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61/829,137 30 May 2013 (30.05.2013) US(71) Applicant: INVISTA NORTH AMERICA S.A.R.L.
[US/US]; Three Little Falls Centre, 2801 Centerville Road,
Wilmington, Delaware 19808 (US).(72) Inventors: BOTES, Adriana Leonora; Red House Farm,
Daleside Road, Rosedale East, Cleveland YO18 8RL (GB).
CONRADIE, Alex Van Eck; 4 Aberdovey Drive,
Eaglescliffe, Cleveland TS16 9EZ (GB). CHEN,
Changlin; 4 Cradoc Grove, Ingleby Barwick, Cleveland
TS17 5EE (GB). PEARLMAN, Paul S.; 24 Timber Lane,
Thornton, Pennsylvania 19373-1049 (US).(74) Agents: MCCORMICK GRAHAM, Monica et al.; Fish
& Richardson P.C., P.O. Box 1022, Minneapolis, Min-
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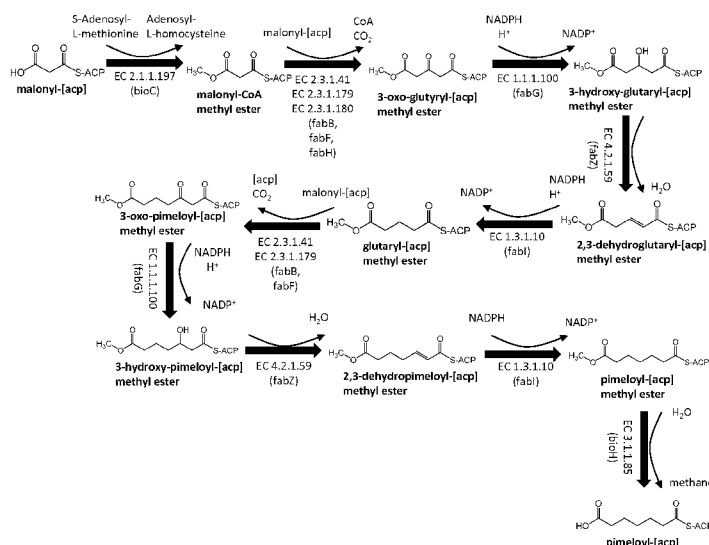
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[Continued on next page]

(54) Title: METHODS OF PRODUCING 7-CARBON CHEMICALS VIA METHYL-ESTER SHIELDED CARBON CHAIN
ELONGATION

FIGURE 1A



(57) Abstract: This document describes biochemical pathways for producing pimelic acid, 7-aminoheptanoic acid, 7-hydroxy-heptanoic acid, heptamethylenediamine or 1,7-heptanediol by forming two terminal functional groups, comprised of carboxyl, amine or hydroxyl group, in a C7 aliphatic backbone substrate. These pathways, metabolic engineering and cultivation strategies described herein rely on enzymes or homologs accepting methyl ester shielded dicarboxylic acid substrates.



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Methods Of Producing 7-Carbon Chemicals Via Methyl-Ester Shielded Carbon Chain Elongation

TECHNICAL FIELD

This invention relates to methods for biosynthesizing one or more of pimelic
5 acid, 7-aminoheptanoic acid, 7-hydroxyheptanoic acid, heptamethylenediamine and
1,7-heptanediol (hereafter “C7 building blocks”) from malonyl-CoA or malonyl-[acp]
and optionally acetyl-CoA using one or more isolated enzymes such as
methyltransferases, β -ketoacyl-[acp] synthases, β -ketothiolases, dehydrogenases,
reductases, hydratases, thioesterases, methylesterases, CoA-transferases, reversible
10 *CoA-ligases* and *transaminases* or using recombinant host cells expressing one or
more such enzymes.

BACKGROUND

Nylons are polyamides which are sometimes synthesized by the condensation
polymerisation of a diamine with a dicarboxylic acid. Similarly, Nylons may be
15 produced by the condensation polymerisation of lactams. A ubiquitous Nylon is
Nylon 6,6, which is produced by reaction of hexamethylenediamine (HMD) and
adipic acid. Nylon 6 is produced by a ring opening polymerisation of caprolactam
(Anton & Baird, Polyamides Fibers, Encyclopedia of Polymer Science and
Technology, 2001).

20 Nylon 7 and Nylon 7,7 represent novel polyamides with value-added
characteristics compared to Nylon 6 and Nylon 6,6. Nylon 7 is produced by
polymerisation of 7-aminoheptanoic acid, whereas Nylon 7,7 is produced by
condensation polymerisation of pimelic acid and heptamethylenediamine. No
economically viable petrochemical routes exist to producing the monomers for Nylon
25 7 and Nylon 7,7.

Given no economically viable petrochemical monomer feedstocks,
biotechnology offers an alternative approach via biocatalysis. Biocatalysis is the use
of biological catalysts, such as enzymes, to perform biochemical transformations of
organic compounds.

30 Both bioderived feedstocks and petrochemical feedstocks are viable starting
materials for the biocatalysis processes.

Accordingly, against this background, it is clear that there is a need for methods for producing pimelic acid, 7-aminoheptanoic acid, heptamethylenediamine, 7-hydroxyheptanoic acid, and 1,7-heptanediol (hereafter “C7 building blocks”) wherein the methods are biocatalyst-based.

5 However, no wild-type prokaryote or eukaryote naturally overproduces or excretes C7 building blocks to the extracellular environment. Nevertheless, the metabolism of pimelic acid has been reported.

 The dicarboxylic acid, pimelic acid, is converted efficiently as a carbon source by a number of bacteria and yeasts via β -oxidation into central metabolites. β -oxidation of CoEnzyme A (CoA) activated pimelate to CoA-activated 3-oxopimelate facilitates further catabolism via, for example, pathways associated with aromatic substrate degradation. The catabolism of 3-oxopimeloyl-CoA to acetyl-CoA and glutaryl-CoA by several bacteria has been characterized comprehensively (Harwood and Parales, *Annual Review of Microbiology*, 1996, 50, 553 – 590).

15 The optimality principle states that microorganisms regulate their biochemical networks to support maximum biomass growth. Beyond the need to express heterologous pathways in a host organism, directing carbon flux towards C7 building blocks that serve as carbon sources rather than to biomass growth constituents, contradicts the optimality principle. For example, transferring the 1-butanol pathway from *Clostridium* species into other production strains has often fallen short by an order of magnitude compared to the production performance of native producers (Shen *et al.*, *Appl. Environ. Microbiol.*, 2011, 77(9), 2905 – 2915).

 The efficient synthesis of the seven carbon aliphatic backbone precursor is a key consideration in synthesizing C7 building blocks prior to forming terminal functional groups, such as carboxyl, amine or hydroxyl groups, on the C7 aliphatic backbone.

SUMMARY

 This document is based at least in part on the discovery that it is possible to construct biochemical pathways for producing a seven carbon chain aliphatic backbone precursor, in which one or two functional groups, i.e., carboxyl, amine, or hydroxyl, can be formed, leading to the synthesis of one or more of pimelic acid, 7-

aminoheptanoate, 7-hydroxyheptanoate, heptamethylenediamine, and 1,7-heptanediol (hereafter “C7 building blocks”). Pimelic acid and pimelate, 7-hydroxyheptanoic acid and 7-hydroxyheptanoate, and 7-aminoheptanoic and 7-aminoheptanoate are used interchangeably herein to refer to the compound in any of its neutral or ionized forms, including any salt forms thereof. It is understood by those skilled in the art that the specific form will depend on pH. These pathways, metabolic engineering and cultivation strategies described herein rely on fatty acid elongation and synthesis enzymes or homologs accepting methyl-ester shielded dicarboxylic acids as substrates.

In the face of the optimality principle, it surprisingly has been discovered that appropriate non-natural pathways, feedstocks, host microorganisms, attenuation strategies to the host’s biochemical network and cultivation strategies may be combined to efficiently produce one or more C7 building blocks.

In some embodiments, the C7 aliphatic backbone for conversion to one or more C7 building blocks is pimeloyl-[acp] or pimeloyl-CoA (also known as 6-carboxyhexanoyl-CoA), which can be formed from malonyl-[acp] or malonyl-CoA, and optionally acetyl-CoA, via two cycles of carbon chain elongation using either NADH or NADPH dependent enzymes. See FIG. 1A, FIG. 1B and FIG. 1C.

In some embodiments, a terminal carboxyl group can be enzymatically formed using a *pimeloyl-[acp] methyl ester methylesterase*, a *thioesterase*, an *aldehyde dehydrogenase*, a *7-oxoheptanoate dehydrogenase*, a *6-oxohexanoate dehydrogenase*, a *reversible CoA ligase* (e.g., *reversible succinyl-CoA ligase*) or a *CoA-transferase* (e.g., a *glutaconate CoA-transferase*). See FIG. 1A, FIG. 1B, FIG. 1C and FIG. 2.

In some embodiments, a terminal amine group can be enzymatically formed using a *ω-transaminase* or a *deacetylase*. See FIG. 3 and FIG. 4.

In some embodiments, a terminal hydroxyl group can be enzymatically formed using a *4-hydroxybutyrate dehydrogenase*, *5-hydroxypentanoate dehydrogenase* or a *6-hydroxyhexanoate dehydrogenase* or an *alcohol dehydrogenase*. See FIG. 5 and FIG. 6.

In one aspect, this document features a method for biosynthesizing a product selected from the group consisting of pimelic acid, 7-aminoheptanoate, 7-hydroxyheptanoate, heptamethylenediamine and 1,7-heptanediol. The method

includes enzymatically synthesizing a seven carbon chain aliphatic backbone from (i) acetyl-CoA and malonyl-CoA via two cycles of methyl ester shielded carbon chain elongation or (ii) malonyl-[acp] via two cycles of methyl-ester shielded carbon chain elongation, and enzymatically forming one or two terminal functional groups selected from the group consisting of carboxyl, amine, and hydroxyl groups in the backbone, thereby forming the product. The seven carbon chain aliphatic backbone can be pimeloyl-[acp] or pimeloyl-CoA. A *malonyl-[acp] O-methyltransferase* can convert malonyl-CoA to a malonyl-CoA methyl ester or can convert malonyl-[acp] to a malonyl-[acp] methyl ester. Each of the two cycles of carbon chain elongation can include using (i) a β -ketoacyl-[acp] synthase or a β -ketothiolase, (ii) a 3-oxoacyl-[acp] reductase, an acetoacetyl-CoA reductase, a 3-hydroxyacyl-CoA dehydrogenase or a 3-hydroxybutyryl-CoA dehydrogenase, (iii) an enoyl-CoA hydratase or a 3-hydroxyacyl-[acp] dehydratase, and (iv) an enoyl-[acp] reductase or a trans-2-enoyl-CoA reductase to produce pimeloyl-[acp] methyl ester from malonyl-[acp] methyl ester or produce pimeloyl-CoA methyl ester from malonyl-CoA methyl ester. A *pimeloyl-[acp] methyl ester methylesterase* can remove the methyl group from pimeloyl-CoA methyl ester or pimeloyl-[acp] methyl ester. The *malonyl-[acp] O-methyltransferase* can have at least 70% sequence identity to the amino acid sequence set forth in SEQ ID NO: 16. The *pimeloyl-[acp] methyl ester methylesterase* can have at least 70% sequence identity to the amino acid sequence set forth in SEQ ID NO: 17.

The two terminal functional groups can be the same (e.g., amine or hydroxyl) or can be different (e.g., a terminal amine and a terminal carboxyl group; or a terminal hydroxyl group and a terminal carboxyl group).

A ω -transaminase or a deacetylase can enzymatically form an amine group. The ω -transaminase can have at least 70% sequence identity to any one of the amino acid sequences set forth in SEQ ID NO. 8 – 13.

A 6-hydroxyhexanoate dehydrogenase, a 5-hydroxypentanoate dehydrogenase, a 4-hydroxybutyrate dehydratase, or an alcohol dehydrogenase can enzymatically form a hydroxyl group.

A thioesterase, an aldehyde dehydrogenase, a 7-oxoheptanoate dehydrogenase, a 6-oxohexanoate dehydrogenase, a CoA-transferase (e.g. a

glutaconate CoA transferase), or a *reversible CoA-ligase* (e.g., a *reversible succinate-CoA ligase*) can enzymatically form a terminal carboxyl group. The *thioesterase* can have at least 70% sequence identity to the amino acid sequence set forth in SEQ ID NO: 1.

5 A *carboxylate reductase* and a *phosphopantetheinyl transferase* can form a terminal aldehyde group as an intermediate in forming the product. The *carboxylate reductase* can have at least 70% sequence identity to any one of the amino acid sequences set forth in SEQ ID NO. 2 – 7.

Any of the methods can be performed in a recombinant host by fermentation.
10 The host can be subjected to a cultivation strategy under aerobic, anaerobic, micro-aerobic or mixed oxygen/denitrification cultivation conditions. The host can be cultured under conditions of nutrient limitation. The host can be retained using a ceramic hollow fiber membrane to maintain a high cell density during fermentation.

In any of the methods, the host's tolerance to high concentrations of a C7
15 building block can be improved through continuous cultivation in a selective environment.

The principal carbon source fed to the fermentation can derive from biological or non-biological feedstocks. In some embodiments, the biological feedstock is, includes, or derives from, monosaccharides, disaccharides,
20 lignocellulose, hemicellulose, cellulose, lignin, levulinic acid and formic acid, triglycerides, glycerol, fatty acids, agricultural waste, condensed distillers' solubles, or municipal waste.

In some embodiments, the non-biological feedstock is or derives from natural gas, syngas, CO₂/H₂, methanol, ethanol, benzoate, non-volatile residue (NVR) or a
25 caustic wash waste stream from cyclohexane oxidation processes, or a terephthalic acid/isophthalic acid mixture waste stream.

This document also features a recombinant host that includes at least one exogenous nucleic acid encoding (i) a *malonyl-[acp] O-methyltransferase*, (ii) a β -*ketoacyl-[acp] synthase* or a β -*ketothiolase*, (iii) a *3-oxoacyl-[acp] reductase*,
30 *acetoacetyl-CoA reductase*, a *3-hydroxyacyl-CoA dehydrogenase* or a *3-hydroxybutyryl-CoA dehydrogenase*, (iv) an *enoyl-CoA hydratase* or *3-hydroxyacyl-[acp] dehydratase*, (v) an *enoyl-[acp] reductase* or a *trans-2-enoyl-CoA reductase*,

and (vi) a *pimeloyl-[acp]methyl ester methylesterase*, the host producing pimeloyl-[acp] or pimeloyl-CoA.

A recombinant host producing pimeloyl-[acp] or pimeloyl-CoA further can include at least one exogenous nucleic acid encoding one or more of a *thioesterase*, an
 5 *aldehyde dehydrogenase*, a *7-oxoheptanoate dehydrogenase*, a *6-oxohexanoate dehydrogenase*, a *glutaconate CoA-transferase*, a reversible *succinyl-CoA ligase*, an *acetylating aldehyde dehydrogenase*, or a *carboxylate reductase*, the host producing pimelic acid or pimelate semialdehyde.

A recombinant host producing pimelate semialdehyde further can include at
 10 least one exogenous nucleic acid encoding a *ω -transaminase*, and producing 7-aminoheptanoate.

A recombinant host producing pimelate semialdehyde further can include at least one exogenous nucleic acid encoding a *4-hydroxybutyrate dehydrogenase*, a *5-hydroxypentanoate dehydrogenase* or a *6-hydroxyhexanoate dehydrogenase*, the host
 15 producing 7-hydroxyheptanoic acid.

A recombinant host producing pimelate semialdehyde, 7-aminoheptanoate, or 7-hydroxyheptanoic acid further can include a *carboxylate reductase*, a *ω -transaminase*, a *deacetylase*, an *N-acetyl transferase*, or an *alcohol dehydrogenase*, the host producing heptamethylenediamine.

A recombinant host producing 7-hydroxyheptanoic acid further can include at
 20 least one exogenous nucleic acid encoding a *carboxylate reductase* or an *alcohol dehydrogenase*, the host producing 1,7-heptanediol.

The recombinant host can be a prokaryote, e.g., from the genus *Escherichia* such as *Escherichia coli*; from the genus *Clostridia* such as *Clostridium ljungdahlii*,
 25 *Clostridium autoethanogenum* or *Clostridium kluyveri*; from the genus *Corynebacteria* such as *Corynebacterium glutamicum*; from the genus *Cupriavidus* such as *Cupriavidus necator* or *Cupriavidus metallidurans*; from the genus *Pseudomonas* such as *Pseudomonas fluorescens*, *Pseudomonas putida* or *Pseudomonas oleovorans*; from the genus *Delftia acidovorans*, from the genus
 30 *Bacillus* such as *Bacillus subtilis*; from the genus *Lactobacillus* such as *Lactobacillus delbrueckii*; from the genus *Lactococcus* such as *Lactococcus lactis* or from the genus *Rhodococcus* such as *Rhodococcus equi*.

The recombinant host can be a eukaryote, e.g., a eukaryote from the genus *Aspergillus* such as *Aspergillus niger*; from the genus *Saccharomyces* such as *Saccharomyces cerevisiae*; from the genus *Pichia* such as *Pichia pastoris*; from the genus *Yarrowia* such as *Yarrowia lipolytica*, from the genus *Issatchenkia* such as
 5 *Issatchenkia orientalis*, from the genus *Debaryomyces* such as *Debaryomyces hansenii*, from the genus *Arxula* such as *Arxula adeninivorans*, or from the genus *Kluyveromyces* such as *Kluyveromyces lactis*.

Any of the recombinant hosts described herein further can include one or more of the following attenuated enzymes: *polyhydroxyalkanoate synthase*, an *acetyl-CoA thioesterase*, an *acetyl-CoA specific β -ketothiolase*, a *phosphotransacetylase forming acetate*, an *acetate kinase*, a *lactate dehydrogenase*, a *menaquinol-fumarate oxidoreductase*, a *2-oxoacid decarboxylase* producing isobutanol, an *alcohol dehydrogenase* forming ethanol, a *triose phosphate isomerase*, a *pyruvate decarboxylase*, a *glucose-6-phosphate isomerase*, a *transhydrogenase* dissipating the
 10 NADH or NADPH imbalance, an *glutamate dehydrogenase* dissipating the NADH or NADPH imbalance, a NADH/NADPH-utilizing *glutamate dehydrogenase*, a *pimeloyl-CoA dehydrogenase*; an *acyl-CoA dehydrogenase* accepting C7 building blocks and central precursors as substrates; a *glutaryl-CoA dehydrogenase*; or a *pimeloyl-CoA synthetase*.

Any of the recombinant hosts described herein further can overexpress one or more genes encoding: an *acetyl-CoA synthetase*, a *6-phosphogluconate dehydrogenase*; a *transketolase*; a *puridine nucleotide transhydrogenase*; a *formate dehydrogenase*; a *glyceraldehyde-3P-dehydrogenase*; a *malic enzyme*; a *glucose-6-phosphate dehydrogenase*; a *fructose 1,6 diphosphatase*; a *L-alanine dehydrogenase*;
 20 a *L-glutamate dehydrogenase* specific to the NADH or NADPH used to generate a co-factor imbalance; a *methanol dehydrogenase*, a *formaldehyde dehydrogenase*, a *diamine transporter*; a *dicarboxylate transporter*; an *S-adenosylmethionine synthetase*, and/or a *multidrug transporter*.

The reactions of the pathways described herein can be performed in one or
 30 more cell (e.g., host cell) strains (a) naturally expressing one or more relevant enzymes, (b) genetically engineered to express one or more relevant enzymes, or (c) naturally expressing one or more relevant enzymes and genetically engineered to

express one or more relevant enzymes. Alternatively, relevant enzymes can be extracted from any of the above types of host cells and used in a purified or semi-purified form. Extracted enzymes can optionally be immobilized to a solid substrate such as the floors and/or walls of appropriate reaction vessels. Moreover, such
5 extracts include lysates (e.g. cell lysates) that can be used as sources of relevant enzymes. In the methods provided by the document, all the steps can be performed in cells (e.g., host cells), all the steps can be performed using extracted enzymes, or some of the steps can be performed in cells and others can be performed using extracted enzymes.

10 Many of the enzymes described herein catalyze reversible reactions, and the reaction of interest may be the reverse of the described reaction. The schematic pathways shown in FIGs. 1-6 illustrate the reaction of interest for each of the intermediates.

In some embodiments, the host microorganism's endogenous biochemical
15 network is attenuated or augmented to (1) ensure the intracellular availability of 2-oxoglutarate and 2-oxoadipate, (2) create an NAD^+ imbalance that may only be balanced via the formation of a C7 building block, (3) prevent degradation of central metabolites, central precursors leading to and including C7 building blocks and (4) ensure efficient efflux from the cell.

20 Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and
25 other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the
30 accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and the drawings, and from the claims. The word "comprising" in the claims may be replaced by

“consisting essentially of” or with “consisting of,” according to standard practice in patent law.

DESCRIPTION OF DRAWINGS

FIG. 1A is a schematic of an exemplary biochemical pathway leading to pimeloyl-[acp] using NADPH-dependent enzymes and malonyl-[acp] as central metabolite.

FIG. 1B is a schematic of an exemplary biochemical pathway leading to pimeloyl-CoA using NADPH-dependent enzymes and acetyl-CoA and malonyl-CoA as central metabolites.

FIG. 1C is a schematic of an exemplary biochemical pathway leading to pimeloyl-CoA using NADH-dependent enzymes and acetyl-CoA and malonyl-CoA as central metabolites.

FIG. 2 is a schematic of exemplary biochemical pathways leading to pimelate using pimeloyl-[acp], pimeloyl-CoA, or pimelate semialdehyde as central precursors.

FIG. 3 is a schematic of exemplary biochemical pathways leading to 7-aminoheptanoate using pimeloyl-CoA, pimelate, or pimelate semialdehyde as central precursors.

FIG. 4 is a schematic of exemplary biochemical pathways leading to heptamethylenediamine using 7-aminoheptanoate, 7-hydroxyheptanoate or pimelate semialdehyde as central precursors.

FIG. 5 is a schematic of exemplary biochemical pathways leading to 7-hydroxyheptanoate using pimelate, pimeloyl-CoA, or pimelate semialdehyde as central precursors.

FIG. 6 is a schematic of an exemplary biochemical pathway leading to 1,7-heptanediol using 7-hydroxyheptanoate as a central precursor.

FIG. 7 contains the amino acid sequences of an *Escherichia coli* thioesterase encoded by *tesB* (see GenBank Accession No. AAA24665.1, SEQ ID NO: 1), a *Mycobacterium marinum* carboxylate reductase (see Genbank Accession No. ACC40567.1, SEQ ID NO: 2), a *Mycobacterium smegmatis* carboxylate reductase (see Genbank Accession No. ABK71854.1, SEQ ID NO: 3), a *Segniliparus rugosus* carboxylate reductase (see Genbank Accession No. EFV11917.1, SEQ ID NO: 4), a *Mycobacterium smegmatis* carboxylate reductase (see Genbank Accession No.

ABK75684.1, SEQ ID NO: 5), a *Mycobacterium massiliense* carboxylate reductase (see Genbank Accession No. EIV11143.1, SEQ ID NO: 6), a *Segniliparus rotundus* carboxylate reductase (see Genbank Accession No. ADG98140.1, SEQ ID NO: 7), a *Chromobacterium violaceum* ω -transaminase (see Genbank Accession No. AAQ59697.1, SEQ ID NO: 8), a *Pseudomonas aeruginosa* ω -transaminase (see Genbank Accession No. AAG08191.1, SEQ ID NO: 9), a *Pseudomonas syringae* ω -transaminase (see Genbank Accession No. AAY39893.1, SEQ ID NO: 10), a *Rhodobacter sphaeroides* ω -transaminase (see Genbank Accession No. ABA81135.1, SEQ ID NO: 11), an *Escherichia coli* ω -transaminase (see Genbank Accession No. AAA57874.1, SEQ ID NO: 12), a *Vibrio fluvialis* ω -transaminase (see Genbank Accession No. AEA39183.1, SEQ ID NO: 13), a *Bacillus subtilis* phosphopantetheinyl transferase (see Genbank Accession No. CAA44858.1, SEQ ID NO: 14), a *Nocardia* sp. NRRL 5646 phosphopantetheinyl transferase (see Genbank Accession No. ABI83656.1, SEQ ID NO: 15), a *Bacillus cereus* malonyl-CoA methyltransferase (see GenBank Accession No. AAS43086.1, SEQ ID NO: 16) or an *Escherichia coli* pimelyl-[acp] methyl ester esterase (see GenBank Accession No. AAC76437.1, SEQ ID NO: 17).

FIG. 8 is a bar graph of the relative absorbance at 412 nm after 20 minutes of released CoA as a measure of the activity of a *thioesterase* for converting pimeloyl-CoA to pimelate relative to the empty vector control.

FIG. 9 is a bar graph summarizing the change in absorbance at 340 nm after 20 minutes, which is a measure of the consumption of NADPH and activity of *carboxylate reductases* relative to the enzyme only controls (no substrate).

FIG. 10 is a bar graph of the change in absorbance at 340 nm after 20 minutes, which is a measure of the consumption of NADPH and the activity of *carboxylate reductases* for converting pimelate to pimelate semialdehyde relative to the empty vector control.

FIG. 11 is a bar graph of the change in absorbance at 340 nm after 20 minutes, which is a measure of the consumption of NADPH and the activity of *carboxylate reductases* for converting 7-hydroxyheptanoate to 7-hydroxyheptanal relative to the empty vector control.

FIG. 12 is a bar graph of the change in absorbance at 340 nm after 20 minutes, which is a measure of the consumption of NADPH and the activity of *carboxylate reductases* for converting N7-acetyl-7-aminoheptanoate to N7-acetyl-7-aminoheptanal relative to the empty vector control.

5 FIG. 13 is a bar graph of the change in absorbance at 340 nm after 20 minutes, which is a measure of the consumption of NADPH and activity of *carboxylate reductases* for converting pimelate semialdehyde to heptanedial relative to the empty vector control.

10 FIG. 14 is a bar graph summarizing the percent conversion after 4 hours of pyruvate to L-alanine (mol/mol) as a measure of the *ω-transaminase* activity of the enzyme only controls (no substrate).

FIG. 15 is a bar graph of the percent conversion after 4 hours of pyruvate to L-alanine (mol/mol) as a measure of the *ω-transaminase* activity for converting 7-aminoheptanoate to pimelate semialdehyde relative to the empty vector control.

15 FIG. 16 is a bar graph of the percent conversion after 4 hours of L-alanine to pyruvate (mol/mol) as a measure of the *ω-transaminase* activity for converting pimelate semialdehyde to 7-aminoheptanoate relative to the empty vector control.

20 FIG. 17 is a bar graph of the percent conversion after 4 hours of pyruvate to L-alanine (mol/mol) as a measure of the *ω-transaminase* activity for converting heptamethylenediamine to 7-aminoheptanal relative to the empty vector control.

FIG. 18 is a bar graph of the percent conversion after 4 hours of pyruvate to L-alanine (mol/mol) as a measure of the *ω-transaminase* activity for converting N7-acetyl-1,7-diaminoheptane to N7-acetyl-7-aminoheptanal relative to the empty vector control.

25 FIG. 19 is a bar graph of the percent conversion after 4 hours of pyruvate to L-alanine (mol/mol) as a measure of the *ω-transaminase* activity for converting 7-aminoheptanol to 7-oxoheptanol relative to the empty vector control.

FIG. 20 is a table of the conversion after 1 hour of pimeloyl-CoA methyl ester to pimeloyl-CoA by a *pimeloyl-[acp] methyl ester methylesterase*.

DETAILED DESCRIPTION

This document provides enzymes, non-natural pathways, cultivation strategies, feedstocks, host microorganisms and attenuations to the host's biochemical network, which generates a seven carbon chain aliphatic backbone from central metabolites in which two terminal functional groups may be formed leading to the synthesis of pimelic acid, 7-aminoheptanoic acid, heptamethylenediamine or 1,7-heptanediol (referred to as "C7 building blocks" herein). As used herein, the term "central precursor" is used to denote any metabolite in any metabolic pathway shown herein leading to the synthesis of a C7 building block. The term "central metabolite" is used herein to denote a metabolite that is produced in all microorganisms to support growth.

Host microorganisms described herein can include endogenous pathways that can be manipulated such that one or more C7 building blocks can be produced. In an endogenous pathway, the host microorganism naturally expresses all of the enzymes catalyzing the reactions within the pathway. A host microorganism containing an engineered pathway does not naturally express all of the enzymes catalyzing the reactions within the pathway but has been engineered such that all of the enzymes within the pathway are expressed in the host.

The term "exogenous" as used herein with reference to a nucleic acid (or a protein) and a host refers to a nucleic acid that does not occur in (and cannot be obtained from) a cell of that particular type as it is found in nature or a protein encoded by such a nucleic acid. Thus, a non-naturally-occurring nucleic acid is considered to be exogenous to a host once in the host. It is important to note that non-naturally-occurring nucleic acids can contain nucleic acid subsequences or fragments of nucleic acid sequences that are found in nature provided the nucleic acid as a whole does not exist in nature. For example, a nucleic acid molecule containing a genomic DNA sequence within an expression vector is non-naturally-occurring nucleic acid, and thus is exogenous to a host cell once introduced into the host, since that nucleic acid molecule as a whole (genomic DNA plus vector DNA) does not exist in nature. Thus, any vector, autonomously replicating plasmid, or virus (e.g., retrovirus, adenovirus, or herpes virus) that as a whole does not exist in nature is considered to be non-naturally-occurring nucleic acid. It follows that genomic DNA fragments

produced by PCR or restriction endonuclease treatment as well as cDNAs are considered to be non-naturally-occurring nucleic acid since they exist as separate molecules not found in nature. It also follows that any nucleic acid containing a promoter sequence and polypeptide-encoding sequence (e.g., cDNA or genomic DNA) in an arrangement not found in nature is non-naturally-occurring nucleic acid. A nucleic acid that is naturally-occurring can be exogenous to a particular host microorganism. For example, an entire chromosome isolated from a cell of yeast x is an exogenous nucleic acid with respect to a cell of yeast y once that chromosome is introduced into a cell of yeast y.

In contrast, the term “endogenous” as used herein with reference to a nucleic acid (e.g., a gene) (or a protein) and a host refers to a nucleic acid (or protein) that does occur in (and can be obtained from) that particular host as it is found in nature. Moreover, a cell “endogenously expressing” a nucleic acid (or protein) expresses that nucleic acid (or protein) as does a host of the same particular type as it is found in nature. Moreover, a host “endogenously producing” or that “endogenously produces” a nucleic acid, protein, or other compound produces that nucleic acid, protein, or compound as does a host of the same particular type as it is found in nature.

For example, depending on the host and the compounds produced by the host, one or more of the following enzymes may be expressed in the host in addition to a *malonyl-[acp] O-methyltransferase* and a *pimeloyl-[acp] methyl ester methylesterase*: a β -ketoacyl-[acp] synthase, a β -ketothiolase, a 3-oxoacyl-[acp] reductase, acetoacetyl-CoA reductase, a 3-hydroxyacyl-CoA dehydrogenase, a 3-hydroxybutyryl-CoA dehydrogenase, an enoyl-CoA hydratase, 3-hydroxyacyl-[acp] dehydratase, an enoyl-[acp] reductase, a *trans*-2-enoyl-CoA reductase, a thioesterase, a reversible CoA ligase, a CoA-transferase, an acetylating aldehyde dehydrogenase, a 6-oxohexanoate dehydrogenase, a 7-oxoheptanoate dehydrogenase, an aldehyde dehydrogenase, a carboxylate reductase, a ω -transaminase, a *N*-acetyl transferase, an alcohol dehydrogenase, a deacetylase, a 6-hydroxyhexanoate dehydrogenase, a 5-hydroxypentanoate dehydrogenase, or a 4-hydroxybutyrate dehydrogenase. In recombinant hosts expressing a carboxylate reductase, a phosphopantetheinyl transferase also can be expressed as it enhances activity of the carboxylate reductase.

For example, a recombinant host can include at least one exogenous nucleic acid encoding (i) a *malonyl-[acp] O-methyltransferase*, (ii) a β -ketoacyl-[acp] synthase

or a β -ketothiolase, (iii) a 3-oxoacyl-[acp] reductase, acetoacetyl-CoA reductase, a 3-hydroxyacyl-CoA dehydrogenase or a 3-hydroxybutyryl-CoA dehydrogenase, (iv) an enoyl-CoA hydratase or 3-hydroxyacyl-[acp] dehydratase, (v) an enoyl-[acp] reductase or a trans-2-enoyl-CoA reductase and (vi) a pimeloyl-[acp]methyl ester methylesterase,
 5 and produce pimeloyl-[acp] or pimeloyl-CoA.

Such recombinant hosts producing pimeloyl-[acp] or pimeloyl-CoA further can include at least one exogenous nucleic acid encoding one or more of a thioesterase, an aldehyde dehydrogenase, a 7-oxoheptanoate dehydrogenase, a 6-oxohexanoate dehydrogenase, a glutaconate CoA-transferase, a reversible succinyl-
 10 CoA ligase, an acetylating aldehyde dehydrogenase, or a carboxylate reductase and produce pimelic acid or pimelate semialdehyde. For example, a recombinant host producing pimeloyl-[acp] or pimeloyl-CoA further can include a thioesterase, a reversible Co-ligase (e.g., a reversible succinyl-CoA ligase), or a CoA transferase (e.g., a glutaconate CoA-transferase) and produce pimelic acid. For example, a
 15 recombinant host producing pimeloyl-CoA further can include an acetylating aldehyde dehydrogenase and produce pimelate semialdehyde. For example, a recombinant host producing pimelate further can include a carboxylate reductase and produce pimelate semialdehyde.

A recombinant hosts producing pimelate semialdehyde further can include at
 20 least one exogenous nucleic acid encoding a ω -transaminase and produce 7-aminoheptanoate. In some embodiments, a recombinant host producing pimeloyl-CoA includes a carboxylate reductase and a ω -transaminase to produce 7-aminoheptanoate.

A recombinant host producing pimelate or pimelate semialdehyde further can
 25 include at least one exogenous nucleic acid encoding a 6-hydroxyhexanoate dehydrogenase, a 5-hydroxypentanoate dehydrogenase or a 4-hydroxybutyrate dehydrogenase, and produce 7-hydroxyheptanoic acid. In some embodiments, a recombinant host producing pimeloyl-CoA includes an acetylating aldehyde dehydrogenase, and a 6-hydroxyhexanoate dehydrogenase, a 5-hydroxypentanoate
 30 dehydrogenase or a 4-hydroxybutyrate dehydrogenase to produce 7-hydroxyheptanoate. In some embodiments, a recombinant host producing pimelate includes a carboxylate reductase and a 6-hydroxyhexanoate dehydrogenase, a 5-

hydroxypentanoate dehydrogenase or a *4-hydroxybutyrate dehydrogenase* to produce 7-hydroxyheptanoate.

5 A recombinant hosts producing 7-aminoheptanoate, 7-hydroxyheptanoate or pimelate semialdehyde further can include at least one exogenous nucleic acid encoding a *ω*-transaminase, a *deacetylase*, a *N-acetyl transferase*, or an *alcohol dehydrogenase*, and produce heptamethylenediamine. For example, a recombinant host producing 7-hydroxyheptanoate can include a *carboxylate reductase* with a *phosphopantetheine transferase* enhancer, a *ω*-transaminase and an *alcohol dehydrogenase*.

10 A recombinant host producing 7-hydroxyheptanoic acid further can include one or more of a *carboxylate reductase* with a *phosphopantetheine transferase* enhancer and an *alcohol dehydrogenase*, and produce 1,7-heptanediol.

15 Within an engineered pathway, the enzymes can be from a single source, i.e., from one species or genus, or can be from multiple sources, i.e., different species or genera. Nucleic acids encoding the enzymes described herein have been identified from various organisms and are readily available in publicly available databases such as GenBank or EMBL.

20 Any of the enzymes described herein that can be used for production of one or more C7 building blocks can have at least 70% sequence identity (homology) (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) to the amino acid sequence of the corresponding wild-type enzyme. It will be appreciated that the sequence identity can be determined on the basis of the mature enzyme (e.g., with any signal sequence removed).

25 For example, a *thioesterase* described herein can have at least 70% sequence identity (homology) (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) to the amino acid sequence of an *Escherichia coli* *thioesterase* encoded by *tesB* (see GenBank Accession No. AAA24665.1, SEQ ID NO: 1). See FIG. 7.

30 For example, a *carboxylate reductase* described herein can have at least 70% sequence identity (homology) (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) to the amino acid sequence of a *Mycobacterium marinum* (see Genbank Accession No. ACC40567.1, SEQ ID NO: 2), a *Mycobacterium smegmatis* (see Genbank Accession No. ABK71854.1, SEQ ID NO: 3), a *Segniliparus rugosus*

(see Genbank Accession No. EFV11917.1, SEQ ID NO: 4), a *Mycobacterium smegmatis* (see Genbank Accession No. ABK75684.1, SEQ ID NO: 5), a *Mycobacterium massiliense* (see Genbank Accession No. EIV11143.1, SEQ ID NO: 6), or a *Segniliparus rotundus* (see Genbank Accession No. ADG98140.1, SEQ ID NO: 7) *carboxylate reductase*. See, FIG. 7.

For example, a ω -*transaminase* described herein can have at least 70% sequence identity (homology) (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) to the amino acid sequence of a *Chromobacterium violaceum* (see Genbank Accession No. AAQ59697.1, SEQ ID NO: 8), a *Pseudomonas aeruginosa* (see Genbank Accession No. AAG08191.1, SEQ ID NO: 9), a *Pseudomonas syringae* (see Genbank Accession No. AAY39893.1, SEQ ID NO: 10), a *Rhodobacter sphaeroides* (see Genbank Accession No. ABA81135.1, SEQ ID NO: 11), an *Escherichia coli* (see Genbank Accession No. AAA57874.1, SEQ ID NO: 12), or a *Vibrio fluvialis* (see Genbank Accession No. AEA39183.1, SEQ ID NO: 13) ω -*transaminase*. Some of these ω -*transaminases* are *diamine ω -transaminases*. See, FIG. 7.

For example, a *phosphopantetheinyl transferase* described herein can have at least 70% sequence identity (homology) (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) to the amino acid sequence of a *Bacillus subtilis* *phosphopantetheinyl transferase* (see Genbank Accession No. CAA44858.1, SEQ ID NO:14) or a *Nocardia* sp. NRRL 5646 *phosphopantetheinyl transferase* (see Genbank Accession No. ABI83656.1, SEQ ID NO:15). See FIG. 7.

For example, a *malonyl-CoA methyltransferase* described herein can have at least 70% sequence identity (homology) (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) to the amino acid sequence of a *Bacillus cereus* *malonyl-CoA methyltransferase* (see GenBank Accession No. AAS43086.1, SEQ ID NO:16). See, FIG. 7.

For example, a *pimelyl-[acp] methyl ester esterase* described herein can have at least 70% sequence identity (homology) (e.g., at least 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%) to the amino acid sequence of an *Escherichia coli* *pimelyl-[acp] methyl ester esterase* (see GenBank Accession No. AAC76437.1, SEQ ID NO:17). See, FIG. 7.

The percent identity (homology) between two amino acid sequences can be determined as follows. First, the amino acid sequences are aligned using the BLAST 2 Sequences (BL2seq) program from the stand-alone version of BLASTZ containing BLASTP version 2.0.14. This stand-alone version of BLASTZ can be obtained from
5 Fish & Richardson's web site (e.g., www.fr.com/blast/) or the U.S. government's National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov). Instructions explaining how to use the BL2seq program can be found in the readme file accompanying BLASTZ. BL2seq performs a comparison between two amino acid sequences using the BLASTP algorithm. To compare two amino acid sequences, the
10 options of BL2seq are set as follows: -i is set to a file containing the first amino acid sequence to be compared (e.g., C:\seq1.txt); -j is set to a file containing the second amino acid sequence to be compared (e.g., C:\seq2.txt); -p is set to blastp; -o is set to any desired file name (e.g., C:\output.txt); and all other options are left at their default setting. For example, the following command can be used to generate an output file
15 containing a comparison between two amino acid sequences: C:\BL2seq -i c:\seq1.txt -j c:\seq2.txt -p blastp -o c:\output.txt. If the two compared sequences share homology (identity), then the designated output file will present those regions of homology as aligned sequences. If the two compared sequences do not share homology (identity), then the designated output file will not present aligned
20 sequences. Similar procedures can be following for nucleic acid sequences except that blastn is used.

Once aligned, the number of matches is determined by counting the number of positions where an identical amino acid residue is presented in both sequences. The percent identity (homology) is determined by dividing the number of matches by the
25 length of the full-length polypeptide amino acid sequence followed by multiplying the resulting value by 100. It is noted that the percent identity (homology) value is rounded to the nearest tenth. For example, 78.11, 78.12, 78.13, and 78.14 is rounded down to 78.1, while 78.15, 78.16, 78.17, 78.18, and 78.19 is rounded up to 78.2. It also is noted that the length value will always be an integer.

30 It will be appreciated that a number of nucleic acids can encode a polypeptide having a particular amino acid sequence. The degeneracy of the genetic code is well known to the art; i.e., for many amino acids, there is more than one nucleotide triplet

that serves as the codon for the amino acid. For example, codons in the coding sequence for a given enzyme can be modified such that optimal expression in a particular species (e.g., bacteria or fungus) is obtained, using appropriate codon bias tables for that species.

5 Functional fragments of any of the enzymes described herein can also be used in the methods of the document. The term “functional fragment” as used herein refers to a peptide fragment of a protein that has at least 25% (e.g., at least: 30%; 40%; 50%; 60%; 70%; 75%; 80%; 85%; 90%; 95%; 98%; 99%; 100%; or even greater than 100%) of the activity of the corresponding mature, full-length, wild-type protein.
10 The functional fragment can generally, but not always, be comprised of a continuous region of the protein, wherein the region has functional activity.

 This document also provides (i) functional variants of the enzymes used in the methods of the document and (ii) functional variants of the functional fragments described above. Functional variants of the enzymes and functional fragments can
15 contain additions, deletions, or substitutions relative to the corresponding wild-type sequences. Enzymes with substitutions will generally have not more than 50 (e.g., not more than one, two, three, four, five, six, seven, eight, nine, ten, 12, 15, 20, 25, 30, 35, 40, or 50) amino acid substitutions (e.g., conservative substitutions). This applies to any of the enzymes described herein and functional fragments. A conservative
20 substitution is a substitution of one amino acid for another with similar characteristics. Conservative substitutions include substitutions within the following groups: valine, alanine and glycine; leucine, valine, and isoleucine; aspartic acid and glutamic acid; asparagine and glutamine; serine, cysteine, and threonine; lysine and arginine; and phenylalanine and tyrosine. The nonpolar hydrophobic amino acids include alanine,
25 leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Any substitution of one member of the above-
30 mentioned polar, basic or acidic groups by another member of the same group can be deemed a conservative substitution. By contrast, a nonconservative substitution is a substitution of one amino acid for another with dissimilar characteristics.

Deletion variants can lack one, two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acid segments (of two or more amino acids) or non-contiguous single amino acids. Additions (addition variants) include fusion proteins containing: (a) any of the enzymes described herein or a
5 fragment thereof; and (b) internal or terminal (C or N) irrelevant or heterologous amino acid sequences. In the context of such fusion proteins, the term "heterologous amino acid sequences" refers to an amino acid sequence other than (a). A heterologous sequence can be, for example a sequence used for purification of the recombinant protein (e.g., FLAG, polyhistidine (e.g., hexahistidine), hemagglutinin (HA), glutathione-S-transferase (GST), or maltosebinding protein (MBP)).
10 Heterologous sequences also can be proteins useful as detectable markers, for example, luciferase, green fluorescent protein (GFP), or chloramphenicol acetyl transferase (CAT). In some embodiments, the fusion protein contains a signal sequence from another protein. In certain host cells (e.g., yeast host cells), expression and/or secretion of the target protein can be increased through use of a heterologous
15 signal sequence. In some embodiments, the fusion protein can contain a carrier (e.g., KLH) useful, e.g., in eliciting an immune response for antibody generation) or ER or Golgi apparatus retention signals. Heterologous sequences can be of varying length and in some cases can be a longer sequences than the full-length target proteins to
20 which the heterologous sequences are attached.

Engineered hosts can naturally express none or some (e.g., one or more, two or more, three or more, four or more, five or more, or six or more) of the enzymes of the pathways described herein. Thus, a pathway within an engineered host can include all exogenous enzymes, or can include both endogenous and exogenous
25 enzymes. Endogenous genes of the engineered hosts also can be disrupted to prevent the formation of undesirable metabolites or prevent the loss of intermediates in the pathway through other enzymes acting on such intermediates. Engineered hosts can be referred to as recombinant hosts or recombinant host cells. As described herein recombinant hosts can include nucleic acids encoding one or more of a
30 *methyltransferase*, a *synthase*, *β -ketothiolase*, a *dehydratase*, a *hydratase*, a *dehydrogenase*, a *methylesterase*, a *thioesterase*, a *reversible CoA-ligase*, a *CoA-*

transferase, a reductase, deacetylase, N-acetyl transferase or a ω -transaminase as described in more detail below.

In addition, the production of one or more C7 building blocks can be performed *in vitro* using the isolated enzymes described herein, using a lysate (e.g., a
 5 cell lysate) from a host microorganism as a source of the enzymes, or using a plurality of lysates from different host microorganisms as the source of the enzymes.

Enzymes generating the C7 aliphatic backbone for conversion to C7 Building Blocks

As depicted in FIG. 1A, FIG. 1B and FIG. 1C, a C7 aliphatic backbone for
 10 conversion to one or more C7 building blocks can be formed from malonyl-[acp], or acetyl-CoA and malonyl-CoA, via two cycles of methyl-ester shielded carbon chain elongation associated with biotin synthesis using either NADH or NADPH dependent enzymes.

In some embodiments, a methyl ester shielded carbon chain elongation
 15 associated with biotin biosynthesis route comprises using a *malonyl-[acp] O-methyltransferase* to form a malonyl-[acp] methyl ester, and then performing two cycles of carbon chain elongation using a *β -ketoacyl-[acp] synthase*, a *3-oxoacyl-[acp] reductase*, a *3-hydroxyacyl-[acp] dehydratase*, and an *enoyl-[acp] reductase*. A *pimeloyl-[acp] methyl ester esterase* can be used to cleave the resulting pimeloyl-
 20 [acp] methyl ester.

In some embodiments, a methyl ester shielded carbon chain elongation route comprises using a *malonyl-[acp] O-methyltransferase* to form a malonyl-CoA methyl ester, and then performing two cycles of carbon chain elongation using (i) a *β -ketothiolase* or a *β -ketoacyl-[acp] synthase*, (ii) an *acetoacetyl-CoA reductase*, a *3-oxoacyl-[acp] reductase*, or a *3-hydroxybutyryl-CoA dehydrogenase*, (iii) *enoyl-CoA hydratase*, and (iv) a *trans-2-enoyl-CoA reductase*. A *pimeloyl-[acp] methyl ester esterase* can be used to cleave the resulting pimeloyl-CoA methyl ester.
 25

In some embodiments, a *methyltransferase* can be a *malonyl-[acp] O-methyltransferase* classified, for example, under EC 2.1.1.197 such as the gene
 30 product of *bioC* from *Bacillus cereus* (see Genbank Accession No. AAS43086.1, SEQ ID NO:16) (see, for example, Lin, 2012, *Biotin Synthesis in Escherichia coli*, Ph.D. Dissertation, University of Illinois at Urbana-Champaign).

In some embodiments, a β -ketothiolase may be classified, for example, under EC 2.3.1.16, such as the gene product of *bktB*. The β -ketothiolase encoded by *bktB* from *Cupriavidus necator* accepts propanoyl-CoA and pentanoyl-CoA as substrates, forming the CoA-activated C7 aliphatic backbone (see, e.g., Haywood *et al.*, *FEMS Microbiology Letters*, 1988, 52:91-96; Slater *et al.*, *J. Bacteriol.*, 1998, 180(8):1979 - 1987).

In some embodiments, a β -ketoacyl-[*acp*] synthase may be classified, for example, under EC 2.3.1.- (e.g., EC 2.3.1.41, EC 2.3.1.179 or EC 2.3.1.180) such as the gene product of *fabB*, *fabF*, or *fabH*.

In some embodiments, a 3-hydroxyacyl-CoA dehydrogenase may be classified under EC 1.1.1.35, such as the gene product of *fadB*, or classified under EC 1.1.1.157, such as the gene product of *hbd* (can be referred to as a 3-hydroxybutyryl-CoA dehydrogenase), or classified under EC 1.1.1.36, such as the gene product of *phaB* (see, for example, Liu & Chen, *Appl. Microbiol. Biotechnol.*, 2007, 76(5), 1153 – 1159; Shen *et al.*, *Appl. Environ. Microbiol.*, 2011, 77(9), 2905 – 2915; or Budde *et al.*, *J. Bacteriol.*, 2010, 192(20), 5319 – 5328).

In some embodiments, a 3-oxoacyl-CoA reductase may be classified under EC 1.1.1.100, such as the gene product of *fabG* (Budde *et al.*, 2010, *supra*; Nomura *et al.*, *Appl. Environ. Microbiol.*, 2005, 71(8), 4297 – 4306).

In some embodiments, an enoyl-CoA hydratase may be classified under EC 4.2.1.17, such as the gene product of *crt*, or classified under EC 4.2.1.119, such as the gene product of *phaJ* (Shen *et al.*, 2011, *supra*; or Fukui *et al.*, *J. Bacteriol.*, 1998, 180(3), 667 – 673).

In some embodiments, an enoyl-[*acp*] dehydratase such as a 3-hydroxyacyl-[*acp*] dehydratase may be classified under EC 4.2.1.59, such as the gene product of *fabZ*.

In some embodiments, a *trans*-2-enoyl-CoA reductase may be classified under EC 1.3.1.- (e.g., EC 1.3.1.38, EC 1.3.1.8, EC 1.3.1.44), such as the gene product of *ter* (Nishimaki *et al.*, *J. Biochem.*, 1984, 95, 1315 – 1321; Shen *et al.*, 2011, *supra*) or *tdter* (Bond-Watts *et al.*, *Biochemistry*, 2012, 51, 6827 – 6837).

In some embodiments, an enoyl-[*acp*] reductase may be classified under EC 1.3.1.10 such as the gene product of *fabI*.

In some embodiments, a *pimelyl-[acp] methyl ester esterase* may be classified, for example, under EC 3.1.1.85 such as the gene product of *bioH* from *E. coli*. See Genbank Accession No. AAC76437.1, SEQ ID NO:17.

Enzymes generating the terminal carboxyl groups in the biosynthesis of C7

5 Building Blocks

As depicted in FIG. 2, a terminal carboxyl group can be enzymatically formed using a *thioesterase*, an *aldehyde dehydrogenase*, a *7-oxoheptanoate dehydrogenase*, a *6-oxohexanoate dehydrogenase*, a *CoA-transferase* or a reversible *CoA-ligase*.

In some embodiments, the second terminal carboxyl group leading to the
10 synthesis of a C7 building block is enzymatically formed by a *thioesterase* classified, for example, under EC 3.1.2.-, such as the gene product of *YciA*, *tesB* (Genbank Accession No. AAA24665.1, SEQ ID NO: 1) or *Acot13* (see, for example, Cantu *et al.*, *Protein Science*, 2010, 19, 1281 – 1295; Zhuang *et al.*, *Biochemistry*, 2008, 47(9), 2789 – 2796; or Naggert *et al.*, *J. Biol. Chem.*, 1991, 266(17), 11044 – 11050).

In some embodiments, the second terminal carboxyl group leading to the
15 synthesis of a C7 building block is enzymatically formed by an *acyl-[acp] thioesterase* classified under EC 3.1.2.-, such as the gene product of *fatB* or *tesA*. The *acyl-[acp] thioesterases* encoded by Genbank Accession Nos. ABJ63754.1 and CCC78182.1 have C6 – C8 chain length specificity (Jing *et al.*, 2011, *BMC*
20 *Biochemistry*, 12(44)).

In some embodiments, the second terminal carboxyl group leading to the synthesis of pimelic acid is enzymatically formed by an *aldehyde dehydrogenase* classified, for example, under EC 1.2.1.3 (see, for example, Guerrillot & Vandecasteele, *Eur. J. Biochem.*, 1977, 81, 185 – 192).

In some embodiments, the second terminal carboxyl group leading to the
25 synthesis of pimelic acid is enzymatically formed by a *dehydrogenase* classified under EC 1.2.1.- such as a *6-oxohexanoate dehydrogenase* (e.g., the gene product of *ChnE* from *Acinetobacter sp.*) or a *7-oxoheptanoate dehydrogenase* (e.g., such as the gene product of *ThnG* from *Sphingomonas macroglutabida*). See, for example, Iwaki
30 *et al.*, *Appl. Environ. Microbiol.*, 1999, 65(11), 5158 – 5162; or López-Sánchez *et al.*, *Appl. Environ. Microbiol.*, 2010, 76(1), 110 - 118. For example, a *6-oxohexanoate*

dehydrogenase can be classified under EC 1.2.1.63. For example, a *7-oxoheptanoate dehydrogenase* can be classified under EC 1.2.1.-.

In some embodiments, the second terminal carboxyl group leading to the synthesis of pimelic acid is enzymatically formed by a *CoA-transferase* (e.g., a
 5 *glutaconate CoA-transferase*) classified, for example, under EC 2.8.3.12 such as from *Acidaminococcus fermentans*. See, for example, Buckel *et al.*, 1981, *Eur. J. Biochem.*, 118:315 – 321.

In some embodiments, the second terminal carboxyl group leading to the synthesis of pimelic acid is enzymatically formed by a reversible *CoA-ligase* (e.g., a
 10 *succinate-CoA ligase*) classified, for example, under EC 6.2.1.5 such as from *Thermococcus kodakaraensis*. See, for example, Shikata *et al.*, 2007, *J. Biol. Chem.*, 282(37):26963 – 26970.

Enzymes generating the terminal amine groups in the biosynthesis of C7

15 Building Blocks

As depicted in FIG. 3 and FIG. 4, terminal amine groups can be enzymatically formed using a *ω-transaminase* or a *deacetylase*.

In some embodiments, the first or second terminal amine group leading to the synthesis of 7-aminoheptanoic acid is enzymatically formed by a *ω-transaminase*
 20 classified, for example, under EC 2.6.1.18, EC 2.6.1.19, EC 2.6.1.29, EC 2.6.1.48, or EC 2.6.1.82 such as that obtained from *Chromobacterium violaceum* (Genbank Accession No. AAQ59697.1, SEQ ID NO: 8), *Pseudomonas aeruginosa* (Genbank Accession No. AAG08191.1, SEQ ID NO: 9), *Pseudomonas syringae* (Genbank Accession No. AAY39893.1, SEQ ID NO: 10), *Rhodobacter sphaeroides* (Genbank
 25 Accession No. ABA81135.1, SEQ ID NO: 11), *Escherichia coli* (Genbank Accession No. AAA57874.1, SEQ ID NO: 12), *Vibrio Fluvialis* (Genbank Accession No. AAA57874.1, SEQ ID NO: 13), *Streptomyces griseus*, or *Clostridium viride*. Some of these *ω-transaminases* are *diamine ω-transaminases* (e.g., SEQ ID NO:12). For example, the *ω-transaminases* classified, for example, under EC 2.6.1.29 or EC
 30 2.6.1.82 may be *diamine ω-transaminases*.

The reversible *ω-transaminase* from *Chromobacterium violaceum* (Genbank Accession No. AAQ59697.1, SEQ ID NO: 8) has demonstrated analogous activity

accepting 6-aminohexanoic acid as amino donor, thus forming the first terminal amine group in adipate semialdehyde (Kaulmann *et al.*, *Enzyme and Microbial Technology*, 2007, 41, 628 – 637).

5 The reversible *4-aminobutyrate:2-oxoglutarate transaminase* from *Streptomyces griseus* has demonstrated analogous activity for the conversion of 6-aminohexanoate to adipate semialdehyde (Yonaha *et al.*, *Eur. J. Biochem.*, 1985, 146:101 - 106).

10 The reversible *5-aminovalerate transaminase* from *Clostridium viride* has demonstrated analogous activity for the conversion of 6-aminohexanoate to adipate semialdehyde (Barker *et al.*, *J. Biol. Chem.*, 1987, 262(19), 8994 – 9003).

In some embodiments, a terminal amine group leading to the synthesis of 7-aminoheptanoate or heptamethylenediamine is enzymatically formed by a *diamine ω -transaminase*. For example, the second terminal amino group can be enzymatically formed by a *diamine ω -transaminase* classified, for example, under EC 2.6.1.29 or
15 classified, for example, under EC 2.6.1.82, such as the gene product of *YgjG* from *E. coli* (Genbank Accession No. AAA57874.1, SEQ ID NO: 12).

The gene product of *ygjG* accepts a broad range of diamine carbon chain length substrates, such as putrescine, cadaverine and spermidine (see, for example, Samsonova *et al.*, *BMC Microbiology*, 2003, 3:2).

20 The *diamine ω -transaminase* from *E. coli* strain B has demonstrated activity for 1,7 diaminohexane (Kim, *The Journal of Chemistry*, 1964, 239(3), 783 – 786).

In some embodiments, the second terminal amine group leading to the synthesis of heptamethylenediamine is enzymatically formed by a *deacetylase* such as *acetylputrescine deacetylase* classified, for example, under EC 3.5.1.62. The
25 *acetylputrescine deacetylase* from *Micrococcus luteus* K-11 accepts a broad range of carbon chain length substrates, such as acetylputrescine, acetylcadaverine and N⁸-acetylspermidine (see, for example, Suzuki *et al.*, 1986, *BBA – General Subjects*, 882(1):140 – 142).

Enzymes generating the terminal hydroxyl groups in the biosynthesis of C7

Building Blocks

As depicted in FIG. 5 and FIG. 6, a terminal hydroxyl group can be enzymatically formed using an *alcohol dehydrogenase*.

5 In some embodiments, a terminal hydroxyl group leading to the synthesis of 1,7 heptanediol is enzymatically formed by an *alcohol dehydrogenase* classified, for example, under EC 1.1.1.- (e.g., 1, 2, 21, or 184) such as the gene product of *YMR318C* (classified, for example, under EC 1.1.1.2, see Genbank Accession No. CAA90836.1) (Larroy *et al.*, 2002, *Biochem J.*, 361(Pt 1), 163 – 172), the gene
10 product of the gene product of *YghD*, the gene product of *cpnD* (Iwaki *et al.*, 2002, *Appl. Environ. Microbiol.*, 68(11):5671 – 5684), the gene product of *gabD* (Lütke-Eversloh & Steinbüchel, 1999, *FEMS Microbiology Letters*, 181(1):63 – 71), or a 6-*hydroxyhexanoate dehydrogenase* classified, for example, under EC 1.1.1.258 such as the gene product of *ChnD* (Iwaki *et al.*, *Appl. Environ. Microbiol.*, 1999, *supra*).

15 Biochemical pathways

Pathways using NADPH-specific enzymes to pimeloyl-[acp] as central precursor leading to C7 Building Blocks

In some embodiments, pimeloyl-[acp] is synthesized from the central metabolite malonyl-[acp], by conversion of malonyl-[acp] to malonyl-[acp] methyl
20 ester by a *malonyl-CoA O-methyltransferase* classified, for example, under EC 2.1.1.197 such as the gene product of *bioC*; followed by conversion with malonyl-[acp] to 3-oxoglutyryl-[acp] methyl ester by a *β -ketoacyl-[acp] synthase* classified, for example, under EC 2.3.1.- (e.g., EC 2.3.1.41, EC 2.3.1.179 or EC 2.3.1.180) such as the gene product of *fabB*, *fabF* or *fabH*; followed by conversion to 3-hydroxy-
25 glutaryl-[acp] methyl ester by a *3-oxoacyl-[acp] reductase* classified, for example, under EC 1.1.1.100 such as the gene product of *fabG*; followed by conversion to 2,3-dehydroglutaryl-[acp] methyl ester by a *3-hydroxyacyl-[acp] dehydratase* classified, for example, under EC 4.2.1.59 such as the gene product of *fabZ*; followed by conversion to glutaryl-[acp] methyl ester by an *enoyl-[acp] reductase* classified, for
30 example, under EC 1.3.1.10 such as the gene product of *fabI*; followed by conversion to 3-oxopimeloyl-[acp] methyl ester by a *β -ketoacyl-[acp] synthase* classified, for

example, under EC 2.3.1. – (e.g., EC 2.3.1.41 or EC 2.3.1.179) such as the gene product of *fabB* or *fabF*; followed by conversion to 3-hydroxy-pimeloyl-[acp] methyl ester by a *3-oxoacyl-[acp] reductase* classified, for example, under EC 1.1.1.100 such as the gene product of *fabG*; followed by conversion to 2,3-dehydropimeloyl-[acp] methyl ester by a *3-hydroxyacyl-[acp] dehydratase* classified, for example, under EC 4.2.1.59 such as the gene product of *fabZ*; followed by conversion to pimeloyl-[acp] methyl ester by an *enoyl-[acp] reductase* classified, for example, under EC 1.3.1.10 such as the gene product of *fabI*; followed by conversion to pimeloyl-[acp] by a *pimeloyl-[acp] methyl ester esterase* classified, for example, under EC 3.1.1.85 such as the gene product of *bioH*. See FIG. 1A.

Pathways using NADPH-specific enzymes to pimeloyl-CoA as central precursor leading to C7 Building Blocks

In some embodiments, pimeloyl-CoA is synthesized from the central metabolite malonyl-CoA, by conversion of malonyl-CoA to malonyl-CoA methyl ester by a *malonyl-CoA O-methyltransferase* classified, for example, under EC 2.1.1.197 such as the gene product of *bioC*; followed by conversion with acetyl-CoA to 3-oxoglutaryl-CoA methyl ester by a *β -ketothiolase* classified, for example, under EC 2.3.1.16 such as the gene product of *bktB* or by conversion with malonoyl-CoA by a *β -ketoacyl-[acp] synthase* classified, for example, under EC 2.3.1.180 such as the gene product of *fabH*; followed by conversion to 3-hydroxy-glutaryl-CoA methyl ester by a *3-oxoacyl-[acp] reductase* classified, for example, under EC 1.1.1.100 such as the gene product of *fabG* or an *acetoacetyl-CoA reductase* classified, for example, under EC 1.1.1.36 such as the gene product of *phaB*; followed by conversion to 2,3-dehydroglutaryl-CoA methyl ester by an *enoyl-CoA hydratase* classified, for example, under EC 4.2.1.119 such as the gene product of *phaJ*; followed by conversion to glutaryl-CoA methyl ester by a *reductase* classified, for example, under EC 1.3.1.- such as an *enoyl-[acp] reductase* classified under EC 1.3.1.10 such as the gene product of *fabI* or a *trans-2-enoyl-CoA reductase* (classified under EC 1.3.1.38, EC 1.3.1.8, or EC 1.3.1.44) such as the gene product of *ter* or *tdter*; followed by conversion to 3-oxopimeloyl-CoA methyl ester by a *β -ketoacyl-[acp] synthase* classified, for example, under EC 2.3.1.- (e.g., EC 2.3.1.41 or EC 2.3.1.179) such as the gene product of *fabB* or *fabF*, or a *β -ketothiolase* classified, for example, under

EC 2.3.1.16 such as the gene product of *bktB*; followed by conversion to 3-hydroxy-pimeloyl-CoA methyl ester by a *3-oxoacyl-[acp] reductase* classified, for example, under EC 1.1.1.100 such as the gene product of *fabG* or an *acetoacetyl-CoA reductase* classified, for example, under EC 1.1.1.36 such as the gene product of *phaB*; followed
 5 by conversion to 2,3-dehydropimeloyl-CoA methyl ester by an *enoyl-CoA hydratase* classified, for example, under EC 4.2.1.119 such as the gene product of *phaJ*; followed by conversion to pimeloyl-CoA methyl ester by a *reductase* classified, for example, under EC 1.3.1.- such as an *enoyl-[acp] reductase* (EC 1.3.1.10) such as the gene product of *fabI* or a *trans-2-enoyl-CoA reductase* (EC 1.3.1.38, EC 1.3.1.8, or
 10 EC 1.3.1.44) such as the gene product of *ter* or *tdter*; followed by conversion to pimeloyl-CoA by a *pimelyl-[acp] methyl ester esterase* classified, for example, under EC 3.1.1.85 such as the gene product of *bioH*. See FIG. 1B.

Pathways using NADH-specific enzymes to pimeloyl-CoA as central precursor leading to C7 Building Blocks

15 In some embodiments, pimeloyl-CoA is synthesized from the central metabolite, malonyl-CoA, by conversion of malonyl-CoA to malonyl-CoA methyl ester by a *malonyl-CoA O-methyltransferase* classified, for example, under EC 2.1.1.197 such as the gene product of *bioC*; followed by conversion with acetyl-CoA to 3-oxoglutaryl-CoA methyl ester by a *β -ketothiolase* classified, for example, under
 20 EC 2.3.1.16 such as the gene product of *bktB* or by conversion with malonyl-CoA by a *β -ketoacyl-[acp] synthase* classified, for example, under EC 2.3.1.180 such as the gene product of *fabH*; followed by conversion to 3-hydroxy-glutaryl-CoA methyl ester by a *3-hydroxyacyl-CoA dehydrogenase* classified, for example, under EC 1.1.1.- (e.g., EC 1.1.1.35 or EC 1.1.1.157) such as the gene product of *fadB* or *hbd*;
 25 followed by conversion to 2,3-dehydroglutaryl-CoA methyl ester by an *enoyl-CoA hydratase* classified, for example, under EC 4.2.1.17 such as the gene product of *crt*; followed by conversion to glutaryl-CoA methyl ester by a *trans-2-enoyl-CoA reductase* classified, for example, under EC 1.3.1.44 such as the gene product of *ter* or *tdter*; followed by conversion to 3-oxopimeloyl-CoA methyl ester by a *β -ketoacyl-[acp] synthase* classified, for example, under EC 2.3.1.- (e.g., EC 2.3.1.41 or EC
 30 2.3.1.179) such as the gene product of *fabB* or *fabF* or a *β -ketothiolase* classified, for example, under EC 2.3.1.16 such as the gene product of *bktB*; followed by conversion

to 3-hydroxy-pimeloyl-CoA methyl ester by a *3-hydroxyacyl-CoA dehydrogenase* classified, for example, under EC 1.1.1.35 such as the gene product of *fadB* or under EC 1.1.1.157 such as the gene product of *hbd*; followed by conversion to 2,3-dehydropimeloyl-CoA methyl ester by an *enoyl-CoA hydratase* classified, for example, under EC 4.2.1.17 such as the gene product of *crt*; followed by conversion to pimeloyl-CoA methyl ester by a *trans-2-enoyl-CoA reductase* classified, for example, under EC 1.3.1.44 such as the gene product of *ter* or *tdter*; followed by conversion to pimeloyl-CoA by a *pimeloyl-[acp] methyl ester esterase* classified under EC 3.1.1.85 such as the gene product of *bioH*. See FIG. 1C.

10 Pathways using pimeloyl-CoA or pimeloyl-[acp] as central precursors to pimelate

In some embodiments, pimelic acid is synthesized from the central precursor, pimeloyl-CoA, by conversion of pimeloyl-CoA to pimelate semialdehyde by an *acetylating aldehyde dehydrogenase* classified, for example, under EC 1.2.1.10 such as the gene product of *PduB* or *PduP* (see, for example, Lan *et al.*, 2013, *Energy Environ. Sci.*, 6:2672 – 2681); followed by conversion to pimelic acid by a *7-oxoheptanoate dehydrogenase* classified, for example, under EC 1.2.1.- such as the gene product of *ThnG*, a *6-oxohexanoate dehydrogenase* classified, for example, under EC 1.2.1.- such as the gene product of *ChnE*, or an *aldehyde dehydrogenase* classified, for example, under C 1.2.1.3. See FIG. 2.

In some embodiments, pimelic acid is synthesized from the central precursor, pimeloyl-CoA, by conversion of pimeloyl-CoA to pimelate by a *thioesterase* classified, for example, under EC 3.1.2.- such as the gene products of *YciA*, *tesB* (Genbank Accession No. AAA24665.1, SEQ ID NO: 1) or *Acot13*. See FIG. 2.

25 In some embodiments, pimelic acid is synthesized from the central precursor, pimeloyl-[acp], by conversion of pimeloyl-[acp] to pimelate by a *thioesterase* classified, for example, under EC 3.1.2.- such as the gene products encoded by Genbank Accession No. ABJ63754.1, Genbank Accession No. CCC78182.1, *tesA* or *fatB*. See FIG. 2.

30 In some embodiments, pimelate is synthesized from the central precursor, pimeloyl-CoA, by conversion of pimeloyl-CoA to pimelate by a *CoA-transferase*

such as a *glutaconate CoA-transferase* classified, for example, under EC 2.8.3.12. See FIG. 2.

In some embodiments, pimelate is synthesized from the central precursor, pimeloyl-CoA, by conversion of pimeloyl-CoA to pimelate by a reversible *CoA-ligase* such as a reversible *succinate-CoA ligase* classified, for example, under EC 6.2.1.5. See FIG. 2.

In some embodiments, pimelate is synthesized from the central precursor, pimelate semialdehyde, by conversion of pimelate semialdehyde to pimelate by a *6-oxohexanoate dehydrogenase* or a *7-oxoheptanoate dehydrogenase* (classified, for example, under EC 1.2.1.-) such as the gene product of *ThnG* or *ChnE*, or an *aldehyde dehydrogenase* classified, for example, under EC 1.2.1.3. See FIG. 2.

Pathways using pimeloyl-CoA or pimelate semialdehyde as central precursor to 7-aminoheptanoate

In some embodiments, 7-aminoheptanoate is synthesized from the central precursor, pimeloyl-CoA, by conversion of pimeloyl-CoA to pimelate semialdehyde by an *acetylating aldehyde dehydrogenase* classified, for example, EC 1.2.1.10, such as the gene product of *PduB* or *PduP*; followed by conversion of pimelate semialdehyde to 7-aminoheptanoate by a *ω-transaminase* classified, for example, under EC 2.6.1.18, EC 2.6.1.19, EC 2.6.1.29, EC 2.6.1.48 or EC 2.6.1.82. See FIG. 3.

In some embodiments, 7-aminoheptanoate is synthesized from the central precursor, pimelate semialdehyde, by conversion of pimelate semialdehyde to 7-aminoheptanoate by a *ω-transaminase* (e.g., EC 2.6.1.18, EC 2.6.1.19, or EC 2.6.1.48). See FIG. 3.

In some embodiments, 7-aminoheptanoate is synthesized from the central precursor, pimelate, by conversion of pimelate to pimelate semialdehyde by a *carboxylate reductase* classified, for example, under EC 1.2.99.6 such as the gene product of *car* in combination with a *phosphopantetheine transferase* enhancer (e.g., encoded by a *sfp* (Genbank Accession No. CAA44858.1, SEQ ID NO:14) gene from *Bacillus subtilis* or *npt* (Genbank Accession No. ABI83656.1, SEQ ID NO:15) gene from *Nocardia*) or the gene products of *GriC* and *GriD* from *Streptomyces griseus* (Suzuki *et al.*, *J. Antibiot.*, 2007, 60(6), 380 – 387); followed by conversion of

pimelate semialdehyde to 7-aminoheptanoate by a ω -transaminase (e.g., EC 2.6.1.18, EC 2.6.1.19, EC 2.6.1.48, EC 2.6.1.29, EC 2.6.1.82 such as SEQ ID NOs:8-13). The *carboxylate reductase* can be obtained, for example, from *Mycobacterium marinum* (Genbank Accession No. ACC40567.1, SEQ ID NO: 2), *Mycobacterium smegmatis* (Genbank Accession No. ABK71854.1, SEQ ID NO: 3), *Segniliparus rugosus* (Genbank Accession No. EFV11917.1, SEQ ID NO: 4), *Mycobacterium smegmatis* (Genbank Accession No. ABK75684.1, SEQ ID NO: 5), *Mycobacterium massiliense* (Genbank Accession No. EIV11143.1, SEQ ID NO: 6), or *Segniliparus rotundus* (Genbank Accession No. ADG98140.1, SEQ ID NO: 7). See FIG. 3.

10 **Pathway using 7-aminoheptanoate, 7-hydroxyheptanoate or pimelate semialdehyde as central precursor to heptamethylenediamine**

In some embodiments, heptamethylenediamine is synthesized from the central precursor, 7-aminoheptanoate, by conversion of 7-aminoheptanoate to 7-aminoheptanal by a *carboxylate reductase* classified, for example, under EC 1.2.99.6 such as the gene product of *car* (see above) in combination with a *phosphopantetheine transferase* enhancer (e.g., encoded by a *sfp* (Genbank Accession No. CAA44858.1, SEQ ID NO:14) gene from *Bacillus subtilis* or *npt* (Genbank Accession No. ABI83656.1, SEQ ID NO:15) gene from *Nocardia*) or the gene product of *GriC* & *GriD*; followed by conversion of 7-aminoheptanal to heptamethylenediamine by a ω -transaminase (e.g., classified, for example, under EC 2.6.1.18, EC 2.6.1.19, EC 2.6.1.29, EC 2.6.1.48, or EC 2.6.1.82 such as SEQ ID NOs:8-13, see above). See FIG. 4.

The *carboxylate reductase* encoded by the gene product of *car* and the *phosphopantetheine transferase* enhancer *npt* or *sfp* has broad substrate specificity, including terminal difunctional C4 and C5 carboxylic acids (Venkitasubramanian *et al.*, *Enzyme and Microbial Technology*, 2008, 42, 130 – 137).

In some embodiments, heptamethylenediamine is synthesized from the central precursor, 7-hydroxyheptanoate (which can be produced as described in FIG. 5), by conversion of 7-hydroxyheptanoate to 7-hydroxyheptanal by a *carboxylate reductase* classified, for example, under EC 1.2.99.6 such as the gene product of *car* (see above) in combination with a *phosphopantetheine transferase* enhancer (e.g., encoded by a *sfp* gene from *Bacillus subtilis* or *npt* gene from *Nocardia*) or the gene product of

GriC & *GriD* (Suzuki *et al.*, *J. Antibiot.*, 2007, 60(6), 380 – 387); followed by conversion of 7-aminoheptanal to 7-aminoheptanol by a ω -transaminase classified, for example, under EC 2.6.1.18, EC 2.6.1.19, EC 2.6.1.29, EC 2.6.1.48, or EC 2.6.1.82 such as SEQ ID NOs:8-13, see above; followed by conversion to 7-aminoheptanal by an *alcohol dehydrogenase* classified, for example, under EC 1.1.1.- (e.g., EC 1.1.1.1, EC 1.1.1.2, EC 1.1.1.21, or EC 1.1.1.184) such as the gene product of *YMR318C* (classified, for example, under EC 1.1.1.2, see Genbank Accession No. CAA90836.1) or *YqhD* (from *E. coli*, GenBank Accession No. AAA69178.1) (Liu *et al.*, *Microbiology*, 2009, 155, 2078 – 2085; Larroy *et al.*, 2002, *Biochem J.*, 361(Pt 1), 163 – 172; Jarboe, 2011, *Appl. Microbiol. Biotechnol.*, 89(2), 249 - 257) or the protein having GenBank Accession No. CAA81612.1 (from *Geobacillus stearothermophilus*); followed by conversion to heptamethylenediamine by a ω -transaminase classified, for example, under EC 2.6.1.18, EC 2.6.1.19, EC 2.6.1.29, EC 2.6.1.48, or EC 2.6.1.82 such as SEQ ID NOs:8-13, see above. See FIG. 4.

In some embodiments, heptamethylenediamine is synthesized from the central precursor, 7-aminoheptanoate, by conversion of 7-aminoheptanoate to N7-acetyl-7-aminoheptanoate by a *N-acetyltransferase* such as a *lysine N-acetyltransferase* classified, for example, under EC 2.3.1.32; followed by conversion to N7-acetyl-7-aminoheptanal by a *carboxylate reductase* classified, for example, under EC 1.2.99.6 such as the gene product of *car* (see above) in combination with a *phosphopantetheine transferase* enhancer (e.g., encoded by a *sfp* gene from *Bacillus subtilis* or *npt* gene from *Nocardia*) or the gene product of *GriC* & *GriD*; followed by conversion to N7-acetyl-1,7-diaminoheptane by a ω -transaminase classified, for example, under EC 2.6.1.18, EC 2.6.1.19, EC 2.6.1.29, EC 2.6.1.48, EC 2.6.1.46, or EC 2.6.1.82 such as SEQ ID NOs:8-13, see above; followed by conversion to heptamethylenediamine by an *acetylputrescine deacylase* classified, for example, under EC 3.5.1.62. See, FIG. 4.

In some embodiments, heptamethylenediamine is synthesized from the central precursor, pimelate semialdehyde, by conversion of pimelate semialdehyde to heptanedial by a *carboxylate reductase* classified, for example, under EC 1.2.99.6 such as the gene product of *car* (see above) in combination with a *phosphopantetheine transferase* enhancer (e.g., encoded by a *sfp* gene from *Bacillus*

subtilis or *npt* gene from *Nocardia*) or the gene product of *GriC* & *GriD*; followed by conversion to 7-aminoheptanal by a ω -transaminase classified, for example, under EC 2.6.1.18, EC 2.6.1.19, or EC 2.6.1.48; followed by conversion to heptamethylenediamine by a ω -transaminase classified, for example, under EC 2.6.1.18, EC 2.6.1.19, EC 2.6.1.29, EC 2.6.1.48, EC 2.6.1.46, or EC 2.6.1.82 such as SEQ ID NOs:8-13, see above. See FIG. 4.

Pathways using pimelate or pimelate semialdehyde as central precursor to 1,7-heptanediol

In some embodiments, 7-hydroxyheptanoate is synthesized from the central precursor, pimelate, by conversion of pimelate to pimelate semialdehyde by a *carboxylate reductase* classified, for example, under EC 1.2.99.6 such as the gene product of *car* (see above) in combination with a *phosphopantetheine transferase* enhancer (e.g., encoded by a *sfp* gene from *Bacillus subtilis* or *npt* gene from *Nocardia*) or the gene product of *GriC* & *GriD*; followed by conversion to 7-hydroxyheptanoate by a *dehydrogenase* classified, for example, under EC 1.1.1.- such as a *6-hydroxyhexanoate dehydrogenase* classified, for example, under EC 1.1.1.258 such as the gene from *ChnD* or a *5-hydroxypentanoate dehydrogenase* classified, for example, under EC 1.1.1.- such as the gene product of *CpnD* (see, for example, Iwaki et al., 2002, *Appl. Environ. Microbiol.*, 68(11):5671 – 5684) or a *4-hydroxybutyrate dehydrogenase* such as *gabD* (see, for example, Lütke-Eversloh & Steinbüchel, 1999, *FEMS Microbiology Letters*, 181(1):63 – 71). See FIG. 5. Pimelate semialdehyde also can be produced from pimeloyl-CoA using an *acetylating aldehyde dehydrogenase* as described above. See, also FIG. 5.

In some embodiments, 1,7 heptanediol is synthesized from the central precursor, 7-hydroxyheptanoate, by conversion of 7-hydroxyheptanoate to 7-hydroxyheptanal by a *carboxylate reductase* classified, for example, under EC 1.2.99.6 such as the gene product of *car* (see above) in combination with a *phosphopantetheine transferase* enhancer (e.g., encoded by a *sfp* gene from *Bacillus subtilis* or *npt* gene from *Nocardia*) or the gene product of *GriC* & *GriD*; followed by conversion of 7-hydroxyheptanal to 1,7 heptanediol by an *alcohol dehydrogenase* classified, for example, under EC 1.1.1.- such as EC 1.1.1.1, EC 1.1.1.2, EC 1.1.1.21,

or EC 1.1.1.184) such as the gene product of *YMR318C* or *YqhD* (see, e.g., Liu *et al.*, *Microbiology*, 2009, 155, 2078 – 2085; Larroy *et al.*, 2002, *Biochem J.*, 361(Pt 1), 163 – 172; or Jarboe, 2011, *Appl. Microbiol. Biotechnol.*, 89(2), 249 - 257) or the protein having GenBank Accession No. CAA81612.1 (from *Geobacillus*
5 *stearothermophilus*). See, FIG. 6.

Cultivation strategy

In some embodiments, the cultivation strategy entails achieving an aerobic, anaerobic or micro-aerobic cultivation condition.

10 In some embodiments, the cultivation strategy entails nutrient limitation such as nitrogen, phosphate or oxygen limitation.

In some embodiments, a cell retention strategy using, for example, ceramic hollow fiber membranes can be employed to achieve and maintain a high cell density during either fed-batch or continuous fermentation.

15 In some embodiments, the principal carbon source fed to the fermentation in the synthesis of one or more C7 building blocks can derive from biological or non-biological feedstocks.

In some embodiments, the biological feedstock can be, can include, or can derive from, monosaccharides, disaccharides, lignocellulose, hemicellulose, cellulose,
20 lignin, levulinic acid and formic acid, triglycerides, glycerol, fatty acids, agricultural waste, condensed distillers' solubles, or municipal waste.

The efficient catabolism of crude glycerol stemming from the production of biodiesel has been demonstrated in several microorganisms such as *Escherichia coli*, *Cupriavidus necator*, *Pseudomonas oleovorans*, *Pseudomonas putida* and *Yarrowia*
25 *lipolytica* (Lee *et al.*, *Appl. Biochem. Biotechnol.*, 2012, 166, 1801 – 1813; Yang *et al.*, *Biotechnology for Biofuels*, 2012, 5:13; Meijnen *et al.*, *Appl. Microbiol. Biotechnol.*, 2011, 90, 885 - 893).

The efficient catabolism of lignocellulosic-derived levulinic acid has been demonstrated in several organisms such as *Cupriavidus necator* and *Pseudomonas*
30 *putida* in the synthesis of 3-hydroxyvalerate via the precursor propanoyl-CoA (Jaremko and Yu, *Journal of Biotechnology*, 2011, 155, 2011, 293 – 298; Martin and Prather, *Journal of Biotechnology*, 2009, 139, 61 – 67).

The efficient catabolism of lignin-derived aromatic compounds such as benzoate analogues has been demonstrated in several microorganisms such as *Pseudomonas putida*, *Cupriavidus necator* (Bugg *et al.*, *Current Opinion in Biotechnology*, 2011, 22, 394 – 400; Pérez-Pantoja *et al.*, *FEMS Microbiol. Rev.*, 5 2008, 32, 736 – 794).

The efficient utilization of agricultural waste, such as olive mill waste water has been demonstrated in several microorganisms, including *Yarrowia lipolytica* (Papanikolaou *et al.*, *Bioresour. Technol.*, 2008, 99(7), 2419 - 2428).

The efficient utilization of fermentable sugars such as monosaccharides and 10 disaccharides derived from cellulosic, hemicellulosic, cane and beet molasses, cassava, corn and other agricultural sources has been demonstrated for several microorganism such as *Escherichia coli*, *Corynebacterium glutamicum* and *Lactobacillus delbrueckii* and *Lactococcus lactis* (see, e.g., Hermann *et al.*, *Journal of Biotechnology*, 2003, 104, 155 – 172; Wee *et al.*, *Food Technol. Biotechnol.*, 2006, 15 44(2), 163 – 172; Ohashi *et al.*, *Journal of Bioscience and Bioengineering*, 1999, 87(5), 647 - 654).

The efficient utilization of furfural, derived from a variety of agricultural lignocellulosic sources, has been demonstrated for *Cupriavidus necator* (Li *et al.*, *Biodegradation*, 2011, 22, 1215 – 1225).

20 In some embodiments, the non-biological feedstock can be or can derive from natural gas, syngas, CO₂/H₂, methanol, ethanol, benzoic acid, non-volatile residue (NVR), a caustic wash waste stream from cyclohexane oxidation processes, or terephthalic acid / isophthalic acid mixture waste streams.

The efficient catabolism of methanol has been demonstrated for the 25 methylotrophic yeast *Pichia pastoris*.

The efficient catabolism of ethanol has been demonstrated for *Clostridium kluyveri* (Seedorf *et al.*, *Proc. Natl. Acad. Sci. USA*, 2008, 105(6) 2128 - 2133).

The efficient catabolism of CO₂ and H₂, which may be derived from natural gas and other chemical and petrochemical sources, has been demonstrated for 30 *Cupriavidus necator* (Prybylski *et al.*, *Energy, Sustainability and Society*, 2012, 2:11).

The efficient catabolism of syngas has been demonstrated for numerous microorganisms, such as *Clostridium ljungdahlii* and *Clostridium autoethanogenum* (Köpke *et al.*, *Applied and Environmental Microbiology*, 2011, 77(15), 5467 – 5475).

5 The efficient catabolism of the non-volatile residue waste stream from cyclohexane processes has been demonstrated for numerous microorganisms, such as *Delftia acidovorans* and *Cupriavidus necator* (Ramsay *et al.*, *Applied and Environmental Microbiology*, 1986, 52(1), 152 – 156).

In some embodiments, the host microorganism is a prokaryote. For example, the prokaryote can be a bacterium from the genus *Escherichia* such as *Escherichia coli*; from the genus *Clostridia* such as *Clostridium ljungdahlii*, *Clostridium autoethanogenum* or *Clostridium kluyveri*; from the genus *Corynebacteria* such as *Corynebacterium glutamicum*; from the genus *Cupriavidus* such as *Cupriavidus necator* or *Cupriavidus metallidurans*; from the genus *Pseudomonas* such as *Pseudomonas fluorescens*, *Pseudomonas putida* or *Pseudomonas oleovorans*; from
10 the genus *Delftia* such as *Delftia acidovorans*; from the genus *Bacillus* such as *Bacillus subtilis*; from the genus *Lactobacillus* such as *Lactobacillus delbrueckii*; or from the genus *Lactococcus* such as *Lactococcus lactis*. Such prokaryotes also can be a source of genes to construct recombinant host cells described herein that are capable of producing one or more C7 building blocks.

20 In some embodiments, the host microorganism is a eukaryote. For example, the eukaryote can be a filamentous fungus, e.g., one from the genus *Aspergillus* such as *Aspergillus niger*. Alternatively, the eukaryote can be a yeast, e.g., one from the genus *Saccharomyces* such as *Saccharomyces cerevisiae*; from the genus *Pichia* such as *Pichia pastoris*; or from the genus *Yarrowia* such as *Yarrowia lipolytica*; from the
25 genus *Issatchenkia* such as *Issatchenkia orientalis*; from the genus *Debaryomyces* such as *Debaryomyces hansenii*; from the genus *Arxula* such as *Arxula adenoinivorans*; or from the genus *Kluyveromyces* such as *Kluyveromyces lactis*. Such eukaryotes also can be a source of genes to construct recombinant host cells described herein that are capable of producing one or more C7 building blocks.

30

Metabolic engineering

The present document provides methods involving less than all the steps described for all the above pathways. Such methods can involve, for example, one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or more of such steps.

5 Where less than all the steps are included in such a method, the first, and in some embodiments the only, step can be any one of the steps listed.

Furthermore, recombinant hosts described herein can include any combination of the above enzymes such that one or more of the steps, e.g., one, two, three, four, five, six, seven, eight, nine, ten, or more of such steps, can be performed within a recombinant host. This document provides host cells of any of the genera and species listed and genetically engineered to express one or more (e.g., two, three, four, five, six, seven, eight, nine, 10, 11, 12 or more) recombinant forms of any of the enzymes recited in the document. Thus, for example, the host cells can contain exogenous nucleic acids encoding enzymes catalyzing one or more of the steps of any of the pathways described herein.

15 In addition, this document recognizes that where enzymes have been described as accepting CoA-activated substrates, analogous enzyme activities associated with [acp]-bound substrates exist that are not necessarily in the same enzyme class.

Also, this document recognizes that where enzymes have been described accepting (R)-enantiomers of substrate, analogous enzyme activities associated with (S)-enantiomer substrates exist that are not necessarily in the same enzyme class.

This document also recognizes that where an enzyme is shown to accept a particular co-factor, such as NADPH, or a co-substrate, such as acetyl-CoA, many enzymes are promiscuous in terms of accepting a number of different co-factors or co-substrates in catalyzing a particular enzyme activity. Also, this document recognizes that where enzymes have high specificity for e.g., a particular co-factor such as NADH, an enzyme with similar or identical activity that has high specificity for the co-factor NADPH may be in a different enzyme class.

30 In some embodiments, the enzymes in the pathways outlined herein are the result of enzyme engineering via non-direct or rational enzyme design approaches with aims of improving activity, improving specificity, reducing feedback inhibition,

reducing repression, improving enzyme solubility, changing stereo-specificity, or changing co-factor specificity.

In some embodiments, the enzymes in the pathways outlined herein can be gene dosed (i.e., overexpressed by having a plurality of copies of the gene in the host
5 organism), into the resulting genetically modified organism via episomal or chromosomal integration approaches.

In some embodiments, genome-scale system biology techniques such as Flux Balance Analysis can be utilized to devise genome scale attenuation or knockout strategies for directing carbon flux to a C7 building block.

10 Attenuation strategies include, but are not limited to; the use of transposons, homologous recombination (double cross-over approach), mutagenesis, enzyme inhibitors and RNA interference (RNAi).

In some embodiments, fluxomic, metabolomic and transcriptomal data can be utilized to inform or support genome-scale system biology techniques, thereby
15 devising genome scale attenuation or knockout strategies in directing carbon flux to a C7 building block.

In some embodiments, the host microorganism's tolerance to high concentrations of a C7 building block can be improved through continuous cultivation in a selective environment.

20 In some embodiments, the host microorganism's endogenous biochemical network can be attenuated or augmented to (1) ensure the intracellular availability of acetyl-CoA and malonyl-CoA, (2) create an NADH or NADPH imbalance that may only be balanced via the formation of one or more C7 building blocks, (3) prevent degradation of central metabolites, central precursors leading to and including one or
25 more C7 building blocks and/or (4) ensure efficient efflux from the cell.

In some embodiments requiring intracellular availability of acetyl-CoA for C7 building block synthesis, endogenous enzymes catalyzing the hydrolysis acetyl-CoA such as short-chain length *thioesterases* can be attenuated in the host organism.

In some embodiments requiring condensation of acetyl-CoA and propanoyl-
30 CoA for C7 building block synthesis, one or more endogenous β -ketothiolases catalyzing the condensation of only acetyl-CoA to acetoacetyl-CoA such as the endogenous gene products of *AtoB* or *phaA* can be attenuated.

In some embodiments requiring the intracellular availability of acetyl-CoA for C7 building block synthesis, an endogenous *phosphotransacetylase* generating acetate such as *pta* can be attenuated (Shen *et al.*, *Appl. Environ. Microbiol.*, 2011, 77(9):2905–2915).

5 In some embodiments requiring the intracellular availability of acetyl-CoA for C7 building block synthesis, an endogenous gene in an acetate synthesis pathway encoding an *acetate kinase*, such as *ack*, can be attenuated.

In some embodiments requiring the intracellular availability of acetyl-CoA and NADH for C7 building block synthesis, an endogenous gene encoding an enzyme
10 that catalyzes the degradation of pyruvate to lactate such as a *lactate dehydrogenase* encoded by *ldhA* can be attenuated (Shen *et al.*, 2011, *supra*).

In some embodiments requiring the intracellular availability of acetyl-CoA and NADH for C7 building block synthesis, endogenous genes encoding enzymes, such as *menaquinol-fumarate oxidoreductase*, that catalyze the degradation of
15 phophoenolpyruvate to succinate such as *frdBC* can be attenuated (see, e.g., Shen *et al.*, 2011, *supra*).

In some embodiments requiring the intracellular availability of acetyl-CoA and NADH for C7 building block synthesis, an endogenous gene encoding an enzyme that catalyzes the degradation of acetyl-CoA to ethanol such as the *alcohol*
20 *dehydrogenase* encoded by *adhE* can be attenuated (Shen *et al.*, 2011, *supra*).

In some embodiments, where pathways require excess NADH co-factor for C7 building block synthesis, a recombinant *formate dehydrogenase* gene can be overexpressed in the host organism (Shen *et al.*, 2011, *supra*).

In some embodiments, where pathways require excess NADH or NADPH co-factor for C7 building block synthesis, a endogenous *transhydrogenase* dissipating the
25 co-factor imbalance can be attenuated.

In some embodiments, an endogenous gene encoding an enzyme that catalyzes the degradation of pyruvate to ethanol such as *pyruvate decarboxylase* can be attenuated.

30 In some embodiments, an endogenous gene encoding an enzyme that catalyzes the generation of isobutanol such as a *2-oxoacid decarboxylase* can be attenuated.

In some embodiments requiring the intracellular availability of acetyl-CoA for C7 building block synthesis, a recombinant *acetyl-CoA synthetase* such as the gene product of *acs* can be overexpressed in the microorganism (Satoh et al., *J. Bioscience and Bioengineering*, 2003, 95(4):335 – 341).

5 In some embodiments, carbon flux can be directed into the pentose phosphate cycle to increase the supply of NADPH by attenuating an endogenous *glucose-6-phosphate isomerase* (EC 5.3.1.9).

In some embodiments, carbon flux can be redirected into the pentose phosphate cycle to increase the supply of NADPH by overexpression a 6-
10 *phosphogluconate dehydrogenase* and/or a *transketolase* (Lee et al., 2003, *Biotechnology Progress*, 19(5), 1444 – 1449).

In some embodiments, where pathways require excess NADPH co-factor in the synthesis of a C7 building block, a gene such as *UdhA* encoding a *puridine nucleotide transhydrogenase* can be overexpressed in the host organisms (Brigham et
15 al., *Advanced Biofuels and Bioproducts*, 2012, Chapter 39, 1065 - 1090).

In some embodiments, where pathways require excess NADPH co-factor in the synthesis of a C7 Building Block, a recombinant *glyceraldehyde-3-phosphate-dehydrogenase* gene such as *GapN* can be overexpressed in the host organisms (Brigham et al., 2012, *supra*).

20 In some embodiments, where pathways require excess NADPH co-factor in the synthesis of a C7 building block, a recombinant *malic enzyme* gene such as *maeA* or *maeB* can be overexpressed in the host organisms (Brigham et al., 2012, *supra*).

In some embodiments, where pathways require excess NADPH co-factor in the synthesis of a C7 building block, a recombinant *glucose-6-phosphate dehydrogenase* gene such as *zwf* can be overexpressed in the host organisms (Lim et
25 al., *J. Bioscience and Bioengineering*, 2002, 93(6), 543 - 549).

In some embodiments, where pathways require excess NADPH co-factor in the synthesis of a C7 building block, a recombinant *fructose 1,6 diphosphatase* gene such as *fbp* can be overexpressed in the host organisms (Becker et al., *J. Biotechnol.*,
30 2007, 132:99 - 109).

In some embodiments, where pathways require excess NADPH co-factor in the synthesis of a C7 building block, endogenous *triose phosphate isomerase* (EC 5.3.1.1) can be attenuated.

5 In some embodiments, where pathways require excess NADPH co-factor in the synthesis of a C7 building block, a recombinant *glucose dehydrogenase* such as the gene product of *gdh* can be overexpressed in the host organism (Sato *et al.*, *J. Bioscience and Bioengineering*, 2003, 95(4):335 – 341).

10 In some embodiments, endogenous enzymes facilitating the conversion of NADPH to NADH can be attenuated, such as the NADH generation cycle that may be generated via inter-conversion of *glutamate dehydrogenases* classified under EC 1.4.1.2 (NADH-specific) and EC 1.4.1.4 (NADPH-specific). For example, avoiding dissipation of an NADH imbalance towards C7 building blocks, a NADPH-specific *glutamate dehydrogenase* can be attenuated.

15 In some embodiments, an endogenous *glutamate dehydrogenase* (EC 1.4.1.3) that utilizes both NADH and NADPH as co-factors can be attenuated.

In some embodiments, a membrane-bound *enoyl-CoA reductases* can be solubilized via expression as a fusion protein to a small soluble protein such as a *maltose binding protein* (Gloerich *et al.*, *FEBS Letters*, 2006, 580, 2092 – 2096).

20 In some embodiments using hosts that naturally accumulate polyhydroxyalkanoates, the endogenous *polymer synthase* enzymes can be attenuated in the host strain.

In some embodiments, a *L-alanine dehydrogenase* can be overexpressed in the host to regenerate L-alanine from pyruvate as an amino donor for ω -*transaminase* reactions.

25 In some embodiments, a *L-glutamate dehydrogenase* specific for the co-factor used to achieve co-factor imbalance can be overexpressed in the host to regenerate L-glutamate from 2-oxoglutarate as an amino donor for ω -*transaminase* reactions. For example, promoting dissipation of the NADH imbalance towards C7 building blocks, a NADH-specific *glutamate dehydrogenase* can be overexpressed.

30 In some embodiments, enzymes such as *pimeloyl-CoA dehydrogenase* classified under, EC 1.3.1.62; an *acyl-CoA dehydrogenase* classified, for example, under EC 1.3.8.7 or EC 1.3.8.1; and/or a *glutaryl-CoA dehydrogenase* classified, for

example, under EC 1.3.8.6 that degrade central metabolites and central precursors leading to and including C7 building blocks can be attenuated.

In some embodiments, endogenous enzymes activating C7 building blocks via Coenzyme A esterification such as *CoA-ligases* (e.g., a *pimeloyl-CoA synthetase*)
5 classified under, for example, EC 6.2.1.14 can be attenuated.

In some embodiments, a *methanol dehydrogenase* and a *formaldehyde dehydrogenase* can be overexpressed in the host to allow methanol catabolism via formate.

In some embodiments, an *S-adenosylmethionine synthetase* can be
10 overexpressed in the host to generate S-Adenosyl-L-methionine as a co-factor for *malonyl-[acp] O-methyltransferase*.

In some embodiments, the efflux of a C7 building block across the cell membrane to the extracellular media can be enhanced or amplified by genetically engineering structural modifications to the cell membrane or increasing any
15 associated transporter activity for a C7 building block.

The efflux of heptamethylenediamine can be enhanced or amplified by overexpressing broad substrate range multidrug transporters such as *Blt* from *Bacillus subtilis* (Woolridge *et al.*, 1997, *J. Biol. Chem.*, 272(14):8864 – 8866); *AcrB* and *AcrD* from *Escherichia coli* (Elkins & Nikaido, 2002, *J. Bacteriol.*, 184(23), 6490 –
20 6499), *NorA* from *Staphylococcus aureus* (Ng *et al.*, 1994, *Antimicrob Agents Chemother*, 38(6), 1345 – 1355), or *Bmr* from *Bacillus subtilis* (Neyfakh, 1992, *Antimicrob Agents Chemother*, 36(2), 484 – 485).

The efflux of 7-aminoheptanoate and heptamethylenediamine can be enhanced or amplified by overexpressing the solute transporters such as the *lysE* transporter
25 from *Corynebacterium glutamicum* (Bellmann *et al.*, 2001, *Microbiology*, 147, 1765 – 1774).

The efflux of pimelic acid can be enhanced or amplified by overexpressing a dicarboxylate transporter such as the *SucE* transporter from *Corynebacterium glutamicum* (Huhn *et al.*, *Appl. Microbiol. & Biotech.*, 89(2), 327 – 335).

30

Producing C7 Building Blocks Using a Recombinant Host

Typically, one or more C7 building blocks can be produced by providing a

host microorganism and culturing the provided microorganism with a culture medium containing a suitable carbon source as described above. In general, the culture media and/or culture conditions can be such that the microorganisms grow to an adequate density and produce a C7 building block efficiently. For large-scale production processes, any method can be used such as those described elsewhere (Manual of Industrial Microbiology and Biotechnology, 2nd Edition, Editors: A. L. Demain and J. E. Davies, ASM Press; and Principles of Fermentation Technology, P. F. Stanbury and A. Whitaker, Pergamon). Briefly, a large tank (e.g., a 100 gallon, 200 gallon, 500 gallon, or more tank) containing an appropriate culture medium is inoculated with a particular microorganism. After inoculation, the microorganism is incubated to allow biomass to be produced. Once a desired biomass is reached, the broth containing the microorganisms can be transferred to a second tank. This second tank can be any size. For example, the second tank can be larger, smaller, or the same size as the first tank. Typically, the second tank is larger than the first such that additional culture medium can be added to the broth from the first tank. In addition, the culture medium within this second tank can be the same as, or different from, that used in the first tank.

Once transferred, the microorganisms can be incubated to allow for the production of a C7 building block. Once produced, any method can be used to isolate C7 building blocks. For example, C7 building blocks can be recovered selectively from the fermentation broth via adsorption processes. In the case of pimelic acid and 7-aminoheptanoic acid, the resulting eluate can be further concentrated via evaporation, crystallized via evaporative and/or cooling crystallization, and the crystals recovered via centrifugation. In the case of heptamethylenediamine and 1,7-heptanediol, distillation may be employed to achieve the desired product purity.

The invention is further described in the following example, which does not limit the scope of the invention described in the claims.

EXAMPLES

EXAMPLE 1

Enzyme activity of *thioesterases* using pimeloyl-CoA as a substrate and forming pimelic acid

5 A sequence encoding an N-terminal His tag was added to the *tesB* gene from *Escherichia coli* that encodes a *thioesterase* (SEQ ID NO 1, see FIG. 7), such that an N-terminal HIS tagged *thioesterase* could be produced. The modified *tesB* gene was cloned into a pET15b expression vector under control of the T7 promoter. The expression vector was transformed into a BL21[DE3] *E. coli* host. The resulting
10 recombinant *E. coli* strain was cultivated at 37°C in a 500mL shake flask culture containing 50 mL Luria Broth (LB) media and antibiotic selection pressure, with shaking at 230 rpm. The culture was induced overnight at 17°C using 0.5 mM IPTG.

 The pellet from the induced shake flask culture was harvested via centrifugation. The pellet was resuspended and lysed in Y-per™ solution
15 (ThermoScientific, Rockford, IL). The cell debris was separated from the supernatant via centrifugation. The *thioesterase* was purified from the supernatant using Ni-affinity chromatography and the eluate was buffer exchanged and concentrated via ultrafiltration.

 The enzyme activity assay was performed in triplicate in a buffer composed of
20 50 mM phosphate buffer (pH = 7.4), 0.1 mM Ellman's reagent, and 667 μM of pimeloyl-CoA (as substrate). The enzyme activity assay reaction was initiated by adding 0.8 μM of the *tesB* gene product to the assay buffer containing the pimeloyl-CoA and incubating at 37 °C for 20 min. The release of Coenzyme A was monitored by absorbance at 412 nm. The absorbance associated with the substrate only control,
25 which contained boiled enzyme, was subtracted from the active enzyme assay absorbance and compared to the empty vector control. The gene product of *tesB* accepted pimeloyl-CoA as substrate as confirmed via relative spectrophotometry (see FIG. 8) and synthesized pimelate as a reaction product.

EXAMPLE 2

Enzyme activity of ω -transaminase using pimelate semialdehyde as substrate and forming 7-aminoheptanoate

A sequence encoding an N-terminal His-tag was added to the genes from
5 *Chromobacterium violaceum*, *Pseudomonas syringae*, *Rhodobacter sphaeroides*, and
Vibrio Fluvialis encoding the ω -transaminases of SEQ ID NOs: 8, 10, 11 and 13,
respectively (see FIG. 7) such that N-terminal HIS tagged ω -transaminases could be
produced. Each of the resulting modified genes was cloned into a pET21a expression
vector under control of the T7 promoter and each expression vector was transformed
10 into a BL21[DE3] *E. coli* host. The resulting recombinant *E. coli* strains were
cultivated at 37°C in a 250mL shake flask culture containing 50 mL LB media and
antibiotic selection pressure, with shaking at 230 rpm. Each culture was induced
overnight at 16 °C using 1 mM IPTG.

The pellet from each induced shake flask culture was harvested via
15 centrifugation. Each pellet was resuspended and lysed via sonication. The cell debris
was separated from the supernatant via centrifugation and the cell free extract was
used immediately in enzyme activity assays.

Enzyme activity assays in the reverse direction (i.e., 7-aminoheptanoate to
pimelate semialdehyde) were performed in a buffer composed of a final concentration
20 of 50 mM HEPES buffer (pH = 7.5), 10 mM 7-aminoheptanoate, 10 mM pyruvate
and 100 μ M pyridoxyl 5' phosphate. Each enzyme activity assay reaction was
initiated by adding cell free extract of the ω -transaminase gene product or the empty
vector control to the assay buffer containing the 7-aminoheptanoate and incubated at
25°C for 4 h, with shaking at 250 rpm. The formation of L-alanine from pyruvate was
25 quantified via RP-HPLC.

Each enzyme only control without 7-aminoheptanoate demonstrated low base
line conversion of pyruvate to L-alanine. See FIG. 14. The gene product of SEQ ID
NO 8, SEQ ID NO 10, SEQ ID NO 11 and SEQ ID NO 13 accepted 7-
aminoheptanoate as substrate as confirmed against the empty vector control. See FIG.
30 15.

Enzyme activity in the forward direction (i.e., pimelate semialdehyde to 7-
aminoheptanoate) was confirmed for the *transaminases* of SEQ ID NO 10, SEQ ID

NO 11 and SEQ ID NO 13. Enzyme activity assays were performed in a buffer composed of a final concentration of 50 mM HEPES buffer (pH = 7.5), 10 mM pimelate semialdehyde, 10 mM L-alanine and 100 μ M pyridoxyl 5' phosphate. Each enzyme activity assay reaction was initiated by adding a cell free extract of the *ω -transaminase* gene product or the empty vector control to the assay buffer containing the pimelate semialdehyde and incubated at 25°C for 4 h, with shaking at 250 rpm. The formation of pyruvate was quantified via RP-HPLC.

The gene product of SEQ ID NO 10, SEQ ID NO 11 and SEQ ID NO 13 accepted pimelate semialdehyde as substrate as confirmed against the empty vector control. See FIG. 16. The reversibility of the *ω -transaminase* activity was confirmed, demonstrating that the *ω -transaminases* of SEQ ID NO 8, SEQ ID NO 10, SEQ ID NO 11, and SEQ ID NO 13 accepted pimelate semialdehyde as substrate and synthesized 7-aminoheptanoate as a reaction product.

EXAMPLE 3

Enzyme activity of *carboxylate reductase* using pimelate as substrate and forming pimelate semialdehyde

A sequence encoding a HIS-tag was added to the genes from *Segniliparus rugosus* and *Segniliparus rotundus* that encode the *carboxylate reductases* of SEQ ID NOs: 4 and 7, respectively (see FIG. 7), such that N-terminal HIS tagged *carboxylate reductases* could be produced. Each of the modified genes was cloned into a pET Duet expression vector along with a *sfp* gene encoding a HIS-tagged *phosphopantetheine transferase* from *Bacillus subtilis*, both under the T7 promoter. Each expression vector was transformed into a BL21[DE3] *E. coli* host and the resulting recombinant *E. coli* strains were cultivated at 37°C in a 250mL shake flask culture containing 50 mL LB media and antibiotic selection pressure, with shaking at 230 rpm. Each culture was induced overnight at 37 °C using an auto-induction media.

The pellet from each induced shake flask culture was harvested via centrifugation. Each pellet was resuspended and lysed via sonication, and the cell debris was separated from the supernatant via centrifugation. The *carboxylate reductases* and *phosphopantetheine transferases* were purified from the supernatant

using Ni-affinity chromatography, diluted 10-fold into 50mM HEPES buffer (pH = 7.5), and concentrated via ultrafiltration.

Enzyme activity assays (i.e., from pimelate to pimelate semialdehyde) were performed in triplicate in a buffer composed of a final concentration of 50 mM HEPES buffer (pH = 7.5), 2 mM pimelate, 10 mM MgCl₂, 1 mM ATP and 1 mM NADPH. Each enzyme activity assay reaction was initiated by adding purified *carboxylate reductase* and *phosphopantetheine transferase* gene products or the empty vector control to the assay buffer containing the pimelate and then incubated at room temperature for 20 min. The consumption of NADPH was monitored by absorbance at 340 nm. Each enzyme only control without pimelate demonstrated low base line consumption of NADPH. See FIG. 9.

The gene products of SEQ ID NO 4 and SEQ ID NO 7, enhanced by the gene product of *sfp*, accepted pimelate as substrate, as confirmed against the empty vector control (see FIG. 10), and synthesized pimelate semialdehyde.

EXAMPLE 4

Enzyme activity of *carboxylate reductase* using 7-hydroxyheptanoate as substrate and forming 7-hydroxyheptanal

A sequence encoding a His-tag was added to the genes from *Mycobacterium marinum*, *Mycobacterium smegmatis*, *Segniliparus rugosus*, *Mycobacterium smegmatis*, *Mycobacterium massiliense*, and *Segniliparus rotundus* that encode the *carboxylate reductases* of SEQ ID NOs: 2 - 7- respectively (see FIG. 7) such that N-terminal HIS tagged *carboxylate reductases* could be produced. Each of the modified genes was cloned into a pET Duet expression vector alongside a *sfp* gene encoding a His-tagged *phosphopantetheine transferase* from *Bacillus subtilis*, both under control of the T7 promoter.

Each expression vector was transformed into a BL21[DE3] *E. coli* host and the resulting recombinant *E. coli* strains were cultivated at 37°C in a 250mL shake flask culture containing 50 mL LB media and antibiotic selection pressure, with shaking at 230 rpm. Each culture was induced overnight at 37 °C using an auto-induction media.

The pellet from each induced shake flask culture was harvested via centrifugation. Each pellet was resuspended and lysed via sonication. The cell debris was separated from the supernatant via centrifugation. The *carboxylate reductases* and *phosphopantetheine transferase* were purified from the supernatant using Ni-affinity chromatography, diluted 10-fold into 50 mM HEPES buffer (pH = 7.5) and concentrated via ultrafiltration.

Enzyme activity (i.e., 7-hydroxyheptanoate to 7-hydroxyheptanal) assays were performed in triplicate in a buffer composed of a final concentration of 50 mM HEPES buffer (pH = 7.5), 2 mM 7-hydroxyheptanal, 10 mM MgCl₂, 1 mM ATP, and 1 mM NADPH. Each enzyme activity assay reaction was initiated by adding purified *carboxylate reductase* and *phosphopantetheine transferase* or the empty vector control to the assay buffer containing the 7-hydroxyheptanoate and then incubated at room temperature for 20 min. The consumption of NADPH was monitored by absorbance at 340 nm. Each enzyme only control without 7-hydroxyheptanoate demonstrated low base line consumption of NADPH. See FIG. 9.

The gene products of SEQ ID NO 2 - 7, enhanced by the gene product of *sfp*, accepted 7-hydroxyheptanoate as substrate as confirmed against the empty vector control (see FIG. 11), and synthesized 7-hydroxyheptanal.

EXAMPLE 5

Enzyme activity of ω -transaminase for 7-aminoheptanol, forming 7-oxoheptanol

A nucleotide sequence encoding an N-terminal His-tag was added to the *Chromobacterium violaceum*, *Pseudomonas syringae* and *Rhodobacter sphaeroides* genes encoding the ω -transaminases of SEQ ID NOs: 8, 10 and 11, respectively (see FIG. 7) such that N-terminal HIS tagged ω -transaminases could be produced. The modified genes were cloned into a pET21a expression vector under the T7 promoter. Each expression vector was transformed into a BL21[DE3] *E. coli* host. Each resulting recombinant *E. coli* strain were cultivated at 37°C in a 250mL shake flask culture containing 50 mL LB media and antibiotic selection pressure, with shaking at 230 rpm. Each culture was induced overnight at 16°C using 1 mM IPTG.

The pellet from each induced shake flask culture was harvested via centrifugation. Each pellet was resuspended and lysed via sonication. The cell debris

was separated from the supernatant via centrifugation and the cell free extract was used immediately in enzyme activity assays.

Enzyme activity assays in the reverse direction (i.e., 7-aminoheptanol to 7-oxoheptanol) were performed in a buffer composed of a final concentration of 50 mM HEPES buffer (pH = 7.5), 10 mM 7-aminoheptanol, 10 mM pyruvate, and 100 μ M pyridoxyl 5' phosphate. Each enzyme activity assay reaction was initiated by adding cell free extract of the *ω -transaminase* gene product or the empty vector control to the assay buffer containing the 7-aminoheptanol and then incubated at 25 °C for 4 h, with shaking at 250 rpm. The formation of L-alanine was quantified via RP-HPLC.

Each enzyme only control without 7-aminoheptanol had low base line conversion of pyruvate to L-alanine. See FIG. 14.

The gene products of SEQ ID NO 8, 10 & 11 accepted 7-aminoheptanol as substrate as confirmed against the empty vector control (see FIG. 19) and synthesized 7-oxoheptanol as reaction product. Given the reversibility of the *ω -transaminase* activity (see Example 2), it can be concluded that the gene products of SEQ ID 8, 10 & 11 accept 7-oxoheptanol as substrate and form 7-aminoheptanol.

EXAMPLE 6

Enzyme activity of *ω -transaminase* using heptamethylenediamine as substrate and forming 7-aminoheptanal

A sequence encoding an N-terminal His-tag was added to the *Chromobacterium violaceum*, *Pseudomonas aeruginosa*, *Pseudomonas syringae*, *Rhodobacter sphaeroides*, *Escherichia coli*, and *Vibrio fluvialis* genes encoding the *ω -transaminases* of SEQ ID NOS: 8 - 13, respectively (see FIG. 7) such that N-terminal HIS tagged *ω -transaminases* could be produced. The modified genes were cloned into a pET21a expression vector under the T7 promoter. Each expression vector was transformed into a BL21[DE3] *E. coli* host. Each resulting recombinant *E. coli* strain were cultivated at 37°C in a 250mL shake flask culture containing 50 mL LB media and antibiotic selection pressure, with shaking at 230 rpm. Each culture was induced overnight at 16°C using 1 mM IPTG.

The pellet from each induced shake flask culture was harvested via centrifugation. Each pellet was resuspended and lysed via sonication. The cell debris

was separated from the supernatant via centrifugation and the cell free extract was used immediately in enzyme activity assays.

Enzyme activity assays in the reverse direction (i.e., heptamethylenediamine to 7-aminoheptanal) were performed in a buffer composed of a final concentration of 50 mM HEPES buffer (pH = 7.5), 10 mM heptamethylenediamine, 10 mM pyruvate, and 100 μ M pyridoxyl 5' phosphate. Each enzyme activity assay reaction was initiated by adding cell free extract of the ω -transaminase gene product or the empty vector control to the assay buffer containing the heptamethylenediamine and then incubated at 25 °C for 4 h, with shaking at 250 rpm. The formation of L-alanine was quantified via RP-HPLC.

Each enzyme only control without heptamethylenediamine had low base line conversion of pyruvate to L-alanine. See FIG. 14.

The gene products of SEQ ID NO 8 - 13 accepted heptamethylenediamine as substrate as confirmed against the empty vector control (see FIG. 17) and synthesized 7-aminoheptanal as reaction product. Given the reversibility of the ω -transaminase activity (see Example 2), it can be concluded that the gene products of SEQ ID 8 – 13 accept 7-aminoheptanal as substrate and form heptamethylenediamine.

EXAMPLE 7

Enzyme activity of *carboxylate reductase* for N7-acetyl-7-aminoheptanoate, forming N7-acetyl-7-aminoheptanal

The activity of each of the N-terminal His-tagged *carboxylate reductases* of SEQ ID NOs: 3, 6, and 7 (see Examples 4, and FIG. 7) for converting N7-acetyl-7-aminoheptanoate to N7-acetyl-7-aminoheptanal was assayed in triplicate in a buffer composed of a final concentration of 50 mM HEPES buffer (pH = 7.5), 2 mM N7-acetyl-7-aminoheptanoate, 10 mM MgCl₂, 1 mM ATP, and 1 mM NADPH. The assays were initiated by adding purified *carboxylate reductase* and *phosphopantetheine transferase* or the empty vector control to the assay buffer containing the N7-acetyl-7-aminoheptanoate then incubated at room temperature for 20 min. The consumption of NADPH was monitored by absorbance at 340 nm. Each enzyme only control without N7-acetyl-7-aminoheptanoate demonstrated low base line consumption of NADPH. See FIG. 9.

The gene products of SEQ ID NO 3, 6, and 7, enhanced by the gene product of *sfp*, accepted N7-acetyl-7-aminoheptanoate as substrate as confirmed against the empty vector control (see FIG. 12), and synthesized N7-acetyl-7-aminoheptanal.

EXAMPLE 8

Enzyme activity of ω -transaminase using N7-acetyl-1,7-diaminoheptane, and forming N7-acetyl-7-aminoheptanal

The activity of the N-terminal His-tagged ω -transaminases of SEQ ID NOs: 8 – 13 (see Example 6, and FIG. 7) for converting N7-acetyl-1,7-diaminoheptane to N7-acetyl-7-aminoheptanal was assayed using a buffer composed of a final concentration of 50 mM HEPES buffer (pH = 7.5), 10 mM N7-acetyl-1,7-diaminoheptane, 10 mM pyruvate and 100 μ M pyridoxyl 5' phosphate. Each enzyme activity assay reaction was initiated by adding a cell free extract of the ω -transaminase or the empty vector control to the assay buffer containing the N7-acetyl-1,7-diaminoheptane then incubated at 25 °C for 4 h, with shaking at 250 rpm. The formation of L-alanine was quantified via RP-HPLC.

Each enzyme only control without N7-acetyl-1,7-diaminoheptane demonstrated low base line conversion of pyruvate to L-alanine. See FIG. 14.

The gene product of SEQ ID NO 8 - 13 accepted N7-acetyl-1,7-diaminoheptane as substrate as confirmed against the empty vector control (see FIG. 18) and synthesized N7-acetyl-7-aminoheptanal as reaction product.

Given the reversibility of the ω -transaminase activity (see example 2), the gene products of SEQ ID 8 – 13 accept N7-acetyl-7-aminoheptanal as substrate forming N7-acetyl-1,7-diaminoheptane.

EXAMPLE 9

Enzyme activity of *carboxylate reductase* using pimelate semialdehyde as substrate and forming heptanedial

The N-terminal His-tagged *carboxylate reductase* of SEQ ID NO 7 (see Example 4 and FIG. 7) was assayed using pimelate semialdehyde as substrate. The enzyme activity assay was performed in triplicate in a buffer composed of a final concentration of 50 mM HEPES buffer (pH = 7.5), 2 mM pimelate semialdehyde, 10

mM MgCl₂, 1 mM ATP and 1 mM NADPH. The enzyme activity assay reaction was initiated by adding purified *carboxylate reductase* and *phosphopantetheine transferase* or the empty vector control to the assay buffer containing the pimelate semialdehyde and then incubated at room temperature for 20 min. The consumption of NADPH was monitored by absorbance at 340 nm. The enzyme only control without pimelate semialdehyde demonstrated low base line consumption of NADPH. See FIG. 9.

The gene product of SEQ ID NO 7, enhanced by the gene product of *sfp*, accepted pimelate semialdehyde as substrate as confirmed against the empty vector control (see FIG. 13) and synthesized heptanedial.

EXAMPLE 10

Enzyme activity of *pimeloyl-[acp] methyl ester methylesterase* using *pimeloyl-CoA methyl ester* as substrate and forming *pimeloyl-CoA*

A nucleotide sequence encoding a C-terminal His-tag was added to the gene from *Escherichia coli* encoding the *pimeloyl-[acp] methyl ester methylesterase* of SEQ ID NO: 17 (see FIG. 7) such that a C-terminal HIS tagged *pimeloyl-[acp] methyl ester methylesterase* could be produced. The resulting modified gene was cloned into a pET28b+ expression vector under control of the T7 promoter and the expression vector was transformed into a BL21[DE3] *E. coli* host. The resulting recombinant *E. coli* strain was cultivated at 37°C in a 500mL shake flask culture containing 100 mL LB media and antibiotic selection pressure, with shaking at 230 rpm. Each culture was induced overnight at 18 °C using 0.3 mM IPTG.

The pellet from each induced shake flask culture was harvested via centrifugation. Each pellet was resuspended and lysed via sonication. The cell debris was separated from the supernatant via centrifugation. The *pimeloyl-[acp] methyl ester methylesterase* was purified from the supernatant using Ni-affinity chromatography, buffer exchanged and concentrated into 20 mM HEPES buffer (pH = 7.5) via ultrafiltration and stored at 4 °C.

Enzyme activity assays converting *pimeloyl-CoA methyl ester* to *pimeloyl-CoA* were performed in triplicate in a buffer composed of a final concentration of 25 mM Tris·HCl buffer (pH = 7.0) and 5 [mM] *pimeloyl-CoA methyl ester*. The enzyme

activity assay reaction was initiated by adding *pimeloyl-[acp] methyl ester methylesterase* to a final concentration of 10 [μ M] to the assay buffer containing the pimeloyl-CoA methyl ester and incubated at 30°C for 1 h, with shaking at 250 rpm. The formation of pimeloyl-CoA was quantified via LC-MS.

5 The substrate only control without enzyme demonstrated no conversion of the substrate pimeloyl-CoA methyl ester to pimeloyl-CoA. See FIG. 20. The *pimeloyl-[acp]methyl ester methylesterase* of SEQ ID NO. 17 accepted pimeloyl-CoA methyl ester as substrate and synthesized pimeloyl-CoA as reaction product as confirmed via LC-MS. See FIG. 20.

10

OTHER EMBODIMENTS

 It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the
15 scope of the following claims.

1 **WHAT IS CLAIMED IS:**

- 2 1. A method for biosynthesizing a product selected from the group consisting of
3 pimelic acid, 7-aminoheptanoate, 7-hydroxyheptanoate, heptamethylenediamine and
4 1,7-heptanediol, said method comprising enzymatically synthesizing a seven carbon
5 chain aliphatic backbone from (i) acetyl-CoA and malonyl-CoA via two cycles of
6 methyl ester shielded carbon chain elongation or (ii) malonyl-[acp] via two cycles of
7 methyl-ester shielded carbon chain elongation, and enzymatically forming two
8 terminal functional groups selected from the group consisting of carboxyl, amine, and
9 hydroxyl groups in said backbone, thereby forming the product.
- 10 2. The method of claim 1, wherein the seven carbon chain aliphatic backbone is
11 pimeloyl-[acp] or pimeloyl-CoA.
- 12 3. The method of claim 1 or 2, wherein a *malonyl-[acp] O-methyltransferase*
13 converts malonyl-CoA to malonyl-CoA methyl ester or converts malonyl-[acp] to
14 malonyl-[acp] methyl ester.
- 15 4. The method of claim 3, wherein each of said two cycles of carbon chain
16 elongation comprises using (i) a β -ketoacyl-[acp] synthase or a β -ketothiolase, (ii) a
17 3-oxoacyl-[acp] reductase, an acetoacetyl-CoA reductase, a 3-hydroxyacyl-CoA
18 dehydrogenase or a 3-hydroxybutyryl-CoA dehydrogenase, (iii) an enoyl-CoA
19 hydratase or a 3-hydroxyacyl-[acp] hydratase, and (iv) an enoyl-[acp] reductase or
20 a trans-2-enoyl-CoA reductase to produce pimeloyl-[acp] methyl ester from malonyl-
21 [acp] methyl ester or produce pimeloyl-CoA methyl ester from malonyl-CoA methyl
22 ester.
- 23 5. The method of claim 4, wherein a *pimeloyl-[acp] methyl ester methylesterase*
24 removes the methyl group from pimeloyl-CoA methyl ester or pimeloyl-[acp] methyl
25 ester.
- 26 6. The method of any one of claims 3-5, wherein the *malonyl-[acp]O-*
27 *methyltransferase* has at least 70% sequence identity to the amino acid sequence set
28 forth in SEQ ID NO: 16.
- 29 7. The method of claim 5 or claim 6, wherein the *pimeloyl-[acp] methyl ester*
30 *methylesterase* has at least 70% sequence identity to the amino acid sequence set forth
31 in SEQ ID NO: 17.

- 32 8. The method of any one of claims 1-7, wherein said two terminal functional
33 groups are the same.
- 34 9. The method of any one of claims 1-7, wherein said two terminal functional
35 groups are different.
- 36 10. The method of claim 9, wherein said product comprises a terminal amine and
37 a terminal carboxyl group.
- 38 11. The method of claim 9, wherein said product comprises a terminal hydroxyl
39 group and a terminal carboxyl group.
- 40 12. The method of any one of claims 1-8, wherein said two terminal functional
41 groups are amine.
- 42 13. The method of any one of claims 1-8, wherein said two terminal functional
43 groups are hydroxyl groups.
- 44 14. The method of claim 11 or claim 13, wherein a *6-hydroxyhexanoate*
45 *dehydrogenase*, a *5-hydroxypentanoate dehydrogenase*, a *4-hydroxybutyrate*
46 *dehydratase*, or an *alcohol dehydrogenase* enzymatically forms a hydroxyl group.
- 47 15. The method of any one of claims 1-10, wherein a *thioesterase*, an *aldehyde*
48 *dehydrogenase*, a *7-oxoheptanoate dehydrogenase*, a *6-oxohexanoate dehydrogenase*,
49 a *glutaconate CoA-transferase*, or a *reversible succinyl-CoA ligase* enzymatically
50 forms a terminal carboxyl group.
- 51 16. The method of claim 15, wherein said *thioesterase* has at least 70% sequence
52 identity to the amino acid sequence set forth in SEQ ID NO: 1.
- 53 17. The method of claim 10 or 12, wherein a *ω-transaminase* or a *deacetylase*
54 enzymatically forms the amine group.
- 55 18. The method of claim 17, wherein said *ω-transaminase* has at least 70%
56 sequence identity to any one of the amino acid sequences set forth in SEQ ID NOs: 8
57 – 13.
- 58 19. The method of any one of claims 1 - 11, wherein a *carboxylate reductase*,
59 enhanced by a *phosphopantetheinyl transferase*, forms a terminal aldehyde group as
60 an intermediate in forming the product.
- 61 20. The method of claim 19, wherein said *carboxylate reductase* has at least 70%
62 sequence identity to any one of the amino acid sequences set forth in SEQ ID NO. 2 –
63 7.

- 64 21. The method of any of the preceding claims, wherein said method is performed
65 in a recombinant host by fermentation.
- 66 22. The method of claim 21, wherein said host is subjected to a cultivation
67 strategy under aerobic, anaerobic, micro-aerobic or mixed oxygen/denitrification
68 cultivation conditions.
- 69 23. The method of claim 21 or claim 22, wherein said host is cultured under
70 conditions of nutrient limitation.
- 71 24. The method according to any one of claims 21-23, wherein said host is
72 retained using a ceramic hollow fiber membrane to maintain a high cell density during
73 fermentation.
- 74 25. The method of any one of claims 21-24, wherein the principal carbon source
75 fed to the fermentation derives from biological or non-biological feedstocks.
- 76 26. The method of claim 25, wherein the biological feedstock is, or derives from,
77 monosaccharides, disaccharides, lignocellulose, hemicellulose, cellulose, lignin,
78 levulinic acid, formic acid, triglycerides, glycerol, fatty acids, agricultural waste,
79 condensed distillers' solubles, or municipal waste.
- 80 27. The method of claim 25, wherein the non-biological feedstock is, or derives
81 from, natural gas, syngas, CO₂/H₂, methanol, ethanol, benzoate, non-volatile residue
82 (NVR) caustic wash waste stream from cyclohexane oxidation processes, or
83 terephthalic acid / isophthalic acid mixture waste streams.
- 84 28. The method of any one of claims 21-27, wherein the host is a prokaryote.
- 85 29. The method of claim 28, wherein said prokaryote is from the genus
86 *Escherichia* such as *Escherichia coli*; from the genus *Clostridia* such as *Clostridium*
87 *ljungdahlii*, *Clostridium autoethanogenum* or *Clostridium kluyveri*; from the genus
88 *Corynebacteria* such as *Corynebacterium glutamicum*; from the genus *Cupriavidus*
89 such as *Cupriavidus necator* or *Cupriavidus metallidurans*; from the genus
90 *Pseudomonas* such as *Pseudomonas fluorescens*, *Pseudomonas putida* or
91 *Pseudomonas oleovorans*; from the genus *Delftia acidovorans*, from the genus
92 *Bacillus* such as *Bacillus subtilis*; from the genus *Lactobacillus* such as *Lactobacillus*
93 *delbrueckii*; from the genus *Lactococcus* such as *Lactococcus lactis* or from the genus
94 *Rhodococcus* such as *Rhodococcus equi*.
- 95 30. The method of any one of claims 21-27, wherein the host is a eukaryote.

- 96 31. The method of claim 30, wherein said eukaryote is from the genus *Aspergillus*
97 such as *Aspergillus niger*; from the genus *Saccharomyces* such as *Saccharomyces*
98 *cerevisiae*; from the genus *Pichia* such as *Pichia pastoris*; from the genus *Yarrowia*
99 such as *Yarrowia lipolytica*, from the genus *Issatchenkia* such as *Issatchenkia*
100 *orientalis*, from the genus *Debaryomyces* such as *Debaryomyces hansenii*, from the
101 genus *Arxula* such as *Arxula adeninivorans*, or from the genus *Kluyveromyces* such
102 as *Kluyveromyces lactis*.
- 103 32. The method of claim 21, wherein the host's tolerance to high concentrations of
104 a C7 building block is improved through continuous cultivation in a selective
105 environment.
- 106 33. The method of any one of claims 21 - 32, wherein said host comprises one or
107 more of the following attenuated enzymes: *polyhydroxyalkanoate synthase*, an *acetyl-*
108 *CoA thioesterase*, an *acetyl-CoA specific β -ketothiolase*, a *phosphotransacetylase*
109 *forming acetate*, an *acetate kinase*, a *lactate dehydrogenase*, a *menaquinol-fumarate*
110 *oxidoreductase*, a *2-oxoacid decarboxylase* producing isobutanol, an *alcohol*
111 *dehydrogenase* forming ethanol, a *triose phosphate isomerase*, a *pyruvate*
112 *decarboxylase*, a *glucose-6-phosphate isomerase*, a *transhydrogenase* dissipating the
113 NADH or NADPH imbalance, an *glutamate dehydrogenase* dissipating the NADH or
114 NADPH imbalance, a NADH/NADPH-utilizing *glutamate dehydrogenase*, a
115 *pimeloyl-CoA dehydrogenase*; an *acyl-CoA dehydrogenase* accepting C7 building
116 blocks and central precursors as substrates; a *glutaryl-CoA dehydrogenase*; or a
117 *pimeloyl-CoA synthetase*.
- 118 34. The method of any one of claims 21, 32 and 33, wherein said host
119 overexpresses one or more genes encoding: an *acetyl-CoA synthetase*, a 6-
120 *phosphogluconate dehydrogenase*; a *transketolase*; a *puridine nucleotide*
121 *transhydrogenase*; a *formate dehydrogenase*; a *glyceraldehyde-3P-dehydrogenase*; a
122 *malic enzyme*; a *glucose-6-phosphate dehydrogenase*; a *fructose 1,6 diphosphatase*; a
123 *L-alanine dehydrogenase*; a *L-glutamate dehydrogenase* specific to the NADH or
124 NADPH used to generate a co-factor imbalance; a *methanol dehydrogenase*, a
125 *formaldehyde dehydrogenase*, a *diamine transporter*; a *dicarboxylate transporter*; an
126 *S-adenosylmethionine synthetase*, and/or a *multidrug transporter*.

- 127 35. A recombinant host comprising at least one exogenous nucleic acid encoding
128 (i) a *malonyl-[acp] O-methyltransferase*, (ii) a *β -ketoacyl-[acp] synthase* or a *β -*
129 *ketothiolase*, (iii) a *3-oxoacyl-[acp] reductase*, *acetoacetyl-CoA reductase*, a *3-*
130 *hydroxyacyl-CoA dehydrogenase* or a *3-hydroxybutyryl-CoA dehydrogenase*, (iv) an
131 *enoyl-CoA hydratase* or *3-hydroxyacyl-[acp] dehydratase*, (v) an *enoyl-[acp]*
132 *reductase* or a *trans-2-enoyl-CoA reductase*, and (vi) a *pimeloyl-[acp] methyl ester*
133 *methylesterase*, said host producing pimeloyl-[acp] or pimeloyl-CoA.
- 134 36. The recombinant host of any one of claim 35, said host comprising at least one
135 exogenous nucleic acid encoding one or more of a *thioesterase*, an *aldehyde*
136 *dehydrogenase*, a *7-oxoheptanoate dehydrogenase*, a *6-oxohexanoate dehydrogenase*,
137 a *glutaconate CoA-transferase*, a reversible *succinyl-CoA ligase*, an *acetylating*
138 *aldehyde dehydrogenase*, or a *carboxylate reductase*, said host producing pimelic acid
139 or pimelate semialdehyde.
- 140 37. The recombinant host of claim 36, said host comprising at least one exogenous
141 nucleic acid encoding a *ω -transaminase*, said host producing 7-aminoheptanoate.
- 142 38. The recombinant host of claim 36, said host further comprising one or more of
143 a *4-hydroxybutyrate dehydrogenase*, a *5-hydroxypentanoate dehydrogenase* or a *6-*
144 *hydroxyhexanoate dehydrogenase*, said host producing 7-hydroxyheptanoic acid.
- 145 39. The recombinant host of any one of claims 35-38, said host further comprising
146 at least one exogenous nucleic acid encoding a *ω -transaminase*, a *deacetylase*, an *N-*
147 *acetyl transferase* or an *alcohol dehydrogenase*, said host producing
148 heptamethylenediamine.
- 149 40. The recombinant host of claim 38, said host further comprising at least one
150 exogenous nucleic acid encoding a (i) *carboxylate reductase* enhanced by a
151 *phosphopantetheinyl transferase* or (ii) an *alcohol dehydrogenase*, said host
152 producing 1,7-heptanediol.
153 .

FIGURE 1A

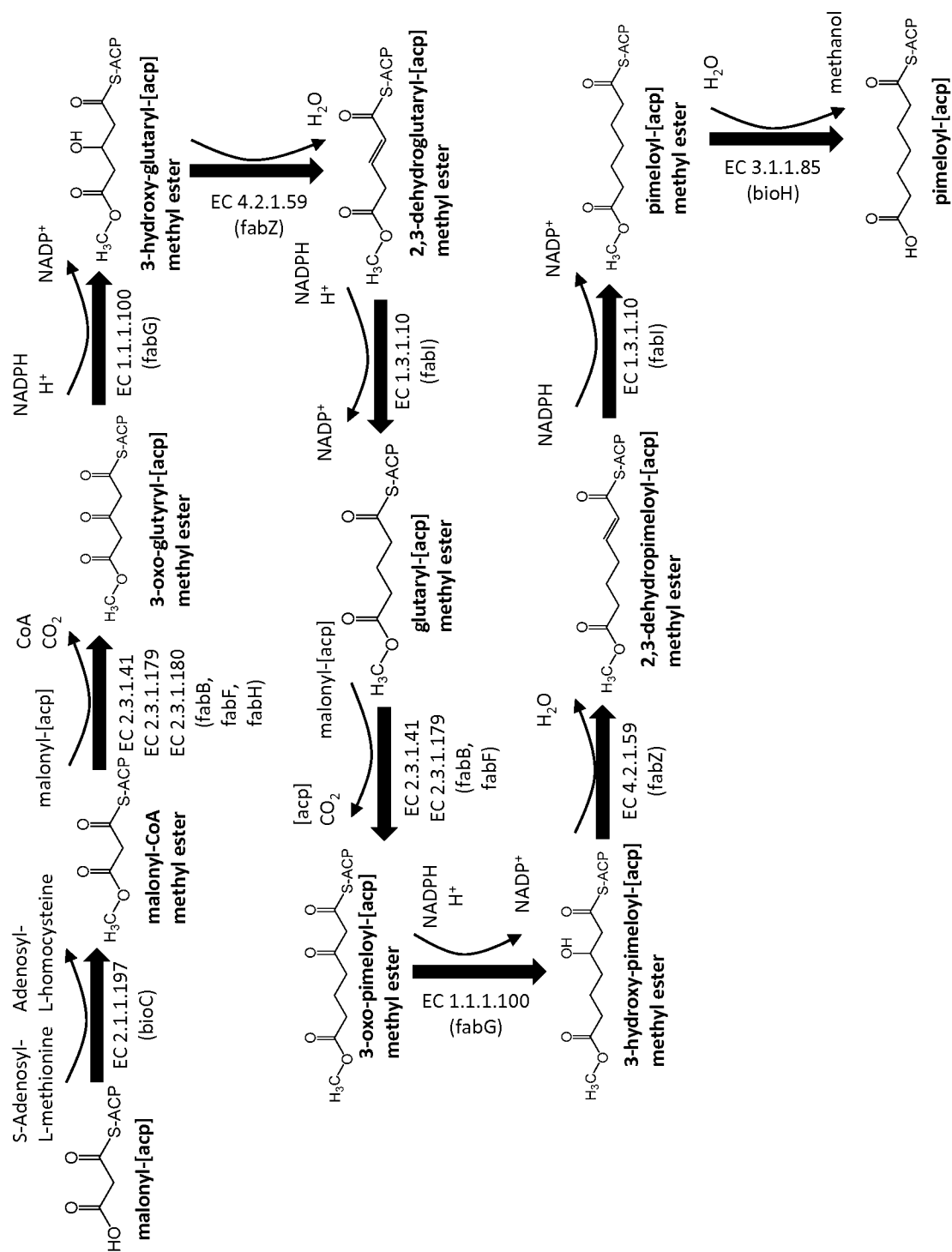


FIGURE 1B

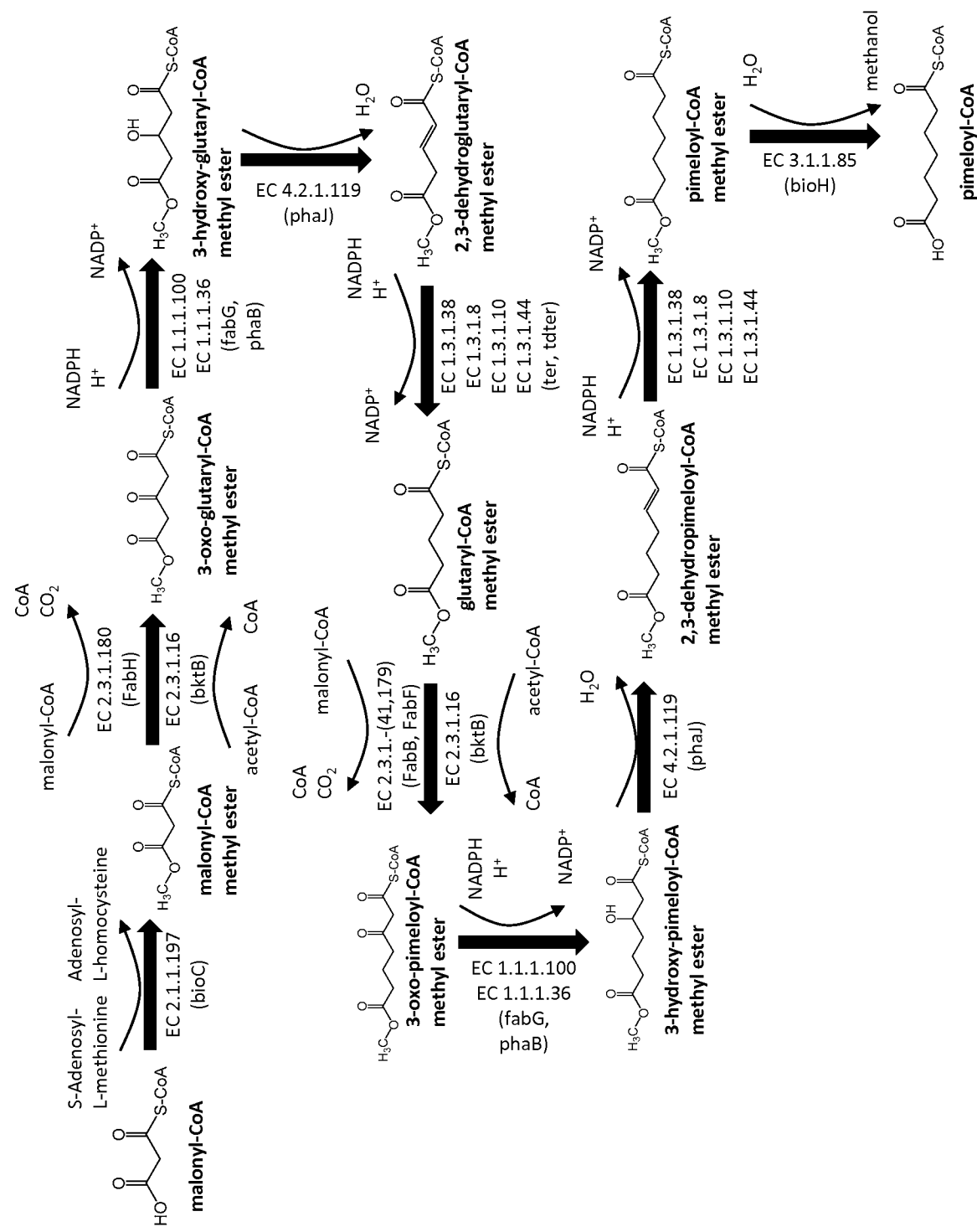
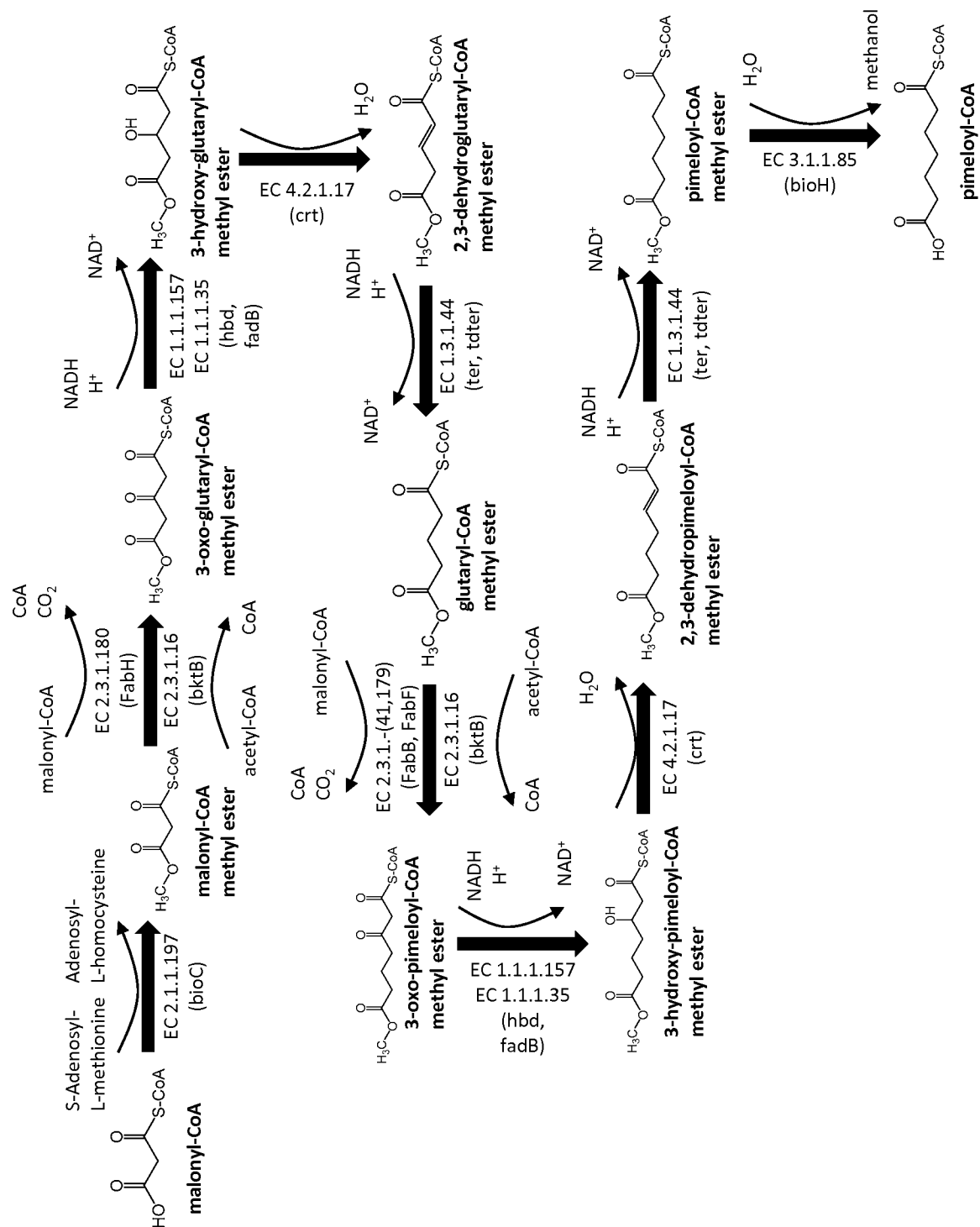


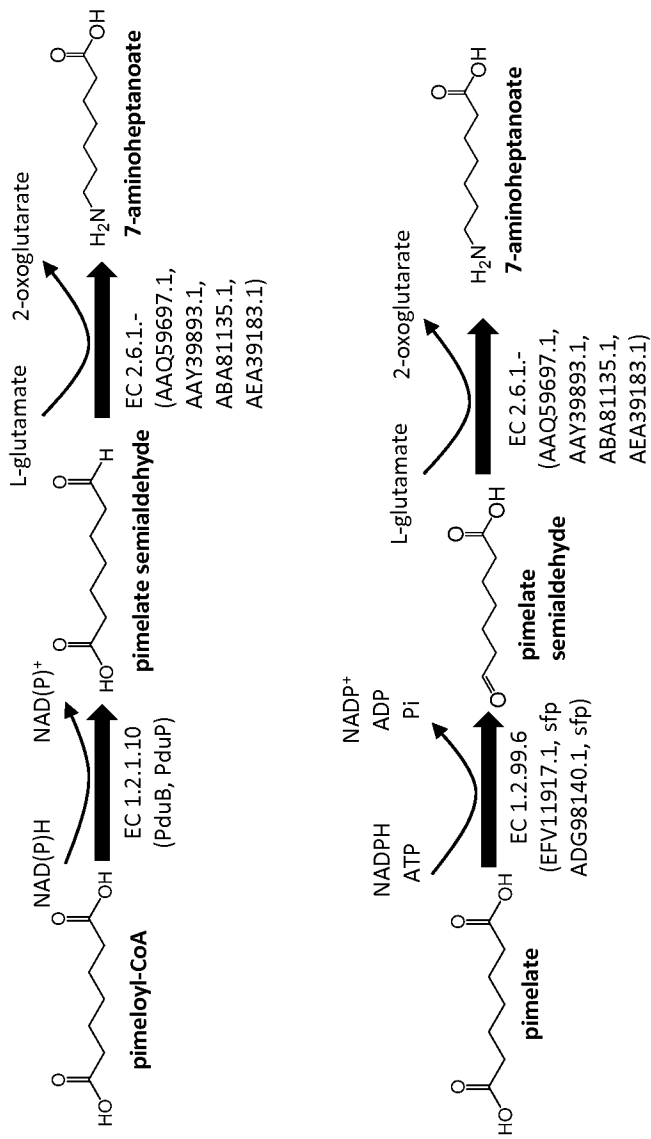
FIGURE 1C



The diagram illustrates the metabolic pathway of pimelate semialdehyde to pimelic acid, involving several enzymatic steps and cofactors:

- Step 1:** Pimeloyl-[acp] (a thioester) is converted to pimeloyl-CoA by the enzyme **EC 3.1.2.- (ABJ63754.1, CCC78182.1, fatB, tesA)**. This step is coupled with the hydrolysis of H₂O and the release of acyl carrier protein (acp).
- Step 2:** Pimeloyl-CoA is converted to pimelic acid by the enzyme **EC 3.1.2.- (YciA, tesB, Acot13)**. This step is coupled with the hydrolysis of H₂O and the release of CoA.
- Step 3:** Pimeloyl-CoA is converted to pimeloyl-CoA (a different thioester) by the enzyme **EC 6.2.1.5 (EC 2.8.3.12)**. This step is coupled with the hydrolysis of ATP to ADP and Pi, and the release of CoA.
- Step 4:** Pimeloyl-CoA is converted to pimelate semialdehyde by the enzyme **EC 1.2.1.10 (PduB)**. This step is coupled with the hydrolysis of NAD(P)H to NAD(P)⁺.
- Step 5:** Pimelate semialdehyde is converted to pimelic acid by the enzyme **EC 1.2.1.3 (ThnG, ChnE)**. This step is coupled with the hydrolysis of NADPH to NADP⁺ and H₂O.

FIGURE 3



NADPH
ADP
ATP
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanoate
 EC 1.2.99.6
*(car, sfp
GriC, GriD)*

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

NAD⁺
ADP
ATP
 Pi
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanoate
 EC 1.2.99.6
*(ACC40567.1, sfp
ABK71854.1, sfp
EFV11917.1, sfp
ABK75684.1, sfp
EIV11143.1, sfp
ADG98140.1, sfp)*

NADPH
ADP
ATP
 Pi
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanoate
 EC 2.3.1.32
 N7-acetyl-7-aminoheptanoate
 EC 1.2.99.6
 (ABK71854.1, *sfp*
EIV11143.1, *sfp*
ADG98140.1, *sfp*)

NAD⁺
ADP
ATP
 Pi
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanoate
 EC 1.2.99.6
 N7-acetyl-7-aminoheptanal
 (AAG08191.1, *N7-acetyl-1,7-diaminoheptane*
AAY39893.1,
ABA81135.1,
AAA57874.1,
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

NAD(P)⁺
NAD(P)H
H⁺
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 1.1.1.-(1, 2, 21, 184)
 (YMP318C,
YqhD,
CAA81612.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697.1,
AAG08191.1
AAY39893.1
ABA81135.1
AAA57874.1
AEA39183.1)

L-glutamate
(L-alanine)
 2-oxoglutarate
(pyruvate)
NC(CCC(CCC(CCC(=O)O)O)O)O
7-aminoheptanal
 EC 2.6.1.-
 (AAQ59697

FIGURE 5

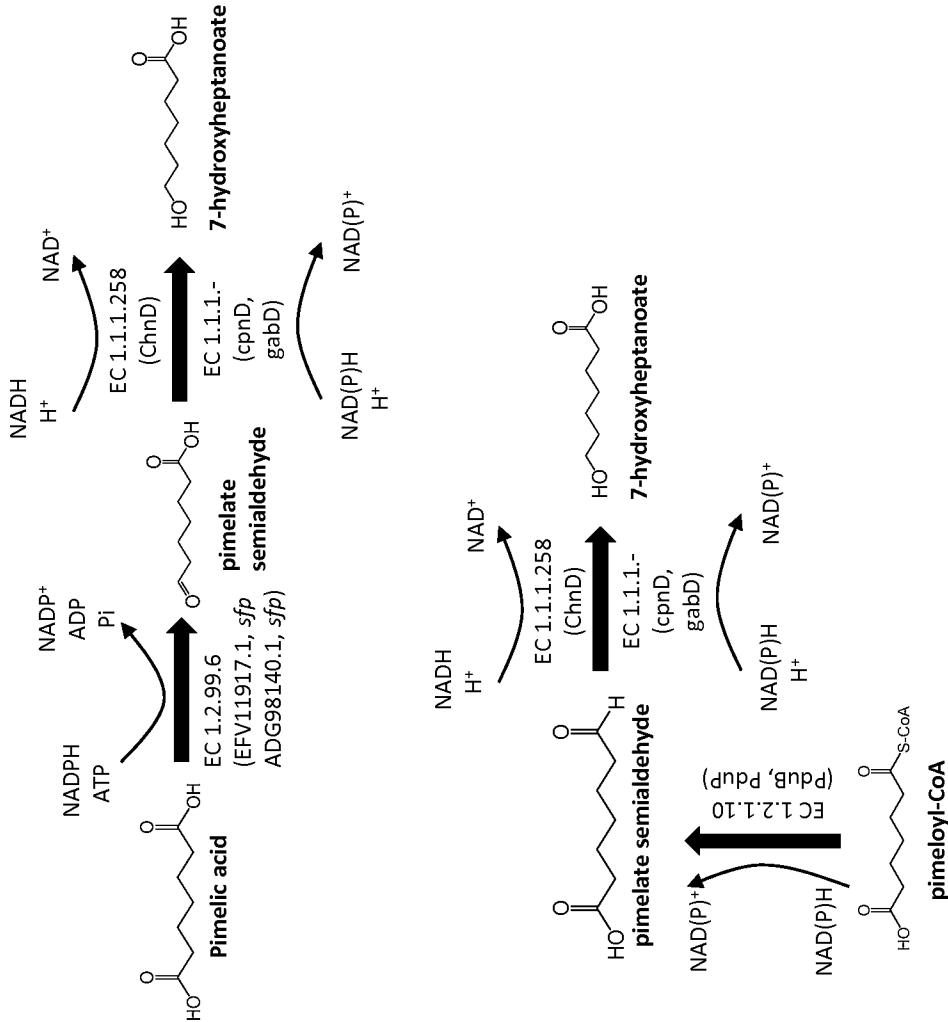
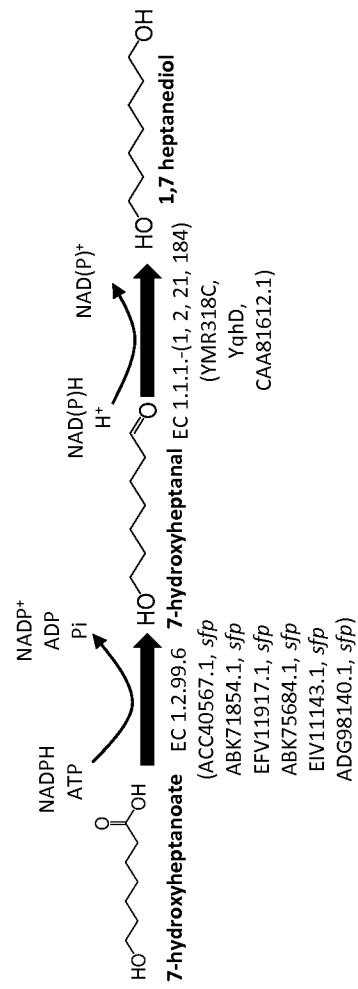


FIGURE 6



9/29

FIGURE 7

SEQ ID NO	Organism	GENBANK reference	Amino acid sequence
1	<i>Escherichia coli</i>	AAA24665.1	MSQALKNLLTLLNLEKIEEGLFRQSEDGLRQVFGGVVQOALYAAKETVPEERLVHSF HSYFLRPGDSKKPIYDVETLRDGNFSARRVAAIQNGKPIFYMTASFQAEAGFEHQKT MPSAPAPDGLPSETQAQSLAHLPPVLKDKFCIDRPLEVRPVEFHNPLKGHVAEPHRQV WIRANGSVDDLRVHQYLLGYASDLNPLVALQPHGIGLEPIGQIATIDHSMWFRPFN LNEWLLYSVESTSASSARGFVRGEFYTDGVLVASTVQEGVMRNHN
2	<i>Mycobacterium marinum</i>	ACC40567.1	MSPITRELERLRIQDLYANDPQFAAAKATATAIERPGLPLQIETVMTGYADRP LAQRSVEFVTDAGTHTTLRLPHFETISYGEWDRISALADVLSTEQTVKPGDRVCLLG FNSVDYATIDMTLARLGAVAVPLQTSAAITQLQPIVAETQPTMIAASVDALADATLALS GQTATRVLVFDHHRQVDAHRAAVESARERLAGSAVETLAEIARGDVPRGASAGSAPGT DVSDSLALIYTSGSTGAPKGAMYPRRNVAFTWRKRTWFEQGYEPSITLNFMPMSHVMG RQILYGTLCNGGTAYFAKSDLSLTFEDLALVRPTELTFRVPRVWDMVDFEQSEVDRRLV DGADRVALEAQVKAIRNDVLGGRYTSALTGSAPISDEMKAWVEELDMHLVEGYGSTE GMILIDGAIRPAVL DYKLVDPDLGYFLTDPRHPRGELLVKTDSLFPGYQRAEVTADV FDADGFYRTGDIMAEVGPQFVYLD RRNNVLKLSQGEFVTVSKLEAVFGDSPLV RQIYY GNSARAYLLAVIVPTQEALDAPVVEELKARLGDSLQEVAKAAGLSYEIPRDFIETTPW TLENGLLTGIRKLARPQLKKHYGELLEQIYTDLAHGQADELSLRQSGADAPVLVTVCR AAALLGGSASDVQPDHFDTLGGDSLALSFTNLLHEIFDIEVPVGVIVSPANDLQALAD YVEAARKPGSSRPTFASVHGASNGQVTEVHAGDLSLDKFIDAATLAEAPRLPAAANTQVRT VLLTGATGFLGRYLALEWLERMDLVGKLCILVRAKSDTEARARLDKTFDSGDPELLAHY RALAGDHLEVLGDKGEADGLDRQVTWQRLADTVDLIVDPAALVNHVLPYSQLFGPNALG TAE LLRLALTSKIKPYSYTSITGIVADQIPPSAFTEDADIRVISATRAVDDSYANGYSNSK WAGEVLLREAHDLGCLPVAVFRCDMILADTTWAGQLNVPDMFTRMILSLAATGIAPGSFY ELAADGARQRAHYDGLPVEFIAEAI STLGAQSQDGFHTYHVMNPYDDGIGLDEFVDWLNE SGCPIQRIADYGDWLQRFETALRALPDRQRHSSLLPLLLHNYRQPERPVRGSIAPTRFRA AVQEAKIGPDKDIPHVGAIIKVVSDLRLLGLL

10/29

SEQ ID NO	Organism	GENBANK reference	Amino acid sequence
3	<i>Mycobacterium smegmatis</i>	ABK71854.1	MTSDVHDATDGVTTETALDDEQSTRRIAEIYATDPEFAAAAPLPAVVDAAHKPGLRLAEIL QTLFTGYGDRPALGYRARELATDEGGRTVTRLLPRFDLTLYAQVWSRVQAAVAAALRHNF QPIYPGDAVATIGFASPDYLTLDLVCAYLGLVSVPLQHNAPVSRILAEVEPRILTVS AEYLDLAVESVRDVNSVSQLVVFDHHPVEDDHRDALARAREQLAGKGIAVTTLDIAIDEG AGLPAEPIYTADHQRLAMILYTSGSTGAPKGMYTEAMVARLWTMSFITGDPTVINVN FMPLNLGGRIPISTAVQNGGTSYFVPESDMSTLFEDLALVRPTLGLVPRVADMLYQHH LATVDRLVTQGADELTAEKQAGAELEQVLGGRVITGFVSTAPLAAEMRAFLDITLGAHI VDGYLTETGAVTRDGVIVRPPVIDYKLIDVPELGYFSTDKPYPRGELLVRSQTLTPGY KRPEVTASVFD RDGYHTGDVMAETAPDHLVYVDRNNVLKLAQGEFVAVANLEAVFSGA ALVRQIFVYGNSESRFLAVVPTPEALEQYDPAALKAALADSLQRTARDAELOSVEVPA DFIVETEPFSAANGLLSGVGKLLRPNLKDRYQRLQEQMYADIAATQANQLRELRRAAATQ PVIDTLTQAAATILGTGSEVASDAHFTDLGGDSLSALTLSNLSDFGFEVPVGTIVNPA TNLAQLAQHIEAQRTAGDRRPSFTTVHGADATEIRASELTLDKFIDAEITLRAAPGLPKVT TEPRTVLLSGANGWLGRFLTQWLERLAPVGGTLITIVGRDDAAARARLTQAYDTPDEL SRRFAELADRLRVVAGDIGNLGLTPEIWHRLAAEVDLVVHPAALVNHVLPYRQLFGP NVVGTAEVIKALATERIKPVTYLTSTVSAMGIPDFEEDGDIRTVSPVRPLDGGYANGYGN SKWAGEVLLREAHDLCLGPVATFRSDMILAHPRYRGQVNVPMFTRLLLSLITGVAPRS FYIGDGERPRAHYPGLTVDFAEAVTTLGAQQREGYVSYDVMNPHDDGISLDVFDWLIR AGHPIDRVDDYDDWVRRFETALTALPEKRRRAQTLPILLHAFRAQAPLRGAPEPTEVFHA AVRTAKVGP GDIPHLDEALIDKYIRDIREFGLI

SEQ ID NO	Organism	GENBANK reference	Amino acid sequence
4	<i>Segniliparus rugosus</i>	EFV11917.1	MGDGEERAKRFFQRIGELSATDQFAAAAPDPAVVEAVSDPSLSFTRVLDTLMRGYAERP ALAHRVGAGYETISYGELWARVGAIAAAWQADGLAPGDFVATVGTSPDYVAVDLAAARS GLVSVPLQAGASLAQLVGILEETEPKVLAAASASSLEGAVACALAAPSVQRLVVFDLRGPD ASESADERRGALADAEELQARAGRAWVETLADLAARGEALPEAPLFEPAEGEDPLALL IYTSGSTGAPKGAMYSQRLVSQWLWGRTPVVPMPNLSLHYMPLSHSYGRAVLAGALSAGG TAHFTANSDLSTLFEDIALARPTFLALVPRVCEMLFQESRQGDVAELRERVLGGRLLVA VCGSAPLSPEMRAFMEEVLGFPLLDGYGSTEALGVMRNGIQRPPVIDYKLVDPVPELGYR TTDKPYPRGELCIRSTSLISGYKRPETAEVFDAAQGYKTGDVMAEAPDHLVYVDRSK NVLKSQGEFVAVAKLEAAYGTSPYVKQIFYVGNRSERFLLAVVVPNAEVLGARDQEEAK PLIAASLQKIAKEAGLSYEVPRDFLIETEPFTTQNGLLSEVGKLLRPKLKARYGEALEA RYDEIAHGQADELRALRDGAGQRPVETVRAAVAISGSEGAEGPEANFADLGGDSLSA LSLANLLHDVFEVEVPVRIIIGPTASLAGIAKHIEAERAGASAPTAASVHGAGATRIRAS ELTLEKLPEDLLAAAKGLPAADQVTVLLTGANGWLGRFLALEQLERLARSGQDGGKLI CLVRGKDAARRRIEETLGTDPALAAARFAELAEGRLEVPGDVGEKFGLDLDDAAWDRLA EEVDVIVHPAALVNHVLPYHQLFGPNVVGTAIEIRLITAKRKPVTYLSVAVAAAGVEPS SFEEDGDIRAVVPERPLGDGYANGYNSKWAGEVLLREAHVGLPVAVFRSDMILAHTR YTGQINVDPQFTRLVLSLATGIAPKSFYQQGAAGERQRAHYDGIPVDFTAETTLGAE PSWFDGGAGFRSFDVFNPHHDGVGLDEFVDWLIAGHPISRIDDKKEWFARFETAVRGLP EAQRQHSLLPLLRAYSFPHPPVDGVSYYPTGKFQGA VKAAQVGSDDHDPVHLKALIVKYAD DLKALGILL

SEQ ID NO	Organism	GENBANK reference	Amino acid sequence
5	<i>Mycobacterium smegmatis</i>	ABK75684.1	MTIETREDRFNRRIDHLEFETDPQFAAARPDEISAASAAAPELRLPAAVKQILAGYADRPALGKRAVEFVTDEEGRTTAKLLPREDTITYRQLAGRIQAVTNAWNHNHPVNAGDRVAILGFTSVDYTTIDIALLELGAVSVPLQTSAPVAQLQPIVAETEPKVIASSVDFLADAVLVESGPAPSRLVFDYSHEVDQREAFEAAGKLAGTGVVWETITDALDRGRSLADAPLYVPDEADPLTLIYTSGSTGTPKGAMYPESKATMWQAGSKARWDETLGVMPSTILNFMPSHVMGRGILCSTLASGGTAYFAARSDLSLFLEDLALVRPTQLNFVPRIWDMLFQEYQSRLDNRRAE GSEDRAEAAVLEEVRTQLLGGRFVSALTGSAPISAEKMSWVEDLLDMHLEGYGSTEAGAVFIDGQIQRPPIVYKLVDPDLGYFATDRPYPRGELLVKSEQMIFPGYYKRPEITAEMFDEDGYRTGDIVAEELGPDHLEYLDRRNINVLKLSQGEFVTVSKLEAVFGDSPLVQRQIYVYGN SARYLLAVVPTEEALSRWDGDELKSRISDSLQDAARAAGLQSYEIPRDFLVETTPFTLENGLLTGIRKLARPKLAHYGERLEQLYTDLAEGQANELRELRNGADRPVETVSRAAV ALLGASVTDLRSDAHFTDLGGDSLALSFSNLLHEIFDVDPVGVIVSPATDLAGVAAVIEGELRGSKRPTYASVHGRDATEVRARDIALGKFIDAKTLSAAPGLPRSGTEIRTVLLTGA TGFLGRYLALWLERMDLVGKVICLVARSDDDEARLDTFTDGTATLLEHYRALAAD HLEVIAGDKGEADLGLDHDWTQRLADTVLIDPAALVNHVLPYSQMFGPNALGTAEILRIALTTTIKPYVYVSTIGVGGISPEAFVEDADIREISATRRVDDSYANGYGNKSWAGEVL LREAHDWCGLPVSVFRCDMILADTTYSGQLNLPDMFTRLMLSLVATGIAPGSFYELDADGNRQRAHYDGLPVEFIAEAISTIGSQVTDGFETHVMNPYDDGIGLDEYVDWLEAGYPVHRVDDYATWLSRFETALRALPERQRQASLLPLLHNYQQPSPPVCGAMAPTRFRAAVQDAKIGPDKDIPHVTADVIVKYISNLQMLGLL

SEQ ID NO	Organism	GENBANK reference	Amino acid sequence
6	<i>Mycobacterium massiliense</i>	E111143.1	MTNETNPQEEQLSRRESLQFRAAQPDPAAVEQVLRPGLHSEIAALMTGYAER PALGERARELVIDQDGRTRLRLPRFDITTYGELWSRTTSVAAAWHHDATHPVKAGDLVA TLGFTSIDYTVLDLAIMILGGVAVPLQTSAPASQWTTILAEAPNTLAVSIELJGAAMES VRATPSIKQVWFYDTPVEDDQREAFEAASQLAGTGIALETLDAVIARGAALPAAPLYA PSAGDDPLALLIYTSGSTGAPKGMHSENIWRRWWIREDVMAGTENLPMGLNFMPSHI MGRGTLTSLTSGTGFAASDMSTLFEDMELIRPTALALVPRVCDMVFRFQTEVDRR LASGDTASAEAAEAEVKADIRDNLFGGRVSAMVMSGAPLSEELGEFIESCFELNLTGYG STEAGMVFRRDGIQRPVVIDYKLVDPVPELGYFSTDKPHPRGELLKTDGMFLGYKRPV TASVFDADGFYMTGDIVAEALAHNIEIIDRRNNVLKLSQGEFVAVATLEAEYANSPVWHQ IYVYSSERSYLLAVVPTPEAAVAAKGAALKTTIADSLQDIAKEIQLQSYEVPRDFI IEPQFTQGNGLTGIAKLRPNLKAHYGPRLEQMYAEIAEQAAEIRALHGVDPDKPAL ETVLKAAQALLGVSSAEIADAHFTDLGGDSLSALSFSDLLRDIFAVEVPVGVVSAAND LGGVAKFVDEQRHSGGTRPTAETVHGAGHTEIRAADLTLDKFIDEATLHAAPSLPKAAGI PHTVLLTGSNGYLGHYLALEWLERLDKTDGKLIVIRGKNAEAYGRLEEAFDTGDTTELL AHFRSLADKHLEVLADIGDNPGLDADTWQRLADTVDIVHPAALVNHVLPYNQLFGPN VVGTAIEIKLAIITTKIKPVYTLSTVAVAAVDPPTTFDEESDIRLISAVRPIDDGYANGYG NAKWAGEVLLREAHDLGCLPVAVFRSDMILAHSRYTGQLNVPDQFTRLISLIATGIAPG SFYQAQTTGERPLAHYDGLPGDFTAEAITLGTQVPEGSEGFVYDCVNPHADGISLDNF VDWLEAGYPIARIDNYTEWFTFRDFAIRGLSEKQKQHSLLPLHAFEQPSAAENHGVVP AKRFQHAVQAAGIGPVGGDGTDDIPHLRRLIVKYAKDLEQLGLL

SEQ ID NO	Organism	GENBANK reference	Amino acid sequence
7	<i>Segniliparus rotundus</i>	ADG98140.1	MTQSHQTQGPQASAAHSRLARRAAELLATDPQAAATLPDPEVVQRATRPGLRLAERVDAIL SGYADRPALGQRSFQTVKDPITGRSSVELLPFTDITTYRELREATAIASDLAHPQAPA KPGDFLASIGFISVDVVAIDVAGVAGLTAVPLQTGATLATITAITAETAPTLEAASIEH LPTAVDAVLATPSVRRLLVFDYRAGSDEDEAVEAAKRIADAGSSVLVDVDEVIARGK SAPKAPLPATDAGDDSLIYTSGSTGTPKGAMYPERNVAFHWGGVWAAAFEDEDAAPP VPAINITFLPSHVASRLSMLPTLARGGLMHFAKSDLSLTFEDLKLRPTNLFVPRVV EMLYHQSELDRRGVQDGTREAEAVKDDLRITGLLGGRLTAGFGSAPLSAEIAGFIESL LQIHLVDGYGSTEAGPVWRDGYLVKPPVTDYKIIDVPELGYFSTDSPHPRGELAIKTQTI LPGYKRPETTAEVEDDEDFYLTGDVVAQIGPEQFAYVDRRNVLKLSQGEFVTLAKLEA AYSSPLVRQLFVYSSERSYLLAVIVPTPDALKKFGVGEAAKALGESLQKIARDEGLQ SYEVRDFIETDPTVENGLLSDARKSLRPKKEHYGERLEAMYKELADGGQANELRDIR RGVQQRPTLETVRRAAAAMLGASAAEIKPDHFTDLGGDSLSALTFSNLFHDLFEVDVPV GVIVSAANTLGSVAEHIDQLAGGRARPTFATVHGKSTTIKASDLTLDKFIDEQTLKLEA KHLKPADPPRTVLTGANGWLGRFLALEWLERLAPAGGKLITVRGKDAQAQAKARLDA YESGDKLAGHYQDLAATTLEVLADGSEPLGLDEATWNLRLADEVDFISHPGALVNHVL PYNQLFGPNVAGVAEIKLAITTRIKPVYTLSTVAVAGVEPSALDEDDGDIRTVSAERSV DEGYANGYNSKWGVEVLLREAHDRGTGLPVRVFRSDMILAHQKYTGQVNTDQFTRLVQS LLATGLAPKSFYELDAQGNQRAHYDGIPVDFTAESITTLGGDLEGYRSYNVFNPHRDG VGLDEFVDWLEAGHPITRIDDDYDQWLSRFETSLRGLPESKRQASVLPLLHAFARGPVAV DGSFPRNTVFRDQVQKAKIGAEHDIPHLGKALVLYADDIKQLGLL
8	<i>Chromobacterium violaceum</i>	AAQ59697.1	MQKQRTTSQWRELDAAHLLHPFTDTASLNQAGARVMTRGEGVYLWDSEGNKIIDGMAGLW CVNVGYGRKDFAEAAARRQMEELPFYNTFFKTTHPAVVELSLLAEVTPAGFDRVFYTNNG SESVDTMIRMVRRYWDVQGKPEKKTIGRWNGYHGSTIGGSLGGMKYMHEQGDLPPIGM AHIEQPWWYKHGKDMTPDEFGVVAARWLEEKILEIGADKVAAFVGEPIQGAGGVVPPAT YWPEIERICRYDVLVLADEVICGFGRTGEWFGHQHFGQPDLTAAKGLSSGYLPIGAV FVGKRVAEGLIAGGDFNHGFTYSGHPVCAAVAHANVAALRDEGIVQRVKDDIGPYMQKRW RETFSRFEHVDDVRGVGMVQAFTLVKNKAKRELFPDFGEIGTLCRDIFRNNLIMRACGD HIVSAPPLVMTRAEVDEMLAVERCLEEFEEQTLKARGLA
9	<i>Pseudomonas aeruginosa</i>	AAG08191.1	MNARLHATSPGLDADLVADQAHYMHGYHVFDHVRVNGSLNIAAGDGYIYDTAGNRYLD AVGGMWCTNIGLGREEMARTVAEQTRLLAYSNPFCDMANPAIELCRKLAELAPGDLHDV FLTTGGSTAVDTAIRLMHYQNCRGKRAKHVITRINAYHSGTFLGMSLGGKSADRPAAEF DFLDERIHHLACPYYYRAPEGLGEAEFLDGLVDEFERKILELGADRVGAFISEPVFGSGG VIVPPAGYHRRMWELCQRYDVLISDEVVTSFGRIGHFFASQAVFGVQPDILTAKGLTS GYQPLGACIFSRRIWEVIAEPDKGRCSFHGFTYSGHPVACAAALKNIEIEREGLLAHAD EVGRYFEERLQSLRDLPIVGDVRGMRMACVEFVADKASKALFPESLNIGEWVHLRAQKR GLLVRPIVHLNVMSPPLITREQVDTVVRVRESIEETVEDLVRAHR

SEQ ID NO	Organism	GENBANK reference	Amino acid sequence
10	<i>Pseudomonas syringae</i>	AA539893.1	MSANNPQTLEWQALSSSEHHLAPFSDYKQLKEGKPRIITRAEGVYLWDSEGNKILDMGMSGLWCVAIGYGREELADAASKQMRELPPYNNLFFQTAHPVLELAKAISDIAPEGMNHHVFTGSGSENDTMLRMVRYHWALKGPQNKTIISRVNGYHGSTVAGASLGGMTYMHEQGDLPIPGVWHIPQYWFGEGGDMTPDEFGIWAEEQLEKILELVENVGAFIAEPIQGAGGVVPPDSYWPKEILSRYDILFAADEVICFGRTSEWFGSDFYGLRPMMTIAKGLTSGVYPMGGLVIRDEIVAVLNEGDFNHGFTSYGHPVAAVALENIRILREEKIVERVSRSETAPYLQKRLRELSHDPLVGEVGVGLLGAIELVKDKTTTTRERTYDKGAGMICRTFCFDNGLIMRAVGDTMIIAPLVISFAQIDELVEKARTCLDLTLAVLQG
11	<i>Rhodobacter sphaeroides</i>	ABA81135.1	MTRNDATNAAGAVGAAMRDHILLPAQEMAKLIGKSAQPVLTAEIGYVHTEDGRRLLDGPAGMWCAQVYGRREIVDAMAHQAMVLPYASWPYMATSPAARLAEKIALTLTPGDLNRIFFTTGGSTAVDSALRSEFYNNVGLRPGQKRIIVRDYGHGSTALTAACGTGRTGNWPNFDIAQDRISFLSPNPRHAGNRSQEAFLDDLQVEFEDRIESLGPDTIAFLAEPILAGSGGVIIPPAYGHARFKAICEKHILYISDEVVTGFGRCGEWFASEKVFVWVDPDIITFAKGVTSGVVPLGLAISEAVLARISGENAKGSWFTNGTYTNSQPVACAAALANIELMEREGIVDQAREMADYFAAALASRLDLPVGAETRSVGLVGCVCQLDPTRADGTAEADKFTLKIDERCFELGLIVRPLGLDLCVISPLIISRAQIDEMVAIMRQAITEVSAAHGLTAKEPAAV
12	<i>Escherichia coli</i>	AAA57874.1	MNRLPSSASALACSAHALNLIKRTLDEHEMKALNREVIEYKEHVNPGFLEYRKSVTAGGDYGAVIEWQAGSLNTLVDTGQGFIDCLGGGFVNGHNPVWVSAVQNQLAKQLHSQELLDPLRAMLAKTLAALTGPKLYSFFCNSGTSVEAALKAKAYQSPPRGKFTFIATSGAFHGKSLGALSATAKSTFRKPFMPLPGFRHVFPFGNIEAMRTALNECKKTGGDDVAAVILEPIQEGGVILPPPGYLTAVRKLCEFGALMILDEVQTMGRTGKMFACHEHNVQPDILCLAKALGGGVMPIGATIATEEVFSVLFDNPFLLHTTFFGNGPLACAAALATINVLLEQNLPAAQAEQKGDMLLDGFRQLAREYPDLVQEARGKGMLMAIEFVDNEIGYNFASEMFRQRVLVAGTLNNAKTIRIEPPLTLTIEQCELVKAARKALAAMRVSEEA
13	<i>Vibrio Fluvialis</i>	AEA39183.1	MNKPOSWEARAETVSLYGTDMPSLHQRTGVVVTTHGEGPIYDVNGRRYLDANSGLWNMVAGFDHKGILDAAKAQYERFPGYHAFFGRMSDQTMVLSKLVESVSPFSGRVSFTNSGSEANDTMVKMLWFLHAAEGPKQKRLTRWNAHYHGVTAVSASMTGKPNYSVFGPLPGFVHLTCPHYWRYGEEGETEEQFVARLARELEETIQREGADTIAGFAEPMVGAGVIPPAGKYFQAAILPILRYDIPVISDEVICGFGRTGNTWGCVTYDFTPDIISSKNLTAGFFPMGAVILGPELSKRLTAIEAIEEFPFHGFTASGHPVGCAIALKAIDVVMNEGLAENVRLRAPRFEERLKHIAERPNIIGEYRGIGFMWALEAVKDKASKTPFDGNLSVSERIANTCTDLGLICRPLGQSVVLCPPFILTEAQMDMEFMDKLEKALDKVFAEVA
14	<i>Bacillus subtilis</i>	CAA44858.1	MKIYGYMDRPLSQEENEFMSFISPEKRCRRFYHKEDAHRTLLGDVLVRSVISRQYQLDKSDIRFSTQEYGPCKIPDLPAHFNISHSGRWVWICAFDSQPIGIDIEKTKPISLEIAKRRFSKTEYSDLLAKDKDEQTDYFYHLWSMKESFIKQEGKGLSLPLDSFVRLHQDGGQVSI FI PDSHSPCYIKTYEVDPGKMAVCAAHPEDFPDITMVSYEII

SEQ ID NO	Organism	GENBANK reference	Amino acid sequence
15	<i>Nocardia</i> sp. NRRL 5646	ABI83656.1	MIETILPAGVESAEELLEYPEDLKAHPAEELHIAKSVKRRRDFIGARHCARLALAEELGEP PVAIGKGERGAPIWPRGVVGSLLTHCDGYRAAAVAHKMRFRSIGIDAEPHATLPEGVLDSV SLPPEREWLKTDDSAHLDRLLFCAKEATYKAWWPLTARWLGFEEAHITFEIDGSADSG NGTFHSELLVPGQTNDGGTPLLSDGRWLIADGFILTAIAYA
16	<i>Bacillus cereus</i>	AAS43086.1	MINKTLLQKRFNVAASVDQYANVQKKMAHSLSTLNRRYSTNSSIRILELGCCTGVTE QLSNLFPKAQITAIIDFAESMIAVAKTRQNNVNVTFYCEDIERLRLEETYDVIISNATFQW LNDLKQVITNLFRHLSIEGILLFSTFGQETFQELHASFORAKEEKNIQNETSIGQRFYSK NQLRHICEIETGDVHVSETCYIERFTEVREFLHSIRKVGATNSNEESYCQSPSLFRAMLR IYERDFTGNEGIMATYHALFVHITKEGKR
17	<i>Escherichia coli</i>	AAC76437.1	MNNIWWQTKGGNVHLVLLHGWGLNAEVWRCIDEELSSHFTLHLVDLPGFGRSRGFGALS LADMAEAVLQQAPDKAIWGLWSLGLVASQIALTHPERVQALVTVASSPCFSARDEWPFI KPDVLAGFQQQLSDDFQRTVERFLALQTMGTETARQDARALKKTVLALPMPEVDVNLNGGL EILKTVDLRQPLQNVSMPLRLYGYLDGLVPRKVPMLDKLWPHSESYIFAKAAHAPFIS HPAEFCHLLVALKQRV

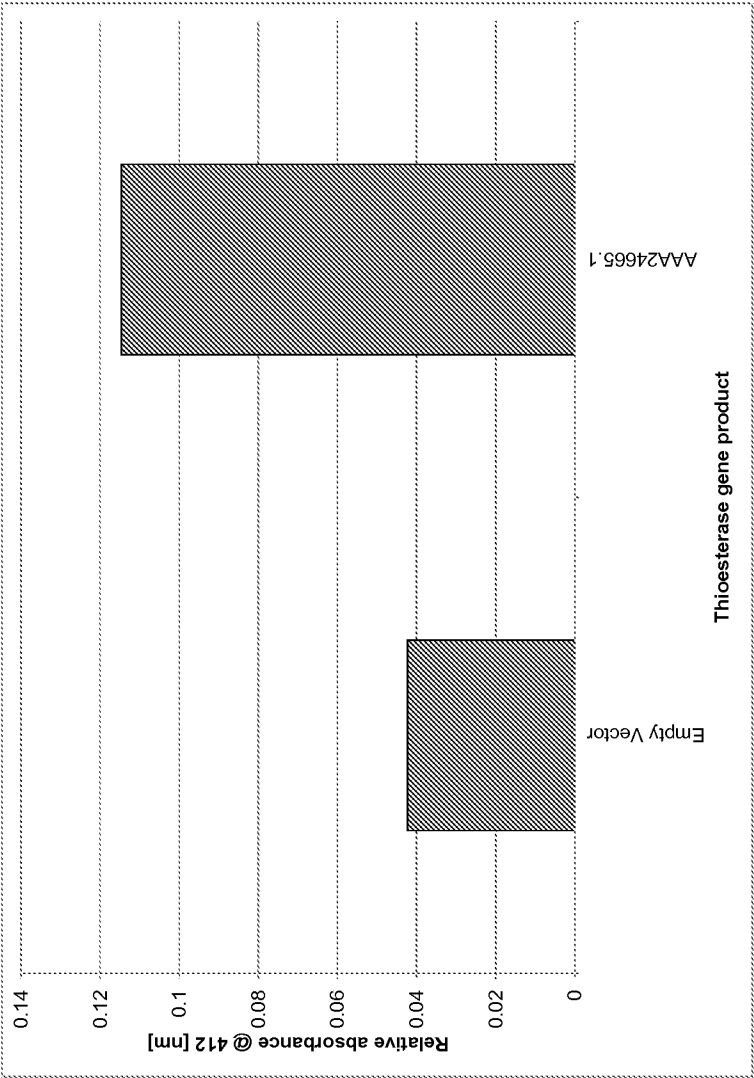


FIGURE 8

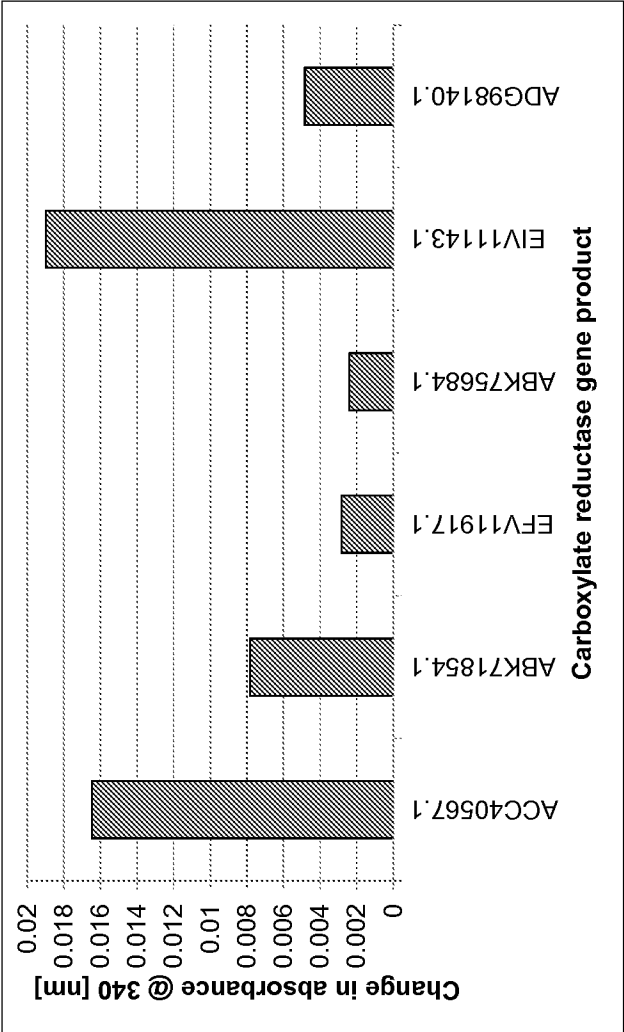


FIGURE 9

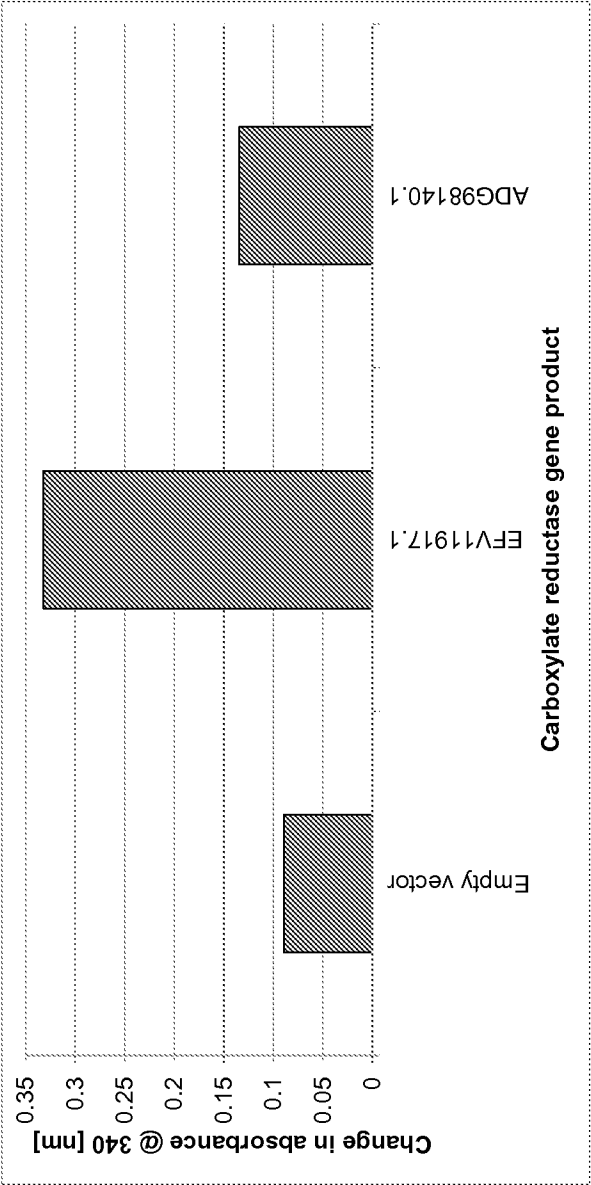


FIGURE 10

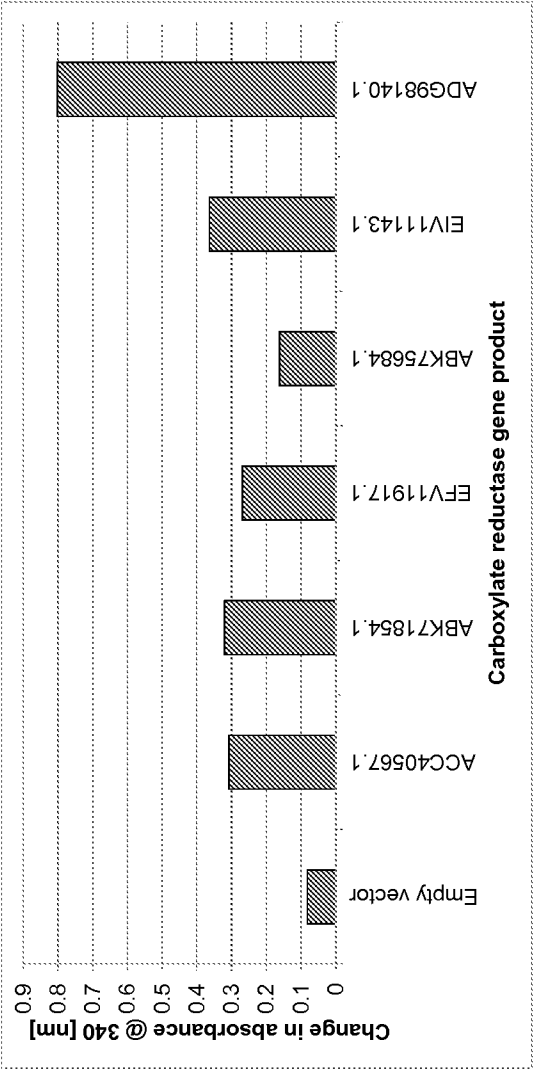


FIGURE 11

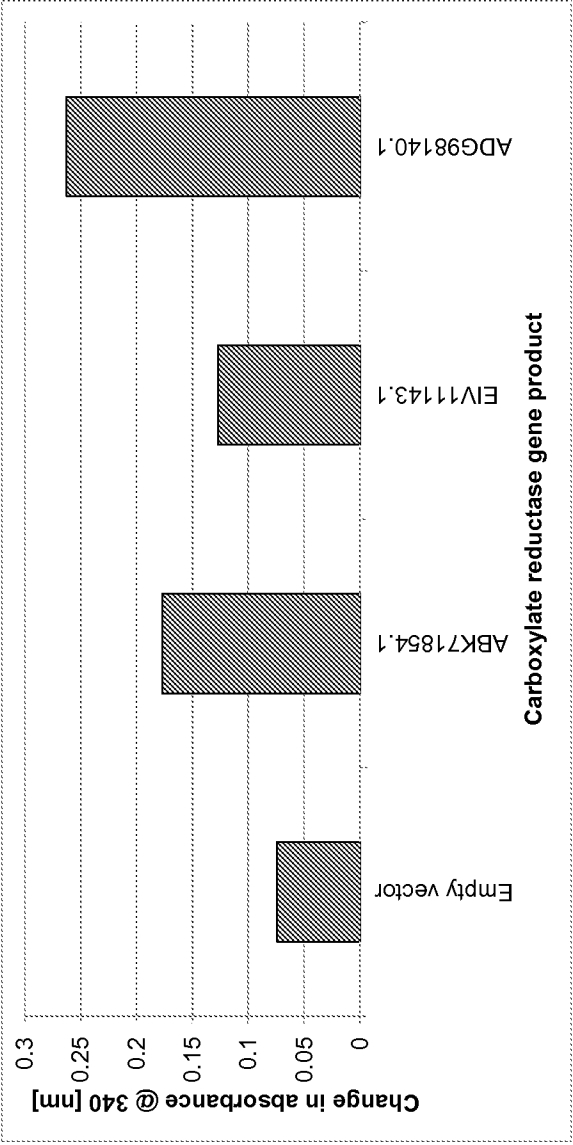


FIGURE 12

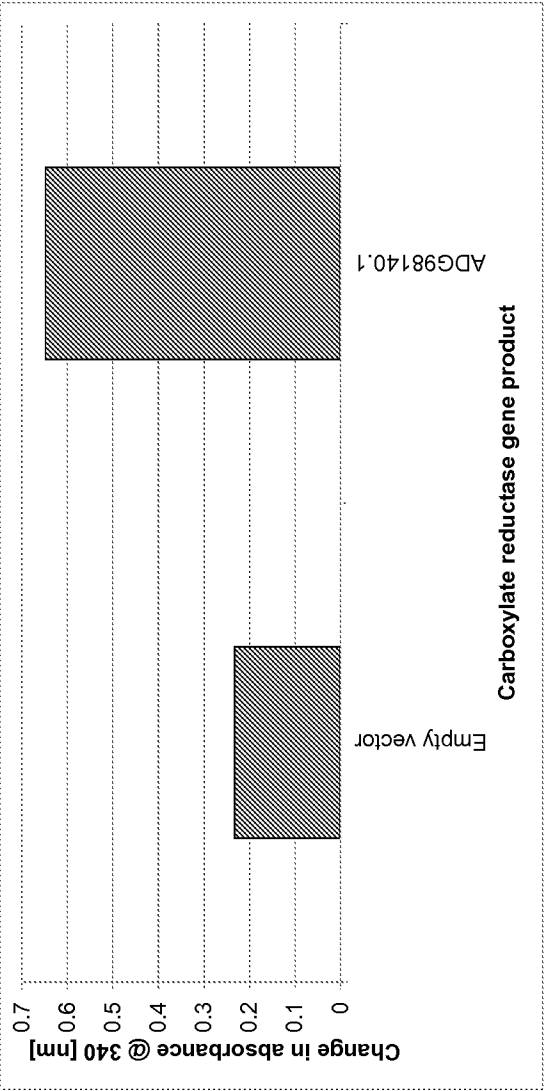


FIGURE 13

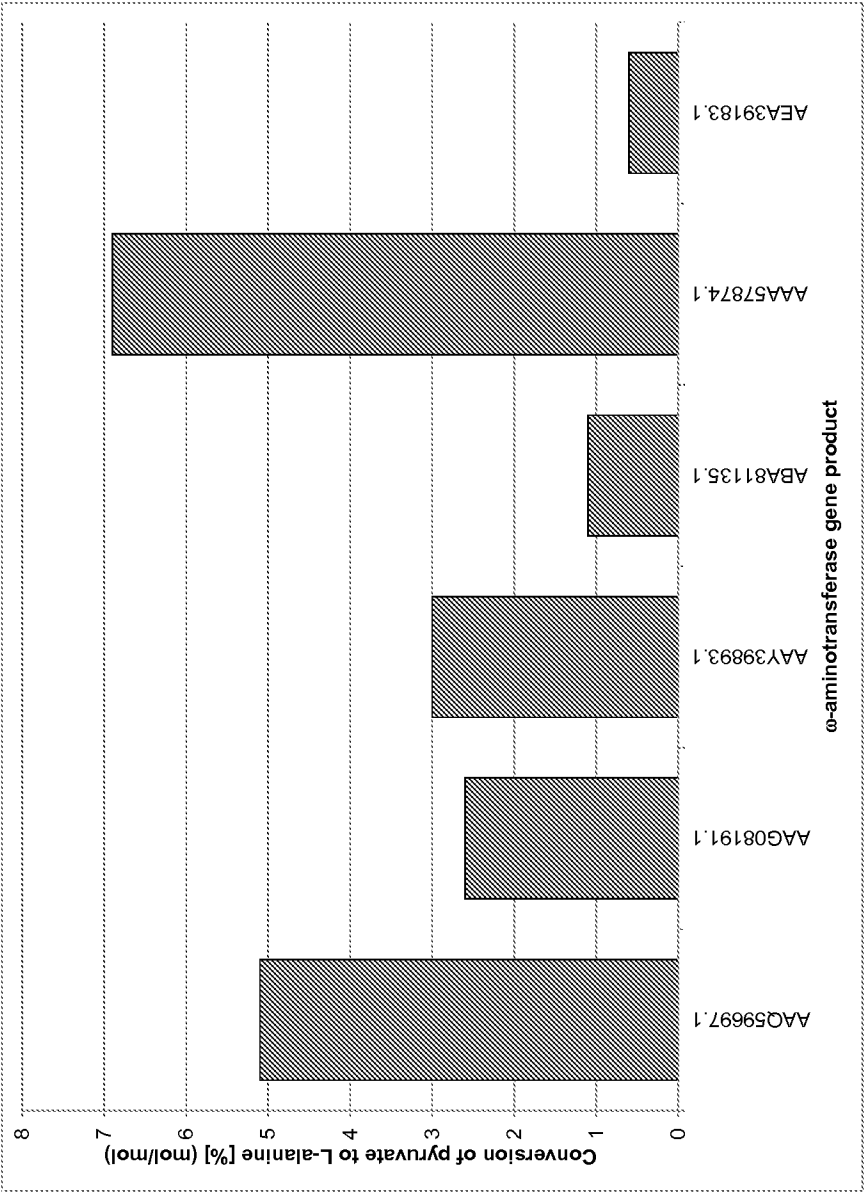


FIGURE 14

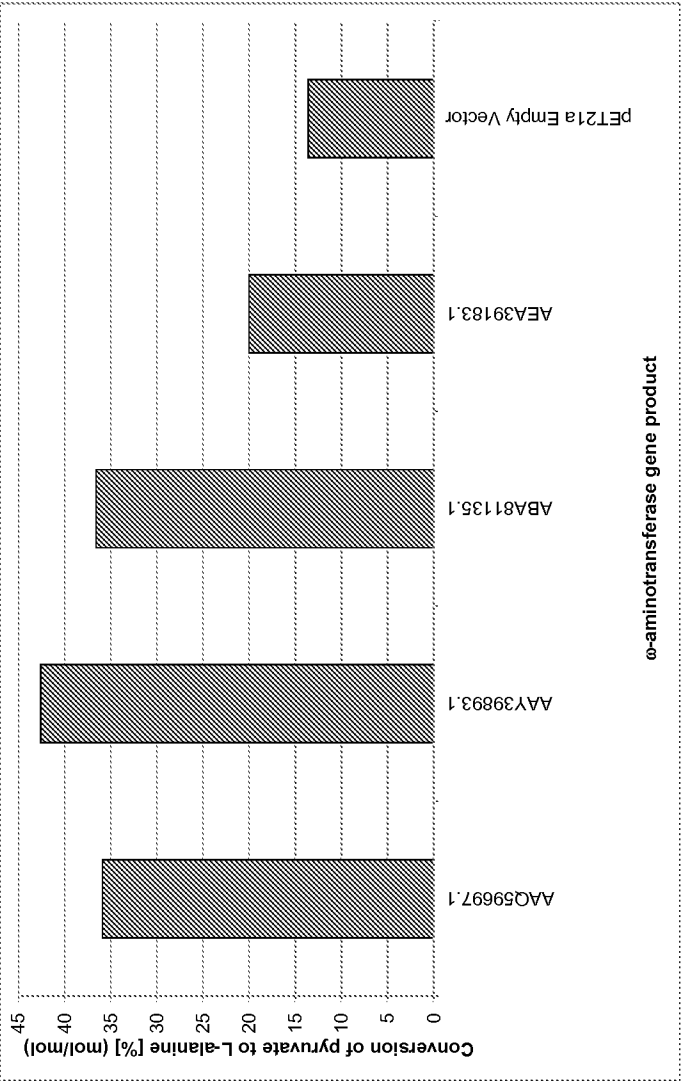


FIGURE 15

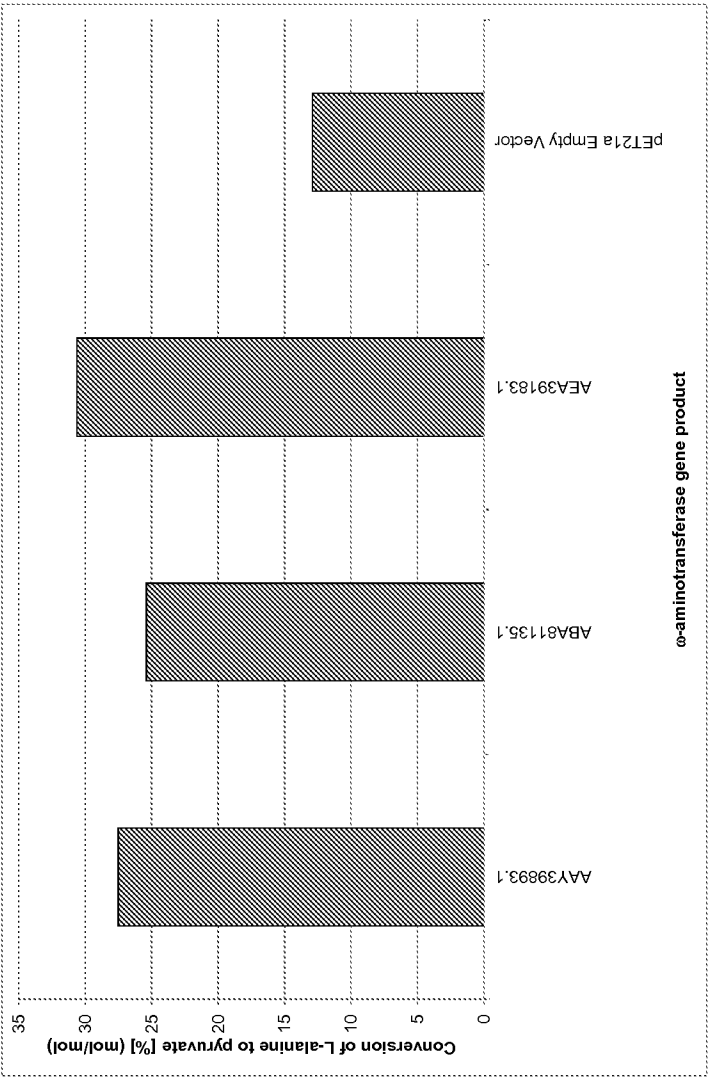


FIGURE 16

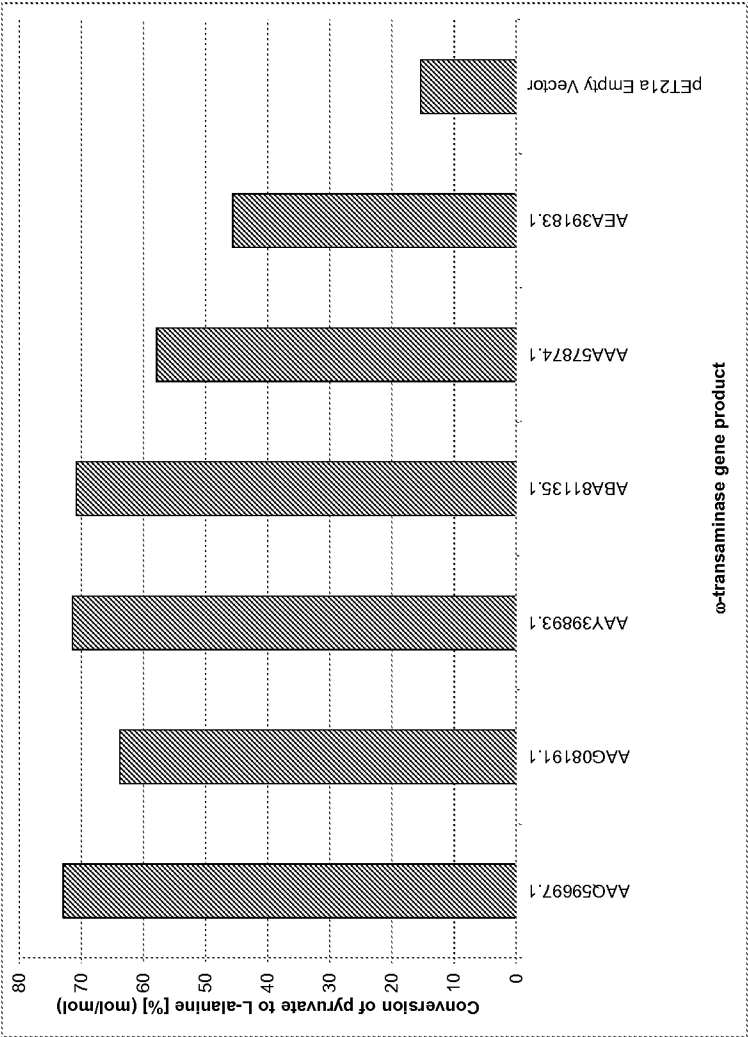


FIGURE 17

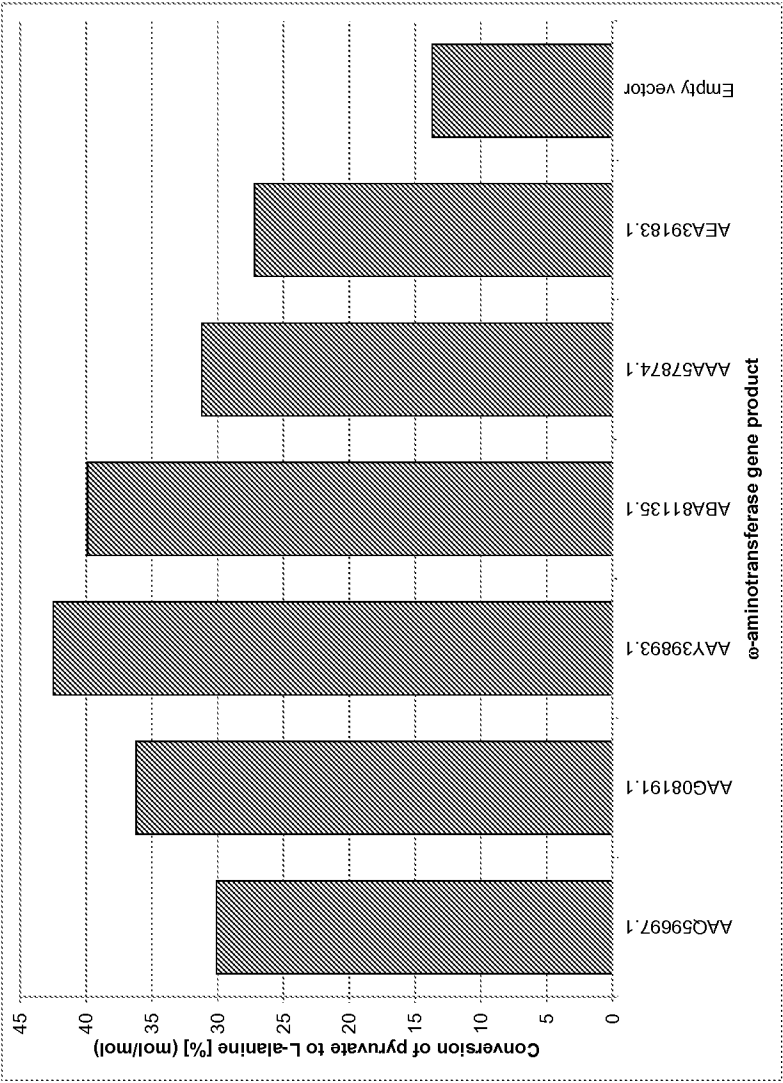


FIGURE 18

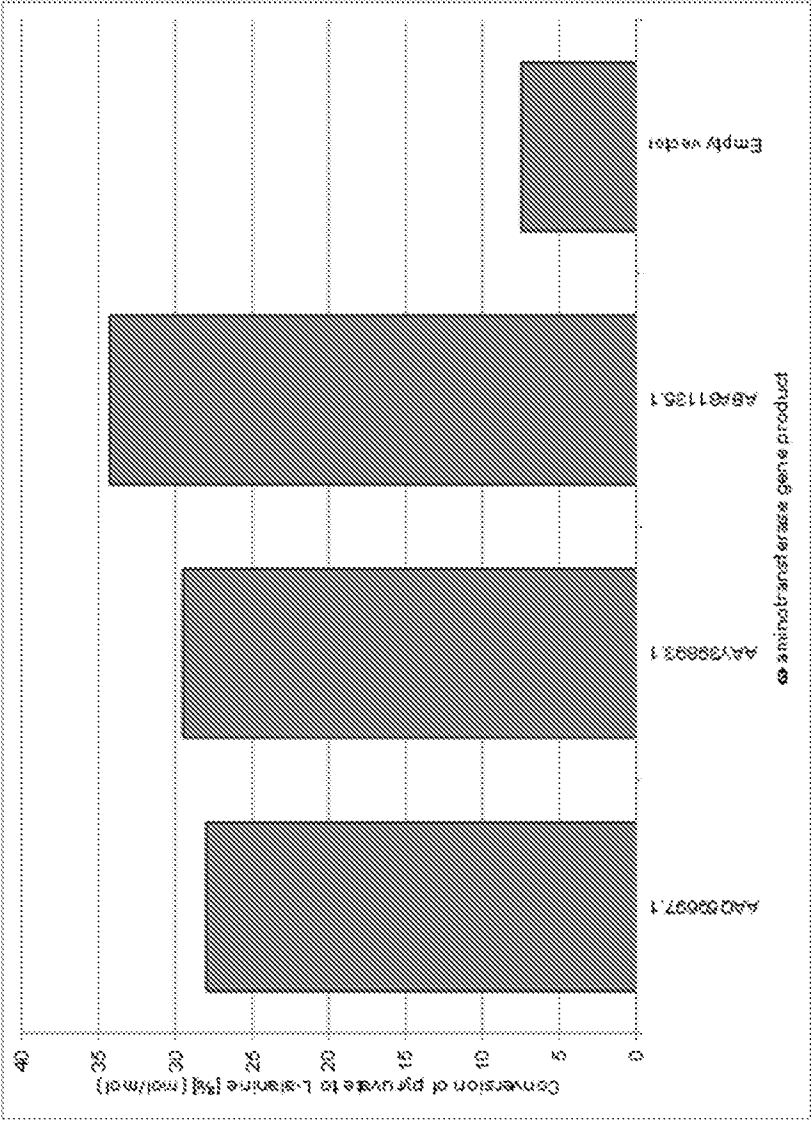


FIGURE 19

FIGURE 20

Sample ID	Analyte	Mwt [g/mol]	Peak Retention Time [min]	Peak Area @ 260nm [mAu]	Observed Mass (m/z)		Comments
					Negative mode (M-H)	Positive mode (M+H)	
Reference Standard	pimeloyl-CoA methyl ester	923	6.161	31172.1	922	924	
Biotransformation at 1 [h] time point #1	pimeloyl-CoA methyl ester	923	nd	nd	nd	nd	No substrate detected after 1 [h]
	pimeloyl-CoA	910	5.577	3956.1	908	910	
Biotransformation at 1 [h] time point #2	pimeloyl-CoA methyl ester	923	nd	nd	nd	nd	No substrate detected after 1 [h]
	pimeloyl-CoA	910	5.579	3974.1	908	910	
Biotransformation at 1 [h] time point #3	pimeloyl-CoA methyl ester	923	nd	nd	nd	nd	No substrate detected after 1 [h]
	pimeloyl-CoA	910	5.577	4093.3	908	910	
Substrate only control (no enzyme) at 1 [h] time point	pimeloyl-CoA methyl ester	923	6.333	4577.9	922	924	No product detected after 1 [h]
	pimeloyl-CoA	910	nd	nd	nd	nd	