSGVVEG SEISGQFDSM LAKLIVWGDT REQALQRSRR ALAEYVVEGM 421 PTVIPFHQHI VENPAFVGND EGFEIYTKWI EEVWDNPIAP YVDASELDED EDKTPAQKVV 481 VEINGRRVEV ALPGDLALGG TAGPKKKAKK RRAGGAKAGV SGDAVAAPMQ GTVIKVNVEE 541 GAEVNEGDTV VVLEAMKMEN PVKAHKSGTV TGLTVAAGEG VNKGVVLLEI K
62	OP80 trc3 + accDA1CB + birA (pAS119.50D)	FH Key Location/Qualifiers FH FT misc_feature 6622 . . . 6713 FT /note = "lacZalpha" FT /translation = " "A.CGIFSLRICAVFHTAYGALSVQSALMPH FT misc_feature complement(7003 . . . 8013) FT /note = "aadA1- aminoglycoside 3'- adenylyltransferase" FT /translation = "MRSRNWSRTLTERSGGNGAVAVFMACYDCFFGVQSMPRASKQQA FT

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
		RYAVGRCLMLWSSNDVTQQGSRPKTKLNIMREAVIAEVSTQLSEVVGVIERHLEPTLL
		FT
		AVHLYGSAVDGGLKPHSDIDLLVTVTVRLDETTRRALINDLLETSASPGESEILRAVE
		FT
		VTIVVHDDIIPWRYPAKRELQFGEWQRNDILAGIFEPATIDIDLAILLTKAREHSVAL
		FT
		VGPAAEELFDPVPEQDLFEALNETLTLWNSPPDWAGDERNVVLTLSRIWYSAVTGKIA
		FT
		PKDVAADWAMERLPAQYQPVILEARQAYLGQEEDRLASRADQLEEFVHYVKGEITKVV
		FT GK"
		FT misc_feature complement(9046 . . . 9996)
		FT /note = "repA protein"
		FT
		/translation = "MSELVVFKANELAISRYDLTEHETKLILCCVALLNPTIENPTRK
		FT
		ERTVSFTYNQYAQMMNISRENAYGVLAKATRELMTRTVEIRNPLVKGFEIFQWTNYAK
		FT
		FSSEKLELVFSEEILPYLFQLKKFIKYNLEHVKSFENKYSMRIYEWLLKELTQKKTHK
		FT
		ANIEISLDEFKFMLMLENNYHEFKRLNQWVLKPISKDLNTYSNMKLVVDKRGRPTDTL
		FT
		IFQVELDRQMDLVTELENNQIKMNGDKIPTTITSDSYLHNGLRKTLHDALTAKIQLTS
		FT FEAKFLSDMQSKYDLNGSFSWLTQKQRTTLENILAKYGRI"
		FT vector join(1 . . . 329, 6619 . . . 10025)
		FT /source = "pCL1920revised"
		FT /type = "Custom cloned vector"
		FT insert 330 . . . 6621
		FT /source = "pCL1920Ptrc"
		FT /type = "Custom cloned insert"
		FT misc_feature 6425 . . . 6582
		FT /note = "TERM rrnB T1 and T2 transcriptional terminators"
		FT misc_feature 2037 . . . 2063
		FT /note = "mini cistron ORF"
		FT misc_feature 2052 . . . 2057
		FT /note = "RBS (Reinitiation)"
		FT misc_feature 1847 . . . 2036
		FT /note = "Ptrc"
		FT misc_feature 1847 . . . 1852
		FT /note = "-35 region"
		FT misc_feature 1870 . . . 1874
		FT /note = "-10 region"
		FT misc_feature 1882 . . . 1902
		FT /note = "lacO- lac operator"
		FT misc_feature 1918 . . . 1987
		FT /note = "rrnB antitermination signal"
		FT misc_feature 2000 . . . 2008
		FT /note = "g10 RBS (gene 10 region)"
		FT misc_feature 2023 . . . 2027
		FT /note = "RBS"
		FT misc_feature 543 . . . 1625
		FT /note = "Lac Repressor lacIq ORF"
		FT
		/translation = "VKPVTLYDVAEYAGVSYQTVSRVVNQASHVSAKTREKVEAAMAE
		FT
		LNYIPNRVAQQLAGKQSLLIGVATSSLALHAPSQIVAAIKSRADQLGASVVVSMVERS
		FT
		GVEACKAAVHNLLAQRVSGLIINYPLDDQDAIAVEAACTNVPALFLDVSDQTPINSII
		FT
		FSHEDGTRLGVEHLVALGHQQIALLAGPLSSVSARLRLAGWHKYLTRNQIQPIAEREG
		FT
		DWSAMSGFQQTMQMLNEGIVPTAMLVANDQMALGAMRAITESGLRVGADISVVGYDDT
		FT
		EDSSCYIPPLTTIKQDFRLLGQTSVDRLLQLSQGQAVKGNQLLPVSLVKRKTTLAPNT
		FT QTASPRALADSLMQLARQVSRLESGQ"
		FT modified_base 1399 . . . 1399
		FT /note = "Change from C to T"
		FT modified_base 1644 . . . 1644
		FT /note = "Change from G to A"

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
		FT misc_feature 1875 . . . 1875 FT /note = "trc3" FT gene 2067 . . . 3698 FT /note = "dtsR1(accDA1)" FT /translation = "MTISSPLIDVANLPDINTTAGKIADLKARRAEAHFPMGEKAVEK FT VHAAGRLTARERLDYLLDEGSFIETDQLARHRTTAFGLGAKRPATDGIVTGWGTIDGR FT EVCIFSQDGTVFGGALGEVYGEKMIKIMELAIDTGRPLIGLYEGAGARIQDGAVSLDF FT ISQTFYQNIQASGVIPQISVIMGACAGGNAYGPALTDFVVMVDKTSKMFVTGPDVIKT FT VTGEEITQEELGGATTHMVTAGNSHYTAATDEEALDWVQDLVSFLPSNNRSYAPMEDF FT DEEEGGVEENITADDLKLDEIIPDSATVPYDVRDVIECLTDDGEYLEIQADRAENVVI FT APGRIEGQSVGFVANQPTQFAGCLDIDSSEKAARFVRTCDAFNIPIVMLVDVPGFLPG FT AGQEYGGILRRGAKLLYAYGEATVPKITVTMRKAYGGAYCVMGSKGLGSDINLAWPTA FT QIAVMGAAGAVGFIYRKELMAADAKGLDTVALAKSFEREYEDHMLNPYHAAERGLIDA FT VILPSETRGQISRNLRLLKHKNVTRPARKHGNMPL" FT gene 3712 . . . 5487 FT /note = "*C. glutamicum* accCB" FT /translation = "MSVETRKITKVLVANRGEIAIRVFRAARDEGIGSVAVYAEPDAD FT APFVSYADEAFALGGQTSAESYLVIDKIIDAARKSGADAIHPGYGFLAENADFAEAVI FT NEGLIWIGPSPESIRSLGDKVTARHIADTAKAPMAPGTKEPVKDAAEVVAFAEEFGLP FT IAIKAAFGGGGRGMKVAYKMEEVADLFESATREATAAFGRGECFVERYLDKARHVEAQ FT VIADKHGNVVVAGTRDCSLQRRFQKLVEEAPAPFLTDDQRERLHSSAKAICKEAGYYG FT AGTVEYLVGSDGLISFLEVNTRLQVEHPVTEETTGIDLVREMFRIAEGHELSIKEDPA FT PRGHAFEFRINGEDAGSNFMPAPGKITSYREPQGPGVRMDSGVVEGSEISGQFDSMLA FT KLIVWGDTREQALQRSRRALAEYVVEGMPTVIPFHQHIVENPAFVGNDEGFEIYTKWI FT EEVWDNPIAPYVDASELDEDEDKTPAQKVVVEINGRRVEVALPGDLALGGTAGPKKKA FT KKRRAGGAKAGVSGDAVAAPMQGTVIKVNVEEGAEVNEGDTVVVLEAMKMENPVKAHK FT SGTVTGLTVAAGEGVNKGVVLLEIK" FT misc_feature 3727 . . . 3729 FT /note = "rare Arg codon, change to CGT or CGC" FT misc_feature 3712 . . . 3714 FT /note = "GTG start codon, change to ATG" FT gene 5507 . . . 6316 FT /note = "birA_Cg_opt" FT /translation = "MNVDISRSREPLNVELLKEKLLQNGDFGQVIYEKVTGSTNADLL FT ALAGSGAPNWTVKTVEFQDHARGRLGRPWSAPEGSQTIVSVLVQLSIDQVDRIGTIPL FT AAGLAVMDALNDLGVEGAGLKWPNDVQIHGKKLCGILVEATGFDSTPTVVIGWGTNIS FT LTKEELPVPHATSLALEGVEVDRTTFLINMLTHLHTRLDQWQGPSVDWLDDYRAVCSS FT IGQDVRVLLPGDKELLGEAIGVATGGEIRVRDASGTVHTLNAGEITHLRLQ"

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
		FT misc_feature 5493 . . . 5498
		FT /note = "RBS"
		FT source 1 . . . 10025
		FT /dnas_title = "OP80 trc3 + accDA1CB + birA"
		SQ 10025 BP; 2326 A; 2661 C; 2606 G; 2432 T;
		CACTATACCA ATTGAGATGG GCTAGTCAAT GATAATTACT AGTCCTTTTC CTTTGAGTTG
		60
		TGGGTATCTG TAAATTCTGC TAGACCTTTG CTGGAAAACT TGTAAATTCT GCTAGACCCT
		120
		CTGTAAATTC CGCTAGACCT TTGTGTGTTT TTTTTGTTTA TATTCAAGTG GTTATAATTT
		180
		ATAGAATAAA GAAAGAATAA AAAAAGATAA AAAGAATAGA TCCCAGCCCT GTGTATAACT
		240
		CACTACTTTA GTCAGTTCCG CAGTATTACA AAAGGATGTC GCAAACGCTG TTTGCTCCTC
		300
		TACAAAACAG ACCTTAAAAC CCTAAAGGCg tcGGCATCCG CTTACAGACA AGCTGTGACC
		360
		GTCTCCGGGA GCTGCATGTG TCAGAGGTTT TCACCGTCAT CACCGAAACG CGCGAGGCAG
		420
		CAGATCAATT CGCGCGCGAA GGCGAAGCGG CATGCATTTA CGTTGACACC ATCGAATGGT
		480
		GCAAAACCTT TCGCGGTATG GCATGATAGC GCCCGGAAGA GAGTCAATTC AGGGTGGTGA
		540
		ATGTGAAACC AGTAACGTTA TACGATGTCG CAGAGTATGC CGGTGTCTCT TATCAGACCG
		600
		TTTCCCGCGT GGTGAACCAG GCCAGCCACG TTTCTGCGAA AACGCGGGAA AAAGTGGAAG
		660
		CGGCGATGGC GGAGCTGAAT TACATTCCCA ACCGCGTGGC ACAACAACTG GCGGGCAAAC
		720
		AGTCGTTGCT GATTGGCGTT GCCACCTCCA GTCTGGCCCT GCACGCGCCG TCGCAAATTG
		780
		TCGCGGCGAT TAAATCTCGC GCCGATCAAC TGGGTGCCAG CGTGGTGGTG TCGATGGTAG
		840
		AACGAAGCGG CGTCGAAGCC TGTAAAGCGG CGGTGCACAA TCTTCTCGCG CAACGCGTCA
		900
		GTGGGCTGAT CATTAACTAT CCGCTGGATG ACCAGGATGC CATTGCTGTG GAAGCTGCCT
		960
		GCACTAATGT TCCGGCGTTA TTTCTTGATG TCTCTGACCA GACACCCATC AACAGTATTA
		1020
		TTTTCTCCCA TGAAGACGGT ACGCGACTGG GCGTGGAGCA TCTGGTCGCA TTGGGTCACC
		1080
		AGCAAATCGC GCTGTTAGCG GGCCCATTAA GTTCTGTCTC GGCGCGTCTG CGTCTGGCTG
		1140
		GCTGGCATAA ATATCTCACT CGCAATCAAA TTCAGCCGAT AGCGGAACGG GAAGGCGACT
		1200
		GGAGTGCCAT GTCCGGTTTT CAACAAACCA TGCAAATGCT GAATGAGGGC ATCGTTCCCA
		1260
		CTGCGATGCT GGTTGCCAAC GATCAGATGG CGCTGGGCGC AATGCGCGCC ATTACCGAGT
		1320
		CCGGGCTGCG CGTTGGTGCG GATATCTCGG TAGTGGGATA CGACGATACC GAAGACAGCT
		1380
		CATGTTATAT CCCGCCGTtA ACCACCATCA AACAGGATTT TCGCCTGCTG GGGCAAACCA
		1440
		GCGTGGACCG CTTGCTGCAA CTCTCTCAGG GCCAGGCGGT GAAGGGCAAT CAGCTGTTGC
		1500
		CCGTCTCACT GGTGAAAAGA AAAACCACCC TGGCGCCCAA TACGCAAACC GCCTCTCCCC
		1560
		GCGCGTTGGC CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG GAAAGCGGGC
		1620
		AGTGAGCGCA ACGCAATTAA TGTaAGTTAG CGCGAATTGA TCTGGTTTGA CAGCTTATCA
		1680
		TCGACTGCAC GGTGCACCAA TGCTTCTGGC GTCAGGCAGC CATCGGAAGC TGTGGTATGG
		1740
		CTGTGCAGGT CGTAAATCAC TGCATAATTC GTGTCGCTCA AGGCGCACTC CCGTTCTGGA
		1800
		TAATGTTTTT TGCGCCGACA TCATAACGGT TCTGGCAAAT ATTCTGTTGA CAATTAATCA
		1860
		TCCGGCTCGT ATAAaGTGTG GAATTGTGAG CGGATAACAA TTTCACACAG GAAACAGCGC
		1920

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
		CGCTGAGAAA AAGCGAAGCG GCACTGCTCT TTAACAATTT ATCAGACAAT CTGTGTGGGC
1980		ACTCGACCGG AATTATCGAT TAACTTTATT ATTAAAAATT AAAGAGGTAT ATATTAATGT
2040		ATCGATTAAA TAAGGAGGAA TAAACCATGA CCATTTCCTC ACCTTTGATT GACGTCGCCA
2100		ACCTTCCAGA CATCAACACC ACTGCCGGCA AGATCGCCGA CCTTAAGGCT CGCCGCGCGG
2160		AAGCCCATTT CCCCATGGGT GAAAAGGCAG TAGAGAAGGT CCACGCTGCT GGACGCCTCA
2220		CTGCCCGTGA GCGCTTGGAT TACTTACTCG ATGAGGGCTC CTTCATCGAG ACCGATCAGC
2280		TGGCTCGCCA CCGCACCACC GCTTTCGGCC TGGGCGCTAA GCGTCCTGCA ACCGACGGCA
2340		TCGTGACCGG CTGGGGCACC ATTGATGGAC GCGAAGTCTG CATCTTCTCG CAGGACGGCA
2400		CCGTATTCGG TGGCGCGCTT GGTGAGGTGT ACGGCGAAAA GATGATCAAG ATCATGGAGC
2460		TGGCAATCGA CACCGGCCGC CCATTGATCG GTCTTTACGA AGGCGCTGGC GCTCGTATTC
2520		AGGACGGCGC TGTCTCCCTG GACTTCATTT CCCAGACCTT CTACCAAAAC ATTCAGGCTT
2580		CTGGCGTTAT CCCACAGATC TCCGTCATCA TGGGCGCATG TGCAGGTGGC AACGCTTACG
2640		GCCCAGCTCT GACCGACTTC GTGGTCATGG TGGACAAGAC CTCCAAGATG TTCGTTACCG
2700		GCCCAGACGT GATCAAGACC GTCACCGGCG AGGAAATCAC CCAGGAAGAG CTTGGCGGAG
2760		CAACCACCCA CATGGTGACC GCTGGTAACT CCCACTACAC CGCTGCGACC GATGAGGAAG
2820		CACTGGATTG GGTACAGGAC CTGGTGTCCT TCCTCCCATC CAACAATCGC TCCTACGCAC
2880		CGATGGAAGA CTTCGACGAG GAAGAAGGCG GCGTTGAAGA AAACATCACC GCTGACGATC
2940		TGAAGCTCGA CGAGATCATC CCAGATTCCG CGACCGTTCC TTACGACGTC CGCGATGTCA
3000		TCGAATGCCT CACCGACGAT GGCGAATACC TGGAAATCCA GGCAGACCGC GCAGAAAACG
3060		TTGTTATTGC ATTCGGCCGC ATCGAAGGCC AGTCCGTTGG CTTTGTTGCC AACCAGCCAA
3120		CCCAGTTCGC TGGCTGCCTG GACATCGACT CCTCTGAGAA GGCAGCTCGC TTCGTCCGCA
3180		CCTGCGACGC GTTCAACATC CCAATCGTCA TGCTTGTCGA CGTCCCCGGC TTCCTCCCAG
3240		GCGCAGGCCA GGAGTACGGT GGCATTCTGC GTCGTGGCGC AAAGCTGCTC TACGCATACG
3300		GCGAAGCAAC CGTTCCAAAG ATCACCGTCA CCATGCGTAA GGCTTACGGC GGAGCGTACT
3360		GCGTGATGGG TTCCAAGGGC TTGGGCTCTG ACATCAACCT TGCATGGCCA ACCGCACAGA
3420		TCGCCGTCAT GGGCGCTGCT GGCGCAGTTG GATTCATCTA CCGCAAGGAG CTCATGGCAG
3480		CTGATGCCAA GGGCCTCGAT ACCGTAGCTC TGGCTAAGTC CTTCGAGCGC GAGTATGAAG
3540		ACCACATGCT CAACCCGTAC CACGCTGCAG AACGTGGCCT GATCGACGCC GTGATCCTGC
3600		CAAGCGAAAC CCGCGGACAG ATTTCCCGCA ACCTTCGCCT GCTCAAGCAC AAGAACGTCA
3660		CTCGCCCTGC TCGCAAGCAC GGCAACATGC CACTGTAAgg aggaaaacta aatgtcagtc
3720		gagactcgca agatcaccaa ggttcttgtc gctaaccgtg gtgagattgc aatccgcgtg
3780		ttccgtgcag ctcgagatga aggcatcgga tctgtcgccg tctacgcaga gccagatgca
3840		gatgcaccat tcgtgtcata tgcagacgag gcttttgccc tcggtggcca aacatccgct
3900		gagtcctacc ttgtcattga caagatcatc gatgcggccc gcaagtccgg cgccgacgcc
3960		atccaccccg gctacggctt cctcgcagaa aacgctgact tcgcagaagc agtcatcaac
4020		gaaggcctga tctggattgg accttcacct gagtccatcc gctccctcgg cgacaaggtc
4080		

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
		accgctcgcc acatcgcaga taccgccaag gctccaatgg ctcctggcac caaggaacca
4140		
		gtaaaagacg cagcagaagt tgtggctttc gctgaagaat tcggtctccc aatcgccatc
4200		
		aaggcagctt tcggtggcgg cggacgtggc atgaaggttg cctacaagat ggaagaagtc
4260		
		gctgacctct tcgagtccgc aacccgtgaa gcaaccgcag cgttcggccg cggcgagtgc
4320		
		ttcgtggagc gctacctgga caaggcacgc cacgttgagg ctcaggtcat cgccgataag
4380		
		cacggcaacg ttgttgtcgc cggaacccgt gactgctccc tgcagcgccg tttccagaag
4440		
		ctcgtcgaag aagcaccagc accattcccc accgatgacc agcgcgagcg tctccactcc
4500		
		tccgcgaagg ctatctgtaa ggaagctggc tactacggtg caggcaccgt tgagtacctc
4560		
		gttggctccg acggcctgat ctccttcctc gaggtcaaca cccgccccca ggtggaacac
4620		
		ccagtcaccg aagagaccac cggcatcgac ctggtccgcg aaatgttccg catcgcagaa
4680		
		ggccacgagc tctccatcaa ggaagatcca gctccacgcg gccacgcatt cgagttccgc
4740		
		atcaacggcg aagacgctgg ctccaacttc atgcctgcac caggcaagat caccagctac
4800		
		cgcgagccac agggcccagg cgtccgcatg gactccggtg tcgttgaagg ttccgaaatc
4860		
		tccggacagt tcgactccat gctggcaaag ctgatcgttt ggggcgacac ccgcgagcag
4920		
		gctctccagc gctcccgccg tgcacttgca gagtacgttg tcgagggcat gccaaccgtt
4980		
		atcccattcc accagcacat cgtggaaaac ccagcattcg tgggcaacga cgaaggcttc
5040		
		gagatctaca ccaagtggat cgaagaggtt tgggataacc caatcgcacc ttacgttgac
5100		
		gcttccgagc tcgacgaaga tgaggacaag accccagcac agaaggttgt tgtggagatc
5160		
		aacggccgtc gcgttgaggt tgcactccca ggcgatctgg cactcggtgg caccgctggt
5220		
		cctaagaaga aggccaagaa gcgtcgcgca ggtggtgcaa aggctggcgt atccggcgat
5280		
		gcagtggcag ctccaatgca gggcactgtc atcaaggtca acgtcgaaga aggcgctgaa
5340		
		gtcaacgaag gcgacaccgt tgttgtcctc gaggctatga agatggaaaa ccctgtgaag
5400		
		gctcataagt ccggaaccgt aaccggcctt actgtcgctg caggcgaggg tgtcaacaag
5460		
		ggcgttgttc tcctcgagat caagtaaTCT AGAGGAGGAA AACTAAATGA ATGTTGACAT
5520		
		TAGCCGCTCT CGTGAACCGT TGAACGTGGA ACTGTTGAAA GAAAAACTGC TGCAGAACGG
5580		
		TGATTTCGGT CAAGTGATCT ACGAGAAGGT CACCGGCTCT ACCAATGCGG ACCTGCTGGC
5640		
		TCTGGCGGGC AGCGGCGCTC CAAACTGGAC CGTCAAGACT GTTGAATTTC AGGACCACGC
5700		
		CCGTGGCCGT CTGGGTCGTC CGTGGAGCGC ACCGGAGGGT TCCCAAACCA TCGTCAGCGT
5760		
		TCTGGTCCAA CTGAGCATTG ATCAGGTGGA CCGTATTGGT ACGATCCCGC TGGCCGCAGG
5820		
		CTTGGCTGTT ATGGATGCGC TGAATGATCT GGGCGTGGAG GGTGCAGGCC TGAAATGGCC
5880		
		GAACGATGTT CAGATCCACG GTAAGAAGTT GTGCGGTATT CTGGTTGAAG CAACCGGCTT
5940		
		CGACTCCACT CCGACCGTGG TTATCGGTTG GGGTACGAAT ATCTCGTTGA CGAAAGAAGA
6000		
		GCTGCCGGTC CCGCACGCGA CCAGCCTGGC CCTGGAGGGT GTTGAAGTTG ACCGTACGAC
6060		
		GTTCCTGATT AACATGCTGA CCCATCTGCA TACCCGTCTG GATCAGTGGC AGGGTCCGTC
6120		
		TGTGGACTGG CTGGATGACT ATCGCGCGGT TTGTAGCAGC ATTGGCCAAG ATGTGCGTGT
6180		
		CCTGCTGCCT GGTGACAAAG AGCTGCTGGG CGAGGCGATT GGCGTGGCGA CCGGTGGTGA
6240		

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
		GATCCGTGTG CGCGACGCCA GCGGCACGGT CCACACGCTG AATGCGGGTG AAATCACGCA
		6300
		TCTGCGTTTG CAATAAAAGC TTGTTTAAAC GGTCTCCAGC TTGGCTGTTT TGGCGGATGA
		6360
		GAGAAGATTT TCAGCCTGAT ACAGATTAAA TCAGAACGCA GAAGCGGTCT GATAAAACAG
		6420
		AATTTGCCTG GCGGCAGTAG CGCGGTGGTC CCACCTGACC CCATGCCGAA CTCAGAAGTG
		6480
		AAACGCCGTA GCGCCGATGG TAGTGTGGGG TCTCCCCATG CGAGAGTAGG GAACTGCCAG
		6540
		GCATCAAATA AAACGAAAGG CTCAGTCGAA AGACTGGGCC TTTCGTTTTA TCTGTTGTTT
		6600
		GTCGGTGAAC GCTCTCCTga cGCCTGATGC GGTATTTTCT CCTTACGCAT CTGTGCGGTA
		6660
		TTTCACACCG CATATGGTGC ACTCTCAGTA CAATCTGCTC TGATGCCGCA TAGTTAAGCC
		6720
		AGCCCCGACA CCCGCCAACA CCCGCTGACG AGCTTAGTAA AGCCCTCGCT AGATTTTAAT
		6780
		GCGGATGTTG CGATTACTTC GCCAACTATT GCGATAACAA GAAAAAGCCA GCCTTTCATG
		6840
		ATATATCTCC CAATTTGTGT AGGGCTTATT ATGCACGCTT AAAAATAATA AAAGCAGACT
		6900
		TGACCTGATA GTTTGGCTGT GAGCAATTAT GTGCTTAGTG CATCTAACGC TTGAGTTAAG
		6960
		CCGCGCCGCG AAGCGGCGTC GGCTTGAACG AATTGTTAGA CATTATTTGC CGACTACCTT
		7020
		GGTGATCTCG CCTTTCACGT AGTGGACAAA TTCTTCCAAC TGATCTGCGC GCGAGGCCAA
		7080
		GCGATCTTCT TCTTGTCCAA GATAAGCCTG TCTAGCTTCA AGTATGACGG GCTGATACTG
		7140
		GGCCGGCAGG CGCTCCATTG CCCAGTCGGC AGCGACATCC TTCGGCGCGA TTTTGCCGGT
		7200
		TACTGCGCTG TACCAAATGC GGGACAACGT AAGCACTACA TTTCGCTCAT CGCCAGCCCA
		7260
		GTCGGGCGGC GAGTTCCATA GCGTTAAGGT TTCATTTAGC GCCTCAAATA GATCCTGTTC
		7320
		AGGAACCGGA TCAAAGAGTT CCTCCGCCGC TGGACCTACC AAGGCAACGC TATGTTCTCT
		7380
		TGCTTTTGTC AGCAAGATAG CCAGATCAAT GTCGATCGTG GCTGGCTCGA AGATACCTGC
		7440
		AAGAATGTCA TTGCGCTGCC ATTCTCCAAA TTGCAGTTCG CGCTTAGCTG GATAACGCCA
		7500
		CGGAATGATG TCGTCGTGCA CAACAATGGT GACTTCTACA GCGCGGAGAA TCTCGCTCTC
		7560
		TCCAGGGGAA GCCGAAGTTT CCAAAAGGTC GTTGATCAAA GCTCGCCGCG TTGTTTCATC
		7620
		AAGCCTTACG GTCACCGTAA CCAGCAAATC AATATCACTG TGTGGCTTCA GGCCGCCATC
		7680
		CACTGCGGAG CCGTACAAAT GTACGGCCAG CAACGTCGGT TCGAGATGGC GCTCGATGAC
		7740
		GCCAACTACC TCTGATAGTT GAGTCGATAC TTCGGCGATC ACCGCTTCCC TCATGATGTT
		7800
		TAACTTTGTT TTAGGGCGAC TGCCCTGCTG CGTAACATCG TTGCTGCTCC ATAACATCAA
		7860
		ACATCGACCC ACGGCGTAAC GCGCTTGCTG CTTGGATGCC CGAGGCATAG ACTGTACCCC
		7920
		AAAAAAACAG TCATAACAAG CCATGAAAAC CGCCACTGCG CCGTTACCAC CGCTGCGTTC
		7980
		GGTCAAGGTT CTGGACCAGT TGCGTGAGCG CATACGCTAC TTGCATTACA GCTTACGAAC
		8040
		CGAACAGGCT TATGTCCACT GGGTTCGTGC CTTCATCCGT TTCCACGGTG TGCGTCACCC
		8100
		GGCAACCTTG GGCAGCAGCG AAGTCGAGGC ATTTCTGTCC TGGCTGGCGA ACGAGCGCAA
		8160
		GGTTTCGGTC TCCACGCATC GTCAGGCATT GGCGGCCTTG CTGTTCTTCT ACGGCAAGGT
		8220
		GCTGTGCACG GATCTGCCCT GGCTTCAGGA GATCGGAAGA CCTCGGCCGT CGCGGCGCTT
		8280
		GCCGGTGGTG CTGACCCCGG ATGAAGTGGTTCGCATCCTCG GTTTTCTGG AAGGCGAGCA
		8340
		TCGTTTGTTC GCCCAGCTTC TGTATGGAAC GGGCATGCGG ATCAGTGAGG GTTTGCAACT
		8400

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
		GCGGGTCAAG GATCTGGATT TCGATCACGG CACGATCATC GTGCGGGAGG GCAAGGGCTC
		8460
		CAAGGATCGG GCCTTGATGT TACCCGAGAG CTTGGCACCC AGCCTGCGCG AGCAGGGGAA
		8520
		TTAATTCCCA CGGGTTTTGC TGCCCGCAAA CGGGCTGTTC TGGTGTTGCT AGTTTGTTAT
		8580
		CAGAATCGCA GATCCGGCTT CAGCCGGTTT GCCGGCTGAA AGCGCTATTT CTTCCAGAAT
		8640
		TGCCATGATT TTTTCCCCAC GGGAGGCGTC ACTGGCTCCC GTGTTGTCGG CAGCTTTGAT
		8700
		TCGATAAGCA GCATCGCCTG TTTCAGGCTG TCTATGTGTG ACTGTTGAGC TGTAACAAGT
		8760
		TGTCTCAGGT GTTCAATTTC ATGTTCTAGT TGCTTTGTTT TACTGGTTTC ACCTGTTCTA
		8820
		TTAGGTGTTA CATGCTGTTC ATCTGTTACA TTGTCGATCT GTTCATGGTG AACAGCTTTG
		8880
		AATGCACCAA AAACTCGTAA AAGCTCTGATGTATCTATCTT TTTTACACC GTTTTCATCT
		8940
		GTGCATATGG ACAGTTTTCC CTTTGATATG TAACGGTGAA CAGTTGTTCT ACTTTTGTTT
		9000
		GTTAGTCTTG ATGCTTCACT GATAGATACA AGAGCCATAA GAACCTCAGA TCCTTCCGTA
		9060
		TTTAGCCAGT ATGTTCTCTA GTGTGGTTCG TTGTTTTTGC GTGAGCCATG AGAACGAACC
		9120
		ATTGAGATCA TACTTACTTT GCATGTCACT CAAAAATTTT GCCTCAAAAC TGGTGAGCTG
		9180
		AATTTTTGCA GTTAAAGCAT CGTGTAGTGT TTTTCTTAGT CCGTTATGTA GGTAGGAATC
		9240
		TGATGTAATG GTTGTTGGTA TTTTGTCACC ATTCATTTTT ATCTGGTTGT TCTCAAGTTC
		9300
		GGTTACGAGA TCCATTTGTC TATCTAGTTC AACTTGGAAA ATCAACGTAT CAGTCGGGCG
		9360
		GCCTCGCTTA TCAACCACCA ATTTCATATT GCTGTAAGTG TTTAAATCTT TACTTATTGG
		9420
		TTTCAAAACC CATTGGTTAA GCCTTTTAAA CTCATGGTAG TTATTTTCAA GCATTAACAT
		9480
		GAACTTAAAT TCATCAAGGC TAATCTCTAT ATTTGCCTTG TGAGTTTTCT TTTGTGTTAG
		9540
		TTCTTTTAAT AACCACTCAT AAATCCTCAT AGAGTATTTG TTTTCAAAAG ACTTAACATG
		9600
		TTCCAGATTA TATTTTATGA ATTTTTTTAA CTGGAAAAGA TAAGGCAATA TCTCTTCACT
		9660
		AAAAACTAAT TCTAATTTTT CGCTTGAGAA CTTGGCATAG TTTGTCCACT GGAAAATCTC
		9720
		AAAGCCTTTA ACCAAAGGAT TCCTGATTTC CACAGTTCTC GTCATCAGCT CTCTGGTTGC
		9780
		TTTAGCTAAT ACACCATAAG CATTTTCCCT ACTGATGTTC ATCATCTGAG CGTATTGGTT
		9840
		ATAAGTGAAC GATACCGTCC GTTCTTTCCT TGTAGGGTTT TCAATCGTGG GGTTGAGTAG
		9900
		TGCCACACAG CATAAAATTA GCTTGGTTTC ATGCTCCGTT AAGTCATAGC GACTAATCGC
		9960
		TAGTTCATTT GCTTTGAAAA CAACTAATTC AGACATACAT CTCAATTGGT CTAGGTGATT
		10020
		TTAAT
		10030
63	pDS57 +	FH Key Location/Qualifiers
	accDA1CB + bir (pTB.74) circular DNA; 11469 BP	FH FT misc_feature 8066 . . . 8157 FT /note = "lacZalpha" FT /translation = "MTMITPSLHACRSTLEDPRVPSSNSLAVVLQRRDWENPGVTQLN FT RLAAHPPFASWRNSEEARTDRPSQQLRSLNGEWRLMRYFLLTHLCGISHRIWCTLSTI FT CSDAA" FT misc_feature complement(8447 . . . 9457) FT /note = "aadA1- aminoglycoside 3'- adenylyltransferase" FT /translation = "MRSRNWSRTLTERSGGNGAVAVFMACYDCFFGVQSMPRASKQQA FT

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
		RYAVGRCLMLWSSNDVTQQGSRPKTKLNIMREAVIAEVSTQLSEVVGVIERHLEPTLL
		FT
		AVHLYGSAVDGGLKPHSDIDLLVTVTVRLDETTRRALINDLLETSASPGESEILRAVE
		FT
		VTIVVHDDIIPWRYPAKRELQFGEWQRNDILAGIFEPATIDIDLAILLTKAREHSVAL
		FT
		VGPAAEELFDPVPEQDLFEALNETLTLWNSPPDWAGDERNVVLTLSRIWYSAVTGKIA
		FT
		PKDVAADWAMERLPAQYQPVILEARQAYLGQEEDRLASRADQLEEFVHYVKGEITKVV
		FT GK"
		FT misc_feature complement(10490 . . . 11440)
		FT /note = "repA protein"
		FT
		/translation = "MSELVVFKANELAISRYDLTEHETKLILCCVALLNPTIENPTRK
		FT
		ERTVSFTYNQYAQMMNISRENAYGVLAKATRELMTRTVEIRNPLVKGFEIFQWTNYAK
		FT
		FSSEKLELVFSEEILPYLFQLKKFIKYNLEHVKSFENKYSMRIYEWLLKELTQKKTHK
		FT
		ANIEISLDEFKFMLMLENNYHEFKRLNQWVLKPISKDLNTYSNMKLVVDKRGRPTDTL
		FT
		IFQVELDRQMDLVTELENNQIKMNGDKIPTTITSDSYLHNGLRKTLHDALTAKIQLTS
		FT FEAKFLSDMQSKYDLNGSFSWLTQKQRTTLENILAKYGRI"
		FT vector join(1 . . . 329, 8059 . . . 11465)
		FT /source = "pCL1920revised"
		FT /type = "Custom cloned vector"
		FT insert join(330 . . . 1840, 3500 . . . 8061)
		FT /source = "pCL1920Ptrc"
		FT /type = "Custom cloned insert"
		FT misc_feature 7869 . . . 8026
		FT /note = "TERM rrnB T1 and T2 transcriptional terminators"
		FT misc_feature 543 . . . 1625
		FT /note = "Lac Repressor lacI ORF"
		FT
		/translation = "VKPVTLYDVAEYAGVSYQTVSRVVNQASHVSAKTREKVEAAMAE
		FT
		LNYIPNRVAQQLAGKQSLLIGVATSSLALHAPSQIVAAIKSRADQLGASVVVSMVERS
		FT
		GVEACKAAVHNLLAQRVSGLIINYPLDDQDAIAVEAACTNVPALFLDVSDQTPINSII
		FT
		FSHEDGTRLGVEHLVALGHQQIALLAGPLSSVSARLRLAGWHKYLTRNQIQPIAEREG
		FT
		DWSAMSGFQQTMQMLNEGIVPTAMLVANDQMALGAMRAITESGLRVGADISVVGYDDT
		FT
		EDSSCYIPPSTTIKQDFRLLGQTSVDRLLQLSQGQAVKGNQLLPVSLVKRKTTLAPNT
		FT QTASPRALADSLMQLARQVSRLESGQ"
		FT /note = "Change from C to T"
		FT modified_base 1644 . . . 1644
		FT /note = "Change from G to A"
		FT insert 1841 . . . 3499
		FT /source = "pds57ctg.seq"
		FT /type = "Custom cloned insert"
		FT promoter 1854 . . . 2046
		FT /note = "Ptrc"
		FT gene 2077 . . . 3498
		FT /note = "ES9(WS377)"
		FT
		/translation = "MKRLGTLDASWLAVESEDTPMHVGTLQIFSLPEGAPETFLRDMV
		FT
		TRMKEAGDVAPPWGYKLAWSGFLGRVIAPAWKVDKDIDLDYHVRHSALPRPGGERELG
		FT
		ILVSRLHSNPLDFSRPLWECHVIEGLENNRFALYTKMHHSMIDGISGVRLMQRVLTTD
		FT
		PERCNMPPPWTVRPHQRRGAKTDKEASVPAAVSQAMDALKLQADMAPRLWQAGNRLVH
		FT
		SVRHPEDGLTAPFTGPVSVLNHRVTAQRRFATQHYQLDRLKNLAHASGGSLNDIVLYL
		FT
		CGTALRRFLAEQNNLPDTPLTAGIPVNIRPADDEGTGTQISFMIASLATDEADPLNRL
		FT

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
		QQIKTSTRRAKEHLQKLPKSALTQYTMLLMSPYILQLMSGLGGRMRPVFNVTISNVPG
		FT
		PEGTLYYEGARLEAMYPVSLIAHGGALNITCLSYAGSLNFGFTGCRDTLPSMQKLAVY
		FT TGEALDELESLILPPKKRARTRK"
		FT misc_feature 1892 . . . 1912
		FT /note = "lac operator"
		FT -35_signal 1857 . . . 1862
		FT /note = "-35"
		FT -10_signal 1880 . . . 1885
		FT /note = "-10"
		FT misc_feature 1928 . . . 1997
		FT /note = "rrnB antitermination"
		FT RBS 2065 . . . 2069
		FT /note = "RBS"
		FT /note = "Seq-lacI-1"
		FT gene 3517 . . . 5148
		FT /note = "dtsR1(accDA1)"
		FT
		/translation = "MTISSPLIDVANLPDINTTAGKIADLKARRAEAHFPMGEKAVEK
		FT
		VHAAGRLTARERLDYLLDEGSFIETDQLARHRTTAFGLGAKRPATDGIVTGWGTIDGR
		FT
		EVCIFSQDGTVFGGALGEVYGEKMIKIMELAIDTGRPLIGLYEGAGARIQDGAVSLDF
		FT
		ISQTFYQNIQASGVIPQISVIMGACAGGNAYGPALTDFVVMVDKTSKMFVTGPDVIKT
		FT
		VTGEEITQEELGGATTHMVTAGNSHYTAATDEEALDWVQDLVSFLPSNNRSYAPMEDF
		FT
		DEEEGGVEENITADDLKLDEIIPDSATVPYDVRDVIECLTDDGEYLEIQADRAENVVI
		FT
		AFGRIEGQSVGFVANQPTQFAGCLDIDSSEKAARFVRTCDAFNIPIVMLVDVPGFLPG
		FT
		AGQEYGGILRRGAKLLYAYGEATVPKITVTMRKAYGGAYCVMGSKGLGSDINLAWPTA
		FT
		QIAVMGAAGAVGFIYRKELMAADAKGLDTVALAKSFEREYEDHMLNPYHAAERGLIDA
		FT VILPSETRGQISRNLRLLKHKNVTRPARKHGNMPL"
		FT gene 5162 . . . 6937
		FT /note = "*C. glutamicum* accCB"
		FT
		/translation = "MSVETRKITKVLVANRGEIAIRVFRAARDEGIGSVAVYAEPDAD
		FT
		APFVSYADEAFALGGQTSAESYLVIDKIIDAARKSGADAIHPGYGFLAENADFAEAVI
		FT
		NEGLIWIGPSPESIRSLGDKVTARHIADTAKAPMAPGTKEPVKDAAEVVAFAEEFGLP
		FT
		IAIKAAFGGGGRGMKVAYKMEEVADLFESATREATAAFGRGECFVERYLDKARHVEAQ
		FT
		VIADKHGNVVVAGTRDCSLQRRFQKLVEEAPAPFLTDDQRERLHSSAKAICKEAGYYG
		FT
		AGTVEYLVGSDGLISFLEVNTRLQVEHPVTEETTGIDLVREMFRIAEGHELSIKEDPA
		FT
		PRGHAFEFRINGEDAGSNFMPAPGKITSYREPQGPGVRMDSGVVEGSEISGQFDSMLA
		FT
		KLIVWGDTREQALQRSRRALAEYVVEGMPTVIPFHQHIVENPAFVGNDEGFEIYTKWI
		FT
		EEVWDNPIAPYVDASELDEDEDKTPAQKVVVEINGRRVEVALPGDLALGGTAGPKKKA
		FT
		KKRRAGGAKAGVSGDAVAAPMQGTVIKVNVEEGAEVNEGDTVVVLEAMKMENPVKAHK
		FT SGTVTGLTVAAGEGVNKGVVLLEIK"
		FT misc_feature 5177 . . . 5179
		FT /note = "rare Arg codon, change to CGT or CGC"
		FT misc_feature 5162 . . . 5164
		FT /note = "GTG start codon, change to ATG"
		FT gene 6957 . . . 7766
		FT /note = "birA_Cg_opt"
		FT
		/translation = "MNVDISRSREPLNVELLKEKLLQNGDFGQVIYEKVTGSTNADLL
		FT
		ALAGSGAPNWTVKTVEFQDHARGRLGRPWSAPEGSQTIVSVLVQLSIDQVDRIGTIPL
		FT

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
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		FT
		LTKEELPVPHATSLALEGVEVDRTTFLINMLTHLHTRLDQWQGPSVDWLDDYRAVCSS
		FT
		IGQDVRVLLPGDKELLGEAIGVATGGEIRVRDASGTVHTLNAGEITHLRLQ"
		FT misc_feature 6943 . . . 6948
		FT /note = "RBS"
		FT misc_feature 7771 . . . 7785
		FT /dnas_title = "pDS57 + accDA1CBbir"
		SQ 11469 BP; 2640 A; 3106 C; 3007 G; 2716 T;
		CACTATACCA ATTGAGATGG GCTAGTCAAT GATAATTACT AGTCCTTTTC CTTTGAGTTG
		60
		TGGGTATCTG TAAATTCTGC TAGACCTTTG CTGGAAAACT TGTAAATTCT GCTAGACCCT
		120
		CTGTAAATTC CGCTAGACCT TTGTGTGTTT TTTTTGTTTA TATTCAAGTG GTTATAATTT
		180
		ATAGAATAAA GAAAGAATAA AAAAAGATAA AAAGAATAGA TCCCAGCCCT GTGTATAACT
		240
		CACTACTTTA GTCAGTTCCG CAGTATTACA AAAGGATGTC GCAAACGCTG TTTGCTCCTC
		300
		TACAAAACAG ACCTTAAAAC CCTAAAGGCg tcGGCATCCG CTTACAGACA AGCTGTGACC
		360
		GTCTCCGGGA GCTGCATGTG TCAGAGGTTT TCACCGTCAT CACCGAAACG CGCGAGGCAG
		420
		CAGATCAATT CGCGCGCGAA GGCGAAGCGG CATGCATTTA CGTTGACACC ATCGAATGGT
		480
		GCAAAACCTT TCGCGGTATG GCATGATAGC GCCCGGAAGA GAGTCAATTC AGGGTGGTGA
		540
		ATGTGAAACC AGTAACGTTA TACGATGTCG CAGAGTATGC CGGTGTCTCT TATCAGACCG
		600
		TTTCCCGCGT GGTGAACCAG GCCAGCCACG TTTCTGCGAA AACGCGGGAA AAAGTGGAAG
		660
		CGGCGATGGC GGAGCTGAAT TACATTCCCA ACCGCGTGGC ACAACAACTG GCGGGCAAAC
		720
		AGTCGTTGCT GATTGGCGTT GCCACCTCCA GTCTGGCCCT GCACGCGCCG TCGCAAATTG
		780
		TCGCGGCGAT TAAATCTCGC GCCGATCAAC TGGGTGCCAG CGTGGTGGTG TCGATGGTAG
		840
		AACGAAGCGG CGTCGAAGCC TGTAAAGCGG CGGTGCACAA TCTTCTCGCG CAACGCGTCA
		900
		GTGGGCTGAT CATTAACTAT CCGCTGGATG ACCAGGATGC CATTGCTGTG GAAGCTGCCT
		960
		GCACTAATGT TCCGGCGTTA TTTCTTGATG TCTCTGACCA GACACCCATC AACAGTATTA
		1020
		TTTTCTCCCA TGAAGACGGT ACGCGACTGG GCGTGGAGCA TCTGGTCGCA TTGGGTCACC
		1080
		AGCAAATCGC GCTGTTAGCG GGCCCATTAA GTTCTGTCTC GGCGCGTCTG CGTCTGGCTG
		1140
		GCTGGCATAA ATATCTCACT CGCAATCAAA TTCAGCCGAT AGCGGAACGG GAAGGCGACT
		1200
		GGAGTGCCAT GTCCGGTTTT CAACAAACCA TGCAAATGCT GAATGAGGGC ATCGTTCCCA
		1260
		CTGCGATGCT GGTTGCCAAC GATCAGATGG CGCTGGGCGC AATGCGCGCC ATTACCGAGT
		1320
		CCGGGCTGCG CGTTGGTGCG GATATCTCGG TAGTGGGATA CGACGATACC GAAGACAGCT
		1380
		CATGTTATAT CCCGCCGTtA ACCACCATCA AACAGGATTT TCGCCTGCTG GGGCAAACCA
		1440
		GCGTGGACCG CTTGCTGCAA CTCTCTCAGG GCCAGGCGGT GAAGGGCAAT CAGCTGTTGC
		1500
		CCGTCTCACT GGTGAAAAGA AAAACCACCC TGGCGCCCAA TACGCAAACC GCCTCTCCCC
		1560
		GCGCGTTGGC CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG GAAAGCGGGC
		1620
		AGTGAGCGCA ACGCAATTAA TGTaAGTTAG CGCGAATTGA TCTGGTTTGA CAGCTTATCA
		1680
		TCGACTGCAC GGTGCACCAA TGCTTCTGGC GTCAGGCAGC CATCGGAAGC TGTGGTATGG
		1740
		CTGTGCAGGT CGTAAATCAC TGCATAATTC GTGTCGCTCA AGGCGCACTC CCGTTCTGGA
		1800
		TAATGTTTTT TGCGCCGACA TCATAACGGT TCTGGCAAAT ATTCTGAAAT GAGCTGTTGA
		1860

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
		CAATTAATCA TCCGGCTCGT ATAATGTGTG GAATTGTGAG CGGATAACAA TTTCACACAG
		1920
		GAAACAGCGC CGCTGAGAAA AAGCGAAGCG GCACTGCTCT TTAACAATTT ATCAGACAAT
		1980
		CTGTGTGGGC ACTCGACCGG AATTATCGAT TAACTTTATT ATTAAAAATT AAAGAGGTAT
		2040
		ATATTAATGT ATCGATTAAA TAAGGAGGAA TAAACCATGA AACGTCTCGG AACCCTGGAC
		2100
		GCCTCCTGGC TGGCGGTTGA ATCTGAAGAC ACCCCGATGC ATGTGGGTAC GCTTCAGATT
		2160
		TTCTCACTGC CGGAAGGCGC ACCAGAAACC TTCCTGCGTG ACATGGTCAC TCGAATGAAA
		2220
		GAGGCCGGCG ATGTGGCACC ACCCTGGGGA TACAAACTGG CCTGGTCTGG TTTCCTCGGG
		2280
		CGCGTGATCG CCCCGGCCTG GAAAGTCGAT AAGGATATCG ATCTGGATTA TCACGTCCGG
		2340
		CACTCAGCCC TGCCTCGCCC CGGCGGGGAG CGCGAACTGG GTATTCTGGT ATCCCGACTG
		2400
		CACTCTAACC CCCTGGATTT TTCCCGCCCT CTTTGGGAAT GCCACGTTAT TGAAGGCCTG
		2460
		GAGAATAACC GTTTTGCCCT TTACACCAAA ATGCACCACT CGATGATTGA CGGCATCAGC
		2520
		GGCGTGCGAC TGATGCAGAG GGTGCTCACC ACCGATCCCG AACGCTGCAATATGCCACCG
		2580
		CCCTGGACGG TACGCCCACA CCAACGCCGT GGTGCAAAAA CCGACAAAGA GGCCAGCGTG
		2640
		CCCGCAGCGG TTTCCCAGGC AATGGACGCC CTGAAGCTCC AGGCAGACAT GGCCCCCAGG
		2700
		CTGTGGCAGG CCGGCAATCG CCTGGTGCAT TCGGTTCGAC ACCCGGAAGA CGGACTGACC
		2760
		GCGCCCTTCA CTGGACCGGT TTCGGTGCTC AATCACCGGG TTACCGCGCA GCGACGTTTT
		2820
		GCCACCCAGC ATTATCAACT GGACCGGCTG AAAAACCTGG CCCATGCTTC CGGCGGTTCC
		2880
		TTGAACGACA TCGTGCTTTA CCTGTGTGGC ACCGCATTGC GGCGCTTTCT GGCTGAGCAG
		2940
		AACAATCTGC CAGACACCCC GCTGACGGCT GGTATACCG GTGAATATCCG GCCGGCAGAC
		3000
		GACGAGGGTA CGGGCACCCA GATCAGTTTT ATGATTGCCT CGCTGGCCAC CGACGAAGCT
		3060
		GATCCGTTGA ACCGCCTGCA ACAGATCAAA ACCTCGACCC GACGGGCCAA GGAGCACCTG
		3120
		CAGAAACTTC CAAAAAGTGC CCTGACCCAG TACACCATGC TGCTGATGTC ACCCTACATT
		3180
		CTGCAATTGA TGTCAGGTCT CGGGGGGAGG ATGCGACCAG TCTTCAACGT GACCATTTCC
		3240
		AACGTGCCCG GCCCGGAAGG CACGCTGTAT TATGAAGGAG CCCGGCTTGA GGCCATGTAT
		3300
		CCGGTATCGC TAATCGCTCA CGGCGGCGCC CTGAACATCA CCTGCCTGAG CTATGCCGGA
		3360
		TCGCTGAATT TCGGTTTTAC CGGCTGTCGG GATACGCTGC CGAGCATGCA GAAACTGGCG
		3420
		GTTTATACCG GTGAAGCTCT GGATGAGCTG GAATCGCTGA TTCTGCCACC CAAGAAGCGC
		3480
		GCCCGAACCC GCAAGTAACT CGAggaggaa aactaaATGA CCATTTCCTC ACCTTTGATT
		3540
		GACGTCGCCA ACCTTCCAGA CATCAACACC ACTGCCGGCA AGATCGCCGA CCTTAAGGCT
		3600
		CGCCGCGCGG AAGCCCATTT CCCCATGGGT GAAAAGGCAG TAGAGAAGGT CCACGCTGCT
		3660
		GGACGCCTCA CTGCCCGTGA GCGCTTGGAT TACTTACTCG ATGAGGGCTC CTTCATCGAG
		3720
		ACCGATCAGC TGGCTCGCCA CCGCACCACC GCTTTCGGCC TGGGCGCTAA GCGTCCTGCA
		3780
		ACCGACGGCA TCGTGACCGG CTGGGGCACC ATTGATGGAC GCGAAGTCTG CATCTTCTCG
		3840
		CAGGACGGCA CCGTATTCGG TGGCGCGCTT GGTGAGGTGT ACGGCGAAAA GATGATCAAG
		3900
		ATCATGGAGC TGGCAATCGA CACCGGCCGC CCATTGATCG GTCTTTACGA AGGCGCTGGC
		3960
		GCTCGTATTC AGGACGGCGC TGTCTCCCTG GACTTCATTT CCCAGACCTT CTACCAAAAC
		4020

TABLE 14-continued

Table of Sequences.

SEQ ID	Designation	Sequence
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		4740
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TABLE 14-continued

Table of Sequences.

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		6360
		ccgcgagcag gctctccagc gctcccgccg tgcacttgca gagtacgttg tcgagggcat
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		6600
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		6780
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		6900
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TABLE 14-continued

Table of Sequences.

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		CCAAGCGATC TTCTTCTTGT CCAAGATAAG CCTGTCTAGC TTCAAGTATG ACGGGCTGAT
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		ACTGGGCCGG CAGGCGCTCC ATTGCCCAGT CGGCAGCGAC ATCCTTCGGC GCGATTTTGC
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		TCAAACATCG ACCCACGGCG TAACGCGCTT GCTGCTTGGA TGCCCGAGGC ATAGACTGTA
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		CCCCAAAAAA ACAGTCATAA CAAGCCATGA AAACCGCCAC TGCGCCGTTA CCACCGCTGC
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TABLE 14-continued

Table of Sequences.

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		10680
		AATCTGATGT AATGGTTGTT GGTATTTTGT CACCATTCAT TTTTATCTGG TTGTTCTCAA
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		GTTCGGTTAC GAGATCCATT TGTCTATCTA GTTCAACTTG GAAAATCAAC GTATCAGTCG
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		10860
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		CATGTTCCAG ATTATATTTT ATGAATTTTT TTAACTGGAA AAGATAAGGC AATATCTCTT
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		CACTAAAAAC TAATTCTAAT TTTTCGCTTG AGAACTTGGC ATAGTTTGTC CACTGGAAAA
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		11220
		TTGCTTTAGC TAATACACCA TAAGCATTTT CCCTACTGAT GTTCATCATC TGAGCGTATT
		11280
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		11340
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acttcgtata gcatacatta tacgaagtta tggatccagc ttatcgatac cgtcaaacaa 1140

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Synthetic polynucleotide"

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Val Glu Val Lys Val Glu Tyr Cys Gly Leu Cys His Ser Asp Leu Ser
35 40 45

Val Ile Asn Asn Glu Trp Gln Ser Ser Val Tyr Pro Ala Val Ala Gly
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His Glu Ile Ile Gly Thr Ile Ile Ala Leu Gly Ser Glu Ala Lys Gly
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Leu Lys Leu Gly Gln Arg Val Gly Ile Gly Trp Thr Ala Glu Thr Cys
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Gln Ala Cys Asp Pro Cys Ile Gly Gly Asn Gln Val Leu Cys Thr Gly
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Glu Lys Lys Ala Thr Ile Ile Gly His Ala Gly Gly Phe Ala Asp Lys
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Pro Leu Leu Lys His Lys Ile Gln Ala Thr His His Val Gly Val Ile
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Gly Ile Gly Gly Leu Gly His Ile Ala Ile Lys Leu Leu Lys Ala Trp
            180                 185                 190

Gly Cys Glu Ile Thr Ala Phe Ser Ser Asn Pro Asp Lys Thr Glu Glu
        195                 200                 205

Leu Lys Ala Asn Gly Ala Asp Gln Val Val Asn Ser Arg Asp Ala Gln
    210                 215                 220

Ala Ile Lys Gly Thr Arg Trp Lys Leu Ile Ile Leu Ser Thr Ala Asn
225                 230                 235                 240

Gly Thr Leu Asn Val Lys Ala Tyr Leu Asn Thr Leu Ala Pro Lys Gly
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Ser Leu His Phe Leu Gly Val Thr Leu Glu Pro Ile Pro Val Ser Val
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Gly Ala Ile Met Gly Gly Ala Lys Ser Val Thr Ser Ser Pro Thr Gly
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Ser Pro Leu Ala Leu Arg Gln Leu Leu Gln Phe Ala Ala Arg Lys Asn
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Ile Ala Pro Gln Val Glu Leu Phe Pro Met Ser Gln Leu Asn Glu Ala
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Ser Val Leu Asn Asn Glu Trp His Ser Ser Val Tyr Pro Val Val Ala
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Gly His Glu Val Ile Gly Thr Ile Thr Gln Leu Gly Ser Glu Ala Lys
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Gly Leu Lys Ile Gly Gln Arg Val Gly Ile Gly Trp Thr Ala Glu Ser
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Cys Gln Ala Cys Asp Gln Cys Ile Ser Gly Gln Gln Val Leu Cys Thr
            100                 105                 110

Gly Glu Asn Thr Ala Thr Ile Ile Gly His Ala Gly Gly Phe Ala Asp
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Lys Val Arg Ala Gly Trp Gln Trp Val Ile Pro Leu Pro Asp Glu Leu
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                165                 170                 175

Ile Gly Ile Gly Gly Leu Gly His Met Ala Ile Lys Leu Leu Lys Ala
            180                 185                 190

Trp Gly Cys Glu Ile Thr Ala Phe Ser Ser Asn Pro Asn Lys Thr Asp
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Glu Leu Lys Ala Met Gly Ala Asp His Val Val Asn Ser Arg Asp Asp
    210                 215                 220

Ala Glu Ile Lys Ser Gln Gln Gly Lys Phe Asp Leu Leu Leu Ser Thr
225                 230                 235                 240

Val Asn Val Pro Leu Asn Trp Asn Ala Tyr Leu Asn Thr Leu Ala Pro
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Asn Gly Thr Phe His Phe Leu Gly Val Val Met Glu Pro Ile Pro Val
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Pro Val Gly Ala Leu Leu Gly Gly Ala Lys Ser Leu Thr Ala Ser Pro
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gcctgtatta gcggtaatca gatcaactgc gagcaaggtg cggtgccgac gattatgaat    360

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agcgtctctg gttctgctac cggcacgcct tatgagctgc gtaagctgat gcgttttgcc    900

gcccgcagca aggttgcgcc gaccaccgaa ctgttcccga tgtcgaaaat taacgacgcc    960

atccagcatg tgcgcgacgg taaggcgcgt taccgcgtgg tgttgaaagc cgatttttga   1020
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<210> SEQ ID NO 6
<211> LENGTH: 473
<212> TYPE: PRT
<213> ORGANISM: Marinobacter hydrocarbonoclasticus

<400> SEQUENCE: 6

Met Lys Arg Leu Gly Thr Leu Asp Ala Ser Trp Leu Ala Val Glu Ser
1 5 10 15

Glu Asp Thr Pro Met His Val Gly Thr Leu Gln Ile Phe Ser Leu Pro
20 25 30

Glu Gly Ala Pro Glu Thr Phe Leu Arg Asp Met Val Thr Arg Met Lys
35 40 45

Glu Ala Gly Asp Val Ala Pro Pro Trp Gly Tyr Lys Leu Ala Trp Ser
50 55 60

Gly Phe Leu Gly Arg Val Ile Ala Pro Ala Trp Lys Val Asp Lys Asp
65 70 75 80

Ile Asp Leu Asp Tyr His Val Arg His Ser Ala Leu Pro Arg Pro Gly
85 90 95

Gly Glu Arg Glu Leu Gly Ile Leu Val Ser Arg Leu His Ser Asn Pro
100 105 110

Leu Asp Phe Ser Arg Pro Leu Trp Glu Cys His Val Ile Glu Gly Leu
115 120 125

Glu Asn Asn Arg Phe Ala Leu Tyr Thr Lys Met His His Ser Met Ile
130 135 140

Asp Gly Ile Ser Gly Val Arg Leu Met Gln Arg Val Leu Thr Thr Asp
145 150 155 160

Pro Glu Arg Cys Asn Met Pro Pro Pro Trp Thr Val Arg Pro His Gln
165 170 175

Arg Arg Gly Ala Lys Thr Asp Lys Glu Ala Ser Val Pro Ala Ala Val
180 185 190

Ser Gln Ala Met Asp Ala Leu Lys Leu Gln Ala Asp Met Ala Pro Arg
195 200 205

Leu Trp Gln Ala Gly Asn Arg Leu Val His Ser Val Arg His Pro Glu
210 215 220

Asp Gly Leu Thr Ala Pro Phe Thr Gly Pro Val Ser Val Leu Asn His
225 230 235 240

Arg Val Thr Ala Gln Arg Arg Phe Ala Thr Gln His Tyr Gln Leu Asp
245 250 255

Arg Leu Lys Asn Leu Ala His Ala Ser Gly Gly Ser Leu Asn Asp Ile
260 265 270

Val Leu Tyr Leu Cys Gly Thr Ala Leu Arg Arg Phe Leu Ala Glu Gln
275 280 285

Asn Asn Leu Pro Asp Thr Pro Leu Thr Ala Gly Ile Pro Val Asn Ile
290 295 300

Arg Pro Ala Asp Asp Glu Gly Thr Gly Thr Gln Ile Ser Phe Met Ile
305 310 315 320

Ala Ser Leu Ala Thr Asp Glu Ala Asp Pro Leu Asn Arg Leu Gln Gln
325 330 335

Ile Lys Thr Ser Thr Arg Arg Ala Lys Glu His Leu Gln Lys Leu Pro
340 345 350

Lys Ser Ala Leu Thr Gln Tyr Thr Met Leu Leu Met Ser Pro Tyr Ile

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        355                 360                 365

Leu Gln Leu Met Ser Gly Leu Gly Gly Arg Met Arg Pro Val Phe Asn
    370                 375                 380

Val Thr Ile Ser Asn Val Pro Gly Pro Glu Gly Thr Leu Tyr Tyr Glu
385                 390                 395                 400

Gly Ala Arg Leu Glu Ala Met Tyr Pro Val Ser Leu Ile Ala His Gly
                405                 410                 415

Gly Ala Leu Asn Ile Thr Cys Leu Ser Tyr Ala Gly Ser Leu Asn Phe
            420                 425                 430

Gly Phe Thr Gly Cys Arg Asp Thr Leu Pro Ser Met Gln Lys Leu Ala
        435                 440                 445

Val Tyr Thr Gly Glu Ala Leu Asp Glu Leu Glu Ser Leu Ile Leu Pro
    450                 455                 460

Pro Lys Lys Arg Ala Arg Thr Arg Lys
465                 470


<210> SEQ ID NO 7
<211> LENGTH: 455
<212> TYPE: PRT
<213> ORGANISM: Marinobacter hydrocarbonoclasticus

<400> SEQUENCE: 7

Met Thr Pro Leu Asn Pro Thr Asp Gln Leu Phe Leu Trp Leu Glu Lys
1               5                   10                  15

Arg Gln Gln Pro Met His Val Gly Gly Leu Gln Leu Phe Ser Phe Pro
            20                  25                  30

Glu Gly Ala Pro Asp Asp Tyr Val Ala Gln Leu Ala Asp Gln Leu Arg
        35                  40                  45

Gln Lys Thr Glu Val Thr Ala Pro Phe Asn Gln Arg Leu Ser Tyr Arg
    50                  55                  60

Leu Gly Gln Pro Val Trp Val Glu Asp Glu His Leu Asp Leu Glu His
65                  70                  75                  80

His Phe Arg Phe Glu Ala Leu Pro Thr Pro Gly Arg Ile Arg Glu Leu
                85                  90                  95

Leu Ser Phe Val Ser Ala Glu His Ser His Leu Met Asp Arg Glu Arg
            100                 105                 110

Pro Met Trp Glu Val His Leu Ile Glu Gly Leu Lys Asp Arg Gln Phe
        115                 120                 125

Ala Leu Tyr Thr Lys Val His His Ser Leu Val Asp Gly Val Ser Ala
    130                 135                 140

Met Arg Met Ala Thr Arg Met Leu Ser Glu Asn Pro Asp Glu His Gly
145                 150                 155                 160

Met Pro Pro Ile Trp Asp Leu Pro Cys Leu Ser Arg Asp Arg Gly Glu
                165                 170                 175

Ser Asp Gly His Ser Leu Trp Arg Ser Val Thr His Leu Leu Gly Leu
            180                 185                 190

Ser Asp Arg Gln Leu Gly Thr Ile Pro Thr Val Ala Lys Glu Leu Leu
        195                 200                 205

Lys Thr Ile Asn Gln Ala Arg Lys Asp Pro Ala Tyr Asp Ser Ile Phe
    210                 215                 220

His Ala Pro Arg Cys Met Leu Asn Gln Lys Ile Thr Gly Ser Arg Arg
225                 230                 235                 240
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Phe Ala Ala Gln Ser Trp Cys Leu Lys Arg Ile Arg Ala Val Cys Glu
                245                 250                 255

Ala Tyr Gly Thr Thr Val Asn Asp Val Val Thr Ala Met Cys Ala Ala
            260                 265                 270

Ala Leu Arg Thr Tyr Leu Met Asn Gln Asp Ala Leu Pro Glu Lys Pro
        275                 280                 285

Leu Val Ala Phe Val Pro Val Ser Leu Arg Arg Asp Asp Ser Ser Gly
    290                 295                 300

Gly Asn Gln Val Gly Val Ile Leu Ala Ser Leu His Thr Asp Val Gln
305                 310                 315                 320

Asp Ala Gly Glu Arg Leu Leu Lys Ile His His Gly Met Glu Glu Ala
                325                 330                 335

Lys Gln Arg Tyr Arg His Met Ser Pro Glu Glu Ile Val Asn Tyr Thr
            340                 345                 350

Ala Leu Thr Leu Ala Pro Ala Ala Phe His Leu Leu Thr Gly Leu Ala
        355                 360                 365

Pro Lys Trp Gln Thr Phe Asn Val Val Ile Ser Asn Val Pro Gly Pro
    370                 375                 380

Ser Arg Pro Leu Tyr Trp Asn Gly Ala Lys Leu Glu Gly Met Tyr Pro
385                 390                 395                 400

Val Ser Ile Asp Met Asp Arg Leu Ala Leu Asn Met Thr Leu Thr Ser
                405                 410                 415

Tyr Asn Asp Gln Val Glu Phe Gly Leu Ile Gly Cys Arg Arg Thr Leu
            420                 425                 430

Pro Ser Leu Gln Arg Met Leu Asp Tyr Leu Glu Gln Gly Leu Ala Glu
        435                 440                 445

Leu Glu Leu Asn Ala Gly Leu
    450                 455


<210> SEQ ID NO 8
<211> LENGTH: 457
<212> TYPE: PRT
<213> ORGANISM: Alcanivorax borkumensis

<400> SEQUENCE: 8

Met Lys Ala Leu Ser Pro Val Asp Gln Leu Phe Leu Trp Leu Glu Lys
1               5                   10                  15

Arg Gln Gln Pro Met His Val Gly Gly Leu Gln Leu Phe Ser Phe Pro
            20                  25                  30

Glu Gly Ala Gly Pro Lys Tyr Val Ser Glu Leu Ala Gln Gln Met Arg
        35                  40                  45

Asp Tyr Cys His Pro Val Ala Pro Phe Asn Gln Arg Leu Thr Arg Arg
    50                  55                  60

Leu Gly Gln Tyr Tyr Trp Thr Arg Asp Lys Gln Phe Asp Ile Asp His
65                  70                  75                  80

His Phe Arg His Glu Ala Leu Pro Lys Pro Gly Arg Ile Arg Glu Leu
                85                  90                  95

Leu Ser Leu Val Ser Ala Glu His Ser Asn Leu Leu Asp Arg Glu Arg
            100                 105                 110

Pro Met Trp Glu Ala His Leu Ile Glu Gly Ile Arg Gly Arg Gln Phe
        115                 120                 125

Ala Leu Tyr Tyr Lys Ile His His Ser Val Met Asp Gly Ile Ser Ala
    130                 135                 140
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Met Arg Ile Ala Ser Lys Thr Leu Ser Thr Asp Pro Ser Glu Arg Glu
145                 150                 155                 160

Met Ala Pro Ala Trp Ala Phe Asn Thr Lys Lys Arg Ser Arg Ser Leu
                165                 170                 175

Pro Ser Asn Pro Val Asp Met Ala Ser Ser Met Ala Arg Leu Thr Ala
            180                 185                 190

Ser Ile Ser Lys Gln Ala Ala Thr Val Pro Gly Leu Ala Arg Glu Val
        195                 200                 205

Tyr Lys Val Thr Gln Lys Ala Lys Lys Asp Glu Asn Tyr Val Ser Ile
    210                 215                 220

Phe Gln Ala Pro Asp Thr Ile Leu Asn Asn Thr Ile Thr Gly Ser Arg
225                 230                 235                 240

Arg Phe Ala Ala Gln Ser Phe Pro Leu Pro Arg Leu Lys Val Ile Ala
                245                 250                 255

Lys Ala Tyr Asn Cys Thr Ile Asn Thr Val Val Leu Ser Met Cys Gly
            260                 265                 270

His Ala Leu Arg Glu Tyr Leu Ile Ser Gln His Ala Leu Pro Asp Glu
        275                 280                 285

Pro Leu Ile Ala Met Val Pro Met Ser Leu Arg Gln Asp Asp Ser Thr
    290                 295                 300

Gly Gly Asn Gln Ile Gly Met Ile Leu Ala Asn Leu Gly Thr His Ile
305                 310                 315                 320

Cys Asp Pro Ala Asn Arg Leu Arg Val Ile His Asp Ser Val Glu Glu
                325                 330                 335

Ala Lys Ser Arg Phe Ser Gln Met Ser Pro Glu Glu Ile Leu Asn Phe
            340                 345                 350

Thr Ala Leu Thr Met Ala Pro Thr Gly Leu Asn Leu Leu Thr Gly Leu
        355                 360                 365

Ala Pro Lys Trp Arg Ala Phe Asn Val Val Ile Ser Asn Ile Pro Gly
    370                 375                 380

Pro Lys Glu Pro Leu Tyr Trp Asn Gly Ala Gln Leu Gln Gly Val Tyr
385                 390                 395                 400

Pro Val Ser Ile Ala Leu Asp Arg Ile Ala Leu Asn Ile Thr Leu Thr
                405                 410                 415

Ser Tyr Val Asp Gln Met Glu Phe Gly Leu Ile Ala Cys Arg Arg Thr
            420                 425                 430

Leu Pro Ser Met Gln Arg Leu Leu Asp Tyr Leu Glu Gln Ser Ile Arg
        435                 440                 445

Glu Leu Glu Ile Gly Ala Gly Ile Lys
    450                 455


<210> SEQ ID NO 9
<211> LENGTH: 70
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
      Synthetic oligonucleotide"

<400> SEQUENCE: 9

aaaaacagca acaatgtgag ctttgttgta attatattgt aaacatattg attccgggga     60

tccgtcgacc                                                           70
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<210> SEQ ID NO 10
<211> LENGTH: 68
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 10

aaacggagcc tttcggctcc gttattcatt tacgcggctt caactttcct gtaggctgga 60

gctgcttc 68

<210> SEQ ID NO 11
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 11

cgggcaggtg ctatgaccag gac 23

<210> SEQ ID NO 12
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 12

cgcggcgttg accggcagcc tgg 23

<210> SEQ ID NO 13
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 13

ccttggcatt ggcaatttga gaattcgagg aggaaaacta aatgaccatt tcctcacctt 60

<210> SEQ ID NO 14
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 14

ttttgttcgg gcccaagctt ttattgcaaa cgcagatgcg tgatttcacc cgcattcagc 60

<210> SEQ ID NO 15
<211> LENGTH: 60

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<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 15

cgggcccaag cttcgaattc ttattgcaaa cgcagatgcg tgatttcacc cgcattcagc 60

<210> SEQ ID NO 16
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 16

gaatagcgcc gtcgacgagg aggaaaacta aatgaccatt tcctcacctt tgattgacgt 60

<210> SEQ ID NO 17
<211> LENGTH: 60
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 17

tgatgatgat gatggtcgac ttattgcaaa cgcagatgcg tgatttcacc cgcattcagc 60

<210> SEQ ID NO 18
<211> LENGTH: 4250
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<400> SEQUENCE: 18

atgaccattt cctcaccttt gattgacgtc gccaaccttc cagacatcaa caccactgcc 60

ggcaagatcg ccgaccttaa ggctcgccgc gcggaagccc atttccccat gggtgaaaag 120

gcagtagaga aggtccacgc tgctggacgc ctcactgccc gtgagcgctt ggattactta 180

ctcgatgagg gctccttcat cgagaccgat cagctggctc gccaccgcac caccgctttc 240

ggcctgggcg ctaagcgtcc tgcaaccgac ggcatcgtga ccggctgggg caccattgat 300

ggacgcgaag tctgcatctt ctcgcaggac ggcaccgtat tcggtggcgc gcttggtgag 360

gtgtacggcg aaaagatgat caagatcatg gagctggcaa tcgacaccgg ccgcccattg 420

atcggtcttt acgaaggcgc tggcgctcgt attcaggacg gcgctgtctc cctggacttc 480

atttcccaga ccttctacca aaacattcag gcttctggcg ttatcccaca gatctccgtc 540

atcatgggcg catgtgcagg tggcaacgct tacggcccag ctctgaccga cttcgtggtc 600

atggtggaca agacctccaa gatgttcgtt accggcccag acgtgatcaa gaccgtcacc 660

ggcgaggaaa tcacccagga agagcttggc ggagcaacca cccacatggt gaccgctggt 720

aactcccact acaccgctgc gaccgatgag gaagcactgg attgggtaca ggacctggtg 780

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tccttcctcc catccaacaa tcgctcctac gcaccgatgg aagacttcga cgaggaagaa 840

ggcggcgttg aagaaaacat caccgctgac gatctgaagc tcgacgagat catcccagat 900

tccgcgaccg ttccttacga cgtccgcgat gtcatcgaat gcctcaccga cgatggcgaa 960

tacctggaaa tccaggcaga ccgcgcagaa aacgttgtta ttgcattcgg ccgcatcgaa 1020

ggccagtccg ttggctttgt tgccaaccag ccaacccagt tcgctggctg cctggacatc 1080

gactcctctg agaaggcagc tcgcttcgtc cgcacctgcg acgcgttcaa catcccaatc 1140

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gttggattca tctaccgcaa ggagctcatg gcagctgatg ccaagggcct cgataccgta 1440

gctctggcta agtccttcga gcgcgagtat gaagaccaca tgctcaaccc gtaccacgct 1500

gcagaacgtg gcctgatcga cgccgtgatc ctgccaagcg aaacccgcgg acagatttcc 1560

cgcaaccttc gcctgctcaa gcacaagaac gtcactcgcc ctgctcgcaa gcacggcaac 1620

atgccactgt aaggaggaaa actaaatgtc agtcgagact cgcaagatca ccaaggttct 1680

tgtcgctaac cgtggtgaga ttgcaatccg cgtgttccgt gcagctcgag atgaaggcat 1740

cggatctgtc gccgtctacg cagagccaga tgcagatgca ccattcgtgt catatgcaga 1800

cgaggctttt gccctcggtg gccaaacatc cgctgagtcc taccttgtca ttgacaagat 1860

catcgatgcg gcccgcaagt ccggcgccga cgccatccac cccggctacg gcttcctcgc 1920

agaaaacgct gacttcgcag aagcagtcat caacgaaggc ctgatctgga ttggaccttc 1980

acctgagtcc atccgctccc tcggcgacaa ggtcaccgct cgccacatcg cagataccgc 2040

caaggctcca atggctcctg gcaccaagga accagtaaaa gacgcagcag aagttgtggc 2100

tttcgctgaa gaattcggtc tcccaatcgc catcaaggca gctttcggtg gcggcggacg 2160

tggcatgaag gttgcctaca agatggaaga agtcgctgac ctcttcgagt ccgcaacccg 2220

tgaagcaacc gcagcgttcg gccgcggcga gtgcttcgtg gagcgctacc tggacaaggc 2280

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ccgtgactgc tccctgcagc gccgtttcca gaagctcgtc gaagaagcac cagcaccatt 2400

cctcaccgat gaccagcgcg agcgtctcca ctcctccgcg aaggctatct gtaaggaagc 2460

tggctactac ggtgcaggca ccgttgagta cctcgttggc tccgacggcc tgatctcctt 2520

cctcgaggtc aacacccgcc tccaggtgga acacccagtc accgaagaga ccaccggcat 2580

cgacctggtc cgcgaaatgt tccgcatcgc agaaggccac gagctctcca tcaaggaaga 2640

tccagctcca cgcggccacg cattcgagtt ccgcatcaac ggcgaagacg ctggctccaa 2700

cttcatgcct gcaccaggca agatcaccag ctaccgcgag ccacagggcc caggcgtccg 2760

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aaagctgatc gtttggggcg acacccgcga gcaggctctc cagcgctccc gccgtgcact 2880

tgcagagtac gttgtcgagg gcatgccaac cgttatccca ttccaccagc acatcgtgga 2940

aaacccagca ttcgtgggca acgacgaagg cttcgagatc tacaccaagt ggatcgaaga 3000

ggtttgggat aacccaatcg caccttacgt tgacgcttcc gagctcgacg aagatgagga 3060

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caagacccca gcacagaagg ttgttgtgga gatcaacggc cgtcgcgttg aggttgcact 3120

cccaggcgat ctggcactcg gtggcaccgc tggtcctaag aagaaggcca agaagcgtcg 3180

cgcaggtggt gcaaaggctg gcgtatccgg cgatgcagtg gcagctccaa tgcagggcac 3240

tgtcatcaag gtcaacgtcg aagaaggcgc tgaagtcaac gaaggcgaca ccgttgttgt 3300

cctcgaggct atgaagatgg aaaaccctgt gaaggctcat aagtccggaa ccgtaaccgg 3360

ccttactgtc gctgcaggcg agggtgtcaa caagggcgtt gttctcctcg agatcaagta 3420

atctagagga ggaaaactaa atgaatgttg acattagccg ctctcgtgaa ccgttgaacg 3480

tggaactgtt gaaagaaaaa ctgctgcaga acggtgattt cggtcaagtg atctacgaga 3540

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agttgtgcgg tattctggtt gaagcaaccg gcttcgactc cactccgacc gtggttatcg 3900

gttggggtac gaatatctcg ttgacgaaag aagagctgcc ggtcccgcac gcgaccagcc 3960

tggccctgga gggtgttgaa gttgaccgta cgacgttcct gattaacatg ctgacccatc 4020

tgcatacccg tctggatcag tggcagggtc cgtctgtgga ctggctggat gactatcgcg 4080

cggtttgtag cagcattggc caagatgtgc gtgtcctgct gcctggtgac aaagagctgc 4140

tgggcgaggc gattggcgtg gcgaccggtg gtgagatccg tgtgcgcgac gccagcggca 4200

cggtccacac gctgaatgcg ggtgaaatca cgcatctgcg tttgcaataa 4250

<210> SEQ ID NO 19
<211> LENGTH: 5659
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic polynucleotide"

<400> SEQUENCE: 19

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gaagcgaacg taaaagaaca aattgcctac acaaaagcac aaggtccgat caaaaacgca 120

cctaagcgcg tgttggttgt cggatcgtct agcggctatg gtctgtcatc acgcatcgct 180

gcggcgtttg gcggtggtgc ggcgacgatc ggcgtatttt tcgaaaagcc gggcactgac 240

aaaaaaccag gtactgcggg tttctacaat gcagcagcgt ttgacaagct agcgcatgaa 300

gcgggcttgt acgcaaaaag cctgaacggc gatgcgttct cgaacgaagc gaagcaaaaa 360

gcgattgagc tgattaagca agacctcggc cagattgatt tggtggttta ctcgttggct 420

tctccagtgc gtaagatgcc agacacgggt gagctagtgc gctctgcact aaaaccgatc 480

ggcgaaacgt acacctctac cgcggtagat accaataaag atgtgatcat tgaagccagt 540

gttgaacctg cgaccgagca agaaatcgct gacactgtca ccgtgatggg cggtcaagat 600

tgggaactgt ggatccaagc actggaagag gcgggtgttc ttgctgaagg ttgcaaaacc 660

gtggcgtaca gctacatcgg tactgaattg acttggccaa tttactggga tggcgcttta 720

-continued

ggccgtgcca agatggacct agatcgcgca gcgacagcgc tgaacgaaaa gctggcagcg 780

aaaggtggta ccgcgaacgt tgcagttttg aaatcagtgg tgactcaagc aagctctgcg 840

attcctgtga tgccgctcta catcgcaatg gtgttcaaga agatgcgtga acagggcgtg 900

catgaaggct gtatggagca gatctaccgc atgttcagtc aacgtctgta caaagaagat 960

ggttcagcgc cggaagtgga tgatcacaat cgtctgcgtt tggatgactg ggaactgcgt 1020

gatgacattc agcagcactg ccgtgatctg tggccacaaa tcaccacaga gaacctgcgt 1080

gagctgaccg attacgacat gtacaaagaa gagttcatca agctgtttgg ctttggcatt 1140

gaaggcattg attacgatgc tgacgtcaat ccagaagtcg aatttgatgt gattgatatc 1200

gagtaattta gtgactgagc gtacatgtat acgaagatta ttggtactgg cagctatctg 1260

cccgaacaag tgcggactaa cgccgatctg gaaaaaatgg ttgagacctc tgacgagtgg 1320

attgtcactc gtacaggtat tcgtaaacgc catatcgccg cgccgaatga aactgtcgcg 1380

acgatgggct ttaccgctgc gaatcgcgcg attgagatgg cggggatcga taaagaccaa 1440

attggcttga ttgtggtggc taccacatca gcaacgcatg catttccaag cgcggcatgt 1500

cagattcaaa gtatgctcgg tattaaaggt tgcccggcgt ttgatgtcgc ggcagcgtgc 1560

gcaggtttca cctacgcgtt aagcatcgcc gaccagtacg ttaaatccgg cgcggttaaa 1620

cacgcgctgg tggtcggttc cgatgtatta gcccgcactt gcgatcctgg cgatcgcggt 1680

acgatcatta ttttcggcga tggcgcaggc gcggccgtac tgagcgcttc tgaagaaccg 1740

ggtattatct ccactcatct tcatgccgat ggccgttacg gtgaattact gaccctgccg 1800

aatgccgatc gcgtaaatcc ggataacccg atttacctga caatggcggg caatgaagtc 1860

tttaaagtgg cggtcactga actggcgcat attgtcgatg agacgctggc ggctaataac 1920

ctggatcgct cagaactcga ttggctggtg ccgcatcagg ctaacctgcg tatcattagc 1980

gcgacagcga aaaaactcgg catgtcgatg gacaatgtcg tcgtcacgct ggacaggcac 2040

ggcaatacct ccgcggcttc tgtgccgtgc gcgctggatg aagccgtgcg tgacgggcga 2100

attaaagccg gtcagctggt attgcttgaa gccttcgggg gtggattcac ctggggctcc 2160

gcgctgattc gtttctagta taaggattta aacatgacgc aatttgcatt tgtgttcccc 2220

ggtcagggtt ctcagagcgt tgggatgttg gccgagatgg cggcaaatta ccctatcgta 2280

gaagaaacgt ttgctgaagc ttctgcggct ctgggatatg atctgtgggc gctcacccag 2340

caaggtccag cggaagaact gaataaaacc tggcagacgc agccggcgtt attaaccgct 2400

tccgtcgcgc tttggcgcgt ttggcagcag cagggcggta aaatgcctgc gttaatggca 2460

ggtcacagcc tgggcgaata ttccgcgctg gtttgcgctg gcgtcatcaa ctttgctgat 2520

gccgttcgtc tggtggaaat gcgcggtaaa ttcatgcagg aagcggttcc ggaaggcact 2580

ggcggcatgt ctgcgatcat cgggctggat gatgcctcta ttgctaaagc ctgtgaagaa 2640

tctgccgaag ggcaggttgt ttcgccggtt aactttaact cgccgggaca ggtggttatc 2700

gccgggcata aagaggcggt agaacgtgcg ggcgcagcct gtaaagccgc tggcgcgaaa 2760

cgcgcgctgc cgctgccggt gagcgtaccg tcgcactgcg cgctgatgaa accagcggca 2820

gataagctgg cggttgaatt agccaaaatt acctttagcg cgccaacggt gccggtagtg 2880

aacaacgttg acgtgaaatg tgaaaccgat gccgccgcta tccgcgatgc gctggttcgc 2940

cagttgtaca atccggtaca gtggacgaag agcgtggaat ttatcgcggc gcagggcgtt 3000

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gaacatcttt atgaagtggg tccaggtaaa gtcctcactg gtctgacgaa acgtattgtc 3060

gacaccctga cagcgtcggc gctgaacgag ccggcggcgc tgtctgcggc acttacgcaa 3120

taaaagagga aaaccatgag ctttgaagga aagattgcgc tggtgactgg tgcaagccgt 3180

ggcataggcc gcgcaattgc agagactctc gttgcccgcg gcgcgaaagt tatcgggact 3240

gcgaccagtg aaaatggtgc gaagaacatt agcgactatt taggtgctaa cgggaaaggt 3300

ttgatgttga atgtgaccga tcctgcatct attgaatctg ttctggaaaa tattcgcgca 3360

gaatttggtg aagtggatat cctggttaat aatgccggta tcactcgtga taatctgttg 3420

atgcgaatga aagatgatga gtggaacgat attatcgaaa ccaacttatc atccgttttc 3480

cgcctgtcaa aagcggtaat gcgcgctatg atgaaaaagc gttgtggtcg cattatcact 3540

attggttctg tggttggtac catgggaaat gcaggtcagg caaactacgc tgcggcgaaa 3600

gcgggcctga tcggtttcag taaatcactg gcgcgtgaag ttgcgtcccg tggtattact 3660

gtcaatgttg tggctccggg ttttattgaa acggacatga cgcgtgcgct gtctgacgat 3720

cagcgtgcgg gtatcctggc gcaggtgcct gcgggtcgcc tcggcggcgc tcaggaaatc 3780

gccagtgcgg ttgcattttt agcctctgac gaagcgagtt acatcactgg tgagactctg 3840

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aagtcaccta tcgtattcat ttcaaacgta tcgtaaaccg tcgcctgatc atgggcctgg 4320

cggacggtga ggttctggtg gatggtcgcc tgatctatac cgcacacgat ttgaaagtcg 4380

gtttgttcca ggatacttcc gcgttctaaa aggaggcaac aaaatgaatc gccgcgttgt 4440

cattacgggt attggtgcag tgacgccggt gggtaacaac gctgatagct tctggtgcag 4500

catcaaagag ggtaaatgtg gcattgacaa gatcaaagcg tttgacgcaa ccgatttcaa 4560

agttaagctg gctgccgaag tgaaggactt caccccggag gactttatcg acaagcgtga 4620

ggcgaaccgt atggaccgtt ttagccagtt tgcgatcgtt gcggcggatg aggcaatcaa 4680

ggacagcaaa ctggacctgg agtcgattga taagaatcgt ttcggcgtca ttgttggtag 4740

cggcattggc ggcatcggca ccattgagaa gcaggatgaa aagctgatta ccaaaggtcc 4800

gggtcgtgtg agccctatga ctattccgat gatcattgcg aatatggcaa gcggtaatct 4860

ggcgattcgt tatggcgcta aaggtatttg cacgaccatt gtcaccgcat gtgcgagcgc 4920

gaacaacagc attggtgagt ccttccgtaa cattaagttt ggttatagcg acgttatgat 4980

ctctggtggt agcgaagcag gtatcacccc gttgagcctg gcgggttttg cctcgatgaa 5040

ggccgtgacc aaatctgagg acccgaagcg cgccagcatc ccgttcgata aggatcgcag 5100

cggttttgtg atgggcgagg gcagcggtat cgttatcttg gaagagttgg agcacgcgct 5160

gaagcgtggt gccaaaatct atgccgagat cgttggctat ggtgcgacct gcgacgcata 5220

tcatatcacg agcccagcgc cgaatggtga aggtggtgca cgtgcaatga aactggcaat 5280

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ggaagaagat aatgtccgcc cagaggacat ttcctatatc aacgcgcacg gtacgagcac 5340

ggcgtacaat gacagcttcg aaacccaagc gatcaagacg gtcctgggtg aatacgccta 5400

caaagtgccg gtgtctagca ccaagagcat gaccggccac ctgctgggcg ctggcggtgc 5460

agtcgaagcg attatctgtg ccaaagctat tgaagagggt ttcattccgc cgaccatcgg 5520

ctacaaagag gcggatccgg aatgcgacct ggattacgtt cctaacgagg gccgtaatgc 5580

agaagtcaac tacgttctgt ccaacagcct gggcttcggt ggccataatg cgactctgct 5640

gttcaaaaag tacaaatga 5659

<210> SEQ ID NO 20
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 20

ttgtccatct ttatataatt tgggggtagg gtgttcttta tgtaaaaaaa acgttttagg 60

atgcatatgg cggcc 75

<210> SEQ ID NO 21
<211> LENGTH: 75
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 21

gataaatcca cgaattttag gtttgatgat cattggtctc ctcctgcagg tgcgtgttcg 60

tcgtcatcgc aattg 75

<210> SEQ ID NO 22
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 22

actcaccgca ttggtgtagt aaggcgcacc 30

<210> SEQ ID NO 23
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 23

tgaatgtcat cacgcagttc ccagtcatcc 30

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<210> SEQ ID NO 24
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 24

ccatcttctt tgtacagacg ttgactgaac atg 33

<210> SEQ ID NO 25
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 25

gcaccatagc cgtaatccca caggttatag 30

<210> SEQ ID NO 26
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 26

tgtcattaat ggttaataat gttga 25

<210> SEQ ID NO 27
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 27

gcagttattg gtgcccttaa acgcctggtt gctacgcctg 40

<210> SEQ ID NO 28
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 28

gagccaatat gcgagaacac ccgagaa 27

<210> SEQ ID NO 29
<211> LENGTH: 48
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source

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<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 29

cgctgaacgt attgcaggcc gagttgctgc accgctcccg ccaggcag 48

<210> SEQ ID NO 30
<211> LENGTH: 51
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 30

ggaattgcca cggtgcggca ggctccatac gcgaggccag gttatccaac g 51

<210> SEQ ID NO 31
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 31

aatcaccagc actaaagtgc gcggttcgtt acccg 35

<210> SEQ ID NO 32
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
Synthetic oligonucleotide"

<400> SEQUENCE: 32

atctgccgtg gattgcagag tctattcagc tacg 34

<210> SEQ ID NO 33
<211> LENGTH: 1168
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium smegmatis

<400> SEQUENCE: 33

Met Thr Ile Glu Thr Arg Glu Asp Arg Phe Asn Arg Arg Ile Asp His
1 5 10 15

Leu Phe Glu Thr Asp Pro Gln Phe Ala Ala Ala Arg Pro Asp Glu Ala
20 25 30

Ile Ser Ala Ala Ala Ala Asp Pro Glu Leu Arg Leu Pro Ala Ala Val
35 40 45

Lys Gln Ile Leu Ala Gly Tyr Ala Asp Arg Pro Ala Leu Gly Lys Arg
50 55 60

Ala Val Glu Phe Val Thr Asp Glu Glu Gly Arg Thr Thr Ala Lys Leu
65 70 75 80

Leu Pro Arg Phe Asp Thr Ile Thr Tyr Arg Gln Leu Ala Gly Arg Ile
85 90 95

Gln Ala Val Thr Asn Ala Trp His Asn His Pro Val Asn Ala Gly Asp

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            100                 105                 110

Arg Val Ala Ile Leu Gly Phe Thr Ser Val Asp Tyr Thr Thr Ile Asp
        115                 120                 125

Ile Ala Leu Leu Glu Leu Gly Ala Val Ser Val Pro Leu Gln Thr Ser
    130                 135                 140

Ala Pro Val Ala Gln Leu Gln Pro Ile Val Ala Glu Thr Glu Pro Lys
145                 150                 155                 160

Val Ile Ala Ser Ser Val Asp Phe Leu Ala Asp Ala Val Ala Leu Val
                165                 170                 175

Glu Ser Gly Pro Ala Pro Ser Arg Leu Val Val Phe Asp Tyr Ser His
            180                 185                 190

Glu Val Asp Asp Gln Arg Glu Ala Phe Glu Ala Ala Lys Gly Lys Leu
        195                 200                 205

Ala Gly Thr Gly Val Val Val Glu Thr Ile Thr Asp Ala Leu Asp Arg
    210                 215                 220

Gly Arg Ser Leu Ala Asp Ala Pro Leu Tyr Val Pro Asp Glu Ala Asp
225                 230                 235                 240

Pro Leu Thr Leu Leu Ile Tyr Thr Ser Gly Ser Thr Gly Thr Pro Lys
                245                 250                 255

Gly Ala Met Tyr Pro Glu Ser Lys Thr Ala Thr Met Trp Gln Ala Gly
            260                 265                 270

Ser Lys Ala Arg Trp Asp Glu Thr Leu Gly Val Met Pro Ser Ile Thr
        275                 280                 285

Leu Asn Phe Met Pro Met Ser His Val Met Gly Arg Gly Ile Leu Cys
    290                 295                 300

Ser Thr Leu Ala Ser Gly Gly Thr Ala Tyr Phe Ala Ala Arg Ser Asp
305                 310                 315                 320

Leu Ser Thr Phe Leu Glu Asp Leu Ala Leu Val Arg Pro Thr Gln Leu
                325                 330                 335

Asn Phe Val Pro Arg Ile Trp Asp Met Leu Phe Gln Glu Tyr Gln Ser
            340                 345                 350

Arg Leu Asp Asn Arg Arg Ala Glu Gly Ser Glu Asp Arg Ala Glu Ala
        355                 360                 365

Ala Val Leu Glu Glu Val Arg Thr Gln Leu Leu Gly Gly Arg Phe Val
    370                 375                 380

Ser Ala Leu Thr Gly Ser Ala Pro Ile Ser Ala Glu Met Lys Ser Trp
385                 390                 395                 400

Val Glu Asp Leu Leu Asp Met His Leu Leu Glu Gly Tyr Gly Ser Thr
                405                 410                 415

Glu Ala Gly Ala Val Phe Ile Asp Gly Gln Ile Gln Arg Pro Pro Val
            420                 425                 430

Ile Asp Tyr Lys Leu Val Asp Val Pro Asp Leu Gly Tyr Phe Ala Thr
        435                 440                 445

Asp Arg Pro Tyr Pro Arg Gly Glu Leu Leu Val Lys Ser Glu Gln Met
    450                 455                 460

Phe Pro Gly Tyr Tyr Lys Arg Pro Glu Ile Thr Ala Glu Met Phe Asp
465                 470                 475                 480

Glu Asp Gly Tyr Tyr Arg Thr Gly Asp Ile Val Ala Glu Leu Gly Pro
                485                 490                 495

Asp His Leu Glu Tyr Leu Asp Arg Arg Asn Asn Val Leu Lys Leu Ser
            500                 505                 510
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Gln Gly Glu Phe Val Thr Val Ser Lys Leu Glu Ala Val Phe Gly Asp
        515                 520                 525

Ser Pro Leu Val Arg Gln Ile Tyr Val Tyr Gly Asn Ser Ala Arg Ser
    530                 535                 540

Tyr Leu Leu Ala Val Val Val Pro Thr Glu Glu Ala Leu Ser Arg Trp
545                 550                 555                 560

Asp Gly Asp Glu Leu Lys Ser Arg Ile Ser Asp Ser Leu Gln Asp Ala
                565                 570                 575

Ala Arg Ala Ala Gly Leu Gln Ser Tyr Glu Ile Pro Arg Asp Phe Leu
            580                 585                 590

Val Glu Thr Thr Pro Phe Thr Leu Glu Asn Gly Leu Leu Thr Gly Ile
        595                 600                 605

Arg Lys Leu Ala Arg Pro Lys Leu Lys Ala His Tyr Gly Glu Arg Leu
    610                 615                 620

Glu Gln Leu Tyr Thr Asp Leu Ala Glu Gly Gln Ala Asn Glu Leu Arg
625                 630                 635                 640

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

Arg Ala Ala Val Ala Leu Leu Gly Ala Ser Val Thr Asp Leu Arg Ser
            660                 665                 670

Asp Ala His Phe Thr Asp Leu Gly Gly Asp Ser Leu Ser Ala Leu Ser
        675                 680                 685

Phe Ser Asn Leu Leu His Glu Ile Phe Asp Val Asp Val Pro Val Gly
    690                 695                 700

Val Ile Val Ser Pro Ala Thr Asp Leu Ala Gly Val Ala Ala Tyr Ile
705                 710                 715                 720

Glu Gly Glu Leu Arg Gly Ser Lys Arg Pro Thr Tyr Ala Ser Val His
                725                 730                 735

Gly Arg Asp Ala Thr Glu Val Arg Ala Arg Asp Leu Ala Leu Gly Lys
            740                 745                 750

Phe Ile Asp Ala Lys Thr Leu Ser Ala Ala Pro Gly Leu Pro Arg Ser
        755                 760                 765

Gly Thr Glu Ile Arg Thr Val Leu Leu Thr Gly Ala Thr Gly Phe Leu
    770                 775                 780

Gly Arg Tyr Leu Ala Leu Glu Trp Leu Glu Arg Met Asp Leu Val Asp
785                 790                 795                 800

Gly Lys Val Ile Cys Leu Val Arg Ala Arg Ser Asp Asp Glu Ala Arg
                805                 810                 815

Ala Arg Leu Asp Ala Thr Phe Asp Thr Gly Asp Ala Thr Leu Leu Glu
            820                 825                 830

His Tyr Arg Ala Leu Ala Ala Asp His Leu Glu Val Ile Ala Gly Asp
        835                 840                 845

Lys Gly Glu Ala Asp Leu Gly Leu Asp His Asp Thr Trp Gln Arg Leu
    850                 855                 860

Ala Asp Thr Val Asp Leu Ile Val Asp Pro Ala Ala Leu Val Asn His
865                 870                 875                 880

Val Leu Pro Tyr Ser Gln Met Phe Gly Pro Asn Ala Leu Gly Thr Ala
                885                 890                 895

Glu Leu Ile Arg Ile Ala Leu Thr Thr Thr Ile Lys Pro Tyr Val Tyr
            900                 905                 910
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Val Ser Thr Ile Gly Val Gly Gln Gly Ile Ser Pro Glu Ala Phe Val
        915                 920                 925

Glu Asp Ala Asp Ile Arg Glu Ile Ser Ala Thr Arg Arg Val Asp Asp
    930                 935                 940

Ser Tyr Ala Asn Gly Tyr Gly Asn Ser Lys Trp Ala Gly Glu Val Leu
945                 950                 955                 960

Leu Arg Glu Ala His Asp Trp Cys Gly Leu Pro Val Ser Val Phe Arg
                965                 970                 975

Cys Asp Met Ile Leu Ala Asp Thr Thr Tyr Ser Gly Gln Leu Asn Leu
            980                 985                 990

Pro Asp Met Phe Thr Arg Leu Met  Leu Ser Leu Val Ala  Thr Gly Ile
        995                 1000                1005

Ala Pro  Gly Ser Phe Tyr Glu  Leu Asp Ala Asp Gly  Asn Arg Gln
    1010                1015                1020

Arg Ala  His Tyr Asp Gly Leu  Pro Val Glu Phe Ile  Ala Glu Ala
    1025                1030                1035

Ile Ser  Thr Ile Gly Ser Gln  Val Thr Asp Gly Phe  Glu Thr Phe
    1040                1045                1050

His Val  Met Asn Pro Tyr Asp  Asp Gly Ile Gly Leu  Asp Glu Tyr
    1055                1060                1065

Val Asp  Trp Leu Ile Glu Ala  Gly Tyr Pro Val His  Arg Val Asp
    1070                1075                1080

Asp Tyr  Ala Thr Trp Leu Ser  Arg Phe Glu Thr Ala  Leu Arg Ala
    1085                1090                1095

Leu Pro  Glu Arg Gln Arg Gln  Ala Ser Leu Leu Pro  Leu Leu His
    1100                1105                1110

Asn Tyr  Gln Gln Pro Ser Pro  Pro Val Cys Gly Ala  Met Ala Pro
    1115                1120                1125

Thr Asp  Arg Phe Arg Ala Ala  Val Gln Asp Ala Lys  Ile Gly Pro
    1130                1135                1140

Asp Lys  Asp Ile Pro His Val  Thr Ala Asp Val Ile  Val Lys Tyr
    1145                1150                1155

Ile Ser  Asn Leu Gln Met Leu  Gly Leu Leu
    1160                1165


<210> SEQ ID NO 34
<211> LENGTH: 1168
<212> TYPE: PRT
<213> ORGANISM: Mycobacterium tuberculosis

<400> SEQUENCE: 34

Met Ser Ile Asn Asp Gln Arg Leu Thr Arg Arg Val Glu Asp Leu Tyr
1               5                   10                  15

Ala Ser Asp Ala Gln Phe Ala Ala Ala Ser Pro Asn Glu Ala Ile Thr
            20                  25                  30

Gln Ala Ile Asp Gln Pro Gly Val Ala Leu Pro Gln Leu Ile Arg Met
        35                  40                  45

Val Met Glu Gly Tyr Ala Asp Arg Pro Ala Leu Gly Gln Arg Ala Leu
    50                  55                  60

Arg Phe Val Thr Asp Pro Asp Ser Gly Arg Thr Met Val Glu Leu Leu
65                  70                  75                  80

Pro Arg Phe Glu Thr Ile Thr Tyr Arg Glu Leu Trp Ala Arg Ala Gly
                85                  90                  95
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Thr Leu Ala Thr Ala Leu Ser Ala Glu Pro Ala Ile Arg Pro Gly Asp
            100                 105                 110

Arg Val Cys Val Leu Gly Phe Asn Ser Val Asp Tyr Thr Thr Ile Asp
        115                 120                 125

Ile Ala Leu Ile Arg Leu Gly Ala Val Ser Val Pro Leu Gln Thr Ser
    130                 135                 140

Ala Pro Val Thr Gly Leu Arg Pro Ile Val Thr Glu Thr Glu Pro Thr
145                 150                 155                 160

Met Ile Ala Thr Ser Ile Asp Asn Leu Gly Asp Ala Val Glu Val Leu
                165                 170                 175

Ala Gly His Ala Pro Ala Arg Leu Val Val Phe Asp Tyr His Gly Lys
            180                 185                 190

Val Asp Thr His Arg Glu Ala Val Glu Ala Ala Arg Ala Arg Leu Ala
        195                 200                 205

Gly Ser Val Thr Ile Asp Thr Leu Ala Glu Leu Ile Glu Arg Gly Arg
    210                 215                 220

Ala Leu Pro Ala Thr Pro Ile Ala Asp Ser Ala Asp Asp Ala Leu Ala
225                 230                 235                 240

Leu Leu Ile Tyr Thr Ser Gly Ser Thr Gly Ala Pro Lys Gly Ala Met
                245                 250                 255

Tyr Arg Glu Ser Gln Val Met Ser Phe Trp Arg Lys Ser Ser Gly Trp
            260                 265                 270

Phe Glu Pro Ser Gly Tyr Pro Ser Ile Thr Leu Asn Phe Met Pro Met
        275                 280                 285

Ser His Val Gly Gly Arg Gln Val Leu Tyr Gly Thr Leu Ser Asn Gly
    290                 295                 300

Gly Thr Ala Tyr Phe Val Ala Lys Ser Asp Leu Ser Thr Leu Phe Glu
305                 310                 315                 320

Asp Leu Ala Leu Val Arg Pro Thr Glu Leu Cys Phe Val Pro Arg Ile
                325                 330                 335

Trp Asp Met Val Phe Ala Glu Phe His Ser Glu Val Asp Arg Arg Leu
            340                 345                 350

Val Asp Gly Ala Asp Arg Ala Ala Leu Glu Ala Gln Val Lys Ala Glu
        355                 360                 365

Leu Arg Glu Asn Val Leu Gly Gly Arg Phe Val Met Ala Leu Thr Gly
    370                 375                 380

Ser Ala Pro Ile Ser Ala Glu Met Thr Ala Trp Val Glu Ser Leu Leu
385                 390                 395                 400

Ala Asp Val His Leu Val Glu Gly Tyr Gly Ser Thr Glu Ala Gly Met
                405                 410                 415

Val Leu Asn Asp Gly Met Val Arg Arg Pro Ala Val Ile Asp Tyr Lys
            420                 425                 430

Leu Val Asp Val Pro Glu Leu Gly Tyr Phe Gly Thr Asp Gln Pro Tyr
        435                 440                 445

Pro Arg Gly Glu Leu Leu Val Lys Thr Gln Thr Met Phe Pro Gly Tyr
    450                 455                 460

Tyr Gln Arg Pro Asp Val Thr Ala Glu Val Phe Asp Pro Asp Gly Phe
465                 470                 475                 480

Tyr Arg Thr Gly Asp Ile Met Ala Lys Val Gly Pro Asp Gln Phe Val
                485                 490                 495
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Tyr Leu Asp Arg Arg Asn Asn Val Leu Lys Leu Ser Gln Gly Glu Phe
            500                 505                 510

Ile Ala Val Ser Lys Leu Glu Ala Val Phe Gly Asp Ser Pro Leu Val
        515                 520                 525

Arg Gln Ile Phe Ile Tyr Gly Asn Ser Ala Arg Ala Tyr Pro Leu Ala
    530                 535                 540

Val Val Val Pro Ser Gly Asp Ala Leu Ser Arg His Gly Ile Glu Asn
545                 550                 555                 560

Leu Lys Pro Val Ile Ser Glu Ser Leu Gln Glu Val Ala Arg Ala Ala
                565                 570                 575

Gly Leu Gln Ser Tyr Glu Ile Pro Arg Asp Phe Ile Ile Glu Thr Thr
            580                 585                 590

Pro Phe Thr Leu Glu Asn Gly Leu Leu Thr Gly Ile Arg Lys Leu Ala
        595                 600                 605

Arg Pro Gln Leu Lys Lys Phe Tyr Gly Glu Arg Leu Glu Arg Leu Tyr
    610                 615                 620

Thr Glu Leu Ala Asp Ser Gln Ser Asn Glu Leu Arg Glu Leu Arg Gln
625                 630                 635                 640

Ser Gly Pro Asp Ala Pro Val Leu Pro Thr Leu Cys Arg Ala Ala Ala
                645                 650                 655

Ala Leu Leu Gly Ser Thr Ala Ala Asp Val Arg Pro Asp Ala His Phe
            660                 665                 670

Ala Asp Leu Gly Gly Asp Ser Leu Ser Ala Leu Ser Leu Ala Asn Leu
        675                 680                 685

Leu His Glu Ile Phe Gly Val Asp Val Pro Val Gly Val Ile Val Ser
    690                 695                 700

Pro Ala Ser Asp Leu Arg Ala Leu Ala Asp His Ile Glu Ala Ala Arg
705                 710                 715                 720

Thr Gly Val Arg Arg Pro Ser Phe Ala Ser Ile His Gly Arg Ser Ala
                725                 730                 735

Thr Glu Val His Ala Ser Asp Leu Thr Leu Asp Lys Phe Ile Asp Ala
            740                 745                 750

Ala Thr Leu Ala Ala Ala Pro Asn Leu Pro Ala Pro Ser Ala Gln Val
        755                 760                 765

Arg Thr Val Leu Leu Thr Gly Ala Thr Gly Phe Leu Gly Arg Tyr Leu
    770                 775                 780

Ala Leu Glu Trp Leu Asp Arg Met Asp Leu Val Asn Gly Lys Leu Ile
785                 790                 795                 800

Cys Leu Val Arg Ala Arg Ser Asp Glu Glu Ala Gln Ala Arg Leu Asp
                805                 810                 815

Ala Thr Phe Asp Ser Gly Asp Pro Tyr Leu Val Arg His Tyr Arg Glu
            820                 825                 830

Leu Gly Ala Gly Arg Leu Glu Val Leu Ala Gly Asp Lys Gly Glu Ala
        835                 840                 845

Asp Leu Gly Leu Asp Arg Val Thr Trp Gln Arg Leu Ala Asp Thr Val
    850                 855                 860

Asp Leu Ile Val Asp Pro Ala Ala Leu Val Asn His Val Leu Pro Tyr
865                 870                 875                 880

Ser Gln Leu Phe Gly Pro Asn Ala Ala Gly Thr Ala Glu Leu Leu Arg
                885                 890                 895

Leu Ala Leu Thr Gly Lys Arg Lys Pro Tyr Ile Tyr Thr Ser Thr Ile
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```
           900                 905                 910

Ala Val Gly Glu Gln Ile Pro Pro Glu Ala Phe Thr Glu Asp Ala Asp
        915                 920                 925

Ile Arg Ala Ile Ser Pro Thr Arg Arg Ile Asp Asp Ser Tyr Ala Asn
    930                 935                 940

Gly Tyr Ala Asn Ser Lys Trp Ala Gly Glu Val Leu Leu Arg Glu Ala
945                 950                 955                 960

His Glu Gln Cys Gly Leu Pro Val Thr Val Phe Arg Cys Asp Met Ile
                965                 970                 975

Leu Ala Asp Thr Ser Tyr Thr Gly Gln Leu Asn Leu Pro Asp Met Phe
            980                 985                 990

Thr Arg Leu Met Leu Ser Leu Ala  Ala Thr Gly Ile Ala  Pro Gly Ser
        995                 1000                1005

Phe Tyr  Glu Leu Asp Ala His  Gly Asn Arg Gln Arg  Ala His Tyr
    1010                1015                1020

Asp Gly  Leu Pro Val Glu Phe  Val Ala Glu Ala Ile  Cys Thr Leu
    1025                1030                1035

Gly Thr  His Ser Pro Asp Arg  Phe Val Thr Tyr His  Val Met Asn
    1040                1045                1050

Pro Tyr  Asp Asp Gly Ile Gly  Leu Asp Glu Phe Val  Asp Trp Leu
    1055                1060                1065

Asn Ser  Pro Thr Ser Gly Ser  Gly Cys Thr Ile Gln  Arg Ile Ala
    1070                1075                1080

Asp Tyr  Gly Glu Trp Leu Gln  Arg Phe Glu Thr Ser  Leu Arg Ala
    1085                1090                1095

Leu Pro  Asp Arg Gln Arg His  Ala Ser Leu Leu Pro  Leu Leu His
    1100                1105                1110

Asn Tyr  Arg Glu Pro Ala Lys  Pro Ile Cys Gly Ser  Ile Ala Pro
    1115                1120                1125

Thr Asp  Gln Phe Arg Ala Ala  Val Gln Glu Ala Lys  Ile Gly Pro
    1130                1135                1140

Asp Lys  Asp Ile Pro His Leu  Thr Ala Ala Ile Ile  Ala Lys Tyr
    1145                1150                1155

Ile Ser  Asn Leu Arg Leu Leu  Gly Leu Leu
    1160                1165


<210> SEQ ID NO 35
<211> LENGTH: 696
<212> TYPE: DNA
<213> ORGANISM: Synechococcus elongatus

<400> SEQUENCE: 35

atgccgcagc ttgaagccag ccttgaactg gactttcaaa gcgagtccta caaagacgct     60

tacagccgca tcaacgcgat cgtgattgaa ggcgaacaag aggcgttcga caactacaat    120

cgccttgctg agatgctgcc cgaccagcgg gatgagcttc acaagctagc caagatggaa    180

cagcgccaca tgaaaggctt tatggcctgt ggcaaaaatc tctccgtcac tcctgacatg    240

ggttttgccc agaaattttt cgagcgcttg cacgagaact tcaaagcggc ggctgcggaa    300

ggcaaggtcg tcacctgcct actgattcaa tcgctaatca tcgagtgctt tgcgatcgcg    360

gcttacaaca tctacatccc agtggcggat gcttttgccc gcaaaatcac ggaggggggtc    420

gtgcgcgacg aatacctgca ccgcaacttc ggtgaagagt ggctgaaggc gaattttgat    480
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gcttccaaag ccgaactgga agaagccaat cgtcagaacc tgcccttggt ttggctaatg 540

ctcaacgaag tggccgatga tgctcgcgaa ctcgggatgg agcgtgagtc gctcgtcgag 600

gactttatga ttgcctacgg tgaagctctg gaaaacatcg gcttcacaac gcgcgaaatc 660

atgcgtatgt ccgcctatgg ccttgcggcc gtttga 696

<210> SEQ ID NO 36
<211> LENGTH: 231
<212> TYPE: PRT
<213> ORGANISM: Synechococcus elongatus

<400> SEQUENCE: 36

Met Pro Gln Leu Glu Ala Ser Leu Glu Leu Asp Phe Gln Ser Glu Ser
1 5 10 15

Tyr Lys Asp Ala Tyr Ser Arg Ile Asn Ala Ile Val Ile Glu Gly Glu
20 25 30

Gln Glu Ala Phe Asp Asn Tyr Asn Arg Leu Ala Glu Met Leu Pro Asp
35 40 45

Gln Arg Asp Glu Leu His Lys Leu Ala Lys Met Glu Gln Arg His Met
50 55 60

Lys Gly Phe Met Ala Cys Gly Lys Asn Leu Ser Val Thr Pro Asp Met
65 70 75 80

Gly Phe Ala Gln Lys Phe Phe Glu Arg Leu His Glu Asn Phe Lys Ala
85 90 95

Ala Ala Ala Glu Gly Lys Val Val Thr Cys Leu Leu Ile Gln Ser Leu
100 105 110

Ile Ile Glu Cys Phe Ala Ile Ala Ala Tyr Asn Ile Tyr Ile Pro Val
115 120 125

Ala Asp Ala Phe Ala Arg Lys Ile Thr Glu Gly Val Val Arg Asp Glu
130 135 140

Tyr Leu His Arg Asn Phe Gly Glu Glu Trp Leu Lys Ala Asn Phe Asp
145 150 155 160

Ala Ser Lys Ala Glu Leu Glu Glu Ala Asn Arg Gln Asn Leu Pro Leu
165 170 175

Val Trp Leu Met Leu Asn Glu Val Ala Asp Asp Ala Arg Glu Leu Gly
180 185 190

Met Glu Arg Glu Ser Leu Val Glu Asp Phe Met Ile Ala Tyr Gly Glu
195 200 205

Ala Leu Glu Asn Ile Gly Phe Thr Thr Arg Glu Ile Met Arg Met Ser
210 215 220

Ala Tyr Gly Leu Ala Ala Val
225 230

<210> SEQ ID NO 37
<211> LENGTH: 1029
<212> TYPE: DNA
<213> ORGANISM: Synechococcus elongatus

<400> SEQUENCE: 37

atggcattcg gtcttatcgg tcatctcacc agtttggagc aggcccgcga cgtttctcgc 60

aggatgggct acgacgaata cgccgatcaa ggattggagt tttggagtag cgctcctcct 120

caaatcgttg atgaaatcac agtcaccagt gccacaggca aggtgattca cggtcgctac 180

-continued

atcgaatcgt gtttcttgcc ggaaatgctg gcggcgcgcc gcttcaaaac agccacgcgc 240

aaagttctca atgccatgtc ccatgcccaa aaacacggca tcgacatctc ggccttgggg 300

ggctttacct cgattatttt cgagaatttc gatttggcca gtttgcggca agtgcgcgac 360

actaccttgg agtttgaacg gttcaccacc ggcaatactc acacggccta cgtaatctgt 420

agacaggtgg aagccgctgc taaaacgctg ggcatcgaca ttacccaagc gacagtagcg 480

gttgtcggcg cgactggcga tatcggtagc gctgtctgcc gctggctcga cctcaaactg 540

ggtgtcggtg atttgatcct gacggcgcgc aatcaggagc gtttggataa cctgcaggct 600

gaactcggcc ggggcaagat tctgcccttg gaagccgctc tgccggaagc tgactttatc 660

gtgtgggtcg ccagtatgcc tcagggcgta gtgatcgacc cagcaaccct gaagcaaccc 720

tgcgtcctaa tcgacggggg ctaccccaaa aacttgggca gcaaagtcca aggtgagggc 780

atctatgtcc tcaatggcgg ggtagttgaa cattgcttcg acatcgactg gcagatcatg 840

tccgctgcag agatggcgcg gcccgagcgc cagatgtttg cctgctttgc cgaggcgatg 900

ctcttggaat ttgaaggctg gcatactaac ttctcctggg gccgcaacca aatcacgatc 960

gagaagatgg aagcgatcgg tgaggcatcg gtgcgccacg gcttccaacc cttggcattg 1020

gcaatttga 1029

<210> SEQ ID NO 38
<211> LENGTH: 342
<212> TYPE: PRT
<213> ORGANISM: Synechococcus elongatus

<400> SEQUENCE: 38

Met Ala Phe Gly Leu Ile Gly His Leu Thr Ser Leu Glu Gln Ala Arg
1 5 10 15

Asp Val Ser Arg Arg Met Gly Tyr Asp Glu Tyr Ala Asp Gln Gly Leu
20 25 30

Glu Phe Trp Ser Ser Ala Pro Pro Gln Ile Val Asp Glu Ile Thr Val
35 40 45

Thr Ser Ala Thr Gly Lys Val Ile His Gly Arg Tyr Ile Glu Ser Cys
50 55 60

Phe Leu Pro Glu Met Leu Ala Ala Arg Arg Phe Lys Thr Ala Thr Arg
65 70 75 80

Lys Val Leu Asn Ala Met Ser His Ala Gln Lys His Gly Ile Asp Ile
85 90 95

Ser Ala Leu Gly Gly Phe Thr Ser Ile Ile Phe Glu Asn Phe Asp Leu
100 105 110

Ala Ser Leu Arg Gln Val Arg Asp Thr Thr Leu Glu Phe Glu Arg Phe
115 120 125

Thr Thr Gly Asn Thr His Thr Ala Tyr Val Ile Cys Arg Gln Val Glu
130 135 140

Ala Ala Ala Lys Thr Leu Gly Ile Asp Ile Thr Gln Ala Thr Val Ala
145 150 155 160

Val Val Gly Ala Thr Gly Asp Ile Gly Ser Ala Val Cys Arg Trp Leu
165 170 175

Asp Leu Lys Leu Gly Val Gly Asp Leu Ile Leu Thr Ala Arg Asn Gln
180 185 190

Glu Arg Leu Asp Asn Leu Gln Ala Glu Leu Gly Arg Gly Lys Ile Leu
195 200 205

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Pro Leu Glu Ala Ala Leu Pro Glu Ala Asp Phe Ile Val Trp Val Ala
    210                 215                 220

Ser Met Pro Gln Gly Val Val Ile Asp Pro Ala Thr Leu Lys Gln Pro
225                 230                 235                 240

Cys Val Leu Ile Asp Gly Gly Tyr Pro Lys Asn Leu Gly Ser Lys Val
                245                 250                 255

Gln Gly Glu Gly Ile Tyr Val Leu Asn Gly Gly Val Val Glu His Cys
            260                 265                 270

Phe Asp Ile Asp Trp Gln Ile Met Ser Ala Ala Glu Met Ala Arg Pro
        275                 280                 285

Glu Arg Gln Met Phe Ala Cys Phe Ala Glu Ala Met Leu Leu Glu Phe
    290                 295                 300

Glu Gly Trp His Thr Asn Phe Ser Trp Gly Arg Asn Gln Ile Thr Ile
305                 310                 315                 320

Glu Lys Met Glu Ala Ile Gly Glu Ala Ser Val Arg His Gly Phe Gln
                325                 330                 335

Pro Leu Ala Leu Ala Ile
            340
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<210> SEQ ID NO 39
<211> LENGTH: 717
<212> TYPE: DNA
<213> ORGANISM: Prochlorococcus mariunus

<400> SEQUENCE: 39

```
atgcaaacac tcgaatctaa taaaaaaact aatctagaaa attctattga tttacccgat     60

tttactactg attcttacaa agacgcttat agcaggataa atgcaatagt tattgaaggt    120

gaacaagagg ctcatgataa ttacatttcc ttagcaacat taattcctaa cgaattagaa    180

gagttaacta aattagcgaa aatggagctt aagcacaaaa gaggctttac tgcatgtgga    240

agaaatctag gtgttcaagc tgacatgatt tttgctaaag aattcttttc caaattacat    300

ggtaattttc aggttgcgtt atctaatggc aagacaacta catgcctatt aatacaggca    360

attttaattg aagcttttgc tatatccgcg tatcacgttt acataagagt tgctgatcct    420

ttcgcgaaaa aaattaccca aggtgttgtt aaagatgaat atcttcattt aaattatgga    480

caagaatggc taaaagaaaa tttagcgact tgtaaagatg agctaatgga agcaaataag    540

gttaaccttc cattaatcaa gaagatgtta gatcaagtct cggaagatgc ttcagtacta    600

gctatggata gggaagaatt aatggaagaa ttcatgattg cctatcagga cactctcctt    660

gaaataggtt tagataatag agaaattgca agaatggcaa tggctgctat agtttaa       717
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<210> SEQ ID NO 40
<211> LENGTH: 238
<212> TYPE: PRT
<213> ORGANISM: Prochlorococcus mariunus

<400> SEQUENCE: 40

```
Met Gln Thr Leu Glu Ser Asn Lys Lys Thr Asn Leu Glu Asn Ser Ile
1               5                   10                  15

Asp Leu Pro Asp Phe Thr Thr Asp Ser Tyr Lys Asp Ala Tyr Ser Arg
            20                  25                  30

Ile Asn Ala Ile Val Ile Glu Gly Glu Gln Glu Ala His Asp Asn Tyr
        35                  40                  45
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Ile Ser Leu Ala Thr Leu Ile Pro Asn Glu Leu Glu Glu Leu Thr Lys
    50                  55                  60

Leu Ala Lys Met Glu Leu Lys His Lys Arg Gly Phe Thr Ala Cys Gly
65                  70                  75                  80

Arg Asn Leu Gly Val Gln Ala Asp Met Ile Phe Ala Lys Glu Phe Phe
                85                  90                  95

Ser Lys Leu His Gly Asn Phe Gln Val Ala Leu Ser Asn Gly Lys Thr
            100                 105                 110

Thr Thr Cys Leu Leu Ile Gln Ala Ile Leu Ile Glu Ala Phe Ala Ile
        115                 120                 125

Ser Ala Tyr His Val Tyr Ile Arg Val Ala Asp Pro Phe Ala Lys Lys
    130                 135                 140

Ile Thr Gln Gly Val Val Lys Asp Glu Tyr Leu His Leu Asn Tyr Gly
145                 150                 155                 160

Gln Glu Trp Leu Lys Glu Asn Leu Ala Thr Cys Lys Asp Glu Leu Met
                165                 170                 175

Glu Ala Asn Lys Val Asn Leu Pro Leu Ile Lys Lys Met Leu Asp Gln
            180                 185                 190

Val Ser Glu Asp Ala Ser Val Leu Ala Met Asp Arg Glu Glu Leu Met
        195                 200                 205

Glu Glu Phe Met Ile Ala Tyr Gln Asp Thr Leu Leu Glu Ile Gly Leu
    210                 215                 220

Asp Asn Arg Glu Ile Ala Arg Met Ala Met Ala Ala Ile Val
225                 230                 235


<210> SEQ ID NO 41
<211> LENGTH: 1044
<212> TYPE: DNA
<213> ORGANISM: Prochlorococcus mariunus

<400> SEQUENCE: 41

atggcatttg ggcttatagg tcattcaact agttttgaag atgcaaaaag aaaggcttca     60

ttattgggct ttgatcatat tgcggatggt gatttagatg tttggtgcac agctccacct    120

caactagttg aaaatgtaga ggttaaaagt gctataggta tatcaattga aggttcttat    180

attgattcat gtttcgttcc tgaaatgctt tcaagattta aaacggcaag aagaaaagta    240

ttaaatgcaa tggaattagc tcaaaaaaaa ggtattaata ttaccgcttt ggggggggttc    300

acttctatca tctttgaaaa ttttaatctc cttcaacata agcagattag aaacacttca    360

ctagagtggg aaaggtttac aactggtaat actcatactg cgtgggttat ttgcaggcaa    420

ttagagatga atgctcctaa aataggtatt gatcttaaaa gcgcaacagt tgctgtagtt    480

ggtgctactg gagatatagg cagtgctgtt tgtcgatggt taatcaataa aacaggtatt    540

ggggaacttc ttttggtagc taggcaaaag gaacccttgg attctttgca aaaggaatta    600

gatggtggaa ctatcaaaaa tctagatgaa gcattgcctg aagcagatat tgttgtatgg    660

gtagcaagta tgccaaagac aatggaaatc gatgctaata atcttaaaca accatgttta    720

atgattgatg gaggttatcc aaagaatcta gatgaaaaat ttcaaggaaa taatatacat    780

gttgtaaaag gaggtatagt aagattcttc aatgatatag gttggaatat gatggaacta    840

gctgaaatgc aaaatcccca gagagaaatg tttgcatgct ttgcagaagc aatgatttta    900

gaatttgaaa aatgtcatac aaactttagc tggggaagaa ataatatatc tctcgagaaa    960
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atggagttta ttggagctgc ttctgtaaag catggcttct ctgcaattgg cctagataag 1020

catccaaaag tactagcagt ttga 1044

<210> SEQ ID NO 42
<211> LENGTH: 347
<212> TYPE: PRT
<213> ORGANISM: Prochlorococcus mariunus

<400> SEQUENCE: 42

Met Ala Phe Gly Leu Ile Gly His Ser Thr Ser Phe Glu Asp Ala Lys
1 5 10 15

Arg Lys Ala Ser Leu Leu Gly Phe Asp His Ile Ala Asp Gly Asp Leu
20 25 30

Asp Val Trp Cys Thr Ala Pro Pro Gln Leu Val Glu Asn Val Glu Val
35 40 45

Lys Ser Ala Ile Gly Ile Ser Ile Glu Gly Ser Tyr Ile Asp Ser Cys
50 55 60

Phe Val Pro Glu Met Leu Ser Arg Phe Lys Thr Ala Arg Arg Lys Val
65 70 75 80

Leu Asn Ala Met Glu Leu Ala Gln Lys Lys Gly Ile Asn Ile Thr Ala
85 90 95

Leu Gly Gly Phe Thr Ser Ile Ile Phe Glu Asn Phe Asn Leu Leu Gln
100 105 110

His Lys Gln Ile Arg Asn Thr Ser Leu Glu Trp Glu Arg Phe Thr Thr
115 120 125

Gly Asn Thr His Thr Ala Trp Val Ile Cys Arg Gln Leu Glu Met Asn
130 135 140

Ala Pro Lys Ile Gly Ile Asp Leu Lys Ser Ala Thr Val Ala Val Val
145 150 155 160

Gly Ala Thr Gly Asp Ile Gly Ser Ala Val Cys Arg Trp Leu Ile Asn
165 170 175

Lys Thr Gly Ile Gly Glu Leu Leu Leu Val Ala Arg Gln Lys Glu Pro
180 185 190

Leu Asp Ser Leu Gln Lys Glu Leu Asp Gly Gly Thr Ile Lys Asn Leu
195 200 205

Asp Glu Ala Leu Pro Glu Ala Asp Ile Val Val Trp Val Ala Ser Met
210 215 220

Pro Lys Thr Met Glu Ile Asp Ala Asn Asn Leu Lys Gln Pro Cys Leu
225 230 235 240

Met Ile Asp Gly Gly Tyr Pro Lys Asn Leu Asp Glu Lys Phe Gln Gly
245 250 255

Asn Asn Ile His Val Val Lys Gly Gly Ile Val Arg Phe Phe Asn Asp
260 265 270

Ile Gly Trp Asn Met Met Glu Leu Ala Glu Met Gln Asn Pro Gln Arg
275 280 285

Glu Met Phe Ala Cys Phe Ala Glu Ala Met Ile Leu Glu Phe Glu Lys
290 295 300

Cys His Thr Asn Phe Ser Trp Gly Arg Asn Asn Ile Ser Leu Glu Lys
305 310 315 320

Met Glu Phe Ile Gly Ala Ala Ser Val Lys His Gly Phe Ser Ala Ile
325 330 335

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Gly Leu Asp Lys His Pro Lys Val Leu Ala Val
340 345

<210> SEQ ID NO 43
<211> LENGTH: 255
<212> TYPE: DNA
<213> ORGANISM: Nostoc punctiforme

<400> SEQUENCE: 43

atgagccaaa cggaactttt tgaaaaggtc aagaaaatcg tcatcgaaca actgagtgtt 60

gaagatgctt ccaaaatcac tccacaagct aagtttatgg aagatttagg agctgattcc 120

ctggatactg ttgaactcgt gatggctttg gaagaagaat ttgatatcga aattcccgac 180

gaagctgccg agcagattgt atcggttcaa gacgcagtag attacatcaa taacaaagtt 240

gctgcatcag cttaa 255

<210> SEQ ID NO 44
<211> LENGTH: 84
<212> TYPE: PRT
<213> ORGANISM: Nostoc punctiforme

<400> SEQUENCE: 44

Met Ser Gln Thr Glu Leu Phe Glu Lys Val Lys Lys Ile Val Ile Glu
1 5 10 15

Gln Leu Ser Val Glu Asp Ala Ser Lys Ile Thr Pro Gln Ala Lys Phe
20 25 30

Met Glu Asp Leu Gly Ala Asp Ser Leu Asp Thr Val Glu Leu Val Met
35 40 45

Ala Leu Glu Glu Glu Phe Asp Ile Glu Ile Pro Asp Glu Ala Ala Glu
50 55 60

Gln Ile Val Ser Val Gln Asp Ala Val Asp Tyr Ile Asn Asn Lys Val
65 70 75 80

Ala Ala Ser Ala

<210> SEQ ID NO 45
<211> LENGTH: 234
<212> TYPE: DNA
<213> ORGANISM: Synechocystis sp.

<400> SEQUENCE: 45

atgaatcagg aaatttttga aaaagtaaaa aaaatcgtcg tggaacagtt ggaagtggat 60

cctgacaaag tgacccccga tgccaccttt gccgaagatt taggggctga ttccctcgat 120

acagtggaat tggtcatggc cctggaagaa gagtttgata ttgaaattcc cgatgaagtg 180

gcggaaacca ttgataccgt gggcaaagcc gttgagcata tcgaaagtaa ataa 234

<210> SEQ ID NO 46
<211> LENGTH: 77
<212> TYPE: PRT
<213> ORGANISM: Synechocystis sp.

<400> SEQUENCE: 46

Met Asn Gln Glu Ile Phe Glu Lys Val Lys Lys Ile Val Val Glu Gln
1 5 10 15

Leu Glu Val Asp Pro Asp Lys Val Thr Pro Asp Ala Thr Phe Ala Glu
20 25 30

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```
Asp Leu Gly Ala Asp Ser Leu Asp Thr Val Glu Leu Val Met Ala Leu
        35                  40                  45

Glu Glu Glu Phe Asp Ile Glu Ile Pro Asp Glu Val Ala Glu Thr Ile
    50                  55                  60

Asp Thr Val Gly Lys Ala Val Glu His Ile Glu Ser Lys
65                  70                  75


<210> SEQ ID NO 47
<211> LENGTH: 243
<212> TYPE: DNA
<213> ORGANISM: Prochlorococcus marinus

<400> SEQUENCE: 47

atgtcacaag aagaaatcct tcaaaaagta tgctctattg tttctgagca actaagtgtt     60

gaatcagccg aagtaaaatc tgattcaaac tttcaaaatg atttaggtgc agactcccta    120

gacaccgtag agctagttat ggctcttgaa gaagcatttg atatcgagat acctgatgaa    180

gcagctgaag gtatcgcaac agtaggagat gctgttaaat tcatcgaaga aaaaaaaggt    240

taa                                                                  243


<210> SEQ ID NO 48
<211> LENGTH: 80
<212> TYPE: PRT
<213> ORGANISM: Prochlorococcus marinus

<400> SEQUENCE: 48

Met Ser Gln Glu Glu Ile Leu Gln Lys Val Cys Ser Ile Val Ser Glu
1               5                   10                  15

Gln Leu Ser Val Glu Ser Ala Glu Val Lys Ser Asp Ser Asn Phe Gln
            20                  25                  30

Asn Asp Leu Gly Ala Asp Ser Leu Asp Thr Val Glu Leu Val Met Ala
        35                  40                  45

Leu Glu Glu Ala Phe Asp Ile Glu Ile Pro Asp Glu Ala Ala Glu Gly
    50                  55                  60

Ile Ala Thr Val Gly Asp Ala Val Lys Phe Ile Glu Glu Lys Lys Gly
65                  70                  75                  80


<210> SEQ ID NO 49
<211> LENGTH: 243
<212> TYPE: DNA
<213> ORGANISM: Synechococcus elongatus

<400> SEQUENCE: 49

atgagccaag aagacatctt cagcaaagtc aaagacattg tggctgagca gctgagtgtg     60

gatgtggctg aagtcaagcc agaatccagc ttccaaaacg atctgggagc ggactcgctg    120

gacaccgtgg aactggtgat ggctctggaa gaggctttcg atatcgaaat ccccgatgaa    180

gccgctgaag gcattgcgac cgttcaagac gccgtcgatt tcatcgctag caaagctgcc    240

tag                                                                  243


<210> SEQ ID NO 50
<211> LENGTH: 80
<212> TYPE: PRT
<213> ORGANISM: Synechococcus elongatus

<400> SEQUENCE: 50
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Met Ser Gln Glu Asp Ile Phe Ser Lys Val Lys Asp Ile Val Ala Glu
1               5                   10                  15

Gln Leu Ser Val Asp Val Ala Glu Val Lys Pro Glu Ser Ser Phe Gln
            20                  25                  30

Asn Asp Leu Gly Ala Asp Ser Leu Asp Thr Val Glu Leu Val Met Ala
        35                  40                  45

Leu Glu Glu Ala Phe Asp Ile Glu Ile Pro Asp Glu Ala Ala Glu Gly
    50                  55                  60

Ile Ala Thr Val Gln Asp Ala Val Asp Phe Ile Ala Ser Lys Ala Ala
65                  70                  75                  80


<210> SEQ ID NO 51
<211> LENGTH: 255
<212> TYPE: DNA
<213> ORGANISM: Nostoc sp.

<400> SEQUENCE: 51

atgagccaat cagaaacttt tgaaaaagtc aaaaaaattg ttatcgaaca actaagtgtg     60

gagaaccctg acacagtaac tccagaagct agttttgcca acgatttaca ggctgattcc    120

ctcgatacag tagaactagt aatggctttg gaagaagaat ttgatatcga aattcccgat    180

gaagccgcag agaaaattac cactgttcaa gaagcggtgg attacatcaa taaccaagtt    240

gccgcatcag cttaa                                                     255


<210> SEQ ID NO 52
<211> LENGTH: 84
<212> TYPE: PRT
<213> ORGANISM: Nostoc sp.

<400> SEQUENCE: 52

Met Ser Gln Ser Glu Thr Phe Glu Lys Val Lys Lys Ile Val Ile Glu
1               5                   10                  15

Gln Leu Ser Val Glu Asn Pro Asp Thr Val Thr Pro Glu Ala Ser Phe
            20                  25                  30

Ala Asn Asp Leu Gln Ala Asp Ser Leu Asp Thr Val Glu Leu Val Met
        35                  40                  45

Ala Leu Glu Glu Glu Phe Asp Ile Glu Ile Pro Asp Glu Ala Ala Glu
    50                  55                  60

Lys Ile Thr Thr Val Gln Glu Ala Val Asp Tyr Ile Asn Asn Gln Val
65                  70                  75                  80

Ala Ala Ser Ala


<210> SEQ ID NO 53
<211> LENGTH: 675
<212> TYPE: DNA
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 53

atgaagattt acggaattta tatggaccgc ccgctttcac aggaagaaaa tgaacggttc     60

atgactttca tatcacctga aaaacgggag aaatgccgga gattttatca taaagaagat    120

gctcaccgca ccctgctggg agatgtgctc gttcgctcag tcataagcag gcagtatcag    180

ttggacaaat ccgatatccg ctttagcacg caggaatacg ggaagccgtg catccctgat    240

cttcccgacg ctcatttcaa catttctcac tccggccgct gggtcattgg tgcgtttgat    300
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tcacagccga tcggcataga tatcgaaaaa acgaaaccga tcagccttga gatcgccaag 360

cgcttctttt caaaaacaga gtacagcgac cttttagcaa aagacaagga cgagcagaca 420

gactattttt atcatctatg gtcaatgaaa gaaagcttta tcaaacagga aggcaaaggc 480

ttatcgcttc cgcttgattc cttttcagtg cgcctgcatc aggacggaca agtatccatt 540

gagcttccgg acagccattc cccatgctat atcaaaacgt atgaggtcga tcccggctac 600

aaaatggctg tatgcgccgc acaccctgat ttccccgagg atatcacaat ggtctcgtac 660

gaagagcttt tataa 675

<210> SEQ ID NO 54
<211> LENGTH: 224
<212> TYPE: PRT
<213> ORGANISM: Bacillus subtilis

<400> SEQUENCE: 54

Met Lys Ile Tyr Gly Ile Tyr Met Asp Arg Pro Leu Ser Gln Glu Glu
1 5 10 15

Asn Glu Arg Phe Met Thr Phe Ile Ser Pro Glu Lys Arg Glu Lys Cys
20 25 30

Arg Arg Phe Tyr His Lys Glu Asp Ala His Arg Thr Leu Leu Gly Asp
35 40 45

Val Leu Val Arg Ser Val Ile Ser Arg Gln Tyr Gln Leu Asp Lys Ser
50 55 60

Asp Ile Arg Phe Ser Thr Gln Glu Tyr Gly Lys Pro Cys Ile Pro Asp
65 70 75 80

Leu Pro Asp Ala His Phe Asn Ile Ser His Ser Gly Arg Trp Val Ile
85 90 95

Gly Ala Phe Asp Ser Gln Pro Ile Gly Ile Asp Ile Glu Lys Thr Lys
100 105 110

Pro Ile Ser Leu Glu Ile Ala Lys Arg Phe Phe Ser Lys Thr Glu Tyr
115 120 125

Ser Asp Leu Leu Ala Lys Asp Lys Asp Glu Gln Thr Asp Tyr Phe Tyr
130 135 140

His Leu Trp Ser Met Lys Glu Ser Phe Ile Lys Gln Glu Gly Lys Gly
145 150 155 160

Leu Ser Leu Pro Leu Asp Ser Phe Ser Val Arg Leu His Gln Asp Gly
165 170 175

Gln Val Ser Ile Glu Leu Pro Asp Ser His Ser Pro Cys Tyr Ile Lys
180 185 190

Thr Tyr Glu Val Asp Pro Gly Tyr Lys Met Ala Val Cys Ala Ala His
195 200 205

Pro Asp Phe Pro Glu Asp Ile Thr Met Val Ser Tyr Glu Glu Leu Leu
210 215 220

<210> SEQ ID NO 55
<211> LENGTH: 867
<212> TYPE: DNA
<213> ORGANISM: Corynebacterium glutamicum

<400> SEQUENCE: 55

ttgggcgtgt cgcccttaaa gcgcgctttt cgacgcgacc ccactacatt ggcttccatg 60

aacgttgaca tttcacgatc cagagagccg ctaaacgttg agctcctgaa ggaaaaattg 120

-continued

ctccaaaacg gtgactttgg ccaggtcatt tacgaaaaag tgacaggctc cactaatgct 180

gacttgctgg cacttgcagg ttctggcgct ccaaactgga cggtgaaaac tgtcgagttt 240

caagatcatg cgcgtgggcg actcggccgc ccgtggtctg cccctgaggg ttcccaaaca 300

atcgtgtctg tgctcgttca actatctatt gatcaagtgg accggattgg cactattcca 360

ctcgcggcgg gactcgctgt catggatgcg ttgaatgacc tcggtgtgga aggtgccgga 420

ctgaaatggc ccaacgatgt tcaaatccac ggcaagaaac tctgcggcat cctggtggaa 480

gccaccggct ttgattccac cccaacagtt gtcatcggtt ggggcactaa tatcagcctg 540

actaaagagg agcttcctgt tcctcatgca acttccctcg cattggaagg tgttgaagtc 600

gacagaacca cattccttat taatatgctc acacatctgc atactcgact ggaccagtgg 660

cagggtccaa gtgtggattg gctcgatgat taccgtgcgg tatgttccag tattggccaa 720

gatgttcgag tgcttctacc tggggataaa gaactcttag gtgaagcgat cggtgtcgcg 780

actggcggag aaattcgtgt tcgcgatgct tcgggcaccg ttcacaccct caacgccggt 840

gaaattacgc accttcgcct gcagtaa 867

<210> SEQ ID NO 56
<211> LENGTH: 810
<212> TYPE: DNA
<213> ORGANISM: Corynebacterium glutamicum

<400> SEQUENCE: 56

atgaatgttg acattagccg ctctcgtgaa ccgttgaacg tggaactgtt gaaagaaaaa 60

ctgctgcaga acggtgattt cggtcaagtg atctacgaga aggtcaccgg ctctaccaat 120

gcggacctgc tggctctggc gggcagcggc gctccaaact ggaccgtcaa gactgttgaa 180

tttcaggacc acgcccgtgg ccgtctgggt cgtccgtgga gcgcaccgga gggttcccaa 240

accatcgtca gcgttctggt ccaactgagc attgatcagg tggaccgtat tggtacgatc 300

ccgctggccg caggcttggc tgttatggat gcgctgaatg atctgggcgt ggagggtgca 360

ggcctgaaat ggccgaacga tgttcagatc cacggtaaga agttgtgcgg tattctggtt 420

gaagcaaccg gcttcgactc cactccgacc gtggttatcg gttggggtac gaatatctcg 480

ttgacgaaag aagagctgcc ggtcccgcac gcgaccagcc tggccctgga gggtgttgaa 540

gttgaccgta cgacgttcct gattaacatg ctgacccatc tgcatacccg tctggatcag 600

tggcagggtc cgtctgtgga ctggctggat gactatcgcg cggtttgtag cagcattggc 660

caagatgtgc gtgtcctgct gcctggtgac aaagagctgc tgggcgaggc gattggcgtg 720

gcgaccggtg gtgagatccg tgtgcgcgac gccagcggca cggtccacac gctgaatgcg 780

ggtgaaatca cgcatctgcg tttgcaataa 810

<210> SEQ ID NO 57
<211> LENGTH: 269
<212> TYPE: PRT
<213> ORGANISM: Corynebacterium glutamicum

<400> SEQUENCE: 57

Met Asn Val Asp Ile Ser Arg Ser Arg Glu Pro Leu Asn Val Glu Leu
1 5 10 15

Leu Lys Glu Lys Leu Leu Gln Asn Gly Asp Phe Gly Gln Val Ile Tyr
20 25 30

-continued

```
Glu Lys Val Thr Gly Ser Thr Asn Ala Asp Leu Leu Ala Leu Ala Gly
        35                  40                  45

Ser Gly Ala Pro Asn Trp Thr Val Lys Thr Val Glu Phe Gln Asp His
    50                  55                  60

Ala Arg Gly Arg Leu Gly Arg Pro Trp Ser Ala Pro Glu Gly Ser Gln
65                  70                  75                  80

Thr Ile Val Ser Val Leu Val Gln Leu Ser Ile Asp Gln Val Asp Arg
                85                  90                  95

Ile Gly Thr Ile Pro Leu Ala Ala Gly Leu Ala Val Met Asp Ala Leu
            100                 105                 110

Asn Asp Leu Gly Val Glu Gly Ala Gly Leu Lys Trp Pro Asn Asp Val
        115                 120                 125

Gln Ile His Gly Lys Lys Leu Cys Gly Ile Leu Val Glu Ala Thr Gly
    130                 135                 140

Phe Asp Ser Thr Pro Thr Val Val Ile Gly Trp Gly Thr Asn Ile Ser
145                 150                 155                 160

Leu Thr Lys Glu Glu Leu Pro Val Pro His Ala Thr Ser Leu Ala Leu
                165                 170                 175

Glu Gly Val Glu Val Asp Arg Thr Thr Phe Leu Ile Asn Met Leu Thr
            180                 185                 190

His Leu His Thr Arg Leu Asp Gln Trp Gln Gly Pro Ser Val Asp Trp
        195                 200                 205

Leu Asp Asp Tyr Arg Ala Val Cys Ser Ser Ile Gly Gln Asp Val Arg
    210                 215                 220

Val Leu Leu Pro Gly Asp Lys Glu Leu Leu Gly Glu Ala Ile Gly Val
225                 230                 235                 240

Ala Thr Gly Gly Glu Ile Arg Val Arg Asp Ala Ser Gly Thr Val His
                245                 250                 255

Thr Leu Asn Ala Gly Glu Ile Thr His Leu Arg Leu Gln
            260                 265
```

<210> SEQ ID NO 58
<211> LENGTH: 1632
<212> TYPE: DNA
<213> ORGANISM: Corynebacterium glutamicum

<400> SEQUENCE: 58

```
atgaccattt cctcaccttt gattgacgtc gccaaccttc cagacatcaa caccactgcc     60

ggcaagatcg ccgaccttaa ggctcgccgc gcggaagccc atttccccat gggtgaaaag    120

gcagtagaga aggtccacgc tgctggacgc ctcactgccc gtgagcgctt ggattactta    180

ctcgatgagg gctccttcat cgagaccgat cagctggctc gccaccgcac caccgctttc    240

ggcctgggcg ctaagcgtcc tgcaaccgac ggcatcgtga ccggctgggg caccattgat    300

ggacgcgaag tctgcatctt ctcgcaggac ggcaccgtat tcggtggcgc gcttggtgag    360

gtgtacggcg aaaagatgat caagatcatg gagctggcaa tcgacaccgg ccgcccattg    420

atcggtcttt acgaaggcgc tggcgctcgt attcaggacg gcgctgtctc cctggacttc    480

atttcccaga ccttctacca aaacattcag gcttctggcg ttatcccaca gatctccgtc    540

atcatgggcg catgtgcagg tggcaacgct tacggcccag ctctgaccga cttcgtggtc    600

atggtggaca agacctccaa gatgttcgtt accggcccag acgtgatcaa gaccgtcacc    660

ggcgaggaaa tcacccagga agagcttggc ggagcaacca cccacatggt gaccgctggt    720
```

-continued

```
aactcccact acaccgctgc gaccgatgag gaagcactgg attgggtaca ggacctggtg    780

tccttcctcc catccaacaa tcgctcctac gcaccgatgg aagacttcga cgaggaagaa    840

ggcggcgttg aagaaaacat caccgctgac gatctgaagc tcgacgagat catcccagat    900

tccgcgaccg ttccttacga cgtccgcgat gtcatcgaat gcctcaccga cgatggcgaa    960

tacctggaaa tccaggcaga ccgcgcagaa aacgttgtta ttgcattcgg ccgcatcgaa   1020

ggccagtccg ttggctttgt tgccaaccag ccaacccagt tcgctggctg cctggacatc   1080

gactcctctg agaaggcagc tcgcttcgtc cgcacctgcg acgcgttcaa catcccaatc   1140

gtcatgcttg tcgacgtccc cggcttcctc ccaggcgcag gccaggagta cggtggcatt   1200

ctgcgtcgtg gcgcaaagct gctctacgca tacggcgaag caaccgttcc aaagatcacc   1260

gtcaccatgc gtaaggctta cggcggagcg tactgcgtga tgggttccaa gggcttgggc   1320

tctgacatca accttgcatg gccaaccgca cagatcgccg tcatgggcgc tgctggcgca   1380

gttggattca tctaccgcaa ggagctcatg gcagctgatg ccaagggcct cgataccgta   1440

gctctggcta agtccttcga gcgcgagtat gaagaccaca tgctcaaccc gtaccacgct   1500

gcagaacgtg gcctgatcga cgccgtgatc ctgccaagcg aaacccgcgg acagatttcc   1560

cgcaaccttc gcctgctcaa gcacaagaac gtcactcgcc ctgctcgcaa gcacggcaac   1620

atgccactgt aa                                                       1632
```

<210> SEQ ID NO 59
<211> LENGTH: 543
<212> TYPE: PRT
<213> ORGANISM: Corynebacterium glutamicum

<400> SEQUENCE: 59

```
Met Thr Ile Ser Ser Pro Leu Ile Asp Val Ala Asn Leu Pro Asp Ile
1               5                   10                  15

Asn Thr Thr Ala Gly Lys Ile Ala Asp Leu Lys Ala Arg Arg Ala Glu
            20                  25                  30

Ala His Phe Pro Met Gly Glu Lys Ala Val Glu Lys Val His Ala Ala
        35                  40                  45

Gly Arg Leu Thr Ala Arg Glu Arg Leu Asp Tyr Leu Leu Asp Glu Gly
    50                  55                  60

Ser Phe Ile Glu Thr Asp Gln Leu Ala Arg His Arg Thr Thr Ala Phe
65                  70                  75                  80

Gly Leu Gly Ala Lys Arg Pro Ala Thr Asp Gly Ile Val Thr Gly Trp
                85                  90                  95

Gly Thr Ile Asp Gly Arg Glu Val Cys Ile Phe Ser Gln Asp Gly Thr
            100                 105                 110

Val Phe Gly Gly Ala Leu Gly Glu Val Tyr Gly Glu Lys Met Ile Lys
        115                 120                 125

Ile Met Glu Leu Ala Ile Asp Thr Gly Arg Pro Leu Ile Gly Leu Tyr
    130                 135                 140

Glu Gly Ala Gly Ala Arg Ile Gln Asp Gly Ala Val Ser Leu Asp Phe
145                 150                 155                 160

Ile Ser Gln Thr Phe Tyr Gln Asn Ile Gln Ala Ser Gly Val Ile Pro
                165                 170                 175

Gln Ile Ser Val Ile Met Gly Ala Cys Ala Gly Gly Asn Ala Tyr Gly
            180                 185                 190
```

-continued

```
Pro Ala Leu Thr Asp Phe Val Val Met Val Asp Lys Thr Ser Lys Met
        195                 200                 205

Phe Val Thr Gly Pro Asp Val Ile Lys Thr Val Thr Gly Glu Glu Ile
    210                 215                 220

Thr Gln Glu Glu Leu Gly Gly Ala Thr Thr His Met Val Thr Ala Gly
225                 230                 235                 240

Asn Ser His Tyr Thr Ala Ala Thr Asp Glu Glu Ala Leu Asp Trp Val
                245                 250                 255

Gln Asp Leu Val Ser Phe Leu Pro Ser Asn Asn Arg Ser Tyr Ala Pro
            260                 265                 270

Met Glu Asp Phe Asp Glu Glu Glu Gly Gly Val Glu Glu Asn Ile Thr
        275                 280                 285

Ala Asp Asp Leu Lys Leu Asp Glu Ile Ile Pro Asp Ser Ala Thr Val
    290                 295                 300

Pro Tyr Asp Val Arg Asp Val Ile Glu Cys Leu Thr Asp Asp Gly Glu
305                 310                 315                 320

Tyr Leu Glu Ile Gln Ala Asp Arg Ala Glu Asn Val Val Ile Ala Phe
                325                 330                 335

Gly Arg Ile Glu Gly Gln Ser Val Gly Phe Val Ala Asn Gln Pro Thr
            340                 345                 350

Gln Phe Ala Gly Cys Leu Asp Ile Asp Ser Ser Glu Lys Ala Ala Arg
        355                 360                 365

Phe Val Arg Thr Cys Asp Ala Phe Asn Ile Pro Ile Val Met Leu Val
    370                 375                 380

Asp Val Pro Gly Phe Leu Pro Gly Ala Gly Gln Glu Tyr Gly Gly Ile
385                 390                 395                 400

Leu Arg Arg Gly Ala Lys Leu Leu Tyr Ala Tyr Gly Glu Ala Thr Val
                405                 410                 415

Pro Lys Ile Thr Val Thr Met Arg Lys Ala Tyr Gly Gly Ala Tyr Cys
            420                 425                 430

Val Met Gly Ser Lys Gly Leu Gly Ser Asp Ile Asn Leu Ala Trp Pro
        435                 440                 445

Thr Ala Gln Ile Ala Val Met Gly Ala Ala Gly Ala Val Gly Phe Ile
    450                 455                 460

Tyr Arg Lys Glu Leu Met Ala Ala Asp Ala Lys Gly Leu Asp Thr Val
465                 470                 475                 480

Ala Leu Ala Lys Ser Phe Glu Arg Glu Tyr Glu Asp His Met Leu Asn
                485                 490                 495

Pro Tyr His Ala Ala Glu Arg Gly Leu Ile Asp Ala Val Ile Leu Pro
            500                 505                 510

Ser Glu Thr Arg Gly Gln Ile Ser Arg Asn Leu Arg Leu Leu Lys His
        515                 520                 525

Lys Asn Val Thr Arg Pro Ala Arg Lys His Gly Asn Met Pro Leu
    530                 535                 540


<210> SEQ ID NO 60
<211> LENGTH: 1776
<212> TYPE: DNA
<213> ORGANISM: Corynebacterium glutamicum

<400> SEQUENCE: 60

atgtcagtcg agactcgcaa gatcaccaag gttcttgtcg ctaaccgtgg tgagattgca     60
```

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```
atccgcgtgt tccgtgcagc tcgagatgaa ggcatcggat ctgtcgccgt ctacgcagag    120

ccagatgcag atgcaccatt cgtgtcatat gcagacgagg cttttgccct cggtggccaa    180

acatccgctg agtcctacct tgtcattgac aagatcatcg atgcggcccg caagtccggc    240

gccgacgcca tccaccccgg ctacggcttc ctcgcagaaa acgctgactt cgcagaagca    300

gtcatcaacg aaggcctgat ctggattgga ccttcacctg agtccatccg ctccctcggc    360

gacaaggtca ccgctcgcca catcgcagat accgccaagg ctccaatggc tcctggcacc    420

aaggaaccag taaaagacgc agcagaagtt gtggctttcg ctgaagaatt cggtctccca    480

atcgccatca aggcagcttt cggtggcggc ggacgtggca tgaaggttgc ctacaagatg    540

gaagaagtcg ctgacctctt cgagtccgca acccgtgaag caaccgcagc gttcggccgc    600

ggcgagtgct tcgtggagcg ctacctggac aaggcacgcc acgttgaggc tcaggtcatc    660

gccgataagc acggcaacgt tgttgtcgcc ggaacccgtg actgctccct gcagcgccgt    720

ttccagaagc tcgtcgaaga agcaccagca ccattcctca ccgatgacca gcgcgagcgt    780

ctccactcct ccgcgaaggc tatctgtaag gaagctggct actacggtgc aggcaccgtt    840

gagtacctcg ttggctccga cggcctgatc tccttcctcg aggtcaacac ccgcctccag    900

gtggaacacc cagtcaccga agagaccacc ggcatcgacc tggtccgcga aatgttccgc    960

atcgcagaag gccacgagct ctccatcaag gaagatccag ctccacgcgg ccacgcattc   1020

gagttccgca tcaacggcga agacgctggc tccaacttca tgcctgcacc aggcaagatc   1080

accagctacc gcgagccaca gggcccaggc gtccgcatgg actccggtgt cgttgaaggt   1140

tccgaaatct ccggacagtt cgactccatg ctggcaaagc tgatcgtttg gggcgacacc   1200

cgcgagcagg ctctccagcg ctcccgccgt gcacttgcag agtacgttgt cgagggcatg   1260

ccaaccgtta tcccattcca ccagcacatc gtggaaaacc cagcattcgt gggcaacgac   1320

gaaggcttcg agatctacac caagtggatc gaagaggttt gggataaccc aatcgcacct   1380

tacgttgacg cttccgagct cgacgaagat gaggacaaga ccccagcaca gaaggttgtt   1440

gtggagatca acggccgtcg cgttgaggtt gcactcccag gcgatctggc actcggtggc   1500

accgctggtc ctaagaagaa ggccaagaag cgtcgcgcag gtggtgcaaa ggctggcgta   1560

tccggcgatg cagtggcagc tccaatgcag ggcactgtca tcaaggtcaa cgtcgaagaa   1620

ggcgctgaag tcaacgaagg cgacaccgtt gttgtcctcg aggctatgaa gatggaaaac   1680

cctgtgaagg ctcataagtc cggaaccgta accggcctta ctgtcgctgc aggcgagggt   1740

gtcaacaagg gcgttgttct cctcgagatc aagtaa                              1776
```

<210> SEQ ID NO 61
<211> LENGTH: 591
<212> TYPE: PRT
<213> ORGANISM: Corynebacterium glutamicum

<400> SEQUENCE: 61

```
Met Ser Val Glu Thr Arg Lys Ile Thr Lys Val Leu Val Ala Asn Arg
1               5                   10                  15

Gly Glu Ile Ala Ile Arg Val Phe Arg Ala Ala Arg Asp Glu Gly Ile
            20                  25                  30

Gly Ser Val Ala Val Tyr Ala Glu Pro Asp Ala Asp Ala Pro Phe Val
        35                  40                  45
```

-continued

```
Ser Tyr Ala Asp Glu Ala Phe Ala Leu Gly Gly Gln Thr Ser Ala Glu
    50                  55                  60

Ser Tyr Leu Val Ile Asp Lys Ile Ile Asp Ala Ala Arg Lys Ser Gly
65                  70                  75                  80

Ala Asp Ala Ile His Pro Gly Tyr Gly Phe Leu Ala Glu Asn Ala Asp
                85                  90                  95

Phe Ala Glu Ala Val Ile Asn Glu Gly Leu Ile Trp Ile Gly Pro Ser
            100                 105                 110

Pro Glu Ser Ile Arg Ser Leu Gly Asp Lys Val Thr Ala Arg His Ile
        115                 120                 125

Ala Asp Thr Ala Lys Ala Pro Met Ala Pro Gly Thr Lys Glu Pro Val
    130                 135                 140

Lys Asp Ala Ala Glu Val Val Ala Phe Ala Glu Glu Phe Gly Leu Pro
145                 150                 155                 160

Ile Ala Ile Lys Ala Ala Phe Gly Gly Gly Gly Arg Gly Met Lys Val
                165                 170                 175

Ala Tyr Lys Met Glu Glu Val Ala Asp Leu Phe Glu Ser Ala Thr Arg
            180                 185                 190

Glu Ala Thr Ala Ala Phe Gly Arg Gly Glu Cys Phe Val Glu Arg Tyr
        195                 200                 205

Leu Asp Lys Ala Arg His Val Glu Ala Gln Val Ile Ala Asp Lys His
    210                 215                 220

Gly Asn Val Val Val Ala Gly Thr Arg Asp Cys Ser Leu Gln Arg Arg
225                 230                 235                 240

Phe Gln Lys Leu Val Glu Glu Ala Pro Ala Pro Phe Leu Thr Asp Asp
                245                 250                 255

Gln Arg Glu Arg Leu His Ser Ser Ala Lys Ala Ile Cys Lys Glu Ala
            260                 265                 270

Gly Tyr Tyr Gly Ala Gly Thr Val Glu Tyr Leu Val Gly Ser Asp Gly
        275                 280                 285

Leu Ile Ser Phe Leu Glu Val Asn Thr Arg Leu Gln Val Glu His Pro
    290                 295                 300

Val Thr Glu Glu Thr Thr Gly Ile Asp Leu Val Arg Glu Met Phe Arg
305                 310                 315                 320

Ile Ala Glu Gly His Glu Leu Ser Ile Lys Glu Asp Pro Ala Pro Arg
                325                 330                 335

Gly His Ala Phe Glu Phe Arg Ile Asn Gly Glu Asp Ala Gly Ser Asn
            340                 345                 350

Phe Met Pro Ala Pro Gly Lys Ile Thr Ser Tyr Arg Glu Pro Gln Gly
        355                 360                 365

Pro Gly Val Arg Met Asp Ser Gly Val Val Glu Gly Ser Glu Ile Ser
    370                 375                 380

Gly Gln Phe Asp Ser Met Leu Ala Lys Leu Ile Val Trp Gly Asp Thr
385                 390                 395                 400

Arg Glu Gln Ala Leu Gln Arg Ser Arg Arg Ala Leu Ala Glu Tyr Val
                405                 410                 415

Val Glu Gly Met Pro Thr Val Ile Pro Phe His Gln His Ile Val Glu
            420                 425                 430

Asn Pro Ala Phe Val Gly Asn Asp Glu Gly Phe Glu Ile Tyr Thr Lys
        435                 440                 445

Trp Ile Glu Glu Val Trp Asp Asn Pro Ile Ala Pro Tyr Val Asp Ala
```

-continued

```
    450                 455                 460

Ser Glu Leu Asp Glu Asp Glu Asp Lys Thr Pro Ala Gln Lys Val Val
465                 470                 475                 480

Val Glu Ile Asn Gly Arg Arg Val Glu Val Ala Leu Pro Gly Asp Leu
                485                 490                 495

Ala Leu Gly Gly Thr Ala Gly Pro Lys Lys Lys Ala Lys Lys Arg Arg
            500                 505                 510

Ala Gly Gly Ala Lys Ala Gly Val Ser Gly Asp Ala Val Ala Ala Pro
        515                 520                 525

Met Gln Gly Thr Val Ile Lys Val Asn Val Glu Glu Gly Ala Glu Val
    530                 535                 540

Asn Glu Gly Asp Thr Val Val Val Leu Glu Ala Met Lys Met Glu Asn
545                 550                 555                 560

Pro Val Lys Ala His Lys Ser Gly Thr Val Thr Gly Leu Thr Val Ala
                565                 570                 575

Ala Gly Glu Gly Val Asn Lys Gly Val Val Leu Leu Glu Ile Lys
            580                 585                 590


<210> SEQ ID NO 62
<211> LENGTH: 10025
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<221> NAME/KEY: source
<223> OTHER INFORMATION: /note="Description of Artificial Sequence:
      Synthetic polynucleotide"

<400> SEQUENCE: 62

cactatacca attgagatgg gctagtcaat gataattact agtccttttc ctttgagttg     60

tgggtatctg taaattctgc tagacctttg ctggaaaact tgtaaattct gctagaccct    120

ctgtaaattc cgctagacct ttgtgtgttt tttttgttta tattcaagtg gttataattt    180

atagaataaa gaaagaataa aaaaagataa aaagaataga tcccagccct gtgtataact    240

cactacttta gtcagttccg cagtattaca aaaggatgtc gcaaacgctg tttgctcctc    300

tacaaaacag accttaaaac cctaaaggcg tcggcatccg cttacagaca agctgtgacc    360

gtctccggga gctgcatgtg tcagaggttt tcaccgtcat caccgaaacg cgcgaggcag    420

cagatcaatt cgcgcgcgaa ggcgaagcgg catgcattta cgttgacacc atcgaatggt    480

gcaaaacctt tcgcggtatg gcatgatagc gcccggaaga gagtcaattc agggtggtga    540

atgtgaaacc agtaacgtta tacgatgtcg cagagtatgc cggtgtctct tatcagaccg    600

tttcccgcgt ggtgaaccag gccagccacg tttctgcgaa aacgcgggaa aaagtggaag    660

cggcgatggc ggagctgaat tacattccca accgcgtggc acaacaactg gcgggcaaac    720

agtcgttgct gattggcgtt gccacctcca gtctggccct gcacgcgccg tcgcaaattg    780

tcgcggcgat taaatctcgc gccgatcaac tgggtgccag cgtggtggtg tcgatggtag    840

aacgaagcgg cgtcgaagcc tgtaaagcgg cggtgcacaa tcttctcgcg caacgcgtca    900

gtgggctgat cattaactat ccgctggatg accaggatgc cattgctgtg gaagctgcct    960

gcactaatgt tccggcgtta tttcttgatg tctctgacca gacacccatc aacagtatta   1020

ttttctccca tgaagacggt acgcgactgg gcgtggagca tctggtcgca ttgggtcacc   1080

agcaaatcgc gctgttagcg ggcccattaa gttctgtctc ggcgcgtctg cgtctggctg   1140
```

-continued

gctggcataa atatctcact cgcaatcaaa ttcagccgat agcggaacgg gaaggcgact 1200

ggagtgccat gtccggtttt caacaaacca tgcaaatgct gaatgagggc atcgttccca 1260

ctgcgatgct ggttgccaac gatcagatgg cgctgggcgc aatgcgcgcc attaccgagt 1320

ccgggctgcg cgttggtgcg gatatctcgg tagtgggata cgacgatacc gaagacagct 1380

catgttatat cccgccgtta accaccatca aacaggattt tcgcctgctg gggcaaacca 1440

gcgtggaccg cttgctgcaa ctctctcagg gccaggcggt gaagggcaat cagctgttgc 1500

ccgtctcact ggtgaaaaga aaaaccaccc tggcgcccaa tacgcaaacc gcctctcccc 1560

gcgcgttggc cgattcatta atgcagctgg cacgacaggt ttcccgactg gaaagcgggc 1620

agtgagcgca acgcaattaa tgtaagttag cgcgaattga tctggtttga cagcttatca 1680

tcgactgcac ggtgcaccaa tgcttctggc gtcaggcagc catcggaagc tgtggtatgg 1740

ctgtgcaggt cgtaaatcac tgcataattc gtgtcgctca aggcgcactc ccgttctgga 1800

taatgttttt tgcgccgaca tcataacggt tctggcaaat attctgttga caattaatca 1860

tccggctcgt ataaagtgtg gaattgtgag cggataacaa tttcacacag gaaacagcgc 1920

cgctgagaaa aagcgaagcg gcactgctct ttaacaattt atcagacaat ctgtgtgggc 1980

actcgaccgg aattatcgat taactttatt attaaaaatt aaagaggtat atattaatgt 2040

atcgattaaa taaggaggaa taaaccatga ccatttcctc acctttgatt gacgtcgcca 2100

accttccaga catcaacacc actgccggca agatcgccga ccttaaggct cgccgcgcgg 2160

aagcccattt ccccatgggt gaaaaggcag tagagaaggt ccacgctgct ggacgcctca 2220

ctgcccgtga gcgcttggat tacttactcg atgagggctc cttcatcgag accgatcagc 2280

tggctcgcca ccgcaccacc gctttcggcc tgggcgctaa gcgtcctgca accgacggca 2340

tcgtgaccgg ctggggcacc attgatggac gcgaagtctg catcttctcg caggacggca 2400

ccgtattcgg tggcgcgctt ggtgaggtgt acggcgaaaa gatgatcaag atcatggagc 2460

tggcaatcga caccggccgc ccattgatcg gtctttacga aggcgctggc gctcgtattc 2520

aggacggcgc tgtctccctg gacttcattt cccagacctt ctaccaaaac attcaggctt 2580

ctggcgttat cccacagatc tccgtcatca tgggcgcatg tgcaggtggc aacgcttacg 2640

gcccagctct gaccgacttc gtggtcatgg tggacaagac ctccaagatg ttcgttaccg 2700

gcccagacgt gatcaagacc gtcaccggcg aggaaatcac ccaggaagag cttggcggag 2760

caaccaccca catggtgacc gctggtaact cccactacac cgctgcgacc gatgaggaag 2820

cactggattg ggtacaggac ctggtgtcct tcctcccatc caacaatcgc tcctacgcac 2880

cgatggaaga cttcgacgag gaagaaggcg gcgttgaaga aaacatcacc gctgacgatc 2940

tgaagctcga cgagatcatc ccagattccg cgaccgttcc ttacgacgtc cgcgatgtca 3000

tcgaatgcct caccgacgat ggcgaatacc tggaaatcca ggcagaccgc gcagaaaacg 3060

ttgttattgc attcggccgc atcgaaggcc agtccgttgg ctttgttgcc aaccagccaa 3120

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        35                  40                  45

Ala His Lys Pro Gly Leu Arg Leu Ala Glu Ile Leu Gln Thr Leu Phe
    50                  55                  60

Thr Gly Tyr Gly Asp Arg Pro Ala Leu Gly Tyr Arg Ala Arg Glu Leu
65                  70                  75                  80

Ala Thr Asp Glu Gly Gly Arg Thr Val Thr Arg Leu Leu Pro Arg Phe
                85                  90                  95

Asp Thr Leu Thr Tyr Ala Gln Val Trp Ser Arg Val Gln Ala Val Ala
            100                 105                 110

Ala Ala Leu Arg His Asn Phe Ala Gln Pro Ile Tyr Pro Gly Asp Ala
        115                 120                 125

Val Ala Thr Ile Gly Phe Ala Ser Pro Asp Tyr Leu Thr Leu Asp Leu
    130                 135                 140

Val Cys Ala Tyr Leu Gly Leu Val Ser Val Pro Leu Gln His Asn Ala
145                 150                 155                 160

Pro Val Ser Arg Leu Ala Pro Ile Leu Ala Glu Val Glu Pro Arg Ile
                165                 170                 175

Leu Thr Val Ser Ala Glu Tyr Leu Asp Leu Ala Val Glu Ser Val Arg
            180                 185                 190

Asp Val Asn Ser Val Ser Gln Leu Val Val Phe Asp His His Pro Glu
        195                 200                 205

Val Asp Asp His Arg Asp Ala Leu Ala Arg Ala Arg Glu Gln Leu Ala
    210                 215                 220

Gly Lys Gly Ile Ala Val Thr Thr Leu Asp Ala Ile Ala Asp Glu Gly
225                 230                 235                 240

Ala Gly Leu Pro Ala Glu Pro Ile Tyr Thr Ala Asp His Asp Gln Arg
                245                 250                 255

Leu Ala Met Ile Leu Tyr Thr Ser Gly Ser Thr Gly Ala Pro Lys Gly
            260                 265                 270

Ala Met Tyr Thr Glu Ala Met Val Ala Arg Leu Trp Thr Met Ser Phe
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Asn His Leu Gly Gly Arg Ile Pro Ile Ser Thr Ala Val Gln Asn Gly
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385                 390                 395                 400

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Gly Ala His Ile Val Asp Gly Tyr Gly Leu Thr Glu Thr Gly Ala Val
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Ile Asp Val Pro Glu Leu Gly Tyr Phe Ser Thr Asp Lys Pro Tyr Pro
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Arg Gly Glu Leu Leu Val Arg Ser Gln Thr Leu Thr Pro Gly Tyr Tyr
465                 470                 475                 480

Lys Arg Pro Glu Val Thr Ala Ser Val Phe Asp Arg Asp Gly Tyr Tyr
                485                 490                 495

His Thr Gly Asp Val Met Ala Glu Thr Ala Pro Asp His Leu Val Tyr
            500                 505                 510

Val Asp Arg Arg Asn Asn Val Leu Lys Leu Ala Gln Gly Glu Phe Val
        515                 520                 525

Ala Val Ala Asn Leu Glu Ala Val Phe Ser Gly Ala Ala Leu Val Arg
    530                 535                 540

Gln Ile Phe Val Tyr Gly Asn Ser Glu Arg Ser Phe Leu Leu Ala Val
545                 550                 555                 560

Val Val Pro Thr Pro Glu Ala Leu Glu Gln Tyr Asp Pro Ala Ala Leu
                565                 570                 575

Lys Ala Ala Leu Ala Asp Ser Leu Gln Arg Thr Ala Arg Asp Ala Glu
            580                 585                 590

Leu Gln Ser Tyr Glu Val Pro Ala Asp Phe Ile Val Glu Thr Glu Pro
        595                 600                 605

Phe Ser Ala Ala Asn Gly Leu Leu Ser Gly Val Gly Lys Leu Leu Arg
    610                 615                 620

Pro Asn Leu Lys Asp Arg Tyr Gly Gln Arg Leu Glu Gln Met Tyr Ala
625                 630                 635                 640

Asp Ile Ala Ala Thr Gln Ala Asn Gln Leu Arg Glu Leu Arg Arg Ala
                645                 650                 655

Ala Ala Thr Gln Pro Val Ile Asp Thr Leu Thr Gln Ala Ala Ala Thr
            660                 665                 670

Ile Leu Gly Thr Gly Ser Glu Val Ala Ser Asp Ala His Phe Thr Asp
        675                 680                 685

Leu Gly Gly Asp Ser Leu Ser Ala Leu Thr Leu Ser Asn Leu Leu Ser
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               725                 730                 735

Gly Asp Arg Arg Pro Ser Phe Thr Thr Val His Gly Ala Asp Ala Thr
            740                 745                 750

Glu Ile Arg Ala Ser Glu Leu Thr Leu Asp Lys Phe Ile Asp Ala Glu
        755                 760                 765

Thr Leu Arg Ala Ala Pro Gly Leu Pro Lys Val Thr Thr Glu Pro Arg
    770                 775                 780

Thr Val Leu Leu Ser Gly Ala Asn Gly Trp Leu Gly Arg Phe Leu Thr
785                 790                 795                 800

Leu Gln Trp Leu Glu Arg Leu Ala Pro Val Gly Gly Thr Leu Ile Thr
                805                 810                 815

Ile Val Arg Gly Arg Asp Asp Ala Ala Ala Arg Ala Arg Leu Thr Gln
            820                 825                 830

Ala Tyr Asp Thr Asp Pro Glu Leu Ser Arg Arg Phe Ala Glu Leu Ala
        835                 840                 845

Asp Arg His Leu Arg Val Val Ala Gly Asp Ile Gly Asp Pro Asn Leu
    850                 855                 860

Gly Leu Thr Pro Glu Ile Trp His Arg Leu Ala Ala Glu Val Asp Leu
865                 870                 875                 880

Val Val His Pro Ala Ala Leu Val Asn His Val Leu Pro Tyr Arg Gln
                885                 890                 895

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            900                 905                 910

Leu Thr Glu Arg Ile Lys Pro Val Thr Tyr Leu Ser Thr Val Ser Val
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    1040                1045                1050

Glu Gly  Tyr Val Ser Tyr Asp  Val Met Asn Pro His  Asp Asp Gly
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Ile Ser  Leu Asp Val Phe Val  Asp Trp Leu Ile Arg  Ala Gly His
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Leu Ile  Asp Lys Tyr Ile Arg  Asp Leu Arg Glu Phe  Gly Leu Ile
    1160                1165                1170
```

What is claimed is:

1. A cultured genetically engineered host cell comprising:

(a) a polynucleotide sequence encoding one or more of: (i) an acetyl-CoA carboxylase (EC 6.4.1.2) polypeptide, (ii) a FadR polypeptide, (iii) a heterologous iFAB polypeptide, (iv) a sequence having a transposon insertion in the yijP gene, and (v) a heterologous ACP protein; and

(b) a polynucleotide sequence encoding a fatty acid derivative biosynthetic polypeptide, wherein the genetically engineered host cell produces a fatty acid derivative composition at a higher titer, yield or productivity when cultured in medium containing a carbon source under conditions effective to overexpress the polynucleotide (s) relative to a corresponding wild type host cell propagated under the same conditions as the genetically engineered host cell.

2. The genetically engineered host cell of claim **1**, wherein the fatty acid derivative composition comprises a fatty acid derivative selected from the group consisting of a fatty acid, a fatty aldehyde, a fatty alcohol, a fatty ester, an alkane, a terminal olefin, an internal olefin and a ketone.

3. The genetically engineered host cell of claim **1** or **2**, wherein the fatty acid derivative composition is produced at a titer that is at least 3 times greater, at least 5 times greater, at least 8 times greater, or at least 10 times greater than the titer of a fatty acid derivative composition produced by a corresponding wild type host cell cultured under the same conditions as the genetically engineered host cell.

4. The genetically engineered host cell of claim **1** or **2**, wherein the fatty acid derivative composition is produced at a titer of at least 100 mg/L.

5. The genetically engineered host cell of claim **1** or **2**, wherein the fatty acid derivative composition is produced at a titer of from 30 g/L to 250 g/L.

6. The genetically engineered host cell of claim **1** or **2**, wherein the fatty acid derivative composition is produced at a yield that is at least 3 times greater, at least 5 times greater, at least 8 times greater, or at least 10 times greater than the yield of a fatty acid derivative composition produced by a corresponding wild type host cell cultured under the same conditions as the genetically engineered host cell.

7. The genetically engineered host cell of claim **1** or **2**, wherein the fatty acid derivative composition has a yield of from 10% to 40%.

8. The genetically engineered host cell of claim **1** or **2**, wherein the fatty acid derivative composition is produced at a productivity that is at least 3 times greater, at least 5 times greater, at least 8 times greater, or at least 10 times greater than the productivity of a fatty acid derivative composition produced by a corresponding wild type host cell cultured under the same conditions as the genetically engineered host cell.

9. The genetically engineered host cell of claim **1** or **2**, wherein the fatty acid derivative composition is produced at a productivity of from 0.7 mg/L/hr to 3 g/L/hr.

10. The genetically engineered host cell of claim **1** or **2**, wherein the acetyl-CoA carboxylase (EC 6.4.1.2) polypeptide is overexpressed.

11. The genetically engineered host cell of claim **10**, wherein the acetyl-CoA carboxylase (EC 6.4.1.2) polypeptide is accD+.

12. The genetically engineered host cell of any one of claims **1** to **11**, wherein the FadR polypeptide is overexpressed.

13. The genetically engineered host cell of any one of claims **1** to **12**, wherein the heterologous iFAB polypeptide is overexpressed.

14. The genetically engineered host cell of claim **13**, wherein the heterologous iFAB polypeptide is iFAB 138.

15. The genetically engineered host cell of any one of claims **1** to **14**, wherein the host cell comprises a transposon insertion in the yijP gene.

16. The genetically engineered host cell of any one of claims **1** to **15**, wherein the host cell comprises a heterologous acp sequence.

17. The genetically engineered host cell of claim **16**, further comprising an sfp gene.

18. The genetically engineered host cell of any one of claims **1** to **17**, wherein the polynucleotide sequence encoding a fatty acid derivative biosynthetic polypeptide is selected from the group consisting of a polypeptide:

(a) having thioesterase activity, wherein the recombinant host cell synthesizes fatty acids;

(b) having thioesterase activity and carboxylic acid reductase ("CAR") activity, wherein the recombinant host cell synthesizes fatty aldehydes and fatty alcohols;

(c) having thioesterase activity, carboxylic acid reductase activity and alcohol dehydrogenase activity wherein the recombinant host cell synthesizes fatty alcohols;

(d) having acyl-CoA reductase ("AAR") activity wherein the recombinant host cell synthesizes fatty aldehydes and fatty alcohols;

(e) having acyl-CoA reductase ("AAR") activity and alcohol dehydrogenase activity wherein the recombinant host cell synthesizes fatty alcohols;

(f) having fatty alcohol forming acyl-CoA reductase ("FAR") activity, wherein the recombinant host cell synthesizes fatty alcohols;

(g) having thioesterase activity, carboxylic acid reductase activity and aldehyde decarbonylase activity, wherein the recombinant host cell synthesizes alkanes;

(h) having acyl-CoA reductase ("AAR") activity and aldehyde decarbonylase activity, wherein the recombinant host cell synthesizes alkanes;

(i) having ester synthase activity wherein the recombinant host cell synthesizes fatty esters;

(j) having thioesterase activity, acyl-CoA synthase activity and ester synthase activity wherein the recombinant host cell synthesizes fatty esters;

(k) having OleA activity, wherein the recombinant host cell synthesizes aliphatic ketones;

(l) having OleABCD activity, wherein the recombinant host cell synthesizes internal olefins; and

(m) having thioesterase activity and decarboxylase activity, wherein the recombinant host cell synthesizes terminal olefins.

19. The genetically engineered host cell of any one of claims **1** to **16**, wherein the fatty acid derivative composition is produced extracellularly.

20. A cell culture comprising the genetically engineered host cell of any one of claims **1** to **19**.

21. The cell culture of claim **20**, wherein the culture medium comprises a fatty acid derivative composition.

22. The cell culture of claim **20**, wherein the fatty acid derivative composition comprises at least one fatty acid derivative selected from the group consisting of a fatty acid, a fatty aldehyde, a fatty alcohol, a fatty ester, an alkane, a terminal olefin, an internal olefin and a ketone.

23. The cell culture of claim **20**, wherein the fatty acid derivative is a C6, C8, C10, C12, C13, C14, C15, C16, C17, or C18 fatty acid derivative.

24. The cell culture of claim **20**, wherein the fatty acid derivative is a C10:1, C12:1, C14:1, C16:1, or C18:1 unsaturated fatty acid derivative.

25. The cell culture of claim **20**, wherein the fatty acid derivative composition comprises one or more of C_8, C_{10}, C_{12}, C_{14}, C_{16}, and C_{18} fatty acid derivatives.

26. The cell culture of claim **22**, wherein the fatty acid derivative composition comprises fatty acids.

27. The cell culture of claim **22**, wherein the fatty acid derivative composition comprises fatty aldehydes.

28. The cell culture of claim **22**, wherein the fatty acid derivative composition comprises fatty alcohols.

29. The cell culture of claim **22**, wherein the fatty acid derivative composition comprises fatty esters.

30. The cell culture of claim **22**, wherein the fatty acid derivative composition comprises an alkane.

31. The cell culture of claim **22**, wherein the fatty acid derivative composition comprises a terminal olefin.

32. The cell culture of claim **22**, wherein the fatty acid derivative composition comprises an internal olefin.

33. The cell culture of claim **22**, wherein the fatty acid derivative composition comprises a ketone.

34. The cell culture of claim **20**, wherein the fatty acid derivative composition comprises a fatty acid derivative having a double bond at position 7 in the carbon chain (between C_7 and C_8) from the reduced end of the fatty alcohol.

35. The cell culture of claim **20**, wherein the fatty acid derivative composition comprises unsaturated fatty acid derivatives.

36. The cell culture of claim **20**, wherein the fatty acid derivative composition comprises saturated fatty acid derivatives.

37. The cell culture of claim **20**, wherein the fatty acid derivative composition comprises branched chain fatty acid derivatives.

38. The cell culture of claim **20**, wherein the fatty acid derivative has a fraction of modern carbon of about 1.003 to about 1.5.

39. The cell culture of claim **20**, wherein the fatty acid derivative has a $\delta^{13}C$ of from about −10.9 to about −15.4.

40. A cultured recombinant host cell, engineered to increase the production of malonyl CoA, comprising:

a polynucleotide sequence encoding one or more of: (i) an acetyl-CoA carboxylase (EC 6.4.1.2) polypeptide, (ii) a FadR polypeptide, (iii) a heterologous iFAB polypeptide, or (iv) a sequence having a transposon insertion in the yijP gene, wherein the engineered host cells produces a fatty acid derivative composition at a higher titer, yield or productivity when cultured in medium containing a carbon source under conditions effective to overexpress the polynucleotide(s) relative to a corresponding wild type host cell propagated under the same conditions as the genetically engineered host cell.

41. The cultured recombinant host cell of claim **40**, wherein the host cell further comprises a polynucleotide sequence encoding a fatty acid derivative biosynthetic polypeptide.

42. The cultured recombinant host cell of claim **40**, wherein the fatty acid derivative composition produced by the cultured genetically engineered host cell has a titer that is at least 3 times greater, at least 5 times greater, at least 8 times greater, or at least 10 times greater than the titer of a fatty acid derivative composition produced by a corresponding wild type host cell cultured under the same conditions as the genetically engineered host cell.

43. The cultured recombinant host cell of claim **40**, wherein the host cell has a titer of at least 100 mg/L.

44. The cultured recombinant host cell of claim **40**, wherein the fatty acid derivative composition produced by the cultured genetically engineered host cell has a titer of from 30 g/L to 250 g/L.

45. The cultured recombinant host cell of claim **40**, wherein the fatty acid derivative composition produced by a cultured genetically engineered host cell has a yield that is at least 3 times greater, at least 5 times greater, at least 8 times greater, or at least 10 times greater than the yield of a fatty acid derivative composition produced by a corresponding wild type host cell cultured under the same conditions as the genetically engineered host cell.

46. The cultured recombinant host cell of claim **40**, wherein the fatty acid derivative composition produced by the cultured genetically engineered host cell has a yield of from 10% to 40%.

47. The cultured recombinant host cell of claim **40**, wherein the fatty acid derivative composition produced by a cultured genetically engineered host cell has a productivity that is at least 3 times greater, at least 5 times greater, at least 8 times greater, or at least 10 times greater than the productivity of a fatty acid derivative composition produced by a corresponding wild type host cell cultured under the same conditions as the genetically engineered host cell.

48. The cultured recombinant host cell of claim **40**, wherein the fatty acid derivative composition produced by a cultured genetically engineered host cell has a productivity of from 0.7 mg/L/hr to 3 g/L/hr.

49. The cultured recombinant host cell of any one of claims **40** to **49**, wherein the acetyl-CoA carboxylase (EC 6.4.1.2) polypeptide is overexpressed.

50. The cultured recombinant host cell of claim **49**, wherein the acetyl-CoA carboxylase (EC 6.4.1.2) polypeptide is accD+.

51. The cultured recombinant host cell of any one of claims **40** to **50**, wherein the FadR polypeptide is overexpressed.

52. The cultured recombinant host cell of any one of claims **40** to **51**, wherein the heterologous iFAB polypeptide is overexpressed.

53. The cultured recombinant host cell of claim **52** wherein the heterologous iFAB polypeptide is iFAB 138.

54. The cultured recombinant host cell of any one of claims **40** to **53**, wherein the host cell comprises a transposon insertion in the yijP gene.

55. A cell culture comprising the cultured recombinant host cell of any one of claims **40** to **54**

56. A method of making a fatty acid derivative composition, comprising the steps of:

(a) engineering a parental host cell to obtain a recombinant host cell which comprises an acetyl-CoA carboxylase (EC 6.4.1.2) polypeptide, (ii) a FadR polypeptide, (iii) a heterologous iFAB polypeptide, and (iv) a sequence having a transposon insertion in the yijP gene;

(b) further engineering the cell to comprise polynucleotide sequence encoding a fatty acid derivative biosynthetic polypeptide;

(c) culturing the recombinant host cell in the presence of a carbon source under conditions effective to result in a yield, titer or productivity of the fatty acid derivative composition that is at least 3 times the yield, titer or productivity of fatty acid derivative composition produced by the parental microbial cell cultured under the same conditions; and

(d) optionally isolating the fatty acid derivative composition.

57. The method of claim **56**, wherein the host cell is further engineered to comprise a polynucleotide sequence encoding a heterologous acp protein.

58. The method of claim **57**, wherein the host cell is further engineered to comprise an sfp gene.

59. The method of claim **56**, wherein the fatty acid derivative biosynthetic polypeptide is selected from the group consisting of a polypeptide:

(a) having thioesterase activity, wherein the recombinant host cell synthesizes fatty acids;

(b) having thioesterase activity and carboxylic acid reductase ("CAR") activity, wherein the recombinant host cell synthesizes fatty aldehydes and fatty alcohols;

(c) having thioesterase activity, carboxylic acid reductase activity and alcohol dehydrogenase activity wherein the recombinant host cell synthesizes fatty alcohols;

(d) having acyl-CoA reductase ("AAR") activity wherein the recombinant host cell synthesizes fatty aldehydes and fatty alcohols;

(e) having acyl-CoA reductase ("AAR") activity and alcohol dehydrogenase activity wherein the recombinant host cell synthesizes fatty alcohols;

(f) having fatty alcohol forming acyl-CoA reductase ("FAR") activity, wherein the recombinant host cell synthesizes fatty alcohols;

(g) having thioesterase activity, carboxylic acid reductase activity and aldehyde decarbonylase activity, wherein the recombinant host cell synthesizes alkanes;

(h) having acyl-CoA reductase ("AAR") activity and aldehyde decarbonylase activity, wherein the recombinant host cell synthesizes alkanes;

(i) having ester synthase activity wherein the recombinant host cell synthesizes fatty esters;

(j) having thioesterase activity, acyl-CoA synthase activity and ester synthase activity wherein the recombinant host cell synthesizes fatty esters;

(k) having OleA activity, wherein the recombinant host cell synthesizes aliphatic ketones;

(l) having OleABCD activity, wherein the recombinant host cell synthesizes internal olefins; and

(m) having thioesterase activity and decarboxylase activity, wherein the recombinant host cell synthesizes terminal olefins.

60. The method of claim **56**, where in the fatty acid derivative is selected from the group consisting of a fatty acid, a fatty alcohol, a fatty aldehyde, a fatty acid ester, a hydrocarbon, a ketone, and an olefin.

61. The method of claim **56**, where in the fatty acid derivative is a C6, C8, C10, C12, C13, C14, C15, C16, C17, or C18 fatty acid derivative.

62. The method of claim **56**, where in the fatty acid derivative is a C10:1, C12:1, C14:1, C16:1, or C18:1 unsaturated fatty acid derivative.

63. A method of making a fatty acid derivative composition with a higher titer, yield or productivity of fatty acid derivatives than produced by a parental host cell, the method comprising:

(a) engineering a parental host cell to obtain a recombinant host cell which comprises one or more of: (i) a polynucleotide encoding an acetyl-CoA carboxylase (EC 6.4.1.2) polypeptide, (ii) a polynucleotide encoding a FadR polypeptide, (iii) a polynucleotide encoding a heterologous iFAB polypeptide, (iv) a sequence having a transposon insertion in the yijP gene, and (v) a polynucleotide encoding a heterologous ACP protein;

(b) further engineering the cell to comprise a polynucleotide sequence encoding a fatty acid derivative biosynthetic polypeptide;

(c) culturing the recombinant host cell in the presence of a carbon source under conditions effective to result in a yield, titer or productivity of fatty acid derivatives that is at least 3 times the yield, titer or productivity of fatty acid derivatives produced by the parental microbial cell cultured under the same conditions; and

(d) optionally isolating the fatty acid derivative composition.

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