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(54) Title: BIOPRODUCTION OF CHEMICALS

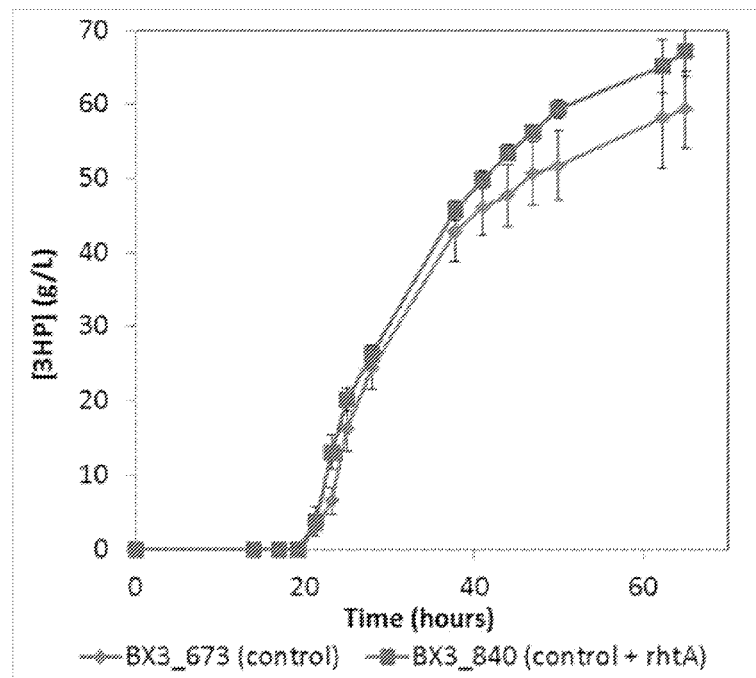


FIG. 7

[Continued on next page]



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(57) Abstract: The present invention provides various combinations of genetic modifications to a transformed host cell that provide increase conversion of carbon to a chemical product. The present invention also provides methods of fermentation and methods of making various chemical products.

BIOPRODUCTION OF CHEMICALS**CROSS-REFERENCE TO RELATED APPLICATIONS**

[001] This application claims priority to U.S. Provisional Patent Application No. 61/852,387, filed on March 15, 2013; and U.S. Provisional Patent Application No. 61/791,743, filed on March 15, 2013; which are herein incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

[002] There is a need for alternative production methods of industrial chemicals used for various consumer products and fuels that are currently made from petroleum. One alternative method is the use of engineered microorganisms to produce industrial chemicals. Currently, in the field of bioproduced chemicals there is a need to improve microbial enzyme performance, enhanced production rate in order to reach the goal of becoming an at-cost replacement basis for petro-based chemicals.

[003] A common challenge faced in field of bio-produced chemicals in microorganisms is that any one modification to a host cell may require coordination with other modifications in order to successfully enhance chemical bioproduction.

[004] Provided herein are, *e.g.*, methods, systems of fermentation, genetically modified microorganisms, and modified enhanced enzymes for chemical production, all of which may be used *e.g.*, in various combinations to increase chemical production of a desired chemical product.

INCORPORATION BY REFERENCE

[005] All publications, patents, and patent applications herein are incorporated by reference in their entireties. In the event of a conflict between a term herein and a term in an incorporated reference, the term herein controls.

SUMMARY OF THE INVENTION

[006] The inventive embodiments provided in this Summary of the Invention are meant to be illustrative only and to provide an overview of selected embodiments disclosed herein. The Summary of the Invention, being illustrative and selective, does not limit the scope of any claim, does not provide the entire scope of inventive embodiments disclosed or contemplated herein, and should not be construed as limiting or constraining the scope of this disclosure or any claimed inventive embodiment.

[007] Provided herein are genetically modified organisms capable of producing an industrial chemical product of interest, wherein the genetic modification can include introduction of nucleic acid sequences coding for polynucleotides encoding one or more of the following: (1)

an acetyl-CoA carboxylase gene with one or more of its subunits fused together in the genetic structure of the organism; and/or (2) an acetyl-CoA carboxylase gene having a predefined stoichiometric ratio of each of the four ACCase subunits relative to one another.

[008] Also provided herein are methods of producing a chemical product using a genetically modified organism capable of producing an industrial chemical product of interest, wherein the genetic modification can include introduction of nucleic acid sequences coding for polynucleotides encoding one or more of the following: (1) an acetyl-CoA carboxylase gene with one or more of its subunits fused together in the genetic structure of the organism; and/or (2) an acetyl-CoA carboxylase gene having a predefined stoichiometric ratio of each of the four ACCase subunits relative to one another.

[009] Further provided herein are products made from a genetically modified organisms capable of producing an industrial chemical product of interest, wherein the genetic modification includes introduction of nucleic acid sequences coding for polynucleotides encoding one or more of the following: (1) an acetyl-CoA carboxylase gene with one or more of its subunits fused together in the genetic structure of the organism; and/or (2) an acetyl-CoA carboxylase gene having a predefined stoichiometric ratio of each of the four ACCase subunits relative to one another. The present invention also relates to products made from such methods.

[0010] Provided herein are genetically modified organisms (GMOs) capable of producing an industrial chemical product of interest, wherein the GMOs comprise a genetic modification including introduction of one or more nucleic acid sequences encoding one or more of the following: (1) an acetyl-CoA carboxylase (ACCase) with one or more of its subunits fused together; and/or (2) an acetyl-CoA carboxylase having a predefined stoichiometric ratio of each of the four ACCase subunits relative to one another.

[0011] Also provided herein are methods of producing a chemical product using a genetically modified organism capable of producing an industrial chemical product of interest, wherein the genetic modification includes introduction of at least one nucleic acid sequence encoding one or more enzymes selected from the group consisting of: (1) a malonyl-CoA reductase that is mutated to enhance its activity at lower temperatures; (2) a salt-tolerant enzyme; (3) a polypeptide that facilitates the exportation of a chemical product of interest or the export of an inhibitory chemical from the cell; and/or (4) a polypeptide that facilitates the importation into a cell of a reactant, precursor, and/or metabolite used for producing a chemical product of interest.

[0012] Further provided herein are products made from a genetically modified organism capable of producing an industrial chemical product of interest, wherein the genetic modification includes introduction of at least one nucleic acid sequence encoding one or more enzymes selected from the group consisting of: (1) a malonyl-CoA reductase that is mutated to enhance its activity at lower temperatures; (2) a salt-tolerant enzyme; (3) a polypeptide that facilitates the exportation of a chemical product of interest or the export of an inhibitory chemical from the cell; and/or (4) a polypeptide that facilitates the importation into a cell of a reactant, precursor, and/or metabolite used for producing a chemical product of interest.

[0013] Provided herein are (1) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more of the following enzymes: ydfG, mmsB, NDSD, rutE, and/or nemA; (2) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more enzymes capable of converting malonate semialdehyde keto form to 3-HP, and one or more genes encoding one or more enzymes capable of converting either the malonate semialdehyde enol form to 3-HP and/or the malonate semialdehyde hydrated form to 3-HP; (3) a monofunctional malonyl-CoA reductase enzyme fused to a dehydrogenase enzyme that is either: (a) NADPH-dependent, (b) NADH-independent, (c) has the capability of using NADPH, (d) flavin-dependent, (e), flavin-independent, (f) has the capability of using flavin, (g) less susceptible to 3-HP inhibition at high concentration, and/or (h) catalyzes a reaction pathway to 3-HP that is substantially irreversible; and/or (4) a monofunctional malonyl-CoA reductase enzyme fused to one or more malonate semialdehyde dehydrogenase enzymes.

[0014] Also provided herein are genetically modified organisms capable of producing an industrial chemical product of interest, wherein the genetically modified organisms comprise (1) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more of the following enzymes: ydfG, mmsB, NDSD, rutE, and nemA; (2) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more enzymes capable of converting malonate semialdehyde keto form to 3-HP, and one or more genes encoding one or more enzymes capable of converting either the malonate semialdehyde enol form to 3-HP and/or the malonate semialdehyde hydrated form to 3-HP; (3) a monofunctional malonyl-CoA reductase enzyme fused to a dehydrogenase enzyme that is either: (a) NADPH-dependent, (b)

NADH-independent, (c) has the capability of using NADPH, (d) flavin-dependent, (e), flavin-independent, (f) has the capability of using flavin, (g) less susceptible to 3-HP inhibition at high concentration, and/or (h) catalyzes a reaction pathway to 3-HP that is substantially irreversible; and/or (4) a monofunctional malonyl-CoA reductase enzyme fused to one or more malonate semialdehyde dehydrogenase enzymes.

[0015] Further provided herein are methods of producing a chemical product using a genetically modified organism capable of producing an industrial chemical product of interest, wherein the genetically modified organism can comprise (1) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more of the following enzymes: ydfG, mmsB, NDSD, rutE, and/or nemA; (2) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more enzymes capable of converting malonate semialdehyde keto form to 3-HP, and one or more genes encoding one or more enzymes capable of converting either the malonate semialdehyde enol form to 3-HP and/or the malonate semialdehyde hydrated form to 3-HP; (3) a monofunctional malonyl-CoA reductase enzyme fused to a dehydrogenase enzyme that is either: (a) NADPH-dependent, (b) NADH-independent, (c) has the capability of using NADPH, (d) flavin-dependent, (e), flavin-independent, (f) has the capability of using flavin, (g) less susceptible to 3-HP inhibition at high concentration, and/or (h) catalyzes a reaction pathway to 3-HP that is substantially irreversible; and/or (4) a monofunctional malonyl-CoA reductase enzyme fused to one or more malonate semialdehyde dehydrogenase enzymes.

[0016] Further provided herein are products made from a genetically modified organism capable of producing an industrial chemical product of interest, wherein the genetically modified organism can comprise (1) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more of the following enzymes: ydfG, mmsB, NDSD, rutE, and/or nemA; (2) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde and one or more genes encoding one or more enzymes capable of converting malonate semialdehyde keto form to 3-HP, and one or more genes encoding one or more enzymes capable of converting either the malonate semialdehyde enol form to 3-HP and/or the malonate semialdehyde hydrated form to 3-HP; (3) a monofunctional malonyl-CoA reductase enzyme fused to a dehydrogenase enzyme that is either: (a) NADPH-dependent, (b) NADH-independent, (c) has the capability of using

NADPH, (d) flavin-dependent, (e), flavin-independent, (f) has the capability of using flavin, (g) less susceptible to 3-HP inhibition at high concentration, and/or (h) catalyzes a reaction pathway to 3-HP that is substantially irreversible; and/or (4) a monofunctional malonyl-CoA reductase enzyme fused to one or more malonate semialdehyde dehydrogenase enzymes.

[0017] Provided herein are genetically modified organisms (“GMOs”) capable of producing an industrial chemical product of interest, wherein the genetic modification can include introduction of at least one nucleic acid sequence encoding one or more enzymes selected from the group consisting of: (1) a malonyl-CoA reductase that is mutated to enhance its activity at lower temperatures; (2) a salt-tolerant enzyme; (3) a polypeptide that facilitates the exportation of a chemical product of interest or the export of an inhibitory chemical from the cell; and/or (4) a polypeptide that facilitates the importation into a cell of a reactant, precursor, and/or metabolite used for producing a chemical product of interest.

[0018] Also provided herein are methods of producing a chemical product using the genetically modified organisms herein. Further provided herein are products made from these methods. In certain embodiments, the products can be acetyl-CoA, malonyl-CoA, malonate semialdehyde, 3-hydroxypropionic acid (3-HP), acrylic acid, 1,3 propanediol, malonic acid, ethyl 3-HP, propiolactone, acrylonitrile, acrylamide, methyl acrylate, a polymer including super absorbent polymers and polyacrylic acid, and/or a consumer product.

[0019] Provided herein are methods of producing a chemical product from a renewable carbon source through a bioproduction process that can comprise a controlled multi-phase production process wherein the initiation and/or completion of one or more phases of the production process can be controlled by genetic modifications to the organism producing the chemical product and/or can be controlled by changes made to the cell environment. The bioproduction process may include two or more of the following phases: (1) growth phase; (2) induction phase; and (3) production phase. Also provided are products made from these methods.

[0020] Also provided herein are methods of making GMOs herein.

[0021] Additionally, provided herein are methods of producing a chemical product using a GMO herein.

[0022] Further provided herein are products produced using a GMO herein which can be, *e.g.*, capable of producing an industrial chemical product of interest.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be

obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0024] **FIG. 1** Depicts some embodiments of the metabolic pathways to produce 3-hydroxypropionic acid (“3-HP”).

[0025] **FIG. 2** Depicts some embodiments of the of various equilibrium states in the malonate semialdehyde to 3-HP reaction in a cell environment.

[0026] **FIG. 3** Depicts some embodiments of the reaction catalyzed by acetyl-CoA carboxylase (ACCase)

[0027] **FIG. 4** Shows the inhibition of ACCase enzyme activity by high salt concentration

[0028] **FIG. 5** Depicts some embodiments of the fusion ACCase subunit gene constructs overexpressed in *E. coli*. CAT = chloramphenicol resistance marker; p15a rep = replication origin; red arrow = promoter.

[0029] **FIG. 6** Show improved production of 3-HP by genetically modified organism with DA fusion ACCase.

[0030] **FIG. 7** Shows improved production of 3-HP by genetically modified organism with overexpression of *rhtA* exporter.

[0031] **FIG. 8** Shows various embodiments of the genetic modules used for optimizing expression in host cells.

[0032] **FIG. 9** Shows various chemical products that can made from various embodiments of the invention.

[0033] **FIG. 10** Shows an enzymatic reaction of the transport of homoserine (threonine and homoserine exporters).

[0034] **FIG. 11** Shows results from the overexpression of 33 transporters (adapted from Yamada S, Awano N, Inubushi K, Maeda E, Nakamori S, Nishino K, Yamaguchi A, Takagi H. (2006))

[0035] **FIG. 12** Shows the average OD after 24 hours of cultures grown in M9 in the presence of different levels of 3-HP at different pHs.

[0036] **FIGs. 13A-D** Shows multiple sequence alignments for *rhtA*.

[0037] **FIGs. 14A-K** Shows pileup sequence comparisons for *rhtA*.

[0038] **Table 1.** Lists the accession numbers for genes encoding ACCase subunits from *Halomonas elongate* (“*H. elongate*”).

[0039] **Table 2.** Depicts some embodiments of the RBS sequences used to enhance expression of *H. elongate* ACCase subunits.

[0040] **Table 3.** Shows improvement in 3-HP production by RBS-optimized expression of *H. elongata* ACCase subunits.

[0041] **Table 4.** Shows some embodiments of the ACCase subunit fusions that increase and ACCase enzyme complex activity.

DETAILED DESCRIPTION OF THE INVENTION

[0042] DEFINITIONS

[0043] As used herein, the term "homology" can refer to the optimal alignment of sequences (either nucleotides or amino acids), which may be conducted by computerized implementations of algorithms. "Homology", with regard to polynucleotides, for example, may be determined by analysis with BLASTN version 2.0 using the default parameters. "Homology", with respect to polypeptides (*i.e.*, amino acids), may be determined using a program, such as BLASTP version 2.2.2 with the default parameters, which aligns the polypeptide or fragments (and can also align nucleotide fragments) being compared and determines the extent of amino acid identity or similarity between them. It will be appreciated that amino acid "homology" includes conservative substitutions, *i.e.* those that substitute a given amino acid in a polypeptide by another amino acid of similar characteristics. Typically seen as conservative substitutions are the following replacements: replacements of an aliphatic amino acid such as Ala, Val, Leu and Ile with another aliphatic amino acid; replacement of a Ser with a Thr or vice versa; replacement of an acidic residue such as Asp or Glu with another acidic residue; replacement of a residue bearing an amide group, such as Asn or Gln, with another residue bearing an amide group; exchange of a basic residue such as Lys or Arg with another basic residue; and replacement of an aromatic residue such as Phe or Tyr with another aromatic residue. For example, homologs can have about: 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%; or at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% overall amino acid or nucleotide identity to the gene or proteins of the invention; or can have about: 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, or 70% overall amino acid or nucleotide identity to the essential protein functional domains of the gene or proteins of the invention; or at least: 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%,

71%, or 70%, overall amino acid or nucleotide to the essential binding amino acids within an essential functional domain of the gene or proteins of the invention.

[0044] The above descriptions and methods for sequence homology are intended to be exemplary and it is recognized that this concept is well-understood in the art. Further, it is appreciated that nucleic acid sequences may be varied and still provide a functional enzyme, and such variations are within the scope of the present invention. The term “enzyme homolog” can also mean a functional variant.

[0045] The term “functional homolog” can describe a polypeptide that is determined to possess an enzymatic activity and specificity of an enzyme of interest but which has an amino acid sequence different from such enzyme of interest. A corresponding “homolog nucleic acid sequence” may be constructed that is determined to encode such an identified enzymatic functional variant.

[0046] The term “3-HP” means 3-hydroxypropionic acid.

[0047] The term “heterologous DNA,” “heterologous nucleic acid sequence,” and the like as used herein can refer to a nucleic acid sequence wherein at least one of the following is true: (a) the sequence of nucleic acids is foreign to (*i.e.*, not naturally found in) a given host microorganism; (b) the sequence may be naturally found in a given host microorganism, but in an unnatural (*e.g.*, greater than expected) amount; and/or (c) the sequence of nucleic acids comprises two or more subsequences that are not found in the same relationship to each other in nature. For example, regarding instance (c), a heterologous nucleic acid sequence that is recombinantly produced can *e.g.*, have two or more sequences from unrelated genes arranged to make a new functional nucleic acid. Embodiments of the present invention may result from introduction of an expression vector into a host microorganism, wherein the expression vector contains a nucleic acid sequence coding for an enzyme that is, or is not, normally found in a host microorganism. With reference to the host microorganism's genome prior to the introduction of the heterologous nucleic acid sequence, then, the nucleic acid sequence that codes for the enzyme is heterologous (whether or not the heterologous nucleic acid sequence is introduced into that genome). The term “heterologous” is intended to include the term “exogenous” as the latter term is generally used in the art as well as “endogenous”.

[0048] As used herein and unless otherwise indicated, the singular forms “a,” “an,” and “the” include plural referents (*e.g.*, mean one or more). Thus, for example, reference to an “expression vector” includes a single expression vector as well as a plurality of expression vectors, either the same (*e.g.*, the same operon) or different; reference to “microorganism” includes a single microorganism as well as a plurality of microorganisms; and the like.

[0049] As used herein and unless otherwise indicated, the term “organism” refers to any contiguous living system. Examples of organisms can include, but are not limited to, animals, fungus, microorganisms, and/or plants. The term organism is meant to encompass unicellular and/or multicellular entities, including but not limited to, prokaryotes (including but not limited to bacteria and fungus) and/or eukaryotes (including, but not limited to, viruses).

[0050] As used herein and unless otherwise indicated, terms such as “contain”, “containing”, “include”, “including”, and the like mean comprising.

[0051] Some embodiments herein contemplate numerical ranges. When ranges are provided, the ranges include the range endpoints unless otherwise indicated. Unless otherwise indicated, numerical ranges include all values and subranges therein as if explicitly written out.

[0052] Some values herein are modified by the term “about.” In some instances, the term “about” in relation to a reference numerical value can include a range of values plus or minus 10% from that value. For example the amount “about 10” can include amounts from 9 to 11. In other embodiments, the term “about” in relation to a reference numerical value can include a range of values plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% from that value.

[0053] Carbon sources can include *e.g.*, sugars such as *e.g.*, glucose, fructose, galactose, dextrose, maltose, glycerol, and/or a solution containing equal to or less than 50% glycerol.

[0054] A disruption of gene function may also be effectuated, in which the normal encoding of a functional enzyme by a nucleic acid sequence has been altered so that the production of the functional enzyme in a microorganism cell has been reduced or eliminated. A disruption may broadly include a gene deletion, and also includes, but is not limited to gene modification (*e.g.*, introduction of stop co dons, frame shift mutations, introduction or removal of portions of the gene, introduction of a degradation signal), affecting mRNA transcription levels and/or stability, and altering the promoter or repressor upstream of the gene encoding the polypeptide. In some embodiments, a gene disruption is taken to mean any genetic modification to the DNA, mRNA encoded from the DNA, and the amino acid sequence resulting there from that result in at least a 50 percent reduction of enzyme function of the encoded gene in the microorganism cell.

[0055] I. INTRODUCTION

[0056] Provided herein are, *e.g.*, genetically modified microorganisms, methods for making the same, and use of the same in making industrial products. Any and all of the microorganisms herein may include a combination of genetic alterations as described herein.

The present invention contemplates, for example, a genetically modified microorganism having one or more of the following genetic modifications: (i) an alteration that affects the stoichiometric ratio, expression or production of one or more ACCase enzyme genes (ii) a recombinant ACCase gene having at least: 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence homology to an ACCase gene from a salt tolerant organism and/or (iii) a genetic alteration in one or more non-ACCase genes.

[0057] The present invention contemplates, for example, a genetically modified microorganism having one or more genetic alterations that encode for one or more exporters capable of exporting 3-HP out of a cell.

[0058] The present invention also relates to methods of fermentation. The genetically modified microorganisms can be cultured under conditions that optimized a host cell for increase chemical production. The bio-production process may include two or more of the following phases of fermentation: (1) growth phase where the culture organism replicates itself and the carbon intermediate product is built up; (2) the induction phase, where the expression of key enzymes critical to the chemical production is induced and the enzymes accumulate within the organism to carry out the engineered pathway reactions required to further produce the chemical product (3) production phase is where the organism expresses proteins that provide for continuously production the desired chemical product. The above phases can be further controlled by (1) addition and amount of the initiating reactant added to the reaction vessel and (2) key enzymes engineered into the organism using promoters that are sensitive to (*e.g.*, activated by) the depletion of the initiating reactant. Additional details about the fermentation process of the invention are disclosed below.

[0059] Provided herein are, *e.g.*, genetically modified microorganisms, methods for making the same, and use of the same in making industrial products. Any and all of the microorganisms herein may include a combination of genetic alterations as described herein. The present invention contemplates, for example, (i) new hybrid molecules or co-expressed molecules of a mono-functional malonyl-CoA reductase enzyme with various 3-HP dehydrogenase proteins that: (a) exhibit less inhibition by high 3-HP concentrations (b) can catalyze a reaction pathway to 3-HP that is less reversible, substantially irreversible and/or irreversible (c) can utilize NADH (d) enzymes that utilize and/or can utilize flavin; and (ii) can have one or more genetic alterations that can be used to channel a carbon within in standard cellular metabolic pathway into a pathway engineered to produce a desired chemical.

[0060] When utilizing certain organisms to create certain products, it may be advantageous to control each phase discretely. For example, depending on the pathway involved, reactions,

reactants, intermediates and byproducts created during cell growth can inhibit enzyme induction and/or the organism's ability to produce the desired chemical product. Similarly, reactions, reactants, intermediates and byproducts created as part of the production pathway can impact cell growth, and even the increased concentration of the chemical product as it is produced can impede cell replication.

[0061] II. ACETYL-CoA CARBOXYLASE

[0062] Malonyl-CoA Flux

[0063] One of the steps in the biosynthesis of 3-HP involves the reaction catalyzed by acetyl-CoA carboxylase enzyme. ACCase is a primary control point in the 3-HP pathway shown in **FIG. 1** (previously described in) for the converting acetyl-CoA to malonyl-CoA and hence to malonate semialdehyde and 3-HP. Inventive embodiments herein contemplate the use of genetic modifications that increase activity of ACCase complex enzymes to thereby increase 3-HP production in a host cell.

[0064] Fused Subunits

[0065] The acetyl-CoA carboxylase complex is a multi-subunit protein. Prokaryotes and plants have multi-subunit acetyl-CoA carboxylase complexes composed of several polypeptides encoded by distinct genes. However, humans and most other eukaryotes, such as yeast, have evolved an acetyl-CoA carboxylase complex with CT and BC catalytic domains and biotin carboxyl carrier domains on a single polypeptide. The biotin carboxylase (BC) activity, biotin carboxyl carrier protein (BCCP), and carboxyl transferase (CT) activity are each contained on a different subunit. In *E. coli* the ACCase complex is derived from multi polypeptide transcribed by distinct, separable protein components known as accA, accB, accC, and accD.

[0066] Acetyl-CoA carboxylase is a biotin-dependent enzyme that catalyzes the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA through its two catalytic activities, biotin carboxylase and carboxyltransferase. The first reaction is carried out by BC and involves the ATP-dependent carboxylation of biotin with bicarbonate. The carboxyl group is transferred from biotin to acetyl-CoA to form malonyl-CoA in the second reaction, which is catalyzed by CT. The main function of ACCase complex in the cell is to provide the malonyl-CoA substrate for the biosynthesis of fatty acids.

[0067] The conversion of acetyl-CoA to malonyl-CoA is an important step in the bioconversion of a renewable carbon source (such as, for example, sugar or natural gas) to a useful industrial chemical (such as, for example, 3-hydroxypropionic acid (3-HP)). In certain organisms, such as *E. coli* or yeast, the native ACCase expression from the chromosome

alone is insufficient to enable the organism to produce chemicals such as 3-HP at a rate to support a commercial scale operation. Overexpression of the ACCase complex has been shown to provide some advantage. *See, e.g.*, US Patent Application Nos.: 12/891,760; 12/891,790; and 13/055,138.

[0068] Applicants have discovered that the introduction of an acetyl-CoA carboxylase gene with one or more of its subunits fused into *e.g.*, an organism or GMO is beneficial to the production of a chemical product in a host cell (*e.g.*, the organism or GMO). In any embodiment herein, fusion can be of two gene products produced from a single polynucleotide controlled by a single promoter, which *e.g.*, can further enhance an organism's bioproduction of an industrial chemical. In any embodiment herein, fusion can be of two gene products, enhanced by at least one promoter, which can further enhance an organism's bioproduction of an industrial chemical. In any embodiment herein, fusion can be of two gene products produced from a single polynucleotide controlled by at least one inducible promoter, which can further enhance an organism's bioproduction of an industrial chemical. Keeping components of the ACCase complex fused together in the genetic structure of an organism can be advantageous because *e.g.*, it can enhance the stability of the non-native ACCase genetic modification and it can facilitate equimolar expression of the fused acc subunits.

[0069] In any embodiment herein, the subunit-fused ACCase (*e.g.*, in the organism, cell or GMO) *e.g.*, may be or contain an accA-accB, accA-accC, accA-accD, accB-accC, accB-accD, accC-accD, accA-accB-accC, accA-accB-accD, accA-accC-accD, accB-accC-accD, or accA-accB-accC-accD fused subunit having *e.g.*, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence homology to the same combination of *E.coli* accA, accB, accC and accD, or their functional homologs. In addition, in any embodiment herein, the organism may include any combination of these fused subunits, or any combination of these fused subunits together with one or more of the four non-fused subunits. When such combinations are used, the subunits

(fused and non-fused) may be expressed on the same plasmid or on different plasmids or on one or more of the organism's chromosome.

[0070] In any embodiment herein, the subunit-fused ACCase (*e.g.*, in the organism, cell or GMO) *e.g.*, may be or contain an accA-accB, accA-accC, accA-accD, accB-accC, accB-accD, accC-accD, accA-accB-accC, accA-accB-accD, accA-accC-accD, accB-accC-accD, or accA-accB-accC-accD fused subunit having *e.g.*, about: 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 100%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence homology to the same combination of *E.coli* accA, accB, accC and accD, or their functional homologs. In addition, in any embodiment herein, the organism may include any combination of these fused subunits, or any combination of these fused subunits together with one or more of the four non-fused subunits. When such combinations are used, the subunits (fused and non-fused) may be expressed on the same plasmid or on different plasmids or on one or more of the organism's chromosome. Further, in any embodiment herein, the organism, cell, or GMO which can include the subunit-fused ACCase can be capable of reducing malonyl-CoA. Further, the organism, cell, or GMO can comprise a polynucleotide encoding a protein that reduces or is capable of reducing malonyl-CoA. In any embodiment herein, the protein that reduces or is capable of reducing malonyl-CoA can be NADPH dependent, NADH dependent, or a combination of these.

[0071] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule encoding a protein that is or can be comprised in an ACCase, wherein the encoded protein comprises 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 contiguous amino acid residues from any one of SEQ ID NOs: 1, 3, 5, 7, 9, or 11.

[0072] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule encoding a protein that is or can be comprised

in an ACCase complex, wherein the encoded protein comprises 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 contiguous amino acid residues from any one of SEQ ID NOs: 1, 3, 5, 7, 9, or 11.

[0073] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule encoding a protein that is or can be comprised in an ACCase domain, wherein the encoded protein comprises 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 contiguous amino acid residues from any one of SEQ ID NOs: 1, 3, 5, 7, 9, or 11.

[0074] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule encoding a protein that is or can be comprised in an ACCase subunit, wherein the encoded protein comprises 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 contiguous amino acid residues from any one of SEQ ID NDs: 1, 3, 5, 7, 9, or 11.

[0075] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule comprising 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 contiguous polynucleotide residues from any one of SEQ ID NDs: 2, 4, 6, 8, or 10.

[0076] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule comprising 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 contiguous polynucleotide residues from any one of SEQ ID NDs: 2, 4, 6, 8, or 10, wherein the polynucleotide encodes a protein that is or can be comprised in an ACCase.

[0077] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule comprising 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 contiguous polynucleotide residues from any one of SEQ ID NDs: 2, 4, 6, 8, or 10, wherein the polynucleotide encodes a protein that is or can be comprised in an ACCase complex.

[0078] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule comprising 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 contiguous polynucleotide residues from any one of SEQ ID NOs: 2, 4, 6, 8, or 10, wherein the polynucleotide encodes a protein that is or can be comprised in an ACCase domain.

[0079] In any embodiment herein, the organism or GMO can produce 3-HP, and can comprise an exogenous nucleic acid molecule comprising 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 contiguous polynucleotide residues from any one of SEQ ID NDs: 2, 4, 6, 8, or 10, wherein the polynucleotide encodes a protein that is or can be comprised in an ACCase subunit.

[0080] In any embodiment herein, an accA-accD fused subunit can be introduced into an organism or GMO either alone or in combination with the accB-accC fused subunit, the accB gene, and/or the accC gene. In any embodiment herein, the organism or GMO can be a bacteria, *e.g.*, *E. coli* or *Cupriavidus necator*.

[0081] Composition stoichiometry

[0082] Composition stoichiometry can be, *e.g.*, the quantitative relationships among elements or parts that comprise a compound or thing (*e.g.*, such as an enzyme or enzyme complex, for example an ACCase or ACCase complex). Although stoichiometric terms are traditionally reserved for chemical compounds, theoretical consideration of stoichiometry are relevant when considering the optimal function of heterologous multi-subunit protein in a host cell.

[0083] For example, in any embodiment herein, a stoichiometric ratio of each of the four ACCase subunits relative to one another can be, for example, between or from: 0 and about 10, 0 and about 9, 0 and about 8, 0 and about 7, 0 and about 6, 0 and about 5, 0 and about 4, 0 and about 3, 0 and about 2, 0 and about 1, about 1 and about 10, about 2 and about 10,

about 3 and about 10, about 4 and about 10, about 5 and about 10, about 6 and about 10, about 7 and about 10, about 8 and about 10, about 9 and about 10, or between or from about 0.5 to about 2, or about 7 to about 9. For example, the stoichiometric ratios, in any embodiment herein, for the protein subunits accA:accB:accC:accD can be about: 1:2:1:1. In any embodiment herein, an organism can be genetically modified to include an accA-accD fused subunit, an accB non-fused subunit, and an accC non-fused subunit, with the ratios or molar ratios of the accDA fusion:accB:accC being about 1:2:1, which can be close to the optimum for enzymatic activity.

[0084] In any embodiment herein, where an organism can be engineered to make 3-HP, in order to get optimal function in a host cell of a heterologous ACCase enzyme complex it can be important to engineer the stoichiometry of these subunits in such a way that provides maximal production of 3-HP such that the subunit can make a more stable enzyme complex when overexpressed in the cell.

[0085] In any embodiment herein can be provided for the controlled expression of the natural accA, accB, accC, and accD subunits of *E.coli* or having at least: 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology to *E.coli* accA, accB, accC and accD. In any embodiment herein can be provided for the inducible expression of the natural accA, accB, accC, and accD subunits of *E.coli* or having at least 80% sequence homology to *E.coli* accA, accB, accC, and accD. In certain aspects the invention provides for the low, medium, high and/or inducible expression of the natural accA, accB, accC, and accD subunits of *E.coli* or having at least 80% sequence homology to *E.coli* accA, accB, accC and accD.

[0086] In any embodiment herein can be provided for the expression of the natural accC and accD subunits of *E.coli* or having at least 80% sequence homology to *E.coli* accA, accB, accC and accD in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for the expression of the natural accB and accA subunits of *E.coli* or having at least 80% sequence homology to *E.coli* accA, accB, accC, and accD in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for the expression of the natural accC and accD subunits with the accA subunit of *E.coli* or having at least 80% sequence homology to *E.coli* accA, accB, accC, and accD in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for the expression of the natural accC and accD subunits with the accB subunit of *E.coli* or having at least about: 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, or 99%, sequence homology to *E.coli* accA, accB, accC, and accD in low, medium, high or inducible expression vectors.

[0087] In any embodiment herein can be provided for the expression of a fusion of two, three, or all of the four ACCase subunits in one polypeptide in low, medium, high or inducible expression vectors. Such fusion may include any of the following combinations of the ACCase subunits: accA-accB, accA-accC, accA-accD, accB-accC, accB-accD, accC-accD, accA-accB-accC, accA-accB-accD, accA-accC-accD, accB-accC-accD, and accA-accB-accC-accD having at least 80% sequence homology to the same combinations of *E.coli* accA, accB, accC and accD or functional homologs thereof.

[0088] In any embodiment herein can be provided for an ACC complex in the stoichiometry of these subunits of the accCB and accDA in a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors. In any embodiment herein can be provide for an ACC complex in the stoichiometry of these subunits of the accCB and accDA in about a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for an ACC complex in the stoichiometry of these subunits of the accDA and accCB in a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors. In any embodiment herein can be provided for an ACC complex in the stoichiometry of these subunits of the accDA and accCB in about a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors.

[0089] In any embodiment herein can be provided for the stoichiometry of the accD-A subunits in a 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 2:1, 2:3, 2:4, 2:5, 2:6, 2:7, 2:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 3:1, 3:3, 3:4, 3:5, 3:6, 3:7, 3:8, 4:1, 4:3, 4:4, 4:5, 4:6, 4:7, 4:8, 5:1, 5:3, 5:4, 5:5, 5:6, 5:7, 5:8, 6:1, 6:3, 6:4, 6:5, 6:6, 6:7, 6:8, 7:1, 7:3, 7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4,

[illegible]

7:4, 7:5, 7:6, 7:7, 7:8, 8:1, 8:3, 8:4, 8:5, 8:6, 8:7, or 8:8 in low, medium, high or inducible expression vectors.

[0090] Bioproduction Methods

[0091] In any inventive embodiment herein, the host cell can be genetically modified for increased malonyl-CoA flux by at least one heterologous ACCase complex, such as **Table 4** to further increase chemical bio-production in host cell.

[0092] III. CONVERSION OF MALONYL-COA TO MALONATE SEMIALDEHYDE

[0093] One of the steps in the biosynthesis of 3-HP involves the conversion of malonyl-CoA (MCA) to malonate semialdehyde (MSA) and the conversion of malonate semialdehyde (MSA) to 3-HP (*see, e.g.*, WO2011/038364). Inventive embodiments herein contemplate the use of novel enzymes and/or combinations of enzymes to catalyze the reaction in a microorganism from MCA to MSA, which results in enhanced cellular bioproduction of 3-HP in the host cell.

[0094] In some embodiments, the invention provides novel enzyme compositions or co-expression of a combinations of enzyme compositions to catalyze the conversion of malonyl-CoA to 3-HP. A general overview of the enzymes and the relevant reaction pathways methods are shown in **FIG.1**. Any and all combinations of the relevant enzymes shown in **FIG.1** are contemplated in this invention.

[0095] In one embodiment, malonyl-CoA is converted to malonate semialdehyde by a malonyl-CoA reductase and malonate semialdehyde is converted to 3-HP through separate or both alternative pathways.

[0096] In another embodiment, malonyl-CoA is converted to malonate semialdehyde by a monofunctional malonyl-CoA reductase that catalyzes the malonyl-CoA conversion, but does not catalyze the malonate semialdehyde conversion.

[0097] In one embodiment, the microorganism herein comprises a monofunctional malonyl-CoA reductase derived from, *e.g.*, *Sulfolobus tokodaii* (stMCR) (SEQ ID NOs. 15 and 16); a functional homolog of stMCR; an enzyme with at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity thereto.

[0098] In some embodiments, the microorganism herein comprises a bi-functional malonyl-CoA reductase having at least two protein fragments, with one fragment having malonyl-CoA reductase activity and the other fragment having malonate semialdehyde dehydrogenase activity derived from, *e.g.*, *Chloroflexus aurantiacus* (caMCR).

[0099] MCR-Dehydrogenase Enzymes for Conversion of 3-HP ions

[00100] Following the conversion of the malonyl-CoA to malonate semialdehyde, the malonate semialdehyde is converted to 3-HP through separate or both of pathways. Malonate semialdehyde may exist in at least three states; the keto form, the enol form, and hydrate form, as shown in **FIG. 2**. Malonate semialdehyde in the enol form, which will stabilize this form when compared to other aldehydes where the enol form is highly unfavored in the equilibrium among the three forms.

[00101] The malonate semialdehyde keto form can be converted to 3-HP utilizing a 3-hydroxy acid dehydrogenase enzyme (*e.g.*, *ydfG*; SEQ ID NOs. 21 and 22), a 3-hydroxyisobutyrate dehydrogenase enzyme (*e.g.*, *Pseudomonas aeruginosa mmsB*; SEQ ID NOs. 23 and 24), and/or NAD⁺-dependent serine dehydrogenase (*e.g.*, *Pseudomonas NDSD*; SEQ ID NOs. 25 and 26). In an embodiment, *Pseudomonas mmsB*, *Pseudomonas NDSD*, and/or *E. coli ydfG* are used. The gene, *ydfG* from *E. coli* can be largely NADPH dependent, whereas *mmsB* and *NDSD* from *Pseudomonas* can utilize either NADPH or NADH.

[00102] The malonate semialdehyde enol form can be converted to 3-HP utilizing an N-ethylmaleimide reductase (*e.g.*, *nemA*; SEQ ID NOs. 17 and 18), and/or a malonic semialdehyde reductase (*e.g.*, *rutE*, SEQ ID NOs. 19 and 20) from *E. coli*. These enzymes do not directly utilize NADPH or NADH. Instead, these enzymes utilize a flavin mononucleotide that is cycled between oxidized and reduced states by NADPH or NADH. The enol pathway also has advantages over the keto pathway in that the equilibrium between the malonate semialdehyde enol form and 3-HP significantly favors the formation of 3-HP. Thus, the reaction may be considered less reversible, and/or essentially irreversible (*e.g.*, drives the reaction toward the formation of 3-HP).

[00103] The malonate semialdehyde hydrated form may also be converted to 3-HP by either 3-HP dehydrogenase or malonate semialdehyde reductase enzymes, although the hydrated form is more likely to be converted to the enol form as the equilibrium continuously re-adjusts.

[00104] In one embodiment, the microorganism comprises a polynucleotide encoding: (1) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde; and (2) one or more genes encoding for at least one enzymes selected from the group consisting of: *ydfG*, *mmsB*, *NDSD*, *rutE*, and *nemA* or a functional homolog or a homolog with at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity .

[00105] In accordance with another aspect of the invention, there is provided an organism that is genetically modified to make 3-HP, wherein the genetic modification includes a polynucleotide encoding: (1) a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde; (2) one or more genes encoding one or more enzymes capable of converting malonate semialdehyde keto form to 3-HP; and/or (3) one or more genes encoding one or more enzymes capable of converting either the malonate semialdehyde enol form or the malonate semialdehyde hydrated form to 3-HP.

[00106] In certain aspects, the invention provides a monofunctional malonyl-CoA reductase enzyme fused to a dehydrogenase enzyme that is either: (a) NADPH-dependent, (b) NADH-independent, (c) has the capability of using NADPH, (d) flavin-dependent, (e), flavin-independent, (f) has the capability of using flavin, (g) less susceptible to 3-HP inhibition at high concentration, and/or (h) catalyzes a reaction pathway to 3-HP that is substantially irreversible.

[00107] In certain aspects the invention also provides a monofunctional malonyl-CoA reductase enzyme fused to a dehydrogenase enzyme that is NADPH-dependent or has the capability of using NADPH.

[00108] Suitable 3-HP dehydrogenase enzymes that are largely NADH-dependent that can be used with the claimed invention include, but are not limited to, *mmsB* or *NDSD*. Suitable malonate reductase enzymes that are flavin-dependent include, but are not limited to, *rutE* and *nemA*. Suitable 3-HP dehydrogenase enzymes that are less susceptible 3-HP inhibition at high concentration that can be used with the claimed invention include, but are not limited to, *ydfG* and *NDSD*. Suitable 3-HP dehydrogenase or malonate semialdehyde dehydrogenase enzymes that catalyze a reaction pathway to 3-HP that is substantially irreversible are *rutE* and *nemA*.

[00109] In certain aspects the invention provides a monofunctional malonyl-CoA reductase enzyme fused to one or more dehydrogenase enzymes. Malonate semialdehyde, which is the intermediate product in the conversion of malonyl-CoA to 3-HP can be very reactive. Therefore, it can be advantageous to have a reaction pathway wherein the residence time of malonate semialdehyde within the cell is minimized, *e.g.*, its conversion to 3-HP occurs quickly. By fusing the malonyl-CoA reductase with the malonate semialdehyde dehydrogenase to create a multi-domain protein (*e.g.*, two domain protein) and having the MCR and dehydrogenase domains adjacent in the sequence, the the malonate semialdehyde can be quickly converted to 3-HP.

[00110] In certain aspects, the invention provides a first monofunctional malonyl-CoA reductase enzyme fused to a first dehydrogenase enzyme of one type and second monofunctional malonyl-CoA reductase enzyme fused to a dehydrogenase enzyme of a different type than the first dehydrogenase enzyme. Suitable different dehydrogenase enzymes include, but are not limited to, enzymes that use as a substrate, different forms of malonate semialdehyde.

[00111] In some embodiments, the invention provides for microorganisms comprising a genetic modification that include, but are not limited to, a malonyl-CoA reductase from *S. tokadaei* fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, and/or *nemA* (or some combination thereof). The fused enzyme may include any of the following configurations: *mcr - ydfG*, *mcr - mmsB*, *mcr - NDSD*, *mcr - rutE*, *mcr - nemA*, *mcr - ydfG - mmsB*, *mcr - ydfG - NDSD*, *mcr - ydfG - rutE*, *mcr - ydfG - nemA*, *mcr - mmsB - ydfG*, *mcr - mmsB - NDSD*, *mcr - mmsB - rutE*, *mcr - mmsB - nemA*, *mcr - NDSD - ydfG*, *mcr - NDSD - mmsB*, *mcr - NDSD - rutE*, *mcr - NDSD - nemA*, *mcr - rutE - ydfG*, *mcr - rutE - mmsB*, *mcr - rutE - NDSD*, *mcr - rutE - nemA*, *mcr - nemA - ydfG*, *mcr - nemA - mmsB*, *mcr - nemA - NDSD*, *mcr - nemA - rutE*, functional homologs, and/or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00112] In certain aspects the invention provides for microorganisms comprising a genetic modification that include, but are not limited to, malonyl-CoA reductase from *C. aggregans* fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, and/or *nemA* (or some combination thereof). The fused enzyme may include any of the following configurations: *mcr - ydfG*, *mcr - mmsB*, *mcr - NDSD*, *mcr - rutE*, *mcr - nemA*, *mcr - ydfG - mmsB*, *mcr - ydfG - NDSD*, *mcr - ydfG - rutE*, *mcr - ydfG - nemA*, *mcr - mmsB - ydfG*, *mcr - mmsB - NDSD*, *mcr - mmsB - rutE*, *mcr - mmsB - nemA*, *mcr - NDSD - ydfG*, *mcr - NDSD - mmsB*, *mcr - NDSD - rutE*, *mcr - NDSD - nemA*, *mcr - rutE - ydfG*, *mcr - rutE - mmsB*, *mcr - rutE - NDSD*, *mcr - rutE - nemA*, *mcr - nemA - ydfG*, *mcr - nemA - mmsB*, *mcr - nemA - NDSD*, *mcr - nemA - rutE*, functional homologs, and/or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity thereof.

[00113] In certain aspects the invention provides for microorganisms comprising a genetic modification that include, but are not limited to, a malonyl-CoA reductase from *O. trichoides* fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, and/or *nemA* (or some combination thereof). The fused enzyme may include, but are not limited to, any of the following configurations: *mcr - ydfG*, *mcr - mmsB*, *mcr - NDSD*, *mcr - rutE*, *mcr - nemA*, *mcr - ydfG - mmsB*, *mcr - ydfG - NDSD*, *mcr - ydfG - rutE*, *mcr - ydfG - nemA*, *mcr - mmsB - ydfG*, *mcr - mmsB - NDSD*, *mcr - mmsB*

- *rutE*, *mcr* - *mmsB* - *nemA*, *mcr* - *NDSD* - *ydfG*, *mcr* - *NDSD* - *mmsB*, *mcr* - *NDSD* - *rutE*, *mcr* - *NDSD* - *nemA*, *mcr* - *rutE* - *ydfG*, *mcr* - *rutE* - *mmsB*, *mcr* - *rutE* - *NDSD*, *mcr* - *rutE* - *nemA*, *mcr* - *nemA* - *ydfG*, *mcr* - *nemA* - *mmsB*, *mcr* - *nemA* - *NDSD*, *mcr* - *nemA* - *rutE*, functional homologs, and/or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00114] Enhanced Mutated Monofunctional MCR for Bioproduction

[00115] In certain aspects, the invention provides for microorganisms comprising a genetic modification having a mutated form of *stMCR* that has enhanced activity at about 0 °C to about 10°C, at about 8°C to about 15°C, at about 12°C to about 21°C, at 20°C to about 44°C, at about 30°C to about 37°C, at about 32°C to about 38°C, at about 35°C to about 42°C, at about 40°C to about 50°C, at about 50°C to about 60°C, and/or at about 59°C to less than equal to about 72°C. Such mutated forms may be designed based on the crystal structure for *stMCR*; see Demmer *et al.*, J. Biol. Chem. 288:6363-6370, 2013.

[00116] The carboxylase domains of the malonyl-CoA reductase derived from *Chloroflexus aggregans* and/or *Oscillochloris trichoides* can be enhanced by mutations in the carboxylase binding domain to provide increased 3-HP production over the natural occurring enzyme.

[00117] The carboxylase activity of the malonyl-CoA reductase derived from *Chloroflexus aurantiacus* can be enhanced to increase activity. In certain aspects the invention provides for a mutated form of *Chloroflexus aurantiacus* malonyl-CoA reductase having a mutated carboxylase domain to provide increased 3-HP production over the natural occurring enzyme.

[00118] In certain aspects the invention provides for microorganisms comprising a genetic modification that include carboxylase domains of the malonyl-CoA reductase derived from *C. aggregans* fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, and/or *nemA* (or some combination thereof). It is contemplated that the any of the enhanced MCR by mutation, as provide above, may be fused in any of the following configurations including, but not limited to, *mcr* - *ydfG*, *mcr* - *mmsB*, *mcr* - *NDSD*, *mcr* - *rutE*, *mcr* - *nemA*, *mcr* - *ydfG* - *mmsB*, *mcr* - *ydfG* - *NDSD*, *mcr* - *ydfG* - *rutE*, *mcr* - *ydfG* - *nemA*, *mcr* - *mmsB* - *ydfG*, *mcr* - *mmsB* - *NDSD*, *mcr* - *mmsB* - *rutE*, *mcr* - *mmsB* - *nemA*, *mcr* - *NDSD* - *ydfG*, *mcr* - *NDSD* - *mmsB*, *mcr* - *NDSD* - *rutE*, *mcr* - *NDSD* - *nemA*, *mcr* - *rutE* - *ydfG*, *mcr* - *rutE* - *mmsB*, *mcr* - *rutE* - *NDSD*, *mcr* - *rutE* - *nemA*, *mcr* - *nemA* - *ydfG*, *mcr* - *nemA* - *mmsB*, *mcr* - *nemA* - *NDSD*, *mcr* - *nemA* - *rutE*, functional homologs, and/or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00119] IV. SALT-TOLERANT ENZYMES

[00120] The growth of engineered microorganisms for enhanced production of a chemical product, such as *E. coli* can be inhibited by high salt concentrations, *e.g.*, when the chemical product accumulates when the organism produces large amounts of the chemical product.

[00121] Dose-dependent studies with increasing amounts of NaCl and Na-3-HP show that salt can have inhibitory effects on ACCase activity which can be essential to fatty acid biosynthesis of membranes required for growth and propagation and for the production of 3-HP (see **EXAMPLE 1**). Thus, the use of salt-tolerant enzymes in 3-HP production should increase 3-HP production in a host cell.

[00122] A. Enzymes from Halophilic Organisms

[00123] Halophiles are characterized as organisms with a great affinity for salt. In some embodiments, a halophilic organism can require at least 0.01M, 0.05M, 0.1M, 0.2M, 0.3M, 0.4M, 0.5M, 0.6M, 0.7M, 0.8M, 0.9M, 1.0M, 1.25M, or 1.5M concentrations of salt (NaCl) for growth. Halophiles can live in hypersaline environments that are generally defined occurring to their salt concentration of their habitats. Halophilic organisms that are defined as having “slight salt affinity” can grow optimally at 2-5% NaCl, moderate halophiles can grow optimally at 5-20% NaCl, and extreme halophiles can grow optimally at 20-30% NaCl.

[00124] In an embodiment, a genetically engineered microorganism can be made to express homologous enzymes of a specific halophile, for example, from a moderate halophile or an extreme halophile.

[00125] In an embodiment, microorganisms can comprise a genetic modification that includes or codes for enzymes from slight halophile organisms. In an embodiment, microorganisms can comprise a genetic modification that includes or codes for enzymes from moderate halophile organisms. In an embodiment, microorganisms can comprise a genetic modification that includes or codes for enzymes from extreme halophile organisms.

[00126] Homology with genes may be determined by analysis with BLASTN version 2.0 provided through the NCBI website. Homology with proteins may be determined by analysis with BLASTP version 2.2.2 provided through the NCBI website. These programs can align disclosed fragments being compared and can determine the extent of identity or similarity between them.

[00127] To date there are many sequenced halophilic organisms that can be used with the claimed invention. Examples of some sequenced halophilic organisms include, but are not limited to, *Chromohalobacter salexigens*, *Flexistipes sinuarabici* strain (MAS10T), *Halobacterium* sp. NRC-1, *Haloarcula marismortui*, *Natronomonas pharaonis*,

Haloquadratum walsbyi, *Haloferax volcanii*, *Halorubrum lacusprofundi*, *Halobacterium* sp. R-1, *Halomicrobium mukohataei*, *Halorhabdus utahensis*, *Halogeometricum borinquense*, *Haloterrigena turkmenica*, *Natronobacterium gregoryi* SP2, *Halalkalicoccus jeotgali*, *Natrialba magadii*, *Haloarcula hispanica*, *Halopiger xanaduensis*, *Halophilic archaeon* DL31, *Haloferax mediterranei*, *Halovivax ruber*, *Natronococcus gregoryi*, and *Natronococcus occultus*.

[00128] Examples of suitable moderate halophilic organisms in which homologous enzymes of the invention can be derived from include, but are not limited to, eukaryotes such as crustaceans (e.g., *Artemia salina*), insects (e.g., *Ephydra hians*), certain plants from the genera *Salicornia* spp, algae (e.g., *Dunaliella viridis*), fungi, and protozoa (e.g., *Fabrea salina*), phototrophic organisms, such as planktonic and microbial mat-formers cyanobacteria as well as other anaerobic red and green sulphur bacteria from the genera *Ectothiorhodospira* spp.) and non-sulphur bacteria from the genera *Chromatium* spp.; gram-negative anaerobic bacteria, for example from the genera *Haloanaerobacter* spp. some of which are methanogenic, for example from the genera *Methanohalophilus* spp. and either aerobic or facultative such as species from the genera *Halomonas*, *Chromohalobacter*, *Salinovibrio*, *Pseudomonas*, for example (e.g., *Halomonas elongate*); gram-positive bacteria from genera such as *Halobacillus*, *Bacillus*, *Marinococcus*, etc. as well as some actinomycetes, for example, *Actinopolyspora halophila*.

[00129] Genomic and Proteomic Hallmarks of Halophilic Organisms

[00130] Comparative genomic and proteomic studies of halophiles and non-halophiles can reveal some common trends in the genomes and proteomes of halophiles. At the protein level, halophilic organisms can be characterized by low hydrophobicity, over-representation of acidic residues, especially Asp, under-representation of Cys, lower propensities for helix formation and higher propensities for coil structure.

[00131] At the DNA level, halophilic organisms can be characterized by the dinucleotide abundant profiles of halophilic genomes that bear some common characteristics, which are distinct from non-halophiles, and hence may be regarded as specific genomic signatures for salt-adaptation. The synonymous codon usage in halophiles may also exhibit similar patterns regardless of their long-term evolutionary history.

[00132] In one embodiment, microorganisms can be synthesized to comprise proteins that are modified for salt tolerance such that they have low hydrophobicity, over-representation of acidic residues, especially Asp, under-representation of Cys, lower propensities for helix formation and higher propensities for coil structure.

[00133] Suitable salt-tolerant enzymes can include enzymes from salt-tolerant organisms. Salt-tolerant organisms (such as, for example, halophiles) can include any living organisms that are adapted to living in conditions of high salinity. Suitable salt-tolerant enzymes can include enzymes from salt-tolerant organisms that are homologs of the following enzymes: Sucrose-6-phosphate hydrolase (cscA from *E. coli*), glucose-6-phosphate isomerase (pgi from *E. coli*), fructokinase (cscK from *E. coli*), fructose-1,6-bisphosphatase (yggF from *E. coli*), fructose 1,6-bisphosphatase (ybhA from *E. coli*), fructose 1,6-bisphosphatase II (glpX from *E. coli*), fructose-1,6-bisphosphatase monomer (fbp from *E. coli*), 6-phosphofructokinase-1 monomer (pfkA from *E. coli*), 6-phosphofructokinase-2 monomer (pfkB from *E. coli*), fructose bisphosphate aldolase monomer (fbaB from *E. coli*), fructose bisphosphate aldolase monomer (fbaA from *E. coli*), triose phosphate isomerase monomer (tpiA), glyceraldehyde 3-phosphate dehydrogenase-A monomer (gapA from *E. coli*), phosphoglycerate kinase (pgk), 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (gpmM from *E. coli*), 2,3-bisphosphoglycerate-dependent or tdcE (from *E. coli*), phosphoglycerate mutase (gpmA), enolase (eno from *E. coli*), phosphoenolpyruvate carboxylase (ppc from *E. coli*), malate dehydrogenase (mdh), fumarase A (fum from *E. coli*), fumarase B (fumB), fumarase C (fumC from *E. coli*), phosphoenolpyruvate synthetase (ppsA from *E. coli*), pyruvate kinase I monomer (pykF from *E. coli*), pyruvate kinase II monomer (pykA from *E. coli*), fumarate reductase (frdABCD from *E. coli*), lipoamide dehydrogenase (lpd from *E. coli*), pyruvate dehydrogenase (aceE from *E. coli*), pyruvate dehydrogenase (aceF from *E. coli*), pyruvate formate-lyase (pflB from *E. coli*), acetyl-CoA carboxylase (accABCD from *E. coli*), malonyl CoA reductase (mcr), 3HP dehydrogenase (mmsB, NDSD, or ydfG), malonate semialdehyde reductase (nemA, rutE from *E. coli*), or a combination thereof.

[00134] Suitable salt-tolerant enzyme homologs that can be used with the claimed invention can have at least or at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% overall amino acid or nucleotide identity and/or homology to the above enzymes. Suitable salt-tolerant enzyme homologs that can be used with the claimed invention can have at least or at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% amino acid or nucleotide identity and/or homology to the essential protein function domains of the enzymes above. Suitable salt-tolerant enzyme homologs that can be used with the claimed invention can have at least or at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%,

81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% overall amino acid or nucleotide identity and/or homology to the essential binding amino acids within an essential protein function domain of the enzymes above.

[00135] In an embodiment, suitable salt-tolerant enzyme homologs can be enzymes from one of the following organisms: *Halomonas elongata*, *Salinibacter ruber*, or *Halobacterium* species (Archaea).

[00136] In an embodiment, there can be provided a non-salt-tolerant organism that can be genetically modified to make 3-HP, wherein the genetic modification can include introducing into the organism a polynucleotide encoding for an acetyl-CoA carboxylase from a salt-tolerant organism. In an embodiment, the acetyl-CoA carboxylase subunits accA, accB, accC and accD can be from *Halomonas elongata*.

[00137] V. CHEMICAL TRANSPORTER

[00138] In one aspect of this disclosure, any of the microorganisms may be genetically modified to introduce a nucleic acid sequence encoding a polypeptide that: (1) facilitates the exportation of the chemical of interest or the export of an inhibitory chemical from the cell; and/or (2) facilitates the importation into the cell, a reactant, precursor, and/or metabolite used in the organism's production pathway for producing the chemical of interest.

[00139] 3-HP Exporter

[00140] In one aspect of this disclosure, 3-HP can be produced using a genetically modified *E. coli* organism. Thus, the present invention contemplates a host cell genetically modified to express or increase expression of an exporter that can function to transfer 3HP from the cellular environment extracellularly.

[00141] Bacterial cells, such as *E. coli*, have at least five different types of exporters: the major facilitator superfamily (MFS); the ATP-binding cassette superfamily (ABC); the small multidrug resistance family (SMR); the resistance-nodulation-cell division superfamily (RND); and the multi antimicrobial extrusion protein family (MATE). In addition, amino acid exporters, which are common to almost all host cells, may be made to export 3-HP. Additionally, solvent tolerant transporters, for example, bromoacetate, butanol, isobutanol, and the like, may be used to export 3-HP.

[00142] In certain aspects the invention provides a host cell with a recombinant exporter wherein the exporter is an MFS exporter, ABC exporter, SMR exporter, RND exporter, MATE exporter, amino acid exporter, solvent tolerance transporter, or a combination thereof.

[00143] Suitable exporters that can be used include, but are not limited to, *acrD*, *bcr*, *cusA*, *dedA*, *eamA*, *eamB*, *eamH*, *emaA*, *emaB*, *emrB*, *emrD*, *emrKY*, *emrY*, *garP*, *gudP*, *hsrA*, *leuE*,

mdlB, mdtD, mdtG, mdtL, mdtM, mhpT, rhtA, rhtB, rhtC, thtB, yahN, yajR, ybbP, ybiF, ybjJ, ycaP, ydcO, yddG, ydeD, ydgE, yddG, ydhC, ydhP, ydiN, ydiM, ydjE, ydjI, ydjK, yeaS, yedA, yeeO, yegH, yggA, yfcJ, yfiK, yhjE, yidE, yigK, yigJ, yijE, yjiI, yjiJ, yjiO, ykgH, ypjD, ytfF, ytfL, or functional homolog or homolog having at least or at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% sequence identity and/or homology. Other potential transporter proteins may be identified using topology analysis as illustrated in [Daley *et al.*, Science 308: 1321-1323, 2005].

[00144] In certain aspects the invention can provide the exporter to be improved for binding to 3-HP. In certain aspects the invention can provide the exporters named to be further enhance by genetic modification of the predicted cytoplasmic domain to increase 3-HP binding. In certain aspects the invention can provide the exporter to be improved for binding to 3-HP. In certain aspects the invention can provide the exporters named to be further enhance by genetic modification of the predicted transmembrane binding domain to increase 3-HP binding or incorporation into the host cell membrane.

[00145] Suitable exporter homologs that can be used with the claimed invention can have at least or about at least 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% overall amino acid or nucleotide identity and/or homology to the previously mentioned exporters. Suitable exporter homologs that can be used with the claimed invention can have at least or at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% amino acid or nucleotide identity and/or homology to the essential protein function domains of the exporters above. Suitable exporter homologs that can be used with the claimed invention can have at least or at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% amino acid or nucleotide identity and/or homology to the essential binding amino acids within an essential exporter domain of the enzymes above.

[00146] In certain aspects the invention can provide for at least one of the exporters provided herein to be expressed in a host cell to increase the chemical production of 3-HP. In certain aspects the invention can provide for at least one of the exporters provided herein to be expressed in a host cell and with a genetic modification of *tig* to increase the chemical production of 3-HP.

[00147] In certain aspects the invention can provide for one exporter to be further modified by one or more genetic modulates so that the expression level and timing of expression of the exporter can be controlled in the host cell. In certain aspects the invention can provide for one exporter to be further modified by an inducible promoter, RBS, high, multicopy plasmid or combination thereof.

[00148] In certain aspects the invention can provide exporters to be expressed in a host cell in an equal ratio. In certain aspects the invention can provide exporters to be expressed in a host cell in a 1:2 ratio. In certain aspects the invention can provide exporters to be expressed in a host cell in a 1:3 ratio. In certain aspects the invention can provide exporters to be expressed in a host cell in a 1:4 ratio. In certain aspects the invention can provide exporters to be expressed in a host cell in a 2:3 ratio.

[00149] In certain aspects the invention can provide for the exporter to maintain the host cell at pH 7.0-7.4 during the continuous production phase. In certain aspects the invention provides for an exporter and a base importer, wherein the cell can maintain a pH 7.0-7.4 during the continuous production phase. In certain aspects the invention can provide for the exporter to maintain the host cell at pH 3.0 to pH 4.0, pH 4.0 to pH 5.0, pH 5.0 to pH 6.0, pH 6.0 to pH 7.0, pH 7.0 to pH 8.0, pH 8.0 to pH 9.0, pH 9.0 to pH 10.0, and/or pH 7.0-7.3 during the continuous production phase. In certain aspects the invention provides for an exporter and base importer, wherein the cell can maintain a pH 3.0 to pH 4.0, pH 4.0 to pH 5.0, pH 5.0 to pH 6.0, pH 6.0 to pH 7.0, pH 7.0 to pH 8.0, pH 8.0 to pH 9.0, pH 9.0 to pH 10.0, and/or pH 7.0-7.3 during the continuous production phase.

[00150] In some embodiments, additional modifications to the host cell may be made to further enhance the transporter's function. In particular, deletion of the *tig* gene from the genome of the host cell may enhance expression and total activity of integral membrane proteins such as exporters and importers.

[00151] **Bicarbonate Importer**

[00152] One of the steps in the conversion of biomass to 3-HP can be the conversion of acetyl-CoA to malonyl-CoA, which is illustrated in **FIG. 3**.

[00153] As shown in **FIG. 3**, this reaction can be catalyzed by the acetyl-CoA carboxylase, and bicarbonate is a reactant that can drive the reaction. One of the primary sources of bicarbonate to drive this reaction can be carbon dioxide within the cell. Carbon dioxide can readily diffuse through a cell's membrane, and a natural equilibrium will be reached between the intracellular and extracellular carbon dioxide. As a cell produces carbon dioxide it can migrate through the cell, and since it tends not to be very soluble in the media, it may bubble

out of the system and more intracellular carbon dioxide may migrate out of the cell to maintain the equilibrium. This process can impede the production of 3-HP since bicarbonate (which is in equilibrium with the dissolved carbon dioxide in the form of carbonic acid) can be needed to drive the acetyl-CoA $\rightarrow$ malonyl-CoA reaction, and the intracellular carbon dioxide can be the primary source for intracellular bicarbonate.

[00154] In some embodiments, an organism can be provided that can include a heterologous gene encoding for a polypeptide that can act as a carbon dioxide importer (*e.g.*, it enhances the importation of carbon dioxide into the cell or inhibits the exportation of carbon dioxide from the cell), which can result in increased intracellular carbon dioxide. Use of a CO₂ importer can mitigate against the natural outflow of carbon dioxide.

[00155] In some embodiments, there can be provided an organism that can be genetically modified, wherein the genetic modification can include a polynucleotide encoding a polypeptide that is capable of importing extracellular carbon dioxide from the media to within the cell membrane or inhibiting the exportation of intracellular carbon dioxide from within the cell membrane to the media. In some embodiments, a microorganism can be genetically modified to encode one or more of the following heterologous genes: *bicA* from *Synechococcus* species, *ychM* gene product of *E. coli*, *yidE* gene product of *E. coli*, any of the bicarbonate transporters as described in [Felce and Saier, J. Mol. Microbiol. Biotechnol. 8: 169-176, 2004] or any amino acid sequences homologous thereof (*e.g.*, at least or at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70% homologous and/or identical to the amino acid or nucleotide sequences of the CO₂ importer/exporters described herein].

[00156] Bioproduction Methods

[00157] In some embodiments, the host cell can be genetically modified by at least one heterologous gene and/or salt tolerant heterologous gene of **FIG. 1** or **Table 1** and at least one 3-HP exporter provided herein to further increase chemical bioproduction in a host cell.

[00158] In some embodiments, the host cell can be genetically modified with a heterologous gene for increased malonyl-CoA flux, 3-HP export, and to increase chemical bio-production. In some embodiments, the host cell can be genetically modified to increase malonyl-CoA flux, 3-HP export to increase chemical bioproduction.

VI. MULTI-PHASE FERMENTATION

[00159] In accordance with another aspect of the present invention, there is provided a method of producing a chemical product from a carbon source through a bioproduction process that comprises a controlled multi-phase production process. The multi-phase production process includes an *e.g.*, an initiation phase and a production phase, wherein the production phase and/or process can be controlled by genetic modifications to the organism producing the chemical product and/or can be controlled by changes made to the cell environment.

[00160] In one aspect, the bioproduction process may include two or more of the following phases: (1) growth phase; (2) induction phase; and (3) production phase. During the growth phase, the organism replicates itself and the biocatalyst needed to produce the chemical product is built up. During the induction phase, expression of key enzymes critical to the production of the chemical is induced and the enzymes accumulate within the biocatalyst to carry out the reactions required to produce the product. During the production phase organism produces the desired chemical product.

[00161] The initiation and/or completion of the growth, induction and/or production phases can be controlled. In accordance with the present invention, the growth phase can be dependent on the presence of a critical external reactant that will initiate growth. The initiation and completion of the growth phase can be controlled by the addition and amount of the initiating reactant added to the reaction vessel.

[00162] In accordance with certain embodiments, the chemical product can be 3-HP and the production organism can be *E. coli* or yeast. The critical external reactant may be phosphate, which may be needed for replication of *E. coli* cells. In one embodiment, the growth phase can be initiated by the addition of phosphate to a reaction vessel (together with a carbon source such as sugar and the *E. coli* cells), and the duration of the growth phase can be controlled by the amount of phosphate added to the system.

[00163] The induction phase can be controlled by genetic modifications to the producing organism. The key enzymes triggered during this phase can be engineered into the organism using promoters that can be sensitive to (*e.g.*, activated by) the depletion of the initiating reactant. As a result, once the initiating reactant is depleted, the growth phase ends, the key enzymes are activated and the induction phase begins.

[00164] In another embodiment, the induction phase can be controlled by key genes that encode for enzymes in the biosynthetic pathway for the product into the production organism using promoters that are activated by phosphate depletion. In one embodiment where the

chemical product is 3-HP and the production organism is *E. coli*, the key genetic modifications may include one or more of the following: *mcr*, *mmsB*, *ydfG*, *rutE*, *nemA* and *NDSD*; genes that encode individual or fused subunits of ACCase, such as *accA*, *accB*, *accC*, *accD*, *accDA* fusion, and *accCB* fusion, and the promoters may include one or more of the promoters that direct expression of the following *E. coli* genes: *amn*, *tktB*, *xasA*, *yibD*, *ytfK*, *pstS*, *phoH*, *phnC*, or other phosphate-regulated genes as described in Baek and Lee, FEMS Microbiol Lett 264: 104-109, 2006. In accordance with this embodiment, once the phosphate is depleted, expression of the key enzymes can be activated by their promoters and the induction phase begins.

[00165] The production phase may also be controlled by genetic modifications. For example, the organism can be engineered to included mutated forms of enzymes critical to the initiation of production of the chemical product. These initiation enzymes may facilitate initiation of production either by: (1) becoming active and serving a key function in the production pathway; and/or (2) becoming inactive and/or disrupted thereby turning off a branch pathway or other competitive pathway that prevents or limits the production pathway leading to the chemical product. In accordance with an embodiment, initiation enzymes can be mutated to form temperature sensitive variants of the enzymes that can be either activated by or deactivated at certain temperatures. As a result, the production phase can be initiated by changing the changing the temperature within the reaction vessel.

[00166] In one embodiment, the production phase can be controlled by genetically modifying the microorganism with a heterologous nucleotide sequence encoding one or more of the following temperature sensitive enzymes: *fabI*^{ts} (SEQ ID NO. 27), *fabB*^{ts} (SEQ ID NO.28) and *fabD*^{ts} (SEQ ID NO. 29). These enzymes can be deactivated or shut-off or altered to have decreased activity at the desired temperature for production of the chemical product. These enzymes can play a key role shuttling carbon atoms into the fatty acid synthesis pathway. Although fatty acid synthesis pathway can be critical during the growth phase, it can inhibit production of the chemical product. Once the reaction vessel temperature is changed, the temperature sensitive enzymes are deactivated and the fatty acid synthesis pathway shuts down thereby allowing the production pathway of the chemical product to ramp up.

[00167] In accordance with the present invention, the growth phase can last be between 10 to 40 hours, or about 15 to about 35 hours, or about 20 to about 30 hours. The induction phase may be for about 1 to about 6 hours, about 1 to about 5 hours, or about 2 to about 4 hours. The production phase may be greater than about: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50,

55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 hours depending on the amount of chemical product that is desired.

[00168] In accordance with the present invention, the growth phase and induction phase can be conducted at a temperature of about 25°C to about 35°C, about 28°C to about 32°C, or about 30 °C. The production phase can be conducted at a temperature of about 35°C to about 45°C, about 35°C to about 40°C, or about 36°C to about 38 °C. The production phase temperature can be higher than the induction phase temperature, and the increase in temperature that initiates the production phase can occur over a period of about 1 to about 5 hours, about 1 to about 3 hours, about 2 hours, or about 1 hour.

[00169] In accordance with the present invention, there is provided a method of producing a chemical product from a renewable carbon source through a bioproduction process comprising:

- (1) constructing a genetically modified organism capable of converting said renewable carbon source to said chemical product, wherein said genetically modified organism requires inorganic phosphate for growth and comprises: (a) at least one heterologous gene whose expression is regulated by a promoter sensitive to inorganic phosphate levels within a culture system, wherein said gene provides a critical function in converting said carbon source to said chemical product and is not required for the genetically modified organism to replicate; and (b) a gene encoding a temperature-sensitive enzyme;
- (2) forming a culture system comprising said carbon source in an aqueous medium and said genetically modified microorganism;
- (3) maintaining the culture system under conditions that allow the genetically modified microorganism to replicate comprising maintaining a sufficient level of inorganic phosphate within said culture system;
- (4) allowing the inorganic phosphate to deplete thereby triggering the expression of the gene regulated by a promoter sensitive to inorganic phosphate levels; and
- (5) changing the temperature of the culture system thereby activating or deactivating said temperature-sensitive enzyme and initiating the production of said chemical product.

[00170] In accordance with the present invention, there is provided a method of producing 3-hydropropionic acid (3-HP) from a renewable carbon source, comprising:

- (1) constructing a genetically modified organism capable of converting said renewable carbon source to 3-HP, wherein said genetically modified organism requires inorganic phosphate for growth and comprises: (a) at least one heterologous gene whose

expression is regulated by a promoter sensitive to inorganic phosphate levels within a culture system, wherein said gene is selected from the group consisting of mcr, mmsB, ydfG, rutE, nemA, NDSD, accA, accB, accC, accD, accDA fusion, and accCB fusion; and (b) a gene encoding a temperature-sensitive enzyme selected from the group consisting of fabI, fabB, fabD, and combinations thereof;

- (2) forming a culture system comprising said carbon source in an aqueous medium, phosphate and said genetically modified microorganism, and thereby initiating a growth phase during which the genetically modified microorganism replicates;
- (3) maintaining a sufficient level of inorganic phosphate within said culture system until the desired level of cell growth is achieved;
- (4) allowing the inorganic phosphate to deplete thereby initiating an induction phase which begins the expression of said gene regulated by a promoter sensitive to inorganic phosphate levels; and
- (5) changing the temperature of the culture system thereby activating or deactivating said temperature-sensitive enzyme and initiating a growth phase during which said genetically modified microorganism produces 3-HP.

[00171] Fermentation Conditions

[00172] Depending on the host cell fermentation may be performed under aerobic, microaerobic, or anaerobic conditions, with or without agitation. The operation of culture systems to achieve aerobic, microaerobic and anaerobic conditions are well known to those of ordinary skill in the art.

[00173] Suitable pH ranges for fermentation depend on the multiple factors such as the host cell. In some applications of the invention fermentation can occur between various pH ranges for example, pH 3.0 to pH 4.0, pH 4.0 to pH 5.0, pH 5.0 to pH 6.0, pH 6.0 to pH 7.0, pH 7.0 to pH 8.0, pH 8.0 to pH 9.0, or pH 9.0 to pH 10.0. However, the actual pH conditions for a particular application are not meant to be limited by these ranges and can be between the expressed pH ranges if it provides more optimal production of the fermentation process, such as increased 3-HP production.

[00174] VII. GENES AND PROTEINS FOR THE BIOPRODUCTION OF CHEMICALS

[00175] An overview of the engineered pathways provided by the invention in a host cell is shown in **FIG.1**. Various combinations of the pathways shown can be carried out by various combinations of genetic modifications to key enzymes either in the intrinsic pathways or supplied through the transformation of a heterologous gene.

[00176] In some applications of the genetically modified microorganism of the invention may comprise a single genetic modification, or one or more genetic modifications. Various types of genetic modifications that can be used with the invention are disclosed herein.

[00177] In some embodiments the genetic modified organism of the invention can comprise a genetic modification to the following gene/proteins or a homolog with at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homolog/identity to or a functional homolog of: bifunctional malonyl-CoA reductase (MCR from *Chloroflexus aurantiacus*), monofunctional malonyl-CoA reductase (caMCR from *Chloroflexus aurantiacus*), malonyl-CoA reductase (stMCR from *Sulfolobus tokodaii*), Enzyme: malonyl-CoA reductase (cgMCR from *Chloroflexus aggregans*), Enzyme: malonyl-CoA reductase (otMCR from *Oscillochloris trichoides*), Polypeptide: host restriction; endonuclease R (hsdR from *E. coli*), lactose metabolism (lac from *E. coli*), L-rhamnulose kinase (rhaB from *E. coli*), rhamnulose-1-phosphate aldolase (rhaD from *E. coli*), Enzyme: β -galactosidase (lacZ from *E. coli*), L-ribulose 5-phosphate 4-epimerase (araD from *E. coli*), L-ribulokinase (araB from *E. coli*), Enzyme: D-lactate dehydrogenase - fermentative (ldhA from *E. coli*), enzyme: pyruvate formate-lyase (pflB from *E. coli*), Enzyme: phosphate acetyltransferase / phosphate propionyltransferase (pta from *E. coli*), Enzyme: pyruvate oxidase (poxB from *E. coli*), Enzyme: methylglyoxal synthase (mgsA from *E. coli*), enzyme: Acetate kinase (ackA from *E. coli*), enzymes: phosphotransacetylase-acetate kinase (pta-ack from *E. coli*), Enzyme: enoyl-[acyl-carrier-protein] reductase (fabI from *E. coli*), Protein: zeocin binding protein (zeoR from *Streptoalloteichus Hindustanus*), Enzyme: carboxytransferase moiety of acetyl-CoA carboxylase (accAD from *E. coli*), Enzyme: triose phosphate isomerase (tpiA from *E. coli*), Enzyme: biotin carboxylase moiety of acetyl-CoA carboxylase (accBC from *E. coli*), Enzyme: transhydrogenase (pntAB from *E. coli*), Polypeptide: LacI DNA-binding transcriptional repressor (lacI from *E. coli*), Enzyme: β -ketoacyl-ACP synthases I (fabB from *E. coli*), Enzyme: β -ketoacyl-ACP synthases II (fabF from *E. coli*), Enzyme: malonyl-CoA-ACP transacylase (fabD from *E. coli*), Enzyme: pantothenate kinase (coaA from *E. coli*), Enzyme: pyruvate dehydrogenase complex (aceEF from *E. coli*), Enzyme: 3-hydroxyisobutyrate/3-HP dehydrogenase (mmsB from *Pseudomonas aeruginosa*), Enzyme: lipoamide dehydrogenase (lpd from *E. coli*), Enzyme: γ -glutamyl- γ -aminobutyraldehyde dehydrogenase (puuC from *E. coli*), Enzyme: malate synthase A (aceB from *E. coli*), Enzyme: isocitrate lyase (aceA from *E. coli*), Enzyme: isocitrate dehydrogenase phosphatase/kinase (aceK from *E. coli*), Enzyme: 3-hydroxy acid dehydrogenase (ydfG from *E. coli*), Enzyme: acetyl CoA carboxylase (accADBC from

E. coli), Polypeptide: predicted transcriptional regulator (yieP from *E. coli*), Blastocycin resistance gene (BSD from *Schizosaccharomyces pombe*), Enzyme: pyridine nucleotide transhydrogenase (udha from *E. coli*), Protein: Cra DNA-binding transcriptional dual regulator (fruR from *E. coli*), (SCB from *E. coli*), enzyme: aldehyde dehydrogenase B (aldB from *E. coli*), Enzyme: carbonic anhydrase (cynT from *E. coli*), Enzyme: cyanase (cynS from *E. coli*), DNA gyrase toxin-antitoxin system (ccdAB from *E. coli*), Enzyme: phosphoglycerate mutase (pgi from *E. coli*), ArcA transcriptional dual regulator or Aerobic respiration control (arcA from *E. coli*), Enzyme: 6-phosphofructokinase (pfk from *E. coli*), Enzyme: glyceraldehyde 3-phosphate dehydrogenase-A complex (gapA from *E. coli*), aldehyde dehydrogenase A (alda from *E. coli*), Enzyme: glutamate dehydrogenase (gdhA from *E. coli*), Enzyme: NADH-dependent serine dehydrogenase (NDSD from *Pseudomonas aeruginosa*), Protein: threonine/homoserine efflux transporter (rhtA from *E. coli*), Enzyme: glyceraldehyde 3-phosphate dehydrogenase (gapN from *E. coli*), Phosphotransferase system (pts from *E. coli*), Enzyme: 6-phosphofructokinase II (pfkB from *E. coli*), Enzyme: methylmalonate-semialdehyde dehydrogenase (mmsA from *Pseudomonas aeruginosa*), Oxaloacetate:beta-alanine aminotransferase (OAT-1 from *Bacillus cereus*), Enzyme: aspartate 1-decarboxylase (panD from *E. coli*), Gene that confers resistance to valine (ValR from *E. coli*), Enzyme: glucokinase (glk from *E. coli*), Polypeptide: 30 S ribosomal sununit protein S12 (rpsL from *E. coli*), Polypeptide: CynR DNA-binding transcriptional repressor (cynR from *E. coli*), Transporter: galactose:H⁺ symporter (galP from *E. coli*), aspartate aminotransferase (aspC from *E. coli*), Enzyme: alpha-ketoglutarate reductase (serA from *E. coli*), Enzyme: 6-phosphofructokinase I (pfkA from *E. coli*), Enzyme: phosphoenolpyruvate carboxylase (ppc from *E. coli*), Enzyme: succinate-semialdehyde dehydrogenase (NADP⁺) (gabD from *E. coli*), Enzyme: pyruvate kinase (pyk from *E. coli*), Enzyme: oxaloacetate 4-decarboxylase (OAD from *Leuconostoc mesenteroides*), Enzyme: trigger factor; a molecular chaperone involved in cell division (tig from *E. coli*), Transcription Unit (ptsHIcrr from *E. coli*), Enzyme: acetyl-CoA acetaldehyde dehydrogenase/alcohol dehydrogenase (adhE from *E. coli*), Enzyme: fattyacyl thioesterase I (tesA from *E. coli*), Enzyme: guanosine 3'-diphosphate 5'-triphosphate 3'-diphosphatase (spoT from *E. coli*), combination of genes encoding accABCD subunits (from *E. coli* and *Halomonas elongata*), pol (from *E. coli*), Enzyme: GDP pyrophosphokinase / GTP pyrophosphokinase (relA from *E. coli*), [Enzyme Name] (me from *E. coli*), Enzyme: citrate synthase (gltA from *E. coli*), Polypeptide: DNA gyrase, subunit A (gyrA from *E. coli*), Enzyme: multifunctional 2-keto-3-deoxygluconate 6-phosphate aldolase and 2-keto-4-

hydroxyglutarate aldolase and oxaloacetate decarboxylase (eda from *E. coli*), thiamin biosynthesis (thi from *E. coli*), Polypeptide: acetolactate synthase II (ilvG from *E. coli*), acetyl CoA carboxylase (accDACB from *E. coli*), Citrate synthase (ArCS from *Arthrobacter aureescens*), Acetyl-CoA carboxylase from *Corynebacter glutamicum* (CgACC from *Corynebacter glutamicum*), Polypeptide: ferrichrome / phage / antibiotic outer membrane porin FhuA (fhuA from *E. coli*), Transporter: phosphate:H⁺ symporter PitA (pitA from *E. coli*), Transporter: uracil:H⁺ symporter (uraA from *E. coli*), Enzyme: uracil phosphoribosyltransferase (upp from *E. coli*), Enzyme: acylphosphatase (yccX from *E. coli*), acetyl-CoA synthetase (acsA from *E. coli*), Polypeptide: restriction of methylated adenine (mrr from *E. coli*), Protein: TrpR transcriptional repressor (trpR from *E. coli*), Enzymes: glutamate 5-semialdehyde dehydrogenase / gamma-glutamyl kinase (proAB from *E. coli*), methylcytosine restriction system (mcrBC from *E. coli*), Protein: citrate lyase, citrate-ACP transferase component (citF from *E. coli*), Enzyme: thioesterase II (tesB from *E. coli*), Enzyme: DNA-specific endonuclease I (endA from *E. coli*), Enzyme: phosphate acetyltransferase (cutD from *E. coli*), Enzyme: propionate kinase (tdcD from *E. coli*), tRNA: tRNA glnV (supE from *E. coli*), Enzyme: DNA-binding, ATP-dependent protease La (lon from *E. coli*), Polypeptide: DNA strand exchange and recombination protein with protease and nuclease activity (recA from *E. coli*), Transcription Unit: restriction endonuclease component of EcoKI restriction-modification system (hsdRMS from *E. coli*), Enzyme: restriction of DNA at 5-methylcytosine residues (mcrA from *E. coli*) araD (from *E. coli*), araB (from *E. coli*), rhaD (from *E. coli*), rhaB (from *E. coli*), ack (from *E. coli*), fruR (from *E. coli*), gapA (from *E. coli*), lacI (from *E. coli*), lacZ (from *E. coli*), ldhA (from *E. coli*), mgsA (from *E. coli*), pfkA (from *E. coli*), pflB (from *E. coli*), pgi (from *E. coli*), poxB (from *E. coli*), pta-ack (from *E. coli*), ptsH (from *E. coli*), glut1 (from *E. coli*) and/ or ack (from *E. coli*) or any combination thereof.

[00178] The use of genetic modifications in genetic elements, genes, proteins or the use of compounds, such as siRNA technology, anti-sense technology, and small molecule inhibitors supplied to the host cell that modulate the expression of gene and proteins provided by the present invention are also contemplated.

[00179] In some embodiments the genetic modified organism of the invention uses genetic elements such as siRNA or equivalent (including but not limited to shRNA, RNAi, miRNA, etc.), genes, proteins or compounds supplied to the host cell to modulate one or more of the following: bifunctional malonyl-CoA reductase (MCR from *Chloroflexus aurantiacus*), monofunctional malonyl-CoA reductase (caMCR from *Chloroflexus aurantiacus*), malonyl-

CoA reductase (stMCR from *Sulfolobus tokodaii*), Enzyme: malonyl-CoA reductase (cgMCR from *Chloroflexus aggregans*), Enzyme: malonyl-CoA reductase (otMCR from *Oscillochloris trichoides*), Polypeptide: host restriction; endonuclease R (hsdR from *E. coli*), lactose metabolism (lac from *E. coli*), L-rhamnulose kinase (rhaB from *E. coli*), rhamnulose-1-phosphate aldolase (rhaD from *E. coli*), Enzyme: β -galactosidase (lacZ from *E. coli*), L-ribulose 5-phosphate 4-epimerase (araD from *E. coli*), L-ribulokinase (araB from *E. coli*), Enzyme: D-lactate dehydrogenase - fermentative (ldhA from *E. coli*), enzyme: pyruvate formate-lyase (pflB from *E. coli*), Enzyme: phosphate acetyltransferase / phosphate propionyltransferase (pta from *E. coli*), Enzyme: pyruvate oxidase (poxB from *E. coli*), Enzyme: methylglyoxal synthase (mgsA from *E. coli*), enzyme: Acetate kinase (ackA from *E. coli*), enzymes: phosphotransacetylase-acetate kinase (pta-ack from *E. coli*), Enzyme: enoyl-[acyl-carrier-protein] reductase (fabI from *E. coli*), Protein: zeocin binding protein (zeoR from *Streptoalloteichus Hindustanus*), Enzyme: carboxytransferase moiety of acetyl-CoA carboxylase (accAD from *E. coli*), Enzyme: triose phosphate isomerase (tpiA from *E. coli*), Enzyme: biotin carboxylase moiety of acetyl-CoA carboxylase (accBC from *E. coli*), Enzyme: transhydrogenase (pntAB from *E. coli*), Polypeptide: LacI DNA-binding transcriptional repressor (lacI from *E. coli*), Enzyme: β -ketoacyl-ACP synthases I (fabB from *E. coli*), Enzyme: β -ketoacyl-ACP synthases II (fabF from *E. coli*), Enzyme: malonyl-CoA-ACP transacylase (fabD from *E. coli*), Enzyme: pantothenate kinase (coaA from *E. coli*), Enzyme: pyruvate dehydrogenase complex (aceEF from *E. coli*), Enzyme: 3-hydroxyisobutyrate/3-HP dehydrogenase (mmsB from *Pseudomonas aeruginosa*), Enzyme: lipoamide dehydrogenase (lpd from *E. coli*), Enzyme: γ -glutamyl- γ -aminobutyraldehyde dehydrogenase (puuC from *E. coli*), Enzyme: malate synthase A (aceB from *E. coli*), Enzyme: isocitrate lyase (aceA from *E. coli*), Enzyme: isocitrate dehydrogenase phosphatase/kinase (aceK from *E. coli*), Enzyme: 3-hydroxy acid dehydrogenase (ydfG from *E. coli*), Enzyme: acetyl CoA carboxylase (accADBC from *E. coli*), Polypeptide: predicted transcriptional regulator (yieP from *E. coli*), Blastocycin resistance gene (BSD from *Schizosaccharomyces pombe*), Enzyme: pyridine nucleotide transhydrogenase (udha from *E. coli*), Protein: Cra DNA-binding transcriptional dual regulator (fruR from *E. coli*), (SCB from *E. coli*), enzyme: aldehyde dehydrogenase B (aldB from *E. coli*), Enzyme: carbonic anhydrase (cynT from *E. coli*), Enzyme: cyanase (cynS from *E. coli*), DNA gyrase toxin-antitoxin system (ccdAB from *E. coli*), Enzyme: phosphoglycerate mutase (pgi from *E. coli*), ArcA transcriptional dual regulator or Aerobic respiration control (arcA from *E. coli*), Enzyme: 6-phosphofructokinase (pfk from *E. coli*), Enzyme: glyceraldehyde 3-phosphate dehydrogenase-A complex (gapA

from *E. coli*), aldehyde dehydrogenase A (alda from *E. coli*), Enzyme: glutamate dehydrogenase (gdhA from *E. coli*), Enzyme: NADH-dependent serine dehydrogenase (NDSH from *Pseudomonas aeruginosa*), Protein: threonine/homoserine efflux transporter (rhtA from *E. coli*), Enzyme: glyceraldehyde 3-phosphate dehydrogenase (gapN from *E. coli*), Phosphotransferase system (pts from *E. coli*), Enzyme: 6-phosphofructokinase II (pfkB from *E. coli*), Enzyme: methylmalonate-semialdehyde dehydrogenase (mmsA from *Pseudomonas aeruginosa*), Oxaloacetate:beta-alanine aminotransferase (OAT-1 from *Bacillus cereus*), Enzyme: aspartate 1-decarboxylase (panD from *E. coli*), Gene that confers resistance to valine (ValR from *E. coli*), Enzyme: glucokinase (glk from *E. coli*), Polypeptide: 30 S ribosomal subunit protein S12 (rpsL from *E. coli*), Polypeptide: CynR DNA-binding transcriptional repressor (cynR from *E. coli*), Transporter: galactose:H⁺ symporter (galP from *E. coli*), aspartate aminotransferase (aspC from *E. coli*), Enzyme: alpha-ketoglutarate reductase (serA from *E. coli*), Enzyme: 6-phosphofructokinase I (pfkA from *E. coli*), Enzyme: phosphoenolpyruvate carboxylase (ppc from *E. coli*), Enzyme: succinate-semialdehyde dehydrogenase (NADP⁺) (gabD from *E. coli*), Enzyme: pyruvate kinase (pyk from *E. coli*), Enzyme: oxaloacetate 4-decarboxylase (OAD from *Leuconostoc mesenteroides*), Enzyme: trigger factor; a molecular chaperone involved in cell division (tig from *E. coli*), Transcription Unit (ptsHICrr from *E. coli*), Enzyme: acetyl-CoA acetaldehyde dehydrogenase/alcohol dehydrogenase (adhE from *E. coli*), Enzyme: fattyacyl thioesterase I (tesA from *E. coli*), Enzyme: guanosine 3'-diphosphate 5'-triphosphate 3'-diphosphatase (spoT from *E. coli*), combination of genes encoding accABCD subunits (from *E. coli* and *Halomonas elongata*), pol (from *E. coli*), Enzyme: GDP pyrophosphokinase / GTP pyrophosphokinase (relA from *E. coli*), [Enzyme Name] (me from *E. coli*), Enzyme: citrate synthase (gltA from *E. coli*), Polypeptide: DNA gyrase, subunit A (gyrA from *E. coli*), Enzyme: multifunctional 2-keto-3-deoxygluconate 6-phosphate aldolase and 2-keto-4-hydroxyglutarate aldolase and oxaloacetate decarboxylase (eda from *E. coli*), thiamin biosynthesis (thi from *E. coli*), Polypeptide: acetolactate synthase II (ilvG from *E. coli*), acetyl CoA carboxylase (accDACB from *E. coli*), Citrate synthase (ArCS from *Arthrobacter aureus*), Acetyl-CoA carboxylase from *Corynebacter glutamicum* (CgACC from *Corynebacter glutamicum*), Polypeptide: ferrichrome / phage / antibiotic outer membrane porin FhuA (fhuA from *E. coli*), Transporter: phosphate:H⁺ symporter PitA (pitA from *E. coli*), Transporter: uracil:H⁺ symporter (uraA from *E. coli*), Enzyme: uracil phosphoribosyltransferase (upp from *E. coli*), Enzyme: acylphosphatase (yccX from *E. coli*), acetyl-CoA synthetase (acsA from *E. coli*), Polypeptide: restriction of methylated adenine

(mrr from *E. coli*), Protein: TrpR transcriptional repressor (trpR from *E. coli*), Enzymes: glutamate 5-semialdehyde dehydrogenase / gamma-glutamyl kinase (proAB from *E. coli*), methylcytosine restriction system (mcrBC from *E. coli*), Protein: citrate lyase, citrate-ACP transferase component (citF from *E. coli*), Enzyme: thioesterase II (tesB from *E. coli*), Enzyme: DNA-specific endonuclease I (endA from *E. coli*), Enzyme: phosphate acetyltransferase (cutD from *E. coli*), Enzyme: propionate kinase (tdcD from *E. coli*), tRNA: tRNA glnV (supE from *E. coli*), Enzyme: DNA-binding, ATP-dependent protease La (lon from *E. coli*), Polypeptide: DNA strand exchange and recombination protein with protease and nuclease activity (recA from *E. coli*), Transcription Unit: restriction endonuclease component of EcoKI restriction-modification system (hsdRMS from *E. coli*), Enzyme: restriction of DNA at 5-methylcytosine residues (mcrA from *E. coli*). In some embodiments the genetic modifications listed above are modified further with the genetic modules provided herein.

[00180] In some embodiments the genetic modification of the genes, proteins and enzymes of the invention can be for the method of bioproduction of various chemicals which can be used to make various consumer products, including but not limited to those described herein.

[00181] In some embodiments the genetic modification of the genes, proteins and enzymes of the invention can be for the bioproduction of 1,4-butanediol (1,4-BDO) . *See, e.g.*, U.S. Pub. No. 20110190513. In some embodiments the genetic modification of the genes, proteins and enzymes of the invention can be for the bioproduction of butanol. *See, e.g.*, U.S. App. No. 13/057,359. In some embodiments, the genetic modification of the genes, proteins and enzymes of the invention can be for the bioproduction of isobutanol. *See, e.g.*, U.S. App. No. 13/057,359.

[00182] In some embodiments the genetic modification of the genes, proteins and enzymes of the invention can be for the bioproduction of 3-HP such and its aldehyde metabolites. *See, e.g.*, U.S. App. No. 13/062,917.

[00183] In some embodiments the genetic modification of the genes, proteins and enzymes of the invention can be for the bioproduction of polyketide chemical products. *See, e.g.*, U.S. App. No. 13/575,581.

[00184] In some embodiments the genetic modification of the genes, proteins and enzymes of the invention can be for the bioproduction of fatty acid methyl esters. *See e.g.*, U.S. Pub. No. 20110124063. In some embodiments the genetic modification of the genes, proteins and enzymes of the invention can be for the bioproduction of C4-C18 fatty acids. *See, e.g.*, U.S. App No. 61/682,127.

[00185] Genetic Modifications

[00186] Various methods to achieve such genetic modification in a host strain are known to one skilled in the art. Example of genetic modifications that can be used by the claimed invention include, but are not limited to, increasing expression of an endogenous genetic element; increasing expression of an exogenous genetic element; decreasing functionality of a repressor gene; increasing functionality of a repressor gene; increasing functionality of an activator gene; decreasing functionality of an activator gene; introducing a genetic change or element integrated in the host genome, introducing a heterologous genetic element permanently, by integration into the genome or transiently by transformation with plasmid; increasing copy number of a nucleic acid sequence encoding a polypeptide catalyzing an enzymatic conversion step; mutating a genetic element to provide a mutated protein to increase specific enzymatic activity; mutating a genetic element to provide a mutated protein to decrease specific enzymatic activity; over-expressing of a gene; reducing the expression of a gene; knocking out or deleting a gene; altering or modifying feedback inhibition; providing an enzyme variant comprising one or more of an impaired binding sites or active sites; increasing functionality of a siRNA or equivalent, decreasing functionality of a siRNA or equivalent, increasing functionality of an antisense molecule, decreasing functionality of an antisense molecule, addition of genetic modules such as RBS, '3 UTR elements to increase mRNA stability or translation; deletion of genetic modules such as RBS, '3 UTR elements to decrease mRNA stability or translation; addition or modification of genetic modules such as '5 UTR elements to increase transcription; deletion or modification of genetic modules such as '5 UTR and elements to increase transcription. In addition other genetic modules, provide herein, such a multicopy plasmids and various promoters can be used to further modify of the genetic modifications provide herein. Additionally, as known to those of ordinary skill in the art compounds such as siRNA technology, anti-sense technology, and small molecule inhibitors can be used to alter gene expression in the same manner as a genetic modification.

[00187] Screening methods, such as SCALE in combination with random mutagenesis may be practiced to provide genetic modifications that provide a benefit to increased production of 3-HP in a host cell. Examples of random mutagenesis can include insertions, deletions and substitutions of one or more nucleic acids in a nucleic acid of interest. In various embodiments, a genetic modification results in improved enzymatic specific activity and/or turnover number of an enzyme. Without being limited in any way, changes may be measured by one or more of the following: KM; Kcat, Kavidity, gene expression level, protein

expression level, level of a product known to be produced by the enzyme, 3-HP tolerance, or by 3-HP production or by any other known means.

[00188] Host Cells

[00189] In some applications of the invention the host cell can be a gram-negative bacterium. In some applications of the invention the host cell can be from *Zymomonas*, *Escherichia*, *Pseudomonas*, *Alcaligenes*, or *Klebsiella*. In some applications of the invention the host cell can be *Escherichia coli*, *Cupriavidus necator*, *Oligotropha carboxidovorans*, or *Pseudomonas putida*. In some applications of the invention the host cell is one or more *E. coli* strains.

[00190] In some applications of the invention the host cell can be a gram-positive bacterium. In some applications of the invention the host cell can be from *Clostridium*, *Salmonella*, *Rhodococcus*, *Bacillus*, *Lactobacillus*, *Enterococcus*, *Paenibacillus*, *Arthrobacter*, *Corynebacterium*, or *Brevibacterium*. In some applications of the invention the host cell is *Bacillus licheniformis*, *Paenibacillus macerans*, *Rhodococcus erythropolis*, *Lactobacillus plantarum*, *Enterococcus faecium*, *Enterococcus gallinarum*, *Enterococcus faecalis*, or *Bacillus subtilis*. In some applications of the invention the host cell is *B. subtilis* strain.

[00191] In some applications of the invention the host cell can be yeast. In some applications of the invention the host cell can be from *Pichia*, *Candida*, *Hansenula* or *Saccharomyces*. In some applications of the invention the host cell is *Saccharomyces cerevisiae*. In some applications of the invention the host cell is *Saccharomyces pombe*.

[00192] In some applications of the invention the host cell can be an alga. In some applications of the invention the host cell can be a halophile. In some applications of the invention the host cell can be an alga. In some applications of the invention the host cell is a chemolithotrophic bacterium.

[00193] In some applications of the invention the host cell can be comprised of multiple host cell types. In some applications of the invention the host cell is comprised of one host cell type. In some applications of the invention the host cell is comprised of one more species or strain of a host cell type.

[00194] VIII. DOWNSTREAM CONSUMER PRODUCTS CHEMICALS

[00195] 3-HP purified according to the methods provided in this disclosure may be converted to various other products having industrial uses including, but not limited to, acrylamide, acrylic acid, esters of acrylic acid, 1,3-propanediol, and other chemicals, collectively referred to as “downstream chemical products” or “downstream products.” In some instances, the conversion can be associated with a separation and/or purification process. These

downstream chemical products can be useful for producing a variety of consumer products which are described in more detail below. The methods of the present invention can include steps to produce downstream products of 3-HP.

[00196] As a C3 building block, 3-HP can offer the potential for a variety of chemical conversions into commercially important intermediates, industrial end products, and consumer products. For example, 3-HP may be converted to acrylic acid, acrylates (*e.g.*, acrylic acid salts and esters), 1,3-propanediol, malonic acid, ethyl-3-hydroxypropionate, ethyl ethoxy propionate, propiolactone, acrylamide, or acrylonitrile.

[00197] Additionally, 3-HP may be oligomerized or polymerized to form poly(3-hydroxypropionate) homopolymers, or co-polymerized with one or more other monomers to form various co-polymers. Because 3-HP has a single stereoisomer, polymerization of 3-HP may not be complicated by the stereo-specificity of monomers during chain growth. This can be contrasted with (S)-2-hydroxypropanoic acid (also known as lactic acid), which has two (D, L) stereoisomers that should be considered during its polymerizations.

[00198] As will be further described, 3-HP can be converted into derivatives starting (i) substantially as the protonated form of 3-hydroxypropionic acid; (ii) substantially as the deprotonated form, 3-hydroxypropionate; or (iii) as mixtures of the protonated and deprotonated forms. Generally, the fraction of 3-HP present as the acid versus the salt can depend on the pH, the presence of other ionic species in solution, temperature (which can change the equilibrium constant relating the acid and salt forms), and, to some extent, pressure. Many chemical conversions may be carried out from either of the 3-HP forms, and overall process economics can likely dictate the form of 3-HP for downstream conversion.

[00199] Acrylic acid obtained from 3-HP purified by the methods described in this disclosure may be further converted to various polymers. For example, the free-radical polymerization of acrylic acid can take place by polymerization methods known to the skilled worker and can be carried out, for example, in an emulsion or suspension in aqueous solution or another solvent. Initiators, such as but not limited to organic peroxides, can be often added to aid in the polymerization. Among the classes of organic peroxides that may be used as initiators can be diacyls, peroxydicarbonates, monoperoxy carbonates, peroxyketals, peroxyesters, dialkyls, and hydroperoxides. Another class of initiators can be azo initiators, which may be used for acrylate polymerization as well as co-polymerization with other monomers. U.S. Patent Nos. 5,470,928; 5,510,307; 6,709,919; and 7,678,869 teach various approaches to polymerization using a number of initiators, including organic peroxides, azo compounds, and other chemical types.

[00200] Accordingly, it can be further possible for co-monomers, such as crosslinkers, to be present during the polymerization. The free-radical polymerization of the acrylic acid obtained from dehydration of 3-HP, as produced herein, in at least partly neutralized form and in the presence of crosslinkers can be practiced in certain embodiments. This polymerization may result in hydrogels which can then be comminuted, ground and, where appropriate, surface-modified, by known techniques.

[00201] An important commercial use of polyacrylic acid can be for superabsorbent polymers. *See e.g.*, Modern Superabsorbent Polymer Technology, Buchholz and Graham (Editors), Wiley-VCH, 1997. Superabsorbent polymers can be used as absorbents for water and aqueous solutions for diapers, adult incontinence products, feminine hygiene products, and similar consumer products. In such consumer products, superabsorbent materials can replace traditional absorbent materials such as cloth, cotton, paper wadding, and cellulose fiber. Superabsorbent polymers can absorb, and retain under a slight mechanical pressure, up to 25 times or more their weight in liquid. The swollen gel can hold the liquid in a solid, rubbery state and can prevent the liquid from leaking. Superabsorbent polymer particles can be surface-modified to produce a shell structure with the shell being more highly cross-linked than the rest of the particle. This technique can improve the balance of absorption, absorption under load, and resistance to gel-blocking. It is recognized that superabsorbent polymers can have uses in fields other than consumer products, including agriculture, horticulture, and medicine.

[00202] Superabsorbent polymers can be prepared from acrylic acid (such as acrylic acid derived from 3-HP provided herein) and a crosslinker, by solution or suspension polymerization. Exemplary methods include those provided in U.S. Patent Nos. 5,145,906; 5,350,799; 5,342,899; 4,857,610; 4,985,518; 4,708, 997; 5,180,798; 4,666,983; 4,734,478; and 5,331,059.

[00203] Among consumer products, a diaper, a feminine hygiene product, and an adult incontinence product can be made with superabsorbent polymer that itself can be made substantially from acrylic acid converted from 3-HP made in accordance with the present invention.

[00204] Diapers and other personal hygiene products may be produced that incorporate superabsorbent polymers that can be made from acrylic acid that can be made from 3-HP which can be produced and purified by the teachings of the present application. The following can provide general guidance for making a diaper that incorporates such superabsorbent polymer. The superabsorbent polymer first can be molded into an absorbent

pad that may be vacuum formed, and in which other materials, such as a fibrous material (*e.g.*, wood pulp) can be added. The absorbent pad then can be assembled with sheet(s) of fabric, generally a nonwoven fabric (*e.g.*, that can be made from one or more of nylon, polyester, polyethylene, and polypropylene plastics) to form diapers.

[00205] More particularly, in one non-limiting process, multiple pressurized nozzles, located above a conveyer belt, can spray superabsorbent polymer particles (*e.g.*, about 400 micron size or larger), fibrous material, and/or a combination of these onto the conveyer belt at designated spaces/intervals. The conveyer belt can be perforated and under vacuum from below, so that the sprayed on materials can be pulled toward the belt surface to form a flat pad. In various embodiments, fibrous material can be applied first on the belt, followed by a mixture of fibrous material and the superabsorbent polymer particles, followed by fibrous material, so that the superabsorbent polymer can be concentrated in the middle of the pad. A leveling roller may be used toward the end of the belt path to yield pads of uniform thickness. Each pad thereafter may be further processed, such as to cut it to a proper shape for the diaper, or the pad may be in the form of a long roll sufficient for multiple diapers. Thereafter, the pad can be sandwiched between a top sheet and a bottom sheet of fabric (one generally being liquid pervious, the other liquid impervious), for example, on a conveyor belt, and these can be attached together, for example by gluing, heating or ultrasonic welding, and cut into diaper-sized units (if not previously so cut). Additional features may be provided, such as elastic components, strips of tape, etc., for fit and ease of wearing by a person.

[00206] The ratio of the fibrous material to polymer particles can affect performance characteristics. In some cases, this ratio can be between 75:25 and 90:10 (see *e.g.*, U.S. Patent No. 4,685,915). Other disposable absorbent articles may be constructed in a similar fashion, such as absorbent articles for adult incontinence, feminine hygiene (sanitary napkins), tampons, etc. (see, for example, U.S. Patent Nos. 5,009,653; 5,558,656; and 5,827,255).

[00207] Low molecular weight polyacrylic acid can have uses for water treatment, and as a flocculant and thickener for various applications including cosmetics and pharmaceutical preparations. For these applications, the polymer may be uncrosslinked or lightly cross-linked, depending on the specific application. The molecular weights can be typically from about 200 to about 1,000,000 g/mol. An example of the preparation of these low molecular weight polyacrylic acid polymers is described in U.S. Patent Nos. 3,904,685; 4,301,266; 2,798,053; and 5,093,472.

[00208] Acrylic acid may be co-polymerized with one or more other monomers selected from the group consisting of: acrylamide, 2-acrylamido-2-methylpropanesulfonic acid, N,N-

dimethylacrylamide, N-isopropylacrylamide, methacrylic acid, and methacrylamide, to name a few. The relative reactivity of the monomers can affect the microstructure and thus the physical properties of the polymer. Co-monomers may be derived from 3-HP, or otherwise provided, to produce co-polymers. *See e.g.*, Ullmann's Encyclopedia of Industrial Chemistry, Polyacrylamides and Poly(Acrylic Acids), WileyVCH Verlag GmbH, Weinheim (2005).

[00209] Acrylic acid can in principle be copolymerized with almost any free-radically polymerizable monomers including styrene, butadiene, acrylonitrile, acrylic esters, maleic acid, maleic anhydride, vinyl chloride, acrylamide, itaconic acid, and so on. End-use applications typically dictate the co-polymer composition, which can influence properties. Acrylic acid also may have a number of optional substitutions and, after such substitutions, may be used as a monomer for polymerization, or co-polymerization reactions. As a general rule, acrylic acid (or one of its co-polymerization monomers) may be substituted by any substituent that does not interfere with the polymerization process, such as alkyl, alkoxy, aryl, heteroaryl, benzyl, vinyl, allyl, hydroxy, epoxy, amide, ethers, esters, ketones, maleimides, succinimides, sulfoxides, glycidyl, and silyl (*see e.g.*, U.S. Patent No. 7,678,869). The following paragraphs provide a few non-limiting examples of copolymerization applications.

[00210] Paints that comprise polymers and copolymers of acrylic acid and its esters are in wide use as industrial and consumer products. Aspects of the technology for making such paints can be found in *e.g.*, U.S. Patent Nos. 3,687,885 and 3,891,591. Generally, acrylic acid and its esters may form homopolymers or copolymers among themselves or with other monomers, such as amides, methacrylates, acrylonitrile, vinyl, styrene and butadiene. A desired mixture of homopolymers and/or copolymers, referred to in the paint industry as "vehicle" (or "binder") can be added to an aqueous solution and agitated sufficiently to form an aqueous dispersion that can include sub-micrometer sized polymer particles. The paint can cure by coalescence of these vehicle particles as the water and any other solvent evaporate. Other additives to the aqueous dispersion may include pigment, filler (*e.g.*, calcium carbonate, aluminum silicate), solvent (*e.g.*, acetone, benzol, alcohols, etc., although these are not found in certain no VOC paints), thickener, and additional additives depending on the conditions, applications, intended surfaces, etc. In many paints, the weight percent of the vehicle portion may range from about nine to about 26 percent, but for other paints the weight percent may vary beyond this range.

[00211] Acrylic-based polymers can be used for many coatings in addition to paints. For example, for paper coating latexes, acrylic acid can be used from 0.1-5.0%, along with styrene and butadiene, to enhance binding to the paper and modify rheology, freeze-thaw

stability and shear stability. *See e.g.*, U.S. Patent Nos. 3,875,101 and 3,872,037. Acrylate-based polymers can be used in many inks, particularly UV curable printing inks. For water treatment, acrylamide and/or hydroxy ethyl acrylate can be co-polymerized with acrylic acid to produce low molecular-weight linear polymers. *See e.g.*, U.S. Patent Nos. 4,431,547 and 4,029,577. Co-polymers of acrylic acid with maleic acid or itaconic acid can also be produced for water-treatment applications, as described in U.S. Patent No. 5,135,677. Sodium acrylate (the sodium salt of glacial acrylic acid) can be co-polymerized with acrylamide (which may be derived from acrylic acid via amidation chemistry) to make an anionic co-polymer that can be used as a flocculant in water treatment.

[00212] For thickening agents, a variety of co-monomers can be used, such as those described in U.S. Patent Nos. 4,268,641 and 3,915,921. U.S. Patent No. 5,135,677 describes a number of co-monomers that can be used with acrylic acid to produce water-soluble polymers.

[00213] In some cases, conversion to downstream products may be made enzymatically. For example, 3-HP may be converted to 3-HP-CoA, which then may be converted into polymerized 3-HP with an enzyme having polyhydroxy acid synthase activity (EC 2.3.1.-). Also, 1,3-propanediol can be made using polypeptides having oxidoreductase activity or reductase activity (*e.g.*, enzymes in the EC 1.1.1.- class of enzymes). Alternatively, when creating 1,3-propanediol from 3-HP, a combination of (1) a polypeptide having aldehyde dehydrogenase activity (*e.g.*, an enzyme from the 1.1.1.34 class) and (2) a polypeptide having alcohol dehydrogenase activity (*e.g.*, an enzyme from the 1.1.1.32 class) can be used. Polypeptides having lipase activity may be used to form esters. Enzymatic reactions such as these may be conducted *in vitro*, such as using cell-free extracts, or *in vivo*.

[00214] Thus, various embodiments described in this disclosure, such as methods of making a chemical, can include conversion steps to any downstream products of microbially produced 3-HP, including but not limited to those chemicals described herein and known in the art. For example, in some cases, 3-HP can be produced and can be converted to polymerized-3-HP (poly-3-HP) or acrylic acid. In some cases, 3-HP or acrylic acid can be used to produce polyacrylic acid (polymerized acrylic acid, in various forms), methyl acrylate, acrylamide, acrylonitrile, propiolactone, ethyl 3-HP, malonic acid, 1,3-propanediol, ethyl acrylate, n-butyl acrylate, hydroxypropyl acrylate, hydroxyethyl acrylate, isobutyl acrylate, 2-ethylhexyl acrylate, and acrylic acid or an acrylic acid ester to which an alkyl or aryl addition may be made, and/or to which halogens, aromatic amines or amides, and aromatic hydrocarbons may be added.

[00215] Reactions that form downstream compounds such as acrylates or acrylamides can be conducted in conjunction with use of suitable stabilizing agents or inhibiting agents reducing the likelihood of polymer formation. *See*, for example, U.S. Publication No. 2007/0219390. Stabilizing agents and/or inhibiting agents include, but are not limited to, *e.g.*, phenolic compounds (*e.g.*, dimethoxyphenol (DMP) or alkylated phenolic compounds such as di-*tert*-butyl phenol), quinones (*e.g.*, *t*-butyl hydroquinone or the monomethyl ether of hydroquinone (MEHQ)), and/or metallic copper or copper salts (*e.g.*, copper sulfate, copper chloride, or copper acetate). Inhibitors and/or stabilizers can be used individually or in combinations as will be known by those of skill in the art.

[00216] In some cases, the one or more downstream compounds can be recovered at a molar yield of up to about 100 percent, or a molar yield in the range from about 70 percent to about 90 percent, or a molar yield in the range from about 80 percent to about 100 percent, or a molar yield in the range from about 90 percent to about 100 percent. Such yields may be the result of single-pass (batch or continuous) or iterative separation and purification steps in a particular process.

[00217] The methods described in this disclosure can also be used to produce downstream compounds derived from 3-HP, such as but not limited to, polymerized-3-HP (poly-3-HP), acrylic acid, polyacrylic acid (polymerized acrylic acid, in various forms), copolymers of acrylic acid and acrylic esters, acrylamide, acrylonitrile, propiolactone, ethyl 3-HP, malonic acid, and 1,3-propanediol. Also, among esters that are formed are methyl acrylate, ethyl acrylate, *n*-butyl acrylate, hydroxypropyl acrylate, hydroxyethyl acrylate, isobutyl acrylate, and 2-ethylhexyl acrylate. These and/or other acrylic acid and/or other acrylate esters may be combined, including with other compounds, to form various known acrylic acid-based polymers. Numerous approaches may be employed for such downstream conversions, generally falling into enzymatic, catalytic (chemical conversion process using a catalyst), thermal, and combinations thereof (including some wherein a desired pressure is applied to accelerate a reaction). For example, without being limiting, acrylic acid may be made from 3-HP via a dehydration reaction, methyl acrylate may be made from 3-HP via dehydration and esterification, the latter to add a methyl group (such as using methanol), acrylamide may be made from 3-HP via dehydration and amidation reactions, acrylonitrile may be made via a dehydration reaction and forming a nitrile moiety, propiolactone may be made from 3-HP via a ring-forming internal esterification reaction, ethyl-3-HP may be made from 3-HP via esterification with ethanol, malonic acid may be made from 3-HP via an oxidation reaction, and 1,3-propanediol may be made from 3-HP via a reduction reaction. Additionally, it is

appreciated that various derivatives of the derivatives of 3-HP and acrylic acid may be made, such as the various known polymers of acrylic acid and its derivatives. Production of such polymers can be considered within the scope of the present invention. Copolymers containing acrylic acid and/or esters have been widely used in the pharmaceutical formulation to achieve extended or sustained release of active ingredients, for example as coating material. Downstream compounds may also be converted to consumer products such as diapers, carpet, paint, and adhesives.

[00218] Another important product, acrylamide, has been used in a number of industrial applications. Acrylamide may be produced from 3-HP, for example, without being limiting, via an esterification-amidation-dehydration sequence. Refluxing an alcohol solution of 3-HP in the presence of an acid or Lewis acid catalyst described herein can lead to a 3-HP ester. Treatment of the 3-HP ester with either an ammonia gas or an ammonium ion could yield 3-HP amide. Finally, dehydration of the 3-HP amide with dehydration reagents described elsewhere in this disclosure could produce acrylamide. The steps mentioned herein may be rearranged to produce the same final product acrylamide. Polymerization of acrylamide can be achieved, for example, and without being limiting, by radical polymerization.

Polyacrylamide polymers have been widely used as additives for treating municipal drinking water and waste water. In addition, they have found applications in gel electrophoresis, oil-drilling, papermaking, ore processing, and the manufacture of permanent press fabrics.

[00219] IX. EXPRESSION SYSTEMS GENERAL CONCEPTS

[00220] The following general concepts can be applicable to the embodiments of the invention described above.

[00221] Multicopy Plasmids

[00222] The researcher is faced with a myriad of genetic module options when designing a plasmid for expression of a heterologous protein in a host cell. How to optimize an expression plasmid system often depends on the downstream use of the expressed protein.

[00223] Different cloning vectors or plasmids are maintained at different copy numbers, dependent on the replicon of the plasmid. Most general cloning plasmids can carry a DNA insert up to about 15 kb in size.

[00224] Multicopy plasmids can be used for the expression of recombinant genes in *Escherichia coli*. Examples of include multicopy plasmids include high-copy, medium-copy and low-copy plasmids (see *e.g.*, FIG. 8). The high copy number plasmid is generally desired for maximum gene expression. However, the metabolic burden effects can result from multiple plasmid copies could prove to be detrimental for maximum productivity in

certain metabolic engineering applications by adding significant metabolic burden to the system.

[00225] The low-copy plasmids, for example, pBR322 is based on the original ColE1 replicon and thus has a copy number of about 15-20. The pACYC series of plasmids are based on the p15A replicon, which has a copy number of about 18-22, whereas pSC101 has even a lower copy number of about 5, and BACs are typically maintained at one copy per cell. Such low copy plasmids may be useful in metabolic engineering applications, particularly when one or more of the substrates used in the recombinant pathway are required for normal cellular metabolism and can be toxic to the cell at high levels.

[00226] However, the use of high-copy plasmids may be useful in enhanced cellular metabolism contexts. The mutant ColEI replicon, as found in the pUC series of plasmids produces a copy number of about 500-700 as a result of a point mutation within the RNAII regulatory molecule.

[00227] There are transcription and translation vectors. Transcription vectors are utilized when the DNA to be cloned has an ATG start codon and a prokaryotic ribosome-binding site. Translation vectors contain an efficient ribosome-binding site and, therefore, it is not necessary for the target DNA to contain one. This is particularly useful in cases where the initial portion of the gene may be cleaved in an effort to improve solubility. Another consideration when choosing a transcription or translation vector is the source of the DNA to be expressed. Prokaryotic genes usually have a ribosome-binding site that is compatible with the host *E. coli* translation machinery, whereas eukaryotic genes do not. Normal prokaryotic gene expression may be enhanced by use of an engineered promoter and ribosome-binding site.

[00228] Promoters

[00229] A promoter is a region of DNA that initiates transcription of a particular gene. In bacteria, transcription is initiated by the promoter being recognized by RNA polymerase and an associated sigma factor, which are often brought to the promoter site by an activator protein's binding to its own DNA binding site located proximal or distal (*e.g.*, near) to the promoter.

[00230] Promoter selection is an important factor when designing an expression plasmid system. A promoter is located upstream of the ribosome-binding site. Owing to the fact that many heterologous protein products are toxic to the cell, the promoter can be regulated so that the heterologous protein is expressed at the appropriate amount and time to reduce the burden on the cell host.

[00231] Historically, the most commonly used promoters have been the lactose (lac) and tryptophan (trp) promoters. These two promoters were combined to create the hybrid promoters tac and trc that are also commonly used. Other common promoters are the phage lambda promoters, the phage T7 promoter (T7), and the alkaline phosphatase promoter (phoA).

[00232] Promoters can be constitutive and inducible. Constitutive promoters are active in all circumstances in the cell, while regulated or inducible promoters become active in response to specific stimuli. In addition, the strength of the promoter can also differ. A strong promoter has a high frequency of transcription and can generate a heterologous protein that is about 10-30% of the total cellular protein production (for examples see **FIG. 8**). Chemically-inducible promoters that can be used in various aspects of the invention include, but are not limited to, promoters whose transcriptional activity is regulated by the presence or absence of alcohol, tetracycline, steroids, metal and other compounds. Physically-inducible promoters that can be used in various aspects of the invention include, but are not limited to, promoters whose transcriptional activity is regulated by the presence or absence of light and low or high temperatures.

[00233] In order to be an inducible promoter, the promoter should ideally initially be completely repressed to transcription. Although, most inducible promoters are known to have some leakage, these inducible promoters are also contemplated. Transcription is subsequently induced at any given time with the addition of an inducer into the desired host cell. Alternatively, an inducible promoter may be responsive to the lack of a substance, such as inorganic phosphate, such that the absence of inorganic phosphate will allow expression at a desired time in the host cell (for examples see **FIG. 8**).

[00234] Ribosome Binding Sites

[00235] A Ribosome Binding Sites (RBS) is an RNA sequence upstream of the start codon that affects the rate at which a particular gene or open reading frame (ORF) is translated. A person of skill in the art can tailor an RBS site to a particular gene. Ribosome Binding Sites (RBSs) are typically short sequences, often less than 20 base pairs. Various aspects of RBS design are known to affect the rate at which the gene is translated in the cell. The RBS module can influence the translation rate of a gene largely by two known mechanisms. First, the rate at which ribosomes are recruited to the mRNA and initiate translation is dependent on the sequence of the RBS. Secondly, the sequence of the RBS can also affect the stability of the mRNA in the cell, which in turn affects the overall level of proteins. Through the use of genetic expression modules, the expression of desired genes, such as genes encoding

enzymes in the biosynthetic pathway for 3-HP, can be tailored either at the transcriptional and translational level.

[00236] One can access the registry RBS collection as a starting point for designing an RBS <<http://partsregistry.org/Ribosome_Binding_Sites/Catalog>> (“Registry”). As of the date of application, the Registry has collections of RBSs that are recommended for general protein expression in *E. coli* and other prokaryotic hosts. In addition, each family of RBSs has multiple members covering a range of translation initiation rates. There are also several consensus RBS sequence for *E. coli* that have been described. However, it is important to keep in mind the known RBS functions and mechanisms in a larger context. For example, in certain contexts the RBS can interact with upstream sequences, such as sequence that comprise the promoter or an upstream ORF. In other contexts, the RBS may interact with downstream sequences, for example, the ribosome enzyme binds jointly to the RBS and start codon at about the same time. These potential interactions should be considered in the overall RBS sequence design. The degree of secondary structure near the RBS can affect the translation initiation rate. This fact can be used to produce regulated translation initiation rates.

[00237] The Shine-Dalgarno portion of the RBS is critical to the strength of the RBS. The Shine-Dalgarno is found at the end of the 16s rRNA and is the portion that binds with the mRNA and includes the sequence 5' - ACCUCC - 3'. RBSs will commonly include a portion of the Shine-Dalgarno sequence. One of the ribosomal proteins, S1, is known to bind to adenine bases upstream from the Shine-Dalgarno sequence. As a result, it is hypothesized that the RBS can be made stronger by adding more adenines in the sequence upstream of the RBS.

[00238] When considering the design of the spacing between the RBS and the start codon, it is important to think of the aligned spacing rather than just the absolute spacing. While the Shine-Dalgarno portion of the RBS is critical to the strength of the RBS, the sequence upstream of the Shine-Dalgarno sequence may also be important. Note that the promoter may add some bases onto the start of the mRNA that may affect the strength of the RBS by affecting S1 binding.

[00239] Computer programs that design RBS sequence to match protein coding sequences, desired upstream sequences including regulatory mRNA sequences, and account of secondary structure are known [see, e.g., Salis, Mirsky, and Voight, Nature Biotechnology 27:

946-950, 2009] and were used to optimize RBSs for the ACCase subunit genes as described in (see **EXAMPLE 2**).

[00240] X. EXPORT OF 3-HYDROXYPROPIONIC ACID FROM CELLS

[00241] 3-HP is structurally and chemically similar to a number of amino acids (*e.g.*, serine, threonine, and to a lesser degree cysteine). These amino acids can be removed from cells by amino acid-specific flux proteins that belong to the major facilitator family (MF). *See, e.g.*, Kramer, R. (1994) Secretion of amino acids by bacteria · physiology and mechanism FEMS Microbiol. Rev. 13, 75-93; *see also e.g.*, Kramer, R. (1994) Systems and mechanisms of amino acid uptake and secretion in prokaryotes Arch. Microbiol. 162, 1-1. This protein family is part of a larger family of previously described and inferred drug extrusion translocases that also include SMR (small multidrug resistance) family, RND (resistance nodulation cell division), ABC (ATP-binding cassette) family, and MATE (multidrug and toxic compound extrusion) family. *See, e.g.*, Yamada S, Awano N, Inubushi K, Maeda E, Nakamori S, Nishino K, Yamaguchi A, Takagi H. (2006) Effect of drug transporter genes on cysteine export and overproduction in *Escherichia coli*. Appl Environ Microbiol. 2006 Jul;72(7):4735-42. Most of these proteins (all except the ABC family) can use the proton gradient or sodium gradient across the inner bacterial membrane to facilitate, *e.g.*, power, translocation. Discussed herein, the proton gradient directed translocases are elucidated as increasing. Furthermore, modifying the effect of the proton gradient directed translocases is expected to preserve as much metabolic energy as possible. However, it can be appreciated that in some embodiments, ATP, sodium, or other metabolite gradient-directed translocases could also be used.

[00242] In some embodiments, the invention may be practiced in a range of microorganisms, such as those bacteria and yeast common use for industrial bioproduction of chemicals. Culture systems can comprise a population of a genetically modified microorganism according to an embodiment of the present invention placed in a media comprising suitable nutrients for growth.

[00243] In some embodiments, the genetically modified microorganisms according to the present invention may be cultured under aerobic, anaerobic, or microaerobic conditions. *See e.g., FIG. 10*. Although some of these proteins have been shown to be fairly specific, members of this protein family have been shown to transport several different amino acids. There are more than 30 identified and putative transporters in the *E. coli* genome. However, the specificities of most of these proteins are unknown. In the MF family, the following specificities have been reported.

Serine	Threonine	Cysteine
rhtA	rhtA	eamA
rhtB	rhtC	eamB

[00244] This list does not necessarily reflect the full set of proteins involved in the transportation of these amino acids. Overexpression of 33 transporters have identified seven transporters that resulted in a significant increase of cysteine efflux (*see e.g.*, FIG. 11, adapted from Yamada S, Awano N, Inubushi K, Maeda E, Nakamori S, Nishino K, Yamaguchi A, Takagi H. (2006) Effect of drug transporter genes on cysteine export and overproduction in *Escherichia coli*. Appl Environ Microbial. 2006 Jul;72(7):4735-42).

[00245] The table below (Table 1) lists paralogs for the identified transport proteins listed in Biocyc. The first column shows the serine and threonine transports, the second column shows the known cysteine transporters, and the third column shows other predicted transporters with operon information or expected functional information:

[00246] Table 1

rhtA, rhtB, rhtC, eamA, or eamB family	Bcrfamily	bcrfamily with unknown functions
yedA	emrB	garP (in operon with garRLK) likely importer
yijE	emrD	gudP(in operon with gudPX) likely importer
ytfF	emrY	hsrA (in operon with yieP) likely exporter
leuE	ydeD (known cysteine)	mdtD (in operon with mdtABC) likely exporter
rhtC	yfiK (known cysteine)	mdtG likely exporter
yahN		mdtI (next to tnaB - tryptophan transporter) likely exporter
		mdtM

		mhpT(3-(3-hydroxyphenyl)propionate transporter) likely exporter
		yajR
		ybjJ (next to riboflavin phosphates)
		ydhC (next to fatty acid synthesis gene) likely exporter
		ydjE
		ydjK(in an operon with putative threonine dehydrogenase ydjL)
		yfd (next to fatty acid synthesis gene) likely exporter
		yhjE
		yidE
		yjiJ

[00247] In a first evaluation, a subset of these proteins can be selected and tested for 3-HP export. As noted, the focus can concentrate on the proton gradient driven translocases, but in general sodium driven, ATP-driven, or other metabolite gradient driven translocases can be evaluated.

[00248] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the spirit of the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EXAMPLES

[00249] EXAMPLE 1: SALT INHIBITION STUDIES IN *E. COLI*

[00250] The activity of ACCase complex, a critical enzyme in the conversion of acetyl-CoA to malonyl-CoA, the immediate precursor for 3-HP, is severely inhibited by salt. Dose-dependent effects on ACCase activity was observed in the presence of NaCl, NH₄Cl, Na-3-HP, or NH₄-3-HP such that salt levels near 0.44M resulted in decreasing the activity of the ACCase enzyme by approximately 80%, while salts of 3-HP levels near 0.66M decreased the activity of the ACCase enzyme by approximately 80% relative to control (**FIG. 4**). Levels of greater than 0.66M (60 g/L) are expected to be present for commercially viable commercial production of 3-HP.

[00251] EXAMPLE 2: OTHER SALT-TOLERANT ENZYMES

[00252] Microorganisms comprising a genetic modifications to enzymes from slight, moderate, and/or extreme halophiles organisms are made. Homology with genes are determined by analysis with BLASTN version 2.0 provided through the NCBI website. Homology with proteins are determined by analysis with BLASTP version 2.2.2 provided through the NCBI website.

[00253] The enzymes from the following halophilic organisms are used: *Chromohalobacter salexigens*, *Flexistipes sinusarabici* strain (MAS10T), *Halobacterium* sp. NRC-1, *Haloarcula marismortui*, *Natronomonas pharaonis*, *Haloquadratum walsbyi*, *Haloferax volcanii*, *Halorubrum lacusprofundi*, *Halobacterium* sp. R-1, *Halomicrobium mukohataei*, *Halorhabdus utahensis*, *Halogeometricum borinquense*, *Haloterrigena turkmenica*, *Natronobacterium gregoryi* SP2, *Halalkalicoccus jeotgali*, *Natrialba magadii*, *Haloarcula hispanica*, *Halopiger xanaduensis*, *Halophilic archaeon DL31*, *Haloferax mediterranei*, *Halovivax ruber*, *Natronococcus gregoryi*, and *Natronococcus occultus*.

[00254] Moderate halophilic organisms in which homologous enzymes are derived are: eukaryotes such as crustaceans (e.g., *Artemia salina*), insects (e.g., *Ephydra hians*), certain plants from the genera *Salicornia* spp, algae (e.g., *Dunaliella viridis*), fungi, and protozoa (e.g., *Fabrea salina*), phototrophic organisms, such as planktonic and microbial mat-formers cyanobacteria as well as other anaerobic red and green sulphur bacteria from the genera *Ectothiorhodospira* spp.) and non-sulphur bacteria from the genera *Chromatium* spp.; gram-negative anaerobic bacteria, for example from the genera *Haloanaerobacter* spp. some of which are methanogenic, for example from the genera *Methanohalophilus* spp. and either aerobic or facultative such as species from the genera *Halomonas*, *Chromohalobacter*, *Salinovibrio*, *Pseudomonas* (e.g., *Halomonas elongate*); gram-positive bacteria from genera

such as *Halobacillus*, *Bacillus*, *Marinococcus*, etc. as well as some actinomycetes, *e.g.*, *Actinopolyspora halophila*.

[00255] Genomic and Proteomic Hallmarks of Halophilic Organisms

[00256] Proteins of microorganisms are genetically modified for salt tolerance such that they have low hydrophobicity, over-representation of acidic residues, especially Asp, under-representation of Cys, lower propensities for helix formation and higher propensities for coil structure.

[00257] Suitable salt-tolerant enzymes from salt-tolerant organisms are used. Salt-tolerant organisms (such as, *e.g.*, halophiles) include any living organism that are adapted to living in conditions of high salinity. Suitable salt-tolerant enzymes can include enzymes from salt-tolerant organism that are homologs of the following enzymes: Sucrose-6-phosphate hydrolase (*e.g.*, *cscA* from *E. coli*), glucose-6-phosphate isomerase (*e.g.*, *pgi* from *E. coli*), fructokinase (*e.g.*, *cscK* from *E. coli*), fructose-1,6-bisphosphatase (*e.g.*, *yggF* from *E. coli*), fructose 1,6-bisphosphatase (*e.g.*, *ybhA* from *E. coli*), fructose 1,6-bisphosphatase II (*e.g.*, *glpX* from *E. coli*), fructose-1,6-bisphosphatase monomer (*e.g.*, *fbp* from *E. coli*), 6-phosphofructokinase-1 monomer (*e.g.*, *pfkA* from *E. coli*), 6-phosphofructokinase-2 monomer (*e.g.*, *pfkB* from *E. coli*), fructose biphosphate aldolase monomer (*e.g.*, *fbaB* from *E. coli*), fructose biphosphate aldolase monomer (*e.g.*, *fbaA* from *E. coli*), triose phosphate isomerase monomer (*e.g.*, *tpiA*), glyceraldehyde 3-phosphate dehydrogenase-A monomer (*e.g.*, *gapA* from *E. coli*), phosphoglycerate kinase (*e.g.*, *pgk*), 2,3-bisphosphoglycerate-independent phosphoglycerate mutase (*e.g.*, *gpmM* from *E. coli*), 2,3-bisphosphoglycerate-dependent or *tdcE* (*e.g.*, from *E. coli*), phosphoglycerate mutase (*e.g.*, *gpmA*), enolase (*e.g.*, *eno* from *E. coli*), phosphoenolpyruvate carboxylase (*e.g.*, *ppc* from *E. coli*), malate dehydrogenase (*e.g.*, *mdh*), fumarase A (*e.g.*, *fum* from *E. coli*), fumarase B (*e.g.*, *fumB*), fumarase C (*e.g.*, *fumC* from *E. coli*), phosphoenolpyruvate synthetase (*e.g.*, *ppsA* from *E. coli*), pyruvate kinase I monomer (*e.g.*, *pykF* from *E. coli*), pyruvate kinase II monomer (*e.g.*, *pykA* from *E. coli*), fumarate reductase (*e.g.*, *frdABCD* from *E. coli*), lipoamide dehydrogenase (*e.g.*, *lpd* from *E. coli*), pyruvate dehydrogenase (*e.g.*, *aceE* from *E. coli*), pyruvate dehydrogenase (*e.g.*, *aceF* from *E. coli*), pyruvate formate-lyase (*e.g.*, *pflB* from *E. coli*), acetyl-CoA carboxylase (*e.g.*, *accABCD* from *E. coli*), malonyl CoA reductase (*e.g.*, *mcr*), 3HP dehydrogenase (*e.g.*, *mmsB*, *NDSD*, or *ydfG*), malonate semialdehyde reductase (*e.g.*, *nemA*, *rutE* from *E. coli*), or a combination thereof.

[00258] Suitable salt-tolerant enzyme homologs that can be used with the claimed invention can have at least and at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%,

89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 75%, or 70% overall amino acid or nucleotide identity to the above enzymes. Suitable salt-tolerant enzyme homologs that can be used with the claimed invention can have at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 75%, or 70% amino acid or nucleotide to the essential protein function domains of the enzymes above.

Suitable salt-tolerant enzyme homologs that can be used with the claimed invention can have at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 75%, or 70% overall amino acid or nucleotide to the essential binding amino acids within an essential protein function domain of the enzymes above.

[00259] In accordance with a preferred embodiment of the invention, suitable salt-tolerant enzyme homologs are enzymes from one of the following organisms: *Halomonas elongata*, *Salinibacter ruber*, or *Halobacterium* species (Archaea).

[00260] In accordance with a preferred embodiment of the present invention, there is provided a non-salt-tolerant organism that is genetically modified to make 3-HP, wherein the genetic modification includes a polynucleotide encoding an acetyl-CoA carboxylase from a salt-tolerant organism. In accordance with a preferred embodiment, the acetyl-CoA carboxylase subunits accA, accB, accC and accD is from *Halomonas elongata*.

[00261] EXAMPLE 3: ACCase FROM HALOPHILIC ORGANISM

[00262] Halophilic organisms, such as *Halomonas elongata*, are found in environments with high salt concentrations and, in general, have a salt internal concentration >2.5-3M. It is hypothesized that enzymes derived from any salt-tolerant species should be more resistant to enzyme inhibition by salts, such as 3-HP. Further, these enzymes that have greater salt tolerance should in turn have extended production under high salt conditions than enzymes with lower salt tolerance.

[00263] Accordingly, the genes encoding the accA, accB, accC, accD of *H. elongata* described in Table 1 were synthesized for expression in *E. coli* using codons optimized for this organism and supplied individually on pUC57 plasmids without promoters. Synthetic operons comprising the subunits were assembled using the Gibson assembly method.

Gene	Accession number	SEQ ID NO.
accA	YP_003898857.1	SEQ ID NO. 1, 2
accB	YP_003897250.1	SEQ ID NO. 3, 4
accC	YP_003897249.1	SEQ ID NO. 5, 6
accD	YP_003897309.1	SEQ ID NO. 7, 8

Table 1. Accession numbers for genes encoding ACCase subunits from *Halomonas elongate*.

[00264] Each gene was amplified by PCRs with *Pfu* Ultra II HS according to the manufacturer's instructions, and the PCR products were purified using the Zymo PCR Cleanup kit. Concentrations of products were measured using the Nanodrop spectrophotometer. The Gibson Assembly kit (NEB) was used to construct plasmids expressing the ACCase subunit genes as directed by the manufacturer. The effect of NH₄-3-HP and NH₄Cl on *H. elongata* ACCase was tested and compared to the *E. coli* ACCase. As shown in **Fig. 4**, whereas the *E. coli* ACCase is significantly inhibited by the salts, the ACCase from the halophile is less affected by either NH₄-3-HP or by NH₄Cl. This result indicated that use of the *H. elongata* ACCase in 3-HP production strains would be beneficial in relieving 3-HP inhibition of the conversion of acetyl-CoA to malonyl-CoA, a critical step in the pathway.

[00265] EXAMPLE 4: RBS-OPTIMIZED GENES

[00266] Enzyme expression is regulated at transcriptional and translational levels in prokaryotes. Ribosome Binding Sites (RBS) are 15 nucleotide segments which are known to control the level of protein expression in microorganisms. To enhance *H. elongata* ACCase expression various customized RBS were constructed and optimized for *E. coli* translation expression. **Table 2** shows the RBS sequences used to increase expression of the individual subunits.

Table 2:

<i>H. elongata</i> ACC expression on plasmid	Modified RBS sequences preceeding ATG (underlined)			
	<i>He_accD</i>	<i>He_accA</i>	<i>He_accC</i>	<i>He_accB</i>

P arent 2- 4	5' - GCGTAGTAAAG GA GGTAAC ATATG	5' - CAATTTATTTA AGGAGGACTCT TAAGATG	5' - GAAATTTTCAT ACCACAGGCG AAGGAGGAAA AACCATG	5' - GGAAGAACAA GGGGTGTACA TG
B 2	Same as 2-4	Same as 2-4	Same as 2-4	5' - ggaagaatta agggggacaa gggggaataA TG
1 3A	5' - gcgtagtagcc gggtgataagg agccgtaacAT G	Same as 2-4	Same as 2-4	
1 4C	5' - gcgtagtagct gatataaaagg aggtaacggAT G	Same as 2-4	Same as 2-4	Same as 2-4
1 5C	Same as 2-4	5' - caatttatTTTT tgttcacccaa ggagtattgct aATG	Same as 2-4	Same as 2-4
1 7C	Same as 2-4	5' - caatttatTTA ccgaaataaaa ggagggatgcg aATG	Same as 2-4	Same as 2-4
3 5C	5' - gcgtagtagcc gggtgataagg agccgtaacAT	5' - caatttatTTTT tgttcacccaa ggagtattgct	Same as 2-4	Same as 2-4

	<u>G</u>	a <u>ATG</u>		
3	5' -	5' -	Same	Same
6C	gcgtagtagcc gggtgataagg agccgtaac <u>AT</u> <u>G</u>	caattttatttta ccgaaataaaaa ggaggggatgcg a <u>ATG</u>	as 2-4	as 2-4
3	5' -	5' -	Same	5' -
6C-8	gcgtagtagcc gggtgataagg agccgtaac <u>AT</u> <u>G</u>	caattttatttta ccgaaataaaaa ggaggggatgcg a <u>ATG</u>	as 2-4	ggaagaatta aggggggacaa gggggaata <u>A</u> <u>TG</u>
7	5' -	5' -	5' -	5' -
2B	gcgtagtagcc gggtgataagg agccgtaac <u>AT</u> <u>G</u>	caattttatttta ccgaaataaaaa ggaggggatgcg a <u>ATG</u>	TCTTCCCACA ACACTGGCGG ACTCCATCAT <u>G</u>	GAAATTTTCAT ACCACAGGCG AAGGAGGAAA AACCAT <u>G</u>
1	5' -	5' -	5' -	5' -
05F	gcgtagtagcc gggtgataagg agccgtaac <u>AT</u> <u>G</u>	caattttattttt tgtttacccaa ggagtatttgct a <u>ATG</u>	TCTTCCCACA ACACTGGCGG ACTCCATCAT <u>G</u>	GAAATTTTCAT ACCACAGGCG AAGGAGGAAA AACCAT <u>G</u>

Table 2. RBS sequences used to enhance expression of *H. elongate* ACCase subunits.

[00267] The expression performance of the RBS-optimized *H. elongata* ACCases was evaluated by 3-HP production in a 96-well format, each in triplicate wells, and the averaged results shown in **Table 3**. Specific 3HP production is shown as g/L per OD₆₀₀. As may be seen in Table 3, enhancing the efficiency of the RBS in strains B2, 35C, and 72 B clearly resulted in increased malonyl-CoA production leading to increased 3-HP production. It is evident from these results that combinations of enhanced RBS's before each of the individual genes *accA*, *accB*, *accC*, and *accD* may result in strains with even higher ACCase expression and activity.

<i>H. elongata</i> ACCase expression plasmid	3HP (g/l.OD)
Parent 2-4	0.06
B2	0.81
13A	0.01
14C	0.54
15C	0.14
17C	0.08
35C	0.68
36C	0.31
36C-8	0.31
72B	0.57
105F	0.19

Table 3. Improvement in 3-HP production by RBS-optimized expression of *H. elongata* ACCase subunits.

[00268] EXAMPLE 5: COORDINATED EXPRESSION BY SUBUNIT FUSIONS

[00269] In nature ACCase subunit genes from prokaryotes such as *E. coli* and *H. elongata* have been shown to have a quaternary structure: $accA_2:accD_2:accB_4:accC_2$. However, the intrinsic levels of the ACCase subunit genes are too low for optimal production. Therefore, for optimal production it is ideal to have overexpression to be coordinated in a similar manner.

[00270] Expression of the genes encoding each ACCase subunit is regulated at transcriptional and translational levels. Coordinated overexpression of the ACCase subunit genes, *accA*, *accB*, *accC*, *accD* should give better ACCase activity. Examples of fusions of *accC-B* proteins exist in bacteria. It is hypothesized that coordinated overexpression may be achieved by fusion of subunit genes should ensures equimolar expression of the subunit genes at the optimal time.

[00271] The following ACCase subunit gene fusion were constructed and the constructs overexpressed in *E. coli*: (A) Control ABCD, (B) fusion of *accC-B* (SEQ ID NOs. 9, 10) subunit genes as seen in bacteria, (C) fusion of *accD-A* subunit genes using a flexible 15-amino acid linker (Linker sequence LSGGGGSGGGGSGGGGSGGGGSAAA; SEQ ID NOs. 11, 12) as depicted in **FIG. 5**.

[00272] The performance of the ACC fusions were tested for their ACCase activity and for various 3-HP production metrics in **Table 4**. ACCase activity was determined in cell lysates

using an assay for malonyl-CoA production as described in Kroeger, Zarzycki, and Fuchs, Analytical Biochem. 411:100-105, 2011, incorporated herein by reference in its entirety. Production of 3-HP was determined in cells co-transformed with a plasmid bearing the genes encoding the malonyl-CoA reductase from *S. tokodaii* and *E. coli ydfG* providing a 3-HP dehydrogenase to complete the metabolic pathway from malonyl-CoA to 3HP. These results show that the strain with the fused *accDA* genes had higher average specific productivity of 3-HP compared to the parental strain in which the overexpressed ACCase is not fused. **Fig. 6** shows that the benefit of the *accDA* fusion were also manifested in 3-HP production in fermentors with environmental controls of nutrient feed, pH, aeration, and temperature.

Strain designation	Plasmid	Avg specific prodn rate (g/gDCW.h) at TS+6	Avg specific prodn rate (g/gDCW.hr) at TS+20	ACCcase specific activity at TS+6 (U/mg)
BX3_783	Parent (unfused ACCase)	0.160	0.146	0.057
BX3_829	No ACC	0.069	0.062	0.000
BX3_837	EC ACC DA fusion	0.209	0.201	0.054

Table 4: ACC Fusions and ACCase activity

[00273] EXAMPLE 6 3-HP EXPORTER

[00274] Growth inhibition has been demonstrated for *E. coli* strains grown in the presence of 3-HP at levels as low as 20 g/L. To produce high titers of 3-HP the production host is required to balance production with overcoming inhibition. A known chemical exporter from *E. coli* that has been previously characterized for homoserine transport, *rhtA*, was evaluated for increased production of 3-HP. A mutant version of the exporter, *rhtA*(P2S) (SEQ ID NOs. 30 and 31) was synthesized behind the PtpiA promoter and inserted into the pTRC-PyibD-MCR plasmid behind a terminator using the Gibson Assembly kit (NEB) according to manufacturer's instructions. The effects of overexpression of *rhtA* were evaluated in 1L fermentation compared to the control plasmid without *rhtA*. As shown in **FIG 7**, overexpression of *rhtA* resulted in a significant improvement in 3HP titer compared to the control production strain. Construction of plasmids expressing another putative transporter, *ydcO* (SEQ ID NOs. 32 and 33) is carried out in the same manner.

[00275] EXAMPLE 7 BICARBONATE IMPORTER

[00276] Increased import of bicarbonate to increase availability of bicarbonate for the ACCase reaction will increase production of malonyl-CoA and hence products derived metabolically from malonyl-CoA, such as 3-HP. The gene encoding the *bicA* bicarbonate

transporter (SEQ ID NO. 13) of *Synechococcus* sp. was synthesized using codons optimized for expression in *E. coli* (SEQ ID NO. 14) and expressed using the *E. coli* tal promoter in a strain co-transformed with plasmids encoding ACCase and MCR functions. Production of 3-HP by this strain is compared to that achieved by a control strain without overexpressed bicA.

[00277] EXAMPLE 8: MONOFUNCTIONAL MCR

[00278] One of the steps in the biosynthesis of 3-HP involves the conversion of malonyl-CoA (MCA) to malonate semialdehyde (MSA) and the conversion of malonate semialdehyde (MSA) to 3-HP. In order to enhance cellular bioproduction of 3-HP in a host cell, a genetically modified organism having novel enzymes and/or combinations of enzymes to catalyze the MCA to MSA reaction are made.

[00279] Organisms that comprise novel enzyme compositions or that co-express a combinations of enzymes to catalyze the conversion of malonyl-CoA to 3-HP are made. For example, malonyl-CoA is converted to malonate semialdehyde by a monofunctional malonyl-CoA reductase that catalyzes the malonyl-CoA conversion, but does not catalyze the malonate semialdehyde conversion.

[00280] The organism is genetically modified to comprise and/or express a monofunctional malonyl-CoA reductase from *Sulfolobus tokodaii* (stMCR) (SEQ ID NOs. 15 and 16) or a functional homolog of stMCR or a homolog with at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity. Other organisms are produced to comprise a genetic modification that include a bi-functional malonyl-CoA reductase comprising two protein fragments with at least one fragment that has malonyl-CoA reductase activity and the other fragment has malonate semialdehyde dehydrogenase activity that is derived from *Chloroflexus aurantiacus* (caMCR).

[00281] EXAMPLE 9: MCR-DEHYDROGENASE ENZYMES FOR CONVERSION OF 3-HP IONS

[00282] Following the conversion of the malonyl-CoA to malonate semialdehyde, the malonate semialdehyde is converted to 3-HP through either or both of two alternative pathways. Malonate semialdehyde may exist in at least three states; the keto form, the enol form, and hydrate form.

[00283] A genetically modified organism comprising and/or expressing a 3-hydroxy acid dehydrogenase enzyme (e.g., *ydfG*; SEQ ID NOs. 21 and 22), a 3-hydroxyisobutyrate dehydrogenase enzyme (e.g., *Pseudomonas aeruginosa mmsB*; SEQ ID NOs. 23 and 24), and/or NAD⁺-dependent serine dehydrogenase (e.g., *Pseudomonas NDSD*; SEQ ID NOs. 25 and 26) are made.

[00284] Genetically modified organisms are made that also comprise and/or express an N-ethylmaleimide reductase (*e.g.*, *nemA*; SEQ ID NOs. 17 and 18), and/or a malonic semialdehyde reductase (*e.g.*, *rutE*, SEQ ID NOs. 19 and 20) from *E. coli*, with or without the previous aforementioned genes.

[00285] EXAMPLE 10: GENETICALLY MODIFIED ORGANISMS AND/OR MODIFIED/FUSED ENZYMES

[00286] The genetically modified organisms are made to comprise and/or express a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde; and one or more genes encoding one or more of the following enzymes: *ydfG*, *mmsB*, *NDSD*, *rutE*, and *nemA* or a functional homolog or a homolog with at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00287] A genetically modified organism capable of making 3-HP is also created. The genetically modified organism comprises and/or expresses a polynucleotide encoding a monofunctional malonyl-CoA reductase gene capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde; one or more genes encoding one or more enzymes capable of converting malonate semialdehyde keto form to 3-HP; and one or more genes encoding one or more enzymes capable of converting either the malonate semialdehyde enol form or the malonate semialdehyde hydrated form to 3-HP.

[00288] Also made are monofunctional malonyl-CoA reductase enzymes fused to a dehydrogenase enzyme that is either: (a) NADPH-dependent, (b) NADH-independent, (c) has the capability of using NADPH, (d) flavin-dependent, (e), flavin-independent, (f) has the capability of using flavin, (g) less susceptible to 3-HP inhibition at high concentration, and/or (h) catalyzes a reaction pathway to 3-HP that is substantially irreversible. Also made are monofunctional malonyl-CoA reductase enzymes fused to a dehydrogenase enzyme that is NADPH-dependent.

[00289] Also made are genetically modified organisms containing and/or expressing 3-HP dehydrogenase enzymes that are largely NADH-dependent, *e.g.*, *mmsB* and/or *NDSD*. Also made are genetically modified organism containing and/or expressing malonate reductase enzymes that are flavin-dependent, *e.g.*, *rutE* and/or *nemA*. Also made are genetically modified organism containing and/or expressing 3-HP dehydrogenase enzymes that are less susceptible 3-HP inhibition at high concentrations, including *e.g.*, *ydfG* and/or *NDSD*. Also made are genetically modified organism containing and/or expressing 3-HP dehydrogenase or

malonate semialdehyde dehydrogenase enzymes that catalyze a reaction pathway to 3-HP that is substantially irreversible, *e.g.*, *rutE* and/or *nemA*.

[00290] Monofunctional malonyl-CoA reductase enzyme are also fused to one or more dehydrogenase enzymes. For example, malonyl-CoA reductase is fused with malonate semialdehyde dehydrogenase to create a multi-domain protein (*e.g.*, two domain protein) and having the MCR and dehydrogenase domains adjacent in the sequence.

[00291] A first monofunctional malonyl-CoA reductase enzyme is fused to a first dehydrogenase enzyme of one type and second monofunctional malonyl-CoA reductase enzyme is fused to a dehydrogenase enzyme of a different type than the first dehydrogenase enzyme.

[00292] In certain fusions, the malonyl-CoA reductase from *S. tokadaii* is fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, and/or *nemA* (or some combination thereof). The fused enzyme includes one of the following the following configurations: *mcr - ydfG*, *mcr - mmsB*, *mcr - NDSD*, *mcr - rutE*, *mcr - nemA*, *mcr - ydfG - mmsB*, *mcr - ydfG - NDSD*, *mcr - ydfG - rutE*, *mcr - ydfG - nemA*, *mcr - mmsB - ydfG*, *mcr - mmsB - NDSD*, *mcr - mmsB - rutE*, *mcr - mmsB - nemA*, *mcr - NDSD - ydfG*, *mcr - NDSD - mmsB*, *mcr - NDSD - rutE*, *mcr - NDSD - nemA*, *mcr - rutE - ydfG*, *mcr - rutE - mmsB*, *mcr - rutE - NDSD*, *mcr - rutE - nemA*, *mcr - nemA - ydfG*, *mcr - nemA - mmsB*, *mcr - nemA - NDSD*, *mcr - nemA - rutE*, functional homologs, and/or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00293] In other fusions, the malonyl-CoA reductase from *C. aggregans* is fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, or *nemA* (or some combination thereof). The fused enzyme includes one of the following configurations: *mcr - ydfG*, *mcr - mmsB*, *mcr - NDSD*, *mcr - rutE*, *mcr - nemA*, *mcr - ydfG - mmsB*, *mcr - ydfG - NDSD*, *mcr - ydfG - rutE*, *mcr - ydfG - nemA*, *mcr - mmsB - ydfG*, *mcr - mmsB - NDSD*, *mcr - mmsB - rutE*, *mcr - mmsB - nemA*, *mcr - NDSD - ydfG*, *mcr - NDSD - mmsB*, *mcr - NDSD - rutE*, *mcr - NDSD - nemA*, *mcr - rutE - ydfG*, *mcr - rutE - mmsB*, *mcr - rutE - NDSD*, *mcr - rutE - nemA*, *mcr - nemA - ydfG*, *mcr - nemA - mmsB*, *mcr - nemA - NDSD*, *mcr - nemA - rutE*, functional homologs, and/or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00294] In other fusions, the malonyl-CoA reductase from *O. trichoides* is fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, or *nemA* (or some combination thereof). The fused enzyme may include one of the following configurations: *mcr - ydfG*, *mcr - mmsB*, *mcr - NDSD*, *mcr - rutE*, *mcr - nemA*, *mcr - ydfG - mmsB*, *mcr - ydfG - NDSD*, *mcr - ydfG - rutE*, *mcr - ydfG - nemA*, *mcr -*

mmsB - *ydfG*, *mcr* - *mmsB* - *NDSD*, *mcr* - *mmsB* - *rutE*, *mcr* - *mmsB* - *nemA*, *mcr* - *NDSD* - *ydfG*, *mcr* - *NDSD* - *mmsB*, *mcr* - *NDSD* - *rutE*, *mcr* - *NDSD* - *nemA*, *mcr* - *rutE* - *ydfG*, *mcr* - *rutE* - *mmsB*, *mcr* - *rutE* - *NDSD*, *mcr* - *rutE* - *nemA*, *mcr* - *nemA* - *ydfG*, *mcr* - *nemA* - *mmsB*, *mcr* - *nemA* - *NDSD*, or *mcr* - *nemA* - *rutE*, functional homologs, or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00295] EXAMPLE 11: ENHANCED MUTATED MONOFUNCTIONAL MCR FOR BIOPRODUCTION

[00296] Microorganisms comprising a genetic modification having a mutated form of *stMCR* that have enhanced activity at about 0 °C to about 10°C, at about 8°C to about 15°C, at about 12°C to about 21°C, at 20°C to about 44°C, at about 30°C to about 37°C, at about 32°C to about 38°C, at about 35°C to about 42°C, at about 40°C to about 50°C, at about 50°C to about 60°C, and/or at about 59°C to less than equal to about 72°C, are made.

[00297] Other mutations are also created. The carboxylase domains of the malonyl-CoA reductase derived from *Chloroflexus aggregans* and/or *Oscillochloris trichoides* are enhanced by mutations in the carboxylase binding domain to provide increased 3-HP production over the natural occurring enzyme. Additional mutation are also made. For example, mutations where the carboxylase activity of the malonyl-CoA reductase derived from *Chloroflexus aurantiacus* is enhanced, are made.

[00298] Other fusions include microorganisms comprising a genetic modification that include carboxylase domains of the malonyl-CoA reductase derived from *C. aggregans* fused to *ydfG*, *mmsB*, *NDSD*, *rutE*, and/or *nemA* (or some combination thereof). Any of the enhanced mutated MCR are also fused one of the following configurations including *mcr* - *ydfG*, *mcr* - *mmsB*, *mcr* - *NDSD*, *mcr* - *rutE*, *mcr* - *nemA*, *mcr* - *ydfG* - *mmsB*, *mcr* - *ydfG* - *NDSD*, *mcr* - *ydfG* - *rutE*, *mcr* - *ydfG* - *nemA*, *mcr* - *mmsB* - *ydfG*, *mcr* - *mmsB* - *NDSD*, *mcr* - *mmsB* - *rutE*, *mcr* - *mmsB* - *nemA*, *mcr* - *NDSD* - *ydfG*, *mcr* - *NDSD* - *mmsB*, *mcr* - *NDSD* - *rutE*, *mcr* - *NDSD* - *nemA*, *mcr* - *rutE* - *ydfG*, *mcr* - *rutE* - *mmsB*, *mcr* - *rutE* - *NDSD*, *mcr* - *rutE* - *nemA*, *mcr* - *nemA* - *ydfG*, *mcr* - *nemA* - *mmsB*, *mcr* - *nemA* - *NDSD*, *mcr* - *nemA* - *rutE*, functional homologs, and/or homologs with 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, sequence homology/identity.

[00299] EXAMPLE 12: TEMPERATURE SENSITIVE MALONYL-CoA REDUCTASE

[00300] In this example, a malonyl-CoA reductase (MCR) gene is mutated to enhance its activity at lower temperatures or enhance its activity within a specific temperature range.

Any MCR can be used, including, an MCR gene derived from *Sulfolobus tokodaii*.

[00301] Mutation is achieved by site directed mutagenesis and/or random mutagenesis.

Subsequent screen methods are used to isolate lower temperature enhanced MCRs and/or specific temperature enhanced MCRs. Screening methods can include any methods to measure any of the substrates and/or products of MCR, *e.g.*, malonate semialdehyde, malonyl-CoA, CoA, NADP⁺, NADPH, Mg²⁺, and/or H⁺. The substrates and/or products can be labeled, *e.g.*, fluorescence or radioactive.

[00302] By repeating this procedure, MCRs with further enhanced activity at lower temperatures and/or MCRs with enhanced activity within a specific temperature range, can be isolated. For example, a mutated MCR having enhanced activity at or about less than 0°C to at or about at 37°C; at or about at 20°C to at or about at 44°C; at or about at 30°C to at or about at 37°C; at or about at 32°C to at or about at 38°C, can be isolated.

[00303] EXAMPLE 13: THE GROWTH PHASE

[00304] A genetically modified organism is used in a controlled multi-phase production process which includes an initiation and/or completion of one or more phases of the production process. For example, the bioproduction process includes one or more of: (1) a growth phase; (2) an induction phase; and (3) a production phase.

[00305] During the growth phase, the genetically modified organism is replicated while a biocatalyst is built up. During the induction phase, key enzymes are expressed, which are used in production. During the production phase, the genetically modified organism produces the desired chemical product.

[00306] During the entire process, the initiation and/or completion of the growth, induction and/or production phases are controlled. Because in some cases, the growth phase is dependent on the presence of a critical external reactant that will initiate growth, the initiation and completion of the growth phase is controlled by the addition and amount of the initiating reactant added to the reaction vessel. For example, the reactant may be phosphate, which is needed for replication of genetically modified organisms, such as, *E. coli* cells. In some cases, the growth phase is initiated by the addition of phosphate to a reaction vessel (together with a carbon source such as sugar and a genetically modified organism such as *E. coli* cell),

and the duration of the growth phase is controlled by the amount of phosphate added to the system.

[00307] EXAMPLE 14: THE INDUCTION PHASE

[00308] The induction phase can be controlled by genetic modifications additional genetic modifications. The key enzymes triggered during this phase are engineered using promoters that are sensitive to (*e.g.*, activated by or deactivated by) the depletion of the initiating reactant. As a result, once the initiating reactant is depleted, the growth phase ends, and the additional enzymes are activated and the induction phase begins.

[00309] The induction phase can be controlled by genes that encode for enzymes in the biosynthetic pathway for the product by using promoters that are activated by for example, phosphate depletion. When desiring to produce 3-HP, *E. coli* can be genetically modified by manipulating one or more of the following genes: *mcr*, *mmsB*, *ydfG*, *rutE*, *nemA* and *NDSD*; genes that encode individual or fused subunits of ACCase, such as *accA*, *accB*, *accC*, *accD*, *accDA* fusion, and *accCB* fusion, and the promoters may include one or more of the promoters that direct expression of the following *E. coli* genes: *amn*, *tktB*, *xasA*, *yibD*, *ytfK*, *pstS*, *phoH*, *phnC*, or other phosphate-regulated genes.

[00310] EXAMPLE 15: THE PRODUCTION PHASE

[00311] The production phase may also be controlled by genetic modifications. For example, the organism is engineered to include mutated forms of enzymes for initiating production of the chemical product. These initiation enzymes facilitate initiation of production either by: (1) becoming active and serving a key function in the production pathway; and/or (2) becoming inactive and thereby turning off a branch pathway or other competitive pathway that prevents or limits the production pathway leading to the chemical product, for example, 3-HP.

[00312] For example, the initiation enzymes are mutated to form temperature sensitive variants of the enzymes that are either activated by or deactivated at certain temperatures. As a result, the production phase is initiated by changing the changing the temperature within the reaction vessel. For example, the production phase is controlled by genetically modifying the organism with a heterologous nucleotide sequence encoding for one or more of the following temperature sensitive enzymes: *fabI*^{ts} (SEQ ID NO. 27), *fabB*^{ts} (SEQ ID NO.28) and *fabD*^{ts} (SEQ ID NO. 29). These enzymes are deactivated or shut-off at the desired temperature for production of the chemical product. Once the reaction vessel temperature is changed, the temperature sensitive enzymes are deactivated and the production pathway of the chemical product is ramped up.

[00313] EXAMPLE 16: CHEMICAL PRODUCTION

[00314] In this example, a genetically modified organism is produced, that is capable of converting a renewable carbon source to a chemical product. The genetically modified organism requires inorganic phosphate for growth and comprises at least one heterologous gene whose expression is regulated by a promoter sensitive to inorganic phosphate levels within a culture system. The at least one heterologous gene converts a carbon source to a chemical product and is not required for the genetically modified organism to replicate. The genetically modified organism also comprises a gene encoding a temperature-sensitive enzyme.

[00315] In this example, a culture system comprising a carbon source in an aqueous medium and the genetically modified organism is created. The culture system is maintained under conditions that allow the genetically modified microorganism to replicate. A sufficient level of inorganic phosphate within said culture system is also maintained. However, once the inorganic phosphate is allowed to be depleted, the expression of the gene regulated by a promoter sensitive to inorganic phosphate levels is triggered. The temperature of the culture system is also altered thereby activating or deactivating said temperature-sensitive enzyme and initiating the production of the desired chemical product.

[00316] EXAMPLE 17: PRODUCING 3-HP

[00317] A genetically modified organism capable of converting a renewable carbon source to 3-HP is produced. The genetically modified organism comprises at least one heterologous gene whose expression is regulated by a promoter sensitive to inorganic phosphate levels within a culture system. The specific gene regulated by the phosphate sensitive promoter is selected from the group consisting of *mcr*, *mmsB*, *ydfG*, *rutE*, *nemA*, *NDSD*, *accA*, *accB*, *accC*, *accD*, *accDA* fusion, and *accCB* fusion. Also included in the genetically modified organism is a gene encoding a temperature-sensitive enzyme selected from the group consisting of *fabI*, *fabB* and *fabD*.

[00318] The genetically modified organism is combined with a carbon source in an aqueous medium comprising phosphate. This initiates the growth phase during which the genetically modified microorganism replicates. The level of inorganic phosphate is maintained until the desired level of cell growth is achieved. By allowing the inorganic phosphate to become depleted, initiates an induction phase which begins the expression of said gene regulated by a promoter sensitive to inorganic phosphate levels. Additionally, changing the temperature of the culture system, activates or deactivates said temperature-sensitive enzyme and initiating a growth phase during which said genetically modified microorganism produces 3-HP.

[00319] EXAMPLE 18: FERMENTATION CONDITIONS

[00320] The growth phase can last between about 10 to about 40 hours, or about 15 to about 35 hours, or about 20 to about 30 hours. The induction phase can last between about 1 to about 6 hours, about 1 to about 5 hours, or about 2 to about 4 hours. The production phase can last from and from about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 hours depending on the amount of chemical product that is desired.

[00321] The growth phase and induction phase are conducted at a temperature of about 25°C to about 35°C, about 28°C to about 32°C, or about 30 °C. The production phase is conducted at a temperature of about 35°C to about 45°C, about 35°C to about 40°C, or about 36°C to about 38 °C. The production phase temperature is typically higher than the induction phase temperature, and the increase in temperature that initiates the production phase occurs over a period of about 1 to about 5 hours, about 1 to about 3 hours, about 2 hours, or about 1 hour.

[00322] Depending on the host cell fermentation may be performed under aerobic, microaerobic, or anaerobic conditions, with or without agitation.

[00323] Suitable pH ranges for fermentation depend on the multiple factors such as the host cell. In some applications of the invention fermentation can occur between various pH ranges for example, pH 3.0 to pH 4.0, pH 4.0 to pH 5.0, pH 5.0 to pH 6.0, pH 6.0 to pH 7.0, pH 7.0 to pH 8.0, pH 8.0 to pH 9.0, or pH 9.0 to pH 10.0.

[00324] EXAMPLE 19: pH VALUES ON 3-HP INHIBITION

[00325] At pH 7.0, growth occurs up to about 30gr/L3-HP. However, at lower pHs growth in 3-HP becomes more inhibitory. As a result several pH and 3-HP values were screened in this example and compared to controls in order to possibly give greater sensitivity within these assays. In order to do this part of the evaluation, buffered media were evaluated for their overall effect, and then selected for use to evaluate the effect of different pH values on 3-HP inhibition on a microorganism (with or without the genetic modification introducing one or more nucleic acids encoding the proteins described herein). *See* FIG. 12.

[00326] EXAMPLE 20: STRUCTURAL CONSIDERATIONS

[00327] MF family proteins typically exhibit common transmembrane spanning structures, although there are varying reports of whether these protein work as monomers, dimer, or trimers. In the case of *rhtA*, multiple sequence alignments appear to show 10 transmembrane stretches (FIGs. 13A-D). Pileup sequence comparisons for directing protein evolution experiments were conducted (FIGs. 14A-K). Thus, site-saturated mutagenesis for perturbing functional aspects of the protein at specific spots without altering essential residues, are

performed. Random mutagenesis are also performed to yield additional mutations for increasing function.

[00328] EXAMPLE 21: EFFLUX PROTEINS

[00329] Accessory protein(s) known to work with these efflux proteins, are increased (*e.g.*, increased performance, expression and/or levels). For example, some accessory proteins include, but are not limited to, out membrane proteins belonging to the Tol family (such as talC) and Omp family (such as oprM).

[00330] Cloning of potential efflux protein genes

[00331] The following genes were PCR amplified from genomic *E.coli* K12 DNA using a phosphorylated 5' containing forward primer and reverse primer containing both SnaBI and SphI sites:

rhtA	emaA	cusA
rhtB	emaB	Bcr
rhtC	emrKY	

[00332] Two backbones were prepared by PCR amplification. The first contained a Ptac promoter with ribosome binding site using a pKK113 vector as template. The second backbone contains Psh and Ptrc promoters and a ribosome binding site at one end and a SphI site on the opposing end. The template for this PCR was a pJ284:29990 vector produced by the gene synthesis services of DNA2.0.

[00333] For the pKK223 backbone, inserts and backbone were gel purified and blunt end ligated together. Colonies were screened, and plasmids showing positive insertions were cultured and mini prepped. Finally the insets were check for proper orientation and will be sent for sequencing.

[00334] For pJ284 backbones, inserts and backbone pieces were digested with SphI, gel purified, and semi-directly ligated together. Colonies were screened, and plasmids showing positive insertions were cultured and miniprepped. Finally the insets were check for proper orientation and will be sent for sequencing.

[00335] Plasmids of each efflux protein are transformed into BW25113 strains, as well as their corresponding Kieo collection strain containing the Knockout of these genes. In other words, efflux proteins able to transport 3-HP are indentified.

[00336] Strains carrying the plasmids, and control strain (wild type and Keio deletion strains) are cultured in M9 media at several (2 to 3) of 3-HP and pH (2 to 3 levels). OD is checked at 600nm to evaluate growth (using IPTG were appropriate). Cultures showing growth are plated and isolated.

[00337] EXAMPLE 22: MUTAGENESIS FOR INCREASED TOLERANCE TO 3-HP

[00338] Efflux proteins showing the most initial tolerance for 3-HP are subject to random mutagenesis and/or site-directed enzyme evolution to increase functionality for increased tolerance to 3-HP. Random mutagenesis are conducted by epPCR (error prone) and site specific mutagenesis is performed by site-saturation mutagenesis.

[00339] Efficient screening is accomplished by plating transformed cultures on minimal media plates prepared at increasing 3-HP levels and various pH values.

[00340] The best growing colonies are cultured and mutations are sequenced to determine the set of mutations conferring better fitness to 3-HP tolerance. The plasmids carrying gene conferring increased fitness are carried forward to repeated screen to develop greater tolerance in strains, and multiple combinations of the isolated mutations are combined.

[00341] Efflux proteins are also evaluated *in vitro*. In such a case, a simple membrane extraction protocol is used where by bacterial membranes are isolated and extruded into vesicles. These vesicles are used to collect 3-HP under the right conditions in an assay similar to that disclosed in Rayman MK, Lo TC, Sanwal BD. Transport of succinate in *Escherichia coli*. II. Characteristics of uptake and energy coupling with transport in membrane preparations. *J. Biol. Chem.* 1972 Oct 10;247(19):6332-9.

[00342] EXAMPLE 23: DOWNSTREAM CHEMICAL PRODUCTS AND CONSUMER PRODUCTS

[00343] 3-HP purified according to the methods provided in this disclosure are converted to various other products having industrial uses including, but not limited to, acrylic acid, acrylates (*e.g.*, acrylic acid salts and esters), 1,3-propanediol, malonic acid, ethyl-3-hydroxypropionate, ethyl ethoxy propionate, propiolactone, acrylamide, or acrylonitrile, and other chemicals, collectively referred to as “downstream chemical products” or “downstream products.” For example, 3-HP is produced and converted to polymerized-3-HP (poly-3-HP) or acrylic acid. 3-HP or acrylic acid are also used to produce polyacrylic acid (polymerized acrylic acid, in various forms), methyl acrylate, acrylamide, acrylonitrile, propiolactone, ethyl 3-HP, malonic acid, 1,3-propanediol, ethyl acrylate, n-butyl acrylate, hydroxypropyl acrylate, hydroxyethyl acrylate, isobutyl acrylate, 2-ethylhexyl acrylate, and acrylic acid or an acrylic acid ester to which an alkyl or aryl addition are made, and/or to which halogens, aromatic amines or amides, and aromatic hydrocarbons are optionally added. In some instances the conversion is associated with the separation and/or purification steps.

[00344] The downstream chemical products are useful for producing a variety of consumer products. The methods of the present invention include steps to produce downstream products of 3-HP.

[00345] Numerous approaches are employed for such downstream conversions, generally falling into enzymatic, catalytic (chemical conversion process using a catalyst), thermal, and combinations thereof (including some wherein a desired pressure is applied to accelerate a reaction). For example, without being limiting, acrylic acid is made from 3-HP via a dehydration reaction, methyl acrylate is made from 3-HP via dehydration and esterification, the latter to add a methyl group (such as using methanol), acrylamide is made from 3-HP via dehydration and amidation reactions, acrylonitrile is made via a dehydration reaction and forming a nitrile moiety, propiolactone is made from 3-HP via a ring-forming internal esterification reaction, ethyl-3-HP is made from 3-HP via esterification with ethanol, malonic acid is made from 3-HP via an oxidation reaction, and 1,3-propanediol is made from 3-HP via a reduction reaction. Various derivatives of the derivatives of 3-HP and acrylic acid are also made, such as the various known polymers of acrylic acid and its derivatives.

Additionally, copolymers containing acrylic acid and/or esters have been widely used in the pharmaceutical formulation to achieve extended or sustained release of active ingredients, for example as coating material. Downstream compounds may also be converted to consumer products such as diapers, carpet, paint, and adhesives.

[00346] 3-HP is oligomerized or polymerized to form poly(3-hydroxypropionate) homopolymers, or co-polymerized with one or more other monomers to form various co-polymers. Because 3-HP has a single stereoisomer, polymerization of 3-HP is not complicated by the stereo-specificity of monomers during chain growth.

[00347] 3-HP is converted into derivatives starting (i) substantially as the protonated form of 3-hydroxypropionic acid; (ii) substantially as the deprotonated form, 3-hydroxypropionate; or (iii) as mixtures of the protonated and deprotonated forms. Generally, the fraction of 3-HP present as the acid versus the salt will depend on the pH, the presence of other ionic species in solution, temperature (which changes the equilibrium constant relating the acid and salt forms), and, to some extent, pressure. Chemical conversions are carried out from either of the 3-HP forms.

[00348] Acrylic acid obtained from 3-HP purified by the methods described in this disclosure can be further converted to various polymers. For example, the free-radical polymerization of acrylic acid takes place by polymerization methods known to the skilled worker and is carried out, for example, in an emulsion or suspension in aqueous solution or

another solvent. Initiators, such as but not limited to organic peroxides, are optionally added to aid in the polymerization. Among the classes of organic peroxides that are used as initiators are diacyls, peroxydicarbonates, monoperoxyarbonates, peroxyketals, peroxyesters, dialkyls, and hydroperoxides. Another class of initiators is azo initiators, which are used for acrylate polymerization as well as co-polymerization with other monomers.

[00349] Co-monomers, such as crosslinkers, are also optionally present during the polymerization. The free-radical polymerization of the acrylic acid obtained from the dehydration of 3-HP, as produced herein, in at least partly neutralized form and in the presence of crosslinkers is also prepared. This polymerization may, in some case, result in hydrogels which are then comminuted, ground and, where appropriate, surface-modified.

[00350] Superabsorbent polymers are prepared from acrylic acid (such as acrylic acid derived from 3-HP provided herein) and a crosslinker, by solution or suspension polymerization. This superabsorbent polymer is used in the manufacture of products requiring absorbents for water and aqueous solutions, such as for diapers, adult incontinence products, feminine hygiene products, and similar consumer products. The superabsorbent polymer particles are also surface-modified to produce a shell structure with the shell being more highly cross-linked than the rest of the particle. This technique improves the balance of absorption, absorption under load, and resistance to gel-blocking. Superabsorbent polymers are also adapted for use in agriculture, horticulture, and medicine.

[00351] When making diapers and other personal hygiene products, the superabsorbent polymer first is molded into an absorbent pad that are vacuum formed, and in which other materials, such as a fibrous material (*e.g.*, wood pulp) are added. The absorbent pad then is assembled with sheet(s) of fabric, generally a nonwoven fabric (*e.g.*, made from one or more of nylon, polyester, polyethylene, and polypropylene plastics) to form diapers.

[00352] Multiple pressurized nozzles, located above a conveyer belt, spray superabsorbent polymer particles (*e.g.*, about 400 micron size or larger), fibrous material, and/or a combination of these onto the conveyer belt at designated spaces/intervals. The conveyor belt is perforated and under vacuum from below, so that the sprayed on materials are pulled toward the belt surface to form a flat pad. In various embodiments, fibrous material is applied first on the belt, followed by a mixture of fibrous material and the superabsorbent polymer particles, followed by fibrous material, so that the superabsorbent polymer is concentrated in the middle of the pad. A leveling roller is used toward the end of the belt path to yield pads of uniform thickness. Each pad thereafter are further processed, such as to cut it to a proper shape for the diaper, or the pad is in the form of a long roll sufficient for

multiple diapers. Thereafter, the pad is sandwiched between a top sheet and a bottom sheet of fabric (one generally being liquid pervious, the other liquid impervious), for example, on a conveyor belt, and these are attached together, for example by gluing, heating or ultrasonic welding, and cut into diaper-sized units (if not previously so cut). Additional features are optionally provided, such as elastic components, strips of tape, etc., for fit and ease of wearing by a person.

[00353] Other disposable absorbent articles are constructed in a similar fashion, such as absorbent articles for adult incontinence, feminine hygiene (sanitary napkins), tampons, etc.

[00354] Low molecular weight polyacrylic acid made by the methods described herein, are used for water treatment, for example, as a flocculant and thickener for various applications including cosmetics and pharmaceutical preparations. For these applications, the polymer is uncrosslinked or lightly cross-linked. The molecular weights are typically from about 200 to about 1,000,000 g/mol.

[00355] Acrylic acid is also co-polymerized with one or more other monomers selected from the group consisting of acrylamide, 2-acrylamido-2-methylpropanesulfonic acid, N,N-dimethylacrylamide, N-isopropylacrylamide, methacrylic acid, and methacrylamide. Co-monomers are also derived from 3-HP, or otherwise provided, to produce co-polymers.

[00356] Acrylic acid is also copolymerized with any free-radically polymerizable monomers including, but not limited to, styrene, butadiene, acrylonitrile, acrylic esters, maleic acid, maleic anhydride, vinyl chloride, acrylamide, and itaconic acid. Acrylic acid (or one of its co-polymerization monomers) are also substituted by any substituent that does not interfere with the polymerization process, such as alkyl, alkoxy, aryl, heteroaryl, benzyl, vinyl, allyl, hydroxy, epoxy, amide, ethers, esters, ketones, maleimides, succinimides, sulfoxides, glycidyl and silyl.

[00357] Acrylic acid and its esters are also formed into paints. A desired mixture of homopolymers and/or copolymers, referred to in the paint industry as “vehicle” (or “binder”) are added to an aqueous solution and agitated sufficiently to form an aqueous dispersion that includes sub-micrometer sized polymer particles. The paint cures by coalescence of these vehicle particles as the water and any other solvent evaporate. Other additives to the aqueous dispersion include, but are not limited to, pigment, filler (*e.g.*, calcium carbonate, aluminum silicate), solvent (*e.g.*, acetone, benzol, alcohols, etc., although these are not found in certain no VOC paints), thickener, and additional additives depending on the conditions, applications, intended surfaces, etc.

[00358] Acrylic-based polymers are used for many coatings in addition to paints. For example, for paper coating latexes, acrylic acid is used from 0.1-5.0%, along with styrene and butadiene, to enhance binding to the paper and modify rheology, freeze-thaw stability and shear stability. Acrylate-based polymers also are used in many inks, particularly UV curable printing inks. For water treatment, acrylamide and/or hydroxy ethyl acrylate are commonly co-polymerized with acrylic acid to produce low molecular-weight linear polymers. Co-polymers of acrylic acid with maleic acid or itaconic acid are also produced for water-treatment applications. Sodium acrylate (the sodium salt of glacial acrylic acid) are also co-polymerized with acrylamide (which may be derived from acrylic acid via amidation chemistry) to make an anionic co-polymer that is used as a flocculant in water treatment.

[00359] For thickening agents, a variety of co-monomers are used.

[00360] Conversion to downstream products are also made enzymatically. For example, 3-HP are converted to 3-HP-CoA, which then is converted into polymerized 3-HP with an enzyme having polyhydroxy acid synthase activity (EC 2.3.1.-). Also, 1,3-propanediol is made using polypeptides having oxidoreductase activity or reductase activity (*e.g.*, enzymes in the EC 1.1.1.- class of enzymes). Alternatively, when creating 1,3-propanediol from 3-HP, a combination of (1) a polypeptide having aldehyde dehydrogenase activity (*e.g.*, an enzyme from the 1.1.1.34 class) and (2) a polypeptide having alcohol dehydrogenase activity (*e.g.*, an enzyme from the 1.1.1.32 class) are used. Polypeptides having lipase activity are used to form esters. Enzymatic reactions such as these are conducted *in vitro*, such as using cell-free extracts, or *in vivo*.

[00361] Reactions that form downstream compounds such as acrylates or acrylamides are conducted in conjunction with use of suitable stabilizing agents or inhibiting agents reducing the likelihood of polymer formation. Stabilizing agents and/or inhibiting agents including, but not limited to, *e.g.*, phenolic compounds (*e.g.*, dimethoxyphenol (DMP) or alkylated phenolic compounds such as di-tert-butyl phenol), quinones (*e.g.*, t-butyl hydroquinone or the monomethyl ether of hydroquinone (MEHQ)), and/or metallic copper or copper salts (*e.g.*, copper sulfate, copper chloride, or copper acetate), are used. Inhibitors and/or stabilizers are also used individually or in combinations.

[00362] The one or more downstream compounds are also recovered at a molar yield of up to about 100 percent, or a molar yield in the range from about 70 percent to about 90 percent, or a molar yield in the range from about 80 percent to about 100 percent, or a molar yield in the range from about 90 percent to about 100 percent. Such yields are the result of single-pass (batch or continuous) or iterative separation and purification steps in a particular process.

[00363] In any embodiment herein, a microorganism can comprise at least 10, at least 15, at least 20, at least 25, at least 30, about 10, about 15, about 20, about 25, or about 30 contiguous polynucleotides or amino acids of any one of SEQ ID NOs: 1-100. In any embodiment herein, a microorganism can comprise a polynucleotide or polypeptide having at least: 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%; or about: 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homology to a polynucleotide or polypeptide of any one of SEQ ID NOS; 1-100. In any embodiment herein, a microorganism can comprise a polynucleotide coding for an MCR having at least about: 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homology to caMCR, stMCR, oaMCR and/or otMCR. In any embodiment herein, a microorganism can comprise a polynucleotide coding for ACCase or an ACCase and/or any subunit thereof having at least about: 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology to an ACCase or any subunit any subunit thereof. In any embodiment herein, the genetically modified microorganism can have at least one disrupted gene selected from the group consisting of: *araD*, *araB*, *lacZ*, *rhaD*, *rhaB*, *hsdR*, *ldhA*, *pflB*, *mgsA*, *poxB*, *pta-ack*, *fabI*, *fabB*, *fabF*, *fabD*, *aldA*, *aldB*, *puuC*, and any combination thereof. In any embodiment herein, the genetically modified microorganism can comprise an exogenous polynucleotide encoding a malonyl-CoA reductase. In any embodiment herein, the malonyl-CoA reductase can be monofunctional or bifunctional (e.g., monofunctional reduces malonyl-CoA to malonate semialdehyde, and bifunctional reduces malonyl-CoA to 3-HP. In any embodiment herein, the genetically modified microorganism herein can comprise an acetyl-CoA carboxylase or one or more subunits thereof and/or a polynucleotide encoding these.

[00364] Acrylamide is also produced from 3-HP, for example, via an esterification-amidation-dehydration sequence. Refluxing an alcohol solution of 3-HP in the presence of an acid or Lewis acid catalyst described herein leads to an 3-HP ester. Treatment of the 3-HP ester with either an ammonia gas or an ammonium ion yields 3-HP amide. Finally, dehydration of the 3-HP amide with dehydration reagents described produces acrylamide. The steps mentioned herein are optionally rearranged to produce the same final product acrylamide. Polymerization of acrylamide is also achieved, for example, by radical polymerization. Polyacrylamide polymers are used as additives for treating municipal drinking water and waste water, in addition to applications in gel electrophoresis, oil-drilling, papermaking, ore processing, and the manufacture of permanent press fabrics.

SEQUENCE LISTING**SEQ ID NO. 1: ACCA PROTEIN, HALOMONAS ELONGATA**

MNPNYLDFEQPIAELQAKIEELRMVGNDSQVNLSDIEGRLEEKSRKLTES
 IFKDLSAWQVSQSLSRHPQRPYTLDYLEHVFTDFDELHGDRRFADDAIVG
 GVARLDDKPVMVIGHQKGRDVHEKVRRNFGMPRPEGYRKACRLMEMAERF
 HMPVLTFIDTPGAYPGIDAEERGQSEAIAYNLGVMSRLKTIISTVVGEG
 GSGGALAIGVCDELAMLQYSTYSVISPEGCASILWKSADKASEAAQAMGI
 TAERLKLGFVDTLIPEPLGGAHRQPSATAERIKTALLESLDRLETMETD
 ALLERRYERLMSYGAPV

SEQ ID NO. 2: ACCA NUCLEIC ACID, HALOMONAS ELONGATA (SYNTHETIC, CODON OPTIMIZE FOR E. COLI EXPRESSION)

ATGAATCCGAACCTATCTGGACTTTGAACAACCGATCGCTGAACTGCAAGCCAAAATCGAA
 GAACTGCGTATGGTGGGCAACGACTCACAGGTGAACCTGTCTGATGAAATTGGCCGTCTG
 GAAGAAAAAAGTCGCAAACTGACCGAATCCATCTTTAAAGACCTGTCAGCGTGGCAAGTT
 AGCCAACTGTCTCGTCATCCGCAACGCCGTATACCCTGGATTACCTGGAACATGTCTTT
 ACGGATTTTCGACGAACTGCACGGTGACCGTCGCTTTCAGATGACGCGGCCATTGTTGGC
 GGTGTCGCTCGTCTGGATGACAAACCGGTCATGGTGATCGGCCATCAGAAAGGTCGTGAT
 GTGCACGAAAAAGTTCGTCGCAACTTCGGCATGCCGCGCCCGGAAGGTTATCGTAAAGCG
 TGCCGCCTGATGGAAATGGCCGAACGCTTTCACATGCCGGTGCTGACCTTCATTGATACG
 CCGGGCGCATATCCGGGTATCGACGCTGAAGAACGTGGCCAAAGCGAAGCGATTGCCTAC
 AATCTGGGTGTTATGTCGCGCCTGAAAACCCCGATTATCAGCACGGTGGTTGGCGAAGGC
 GGTCTGGCGGTGCACTGGCTATCGGTGTCTGCGATGAACTGGCGATGCTGCAATATAGT
 ACCTACTCCGTGATTTACCGGAAGGCTGTGCCTCGATCCTGTGGAAAAGCGCAGATAAA
 GCTTCTGAAGCAGCTCAAGCGATGGGCATTACCGCCGAACGTCTGAAAGAACTGGGTTTC
 GTTGACACGCTGATCCCGGAACCGCTGGGCGGTGCACATCGTCAGCCGAGTGCGACCGCC
 GAACGCATTAAAACGGCCCTGCTGGAAAGCCTGGATCGCCTGGAAACGATGGAAACGGAT
 GCCCTGCTGGAACGCCGCTATGAACGCCTGATGTCTTACGGTGCCCCGGTCTGA

SEQ ID NO. 3: ACCB PROTEIN, HALOMONAS ELONGATA

MDIRKVKKLIELLEESNISEIEIQEGEESVRISRHPPNGTEHPQPAAPAWP
 ATAAAPAPQPAAAPVESPAEVDEGPAYQGQPIVSPMVGTIFYRAPAPGAKE
 FVELGQSVKKGETVCIVEAMKMMNQIEADRDGVVEAILVEDGEPVEFEQF

MVVIS

SEQ ID NO. 4: ACCB NUCLEIC ACID HALOMONAS ELONGATA (SYNTHETIC, CODON OPTIMIZE FOR E. COLI EXPRESSION)

ATGGACATCCGCAAAGTGAAAAAACTGATCGAACTGCTGGAAGAAAGTAACATCTCTGAA
ATTGAAATCCAAGAAGGCGAAGAAAGCGTGCGTATTAGTCGCCATCCGAACGGCACCAGAA
CACCCGCAGCCGGCGGCACCGGCATGGCCGGCCACGGCAGCTGCGCCGGCGCCGCAACCG
GCCGCAGCTCCGGTGGAAAGCCCGGCAGAAAGTTGATGAAGGCCCGGCTTATCAGGGTCAA
CCGATCGTTTCTCCGATGGTCGGCACCTTTTACCGTGCGCCGGCACCGGGTGCAAAAGCT
TTCGTCGAACTGGGCCAGAGCGTTAAAAAAGGTGAAACGGTCTGCATTGTGGAAGCCATG
AAAATGATGAATCAAATCGAAGCCGATCGCGACGGTGTGGTTGAAGCAATCCTGGTGGAA
GATGGTGAACCGGTGGAATTTGAACAGCCGATGGTGGTGATTAGTTAA

SEQ ID NO. 5: ACCC PROTEIN, HALOMONAS ELONGATA

MLDKVLIANRGEIALRILRACKELGIRTVAVHISKADRELMHVRLADEAVC
IGPASSAQSYLNIPALISAAEVTDTSAIHPGYGFLSENADFAEQVERSGF
TFIGPSAETIRLMGDKVSAINAMKEAGVPTVPGSNGPLGDDEGEILATAR
RIGYPVIAKAAAGGGGRGMRVVHAEGHLLSAVNVTRTEAHSSFGDGTVYM
EKFLENPRHVEVQVLADGQGNIAHLYDRDCSLQRRHQKVLEEAPAPGLDQ
QAREQVFKACRDACVKIGYRGAGTFEFLYENGEEFFIEMNTRVQVEHPVT
EMVTGVDIVREQLRIASGLPLSIRQEDVELSGHAFECRINAEDSRTFMPS
PGRVTLYHPPGGLGVRMDSHVYTGTVPPHYDSLIGKLITWGDDRETALI
RMRNALDELLVEGIKTNTDLHKDLVRDGYFQQGGVNIHYLEKKLGL

SEQ ID NO. 6: ACCC NUCLEIC ACID, HALOMONAS ELONGATA (SYNTHETIC, CODON OPTIMIZE FOR E. COLI EXPRESSION)

ATGCTGGACAAAGTGCTGATTGCGAATCGTGGCGAAATTGCGCTGCGTATCCTGCGTGCG
TGTAAGAAGAACTGGGTATCCGTACCGTCGCTGTTCAATCAAAAGCGGACCGTGAAGTATG
CACGTCCGCCTGGCCGATGAAGCAGTGTGCATTGGTCCGGCTAGCTCTGCGCAGTCGTAT
CTGAACATTCCGGCACTGATCAGTGCGGCCGAAGTGACCGACACGTCCGCGATCCATCCG
GGCTACGGTTTCTCTGAGCGAAAATGCCGATTTTGAGAACAAAGTCGAACGTTTCAGGTTTC
ACCTTTATTGGCCCGTCGGCCGAAACGATCCGCCTGATGGGTGATAAAGTTAGTGCTATT
AACGCGATGAAAGAAGCAGGCGTGCCGACCGTTCCGGGTCCAATGGTCCGCTGGGTGAT
GACGAAGGTGAAATTCTGGCCACCGCACGTCGCATCGGCTATCCGGTTATTATCAAAGCA
GCTGCGGGCGGTGGCGGTCTGGTATGCGTGTGGTTCATGCTGAAGGCCACCTGCTGAGC

GCGGTCAACGTGACCCGTACGGAAGCGCATAGTTCCTTCGGCGATGGCACCGTTTATATG
 GAAAAATTTCTGGAAAACCCGCGTCACGTTGAAGTCCAGGTGCTGGCCGATGGCCAGGGT
 AATGCAATTCATCTGTACGATCGCGACTGCTCTCTGCAACGTGCGCCACCAAAAAGTGCTG
 GAAGAAGCTCCGGCACCGGGTCTGGACCAGCAAGCACGTGAACAGGTTTTTAAAGCCTGC
 CGCGATGCATGTGTCAAAATTGGTTATCGTGGCGCGGGCACCTTCGAATTTCTGTACGAA
 AACGGCGAATTTTTCTTTATCGAAATGAATACGCGCGTTCAGGTGCAACATCCGGTGACC
 GAAATGGTCACGGGTGTGGATATTGTTCTGTGAACAGCTGCGTATCGCATCAGGTCTGCCG
 CTGTCGATTCGCCAAGAAGACGTTGAACTGAGCGGTTCATGCCTTCGAATGTCGTATCAAT
 GCAGAAGATAGCCGCACCTTTATGCCGTCTCCGGGTCTGTGTACGCTGTATCACCCGCCG
 GGCGGTCTGGGTGTCCGTATGGACAGCCATGTGTATACCGGCTACACGGTTCGCCCGCAC
 TACGATTCTCTGATTGGTAAACTGATCACCTGGGGCGATGACCGTGAAACGGCTCTGATT
 CGTATGCGCAACGCCCTGGATGAACTGCTGGTTGAAGGCATCAAAACCAATACGGATCTG
 CACAAAGACCTGGTTCGCGATGGCTACTTTCAGCAAGGCGGTGTCAACATTCACTACCTG
 GAAAAAAACTGGGTCTGTAA

SEQ ID NO. 7: ACCD PROTEIN, HALOMONAS ELONGATA

MSWLDKIVPSVGRIQRKERRTSVPDGLWRKCPKCESVLYLPELEKHHNVC
 PKCDHHLRLTARKRLDWFLDKEGREEIAADLEPVDRLKFRDSKKYKDRLS
 AAQKATGEKDGLVAMRGTLLEGLPVVAVAFEFTFMGGSMGAVVGEKRVRAA
 TQALDEGVPLVCFASGGARMQEALFSLMQMAKTSAALEKLKQAGVPYIS
 VLTDPVFGGVSASLAMLGDLNIAEPNALIGFAGPRVIEQTVREQLPEGFQ
 RSEFLLEHGAVDMIVHRQQIRERLGGVLRKLTHQPASGPAVVENDEPDLV
 DAAEQAEPPQPEAPEAVETSESEAPTEKGVEADSEETDESPRSGDNR

SEQ ID NO. 8: ACCD NUCLEIC ACID, HALOMONAS ELONGATA (SYNTHETIC, CODON OPTIMIZE FOR E. COLI EXPRESSION)

ATGTCTTGGTTAGATAAAATCGTCCCGTCAGTGGGTCGCATTCAACGCAAAGAACGTGCG
 ACCTCGGTCCCGGATGGTCTGTGGCGTAAATGCCCGAAATGTGAATCAGTTCTGTATCTG
 CCGGAACTGGAAAAACATCACACGTCTGCCCAGAAATGTGATCATCACCTGCGTCTGACC
 GCGCGTAAACGCCTGGACTGGTTCCTGGATAAAGAAGGCCGCGAAGAAATTGCGGCCGAC
 CTGGAACCGGTGGATCGTCTGAAATTCGCGACAGCAAAAAATACAAAGATCGTCTGAGC
 GCGGCGCAGAAAGCAACCGGTGAAAAAGACGGTCTGGTGGCCATGCGTGGCACGCTGGA

A

GGTCTGCCGGTGGTTGCAGTTGCTTTTGAATTTACCTTTATGGGCGGTAGCATGGGCGCA

GTCGTGGGTGAAAAATTCGTTCTGCGGCCACGCAGGCTCTGGATGAAGGTGTGCCGCTG
 GTTTGCTTCAGCGCATCTGGCGGTGCCCCGATGCAGGAAGCACTGTTTAGTCTGATGCAA
 ATGGCTAAACCTCCGCAGCTCTGGAAAACTGAAACAGGCGGGCGTGCCGTATATTTCT
 GTTCTGACGGACCCGGTCTTCGGCGGTGTGAGTGCGTCCCTGGCCATGCTGGGTGATCTG
 AACATTGCAGAACCGAATGCTCTGATCGGCTTTGCGGGTCCGCGTGTGCATCGAACAGACC
 GTGCGCGAACAACTGCCGGAAGGCTTCCAGCGTTTCTGCTGGAACATGGTGCC
 GTTGATATGATTGTCCACCGTCAGCAAATCCGTGAACGCCTGGGCGGTGTGCTGCGCAA
 CTGACGCATCAACCGGCATCGGGTCCGGCCGTTGTCGAAAATGATGAACCGGACCTGGTC
 GATGCGGCCGAACAGGCAGAACCGCAACCGGAAGCACCGGAAGCTGTTGAAACCTCAGAA
 TCGGAAGCACCGACGGAAAAAGGCGTGGAAGCAGACTCGGAAGAAACGGATGAATCACC

G

CGCTCAGGCGACAACCGCTAA

SEQ ID NO. 9: FUSION ACCCB PROTEIN, E. COLI

MLDKIVIANRGEIALRILRACKELGIKTVAVHSSADRD LKHVLLADETV
 IGPAPSVKSYLNIPAIISAAEITGAVAIHPGYGFLSENANFAEQVERSGF
 IFIGPKAETIRLMGDKVSAIAAMKKAGVPCVPGSDGPLGDDMDKNRAIAK
 RIGYPVIAKASGGGGGRGMRVVRGDAELAQSISMTRAEAKAASFNDMVYM
 EKYLENPRHVEIQVLADGQGNAIYLAERDCSMQRRHQKVVEEAPAPGITP
 ELRRYIGERCAKACVDIGYRGAGTFEFLFENGIFYFIEMNTRIQVEHPVT
 EMITGVDLIKEQLRIAAGQPLSIKQEEVHVRGHAVECRINAEDPNTFLPS
 PGKITRFHAPGGFGVRWESHIYAGYTVPPYYDSMIGKLICYGENRDVAIA
 RMKNALQELIIDGIKTNDLQIRIMNDENFQHGGTNIHYLEKKLGLQEKD
 IRKIKKLIELVEESGISELEISEGEESVRISRAAPAASFPVMQQAYAAPM
 MQQPAQSNAAPATVPSMEAPAAAEISGHIVRSPMVGTFYRTPSPDAKAF
 IEVGQKVNVDGTLTLCIVEAMKMMNQIEADKSGTVKAILVESGQPVEFDEPL
 VVIE

SEQ ID NO. 10: FUSION ACCCB NUCLEIC ACID, E. COLI

ATGCTGGACAAGATCGTCATCGCCAACCGCGGCGAAATCGCCCTGCGCATCTTGCGCGCG
 TGTAAGAGCTGGGCATTAAGACTGTTGCCGTGCATTCCAGCGCAGACCGCGACCTGAAG
 CATGTTCTGCTGGCCGACGAAACGGTTTGCATCGGTCCGGCACCGAGCGTGAAAAGCTAT
 CTGAACATCCCGGCCATCATCTCTGCGGCAGAGATCACCGGTGCAGTGGCGATTATCCG
 GGCTACGGTTTCCTGAGCGAGAACGCTAACTTTGCTGAACAAGTGGAGCGTAGCGGTTTC

ATCTTCATTGGCCCTAAGGCGGAGACGATTCGCCTGATGGGCGACAAAGTGAGCGCCATT
GCAGCGATGAAAAAGGCCGGTGTGCCGTGTGTTCCGGGCAGCGATGGTCCGCTGGGTGAC
GATATGGACAAGAACCGTGCCATCGCTAAACGTATTGGCTACCCGGTCATTATCAAAGCC
TCTGGTGGTGGCGGTGGCCGTGGTATGCGTGTCTCGTCCGTGGTGTATGCGGAACTGGCGCAA
AGCATCAGCATGACCCGTGCGGAAGCCAAAGCGGCGTTCTCTAACGATATGGTGTATATG
GAGAAGTATCTGGAGAATCCGCGCCACGTTGAAATCCAAGTTCTGGCGGATGGTCAGGGC
AATGCGATCTACTTGGCAGAACGTGATTGCTCCATGCAACGCCGTCATCAGAAGGTGGTG
GAAGAGGCACCGGCTCCGGGTATTACGCCGGAAGTGCCTCGCTACATCGGTGAGCGCTGT
GCGAAAGCGTGTGTGGACATTGGTTACCGTGGTGGCGGTACGTTTGAGTTCCTGTTCGAA
AATGGTGAGTTTTACTTCATTGAAATGAATACCCGCATCCAGGTTGAGCACCCGGTGACC
GAGATGATTACTGGCGTTGATCTGATCAAAGAGCAACTGCGCATTGCGGCTGGTCAGCCG
CTGTCGATCAAGCAAGAAGAGGTGCACGTTCTGGTACGCGGTGAGTGCCGTATCAAT
GCGGAGGACCCGAATACCTTTCTGCCGAGCCCTGGTAAGATCACTCGTTTTACGCGCCA
GGTGGTTTTGGCGTTCGTTGGGAGTCTCACATCTACGCCGTTACACCGTGCCGCCGTAC
TATGACAGCATGATTGGTAACTGATCTGCTATGGCGAAAATCGTGATGTCGCGATCGCC
CGCATGAAAAACGCGCTGCAAGAGCTGATCATTGATGGCATTAAAGACCAATGTGGATTG
CAGATCCGCATTATGAACGACGAGAATTTCCAGCACGGCGGTACGAACATTCACTACCTG
GAAAAGAACTGGGCCTGCAAGAGAAAGACATCCGCAAGATCAAGAAGCTGATCGAACTG
GTGGAAGAGTCTGGCATCAGCGAGCTGGAGATCAGCGAAGGTGAAGAGAGCGTCCGTATT
TCCCGTGCGGCACCGGCAGCGAGCTTTCCGGTTATGCAGCAAGCATACGCCGCTCCGATG
ATGCAACAGCCGGCACAGAGCAACGCCGCTGCACCGGCGACCGTTCCAAGCATGGAGGCA
CCGGCAGCGGCCGAGATTTGGGTGATATCGTGCGTAGCCCGATGGTGGGCACCTTCTAT
CGCACGCCGTCGCCGACGCAAAAGCCTTCATCGAAGTCGGCCAGAAGGTCAATGTGGC
GACACGCTGTGTATCGTTGAGGCAATGAAAATGATGAACCAGATTGAAGCGGATAAGAGC
GGTACTGTAAAGCGATCCTGGTGAATCCGGCCAGCCTGTTGAGTTCGATGAACCGCTG
GTTGTGATCGAGTAA

SEQ ID NO. 11: FUSION ACCDA PROTEIN, E. COLI

MSWIERIKSNITPTRKASIPEGVWTKCDSCGQVLYRAELERNLEVCPKCD
HHMRMTARNRLHSLLEDEGSLVELGSELEPKDVLKFRDSKKYKDRLASAQK
ETGEKDALVVMKGTLYGMPVVAAAFEFMGGSMGVSVVGARFVRAVEQAL
EDNCPLICFSASGGARMQEALMSLMQMAKTSAAALAKMQERGLPYISVLTD
PTMGGVSASFAMLGDLNIAEPKALIGFAGPRVIEQTVREKLPPGFQRSEF

LIEKGAIDMIVRRPEMRLKLASILAKLMNLPAPNPEAPREGVVVPPVPDQ
EPEALSGGGGSGGGGSGGGGSGGGGSAAASLNFLDFEQPIAELEAKIDSL
TAVSRQDEKLDINIDEEVHRLREKSVELTRKIFADLGAWQIAQLARHPQR
PYTLDYVRLAFDEFDELADRAYADDKAIVGGIARLDGRPVMIIIGHQKGR
ETKEKIRRNFGMPAPEGYRKALRLMQMAERFKMPIITFIDTPGAYPGVGA
EERGQSEAIARNLREMSRLGVPVVCTVIGEGSGGALAIGVGDKNMLQY
STYSVISPEGCASILWKSADKAPLAAEAMGIIAPRLKELKLIDSIIPEPL
GGAHRNPEAMAASLKAQLLADLADLDVLSTEDLKNRRYQRLMSYGYA

SEQ ID NO. 12: FUSION ACCDA NUCLEIC ACID; E. COLI

ATGAGCTGGATCGAGCGCATCAAGAGCAACATCACCCGACCCGCAAGGCGAGCATCCCT
GAAGGCGTCTGGACCAAATGCGATAGCTGCGGTGAGGTTTTGTATCGTGCGGAGCTGGAG
CGTAACCTGGAAGTGTGCCCCGAAATGCGACCATCACATGCGTATGACCGCTCGTAATCGT
CTGCATAGCCTGCTGGATGAGGGCAGCCTGGTCGAGCTGGGTAGCGAACTGGAACCGAAA
GATGTTCTGAAATTCCGTGATTCCAAGAAGTATAAGGATCGTTTGGCATCTGCACAAAAA
GAAACCGGTGAGAAGGACGCACTGGTTGTTATGAAAGGCACCCTGTATGGTATGCCGGTT
GTTGCTGCGGCGTTGAGTTTGCCTTATGGGTGGCAGCATGGGTTCCGTGGTGGGCGCA
CGCTTTGTGCGTGCCGTGGAGCAGGCGCTGGAGGATAACTGTCCTCTGATTTGTTTCAGC
GCGAGCGGTGGTGCGCGTATGCAAGAGGCCCTGATGAGCCTGATGCAGATGGCAAAAACC
TCGGCAGCCCTGGCGAAGATGCAAGAACGCGGCCTGCCGTACATTTCCGTCTGACCGAC
CCTACGATGGGCGGTGTCAGCGCCAGCTTTGCGATGCTGGGTGATTTGAACATCGCAGAG
CCGAAGGCTCTGATTGGTTTTGCTGGTCCGCGTGTTATTGAACAGACGGTTCGCGAAAAG
TTGCCGCTGGTTTCCAGCGCAGCGAGTTCCTGATTGAGAAAGGTGCCATCGACATGATC
GTTGCGCGTCCAGAAATGCGTCTGAACTGGCGAGCATTCTGGCGAAATTGATGAATCTG
CCGGCTCCGAATCCTGAAGCACCGCGTGAGGGTGTCGTGGTTCGCGCGGTCCCGGACCAA
GAGCCGGAGGCTCTGAGCGGCGGAGGTGGCTCTGGTGGAGGCGGTTGAGGAGGCGGTGG

C

AGTGGTGGCGGCGGATCTGCGGCAGCTTCTCTGAACTTCCTGGACTTCGAGCAGCCGATC
GCCGAACTGGAGGCGAAGATTGACAGCCTGACCGCGTTAGCCGTCAAGATGAGAACTG
GACATTAACATCGACGAAGAGGTCCACCGTTTTCGTGAGAAGTCTGTTGAACTGACTCGC
AAAATCTTTGCTGATTTGGGCGCATGGCAGATTGCCAGTTGGCTCGCCACCCACAACGC
CCATATACCCTGGACTACGTGCGCCTGGCGTTTGACGAGTTCGACGAACTGGCAGGCGAC
CGCGCCTATGCGGACGATAAAGCAATTGTCGGCGGTATTGCTCGTTTGGATGGCCGTCCG

GTGATGATTATCGGCCATCAAAAAGGCCGCGAGACAAAGGAAAAGATTCGTCGTAATTTTC
GGAATGCCGGCACCGGAGGGCTACCGCAAGGCCCTGCGTCTGATGCAAATGGCCGAACGC
TTTAAGATGCCGATTATCACGTTTATTGATACGCCTGGTGCGTACCCAGGCGTTGGTGCG
GAAGAGCGTGGTCAGAGCGAGGCCATCGCACGTAACCTGCGTGAGATGTCTCGTCTGGGT
GTGCCGGTCTGTTTGACCGTGATTGGCGAGGGCGGTAGCGGTGGTGCGTTGGCGATCGGT
GTCGGTGATAAGGTCAACATGCTGCAATACAGCACGTACAGCGTCATTAGCCCCGGAAGGT
TGCGCTTCCATTCTGTGGAAGAGCGCGGATAAAGCACCATTTGGCAGCGGAAGCGATGGGT
ATCATCGCACCGCGTCTGAAAGAACTGAAGTTGATTGATTCTATCATCCCGGAACCGCTG
GGCGGTGCTACCGTAATCCGGAGGCGATGGCAGCCAGCCTGAAGGCCAGCTGCTGGCG
GACCTGGCGGATCTGGACGTGCTGAGCACGGAGGATCTGAAAAACCGTCGCTATCAGCGC
TTGATGAGCTATGGCTACGCTTAA

SEQ ID NO. 13: BICA PROTEIN, SYNECHOCOCCUS SP.

MQITNKIHFNRNIRGDIFGGLTAAVIALPMALAFGVASGAGAEAGLWGAVL
VGFFAALFGGTPTLISEPTGPMTVVMTAVIAHFTASAATPEEGLAIAFTV
VMMAGVFQIIFGSLKLGKYVTMMPYTVISGFMSGIGIILVILQLAPFLGQ
ASPGGGVIGTLQNLPILLSNIQPGETALALGTVAIIWFMPEKFKKVIPPQ
LVALVLGTVIAFFVFPPEVSDLRRIEIRAGFPELVRPSFSPVEFQRMIL
DAAVLGMLGCIDALLTSVVADSLRTEHNSNKEIGQGLGNLFSGLFGGI
AGAGATMGTVVNIQSGGRTALSGLVRAFVLLVILGAASLTATIPLAVLA
GIAFKVGVDIIDWSFLKRAHEISPKGALIMYGVILLTVLVDLIVAVGVGV
FVANVLTIERMSNLQSEKVQTVSDADDNIRLTTEKRWLDEGQGRVLLFQ
LSGPMIFGVAKAIAREHNAMGDCDALVFDIGEVPHMGVTASLALENAIEE
ALDKERQVYIVGAAGQTRRRLEKLFKRVPPDKCLMSREEALKNAVLGI
YPHLADGVTAPSEMGG

SEQ ID NO. 14: BICA NUCLEIC ACID, SYNECHOCOCCUS SP.

ATGCAGATTACCAACAAGATCCATTTCCGTAACATTCGTGGCGACATTTTTGGTGGCCTG
ACCGCTGCTGTGATTGCGCTGCCGATGGCACTGGCTTTTGGTGTGGCAAGTGGTGCAAGT
GCAGAAGCAGGTCTGTGGGGTGCACTTCTGGTGGGCTTTTTCGCAGCACTGTTCCGTGGT
ACGCCGACCCTGATTTTCAAGAACCGACGGGCCCCGATGACCGTGGTTATGACGGCCGTGATC
GCACATTTTACCGCATCGGCAGCTACGCCGGAAGAAGGCCTGGCTATTGCGTTCACCGTC
GTGATGATGGCCGGTGTTTTTCAGATTATCTTCGGCAGCCTGAAACTGGGCAAGTATGTT
ACCATGATGCCGTACACGGTCATCAGTGGTTTTATGTCCGGTATTGGCATTATCCTGGTG

ATCCTGCAGCTGGCACCGTTCCTGGGTCAAGCCAGTCCGGGCGGTGGCGTTATTGGCACC
 CTGCAGAACCTGCCGACGCTGCTGTCCAATATCCAACCGGGTGAAACCGCCCTGGCACTG
 GGTACGGTCGCGATTATCTGGTTCATGCCGGAAGTTCAAGAAGGTTATCCCGCCGCAG
 CTGGTTGCGCTGGTTCTGGGCACCGTCATCGCGTTTTTCGTGTTTCCGCCGGAAGTTAGC
 GATCTGCGTCGCATTGGCGAAATCCGTGCAGGTTTCCCGGAACTGGTGCGTCCGAGCTTT
 TCTCCGGTTGAATTTACAGCGCATGATTCTGGATGCGGCCGTGCTGGGCATGCTGGGTGTC
 ATCGATGCGCTGCTGACCAGCGTTGTCGCCGACTCTCTGACGCGTACCGAACATAACAGC
 AATAAAGAACTGATTGGTCAGGGCCTGGGTAACTGTTTTCTGGCCTGTTCCGTGGTATT
 GCTGGTGCAAGGTGCAACGATGGGCACCGTGGTTAATATCCAAAGTGGTGGCCGTACCGCA
 CTGTCCGGTCTGGTGCGTGCTTTTGTTCTGCTGGTCTGATTCTGGGTGCAGCTTCTCTG
 ACGGCAACCATTCCGCTGGCTGTGCTGGCAGGCATCGCCTTTAAAGTGGGTGTTGATATT
 ATCGACTGGTCATTCTGAAACGCGCCACGAAATCTCGCCGAAGGGCGCACTGATTATG
 TATGGTGTGATCCTGCTGACCGTCCTGGTGGATCTGATTGTTGCGGTCCGCGTGGGTGTT
 TTTGTCGCCAACGTTCTGACCATCGAACGTATGTCAAATCTGCAGTCGGAAAAAGTCCAA
 ACCGTGAGCGATGCGGATGACAACATTGCCTGACCACGACCGAAAAGCGTTGGCTGGAC
 GAGGGTCAGGGTCGTGTGCTGCTGTTTCAACTGTCTGGCCCGATGATTTTCGGTGTGCA
 AAAGCTATCGCGCGTGAACATAACGCAATGGGTGATTGCGACGCTCTGGTGTGTTGATATT
 GGCGAAGTCCCGCACATGGGTGTGACCGCAAGTCTGGCTCTGGAAAATGCGATTGAAGAA
 GCCCTGGACAAAGAACGCCAGGTTTACATCGTCGGTGCAGCAGGTCAAACCCGTCGCCGT
 CTGGAAAACTGAAGCTGTTTAAACGCGTGCCGCCGGATAAGTGTCTGATGTCCCGTGAA
 GAAGCACTGAAGAATGCTGTTCTGGGTATCTATCCGCATCTGGCTGACGGTGTTACGGCT
 CCGAGTTCCGAAATGGGCTAA

SEQ ID NO. 15: MCR DNA ORGANISM NAME: SULFOLOBUS TOKODAI

ATGTCTCGTC GCACCCTGAA AGCGGCTATC CTGGGCGCCA CCGGCCTGGT
 TGGTATCGAA 60
 TATGTCCGTA TGCTGTCAAA TCATCCGTAT ATCAAACCGG CGTATCTGGC CGGCAAAGGT
 120
 TCAGTTGGCA AACCGTACGG TGAAGTGGTT CGTTGGCAGA CCGTTGGCCA
 AGTCCCGAAA 180
 GAAATCGCCG ATATGGAAAT TAAACCGACG GACCCGAAAC TGATGGATGA
 CGTGGATATT 240

ATCTTTTCGC CGCTGCCGCA GGGTGCGGCC GGTCCGGTTG AAGAACAATT
TGCAAAAGAA 300
GGCTTCCCGG TCATCAGCAA CTCTCCGGAT CATCGTTTCG ATCCGGACGT CCCGCTGCTG
360
GTGCCGGAAC TGAATCCGCA CACCATTAGT CTGATCGATG AACAGCGCAA
ACGTCGCGAA 420
TGGAAAGGTT TTATTGTTAC CACGCCGCTG TGCACGGCAC AAGGTGCAGC
TATCCCGCTG 480
GGTGCTATCT TCAAAGATTA CAAAATGGAC GGC GCGTTCA TTACCACGAT CCAGAGTCTG
540
TCCGGTGCAG GTTACCCGGG TATCCCGTCT CTGGATGTCG TGGACAACAT TCTGCCGCTG
600
GGCGATGGTT ATGACGCGAA AACCATTAAA GAAATCTTCC GTATTCTGTC AGAAGTTAAA
660
CGCAATGTCG ATGAACCGAA ACTGGAAGAC GTTTCGCTGG CGGCCACCAC
GCATCGTATC 720
GCCACCATTG ATGGCCACTA TGAAGTGCTG TACGTTAGTT TTAAAGAAGA AACCGCAGCT
780
GAAAAAGTGA AAGAAACGCT GGAAAACTTC CGCGGTGAAC CGCAGGATCT
GAAACTGCCG 840
ACCGCACCGT CCAAACCGAT TATCGTCATG AATGAAGATA CGCGTCCGCA AGTGTACTTT
900
GATCGCTGGG CTGGCGACAT TCCGGGTATG AGCGTTGTCG TGGGCCGTCT
GAAACAGGTG 960
AACAAACGTA TGATCCGCCT GGTGTCTCTG ATTCACAATA CCGTTCGCGG
TGC GGCGGGC 1020
GGTGGCATCC TGGCTGCTGA ACTGCTGGTT GAAAAAGGTT ACATTGAAAA A
1071

<212> TYPE : DNA

<211> LENGTH : 1071

SEQUENCENAME : SULFOLOBUS TOKODAI MCR DNA

SEQ ID NO. 16: MCR PROTEIN ORGANISM NAME : SULFOLOBUS TOKODAI

MSRRTLKAAI LGATGLVGIE YVRMLSNHPY IKPAYLAGKG SVGKPYGEVV RWQTVGQVPK
60
EIADMEIKPT DPKLMDDVDI IFSPLPQGAA GPVEEQFAKE GFPVISNSPD HRFDPDVPLL
120
VPELNPHTIS LIDEQRRRE WKGFI VTTPL CTAQGAAIPL GAIFKDYKMD GAFITTIQSL
180
SGAGYPGIPS LDVVDNILPL GDGYDAKTIK EIFRILSEVK RNVDEPKLED VSLAATTHRI
240
ATIHGHYEVL YVSFKEETAA EKVKETLENF RGEPQDLKLP TAPSKPIIVM NEDTRPQVYF
300
DRWAGDIPGM SVVVGRLKQV NCRMIRLVSL IHNTVRGAAG GGILAAELLV EKGYIEK
357

<212> TYPE : PRT

<211> LENGTH : 357

SEQUENCENAME : SULFOLOBUS TOKODAI MCR PROTEIN

SEQ ID NO. 17 : NEMA DNA ORGANISM NAME : ESCHERICHIA COLI

ATGTCATCTG AAAAAGTGTG TTCCCCACTG AAAGTGGGCG CGATCACGGC
GGCAAACCGT 60
ATTTTATGG CACCGCTGAC GCGTCTGCGC AGTATTGAAC CGGGTGACAT TCCTACCCCG
120
TTGATGGCGG AATACTATCG CCAACGTGCC AGTGCCGGTT TGATTATTAG
TGAAGCCACG 180
CAAATTTCTG CCCAGGCAAA AGGATATGCA GGTGCGCCTG GCATCCATAG
TCCGGAGCAA 240
ATTGCCGCAT GGAAAAAAT CACCGCTGGC GTTCATGCTG AAAATGGTCA
TATGGCCGTG 300
CAGCTGTGGC ACACCGGACG CATTTCTCAC GCCAGCCTGC AACCTGGCGG
TCAGGCACCG 360
GTAGCGCCTT CAGCACTTAG CGCGGGAACA CGTACTTCTC TGC GCGATGA
AAATGGTCAG 420
GCGATCCGTG TTGAAACATC CATGCCGCGT GCGCTTGAAC TGGAAGAGAT
TCCAGGTATC 480

GTCAATGATT TCCGTCAGGC CATTGCTAAC GCGCGTGAAG CCGGTTTTGA
 TCTGGTAGAG 540
 CTCCACTCTG CTCACGGTTA TTTGCTGCAT CAGTTCCTTT CTCCTTCTTC AAACCATCGT
 600
 ACCGATCAGT ACGGCGGCAG CGTGGAATAAT CGCGCACGTT TGGTACTGGA
 AGTGGTCGAT 660
 GCCGGGATTG AAGAATGGGG TGCCGATCGC ATTGGCATTG GCGTTTCACC
 AATCGGTACT 720
 TTCCAGAACA CAGATAACGG CCCGAATGAA GAAGCCGATG CACTGTATCT
 GATTGAACAA 780
 CTGGGTAAAC GCGGCATTGC TTATCTGCAT ATGTCAGAAC CAGATTGGGC
 GGGGGGTGAA 840
 CCGTATACTG ATGCGTTCCG CGAAAAAGTA CGCGCCCGTT TCCACGGTCC GATTATCGGC
 900
 GCAGGTGCAT ACACAGTAGA AAAAGCTGAA ACGCTGATCG GCAAAGGGTT
 AATTGATGCG 960
 GTGGCATTG GTCGTGACTG GATTGCGAAC CCGGATCTGG TCGCCCGCTT
 GCAGCGCAAA 1020
 GCTGAGCTTA ACCCACAGCG TGCCGAAAGT TTCTACGGTG GCGGCGCGGA
 AGGCTATACC 1080
 GATTACCCGA CGTTGTAA 1098

<212> TYPE : DNA

<211> LENGTH : 1098

SEQUENCENAME : ESCHERICHIA COLI NEMA DNA

SEQ ID NO. 18: NEMA PROTEIN ORGANISM NAME: ESCHERICHIA COLI

MSSEKLYSPL KVGAITAANR IFMAPLTRLR SIEPGDIPTP LMAEYYRQRA SAGLIISEAT 60
 QISAQAKGYA GAPGIHSPEQ IAAWKKITAG VHAENGHMAV QLWHTGRISH
 ASLQPGGQAP 120
 VAPSALSAGT RTSLRDENGQ AIRVETSMPR ALELEEIPGI VNDFRQAIAN AREAGFDLVE
 180
 LNSAHGYLLH QFLSPSSNHR TDQYGGSVEN RARLVLEVVD AGIEEWGADR IGIRVSPIGT
 240

FQNTDNGPNE EADALYLIEQ LGKRGIAYLH MSEP DWAGGE PYTDAFREKV RARFHGPIIG
300
AGAYTVEKAE TLIGKGLIDA VAFGRDWIAN PDLVARLQRK AELNPQRAES FYGGGAEGYT
360
DYPTL 365
<212> TYPE : PRT
<211> LENGTH : 365
SEQUENCENAME : ESCHERICHIA COLI NEMA PROTEIN
SEQ ID NO. 19: RUTE DNA ORGANISM NAME: ESCHERICHIA COLI
ATGAACGAAG CCGTTAGCCC AGGTGCGCTT AGCACCTGT TCACCGATGC CCGCACTCAC
60
AACGGCTGGC GGGAGACACC CGTCAGCGAT GAGACGTTAC GGGAGATTTA
TGCCCTGATG 120
AAATGGGGGC CGACATCAGC TAACTGTTCT CCGGCACGGA TCGTGTTTAC
CCGCACGGCA 180
GAAGGAAAAG AACGTCTGCG CCCGGCACTT TCCAGCGGCA ATCTGCAAAA
AACCTGACC 240
GCGCCCGTCA CCGCTATCGT CGCCTGGGAC AGTGAATTTT ATGAACGGTT ACCACTACTG
300
TTTCCCCACG GTGATGCCCC CAGTTGGTTT ACCTCCAGCC CACAATTGC CGAAGAAACA
360
GCGTTTCGCA ACAGTTCCAT GCAGGCGGCC TATCTGATCG TCGCCTGCCG
GGCGCTGGGA 420
CTGGATACCG GCCCGATGTC GGGCTTTGAC CGTCAACACG TGGACGACGC CTTTTTTACG
480
GGCAGCACGC TGAAGAGCAA TCTGCTGATT AATATCGGCT ATGGCGATAG
CAGCAAGCTT 540
TATGCGCGCC TGCCACGTCT GTCCTTTGAA GAAGCCTGCG GGCTGTTGTA A
591
<212> TYPE : DNA
<211> LENGTH : 591
SEQUENCENAME : ESCHERICHIA COLI RUTE DNA

SEQ ID NO. 20 RUTE PROTEIN ORGANISM NAME : ESCHERICHIA COLI

MNEAVSPGAL STLFTDARTH NGWRETPVSD ETLREIYALM KWGPTSANCS PARIVFTRTA
 60
 EGKERLRPAL SSGNLQKTLT APVTAIVAWD SEFYERLPLL FPHGDARSWF TSSPQLAEET
 120
 AFRNSSMQAA YLIVACRALG LDTGPMSGFD RQHVDDAFFT GSTLKSNNLI NIGYGDSSKL
 180
 YARLPRLSFE EACGLL 196

<212> TYPE : PRT

<211> LENGTH : 196

SEQUENCENAME : ESCHERICHIA COLI RUTE PROTEIN

SEQ ID NO. 21: YDFG DNA ORGANISM NAME : ESCHERICHIA COLI

ATGGTCGTTT TAGTAACTGG AGCAACGGCA GGTTTTGGTG AATGCATTAC TCGTCGTTTT
 60
 ATTCAACAAG GGCATAAAGT TATCGCCACT GGCCGTCGCC AGGAACGGTT
 GCAGGAGTTA 120
 AAAGACGAAC TGGGAGATAA TCTGTATATC GCCCAACTGG ACGTTCGCAA
 CCGCGCCGCT 180
 ATTGAAGAGA TGCTGGCATC GCTTCCTGCC GAGTGGTGCA ATATTGATAT CCTGGTAAAT
 240
 AATGCCGGCC TGGCGTTGGG CATGGAGCCT GCGCATAAAG CCAGCGTTGA
 AGACTGGGAA 300
 ACGATGATTG ATACCAACAA CAAAGGCCTG GTATATATGA CGCGCGCCGT
 CTTACCGGGT 360
 ATGGTTGAAC GTAATCATGG TCATATTATT AACATTGGCT CAACGGCAGG
 TAGCTGGCCG 420
 TATGCCGGTG GTAACGTTTA CGGTGCGACG AAAGCGTTTG TTCGTCAGTT TAGCCTGAAT
 480
 CTGCGTACGG ATCTGCATGG TACGGCGGTG CGCGTCACCG ACATCGAACC
 GGGTCTGGTG 540
 GGTGGTACCG AGTTTTCCAA TGTCCGCTTT AAAGGCGATG ACGGTAAAGC
 AGAAAAAACC 600

TATCAAAATA CCGTTGCATT GACGCCAGAA GATGTCAGCG AAGCCGTCTG
GTGGGTGTCA 660
ACGCTGCCTG CTCACGTCAA TATCAATACC CTGGAAATGA TGCCGGTTAC CCAAAGCTAT
720
GCCGGACTGA ATGTCCACCG TCAGTAA 747
<212> TYPE : DNA
<211> LENGTH : 747

SEQUENCENAME : ESCHERICHIA COLI YDFG DNA

SEQ ID NO. 22: YDFG PROTEIN ORGANISM NAME: ESCHERICHIA COLI

MVVLTGATA GFEGCITRRF IQQGHKVIAT GRRQERLQEL KDELGDNLYI AQLDVRNRAA
60
IEEMLASLPA EWCNIDILVN NAGLALGMEP AHKASVEDWE TMIDTNNKGL VYMTRAVLPG
120
MVERNHHGII NIGSTAGSWP YAGGNVYGAT KAFVRQFSLN LRTDLHGTA V RVTDIEPGLV
180
GGTEFSNVRF KGDDGKAEKT YQNTVALTPE DVSEAVWWVS TLPahVNINT LEMMPVTQSY
240
AGLNVHRQ 248
<212> TYPE : PRT
<211> LENGTH : 248

SEQUENCENAME : ESCHERICHIA COLI YDFG PROTEIN

SEQ ID NO. 23: MMSB DNA ORGANISM NAME : P. AERUGINOSA

ATGGCCGACA TTGCGTTTCT GGGTCTGGGC AATATGGGCG GTCCGATGGC
CGCGAACCTG 60
CTGAAAGCCG GCCACCGTGT GAATGTGTTC GACCTGCAAC CAAAAGCGGT
CCTGGGCTTG 120
GTTGAGCAAG GCGCGCAGGG CGCAGACTCT GCTCTGCAAT GTTGTGAGGG
TGCGGAGGTC 180
GTGATTCTA TGCTGCCAGC AGGCCAGCAT GTGGAAAGCC TGTACCTGGG
CGATGATGGT 240
CTGCTGGCAC GCGTGGCGGG CAAGCCTTTG CTGATTGACT GTAGCACCAT
CGCACC GGAA 300

ACGGCGCGTA AGGTGGCGGA GGCAGCCGCA GCAAAGGGCC TGACGCTGCT
GGATGCCCCG 360
GTTTCGGGCG GTGTCGGTGG TGCCCGTGCA GGTACGCTGT CGTTTATCGT
GGGTGGTCCG 420
GCGGAGGGTT TTGCGCGTGC GCGTCCGGTT CTGGAGAATA TGGGTCGCAA
CATTTTCCAC 480
GCGGGTGATC ACGGCGCTGG TCAGGTGGCG AAAATCTGTA ACAACATGCT
GCTGGGTATC 540
TTGATGGCGG GCACCGCCGA AGCCTTGGCG CTGGGCGTCA AAAACGGTCT
GGACCCGGCA 600
GTGCTGTCCG AAGTGATGAA ACAGAGCAGC GGTGGTAACT GGGCGCTGAA
TCTGTACAAT 660
CCGTGGCCGG GTGTGATGCC GCAGGCCCCA GCCTCTAATG GCTACGCAGG
CGGCTTCCAA 720
GTGCGCCTGA TGAACAAAGA CCTGGGCCTG GCGCTGGCGA ATGCGCAAGC
GGTCCAAGCG 780
AGCACCCCGC TGGGCGCACT GGCCCGTAAC CTGTTTAGCC TGCACGCTCA
AGCCGACGCC 840
GAGCACGAAG GTCTGGACTT CAGCTCTATT CAAAACTGT ATCGCGGTAA GGATTAG
897

<212> TYPE : DNA

<211> LENGTH : 897

SEQUENCE NAME : P. AERUGINOSA MMSB DNA

SEQ ID NO. 24: MMSB PROTEIN ORGANISM NAME: P. AERUGINOSA

MADIAFLGLG NMGGPMAANL LKAGHRVNVF DLQPKAVLGL VEQGAQGADS
ALQCCEGAEV 60
VISMLPAGQH VESLYLGDDG LLARVAGKPL LIDCSTIAPE TARKVAEAAA AKGLTLLDAP
120
VSGGVGGARA GTLSFIVGGP AEGFARARPV LENMGRNIFH AGDHGAGQVA KICNNMLLGI
180
LMAGTAEALA LGVKNGLDPA VLSEVMKQSS GGNWALNLYN PWPGVMPQAP
ASNGYAGGFQ 240

VRLMNKDLGL ALANAQAVQA STPLGALARN LFSLHAQADA EHEGLDFSSI QKLYRGKD

298

<212> TYPE : PRT

<211> LENGTH : 298

SEQUENCENAME : P. AERUGINOSA MMSB PROTEIN

SEQ ID NO. 25: NDSD DNA ORGANISMNAME : PSEUDOMONAS AERUGINOSA

ATGGGCAAAC AGATCGCCTT CATCGGCCTG GGCCATATGG GCGCACCTAT

GGCCACCAAC 60

CTGCTGAAGG CCGGCTACCT GCTGAATGTG TTCGACCTGG TGCAGAGCGC

CGTGGATGGT 120

TTAGTGGCCG CAGGTGCAAG TGCAGCACGC AGTGCACGCG ATGCCGTTCA

GGGTGCCGAC 180

GTGGTGATCA GCATGCTGCC TGCCAGCCAA CACGTGGAGG GTCTGTACCT

GGACGACGAT 240

GGTCTGCTGG CCCACATTGC CCCTGGCACC TTAGTGCTGG AGTGCAGCAC

AATCGCCCCG 300

ACCA GTGCAC GCAAGATTCA TGCAGCAGCC CGCGAGCGTG GTCTGGCAAT

GCTGGACGCA 360

CCGGTTAGCG GTGGTACAGC AGGTGCCGCA GCAGGCACCC TGACCTTCAT

GGTGGGCGGT 420

GACGCCGAAG CCCTGGAAAA AGCACGCCCCG CTGTTTGAGG CAATGGGCCG

TAACATCTTC 480

CATGCCGGCC CTGATGGCGC AGGTCAGGTG GCCAAAGTGT GCAATAACCA

GCTGCTGGCA 540

GTGCTGATGA TCGGTACCGC CGAGGCAATG GCACTGGGCG TGGCAAACGG

CTTAGAGGCC 600

AAGGTGCTGG CAGAAATCAT GCGCCGTAGT AGCGGCGGTA ACTGGGCCCT

GGAGGTGTAC 660

AACCCGTGGC CTGGCGTGAT GGAGAATGCA CCGGCCAGTC GTGACTACAG

CGGCGGTTTC 720

ATGGCACAGC TGATGGCCAA GGACCTGGGC TTAGCCCAAG AGGCAGCCCA

AGCCAGCGCC 780

AGTAGTACCC CGATGGGCAG CTTAGCCCTG AGTCTGTACC GCTTACTGCT
GAAGCAGGGC 840

TACGCCGAAC GCGACTTCAG CGTGGTGCAG AAGCTGTTCG ACCCGACCCA
AGGCCAGTAA 900

<212> TYPE : DNA

<211> LENGTH : 900

SEQUENCENAME : P. AERUGINOSA NDSD DNA

SEQ ID NO. 26: NDSD PROTEIN PSEUDOMONAS AERUGINOSA

MGKQIAFIGL GHMGAPMATN LLKAGYLLNV FDLVQSAVDG LVAAGASAAR
SARDAVQGAD 60
VVISMLPASQ HVEGLYLDDD GLLAHIAPGT LVLECSTIAP TSARKIHAAA RERGLAMLDA
120
PVSGGTAGAA AGTLTFMVGG DAEALEKARP LFEAMGRNIF HAGPDGAGQV
AKVCNNQLLA 180
VLMIGTAEAM ALGVANGLEA KVLAEIMRRS SGGNWALEVY NPWPGVMENA
PASRDYSGGF 240
MAQLMAKDLG LAQEAAQASA SSTPMGSLAL SLRLLLLKQG YAERDFSVVQ KLFDPTQGQ
299

<212> TYPE : PRT

<211> LENGTH : 299

SEQUENCENAME : P. AERUGINOSA NDSD PROTEIN

SEQ ID NO. 27: FABI(TS) PROTEIN ORGANISM NAME : ESCHERICHIA COLI

MGFLSGKRIL VTGVASKLSI AYGIAQAMHR EGAELAFTYQ NDKLKGRVEE FAAQLGSDIV
60
LQCDVAEDAS IDTMFAELGK VWPKFDGFVH SIGFAPGDQL DGDYVNAVTR EGFKIAHDIS
120
SYSFVAMAKA CRSMLNPGSA LLTSLYGAE RAIPNYNVMG LAKASLEANV RYMANAMGPE
180
GVRVNAISAG PIRTLAASGI KDFRKMLAHC EAVTPIRRTV TIEDVGNSAA FLCSDLSAGI
240
FGEVVHVDGG FSIAAMNELE LK 262
<212> TYPE : PRT

<211> LENGTH : 262

SEQUENCENAME : ESCHERICHIA COLI FABI(TS) PROTEIN

SEQ ID NO. 28: FABB(TS) PROTEIN ORGANISM NAME : ESCHERICHIA COLI

MKRAVITGLG IVSSIGNNQ EVLASLREGR SGITFSQELK DSGMRSHVWG NVKLDTTGLI

60

DRKVVRFMSD ASIYAFLSME QAIADAGLSP EAYQNNPRVG LIAGSGGGSP RFQVFGADAM

120

RGPRGLKAVG PYVVTKAMAS GVSACLATPF KIHGVNYSIS SACATSAHCI GNAVEQIQLG

180

KQDIVFAGGG EELCWEMACE FDAMGALSTK YNDTPEKASR TYDAHRDGFV

IAGGGGMVVV 240

ELEHALARG AHIYAEIVGY GATSDGADMV APSGEGAVRC MKMAMHGVDT PIDYLNHSGT

300

STPVGDVKEL AAIREVFGDK SPAISATKVM TGHSLGAAGV QEAIYLLML EHGFIAPSIN

360

IEELDEQAAG LNIVTETDDR ELTTVMSNSF GFGGTNATLV MRKLKD 406

<212> TYPE : PRT

<211> LENGTH : 406

SEQUENCENAME : ESCHERICHIA COLI FABB(TS) PROTEIN

SEQ ID NO. 29: FABD(TS) PROTEIN ORGANISM NAME : ESCHERICHIA COLI

MTQFAFVFPG QGSQTVGMLA DMAASYPIVE ETFAEASAAL GYDLWALTQQ

GPAEELNKTW 60

QTQPALLTAS VALYRVWQQQ GKGAPAMMAG HSLGEYSALV CAGVIDFADA

VRLVEMRGKF 120

MQEAVPEGTG AMAAIIGLDD ASIAKACEEA AEGQVVSPVN FNSPGQVVIA GHKEAVERAG

180

AACKAAGAKR ALPLVSVPS HCALMKPAAD KLAVELAKIT FNAPTVPVVN NVDVKCETNG

240

DAIRDALVRQ LYNPVQQTKS VEYMAAQGVE HLYEVGPGKV LTGLTKRIVD TLTASALNEP

300

SAMAAALEL 309

<212> TYPE : PRT

<211> LENGTH : 309

SEQUENCENAME : ESCHERICHIA COLI FABD(TS) PROTEIN

SEQ ID NO. 30: RHTA DNA ORGANISM NAME : ESCHERICHIA COLI

ATGAGCGGTT CATTACGTAA AATGCCGGTC TGGTTACCAA TAGTCATATT GCTCGTTGCC
60
ATGGCGTCTA TTCAGGGTGG AGCCTCGTTA GCTAAGTCAC TTTTCTCTCT GGTGGGCGCA
120
CCGGGTGTCA CTGCGCTGCG TCTGGCATTG GGAACGCTGA TCCTCATCGC GTTCTTTAAG
180
CCATGGCGAC TGCCTTTGCG CAAAGAGCAA CGGTTACCGC TGTTGTTTGA CGGCGTTTCG
240
CTGGGTGGGA TGAATTATCT TTTTATCTT TCTATTCAGA CAGTACCGCT GGGTATTGCG
300
GTGGCGCTGG AGTTCACCGG ACCACTGGCG GTGGCGCTGT TCTCTTCTCG
TCGCCCCGTA 360
GATTCGTCT GGGTTGTGCT GCGGTTCTT GGTCTGTGGT TCCTGCTACC GCTGGGGCAA
420
GACGTTTCCC ATGTCGATTT AACCGGCTGT GCGCTGGCAC TGGGGGCCGG
GGCTTGTTGG 480
GCTATTTACA TTTTAAGTGG GCAACGCGCA GGAGCGGAAC ATGGCCCTGC
GACGGTGGCA 540
ATTGGTTCGT TGATTGCAGC GTTAATTTTC GTGCCAATTG GAGCGCTTCA GGCTGGTGAA
600
GCACTCTGGC ACTGGTCGGT TATTCCATTG GGTCTGGCTG TCGCTATTCT CTCGACCGCT
660
CTGCCTTATT CGCTGGAAAT GATTGCCCTC ACCCGTTTGC CAACACGGAC ATTTGGTACG
720
CTGATGAGCA TGGAACCGGC GCTGGCTGCC GTTCCGGGA TGATTTTCCT
CGGAGAAACA 780
CTGACACCCA TACAGCTACT GCGCTCGGC GCTATCATCG CCGCTTCAAT GGGGTCTACG
840
CTGACAGTAC GCAAAGAGAG CAAAATAAAA GAATTAGACA TTAATTAA 888

<212> TYPE : DNA

<211> LENGTH : 888

SEQUENCENAME : ESCHERICHIA COLI RHTA DNA

SEQ ID NO. 31: RHTA PROTEIN ORGANISM NAME : ESCHERICHIA COLI

MSGSLRKMPV WLPIVILLVA MASIQGGASL AKSLFPLVGA PGVTALRLAL GTLILIAFFK

60

PWRLRFAKEQ RLPLLFYGVV LGGMNYLFYL SIQTVPLGIA VALEFTGPLA VALFSSRRPV

120

DFVWVVLAVL GLWFLPLGQ DVSHVDLTGC ALALGAGACW AIYILSGQRA GAEHGPATVA

180

IGSLIAALIF VPIGALQAGE ALWHWSVIPL GLAVAILSTA LPYSLEMIAL TRLPTRTFGT 240

LMSMEPALAA VSGMIFLGET LTPIQLLAG AIIAASMGST LTVRKESKIK ELDIN 295

<212> TYPE : PRT

<211> LENGTH : 295

SEQUENCENAME : ESCHERICHIA COLI RHTA PROTEIN

SEQ ID NO. 32: YDCO DNA ORGANISM NAME : ESCHERICHIA COLI

ATGCGACTCC TCCGGCAAAA CGGAAGTTTA TCACTTGTGC GTTATAACGG ACAAATGCTA

60

CGGTGCCTGT ACGCTATAAC GCACGAGGTG ACTATGCGTC TGTTTTCTAT TCCTCCACCC

120

ACGCTACTGG CGGGGTTTCT GGCGGTATTA ATTGGCTACG CCAGTTCAGC

GGCAATAATC 180

TGGCAAGCAG CGATTGTCGC CGGAGCCACC ACTGCACAAA TCTCTGGCTG

GATGACGGCG 240

CTGGGGCTGG CAATGGGCGT CAGTACGCTG ACTCTGACAT TATGGTATCG

CGTACCTGTT 300

CTCACCGCAT GGTCAACGCC TGGCGCGGCT TTGTTGGTCA CCGGATTGCA

GGGACTAACA 360

CTTAACGAAG CCATCGGCGT TTTTATTGTC ACCAACGCGC TAATAGTCCT CTGCGGCATA

420

ACGGGACTCT TTGCTCGTCT GATGCGCATT ATTCCGCACT CGCTTGCGGC GGCAATGCTT

480

GCCGGGATTT TATTACGCTT TGGTTTACAG GCGTTTGCCA GTCTGGACGG TCAATTTACG
540
TTGTGTGGAA GTATGTTGCT GGTATGGCTG GCAACCAAGG CCGTTGCGCC
GCGCTATGCG 600
GTAATTGCCG CGATGATTAT TGGGATCGTG ATCGTCATCG CGCAAGGTGA
CGTTGTCACA 660
ACTGATGTTG TCTTTAAACC CGTTCTCCCC ACTTATATTA CCCCTGATTT TTCGTTTGCT
720
CACAGCCTGA GCGTTGCACT CCCCCTTTTT CTGGTGACGA TGGCATCGCA AAACGCACCG
780
GGTATCGCAG CAATGAAAGC AGCTGGATAT TCGGCTCCTG TTTCGCCATT AATTGTATTT
840
ACTGGATTGC TGGCACTGGT TTTTCCCCT TTCGGCGTTT ATTCCGTCGG TATTGCGGCA
900
ATCACCGCGG CTATTTGCCA AAGCCCGGAA GCGCATCCGG ATAAAGATCA
ACGTTGGCTG 960
GCCGCTGCCG TTGCAGGCAT TTTCTATTTG CTCGCAGGTC TGTTTGGTAG TGCCATTACC
1020
GGGATGATGG CTGCCCTGCC CGTAAGTTGG ATCCAGATGC TGGCAGGTCT
GGCGCTGTTA 1080
AGTACCATCG GCGGCAGTTT GTATCAGGCG CTGCATAATG AGCGTGAGCG
AGACGCGGCG 1140
GTGGTGGCAT TTCTGGTAAC GGCAAGTGGA TTGACGCTGG TCGGGATTGG
TTCTGCGTTT 1200
TGGGGATTAA TTGCCGAGG CGTTTGTTAC GTGGTGTTGA ATTTAATCGC
TGACAGAAAC 1260
CGATATTGA 1269

<212> TYPE : DNA

<211> LENGTH : 1269

SEQUENCENAME : ESCHERICHIA COLI YDCO DNA

SEQ ID NO. 33: YDCO PROTEIN ORGANISM NAME : ESCHERICHIA COLI

MRLLRQNGSL SLVRYNGQML RCLYAITHEV TMRLFSIPPP TLLAGFLAVL IGYASSAAII
60
WQAAIVAGAT TAQISGWMTA LGLAMGVSTL TLTLWYRVPV LTAWSTPGAA LLVTGLQGLT
120
LNEAIGVFIV TNALIVLCGI TGLFARLMRI IPHSLAAAML AGILLRFGQLQ AFASLDGQFT
180
LCGSMLLVWL ATKAVAPRYA VIAAMIIGIV IVIAQGDVVT TDVVFKEPVL TYITPDFSFA
240
HSLSVALPLF LVTMASQNAP GIAAMKAAGY SAPVSPLIVF TGLLALVFSP FGVYSGVIAA
300
ITAAICQSPE AHPDKDQRWL AAVAGIFYL LAGLFGSAIT GMMAALPVSW IQMLAGLALL
360
STIGGSLYQA LHNERERDAA VVAFLVTASG LTLVGIGSAF WGLIAGGVCY VVLNLIADRN
420
RY 422

<212> TYPE : PRT

<211> LENGTH : 422

SEQUENCENAME : ESCHERICHIA COLI YDCO PROTEIN

SEQ ID NO. 34: ACRD ORGANISM NAME : ESCHERICHIA COLI

MANFFIDRPI FAWVLAILLC LTGTLAIFSL PVEQYPDLP PNVVRTANYP GASAQTLENT
VTQVIEQNMT GLDNLMYMSS QSSGTGQASV TLSFKAGTDP DEAVQQVQNNQ LQSAMRKLPQ
AVQNQGVTVR KTGDTNILT AFVSTDGSMQ KQDIADYVAS NIQDPLSRVN GVGIDIDAYS
QYSMRIWLDK AKLNSFQMTA KDVTDIESQ NAQIAGVQLG GTPSVDKQAL NATINAQSL
QTPEQFRDIT LRVNQDGSEV RLGDVATVEM GAEKYDYLSR FNGKPASGLG VKLASGANEM
ATAELVLNRL DELAQYFPHG LEYKVAYETT SFVKASIEDV VKTLLEAIAL VFLVMYFLQ
NFRATLIPTI AVPVVLMGTF SVLYAFGYSV NTLTMFAMVL AIGLLVDDAI VVVENVERIM
SEGLTPREA TRKSMGQIQG ALVGIAMVLS AVFVPMAFFG GTTGAIYRQF SITIVAAMVL
SVLVAMILTP ALCATLLKPL KKGEHHGQKG FFAWFNQMFN RNAERYEKGV AKILHRSRLW
IVIYVLLGG MVFLFLRLPT SFLPLEDRGM FTTSVQLPSG STQQQTLKVV EQIEKYFTH
EKDNIMSVFA TVGSGPGGNG QNVARMFIRL KDWSEKDSK GTSFALLERA TKAFNQIKEA
RVIASSPPAI SGLGSSAGFD MELQDHAGAG HDALMAARNQ LLALAAENPE LTRVRHNGLD
DSPQLQIDID QRKAQALGVA IDDINDTLQT AWGSSVYVNDF MDRGRVKKVY VQAAAPYRML

PDDINLWYVR NKDGGMVPFS AFATSRWETG SPRLERYNGY SAVEIVGEAA PGVSTGTAMD
 IMESLVKQLP NGFGLEWTAM SYQERLSGAQ APALY AISLL VVFLCLAALY ESWSVPFSVM
 LVVPLGVIGA LLATWMRGLE NDVYFQVGLL TVIGLSAKNA ILIVEFANEM NQKGHDLEA
 TLHACRQRLR PILMTSLAFI FGVLP MATST GAGSGGQHAV GTGVMGGMIS ATILAIYFVP
 LFFVLVRRRF PLKPRPE

SEQ ID NO. 35: ATTA ORGANISM NAME : ESCHERICHIA COLI

MAHPPLLHLQ DITLSLGGNP LLDGAGFAVG RGERLCLVGR NGSGKSTLLK IAAGVIQPD
 GSVFVQPGAS LRYLPQEPDL SAYATTADYV VGQIGDPDMA WRATPLLDAL GLTGRESTQN
 LSGGEGRRCA IAGVLAAAPD VLLLDEPTNH LDMPTIEWLE RELSLGAMV IISHDRRLS
 TLRSVVWLD RGVTRRLDEG FGRFEAWREE VLEQEERDAH KLDRIAREE DWMRYGVTAR
 RKRNVRRVRE LADLRTARKE AIRAPGTTL NTQLRPHRKL VAVAEDISKA WGEKQVVRHL
 DLRLRGDRL GIVGANGAGK TTLLRMLTGL DQPDSTISL GPSLNMVTL DQQRRTLNPER
 TLADTLTEGG GDMVQVGTEK RHVVGVMKDF LFRPEQARTP VSALSGGERG RLMLACALAK
 PSNLLVLDEP TNDLDLETLD ILQDMLASCE GTVLLVSHDR DFLDRVATSV LATEGDGNWI
 EYAGGYSDML AQRHQKPLTT ASVVENPTK PKETTAARGP TKKLSYKDQF ALDNLPKEME
 KLEAQAANCV KNWQIQIYME KTPRSLRNER LIYRSSKQSW QNLKNAGWNW K

SEQ ID NO. 36: BCR ORGANISM NAME : ESCHERICHIA COLI

MTTRQHSSFA IVFILGLLAM LMPLSIDMYL PALPVISAQF GVPAGSTQMT LSTYLGFAL
 GQLIYGPMAD SFGRKPVVLG GTLVFAAAAV ACALANTIDQ LIVMRFFHGL AAAAASVVIN
 ALMRDIYPKE EFSRMMSFVM LVTTIAPLMA PIVGGWVLVW LSWHYIFWIL ALAAILASAM
 IFFLIKETLP PERRQPFHIR TTIGNFAALF RHKRVLSYML ASGFSFAGMF SFLSAGPFVY
 IEINHVAPEN FGYYFALNIV FLFVMTIFNS RFVRRIGALN MFRSGLWVIF IMAAWMVISA
 LLGLGFWSLV VGVAAFVGCV SMVSSNAMAV ILDEFPHMAG TASSLAGTFR FGIGAIVGAL
 LSLATFNSAW PMIWSIAFCA TSSILFCLYA SRPKKR

SEQ ID NO. 37: CUSA ORGANISM NAME : ESCHERICHIA COLI

MIEWIIRRSV ANRFLVLMGA LFLSIWGTWT IINTPVDALP DLSDVQVIK TSYPGQAPQI
 VENQVTYPLT TTMLSVPGAK TVRGFSQFGD SYVYVIFEDG TDPYWARSRV LEYLNQVQGK
 LPAGVSAELG PDATGVGWYI EYALVDRSGK HDLADLRSLQ DWFLKYELKT IPDVAEVASV
 GGVVKEYQVV IDPQRLAQYG ISLAEVKSAL DASNQEAGGS SIELAEAEYM VRASGYLQTL
 DDFNHIVLKA SENGVPVYLR DVAKVQIGPE MRRGIAELNG EGEVAGGVVI LRSGKNAREV
 IAAVKDKLET LKSSLPEGVE IVTTYDRSQL IDRAIDNLSG KLEEFIVVA VVCALFLWHV
 RSALVAIISL PLGLCIAFIV MHFQGLNANI MSLGGIAIAV GAMVDAAIVM IENAHKRLEE

WQHQPDPATL DNKTRWQVIT DASVEVGPAL FISLLIITLS FIPIFTLEGQ EGRLFGPLAF
 TKTYAMAGAA LLAIVVIPIL MGYWIRGKIP PESSNPLNRF LIRVYHPLLL KVLHWPKTTL
 LVAALSVLTV LWPLNKVGGE FLPQINEGDL LYMPSTLPGI SAAEAASMLQ KTDKLIMSVP
 EVARVFGKTG KAETATDSAP LEMVETTIQL KPQEQWRPGM TMDKIIIEELD NTVRLPGLAN
 LWVPPIRNRI DMLSTGIKSP IGIKVS GTVL ADIDAMAEQI EEVARTVPGV ASALAERLEG
 GRYINVEINR EKAARYGMTV ADVQLFVTSV VGGAMVGETV EGIARYPINL RYPQSWRDSP
 QALRQLPILT PMKQQITLAD VADIKVSTGP SMLKTENARP TSWIYIDARD RDMVSVVHDL
 QKAIAEKVQL KPGTSVAFSG QFELLERANH KLKLMVPMTL MIIFVLLYLA FRRVGEALLI
 ISSVPFALVG GIWLLWWMGF HLSVATGTGF IALAGVAAEF GVVMLMYLRH AIEAVPSLNN
 PQTFSEQKLD EALYHGAVLR VRPKAMTVAV IAGLLPILW GTGAGSEVMS RIAAPMIGGM
 ITAPLLSLFI IPAAAYKLMWL HRHRVRK

SEQ ID NO. 38: CYNX ORGANISM NAME: ESCHERICHIA COLI

MLLVVLIGL NMRPLTSGV PLLPQLRQAS GMSFSVAALL TALPVVTMGG LALAGSWLHQ
 HVSERRSVAI SLLLIavgal MRELYPQSAL LLSSALLGGV GIGIIQAVMP SVIKRRFQQR
 TPLVMGLWSA ALMGGGGLGA AITPWLvQHS ETWYQTLAWW ALPAVVALFA WWWQSAREVA
 SSHKTTTTTPV RVVFTPRAWT LGVYFGLING GYASLIWLP AFYIEIGASA QYSGSLLALM
 TLGQAAGALL MPAMARHQDR RKLLMLALVL QLVGFCGFIW LPMQLPVLWA MVCGLGLGGA
 FPLCLLLALD HSVQPAIAGK LVAFMQGIGF IAGLAPWFS GVLRISISGNY LMDWAFHALC
 VVGLMIITLR FAPVRFPQLW VKEA

SEQ ID NO. 39: DEDA ORGANISM NAME : ESCHERICHIA COLI

MDLIYFLIDF ILHIDVHLAE LVAEYGVWVY AILFLILFCE TGLVVTPFLP GDSLLFVAGA
 LASLETNDLN VHMMVVLMLI AAIVGDAVNY TIGRLFGEKL FSNPNSKIFR RSYLDKTHQF
 YEKHGGKTII LARFVPIVRT FAPFVAGMGH MSYRHFAAYN VIGALLWVLL FTYAGYFFGT
 IPMVQDNLKL LIVGIIVSI LPGVIEIRH KRAAARAAC

SEQ ID NO. 40: EAMA ORGANISM NAME : ESCHERICHIA COLI

MSRKDGV LAL LVVVVWGLNF VVIKVG LHM PPLMLAGLRF MLVAFPAIFF VARPKVPLNL
 LLGYGLTISF AQFAFLFCAL NFGMPAGLAS LVLQAQAFIT IMLGAFTFGE RLHGKQLAGI
 ALAIFGV LVL IEDSLNGQHV AMLGFMLTLA AAFSWACGNI FNKKIMSHST RPAVMSLVIW
 SALIPIIPFF VASLILDGSA TMIHSLVTID MTTILSLMYL AFVATIVGYG IWGTLLGRYE
 TWRVAPLSLL VPVVG LASAA LLLDERLTGL QFLGAVLIMT GLYINVFLGR WRKAVKVGs

SEQ ID NO. 41: EAMB ORGANISM NAME : ESCHERICHIA COLI

MTPTLLSAFW TYTLITAMTP GPNNILALSS ATSHGFRQST RVLAGMSLGF LIVMLLCAGI
SFSLAVIDPA AVHLLSWAGA AYIVWLAWKI ATSPTKEDGL QAKPISFWAS FALQFVNVKI
ILYGV TALST FVLPQTQALS WVVGVSVLLA MIGTFGNVCW ALAGHLFQRL FRQYGRQLNI
VLALLLVYCA VRIFY

SEQ ID NO. 42: EMRB ORGANISM NAME : ESCHERICHIA COLI

MQQQKPLEGA QLVIMTIALS LATFMQVLDS TIANVAIPTI AGNLGSSLSQ GTWVITSFGV
ANAI SIPLTG WLAKRVGEVK LFLWSTIAFA IASWACGVSS SLNMLIFFRV IQGIVAGPLI
PLSQSLLLNN YPPAKRSIAL ALWSMTVIVA PICGPILGGY ISDNYHWGWI FFINVPIGVA
VVLMTLQTLR GRETRTERRR IDAVGLALLV IGIGSLQIML DRGKELDWFS SQEIIILTVV
AVVAICFLIV WELTDDNPIV DLSLFKSRNF TIGCLCISLA YMLYFGAIVL LPQLLQEVYG
YTATWAGLAS APVGIIPVIL SPIIGRFAHK LDMRRLVTFS FIMYAVCFYW RAYTFEPGMD
FGASAWPQFI QGFAVACFFM PLTTITLSGL PPERLAAASS LSNFTRTLAG SIGTSITTTM
WTNRESMHHA QLTESVNPFN PNAQAMYSQL EGLGMTQQQA SGWIAQQITN QGLIISANEI
FWMSAGIFLV LLGLVWFAKP PFGAGGGGGG AH

SEQ ID NO. 43: EMRD ORGANISM NAME : ESCHERICHIA COLI

MKRQRNVNLL LMLVLLVAVG QMAQTIYIPA IADMARDLNV REGAVQSVMG
AYLLTYGVSQ
LFYGPISDRV GRRPVILVGM SIFMLATLVA VTTSSLTVLI AASAMQGMGT GVGGVMARTL
PRDLYERTQL RHANSLNMG ILVSPLLAPL IGGLDTMWN WRACYLFLV LCAGVTFSMA
RWMPETRPVD APRTRLLTSY KTLFGNSGFN CYLLMLIGGL AGIAAFEACS GVLMGAVLGL
SSMTVSILFI LPIPAFFGA WFAGRPNKRF STLMWQSVIC CLLAGLLMWI PDWFGVMNVW
TLLVPAALFF FGAGMLFPLA TSGAMEPFPP LAGTAGALVG GLQNIGSGVL ASLSAMLPQT
GQGSLLMT LMGLLIVLCW LPLATRMHQ GQPV

SEQ ID NO. 44: EMRKY ORGANISM NAME : ESCHERICHIA COLI

MAITKSTPAP LTGGTLWCVT IALSLATFMQ MLDSTISNVA IPTISGFLGA STDEGTWVIT
SFGVANAI AI PVTGRLAQRI GELRLFLLSV TFFSLSSLMC SLSTNLDVLI FFRVVQGLMA
GPLIPLSQL LLRNYPPEKR TFALALWSMT VIIAPICGPI LGGYICDNFS WGWIFLINVP
MGIIVTLCL TLLKGRETET SPVKMNLPLG TLLVLGVGGL QIMLDKGRDL DWFNSSTIII
LTVSVISLI SLVIWESTSE NPILDLSLFK SRNFTIGIVS ITCAYLFYSG AIVLMPQLLQ
ETMGYNAIWA GLAYAPIGIM PLLISPLIGR YGNKIDMRLL VTFSFLMYAV CYYWRSVTFM
PTIDFTGIIL PQFFQGFVA CFFLPLTTIS FSGLPDNKFA NASSMSNFFR TLGSGVGTSL

TMTLWGRRES LHHSQLTATI DQFNPVFNSS SQIMDKYYGS LSGVLNEINN EITQQSLSIS
ANEIFRMAAI AFILLTVLVW FAKPPFTAKG VG

SEQ ID NO. 45: EMRY ORGANISM NAME : ESCHERICHIA COLI

MAITKSTPAP LTGGTLWCVT IALSLATFMQ MLDSTISNVA IPTISGFLGA STDEGTWVIT
SFGVANAIAI PVTGRLAQRI GELRLFLLSV TFFSLSSLMC SLSTNLDVLI FFRVVQGLMA
GPLIPLSQL LLRNYPPEKR TFALALWSMT VIIAPICGPI LGGYICDNFS WGWIFLINVP
MGIIVLTLCL TLLKGRETET SPVKMNLPLG TLLVLGVGGL QIMLDKGRDL DWFNSSTIII
LTVVSVISLI SLVIWESTSE NPILDLSLFK SRNFTIGIVS ITCAYLFYSG AIVLMPQLLQ
ETMGYNAIWA GLAYAPIGIM PLLISPLIGR YGNKIDMRLL VTFSFLMYAV CYYWRSVTFM
PTIDFTGIIL PQFFQGFAVA CFFLPLTTIS FGLPDNKFA NASSMSNFFR TSGSVGTSL
TMTLWGRRES LHHSQLTATI DQFNPVFNSS SQIMDKYYGS LSGVLNEINN EITQQSLSIS
ANEIFRMAAI AFILLTVLVW FAKPPFTAKG VG

SEQ ID NO. 46: GARP ORGANISM NAME : ESCHERICHIA COLI

MILDTVDEKK KGVHTRYLIL LIIFIVTAVN YADRATLSIA GTEVAKELQL SAVSMGYIFS
AFGWAYLLMQ IPGGWLLDKF GSKKVYTYSL FFWSLFTFLQ GFVDMFPLAW AGISMFFMRF
MLGFSEAPSF PANARIVAAW FPTKERGTAS AIFNSAQYFS LALFSPLLGW LTFAWGWEHV
FTVMGVIGFV LTALWIKLIH NPTDHPRMSA EELKFISENG AVVDMDHKKP GSAAASGPKL
HYIKQLLSNR MMLGVFFGQY FINTITWFFL TWFPYLVQE KGMSILKVGL VASIPALCGF
AGGVLGGVFS DYLIKRGSL TLARKLPIVL GMLLASTIIL CNYTNNTTLV VMLMALAFFG
KGFGALGWPV ISDTAPKEIV GLCGGVFNVF GNVASIVTPL VIGYLVSELH SFNAALVFVG
CSALMAMVCY LFVVGDIKRM ELQK

SEQ ID NO. 47: GUDP ORGANISM NAME: ESCHERICHIA COLI

MSSLSQAASS VEKRTNARYW IVVMLFIVTS FNYGDRATLS IAGSEMAKDI GLDPVGMGYV
FSAFSWAYVI GQIPGGWLLD RFGSKRVYFW SIFIWSMFTL LQGFVDIFSG FGIIVALFTL
RFLVGLAEAP SFPGNSRIVA AWFPAQERGT AVSIFNSAQY FATVIFAPIM GWLTHEVGWS
HVFFFMGGLG IVISFIWLKV IHEPNQHPGV NKKELEYIAA GGALINMDQQ NTKVKVPFSV
KWGQIKQLLG SRMMIGVYIG QYCINALTYF FITWFPVYLV QARGMSILKA GFVASVPAVC
GFIGGVLLGI ISDWLMRRTG SLNIARKTPI VMGMLLSMVM VFCNYVNVEW MIIGFMALAF
FGKGIGALGW AVMADTAPKE ISGLSGGLFN MFGNISGIVT PIAIGYIVGT TGSFNGALIY
VGVHALIAVL SYLVLVGDIK RIELKPVAGQ

SEQ ID NO. 48: HSRA ORGANISM NAME: ESCHERICHIA COLI

MSDKKKRSMAGLPWIAAMAF FMQALDATIL NTALPAIAHS LNRSPAMQS AIISYTLTVA
MLIPVSGWLA DRFGTRRIFT LAVSLFTLGS LACALSNSLP QLVVFRVIQG IGGAMMMMPVA
RLALLRAYPR NELLPVLFV AMPGLVGPIL GPVLGGVLVT WATWHWIFLI NIPIGIAGLL
YARKHMPNFT TARRRFDITG FLLFGLSLVL FSSGIELFGE KIVASWIALT VIVTSIGLLL
LYILHARRTP NPLISDLFK TRTFSIGIVG NIATRLGTGC VPFLMPLMLQ VGFGYQAFIA
GCMMAPTALG SIIAKSMVTQ VLRRLGYRHT LVGITVIIGL MIAQFSLQSP AMAIWMLILP
LFILGMAMST QFTAMNTITL ADLTDDNASS GNSVLAVTQQ LSISLGVAVS AAVLRVYEGM
EGTTTVEQFH YTFITMGIIT VASAAMFMLL KTTDGNNLIK RQRKSKPNRV PSESE

SEQ ID NO. 49: LEUE ORGANISM NAME : ESCHERICHIA COLI

MFAEYGVVNY WTYLVGAIFI VLVPGPNTLF VLKNSVSSGM KGGYLAACGV FIGDAVLMFL
AWAGVATLIK TTPILFNIVR YLGAFYLLYL GSKILYATLK GKNSEAKSDE PQYGAIFKRA
LILSLTNPKA ILFYVSFFVQ FIDVNAPHTG ISFFILAATL ELVSFCYLSF LIISGAFVTQ
YIRTKKKLAK VGNSLIGLMF VGFAARLATL QS

SEQ ID NO. 50: MDLB ORGANISM NAME : ESCHERICHIA COLI

MRSFSQLWPT LKRLLAYGSP WRKPLGIAVL MMWVAAAAEV SGPLLISYFI DNMVAKNNLP
LKVVAGLAAA YVGLQLFAAG LHQAQSLLEN RAAVGVVQQL RTDVMDAALR QPLSEFDTQP
VGQVISRVTN DTEVIRDLYV TVVATVLRSA ALVGAMLVAM FSLDWRMALV AIMIFPVVLV
VMVIYQRYST PIVRRVRAYL ADINDGFNEI INGMSVIQQF RQQARFGERM GEASRSHYMA
RMQTLRLDGF LLRPLLSLFS SLILCGLLML FGFSASGTIE VGVLYAFISY LGRLNEPLIE
LTTQQAMLQQ AVVAGERVFE LMDGPRQQYG NDDRPLQSGT IEVDNVSFAY RDDNLVLKNI
NLSVPSRNFV ALVGHTGSGK STLASLLMGY YPLTEGEIRL DGRPLSSLH SALRQGVAMV
QQDPVVLADT FLANVTLGRD ISEERVWQAL ETVQLAELAR SMSDGIYTPL GEQGNNSLVG
QKQLLALARV LVETPQILIL DEATASIDSG TEQAIQHALA AVREHTTLVV IAHRLSTIVD
ADTILVLHRG QAVEQGTHQQ LLAAQGRYWQ MYQLQLAGEE LAASVREEES LSA

SEQ ID NO. 51: MDTD ORGANISM NAME : ESCHERICHIA COLI

MTDLPDSTRW QLVWVAFGFF MQSLDTTIVN TALPSMAQSL GESPLMHMNV IVSYVLTAVV
MLPASGWLAD KVGVRNIFFT AIVLFTLGSF FCALSGTLNE LLLARALQGV GGAMMVPVGR
LTMVKIVPRE QYMAAMTFVT LPGQVGPLL G PALGGLLVEY ASWHWIFLIN IPVGIIGAIA
TLLLMPNYTM QTRRFDLSGF LLLAVGMAVL TLALDGSKGT GLSPLTIAGL VAVGVVALVL
YLLHARNNNR ALFSLKLFRT RTFSLGLAGS FAGRIGSGML PFMTVPFLQI GLGFSPFHAG
LMMIPMVLGS MGMKRIVVQV VNRFGYRRVL VATTGLSLV TLLFMTTALL GWYYVLPFVL

FLQGMVNSTR FSSMNTLTlk DLPDNLASSG NSLLSMIMQL SMSIGVTIAG LLLGLFGSQH
VSVDSGTTQT VFMYTWLSMA LIALPAFIF ARVPNDTHQN VAISRRKRSA Q

SEQ ID NO. 52: MDTG ORGANISM NAME : ESCHERICHIA COLI

MSPCENDTPI NWKRNLIVAW LGCFLTGA AF SLVMPFLPLY VEQLGVTGHS ALNMWSGIVF
SITFLFSAIA SPFWGGLADR KGRKLMMLRS ALGMGIVMVL MGLAQNIWQF LILRALLGLL
GGFVPPANAL IATQVPRNKS GWALGTSTG GVSGALLGPM AGGLLADSYG LRPVFFITAS
VLILCFFVTL FCIREKFQPV SKKEMLMHRE VVTSKLNPKL VLSLFVTTLI IQVATGSIAP
ILTLYVRELA GNVSNAFIS GMIASVPGVA ALLSAPRLGK LGDRIGPEKI LITALIFSVL
LLIPMSYVQT PLQLGILRFL LGAADGALLP AVQTLLVYNS SNQIAGRIFS YNQSF RDIGN
VTGPLMGA AI SANYGFRAVF LVTAGVVLFN AVYSWNSLRR RRIPQVSN

SEQ ID NO. 53: MDTL ORGANISM NAME : ESCHERICHIA COLI

MSRFLICSFA LVLLYPAGID MYLVGLPRIA ADLNASEAQL HIAFSVYLAG MAAAMLFA GK
VADRSGRKPV AIPGAALFII ASVFCSLAET STLFLAGRFL QGLGAGCCYV VAFAILRDTL
DDRRRAKVLS LLNGITCIIP VLAPVLGH LI MLKFPWQSLF WAMAMMGIAV LMLSLFILKE
TRPAAPAASD KPRENSESLL NRFFLSRVVI TTLSVSVILT FVNTSPVLLM EIMGFERGEY
ATIMALTAGV SMTVSFSTPF ALGIFKPRTL MITSQVLFLA AGITLAVSPS HAVSLFGITL
ICAGFSVGFG VAMSQALGPF SLRAGVASST LGIAQVCGSS LWIWLAAVVG IGAWNMLIGI
LIACSIVSLL LIMFVAPGRP VAAHEEIH HH A

SEQ ID NO. 54: MDTM ORGANISM NAME : ESCHERICHIA COLI

MPRFFTRHAA TLFFPMALIL YDFAAYLSTD LIQPGIINVV RDFNADVSLA PAAVSLYLAG
GMALQWLLGP LSDRIGRRPV LITGALIFTL ACAATMFTTS MTQFLIARAI QGTSICFIAT
VGYYTVQEAF GQTKGIKLMA IITSIVLIAP IIGPLSGAAL MHFMHWKVLF AIIAVMGFIS
FVGLLLAMPE TVKRGAVPFS AKSVLRDFRN VFCNRLFLFG AATISLSYIP MMSWVAVSPV
ILIDAGSLTT SQFAWTQVPV FGAVIVANAI VARFVKDPTE PRFIWRAVPI QLVGLSLLIV
GNLLSPHVWL WSVLGTSLYA FGIGLIFPTL FRFTLFSNKL PKGTVSASLN MVILMVMSVS
VEIGRWLWFN GGRLPFHLLA VVAGVIVVFT LAGLLNRVRQ HQAAELVEEQ

SEQ ID NO. 55: MHPT ORGANISM NAME : ESCHERICHIA COLI

MSTRTPSSSS SRLMLTIGLC FLVALMEGLD LQAAGIAAGG IAQAFALDKM QMGWIFSAGI
LGLLP GALVG GMLADRYGRK RILIGSVALF GLFSLATAIA WDFPSLVFAR LMTGVGLGAA
LPNLIALTSE AAGPRFRGTA VSLMYCGVPI GAALAATLGF AGANLAWQTV FWVGGVVPLI
LVPLLMRWLP ESAVFAGEKQ SAPPLRALFA PETATATLLL WLCYFFTL LV VYMLINWLPL
LLVEQGFQPS QAAGVMFALQ MGAASGTLML GALMDKLRPV TMSLLIYSGM LASLLALGTV

SSFNGMMLLAG FVAGLFATGG QSVLYALAPL FYSSQIRATG VGTAVAVGRL GAMSGPLLAG
KMLALGTGTV GVMAASAPGI LVAGLAVFIL MSRRSRIQPC ADA

SEQ ID NO. 56: RHTA ORGANISM NAME : ESCHERICHIA COLI

MPGSLRKMPV WLPIVILLVA MASIQGGASL AKSLFPLVGA PGVTALRLAL GTLILIAFFK
PWRLRFAKEQ RLPLLFYGVSLGGMNYLFYL SIQTVPLGIA VALEFTGPLA VALFSSRRPV
DFVWVVLAVL GLWFLPLGQ DVSHVDLTGC ALALGAGACW AIYILSGQRA GAEHGPATVA
IGSLIAALIF VPIGALQAGE ALWHWSVIPL GLAVAILSTA LPYSLEMIAL TRLPTRTFGT
LMSMEPALAA VSGMIFLGET LTPIQLLAG AIIAASMGST LTVRKESKIK ELDIN

SEQ ID NO. 57: RHTB ORGANISM NAME : ESCHERICHIA COLI

MTLEWWFAYL LTSIILSLSP GSGAINTMTT SLNHGYRGAV ASIAGLQTGL AIHIVLVGVG
LGTLSRSVI AFEVLKWAGA AYLIWLGIIQ WRAAGAILDK SLASTQSRRH LFQRAVFNL
TNPKSIVFLA ALFPQFIMPQ QPQLMQYIVL GVTIVVDII VMIGYATLAQ RIALWIKGPK
QMKALNKIFG SLFMLVGALL ASARHA

SEQ ID NO. 58: RHTC ORGANISM NAME : ESCHERICHIA COLI

MLMLFLTAM VHIVALMSPG PDFFVVSQTA VSRSRKEAMM GVLGITCGVM
VWAGIALGL
HLIEKMAWL HTLIMVGGGL YLCWMGYQML RGALKKEAVS APAPQVELAK SGRSFLKGLL
TNLANPKAII YFGSVFSLFV GDNVGTARW GIFALIIVET LAWFTVVASL FALPQMRRGY
QRLAKWIDGF AGALFAGFGI HLIISR

SEQ ID NO. 59: YAHN ORGANISM NAME : ESCHERICHIA COLI

MMQLVHLFMD EITMDPLHAV YLTVGLFVIT FFNPGANLFV VVQTSLASGR RAGVLTGLGV
ALGDAFYSGL GLFGLATLIT QCEEIFSLIR IVGGAYLLWF AWCSMRRQST PQMSTLQQPI
SAPWYVFFRR GLITDLSNPQ TVLFFSIFS VTLNAETPTW ARLMAWAGIV LASIIWRVFL
SQAFLPAVR RAYGRMQRVA SRVIGAIIGV FALRLIYEGV TQR

SEQ ID NO. 60: YAJR ORGANISM NAME : ESCHERICHIA COLI

MNDYKMTPGE RRATWGLGTV FSLRMLGMFM VLPVLTITYGM ALQGASEALI GIAIGIYGLT
QAVFQIPFGL LSDRIGRKPL IVGGLAVFAA GSVIAALSDS IWGIILGRAL QGSGAIAAAV
MALLSDLTRE QNRKAMAFI GVSFGITFAI AMVLGPIITH KLGLHALFWM IAILATTGIA
LTIWVVPNSS THVLNRESGM VKGSFSKVL AEPRLKLNFG IMCLHILLMS TFVALPGQLA
DAGFPAAEHV KVLATMLIA FGSVVPFIY AEVKRKMVKV FVFCVGLIVV AEIVLWNAQT
QFWQLVVG VQ LFFVAFNLME ALLPSLISKE SPAGYKGTAM GYVSTSQFLG VAIGGSLGGW

INGMFDGQGV FLAGAMLAHV WLTVASTMKE PPYVSSLRIE IPANIAANEA LKVRLLTEG
IKEVLIAEEE HSAYVKIDSK VTNRFIEQA IRQA

SEQ ID NO. 61: YBBP ORGANISM NAME: ESCHERICHIA COLI

MIARWFWREW RSPSLIVWL ALSLAVACVL ALGNISDRME KGLSQQSREF MAGDRALRSS
REVPQAWLEE AQKRGLKVGK QLTFATMTFA GDTPQLANVK AVDDIYPMYG DLQTNPPGLK
PQAGSVLLAP RLMALLNLKT GDTIDVGDT LRIAGEVIQE PDSGFNPFQM APRLMMNLAD
VDKTGAVQPG SRVTWRYKFG GNENQLDGYE KWLLPQLKPE QRWYGLEQDE GALGRSMERS
QQFLLLSALL TLLAVAABA VAMNHYCRSR YDLVAILKTL GAGRAQLRKL IVGQWLMVLT
LSAVTGGAIG LLFENVLMVL LKPVLPALP PASLWPWLWA LGTMTVISLL VGLRPYRLLL
ATQPLRVLRN DVVANVWPLK FYLPVSVVV VLLLAGLMGG SMLLWAVLAG AVVLALLCGV
LGWMLLNVL RMTLKSPLR LAVSRLLRQP WSTLSQLSAF SLSFMLLALL LVLRGDLLDR
WQQQLPPESP NYFLINIATE QVAPLKAFLA EHQIVPESFY PVVRARLTAI NDKPTEGNE
EALNRELNL WQNTRPDHP IVAGNWPPKA DEVSMEEGLA KRLNVALGDT VTFMGDTQEF
RAKVTSLRKV DWESLRPNFY FIFPEGALDG QPQSWLTSFR WENGNGMLTQ LNRQFPTISL
LDIGAILKQV GQVLEQVSRA LEVMVVLVTA CGMLLLLAQV QVGMRQRHQE LVVWRTLGA
KKLLRTTLWC EFAMLGFSV LVAAGAETA LAVLQAKVFD FPWEPDWRLW IVLPCSGALL
LSLFGGWLGA RLVKGKALFR QFAG

SEQ ID NO.62: YBIF (RHTA) ORGANISM NAME : ESCHERICHIA COLI

MPGSLRKMPV WLPIVILLVA MASIQQGASL AKSLFPLVGA PGVTALRLAL GTLILIAFFK
PWRLRFAKEQ RLPLLFYGV LGGMNYLFYL SIQTVPLGIA VALEFTGPLA VALFSSRRPV
DFVWVVLAVL GLWFLLPLGQ DVSHVDLTGC ALALGAGACW AIYILSGQRA GAEHGPATVA
IGSLIAALIF VPICALQAGE ALWHWSVIPL GLAVAILSTA LPYSLEMIAL TRLPTRTFGT
LMSMEPALAA VSGMIFLGET LTPIQLLALG AIIAASMGST LTVRKESKIK ELDIN

SEQ ID NO.63: YBJJ ORGANISM NAME : ESCHERICHIA COLI

MTVNSSRNAL KRRTWALFMF FFLPGLLMAS WATRTPAIRD ILSVSIAEMG GVLFGLSIGS
MSGILCSAWL VKRFGTRNVI LVTMSCALIG MMILSLALWL TSPLFAVGL GVFGASFGSA
EVAINVEGAA VEREMNKTVL PMMHGFYSLG TLAGAGVGMA LTAFGVPATV HILLAALVGI
APIYIAIQAI PDGTGKNAAD GTQHGEKGVP FYRDIQLLLI GVVVLAMAF AEGSANDWLPL
LMVDGHHGFSP TSGSLIYAGF TLGMTVGRFT GGWFIDRYSR VAVVRASALM GALGIGLIIF
VDSAWVAGVS VVLWGLGASL GFPLTISAAS DTGPDAPTRV SVVATTGYLA FLVGPPLGY
LGEHYGLRSA MLVVLALVIL AAIVAKAVAK PDTKTQTAME NS

SEQ ID NO. 64: YCAP ORGANISM NAME: ESCHERICHIA COLI

MKAFDLHRMA FDKVPDFLG EVALRSlyTF VLVFLFKMT GRRGVRQMSL FEVLIILTLG
 SAAGDVAfyD DVPMVPVLIV FITLALLYRL VMWLMahSEK LEDLLEGKPV VIIEGELAW
 SKLNNSNMTE FEFFMELRLR GVEQLGQVRL AILETNGQIS VYFFEDDKVK PGLLILPSCD
 TQRYKVPES ADYACIRCSE IihmkAGEKQ LCPRCANPEW TKASRAKRVt

SEQ ID NO. 65: YDCO ORGANISM NAME : ESCHERICHIA COLI

MRLFSIPPT LLAGFLAVLI GYASSAIIW QAAIVAGATT AQISGWMTAL GLAMGVSTLT
 LTLWYRVPVL TAWSTPGAAL LVTGLQGLTL NEAIGVFIVT NALIVLCGIT GLFARLMRII
 PHSLAAAMLA GILLRFGQLA FASLDGQFTL CGSMMLLVWLA TKAVAPRYAV IAAMIIGIVI
 VIAQGDVVTt DVVFKPVLPT YITPDFSFAH SLSVALPLFL VTMA SQNAPG IAAMKAAGYS
 APVSPLIVFT GLLALVFSPF GVYSVGIAAI TAAICQSPEA HPDKDQRWLA AAVAGIFYLL
 AGLFGSAITG MMAALPVSWI QMLAGLALLS TIGGSlyQAL HNERERDAAV VAFLVtASGL
 TLVGIGSAFW GLIAGGVcyV VLNLIADRNRY

SEQ ID NO. 66: YDDG ORGANISM NAME : ESCHERICHIA COLI

MTRQKATLIG LIAIVLWSTM VGLIRGVSEG LGPVGGAAAI YLSGLLLIF TVGFPRIRQI
 PKGYLLAGSL LFVSYEICLA LSLGYAATHH QAIEVGMVNY LWPSLTILFA ILFNGQKTNW
 LIVPGLLLAL VGVCWVLGGD NGLHYDEIIN NITTSPLSYF LAFIGAFIWA AYCTVTNKYA
 RGFNGITVfV LLTGASLWVY YFLTPQPEMI FSTPVMIKLI SAAFTLGfAY AAWNvGILHG
 NVTIMAVGSY FTPVLSSALA AVLLSAPLSF SFWQGALMVC GGSLLCWLAT RRG

SEQ ID NO. 67: YDED (EAMA) ORGANISM NAME : ESCHERICHIA COLI

MSRKDGVLAL LVVVVWGLNF VVIKVLGHNM PPLMLAGLRF MLVAFPAIFF VARPKVPLNL
 LLGYGLTISF AQFAFLfCAI NFGMPAGLAS LVLQAQAFfT IMLGAFTfGE RLHGKQLAGI
 ALAIFGVLVL IEDSLNGQHv AMLGFMLTLA AAFSWACGNI FNKKIMSHST RPAVMSLVIW
 SALIPIIPFF VASLILDGSA TMIHSLVTID MTTILSLMYL AFVATIVGYG IWGTLLGRYE
 TWRVAPLSLL VPVVG LASAA LLLDERLTGL QFLGAVLIMT GLYINVfGLR WRKAVKVGs

SEQ ID NO. 68: YDGE (MDTI) ORGANISM NAME : ESCHERICHIA COLI

MAQFEWVHAA WLALAIVLEI VANVFLKFSD GFRRKIFGLL SLAAVLAAFS ALSQAVKGID
 LSVAYALWGG FGIAATLAAG WILFGQRLNR KGWIGLVLLL AGMIMVKLA

SEQ ID NO. 69: YDHC ORGANISM NAME : ESCHERICHIA COLI

MQPGKRFLVW LAGLSVLGFL ATDMYLPAPA AIQADLQTPA SAVSASLSLF LAGFAAAQLL
 WGPLSDRYGR KPVLLIGLTI FALGSLGMLW VENAATLLVL RFVQAVGVCA AAVIWQALVT
 DYYPSQKVNR IFAAIMPLVG LSPALAPLLG SWLLVHfSWQ AIFATLfAIT VVLILPIFWL
 KPTTKARNNS QDGLTfTDLL RSKTYRGNVL IYAACSASFF AWLTGSPFIL SEMGYSPAVI

GLSYVPQTIA FLIGGYGCRA ALQKWQ GKQL LPWLLVFAV SVIATWAAGF ISHVSLVEIL
 IPFCVMAIAN GAIYPIVVAQ ALRPFPHATG RAAALQNTLQ LGLCFLASLV VSWLISISTP
 LLTTTSMVLS TVVLVALGYM MQRCEEVGCQ NHGNAEVAHS ESH

SEQ ID NO. 70: YDHP ORGANISM NAME : ESCHERICHIA COLI

MKINYPLAL AIGAFGIGTT EFSPMGLLPV IARGVDVSIP AAGMLISAYA VGVMVGAPLM
 TLLLSHRARR SALIFLMAIF TLGNVLSAIA PDYMTLMLSR ILTSLNHGAF FGLGSVVAAS
 VVPKHKQASA VATMFMGLTL ANIGGVPAAT WLGETIGWRM SFLATAGLGV ISMVSLLFFSL
 PKGGAGARPE VKKELAVLMR PQVLSALLTT VLGAGAMFTL YTYISPVLS ITHATPVFVT
 AMLVLIGVGF SIGNYLGGKL ADRSVNGTLK GFLLLLMVIM LAIPFLARNE FGAAISMVVW
 GAATFAVPP LQMRVMRVS EAPGLSSSVN IGAFNLGNAL GAAAGGAVIS AGLGYSFVPV
 MGAIVAGLAL LLVFMSARKQ PETVCVANS

SEQ ID NO. 71: YDIN ORGANISM NAME: ESCHERICHIA COLI

MSQNKAFTS FILAVLCIYF SYFLHGISVI TLAQNMSSLA EKFTDNAGI AYLISGIGLG
 RLISILFFGV ISDKFGRRRAV ILMAVIMYLL FFFGIPACPN LTLAYGLAVC VGIANSALDT
 GGYPALMECF PKASGSVIL VKAMVSFGQM FYPMLVSYML LNNIWYGYGL IIPGILFVLI
 TLMLLKSKFP SQLVDASVTN ELPQMNSKPL VWLEGVSSVL FGVAAFSTFY VIVVWMPKYA
 MAFAGMSEAE ALKTISYYSM GSLVCVFIFA ALLKKMVRPI WANVFNSALA TITAAIYLY
 PSPLVCNAGA FVIGFSAAGG ILQLGVSVMSEFFPKSKAKV TSIYMMMGGL ANFVIPLITG
 YLSNIGLQYI IVLDFTFALL ALITAIIVFI RYYRVFIPE NDVRFGERKF CTRLNTIKHR

G

SEQ ID NO. 72: YDIM ORGANISM NAME: ESCHERICHIA COLI

MKNPYEPTAL GLYFNVLVHG MGVLMLSLNM ASLETLWQTN AAGVSIVISS LGIGRLSVLL
 FAGLLSDRFG RRPFIMLGMC CYMAFFFGIL QTNNIIAYV FGFLAGMANS FLDAGTYPSTL
 MEAFPRSPGT ANILIKAFVS SGQFLLPLII SLLVWAEWLF GWSFMIAAGI MFINALFLYR
 CTFPPHPGRR LPVIKKTSS TEHRCSIIDL ASYTYGYIS MATFYLVSQW LAQYGQFVAG
 MSYTMSIKLL SIYTVGSLLC VFITAPLIRN TVRPTTLLML YTFISFIALF TVCLHPTFYV
 VIIFAFVIGF TSAGGVVQIG LTLMAERFPY AKGKATGIYY SAGSIATFTI PLITAHLSQR
 SIADIMWFDT AIAAIGFLA LFIGLRSRKK TRHSLKENV APGG

SEQ ID NO. 73: YDJE ORGANISM NAME : ESCHERICHIA COLI

MEQYDQIGAR LDRLPLARFH YRIFGIISFS LLLTGFLSYS GNVVLAKLVS NGWSNNFLNA
 AFTSALMFGY FIGSLTGGFI GDYFGRRRAF RINLLIVGIA ATGAAFVPDM YWLIFRFLM
 GTGMGALIMV GYASFTEFIP ATVRGKWSAR LSFVGNWSPM LSAAIGVVVI AFFSWRIMFL

LGGIGILLAW FLSGKYFIES PRWLAGKGQI AGAECQLREV EQQIEREKSI RLPPLTSYQS
 NSKVVKVIKGT FWLLFKGEML RRTLVAITVL IAMNISLYTI TVWIPTIFVN SGIDVDKSIL
 MTAVIMIGAP VGIFIAALII DHFPRRLFGS TLLIIAVLG YIYSIQTTEW AILIYGLVMI
 FFLYMYVCFA SAVYIPELWP THLRLRGS GF VNAVGRIVAV FTPYGVAALL THYGSITVFM
 VLGVMLLLCA LVLSIFGIET RKVSLEEISE VN

SEQ ID NO. 74: YDJF ORGANISM NAME: ESCHERICHIA COLI

MAAKDRIQAI KQMVANDKKV TVSNLSGIFQ VTEETIRRDLEKLEDEGFLT RTYGGAVLNT
 AMLTENIHFY KRASSFYEEK QLIARKALPF IDNKTTMAAD SSSTVMELLK LLQDRSGLTL
 LTNSAEAIHV LAQSEIKVVS TGGELNKNTL SLQGRITKEI IRRYHVDIMV MSCKGLDINS
 GALDSNEAEA EIKKTMIRQA TEVALLVDHS KFDRKAFVQL ADFSHINYII TDKSPGAEWI
 AFCKDNNIQL VW

SEQ ID NO. 75: YDJI ORGANISM NAME : ESCHERICHIA COLI

MLADIRYWEN DATNKHYAIA HFNWVNAEML MGVIDAAEEA KSPVIISFGT GFVGNTSFED
 FSHMMVSMAQ KATVPVITHW DHGRSMELIH NAWTHGMNSL MRDASAFDFE ENIRLTKEAV
 DFFHPLGIPV EALGHVGN TVYEEALAGY HYTPDQAAE FVERTGCDSL AVAIGNQHGV
 YTSEPQLNFE VVKRVRDAVS VPLVLHGASG ISDADIKTAI SLGIAKINIH TELCQAAMVA
 VKENQDQPFL HLEREVRKAV KERALEKIKL FGSDGKAE

SEQ ID NO. 76: YDJK ORGANISM NAME : ESCHERICHIA COLI

MEQITKPHCG ARLDRLPCR WHSSMFAIVA FGLLCWSNA VGGLILAQLK ALGWTDNSTT
 ATFSAITTAG MFLGALVGGI IGDKTGRRNA FLYEAIHIA SMVVGAFSPN MDFLIACRFV
 MGVLGALLV TLFAGFTEYM PGRNRGTWSS RVSFIGNWSY PLCSLIAMGL TPLISAEWNW
 RVQLLIPAIL SLIATALAWR YFPESPRWLE SRGRYQAEK VMRSIEEGVI RQTGKPLPPV
 VIADDGKAPQ AVPYALLTG VLLKRVILGS CVLIAMNVVQ YTLINWLPTI FMTQGINLKD
 SIVLNTMSMF GAPFGIFIAM LVMDKIPRKT MGVGILLILIA VLGYYISLQT SMLLITLIGF
 FLITFVYMYV CYASAVYVPE IWPTAKLRG SGLANAVGRI SGIAAPYAVA VLLSSYGVTG
 VFILLGAVSI IVAIAIATIG IETKGVSVES LSIDAVANK

SEQ ID NO. 77: YEAN ORGANISM NAME : ESCHERICHIA COLI

MTCSTSLSGK NRIVLIAGIL MIATTLRVTF TGAAPLLDTI RSAYSLTTAQ TGLLTTLPLL
 AFALISPLAA PVARRFGMER SLFAALLLIC AGIAIRSLPS PYLLFGGTAV IGGGIALGNV
 LLPGLIKRDF PHSVARLTGA YSLTMGAAAA LGSAMVVPLA LNGFGWQGAL LMLMCFPLLA
 LFLWLPQWRS QQHANLSTSR ALHTRGIWRS PLAWQVTLFL GINSLVYYVI IGWLPAILIS
 HGYSEAQAGS LHGLLQLATA APGLLIPLFL HHVKDQRGIA AFVALMCAVG AVGLCFMPAH

AITWTLLFGF GSGATMILGL TFIGLRASSA HQAAALSGMA QSVGYLLAAC GPPLMGKIHD
ANGNWSVPLM GVAISSLMA IFGLCAGRDK EIR

SEQ ID NO. 78: YEAS (LEUE) ORGANISM NAME : ESCHERICHIA COLI

MFAEYGVVNY WTYLVGAIFI VLVPGPNTLF VLKNSVSSGM KGGYLAACGV FIGDAVLMFL
AWAGVATLIK TTPILFNIVR YLGAFYLLYL GSKILYATLK GKNSEAKSDE PQYGAIFKRA
LILSLTNPKA ILFYVSFFVQ FIDVNAPHTG ISFFILAATL ELVSFCYLSF LIISGAFVTQ
YIRTKKKLAK VGNSLIGLMF VGFAARLATL QS

SEQ ID NO. 79: YEBS ORGANISM NAME : ESCHERICHIA COLI

MALNTPQITP TKKITVRAIG EELPRGDYQR CPQCDMLFSL PEINSHQSAY CPRCQAKIRD
GRDWSLTRLA AMAFTMILLM PFAWGEPLLH IWLLGIRIDA NVMQGIWQMT KQGDAITGSM
VFFCVIGAPL ILVTSIAYLW FGNRLGMNLR PVLLMLERLK EWMMLDIYLV GIGVASIKVQ
DYAHIQAGVG LFSFVALVIL TTVTLSHLNV EELWERFYPQ RPATRRDEKL RVCLGCHFTG
YPDQRGRCPR CHIPLRLRRR HSLQKCWAAL LASIVLLPA NLLPISIIYL NGGRQEDTIL
SGIMSLASSN IAVAGIVFIA SILVPFTKVI VMFTLLLSIH FKCQQGLRTR ILLLRMTWI
GRWSMLDLFV ISLTMSLINR DQILAFTMGP AAFYFGAAVI LTILAVEWLD SROLLWDAHES
GNARFDD

SEQ ID NO. 80: YEDA ORGANISM NAME : ESCHERICHIA COLI

MRFRQLPLF GALFALYIIW GSTYFVIRIG VESWPPLMMA GVRFLAAGIL LLAFLLLRGH
KLPLRPLLN AALIGLLLLA VGNGMVTVAE HQNVPSGIAA VVVATVPLFT LCFSRLFGIK
TRKLEWVGIA IGLAGIIMLN SGGNLSGNPW GAILILIGSI SWAFGSVYGS RITLPVGMMA
GAIELAAGV VLMIASMIAG EKLALPSLS GFLAVGYLAL FGSIIAINAY MYLIRNVSPA
LATSAYVNP VVAVLLGTGL GGETLSKIEW LALGVIVFAV VLVTLGKYL PAKPVVAPVI
QDASSE

SEQ ID NO. 81: YEE0 ORGANISM NAME : ESCHERICHIA COLI

MLRHILTAKN LLSNPIFKFP NCLPFLSTVC CICRQFVGEN LCSFADSPSL FEMWFHFLQL
RSALNISSAL RQVVHGTRWH AKRKSYSKVLW WREITPLAVP IFMENACVLL MGVLTSTFLVS
WLGKDAMAGV GLADSFNMVI MAFFAAIDL TTVVVAFLSG KRDRRRARVA TRQSLVIMTL
FAVLLATLIH HFGEQIIDFV AGDATTEVKA LALTYLELTV LSYPAAAITL IGSGALRGAG
NTKIPLING SLNIIIIIS GILYGLFSW PGLGFVGAGL GLTISRYIGA VAILWVLAIG
FNPALRSLK SYFKPLNFSI IWEVMGIGIP ASVESVLFTS GRLLTQMFVA GMGTSVIAGN
FIAFSIAALI NLPGSALGSA STIITGRRLG VGQIAQAEIQ LRHVFWLSTL GLTAIAWLTA
PFAGVMASFY TQDPQVKHVV VILIWLNALF MPIWSASWVL PAGFKGARDA RYAMWVSMLS

MWGCRVVVGY VLGIMLGWGV VGVWMGMFAD WAVRAVLFW RMVTGRWLWK YPRPEPQKCE
KKPVVSE

SEQ ID NO. 82: YEGH ORGANISM NAME: ESCHERICHIA COLI

MEWIADPSIW AGLITLIVIE LVLGIDNLVF IAILAEKLPP KQRDRARVTG LLLAMLMRLL
LLASISWLVT LTQPLFSFRS FTFSARDLIM LFGGFFLLFK ATMELNERLE GKDSNNPTQR
KGAKFWGVVT QIVVLDAIFS LDSVITAVGM VDHLVMMMAA VVIAISMLM ASKPLTQFVN
SHPTIVILCL SFLLMIGFSL VAEGFGFVIP KGYLYAAIGF SVMIEALNQL AIFNRRRFLS
ANQTLRQRTT EAVMRLLSGQ KEDAELDAET ASMLVDHGNQ QIFNPQERRM IERVLNLNQR
TVSSIMTSRH DIEHIDLNAP EEEIRQLLER NQHTRLVVD GDDAEDLLGV VHVIDLLQQS
LRGEPLNLRV LIRQPLVFPE TLPLPALEQ FRNARTHFAF VVDEFGSVEG IVTLSDVTET
IAGNLPNEVE EIDARHDIQK NADGSWTANG HMPLEDLVQY VPLPLDEKRE YHTIAGLLME
YLQRIPKPG EYQVGDYLLK TLQVESHRVQ KVQIPLRKD GEMEYEV

SEQ ID NO. 83: YGGA (ARGO) ORGANISM NAME: ESCHERICHIA COLI

MFSYYFQGLA LGAAMILPLG PQNAFVMNQG IRRQYHIMIA LLCAISDLVL ICAGIFGGSA
LLMQSPWLLA LVTWGGVAFL LWYGFAGFT AMSSNIELAS AEVMKQGRWK IIATMLAVTW
LNPHVYLDTF VVLGSLGGQL DVEPKRWFAL GTISASFLWF FGLALLAAWL APRLRATAQ
RIINLVGCV MWFIALQLAR DGIAHAQALF S

SEQ ID NO. 84: YFCJ ORGANISM NAME : ESCHERICHIA COLI

MTAVSQTETR SSANFSLFRI AFAVFLTYMT VGLPLPIPL FVHHELGYGN TMVGIAVGIQ
FLATVLTRGY AGRLADQYGA KRSALQGMLA CGLAGGALL AAILPVSAPF KFALLVVGRL
ILGFGESQLL TGALTWGLGI VGPKHSGKVM SWNGMAIYGA LAVGAPLGLL IHSYGFAL
AITTMVLPVL AWACNGTVRK VPALAGERPS LWSVVGLIWK PGLGLALQGV GFAVIGTFVS
LYFASKGWAM AGFTLTAFGG AFVVMRVMFG WMPDRFGGVK VAIVSLLVET VGLLLLWQAP
GAWVALAGAA LTGAGCSLIF PALGVEVVKR VPSQVRGTAL GGYAAFQDIA LGVSGPLAGM
LATTFGYSSV FLAGAISAVL GIIVTILSFR RG

SEQ ID NO. 85: YFIK (EAMB) ORGANISM NAME: ESCHERICHIA COLI

MTPTLLSAFW TYTLITAMTP GPNNILALSS ATSHGFRQST RVLGMSLGF LIVMLLCAGI
SFSLAVIDPA AVHLLSWAGA AYIVWLAWKI ATSPTKEDGL QAKPISFWAS FALQFVNVKI
ILYGVLTALST FVLPQTQALS WVVGVSVLLA MIGTFGNVCW ALAGHLFQRL FRQYGRQLNI
VLALLLVYCA VRIFY

SEQ ID NO. 86: YHJE ORGANISM NAME: ESCHERICHIA COLI

MQATATTLDH EQEYTPINSR NKVLVASLIG TAIEFFDFYI YATAAVIVFP HIFFPQGDPT
 AATLQSLATF AIAFVARPIG SAVFGHFGDR VGRKATLVAS LLTMGISTVV IGLLPGYATI
 GIFAPLLLAL ARFGQGLGLG GEWGGAALLA TENAPPRKRA LYGSFPQLGA PIGFFFANGT
 FLLLSWLLTD EQFMSWGWRV PFIFSAVLVI IGLYVRVSLH ESPVFEKVAK AKKQVKIPLG
 TLLTKHVRVT VLGTIFIMLAT YTLFYIMTVY SMTFSTAAAP VGLGLPRNEV LWMLMMAVIG
 FGVMVPVAGL LADAFGRRKS MVIITTLIIL FALFAFNPLL GSGNPILVFA FLLGLSLMG
 LTFGPMGALL PELFPTEVRY TGASFSYNVA SILGASVAPY IAAWLQTNYG LGAVGLYLAA
 MAGLTLIALL LTHETRHQSL

SEQ ID NO. 87: YIDE ORGANISM NAME: ESCHERICHIA COLI

MSDIALTVSI LALVAVVGLF IGNVKFRGIG LGIGGVLFGG IIVGHFVSQA GMTLSSDMLH
 VIQEFGLILF VYTIGIQVGP GFFASLRVSG LRLNLFVAVLI VIIGGLVTAI LHKLFDIPLP
 VVLGIFSGAV TNTPALGAGQ QILRDLGTPM EMVDQMGMSY AMAYPFGICG ILFTMWMLRV
 IFRVNVETEA QQHESSRTNG GALIKTINIR VENPNLHDLA IKDVPILNGD KIICSRLKRE
 ETLKVPSPDT IIQLGDLLHL VGQPADLHNA QLVIGQEVDI SLSTKGTDLR VERVVVTNEN
 VLGKRIRDLH FKERYDVVIS RLNRAGVELV ASGDISLQFG DILNLVGRPS AIDAVANVLG
 NAQQKLQVQV MLPVFIGIGL GVLLGSIPVF VPGFPAALKL GLAGGPLIMA LILGRIGSIG
 KLYWFMPPSA NLALRELGIV LFLSVVGLKS GGDFVNTLVN GEGLSWIGYG ALITAVPLIT
 VGILARMLAK MNYLTMCGML AGSMTDPPAL AFANNLHPTS GAAALSYATV YPLVMFLRII
 TPQLLAVLFW SIG

SEQ ID NO. 88: YIGK (RHTB) ORGANISM NAME: ESCHERICHIA COLI

MTLEWWFAYL LTSIILSLSP GSGAINTMTT SLNHGYRGAV ASIAGLQTGL AIHIVLVGVG
 LGTLFSRSVI AFEVLKWAGA AYLIWLGIOQ WRAAGAILDK SLASTQSRRH LFQRAVFVNL
 TNPKSIVFLA ALFPQFIMPQ QPQLMQYIVL GVTTIVVDII VMIGYATLAQ RIALWIKGPK
 QMKALNKIFG SLFMLVGALL ASARHA

SEQ ID NO. 89: YIGJ (RHTC) ORGANISM NAME : ESCHERICHIA COLI

MLMLFLTVM VHIVALMSPG PDFFFVSQTA VSRSRKEAMM GVLGITCGVM
 VWAGIALLGL
 HLIIEKMAWL HTLIMVGGGL YLCWMGYQML RGALKKEAVS APAPQVELAK SGRSFLKGLL
 TNLANPKAII YFGSVFSLFV GDNVGTARW GIFALIIVET LAWFTTVASL FALPQMRRGY
 QRLAKWIDGF AGALFAGFGI HLIISR

SEQ ID NO. 90: YIJE ORGANISM NAME : ESCHERICHIA COLI

MSAAGKSNPL AISGLVVLTL IWSYSWIFMK QVTSYIGAFD FTALRCIFGA LVLFIIVLLR
 GRGMRPTPFK YTLAIALLT CGMVGLAQWA LVSGGAGKVA ILSYTMPFWV VIFAALFLGE
 RLRRGQYFAI LIAAFGLFLV LQPWQLDFSS MKSAMLAILS GVSWGASAIV AKRLYARHPR
 VDLSLTSWQ MLYAALVMSV VALLVPQREI DWQPTVFWAL AYSAILATAL AWSLWLFVLK
 NLPASIASLS TLAVPVCVGL FSWWLLGENP GAVEGSGIVL IVLALALVSR KKKEAVSVKRI

SEQ ID NO. 91: YJII (KPTA) ORGANISM NAME : ESCHERICHIA COLI

MAKYNEKELA DTSKFLSFVL RHKPEAIGIV LDREGWADID KLILCAQKAG KRLTRALLDT
 VVATSDKKRF SYSSDGR CIR AVQGHSTSQV AISFAEKTTP QFLYHGTASR FLDEIKKQGL
 IAGERHYVHL SADEATARKV GARHGSPVIL TVKAQEMAKR GLPFWQAENG VWLTSTVAVE
 FLEW

SEQ ID NO. 92: YJII ORGANISM NAME : ESCHERICHIA COLI

MPSSTHPVER FSFSTALFGM LVLTLMGLG RFLYTPMLPV MMAEGSFSFS QLSWIASGNY
 AGYLAGSLLF SFGAFHQPSR LRPFLASAL ASGLLILAMA WLPPFILVLL IRVLAVASA
 GMLIFGSTLI MQHTRHPFVL AALFSGVGIG IALGNEYVLA GLHFDLSSQT LWQGAGALSG
 MMLIALTLLM PSKKHAITPM PLAKTEQQIM SWWLLAILYG LAGFGYIIVA TYLPLMAKDA
 GSPLTAHLW TLVGLSIVPG CFGWLWAAKR WGALPCLTAN LLVQAICVLL TLASDSPLLL
 IISLGFGGT FMGTTSVMT IARQLSVPGN LNLLGFVTI YGIGQILGPA LTSMLSNGTS
 ALASATLCGA AALFIAALIS TVQLFKLQVV TS

SEQ ID NO. 93: YJIO (MDTM) ORGANISM NAME : ESCHERICHIA COLI

MPRFFTRHAA TLFFPMALIL YDFAAYLSTD LIQPGIINVV RDFNADVSLA PAAVSLYLAG
 GMALQWLLGP LSDRIGRRPV LITGALIFTL ACAATMFTTS MTQFLIARAI QGTSICFIAT
 VGYVTVQEF GQTKGIKMA IITSIVLIAP IIGPLSGAAL MHFMHWKVLF AIIAVMGFIS
 FVGLLLAMPE TVKRGAVPFS AKSVLRDFRN VFCNRLFLFG AATISLSYIP MMSWVAVSPV
 ILIDAGSLTT SQFAWTQVPV FGAVIVANAI VARFVKDPTE PRFIWRAVPI QLVGLSLLIV
 GNLLSPHVWL WSVLGTSLYA FGIGLIFPTL FRFTLFSNKL PKGTVSASLN MVILMVMSVS
 VEIGRWLWFN GGRLPFHLLA VVAGVIVVFT LAGLLNRVRQ HQAAELVEEQ

SEQ ID NO. 94: YKGH ORGANISM NAME : ESCHERICHIA COLI

MREQIKQDID LIEILFYLLK KIRVILFIMA ICMAMVLLFL YINKDNIKVI YSLKINQTP
 GILVSCDSNN NFACQTTMTE DVIQRITTF HTSPDVKNRE IRLEWSGDKR ALPTAEES

RVQASIIKWY ASEYHNGRQV LDEIQTPSAI NSELYTKMIY LTRNWSLYPN GDGCVTISSP
EIKNKYPAAI CLALGFFLSI VISVMFCLVK KMOVDEYQQNS GQ

SEQ ID NO. 95: YPJD ORGANISM NAME : ESCHERICHIA COLI

MQRLEQRSPD AILLFLIAQ TVDITMPVFA LLALVAYSVS LALIVPGLLQ KNNGWRRMAI
ISAVIALVCH AIALEARILP DGDGSGQNLSL LNVGSLVSLM ICTVMTIVAS RNRGWLLPI
VYAFALINLA LATFMPNEYI THLEATPGML VHIGLSLFSY ATLIIAALYA LQLAWIDYQL
KNKKLAFNQE MPPLMSIERK MFHITQIGVV LLTLTLCTGL FYMHNLFSE NIDKAVLSIV
AWFVYIVLLW GHYHEGWRGR RVVWFNVAGA VILTAYFGS RIVQQLIS

SEQ ID NO. 96: YTFF ORGANISM NAME : ESCHERICHIA COLI

MPVMISGVLY ALLAGLMWGL IFVGPLIVPE YPAMLQSMGR YLALGLIALP IAWLGRVRLR
QLARRDWLTA LMLTMMGNLI YYFCLASAIQ RTGAPVSTMI IGTLPVVIPV FANLLYSQRD
GKLAWGKLAP ALICIGIGLA CVNIAELNHG LPDFDWARYT SGIVLALVSV VCWAWYALRN
ARWLRENPKD HPMMWATAQA LVTLPVSLIG YLVACYWLNT QTPDFSLPFG PRPLVFISLM
VAIAVLCSWV GALCWNVASQ LLPTVILGPL IVFETLAGLL YTFLLRQQMP PLMTLSGIAL
LVIGVVIIVR AKPEKPLTES VSES

SEQ ID NO. 97: YTFL ORGANISM NAME: ESCHERICHIA COLI

MLNSILVILC LIAVSAFFSM SEISLAASRK IKLKLLADEG NINAQRVLNM QENPGMFFTV
VQIGLNAVAI LGGIVGDAAF SPAFHSLFSR YMSAELSEQL SFILSFSLVT GMFILFADLT
PKRIGMIAPE AVALRIINPM RFCLYVCTPL VWFFENGLANI IFRIFKLPMV RKDDITSDDI
YAVVEAGALA GVLKQEHLEL IENVFELESR TVPSSMTPRE NVIWFDLHED EQSLKNKVAE
HPHSLFLVCN EDIDHIIGYV DSKDLLNRVL ANQSLALNSG VQIRNTLIVP DTTLTSEALE
SFKTAGEDFA VIMNEYALVV GIITLNDVMT TLMGDLVGQG LEEQIVARDE NSWLIDGGTP
IDDVMRVLDI DEFPOSGNIE TIGGFMMFML RKIPKRTDSV KFAGYKFEVV DIDNYRIDQL
LVTRIDSKAT ALSPKLPDAK DKEESVA

SEQ ID NO. 98: CHLOROFLEXUS AGGREGANS (CGMCR)

<400> 785

MET SER VAL THR GLY ARG LEU ALA GLY LYS ILE ALA LEU ILE THR GLY

1 5 10 15

GLY ALA GLY ASN ILE GLY SER GLU MET THR ARG ARG PHE LEU ALA GLU

20 25 30

GLY ALA THR VAL ILE ILE SER GLY ARG ASN SER ALA LYS LEU ALA ALA

35 40 45

LEU ALA GLU ARG LEU ARG SER GLU ALA GLY VAL PRO ALA LYS ARG ILE
50 55 60
ASP LEU GLU VAL MET ASP GLY SER ASP PRO ALA ALA VAL ARG ALA GLY
65 70 75 80
VAL ALA ALA ILE ILE GLY ARG HIS GLY HIS ILE ASP ILE LEU VAL ASN
85 90 95
ASN ALA GLY SER THR GLY ALA GLN ARG ARG LEU ALA GLU ILE PRO LEU
100 105 110
ASN GLU THR ASP ARG ASP LEU ASP ASP GLU GLU ALA LEU SER THR SER
115 120 125
VAL ALA ASN LEU LEU GLY MET ALA TRP HIS LEU MET ARG ILE LEU SER
130 135 140
PRO HIS MET PRO PRO GLY SER ALA ILE ILE ASN ILE SER THR ILE PHE
145 150 155 160
SER ARG ALA GLU TYR TYR GLY ARG ILE PRO TYR VAL VAL PRO LYS ALA
165 170 175
ALA LEU ASN THR LEU THR GLN ILE ALA ALA ARG GLU LEU GLY ILE ARG
180 185 190
GLY ILE ARG VAL ASN THR ILE PHE PRO GLY PRO ILE GLU SER GLU ARG
195 200 205
ILE GLN THR VAL PHE GLN ARG MET ASP GLN LEU LYS GLY ARG PRO GLU
210 215 220
GLY ASP THR ALA SER GLN PHE LEU ALA THR MET ARG LEU TYR ARG ALA
225 230 235 240
ASN ASP GLN GLY GLN LEU GLU ARG ARG PHE PRO THR ILE CYS ASP VAL
245 250 255
ALA ASP ALA ALA VAL PHE LEU ALA SER ASP GLU ALA ALA ALA LEU THR
260 265 270
GLY GLU THR ILE GLU VAL THR HIS GLY MET GLU LEU PRO THR SER SER
275 280 285
GLU THR SER LEU LEU ALA ARG THR ASP LEU ARG THR ILE ASP ALA ASN
290 295 300

GLY ARG THR THR LEU ILE CYS ALA GLY ASP GLN ILE GLU GLU VAL MET
305 310 315 320
ALA LEU THR GLY MET LEU ARG THR CYS GLY SER GLU VAL ILE ILE GLY
325 330 335
PHE ARG SER GLU ALA ALA LEU ALA GLN PHE GLU GLN ALA ILE GLY GLU
340 345 350
SER ARG ARG LEU ALA GLY GLU SER PHE ILE PRO PRO ILE ALA LEU PRO
355 360 365
ILE ASP LEU ARG ASN PRO SER THR ILE ASP ALA LEU PHE ASP TRP ALA
370 375 380
GLY GLU ASN THR GLY GLY ILE HIS ALA ALA VAL ILE LEU PRO ALA SER
385 390 395 400
GLY ARG GLU PRO ALA THR GLN VAL ILE ASP ILE ASP ASP ALA HIS VAL
405 410 415
GLN ALA PHE LEU ASN ASP GLU ILE VAL GLY SER ILE ILE ILE ALA SER
420 425 430
ARG LEU ALA ARG TYR TRP GLN ALA GLN ARG ILE ALA PRO GLY ALA ARG
435 440 445
ALA ARG GLU PRO ARG VAL ILE PHE LEU SER ASN GLY ALA SER THR ALA
450 455 460
GLY ASN PRO TYR GLY ARG ILE GLN SER ALA ALA ILE GLU GLN LEU ILE
465 470 475 480
ARG VAL TRP ARG HIS GLU ALA ALA LEU ASP TYR GLU ARG ALA THR ALA
485 490 495
ALA GLY GLU ARG VAL LEU PRO ALA VAL TRP ALA SER GLN ILE VAL ARG
500 505 510
PHE ALA ASN ARG SER LEU GLU GLY LEU GLU PHE ALA CYS ALA TRP THR
515 520 525
ALA GLN LEU LEU HIS SER GLN ARG ARG ILE ASN GLU ILE THR LEU THR
530 535 540
ILE PRO ALA ASP ILE SER ALA THR THR GLY ALA ARG SER ALA SER VAL
545 550 555 560

GLY TRP ALA GLU SER LEU ILE GLY LEU HIS LEU GLY LYS VAL ALA LEU
565 570 575

ILE THR GLY GLY SER ALA GLY ILE GLY GLY GLN ILE GLY ARG LEU LEU
580 585 590

ALA LEU SER GLY ALA ARG VAL MET LEU ALA ALA ARG ASP PRO HIS LYS
595 600 605

LEU GLU GLN ILE GLN ALA THR ILE ARG ALA GLU LEU ALA GLU VAL GLY
610 615 620

TYR THR ASP VAL GLU GLU ARG VAL GLN ILE ALA PRO GLY CYS ASP VAL
625 630 635 640

SER SER GLU GLU GLN LEU VAL ASP LEU VAL GLU ARG THR LEU ALA ALA
645 650 655

PHE GLY THR VAL ASP TYR LEU ILE ASN ASN ALA GLY ILE ALA GLY VAL
660 665 670

GLU GLU MET VAL ILE ASP MET PRO VAL GLU GLY TRP ARG ASN THR LEU
675 680 685

TYR ALA ASN LEU ILE SER ASN TYR SER LEU MET ARG LYS LEU ALA PRO
690 695 700

LEU MET LYS LYS GLN GLY SER GLY TYR VAL LEU ASN VAL SER SER TYR
705 710 715 720

PHE GLY GLY GLU LYS ASP ALA ALA ILE PRO TYR PRO ASN ARG ALA ASP
725 730 735

TYR ALA VAL SER LYS ALA GLY GLN ARG ALA MET ALA GLU VAL PHE ALA
740 745 750

ARG PHE LEU GLY PRO GLU ILE GLN ILE ASN ALA ILE ALA PRO GLY PRO
755 760 765

VAL GLU GLY ASP ARG LEU ARG GLY THR GLY GLU ARG PRO GLY LEU PHE
770 775 780

ALA ARG ARG ALA ARG LEU ILE LEU GLU ASN LYS ARG LEU ASN GLU LEU
785 790 795 800

HIS ALA ALA LEU ILE THR ALA ALA ARG THR ASP ASN ARG PRO MET ARG
805 810 815

GLU LEU VAL GLU LEU LEU LEU PRO ASN ASP VAL ALA ALA LEU ALA GLN
820 825 830
HIS PRO ALA ALA PRO ASP VAL LEU ARG THR LEU ALA LYS ARG PHE GLN
835 840 845
SER GLU GLY ASP PRO ALA ALA SER SER SER SER PHE LEU LEU ASN ARG
850 855 860
SER ILE ALA ALA LYS LEU LEU ALA ARG LEU ILE ASN GLY GLY TYR ASP
865 870 875 880
LEU PRO ALA ASP ILE PHE ALA ASN LEU ALA VAL PRO PRO ASP PRO PHE
885 890 895
PHE THR ARG ALA GLN ILE ASP ARG GLU ALA ARG LYS VAL ARG ASP GLY
900 905 910
ILE MET GLY MET LEU TYR LEU GLN ARG MET PRO THR GLU PHE ASP VAL
915 920 925
ALA MET ALA THR VAL TYR TYR LEU ALA ASP ARG ASN VAL SER GLY GLU
930 935 940
THR PHE HIS PRO SER GLY GLY LEU ARG TYR GLU ARG THR PRO THR GLY
945 950 955 960
GLY GLU LEU PHE GLY LEU PRO ALA PRO GLU ARG LEU ALA GLU LEU VAL
965 970 975
GLY SER THR VAL TYR LEU ILE GLY GLU HIS LEU THR GLU HIS LEU ASN
980 985 990
LEU LEU ALA ARG ALA TYR LEU GLU ARG TYR GLY ALA ARG GLN VAL VAL
995 1000 1005
MET ILE VAL GLU THR GLU ALA GLY ALA GLU LYS MET ARG HIS LEU
1010 1015 1020
LEU HIS ASP HIS VAL GLU ALA GLY ARG LEU PRO ILE ILE VAL ALA
1025 1030 1035
GLY ASP GLN ILE GLU ALA ALA ILE ASP GLN ALA ILE ALA ASN TYR
1040 1045 1050
GLY ARG PRO GLY PRO VAL VAL CYS THR PRO PHE ARG PRO LEU PRO
1055 1060 1065

SER ALA PRO LEU VAL GLY ARG LYS ASP SER ASP TRP SER THR VAL

1070 1075 1080

LEU SER GLU ALA GLU PHE ALA GLU LEU CYS GLU HIS GLN LEU THR

1085 1090 1095

HIS HIS PHE ARG VAL ALA ARG LYS ILE ALA LEU SER ASP GLY ALA

1100 1105 1110

SER LEU ALA LEU VAL THR PRO GLU THR THR ALA THR SER SER THR

1115 1120 1125

GLU GLN PHE ALA LEU ALA ASN PHE VAL LYS THR THR LEU HIS ALA

1130 1135 1140

PHE THR ALA THR ILE GLY VAL GLU SER GLU ARG THR ALA GLN ARG

1145 1150 1155

ILE LEU ILE ASN GLN VAL ASP LEU THR ARG ARG ALA ARG ALA GLU

1160 1165 1170

GLU PRO ARG ASP PRO ARG GLU ARG GLN GLN GLU LEU GLU ARG PHE

1175 1180 1185

ILE GLU ALA VAL LEU LEU VAL THR ALA PRO LEU PRO PRO GLU ALA

1190 1195 1200

ASP THR ARG TYR ALA GLY ARG ILE HIS ARG GLY ARG ALA ILE THR VAL

1205 1210 1215

SEQ ID NO. 99: MCR OSCILLOCHLORIS TRICHOIDES (OTMCR)

MFMTRLNDKIALITGGAGTIGEVITRRYLEEGATVVMAGRNRDKLDYRERLITEFHLP

ERVMVVRMDGSSNAEVRMGIAAVVAHFGRIDLVNNAAGSAGARQRLPAIPLLRSELQADE

TETLADSIGNLIGITWNLIRAAAPFMPAGSSVINISTIFARTDYYGRIPYVVPKAAHAL

TLAAATELGERGIRVNQINPGPIDSDRITVFRMDELKGVPEQSTADGFFQMMRLRRPN

AEGDLVKGFPKTLDVANVAVFLGSAESAALSGETLDVTHGMAVPTESRTTLTSRPGRAV

DGSGHTTLICVGDQIEEAAALTGVLRACGAEEVIGFRSRAAIARFDHLIERGRHLPSEQH

VAPVLLYLNPTEPESIDQALRWMATNLDLPTSVIILPAQRQPLPPSVVRASDEEVAYFLR

DELSGMIVLASRLARFWQQATLAPGNAPIQPRVLFMTNPDDGQGNLYAEILRAGVEQLCR

VWRHESQLDYTRLAQMDAHPPIRPVWANQLVRFANNEQENLEYCCAWVAKILLSERTIE

ELNLYLPRQIGSTTGSRQPSFGWAENLIGLHLGKTALITGGSAGIGSQIARLLALSGARV

MLCARDERKLIQMRDMIIAELTEVGYNQVESRVQICAGCDVGEEEQLEIAVQRTLDLFGH

VDYLINNAGIAGAEEMVLDLPLEAWQRTLRTNLISNYSIRKLAPQMKSRGSGYILNVSS
YFGGEKYAAIPYPNRADYAVSKAGQRALGEALARLLGPEVQINAMAPGPVEGERLRGSGD
RPGLFLRRGRILENKRLNDLHATLIAAERETQVGMRDLLARLLHNDVCALIDDPAAPTH
LRLAERIWEQSDPNSYARAFFMNANIATKLLARLFNADQIDAQTFHTSQPNLPPEPFFA
RTQIDREARRVRDGVMSMLYLQRMPTFEDVALATVYYLNDRSVSGETFHPSGGLRHERTP
TGAELYGSPAPQRLASLAGSTVYLIGESMAAHLEALARAYIERYAATRVLICATPAGVE
RFSHHLADHLASGAILLSAEEGIEAALSEALRRFGPPGPVVSTPFQPLPSQPLIGRND
DWSTVLDVAGFSAMCEQQLTHHFRVTRKLSLVAGVSLVLVTPETDSHSSTEQFALANFVK
TTLHAFTATVGVECERTAHRILVNQVDLGRQARAEPRSPAEEQAQEMERFIDAIMLTAP
IPAEEDNRYTGRIYRGRAITV

SEQ ID NO. 100: CHLOROFLEXUS AURANTIACUS (CAMCR)

MSGTGRLAGKIALITGGAGNIGSELTRRFLAEGATVIISGRNRAKLTALAERMQAEAGVP
AKRIDLEVMDGSDPVAVRAGIEAIVARHGQIDILVNNAGSAGAQRRLAEIPLTEAELGPG
AEETLHASIANLLGMGWHLMRIAPHMPVGSVINVTIFSRAEYYGRIPYVTPKAALNA
LSQLAARELGARGIRVNTIFPGPIESDRITVFQRMQDQKGRPEGDTAHHFLNTMRLCRA
NDQGALERFRFSPVGDVADAFLASAESAALSGETIEVTHGMELPACSETSLARTDLRT
IDASGRITLICAGDQIEEVMALTGMLRTCGSEVIIGFRSAAALAQFEQAVNESRRLAGAD
FTPPIALPLDPRPATIDAVFDWGAGENTGGIHAAILPATSHAPAPCVIEVDDERVLNF
LADEITGTIVIASRLARYWQSQRITPGARARGPRVIFLSNGADQNGNVYGRIQSAAIGQL
IRVWRHEAELDYQRASAAGDHVLPVWANQIVRFANRSLEGLEFACAWTAQLLHSQRHIN
EITLNIPANISATTGARSASVGWAESLIGLHLGKVALITGGSAGIGGQIGRLLALSGARV
MLAARDRHKLEQMAMIQSELAEVGYTDVEDRVHIAPGCDVSSEAQLADLVERTLSAFGT
VDYLINNAGIAGVEEMVIDMPVEGWRTLFANLISNYSIMRKLAPLMKKQSGSYILNVSS
YFGGEKDAAIPYPNRADYAVSKAGQRAMAIEVFARFLGPEIQINAIAPGPVEGDRLRGTGE
RPGLFARRARLILENKRLNELHAALIAAARTDERSMHVELLELLPNDVAALEQNPAAPTA
LRELARRFRSEGDPAASSSSALLNRSIAAKLLARLHNGGYVLPADIFANLPNPPDPFFTR
AQIDREARKVRDGMGMMLYLQRMPTFEDVAMATVYYLADRNVSGETFHPSGGLRYERTPT
GGELFLPSPERLAELVGSTVYLIGEHLTEHLNLLARAYLERYGARQVVMIVETETGAET
MRRLLHDHVEAGRLMTIVAGDQIEAAIDQAITRYGRPGPVVCTPFRPLPTVPLVGRKDSD
WSTVLSEAEFAELCEHQLTHHFRVARKIALSDGASLALVTPETTATSTTEQFALANFIKT
TLHAFTATIGVESERTAQRILINQVDLTRRARAEEPRDPHERQQELERFIEAVLLVTAPL
PPEADTRYAGRIHRGRAITV

CLAIMS

We claim:

1. A genetically modified organism capable of producing a chemical product of interest comprising, at least one nucleic acid sequence encoding for a mutated malonyl-CoA reductase having increased activity at lower temperatures.
2. The genetically modified organism of any claim, wherein the mutated malonyl-CoA reductase has increased activity at about 20°C to about 44°C.
3. The genetically modified organism of any claim, wherein the mutated malonyl-CoA reductase has increased activity at about 30°C to about 37°C.
4. The genetically modified organism of any claim, wherein the mutated malonyl-CoA reductase has increased activity at about 32°C to about 38°C.
5. The genetically modified organism of any claim, wherein the mutated malonyl-CoA reductase gene is derived from *Sulfolobus tokodaii*.
6. A method of making a genetically modified organism capable of producing an chemical product of interest comprising, introducing into said organism at least one nucleic acid sequence encoding for a mutated malonyl-CoA reductase having increased activity at lower temperatures.
7. The method of any claim, wherein the mutated malonyl-CoA reductase has increased activity at about 20°C to about 44°C.
8. The method of any claim, wherein the mutated malonyl-CoA reductase has increased activity at about 30°C to about 37°C.
9. The method of any claim, wherein the mutated malonyl-CoA reductase has increased activity at about 32°C to about 38°C.
10. The method of any claim, wherein the mutated malonyl-CoA reductase gene is derived from *Sulfolobus tokodaii*.
11. A method of using the genetically modified organism of any claim, comprising culturing said genetically modified organism with a carbon source to produce a chemical product.
12. A genetically modified organism capable of producing a chemical product of interest comprising, at least one nucleic acid sequence encoding for a salt-tolerant enzyme.
13. The genetically modified organism of any claim, wherein said at least one nucleic acid sequence encodes for one or more enzymes from a salt-tolerant organism selected from the group consisting of: sucrose-6-phosphate hydrolase, glucose-6-phosphate isomerase, fructokinase, fructose-1,6-bisphosphatase, fructose 1,6-bisphosphatase, fructose 1,6-

bisphosphatase II, fructose-1,6-bisphosphatase monomer, 6-phosphofructokinase-1 monomer, 6-phosphofructokinase-2 monomer, fructose bisphosphate aldolase monomer, fructose bisphosphate aldolase monomer, triose phosphate isomerase monomer, glyceraldehyde 3-phosphate dehydrogenase-A monomer, phosphoglycerate kinase, 2,3-bisphosphoglycerate-independent phosphoglycerate mutase, 2,3-bisphosphoglycerate-dependent or tdcE, phosphoglycerate mutase, enolase, phosphoenolpyruvate carboxylase, malate dehydrogenase, fumarase A, fumarase, fumarase C, phosphoenolpyruvate synthetase, pyruvate kinase I monomer, pyruvate kinase II monomer, fumarate reductase, lipoamide dehydrogenase, pyruvate dehydrogenase, pyruvate dehydrogenase, pyruvate formate-lyase, acetyl-CoA carboxylase, malonyl CoA reductase, 3HP dehydrogenase, malonate semialdehyde reductase, and any combination thereof.

14. The genetically modified organism of any claim, wherein said one or more enzymes from a salt-tolerant organism is at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, or 70% identical or homologous to their respective enzyme.

15. The genetically modified organism of any claim, wherein said one or more enzymes from a salt-tolerant organism are at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, or 70% identical or homologous to the functional domains of their respective enzymes.

16. The genetically modified organism of any claim, wherein said one or more enzymes from a salt-tolerant organism are at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, or 70% identical or homologous to the binding domains of their respective enzymes.

17. A genetically modified non-salt-tolerant organism capable of producing a chemical product of interest comprising, at least one nucleic acid sequence encoding for an acetyl-CoA carboxylase from a salt-tolerant organism.

18. The genetically modified organism of any claim, wherein said salt-tolerant organism is selected from the group consisting of: *Halomonas*, *Salinibacter*, and *Halobacterium*.

19. A method of making a genetically modified organism capable of producing a chemical product of interest comprising, introducing into said organism at least one nucleic acid sequence encoding for a salt-tolerant enzyme.

20. The method of any claim, wherein said at least one nucleic acid sequence encodes for one or more enzymes from a salt-tolerant organism selected from the group consisting of: sucrose-6-phosphate hydrolase, glucose-6-phosphate isomerase, fructokinase, fructose-1,6-bisphosphatase, fructose 1,6-bisphosphatase, fructose 1,6-bisphosphatase II, fructose-1,6-bisphosphatase monomer, 6-phosphofructokinase-1 monomer, 6-phosphofructokinase-2 monomer, fructose bisphosphate aldolase monomer, fructose bisphosphate aldolase monomer, triose phosphate isomerase monomer, glyceraldehyde 3-phosphate dehydrogenase-A monomer, phosphoglycerate kinase, 2,3-bisphosphoglycerate-independent phosphoglycerate mutase, 2,3-bisphosphoglycerate-dependent or tdcE, phosphoglycerate mutase, enolase, phosphoenolpyruvate carboxylase, malate dehydrogenase, fumarase A, fumarase, fumarase C, phosphoenolpyruvate synthetase, pyruvate kinase I monomer, pyruvate kinase II monomer, fumarate reductase, lipoamide dehydrogenase, pyruvate dehydrogenase, pyruvate dehydrogenase, pyruvate formate-lyase, acetyl-CoA carboxylase, malonyl CoA reductase, 3HP dehydrogenase, malonate semialdehyde reductase, and combinations thereof.
21. A method of making a modified non-salt-tolerant organism capable of producing a chemical product of interest comprising, introducing into said non-salt-tolerant organism at least one nucleic acid sequence encoding for an acetyl-CoA carboxylase from a salt-tolerant organism.
22. The method of any claim, wherein said salt-tolerant organism is selected from the group consisting of: *Halomonas*, *Salinibacter*, and *Halobacterium*.
23. A method using the organism of any claim, comprising culturing said organism with a carbon source to produce an industrial chemical product.
24. A genetically modified organism capable of producing a chemical product of interest comprising, at least one nucleic acid sequence encoding for a polypeptide that facilitates export of a chemical product or the export of an inhibitory chemical.
25. The genetically modified organism of any claim, wherein said at least one nucleic acid is a member of a family selected from the group consisting of: major facilitator superfamily, ATP-binding cassette superfamily, small multidrug resistance family, resistance-nodulation-cell division superfamily, multi antimicrobial extrusion protein family, and combinations thereof.
26. The genetically modified organism of any claim, wherein said at least one nucleic acid sequence encoding for a polypeptide is a solvent tolerant transporter.

27. The genetically modified organism of any claim, wherein said solvent tolerant transporter transports a chemical selected from the group consisting of: bromoacetate, butanol, and isobutanol.
28. The genetically modified organism of any claim, wherein said gene is selected from the group consisting of: *acrD*, *bcr*, *cusA*, *dedA*, *eamA*, *eamB*, *eamH*, *emaA*, *emaB*, *emrB*, *emrD*, *emrKY*, *emrY*, *garP*, *gudP*, *hsrA*, *leuE*, *mdlB*, *mdtD*, *mdtG*, *mdtL*, *mdtM*, *mhpT*, *rhtA*, *rhtB*, *rhtC*, *thtB*, *yahN*, *yajR*, *ybbP*, *ybiF*, *ybjJ*, *ycaP*, *ydcO*, *yddG*, *ydeD*, *ydhC*, *ydhP*, *ydjE*, *ydjI*, *ydjK*, *yeaS*, *yedA*, *yeeO*, *yegH*, *yfcJ*, *yfiK*, *yhjE*, *yidE*, *yiJE*, *yjiI*, *yjiJ*, *yjiO*, *ykgH*, *ypjD*, *ytfF*, *ytfL*, homologs thereof, and any combination thereof.
29. The genetically modified organism of any claim, wherein said at least one nucleic acid sequence encoding for a gene comprises two or more exporter genes.
30. The genetically modified organism of any claim, further comprising a disruption or reduced expression of a *tig* gene.
31. A method of making a genetically modified organism capable of producing a chemical product of interest comprising, introducing into said organism at least one nucleic acid sequence encoding for a polypeptide that facilitates export of a chemical product or the export of an inhibitory chemical.
32. The method of any claim, wherein said polypeptide is a member of a family selected from the group consisting of: major facilitator superfamily, ATP-binding cassette superfamily, small multidrug resistance family, resistance-nodulation-cell division superfamily, multi antimicrobial extrusion protein family, and combinations thereof.
33. The method of any claim, wherein said polypeptide is a solvent tolerant transporter.
34. The method of any claim, wherein said solvent tolerant transporter transports a chemical selected from the group consisting of: bromoacetate, butanol, and isobutanol.
35. The method of any claim, wherein said gene is selected from the group consisting of: *acrD*, *bcr*, *cusA*, *dedA*, *eamA*, *eamB*, *eamH*, *emaA*, *emaB*, *emrB*, *emrD*, *emrKY*, *emrY*, *garP*, *gudP*, *hsrA*, *leuE*, *mdlB*, *mdtD*, *mdtG*, *mdtL*, *mdtM*, *mhpT*, *rhtA*, *rhtB*, *rhtC*, *thtB*, *yahN*, *yajR*, *ybbP*, *ybiF*, *ybjJ*, *ycaP*, *ydcO*, *yddG*, *ydeD*, *ydhC*, *ydhP*, *ydjE*, *ydjI*, *ydjK*, *yeaS*, *yedA*, *yeeO*, *yegH*, *yfcJ*, *yfiK*, *yhjE*, *yidE*, *yiJE*, *yjiI*, *yjiJ*, *yjiO*, *ykgH*, *ypjD*, *ytfF*, *ytfL*, homologs thereof, and combinations thereof.
36. The method of any claim, wherein said at least one nucleic acid sequence encoding for a gene comprises two or more exporter genes.
37. The method of any claim, further comprising a disruption of a *tig* gene.

38. A method using the organism of any claim, comprising culturing said organism with a carbon source to produce an industrial chemical product.
39. A genetically modified organism capable of producing an chemical product of interest comprising, at least one nucleic acid sequence encoding for a polypeptide that facilitates the importation of a reactant, precursor, or metabolite used for producing a chemical product of interest; and/or inhibits the exportation of a reactant, precursor, or metabolite.
40. The genetically modified organism of any claim, wherein the reactant, precursor or metabolite is carbon dioxide or bicarbonate.
41. The genetically modified organism of any claim, wherein said at least one nucleic acid sequence for a polypeptide is selected from the group consisting of: bicA, ychM, and yidE.
42. A method of making a genetically modified organism capable of producing an chemical product of interest, comprising introducing into said organism at least one nucleic acid sequence encoding for a polypeptide that facilitates the importation of a reactant, precursor, or metabolite used for producing a chemical product of interest and/or inhibits the exportation of a reactant, precursor, or metabolite.
43. The method of any claim, wherein the reactant, precursor or metabolite is carbon dioxide or bicarbonate.
44. The method of any claim, wherein said at least one nucleic acid sequence for a gene is selected from the group consisting of: bicA, ychM, yidE, and combinations thereof.
45. A method of using the organism of any claim, comprising culturing said organism with a carbon source to produce an industrial chemical product.
46. A method of producing 3-HP using the genetically modified organisms of any claim, wherein the organism is capable of converting a carbon source to malonyl-CoA, malonyl-CoA to malonate semialdehyde, and malonate semialdehyde to 3-HP.
47. A method for producing a chemical for a consumer product, said method comprising:
a) combining a carbon source and a genetically modified cell; and
b) culturing said carbon source and genetically modified cell under suitable conditions to sustain cellular proliferation,
wherein said genetically modified cell exhibits increased conversion of said carbon source to a chemical for a consumer product compared to a non-genetically modified cell.
48. The method of any claim, further comprising culturing said genetically modified cell under suitable conditions to sustain continuous cell proliferation.

49. The method of any claim, wherein said genetically modified cell is further modified to increase conversion of said carbon source to acetyl-CoA.
50. The method of any claim, wherein said genetically modified cell is further modified to increase conversion of said carbon source to malonyl-CoA.
51. The method of any claim, wherein said genetically modified cell is further modified to increase conversion of said carbon source to both acetyl-CoA and malonyl-CoA.
52. The method of any claim, further comprising a genetic modification that decreases the production of a chemical selected from the group consisting of: ethanol, lactate, acetate, and combinations thereof.
53. The method of any claim, wherein said genetically modified cell further comprises an inducible gene that decreases the availability of malonyl-CoA reductase for use in the fatty acid production pathway.
54. The method of any claim, wherein said genetically modified cell further comprises at least one genetic modification that decreases or eliminates the production of a chemical selected from the group consisting of: ethanol, lactate, acetate, or combinations thereof.
55. The method of any claim, wherein said genetically modified cell further comprises an inducible genetic module that decreases or eliminates the expression of a gene selected from the group consisting of: aceEF, lpd, pta, ackA, adhE, fabI, fabB, fabD, and combinations thereof.
56. The method of any claim, wherein said inducible genetic module is activated by a chemical or by temperature.
57. The method of any claim, wherein said genetically modified cell further comprises a malonyl-CoA reductase having a dehydrogenase domain that enhances malonate semialdehyde or 3-HP production.
58. The method of any claim, wherein said malonyl-CoA reductase is a monofunctional malonyl-CoA reductase.
59. The method of any claim, wherein said monofunctional malonyl-CoA reductase enzyme has at least about 70% identity and/or homology to the malonyl-CoA reductase from *Sulfolobus tokodaii*, *Chloroflexus aggregans*, and/or *Oscillochloris trichoides*.
60. The method of any claim, wherein the expression of the monofunctional malonyl-CoA reductase is controlled by an inducible genetic module.
61. The method of any claim, wherein said inducible genetic module is activated by a chemical or by temperature.

62. The method of any claim, wherein said malonyl-CoA reductase enzyme is bifunctional.
63. The method of any claim, wherein said bifunctional malonyl-CoA reductase enzyme has at least 70% identity and/or homology to the *Chloroflexus aurantiacus* malonyl-CoA reductase.
64. The method of any claim, wherein the expression of the bifunctional malonyl-CoA reductase is controlled by an inducible genetic module.
65. The method of any claim, wherein said inducible genetic module is activated by a chemical or by temperature.
66. The method of any claim, wherein said dehydrogenase domain prevents the malonyl-CoA reductase enzyme from making malonyl-CoA.
67. The method of any claim, wherein said dehydrogenase domain comprises rutE or nemA.
68. The method of any claim, wherein said dehydrogenase domain allows malonyl-CoA reductase enzyme to use a cofactor in addition to NADPH.
69. The method of any claim, wherein said dehydrogenase domain allows malonyl-CoA reductase enzyme to use NADH.
70. The method of any claim, wherein said dehydrogenase domain is mmsB.
71. The method of any claim, wherein said dehydrogenase domain allows malonyl-CoA reductase enzyme to use falvin.
72. The method of any claim, wherein said dehydrogenase domain is rutE and nemA.
73. The method of any claim, wherein said dehydrogenase domain reduces the 3-hydroxypropionic acid inhibition of malonyl-CoA reductase.
74. The method of any claim, wherein said dehydrogenase domain is ydfG or NAD⁺-dependent serine dehydrogenase.
75. The method of any claim, wherein the genetically modified organism further comprises acetyl-CoA carboxylase.
76. The method of any claim, wherein said malonyl-CoA reductase enzyme is derived from a halophilic organism.
77. The method of any claim, wherein said halophilic organism is *Halomonase*.
78. The method of any claim, wherein the genetically modified organism further comprises at least one nucleic acid sequence having at least 70% homology and/or identity to any sequence in **Table 2**.

79. The method of any claim, wherein the genetically modified organism further comprises at least one efflux transporter capable of exporting 3-HP ions.
80. The method of any claim, wherein said at least one efflux transporter capable of exporting 3-HP ions is selected from the group consisting of: MFS exporter, ABC exporter, SMR exporter, RND exporter, MATE exporter, amino acid exporters, solvent tolerance transporters, and combinations thereof.
81. The method of any claim, wherein said at least one efflux transporter capable of exporting 3-HP ions is encoded by a gene is selected from the group consisting of: *acrD*, *bcr*, *cusA*, *dedA*, *eamA*, *eamB*, *eamH*, *emaA*, *emaB*, *emrB*, *emrD*, *emrKY*, *emrY*, *garP*, *gudP*, *hsrA*, *leuE*, *mdlB*, *mdtD*, *mdtG*, *mdtL*, *mdtM*, *mhpT*, *rhtA*, *rhtB*, *rhtC*, *thtB*, *yahN*, *yajR*, *ybbP*, *ybiF*, *ybjJ*, *ycaP*, *ydcO*, *yddG*, *ydeD*, *ydgE*, *yddG*, *ydhC*, *ydhP*, *ydiN*, *ydiM*, *ydjE*, *ydjI*, *ydjK*, *yeaS*, *yedA*, *yeeO*, *yegH*, *yggA*, *yfcJ*, *yfiK*, *yhjE*, *yidE*, *yigK*, *yigJ*, *yijE*, *yjiI*, *yjiJ*, *yjiO*, *ykgH*, *ypjD*, *ytff*, *ytfL*, homologs thereof, and combinations thereof.
82. The method of any claim, wherein said genetically modified cell is selected from the group consisting of: gram-negative bacterium, gram-positive bacterium, chemolithotrophic bacteria, yeast, and algae.
83. The method of any claim, comprising a gram-negative bacterium that is *Escherichia*.
84. The method of any claim, comprising a gram-positive bacterium that is *Bacillus*.
85. The method of any claim, comprising a yeast that is *Saccharomyces*.
86. A genetically modified organism capable of producing a chemical product of interest comprising, one or more nucleic acid sequences encoding for a polypeptide selected from the group consisting of:
- (1) an acetyl-CoA carboxylase having one or more of its subunits fused;
 - (2) an acetyl-CoA carboxylase having a predefined stoichiometric ratio of four ACCase subunits relative to one another;
 - (3) (a) a monofunctional malonyl-CoA reductase capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde, and (b) one or more nucleic acids selected from the group consisting of: *ydfG*, *mmsB*, *NDSD*, *rutE*, *nemA*, and any combination thereof;
 - (4) (a) a monofunctional malonyl-CoA reductase capable of catalyzing the conversion of malonyl-CoA to malonate semialdehyde, (b) one or more nucleic acids encoding for one or more enzymes capable of converting malonate semialdehyde keto form to 3-HP, and (c) one or more nucleic acids encoding for one or more enzymes capable of converting either malonate semialdehyde enol form to 3-HP or malonate semialdehyde hydrated form to 3-HP;

(5) a monofunctional malonyl-CoA reductase fused to a dehydrogenase that is either: (a) NADPH-dependent, (b) NADH-independent, (c) has the capability of using NADPH, (d) flavin-dependent, (e), flavin-independent, (f) has the capability of using flavin, (g) less susceptible to 3-HP inhibition at high concentration, and/or (h) catalyzes a reaction pathway to 3-HP that is substantially irreversible;

(6) a monofunctional malonyl-CoA reductase fused to one or more dehydrogenases;

(7) a malonyl-CoA reductase that is mutated to enhance its activity at lower temperatures;

(8) salt-tolerant enzyme;

(9) an exporter that facilitates the exportation of a chemical product of interest or the export of an inhibitory chemical from within the organism;

(10) an importer that facilitates the importation into the organism of a reactant, precursor, and/or metabolite used in the organism's production pathway for producing a chemical product of interest;

(11) an enzyme within a biosynthetic pathway for converting the carbon source to the chemical product, wherein the gene encoding the enzyme is activated by phosphate depletion;

(12) a polypeptide encoded by a gene selected from the group consisting of: mcr, mmsB, ydfG, rutE, nemA, NDSD, genes that encode individual or fused subunits of ACCase, and combination thereof, wherein the gene comprises the promoter of a gene selected from the group consisting of: amn, tktB, xasA, yibD, ytfK, pstS, phoH, phnC, and phosphate-regulated genes;

(13) an enzyme that is mutated to become activated or deactivated at a particular temperature and/or temperature range;

(14) an enzyme that is controlled by a mutated gene such that the gene: (a) is active and serves a key function in the conversion of the carbon source to the chemical product; or (b) is inactive and turns off a branch pathway or other competitive pathway that prevents or limits the pathway leading to the conversion of the carbon source to the chemical product; and

(15) fabI, fabB, and/or fabD, that is mutated to become temperature sensitive.

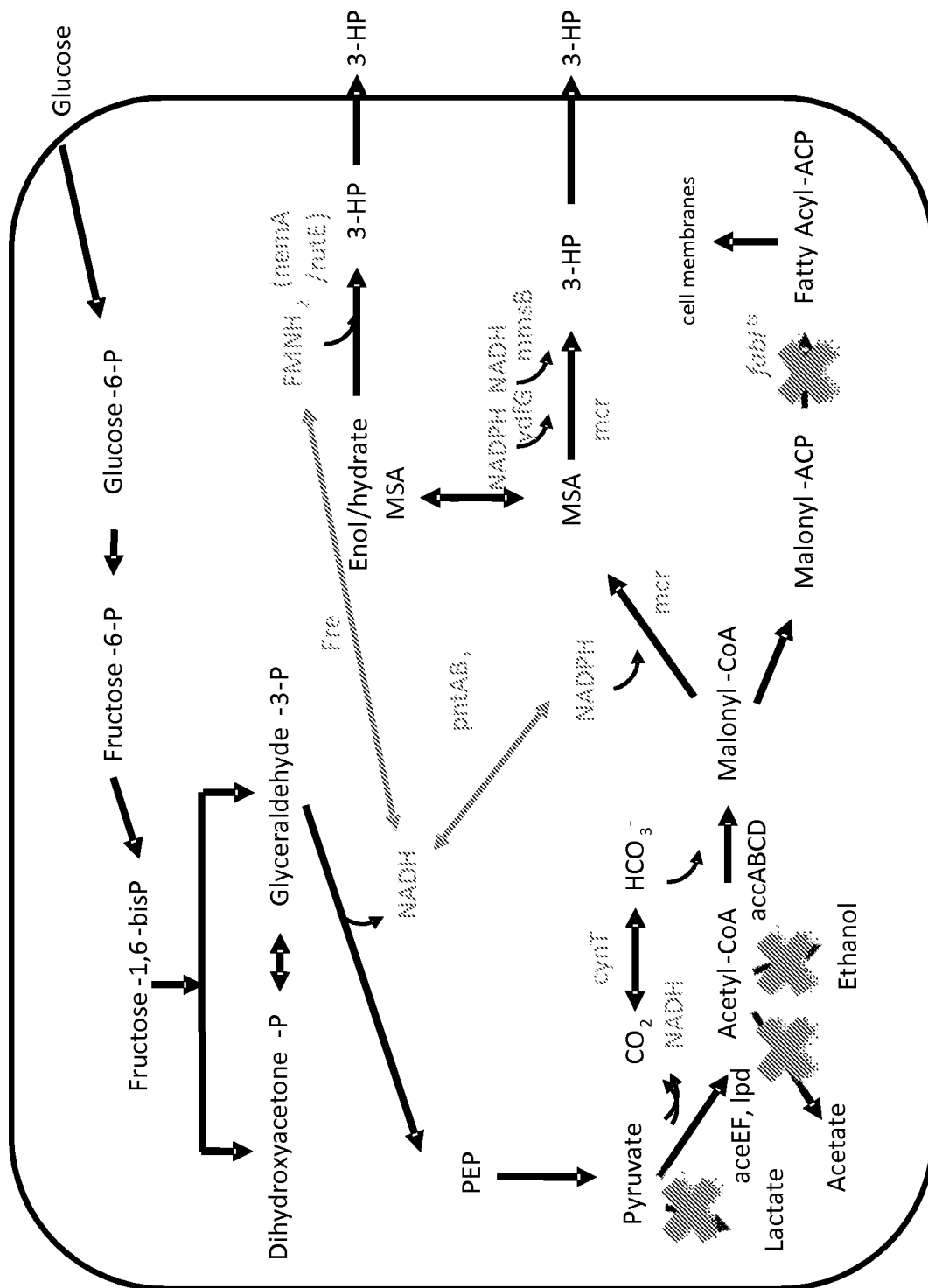


FIG. 1

CONVERSION OF MALONATE SEMIALDEHYDE TO 3-HP

Equilibrium states of Malonate Semialdehyde

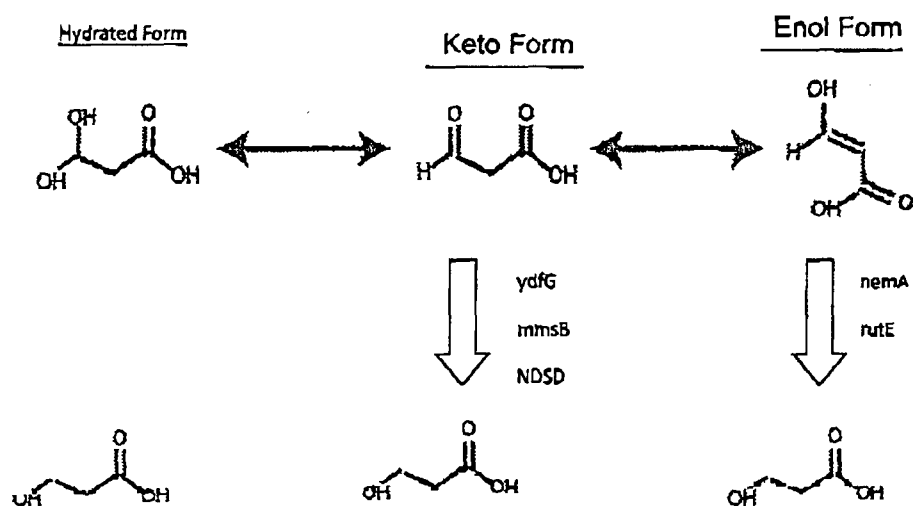


FIG. 2

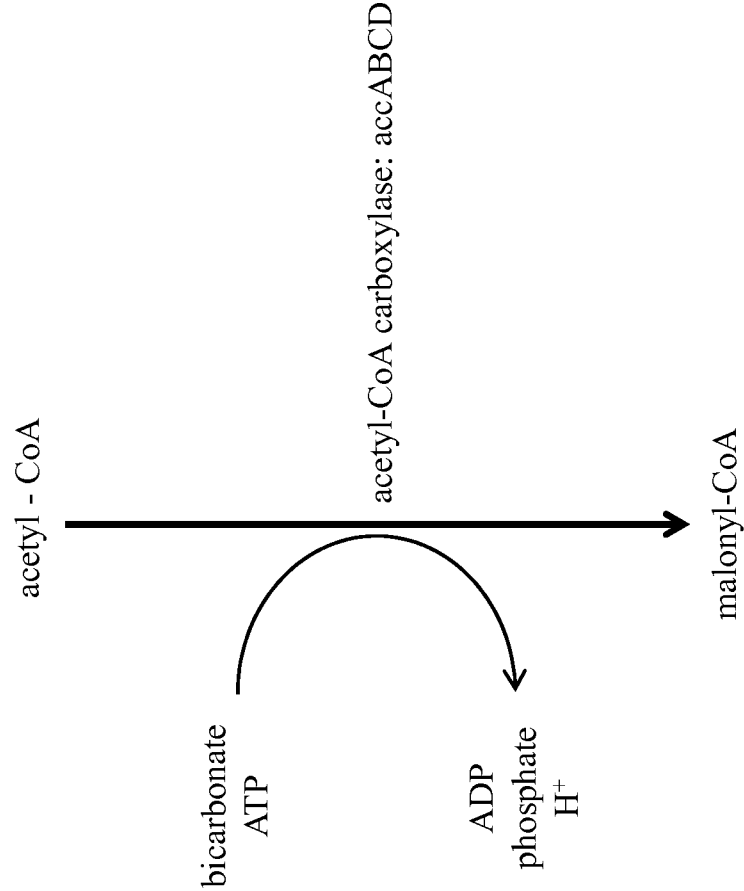


FIG. 3

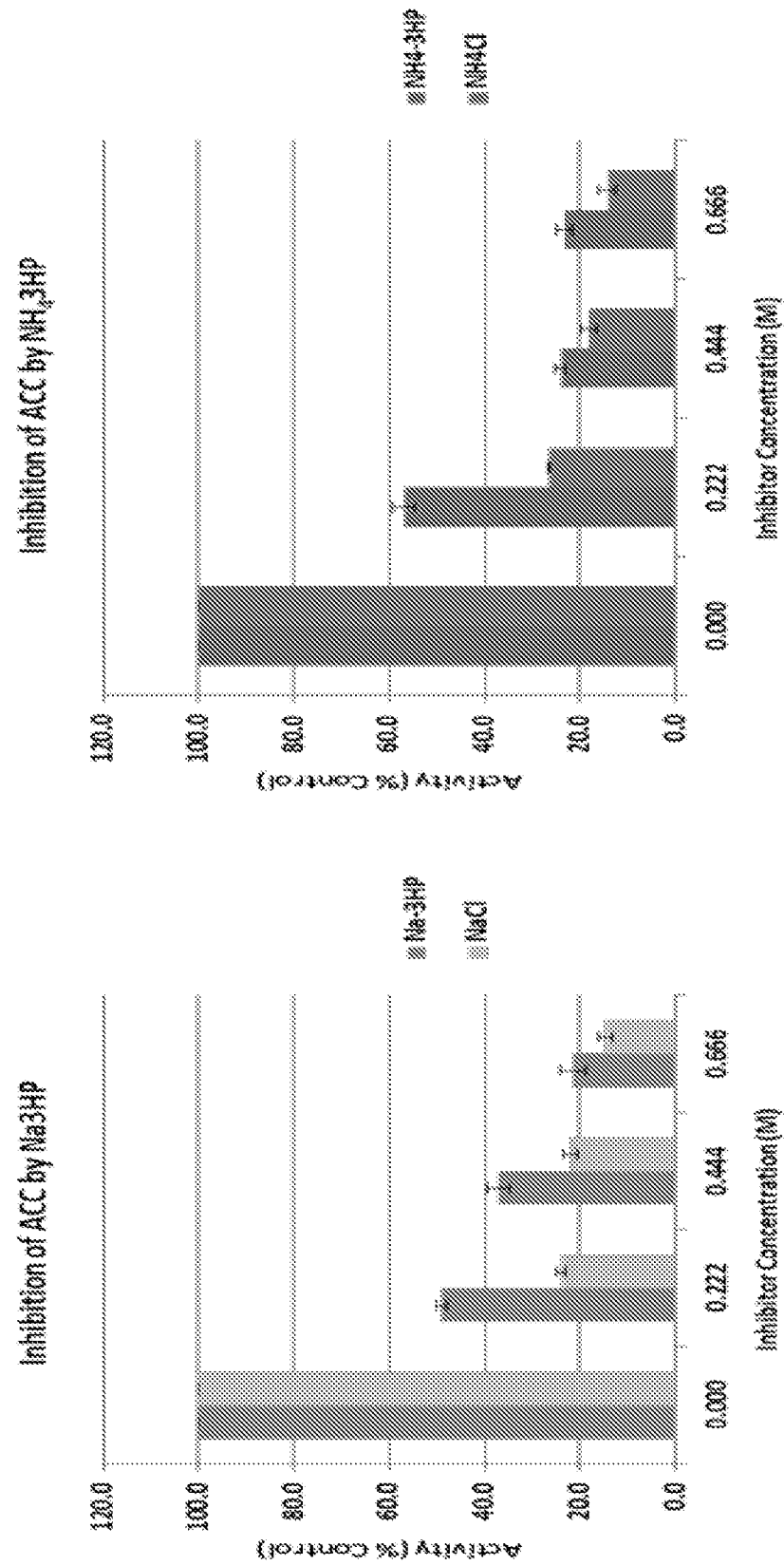


FIG. 4

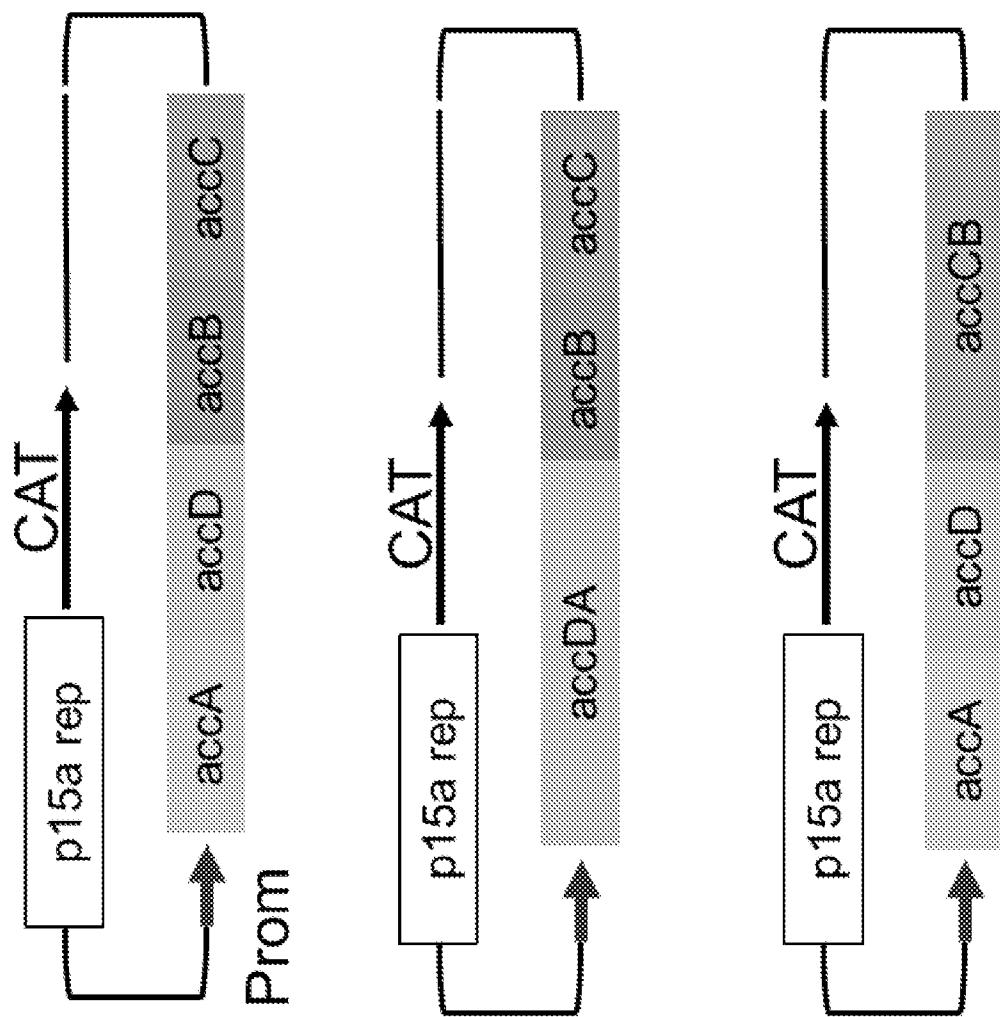


FIG. 5

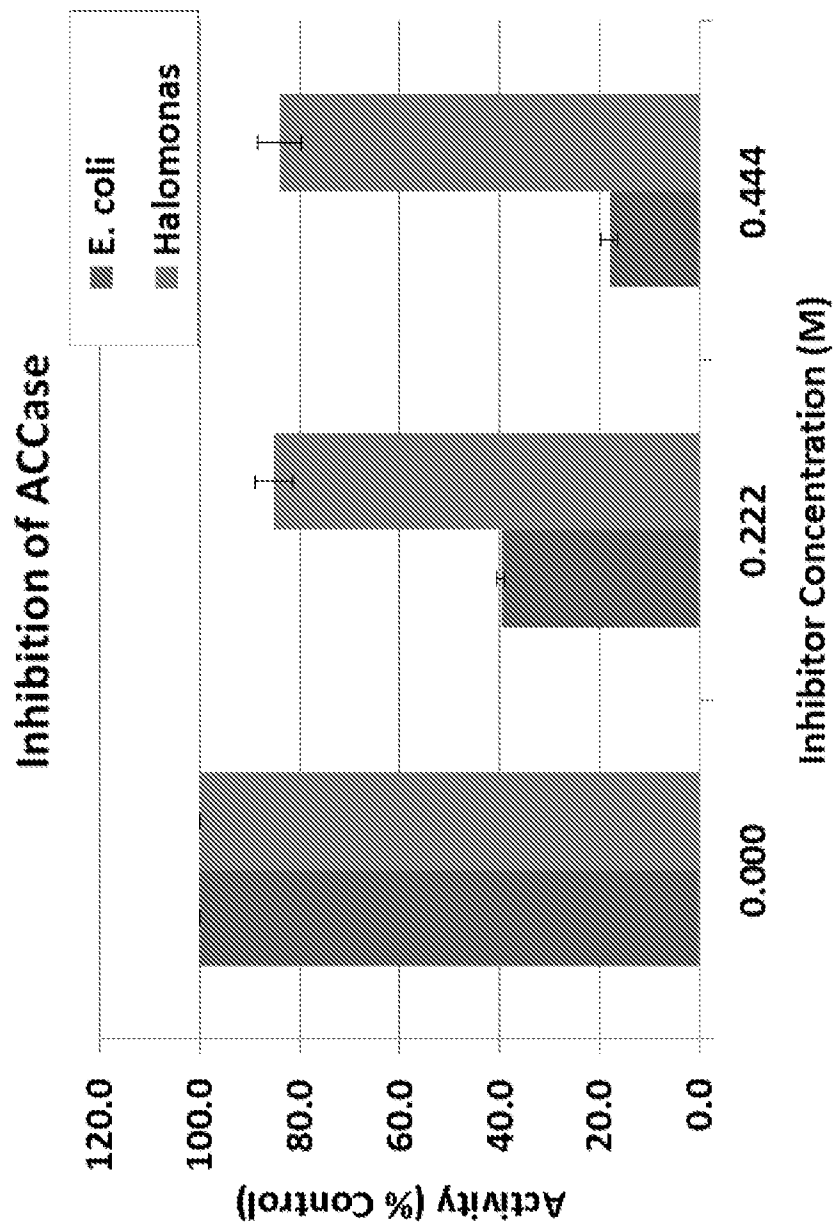


FIG. 6

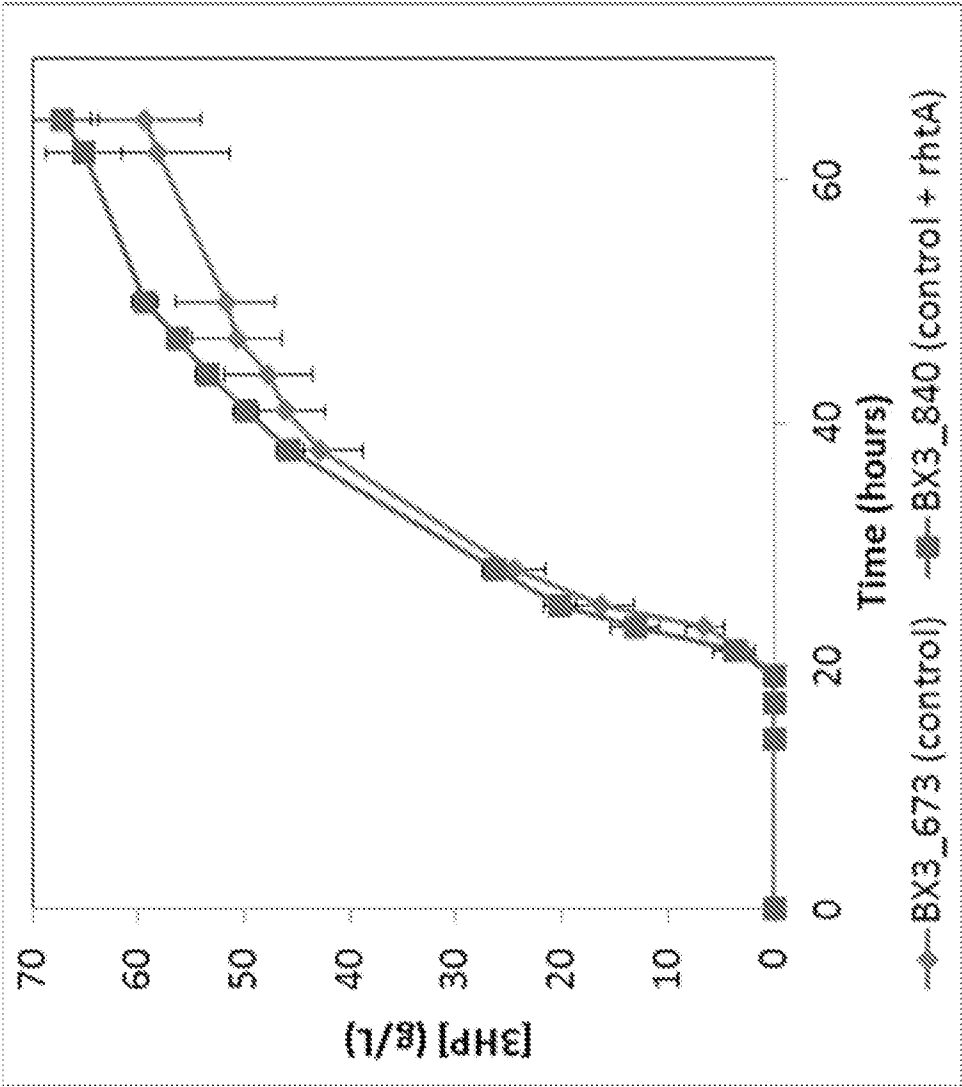


FIG. 7

<u>Promoters:</u>	<u>Regulated:</u>	<u>Plasmids</u>		
	Phosphate PstslH PybD ProD _{phx} ProX _{phx}	<u>Origins: (copy number at 30C)</u> pBBR1 (15-20) pBMT1-4, pBM1-4 pSC101 (5) pSC101 pSC101ts (RepA-A56V) (pKD46, pRed ⁺ p708) P15 (10-12) pACYC, pJ251, pMB1 ⁺ (20-30, >200 at 37°C) pUC, pCR4/Biunt, pJ244 pMB1/ColEI (15-20) pET, pTrc, pBR322 CisDF13 (20-40, ~50 at 37°C) pCDF-Duet RSF1030 (>100?) pRSF-Duet		
	<u>IPTG</u> PT7 P _{trc} Plac			
	<u>Temperature</u> Lambda repressor			
	<u>Constitutive:</u> PT5 _{out} PT5 _{extra} PT5 _{low} Pipia Pipia P _{scp1-4} (ProA-D) Pkan PgapA Library Designs	<u>Plasmid Selection:</u> <u>Antibiotic</u> Amp/Carb Kan Cam Tet Stp Bsd Zeo		
		<u>Other Modules</u> <u>Recombination Sites</u> FRT loxP		
<u>RBS</u>	Many	<u>Non-antibiotic:</u> ccdB gapA CitSyn		
	<u>Transcriptional terminators</u> rmBT1T2 tonB soxR BicbrkTT			

FIG. 8

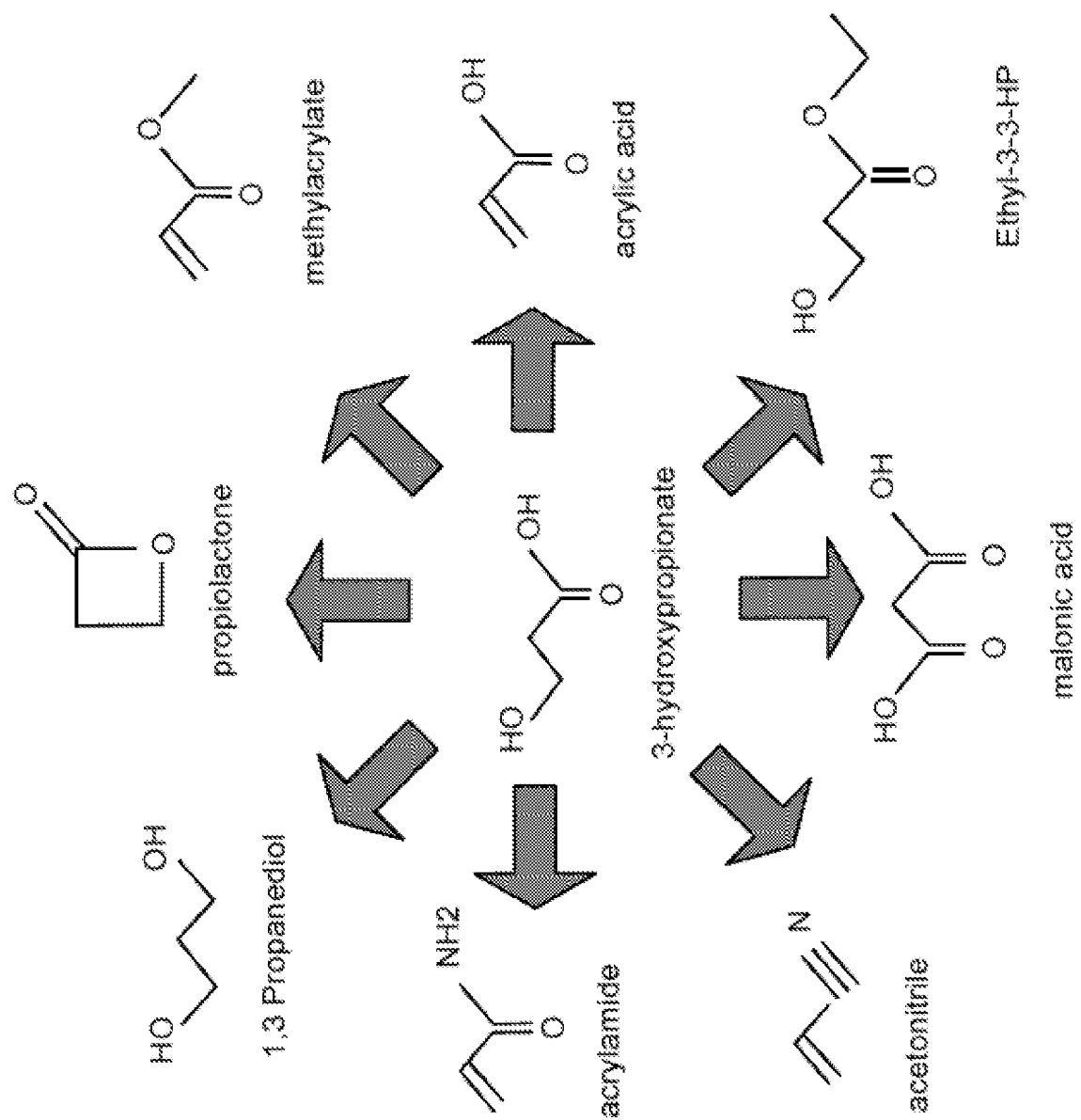


FIG. 9

Examples: Enzymatic reaction of: transport of homoserine (threonine and homoserine exporter)
(Biotec)

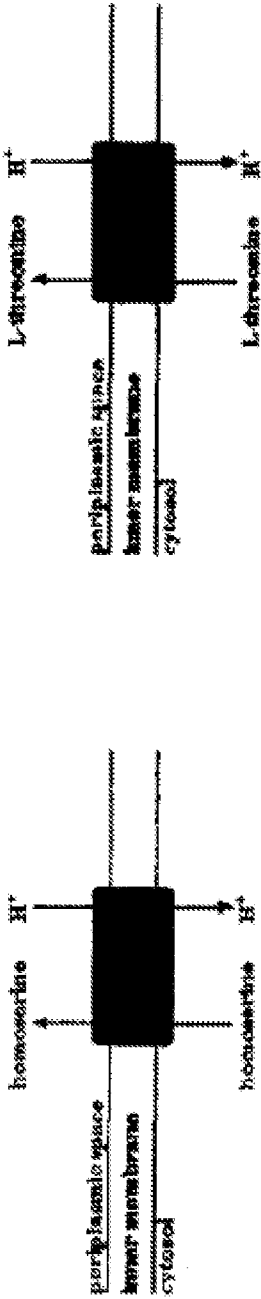


FIG. 10

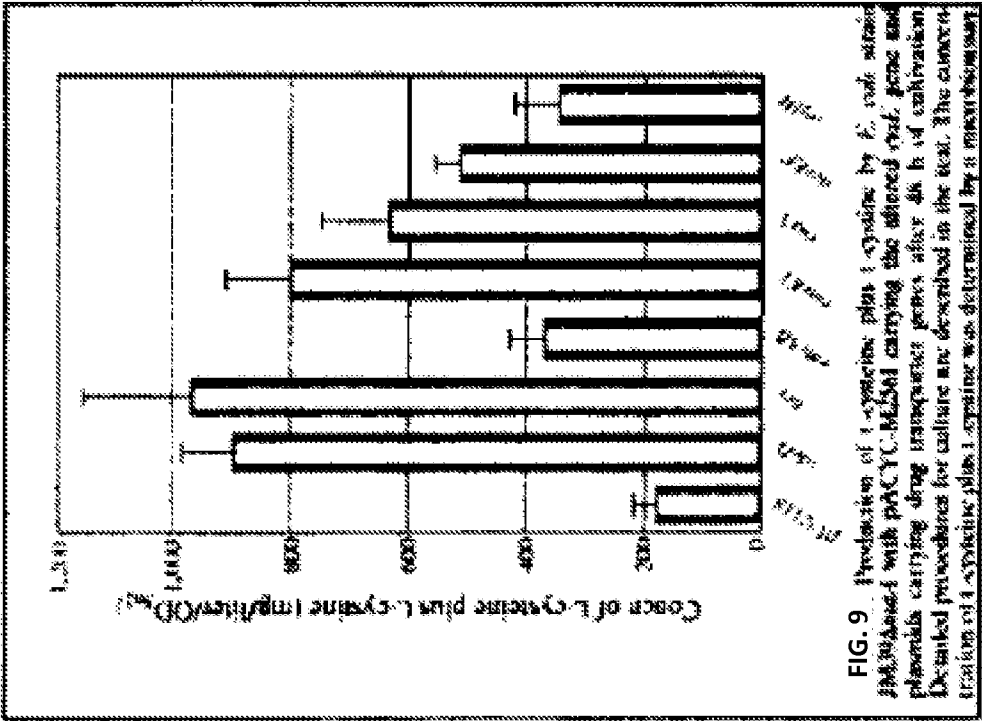


FIG. 11

12/27

Avg 24hr ΔOD600

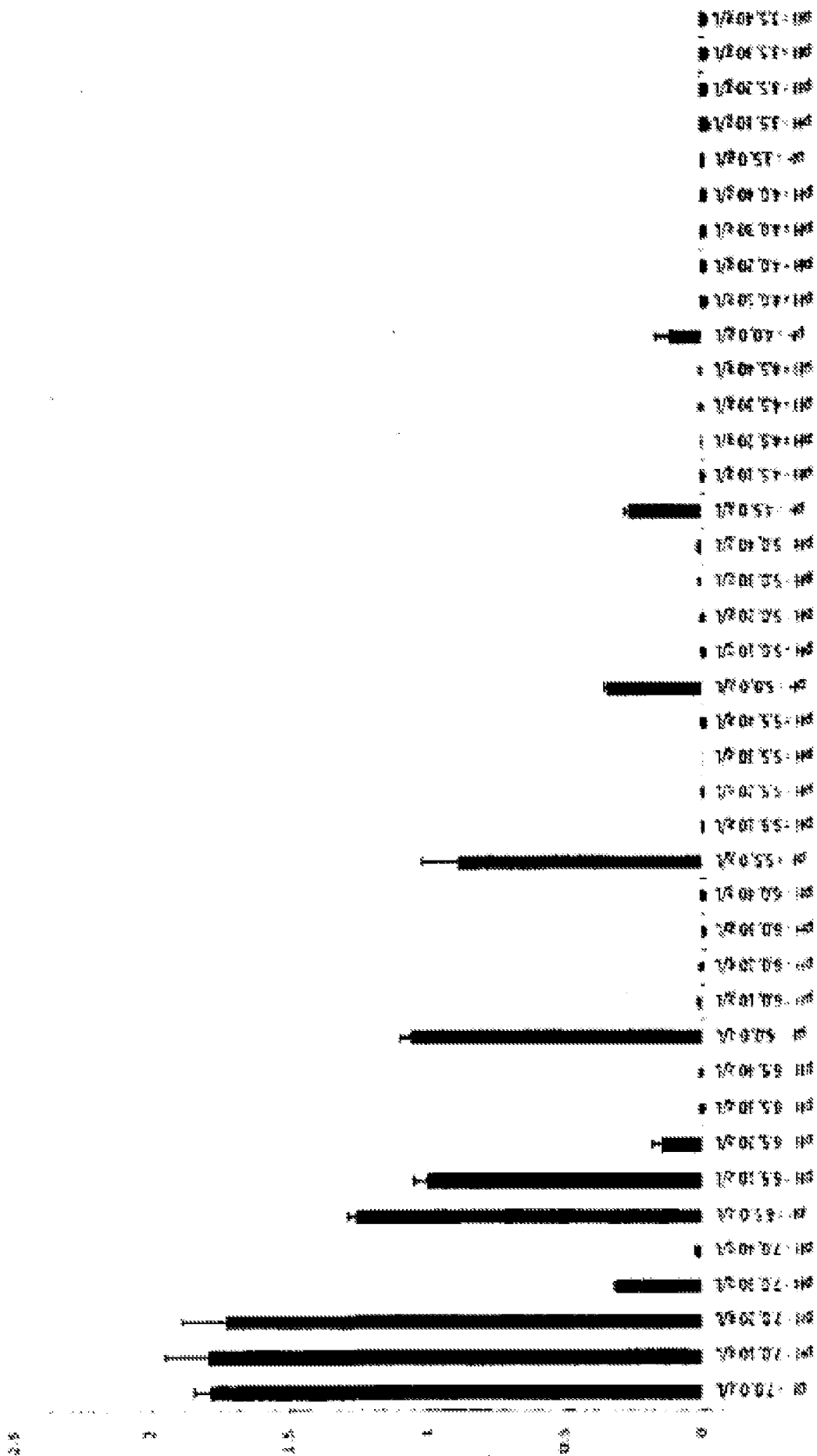


FIG. 12

[illegible]

FIG. 13B

002062463789ref -
0018560340821re 7
0018700238401re -
0018533697281re -
0020624637891re -
002273259231re 3
00253687875re 3
0000131689ref 3
002271151031re 3
00253990387re 6
002587460961re -
002387333801re -
6093 Content 7

FIG. 13D

Plotmap for rhlA.

CLUSTAL 2.0.11 multiple sequence alignment

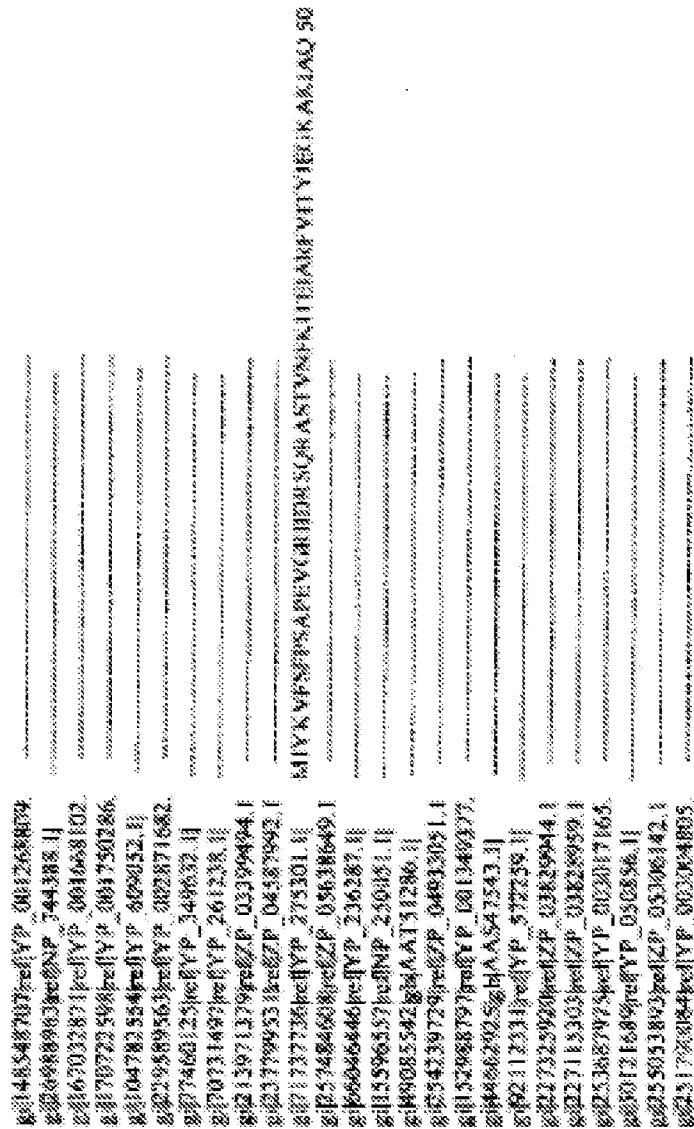


FIG. 14A

FIG. 14B

00774601250e11YF	249032	0	00002	00051	0
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0023779793311e12YF	0445379623	3	MS0	MS0	MS0
007173777550e11YF	2753301	0	MS0	MS0	MS0
002457484100e12YF	003180469	3	MS0	MS0	MS0
001860404446e11YF	236037	3	MS0	MS0	MS0
0011359655779e11YF	2300331	3	MS0	MS0	MS0
004604654035e11YF	041296	3	MS0	MS0	MS0
0025602397259e12YF	040290513	3	MS0	MS0	MS0
0014579087979e11YF	001349377	3	MS0	MS0	MS0
0044067373099e11YF	001349377	3	MS0	MS0	MS0
0021133311e11YF	5722388	3	MS0	MS0	MS0
002213349420e12YF	03849644	3	MS0	MS0	MS0
002227115320e12YF	03849644	3	MS0	MS0	MS0
00230077973e11YF	003017145	3	MS0	MS0	MS0
0013013116899e11YF	040086	3	MS0	MS0	MS0
002517900804e11YF	003004803	3	MS0	MS0	MS0
0023803187979e12YF	057247973	3	MS0	MS0	MS0
0024273998131e11YF	002097994	3	MS0	MS0	MS0
0011234430521e11YF	001007026	3	MS0	MS0	MS0
0023876686511e12YF	046356114	3	MS0	MS0	MS0
001700270464e11YF	001702051	3	MS0	MS0	MS0
002312357750e12YF	0008908	3	MS0	MS0	MS0
0016024188704e11YF	001000257	3	MS0	MS0	MS0
0023879541519e12YF	046370333	3	MS0	MS0	MS0
002387610394e12YF	04022013	3	MS0	MS0	MS0
00238798649e12YF	04641944	3	MS0	MS0	MS0
002387940694e12YF	04023879	3	MS0	MS0	MS0
002387949594e12YF	04631400	3	MS0	MS0	MS0
002387372264e12YF	046164473	3	MS0	MS0	MS0
00238735320e12YF	046147453	3	MS0	MS0	MS0
001573697204e11YF	001477715	3	MS0	MS0	MS0
002386346504e12YF	057260810	3	MS0	MS0	MS0
00118354311e11YF	001008128	3	MS0	MS0	MS0
001579697939e11YF	002338308	3	MS0	MS0	MS0
00206677333e11YF	002338346	3	MS0	MS0	MS0
001959904762e12YF	030831443	3	MS0	MS0	MS0
00209972767e12YF	032817513	3	MS0	MS0	MS0
0014403109504e11YF	001176603	3	MS0	MS0	MS0
002377353404e12YF	030819096	3	MS0	MS0	MS0
00237730791e12YF	04641272	3	MS0	MS0	MS0
001679447949e12YF	459408	3	MS0	MS0	MS0
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001038319064e12YF	032462733	3	MS0	MS0	MS0
00237929864e11YF	215845	3	MS0	MS0	MS0
001867973094e11YF	455367	3	MS0	MS0	MS0
00213022444e12YF	033310083	3	MS0	MS0	MS0
002134336339e12YF	031499453	3	MS0	MS0	MS0
0036118490310e11YF	001571113	3	MS0	MS0	MS0
0015711864040e11YF	001453603	3	MS0	MS0	MS0
004401818384e11YF	001453603	3	MS0	MS0	MS0
0020623779e12YF	832648	3	MS0	MS0	MS0
0021870411939e11YF	002811718	3	MS0	MS0	MS0
002624613994e12YF	735828	3	MS0	MS0	MS0
001700494706e11YF	0017004947	3	MS0	MS0	MS0
0018003553e12YF	286577	3	MS0	MS0	MS0
002702326311e11YF	00127585	3	MS0	MS0	MS0
002154085906e11YF	002328331	3	MS0	MS0	MS0
002276394723e12YF	040200283	3	MS0	MS0	MS0

[illegible]

[illegible]

FIG. 14E

[illegible]

FIG. 14I

[illegible]

FIG. 14J

[illegible]

FIG. 14K

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/030083**A. CLASSIFICATION OF SUBJECT MATTER****C12N 1/21(2006.01)i, C12N 15/53(2006.01)i, C12N 15/63(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N 1/21; C12P 7/52; C07H 21/04; A61L 15/22; C12N 9/20; C12P 7/42; A01H 13/00; C12P 7/00; C12N 1/19; C12N 15/53; C12N 15/63

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: genetically modified organism, mutated malonyl-CoA reductase, salt-tolerant enzyme, acetyl-CoA carboxylase, exportation, importation

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011-0125118 A1 (LYNCH, MICHAEL D.) 26 May 2011. See abstract; claims 1-56; and paragraphs 122, 126 and 184.	12, 17, 19, 21, 47, 86
A		24, 31, 39, 42
X	WO 2011-094457 A1 (OPX BIOTECHNOLOGIES, INC. et al.) 04 August 2011. See abstract; claims 1-33; and paragraph 331.	24, 31, 39, 42
A		12, 17, 19, 21, 47, 86
A	US 2012-0244588 A1 (PARK, S. M., et al.) 27 September 2012. See the whole document.	12, 17, 19, 21, 24, 31 , 39, 42, 47, 86
A	WO 2010-105095 A1 (SAPPHIRE ENERGY, INC.) 16 September 2010. See the whole document.	12, 17, 19, 21, 24, 31 , 39, 42, 47, 86
A	US 2004-0076982 A1 (GOKARN, R. R., et al.) 22 April 2004. See the whole document.	12, 17, 19, 21, 24, 31 , 39, 42, 47, 86

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

25 July 2014 (25.07.2014)

Date of mailing of the international search report

28 July 2014 (28.07.2014)

Name and mailing address of the ISA/KR

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INTERNATIONAL SEARCH REPORTInternational application No.
PCT/US2014/030083**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1-4, 6-9
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The subject matter of claims 1-4, 6-9 is not clear since 'a mutated malonyl-CoA reductase' is defined by the result to be achieved (PCT Article 6).
3. ☒ Claims Nos.: 3-5, 7-11, 13-16, 18, 20, 22-23, 25-30, 32-38, 40-41, 43-46, 48-85
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2014/030083

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US 7393676 B2	01/07/2008
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		US 8076120 B2	13/12/2011
		US 8198066 B2	12/06/2012
		US 8501455 B2	06/08/2013